KIDNEYWEEK 2012
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TH-OR002

Regulation of Renal Cyst Formation by Phosphodiesterase 1A in Zebrafish

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Background: A large body of evidence indicates the importance of elevated cAMP in Polycystic Kidney Disease (PKD). Accumulation of cAMP in cystic tissues may in part be due to enhanced adenylyl cyclase activity, but inhibition of cAMP degradation by phosphodiesterases (PDE) likely plays an important role, as cAMP is inactivated faster than it is synthesized. PDE1 is the only PDE family activated by calcium, which is reduced in PKD cells.

Methods: To assess the contribution of the PDE1A subfamily to renal cyst formation, we examined the expression and function of PDE1A in zebrafish using RT-PCR and antisense morpholinos (MO), and effects of the PDE1 inhibitor, vinpocetine.

Results: We identified two splice isoforms with alternative starts corresponding to human PDE1A1 and PDE1A5. Each has 77% amino acid identity to its human ortholog overall, and 89% in the hydrolase domain. Two splice-blocking (SB) MO caused deletion of exon 7, at the beginning of the hydrolase domain, and increased the percent of embryos with renal cysts and/or tubule dilation (32%±12 (n=4) or 12%±5 (n=3) vs 1%±1, p≤0.002, t-test). One SB MO also caused phenotypes similar to PKD2 mutant zebrafish including hydrocephalus (79%±14 vs. 3%±2, p=3e-6, t-test, n=4) and curvature (44%±12 vs. 10%±5, p=0.004, t-test, n=4). A third MO targeting the start of the PDE1A1 isoform also increased the percent of embryos with renal cysts (42%±13 vs 3±2, p=0.01, t-test, n=4) and hydrocephalus (85%±8 vs 3%±3, p=0.001, t-test, n=4). Vinpocetine caused defects in glomerulus development visualized in a transgenic reporter fish, Tg(*wt1b*:EGFP). Vinpocetine additionally caused edema, dorsal curvature, and inhibited clearance of 10K MW rhodamine-dextran. Differences between vinpocetine and PDE1A MO may be due to inhibition of PDE1B and PDE1C, in addition to PDE1A, by vinpocetine.

Conclusions: Overall, data indicate that PDE1A has potent effects on kidney development and cyst formation, and are consistent with PDE1A function downstream of Polycystin 1/Polycystin 2.

Funding: NIDDK Support

TH-OR003

Defective Glucose Metabolism in Polycystic Kidney Disease Identifies a Novel Therapeutic Paradigm

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Background: ADPKD is a common genetic disorder characterized by bilateral renal cyst formation. Identification of de-regulated cascades has led to the initiation of several clinical trials, but an effective therapy is still lacking. Using *Pkd1* knock-out cells we identified metabolic alterations in ADPKD that could be targeted for therapy.

Methods: We performed a metabolomic screening using NMR analysis followed by biochemical studies, microarrays and real-time analysis of the expression levels of the enzymes involved using cells as well as cystic tissues derived from either a PKD mouse model or human ADPKD kidneys.

Results: Metabolomic profiling of the extracellular medium of $Pkd1^{-/-}$ and $Pkd1^{+/+}$ MEFs indicated an increase in glucose uptake and lactate production in the mutant cells, suggesting an increased aerobic glycolysis.

Consistent with this, intracellular ATP concentration and glycolytic enzymes gene expression was enhanced in the mutant compared to the wt cells whereas the mitochondrial potential was unchanged. Notably, glucose deprivation reduced proliferation and sensitized *PKD1* mutant cells to apoptosis.

Next, we analysed glycolytic enzymes gene expression using microarray databases derived from *PKD1* human renal cysts and found that several genes encoding glycolytic enzymes were up-regulated. A similar upregulation of glycolytic enzymes and ATP content was found in the cystic kidneys of *a* $Pkd1^{flox/-}$*:Ksp-Cre* mouse model.

Notably, treatment of this mouse model with 2DG, an inhibitor of glycolysis, greatly reduced kidney weight and proliferation rates in the cystic epithelia. Importantly, 2DG also increases the number of non-cystic tubules. Finally, we show that these metabolic alterations depend on the ERK pathway acting by inhibiting the LKB1-AMPK axis and activating the mTORC1-glycolytic cascade.

Conclusions: Our data show that defective glucose metabolism is involved in ADPKD and provide the rationale for a novel therapeutic approach.

Funding: Private Foundation Support

TH-OR004

A BET Bromodomains Inhibitor Decreases Cystic Epithelial Cell Proliferation through Targeting c-Myc

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Background: Overexpression of c-Myc is proposed to play an important role in the pathogenesis of polycystic kidney disease (PKD). The increased expression of c-Myc has been reported in all rodent models of PKD and human ADPKD, which makes c-Myc as a potential therapeutic target in ADPKD. JQ1, a selective small-molecular inhibitor of BET bromodomains (BRD), down-regulates c-Myc transcription through inhibition of BRD proteins. We hypothesized that targeting c-Myc with JQ1 might prevent/delay cyst formation.

Methods: To determine whether overexpression of c-Myc in renal cystic epithelial cells is induced by BRD proteins and test whether JQ1 prevents/delays renal cyst formation in ADPKD mouse model, we treated renal cystic epithelial cells and *Pkd1* conditional knockout mice with JQ1.

Results: We found that c-Myc mRNA and protein expression were upregulated in Pkd1 mutant mouse embryonic kidney epithelial (MEK) cells, postnatal *Pkd1* homozygous PN24 cells and kidney tissues from $Pkd1^{flox/flox}$:Ksp-cre mice compared to that in *Pkd1* wild type MEK cells, postnatal *Pkd1* heterozygous PH2 cells and *Pkd1* wild type kidneys respectively. We also found that BRD4 mRNA was increased in *Pkd1* postnatal homozygous PN24 cells compared to that in *Pkd1* postnatal heterozygous PH2 cells. Treatment with JQ1 1) decreased the levels of c-Myc mRNA and protein in a time-dependent manner in *Pkd1* mutant MEK cells and PN24 cells; 2) increased the expression of p21 mRNA and protein, which is transcriptionally down-regulated by the c-Myc; 3) decreased the phosphorylation of Rb in *Pkd1* mutant MEK cells; 4) decrease cystic epithelial cell proliferation indicated by inhibition of S phase entry with FACS analysis. These results suggested that JQ1 might inhibit renal cystic epithelia proliferation through BRD4-c-Myc- p21 pathway. Further, we found that JQ1 strikingly delayed cyst development in the kidneys from $Pkd1^{flox/flox}$:Ksp-cre mice.

Conclusions: In sum, JQ1 produces a potent antiproliferative effect through targeting c-Myc in renal cystic epithelia and delays renal cyst formation, which may function as a therapeutic strategy in ADPKD.

Funding: NIDDK Support

TH-OR005

Novel Insights into the Development of Renal Fibrosis in ADPKD

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by two phases: 1) bilateral kidney enlargement; and 2) diffuse intraparenchymal fibrosis. The C57BL6/J-$Pkd1^{nl/nl}$ (Pkd1-W) is a novel orthologous mouse model of ADPKD which demonstrates both characteristic phases with cyst formation beginning in late gestation and fibrosis beginning at PN28. This model is ideal for studying the pathogenesis and potential therapeutic intervention of both phases. KD019, is a novel multi-kinase inhibitor effective in reducing cyst formation (In Press JASN 2012).

Methods: In this study we tested the hypothesis that KD019 retards ADPKD specific fibrosis. Cystic and control Pkd1-W mice received KD019 (30mg/kg;IP) or vehicle daily from PN-8 to PN 28. Evaluation included target analysis and changes in molecular and standard pathologic markers of fibrosis.

Results: KD019 decreased phosphorylation (activity) of all KD019 targets including ErbB1, ErbB2, c-Src and VEGFR2 (KDR). However, expression of TGF-β, TNF-α, and macrophage recruitment to interstitial areas showed no difference compared to controls. Despite decreasing cystic changes, KD019 therapy did not eliminate early stages of interstitial fibrosis.

Conclusions: These data suggest that in genetically-determined ADPKD, the fibrosis which leads to renal failure may be independent of the pathways that lead to cyst formation. The identification of a RTK-independent pathway of renal interstitial fibrosis in ADPKD may provide new insight into the development of interstitial fibrosis in a number of progressive renal diseases in which fibrosis and disease progression follow an acute insult despite elimination of the initial injury. In conclusion, we speculate that in ADPKD, the two phases of: 1) cyst formation and growth; and 2) renal fibrosis may be independent. If confirmed, treatment of human ADPKD in the future may require anti-fibrotic therapies in addition to therapies which reduce cyst formation.

Funding: NIDDK Support, Private Foundation Support

TH-OR006

A Novel PPARγ Agonist DJ5 Retards Cyst Growth and Preserves Kidney Function in a PKD1 Conditional Knock Out Mouse Model

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Background: Several studies have shown that the TZD class of PPARγ agonists could reduce renal cystogenesis, retard the progression of kidney failure and prolong survival in animal models of ADPKD. However, treatment with TZDs was associated with fluid retention and heart failure. To overcome these side effects, we developed a novel non-TZD PPARγ agonist termed DJ5 which did not cause cardiac side effects in the Han:SPRD rat model of PKD, yet it significantly inhibited cyst epithelial cell proliferation and retarded cyst development in these rats.The purpose of the present study was to investigate the therapeutic effect of DJ5 in a mouse model which is orthologous to human ADPKD.

Methods: Tamoxifen (150 mg/kg/d) was given to nursing mothers of *Pkd1* knockout mice from P10 to P12. From P7-P21, DJ5 (100 mg/kg/day) was administered to the nursing mothers by gavage. After weaning, from P22-P35, DJ5 (100 mg/kg/day) was administered to the mice directly through gavage. At P36, 24-hr urine samples were collected and the kidneys were harvested.

Results: Compared with vehicle treated *Pkd1*$^{-/-}$mice, DJ5 treatment reduced the BUN for 80.5% (P=0.002), the the two-kidney/total body weight ratio for 43.8% (P=0.014) without causing body weight loss. cyst volume density for 30.5% (P=0.001). Ki-67 staining was decreased while TUNEL staining was increased in dilated tubular and cyst epithelial cells in DJ5 treated *Pkd1*$^{-/-}$ mice compared with the vehicle group.

Conclusions: The new PPARγ agonist DJ5 markedly delays the loss of renal function, retards cyst development, inhibits the dilated tubular epithelial cell proliferation, and increases apoptosis in cyst lining epithelial cells in *Pkd1*$^{-/-}$ mice. DJ5 might become a potential drug candidate to retard progressive renal failure in patients with ADPKD in the future. The mechanisms of the pathways which are influenced by DJ5 will be further investigated.

TH-OR007

Glucosidase II Activity Represents a Novel Urine Biomarker in the Detection Autosomal Dominant Polycystic Liver Disease (ADPLD) due to Mutations in *PRKCSH* Sorin V. Fedeles,[1] Xin Tian,[1] Rachel Gallagher,[1] Sohan Lal,[1] Ming Ma,[1] Vicente E. Torres,[2] Stefan Somlo.[1] *[1]Internal Medicine/Nephrology, Yale School of Medicine, New Haven, CT; [2]Internal Medicine/Nephrology, Mayo Clinic, Rochester, MN.*

Background: *PRKCSH* is one of the genes mutated in Autosomal Dominant Polycystic Liver Disease (ADPLD) and it encodes the non-catalytic β-subunit of the ER glucose-trimming enzyme glucosidase II (GII), involved in transmembrane/secreted glycoprotein processing. The GIIβ subunit contains an HDEL motif through which it retains the catalytic GIIα subunit in the ER. In the current study we set forth to test the effect of *Prkcsh* deletion on the activity of GIIα in the context of murine and human ADPLD.

Methods: We performed GII substrate cleavage assays using cell media from GIIβ knockout cells and urine samples from both mice with conditional inactivation of *Prkcsh* and ADPLD patients with heterozygous truncating mutations in *PRKCSH*.

Results: Removal of the HDEL motif in *GIIβ* resulted in increased GIIα activity in the media of transfected Cos7 cells (p<0.001). Additionally, media from GIIβ knockout cells displayed a 2-fold increase in GIIα activity (p=0.0021). Conditional inactivation of *Prkcsh* in the kidney resulted in a gene dosage dependent increase in urine GIIα activity in *Prkcsh*$^{fl/+}$*;KspCre* mice (*p<0.05) compared to control which was further exacerbated in homozygous mutant *Prkcsh*$^{fl/fl}$*;KspCre mice (***p<0.001)*. Between the two experimental groups, urine activity was higher in the *Prkcsh* homozygous vs. heterozygous mice (*p<0.05). As a control, *Sec63*$^{fl/+}$*;KspCre* and *Sec63*$^{fl/fl}$*;KspCre* mice (*Sec63* is the other known ADPLD gene acting in the same pathway with *Prkcsh*) did not differ in urine GIIα activity. Finally, GIIα activity was examined in the urine of 4 ADPLD patients with truncating *PRKCSH* mutations and found to be ~3 fold higher than control (p<0.001).

Conclusions: These data suggest that *in vivo,* GIIα is secreted in the urine in a dose dependent manner with reduction or absence of the ER retention motif present on the *PRKCSH* gene product, GIIβ. These findings suggest the utility of the GII urine assay as a diagnostic tool for ADPLD patients with mutations in *PRKCSH.*

Funding: NIDDK Support

TH-OR008

Immune Cell Derived C3a AND C5a Mediate Human Alloreactive T Cell Immunity Paolo Cravedi, Jeremy S. Leventhal, Parth R. Lakhani, Peter S. Heeger. *Department of Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Mouse studies previously showed immune cell derived C3a/C5a are costimulatory intermediaries required for T cell activation. Herein we tested whether these concepts apply to human alloreactive T cells.

Methods: Human DCs were matured from monocytes and naïve human CD45RA+CD45RO-CD4+ T cells were isolated from peripheral blood by magnetic beads/sorting. Experimental strategies included RT-PCR, flow cytometry with CFSE dilution, ELISA (C3a, C5a, IL-12), and siRNA transfection.

Results: Human DCs produced C3, factor B (fB), and C5 RNA. LPS upregulated C3 and fB (135.8±17.8 and 166.1±14.4 fold, respectively, p<0.05). No C3, fB or C5 RNA was detected in resting or anti-CD3/CD28 stimulated T cells. DCs and naïve CD4 T cells expressed C3aR and C5aR on their surfaces. CD4 cells proliferated in response allogeneic DCs, yielding C3a and C5a in culture supernatants (p<0.05 vs control), indicating alloreactions cause complement production/activation. C3aR- and/or C5aR-blockade (specific antagonists) reduced T cell proliferation (C3aR block: -28.2±25.8%, C5aR block: -60.1±41.5%; p<0.05). We tested effects of increasing local C3a/C5a by downregulating (siRNA) DC expression of decay accelerating factor (DAF), a surface protein that restrains complement activation. In MLRs, DAF deficient DCs produced more C3a/C5a (1.9±0.4/1.4±0.4 fold, respectively; p<0.05) and enhanced T cell proliferation (26.0±2.0% increase, p<0.05). The augmented responses were abrogated by C3aR- and/or C5aR blockade (p<0.05). When we stimulated naïve CD4+ T cells with anti-CD3±C3a we observed a 3.4±0.2 fold increase in proliferation (p<0.05) along with increased p-AKT, the latter biochemically linking C3aR/C5aR to known T cell signaling pathways. C3a/C5a also augmented IL-12p70 production by DCs without altering costimulatory molecule or class II HLA levels.

Conclusions: Our findings that DC-derived and locally produced C3a/C5a mediate human alloreactive T cell responses provide new mechanistic insight, and support the need for testing whether C3aR/C5aR can be exploited as targets for treating T cell mediated allograft rejection in humans.

Funding: Other NIH Support - NIAID

TH-OR009

Critical Role of Notch Receptors in Alloimmunity Ciara N. Magee, Tetsunosuke Shimizu, Nader Najafian, Leonardo V. Riella. *Brigham & Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Notch receptor signaling is known to be crucial in cell development. More recently, Notch signaling has been found to play a key role in T cell activation and differentiation. Using both a genetic and antibody approach, we sought to investigate the role of Notch-1 and Notch-2 in transplantation.

Results: Using flox-cre technology, we first generated Notch1/2$^{flox/flox}$ CD4-Cre mice. We observed that N1N2DKO CD4$^+$ T cells proliferated 10-fold less than control CD4$^+$ T cells upon stimulation with irradiated allo-splenocytes *in vitro* (811±35 *vs* 9185±2360 cpm;p=0.0002), and, furthermore, produced significantly less inflammatory cytokines, as detected by Luminex (e.g. IL1β 3±0.07 *vs* 209±16;p<0.01).

We then investigated the effect of a novel, selective antibody against either Notch-1 (aNotch-1) or Notch-2 (aNotch-2) on allograft survival in a fully MHC mismatched (Balb/c→B6) transplant model. Mice treated with either aNotch-1 or aNotch-2 for 5 days had significantly prolonged graft survival compared to IgG-treated controls (MST 13 and 10 *vs* 7 days;p=0.0019 & 0.0023, respectively). Furthermore, the addition of aNotch-1 to a single dose of CTLA4-Ig synergistically prolonged graft survival from 30 to 66 days (p=0.002).

Using this model, we determined that use of aNotch-1 significantly inhibited CD4$^+$ and CD8$^+$ effector memory T cells, but not splenic CD4$^+$FoxP3$^+$ regulatory T cells (20.4±0.47 *vs* 19.8±1.2% controls;p=0.66). Further characterization of the thymus of aNotch-1 treated mice revealed interruption of the maturation of CD4$^-$CD8$^-$ double negative (DN) to double positive thymocytes at DN stage I (CD44$^+$CD25$^-$). Blockade of Notch1 also significantly affected lymphocyte function with a decrease in the frequency of cells secreting IFN-γ (250±40 *vs* 1014±7.9;p<0.0001) and Granzyme B (114±22 *vs* 390±10;p<0.0001), as well as a decrease in alloantibody production (IgG2>IgG1).

Conclusions: These data reveal an important role of Notch in T cell development and differentiation in alloimmunity. Selective targeting of Notch receptors may have the potential to modify the balance between effector and regulatory T cell subsets, a promising novel approach for immune modulation in transplantation.

TH-OR011

Different Effects of Calcineurin Inhibitor versus mTOR Inhibitor on the Proliferation, Activation and Differentiation of Human B Cells Giovanna La Monica,[1] Luting Xu,[1] Geetha Chalasani,[2] Lorenzo G. Gallon.[1] *[1]Nephrology/Medicine, Northwestern University Feinburg School of Medicine, Chicago, IL; [2]Renal-Electrolyte/Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: In organ transplantation, humoral immunity is increasingly recognized as an important factor in promoting transplant rejection. However, the direct effect of immunosuppressive drugs calcineurin inhibitor (Tacrolimus, TAC) and mTOR inhibitor (Sirolimus, SRL) on B cell proliferation, activation and differentiation is not well documented.

Methods: Purified human B cells from healthy volunteers were stimulated with two different protocols (BCR/CD40 and polyclonal TLR9/CpG) for 6 days. A variety of parameters of B cell activity including proliferation, activation, differentiation, and cytokine productions were monitored by flow cytometry.

Results: SRL at clinically relevant concentrations (e.g. 6 ng/ml) profoundly inhibited CD19+ B cell proliferation (up to ~2 folds) compared to controls whereas TAC at 6-10ng/ml had a minimal effect. SRL decreased total CD27+ memory B cells by 2-3 folds; however, this inhibition was on newly expressed CD27+ B cells but not from CD27+ memory B cells. SRL effectively blocked plasma cell differentiation (% of CD19+CD138+) even at low dose (e.g. 2ng/ml), and totally eliminated this cell population at over 6ng/ml (% of CD19+CD138+ and % of Blimp1+/Pax5(low) transcriptional factors). SRL-treated group decreased absolute B cell accounts, but maintained an activated phenotype (CD25+/CD69+) and increased expression of HLA-DR. Both SRL and TAC decreased % of CD24hiCD38hi Breg subset without affecting CD21hiCD23hi Breg subset. Finally, TAC was more effective than SRL in inhibiting inflammatory cytokine TNFalpha expression in B cells; however, both drugs had no effects on other cytokines tested, which include anti-inflammatory cytokines IL-10 and IL-35.

Conclusions: SRL strongly inhibits B cell responses when compared to TAC. These data can help to guide the use of SRL in humoral alloimmune responses in transplantation.

Funding: Clinical Revenue Support

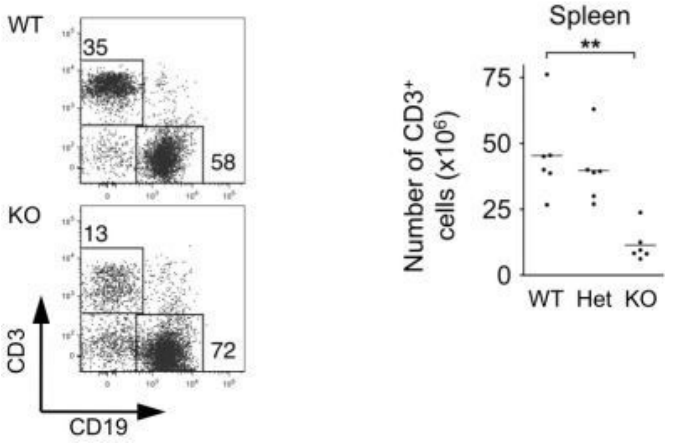
TH-OR012

Identification of microRNAs with Critical Roles in the Differentiation and Function of CD4+ T Helper Cells Kerem Atalar, Graham M. Lord. *MRC Centre for Transplantation, King's College London, London, United Kingdom.*

Background: CD4$^+$ T cells play a central role in allograft rejection. These cells may differentiate to multiple subtypes including T helper type 1 (Th1), Th2, Th17 and regulatory T cells, but the mechanisms that control differentiation are incompletely understood. It has also recently been recognised that interconversion between subsets is possible, but the pathways that regulate lineage stability and plasticity are poorly defined. microRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate gene expression, and T cells that are globally deficient in all miRNAs have been shown to exhibit abnormal development, survival and differentiation. However, current understanding of the specific miRNAs that are responsible for these defects is limited.

Methods: In order to identify candidate miRNAs of importance in CD4+ T helper cell differentiation and function we performed microarray profiling of miRNA expression in T cell subsets. These data have been analysed in combination with genome-wide master regulatory transcription factor binding data to select candidate miRNAs for functional investigation.

Results: We identified a single candidate miRNA for further study from microarray and binding data, and generated a mouse line that is deficient in this miRNA (KO). Analysis of these animals revealed dramatic defects in T cell homeostasis and survival *in vivo* compared with wild-type (WT). In addition, KO T cells exhibit default adoption of the Th1 phenotype following T cell receptor-mediated activation and impaired lineage stability.



Conclusions: The combination of miRNA microarray and ChIP data enabled the identification of a miRNA that is critically important for normal T cell function and homeostasis. We believe that these data have important implications for our understanding of T helper cell biology, and are therefore of relevance to clinical transplantation.

TH-OR013

Overexpression of MiR-126 in the Hematopoietic Compartment Protects against Renal Ischemia Reperfusion Injury Roel Bijkerk,[1,2] Coen van Solingen,[1,2] Pieter van der Pol,[1] Ellen Lievers,[1] Nicole Schlagwein,[1] Danielle Van Gijlswijk,[1] Annemarie Van Oeveren-rietdijk,[1,2] Hetty C. de Boer,[1,2] Antoine A.F. De Vries,[3] Cees van Kooten,[1] Frank Staal,[4] Ton J. Rabelink,[1,2] Anton Jan Van Zonneveld.[1,2] *[1]Nephrology, LUMC; [2]Einthoven Laboratory for Experimental Vascular Medicine, LUMC; [3]Cardiology, LUMC; [4]Immunohematology and Blood Transfusion, LUMC.*

Background: Hematopoietic stem/progenitor cells (HSPC) are known to promote kidney repair after ischemia/reperfusion injury (IRI). Stromal cell-derived factor-1 (SDF-1) has been described to provide protection against IRI and is thought to be an important mediator of mobilization of these HSPC to the site of injury. However, the exact mechanism of how SDF-1 protects against renal IRI remains under debate. We previously identified microRNA-126 (miR-126) to be able to modulate SDF-1 expression and subsequent mobilization of HSPC. Here we investigated the effect of overexpression of miR-126 in the hematopoietic compartment on IRI in the kidney.

Methods: Using a lentiviral construct we overexpressed miR-126 in lineage depleted bone marrow cells. Subsequently these cells were intravenously injected into lethally irradiated mice. Eight weeks after reconstitution of the bone marrow the mice underwent renal bilateral IRI. Mice were sacrificed 3 days after surgery.

Results: Blood urea levels were 40% reduced in mice that overexpressed miR-126 when compared with mice that were transplanted with mock-transduced cells. In addition, we found decreased levels of damage markers KIM-1 and NGAL. Q-PCR analyses of inflammatory cytokines showed a shift in the expression profile towards a protected state. We observed a 40% decrease in neutrophil influx as determined by Gr-1 staining. Surprisingly, we did not find a reduction in the number of CD45 positive cells or F4/80 positive cells. SDF-1 mRNA and protein levels were elevated and HSPC number was increased, suggesting a protective effect of miR-126 through SDF-1 in mobilization of HSPC.

Conclusions: Overexpression of miR-126 in the hematopoietic compartment protects against renal ischemia/reperfusion injury through a mechanism in which SDF-1 may play a central role.

TH-OR014

Multi-Photon Microscopy Based Kidney Live Imaging Identifies a Series of Micro-Circulation Changes in a Rat Acute Kidney Rejection Model Jun-Ya Kaimori,[1] Yoichi Kakuta,[4] Hidetoshi Tsuda,[1] Masaki Hatanaka,[2] Yoshitsugu Obi,[2] Hiromi Rakugi,[2] Masaru Ishii,[3] Shiro Takahara,[1] Yoshitaka Isaka.[2] *[1]ATT, Osaka Univ, Japan; [2]Geront & Neph, Osaka Univ; [3]Osaka Univ iFrec; [4]Urology, Osaka Univ.*

Background: Multi-photon microscopy based kidney live imaging enables us to see on-going phenomena in a living kidney, including microcirculation, and cell dynamics in the deep kidney tissue.

Methods: We constructed kidney live imaging system using an inverted multi-photon microscopy and special attachments for kidney live imaging, whose images were not affected by breathing or heart beating. We employed rat kidney syngeneic and allogeneic transplantation models, using GFP-expressing LEW rats as recipients and wild type LEW rats and wild type DA rats as donors, respectively, with contra-lateral native kidney spared. After 1-4 days post transplantation, graft kidneys were observed by this system and fluorescent dextrans.

Results: We observed more severe endothelial damages, vascular leakages and microcirculation defects in allogeneic kidneys than syngeneic. Administration of serum of allogeneic rat induced similar microcirculation defects in normal rat, suggesting that humoral factors in allogeneic animal serum were implicated in compromised microcirculation. Next we examined GFP-positive cells behavior in syngeneic or allogeneic transplanted kidneys, using GFP-expressing LEW rats as recipients. The time laps video images in allogeneic kidneys revealed that GFP-positive cells attached to and infiltrated between tubular cells. Surprisingly, almost GFP-positive infiltrating cells stayed in the same focal plane when they went across the lumen of tubules. In concordance with these images, cell tracking analysis using IMARIS software identified the decrease in mean velocity [50.4μm/min (syn) vs 22.2μm/min (allo), p<0.001] and confinement ratio [0.81 (syn) vs 0.73 (allo), p<0.001] of allogeneic kidneys, compared with syngeneic kidneys.

Conclusions: The multi photon microscopy based kidney live imaging system enables us to see the series of cortical microcirculation changes in an allograft kidney and will become one of powerful tools for renal transplantation researches.

TH-OR015

Immunomodulation with a Selective Cytopheretic Device (SCD) Improves Myocardial Contractility and Renal Sodium Excretion in a Canine Model of Congestive Heart Failure David Humes,[1,2,3] D. Buffington,[1] Angela J. Westover,[1] Hani N. Sabbah.[4] *[1]Innovative BioTherapies, Inc.; [2]University of Michigan; [3]CytoPherx, Inc.; [4]Henry Ford Hospital.*

Background: Cardiorenal syndrome (CRS), the most severe subset of patients with CHF, is characterized by diuretic resistance in volume overload. Current therapy is limited and new innovative approaches are needed. CHF is characterized by a proinflammatory state. The cytokines, TNF-a and IL-6, inhibit mitochondrial respiration and depress cardiac contractility (CC). Monocytes and tissue macrophages are sources of systemic inflammation in CHF. Systemic monocyte levels correlate with poor outcome in CHF. The SCD, a novel biomimetic device, when placed in an extracorporeal circuit with regional citrate (c) anticoagulation has been shown to be an effective immunomodulatory device in acute multiorgan organ failure.

Methods: To evaluate the acute effects of the SCD in a canine model of CHF, three groups of animals were evaluated during 4 hours of treatment: SCD-C, SCD-Heparin (H), and a sham control (S-C), n=2-5 in each group.

Results: Left ventricle (LV) ejection fraction (EF) increased substantially in the SCD-C group from 34 ± 2.3 to 48 ± 3.7% while SCD-H and S-C (n= 2-5) did not change. This effect was not due to a decline in systemic vascular resistance (SVR) which was similar in all groups. Ventriculograms demonstrated the SCD-C to convert viable but non-contracting myocardium to better contracting myocardium. The renal effects were also substantive. The fractional excretion (FE) Na nearly doubled in the SCD-C compared to SCD-H increasing from 2.2 ± to 0.8 to 5.3 ± 0.8% and FE_{urea}went from 59 ± 3.1 to 81 ± 11.3%. No adverse events of arrhythmia or hypotension were observed during treatment. Isolated peripheral blood monocytes showed a decline in IL-6 and TNF-a secretion at end of treatment compared to baseline demonstrating a change in proinflammatory phenotype.

Conclusions: These results demonstrate that immunomodulation with the SCD improves left ventricular function and improves natriuresis. Removal of the cardio depressant effects of the chronic inflammatory state of CHF may be a new innovative approach to the management of CRS.

Funding: NIDDK Support, Other U.S. Government Support

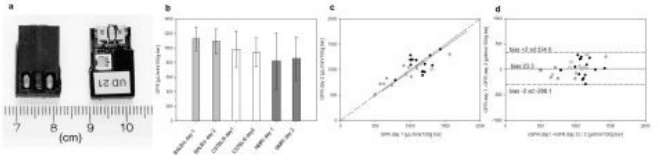
TH-OR016

Reproducibility of Transcutaneous Measurement of Glomerular Filtration Rate in Conscious Mice Daniel Schock-kusch,[1] Esther Ermeling,[2] Yury Shulhevich,[1] Stefania Geraci,[1] Jürgen Werner Hesser,[1] Dzmitry Stsepankou,[1] Sabine Neudecker,[1] Stefan Koenig,[3] Tobias Michael Hoerr,[4] Johannes Pill,[5] Roland Schmitt,[2] Anette Melk.[2] *[1]Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; [2]Hannover Medical School, Hannover, Germany; [3]Freudenberg Forschungsdienste KG, Weinheim, Germany; [4]Mektec Europe GmbH, Weinheim, Germany; [5]Mannheim Pharma & Diagnostics GmbH, Germany.*

Background: Recently developed approaches for the transcutaneous (TC) measurement of glomerular filtration rate (GFR) in lab animals allow GFR assessment without blood and/or urine sampling. Reproducability of measurements in the same animal is missing so far. Therefore, we investigated the reproducibility using our TC GFR assessment method in conscious mice.

Methods: Two TC GFR measurements were performed within three days in three mouse strains. In total 31 mice were examined (Balb/c n=15 (open circles); C57BL/6 n=10 (black circles); NMRI n=6 (grey diamonds). A miniaturized fluorescence detector (Fig.1a) allows the TC measurement of the elimination kinetics of the fluorescent GFR marker FITC-sinistrin. The device was fixed on the depilated back of the mice using an adhesive patch, before FITC-sinistrin (7.5 mg/100g) was injected.

Results: Results are depicted in fig.1.



Mean values of the two measurements show modest day to day variability in all three strains (b). Standard deviations do not reflect imprecision of the measurement but individual variations of GFR (c). Good agreement between both measurements is also reflected in the Bland Altman plot (d) with negligible bias and narrow 95% confidence interval. In total 100% of GFR values measured on day two are within the 30% range of day one, 80.6% are within 20% and 50.6% within 10%.

Conclusions: The data given let expect the TC method appropriate for GFR monitoring in conscious mice over a longer period of time.

TH-OR017

Hypertonicity Maintains a Differentiated Renal Epithelial Monolayer: A Promising Approach for Bioartificial Kidney Sa'ad Al-lahham, Ruud A. Bank. *Department of Pathology & Medical Biology, University Medical Centre Groningen, Groningen, Netherlands.*

Background: The development of a successful bioartificial kidney faces some challenges such as overgrowth and dedifferentiation of epithelia on synthetic membranes. It is known that renal medullary epithelial cells are exposed to hypertonic environment in vivo and it modulates the synthesis of the extracellular matrix proteins, therefore we aim to investigate the effect of hypertonicity treatment on the performance of epithelial cells.

Methods: Human epithelial cells were treated with regular (300 mOsm) and hypertonic media (400 and 500 mOsm). Hypertonic media was made by adding NaCl.

Results: We found that hypertonic media suppressed the mitochondrial activity, suggesting the suppression of the overgrowth of cells. Morphological evaluation revealed that hypertonic media maintained an intact epithelial monolayer, while isotonic medium treatment resulted in a disrupted layer of cells, where some cells looked like mesenchymal cells. On mRNA level, hypertonic media treatment significantly induced certain epithelial markers (e.g. E-cadherin, EPCAM and ZO-1) and renal transporters (e.g. OATP4C1 and MRP4), while it induced slightly N-cadherin and had no effect on α-SMA (mesenchymal markers). Hypertonic media treatment had no effect on the mRNA expression of COL4A1, LAMA1, LAMA5 and FN. Suggesting that hypertonic effect was not due to modulation of extracellular matrix genes.

Conclusions: Our results are promising for bioartificial kidney, since they suggest that hypertonicity inhibits the overgrowth of the cells and maintains an intact differentiated monolayer, which are some of the major obstacles that counteract the development of bioartificial kidney. In the future we will investigate the effect of hypertonicity on certain epithelial functions and the mechanism(s) behind that.

Funding: Government Support - Non-U.S.

TH-OR018

A Computational Drug Prediction Approach Identifies an Additive Renal-Protective Effect between an ACEI and a Histone Deacetylase Inhibitor Yifei Zhong,[1] Ruijie Liu,[2] Peter Y. Chuang,[2] John C. He.[2] *[1]Department of Nephrology, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China; [2]Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York City, NY.*

Background: Because of the complexity of the kidney disease, investigators have attempted to develop the combination therapy with two drugs such as ACEI and ARBs. However, these combination therapies that target on one system (RAS) are not very successful.

Methods: The Connectivity Map (CMAP) dataset was created from over 6000 gene expression microarrays testing the effects of approximately 1300 individual drugs in several human cancer cell-lines. The CMAP web-site provides a querying tool for matching drug induced gene expression signatures with differentially expressed gene lists provided by users. Based on this, we have implemented a Java program called Drug Pair Seeker(DPS) that allows us to predict and prioritize pairs of drugs which are likely reverse or aggravate the gene expression state in a disease condition. We used this approach to analyze the microarray data obtained from the kidney of HIV-1 transgenic mice (Tg26), a model for HIV-associated nephropathy, compared to WT.

Results: We predicted that, among these 1300 drugs, the combination of an ACEI and a histone deacetylase inhibitor (HDACI) was able to reverse the maximal number of genes altered in the diseased kidney while causing the minimal number of genes to be further altered. Then, we experimentally validated in Tg26 mice that the combined therapy with both ACEI and HDACI provided an additive renal protection as suggested by reduction of proteinuria, improvement of renal function, and attenuation of kidney injury. We also validated experimentally the expression of selected genes, which are predicted to be reversed by the therapy, in kidneys of these mice. Finally, we explored potential signaling pathways affected by either ACEI or HDACI or both using combined computational and experimental approach.

Conclusions: In conclusion, our studies suggest that DPS tool could be used to predict the drug combination. ACEI combined with HDACI could be a potential new therapy for kidney disease.

Funding: NIDDK Support, Government Support - Non-U.S.

TH-OR019

Engineering of Vascularized Nephrons from Simple Suspensions of Single Embryonic Cells Christodoulos Xinaris,[1] Valentina Benedetti,[1] Paola Rizzo,[1] Mauro Abbate,[1] Daniela Corna,[1] Nadia Azzollini,[1] Mathieu Unbekandt,[2] Jamie Davies,[2] Marina Morigi,[1] Ariela Benigni,[1] Giuseppe Remuzzi.[1,3] *[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [2]University of Edinburgh, Scotland, United Kingdom; [3]Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy.*

Background: Paucity of organs for transplantation and cost of dialysis render the development of alternative therapeutics a vital task for regenerative nephrology. An engineering method of producing organoids similar to immature kidney from embryonic mouse cells has major potential applications (Unbekandt, Davies KI, 2010), but the avascular *in vitro* environment precluded efficient formation of glomeruli. Here, we combined this method with an *in vivo* approach to obtain vascularized glomeruli, key step toward functional tissue.

Methods: We dissociated enzymatically E11.5 embryonic mouse kidneys into single precursor cells, centrifuged the cell suspensions, and then cultured pellets (5 days) to yield cell-aggregates. We characterized the resulting organoids *in vitro*by IF analysis of renal markers and transplanted them beneath renal capsule of athymic rats to examine morphology and function.

Results: *In vitro* organoids showed typical phenotypes of developing nephrons. Pax-2 and NCAM were transiently expressed in a spatiotemporally specific manner. Podocytes were identified as cells devoid of Pax-2 and expressing WT-1 and synaptopodin. To examine whether the self-organising tissue sustained development *in vivo*, we first tested organoids by varying cell numbers and chose large cell-aggregation cultures (LCA, $4x10^5$ cells). Implanted LCA organoids showed imperfect glomerulogenesis. To stimulate vessel formation and glomerulogenesis, we applied combined pretreatment of LCA and local/systemic administration of VEGF. This protocol greatly improved vascularization of grafted LCA resulting in formation of filtering glomeruli, proximal tubules capable of protein reuptake, and EPO-producing cells.

Conclusions: These results provide a proof of principle that tissue-engineering protocols can be successfully applied to single embryonic kidney cells, to grow vascularized grafts for possible use in basic and translational studies.

Funding: Private Foundation Support

TH-OR020

Evaluation of Bioartificial Renal Epithelial Cell System (BRECS) Therapy in a Porcine Septic Shock-Associated Acute Kidney Injury (SSAKI) Model Angela J. Westover,[1] D. Buffington,[1] P. Smith,[1] K. Johnston,[1] C. Pino,[1] Charu Dewitt,[1] David Humes.[1,2,3] *[1]Innovative BioTherapies, Inc.; [2]University of Michigan Medical School; [3]Cytopherx, Inc.*

Background: Renal cell therapy incorporated into a CRRT circuit has shown therapeutic efficacy in renal failure in preclinical and clinical studies. To improve upon previously used hollow fiber based delivery platforms, and accommodate projected clinical need, an injection molded (IM), freezable BRECS has been developed. BRECS contain approximately 10^8 human renal epithelial cells, obtained from donor discards and grown using an established enhanced propagation (EP) method. EP cells are seeded onto trabeculated carbon disks and maintained within BRECS by continuous perfusion.

Methods: Hemofiltration, established in a SSAKI model, incorporates the BRECS into an ultrafiltrate (UF) loop, with processed UF returned to the animal allowing for maintenance of cell viability and communication between BRECS and host. Hemodynamic parameters including cardiac output (CO), hematocrit (HCT) and renal blood flow (RBF) were monitored through death, as assessed by a mean arterial pressure of less than 10, or up to 16 hours. Systemic inflammation was evaluated by neutrophil (NE) expression of CD11b and evaluation of NE extravasation into lung tissue. Cell viability was verified via in-line O_2 consumption.

Results: 6 IM-EP BRECS and 6 acellular sham treated SSAKI pigs were evaluated. A significant difference in survival time was seen between SSAKI pigs treated with IM-EP BRECS (13.7±1.1 hrs) vs. acellular sham (6.8±0.4 hrs). Of note, 3 of the 6 IM-EP BRECS treated SSAKI pigs survived the entire 16 hour study. Improvements in CO, HCT and RBF were observed with IM-EP BRECS vs. acellular sham treatment. Although significant differences in systemic cytokine levels were not observed, CD11b expression and NE extravasation into lung tissue decreased in the IM-EP BRECS vs. acellular sham group. O_2 consumption indicated cells in the BRECS remained viable for the entire study.

Conclusions: In this SSAKI model, IM-EP BRECS therapy was shown to improve hemodynamic parameters and decrease systemic inflammation, contributing to the increased survival time of the animal.

Funding: NIDDK Support, Other U.S. Government Support

TH-OR021

Development of Sequential Techniques to Correlate Hemodynamics with Subsequent Lumen Geometry Changes in Arteriovenous Fistula Christi M. Terry,[1] Yong He,[4] Yan-Ting E. Shiu,[1] Rupak Banerjee,[3] Prabir Roy-Chaudhury,[3] Scott A. Berceli,[4] Alfred K. Cheung.[1,2] *[1]Medicine, Univ of UT, Salt Lake City, UT; [2]Medicine, VASLCHCS, Salt Lake City, UT; [3]Medicine, U Cinci, Cincinatti, OH; [4]Surgery, Univ of FL, Gainesville, FL.*

Background: Arteriovenous fistulas (AVF) often fail to mature. To assess the influence of hemodynamics on AVF maturation, a sequence of techniques was developed that allows for the serial assessment of blood flow characteristics with later coincident changes in lumen geometry in human AVF.

Methods: Contrast-free black-blood MRI at 1 day, 6 weeks and 6 months after AVF creation yielded lumen geometry. Cine phase-contrast MRI provided volumetric blood flow rates at the inflow and outflow regions. Lumen geometry and flow rates were input for computational fluid dynamic modeling of the pulsatile velocity flow fields to calculate wall shear stress (WSS), WSS gradient, and oscillatory shear index. The hemodynamic parameters and later coincident lumen geometries were co-registered at 1-mm intervals using the anastomosis as an anatomical landmark.

Results: MRI scans required up to 1 hr and were well tolerated but patient body size was sometimes a limiting factor on scan acquisition. Oscillatory and disturbed blood flow within the AVF was typical and often persisted between the first and last scans. Outward lumen expansion was observed but was frequently of limited extent at the anastomoses. This suggests impaired wall distensibility perhaps due to factors unique to the anastomosis such as suture constriction and low-compliance scar formation due to surgical manipulation. Regions in proximity to vein valves also may be associated with restricted wall expansion.

Conclusions: Early findings suggest that AVF regions with unique vein wall characteristics such as the anastomosis and vein valves, have divergent biomechanical characteristics that may need to be considered in correlation analyses separate from other regions. This novel MRI-to-CFD pipeline sets the stage for in-depth serial assessment of AVF hemodynamics and lumen geometry over time. It will be used in a multicenter prospective study to identify critical hemodynamic factors that contribute to AVF maturation failure.

Funding: NIDDK Support, Veterans Administration Support

TH-OR022

24-h Intraocular Pressure Monitoring in Patients Undergoing Chronic Hemodialysis Vassilios Liakopoulos,[2] Evangelia S. Panagiotou,[1] Dimitrios Mikropoulos,[1] Theodoros Giannopoulos,[1] Paraskevi Demirtzi,[2] Eirini C. Voudouragkaki,[1] Eleni Paschalinou,[1] Vasileios Konidaris,[1] Olga Nikitidou,[2] Pavlos Nikolaidis,[2] Anastasios G.P. Konstas.[1] *[1]Glaucoma Unit, 1st University Department of Opthalmology, AHEPA Hospital, Thessaloniki, Greece; [2]Renal Unit, 1st University Department of Internal Medicine, AHEPA Hospital, Thessaloniki, Greece.*

Background: The effect of hemodialysis (HD) upon 24-hour IOP of subjects with normal intraocular pressure (IOP) has not been previously documented. The aim of this study was to document 24-hour IOP changes caused by HD and compare them with 24-hour IOP profile of the same subjects on a day without HD.

Methods: This was a prospective, observational, before-after 24-hour trial performed on consecutive subjects with normal IOP undergoing maintenance HD 3 days a week between 13:00-17:00 hours in an academic unit. Following a comprehensive ocular assessment those with conditions that may have influenced IOP were excluded. One eye was randomly selected and two 24-hour IOP curves were performed (HD day first). The IOP was measured at 10:00, 13:00, 15:00, 17:00, 22:00, 02:00 and 06:00 hours employing Goldmann and Perkins tonometry on habitual position. During the course of one year 23 HD patients were enrolled out of whom 18 patients completed the study.

Results: IOP monitoring on a HD day demonstrated a significantly higher mean 24-hour IOP (15.4±2.7 vs. 14.1±2.2 mm Hg; p=0.025), higher mean peak 24-hour IOP (18.5±3.5 vs. 15.8±2.5 mm Hg; p=0.003) and significantly wider 24-hour fluctuation of IOP (6.2±2.3 vs. 4.0±1.9 mm Hg; p=0.001). When individual timepoints were compared after a Bonferroni correction, IOP was significantly higher only at 17:00 on HD day reflecting gradual IOP elevation during HD (p=0.021). Further, the mean IOP curve evaluated during the HD procedure (13:00, 15:00 and 17:00) was significantly higher on a HD day (16.4±3.0 vs. 14.7±2.4 mm Hg; p=0.004).

Conclusions: This prospective, before-after trial suggests that HD significantly worsens 24-hour IOP characteristics in normotensive eyes. The long-term significance of these findings requires further elucidation with larger, long-term studies in normal and particularly in glaucoma patients undergoing HD.

TH-OR023

Microcirculatory Impairment during Haemodialysis Jean-christophe Szelag, Myriam Pastural, Carlos Cardozo, Alejandra Lenz, Ignace Mpio, Nouredine Boumendjel, Elias Abdullah, Walid Arkouche, Denis Fouque, Maurice Laville. *AURAL.*

Background: The incidence of peripheral arterial occlusive disease (PAOD) is higher in chronic haemodialysis (HD) patients and little is known about the specific effect of the dialysis session. We studied the impact of one single HD session on the microcirculatory function.

Methods: Fifty stable patients were enrolled in the study (mean age 67,4+/-14,6 years, diabetes 45%). Lower limbs were classified according Ankle Brachial Index (ABI) and Toe Brachial Index (TBI) (PAOD <0,9 and < 0,65, respectively). Microcirculation function was assessed using laser Doppler flowmetry (Toe pressure) and transcutaneous oxygen pressure (TcPO2) at the dorsum of the foot. Measurements were started after 20 minutes of rest in supine position, in the first thirty minutes and in the second part (mean: 165 minutes) of the HD session.

Results: PAOD could be suspected in 30 lower limbs using ABI, 69 legs using TBI. TcPO2 was significantly reduced along the dialysis session. The largest drop was observed in the PAOD population (34% reduction, 31,6 +/-15,6 to 20,8+/-15,8 mmHg, p=0,0001*)*. One third of PAOD legs reached the threshold of critical ischemia (<10 mmHg) in the second part of HD session*.* The reduction was either observed in non PAOD patients but remained above the threshold usually defining ischemia (>30mmHg) (16,9% reduction, 36+/-13 to 30+/-13,5 mmHg, p=0,04). The same fall pattern was observed by classifying the lower limbs with TBI (21% reduction) Toe pressure was significantly decreased in PAOD population (30 legs, 53,15+/-20,1 to 38,3+/-24 mmHg, p<0,0001) whereas no change was detected in patient with ABI>0,9 (76,4+/-34 to 71,6+/-34,18 mmHg, p=0,12, 65 legs). The only factors retained in multivariate analysis were: systemic blood pressure variation (TcPO2 and Toe pressure), diabetes (TcPO2 only) and PAOD (Toe pressure only).

Conclusions: Haemodialysis is associated with a significant impairment of lower limbs microcirculation. This phenomenon exacerbates the ischemia of PAOD patients but seems to exist despite the absence of severe macrocirculatory lesions. It is poorly explained by the classical haemodialysis associated factors, patient condition or drugs.

TH-OR024

Cardioprotective Profile of Intradialytic Changes in Arterial Stiffness with Hemodiafiltration Compared to Hemodialysis Albert J. Power,[1] Evangelia E.M. Charitaki,[2] Andrew Davenport.[2] *[1]Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom; [2]UCL Centre for Nephrology, Royal Free Hampstead NHS Trust, London, United Kingdom.*

Background: Intradialytic myocardial ischemia has been associated with adverse outcomes in hemodialysis [HD]. Although hemodiafiltration [HDF] confers better blood pressure stability compared to HD during treatment it is unclear whether these modalities affect vascular tone and cardiac perfusion differently and independently of ultrafiltration.

Methods: 231 maintenance dialysis patients [HD=121; HDF=110, 92% postdilution] were studied [mean age 62.1±16.6yrs, 48% diabetic]. There were no significant differences in age, gender, comorbidities, treatment time between groups. Pulse wave velocity [PWV], aortic augmentation index [AAix], central aortic systolic blood pressure [SBPAo] were measured just before dialysis, at 20 minutes and at the end of treatment [T2] using the Arteriograph device. The diastolic reflection area [DRA], a measure of coronary perfusion was derived from available data.

Results: There were no significant changes in PWV at all time points in both groups. Compared to baseline there were significant reductions in AAix at 20 mins in the HDF group alone [median -5.3%, p<0.001] associated with greater reductions in SBPAo compared to HD [13±2 vs 7±2mmHg, p=0.02]. Significant increases in DRA occurred in both groups at 20 mins [p<0.01] without significant differences in magnitude by modality [p=0.09]. By the end of dialysis and despite similar ultrafiltration [UF] volumes [2.1±0.8 vs 2.0±0.9, p=0.5] the HDF group manifested greater reductions in SBPAo [12±3 vs 23±3mmHg, p=0.007] but had 90% greater increase in DRA compared to HD [p=0.03].

Conclusions: These findings suggest that despite more pronounced early reductions in central aortic systolic blood pressure, HDF facilitates progressive increases in coronary perfusion that may be cardioprotective in the long term.

TH-OR025

Changes in Vascular Tone Occur Early in Hemodialysis Independently of Volume Reduction Albert J. Power,[1] Evangelia E.M. Charitaki,[2] Andrew Davenport.[2] *[1]Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom; [2]UCL Centre for Nephrology, Royal Free Hampstead NHS Trust, London, United Kingdom.*

Background: Cardiovascular disease is the leading cause of death in hemodialysis [HD] patients in the US. Ultrafiltration [UF] leading to intradialytic myocardial ischemia ("stunning") has been proposed as a significant treatment-related mechanism. Whether this is due to intravascular volume depletion or changes in vascular tone during HD remains unclear. We investigated changes in arterial tone shortly after the start of dialysis.

Methods: Arterial stiffness was measured using a brachial oscillometric device [Arteriograph] just prior to HD [T0], 20 minutes in [T1] and at the end of treatment [T2] in 121 stable maintenance HD patients [mean age 60.7±17.5yrs, 59% male, 50% diabetic, 31% with diagnosed ischemic heart disease] over one dialysis session. Variables of interest were aortic pulse wave velocity [PWV] & augmentation index [Aix], aortic systolic blood pressure [SBPAo] and diastolic reflection area [DRA], a measure of coronary perfusion. HD was performed using high-flux polysulfone membranes [Elisio] with mean session length 3.9±0.4hrs, UF volume 2.1±0.8 liters, predialysis BP 145±26/77±15mm.

Results: HD led to early significant reductions in SBPAo [p<0.001] with reciprocal increases in DRA [p=0.009] but no significant change in PWV [p=0.1]. This was associated with significant changes in brachial but not aortic Aix [p=0.02 & 0.2 respectively].

	T0	T1	T2
PWV [m/sec]	9.5±2.1	9.3±1.9	9.7±2.3
Aortic Aix [%]	37.7±17.6	35.1±17.3	30.7±19.1
Brachial Aix [%]	0.68±34.5	-4.94±34.1	-33.3±37.6
SBPAo [mmHg]	152±31	145±31	140±36
DRA	40.9±16.6	45.2±18.8	45.9±19.8

Overall HD reduced SBPAo [p<0.001] and Aix [p=0.001] but no significant change in PWV compared to baseline [p=0.2]. Significant changes to the DRA were restricted to the first 20 minutes of HD.

Conclusions: This suggests that early reductions in vascular tone in HD occur before clinically significant UF. A sustained increase in DRA does not match progressive reductions in SBPAo that are seen and which could lead to relative coronary hypoperfusion mediating cardiac dysfunction during HD.

TH-OR026

The Effects of Limiting Maximum Ultrafiltration Rate in an In-Center Hemodialysis Population James L. Pirkle,[1] Amret T. Hawfield,[1] Gregory B. Russell,[2] John M. Burkart.[1] *[1]Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Department of Biostatistics, Wake Forest School of Medicine.*

Background: Both intradialytic hypotension and high ultrafiltration (UF) rates are independently associated with cardiac morbidity and mortality. An analysis of the HEMO study showed that UF rates higher than 13 ml/kg/h were associated with an increased risk of all-cause and CV mortality. The effects of limiting maximum UF rates are unknown.

Methods: We compared treatment patterns, BPs, and weights in 123 patients in one urban based ICHD unit during the 8 week periods before (pre-policy) and after (post-policy) implementation of a policy limiting UF rates to a maximum of 13 ml/kg/h. Treatment times were extended and/or extra treatments were offered when necessary to achieve target weights (TW).

Results: Pre-policy, 57 patients (46.3%) had >3 treatments with high UF rates (>13 ml/kg/h). The highest quartile of weight gainers (n=31) had an average interdialytic weight gain (IDWG) ≥ 4% of TW. Post-policy implementation, patients who had signed off treatments early (n=21, 17.0%) increased time on dialysis by 8 minutes per treatment (+/-16, p=0.03); treatment times were extended in 62 (2.2%) of 2779 treatments; and 13 patients (10.5%) returned for a total of 28 extra treatments. Post-policy, TWs and pre-dialysis weights for the entire cohort did not change and IDWG decreased -0.3 kg (+/-1.1, p=0.001). Pre-dialysis BPs decreased: systolic -13.9 mmHg (+/-15.0, p<0.0001), diastolic -6.6 mmHg (+/-8.3, p<0.0001); as did post-dialysis BPs. Lowest BPs during dialysis were higher: systolic +1.3 mmHg (+/-8.5 p=0.096), diastolic +1.8 mmHg (+/-5.5, p=0.0006). For the highest quartile of IDWG, the decrease in IDWG was the most pronounced: -1.1 kg (+/-1.7, p=0.0007) as was the increase in lowest dialysis BP: systolic +4.0 mmHg (+/-8.2, p=0.011), diastolic +2.5 mmHg (+/-6.1, p=0.027).

Conclusions: A policy limiting maximum UF rates in an urban ICHD unit resulted in minimal changes in treatment patterns of extended dialysis times and extra treatments. Instead, patients responded to the policy with behavioral changes by reducing IDWG. All BP parameters improved with the policy.

TH-OR027

Consequences of Changing Trends in Buffer Content of Acid Concentrate B. Horowitz, O. Myers, S. Paine, A. Harford, A. Gul, P. Zager. *UNM.*

Background: Many dialysis providers have begun using acid concentrates with increased alkaline buffer. Manufacturers and FDA have cautioned providers about potential increases in serum bicarbonate and related health risks. K/DOQI guidelines recommend pre-dialysis serum bicarbonate 22 mmol/L with no upper limit. This study explores the hypothesis that pre-dialysis serum bicarbonate levels have increased over time and are associated with increased all-casue mortality.

Methods: We conducted a cross-sectional study of pre-dialysis serum bicarbonate in hemodialysis (HD) patients (n=33912) treated in facilities operated by DCI from 2007 to 2012. We computed the percentage of serum bicarbonate values that fell into a given range.

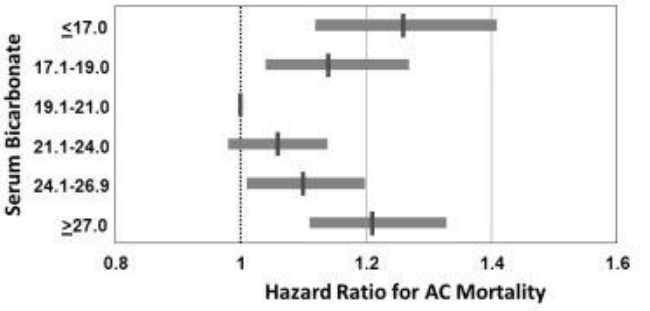
We constructed a Cox model to compute the hazard ratios (HR) for all-cause mortality associated with each category of pre-dialysis serum bicarbonate. We studied incident patients (n=15,774), age >18 years, who began HD between Jan 2004 and June 2011. Median follow-up was 18 months. We adjusted for baseline demographics (race/ethnicity, sex, age, cause ESRD, vintage, and vascular access) and standard time-dependent covariates (albumin, creatinine, potassium (K), hemoglobin, Kt/V, and clinic SMR.

Results: The distributions of serum bicarbonate in 2007 and 2012 are shown. Values >27 mEq/L increased from 10% to 16.7% and the percentage ≤17 decreased from 8% to 4%.

Serum Bicarbonate in 2007 & 2012.

Year	≤17	17.1-19	19-21	21-24	24-27	>27
2007	8	13	21	34	19	10
2012	4	8	16	36	26	17

A Forest plot depicting the hazard ratios for each serum bicarbonate category is shown. There was not a significant K x bicarbonate interaction (P=0.81) and the results were not significantly altered by removing K from the model.



Conclusions: Since 2007, the serum bicarbonate have increased. Serum bicarbonate >27 mEq/L and ≤17 mEq/L were associated with increased mortality. This association was not modified by serum K.

TH-OR028

The Effect of Using Ultrapure Water for Hemodialysis on the Circulating Bacterial Fragment and Vascular Stiffness Chi-bon Leung, Cheuk-Chun Szeto, Bonnie Kwan, Kai Ming Chow, Philip K.T. Li. *Department of Medicine and Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

Background: Cardiovascular disease (CVD) is the major cause of mortality and morbidity in dialysis patients. Recently, circulating endotoxin is found to associate with the systemic inflammatory state and CVD of dialysis patients. Previous studies showed that the use of ultrapure dialysate for hemodialysis could reduce the exposure to exogenous endotoxin. We studied the effect of using ultrapure water for hemodialysis on circulating endotoxin and bacterial DNA fragment levels and vascular stiffness.

Methods: This is an open-labelled prospective study of 25 patients (14 male). Circulating endotoxin and bacterial DNA level, vascular stiffness as represented by arterial pulse wave velocity (PWV), nutrition and hydration status were monitored before and repeatedly with 12 months after the use of ultrapure dialysate for hemodialysis.

Results: The average age was 58.9 ± 10.2 years; 21 patients completed the study. Within 4 weeks of conversion to ultrapure water for hemodialysis, plasma endotoxin level fell from 0.302 ± 0.083 to 0.209 ± 0.044 EU/ml ($p < 0.0001$) and then remained static, while serum bacterial DNA level remained similar. Furthermore, the time-averaged plasma endotoxin level during the study period significantly correlated with the carotid-femoral PWV ($r = 0.455$, $p = 0.033$), malnutrition inflammation score (MIS) ($r = 0.461$, $p = 0.031$), and inversely with lean tissue mass ($r = -0.571$, $p = 0.006$). The time-averaged serum bacterial DNA level significantly correlated with the subjective global assessment score ($r = -0.543$, $p = 0.009$), and inversely with MIS ($r = 0.550$, $p = 0.008$), but not with PWV.

Conclusions: Using ultrapure water for hemodialysis effectively reduces circulating endotoxin in hemodialysis patients, which is associated with less severe vascular stiffness and systemic inflammation. The causal relationship of this change and the long term benefit of using ultrapure water require further study.

Funding: Government Support - Non-U.S.

TH-OR029

Recurrent Acute Kidney Injury: Prevalence and Outcomes Areef Ishani,[1,2] Bipin R. Bista,[3] Craig Solid.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Minneapolis VAMC, Minneapolis, MN; [3]University of MN, Minneapolis, MN.*

Background: Outcomes after an acute kidney injury (AKI) episode are poor. It is unknown what percentage of patients with an AKI episode are re-hospitalized within one year for a recurrent AKI, and if the rate of recurrence varies by race or age.

Methods: Using the General Medicare 5% Random Sample, we identified patients aged 65+ who had an inpatient (IP) hospitalization for AKI during 2009, and then followed them for 1 year to look for recurrent AKI. The percent with a recurrence was calculated by age and race group. Outcomes following the recurrent-AKI were identified using Medicare claims.

Results: We identified 28,389 patients with an index AKI event in 2009, 648 of whom required dialysis during the AKI hospitalization. A total of 28% of all AKI patients and 33% of AKI-dialysis patient experienced a recurrent AKI within 1 year. While rates were consistent across age groups, African-Americans experienced recurrent AKI at a higher rate (34% of all, 49% of AKI-dialysis), compared to Whites (27% of all, 30% of AKI-dialysis).

Percent with a Recurrent AKI

	Original AKI: All (N=28,389)		Original AKI: with dialysis (N=648)	
	N	%	N	%
Recurrent AKI				
All	7990	28.1	216	33.3
Age 66-69	980	28.1	127	33.1
Age 70-74	1398	28.9	152	31.6
Age 75-79	1595	28.0	151	36.4
Age 80-84	1757	28.7	128	32.8
Age 85+	2260	27.4	90	32.2
White	6370	27.0	156	29.7
African American	1188	34.3	43	49.4
Other/Unk	432	32.1	17	48.6

Conclusions: Recurrent AKI within one year of an initial AKI hospitalization is common, occurring in about 30% of patients. Patients requiring dialysis during their index AKI have higher recurrence rates. African Americans appear to have a greater risk of AKI recurrence compared to white patients.

Funding: NIDDK Support

TH-OR030

Electronic Alerting for Acute Kidney Injury Edward Stern,[1] Rebecca Edwards,[1] Mark Harber,[1] Chris Laing,[2] Anne B. Dawnay.[2] *[1]Departments of Nephrology and Clinical Biochemistry, Whittington Health, London, United Kingdom; [2]Departments of Nephrology and Clinical Biochemistry, University College London Hospital, London, United Kingdom.*

Background: Acute kidney Injury (AKI) is associated with greatly increased risk of in-hospital death. Avoidable delay in identification contributes to mortality in a large proportion of these deaths (43% in a UK survey). We piloted electronic alerting for AKI in two hospitals in North Central London as an aid to early identification and response.

Methods: We designed an automated rule for the hospitals' electronic pathology systems to identify patients whose creatinine rose by ≥50% within 90 days. In response, an electronic alert was posted on the pathology report for clinicians, identifying the patient as having AKI and providing a link to the London AKI Network website, which gives relevant clinical guidelines. The biochemistry team used their discretion to subsequently telephone requesting clinicians.

Results: We collected data in Whittington Health for the first 100 adult patients identified by alert (86 inpatients, 7 hospital outpatients and 7 community general practitioner patients). Mean delta creatinine was 1.27 mg/dL (130%). Mean time from baseline to alert creatinine was 27 days. Divided by KDIGO stage: 56 patients had AKI 1, 29 had AKI 2, 15 had AKI 3. 15 patients died within the six-week period (five AKI 1, eight AKI 2, two AKI 3) compared with 1.7% overall mortality for Whittington inpatients.

Conclusions: This automated rule identifies a heterogeneous group: alert creatinines ranged from 0.49 to 11.02 mg/dL. Nevertheless, 24-hour electronic alerting was simple to implement and facilitated early identification of patients with unmet clinical need. In particular, post-op AKI was identified and managed early and we identified a number of unexpected AKI cases among samples sent by community GPs. Our data helped to raise awareness of the high mortality associated with even moderate rises in creatinine. Despite some limitations, this has proved to be a powerful tool in mobilising a response to AKI. We are now introducing the alerting system across five hospitals in North Central London as part of a standardised regional approach to the management of AKI.

TH-OR031

Association of Kidney Biomarkers with Progression of Acute Kidney Injury and Mortality in Patients with Cirrhosis Justin Miles Belcher,[1] Guadalupe Garcia-Tsao,[2] Arun Sanyal,[3] Chirag R. Parikh.[1] *[1]Section of Nephrology, Yale University School of Medicine, New Haven, CT; [2]Section of Digestive Diseases, Yale University School of Medicine, New Haven, CT; [3]Division of Gastroenterology, Virginia Commonwealth University School of Medicine, Richmond, VA.*

Background: Acute kidney injury (AKI) is common in patients with cirrhosis and is independently associated with mortality. We have conducted the first prospective study evaluating the utility of urinary biomarkers of kidney injury and tubular function for the prediction of AKI progression and mortality in patients with cirrhosis.

Methods: Patients with cirrhosis and AKI were identified via hospital-wide screening at four academic centers. AKI was defined via AKIN criteria based on documented outpatient baseline creatinine values. Structural; urinary NGAL, IL-18, KIM-1, L-FABP and albumin, and functional; urinary sodium and FENa, biomarkers were evaluated for association with a composite outcome of AKI progression or mortality. Patients were stratified via IAC criteria into non-HRS (n=159) and HRS (n=26) for analysis.

Results: For non-HRS patients, median injury biomarker values were significantly higher and sodium lower in those who had progressive AKI or death. NGAL, IL-18, L-FABP, and albumin were independently associated with the primary outcome. Remarkably, in patients with HRS, median NGAL, urinary sodium and FENa were lower in patients with the primary outcome.

Figure 1. Association of Biomarkers with the Primary Outcome in non-HRS Patients

Urine Biomarker* (Log transformed)	AKIN Progression or Death	
	Unadjusted RR (95% CI)	Adjusted RR (95% CI)**
Tubular injury markers		
NGAL (ng/ml)	1.71 (1.42-2.07)	1.34 (1.05-1.71)
IL-18 (pg/ml)	1.63 (1.30-2.04)	1.29 (1.01-1.64)
KIM-1 (ng/ml)	1.39 (1.07-1.80)	1.18 (0.93-1.48)
L-FABP (ng/ml)	1.51 (1.27-1.79)	1.33 (1.11-1.60)
Tubular function markers		
Sodium (mmol/L)	0.67 (0.48-0.93)	0.79 (0.56-1.13)
FENa (%)	1.02 (0.77-1.34)	0.98 (0.73-1.31)
Glomerular injury marker		
Albumin (mg/dL)	1.48 (1.21-1.81)	1.41 (1.14-1.74)

*Variables are $\log_{10}$ transformed and RR are per log-unit change
**Adjusted for CKD stage + demographics (race, age, and sex) + MELD score + Serum Sodium

Conclusions: Both structural and functional biomarkers of kidney injury are independently associated with progression of AKI and mortality in patients with cirrhosis. In patients without HRS, an elevation in injury markers was seen in those with worse outcomes. In patients meeting criteria for HRS, this typical association was inverted and those with less tubular injury and evidence of more intact tubular function actually fared worse.

Funding: NIDDK Support

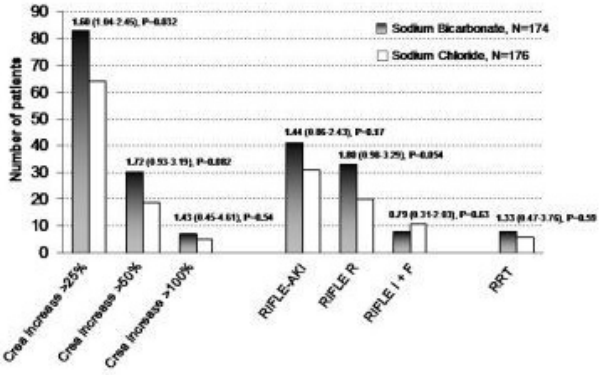
TH-OR032

Sodium Bicarbonate and Renal Function after Cardiac Surgery: Results of a Multicenter Double-Blind Randomized Controlled Trial Michael Haase,[1] Anja Haase-Fielitz,[1] Michael Plass,[2] Patrick T. Murray,[3] Michael J. Bailey,[4] Rinaldo Bellomo,[5] Sean M. Bagshaw.[6] *[1]Nephrology, Otto von-Guericke-University, Magdeburg, Germany; [2]Anesthesiology, German Heart Center, Berlin, Germany; [3]Mater Misericordiae Hospital, University College, Dublin, Ireland; [4]ANZIC, Monash University, Melbourne, Australia; [5]Intensive Care, Austin Health, Melbourne, Australia; [6]Intensive Care, University of Alberta, Canada.*

Background: Preliminary evidence suggests a potentially beneficial reno-protective effect of urinary alkalinization in patients at risk of acute kidney injury (AKI). We tested whether perioperative sodium bicarbonate infusion can prevent postoperative AKI.

Methods: In a multicenter, double-blind, randomized controlled trial we enrolled 350 cardiac surgical patients to receive either 24hrs of iv. infusion of sodium bicarbonate (5mmol/kg) or sodium chloride (5mmol/kg). The primary endpoint was the proportion of patients developing AKI defined as creatinine increase >25% or >0.5 mg/dL of baseline within the first 5 postop. days. Secondary renal outcomes included RIFLE-AKI and renal replacement therapy (RRT).

Results: Patient groups were similar in demographics, comorbidities and cardiac procedures performed. Sodium bicarbonate increased plasma bicarbonate concentration (P<0.001) and urine pH (P<0.001) compared to control. More patients in the bicarbonate group (47.7%, 83/174) developed the primary endpoint compared to control (36.4%, 64/176) (OR 1.60 [95%CI, 1.04-2.45]; unadjusted P=0.032). Multivariate adjustment, adjustment of peak creatinine to corresp. fluid-balance, testing for RIFLE-AKI or RRT revealed no significant group differences. There were no significant side effects.



Conclusions: Sodium bicarbonate, although safe, was not associated with a lower incidence of AKI in cardiac surgical patients. The findings of this study do not justify further investigation. (NCT00672334).

Funding: Private Foundation Support

TH-OR033

Usefulness of Renal Ultrasound in the Evaluation of Hospitalized Patients with Acute Kidney Injury Dipal Patel, Kavitha Ganta, Vidyasagar Reddy Cirra, Andres Serrano. *Mount Sinai Hospital, Chicago, IL.*

Background: Renal Ultrasound (RUS) is routinely ordered in the evaluation of hospitalized patients with Acute Kidney Injury (AKI). The main purpose of the test is to identify obstructive uropathy (UO). We evaluated the usefulness of RUS as a routine test.

Methods: We conducted a retrospective review of all hospitalized patients who underwent renal ultrasound at our institution from January 2005 to June 2011. Among the patients who underwent this test, we selected patients who had AKI (AKIN definition). Patients younger than 18 years-old, pregnant women, and patients with history of kidney transplantation were excluded. Clinical history at the time of presentation, patient demographics (age, sex, and race), serum creatinine, and prostatic specific antigen (PSA) - if available, were collected. Adittionally, the presence of the following medical problems, which are known to cause OU, were identified from the patients past medical history: benign prostatic hypertrophy, abdominal or pelvic surgery, pelvic malignancy, and nephrolithiasis. Patients who had a positive RUS, defined by the presence of bilateral hydronephrosis, were compared against patients who had a negative RUS, trying to find parameters, which will help identify patients with a higher likelihood of having a positive RUS.

Results: 5010 patients underwent RUS during the defined period. Of these, 1121 met inclusion criteria. In this group, the mean age was 62 year-old, 53.8% were male, and the majority of patients were AA (73.5%). 62 (5.5%) patients had a positive RUS. Among the patients with a positive RUS, 43 (69%) were male, and mean age was 65 year-old.

Interestingly, only 28 of the 62 (45%) patients presented with symptoms of urinary retention. The majority of patients, 47 or 76%, had risk factors known to cause OU. Elevated PSA, male gender, and having one or more of the defined medical problems in their history, were significantly associated with a positive RUS.

Conclusions: The incidence of OU is very low to justify the routine use of RUS in the evaluation of hospitalized patients with AKI. We suggest that RUS should be performed in patients who have, based on history, a higher likelihood of OU.

TH-OR034

The Relationship between Intraoperative Mean Arterial Pressure and Acute Kidney Injury after Noncardiac Surgery Michael Walsh,[1] Amit X. Garg,[2] Philip J. Devereaux,[1] Daniel Sessler.[3] *[1]McMaster University; [2]Western University; [3]Cleveland Clinic.*

Background: Worldwide over 200 million patients undergo noncardiac surgery annually and are at risk of acute kidney injury (AKI). Intraoperative hypotension may contribute to AKI however, there is little empiric data to inform anesthetists safe intraoperative blood pressures targets. We studied the relationship between intraoperative mean arterial pressure (MAP) and the risk of acute kidney injury.

Methods: We obtained preoperative characteristics, perioperative variables and postoperative laboratory data for 33,330 noncardiac surgeries that occcurred between January 2005 and September 2010 at the Cleveland Clinic in Ohio, USA. We first evaluated whether there was an association between the time spent at a MAP of <55, 55 to 59, 60 to 64, 65 to 69, and 70 to 74 mmHg and the outcomes of AKI (defined as at least a 1.5-fold rise in serum creatinine within 7 days of surgery) to determine at which MAP risk is increased. We then evaluated the independent risk associated with 1 to 5, 6 to 10, 11 to 20 and >20 minutes below the MAP at which risk increased for AKI and myocardial injury by adjusting for potential confounding variables.

Results: AKI developed in 2478 (7.4%) surgeries. The risk of AKI increased only at MAPs below 55 mmHg and was increased at even 1 to 5 minutes with a MAP <55 mmHg. Compared to never developing a MAP <55 mmHg, those with a MAP < 55 mmHg for 1 to 5, 6 to 10, 11 to 20 and >20 minutes the risk of AKI was 1.18 (95% confidence interval [CI] 1.06 to 1.31), 1.19 (95% CI 1.03 to 1.39), 1.32 (95% CI 1.11 to 1.56) and 1.51 (95% CI 1.24 to 1.84).

Conclusions: A MAP below 55 mmHg is associated with AKI even at very short durations. Further research is required to determine if interventions to keep the MAP >55 mmHg during surgery will improve clinical outcomes.

TH-OR035

Statin Toxicity from Macrolide Antibiotic Co-Prescription: A Population Based Study of Older Adults Amit Patel, Salimah Z. Shariff, David G. Bailey, Sonja Gandhi, David N. Juurlink, Jamie L. Fleet, Tara Gomes, Y. Joseph Hwang, Amit X. Garg. *Institute for Clinical Evaluative Sciences, ON, Canada.*

Background: HMG-CoA reductase inhibitors ("statins") are among the most commonly prescribed medications. Clarithromycin and erythromycin inhibit cytochrome P450 (CYP) 3A4, increasing blood levels of statins metabolized by CYP3A4. The clinical consequences of this drug-drug interaction at the population level are unknown.

Methods: We studied a population-based cohort of older atorvastatin, simvastatin, or lovastatin users in Ontario, Canada who were co-prescribed either clarithromycin or erythromycin between 2003 and 2010. The mean age was 74 years. All outcomes were assessed in the 30 days following a new prescription for these macrolide antibiotics. The primary outcome was hospital admission with rhabdomyolysis. Secondary outcomes included hospital admission with acute kidney injury and all-cause mortality. The comparison group was statin users co-prescribed azithromycin, a related antibiotic that does not inhibit CYP3A4 and does not increase statin blood levels. Risks were expressed in both relative and absolute terms.

Results: Co-prescribing clarithromycin or erythromycin (75,858 patients), or azithromycin (68,479 patients) with a CYP3A4 metabolized statin was common. A co-prescription for clarithromycin or erythromycin was associated with a higher risk of hospital admission with rhabdomyolysis (relative risk (RR) 2.17 (95% confidence interval (CI) 1.04 to 4.53)), acute kidney injury (RR 1.78 (95% CI 1.49 to 2.14)), and a higher risk of all-cause mortality (RR 1.56 (95% CI 1.36 to 1.80)). Results were consistent in adjusted analyses and in a subpopulation with laboratory measurements. When risk was expressed in absolute terms, a co-prescription for clarithromycin or erythromycin was associated with a 1.26% (95% CI 0.58 to 1.95) higher incidence of hospital admission with acute kidney injury and a 0.25% (95% CI 0.17 to 0.33%) higher incidence of all-cause mortality.

Conclusions: In older adults co-prescription of clarithromycin or erythromycin with a CYP3A4 metabolized statin increases the risk of serious statin toxicity and the combination should be avoided.

Funding: Government Support - Non-U.S.

TH-OR036

Muscle Damage, Heat and Exercise: Effects on Acute Kidney Injury Biomarkers and Renal Function Naushad Ali Junglee, Umberto Di Felice, Alberto Dolci, Matthew B. Fortes, Andrew B. Lemmey, Neil Peter Walsh, Jamie Hugo Macdonald. *Extremes Group, College of Health and Behavioural Sciences, Bangor University, Bangor, Gwynedd, United Kingdom.*

Background: Physical activity in the heat can result in acute kidney injury (AKI). This study examined whether muscle-damaging and inflammatory-inducing exercise, as typically induced in military, occupational and sports settings, would result in upregulation of the AKI biomarker neutrophil-gelatinase associated lipocalin (NGAL) and alter kidney function following physical activity in the heat.

Methods: Ten males (mean age±SD, 20±2 years) completed two randomized and counter-balanced treadmill running trials separated by 14 days. The first trial involved running for 60 minutes at 64% of maximal aerobic capacity in 68°F, on a +1% gradient (CON). The second trial was identical but on a -10% gradient to induce muscle damage (MD). Following each trial, subjects performed an exercise heat-stress test that involved running for 40 minutes at 65% of maximal aerobic capacity on a +1% gradient in 91°F. Urine and plasma NGAL, urine flow rate and specific gravity, plasma creatine kinase (CK) and plasma inflammatory cytokine interleukin-6 (IL-6) were measured at baseline, pre- and post-heat stress. Data were analyzed using repeated measures ANOVA and Pearson's correlations. Statistical significance was set at $P \leq 0.05$.

Results: By design the MD trial induced significant muscle damage and inflammation compared to CON, as measured by a greater concentration in plasma CK ($P<0.01$) and IL-6 ($P<0.01$). During heat-stress, MD induced a greater increase in urine NGAL compared to CON (+2291% vs. +113%; $P<0.01$) and similarly with plasma NGAL ($P=0.04$). MD also decreased urine flow rate to a greater degree than CON (-50% vs. -13%; $P=0.05$); consequently specific gravity was elevated in MD ($P=0.04$). Finally, plasma NGAL was positively correlated to plasma IL-6 ($R=0.65$; $P=0.04$) but negatively correlated with urine flow rate in MD ($R=-0.62$; $P=0.05$).

Conclusions: Muscle damaging exercise and its inflammatory effects elevated NGAL and altered kidney function following exercise-heat stress. Muscle injury is thus a novel risk factor for AKI when performing physical activity in the heat.

TH-OR037

Urinary α-Glutathione S-Transferase (GST) May Identify Patients at Highest Risk for Renal Injury (AKI) during Cisplatin Chemotherapy Peter H. O'Donnell,[1] Alicia J. Wyche,[1] Claudia Wing,[1] Jay L. Koyner,[1] Walter M. Stadler,[1] Patrick T. Murray.[2] *[1]University of Chicago; [2]University College Dublin.*

Background: AKI limits use of cisplatin in treating cancers. There are no reliable predictive biomarkers to identify patients (pts) at greatest risk for cisplatin-induced AKI.

Methods: We prospectively enrolled adult cancer pts newly-starting cisplatin at our institution. Urine samples were collected 2 hrs before first cisplatin administration and analyzed for α- and π-GST. Serum creatinine (SCr) was measured at baseline and throughout therapy. Comparison of baseline (pre-cisplatin) GSTs in pts with ≥50% SCr rise (RIFLE "Risk") during cisplatin vs controls (≤25% and ≤0.2mg/dL SCr rise) was the primary endpoint.

Results: From Nov'09—May'12, 87 pts (74% male; 77% White/16% Black) enrolled. Of these, 13 (15%) exhibited RIFLE "Risk" AKI during cisplatin. Using the broader AKIN definition (SCr rise ≥0.3mg/dL or ≥50%), an additional 15 pts (28 total=32%) had AKI. There was no difference in average baseline SCr between cases/controls (1.0 vs 0.9mg/dL, P=NS) however control pts were younger (61 vs 54 yrs, P=0.003). Urinary GSTs were available for 57/87 pts, including 27/28 pts with AKI. Baseline α-GSTs were significantly higher in pts developing subsequent RIFLE "Risk" AKI compared to controls (median=11.8 vs 4.4μg/L, P=0.04). This remained significant when using AKIN. Age did not correlate with higher baseline α-GST. Significant differences in π-GSTs between cases/controls were not observed. Five pts with the highest baseline α-GST (133, 87, 80, 70, 28μg/L) all showed dramatic SCr rises during cisplatin (44%, 131%, 82%, 174%, 214%, respectively). Area under the receiver-operator characteristic curve was 0.81 (P=0.003). A cutpoint of 10μg/L for baseline α-GST provided PPV=75%, NPV=85%, and a likelihood ratio for AKI of 7.7.

Conclusions: Urinary α-GST measured before receipt of cisplatin can identify pts at higher risk of developing cisplatin-related AKI. Since nephrotoxic effects of cisplatin likely involve direct proximal tubule (PT) injury, our results for α-GST (a PT marker) provide clinical evidence that baseline PT dysfunction is detectable and predicts for cisplatin AKI.

Funding: Other NIH Support - K12 CA139160, Private Foundation Support

TH-OR038

Timing of Renal Replacement Therapy and Outcomes in Critically Ill Patients with Acute Kidney Injury in the Randomized Evaluation of Normal versus Augmented Level of Replacement Therapy Trial Min Jun,[1] Rinaldo Bellomo,[2] Alan Cass,[1] Martin P. Gallagher,[1] Serigne N. Lo.[1] *[1]Renal and Metabolic Division, The George Institute for Global Health, Camperdown, NSW, Australia; [2]U of Melbourne, Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG), Austin, VIC, Australia.*

Background: The effect of timing of RRT initiation upon patient outcomes in AKI has not been well defined. We assessed the relationship between the timing of commencement of renal replacement therapy (RRT) in patients with acute kidney injury (AKI) and death at 28 and 90 days.

Methods: As a planned sub-study of the Randomized Evaluation of Normal vs. Augmented Level Replacement Therapy (RENAL) Study, we collected data on timing of onset of AKI using the RIFLE criteria in a nested cohort of patients. The timing of RRT commencement was determined using the time from AKI diagnosis to randomization (proxy to RRT commencement). Cox and logistic regression models were constructed using baseline demographic, disease-severity measures, and biochemical information.

Results: RIFLE criteria were obtained in 439 patients with a median time between renal failure diagnosis and RRT commencement of 17.6hrs. Patients were grouped into 4 categories of increasing time from renal failure to RRT commencement (based on quartiles: <7.1 [reference group], ≥7.1 to <17.6, ≥17.6 to <46.0, ≥46.0hrs). Earlier commencement of RRT was not associated with a lower risk of death at 28 days (HR for ≥7.1 to <17.6: 1.06, 95% CI: 0.62 to 1.81; p=0.83; HR for ≥17.6 to <46.0: 1.23, 95% CI: 0.71 to 2.12; p=0.46; HR for ≥46.0: 1.33, 95% CI: 0.77 to 2.31; p=0.31). Similar findings were observed for death at 90 days. Whilst not statistically significant, multivariate Cox and logistic regression models suggested a step-wise increase in mortality with increasing delay in RRT commencement.

Conclusions: In the RENAL Study, earlier commencement of RRT was not significantly associated with improved mortality rates. A step-wise increase in mortality may suggest progressive harm with delayed RRT and this needs to be tested in sufficiently powered randomized trials.

TH-OR039

Fgfr/Frs2α Signaling Is Required in Nephron Progenitors

Valeria E. Di Giovanni, Sunder Sims-Lucas, Caitlin M. Schaefer, Carlton M. Bates. *Div of Nephro, Dept of Peds, University of Pittsburgh, Pittsburgh, PA.*

Background: Fibroblast growth factor receptors (Fgfrs) signal intracellularly via adapters including fibroblast growth factor substrate 2α (Frs2α). We have shown critical roles for Fgfr/Frs2α signaling in the early metanephric mesenchyme (MM) in compound mice with Pax3cre deletion of *Fgfr1* and global point mutations in the Frs2α binding site of Fgfr2 (PFLR mice). PFLR mice develop ureteric bud (UB) branching defects and ureteric and mesenchyme-derived cysts with ciliary defects.

Methods: To understand the role of Fgfr/Frs2α signaling axis in more restricted populations of the MM, we generated SFLR mice using Six2cre, which delete *Fgfr1* slightly later than in PFLR mice, and specifically in nephron progenitors (NPs).

Results: Unlike PFLR mice, SFLR mice have normal ureteric development. However, SFLR mice have progressively worsening mesenchyme-derived cysts associated with abnormally long cilia. Strikingly, SFLR mice had abnormalities in the NP population. Embryonic day (E) 13.5 SFLR kidneys had normal-appearing NP cells by histology, Six2 and Sall1 antibody staining; however, they lost expression of Cited1, a marker of the "stem-cell like" renewing NP subpopulation, and demonstrated excessive apoptosis. By E14.5, SFLR NP cells were severely depleted and continued to demonstrate excessive apoptosis. Analysis of PFLR mice showed a similar loss of NPs. Despite the loss of NPs, those that survived were able to differentiate into nephrons as noted by H&E and Ncam staining in both SFLR and PFLR mice. Preliminary RNA-Seq analysis in FACS sorted E13.5 SFLR NP cells confirm a loss of expression of early NP markers in SFLR mutants, including Meox1 and Osr1. Also, Egf receptor pathways appeared down regulated and Notch activity appeared to be up regulated in SFLR NP cells, suggesting candidate molecules downstream of Fgfr/Frs2α signaling.

Conclusions: In conclusion, Fgfr/Frs2α signaling in NPs is critical for controlling early NP survival and in preventing mesenchyme-derived cystogenesis, possibly by regulating cilia length. Furthermore, the PFLR and SFLR allelic series reveals temporal and spatial roles of Fgfr/Frs2α signaling in regulating ureteric and MM development.

Funding: NIDDK Support

TH-OR040

Hematopoietic Progenitor Cells and Vascular Development in the Kidney

Yan Hu,[1,2] Minghong Li,[1] Roberto Ariel Gomez,[1,2] Maria Luisa S. Sequeira Lopez.[1] *[1]Department of Pediatrics, University of Virginia, Charlottesville, VA; [2]Department of Biology, University of Virginia, Charlottesville, VA.*

Background: There is currently very limited information regarding the crucial events that govern the morphogenesis of the renal arterial tree, including the origin, lineage relationship and mechanisms involved in the formation of the renal vasculature. It is now accepted that hematopoietic stem cells (HSCs) and endothelial cells (ECs) originate from a common progenitor, the hemangioblast. The following studies were performed to define the presence and contribution of the renal hemangioblast to the formation of the kidney vasulature.

Methods: Fate-tracing studies using transgenic mice (EC-SCL-CRE-ER^T and HSC-SCL-CRE-ER^T) that specifically express inducible cre upon tamoxifen administration in EC progenitors and HSCs, respectively. Histological methods, including X-gal staining and immunohistochemistry. Identification of hematopoietic progenitors using methylcellulose based colony-forming-cell assays using embryonic kidney cells from HSC-SCL-Cre-ER^T; mTmG mice, which express GFP in the cells from the HSC lineage upon tamoxifen induction. Crosstransplantation of embryonic kidneys from EC-SCL-Cre-ER^T;R26R mice under the kidney capsule of adult WT mice.

Results: 1. Cells from the endothelial and HSC lineage contribute to both erythroblasts and ECs in renal arteries, capillaries in between developing tubules and inside the glomeruli in the early embryonic kidney.

2. Renal hemangioblasts only give rise to ECs and blood.

3. The embryonic kidney possesses early hematopoietic progenitors.

4. Blood cells derived from the transplanted embryonic kidneys suggest that the embryonic kidney possesses HSCs that originate *in situ.*

Conclusions: The embryonic kidney possesses hemangioblasts that develop *in situ* and are progenitors of kidney vascular ECs and blood cells, which are distinct from the progenitor of mural cells.

Funding: NIDDK Support, Other NIH Support - RO1 Dk-091330 R37 HL-066242 RO1 HL-096735

TH-OR041

Renal Stroma Derived Endothelial Precursors Are Critical for Renal Development

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Background: Kidney structural abnormalities are amongst the leading causes of pediatric chronic kidney disease, producing significant morbidity and mortality. Understanding how different kidney lineages develop and interact is critical for making an impact on structural kidney disease. While most of the field has focused on ureteric, nephrogenic and stromal lineages, few have interrogated the role of the vasculature in the process of kidney development, except as it relates to the glomerulus. Moreover, the origin of the kidney vasculature has been debated to come from either the nephrogenic mesenchyme (within the kidney) or invading vessels.

Methods: Utilizing a combination of flow sorting and immunostaining both in wildtype and linage tagged renal cortical stroma we have shown a portion of the renal endothelium is derived from the cortical stroma.

Results: Immunofluorescence revealed that the lineage tagged stromal-derived endothelial cells gave rise to a significant portion of the peritubular capillary network, but not glomerular capillaries. In vitro, cultured embryonic stromal cells differentiated into tubular networks that expressed endothelial markers such as Flk1. To determine roles of stroma derived endothelium in vivo, we conditionally deleted Flk1 in the renal stroma with Foxd1cre lines (Flk1$^{ST-/-}$ mice). Flk1$^{ST-/-}$ mice had dilated peritubular capillaries with normal-appearing glomerular capillaries. Flk1$^{ST-/-}$ mice also had renal papillary hypoplasia/fibrosis and urinary concentrating defects. Microarray analysis of Flk1$^{ST-/-}$ kidneys revealed perturbations in key renal developmental pathways including, Vegf, Tgfβ and Notch.

Conclusions: These studies provide new insights into the origins of renal endothelium and their contribution to renal development including the renal medulla. Manipulation of this novel progenitor pool may impact therapeutically on vascular related kidney abnormalities and congenital kidney diseases.

Funding: Private Foundation Support

TH-OR042

Targeted Inactivation of the Prorenin Receptor (*PRR*) in the Ureteric Bud (UB) Inhibits UB Branching Morphogenesis and Collecting Duct Development

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Background: The PRR is the cell-surface receptor for renin and prorenin, and an accessory subunit of the vacuolar proton pump H$^+$-ATPase. In this study, we tested the hypothesis that targeted inactivation of the *PRR* (*Atp6ap2*) in the UB is essential for normal kidney development.

Methods: *PRR*$^{flox+}$ mice were bred with mice that express the Cre recombinase under the control of the UB-specific promoter (*Hoxb7*$^{Cre+}$). The resulting *Hoxb7*$^{Cre+}$/*PRR*$^{flox/flox}$ mice represent UB-specific *PRR*-knockout mice.

Results: RT-PCR demonstrated that isolated intact embryonic (E) day E11.5 UBs and cultured UB cells express PRR mRNA. The number of UB tips was reduced as early as on E12.5 in mutant *Hoxb7*$^{Cre+}$/*PRR*$^{flox/flox}$ (n=4 mice) compared to control *Hoxb7*$^{Cre-}$/*PRR*$^{flox/flox}$, n=4 mice) kidneys (5.2±0.5 vs. 13±0.6, p<0.01). The number of caspase 3-positive apoptotic cells in the UB was higher in mutant compared to control kidneys (7.6±1.2 vs. 0.6±0.1, p<0.01). qRT-PCR showed that Ret, Wnt11, Etv4 and Etv5 mRNA levels were decreased in mutant compared to control kidneys (p<0.001-0.01). Immunohistochemistry demonstrated reduced Erk1/2 phosphorylation in the UB of mutant kidneys. On E17.5, marked kidney hypoplasia, widespread apoptosis of the collecting duct cells, decreased expression of Foxi1, AE1, pendrin, Nos1, and H$^+$-ATPase mRNA levels (p<0.001-0.01), determined by qRT-PCR, were observed in mutant compared to control kidneys. On P1, kidney length (471±15 vs. 711±38 μm, p<0.01) and surface area (50117±3000 vs. 93000±2650 pixels, p<0.001) were reduced in mutant compared to control mice. Histological examination of P1 kidney sections demonstrated moderate dilation and occasional cysts in medullary collecting ducts.

Conclusions: PRR present in the UB epithelia performs essential functions during normal UB branching morphogenesis and collecting duct development *via* control of Ret/Wnt11 gene expression, UB/collecting duct cell survival, activation of Erk1/2 signaling and differentiation of collecting duct cells involved in acid-base homeostasis.

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TH-OR043

Transcription Factor Tcfcp2l1 Is Necessary and Sufficient for Distal Nephron Differentiation

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Background: Transcription factor Tcfcp2l1 belongs to the Grainyhead family and plays important roles in epithelial development in the kidney and other organs (e.g. Max Werth and Kai Schmidt-Ott, *Development* 2010).

Methods: We generated a conditional knockout of Tcfcp2l1 and deleted the gene from metanephric mesenchyme using Pax3ProCre and the whole kidney using EIIA-Cre. We overexpressed Tcfcp2l1 in the metanephric mesenchyme using adenoviral mediated gene transfer and we developed specific ChIP protocols.

Results: The expression pattern of Tcfcp2l1 during kidney development was very dynamic and covered mesonephros, ureteric bud stalk and the distal part of S-shaped bodies. In the adult kidney highest expression level of the Tcfcp2l1 was observed in the distal convoluted tubule, connecting segment and lower expression in the cortical collecting duct. To study the function of Tcfcp2l1 we generated the conditional knockout in the distal tubule. We observed downregulation of major markers of alpha- and beta-intercalated cells in the knockouts such as V-ATPase, Pendrin and AE-1. Gene array analysis confirmed the loss of expression of these major intercalated genes. Using chromatin immunoprecipitation assay we were able to show that Tcfcp2l1 binds to the promoter region of several subunits of V-type ATPase implicating Tcfcp2l1 is a direct regulator. By lentiviral expression, Tcfcp2l1 induced epithelial tubules in metanephric mesenchyme.

Conclusions: Taken together these data demonstrate that Tcfcp2l1 is sufficient and necessary for epithelial development and terminal differentiation particularly in the intercalated cell lineage.

Funding: NIDDK Support

TH-OR044

Role of the PCP Pathway Gene Fuzzy in Ciliary Formation and Signalling: Implications for Kidney Development

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Background: In humans, congenital anomalies of the kidneys and urinary tract (1-3 cases per 500 births) are the leading cause of the end stage renal failure in the pediatric population. Normal kidney development depends on timely activation of key intracellular signaling pathways including the canonical and non-canonical WNT. Currently, it is believed that these pathways are influenced by signals from the primary cilium in response to onset of tubular flow. We recently found that homozygous inactivation of the Fuzzy gene, encoding a protein in the non-canonical WNT planar cell polarity (PCP) pathway, causes profound renal hypoplasia and loss of cilia in kidney cells. We hypothesize that Fuzzy is crucial for normal ciliogenesis and regulation of intracellular signaling during kidney development.

Methods: A gene-trap Fuzzy mouse was generated in our lab; kidneys from E14.5 wildtype and mutant embryos were analyzed by histological and immunological methods with various antibodies. Wildtype and mutant mouse embryonic fibroblasts were established to study ciliogenesis and both WNT/β-catenin and WNT/PCP signaling by immunofluorescent microscopy and reporter assays.

Results: We established that Fuzzy is expressed in developing renal epithelial structures (ureteric buds, comma and S-shaped bodies). In MEFs and renal epithelial cells, native and exogenous Fuzzy protein was detected in the basal body, ciliary membrane and TGN/late endosomal vesicular compartment. We show that loss of Fuzzy leads to the loss of Rab8 small GTPase at the basal body suggesting that Fuzzy regulates recruitment of Rab8 to the primary cilium. We identified an interaction between Fuzzy and the modular PCP protein, Dishevelled. The latter is critically involved in both canonical and PCP pathways. Loss of Fuzzy results in loss of cilium with concomitant hyperactivation of the canonical WNT pathway and loss of PCP signaling.

Conclusions: We propose that Fuzzy is a crucial regulator of early renal development and that loss of Fuzzy causes renal hypoplasia by disrupting normal ciliogenesis and disturbing WNT and PCP signaling.

Funding: Government Support - Non-U.S.

TH-OR045

Ret Docking Tyrosines Y1015 and Y1062 Regulate Ureterovesicular Junction Development through Different Mechanisms by Modulating MAPK Activity

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Background: Connection of the upper and lower urinary tract at the ureterovesicular junction (UVJ) requires precise spatio-temporal regulation of molecular signals in the Wolffian duct (WD) and the surrounding structures that are not well defined. Delayed or defective insertion of WD or abnormalities in ureter remodeling can cause congenital anomalies of kidneys or urinary tract (CAKUT) including hydronephrosis, ureterocele, vesicoureteral reflux and megaureter. Mutations in the receptor tyrosine kinase *RET* are associated with CAKUT. RET signaling is mediated by docking tyrosines that regulate downstream signaling pathways. We previously showed that abrogation of the docking sites for PLCγ (Y1015) or SHC (Y1062) causes markedly different CAKUT.

Methods: We performed ontogenic and molecular analyses of early events in the formation of UVJ in RetY1015F and Y1062F mutant mice.

Results: We found abnormal ureter-bladder fusion in both Y1015F and Y1062F mutant mice, however through quite distinct developmental and cellular mechanisms. The common nephric ducts (CND), the caudal-most WD segment, of Y1015F mutants failed to degenerate normally, had increased MAPK activity and reduced apoptosis at E12.5 compared to controls. In contrast, WDs in RetY1062F mutant mice show failure to reach the cloaca at E10.5, asymmetric caudal growth at E11.5. At later developmental time points the ureters continued to show insertion/fusion defects into the primitive bladder. Both Ret-null and RetY1062F mutants show reduced MAPK activity throughout the entire WD at E10.5. These results show that the mechanism of WD to reach cloaca in Ret-null mice is reduction in the RetY1062-mediated MAPK activity. Inhibiting MAPK activity in half-genitourinary organ cultures in conjunction with time-lapse microscopy and whole mount immunohistochemistry analyses at E10 in wild-type mice revealed a major effect of MAPK pathway in the formation of UVJ.

Conclusions: Our study demonstrates how the same gene *RET* can modulate lower tract development through different mechanisms and provides a possible explanation for the broad range of phenotypes observed in the CAKUT affected individuals.

Funding: NIDDK Support, Private Foundation Support

TH-OR046

Absence of MicroRNA-21 Aggravates Glomerular Damage in Experimental Diabetic Nephropathy

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Background: Diabetic nephropathy (DN) is associated with mesangial expansion, loss of podocytes, and increased extracellular matrix (ECM) deposition in glomeruli and tubulo-interstitium. TGF-beta/Smad signaling is an important mediator of these changes in DN and regulates microRNAs (miRs) expression. The functional role of miRs in DN is not well understood. We had previously reported that miR-21 expression levels strongly correlate with proteinuria in patients with DN and that loss of miR-21 is associated with increased loss of podocytes and accelerated glomerular disease in TGF-beta1 transgenic mice. Now we present our findings on the role of miR-21 in a murine model of DN.

Methods: Experimental DN was induced in 10 weeks old miR-21 mutant DBA mice by administration of 50 mg/kg streptozotocin (STZ) intraperitoneally for 5 consecutive days. Functional and histological analyses were carried out at 10, 16 and 20 weeks after the completion of STZ injection. Furthermore, primary renal tubular epithelial cells (RTECs), fibroblasts and mesangial cells (MCs) were isolated from miR-21 mutant mouse kidneys, and the impact of high-glucose (HG) on the proliferation of the culture cells was detected using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. ECM deposition in the kidney was quantified using Sirius red staining.

Results: MiR-21 knockout (KO) mice developed increased albuminuria, higher blood urea nitrogen (BUN) levels and increased ECM deposition in glomeruli but not in tubule-interstitium compared with wild-type (WT) littermates 16 weeks after STZ-treatment. Whereas proliferation of miR-21 KO MCs and renal fibroblasts was increased, miR-21 KO RTECs proliferated less compared with WT cells. HG further decreased proliferation in miR-21 KO RETCs but increased it miR-21 KO fibroblast and MCs.

Conclusions: Consistent with our previous findings in TGF-beta1 transgenic mice, miR-21 provides protection from glomerular damage in a murine model of DN. Candidate cellular mechanisms includes increased podocyte loss and MC proliferation. Studies exploring the underlying molecular mechanisms are ongoing.

TH-OR047

Cross-Talk between TGF-β1 and 12/15-Lipoxygenase in the Epigenetic Regulation of Pro-Fibrotic Genes Related to Diabetic Nephropathy

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Background: Epigenetic chromatin histone modifications such as H3-lysine methylation (H3KMe) and acetylation (H3KAc) at gene promoters have profound effects on gene expression. Increased expression/activity of TGF-β1 (TGFB) and 12/15-Lipoxygenase (LO) and cross-talk between them play key roles in the upregulation of profibrotic genes (Collagen1a1, PAI-1 and CTGF) in Diabetic Nephropathy. We recently demonstrated the role of H3KMe in high glucose and TGFB induced profibrotic gene expression in mesangial cells (MC). Here, we evaluated the role of LO-TGFB cross-talk in the regulation of histone modifications and expression of a key H3K4-methytransferase (HMT) Set7/9 in MC and renal cortex from diabetic mice.

Methods: Gene expression was determined by QRT-PCR and histone modifications by Chromatin immunoprecipitation (ChIP) assays in rat MC treated with 12(S)-HETE (a LO- product) as well as in MC derived from wild type (WT) or LO-knockout (LOKO) mice after TGFB stimulation. In vivo relevance was tested using renal cortex from STZ injected diabetic LOKO mice, and WT diabetic mice treated with LO siRNAs.

Results: 12(S)-HETE increased the expression of profibrotic genes and SET7/9, and also altered H3K9Ac/H3K4Me1/H3K4Me3 levels at profibrotic gene promoters in rat MC. Interestingly, TGFB induced Set7/9 and profibrotic gene expression as well as H3K9Ac at their promoters was significantly ameliorated in MC from LOKO mice. Further, SET7/9 and profibrotic gene expression were increased in diabetic WT mice and these were significantly attenuated in diabetic LOKO mice, and in diabetic WT mice treated in vivo with LO siRNAs.

Conclusions: These new results demonstrate that oxidized lipids of the LO pathway can regulate key HMTs and histone modifications under diabetic conditions and also modulate TGFB induced epigenetic mechanisms involved in profibrotic gene expression. This can augment extracellular matrix deposition and fibrosis linked to diabetic nephropathy.

TH-OR048

Renal Protection by Knockout of the Atypical Chemokine Receptor D6 in Diabetic OVE Mice

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Background: Monocyte/macrophage recruitment correlates strongly with the progression of renal impairment in diabetic nephropathy. Chemokines and their receptors play a major role in leukocyte recruitment into inflammatory sites. The chemokine receptor D6 is a chemokine scavenging receptor that binds and sequesters many inflammatory CC

chemokines but does not transduce an apparent intracellular signal. It may play an important role in modulating the inflammatory response by scavenging CC chemokines in inflamed tissue. But its function in the diabetic kidney disease has not been tested.

Methods: In this study, we utilized the D6 knockout mice to test whether elimination of D6 will aggravate renal damage in diabetic nephropathy. D6KO mice were back crossed onto FVB background for 10 generations and then mated with OVE26 (OVE) mice to eliminate the D6 gene in this diabetic model. Control mice, OVE mice and OVE-D6$^{-/-}$ mice were tested for albuminuria, renal fibrosis, leukocyte infiltration and gene expression.

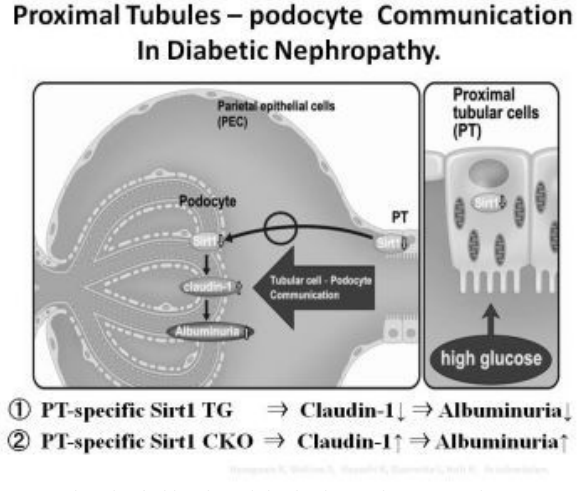
Results: D6 deficiency did not alter the diabetic blood glucose levels. Surprisingly, deletion of the D6 gene drastically reduced all features of diabetic nephropathy. Compared to OVE mice OVE-D6$^{-/-}$ mice had a 5 fold reduction in albuminuria. Renal fibrosis and leukocyte infiltration, which were greatly increased by OVE diabetes, declined to almost non-diabetic levels in OVE-D6$^{-/-}$ mice. Consistent with the changes in phenotype, gene expression in pathways of renal damage, inflammation and fibrosis were more normal in OVE-D6$^{-/-}$ mice. Renal protection by D6 knockout was unexpected but very effective.

Conclusions: The mechanisms by which the loss of inflammatory chemokine scavenging function in the diabetic kidney resulted in reduced leukocyte infiltration and inflammation remains to be investigated but our results reinforce the importance of inflammatory chemokines in modulating the progression of diabetic nephropathy.

TH-OR049

In-Vivo Claudin-1 Ectopic Overexpression in Podocytes Worsens Diabetic Albuminuria Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio University, Shinjuku-ku Shinanomachi-35, Tokyo, Japan.*

Background: We created proximal tubule (PT)-specific Sirt1 TG (JBC 2010) and conditional knockout (CKO) mice (oral presentation in 2010, 2011 ASN). After streptozotocin (STZ) treatment, wild-type (WT) mice had diabetic nephropathy (DN) with prominent albuminuria (Alb), combined with decreased Sirt1 and increased tight-junction protein claudin-1 (Cldn-1) in podocytes (Pods) as compared to saline-treated (Sal) control mice. These changes were prevented in TG and were aggravated in CKO.



However, the role of Cldn-1 in Pods in vivo has not been reported.

Methods: We delivered the gene of NPHS2-Cldn-1 cDNA vector, Cldn-1 siRNA, and control (Cont) plasmid using HVJE vector by tail-vein injection in Sal-treated or STZ-treated mice. The efficiency was tested by Cldn-1 staining and immunoelectron microscopy.

Results: In Cont+Sal, endogenous Cldn-1 was detected in PECs. In Cont+STZ, Cldn-1 was detected in PECs and Pods. Gold particles in Cont+STZ were characteristically located on the bottom side of foot processes (FP), not along with the lateral side. FP might be dragged from the bottom side by the tension power formed by newly emerged Cldn-1, which seems likely as FP become flattened and effaced. In Cldn-1+Sal, Cldn-1 upregulation was detectable in a Pods-specific way, which was concordant with the usage of NPHS2 promoter. In Cldn-1+STZ, further intense staining was seen in Pods. Cont+STZ or Cldn-1+Sal revealed significantly enhanced Alb and FP effacement, which was further enhanced in Cldn-1+STZ. When mice were rendered Cldn-1 siRNA, Cldn-1 was successfully silenced in the kidney. Cldn-1 in Pods and Alb after STZ was dramatically abolished by Cldn-1 siRNA.

Conclusions: Pods-specific Cldn-1 significantly worsened FP effacement and Alb. Cldn-1 siRNA therefore seems to be beneficial for the maintenance of Pods function and is a novel therapeutic approach for DN.

TH-OR050

Overexpression of Heterogeneous Nuclear Ribonucleoprotein F Up-Regulates Angiotensin-Converting Enzyme-2 Expression and Attenuates Systemic Hypertension in Diabetic Akita Transgenic Mouse Kidney Chao-Sheng Lo,[1] Shiao-ying Chang,[1] Yixuan Shi,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hospital, Montreal, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp., Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp, Boston, MA.*

Background: We investigated whether overexpression of heterogeneous nuclear ribonucleoprotein F (hnRNP F) modulates renal angiotensin-converting enzyme-2 (Ace-2) and angiotensin-converting enzyme (ACE) expression and subsequently attenuates hypertension in type 1 diabetic Akita mice.

Methods: Akita transgenic (Tg) mice specifically overexpressing hnRNP F in their renal proximal tubular cells (RPTCs) were studied. Plasma glucose, systolic blood pressure (SBP) and albuminuria were monitored bi-weekly in adult male non-Akita littermates, Akita and Akita hnRNP F-Tg mice from 10 to 20 weeks of age. Kidneys were processed for immunostaining of Ace-2 and ACE. Renal proximal tubular (RPT) Ace2 and ACE mRNA and protein expression were evaluated by respective real time-qPCR and Western blotting. Urinary angiotensin 1-7 (Ang 1-7) and angiotensin II (Ang II) levels were quantified by ELISA.

Results: Akita mice developed hypertension (SBP: 136.8 ±2.60 mm Hg) with increased glomerular filtration rate (GFR: 520.1 ± 44.08 µl/min), and displayed renal hypertrophy as compared to non-Akita controls (SBP: 107.7 ±1.11 mm Hg and GFR: 229.3 ±13.69 µl/min). HnRNP F overexpression normalized SBP, and decreased GFR (408.7 ±17.91 µl/min), renal hypertrophy and the urinary albumin/creatinine ratio without affecting plasma glucose levels in Akita hnRNP-F Tg mice. RPT Ace-2 mRNA and protein expression, and urinary Ang 1-7 levels were significantly decreased in Akita mice but normalized in Akita hnRNP F-Tg mice. In contrast, RPT ACE mRNA and protein expression were similar in Akita and Akita hnRNP F-Tg mice, whereas urinary Ang II levels were significantly increased in Akita mice and normalized in Akita hnRNP F-Tg mice.

Conclusions: Our data suggest that hnRNP F plays a protective role by attenuating SBP and preventing RPTC injury in diabetes,predominantly through increasing intrarenal Ace-2 gene expression.

Funding: Government Support - Non-U.S.

TH-OR051

Inhibition of the MAPK p38 Decreases Nephrin Endocytosis and Protects Mice against Hyperglycemia-Induced Proteinuria Magdalena Woznowski, Sebastian Alexander Potthoff, Eva Koenigshausen, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin, Ivo Quack. *Nephrology, Heinrich Heine University, Duesseldorf, Germany.*

Background: We could recently demonstrate that hyperglycemia leads to PKC? mediated endocytosis of nephrin. It has also been shown that p38 activation leads to loss of nephrin. However, the underlying pathomechanism remains unclear. Thus we were interested whether p38 is involved in nephrin endocytosis.

Methods: GST-fusion proteins of nephrin were used in kinase assay with recombinant p38. HEK293T cells were transfected with nephrin and β-arrestin2 as well as PKCα and PICK1. Co-IP with subsequent western blotting was performed. C57BL/6 mice were treated i.p with streptozotocin to induce hyperglycemia. As p38 inhibitor, SB202190 (i.p.) or BIRB0796 (p.o.) were used. Albuminuria was quantified as albumin/creatinin ratio. Isolated glomeruli were analyzed via western blot for activation of p38 and the interaction of nephrin with β-arrestin2 and nephrin endocytosis.

Results: Treatment of podocytes with high glucose (30 mmol) induced activation of the MAPK p38. Under high glucose conditions p38 phosphorylated nephrin at Ser1146 strenghtening the binding of PICK1 to nephrin. PICK1 facilitated the interaction of PKCα with nephrin promoting the phosphorylation of nephrin Thr 1120/1125 by PKCα. This step enabled the binding of β-arrestin2 to nephrin, initiating nephrin endocytosis. In mice hyperglycemia also led to p38 activation, nephrin endocytosis and proteinuria, which could be nearly completely prevented by p38 inhibition.

Conclusions: Here we propose a model for the role of p38 in nephrin endocytosis: high glucose levels lead to activation of MAPK p38 acting as an upstream mediator of PKCα. Active p38 phosphorylates nephrin at Ser1146 and faciliates the binding of PICK1 and PKCα to nephrin. The phosphorylation of Thr1120/1125 by PKCα is the prerequisite for β-arrestin2 binding and nephrin endocytosis, leading to a leaky filter. Noteworthy,in vivo the inhibition of p38 attenuates high glucose mediated nephrin endocytosis and proteinuria nearly completely. These findings suggest p38 and PKCα as promising targets for prevention and therapy of hyperglycemia induced proteinuria.

Funding: Government Support - Non-U.S.

TH-OR052

The Coagulation Protease Activated Protein C Ameliorates Diabetic Nephropathy by Regulating the Endoplasmic Reticulum Stress Response

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Background: Diabetic nephropathy (DN) is associated with endothelial dysfunction and impaired function of the endothelial derived, cytoprotective thrombomodulin (TM) protein C (PC) system. Impaired PC activation causes glomerular cell dysfunction and DN. The intracellular mechanism through which PC activation modulates DN is not known. Here we show that activated PC (aPC), a central mediator of haemostatic system, regulates cellular homeostasis by inhibiting endoplasmic reticulum (ER)-stress in DN., the cellular components of the glomerular barrier.

Methods: Wild-type (Wt) mice or mice with altered activity of the TM-PC system (loss of function with impaired protein C activation ($TM^{Pro/Pro}$) or gain of function with high aPC plasma levels (APC^{high})) were maintained diabetic for 26 weeks. Subsets of diabetic mice were treated with a chemical ER-chaperone (TUDCA). After 26 weeks markers of DN were determined and tissue samples were isolated for *ex vivo* analysis. *In vitro* assays were performed in podocytes and endothelial cells.

Results: Compared to Wt mice, impaired PC activation in $TM^{Pro/Pro}$ mice increased markers of ER-stress (CHOP/ATF6) in DN, while nuclear translocation of the transcription factor X-box binding protein-1 (XBP1) was impaired. Restoring aPC levels in $TM^{Pro/Pro}$ mice or inhibition of ER-stress with TUDCA normalized nuclear levels of XBP1, inhibited CHOP/ATF6 expression and protected against DN. In addition deletion of CHOP in $TM^{Pro/Pro}$ mice protected against DN. Consistent with these *in vivo* results, aPC promotes the nuclear translocation of XBP1 in endothelial cells and podocytes and inhibits hyperglycaemia induced ER-stress (reduced CHOP/ATF6expression). Podocyte or endothelial specific deletion of XBP1 abolished the cytoprotective effect of aPC.

Conclusions: These studies demonstrate that impaired PC activation is causally linked to ER-stress, establishing a novel link between haemostatic system and endoplasmic reticulum function in regulating cellular homeostasis in kidney disease.

Funding: Government Support - Non-U.S.

TH-OR053

Distal Renal Tubular Acidosis in Sjögren's Syndrome Is Associated with Loss of Differentiated Intercalated Cells

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Background: Sjögren's syndrome is associated with renal manifestations such as distal renal tubular acidosis (dRTA). Few studies have suggested a pathophysiological role for autoantibodies against carbonic anhydrase type II or subunits of the vacuolar H^+-ATPase in the acid secretory type A intercalated cells.

Methods: We aimed to investigate if dRTA in Sjögren's disease is caused by altered expression of acid-base transport proteins and abnormal acid-secretory cells in kidney biopsies of patients with clinically defined dRTA. Immunohistochemistry was performed for anion exchanger AE1, the Cl^-/HCO_3^- exchanger pendrin, the a4 and B1 subunits of the vacuolar H^+-ATPase, and carbonic anhydrase II. Principal cells were identified with AQP2. 15 patients with various types of nephritis served as controls and were compared to 15 Sjögren patients with dRTA.

Results: While control patients showed basolateral staining for AE1, no AE1 staining was detected in Sjögren patients with dRTA. Notably, AE1 was still detectable in red blood cells. Similar findings were made for pendrin. The a4-subunit was detected only in intercalated cells from control patients. In contrast, staining for B1-subunit was still detectable in some Sjögren patients but with lower intensity. The number of cells positive for any intercalated cell specific markers was dramatically reduced in Sjögren patients with dRTA. Next, IgG fractions from patients with Sjögren's disease and dRTA stained intercalated cells in normal kidney tissues and showed reactive bands in human kidney protein lysates that were absent when control IgG fractions were used.

Conclusions: In conclusion, patients with Sjögren's disease and dRTA show autoantibodies against intercalated cells with loss of differentiation markers and reduced numbers of intercalated cell which may contribute to the development of dRTA and serve as a new diagnostic hallmark.

Funding: Government Support - Non-U.S.

TH-OR054

Stimulatory Effect of Insulin on Renal Proximal Na Transport Is Preserved in Insulin Resistance

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Background: To clarify the pathogenesis of hypertension associated with insulin resistance, we examined whether the stimulatory effect of insulin on proximal tubule (PT) transport is preserved in insulin resistance.

Methods: Normal human kidney cortex tissues were obtained during the nephrectomy for renal carcinoma. Cell pH measurement with BCECF was performed to determine the Na-HCO_3 cotransporter NBCe1 activity in freshly isolated human or rat PTs. NBCe1 activity was also determined after isolated PTs were incubated for 24 h in DMEM containing FBS and siRNA at 37 °C. Glucose uptake into adipocytes prepared from perirenal (human) or periepididymal (rat) fat tissues was measured to estimate the degree of insulin resistance.

Results: In isolated PTs from human and Wistar rats, the stimulatory effect of insulin on NBCe1 activity was completely suppressed by a PI3-kinase inhibitor wortmannin. The siRNA treatment against IRS2 but not IRS1 largely (~85%) suppressed the stimulatory effect of insulin in rat PTs. In hyperphagic OLETF rats with obesity and hyperinsulinemia, the stimulated glucose uptake into adipocytes by 10^{-8} M insulin was severely reduced to 21% of that in control LETO rats. By sharp contrast, the stimulation of NBCe1 activity by 10^{-8} M insulin was comparable in OLETF and LETO rats (70 ± 11 vs 74 ± 15%). In insulin resistant human with elevated HOMA-IR (> 3.5), the stimulated glucose uptake into adipocytes by 10^{-8} M insulin was severely reduced to 19% of that in human with normal HOMA-IR. However, the stimulation of NBCe1 activity by 10^{-8} M insulin was comparable in human with or without insulin resistance (93 ± 7 vs 85 ± 13%).

Conclusions: These results indicate that the stimulatory effect of insulin on PT transport, dependent on IRS2/PI3-kinase pathway, was completely preserved in insulin resistance. Because insulin-induced vasodilation is known to be impaired in insulin resistance, hyperinsulinemia associated with obesity may contribute to the occurrence of hypertension by facilitating renal Na absorption.

Funding: Government Support - Non-U.S.

TH-OR055

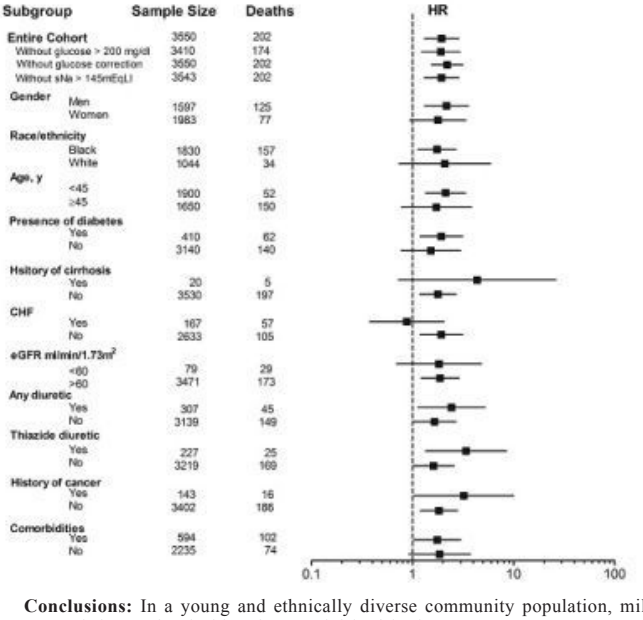
Hyponatremia and Mortality in an Ambulatory Setting: Results from the Dallas Heart Study

Fabrice Gankam Kengne, Colby Ayers, Amit Khera, James Delemos, Naim M. Maalouf. *Internal Medicine - Division of Cardiology, UTSW, Dallas, TX.*

Background: Hyponatremia is a common disorder associated with higher mortality in hospitalized patients, but its impact in an ambulatory setting remains unclear. We sought to determine the prevalence and determinants of hyponatremia and its impact on mortality in a general ambulatory population from a diverse ethnic background.

Methods: Serum sodium was measured in individuals aged 30 to 65 years enrolled in the Dallas Heart Study. Individuals were categorized based on baseline serum sodium levels, with hyponatremia defined as serum sodium below 135 mEq/L. The main outcomes were the prevalence, determinants and all-cause mortality associated with hyponatremia.

Results: 3,551 individuals were included in this analysis. The median age was 43 years (interquartile range 36-52 years) and median follow-up was 8.4 years (interquartile range 8.0-8.9 years). The sample weight-adjusted prevalence of hyponatremia in Dallas County was 6.9%, and age, black ethnicity, presence of cirrhosis or congestive heart failure (CHF) and use of selective serotonin reuptake inhibitors (SSRIs) were significantly associated with hyponatremia. By the end of the follow up period, 202 deaths occurred, including 29 in hyponatremic individuals. The unadjusted HR for hyponatremia and death was 1.94 (95% CI 1.31-2.87, p=0.001). Hyponatremia remained significantly associated with mortality after adjustment for age, gender, ethnicity, diabetes, hypertension, dyslipidemia, smoking, alcohol use, renal function, plasma CRP, use of antiepileptic drugs and SSRIs, and history of CHF, cirrhosis, and cancer (HR: 1.75; 95% CI: 1.08-2.81, p=0.02).



Conclusions: In a young and ethnically diverse community population, mild hyponatremia is associated with an increased risk of death.

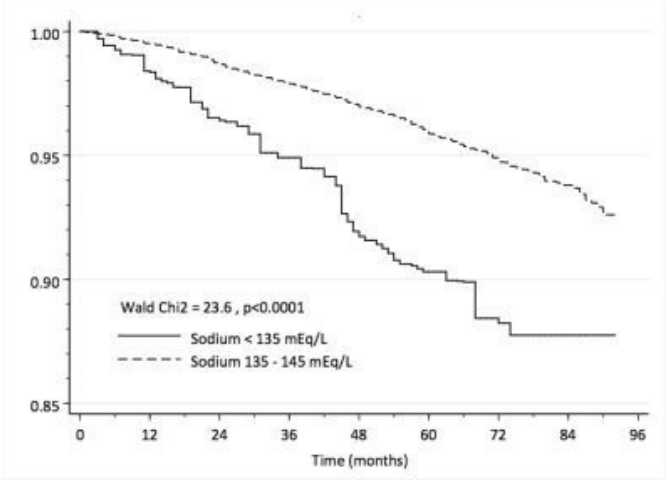
TH-OR056

Prevalence of Hyponatremia in NHANES 1999-2004 and Association with Mortality Sue Gu, Sumit Mohan, Amay Parikh, Jai Radhakrishnan. *Department of Medicine, Division of Nephrology, Columbia University Medical Center, New York, NY.*

Background: Hyponatremia is the most frequently observed electrolyte abnormality in the US, with prevalence reported to be from 2.5% up to 42.6% in the acute care setting, where it is associated with increased mortality, morbidity, and longer hospital stays. Hyponatremia in the ambulatory setting is poorly studied and is likely underdiagnosed due to its often subtle symptomatology. We seek to establish the prevalence of hyponatremia in the general US population and its association with mortality.

Methods: Data from NHANES 1999-2004 was analyzed for the presence of hyponatremia, defined as serum Na < 135 mEq/L, in the US population aged ≥ 18. Survival analysis was performed using linked mortality data through December 31, 2006.

Results: The overall prevalence of hyponatremia in the adult US population was 3.38%, with significantly higher prevalence of hyponatremia among women (4.32% vs 2.37% $p<0.0001$). For subjects aged 18-44, 45-64, and >65, we noted a significant increase in the overall prevalence with increasing age (2.82%, 3,32%, 5.31%, $p<0.0001$) among both men (1.75% 2.63%, 4.13%, $p<0.0001$), and women (3.89%, 3.97%, 6.19%, $p<0.0013$), respectively. Hyponatremia was associated with significantly lower survival rates on univariate analysis (hazard ratio 2.35, $p<0.0001$).



Conclusions: Hyponatremia is common in the US adult population, with significantly higher prevalence rates among women and older adults, and appears to be associated with significantly higher mortality in the general population.

Funding: NIDDK Support

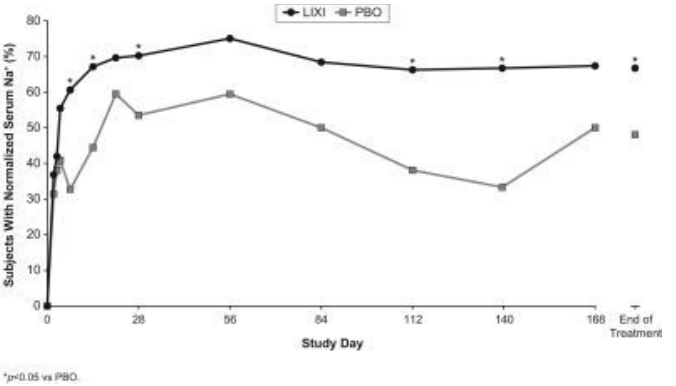
TH-OR057

24-Week Treatment with Lixivaptan for Euvolemic Hyponatremia: Effect on Serum Sodium Levels and Safety in an Outpatient Setting William Abraham,[1] Guy Decaux,[2] Richard Josiassen,[3] Yoram Yagil,[4] Nelson P. Kopyt,[5] Hemant Thacker,[6] Massimo Mannelli,[7] Daniel G. Bichet,[8] Cesare Orlandi.[9] *[1]The Ohio State Univ; [2]Erasme Univ Hosp; [3]Drexel Univ Col of Med; [4]Ben-Gurion Univ; [5]Lehigh Valley Hosp; [6]Bhatia Hosp; [7]Univ of Florence; [8]Université de Montréal; [9]Cardiokine Biopharma, Inc.*

Background: Treatment with lixivaptan (LIXI) for 24 wks was previously shown to increase serum Na^+ levels in subjects with euvolemic hyponatremia (EH) (ASN 2011). We further assessed the rapidity, degree, predictability, and safety of this increase in outpatients with EH.

Methods: In this multicenter, randomized, double-blind study (HARMONY), non-hospitalized subjects received (3:1) oral LIXI 25mg (n=154) or placebo (PBO; n=52) once daily, with subsequent dose titration (25–100mg) based on serum Na^+ levels. Fluid restriction was implemented at the investigator's discretion. The treatment period lasted 24 wks.

Results: Mean baseline serum Na^+ was 131.5mmol/L (LIXI) and 131.6mmol/L (PBO). Least-square mean±SE changes from baseline to Day 7 (primary endpoint) were 3.2±0.5 and 0.8±0.6mmol/L, respectively (p<0.001). The benefit of LIXI was consistent across gender/age groups; the magnitude of change in serum Na^+ increased with increasing baseline severity of hyponatremia. Normalization rates (serum Na^+ levels 135–145mmol/L) were greater with LIXI than PBO (60.6% vs 32.7% on Day 7, p<0.05; Figure). Kaplan-Meier analysis of time to first serum Na^+ level ≥135mmol/L favored LIXI (p=0.051). Few subjects experienced a rapid rise in serum Na^+ levels during titration (2.0% LIXI, 1.9% PBO). LIXI was well tolerated; no subject had signs/symptoms of osmotic demyelination syndrome.



*p<0.05 vs PBO.

Conclusions: Our findings suggest that LIXI effectively increases serum Na^+ levels in non-hospitalized subjects with EH and can be initiated and titrated in an outpatient setting.

Funding: Pharmaceutical Company Support - Cardiokine Biopharma, Inc

TH-OR058

Acyclovir Induced Hypokalemia Paul E. Drawz,[1,2,3] Federico Perez,[1,3] Robert Bonomo.[1,3] *[1]Louis Stokes Cleveland VAMC; [2]MetroHealth Medical Center; [3]Case Western Reserve University, Cleveland, OH.*

Background: Intravenous (IV) acyclovir, a mainstay antiviral for herpesviruses, may cause crystalluria and lead to acute kidney injury. A patient with hypokalemia following treatment with IV acyclovir prompted us to evaluate whether acyclovir also poses an increased risk of hypokalemia.

Methods: We evaluated the rate of hypokalemia (potassium < 3.5 mEq/L) in the four days after receipt of IV acyclovir for all patients treated between Jan 1, 2002 and Jan 1, 2012. As a comparison, we evaluated the rate of hypokalemia in the 14 days after admission for all subjects not treated with acyclovir and admitted to the same ward within 30 days of a patient who received IV acyclovir.

Results: 423 patients received IV acyclovir; 29,634 patients were admitted to the same ward within 30 days of a patient who received IV acyclovir. Hypokalemia developed in 46% of patients treated with IV acyclovir. The rate of significant hypokalemia was more than threefold greater among acyclovir recipients than in the control group, even after excluding subjects with previous hypokalemia, reduced kidney function, and diuretic use.

Rate of hypokalemia in patients treated with IV acyclovir and in untreated patients (%)

	All patients					
			Excluding subjects with K < 3.6mEq/L in week prior to first acyclovir dose (or week prior to admission) or eGFR <60 within one week of acyclovir dose (or one week prior to or two weeks after admission)			
					Excluding subjects who received diuretics	
	Acyclovir	No acyclovir	Acyclovir	No acyclovir	Acyclovir	No acyclovir
	(N=423)	(N=29,634)	(N=154)	(N=16,157)	(N=124)	(N=11,147)
<3.5mEq/L	45.6	21.3	29.2	8.6	26.6	6.9
<3.4mEq/L	38.5	15.9	23.4	5.8	19.4	4.5
<3.3mEq/L	30.0	11.5	16.2	3.7	12.9	2.8
<3.2mEq/L	25.1	8.2	13.6	2.4	10.5	1.6
<3.1mEq/L	19.4	5.7	9.1	1.5	6.5	1.0
<3.0mEq/L	12.1	4.1	5.8	1.0	4.8	0.7
<2.9mEq/L	8.9	2.7	3.2	0.6	2.4	0.4

Potassium levels within 4 days after IV acyclovir (or within 14 days after admission, if untreated)

Conclusions: Our data demonstrates, for the first time, that hypokalemia is common in patients treated with IV acyclovir. Reduced renal plasma flow and crystal induced distal tubular damage may be two mechanisms by which acyclovir induces hypokalemia.

Funding: NIDDK Support

TH-OR059

Hereditary Nephrogenic Diabetes Insipidus in Japanese Patients: Analysis of 73 Families Sei Sasaki, Motoko Chiga, Eriko Kikuchi, Shinichi Uchida. *Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Familial form of nephrogenic diabetes insipidus (NDI) is a rare hereditary disease caused by the AVPR2 and AQP2 gene mutations. It is speculated that about 90% of NDI are due to the AVPR2 gene mutations and the remaining 10% are due to AQP2, but rigid data supporting this relative frequency are lacking. It is also unknown whether this frequency is the same in different ethnic backgrounds.

Methods: Since 1994, gene mutation analyses were performed for 73 Japanese NDI families in our department. All exons and intron-exon boundaries of AVPR2 and AQP2 genes were directly sequenced. Some cases that were reported previously were included in the present analysis (16 families).

Results: Disease-causing mutations were identified in the AVPR2 gene in 60 families (82%), and in the AQP2 gene in 8 families (11%). In the remaining 5 families (7%) no mutation could be detected. This relative frequency of causative genes matches the general speculation. A total of 21 novel disease-causing mutations were newly identified in this study (17 in AVPR2 and 4 in AQP2). Regarding AVPR2, 52 disease-causing mutations were identified in 60 families. Among them, missense mutations were most popular accounting for a half of the mutations, followed by deletion mutations > nonsense mutations > insertions

and splicing mutations. These relativities are comparable with those of a global summary (Spankis 2008). In 49 female subjects who had heterozygous disease-causing AVPR2 mutations, 16 subjects (26%) had polyuria and 4 showed a complete NDI, demonstrating a quarter of female carriers are symptomatic. For AQP2, 8 disease-causing mutations were identified in 8 families (of these, 3 mutations in 3 families were previously reported). Two missense mutations showed a recessive inheritance, while one missense mutation (R254Q) and five deletion mutations (1~10nt) in the C-terminus of AQP2 showed a dominant inheritance, confirming its importance in AQP2 pathophysiology.

Conclusions: In Japanese NDI, most families (82%) are related to AVPR2 and 11% are to AQP2. A small portion of the families (7%) without any obvious mutations may imply the presence of unknown NDI-causing genes.

TH-OR060

Prominent Neointima Formation Is Common in Veins Utilized for AV Fistula Creation: The Hemodialysis Fistula Maturation (HFM) Study

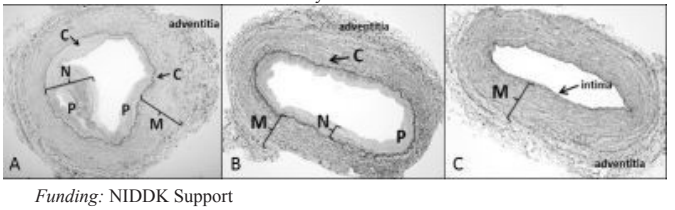
Charles E. Alpers,[1] The HFM Study Group.[2] *[1]Pathology, Univ. of Washington, Seattle, WA; [2]Cleveland Clinic.*

Background: A major cause of native arteriovenous fistula (AVF) failure is stenosis as a result of venous neointimal hyperplasia (VNH). Little is known about the morphology of veins at the time of AVF creation that may contribute to future AVF stenosis and failure.

Methods: Segments of vein used for anastomosis creation were harvested at the time of AVF creation from 303 HFM Study participants with advanced CKD or ESRD and analyzed histologically. Intimal and medial wall thickness and length of the internal and external elastic lamina were quantified by morphometry. Intimal cells were assessed by immunohistochemistry and extracellular matrices (ECM) were analyzed with Movat's stain.

Results: A major finding was a neointima (N) that was present in 87.8% of the samples in which a full circumferential vein structure was present in histologic sections (n=204). The neointima was usually irregularly thickened (Fig. A), sometimes concentric (Fig. B), and all contained α-smooth muscle actin expressing cells of smooth muscle or myofibroblast origin. Proteoglycans (P) constituted the predominant matrix in the neointima. A novel finding was large aggregates of collagen (C), present in 82% of vein samples within the media (M) of vessel walls, in addition to collagen which also was present in the neointima. Figures A and B show abnormalities typical of a large majority of vein samples analyzed; Figure C shows one of the veins without neointima formation.

Conclusions: Among HFM Study participants, we observed pre-existing abnormalities, with neointimas and prominent accumulation of ECM, in the great majority of veins used for AVF creation. Outcome studies to determine the importance of these common abnormalities on fistula maturation and use are underway.



Funding: NIDDK Support

TH-OR061

Notch Signaling Regulates CKD-Induced Endothelial-Mesenchymal Transition and Neointima Formation in a Mouse Model of Arteriovenous Fistula

Yun Wang,[1,2] Anlin Liang,[1] William E. Mitch,[1] Jizhong Cheng.[1] *[1]Division of Nephrology, Baylor College of Medicine, Houston, TX; [2]Department of Cell Biology, Third Military Medical University, Chongqing, China.*

Background: A functional arteriovenous fistula (AVF) is the Achilles heel of dialysis patient. An intact endothelium can prevent neointima formation in AVF because endothelial cells (ECs) dysfunction causes vascular remodeling. Indeed, CKD patients with failed AVFs have biomarkers of endothelial dysfunction in the nonfunctioning AVF.

Methods: To study mechanisms of CKD-induced AVF failure, we created a mouse model of AVF by anastomosing common carotid artery and internal jugular vein. At different times, the molecular events in the AVFs were explored.

Results: The endothelium in AVFs from humans or CKD mice had positive staining of mesenchymal markers (SMA-α and FSP-1), indicating there was endothelial cell to mesenchymal transition (EnMT). ECs treated with serum from CKD mice had increased Jagged 1 and Notch activation VS results in ECs exposed to serum from control mice. With CKD, Notch and its downstream transcription factor, RBP-Jk, were significantly activated in AVF VS controls ($P < 0.05$). Overexpression of Jagged 1 or expose to TGF-β1 decreased VE-cadherin expression in ECs, while the expression of SMA-α and FSP-1 were increased. Pretreatment with the Notch inhibitor, DAPT, blocked these markers of EnMT. Notably, RBP-Jk KO preserved the endothelial barrier function and ameliorated the increased permeability associated with Jagged 1 overexpression or TGF-β1 treatment in ECs. Similarly, RBP-Jk KO suppressed Notch activation- or TGF-β1- induced transmigration of bone marrow inflammatory cells. We also found that EnMT in AVF was suppressed in endothelial-specific RBP-Jk knockdown mice (RBP-Jk$^{+/-}$/VE-cadherin-Cre). Thus, the neointima in AVFs created in RBP-Jk knockdown mice was ~ 40% less VS results in WT mice despite CKD ($P < 0.05$).

Conclusions: We conclude that CKD induces Notch activation as well as EnMT that results in accelerated AVF failure. Blocking Notch in the endothelium suppresses CKD-mediated neointima formation. Notch might be a target for suppressing neointima formation in AVFs.

Funding: NIDDK Support, Other NIH Support - American Heart Association

TH-OR062

Development of Novel Bioengineered Grafts for Hemodialysis Vascular Access

Yan-Ting E. Shiu,[1] Huan Li,[1] Ilya S. Zhuplatov,[1] Christi M. Terry,[1] Alfred K. Cheung,[1] Wei Tan.[2] *[1]Medicine, Univ of UT, Salt Lake City, UT; [2]Mechanical Engineering, Univ of CO, CO.*

Background: Arteriovenous (AV) dialysis grafts made of polytetrafluoroethylene are susceptible to stenosis. Tissue-engineered dialysis grafts using autologous vascular cells and biomaterials have shown success in clinical trials, but their potential is tempered by the shortage of autologous cell sources, long lead time, high costs and storage complications. We thus aimed to design a degradable graft that readily allows grafting of allogeneic mesenchymal stem cells (MSCs) for vessel generation in vivo.

Methods: We have developed an inexpensive biodegradable graft, consisting of a bilayered scaffold composed of a nanofibrous heparin-impregnated polycaprolactone inner layer and a porous collagen-based outer layer. The graft lumen can be seeded with allogeneic MSCs from bone marrow, and the graft possesses compliance (4.5% / mmHg $X10^2$) comparable to native blood vessels, and burst strength (680-800 mm Hg) and permeability (500-600 ml/cm^2.min) values suitable for implantation. Grafts were implanted as carotid artery interposition grafts in sheep. In the pig, a seeded graft was placed as a jugular vein interposition graft and an acellular graft was placed as a carotid-jugular AV graft.

Results: The interposition grafts in sheep remained patent for one month, the latest time point tested. Acellular grafts (n=4) had significant outward remodeling mainly by proliferation and migration of adventitial fibroblasts, and had no PECAM+ cells on the lumen. MSC-seeded grafts (n=2) showed significant reduction in the fibrotic process, and PECAM+ cells were found on the lumen, suggesting the possibility of MSCs transforming into endothelial-like cells. The MSC-seeded interposition graft in pig remained patent for at least 3 weeks after implantation. The acellular AV graft in pig withstood arterial hemodynamic forces and was patent for 2 weeks.

Conclusions: Allogeneic MSCs on the polymeric vascular graft appeared to be non-immunogenic and have anti-fibrotic and pro-regenerative potential in sheep. Preliminary graft results in the pig model are promising warranting further exploration.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support

TH-OR063

True Benefit or Selection Bias?: Vascular Access and Mortality in the Elderly

Vanessa Grubbs,[1] Monnie Wasse,[2] Barbara A. Grimes,[1] Kirsten L. Johansen.[3] *[1]UCSF; [2]Emory; [3]UCSF-VAMC.*

Background: Prior studies suggest a survival benefit from the "fistula (AVF) first" initiative among the elderly, but the extent to which this benefit is driven by selection of healthier patients for AVF has not been fully explored. We hypothesized that functional status and hospital days prior to dialysis initiation partially explain the increased mortality associated with catheter use.

Methods: From the U.S. Renal Data System 2005-2007, we included 117,277 incident hemodialysis patients aged 67-90 years. Using proportional hazards models, we examined the association between initial vascular access and mortality through 2010 after accounting for functional status at dialysis initiation (institutionalized, need assistance with activities of daily living, or inability to ambulate or transfer) and number of hospital days in the two years prior to dialysis initiation. We defined access as AVF, graft (AVG), catheter with maturing AVF, catheter with maturing AVG, or catheter only.

Results: Of those with catheter, 25.5% had limited functional status vs. 10.8% of those with AVF. Those with catheter had 3-fold more prior hospital days (mean 18.0 vs. 5.4) than those with AVF. Median survival was shortest among those with catheter (Table). After adjusting for demographics, co-morbid conditions, and nephrology care prior to dialysis initiation (Model 1), all access types compared to AVF were associated with higher mortality. This finding was modestly attenuated after additionally adjusting for functional status and prior hospital days (Model 2).

Mortality by access type

Access type	Time to death in days, median (IQR)	unadjusted, HR (95% CI)	Model 1, HR (95% CI)	Model 2, HR (95% CI)
AVF	1,070 (456, 1548)	1.0	1.0	1.0
AVG	905 (337, 1533)	1.21 (1.16-1.25)	1.20 (1.15-1.26)	1.17 (1.12-1.22)
catheter with AVF	778 (263, 1447)	1.34 (1.31-1.38)	1.22 (1.19-1.26)	1.17 (1.14-1.21)
catheter with AVG	716 (214, 1437)	1.46 (1.40-1.53)	1.32 (1.26-1.39)	1.23 (1.17-1.29)
catheter only	374 (101, 1078)	1.94 (1.90-1.99)	1.60 (1.55-1.64)	1.48 (1.44-1.53)

Conclusions: Mortality risk among elderly with non-AVF access is attenuated, but not eliminated, after accounting for functional status and prior hospitalization.

TH-OR064

First Year Hospitalization in Incident Hemodialysis versus Peritoneal Dialysis Patients

Eduardo K. Lacson,[1] Nien-chen Li,[1] Raymond M. Hakim,[2] J. Michael Lazarus,[3] Franklin W. Maddux,[1] Joseph P. Pulliam.[1] *[1]Fresenius Medical Care North America, Waltham, MA; [2]Vanderbilt University, Nashville, TN; [3]University of North Carolina, Chapel Hill, NC.*

Background: Recent modality analyses attributed the survival advantage observed in Canadian peritoneal dialysis (PD) patients largely to hemodialysis (HD) catheter use. We sought to compare hospitalization from all causes during the first year between incident PD and HD patients, then evaluating hospitalization risk for patients by access type: HD catheter vs. arteriovenous access.

Methods: Incident patients who started dialysis in Fresenius Medical Care North America facilities in 2009 were compared with regards to time to first hospitalization during their first year using an intention to treat methodology. Cox models were adjusted for case-mix and the first available admission quality indicator labs (albumin, hemoglobin, & phosphorus). HD patients were also stratified by catheter vs. non-catheter access.

Results: 58% of 1,677 PD and 69% of 24,516 HD patients were hospitalized during follow-up. Hospitalization rates were 0.92 (PD) and 1.48 (HD) per patient-year, with HD catheter patients at 1.69 vs 1.03 (no catheter) per patient-year (all p<0.001). PD patients were younger (57 vs. 64 years), admitted later (admission vintage 22 vs. 11 days), had fewer black patients (21% vs. 31%), larger BMI (32 vs. 31 kg/m^2) and had fewer comorbidities (DM: 53% vs. 62%, CHF: 3% vs. 4%, PVD: 6% vs. 10%, & Amputations: 3% vs. 5%) than HD patients all p<0.001), although both cohorts had 56% males. The adjusted hazard ratio for hospitalization was 0.65 (0.61-0.70) favoring PD. The corresponding adjusted hazard ratios were 1.22 (1.13-1.32) for non-catheter and 1.64 (1.53-1.75) for HD catheter patients, compared to PD (all p<0.001).

Conclusions: In this national US cohort, incident PD patients had lower hospitalization risk than HD patients during the first year, despite adjusting for favorable case-mix. Stratifying by access type did not abrogate the difference in hospitalization risk in non-catheter access patients but the associated risk in HD catheter patients was much higher.

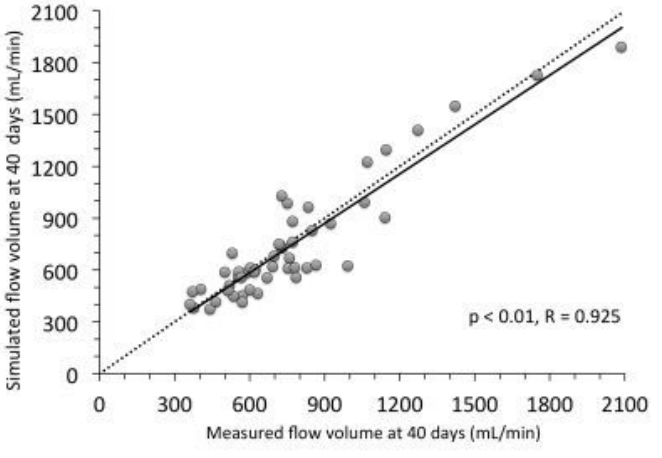
TH-OR065

Validation of a Patient-Specific Hemodynamic Computational Model for Planning Vascular Access Surgery in Hemodialysis Patients Simone Manini,[1] Anna Caroli,[1] Luca Antiga,[1,3] Stefano Rota,[4] Giuseppe Remuzzi,[1,4] Andrea Remuzzi.[1,2] *[1]Biomedical Engineering Department, Mario Negri Institute, Ranica, Bergamo, Italy; [2]Industrial Engineering Department, Bergamo University, Bergamo, Italy; [3]Orobix S.r.l, Bergamo, Italy; [4]Unit of Nephrology and Dialysis, Ospedali Riuniti, Bergamo, Italy.*

Background: Short- and long-term vascular access (VA) dysfunctions, including non-maturation and thrombotic complications, are the major cause of morbidity and hospitalization in hemodialysis (HD) patients. The extent of this major clinical problem points out the need of prediction and prevention of VA dysfunctions.

Methods: We developed specific 1D computational vascular network modelling tools (archTk) to simulate pre- and post-surgery blood flow, including vascular adaptation due to vessel wall remodeling up to 40 days after surgery. The advantage of this computational modeling is the ability to quantitatively estimate hemodynamic results with low computational cost. For model validation, a multicenter longitudinal clinical prospective observational study was performed in patients with end stage renal disease awaiting VA creation for HD treatment, in order to collect longitudinal data on arm vasculature and VA function by ultrasounds.

Results: Sixty-five patients with newly created arteriovenous fistula (AVF) were included in the simulation dataset and divided in 4 groups, based on AVF configuration. Predicted blood flow showed good agreement with measured values 40 days after surgery, regardless of AVF configuration.



Conclusions: These computational tools allowed to reliably predict patient-specific blood flow increase resulting from VA creation and vascular adaptation. This innovative approach may help the surgeon to choose the most appropriate AVF location and configuration, to reduce AVF primary non-function and to obtain a lower incidence of short- and long-term VA dysfunctions.

TH-OR066

Vitamin D Therapy and Arteriovenous Fistula (AVF) Maturation in End Stage Renal Disease (ESRD) Patients M. Salman Singapuri, Monnie Wasse. *Renal Division, Emory University School of Medicine, Atlanta, GA.*

Background: Vitamin D therapy reduces development of neointimal hyperplasia in animal models and attenuates markers of vessel remodeling in vitro. We tested the effect of vitamin D3 on AVF maturation in a double-blinded, placebo-controlled pilot study.

Methods: Fifty-two ESRD patients preparing to undergo AVF creation were randomly assigned to receive either 200,000 IU of oral cholecalciferol once weekly for 3 weeks (total 600,000 IU), or placebo. Blood was collected at study enrollment and 3 weeks after study drug completion, followed by surgical AVF creation within 3 weeks. The outcome was AVF maturation, defined as the ability to cannulate the AVF with two large bore needles at ≥ 6 consecutive dialysis sessions, and achievement of an AVF Qb >300 ml/min, assessed at six months.

Results: At baseline 94% of all subjects were 25(OH) D insufficient (< 30 ng/mL); mean 25 (OH) D was 16 ± 7 ng/mL, and mean 1,25 (OH) D was 17 ± 9 pg/mL. Other baseline characteristics were similar between the study groups, except for hemoglobin levels (p<0.04). Post-treatment, 25(OH) D sufficiency (≥ 30 ng/mL) was achieved in 91% of cholecalciferol-treated patients (mean 25(OH) D= 52 ± 18 ng/mL) and only 14% of the placebo-treated, while 1,25(OH) D significantly increased in cholecalciferol-treated patients (p<0.001). There was no significant change in serum calcium, phosphorus or iPTH. At follow-up, among patients with an AVF outcome (n=44), 41% of cholecalciferol-treated and 50% of placebo-treated patients achieved AVF maturation (p=0.74). Following adjustment, there was no association between AVF maturation and baseline, post-treatment or change in 25 (OH) D levels (p=0.9, 0.5, 0.5, respectively), or 1,25 (OH)D levels (p=0.5, 0.6, 0.4, respectively).

Conclusions: Although high-dose, short-term vitamin D3 supplementation is safe and effective, peri-operative 25(OH) D sufficiency did not improve AVF maturation. Longer-term studies may be valuable in assessing the role of correction and maintenance of vitamin D on AVF outcome.

TH-OR068

Whole Exome Resequencing and Homozygosity Mapping Identify Mutation of ADCK4 as a Novel Single-Gene Cause of Steroid Resistant Nephrotic Syndrome Shazia Ashraf,[1] Heon Yung Gee,[1] Virginia Vega-Warner,[1] Humphrey Fang,[1] Jameela Abdulaziz Kari,[2] Detlef Bockenhauer,[3] Robert Kleta,[3] Letian Xie,[4] Catherine F. Clarke,[4] Friedhelm Hildebrandt.[1,5] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan; [2]Department of Pediatrics, King Abdulaziz University, Saudi Arabia; [3]Pediatric Nephrology, Great Ormond Street Hospital and UCL Institute of Child Health, United Kingdom; [4]Department of Chemistry and Biochemistry, University of California; [5]Howard Hughes Medical Institute, University of Michigan.*

Background: Identification of single-gene causes of steroid resistant nephrotic syndrome (SRNS) has furthered the understanding of its pathogenesis. However, additional genes and disease mechanisms remain obscure. To identify a new causative gene for SRNS, we combined homozygosity mapping and whole exome resequencing (WER).

Methods: In two consanguineous siblings with SRNS, homozygosity mapping yielded 5 segments of homozygosity by descent (~115 Mb). We performed WER in one sibling to identify the underlying disease-causing mutation.

Results: We detected a homozygous missense mutation (p.R178W) in the *ADCK4* (*aarF domain containing kinase 4*) gene in an amino acid residue conserved to prokaryotes and the mutation segregated in this family. ADCK4 participates in the biosynthesis of coenzyme Q_{10} (CoQ_{10}) and has a high sequence similarity to ADCK3, the ortholog of yeast Coq8. Primary CoQ_{10} deficiencies due to mutations in ubiquinone biosynthesis genes have previously been identified in patients with NS. We identified recessive mutations in *COQ6* as a novel cause of SRNS that can be treated with the innocuous food supplement CoQ_{10}. By coimmunoprecipitation, we show that ADCK4 interacts with COQ6 and COQ7 in human cultured podocytes. Knockdown of ADCK4 in cultured podocytes results in the decrease of their migratory phenotype. ADCK4 is strongly expressed in human glomerular podocytes and localizes to mitochondria.

Conclusions: We, thus, identified mutation of *ADCK4* as a novel single-gene cause of SRNS. Better understanding of genes involved in CoQ_{10} biosynthesis will provide a therapeutic basis for the use of CoQ_{10} in nephrotic syndrome.

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TH-OR069

Rhophilin-1 Is a Key Regulator of the Podocyte Actin Cytoskeleton and Is Required for Glomerular Filtration Mark Lal, Ann-charlotte M. Andersson, Karl Tryggvason. *Matrix Division, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*

Background: The podocyte is a critical cellular component of the glomerular filtration barrier and dysfunction of this cell type is implicated in most glomerulopathies. Here, we identify and describe the functional role of a novel podocyte–specific protein, Rhophilin-1.

Results: Immunohistochemical analysis indicates that Rhophilin-1 is uniquely expressed in podocytes of both mouse and human glomeruli. *Rhpn1* knockout mice were phenotypically normal at birth but developed progressive albuminuria from about two weeks of age. Histological examination of kidneys from severely albuminuric mice revealed tubular protein casts and FSGS-like lesions as well as glomerular hypertrophy. Immunochemical analysis of a host of podocyte-marker proteins indicated that glomerular dysfunction in *Rhpn1*$^{-/-}$ was not likely a result of any overt alterations in the pattern of their expression. Widespread podocyte foot process effacement as well as thickening of the glomerular basement membrane were readily detected upon electron microscopy and are indicative of a principal podocyte insult likely involving impaired cytoskeleton dynamics. In vitro culture of primary glomerular explants indicates that Rhophilin-1 is enriched at the plasma membrane leading edge of podocytes where it elicits reorganization of the actin network from the cell body towards lamellipodia. This effect of Rhophilin-1 is mediated by inhibition of RhoA-dependent phosphorylation of myosin light chain and actin stress

fiber formation. Conversely, a lack of Rhophilin-1 in *Rhpn1*$^{-/-}$ mice glomeruli was correlated with increased RhoA signaling and myosin light chain hyper phosphorylation in podocyte foot processes. Interestingly, conditional deletion of RhoA in podocytes in vivo did not result in glomerular damage, but when bred onto a Rhophilin-1 null background, double deletion mutants presented with nephrotic syndrome and death.

Conclusions: Taken together, our results indicate that Rhophilin-1 is essential for the integrity of the glomerular filtration barrier and that this protein is a master regulator of Rho-dependent signaling and cytoskeleton architecture in podocytes.

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TH-OR070

Loss of Robo2 Alleviates Podocyte Structural Defects in Nephrin Null Mice

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Background: Robo2 is the cell surface receptor for the repulsive guidance cue Slit and is involved in axon guidance and neuronal migration. Nephrin is a podocyte slit-diaphragm protein that functions in the kidney glomerular filtration barrier. Previous research has shown that engagement of nephrin ectodomain can induce localized actin polymerization. Nephrin homozygous knockout mice develop characteristic phenotypes including dilation of the Bowman's space, abnormally broad foot processes, and absence of glomerular slit-diaphragms. Our recent in vitro studies show that Robo2 is expressed in podocytes where it forms a complex with nephrin through the adaptor protein Nck and opposes nephrin-induced actin polymerization. However, it is not clear if loss of Robo2 would modify the podocyte phenotype in nephrin null mice in vivo.

Methods: To test the hypothesis of a genetic interaction between Robo2 and nephrin, we generated Robo2-nephrin double knockout mice and analyzed their survival and the glomerular phenotype by light and electron microscopy.

Results: Like nephrinsingle homozygotes, all Robo2-nephrindouble knockout mice died within 10 hours after birth. By histological analysis, we found that the morphology of glomeruli in the Robo2-nephrin double homozygous knockout mice appeared relatively normal compared with the severe phenotype in nephrin single homozygousmice. Detailed analysis of podocyte ultrastructure by scanning electron microscopy revealed that over 90% glomeruli in nephrin single homozygous knockouts lost the interdigitating podocyte foot process structure. Remarkably, the interdigitating pattern of the podocyte foot processes was restored in 75% glomeruli from Robo2-nephrin double homozygous knockout newborn mouse kidneys.

Conclusions: These results suggest that a concomitant loss of Robo2 and nephrin alleviates the podocyte foot process structural defect in mice. Our findings indicate that the Robo2-Nck-nephrin physical interactions have a substantial effect on podocyte foot process morphology in vivo when the levels of expression of Robo2 and nephrin are genetically altered.

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TH-OR071

Homozygosity Mapping and Whole Exome Resequencing Identify Mutation of *MED28* as a Novel Single-Gene Cause of Nephrotic Syndrome

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Background: A large proportion of single-gene causes of nephrotic syndrome (NS) remains genetically unsolved, and the discovery of novel NS genes may shed light on its pathogenesis.

Methods: In order to identify new genes for NS, we performed homozygosity mapping with whole exome resequencing in a cohort of 63 sibling cases of NS. The expression and function of MED28 were examined in podocytes and rat kidney tissues using biochemical and molecular approaches.

Results: In one affected sibling pair, a homozygous truncating mutation (p.Y163X) was found in the *MED28* gene (*mediator complex subunit 28*). This mutation segregated with the affected status of each sibling and was confirmed by Sanger sequencing. MED28 has been previously characterized as a direct interaction partner of the neurofibromatosis 2 tumor supressor protein (NF2/Merlin), Grb2, and Src-family kinases, e.g. FYN, LCK. In addition, MED28 has been shown to localize not only to the nucleus where it regulates transcription, but also to the cytoplasm where it associates with the actin cytoskeleton. By immunofluorescence of MED28 in rat glomeruli, we showed that MED28 localized to both the nucleus and the cytoplasm of podocytes. MED28 also colocalized with PLCE1, which is mutated in NS in humans, and RHOA, which has been implicated in a mouse model of NS. We also show that overexpression of mutant MED28 (p.Y163X) significantly decreased RhoA activation, but had no effect on the activation of Rac1 and CDC42.

Conclusions: We propose that MED28 has a role in RhoA-mediated cellular signaling, and that mutation of MED28 may be a novel single-gene cause of NS.

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TH-OR072

GLEPP1 Enforces the Integrity of the Glomerular Silt Diaphragm by Activation of Src Kinases and Modulation of Focal Adhesions

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Background: Proteinuria is one of the symptoms of inherited and acquired glomerular kidney diseases. Proteinuria evolves out of the damage of the glomerular filter. One major components of the glomerular slit diaphram is nephrin, which is phosphorylated by src kinases. A loss of nephrin out of the glomerular silt, for example by endocytosis, results in proteinuria. GLEPP1 is a receptor tyrosine phosphatase in the podocyte foot process. Recently, mutations in the GLEPP1 gene were shown to be associated with FSGS in childhhood. The precise function of GLEPP1 is not fully understood.

Methods: Cells expressing nephrin, GLEPP1, β-arrestin2 and src-kinasen were used. After lysis Co-immunoprecipitations were done and the binding partners deteced by western blot. To visualize the activation of src-kinases phosphospecific antibodies for Y527 and Y416 were used. Cos7 cells were retrovirally transduces with GLEPP1 or a control. In a wound scratch assay cell migration was documented after 12h and 24h. For immunofluorescence Glepp1 expressing cos7 cells were used. After fixation the cells were stained for phosphorylated tyrosine, FAK and GLEPP1. Proteinuria in GLEPP1 -/- deficient mice on a 129P3/J background with an age of 6 months was analysed by a commassie.

Results: GLEPP1 interacts with podocin, src, fyn and paxillin. GLEPP1 ameliorates the nephrin interaction with β-arrestin2 and inhibits thereby the nephrin endocytosis. GLEPP1 activates src and fyn by dephosphorylation at their Y527. The activation of src and fyn is confirmed by their autophosphorylation at Y416. Cells stabely transduced with GLEPP1 present an increased migration. GLEPP1 expressing cells present more focal adhesions in the immunoflurescence compared to controls. GLEPP1 -/- mice are proteinuric at the age of 6 months.

Conclusions: Glepp1 stabelizes the glomerular slit diaphram by activation of src-kinases and an increased tunrover of focal adhesions. Patients with a GLEPP1 mutation are lacking the GLEPP1 enabled dynamic regulation of the podocyte foot processes and suffer from FSGS.

TH-OR073

A Chemical Chaperone Improves Defective Secretion of a Mutant Laminin Associated with Human Congenital Nephrotic Syndrome

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Background: Laminin beta 2 (LAMB2) is a component of Laminin-521, the major laminin of the glomerular basement membrane (GBM). Null mutations in *LAMB2* cause Pierson syndrome (congenital nephrotic syndrome with severe extrarenal defects). In contrast, patients with missense *LAMB2* mutations, such as C321R, display congenital nephrotic syndrome with much milder extrarenal symptoms. Here we investigated how the C321R mutation causes proteinuria.

Methods: We generated 3 independent lines of C321R-LAMB2 transgenic mice, each with differing podocyte expression levels (Tg^{Lo}, Tg^{Med}, and Tg^{Hi}) and bred them onto the *Lamb2* null background.

Results: In the 1st postnatal month, *Lamb2*$^{-/-}$ mice exhibited proteinuria that was attenuated in a dose-dependent fashion by deposition of C321R-LAMB2 into the GBM. However, proteinuria increased in all 3 lines, leading to renal failure. Analysis of transgene-derived mRNA and protein levels suggested that ample levels of mRNA were associated with unexpectedly low levels of C321R-LAMB2 in the GBM, suggesting a defect in secretion. *In vitro* studies demonstrated an inhibition of secretion of C321R-LAMB2 that was associated with intracellular retention; similarly, significant podocyte endoplasmic reticulum (ER) distension in Tg^{Med} or Tg^{Hi} mutants was observed, presumably due to accumulation of the mutant protein. Additionally, C321R-LAMB2 expression upregulated the ER chaperones Bip and protein disulfide isomerase, suggesting a protein folding defect. Chemical chaperones such as taurodeoxycholic acid (TUDCA) can assist protein folding in the ER and facilitate the trafficking of mutant proteins. TUDCA treatment of cells expressing C321R-LAMB2 greatly increased its secretion into the medium.

Conclusions: These results may have significant therapeutic implications for the treatment of proteinuria in patients carrying the C321R and perhaps other missense LAMB2 mutations.

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TH-OR074

The Role of Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) in ACTN4-Associated Focal Segmental Glomerulosclerosis (FSGS)

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Background: Podocyte UCH-L1 is upregulated in ACTN4-associated FSGS and may promote ubiquitin proteasome system (UPS) and macroautophagy (MA) impairment. Loss of UCH-L1 should improve podocyte injury in ACTN4-associated FSGS.

Methods: UCH-L1+/- and K256E-ACTN4^{pod+} mice were crossed to generate K256E-ACTN4^{pod+}/UCH-L1-/- mice. Glomeruli were scored for sclerosis using PAS stained kidney sections. Albuminuria (albumin/creatinine) was determined by ELISA. Conditionally

immortalized podocytes were infected with adenovirus containing GFP, WT or K256E α-actinin-4 (αA4) for 3 days and treated with LDN57444 (UCH-L1 inhibitor; 50μM; 24h), or mechanically stretched (24h). Protein expression and localization were assessed by western blot and IF.

Results: K256E-ACTN4^{pod+} mice show segmental and global (35.5% and 31.3% of glomeruli) glomerulosclerosis, with 2519μg/mg albuminuria by 10 weeks of age. K256E-ACTN4^{pod+}/UCH-L1-/- mice exhibit reduced segmental (14.2%) and global (16.6%) glomerulosclerosis at 10 weeks with 569μg/mg albuminuria. Renal ubiquitin (Ub) is elevated in K256E-ACTN4^{pod+} mice while levels are normalized in K256E-ACTN4^{pod+}/UCH-L1-/- mice. K256E αA4 levels were not reduced by UCH-L1 inhibition nor was peripheral localization ameliorated. Similar to WT αA4 expressing cells, UCH-L1 inhibition increased poly-Ub proteins and cleaved caspase-3 in K256E αA4 expressing cells. LC3II levels were also elevated, suggesting MA activation. Interestingly, UCH-L1 inhibition led to loss of UCH-L1 expression in podocytes expressing GFP or WT αA4 but not K256E αA4. Preserved UCH-L1 expression may be due to an impaired UPS or MA pathway. Similar reductions of mono-Ub correlated with UCH-L1 expression. Finally, mechanical stretch decreased LC3II levels in K256E αA4 expressing podocytes, suggesting MA impairment.

Conclusions: Our data suggest that K256E αA4 is associated with UPS/ MA dysfunction and that loss of UCH-L1 may relieve these pathways in a mouse model of FSGS, reducing poly-Ub K256E αA4 aggregates.

TH-OR075

Role of p120 Catenin in Glomerular Function Denise K. Marciano,[1] Bing Liu,[1] Chao-zong Lee,[2] Louis Reichardt.[2] *[1]Medicine, Division of Nephrology, University of Texas Southwestern Medical Center, Dallas, TX; [2]Physiology, University of California, San Francisco, San Francisco, CA.*

Background: Diseases of the glomerulus account for more than 50% of all cases of end stage renal disease, yet few targeted therapies exist to date. This is due in part to our limited understanding of the molecules and signaling pathways that develop and maintain glomerular function. Cell-cell adhesive junctions between glomerular podocytes act as a selective barrier for passage of filtrate into Bowman's space. Similarly, adhesive junctions between parietal epithelial cells have been proposed to act as a selective barrier to retain glomerular filtrate within Bowman's space. While there is considerable knowledge of some of the adhesive proteins involved in podocyte cell-cell junctions, little is known about the role of the adherens junction proteins, namely cadherins and p120 catenin (p120ctn) in glomerular function.

Methods: We used a mouse model to conditionally delete p120ctn from developing nephrons.

Results: We found that p120ctn is produced in developing podocytes as well as parietal epithelial cells. Our data showed that mice lacking p120ctn from developing nephrons acquire proteinuria, inflammation, and focal segmental glomerulosclerosis, a common glomerular pathology in humans. Inflammation was a prominent aspect of this phenotype and was associated with cell-autonomous, as well as non-cell autonomous, upregulation of NF-kB and NF-kB targets. We examined this further by performing gene expression analysis in control and p120ctn-depleted renal epithelial cells and found upregulation of NF-kB targets, as well as several additional genes.

Conclusions: Together these results implicate a role for p120ctn in developing podocytes and/or parietal epithelial cells and suggest that dysregulation of NF-kB signaling may play a critical role in the glomerular function.

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TH-OR076

The Effects of Rac1 Deletion on Short and Long-Term Injury to the Podocyte Stephanie Wylie,[1] Masashi Nishio,[2] Kevin B. Atkins,[2] Matthias Kretzler,[2] Jeffrey B. Hodgin.[1] *[1]Pathology, University of Michigan, Ann Arbor, MI; [2]Internal Medicine - Nephrology, University of Michigan, Ann Arbor, MI.*

Background: Podocyte function relies on maintenance of a complex cytoarchitecture through precise regulation of the actin cytoskeleton. The Rho GTPases are a family of 20+ intracellular signaling molecules with multiple cellular effects, but are best known for cytoskeleton assembly and organization. Pharmacologic evidence indicates that inhibition of the RhoGTPase Rac1 imparts a protective podocyte phenotype, in part by abrogating foot process effacement and proteinuria. We hypothesized that podocyte-specific deletion of Rac1 would be protective in mouse models of podocyte and glomerular injury.

Methods: Podocyte-specific Rac1 deletion in the mouse (podo-Rac1-/-), using Cre-lox technology, showed normal podocyte structure and function. In addition, podo-Rac1-/- mice displayed no podocyte foot process effacement with protamine sulfate infusion, a short-term podocyte injury model. To investigate the effect of podocyte Rac1 deletion in the setting of chronic glomerular damage, we employed a hypertensive model using deoxycorticosterone acetate (DOCA) implants after uninephrectomy (UNX) in mice given high-salt drinking water (UNX/DOCA-salt model).

Results: After 4 weeks, both podo-Rac1-/- and control mice showed similar elevations in blood pressure, kidney and left ventricular weight compared to sham groups. As expected, uninephrectomy and DOCA-salt elicited proteinuria and glomerulosclerosis in control mice. However, UNX/DOCA-salt podo-Rac1-/- mice were not protected, but instead had exacerbated albumin to creatinine ratios at 2 (46.3±6.5 vs. 26.4±4.2 mg/g, $P<0.05$) and 4 weeks (54.3±11.7 vs. 32.7±4.5 mg/g, $P=0.19$) after UNX/DOCA, and twice the glomerulosclerosis by 4 weeks (5.8±0.9 vs 2.7±0.0 glomeruli per 100). Podocyte foot process effacement was focal, but evident in both control and podo-Rac1-/- treated mice, suggesting Rac1-independent pathways may mediate podocyte cytoskeleton rearrangement after injury.

Conclusions: Thus the loss of Rac1 appears to impair immediate cytoskeletal responses in acute injury, but this may predispose to chronic progression of disease.

Funding: NIDDK Support

TH-OR077

Conditioned Nanofibers with Embryonic Stem Cells Improve Nephrotoxicity and Survival in Cisplatin-Induced Renal Failure Yin Wang, Farhad R. Danesh. *Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Cisplatin and other platinum derivatives are the most commonly used chemotherapeutic agents to treat solid tumors. Several strategies have been proposed with the potential to improve the nephrotoxicity and improve cancer patient survival without impairing the chemotherapeutic effect of cisplatin. We have previously reported that conditioned nanofibers with embryonic stem cells (ESCs) can protect against kidney injury in several models of AKI (Wang et al, JASN 2011). In this study, we investigated the protective effects of conditioned nanofibers with ESCs on cisplatin-induced nephrotoxicity.

Methods: Conditioning of nanofibers was performed by exposing nanofibers (100μl) to ESCs for 24 hours as previously described (Wang et al, JASN 2011). Secretome from ESCs was concentrated using centrifugal filters with a 3-kD molecular weight cut-off (Amicon Ultra-PL3). Mice were allocated to receive an IP injection of: 1) conditioned nanofibers (100μl/day), 2) concentrated secretome from ESCs (100μl/day) or 3) non-conditioned nanofibers (100μl/day) for 3 consecutive days. At the 4th day, mice received a single IP injection of cisplatin (20mg/kg). Mice were sacrificed at day6 after cisplatin injection.

Results: We found that conditioned nanofibers with ESCs significantly improved the survival rate of cisplatin-treated mice compared with all other groups (91% vs. 57% and 0% respectively, $P<0.001$). Serum levels of BUN and creatinine were also significantly lower in mice treated with conditioned nanofibers. PAS staining and TUNEL assays indicated that tubular damage score and tubular apoptosis were improved by using conditioned nanofibers. We also examined expression levels of BAX and ROCK1 activity in the kidney. Cisplatin increased the levels of BAX protein and ROCK1 activity, whereas these changes were prevented in mice treated with conditioned nanofibers.

Conclusions: This study demonstrates the first evidence for the renoprotective effect of conditioned nanofibers with ESCs on cisplatin-induced AKI. These findings provide a novel therapeutic strategy in preventing cisplatin-induced AKI.

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TH-OR078

The Effect of Nrf2 Activation by CDDO-Im on Progression of Chronic Kidney Disease after Acute Kidney Injury in Mice Elizabeth M. Higbee,[1] Sanjeev Noel,[1] Thomas W. Kensler,[2] Sekhar P. Reddy,[3] Hamid Rabb.[1] *[1]Johns Hopkins Medical Institutions; [2]University of Pittsburgh; [3]University of Illinois.*

Background: The progression of acute kidney injury (AKI) to chronic kidney disease (CKD) is an important problem, involving unresolved oxidative stress and inflammation. Transcription factor Nrf2 is a crucial regulator of antioxidant gene expression that is known to confer protection against AKI (KI, 76:277, 2009). Recently, increased Nrf2 activation by prolonged treatment with CDDO-Me (bardoxolone) delayed the progression of CKD in humans (NEJM, 365:327, 2011). We tested the hypothesis that long term Nrf2 activation using CDDO-Im would prevent CKD progression after AKI.

Methods: Male C57BL/6 mice (6-8 weeks) underwent 45 min unilateral kidney ischemia. CDDO-Im (30 μmol/kg) or vehicle was administered 3 times per week for 6 weeks, beginning 24 h after injury.

Results: Real-time PCR analysis showed that Nrf2 target gene expression (NQO1, HO1, GCLM, GCLC, and GPX3, $p<0.05$, n=5) was significantly impaired in AKI-induced CKD kidneys. NQO1 was significantly upregulated in all CDDO-Im kidneys as compared to vehicle-treated counterparts (fold change CDDO-Im/vehicle, CKD: 3.8, contralateral: 4.1, n=5); however, CDDO-Im failed to broadly upregulate other Nrf2 target genes at six weeks, which contrasts with short-term CDDO-Im treatment. CKD led to a significant reduction in kidney mass both in vehicle-treated (0.16g±0.02 contralateral vs. 0.08g±0.03 CKD) and CDDO-Im-treated mice (0.18g±0.02 contralateral vs. 0.06g±0.02 CKD, n=5); however, this decrease was not improved by CDDO-Im. Cytokine analysis showed a significant increase in proinflammatory mediators (TGF-β1, TGF-β2, IL-1β, IL-10, IFN-γ, TNF-α, MCP-1) in all CKD kidneys. This increase was significantly higher in CDDO-Im mice compared to vehicle-treated mice (TGF-β2, IL-1β, TNF-α, MCP-1, $p\leq0.01$, n=4).

Conclusions: These data suggest that long-term CDDO-Im treatment, as used in our experimental conditions, is insufficient to prevent established AKI from progressing to CKD. Furthermore, prolonged Nrf2 activation could increase proinflammatory cytokines during CKD.

Funding: NIDDK Support

TH-OR079

Delivery of Adipose-Derived Stem Cells Demonstrates Greater Efficacy than Expanded Bone Marrow-Derived Mesenchymal Stem Cells in Prevention and Treatment of AKI, with Potential for Point-of-Care Treatment and Significant Cost Advantage Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Dmitry O. Traktuev,[3] Stephanie Merfeld-clauss,[3] Keith L. March,[3] Christof Westenfelder.[1,2] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Physiology, University of Utah, Salt Lake City, UT; [3]Vascular and Cardiac Center for Adult Stem Cell Therapy, IU SOM, VAMC, Indianapolis, IN.*

Background: Our pre-clinical studies (AJP 2005) and a Phase I Clinical Trial (Nat Rev Neph 2010) showed that administration of expanded allogeneic bone marrow-derived stromal cells (MSCs) is effective in prevention of and recovery from experimental acute kidney injury (AKI), and is safe and apparently renoprotective in on-pump cardiac surgery patients at risk for post-op AKI. However, MSC expansion and banking are expensive and time-consuming. To address these issues, we compared the therapeutic efficacy of MSCs to those of 1. freshly prepared adipose derived Stromal Vascular Fraction (SVF, composed of adipose derived stem cells [ASCs], endothelial cells, and leukocytes), or 2. minimally processed adipose derived stem cells (ASCs) in rats with AKI. ASCs share therapeutic activities with MSCs but are available in sufficient quantities without need for expansion. SVF can be readily prepared at point of care.

Methods: ASCs and SVF were isolated from abdominal fat from Fisher344 (F344) rats. IRI AKI was induced (40' bilateral renal pedicle clamp) in 5 groups of adult F344 rats (n=7 each). Post reflow, rats were infused via suprarenal aorta with $1x10^6$ (a) autologous SVF,(b) ASCs, (c) syngeneic, cultured MSCs, or vehicle (controls and shams).

Results: Renal function (serum Creatinine) was significantly better protected in ASC and SVF treated animals, and recovery was achieved more rapidly vs. vehicle or MSC treated rats.

Conclusions: Our data suggest that treatment of AKI patients with autologous SVF or ASCs, obtained by minimally invasive lipoaspiration and bedside processing, can provide a safe, efficient, inexpensive point-of-care therapy to prevent or treat AKI. Safety and feasibility of freshly isolated SVF and minimally-expanded ASC will be tested in a Phase I Clinical Trial.

Funding: Veterans Administration Support, Private Foundation Support

TH-OR080

Renal Proximal Tubular Epithelial Cell Survivin Deletion Delays Functional and Structural Recovery from Acute Kidney Injury Jianchun Chen,[1] Jian-Kang Chen,[1] Edward M. Conway,[2] Raymond C. Harris.[1] *[1]Nephrology/Medicine, Vanderbilt University, Nashville, TN; [2]Univ. Centre for Blood Research, British Columbia, Vancouver, BC, Canada.*

Background: Renal proximal tubule cells have a remarkable capacity for regeneration. Survivin is an important component of the mitotic apparatus involved in cell proliferation, but its role in response to AKI is incompletely understood.

Methods: We generated renal proximal tubule cell-specific Survivin knockout mice ($Survivin^{ptKO}$) by crossing $Survivin^{flox/flox}$ mice with gGT-Cre mice and these mice and their wild type littermates (*WT*) were subjected to ischemia reperfusion injury (IR).

Results: Balb/c mice were subjected to bilateral renal pedicles clamping for 35 min followed by reperfusion. Survivin protein expression was markedly increased 48h after IR injury and remained elevated at 6 days after IR. *WT* and $Survivin^{ptKO}$ mice were subjected to IR injury and developed similar initial functional and structural impairment estimated by BUN (24h after I/R: *WT* vs $Survivin^{ptKO}$: 114 ± 15.2 mg/dl vs 115 ± 16.4 mg/dl). 7 days after IR, the BUN recovered (22.4 ± 1.2 mg/dl) in *WT* mice, but remained elevated (50 ± 5.3 mg/dl) in $Survivin^{ptKO}$ mice. Histologic data showed more severe proximal tubule dilation and epithelial simplification and cast formation in $Survivin^{ptKO}$ mice than that in *WT* mice at day 14 after IR injury. Phosphorylation of a Signal Transducers and Activators of Transcription 3 (STAT3) was markedly increased in response to IR within 48h, and inhibition of STAT3 phosphorylation by a specific inhibitor S3I-201 inhibited survivin up-regulation and delayed renal functional and structural recovery in response to IR. To simulate the IR model *in vitro*, we treated mouse cortical tubule epithelial cells (MCT) with antimycin and found that both survivin expression and STAT3 Kphosphorylation were up-regulated within 48h. knocking down the expression of STAT3 by specific siRNAs inhibited antimycin treatment-induced survivin expression in MCT cells.

Conclusions: This study demonstrates that STAT3 phosphorylation-mediated survivin expression is an important molecular mechanism underlying renal functional and structural recovery in response to acute kidney injury.

Funding: NIDDK Support, Veterans Administration Support

TH-OR081

CSF-1-Mediated Proliferation and Polarization of Renal Macrophages/ Dendritic Cells Is Essential for Recovery from Acute Kidney Injury Ming-Zhi Zhang, Bing Yao, Shilin Yang, Raymond C. Harris. *Medicine, Vanderbilt University School of Medicine, Nashville, TN.*

Background: We have previously reported an important role for macrophages in the recovery process following acute kidney injury induced by ischemia/reperfusion (I/R) injury or selective apoptotic proximal tubule injury in transgenic mice expressing the human diphtheria toxin (DTR) receptor and exposed to DT.

Methods: For I/R injury, wild type (WT), CSF-1 -/- mice or WT mice treated with GW2580, a CSF-1 receptor inhibitor, were subjected to 30 min of ischemia and contralateral nephrectomy. CSF-1 -/- mice were also crossed with DTR transgenic mice.

Results: Following I/R-induced AKI, there were early increases in renal macrophages derived from circulating monocytes that expressed "M1" (inflammatory) markers, followed by accumulation of renal macrophages/dendritic cells with an "M2" (wound healing) phenotype. In contrast, in renal injury induced by DT, only M2 macrophages/dendritic cells increased. Depletion of M2 macrophages/dendritic cells with clodronate delayed recovery in both models. We used flow cytometry to double label renal F4/80+ cells with the proliferation marker, Ki67. The increases in M2 macrophages/dendritic cells resulted largely from *in situ* proliferation in the kidney in both models. In both CSF-1-/- mice and mice treated with GW2580, there was markedly delayed recovery in both models of AKI. Genetic or pharmacologic inhibition of CSF-1 signaling inhibited renal macrophage/ dendritic cell proliferation and prevented polarization of these cells to an M2 phenotype.

Conclusions: These studies demonstrate that CSF-1-mediated expansion and polarization of resident renal macrophages/dendritic cells is a novel and important mechanism mediating renal tubule epithelial regeneration following acute kidney injury.

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TH-OR082

Selective Proximal Tubular Mitofusin 2 Deficiency Prevents Oxidant Stress and Promotes Cell Proliferation Following Ischemia Reperfusion (I/R) Injury Jonathan M. Gall,[1] Andrea Havasi,[1] Marc Liesa,[2] John H. Schwartz,[1] Steven C. Borkan,[1] Ramon G. Bonegio.[1] *[1]Renal Section, Boston Medical Center, Boston, MA; [2]Obesity Center, Boston Medical Center, Boston, MA.*

Background: Renal ischemia causes dramatic changes in mitochondrial morphology. Mitochondria regulate cell survival after stress. We hypothesized that MFN2, required for mitochondrial fusion, contributes to organ function by altering mitochondrial injury and/or accelerating organ recovery. To test this hypothesis, mice with structure-specific, conditional MFN2-deficiency (MFN2cKO) were subjected to I/R injury and mitochondrial morphology, histologic injury score, oxidant stress, cell proliferation and renal function were quantified.

Methods: To delete MFN2 in renal proximal tubule cells, MFN2f/f mice were crossed to Kap2-Cre mice that express Cre-recombinase exclusively in renal proximal tubular (PT) epithelial cells. Acute kidney injury was induced in Cre-positive males (MFN2cKOs) and Cre-negative littermates (control) by renal pedicle occlusion. Blood urea nitrogen (BUN), renal histology, oxidant stress (anti-nitrotyrosine staining), and proliferation (BrdU uptake) were assessed within 48 hours after transient ischemia/reperfusion.

Results: MFN2cKO caused fragmentation in proximal tubular epithelial cell mitochondria. Despite similar tubular injury scores, levels of apoptosis and necrosis, post-ischemic survival (86% vs. 28%, $P<0.05$) and renal function were significantly improved in MFN2cKO mice compared to control. Cortical nitrotyrosine staining was reduced by 40% ($P< 0.05$) and BrDU positive cortical epithelial cells were increased 3.5-fold ($P< 0.05$) in MFN2cKO mice.

Conclusions: MFN2 deficiency in proximal tubule epithelial cells promotes proximal tubule survival, decreases local oxidant stress and promotes proximal tubular cell proliferation, key determinants of organ injury and recovery after renal I/R. We propose that modulators of mitochondrial dynamics and/or MFN2 function represent rational targets for treating ischemic acute kidney injury that involve critical injury pathways.

Funding: NIDDK Support

TH-OR083

Toll-Like Receptor-4 Activation Induces Interleukin-22 Secretion in Renal Mononuclear Phagocytes Which Stimulates Tubular Epithelial Cell Regeneration upon Kidney Injury Onkar Kulkarni, Ingo Hartter, Shrikant R. Mulay, Murthy Darisipudi, Dana Thomasova, Hans J. Anders. *Nephrology Center, Medizinische Klinik and Poliklinik IV, Munich, Germany.*

Background: Inflammation involves leukocyte activation at sites of injury which contributes to tissue damage and remodelling. For example, the activation of leukocyte Toll-like receptors (TLR) drives kidney inflammation and injury. Here we address the speculative hypothesis that TLR-mediated leukocyte activation also contributes to repair during the healing phase of acute kidney injury (AKI).

Results: We used the isolation of primary tubular epithelial cells (TECs) as an in-vitro assay to evaluate the potential of various cytokines to enhance tubular cell survival as well as regenerative TEC outgrowth from surviving cells. IL22 showed significant improvement in TEC survival and regeneration. Supernatant from necrotic TECs (NS) induced IL22 release in bone marrow-derived mononuclear phagocytes (BMDC). TLR4 inhibition significantly reduced NS-induced IL22 production in these cells. Only TLR4 but not TLR2/9 stimulation of BMDCs produced significant amounts of IL22. To evaluate the physiological significance of TLR4 activation in renal repair, we blocked TLR4 signaling with anti-TLR4 or control IgG from day 2-4 after unilateral renal pedicle clamping for 45 min. Kidneys were analyzed at day 5. TLR4 blockade significantly reduced IL22 expression in the ischemic kidney (IHC) and reduced the numbers of $CD11b^+CD103^-IL22^+$ and $CD11b^+CD103^+IL22^+$ cells (FACS). TLR4 inhibition was associated with more necrotic tubules (PAS stain) and less intact distal tubules (THP stain) or proximal tubules (lectin stain) and increased expression of injury markers such as πGTP, Kim-1, TGF-β, MCP-1 and TNF-α.

Conclusions: We conclude that necrotic tubuli release endogenous agonists for TLR4 on intrarenal CD11b/CD103+ or CD11b/CD103- mononuclear phagocytes, to release IL22. IL22 promotes TEC survival and enhances tubular regeneration. The role of intrarenal mononuclear phagocytes is not redundant because direct TLR activation of surviving TECs cannot affect survival or repair. These data first document a role of TLR signalling in renal regeneration.

TH-OR084

Thiazide-Sensitive Na^+-Cl^- Cotransporter Gene Inactivation Enhances Bone Ca^{2+} Content and Osteoblast Differentiation In Vitro and In Vivo Yu-Juei Hsu, Sung-Sen Yang, Shih-Hua P. Lin. *Tri-Service General Hospital, Neihu, Taiwan.*

Background: Gitelman's syndrome (GS) is caused by inactivating thiazide-sensitive Na^+-Cl^- cotransporter (*NCC*). GS typically manifests with hypocalciuria and increased bone mineral density (BMD), resembling the effects of long-term treatment with thiazide diuretics.

Methods: To elucidate the molecular mechanisms underlying the effects of NCC inhibition and thiazides on bone we have investigated bone metabolism in nonsense Ncc Ser707X (S707X) homozygous knockin mice ($Ncc^{S707X/S707X}$ mice), analyzing bone Ca^{2+} content, bone architecture, expression of osteoblast differentiation markers and osteoblast-specific transcription factors in vitro and in vivo.

Results: The bone Ca^{2+} content in femurs of $Ncc^{S707X/S707X}$ mice was significantly increased compared to wild-type and heterozygous littermates. Bone structure parameters obtained by mCT revealed significantly increased trabecular bone volume, cortical bone thickness and BMD with a slightly increased polar moment of inertia in Ncc S707X homozygous knockin mice. The expression of bone alkaline phosphatase, procollagen I, osteocalcin and osterix were significantly increased in femoral bones of $Ncc^{S707X/S707X}$ mice as determined by real time quantitative PCR and immunohistochemistry. In vitro, osteoblastic differentiation was enhanced and associated with increased phosphorylation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase 1/2 (ERK 1/2) in cells from mutant mice or from wild type mice treated with thiazides.

Conclusions: NCC inhibition stimulates osteoblast differentiation, and enhances bone Ca^{2+} content, possibly through a FAK / ERK dependent mechanism.

Funding: Other U.S. Government Support

TH-OR085

Ankyrin-3 Binds and Potently Inhibits the Shaker-Like Potassium Channel (Kv1.1): Implications for Renal Mg^{2+} Handling Pedro San-Cristobal, Sergio Lainez Vicente, Henrik Dimke, Joost G. Hoenderop, René J. Bindels. *Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: The kidney is the principal organ responsible in regulation of body Mg^{2+} balance. After being identified as a causative factor in isolated dominant hypomagnesemia, Kv1.1 has emerged as a novel key player in the maintenance of systemic Mg^{2+} balance. Kv1.1 is situated in the distal convoluted tubule (DCT) where it generates a favourable electrochemical gradient, which drives Mg^{2+} into the cell via the Transient Receptor Potential Melastatin 6. Little is, however, known about the molecular mechanisms governing Kv1.1 regulation in the kidney.

Methods: Pull-down experiments using total mouse kidney lysates, coupled with mass-spectrometry were performed. Co-immunoprecipitation assays confirmed interaction of Ankyrin-3 (ANK3) with Kv1.1. Furthermore, electrophysiology studies elucidating the functional properties of those candidates were performed. Finally, RT-PCR (mRNA) of magnesiotropic candidates was quantified from C57BL/6 mice that were fed diets with low (0.005 %w/w), normal (0.19 %w/w), or high dietary Mg^{2+} Content (0.48 %w/w) for 10 days.

Results: ANK3 was identified as a novel binding partner of Kv1.1. ANK3 was predominantly expressed in DCT. Co-expression of ANK3 with Kv1.1 had a significant inhibitory effect on currents generated by Kv1.1. The decrease in channel activity was a consequence of a reduction in the conductance of the potassium channel. This effect occurred independently of changes in the membrane abundance of the channel. Finally, to evaluate a potential role of ANK3 in Mg^{2+} handling, we investigated whether changes in dietary Mg^{2+} content could affect the renal abundance of ANK3. Mice exposed to a high Mg^{2+}diet showed an appropriate elevation in their fractional urinary excretion of Mg^{2+}. Moreover, these mice had an increased renal expression of ANK3 compared to mice on a normal or low Mg^{2+} diet.

Conclusions: Our observations demonstrate a novel functional role for the Kv1.1-ANK3 complexin renal Mg^{2+} handling, which is achieved by modifying the biophysical properties of Kv1.1.

Funding: Government Support - Non-U.S.

TH-OR086

The Membrane Cytoskeletal Crosslinker Ezrin Is Essential for the Regulation of Phosphate Homeostasis in the Kidney Ryo Hatano,[1] Hiroko Segawa,[2] Atsushi Tamura,[3] Ken-ichi Miyamoto,[2] Sachiko Tsukita,[3] Shinji Asano.[1] *[1]Department of Molecular Physiology, College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan; [2]Department of Molecular Nutrition, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan; [3]Laboratory of Biological Science, Graduate School of Frontier Biosciences/Graduate School of Medicine, Osaka University, Suita, Osaka, Japan.*

Background: Ezrin is a cross-linker between the plasma membrane proteins and actin cytoskeleton. In the kidney, ezrin mainly localizes at the brush border membrane of proximal tubules with the scaffolding protein, Na^+/H^+ exchanger regulatory factor (NHERF) 1. NHERF1 interacts with the Na^+/phosphate (P_i) cotransporter, Npt2a. Defects in NHERF1 or Npt2a in mice cause hypophosphatemia. Therefore, in the present study, we investigated the physiological role of ezrin in renal P_i reabsorption by using ezrin knockdown mice (*Vil2$^{kd/kd}$*).

Methods: We used *Vil2$^{kd/kd}$* mice and MDCK cells to investigate roles of ezrin in the regulation of phosphate reabsorption *in vivo* and *in vitro*, respectively.

Results: We found that *Vil2$^{kd/kd}$* mice exhibited hypophosphatemia, hypocalcemia, and osteomalacia. The reduced plasma concentrations of P_i were attributable to defects in urinary P_i reabsorption. Immunofluorescence staining and immunoblotting revealed a marked reduction in renal Npt2a and NHERF1 expression at the apical membrane of proximal tubules in *Vil2$^{kd/kd}$* mice whereas total expression levels of these proteins were not different between WT and *Vil2$^{kd/kd}$* mice kidneys. In *Vil2$^{kd/kd}$* mice, Npt2a localizes mainly in the subapical and other vesicular compartments. In MDCK cells, stable expression of dominant negative ezrin also revealed abnormal membrane expression of Npt2a.

Conclusions: These results suggest that ezrin is required for the regulation of systemic P_i homeostasis by the regulation of membrane localization of Npt2a.

TH-OR087

Klotho Upregulates the Calcium Channel TRPV5 by Intra- and Extracellular N-Glycosylation Dependent Mechanisms Matthias Tilmann Florian Wolf,[1] Chou-Long Huang.[2] *[1]Pediatrics, UTSW Medical Center, Dallas, TX; [2]Internal Medicine, UTSW Medical Center, Dallas, TX.*

Background: Klotho (KL) is an anti-aging molecule synthesized in the distal convoluted tubule (DCT) as a type-1 transmembrane protein; the extracellular domain is secreted into urine to regulate ion homeostasis. Secreted KL functions as sialidase by removing terminal sialic acids from N-glycans of TRPV5. This enables TRPV5 to bind extracellular galectin-1 and to avoid endocytosis. Because Klotho and TRPV5 are both expressed in DCT, we examined if Klotho also regulates TRPV5 acting from intracellular.

Methods: HEK293 cells were cotransfected with TRPV5 and secreted or transmembrane KL. Whole-cell current was analyzed by patch-clamp recording.

Results: We distinguished between extracellular and intracellular actions of KL by using secreted KL (lacks transmembrane domain and is easily detected in supernatant of transfected cells) and transmembrane KL (which was not detected in supernatant). Secreted and transmembrane KL upregulated wild-type TRPV5 but not N-glycosylation-defective mutant TRPV5, supporting that KL increases TRPV5 acting at extracellular and intracellular sites. The role as a sialidase for intracellular KL was supported by mutations of crucial residues for sialidase activity. These mutations abolished TRPV5 regulation by secreted and transmembrane KL. Intracellular action of transmembrane KL was supported by the finding that extracellular application of sialidase inhibitor DANA abolished the effect of secreted but not of transmembrane KL. Secreted and transmembrane KL upregulate TRPV5 by different mechanisms; one involves inhibition of endocytosis and the other increases forward trafficking. While dominant-negative dynamin II abrogated the effect of secreted KL, it did not prevent the effect of transmembrane KL. Blocking forward trafficking by brefeldin A prevented the upregulation of TRPV5 by transmembrane KL, but not by secreted KL.

Conclusions: KL upregulates TRPV5 current acting from intracellular and extracellular sites. Both actions require N-glycosylation of TRPV5 and KL sialidase activity. While extracellular KL inhibits endocytosis of the channel, intracellular KL enhances forward trafficking.

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TH-OR088

Urinary Plasmin Inhibits Transient Receptor Potential Cation Channel V5 in Nephrotic-Range Proteinuria Joost G. Hoenderop,[1] Kukiat Tudpor,[1] Sergio Lainez Vicente,[1] Gerjan Navis,[2] René J. Bindels.[1] *[1]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Internal Medicine, Division of Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

Background: In patients with nephrotic syndrome serum plasminogen levels are elevated. After leakage into the urine, plasminogen is converted into active plasmin by tubular urokinase-type plasminogen activator (uPA). Disturbances in Ca^{2+} homeostasis are frequently reported in patients with nephrotic syndrome including hypocalcemia. This study aims to investigate the effect of urinary plasmin on TRPV5-mediated Ca^{2+} reabsorption.

Methods: TRPV5 activity was determined by patch clamp analysis and $^{45}Ca^{2+}$-influx measurements. Immunoblotting was used to detect the expression level of TRPV5 under the various conditions. Urinary calcium and plasmin was measured by a colometric assay and immunoblotting, respectively. The binding of CaM with TRPV5 was studied by NMR spectroscopy.

Results: We showed that purified plasmin from the urine of patients with nephrotic-range proteinuria inhibits Ca^{2+} uptake in HEK293 expressing TRPV5 via activation of protease-activated receptor-1 (PAR-1). PAR-1 was expressed in HEK293 cells and colocalized with TRPV5 in the mouse distal convoluted tubule. Pre-incubation with plasmin inhibitor, PAR-1 antagonist, or PKC inhibitor abolished the effect of plasmin. The S144A-TRPV5 PKC phosphorylation mutant was resistant to plasmin action. The plasmin-mediated reduction in Ca^{2+} uptake was accompanied by decreased TRPV5 pore size and open probability (NPo) as shown by patch-clamp analysis. Because CaM has been found to interact with residues 133-154 in the N-terminus of TRPV5 (N_{ter}), the effect of S144 phosphorylation on CaM-N_{ter} binding affinity was investigated. High-resolution NMR spectroscopy indicated a tighter binding between CaM-N_{ter} when S144 was phosphorylated.

Conclusions: PAR-1 activation by plasmin induced PKC phosphorylation of TRPV5, enhancing the binding affinity of CaM for TRPV5 and decreasing the channel pore size and NPo. These results demonstrate that urinary plasmin can be involved in the downstream effects of proteinuria on the tubulo-interstitium by the negative modulation of TRPV5.

Funding: Government Support - Non-U.S.

TH-OR089

Constitutively Active LXRα Transgenic Mice Demonstrates Inhibition of Intestinal Phosphate Transporter NaPi-2b Hector Giral-Arnal, Yupanqui A. Caldas, Moshe Levi. *Medicine, University of Colorado, Aurora, CO.*

Background: Pi homeostasis is maintained through the activity of synchronized mechanisms involving intestinal absorption, bone retention-release, and kidney reabsorption. Renal Pi reabsorption has been considered the main mechanism controlling Pi homeostasis. Recently, small intestine Pi absorption has strongly emerged as a new putative pharmacological target for the control of hyperphosphatemia. We have recently reported that activation of Liver X Receptor (LXR) with LXR agonists leads to the decreased expression of renal and intestinal NaPi co-transporters which induced an increase in urinary Pi excretion and a decrease in serum Pi concentration. In order to prove that these effects are associated to specific activation of LXR and to unravel the mechanisms behind this regulation we decided to study a transgenic model, VP16-LXRα Tg mouse.

Methods: VP16-LXRα Tg mouse expresses an active form of the LXRα isoform fused with the activation domain of VP16 under the control of the endogenous LXRα promoter.

Results: VP16-LXRα Tg mice showed reduced expression of NaPi-2b protein in the apical membrane of enterocytes compared to wild type animals. This was paralleled by reduced NaPi uptake in BBM. Moreover, a significant reduction of NaPi-2b mRNA expression was found in the VP16-LXRα Tg animals. Additionally, we characterized the CaCo-2_{BBE} cell line as a model to study the regulation of intestinal Pi transport. LXR activation induced a decrease in the expression of the endogenous NaPi-2b, and reduced the transport activity in our intestinal cell model.

Conclusions: These results point to a direct effect of LXR nuclear receptors in the enterocyte to downregulate the expression of NaPi-2b protein and NaPi transport activity in the apical membrane.

Funding: NIDDK Support, Veterans Administration Support

TH-OR090

Gas6 Protein and It's Role in Vascular Calcification Nadine Kaesler, Svenja Immendorf, Chun Ouyang, Thilo Krueger, Peter Carmeliet, Jürgen Floege, Georg Schlieper. *Nephrology, University Hospital of the RWTH Aachen, Aachen, Germany.*

Background: Vascular calcification is reduced by vitamin K and accelerated by warfarin, i.e. a direct inhibitor of the vitamin K regenerating cycle. These vascular effects are believed to largely involve the vitamin K dependent matrix gla protein. Another vitamin K dependent protein, Gas6, is also expressed in vascular smooth muscle cells (VSMC). Recent data indicated that gas6 mediates protective effects in vascular calcification by inhibition of VSMC apoptosis.

Methods: Here we investigated the role of Gas 6 in *in vivo* and *in vitro* calcification models (*in vitro* cell culture; *in vivo* Warfarin diet for 8 weeks, uninephrectomy (UniNx) combined with high phosphate diet, aging mice at the age of 36 weeks). At the end of the feeding period, echocardiography was performed. After sacrifice, the extent of soft tissue calcification, serum and urine biochemistries were measured.

Results: *In vitro* VSMC exposed to warfarin calcified and this was not different between VSMC generated from wildtype (WT) and $Gas6^{-/-}$ mice. *In vivo*, serum phosphate was higher in UniNx $Gas6^{-/-}$ compared to UniNx WT mice but no significant difference in aortic calcium content was observed between these groups. After 8 weeks of warfarin, total protein and phosphate in serum were significantly higher in $Gas6^{-/-}$ than in WT mice. This was accompanied by a higher aortic calcium content in $Gas6^{-/-}$ mice, however von Kossa staining revealed only a weakly positive vascular staining in $Gas6^{-/-}$ mice. In aging, non-manipulated mice, no significant differences in vascular calcification could be identified between $Gas6^{-/-}$ and WTmice. No differences were found in LV mass, stroke volume or pulse wave velocity in all groups.

Conclusions: Our data do not support a major role of gas 6 in the pathogenesis of vascular calcification.

TH-OR091

Chondrocyte-Specific Calcium-Sensing Receptor (Casr) Deficient Mice Display Severe Growth Retardation, Hypercalcemia, Hyperparathyroidism and Early Death Hakan R. Toka,[1,2] Salvatore Dibartolo,[2] David B. Mount,[1] Gary C. Curhan,[1] Edward M. Brown,[3] Martin R. Pollak.[2] *[1]Nephrology, Brigham and Women's Hospital, Boston, MA; [2]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [3]Endocrinology, Brigham and Women's Hospital, Boston, MA.*

Background: The calcium-sensing receptor (Casr) has an important role in regulating calcium homeostasis within several tissues including cartilage. Previous *in vivo* studies suggest that chondrocyte-specific Casr-deficient mice driven by the Collagen 2alpha1 (Col2a1) promoter, in which exon 7 of the Casr was deleted, display a very severe phenotype with embryonic lethality.

Methods: We generated a conditional Casr-deficient mouse model targeting exon 3 using the Cre/Lox system. We obtained Cre transgenic mice driven by the chondrocyte-specific Col2a1 promoter and developed chondrocyte Casr-deficient mice (Ch $Casr^{-/-}$).

Results: Ch $Casr^{-/-}$ mice are viable and born at the expected Mendelian ratio displaying growth retardation beginning at 3 to 4 days of age. Most Ch $Casr^{-/-}$ show significant growth retardation by the age of 3 to 4 weeks with decreased life expectancy. The cause of death is unclear. Studies at ages 3 days and 3 weeks show hypercalcemia (12.4mg/dl ±0.7 vs. 9.2 ±0.4, p=0.02, and 15.2 ±5.5 vs. 11.6 ±0.3, p=0.4, respectively) and hypophosphatemia (3.1 mg/dl ±0.4 vs. 4.0 ±0.6, p=0.06, and 1.8 ±0.4 vs. 3.0 ±0.7, p=0.03, respectively) with significantly elevated PTH levels at age 3 weeks (510 pg/ml ±373 vs. 128 ±18, p=0.01). Ch $Casr^{-/-}$ animals surviving 5 weeks or longer display dwarfism with thick bones.

Conclusions: Chondrocyte-specific Casr-deficient mice driven by the Col2a1 promoter are viable, exhibiting a less severe phenotype than that observed previously by others using conditional deletion of exon 7 in chondrocytes. However, they display significant growth retardation, hypercalcemia, hyperparathyroidism and early death. The cause of the unexpected hypercalcemic hyperparathyroidism is currently unclear. *In vivo* and *in vitro* studies addressing parathyroid function, chondrocyte differentiation and proliferation, and endochondrial ossification in our model are being pursued.

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TH-OR092

Membrane Topology and Intracellular Processing of Cyclin M2 (CNNM2) Jeroen H.F. De Baaij,[1] Marchel Stuiver,[2] Iwan Meij,[3] Sergio Lainez Vicente,[1] Dominik Müller,[2] René J. Bindels,[1] Joost G. Hoenderop.[1] *[1]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Department of Pediatric Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Molecular Medicine, Max Delbrück Centre for Molecular Medicine, Berlin, Germany.*

Background: Recently, mutations in the Cyclin M2 (CNNM2) gene were identified to be causative for severe hypomagnesemia. In kidney, CNNM2 is a basolaterally expressed protein with highest expression in the distal convoluted tubule (DCT). Transcellular magnesium (Mg^{2+}) reabsorption in DCT represents the final step before Mg^{2+} is excreted into the urine. The present study aims to get insight in the structure of CNNM2 and to characterize its post-translational modifications.

Methods: The topology of CNNM2 was evaluated by immunocytochemistry using intramolecular epitopes. In addition, CNNM2 was expressed in HEK293 cells after which expression and modifications of the protein were measured by immunoblot analysis.

Results: Extensive expression profiling shows that CNNM2 is mainly expressed in the DCT. Furthermore, immunocytological membrane topology studies showed that CNNM2 has an extracellular amino-terminus and an intracellular carboxy-terminus. This suggests that one of the predicted transmembrane regions is reentrant. By homology modeling we demonstrated that the loss-of-function mutation as found in patients disturbs potential ATP binding by the intracellular cysthationine β-synthase domains. In addition, the cellular processing pathway of CNNM2 was exposed in detail. In the endoplasmic reticulum, the signal peptidase complex cleaves off a large amino-terminal signal peptide of about 64 amino acids. Mutagenesis screening showed that CNNM2 is glycosylated at N112 stabilizing CNNM2 on the plasma membrane. Interestingly, co-immunoprecipitation studies evidenced that CNNM2a forms heterodimers with the smaller isoform CNNM2b.

Conclusions: In conclusion, we propose a protein topology of CNNM2 consisting of three membrane-spanning domains and a reentrant loop. CNNM2 is post-translationally modified by signal peptide cleavage and glycosylation. Rather than transporting Mg^{2+} itself, we hypothesize CNNM2 indirectly regulates other Mg^{2+} transporters.

Funding: Government Support - Non-U.S.

TH-OR093

New Regulatory Mechanism Explaining the Molecular Basis of Constitutively Active Variants of the Calcium-Sensing Receptor (CaSR) Marianna Ranieri,[1] Grazia Tamma,[1] Annarita Di Mise,[1] Giuseppe Vezzoli,[2] Laura Soldati,[3] Maria Svelto,[1] Giovanna Valenti.[1] *[1]Department of Biosciences, Biotechnologies and Pharmacological Sciences, University of Bari, Bari, Italy; [2]Division of Nephrology and Dialysis (IRCCS), San Raffaele Hospital, Milan, Italy; [3]Department of Health Sciences, University of Milan, Milan, Italy.*

Background: To evaluate some aspects of renal CaSR physiopathology, we analyzed the signaling underling two constitutively active variants of the CaSR.

Methods: Constructs encoding human wild-type CaSR (hCaSR-wt) and its constitutively active (hCaSR-R990G; hCaSR-N124K) and inactive variants (hCaSR-del121) were transiently transfected in HEK cells.

Results: Immunofluorescence studies revealed that both hCaSR-wt and its activating variants were expressed at the plasma membrane, whereas the inactive form localized intracellularly. The physiological agonist, calcium (Ca^{++} 5mM), and the calcimimetic NPS-R568 (5μM) induced a significant increase in cytosolic calcium in cells expressing hCaSR-wt and its active variants, compared to mock. We also found that the basal intracellular calcium was significantly lower in cells expressing hCaSR-wt and its activating variants compared to mock and hCaSR-del121 transfected cells. Low calcium levels are expected to make cells more sensitive to intracellular calcium changes in response to CaSR agonists. In line, FRET studies using D1ER probe, which detects $[Ca^{++}]_{ER}$ directly demonstrated a significant higher calcium accumulation in cells expressing the activating CaSR variants. Since the storage of calcium in the ER is mainly regulated by SERCA, the activity and the expression of this pump were evaluated. Compared to hCaSR-wt expressing cells SERCA expression and activity were found significantly increased in cells expressing activating CaSR variants. An inverse correlation with PMCA was also found.

Conclusions: Together, these findings indicate that for the efficiency of calcium signaling system, cells monitor cytosolic and ER calcium levels regulating the expression of the SERCA and PMCA. To our knowledge this is the first demonstration that a complex parallel adaptive feedback can explain the molecular basis of constitutively active variants of the CaSR.

Funding: Government Support - Non-U.S.

TH-OR094

IV Iron Dosing and Changes in Serum TSAT, Ferritin, and Hemoglobin Levels: Implications of Increasing IV Iron Dosing in the US

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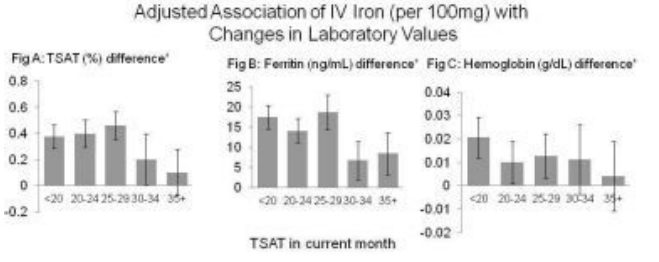
Background: Dialysis Outcomes and Practice Patterns Study (DOPPS) data (8/2010 to 12/2011) indicate that, since the new US bundled payment system in 1/2011, the 1mo period prevalence of IV iron (Fe) use rose in the US by 20% (to 77%) and the median serum ferritin level rose by 25% (to 697ng/mL), but the median TSAT rose by only 3% (to 29%). We evaluated the hypothesis that iron dosing at higher TSAT levels raises ferritin but has little effect on subsequent TSAT.

Methods: Monthly data were from 15,183 HD patients in 12 DOPPS 4 countries (2009-11). Among patients prescribed IV Fe, the effects of Fe dose within strata of current TSAT on the next month's TSAT(N=28,000), ferritin(N=18,818), and hemoglobin(hgb, N=37,677) were estimated using GEE models.

Results: Fe dose was positively associated with next month's TSAT, ferritin, and hgb, but associations were weaker for current TSAT≥30%. Every 100mg of Fe raised next month's TSAT by 0.43%(for TSAT<30%) vs 0.10%(for TSAT≥30%; p<.001 for interaction); ferritin by 17 vs 7ng/mL(p<.001); and hgb by 0.017 vs 0.004g/dL (p<.001).Findings by 5% TSAT increments follow.



*Y axis shows next-month minus current-month values, for the effect of IV iron dose in current month.
Adjusted for: age, gender, black race, vintage, catheter use, BMI, region (Japan, Europe/ANZ, US/Canada), 14 comorbid conditions including GI bleed; current month: ESA dose, hemoglobin, white blood cell count, and serum albumin, creatinine, TSAT, and ferritin; one month prior: IV iron dose, ESA dose, hemoglobin, TSAT and ferritin.
For results presented in the text, 'TSAT in current month' was dichotomized at 30%.

Conclusions: Though targeting TSAT 30-50% is now common in the US, Fe dosing has little effect on TSAT when TSAT is already ≥30%. The effect of Fe on ferritin and hgb levels is also modest at TSAT≥30%. Ongoing Fe dosing when TSAT is ≥30% provides little erythropoietic support and may deleteriously increase parenchymal iron deposition. Studies evaluating this possibility are needed.

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TH-OR095

Comparative Safety of Intravenous Iron Supplementation Practices in Hemodialysis Patients

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Background: Intravenous (IV) iron is in widespread use in hemodialysis patients, but the comparative safety of different approaches to dosing is unknown. We sought to compare different IV iron supplementation practices with respect to risk of various adverse outcomes in hemodialysis patients.

Methods: We conducted a retrospective cohort study of hemodialysis patients using clinical data from a large dialysis organization merged with hospitalization data from the United States Renal Data System, 2004-2008. We compared the effects of bolus dosing (providing a large amount of iron over a short period of time) vs maintenance dosing (smaller administrations designed to maintain iron repletion) and high vs low dose. Semiparametric additive risk models were used to assess the relation between measures of iron exposure and mortality, infection- and cardiovascular-related hospitalizations, and hypersensitivity reactions occurring during a 3-month (mo) follow-up. The models included a range of clinical and laboratory variables assessed prior to exposure.

Results: Of 117,050 patients who met our study entry requirements, 9,033 (16.3%) received a bolus administration, 55,457 (47.4%) received maintenance therapy, and 42,560 (36.4%) received no iron during the 1-mo exposure assessment period. Compared with maintenance iron, those receiving bolus iron were at increased risk of infection-related hospitalization (4.3 additional events/100 patient years(PY), 95% CI 2.0-6.5) during follow-up. Patients receiving over 200mg/mo of iron were at slightly increased risk of infection (2.0 events/100 PY, 95%CI 0.2-3.8). We observed no association between bolus dosing and the other outcomes considered. Compared to no iron, maintenance dosing was not associated with an increased risk of adverse outcomes. The results were robust to changes to the length of the exposure, follow-up period, and the inclusion of additional covariates.

Conclusions: Sequences of large doses of IV iron are associated with an increased risk of infection; smaller, less frequent doses aimed at maintenance of iron reserves appear to be safe.

Funding: Other U.S. Government Support

TH-OR096

FG-4592, an Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor, Corrects Anemia without Iron Supplementation in Incident Dialysis Patients

Anatole Besarab,[1] Elena Na Chernyavskaya,[2] Igor Motylev,[3] Shutov Evgeny,[4] Anatoly F. Yampolskiy,[5] Lalathaksha Murthy Kumbar,[1] Konstantin Gurevich,[6] Daniel Tak Mao Chan,[7] Robert Leong,[8] Ming Zhong,[8] Marietta Franco,[8] Kin-Hung Peony Yu,[8] Thomas B. Neff.[8] *[1]Henry Ford Hosp, Detroit, MI; [2]City Clin Hosp, Omsk, RU; [3]City Hosp, N. Novgorod, RU; [4]City Clin Hosp, Moscow, RU; [5]Regional Nephrology Center, Krasnodar, RU; [6]City Hosp of St Venerable Martyr Elizabeth, St Petersburg, RU; [7]Queen Mary Hosp, HK; [8]FibroGen, Inc., San Francisco, CA.*

Background: Optimal iron management strategy in treating anemia is controversial. We evaluated whether FG-4592, with or without iron supplementation, can correct hemoglobin (Hb) levels in incident dialysis patients.

Methods: In an ongoing, open-label, phase 2 study, erythropoiesis-stimulating-agent-naïve patients on hemodialysis (HD) for 2 wks to 4 mos, with baseline (BL) Hb ≤ 10.0 g/dL, were randomized to 3 arms (N=12/arm) to receive FG-4592 thrice weekly for 12 wks without iron, with oral iron, or with intravenous (IV) iron. Another arm evaluated FG-4592 with oral iron in peritoneal dialysis patients (N=12). A confirmatory arm further assessed FG-4592 without iron in HD patients (N=12). Initial FG-4592 doses were tiered by weight; dose adjustments were allowed every 4 wks.

Results: Results are reported for 36 HD patients completing ≥8 weeks of treatment (Table); a majority were not iron replete at BL. Mean Hb changes from BL at Wk 8 were similar across arms. FG-4592 was well tolerated, with an adverse event profile consistent with the patient population.

FG-4592 Arm (N=12/Arm)	N (%) Not Iron Replete at Baseline (BL)	Mean±SE Ferritin (ng/mL), BL	Mean±SE TSAT (%), BL	Mean±SE Hb (g/dL), BL	Mean±SE Change in Hb (g/dL), Wk 8	Mean±SE Max Change in Hb (g/dL)
No Iron	9 (75)	150±19	20±2	7.8±0.3	2.1±0.4	2.8±1.0
Oral Iron	6 (50)	184±63	21±2	8.5±0.3	2.2±0.4	3.3±1.9
IV Iron	9 (75)	158±25	23±5	8.3±0.3	2.3±0.7	3.2±1.7

Conclusions: Preliminary data indicated that treatment with oral FG-4592 TIW increased mean Hb in incident dialysis patients, regardless of BL iron status or iron supplementation. FG-4592 may prevent iron-deficient erythropoiesis, thus avoiding potential risks associated with parenteral iron.

Funding: Pharmaceutical Company Support - FibroGen, Inc.

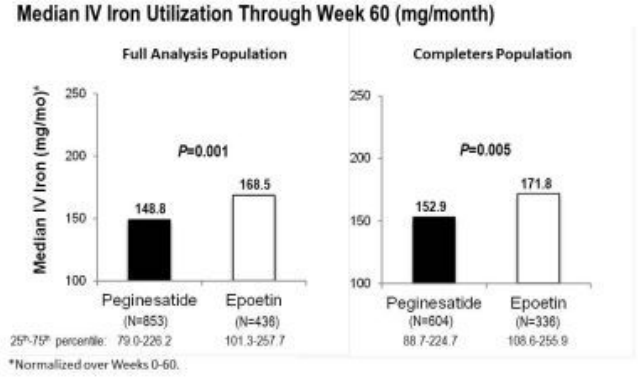
TH-OR097

Intravenous (IV) Iron Use in US Hemodialysis (HD) Patients (Pts) Receiving Peginesatide for Anemia due to Chronic Kidney Disease Robert Provenzano,[1] Bruce S. Spinowitz,[1] Anatole Besarab,[1] Steven Fishbane,[1] Whedy Wang,[2] Alex Yang,[2] Martha Mayo,[2] Iain C. Macdougall.[1] *[1]AFX01-12 & -14 Peginesatide Study Groups; [2]Affymax, Inc, Palo Alto, CA.*

Background: Two randomized open-label, active-controlled trials (EMERALD 1, 2) provide a large (N~1600) database on long-term (median follow-up >60 wk) anemia treatment with peginesatide or epoetin in HD pts. In these trials, peginesatide was noninferior to epoetin in maintaining hemoglobin (Hb) levels, with similar cardiovascular safety. Given the interdependence of iron and ESAs on red cell production, we conducted post-hoc analyses of iron use and status in US EMERALD pts.

Methods: Data were pooled from EMERALD 1 and 2, assessing peginesatide Q4W vs epoetin 1-3X weekly (randomized 2:1) in US HD pts on stable epoetin doses ≥4 wk. IV iron dose, serum ferritin, and TSAT in full analysis population (FAS, randomized pts receiving ≥1 study drug dose; N=853 peginesatide, N=436 epoetin) and in completers (≥60 wks' drug exposure; ferritin, TSAT measured at same visit; N=604 peginesatide; N=336 epoetin) were summarized in 12-wk intervals through week 60.

Results: In both populations, peginesatide pts received less IV iron than epoetin pts (Figure).



TSAT (FAS) was similar at baseline in both arms (30.2% peginesatide; 29.4% epoetin) and increased to 37.9% with peginesatide compared to 30.7% with epoetin by week 12, with similar results in completers *(P*<0.01 wk 12-60, both populations). In both arms, ferritin was similar at baseline and through week 60, and Hb levels were similar for the duration of the study (FAS and completers).

Conclusions: In two post-hoc analyses in US pts, peginesatide pts received less IV iron, had higher TSAT, and had similar ferritin levels over 60 weeks, while maintaining similar Hb levels, vs epoetin pts. The observed difference in iron availability between these agents merits further scientific evaluation.

Funding: Pharmaceutical Company Support - Affymax, Inc., and Takeda Pharmaceutical Company Ltd.

TH-OR098

Sickle Cell Trait in African-American (AA) Hemodialysis Patients Associates with Higher Doses of Erythropoiesis-Stimulating Agents Vimal K. Derebail,[1] Eduardo K. Lacson,[2] Abhijit V. Kshirsagar,[1] Nigel S. Key,[1] Susan L. Hogan,[1] Raymond M. Hakim,[3] Ann Mooney,[2] Chinu M. Jani,[2,4] James Zazra,[4] Yichun Hu,[1] Ronald J. Falk,[1] J. Michael Lazarus.[1,2] *[1]University of North Carolina; [2]Fresenius Medical Care, NA; [3]Vanderbilt University; [4]Spectra Laboratories.*

Background: Based upon initial small studies, we evaluated whether variant hemoglobin (Hb) - sickle cell trait (HbAS) and hemoglobin C trait (HbAC)- associated with higher dose erythropoiesis-stimulating agents (ESAs) in a large, targeted national sample of AA in-center hemodialysis (HD) patients.

Methods: 5,319 AA adult prevalent HD patients were screened by hemoglobin (Hb) electrophoresis. Laboratory data and ESA dose were collected over seven months. Data were evaluated by Hb type (HbAA, HbAS, HbAC). Multivariate linear regression models (adjusted for case-mix and labs) were constructed with log-transformation of ESA dose/ treatment.

Results: Variant Hb was common and present in 671 (12.6%) patients – HbAS in 542 (10.2%) and HbAC in 129 (2.4%). Except ESA dose, variables were similar even when stratified by Hb variant, including vascular access type. Achieved Hb and hospitalization days were also similar among groups. In univariate analysis, median ESA dose was ~8.6% (p=0.02) greater in those with variant Hb and consistent between HbAS and HbAC.(Table) In multivariate models, variant Hb was associated with 13.2% (p=0.001) higher ESA dose, primarily driven by HbAS at 13.2% (p=0.003) with HbAC at 12.9% (p=0.1).

ESA dosing by Hb phenotype.

	HbAA	Variant Hb*	HbAS	HbAC
	N=4,648	N=671	N=542	N=129
Unadjusted				
ESA dose (u/tx)†	4364.1 (2237.4, 7903.4)	4737.4 (2566.0, 8645.5)	4701.1 (2611.9, 8489.2)	5449.2 (2550.5, 9832.4)
Multivariate model				
% Diff. in ESA dose (95% CI)‡	ref	13.2 (5.1, 21.9)	13.2 (4.3, 22.9)	12.9 (-3.1, 31.6)

*HbAS&HbAC, †Median(IQR), ‡Adj for age, sex, vintage, iPTH, albumin, Kt/V, ferritin, Tsat, vascular access, iron dose, missed treatments, hospitalizations

Conclusions: In a large cohort of AA HD patients, variant Hb, particularly HbAS, is common and associated with 13% higher ESA dose. Besides the potential financial impact, the clinical implications of higher ESA dose in these patients require further study.

Funding: Other NIH Support - Duke-UNC Clinical Hematology Research Career Development Program 5K12 HL087097-05, NIH/NHLBI, PI: Dr Marilyn Telen, Pharmaceutical Company Support - Fresenius Medical Care, North America

TH-OR099

Protein Carbamylation Is due to Urea-Amino Acid Imbalance and Predicts Kidney Failure Mortality Anders H. Berg,[1] Christiane Drechsler,[2] Julia Beth Wenger,[3] Christoph Wanner,[2] Ravi I. Thadhani,[3] S. Ananth Karumanchi.[1] *[1]Beth Israel Deaconess Medical Center; [2]University of Wuerzburg, Wuerzburg, Germany; [3]Massachusetts General Hospital.*

Background: Urea carbamylates proteins and amino acids, and carbamylated proteins contribute to atherosclerosis. We hypothesized that serum levels of carbamylated albumin represent a time-averaged index of uremia and risk in hemodialysis patients, and that deficiencies of free amino acid scavengers promote protein carbamylation.

Methods: Serum from 187 participants of the Accelerated Mortality on Renal Replacement study and 1,131 subjects of Die Deutsche Diabetes Dialyze Studie were analyzed. Specimens were collected at the beginning of the 12-month study and analyzed for % carbamylated albumin and free amino acids using LC-MS/MS.

Results: Serum carbamylated albumin (%C-Alb) correlated with average blood urea and was doubled in ESRD patients compared to controls (*P*< 0.0001). %C-Alb was associated with 1-year mortality. In the 4D study cohort, %C-Alb was a similarly strong predictor of mortality. Negative correlations between subjects' %C-Alb and most amino acids suggested that free amino acids inhibit protein carbamylation. Glycylglycine, taurine, and cysteine efficiently inhibited albumin carbamlyation in vitro. Serum myeloperoxidase, thiocyanate, and smoking history were not associated with %C-Alb levels.

ArMORR Study Hazard Ratio Estimates for Mortality by Level of % Carbamylated Albumin.

	Univariate		Multivariable	
Variables	HR (95% CI)	p-value	HR (95% CI)	p-value
% Carbamylated Albumin	3.76 (2.20 - 6.43)	<0.0001*	3.04 (1.70 - 5.42)	0.0002*
Albumin	0.43 (0.26 - 0.71)	0.001*	0.58 (0.34 - 0.99)	0.04*
Hemoglobin	0.79 (0.69 - 0.91)	0.001*	0.95 (0.81 - 1.10)	0.46
History of Hypertension	0.42 (0.25 - 0.71)	0.001*	0.44 (0.26 - 0.74)	0.002*
Systolic Blood Pressure	0.98 (0.97 - 0.99)	<0.0001*	0.99 (0.98 - 1.00)	0.007*

*Students' t-test for significance at p<0.05. Data collected on days 15 - 90 after initiation of dialysis. HR represents risk of death over 12 month period.

Conclusions: Serum %C-Alb represents a novel biomarker associated with mortality in hemodialysis patients. Chronic uremia combined with amino acid deficiencies contributes to protein carbamylation, and may be modifiable by amino acid therapy.

Funding: Private Foundation Support

TH-OR100

Hemodialysis-Induced Regional Left Ventricular Systolic Dysfunction Is Associated with Inflammation Solmaz Assa,[1] Yoran M. Hummel,[2] Adriaan A. Voors,[1] Johanna J. Kuipers,[3] Paul E. de Jong,[1] Ralf Westerhuis,[3] Casper F. Franssen.[1] *[1]Nephrology; [2]Cardiology, University Medical Center Groningen; [3]Dialysis Center Groningen, Netherlands.*

Background: Hemodialysis (HD) may acutely induce regional left ventricular (LV) systolic dysfunction ('cardiac stunning'), which is associated with increased mortality and progressive heart failure. We hypothesize that patients with HD-induced regional LV systolic dysfunction have elevated markers of inflammation, which might affect cardiac function. We, therefore, studied the relation between HD-induced regional LV systolic dysfunction and plasma markers of inflammation.

Methods: In 105 patients with a mean (±SD) age of 62.5±15.6 and median (IQR) dialysis vintage of 21.4 (7.8-48.5) months, echocardiography was performed pre-HD, 60 and 180 min intra-HD, and 30 min post-HD. HD-induced regional LV systolic dysfunction was defined as an increase in wall motion score index in ≥2 segments compared with pre-HD. Plasma levels of hsCRP, IL-6 and IL-10 were measured pre-HD.

Results: Twenty-nine (27%) patients developed HD-induced regional LV systolic dysfunction. These patients had significantly higher CRP and IL-6 levels and higher lL-6/ IL-10 ratio.

Pre-dialysis, median (IQR)	No HD-induced regional LV systolic dysfunction (n=76)	HD-induced regional LV systolic dysfunction (n=29)	P value
hsCRP, mg/l	4.7 (1.6-8.8)	8.8 (6.7-12.7)	0.011
IL-6, pg/ml	4.7 (3.0-7.6)	7.1 (5.2-9.3)	0.021
IL-10, pg/ml	0.40 (0.29-0.57)	0.33 (0.22-0.59)	0.149
IL-6/ IL-10 ratio	10.7 (6.2-21.1)	20.1 (12.4-30.6)	0.002

In multivariate analysis correcting for age, sex and dialysis vintage, higher log-hsCRP (p=0.028), lower log-IL-10 (p=0.038) and higher log-IL-6/IL-10 ratio (p=0.005) were significantly associated with HD-induced regional LV systolic dysfunction. hsCRP increased with an increasing number of abnormal LV segments developing during or after HD: 0-1 abnormal segments: 4.7 (1.6-8.8); 2-3 abnormal segments: 7.8 (6.9-9.1); ≥4 abnormal segments: 9.8 mg/l (3.2-14.5); p=0.035.

Conclusions: Patients with HD-induced regional LV systolic dysfunction have a pro-inflammatory cytokine profile. These findings suggest that inflammation is involved in the pathogenesis of HD-induced regional LV systolic dysfunction.

Funding: Government Support - Non-U.S.

TH-OR101

Myostatin and Atrogin-1 mRNA Are Upregulated with TNF-α/IL-6 Elevated in Skeletal Muscle of Patients Hemodialysis Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: The accelerated muscle wasting is common in advancedchronic kidney disease (CKD). A chronic inflammatory cytokine condition including elevated levels of the proinflammatory cytokine such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), play a pivotal role in muscle wasting in CKD. This study investigates the relationship of inflammation cytokines and muscle wasting in patients with hemodialysis (HD), and the expression of genes involved in the regulation of muscle massin musclebiopsies.

Methods: 40 HD patients were selected(HD)and 30 healthy adults with gender and age matched as control(Ctl). The biochemical, inflammatory cytokine were measured with their serum samples, the muscle mass was measured by magnetic resonance imagin(MRI). Then we analyzed the expression of Myostatin and Atrogin-1in muscle biopsies from 8HD patients and 6 non-kidney patients.

Results: The HD patients had asignificantly lower body mass index (BMI 23.44±2.50 kg/m²Ctl vs 21.36±4.47kg/m² HD, p<0.05), though the plasma albumin showed no difference in HD and Ctl.(44.02±2.33g/L Ctlvs 42.23±3.50g/L HD, p=0.329), the serum inflammation markers such as high sensitive C-protein, TNF-α and IL-6 showed elevated (hs-CRP(2.08±2.14 mg/L Ctl vs 23.95±46.46 mg/L HD, p<0.001),TNF-α(50.03±9.19 pg/mLCtl vs 65.10±12.05pg/mL HD, p<0.05), IL-6(7.29±6.91 pg/mL Ctl vs 38.53±108.99 pg/mL HD, p<0.001) in HD compared with Ctl. The HD patients tend to have a less muscle mass when analyses the cross-sectional area (CSA) of low limb by MRI. The muscle composition: total CSA ratio for all layers of MRI images was significantly decrease (p<0.01). Interestingly, the muscle TNF-α, IL-6, mostatin and Atrogin-1 mRNA levels was elevated 3-6 fold measured by RT-PCR.

Conclusions: Advanced CKD patients present a high level of TNF-α and IL-6 in serum and muscle sample. The loss of muscle mass accompanies the expression of myostatin and Atrogin-1 upregulated. So ameliorate chronic inflammatory disorders might be a reasonable strategy to improve muscle wasting in CKD.

Funding: Government Support - Non-U.S.

TH-OR102

Hypoglycemia-Related Hospitalization in Diabetic Hemodialysis Patients: Risk Factors Mark E. Williams,[1] Weiling Wang,[2] Eduardo K. Lacson.[2] *[1]Renal Division, Joslin Diabetes Center, Boston, MA; [2]Fresenius Medical Care, NA, Waltham, MA.*

Background: The benefit of tight glycemic control on outcomes in diabetic ESRD appears to be weak. Hypoglycemia is associated with increased risk of adverse clinical outcomes in the general diabetic population. We previously reported that the risk of hypoglycemia-related hospitalization was associated with higher, not lower, hemoglobin A1c (HbA1c) values.

Methods: We further analyzed 24,751 diabetic chronic in-center HD patients in Fresenius-North America facilities active on January 1, 2003 with at least one HgA1c in Q4-2002. Baseline patient characteristics (age, gender, race, body surface area or BSA, diabetes type, vascular access), insulin use, and labs (HbA1c, eKt/V, albumin, hemoglobin, calcium, phosphorus, creatinine, and white blood cell count) were recorded. Over the next three years, hospitalization ICD-9 diagnosis codes that specified hypoglycemia were found in 1,017, or 4.1% of patients (cases). Stepwise logistic regression models were used to determine significant associates of hypoglycemia-related hospitalizations. Time-dependent Cox models were used to relate HbA1c and glucose results to the same outcome.

Results: High HbA1c was associated with increased risk of hypoglycemia in the same quarter for Type 1 and, to a lesser extent in Type 2, patients. Glucose variability was strongly associated with hospitalization and mortality by standard Cox model. HbA1c levels drawn one quarter earlier confirmed the greater risk of hypoglycemia-hospitalization for high HbA1c levels. Over 12 quarters, distribution of HbA1c values in the population remained constant, except for a decrease in the lowest HbA1c categories. Use of insulin rose steadily in incident patients throughout the 12 quarters. High-A1c patients were more likely to be on insulin. Greater glucose variability was associated with higher mean glucose levels by trend analysis.

Conclusions: High HbA1c values and glucose variability increase the hazard risk for hypoglycemia requiring hospitalization in diabetic ESRD. Risk of hypoglycemia must be considered when attempts are made to improve high A1c levels with aggressive use of medications such as insulin.

TH-OR103

Effect of Ultrapure Dialysate on Markers of Inflammation, Oxidative Stress, and Nutrition, and Anemia Parameters: A Meta-Analysis Paweena Susantitaphong,[1,2] Cristian Riella,[1] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn University, Bangkok, Thailand.*

Background: Markers of inflammation have been linked to malnutrition and confer an increased mortality risk in hemodialysis patients. Ultrapure dialysate has been hypothesized to have a beneficial effect on markers of inflammation. To systematically review the evidence, we conducted a meta-analysis of all the studies of patients on hemodialysis that examined the effect of ultrapure vs. standard dialysate on markers of inflammation, oxidative stress and nutrition, and anemia parameters, and explored sources of heterogeneity.

Methods: We performed a literature search using MEDLINE, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, scientific abstracts, and bibliographies of retrieved articles. Single-arm cohort studies (pre-/post-study evaluations), non-randomized and randomized controlled trials were included. We conducted random-effects model meta-analyses to assess changes in outcomes of interest.

Results: We identified 16 single-arm or cohort studies, 2 crossover and 3 parallel-arm non-randomized controlled trials, and 5 crossover and 5 parallel-arm randomized controlled trials. In an analysis of 23 study arms or cohorts that assessed CRP (n=2,221), use of ultrapure dialysate resulted in a significant decrease in the CRP level (-0.32 mg/dL; 95% CI -0.46,-0.18; P<0.001). Other inflammatory markers displayed similar significant improvements. There were also a significant increase in the serum albumin (0.11 g/dL; 95% CI 0.02, 0.19; P=0.011) and hemoglobin level (0.40 g/dL; 95% CI 0.06, 0.75; P=0.022), and a decrease in the weekly erythropoietin dose (-273 units; 95% CI -420, -126; P<0.001). The results remained significant when the analyses were restricted to the controlled trials.

Conclusions: Use of ultrapure dialysate in hemodialysis patients results in a decrease in several markers of inflammation and oxidative stress, an increase in serum albumin and hemoglobin, and a decrease in erythropoietin requirement. Although, the improvement in these surrogaste endpoints might confer a cardiovascular benefit, a large trial with hard clinical endpoints is required.

TH-OR104

β2 Integrin-Mediated Cell-Cell Contact Transfers Myeloperoxidase from Neutrophils to Endothelial Cells Uwe Jerke,[1] Susanne Rolle,[1] Bettina Purfürst,[2] Friedrich C. Luft,[1] William Michael Nauseef,[3] Ralph Kettritz.[1,4] *[1]Experimental and Clinical Research Center, a Joint Cooperation between the Charité and the Max-Delbrück Center for Molecular Medicine (MDC); [2]MDC, Berlin; [3]Iowa Inflammation Program and Department of Medicine, Roy and Lucille A. Carver College of Medicine, University of Iowa and VA Medical Center, IA; [4]Nephrology and Intensive Care Medicine, Charité Campus Virchow, Berlin.*

Background: ANCA vasculitis and atherosclerosis both feature inflammation mediated by neutrophil-endothelial-cell (EC) contact. The heme protein myeloperoxidase (MPO) provides an ANCA antigen, but also disrupts normal EC function. MPO has the unique capacity to oxidize chloride and to generate hypochlorous acid, a potent microbicide that contributes to host defense, but also promotes cardiovascular disease. The mechanism by which MPO is transferred to EC are unknown. It is assumed that EC capture MPO that is released into the blood by activated neutrophils. We tested the hypothesis that close, β2-integrin-dependent neutrophil-EC contact also mediates MPO transfer from neutrophils to EC.

Methods: We used sensitive MPO assays, flow cytometry and confocal microscopy to detect MPO in EC. Human and wild-type and CD11b knock-out mouse neutrophils as well as different EC lines were employed.

Results: We demonstrate that EC acquired MPO when direct neutrophil contact was allowed, but not when EC and neutrophils were separated in transwells. The transfer was dependent on neutrophil number, incubation temperature and time. Transfer occurred in several EC types, increased with endotoxin, was not accompanied by MPO release into the medium and was not abrogated by inhibiting degranulation to secretogogues. Confocal microscopy showed MPO internalization by EC. Neutrophils and EC formed intimate contact sites by electron microscopy. Blocking CD11b or CD18 β2-integrin chains, or using neutrophils from CD11b gene-deleted mice, reduced MPO transfer.

Conclusions: The data suggest an alternative to EC uptake of soluble MPO, namely the cell contact-dependent, β2-integrin-mediated transfer from neutrophils. The findings could be of therapeutic relevance in atherosclerosis and vasculitis.

Funding: Government Support - Non-U.S.

TH-OR105

Biomechanical Strain-Mediated Mesangial Filopodial Invasion of Capillary Tuft as the Source of GBM Laminin 211 in Alport Mice: Early Mechanism for Alport Glomerular Pathology Dominic E. Cosgrove, Brianna Johnson, Daniel T. Meehan, Duane C. Delimont, Marisa Zallocchi. *Genetics, Boys Town National Research Hospital, Omaha, NE.*

Background: Alport syndrome results from mutations in type IV collagen COL4A3, COL4A4, or COL4A5 genes. Mutations in any of these genes results in the absence of all 3 in the GBM type IV collagen network. The result is a thinner and less crosslinked GBM collagen network resulting in delayed onset progressive glomerulonephritis. The molecular trigger for disease onset is unknown.

Methods: Alport mice, CD151 knockout mice, laminin α2-deficient Alport mice, Rac1 inhibition in Alport mice, and L-NAME-induced hypertension. Glomeruli analyzed by fluorescence immunohistochemistry and real time qRT-PCR. Mesangial cell migration and mesangial cell stretching assays.

Results: A comparative analysis of glomerular disease progression in Alport mice and CD151 knockout mice revealed a progressive irregular deposition of mesangial laminin 211 in the GBM. Co-localization studies showed that integrin α8β1 also progressively accumulates in the capillary loops of both models, reflecting an invasion of the capillary loops by mesangial cell processes. L-NAME salt-induced hypertension accelerated mesangial filopodial invasion, suggesting biomechanical strain plays a role in this mechanism. Mesangial cells showed reduced migratory potential when treated with either integrin linked kinase inhibitor, focal adhesion kinase inhibitor, or Rac1 inhibitors. Biomechanical stretching of cultured mesangial cells induced promigratory cytokines TGF-β1 and CTGF. Treatment of Alport mice with Rac1 inhibitors reduced mesangial filopodial invasion of the glomerular capillary tuft. Laminin α2-deficient Alport mice show reduced GBM damage and triple the lifespan of Alport mice, indicating a central role for mesangial laminins in progression of Alport glomerular pathogenesis.

Conclusions: Collectively, these findings predict a role for biomechanical insult in the induction of mesangial filopodial invasion of the glomerular capillary tuft leading to the irregular deposition of mesangial laminin 211 as an initiation mechanism of Alport glomerular pathology.

Funding: NIDDK Support

TH-OR106

Model for Idiopathic FSGS: Proteinuria after Injection or Overexpression of Cardiotrophin-Like Cytokine 1 in Mice Virginia J. Savin,[1] Mukut Sharma,[1] Changli Wei,[3] Jochen Reiser,[3] Ellen T. McCarthy,[2] Jean-francois Gauchat,[4] Ram Sharma.[1] *[1]Research Service, Kansas City VAMC, Kansas City, MO; [2]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [3]Nephrology, University of Miami School of Medicine, Miami, FL.*

Background: Idiopathic focal segmental glomerulosclerosis (FSGS) is associated with recurrence after transplantation due to circulating permeability factor(s) (NEJM, 334:878-883, 1996, Nat Med, 17:952-60, 2011). We have shown the effects of FSGS plasma and its fractions on glomerular permeability *in vitro* and *in vivo* and have used state-of-the-art proteomics to identify cardiotrophin-like cytokine-1 (CLCF1), a member of the IL-6 family, as a candidate for the active substance. We propose to develop a unique model of FSGS based on the effects of CLCF1 in mice.

Methods: rCLCF1 (R&D Systems) was injected intraperitoneally (IP), one dose, 10 μg/kg, or infused by minipump for 28 days, 200 ng/day. A construct containing *CLCF1* was administered by electroporation. Studies were done in C57BL6 mice. Urinary albumin/creatinine, pJAK1, pSTAT3, pATK, and pERK in peripheral blood cells (PBC) and in kidney homogenate were measured and glomerular histology was assessed.

Results: Albuminuria of up to 5 times baseline occurred after injection or electroporation. Albuminuria was maximal 6 days after electroporation of *CLCF1* construct. pJAK2 and pSTAT3 of PBC were increased within 15 min. of injection and kidney pJAK1 and pSTAT3 remained upregulated for at least 72 hours after injection. Kidney homogenate pJAK2, pSTAT3, pERK/12 and pAKT were each increased after 28 days of infusion. Glomeruli at 28 days showed increased mesangial matrix.

Conclusions: We conclude that CLCF1 mimics renal effects of the active fraction of plasma from patients with idiopathic FSGS and may play an important role in FSGS in native kidneys and transplant recurrence. Its relationship to other candidates such as circulating urokinase receptor (suPAR) requires further investigation. A murine model based on administration or overexpression of *CLCF1* may permit us to define mechanisms of injury and to test potential therapeutic agents prior to clinical trials.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

TH-OR107

A Transcriptional Network Underlies Susceptibility to Kidney Disease Progression Denise Laouari,[1] Martine Burtin,[1] Frank Bienaime,[1] Christophe M. Legendre,[1] Gerard Friedlander,[1] Marco Pontoglio,[2] Fabiola Terzi.[1] *[1]INSERM U845 - Universite Paris Descartes - APHP, Paris, France; [2]INSERM U1016 - CNRS UMR 8104 - Universite Paris Descartes, Paris, France.*

Background: The molecular networks that control the progression of chronic kidney diseases (CKD) are poorly defined. EGFR stimulates CKD progression, but the molecular networks that activate this signaling pathway remain unknown. We have recently shown that the susceptibility to development of renal lesions after nephron reduction is controlled by a locus (*Ckdp1*) on mouse chromosome 6 and requires EGFR activation through TGF-α. The aim of the present study was to identify the modifier gene(s) encoded by the *Ckdp1* locus.

Methods: For this purpose, we used *in silico, in vivo* and *in vitro* approaches. *In vivo*, we combined an experimental model of nephron reduction to mice from different genetic backgrounds and genetically modified animals. *In vitro*, molecular and cellular studies were conducted to determine the functional consequence of the genetic variant. In addition, human biopsies from renal transplant recipients with different scores of renal lesions were studied.

Results: We identified *Mitfa*, a gene encoding for a bHLH transcription factor, as a candidate modifier of CKD progression. Sequencing revealed a strain-specific hypomorphic variant in the 5' UTR of *Mitfa* that decreased the efficiency of MITF-A translation in FVB/N mice. *In vitro*, we observed that MITF-A acts in a complex genetic network in which it interacts with histone deacetylases to repress the transcription of TGF-α and antagonizes transactivation by its related partner, TFE3. Consistent with the key role of this network in CKD, TFE3 significantly increased after nephron reduction. More importantly, *Tgfa* gene inactivation protected hypomorphic *Mitfa* FVB/N mice from renal deterioration after nephron reduction. These data are relevant to human CKD, as we found that TFE3/MITF-A ratio markedly increased in patients with progressive renal damage.

Conclusions: In conclusion, our study uncovers a novel transcriptional network and unveils novel potential prognostic and therapeutic targets for preventing progression of human CKD.

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TH-OR108

Downregulation of miR-205 Modulates Cell Susceptibility to Oxidative and Endoplasmic Reticulum Stresses in Renal Tubular Cells Shiyo Muratsu, Masaomi Nangaku, Yoichiro Ikeda, Tetsuhiro Tanaka, Takehiko Wada, Reiko Inagi. *Division of Nephrology and Endocrinology, University of Tokyo Hospital, Tokyo, Japan.*

Background: Oxidative stress and endoplasmic reticulum (ER) stress play a crucial role in tubular damage. While the pathophysiological contribution of microRNAs (miR) to renal damage has been highlighted, the effect of miR on renal damage under oxidative and ER stresses remains elusive.

Methods: We assessed the effect of miR expression in cultured human tubular cells (HK-2) exposed to hypoxia-reoxygenation or ER stress by miR microarray analysis. The pathophysiological effect of miR was evaluated by cell survival rate, intracellular reactive oxygen species (ROS) level, and anti-oxidant enzyme expression in miR-modulated HK-2 under these stress conditions. The target gene of miR was identified by 3'UTR-luciferase assay.

Results: In 799 miRs we tested, we identified one miR, miR-205, whose expression was markedly decreased under both stress conditions. Functional analysis revealed that decreased miR-205 enhanced cell susceptibility to both stresses, which was associated with the induction of intracellular ROS. While increased miR-205 by itself made no change in phenotypes of HK-2, cell viability under both stresses was partially restored. Further, miR-205 bound to the 3'UTR of the prolyl hydroxylase 1 (PHD1) and suppressed the transcription level of PHD1. The upregulation of PHD1 by inhibiting miR-205 decreased nuclear amount of HIF, resulting in the reduction of HIF-regulated anti-oxidant enzymes (SOD1, SOD2, and catalase). Moreover, the inhibition of miR-205 decreased nuclear activating transcription factor 4 (ATF4), which was a crucial regulator of ER stress. Targets of ATF4 include genes for redox balance, such as hemoxygenase 1 (HO-1), which mediates an adaptive response to oxidative stress and was indeed decreased by miR-205 inhibition.

Conclusions: PHD1 is one of the novel direct targets of miR-205, and its upregulation by inhibiting miR-205 leads to subsequent downregulation of HIF and ATF4, then finally increases the intracellular ROS via suppression of HIF/ATF4-regulated anti-oxidant enzymes, and renders the cells more vulnerable to these stresses.

TH-OR109

Tubular β-Catenin Signaling Controls Interstitial Fibroblast Fate via Epithelial-Mesenchymal Communication Dong Zhou, Youhua Liu. *Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Activation of β-catenin, the principal mediator of canonical Wnt signaling, is a common finding in a wide variety of chronic kidney diseases (CKD). Inhibition of hyperactive β-catenin in CKD is known to be reno-protective, leading to amelioration of renal fibrotic lesions. β-Catenin is predominantly induced in renal tubular epithelium; however, the role of tubule-specific β-catenin activation in renal fibrogenesis remains elusive.

Methods: We investigated this issue by utilizing mice with tubule-specific ablation of endogenous β-catenin (Ksp-β-cat-/-). These mice are phenotypically normal under normal physiologic conditions.

Results: Surprisingly, we found that loss of tubular β-catenin resulted in little alterations in renal fibrosis after obstructive injury. Both Ksp-β-cat-/- mice and their control littermates displayed similar fibrotic lesions at different time points after unilateral ureteral obstruction (UUO), as shown by the same levels of collagen, fibronectin and PAI-1 expression. No difference was found in renal α-SMA levels in the obstructed kidneys of Ksp-β-cat-/- and control mice, suggesting a similar size of myofibroblast population, despite epithelial-mesenchymal transition was largely inhibited in Ksp-β-cat-/- kidneys. Interestingly, more apoptosis was detected selectively in renal interstitial fibroblasts of control mice, compared to Ksp-β-cat-/- mice, which was accompanied by an increased renal expression of Bax and Fas ligand (FasL). Tubule-specific knockout of β-catenin abolished the induction of MMP-7 in the obstructed kidneys. In vitro, recombinant MMP-7 induced FasL expression in renal interstitial fibroblasts and promoted fibroblast apoptosis.

Conclusions: These results demonstrate that loss of tubular β-catenin resulted in an enhanced interstitial fibroblast survival. Our studies also illustrate a novel role of MMP-7 in mediating epithelial-mesenchymal communication in renal fibrogenesis.

Funding: NIDDK Support

TH-OR110

Nuclear Hormone Receptor Farnesoid X Receptor and G Protein Coupled Receptor TGR5 Exhibit Calorie Restriction Mimetic Effects in Aging Mice Xiaoxin Wang, Hannah Danielle Santamaria, Liru Qiu, Moshe Levi. *Medicine, University of Colorado, Aurora, CO.*

Background: Lifelong calorie restriction (CR) has been shown to have beneficial effects on life and health span in aging rodents and mammals and to prevent age-related decline is renal function. We have found that renal expression of FXR and TGR5 are decreased in ad lib fed aging mice. In contrast in mice with lifelong CR FXR and TGR5 expression are markedly increased. The purpose of the present study was to determine if activation of FXR and TGR5 in the kidneys of ad lib fed aging mice have similar effects to CR.

Methods: We studied 5 month old ad lib fed, 24 month old ad lib fed, 22 months old ad lib fed mice treated with the dual FXR/TGR5 agonist INT-767 for 2 months, and compared them to lifelong 24 month old CR mice.

Results: We found that treatment of 22 month old ad lib fed aging mice with INT-767 decreased urinary albumin excretion, markers of fibrosis, and oxidative stress. INT-767 also prevented age-related decreases in eNOS protein and mRNA. In addition INT-767 treatment augmented energy metabolism and mitochondrial biogenesis in the aging kidney, including increases in Nampt, SIRT1, PGC-1α, ERRα, SIRT3, Nrf1, mitochondrial DNA content, and mitochondrial fatty acid oxidizing enzymes MCAD and PDK4, when compared to ad lib fed 24 month old mice. The changes induced by INT-767 treatment of 22 month old ad lib fed aging mice over a 2 month period were remarkably similar to those induced by lifelong CR in 24 month old mice and also the long-lived Ames Dwarf mice.

Conclusions: Our results therefore indicate that activation of FXR and TGR5 in the aging kidney reverses most of the age-related changes and the effects of FXR and TGR5 are similar to beneficial effects achieved by lifelong calorie restriction.

Funding: NIDDK Support, Other NIH Support - NIA, Veterans Administration Support, Pharmaceutical Company Support - Intercept

TH-OR111

Internalization and Retromer Binding Regulate Vasopressin V2-Receptor Desensitization: Differential Effects of Vasopressin and Oxytocin on cAMP Signaling Richard Bouley,[1] Timothy N. Feinstein,[2] Naofumi Yui,[3] Dennis Brown,[1] Jean-Pierre Vilardaga.[2] *[1]CSB, Program in Membrane Biology and Nephrology Division, Massachusetts General Hospital, Boston, MA; [2]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA; [3]Nephrology Division, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: The peptide hormones vasopressin (VP) and oxytocin (OT) both bind a single G protein-coupled receptor, the vasopressin type 2 receptor (V2R), in renal epithelial cells. The antidiuretic effect of VP, however, is much greater than OT.

Methods: Here we used a combination of pharmacological, biochemical and optical techniques, including FRET-based approaches, to determine the molecular and cellular mechanisms that differentiate for the biological differences between VP and OT acting at the V2R.

Results: We find that VP induced rapid translocation of β-arrestins, V2R internalization and cAMP generation that persisted for 20-30 min after VP washout. In kidney epithelial cells expressing transgenic V2R (MDCK-V2R), VP induced persistent apical localization of the water channel protein aquaporin 2 (AQP2) and phosphorylation at S269. OT induced neither arrestin binding nor V2R internalization. cAMP generation ended quickly after OT washout, and phosphorylation and apical localization of AQP2 did not persist after OT washout. Expression of a dominant-active mutant of β-arrestin 1 further enhanced cAMP generation and cAMP generation was shorter when either V2R or the cAMP biosensor was restricted to the plasma membrane, supporting a model in which arrestin and V2R internalization enhance rather than inhibit cAMP generation by V2R. Desensitization was mediated instead by the endosomal sorting complex retromer. Internalized V2R colocalized and formed a complex with retromer, which in turn inhibited cAMP generation triggered by VP.

Conclusions: We conclude that V2R can signal from early endosomes after challenge with VP but not OT, and that endosomal signaling is terminated by the retromer sorting complex. This ligand-biased signaling could explain distinct physiological effects during the therapeutic use of ligands that target the V2R.

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TH-OR112

Absence of PKC-alpha Attenuates Lithium-Induced Nephrogenic Diabetes Insipidus Jae H. Sim, Sara K. Redd, Seongun M. Hong, Tobias N. von Bergen, Mitsi A. Blount. *Department of Medicine - Renal Division, Emory University, Atlanta, GA.*

Background: Lithium, the most effective treatment for bipolar disorder, induces nephrogenic diabetes insipidus (NDI) in ~ 60% of patients. We and others have shown that the decreased capacity to concentrate urine is likely due to lithium acutely disrupting the cAMP pathway and chronically reducing the urea transporter (UT-A1) and water channel (AQP2) expression in the kidney inner medulla. Targeting an alternative signaling pathway, such as PKC signaling may be an effective method of treating lithium-induced polyuria.

Methods: PKC-alpha KO mice and strain-matched wild type (WT) controls were first treated with 40 mmol/kg of lithium for 0, 3, or 5 days. In separate experiments, animals were treated with lithium for 6 wk.

Results: WT mice had increased 24 h urine output and lowered urine osmolality after 3 and 5 days of treatment whereas PKC-alpha KO mice had no change in basal urine output or concentration at either time point. Western blot analysis revealed that AQP2 expression was lowered 53% after 3 days and 86% after 5 days; however, APQ2 was unchanged in PKC-alpha KO mice. Similar results were observed with UT-A1 expression. Animals were next treated with lithium for 6 wk. Lithium-treated WT mice had 19-fold increased urine output which was significantly different from treated PKC-alpha KO animals which had a 4-fold increase in urine output. Western blot analysis revealed that AQP2 and UT-A1 expression were barely detectable in 6 wk lithium-treated WT animals whereas in treated PKC-alpha KO mice, APQ2 and UT-A1 expression was only reduced by 2-fold.

Conclusions: Our data shows that ablation of PKC-alpha preserved urine concentrating transporter expression in lithium-induced NDI and this likely prevents the development of the severe polyuria associated with therapy.

Funding: NIDDK Support, Private Foundation Support

TH-OR113

Vasopressin V2R-Targeting Peptide Carrier Mediates siRNA Delivery Into Collecting Duct Cells Hyun Jun Jung, Jung-suk Lim, Hyo-jung Choi, Tae-Hwan Kwon. *Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Taegu, Korea.*

Background: Internalization of receptor proteins after interacting with specific ligands has been proposed to facilitate siRNA delivery into the target cells via receptor-mediated siRNA transduction. In this study, we demonstrated a novel method of vasopressin V2 receptor (V2R)-mediated siRNA delivery against AQP2 in primary cultured inner medullary collecting duct (IMCD) cells of rat kidney.

Methods: The dDAVP conjugated with nine D-arginines (dDAVP-9r) as a peptide carrier for siRNA delivery was synthesized. We examined 1) formation and stability of the polyplexes by Electrophoretic mobility shift assay; 2) Cellular uptake of the polyplexes in V2R-expressing cells by flow cytometry analysis and confocal microscopy; 3) Particle size and surface charge of the polyplexes; 4) Phsphorylation of AQP2 (S256) in rat IMCD cells by interaction of V2R and the polyplexes; 5) Protein knockdown of AQP2 via siRNA delivery using AQP2-siRNA/dDAVP-9r polyplexes by semiquantitative immunoblotting.

Results: The results revealed that 1) synthesized dDAVP-9r peptides formed a stable polyplex with siRNA; 2) siRNA/dDAVP-9r polyplex could bind to the V2R of IMCD cells and induced AQP2 phosphorylation (Ser 256); 3) siRNA/dDAVP-9r polyplex was stable in response to the wide range of different osmolalities, pH levels, or to the RNases; 4) fluorescein-labeled siRNA was delivered into V2R-expressing MDCK and LLC-PK1 cells by siRNA/dDAVP-9r polyplex, but not into the V2R-negative Cos-7 cells; and 5) AQP2-siRNA/dDAVP-9r polyplex effectively delivered siRNA into the IMCD cells, resulting in the significant decrease of protein abundance of AQP2, but not AQP4.

Conclusions: We introduced a novel method of V2R-mediated siRNA delivery in the kidney collecting duct cells using a specific peptide ligand to V2R. siRNA/dDAVP-9r polyplex effectively delivered siRNA into the IMCD cells in vitro, resulting in the significant decrease of protein abundance of AQPs. This method could be exploited to deliver siRNAs to regulate abnormal expression of specific target proteins in vivo, potentially associated with disease conditions in the kidney tubule cells expressing V2R.

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TH-OR114

Identification of Proteins Involved in the Trafficking of Aquaporin-2 via Genome-Wide siRNA Screening Doerte Faust,[1] Martin Neuenschwander,[2] Katina Lazarow,[2] Simone M. Graeber,[2] Jens Peter von Kries,[2] Walter Rosenthal,[1] Enno Klussmann.[1] *[1]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany; [2]Leibniz-Institut für Molekulare Pharamakologie (FMP), Berlin, Germany.*

Background: Arginine-vasopressin (AVP) controls water reabsorption in renal collecting duct principal cells and thereby fine tunes body water homeostasis. AVP stimulates V2R receptors on the basolateral surface of the cells, which increases cAMP and leads to activation of protein kinase A (PKA). PKA phosphorylates the water channel aquaporin-2 (AQP2), inducing its redistribution from intracellular vesicles into the plasma membrane. This facilitates water reabsorption from primary urine. Aberrations of AVP secretion or AVP-activated signaling cause nephrogenic diabetes insipidus (NDI). An elevated blood plasma AVP level is associated with chronic heart failure and the syndrome of inappropriate antidiuretic hormone secretion (SIADH).

Methods: We are establishing a genome-wide siRNA screening using mouse collecting duct cells stably expressing human AQP2 (MCD4). The cells are transfected with siRNA in 384 well format to down regulate the expression of 17,000 genes. The localisation of AQP2 is analyzed by automated immunofluorescence microscopy.

Results: By using CellProfiler analysis software detailed image information was extracted. To classify the different complex phenotypes ranging from exclusive intracellular to exclusive plasma membrane localization of AQP2, machine learning was used, combining advanced statistical and computational techniques in learning generative models. The efficiency of reverse siRNA transfection was optimized to minimize adverse effects on cell viability and maximize down regulation of protein expression. Up to 80% reduction of gene expression was achieved. Using this system we aim to identify components of the AQP2 trafficking machinery.

Conclusions: Our approach contributes to understanding the molecular mechanisms underlying the control of AQP2 and to identify potential therapeutic targets for the treatment of diseases that are associated with disturbances of AVP-mediated water reabsorption.

Funding: Government Support - Non-U.S.

TH-OR115

A Novel PKA-Independent Signaling Pathway Controlling AQP2 Trafficking as a Possible Cause for the Syndrome of Inappropriate Antidiuresis Grazia Tamma,[1] Christiane Trimpert,[2] Marianna Ranieri,[1] Annarita Di Mise,[1] Maria Grazia Mola,[1] Olivier Devuyst,[3] Maria Svelto,[1] Peter M.T. Deen,[2] Giovanna Valenti.[1] *[1]Dept. Bioscience Biotechnologies and Pharmacological Sciences I, University of Bari, Bari, Italy; [2]Dept. of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Inst. of Physiology, University of Zurich, Zurich, Switzerland.*

Background: Renal water reabsorption is controlled by vasopressin (AVP) which activates phosphorylation of AQP2 at serine 256 (pS256) and translocation to the plasma membrane. Besides S256, AVP causes dephosphoryation of S261. Recent studies showed that cyclin-dependent kinases can phosphorylate S261 AQP2 peptides in vitro. In an attempt to investigate the possible role of cdk_s on AQP2 phosphorylation, we identified a PKA-independent pathway regulating AQP2 trafficking.

Methods: MDCK cells or kidney slices were left untreated or forskolin stimulated with or without roscovitine (10μM), a specific cdk_s inhibitor.

Results: In *ex-vivo* kidney slices and MDCK cells roscovitine increased pS256 and decreased pS261.The changes in AQP2 phosphorylation were paralleled by an increase in cell surface AQP2 expression and osmotic water permeability in the absence of forskolin stimulation. Of note, roscovitine did alter neither cAMP intracellular level nor PKA activity. Because phosphorylation results from the balance between kinase and phosphatase activity we evaluated the possible contribution of protein phosphatases PP1, PP2A and PP2B. Of these, roscovitine treatment specifically reduced PP2A activity in MDCK cells. Interestingly, in $PKD1^{+/-}$ mice displaying a syndrome of inappropriate antidiuresis with high level of pS256 despite unchanged AVP and cAMP (Alharabi et. al 2007) we found a reduced PP2A expression and activity. Indeed similarly to what previously found in $PKD1^{+/-}$ mice, roscovitine significantly decreased intracellular calcium in MDCK.

Conclusions: Our data indicate that a reduced activity of PP2A, secondary to reduced intracellular Ca2+ levels, promotes AQP2 trafficking independently from the AVP-PKA axis. This pathway may be relevant for explaining pathological states characterized by inappropriate AVP secretion and positive water balance.

Funding: Government Support - Non-U.S.

TH-OR116

Small Molecule Library Screening to Identify Components of the Machinery Controlling Aquaporin-2 in Renal Principal Cells Enno Klussmann,[1] Jana Bogum,[2] Doerte Faust,[1] Jens Furkert,[2] Martin Neuenschwander,[2] Jens Peter von Kries,[2] Burkhard Wiesner,[2] Giovanna Valenti,[3] Walter Rosenthal.[1] *[1]Max Delbrueck Center Berlin-Buch (MDC), Berlin, Germany; [2]Leibniz-Institut für Molekulare Pharmakologie (FMP), Berlin, Germany; [3]Department of General and Environmental Physiology, University of Bari, Bari, Italy.*

Background: Arginine-vasopressin (AVP) regulates water reabsorption of renal collecting duct principal cells. It binds to V2 receptors on the surface of the cells, leading to elevation of cAMP and subsequent activation of protein kinase A (PKA). PKA phosphorylates the water channel aquaporin-2 (AQP2) at serine 256 (S256), which elicits the translocation from intracellular vesicles to the plasma membrane and facilitates water reabsorption from primary urine. Our study aims at the identification of novel players controlling AQP2.

Methods: MCD4 cells stably expressing AQP2 were incubated with each of 17,700 small molecules (ChemBioNet library), stimulated with the cAMP-elevating agent forskolin and AQP2 was detected by automated immunofluorescence microscopy.

Results: One of the small molecules identified by this approach was 4-acetyldiphyllin (4AD), a specific blocker of vacuolar (V)-ATPase. It inhibits the cAMP-dependent increase of AQP2 phosphorylation at S256 and its translocation to the plasma membrane of principal cells. 4AD does not affect cAMP levels or PKA activity; it increases pH levels of intracellular vesicles and causes an accumulation of AQP2 in the Golgi.

Conclusions: The acidification of intracellular vesicles by V-ATPase is not only essential for the exit of AQP2-bearing vesicles from the Golgi but also facilitates the PKA-catalyzed phosphorylation of AQP2 at S256, a prerequisite for its AVP-induced translocation to the plasma membrane of renal collecting duct cells.

In general, the identification of the target proteins of candidate molecules will contribute to understanding the molecular mechanisms underlying AQP2 trafficking and may lead to the development of new therapeutic approaches for the treatment of diseases that are associated with disturbances of AVP-mediated water reabsorption such as chronic heart failure or SIADH.

Funding: Government Support - Non-U.S.

TH-OR117

Deep Sequencing of the Cortical Thick Ascending Limb Transcriptome Jae Wook Lee, Chung Lin Chou, R. Lance Miller, Mark A. Knepper. *Epithelial Systems Biology Laboratory, Systems Biology Center, National Heart, Lung and Blood Institutes, National Institutes of Health, Bethesda, MD.*

Background: Renal tubule microdisseection has been an important tool in the discovery of renal mechanisms for the past 50 years. Here, we extend the use of microdissected renal tubules to transcriptomic analysis using the RNA-seq method with small sample amplification.

Methods: Samples containing 5-25 mm of cortical thick ascending limbs (cTAL) were isolated by hand dissection from collagenase-incubated rat renal cortex. After lysis of cells, poly(A)-targeted reverse transcription and library preparation were performed. Paired-end sequencing of the cDNA library was done using an Illumina HiSeq 2000 sequencer. The generated 50-bp reads were aligned to the UCSC rat reference genome. Reads per kilobase exon model normalized by million mapped reads (RPKM) were calculated.

Results: In an initial sample of 5 mm cTALs, 451,694,588 total reads were obtained of which 235,735,784 reads (52%) were mapped and properly paired. In this experiment, 5410 RefSeq genes were identified. To assess accuracy, we surveyed G-protein coupled receptors (GPCRs) and transport proteins that were identified. Among the GPCRs known to be expressed in cTAL (*Avpr2, Pth1r, Gcgr, Calcr, Casr, and Ptger3*) only the calcitonin receptor was not found. Among the transporter proteins generally accepted to be expressed in cTAL (*Slc12a1, Kcnj1, Clcnkb, Cldn16, Slc9a3, Slc2a4 and Atp1a1*) only *Slc9a3* was not found. In contrast, transporter proteins characteristic of neighboring tubule segments were not detected. Gene-set analysis also showed overrepresentation of mitochondrial genes among transcripts of high RPKM. When longer lengths of cTAL were used, the number of mapped transcripts increased, but not linearly (15 mm, 8455 genes; 25 mm, 12,110 genes). Experiments with a second segment, medullary TALs, gave a profile very similar to that of the cTAL.

Conclusions: These studies establish the feasibility of RNA-seq-based transcriptomic profiling in microdissected renal tubule segments and 'open the door' for profiling of gene expression along the entire renal tubule.

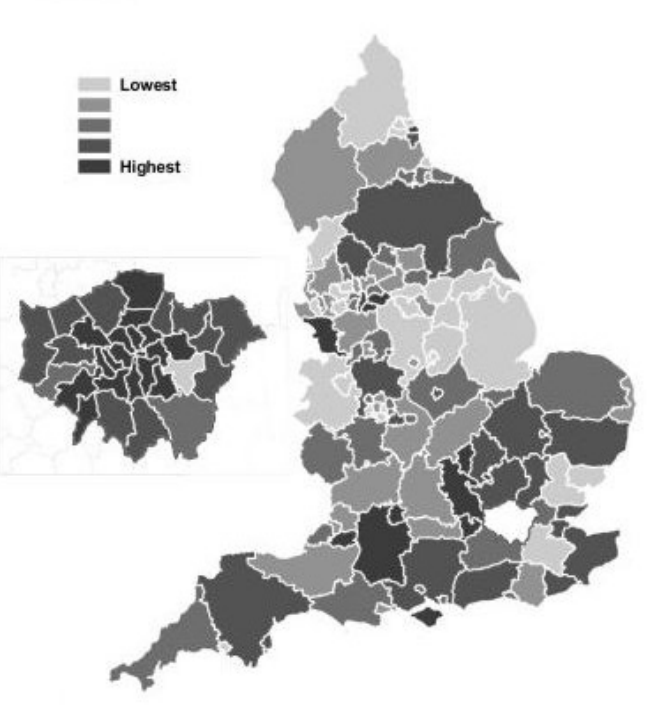
TH-OR118

Mapping Healthcare in England: The Atlas of Variation for People with Kidney Disease Partha Das,[1] Ben Bray,[1] Donal O'Donoghue,[2] Beverley Matthews.[1] *[1]NHS Kidney Care, London, United Kingdom; [2]National Clinical Director for Kidney Care, Department of Health, London, United Kingdom.*

Background: Geographical variations in healthcare will always occur but some variations are unwarranted. The study of unwarranted variation has been revolutionised since the publication of the Dartmouth Atlas of Healthcare in 1996 by John Wennberg. The NHS in England has replicated this with the Atlas of Variation in 2010 and 2011. Nephrology has had no such analysis by regional until now. The service improvement organisations NHS Kidney Care and Right Care present the first Atlas of Variation for People with Kidney Disease.

Methods: 18 indicators have been selected encompassing CKD in primary care, secondary care treatments and patient reported outcome measures. Data have been extracted from a variety of sources including UK Renal Registry data and visualised as maps by geographical region, NHS primary care trust (PCT) or renal service provider.

Results: Significant variations exist across England. Examples include the observed:expected prevalence of CKD which varies from 0.3 to 1.4 (4.5-fold variation.)



The standardised acceptance ratio for incidence of RRT ranges from 0.4 to 2.6 (7-fold variation) and those presenting late (<90 days) range from 11.5% to 35.2% (3.1-fold variation). The percentage of incident patients with definitive dialysis access ranges from 42.5% to 62.3% (1.5-fold variation by network). The rate of kidney transplants from living donors ranges from 11.6 to 22.3 per million population (1.9-fold variation).

Conclusions: This atlas is the first to highlight geographical variations in kidney care in a nation. The causes are many but the fact that unwarranted variation exists in the delivery of renal services should prompt improvements in the organisation of care. The atlas provides a unique opportunity to understand and eliminate unwarranted variation in kidney care.

TH-OR119

Racial Disparity in CKD Care among Medicare CKD Patients

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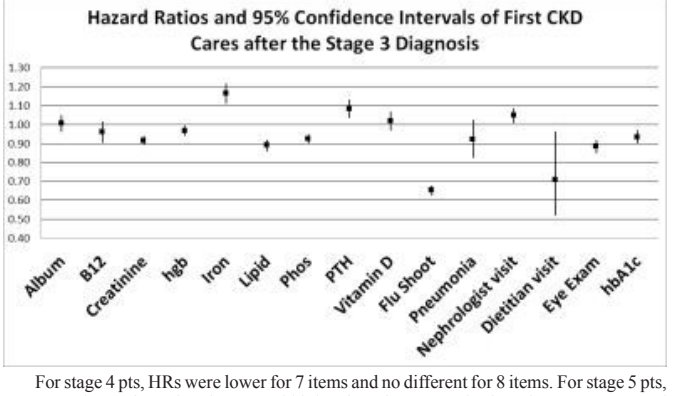
Background: Studies have shown racial disparities in provision of health care in various populations, including CKD patients (pts). However, racial disparities in CKD care among Medicare CKD pts have not been assessed.

Methods: Using the 5% Medicare data, this study compared CKD care among race groups in the Medicare CKD population by stage. Included pts were diagnosed with CKD stage 3, 4, or 5 between January 1, 2007, and December 31, 2009; were not on dialysis; and had not undergone kidney transplant. Pts were followed from the first diagnosis date to the earliest of death, CKD stage change, loss to follow-up, loss of Medicare coverage, or 1 year. CKD care after diagnosis was derived from Medicare claims. Care items included specialist visits (nephrologist, dietitian), lab tests (hgb, PTH, phosphorus, albumin, creatinine, iron, lipids, vitamin D, B12), diabetes care (diabetic patients only: hbA1c tests, diabetic eye exams), and vaccinations (flu, pneumonia). Time to first care event was compared between race groups for each care item using a Cox model adjusted for patient age, sex, socioeconomic status, Medicare/Medicaid dual eligibility, rural/urban residence, and comorbid conditions.

Results: In all, 102,856 pts were included; 67.2%, 22.0%, and 10.8% were in stages 3, 4, and 5, respectively; 84.1% were white, 11% black, and 4.9% other race. Among the 15 CKD care items listed above, compared with whites, HRs were lower for blacks for 8 items, no different for 4 items, and higher for 3 items among stage 3 pts.



For stage 4 pts, HRs were lower for 7 items and no different for 8 items. For stage 5 pts, HRs were lower for only 4 items, and higher for 1 item. Results for other race compared with whites were similar.

Conclusions: Among CKD patients, racial disparity was found in CKD care. The differences were small among stage 5 pts than that among pts in stages 3 and 4.

Funding: NIDDK Support

TH-OR120

Effect of the Implementation of Clinical Practice Guidelines for the Prevention, Diagnosis and Treatment of CKD in Patients with Type2 Diabetes Mellitus at the Primary Health-Care

Petra Martínez, Héctor R. Martínez Ramírez, Laura Cortes-sanabria, Alfonso M. Cueto-Manzano. *U. Investigación Médica en Enfermedades Renales, IMSS, Guadalajara, Jalisco, Mexico.*

Background: The Application of Clinical Practice Guidelines (CPG) could help to improve clinical attitude of family physicians (FP) and renal function of patients with type 2 diabetes mellitus (DM2). This has never been demonstrated in the CKD area.

Methods: Objective. To evaluate the effect of an educative intervention to FP based on CPG published on-line on renal function of patients with DM2 and CKD. Prospective cohort in which an educative-participative intervention based on the CPG for Prevention, Diagnosis and Treatment of Chronic Kidney Disease (CKD) was applied to FP of 3 Family Medicine Units (Mexican Institute of Social Security). Once the educative intervention was concluded, DM2 patients attended by participant FP were evaluated for CKD. Patients with confirmed CKD (K/DOQI classification) were evaluated every six months. Glomerular filtration rate was estimated (simplified MDRD/formula), and albuminuria (nephelometry). Patients were integrally managed by FP.

Results: Two-hundred sixty patients had CKD; they were divided in 2 groups: Early CKD (58 with stage 1 and 55 with stage 2) and Late CKD (23 with stage 3 and 5 with stage 4). In both groups, median follow-up was 12 (9-15) months. Main results are shown.

Variable	Early/CKD n=113		Late/CKD n=28	
	Baseline	Final	Baseline	Final
Age(yrs)	63±11		71±9	
Duration of DM2(yrs)	13(8-18)		16(11-20)	
Presence of hypertension, n(%)	74(66)		27(93)	
Systolic blood pressure (mmHg)	136±23	121±14*	138±24	127±18*
Diastolic blood pressure (mmHg)	81±10	76±9*	81±10	78±7
LDL-Cholesterol (mg/dl)	110(92-131)	106(83-123)*	112(87-133)	103(82-122)
HbA1c(%)	8.9±2.1	8.4±2*	8.0±2.2	7.2±1.3*
eGFR(ml/min)	91(75-109)	88(75-111)	48(35-57)	45 (33-59)
Urine/Albumin/ Creatinine (mg/g)	122(58-260	56 (21-148)*	182(44-433)	92(23-397)*

*p<0.05 vs baseline/same group

Conclusions: CPG for Prevention, Diagnosis and Treatment of CKD to FP, implemented by means of an educative intervention, positively influences on the improvement of renal function and other variables of DM2 patients with CKD.

TH-OR121

Preservation of Renal Function by Thyroid Hormone Replacement Therapy in Chronic Kidney Disease Patients with Subclinical Hypothyroidism

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Background: Subclinical hypothyroidism is not a rare condition, but the replacement of thyroid hormone to treat subclinical hypothyroidism is an issue of debate. This study was undertaken to investigate the impact of thyroid hormone therapy on the changes in estimated glomerular filtration rate (eGFR) in subclinical hypothyroidism patients with chronic kidney disease (CKD).

Methods: A total of 309 CKD patients with subclinical hypothyroidism were included in this study. The changes in eGFR over time were compared between patients with and without thyroid hormone replacement therapy using a linear mixed model. Kaplan-Meier curves were constructed to determine the effect of thyroid hormone on renal outcome; a reduction of eGFR by 50% or end-stage renal disease. The independent prognostic values of subclinical hypothyroidism treatment for renal outcome were also ascertained by multivariate Cox regression analysis.

Results: One hundred and eighty patients (58.3%) took thyroid hormone (treatment group), while 129 (41.7%) did not (non-treatment group). Serum cholesterol and triglyceride concentrations were significantly higher in the treatment group than in the non-treatment group (P<0.01). During the mean follow-up duration of 34.8±24.3 months, the overall rates of decline in eGFR were significantly greater in the non-treatment group compared to the treatment group (-5.93±1.65 vs. -2.11±1.12 mL/min/year/1.73 m^2, P=0.04). In addition, a linear mixed model revealed that there was a significant difference in the rates of eGFR decline over time between the two groups (P<0.01). Kaplan-Meier analysis also showed that renal event-free survival was significantly higher in the treatment group (P<0.01). In multivariate Cox regression analysis, thyroid hormone replacement therapy was found to be an independent predictor of renal outcome (Hazard ratio, 0.28; 95% confidence interval, 0.12-0.68; P=0.01).

Conclusions: Thyroid hormone replacement therapy preserved renal function and was an independent predictor of renal outcome in CKD patients with subclinical hypothyroidism.

TH-OR122

Improved Physical Function in Patients with Chronic Kidney Disease Stage (CKD) 3 and 4 through a Renal Rehabilitation Exercise (RRE) Program

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Background: Patients with CKD have a high prevalence of cardiovascular disease (CVD) associated with or exacerbated by inactivity. Cardiac rehabilitation is proven to have beneficial effects on cardiovascular risk factors and exercise capacity in patients with CVD. The goal of this study was to determine whether a RRE program for patients with stage 3 or 4 CKD would improve their physical function.

Methods: Patients with CKD stage 3 and 4 were randomized to the RRE intervention or usual care (UC). The RRE intervention consisted of guided exercise at a cardiac rehabilitation facility, 3 times per week for 12 weeks. Physical function at baseline and after the intervention period was determined by 3 well-established performance-based physical function tests: 6-minute walk test (6MWT), sit-to-stand test (STST) and gait speed test (GST).

Results: There were 48 patients (mean age 69±12 yr, 69% males) in the UC group and 59 patients (mean age 68±12 yr, 39% males) in the RRE group. At baseline there were no differences in self-reported level of activity, BMI, or 6MWT and STST scores, but the RRE group had a higher GST score than the UC group (173±143 vs. 119±106 cm/sec; p=0.03). After the intervention period, the UC group did not experience improvements in the 3 main outcomes. The RRE group demonstrated significant improvement in the 6MWT (19%, from 1117±320 to 1327±373 ft/6 min; p=<0.0001) and STST (29%, from 1.64±0.85 to 1.16±0.48 % of age predicted; p=0.0004). No improvement in the GST was observed (183±160 vs 171±145 cm/sec; p=0.6). The improvements observed were significant for both CKD stages, males and females, and patients >70 and <70 years of age. The number of exercise sessions attended were positively correlated with improvements in the 6MWT (r=0.38, p=0.009) and STST (r=0.49, p=0.006). Six patients in the RRE group withdrew early; time conflict was the main reason. Overall RRE was well tolerated.

Conclusions: A 12-week RRE program improved the physical capacity of patients with CKD stage 3 and 4. Longer follow-up is needed to determine if this will translate into decrease mortality rates.

TH-OR123

Fluid Intake, Mortality and Kidney Function: A Cohort Study

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Background: Drinking eight glasses of water each day to improve health is a widely-held belief. Observational data exploring the association between fluid intake and mortality or kidney disease are sparse and conflicting. We examined these associations.

Methods: We examined adults ≥49 years in a census population in Sydney, Australia. Daily fluid intake was measured using a self-administered food frequency questionnaire and death obtained from the National Death Index. Cox proportional hazard models were used to assess the relationship between all-cause and cardiovascular mortality and daily fluid consumption controlling for age, gender, smoking, prior myocardial infarction, cancer or cerebrovascular disease, employment, glucose, HDL, cholesterol, triglyceride, platelet, white cell count and fibrinogen. We evaluated the association between fluid intake and change in estimated glomerular filtration rate (eGFR) during follow up. Adjusted Hazard ratios (AHRs) and 95% confidence intervals (CI) with higher water intake (>2.0 l/day, in quartiles) were then compared with those drinking <2.0 l/day.

Results: We had follow-up data for 2897 of 3654 individuals (age 66.2 ± 9.8 years; eGFR 66.9 [17.6] ml/min/1.73 m^2) over 13.1 (11.1-13.9) years. eGFR was available for 1209 individuals. Compared with the lowest fluid intake, the AHR for total mortality did not differ significantly for each quartile of fluid intake (aHR 0.85 (95% CI 0.70-1.03), 1.03 (0.85-1.25), and 0.94 (0.78-1.15) for fluid intakes of 2.0-2.4, 2.5-3.0 and >3.0 l/day when compared to <2.0 l/day, respectively). There was no association between cardiovascular mortality and fluid intake. There was no dose-response relationship between increasing daily fluid consumption (per deciliter) and all-cause (1.01 (95% CI 0.99 to 1.02) or cardiovascular mortality (1.05 [0.89 to 1.12]) compared to less than 2 l/day. Change in eGFR was not associated with fluid intake (p=0.15).

Conclusions: Daily fluid intake is not associated with survival or change in kidney function in this opportunistic analysis from an existing database. An ad hoc designed cohort study and a randomized trial are needed to confirm the findings.

Funding: Government Support - Non-U.S.

TH-OR124

Efficacy and Safety of Combined versus Single Renin Angiotensin Aldosterone System Blockade in Chronic Kidney Disease: A Meta-Analysis

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Background: Although dual blockade of the renin-angiotensin-aldosterone system (RAAS) has gained popularity for the treatment of kidney disease, its benefits and potential risks have not been fully elucidated.We conducted a meta-analysis of all randomized controlled trials comparing the efficacy and safety of combined vs. single RAAS blockade therapy in patients with CKD.

Methods: We performed a literature search using MEDLINE, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, scientific abstracts from meetings, and bibliographies of retrieved articles. Data were extracted independently by 2 authors; random-effects models were used to compute absolute and standardized net changes, and rate differences.

Results: Fifty-nine (25 crossover and 34 parallel-arm) were identified (4,975 patients). Combined RAAS blockade therapy was associated with a significant net decrease in GFR (-1.8 mL/min or mL/min/1.73 m2; 95%CI -3.1,-0.5; P=0.005), albuminuria (-62 mg/day; 95%CI -97,-28; P<0.001), and proteinuria (-339 mg/day; 95%CI -434, -243; P<0.001). Similar results were observed using standardized net changes. Combined RAAS blockade therapy was associated with 9.4% higher rate of regression to normoalbuminuria and 5% higher rate of achieving blood pressure goal. However, combined RAAS blockade therapy was associated with a significant net increase in serum potassium level (0.13 mEq/L; 95% CI 0.09, 0.18; P<0.001), a 3.4% higher rate of hyperkalemia, and a 4.6% higher rate of hypotension. There was no effect on doubling of serum creatinine, hospitalization or mortality.

Conclusions: Although combined RAAS blockade therapy in CKD is associated with a decrease in albuminuria and proteinuria, there is a decrease in GFR, and a higher incidence of hyperkalemia and hypotension relative to single therapy. The potential long-term kidney benefits of this treatment strategy require further study.

TH-OR125

The Relationship of ADHD Symptoms with Literacy, Depression, Quality of Life and Transition/Self-Management among Adolescents and Emerging Adults with Chronic Kidney Disease

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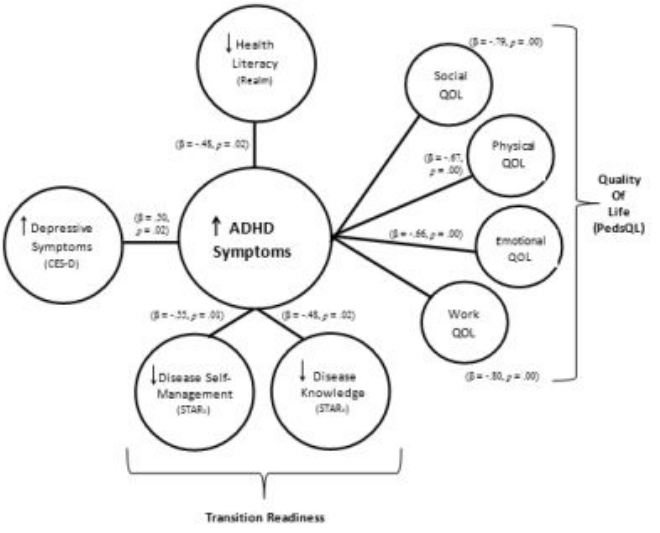
Background: ADHD is an often undiagnosed co-morbidity of CKD that may impact the ability to successfully self-manage CKD. We determined the relationship of ADHD symptoms with transition readiness/self-management, literacy, health-related quality of life (QoL) & depression among adolescents/young adults in pediatric and internal medicine nephrology practices.

Methods: Youth with CKD ages 12-29 completed 5 scales: (1)REALM- literacy (2) Depression (CES-D) scale (3)PedsQL- physical, social, emotional, work (4)PedsQL-ADHD/cognitive (5)STARx- transition readiness. Depression or ADHD were determined by chart review.

Results: 25 patients with CKD stage ≥4 had these characteristics: Females 60%; μ age 21.56(±4.7); μ age at diagnosis 9.47(±8.46); Whites 44%; Blacks 32%; Hispanic 16%. 9 participants were managed by pediatric & 16 by adult nephrology. Three participants self-reported depression (4 on anti-depressants) & 2 participants reported ADHD (1 on stimulants).

Higher prevalence of ADHD symptoms was related to lower transition readiness/self-management (ß=-.55, p=.01), disease knowledge (ß=.48, p=.02), health literacy (ß=-.48, p=0.02), and QoL - social (ß=-0.79, p=.00), physical (ß=-.67, p=.00), emotional (ß=-.66, p=.00) & work-related (ß=-.80, p=.00). In addition, higher ADHD symptoms were related to higher depressive symptoms (ß=.50, p=.02).



Conclusions: Among youth with CKD, self-reported ADHD symptoms were significantly correlated with lower transition/self-management, QoL & health literacy and higher depressive symptoms. Addressing these modifiable factors may improve patient outcomes. Further validation is underway.

Funding: Private Foundation Support

TH-OR126

Effects of Multidisciplinary Care on Outcomes in Chronic Kidney Disease: A Systematic Review and Meta-Analysis

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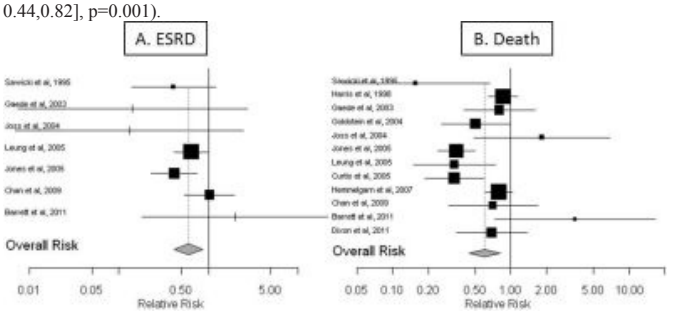
Background: Chronic kidney disease (CKD) is common and often suboptimally managed. Multidisciplinary care (MDC) may improve important clinical outcomes and reduce the public health burden of kidney disease: this study synthesizes the evidence on effects of MDC among patients with CKD.

Methods: We performed a systematic review of available literature in MEDLINE, CINAHL, and the Cochrane trials databases through 2011. Summary effects of MDC interventions in patients with pre-dialysis CKD were obtained using random effects models.

Results: From 8686 search results, 28 studies evaluated MDC in 11143 patients (median 200 [IQR 147,437]) from 12 countries. Two studies used shared-care models to enhance collaboration between primary care and nephrology, while 26 used MDC teams of physicians, nurses, pharmacists, dietitians, and/or social workers. MDC was generally associated with favorable results for many outcomes, such as control of CKD-related risk factors and complications, and planning for dialysis. Only 1 study included a formal cost analysis. In studies with a mean follow-up of 4.2 years, annual change in estimated glomerular filtration rate was -1.79 ml/min/1.73m^2 for MDC vs. -4.70 for usual care (difference -2.91 [95%CI -4.42,-1.39], p<0.001). Compared to usual care, MDC was also

associated with significantly lower cumulative risks of incident ESRD (7 studies [Fig.A]; RR 0.60 [95%CI 0.41,0.87], p=0.008) and death (12 studies [Fig.B]; RR 0.60 [95%CI 0.44,0.82], p=0.001).



Conclusions: MDC may reduce progression of kidney disease, ESRD, and death among patients with CKD. Future studies should evaluate the cost effectiveness and public health impact of broader implementation of MDC, which could significantly improve morbidity and mortality among patients with CKD.

TH-OR127

Early Dialysis Starts Experience Greater Improvements in Survival than Late Starts Despite Higher Comorbidity Burden at Dialysis Initiation

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Background: Recent studies have shown that early dialysis initiation compared with late is associated with poorer survival among patients receiving haemodialysis in the US. This finding has led to skepticism in promoting early start programs. We explored patterns in survival between "early and late dialysis initiation" in a representative sample of US dialysis patients.

Methods: Mortality trends among 'early start' (estimated glomerular filtration rates (eGFR): 10-15, 15-20 and > 20 ml/min/1.73 m²) and 'late start' patients (eGFR: 5-10 and < 5ml/min) were compared from 1995-2005 in 570, 903 incident patients and followed until Oct 2006 using data from the US Renal Data System. Crude mortality rates and adjusted 2-year mortality risks were compared across 3 time periods (1995-98, 1999-01 and 2002-04) within each eGFR category using Cox regression.

Results: Although comorbidity profiles and crude mortality rates were significantly higher for early than late starts, the multivariate analysis found significantly greater improvements in survival over 10 years for early compared with late start patients. No significant improvement was found for patients with eGFR < 5 ml/min.

Crude Mortality Rate (deaths/1000 person yrs)	1995-98	1999-01	2002-04
< 5 ml/min	186	189	186
5-10 ml/min	255	254	250
10-15 ml/min	361	333	305
15-20 ml/min	453	400	369
> 20 ml/min	407	328	308
Relative Risk Death[1],[2]			
< 5 ml/min	1.00	1.02	0.99
5-10 ml/min	1.00	0.98*	0.96**
10-15 ml/min	1.00	0.95**	0.90**
15-20 ml/min	1.00	0.93*	0.90**
>20 ml/min	1.00	0.87*	0.87*

[1]Each eGFR category modeled separately.[2]Adjusted for 17 demographic, clinical and laboratory variables, *P<0.001 **P<0.0001

Conclusions: Although mortality risks are significantly higher for early start compared to late starts, this study found that relative improvements in survival over time were unequal across GFR categories and were far superior for early start patients. The improved survival experience for these "high risk early starts" suggests potential benefits of early start programmes in selected populations.

TH-OR128

Angiotensin II Receptor Blocker Improves the Stenotic Kidney Microvasculature in Swine Unilateral Renal Artery Stenosis

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Background: Renal artery stenosis (RAS) is associated with intra-renal microvascular loss and remodeling. Angiotensin II receptor blockers (ARB) may decrease stenotic kidney (STK) function acutely, but their long-term effect on the STK is incompletely understood.

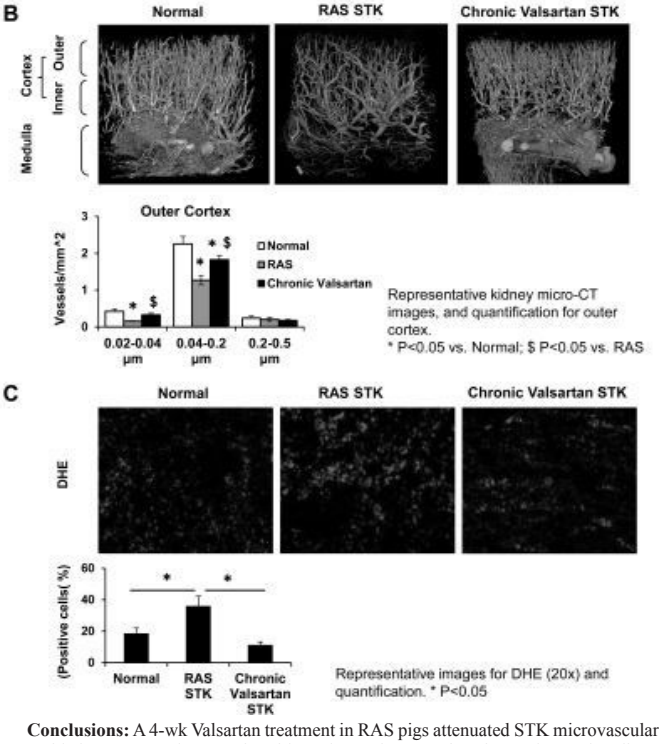
Methods: Domestic pigs were randomized as Normal, RAS, and RAS+Valsartan (n=6 each). The ARB Valsartan (320mg/d PO) was administered for 4 wks starting at 6 wks of unilateral RAS. After 10 wks of RAS, STK GFR was assessed by CT. STK tissue oxidative stress was then evaluated by DHE, apoptosis by TUNEL, and fibrogenic activity by plasminogen activator inhibitor-1 (PAI-1) expression. Density of STK microvessels (diameter <500 μm) in cortex was determined by micro-CT. In 6 additional RAS pigs the acute effects of intra-renal Valsartan infusion (320 mg IV) on GFR was assessed.

Results: RAS and RAS+Valsartan had comparable stenosis. At 10 wks MAP was unchanged in RAS but decreased in chronic Valsartan (from 151.5±12.7 to 128.1±6.2 mmHg, P<0.05). Valsartan infusion caused an acute 14.6% decrease of STK GFR, but not MAP (P=0.23), while chronic treatment did not affect GFR (Figure A). Conversely, chronic valsartan improved density of small microvessels (<200 μm) in the STK in outer cortex (Figure B), attenuated oxidative stress (Figure, C), and TUNEL positive cells (P<0.05 vs. RAS). Valsartan also normalized elevated PAI-1 expression in RAS (P<0.01 vs. Normal and RAS).

A

	Normal	RAS	Chronic Valsartan
Serum creatinine (mg/dl)	1.2±0.1	1.6±0.2*	1.6±0.2*
Single kidney (STK) GFR (ml/min)	81.8±6.5	57.5±9.4*	62.4±5.2*
Degree of stenosis (%)	/	78±7	76±5

STK: stenotic kidney; GFR: glomerular filtration rate. * P<0.05 vs. Normal



Conclusions: A 4-wk Valsartan treatment in RAS pigs attenuated STK microvascular loss, oxidative stress, and apoptosis, without decreasing STK function. Valsartan has direct renoprotective effects that preserve the STK in experimental RAS.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-OR129

Indoxyl Sulfate, a Uremic Toxin, Counteracts Endothelial Effects of Erythropoietin through Suppression of Akt Phosphorylation

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Background: Erythropoietin (EPO) is used to treat anemia in patients with chronic kidney disease (CKD). However, a wide variation in individual response to EPO is often observed, causing EPO resistance. EPO exhibits not only hematopoietic but also extra-hematopoietic functions such as endothelial effects. Indoxyl sulfate, a uremic toxin, is involved in endothelial dysfunction, and consequently the pathogenesis of CKD-associated cardiovascular disease (CVD). Hemodialysis patients with increased serum level of indoxyl sulfate require a higher dosage of EPO. The present study aimed to determine the effect of indoxyl sulfate on the extra-hematopoietic functions of EPO in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were incubated with EPO in the presence or absence of IS. HUVEC proliferation was examined using MTS cell proliferation assay. Cellular apoptosis was examined by caspase 3/7 assay. Expression of Akt, phosphorylated-Akt (p-Akt), extracellular signal-regulated kinases (ERK), p-ERK, endothelial nitric oxide synthase (eNOS), p-eNOS, thrombospondin-1 (TSP-1), EPO receptor (EPOR), and p-EPOR was detected by western blotting. A specific Akt inhibitor was used to determine the role of Akt.

Results: Indoxyl sulfate suppressed EPO-induced survival/proliferation, anti-apoptosis function, phosphorylation of eNOS, and the expression of TSP-1, an erythroid-stimulating factor, in HUVECs. Although EPO induced phosphorylation of both Akt and ERK, indoxyl sulfate suppressed phosphorylation of Akt but not ERK. An Akt kinase inhibitor suppressed all the EPO-induced cellular effects in HUVECs. As a site of action of indoxyl sulfate on EPO signaling, indoxyl sulfate attenuated EPO-induced tyrosine phosphoryaltion of EPOR.

Conclusions: Indoxyl sulfate negatively regulates EPOR-Akt pathway that is crucial for the pathogenesis of endothelial dysfunction and anemia, and might be involved in EPO resistance in patients with CKD.

Funding: Private Foundation Support

TH-OR130

Differential Actions of Angiotensin II on Proximal Tubule Transport Carolina Panico,[1] Earl H. Rudolph,[1] Roland C. Blantz,[3] Giovambattista Capasso,[2] Christopher S. Wilcox,[1] William J. Welch.[1] *[1]Nephrology, Georgetown University; [2]Second University of Naples; [3]University of California.*

Background: Studies in proximal tubule (PT)-specific AT_1R deficient-mice showed that the PT contributes to Ang II-induced hypertension. Ang II levels in PT fluid are 100- to 1000-fold higher than in plasma, yet the function of AT_1-Rs on the luminal side of the PT is unclear. Moreover, the hemodynamic effect of Ang II, which increases filtration fraction and peritubular forces, also increases fluid reabsorption.

Methods: We tested the effects of Ang II and AT_1-Rs on PT fluid uptake in rats.

Results: In Sprague Dawley (SD) rats, Ang II (200 ng/kg/min) delivered for 2 weeks increased MAP (Veh: 95±9 vs Ang II: 140±5 mmHg, p<0.001), single nephron GFR (SNGFR)(Veh: 36±3 vs Ang II: 43±3, p<0.05) and absolute proximal reabsorption (APR) (Veh: 20±3 vs Ang II: 34.5±6.5 nl/min, p<0.01), measured by free-flow collections in the S2 segment. These effects were normalized by acute reduction of MAP via a supra-renal aortic clamp. In Munich Wistar Frömter (MWF) rats, whose kineys allow access to the S1 segment, Ang II increased SNGFR and APR in the S1 segment (Veh: 19±4 vs Ang II: 44±5 nl/min, p<0.01; +125±9%) substantially more than in the S2 segment (Veh: 25±6 vs Ang II 37±7 nl/min, p<0.01; +48±6%). In SD rats, microperfusion *in situ* experiments, which allow for control of SNGFR, Ang II delivered into the lumen of the S2 segment reduced PT fluid reabsorption (Jv) in a dose-dependent manner. Ang II (10^{-9} M) at physiological levels reduced Jv by 60±7%; this effect was blocked by co-perfusion of an AT_1R blocker. Ang II (10^{-9} M), microperfused into the S2 segment reduced Jv in MWF rats by 44±5%. Ang II in cultured PT cells at high concentrations (10^{-7} to 10^{-9} M) also reduced $^{22}Na^+$ uptake by 20-35%.

Conclusions: In conclusion, Ang II acting on luminal AT_1-Rs reduces PT fluid reabsorption in the S2 segment. This effect could be overcome by the ability of systemic Ang II to increase absolute proximal reabsorption via hemodynamic enhancement of peritubular forces, especially in the S1 segment.

Funding: Other NIH Support - NHLBI P01-HL68686; NHLBI R01-HL095796

TH-OR131

Intra-Renal Over-Expression of Heat Shock Protein 90 alfa or beta (Hsp90α or Hsp90β) Protects against Ischemia/Reperfusion Injury Jonatan Barrera-Chimal,[1,2] Rosalba Pérez-villalva,[1,2] Juan-antonio Ortega,[1,2] Norma Bobadilla.[1,2] *[1]Molecular Physiology Unit, Instituto de Investigaciones Biomédicas, UNAM, Mexico; [2]Instituto Nacional de Ciencias Médicas y Nutrición SZ, Mexico.*

Background: We previously reported that Hsp90α and Hsp90β are equally and abundantly expressed in all the nephron segments. Moreover, we have also shown that radicicol (Hsp90 inhibitor) induced a reduction in the renal blood flow (RBF) and GFR, in part due to a reduction in urinary NO2/NO3 excretion, suggesting that Hsp90 regulates RBF in physiological conditions. However, the specific role of Hsp90α or Hsp90β on RBF regulation remains unclear. This study evaluated the effect of Hsp90α or Hsp90β over-expression in normal and ischemic settings.

Methods: Fifty Wistar rats were divided in sham, bilateral renal ischemia (B-I/R), unilateral ischemia (U-I/R) and nephrectomy plus I/R (Nx+I/R) groups. Rat Hsp90α and Hsp90β were cloned into pcDNA3.1(+). Gene identity was corroborated by clone sequencing. Intra-renal transfection was carried out by left renal artery injection of either pcHsp90α, pcHsp90β or empty vector (EV), 48-h before inducing ischemia (30 min). At the end of the experiment, RBF, serum creatinine (SCr) and urinary protein excretion (Uprot) were measured.

Results: I/R injury was characterized by renal dysfunction. In contrast, Hsp90α or Hsp90β over-expression (corroborated by Western Blot) prevented RBF reduction and reduced or prevented Uprot, however, this renoprotective effect was more evident in Nx+I/R transfected rats, because the lack of contra-lateral non-transfected kidney contribution.

	Groups n=5	**RBF** (mL/min)	**Uprot** (mg/24 h)	**SCr** (mg/dL)
Sham		9.5±0.4	14.9±3.3	0.5±0.1
	EV	6.3±0.8	70.4±20.1	2.8±0.3
B-I/R	Hsp90α	9.2±0.5a	39.6±7.4a	2.9±0.2
	Hsp90β	9.3±1a	40.3±13.9a	2.9±0.5
	EV	5.9±0.6	38.7±8.7	0.6±0.1
U-I/R	Hsp90α	8.2±0.3b	21.3±3.1b	0.5±0.1
	Hsp90β	8.1±0.1b	13.8±1.8b	0.6±0.1
	EV	5.8±0.3	33.4±3.4	2.9±0.6
Nx+I/R	Hsp90α	9.2±0.2c	15.5±1.6c	0.7±0.1c
	Hsp90β	9.9±0.7c	13±3.4c	0.9±.0.1c

a p<0.05 vs. B-I/R, b p<0.05 vs. U-I/R, c p<0.05 vs. Nx+I/R

Conclusions: This is the first study to demonstrate that intra-renal transfection of Hsp90α or Hsp90β before ischemia protects against renal injury induced by I/R.

This study was supported by CONACYT and PAPPIT.

Funding: Government Support - Non-U.S.

TH-OR132

Functional Consequences of Calcium-Sensing Receptor (CaSR) Gene-Knockdown in Renin-Secreting Cells in Mice Jan Czogalla,[1] Takahiro Masuda,[1] Maria Gerasimova,[1] Michael A. Rose,[1] Roberto Ariel Gomez,[2] Wenhan Chang,[3] Volker Vallon.[1] *[1]Dept of Medicine, UC San Diego and VA San Diego Healthcare System, San Diego, CA; [2]Dept of Biology, University of Virginia, Charlottesville, VA; [3]Dept of Medicine, UC San Francisco and Veteran Affairs Medical Center, San Francisco, CA.*

Background: Renin-secreting cells express the CaSR, but its function is unclear.

Methods: Utilizing the cre/lox system, we generated a CaSR mutant mouse model with knockdown of the CaSR in cells expressing renin ($renin^{Cre/+}/CaSR^{lox/lox}$), and compared with control mice ($CaSR^{lox/lox}$).

Results: On normal diet (0.8% NaCl) or after 6 days of low-salt diet (0.01% NaCl), systolic blood pressure (SBP, tailcuff in awake mice), heart rate (HR) and plasma renin concentration (PRC) were not different between genotypes. In contrast, 6 days of high-salt diet (4.0% NaCl) increased SBP in CaSR mutants relative to the change observed in controls (Δ mmHg: 3±2 vs -7±2, n=6-9, P<0.05). HR was reduced over the first 48h (-45±2 bpm) only in CaSR mutants, potentially due to a compensatory baroreceptor response. PRC was similar in both genotypes despite lower renal renin mRNA expression in CaSR mutants vs controls (low-salt: 0.95±0.04 vs 1.46±0.19; high-salt: 0.31±0.05 vs 0.60±0.10; P<0.05 each). Considering the strong correlation between renal mRNA and protein expression of renin, this may indicate a "fractional" increase in renin secretion in CaSR mutants. Acute i.p. injections of isoproterenol or furosemide increased PRC to a similar extent in both genotypes, but the effect of the ACE inhibitor, enalapril, on PRC was greater in CaSR mutants (Δ ng angiotensin I/ml per hr: 4471±474 vs 3056±118 in controls, n=5-6, P<0.05).

Conclusions: We propose that enalapril unmasked an enhanced angiotensin II tone in CaSR mutants that feeds back to lower PRC (by reducing renin expression and/or secretion) when the inhibitory influence of the CaSR on renin secretion is reduced. The efficiency of this feedback may make it difficult to detect differences in PRC in the steady state. An inhibitory influence of the CaSR on renin secretion may facilitate NaCl homeostasis and maintain blood pressure in response to a high NaCl diet.

Funding: NIDDK Support, Veterans Administration Support, Government Support - Non-U.S.

TH-OR133

Constrictor Mechanism of the Afferent Arteriole Initiated by Na/H Exchanger in the Nephron Hong Wang, Martin A. D'Ambrosio, Yilin Ren, Jeffrey L. Garvin, Oscar A. Carretero. *Hypertension & Vascular Research Division. Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.*

Background: The afferent arteriole (Af-Art) accounts for most of renal vasculature resistance, thus controlling glomerular filtration rate and renal function. Two mechanisms have been described in which nephron helps control Af-Art resistance, namely tubuloglomerular feedback (TGF), and connecting tubule glomerular feedback (CTGF). TGF is a constrictor mechanism initiated at the macula densa by the Na/K/2Clcotransporter (NKCC2), while CTGF is a dilator mechanism initiated at the connecting tubule by the epithelial Na channel (ENaC). However, when NKCC2 is blocked by furosemide, CTGF-induced Af-Art dilation is not evident, thus we hypothesize that there is a constrictor mechanism that is furosemide-insensitive and counters CTGF.

Methods: To test this, we used *in vivo* micropuncture of single nephrons. Stop-flow pressure (P_{SF}) was measured as an index of glomerular capillary pressure (which decreases when the Af-Art constricts). Two consecutive P_{SF} curves were generated by raising nephron perfusion from 0 to 40 nL/min while adding drugs to the tubular perfusate.

Results: The decrease in P_{SF} induced by increasing nephron perfusion was blocked by furosemide 10^{-4}M (control: 7.9±0.2 mmHg, furosemide: 0.4±0.2 mmHg, P<0.001, n=6), but was partially restored when blocking CTGF with the ENaC inhibitor benzamil 10^{-6}M (furosemide: 0.2±0.1 mmHg, furosemide+benzamil: 4.3±0.3 mmHg, P<0.001, n=6). A possible explanation for these observations is that TGF may be activated to some degree by Na/H exchanger (NHE)-mediated Na entry at the macula densa when NKCC2 is blocked. When we added the NHE blocker dimethylamiloride (DMA, 10^{-4}M) in the presence of furosemide and benzamil, the decrease in P_{SF} was significantly prevented (furosemide+benzamil: 4.6±0.3 mmHg, furosemide+benzamil+DMA: 1.1±0.2 mmHg, P<0.001, n=6).

Conclusions: Therefore, we conclude that a constrictor mechanism of the Af-Art is initiated by NHE in the nephron and is observable when NKCC2-mediated TGF and ENaC-mediated CTGF have been blocked.

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TH-OR134

Activation of mTOR Pathway Induced by Inflammation Accelerates the Progression of Atherosclerosis in Apolipoprotein E Knockout Mice Kun Ling Ma, Jing Liu, Jie Ni, Yang Zhang, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiang Su Province, China.*

Background: Our previous studies demonstrated that Rapamycin overrode the dysregulation of low lipoprotein receptor (LDLR) pathway induced by inflammation. This study was to investigate whether the activation of mammalian target of Rapamycin (mTOR) pathway was involved in the progression of atherosclerosis in apolipoprotein E knockout (apoE KO) mice.

Methods: The apoE KO mice fed with Western diet were divided into four groups for eight weeks (n=8): distilled water injection (Control), 8 mg/kg/q.o.d Sirolimus injection, 10% casein injection, Sirolimus plus casein injection. Plasma cytokine production was measured by ELISA. Foam cell formation and lipid accumulation were checked by hematoxylin-eosin (HE) staining and Oil red O (ORO) staining. The associated protein expressions of mTOR pathway and LDLR pathway were examined by immunohistochemical staining and immunofluorescent staining.

Results: Increased serum amyloid A (SAA) in casein injected mice suggested a successful induction of inflammatory stress. Interestingly, Serum total cholesterol, triglyceride, LDL, and HDL were significantly decreased in casein injected mice, while these were no difference in Rapamycin treated group compared to the controls. Inflammatory stress exacerbated foam cell formation and atherosclerotic plaque, which was correlated with the increased protein expressions of LDLR, sterol regulatory element binding protein-2 (SREBP-2), and SREBP cleavage-activating protein (SCAP). Furthermore, inflammation enhanced the translocation of SCAP escorting SREBP-2 from the endoplasmic reticulum to the Golgi, thereby activating LDLR gene transcription. Further analysis showed that inflammation increased protein expressions of mTOR, phosphated-mTOR, eukaryotic translation initation factor 4E binding protein 1(4EBP1), p-4EBP1, ribosomal protein S6 kinase 1(S6K1), p-S6K1, which were closely associated with the dysregulation of LDLR pathway.

Conclusions: Inflammation accelerated the progression of atherosclerosis in apoE KO mice via dysregulation of LDLR pathway, which could be mediated by the activation of mTOR pathway.

Funding: Government Support - Non-U.S.

FR-OR001

Missense Mutation S427L Impairs NBCe1-A Transport Function by Altering the Orientation of Transmembrane Segment 1

Quansheng Zhu, Rustam Azimov, Liyo Kao, Weixin Liu, Debra Newman, Natalia Abuladze, Ira Kurtz. *Medicine, UCLA, Los Angeles, CA.*

Background: NBCe1-A plays a critical role in renal proximal tubule bicarbonate transport where it is responsible for reabsorption of 80% of the filtered bicarbonate load. Eight missense mutations in NBCe1-A have been identified that cause proximal renal tubular acidosis including S427L that resides in the transmembrane segment 1 (TM1). Recently, three residues in NBCe1-A-TM1 have been shown to line the NBCe1-A ion translocation pathway implying the structural importance of TM1 in NBCe1-A mediated transport.

Methods: In this study, we examined the topology of NBCe1-A-TM1 in detail and determined the structural effect of S427L on the orientation of TM1 in NBCe1-A protein complex, by using the substituted cysteine accessibility method combined with chemical stripping, in situ chemical probing, and functional transport assays.

Results: Our data show that: 1) NBCe1-A-TM1 contains 31 residues starting from amino acid Phe412 to Thr442, which is considerately longer than what was thought previously; 2) the region prior to Phe412 till Arg394 is inaccessible to aqueous except Lys404 and Arg408, suggesting it is in a folded conformation potentially in close contact with the cytoplasmic domain; 3) none of the cysteine substitutions in the region of Cys389-Gln424 is sensitive to MTS inhibition, indicating this region is not involved in forming the ion translocation pathway; 4) aqueous accessibility of T442C is completely blocked in the presence of S427L, and not in the presence of S427A which retains over 50% transport function.

Conclusions: Our findings demonstrate that NBCe1-A-TM1 contains 31 amino acids with its N-terminus in close interaction with the cytoplasmic domain. Missense mutation S427L alters the orientation of NBCe1-A-TM1 significantly changing the configuration of the ion permeation pathway resulting in impaired transport function.

Funding: NIDDK Support

FR-OR002

Extracellular Loop 3 Forms a Domain-Like Structure on the Surface of NBCe1-A

Quansheng Zhu, Liyo Kao, Weixin Liu, Debra Newman, Rustam Azimov, Ira Kurtz. *Medicine, UCLA, Los Angeles, CA.*

Background: All sodium-driven bicarbonate transporters within the SLC4 family share a common feature: the presence of four cysteines (Cys) in the glycosylated extracellular loop 3 (EC-loop 3). Topological studies have determined that EC-loop 3 is the largest highly exposed surface loop in NBCe1-A which contains 82 amino acids. More surprisingly, EC-loop 3 from each of the NBCe1-A monomers resides at the dimer interface.

Methods: In the present study, we analyzed the potential pattern of disulfide bonding among the four Cys in the NBCe1-A EC-loop 3, and the role of glycosylation in assisting the precise bond formation. NBCe1-A constructs carrying one to four Cys residues in various combinations in EC-loop 3 were expressed in HEK-293 cells and subjected to an SDS-PAGE gel shift assay, chemical stripping, DTT treatment, chemical probing and proteolytic analysis. The function of each construct was analyzed in HEK-293 cells.

Results: Our study revealed: 1) the four Cys in EC-loop 3 are in a folded conformation and intra-disulfided; 2) Cys 1, 2 and Cys 3, 4 form two intramonomeric disulfide bonds; 3) the first disulfide bond determines the number of glycosylation sites in the loop, and glycosylation guides the disulfide bond formation between Cys 3 and 4; 4) in the absence of the four Cys residues, NBCe1-A traffics normally to the plasma membrane, however, each monomer becomes triply glycosylated and most of the introduced Cys in loop 3 become self cross-linked; 5) when the four Cys are absent, EC-loop 3 adopts an extended conformation and becomes accessible to proteolytic cleavage.

Conclusions: Our findings indicate that EC-loop 3 in NBCe1-A is highly structured via two disulfide bonds. This unique structure on the extracellular surface of NBCe1-A is reminiscent of G-protein coupled receptors and may interact with specific ligand(s).

Funding: NIDDK Support

FR-OR003

An Atp6v0a4 Knockout Mouse Is a Model of Distal Renal Tubular Acidosis with Hearing Loss, with Additional Extra-Renal Phenotype

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Background: Recessive distal Renal Tubular Acidosis (dRTA) is a severe childhood disorder of acid base homeostasis. It is characterised by hyperchloremic acidosis, hypokalemia, alkaline urine, hypercalciuria, hypocitraturia, nephrocalcinosis and rickets if untreated. Sensorineural deafness is often associated. Mutations in *ATP6V0A4,* which encodes the tissue restricted a4 subunit of the H^+-ATPase, can underlie dRTA. H^+-ATPases are multisubunit pumps found in organellar membranes, and in specialized cells of the distal nephron, inner ear and male reproductive tract, additional plasma membrane pumps contain tissue restricted subunits which replace generic ones.

Methods: An *Atp6v0a4*-null mouse line was created and using β-galactosidase as a reporter, embryonic a4 expression was investigated. Postnatally, biochemical and histological assessments were undertaken before and after dietary manipulation. Auditory brainstem response thresholds (ABRs), endocochlear potential (EP), olfactory behavior and bone mineral density (BMD) were assessed.

Results: a4 expression was detected in developing kidney, ear, bone, nose, eye and skin. At 21 days, *Atp6v0a4*$^{-/-}$ pups were smaller than their littermates, with hyperchloremic acidosis and hypokalemia. Most -/- pups died at weaning unless treated with alkali from birth, which facilitated survival into adulthood. Alkali supplementation could later be withdrawn, but -/- animals remained acidotic with alkaline urine. Null mice were hypocitraturic and nephrocalcinotic, but hypercalciuria was absent. They were severely hearing-impaired by 14 days, with elevated ABR thresholds and absent EPs. They were also hypo-osmic. +/- animals were biochemically normal until acid-loaded, when they became acidotic with reduced BMD.

Conclusions: This is the first faithful mouse model of dRTA. It illustrates the vital role of a4 in both renal and extra-renal acid-base homeostasis and reveals new avenues for clinical investigation of bone physiology and olfaction in patients and mutation carriers.

Funding: Private Foundation Support

FR-OR004

Effect of Collecting Duct-Specific Deletion of Both Rh B Glycoprotein (Rhbg) and Rh C Glycoprotein (Rhcg) on Renal Response to Metabolic Acidosis

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Background: Rhbg and Rhcg are ammonia transporters in distal nephron and collecting duct believed important in net acid excretion. Our purpose was to determine the effect of their combined deletion on the renal response to metabolic acidosis.

Methods: Mice with collecting duct-specific deletion of both Rhbg and Rhcg (CD-KO) were generated using Cre-loxP techniques and the Ksp-cadherin promoter for Cre-recombinase. Control mice (C) did not express Cre-recombinase. Acid-loading was performed by pair-feeding chow mixed with HCl, 0.4 M, for 7 days. N=6/group.

Results: We confirmed kidney-specific deletion with genomic PCR analysis and collecting duct-specific deletion with immunohistochemistry. On regular diet, without HCl, urine ammonia excretion, urine pH, and serum HCO_3^- and K^+ were similar in C and CD-KO. On diet with HCl, CD-KO developed more severe metabolic acidosis than did C (serum HCO_3^-: 4.3 ± 2.5 vs 15.9 ± 2.7 mmol/L, respectively, $P<0.01$), and developed hypokalemia, whereas C did not (serum K^+: 3.9 ± 0.1 vs 4.5 ± 0.2 mmol/L, respectively, $P<0.01$). Acid-loading increased ammonia excretion, but it increased more slowly in CD-KO and was significantly less than in C on days 1-5. Urine pH was lower in CD-KO than in C on days 1 and 3 of acid-loading. Without acid-loading, PEPCK, PDG, NHE-3 and glutamine synthetase (GS) expression were similar in CD-KO and C. Metabolic acidosis increased PEPCK and NHE-3 expression and decreased GS expression, and the changes were significantly greater in CD-KO than in C.

Conclusions: 1) Rhbg and Rhcg are critically important in the renal response to metabolic acidosis; 2) the significantly greater changes in PEPCK, NHE-3 and GS expression in acid-loaded CD-KO compared to C mice indicates the role of Rhbg and Rhcg is underestimated due to exaggerated changes in other proteins involved in renal ammonia metabolism; and 3) in mice with intact Rhbg and Rhcg expression, acidosis-induced changes in PEPCK, NHE-3 and GS expression are submaximal, despite the presence of persistent metabolic acidosis.

Funding: NIDDK Support, Veterans Administration Support

FR-OR005

Phosphotyrosine Signaling during Acid-Base Sensing in the Mouse Proximal Tubule Antonios Charokopos, Lara A. Skelton, Walter F. Boron. *Physiology and Biophysics, Case Western Reserve, Cleveland, OH.*

Background: The renal proximal tubule (PT) is a major site for maintenance of blood pH homeostasis. During acidosis, the PT increases HCO_3- reabsorption and also generates "new HCO_3-", thus neutralizing excess blood protons. The question of how does the PT sense blood pH has been a puzzling one in renal physiology. Previously, we found that the PT modulates its HCO_3- reabsorption in response to blood [CO_2] and [HCO_3-], paradoxically not pH. We also found that this modulation is prevented: i) by inhibiting the ErbB tyrosine kinase family and ii) by knocking out tyrosine phosphatase RPTPγ. We sought to understand the interaction between RPTPγ and ErbB1 and 2 in response to varying [CO_2]. We also looked at the Src tyrosine kinase family, known to synergize with ErbB receptors and also to be activated by acidosis.

Methods: We generated PT suspensions from WT and RPTPγ-KO mice and then subjected the PTs to respiratory alkalosis and acidosis (2.5% or 5% or 10% CO_2, constant HCO_3-) for an acute timeframe (20 min).

Results: By Western Blot, we were able to detect total ErbB1 and its phosphorylation (p) at Y845, Y992, Y1068 and Y1173; total ErbB2 and its phosphorylation at Y1221/2; specific Src family members (Src Fyn Lyn Lck and Yes); Src-family phosphorylation at the Y416 (stimulatory) and Y527 (inhibitory) sites; and the M-calpain protease and its inhibitor calpastatin. As [CO_2] increases we observe, in both genotypes: i) a modest increase in ErbB1 phosphorylation, ii) an increase (as detected with a C-terminal antibody) in total ErbB2 levels, and iii) an increase in calpastatin levels. Compared to the WT, the RPTPγ-KO exhibits: i) increased activation of the Src family (based on increased pY416 and/or decreased pY527), and ii) low levels of total p60Src, but not p55Src. The changes in total protein levels of ErbB2 and p60Src may reflect involvement of the calpain-calpastatin proteolytic system.

Conclusions: In summary, respiratory acidosis/alkalosis in the PT seems to produce changes in total protein or pY levels of ErbB1/2 and the Src family, and these changes appear to be modulated by the presence/absence of RPTPγ.

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FR-OR006

Lipopolysaccharide (LPS) Activates Different TLR4 Signaling Mechanisms in Apical and Basolateral Membranes to Inhibit HCO_3- Absorption in Medullary Thick Ascending Limb (MTAL) Bruns A. Watts, Andrew Badalamenti, David W. Good. *U TX Med Branch, Galveston, TX.*

Background: Previously we demonstrated that bacterial LPS inhibits HCO_3- absorption in the MTAL through activation of its cell-surface receptor TLR4, and that LPS inhibits HCO_3- absorption through different TLR4 signaling pathways in the basolateral and apical membranes. Basolateral LPS inhibits HCO_3- absorption through TLR4-dependent ERK activation. Here we examined signaling mechanisms involved in inhibition by luminal LPS and factors that may contribute to different basolateral and apical TLR4 signaling.

Results: Adding LPS to the lumen decreased HCO_3- absorption by 28% in perfused mouse and rat MTALs. Inhibitors of ERK activation, which eliminate inhibition of HCO_3- absorption by bath LPS, had no effect on inhibition by lumen LPS. Conversely, inhibitors of phosphatidylinositol 3-kinase (PI3K) or its downstream target Akt eliminated inhibition of HCO_3- absorption by lumen LPS but not by bath LPS. Inhibition by lumen LPS also was eliminated by inhibiting mTOR, a downstream effector of PI3K/Akt signaling. Exposure to LPS for 15 min increased Akt and mTOR phosphorylation; these effects were blocked by wortmannin, confirming that Akt and mTOR are activated downstream of PI3K. TLR2 is expressed along with TLR4 in the basolateral membrane, but not in the apical membrane, of MTAL cells. Basolateral LPS failed to activate ERK or inhibit HCO_3- absorption in MTALs from $TLR2^{-/-}$ mice despite normal TLR4 expression. Inhibition of HCO_3- absorption by lumen LPS via PI3K was preserved in $TLR2^{-/-}$ MTALs. Co-immunoprecipitation studies showed that TLR4 and TLR2 are physically associated in inner stripe of outer medulla.

Conclusions: We conclude: 1) lumen LPS inhibits HCO_3- absorption in the MTAL through a TLR4-mediated PI3K/Akt/mTOR pathway; 2)TLR2 is required for LPS-induced TLR4 signaling at the basolateral but not the apical membrane. The ability of LPS to induce different intracellular signals at the two membranes may involve a novel interaction between TLR4 and TLR2 in the basolateral membrane and could provide new mechanisms for control and selective targeting of TLR4-mediated responses that impair renal tubule function.

Funding: NIDDK Support

FR-OR007

Glutamine Synthetase: Expression in Proximal Tubule and Intercalated Cells and Differential Cellular Regulation in Hypokalemia Jill W. Verlander,[1] Hyun-Wook Lee,[1] Diana Chu,[1] Sharon W. Matthews,[1] Mary E. Handlogten,[1] Jesse M. Bishop,[1] I. David Weiner.[1,2] *[1]Renal Division, University of Florida, Gainesville, FL; [2]NF/SGVHS, Gainesville, FL.*

Background: Renal glutamine synthetase (GS) is regulated by acid-base disturbances, hypokalemia, and genetic deletion of renal ammonia transporters, suggesting GS is important in ammonia metabolism. Thus we re-examined GS expression in mice and identified cell-specific changes in expression induced by hypokalemia.

Methods: We examined renal GS expression by immunoblot, RT-PCR and immunohistochemisty (IHC). Hypokalemia was induced by feeding zero-K vs normal K diet X 12 days (n=4/group). 24-hr urines and blood were analyzed for ammonia and potassium. We measured cell-specific GS expression using IHC with quantitative analysis.

Results: Immunoblots showed GS protein in cortex and outer and inner stripe of outer medulla (OMo and OMi). By IHC, GS label intensity was similar in cortical and OMo proximal tubules; GS was also in collecting duct, connecting segment (CNT) and DCT intercalated cells. Double immunolabel for GS with AE-1, H^+ATPase, and NCC showed GS in type A intercalated cells in CNT through IMCD and in nonAB intercalated cells in CNT and DCT. Type B intercalated cells were GS-negative. RT-PCR showed GS mRNA in OMi, which has no proximal tubules. Zero-K diet decreased serum and urine K and increased urine ammonia excretion. By IHC, proximal tubule GS was greatly reduced in cortex and OMo. Conversely, quantitative IHC showed significantly increased GS expression in type A intercalated cells in CCD and OMCD.

Conclusions: 1) We show for the first time that type A intercalated cells from CNT through IMCD and nonAB intercalated cells in CNT and DCT express GS. 2) GS expression is similar in proximal convoluted and straight tubules in mouse kidney. 3) In hypokalemia GS regulation differs in proximal tubule and intercalated cells. Decreased GS in proximal tubules would enhance renal ammonia excretion and promote alkalosis; increased GS in type A intercalated cells may counter the proximal tubule effect on acid-base balance. Thus, intercalated cells not only transport ammonia, but may contribute to regulated ammonia metabolism.

Funding: NIDDK Support, Veterans Administration Support

FR-OR008

Detection of PLA_2R in Glomerular Deposits in Membranous Nephropathy Cases Seronegative for Anti-PLA_2R A. Bernard Collins,[1] Evan A. Farkash,[1] Laurence H. Beck,[2] Rex Neal Smith,[1] Robert B. Colvin.[1] *[1]Pathology, Massachusetts General Hospital, Boston, MA; [2]Medicine, Boston University School of Medicine, Boston, MA.*

Background: Approximately 70% of patients with primary membranous nephropathy (MN) have autoantibodies to the phospholipase A_2 receptor (PLA_2R). In contrast, PLA_2R antibodies are rarely detected in secondary forms of MN (e.g., lupus and de novo transplant MN). The nature of the autoantibody in the 30% of primary MN patients seronegative for anti-PLA_2R is unknown. We hypothesize that some are anti-PLA_2R-associated disease that might be detected by the presence of PLA_2R in immune deposits.

Methods: Double immunofluorescence (IF) studies for IgG4/PLA_2R and IgG/PLA_2R colocalization were performed on frozen tissue sections from the renal biopsies of 11 MN patients seronegative for anti-PLA_2R by Western blot and 4 comparison patients who were seropositive for anti-PLA_2R. IgG4 and IgG were directly labeled with FITC and PLA_2R was detected using a 3 step Cy3 amplified IF technique. Colocalization was assessed morphometrically as % of Cy3 pixels with FITC signal using Image J Software.

Results: All of the patients seropositive for anti-PLA_2R had colocalization (70±15%) by IF (4/4) and were clinically felt to have primary MN. A subset of the patients seronegative for anti-PLA_2R (4/11) also had widespread colocalization (81±34%) by IF. One of these 4 patients had SLE and one had HCV; the other two had no clear etiology of the MN and were considered idiopathic. Seven patients had no colocalization by IF (1.3±1.2%) and a negative Western blot assay. Most of these patients (57%) had morphological evidence of secondary MN (mesangial deposits, TBM deposits and/or penetrating deposits). While morphometric techniques were used in this study, visual inspection alone clearly distinguished cases with or without colocalization.

Conclusions: Dual staining of renal biopsies by IF can detect most or all cases of anti-PLA_2R-associated MN, including a hitherto undetectable subgroup of patients (~35%) who are seronegative for anti-PLA_2R probably due to immunological remission. Such IF techniques will also be useful to distinguish primary from secondary MN.

Funding: Clinical Revenue Support

FR-OR009

Phospholipase A2 Receptor (PLA2R) Staining in the Deposits of Membranous Glomerulopathy Christopher Patrick Larsen,[1,2] Nidia Cordeiro Messias,[1] Patrick D. Walker.[1,2] *[1]Nephropath, Little Rock, AR; [2]Dept of Path, Univ of Ark for Med Sci, Little Rock, AR.*

Background: Anti-PLA2R antibodies are the underlying etiology in most cases of primary membranous glomerulopathy (MG). The data on PLA2R staining in renal biopsies is limited and all reports thus far have utilized confocal microscopy, a technique not readily available in many renal pathology laboratories. The aim of this study was to determine the sensitivity and specificity of PLA2R staining in renal biopsies for detecting primary MG.

Methods: The database at our institution was searched for cases of known primary and secondary MG. 162 cases met inclusion criteria including 83 primary and 79 secondary. Standard renal biopsy processing techniques were used including light, immunofluorescence (IF), and electron microscopy. PLA2R was detected with standard IF.

Results: PLA2R was positive in 63/83 cases of primary MG and 14/79 of secondary MG. The sensitivity and specificity of PLA2R for detection of primary MG was 76% and 82%, respectively. Hepatitis C virus was the most common secondary etiology with PLA2R staining (7/11, 64%) followed by sarcoidosis (3/4, 75%) and neoplasia (3/12, 25%). Autoimmune etiologies showed the least PLA2R staining (1/45, 2%). The morphologic findings examined in all cases included the pattern of deposition, IF staining pattern, and evaluation for subendothelial and mesangial deposits. 8 segmental MG cases were present including 2 idiopathic and 6 secondary and all were negative for PLA2R. 5 cases showed light chain restriction and only one was PLA2R positive. Subendothelial deposits were seen in 17 secondary and no primary biopsies, all were negative for PLA2R. Mesangial deposits were found in 84 cases including 24 primary and 60 secondary. 24 cases with mesangial deposits were positive for PLA2R. 10 cases had 'full house' staining, none of which were positive for PLA2R.

Conclusions: We present the largest series to date detailing the sensitivity and specificity of PLA2R in MG, utilizing a technique that could be immediately adopted by most renal pathology laboratories. PLA2R staining in kidney biopsies is a useful marker of primary MG with a sensitivity of 76% and specificity of 82%.

FR-OR010

Genetical and Clinical Aspects of X-Linked Alport Syndrome in Males with Positive Staining of the α5(IV) Chain Yuya Hashimura,[1] Kandai Nozu,[2] Hiroshi Kaito,[2] Hiromi Ohtsubo,[2] Fusako Hashimoto,[2] Shingo Ishimori,[2] Takeshi Ninchoji,[2] Naoya Morisada,[2] Koichi Nakanishi,[3] Norishige Yoshikawa,[3] Kazumoto Iijima.[2] *[1]Pediatrics, Hyogo Prefectural Kobe Children's Hospital, Japan; [2]Pediatrics, Kobe University Graduate School of Medicine, Japan; [3]Pediatrics, Wakayama Medical University, Japan.*

Background: X-linked Alport syndrome (XLAS) is caused by mutations in *COL4A5* that encodes the type IV collagen α5 chain. In male patients, complete negativity for α5 staining in the renal basal membrane is considered a pathological characteristic; however, it has been demonstrated α5 staining is positive in >20% of patients. Further, the genetic and clinical backgrounds of male XLAS patients presenting with such atypical immunohistological findings have not yet been elucidated.

Methods: We retrospectively studied 52 male patients diagnosed with XLAS on the basis of *COL4A5* analysis, in whom α5 expression had been examined. Patients showing complete negativity for α5 staining were classified as the negative group (NG); all other patients were classified as the positive group (PG). The genetic and clinical backgrounds of patients in both groups were examined using the Kaplan–Meier method and receiver operating characteristic (ROC) analysis.

Results: PG included 15 of the 52 patients (28%). All 15 patients had non-truncating mutations. The renal survival curve for the patients and their affected family members showed that renal failure occurred at a significantly later age in PG than in NG ($p = 0.03$). Further, ROC analysis of patients with missense mutations showed that the cutoff value was exon 25 in both groups and that mutations in PG showed a significant bias to be located before exon 25.

Conclusions: This is the first report on the genetic and clinical backgrounds of male XLAS patients with atypical immunohistological findings. Male XLAS patients who showed α5 chain expression had relatively mild clinical manifestations, and their genotypes matched with those of reported cases presenting with a mild form of the disease. Further, we also showed for the first time that differences in α5 expression among patients with missense mutations resulted from differences in the location of the mutation.

FR-OR011

Decreased Circulating C3 Levels and Mesangial C3 Deposition Predict Renal Outcome in Patients with IgA Nephropathy Seung Hyeok Han,[2] Hye-young Kang,[1] Dae-Suk Han,[2] Dong Ho Shin,[2] Mi Jung Lee,[2] Seong Hun Kim,[1] Shin-Wook Kang.[1,2] *[1]Severance Biomedical Science Institute, Brain Korea 21, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea.*

Background: Mesangial C3 deposition is frequently observed in patients with IgA nephropathy (IgAN). However, the role of complement in the pathogenesis or progression of IgAN is uncertain. In this observational cohort study, we aimed to identify the clinical implication of circulating C3 levels and mesangial C3 deposition and to investigate their potentialities as predictors of renal outcomes in patients with IgAN.

Methods: A total of 343 patients with biopsy-proven IgAN were enrolled between January 2000 and December 2008. Decreased serum C3 levels (hypoC3) was defined as C3<90 mg/dl. The study endpoint was end-stage renal disease (ESRD) or a doubling of the baseline serum creatinine concentrations (D-SCr).

Results: Of the patients, there were 66 patients (19.2%) with hypoC3. During a mean follow-up of 53.7 months, ESRD occurred in 5 patients (7.6%) with hypoC3 compared to 9 patients (3.2%) with normal C3 levels (P=0.11). However, 12 patients (18.2%) with hypoC3 reached D-SCr compared to 17 patients (6.1%) with normal C3 levels [Hazard ratio (HR), 3.59; 95% confidence interval (CI), 1.33-10.36; P=0.018]. In a multivariable model in which serum C3 levels were treated as a continuous variable, hypoC3 significantly predicted the development of D-SCr (per 1 mg/dl increase of C3; HR, 0.95; 95% CI, 0.92-0.99; P=0.011). The risk of reaching renal endpoint was significantly higher in patients with mesangial C3 deposition 2+ to 3+ than in patients without deposition (HR, 9.37; 95% CI, 1.10-80.26; P=0.04).

Conclusions: HypoC3 and mesangial C3 deposition were independent risk factors for progression, suggesting that complement activation may play an unfavorable pathogenic role in patients with IgAN.

FR-OR012

Interobserver Agreement amongst 77 Nephropathologists Using the New Classification System for Diabetic Nephropathy Elisabeth J. Valk,[1] Eloise Magnenat,[2] Ron Wolterbeek,[3] Emile De Heer,[1] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]University of Geneva, Switzerland; [3]Medical Statistics, LUMC, Netherlands.*

Background: In 2010, the new classification system for Diabetic Nephropathy was launched (JASN 2010). In this paper, a pilot study on the interobserver agreement amongst 5 pathologists who participated in the design of the classification system, showed a good correlation for scoring the separate classes (ICC: 0.84). The purpose of the present study was to assess the interobserver agreement of an international group of nephropathologists who were still fairly unfamiliar with the classification system.

Methods: 13 biopsies with Diabetic Nephropathy were selected and sent round on a DVD to all members of the Renal Pathology Society and also, an announcement was made to the members of the ISN to join in this survey. The 2010 publication served as a guideline. Participants had to classify the 13 cases and also had to score: IFTA, interstitial inflammation, amount of hyalinosis and arteriosclerosis. They also had to evaluate their own level of expertise.

Results: 77 nephropathologists from 28 countries participated in the study. The ICC for the classification score was 0.74 amongst all pathologists and 0.76 amongst experienced pathologists. The ICC for IFTA, interstitial inflammation, hyalinosis and arteriosclerosis was 0.72; 0.47; 0.41; 0.53 for all pathologists and 0.73; 0.57; 0.43; 0.42 for experienced pathologists. Comments made by the nephropathologists who joined in the study concerned confusion over the definitions for clasess IIa and IIb (mild versus severe mesangial expansion), scoring inflammation inside or outside areas with IFTA, the assessment of arterial hyalinosis, and definition of vessel size in which arteriosclerosis had to be scored.

Conclusions: The classification system for Diabetic Nephropathy shows good interobserver agreement among nephropathologists worldwide, with experienced pathologists performing similar to less experienced ones. Rephrasing of some of the definitions would be useful, and might ameliorate the interobserver agreement.

FR-OR013

β2-Microglobulin Asearly Predictors of Glucocorticoid Treatment Failure in Adult Patients with Nephrotic Syndrome Jingyuan Xie, Qiongxiu Zhou, Hong Ren, Jing Xu, Xu Hao, Wen Xue, Nan Chen. *Nephrology, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China.*

Background: Urinary α1-microglobulin(uα1-MG) and β2-microglobulin (uβ2-MG) which were reported have predictable value of Steroid-resistant nephrotic syndrome(SRNS) in children. The aim of this study is to examine whether they can predict steroid responses in adult-onset primary nephrotic syndrome(PNS).

Methods: We retrospectively enrolled 113 cases with PNS [78 had focal segmental glomerulosclerosis (FSGS), 35 had minimal-change disease(MCD)], patients with BMI≥30kg/m^2 were excluded. They were divided to SRNS and SSNS based on their response to steroid treatment of 12-16 weeks. We recorded demographic and clinical data from all patients at the time of renal biopsy and during follow up. These included gender, sex, body mass index (BMI), serum creatinine (Scr), albumin, urine protein, uα1-MG and uβ2-MG et al. The association of baseline variables with the primary outcome (response to steroid therapy) was tested using Logistic regression models. Areas under receiver operator characteristics (ROC) curves were also assessed for evaluating predictable valuable of baseline variables include uα1-MG and uβ2-MG.

Results: Of all the 113 participants, 43 were SRNS and 70 were SSNS. There were 79 men and 34 women with a median age of 27 years old in this study. Baseline uα1-MG (9.92vs 2.97 mg/dl, P<0.001) and uβ2-MG(623.85 vs 131.45 ug/l, P<0.001) were significantly increased in SRNS group when compared to the SSNS group. In univariate analysis, elder age [OR=1.03, 1.00-1.06, P=0.049], lower hemoglobulin[OR=0.98, 0.96-0.99, P=0.028], lower albumin [OR=0.94, 0.90-0.99, P=0.01], higher Scr [OR=1.02, 1.01-1.03, P=0.003],higher ln(uα1-MG) [OR=2.12, 1.42-3.15, P=$2.21*10^{-4}$]and ln(uβ2-MG) [OR=3.10, 1.46-6.58, P=$3*10^{-3}$] were associated with increased risk for SRNS. In the multivariate stepwise Logistic regression models, only ln(β2-MG)(OR=3.16, 95%CI=1.24-8.07, P=0.02) had a significant, independent effect on the risk of SRNS. The area under the ROCcurves was estimated at 0.83(95%CI=0.70-0.96) for ln(β2 –MG).

Conclusions: Urine β2-MG can be considered as early and reliable prognostic predictors of adult-onset SRNS.

FR-OR014

Plasma Cell Rich Infiltrate Is an Independent Risk Factor for Allograft Failure in Late Acute Rejection Sharad Sathyan, Thangamani Muthukumar, Choli Hartono, Darshana Dadhania, Manikkam Suthanthiran, Surya Seshan. *Nephrology and Pathology, Weill Medical College of Cornell University.*

Background: Late acute rejection (AR) portends a poor prognosis. AR with plasma cell rich (>20%) infiltrates (Plasma cell-rich AR) tend to occur late after transplant. The clinico-pathological features of late AR and risk factors of allograft failure has not been well characterized.

Methods: We reviewed the records of all kidney recipients who had for-cause biopsies done >1 year after transplant (1999-2010). AR was defined as per the Banff classification. We obtained relevant demography, clinical, laboratory, hisopathological and follow up information. Primary outcome was graft loss (persistent decline of eGFR to <15 ml) or return to dialysis or re-transplant. Patients who did not reach the outcome were censored at

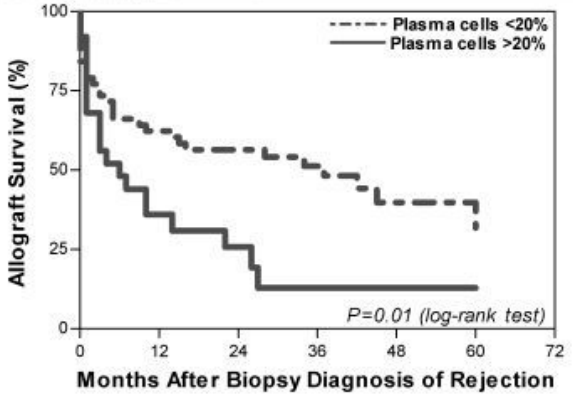
their last follow up. We generated Kaplan-Meier curves and performed Cox-proportional hazard regression to determine the independent risk factors for allograft failure.

Results: There were 82 patients with late AR; 23 (28%) acute T-cell mediated rejection; 23 (28%) C4d+ acute antibody mediated rejection and 11 (13%) mixed. The remaining 25 (30%) had plasma cell-rich AR. Among them 9 (36%) were C4d+.

The salient characteristics are shown in the table.

Patients with plasma cell-rich AR had an inferior allograft outcome compared to those who did not have plasma cell-rich AR.

Category	Acute Rejection >1 year After Transplant		
	Rich in Plasma Cells (>20%) [N=25]	Not Rich in Plasma Cells (<20%) [N=57]	P
Age, years, *median*	40	46	NS
Gender, M:F	10:15	32:25	NS
African American, n	12	17	NS
Deceased donor, n (%)	11 (44%)	39(68%)	0.035
Cold Ischemia time, hours, *median*	16h30min	23h55m	NS
Delayed graft function, n (%)	0 (0%)	11(19%)	0.02
Antithymocyte globulin induction, n (%)	12 (48%)	29(51%)	NS
Steroid based immunosuppression, n (%)	14 (56%)	31(54%)	NS
Time to biopsy, months, *median (IQR)*	38 (46)	28 (24)	NS
Prior rejection, n (%)	9 (36%)	11(19.2%)	NS
Serum Creatinine at biopsy, mg/dl, *median*	4.3	3	NS
Tacrolimus trough at biopsy, ng/ml, *median*	3.35	4.1	NS
3 months prior	4.6	5	NS
6 months prior	5.2	5.9	NS
IF/TA, moderate/severe (>25%), n (%)	9 (36%)	15(26%)	NS
Follow up, months, *median*	49	38	NS
Allograft survival 1-year post biopsy	31%	60%	0.01
Median allograft survival, months	6	37	



By Cox regression, plasma cell-rich AR was a significant risk factor for allograft failure independent of receiving a transplant from a deceased donor, history of delayed graft function, time from transplant to biopsy or interstitial fibrosis/tubular atrophy.

Conclusions: In our single center study of 82 late AR, plasma cell rich AR has an inferior allograft survival as compared to other late AR, independent of the time to biopsy or severity of interstitial fibrosis/tubular atrophy.

FR-OR015

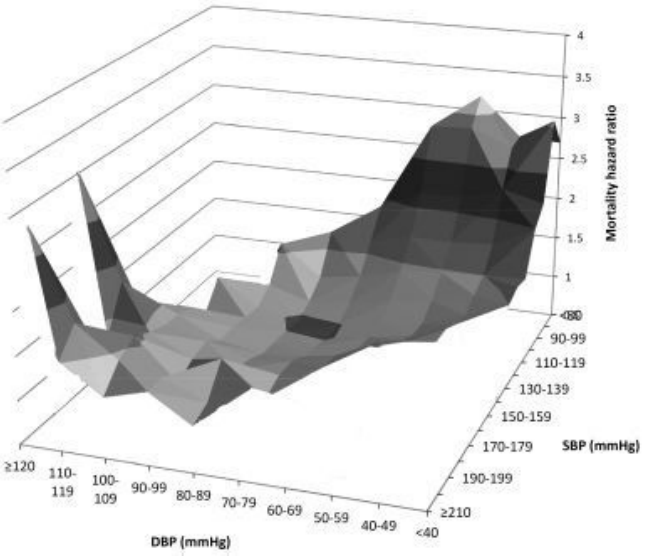
Association of Blood Pressure Levels with Mortality in a Nationally Representative Cohort of US Veterans with Non-Dialysis Dependent CKD

Csaba P. Kovesdy,[1,2] Anthony J. Bleyer,[3] Miklos Zsolt Molnar,[4] Jennie Z. Ma,[5] Leigh Darryl Quarles,[2] Kamyar Kalantar-Zadeh.[4] *[1]Memphis VA Medical Center, Memphis, TN; [2]University of Tennessee Health Science Center, Memphis, TN; [3]Wake Forest University, Winston Salem, NC; [4]Harold Simmons Center at Harbor-UCLA, Torrance, CA; [5]University of Virginia, Charlottesville, VA.*

Background: SBP and DBP display U-shaped associations with mortality in CKD, but the concomitant presence of an ideal SBP and DBP is rare. It is unclear if elevated SBP combined with ideal DBP, or ideal SBP combined with low DBP is better.

Methods: We evaluated the association of BP with all-cause mortality in a national cohort of 650,243 US veterans with CKD stages 1-5. SBP and DBP were examined both separately, and as all possible combinations of each other in 96 categories (from <80/<40 to >210/>120 mmHg, in increments of 10 mmHg). Associations with mortality were examined in time-dependent Cox models, with adjustment for age, sex, race, comorbidities, eGFR, serum K and antihypertensive use.

Results: Over a median follow-up of 4.6 years 185,502 patients died. Both SBP and DBP had U-shaped associations with mortality individually. When considering combinations of SBP and DBP, patients with BP of 130-139/80-89 had the lowest adjusted mortality, and those in whom both SBP and DBP were concomitantly very high or very low had the highest mortality (Figure). Patients with elevated SBP of 140-179 mmHg combined with ideal DBP experienced lower mortality compared to those with ideal SBP of 130-139 mmHg combined with low DBP.



Conclusions: Both low and high SBP and DBP are associated with increased mortality in patients with CKD. The optimal BP appears to be 130-139/80-89 mmHg. It may not be advantageous to lower SBP to ideal levels in patients whose DBP would decrease below ideal as a result.

Funding: NIDDK Support, Veterans Administration Support

FR-OR016

Analysis of Renal Histological Findings Associated with Blood Pressure in Ambulatory Blood Pressure Monitoring of CKD Patients

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Background: Recent studies have demonstrated that ambulatory blood pressure monitoring (ABPM) is useful in the prediction of long-term renal prognosis and cardiovascular events in CKD patients. Currently, however, information is limited regarding the relationship between individual renal pathological findings and blood pressure (BP) in the analyses of ABPM. We therefore aimed to determine renal pathological parameters associated with the findings of ABPM in CKD patients.

Methods: This study included a total of 93 CKD patients in whom both renal biopsy and ABPM were performed. Renal pathological parameters, including global glomerular sclerosis (GS), interstitial fibrosis/tubular atrophy (IF/TA), arterial lesion (AA) and arteriole lesion (AO) were scored (grade 0-2) and analyzed in relation to the findings of BP in ABPM.

Results: The IF/TA grade was significantly associated with the daytime systolic ($p<0.001$) and diastolic ($p=0.002$) BP and nighttime systolic ($p<0.001$) and diastolic ($p=0.002$) BP in ABPM. The AO grade was significantly associated with the daytime systolic ($p=0.011$) and diastolic ($p=0.013$) BP and nighttime diastolic BP ($p=0.039$), but did not associate with systolic BP. In contrast, the grades for GS and AA did not show statistically significant relationships with these ABPM findings. In the analysis of the patients without anti-hypertensive medications, IF/TA was the only pathological parameter significantly associated with the daytime systolic ($p=0.006$) and diastolic ($p=0.004$) BP and nighttime systolic ($p=0.017$) and diastolic ($p=0.033$) BP in ABPM. In the further multivariate analysis, IF/TA (≥25%) showed significant associations with both daytime and nighttime hypertension, which was independent of age, kidney function or use of anti-hypertensive medications. The IF/TA grade was not associated with daytime and nighttime BP ratio of ABPM in these CKD patients.

Conclusions: These results suggest that the severity of IF/TA is a renal pathological parameter that has close relationships to daytime and nighttime hypertension in CKD patients.

FR-OR017

Association of Central Blood Pressure with Kidney Histology in Living Kidney Donors

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Background: Most kidney transplant centers exclude prospective living kidney donors with hypertension from donation because of concerns that nephrectomy may increase any medical risks of hypertension after donation. It is unclear however, whether blood pressure (BP) is independently associated with kidney histology and function in living kidney donors. Markers of central BP, such as aortic stiffness and central wave reflection characteristics, may be better to assess target organ damage. Our objective was to assess association of central BP with kidney histology in living kidney donors.

Methods: 51 donors who donated a kidney between 2009 to 2011 had in-office brachial (peripheral) BP assessed by an automated sphygmomanometer, and central (aortic) BP assessed by the Sphygmocor device, in addition to implant kidney biopsy.

Histological abnormalities were defined as any one or more of the following: a) >5% global glomerulosclerosis, b) >5% interstitial fibrosis with any tubular atrophy, or c) any arteriosclerosis.

Results: Histological abnormalities were present in 19 (37.3%) donors, who were more likely to be men (94.7% vs. 37.5%, $p<0.01$) and older (47.9 ± 8.6 vs. 38.0 ± 10.1 years, $p<0.01$) with lower unadjusted iothalamate GFR (100.5 ± 14.2 vs. 112.6 ± 20.1 ml/min, $p<0.05$) than donors with normal histology. There was no difference in brachial systolic BP (SBP) between these 2 groups (118.1 ± 16.9 vs. 114.9 ± 11.0 mmHg, $p=0.43$). Donors with histological abnormalities had significantly higher aortic SBP (106.2 ± 13.1 vs. 96.7 ± 11.0 mmHg, $p<0.05$), aortic pulse pressure (30.3 ± 7.6 vs. 26.2 ± 5.6 mmHg, $p<0.05$) and augmentation pressure (6.5 ± 4.3 vs. 3.0 ± 4.0 mmHg, $p<0.01$) than donors with normal histology. After adjusting for age and gender, augmentation pressure was an independent risk factor for histological abnormalities (OR, 1.20; 95%CI, 1.03-1.38).

Conclusions: Aortic stiffness and abnormalities in central BP can be associated with histological abnormalities in the kidney. Central augmentation pressure is a parameter of target organ damage in living kidney donors.

FR-OR018

Progressive Renal Injury after Subtotal Nephrectomy (NX) Exhibits Stronger Correlation with BP Fluctuation Patterns during Sleep than Awake Periods

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Background: Clinical data suggest that nocturnal hypertension confers significant independent risk for CKD progression. The responsible mechanisms remain poorly defined. The hypothesis was tested that BP elevations during sleep may be more pathogenic than during active periods.

Methods: The relationship of radiotelemetrically monitored BP parameters during sleep (6AM – 6PM) vs. active (6PM – 6AM) periods with progressive glomerulosclerosis (GS) was examined in the rat 3/4 surgical excision nephrectomy (NX model) which exhibits impaired renal autoregulation and enhanced glomerular BP transmission. Two wks after NX, graded hypertension was superimposed by either substituting diets with 2 and 4% instead of standard 1% salt or by SQ phenylephrine (PE, 50 mg/kg/d, Alzet pumps) and blinded assessment of % GS was done after 4 more wks. Results: Mean±SE.

Results: Linear regression analysis (table) showed strong and significant correlations between BP parameters and % GS in both salt groups ($p < 0.025$) but not in the PE group indicating reduced glomerular BP transmission in PE rats. For most BP parameters and in each of the groups, better correlations and steeper slopes were observed during sleep vs. active periods.

	2% SALT DIET (n=19)		4% SALT DIET (n=11)		PE (n=17)	
% GS	7±2		18±4		7±2	
	Awake	Sleep	Awake	Sleep	Awake	Sleep
Average Systolic BP	151±3.3	144±3.1*	169±3.9	161±3.8*	157±3.5	154±3.5
Correlation with GS (r^2)	0.39	0.47	0.43	0.45	0.15	0.21
BP values > 175 mmHg (%)	14.7±3.5	9.3*±4.5	38.2±6.9	27.8*±6.1	27.5±4.2	25.1±3.9
Correlaton with GS (r^2)	0.47	0.56	0.35	0.43	0.06	0.07

*P < .001 compared to awake BP parameters

Conclusions: These results are consistent with the dependence of progressive GS after NX on glomerular transmission of BP fluctuations, which appears to occur to a greater extent during sleep than active periods and is likely reduced by α1 adrenoreceptor activation as would be expected during activity/exercise.

Funding: NIDDK Support, Veterans Administration Support

FR-OR019

Longterm Variability in Blood Pressure Predicts Outcome in Chronic Hemodialysis Patients

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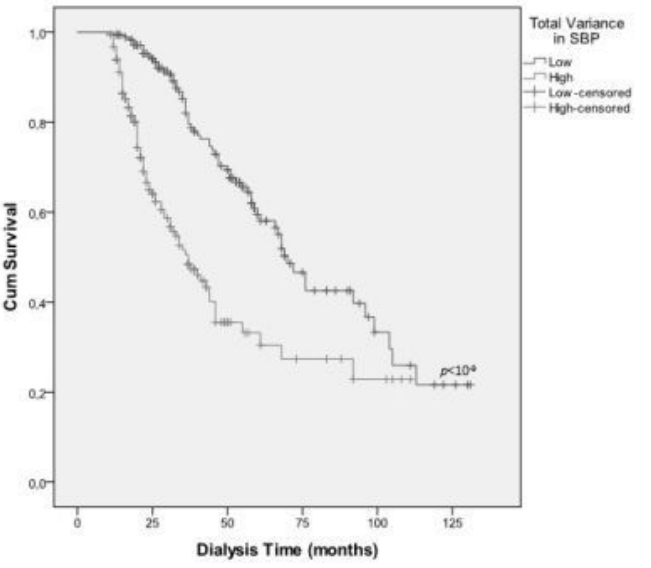
Background: Cardiovascular morbidity and mortality are significantly higher in hemodialysis patients than in the general population. Previous studies have shown that a high short-term variability in blood pressure is an adverse prognostic factor for survival in hemodialysis patients. We studied whether a high variation in long-term, i.e. monthly, systolic blood pressure leads to a higher mortality rate.

Methods: Blood pressure was recorded in every individual patient before and after each dialysis session. Mean values of all recordings of each month of the year were calculated and used for analysis. The differences in survival between 417 patients with either a low or high monthly variability in systolic blood pressure was compared with multivariate Cox survival analysis. Patient characteristics between the groups were compared with either the unpaired t-test or the Chi square-test.

Results: Patients with a high monthly variability for systolic blood pressure (SBP) showed a significantly higher all-cause mortality. Unadjusted hazard ratio for total mortality high vs. low variability in systolic blood pressure was 2.61 (95% CI 1.88-3.64), $p<10^{-9}$. Hazard ratio adjusted for confounders was 2.89 (95% CI 1.96-4.26), $p< 10^{-8}$. Survival in months in the groups low vs. high variability in systolic blood pressure were 78 (±4) vs. 52(±4) months, $p<10^{-9}$.



Kaplain Meier Curve of Survival: Low or High Variability in Systolic Blood Pressure

Conclusions: A high monthly variability in systolic blood pressure is an independent adverse prognostic factor in patients on maintenance hemodialysis. Further study is needed to elucidate the role of contributing factors such as seasonal variation in vascular tone or physical activity.

FR-OR020

Impaired Exercise-Induced Production of Nitric Oxide in Patients with Early Autosomal Dominant Polycystic Kidney Disease

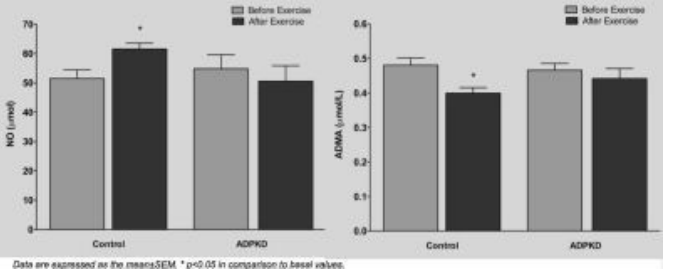
Natália Lopes Reinecke,[1] Thulio Marquez Cunha,[2] Elisa M.S. Higa,[1] Ita Pfeferman Heilberg,[1] Waldemar S. Almeida,[1] Nestor Schor.[1] *[1]Nephrology, UNIFESP, Sao Paulo, Brazil; [2]Pneumology, UNIFESP, Sao Paulo, Brazil.*

Background: It has been suggested that early Autosomal Dominant Polycystic Kidney Disease(ADPKD) patients may exhibit lower Nitric Oxide(NO) and higher Asymmetric Dimethylarginine(ADMA) levels in comparison to healthy subjects, what could lead to endothelial dysfunction and hypertension. It is known that physical exercise increases shear stress and NO. However,the effect of exercise in ADPKD remains unknown. We aimed to evaluate the influence of acute exercise on Blood Pressure(BP),production of NO and ADMA in early ADPKD.

Methods: Sedentary normotensive ADPKD patients (n=26;M/F=8/18; 29.8±7.7yr; CrCl=99.6±16.8ml/min) and healthy subjects(n=30;M/F=9/21;28.7±6.5yr) performed a maximum incremental cardiopulmonary test to determine the peak oxygen uptake(VO2peak). After, the subjects underwent to a 20min of moderate aerobic exercise, with BP measures and blood samples collected before and after for NO and ADMA measurements.

Results: ADPKD patients presented lower VO2peak and maximal workload, and higher mean exercise Systolic BP. Basal NO and ADMA did not differ between groups. However, NO did not increase and ADMA did not decrease after exercise in ADPKD, as observed in controls. Serum NO after exercise correlated with VO2peak (r=0.4,p<0.05).

Variable	Control	ADPKD	p
BMI	23.5±4.4	23.4±3.0	NS
VO2peak	28.8±6.2	22.5±3.5	<0.05
Maximal Workload	156.5±50.8	117.6±32.8	<0.05
Basal SBP	108.9±12	119.5±13.2	NS
Basal DBP	70.4±9	76.9±11.8	NS
Exercise SBP	143.5±12.2	160.6±19.4	<0.05
Exercise DBP	64.9±8.5	72.9±11	NS



Data are expressed as the mean±SEM. * p<0.05 in comparison to basal values.

Conclusions: This study suggests that patients with early ADPKD have an impaired regulation of exercise-related vasodilator system showed by a limited generation of NO and lack of ADMA decrease after exercise.

FR-OR021

Impact of Losartan and Hydrochlorothiazide Combination on Proteinuria in Patients with Chronic Kidney Disease and Hypertension: ILOHA Study Kiichiro Fujisaki,[1] Kazuhiko Tsuruya,[1] Hideki N. Hirakata,[2] Takanari Kitazono.[1] *[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Division of Nephrology, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan.*

Background: It remains unknown whether use of diuretics is more optimal than the other antihypertensive agents in CKD patients whose BP is still uncontrolled despite treatment with RAS inhibitors, because hydrochlorothiazide (HCTZ) showed more effective in reducing albuminuria than calcium channel blocker (CCB), while CCB reduced progression of CKD more effectively than HCTZ in such patients. In the present study, we assessed an additive effect of HCTZ in reducing proteinuria in CKD patients under treatment with losartan (LS).

Methods: We performed a multicenter, open-label randomized trial. One hundred and two CKD patients with hypertension and proteinuria were recruited from nine centers and randomly assigned to either treatment with LS (50 mg, n=51) or combination of LS and HCTZ (12.5 mg) (LS/HCTZ, n=51). The primary outcome was a decrease in the urinary protein to creatinine ratio (Up/Ucr). The target blood pressure (BP) was less than 130/80 mmHg, and antihypertensive agents (other than RAS inhibitors and diuretics) were added if target BP was not attained in the both groups. The primary population was the intent-to-treat population, defined as all randomly assigned patients.

Results: The baseline data of the two groups were similar. After a year of the treatment, the decrease in Up/Ucr was significantly lower in LS/HCTZ group than in LS group (-578±106 vs. -25±133 mg g^{-1}, P <0.05); however, there were no significant differences in BP and estimate GFR between the two groups through the observation period.

Conclusions: The combination therapy with LS/HCTZ showed a greater reduction in proteinuria compared to the treatment with LS even though BP in LS was similar to that in LS/HCTZ using additive antihypertensive agents through the observation period. This finding suggests that addition of diuretics is optimal treatment in CKD patients under treatment with RAS inhibitors and with LS/HCTZ exhibits renoprotective effects through a mechanism independent of BP reduction.

Funding: Pharmaceutical Company Support - Merck Sharp and Dohme

FR-OR023

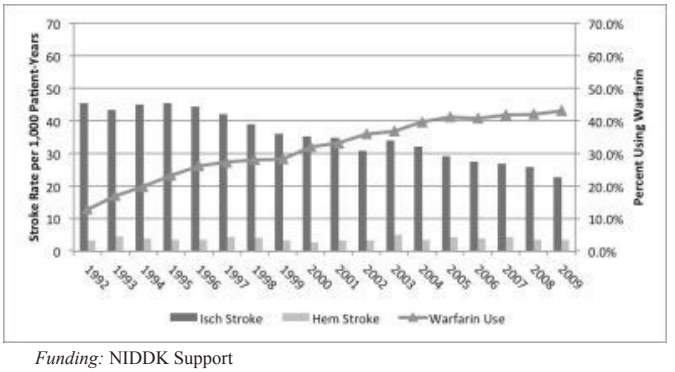
Atrial Fibrillation and Stroke in the U.S. Hemodialysis Population: 1992-2009 Charles A. Herzog,[1] Craig Solid,[1] Wolfgang C. Winkelmayer.[2] *[1]CVSSC, USRDS, MMRF, Minneapolis, MN; [2]Stanford Univ, Palo Alto, CA.*

Background: Non-valvular atrial fibrillation (AF) is a major cause of stroke in the elderly, in whom anticoagulation (AC) is of proven benefit. It is unclear if hemodialysis (HD) pts with AF should receive AC, as these pts were excluded from clinical trials. Observational studies suggest harm in HD AF pts receiving warfarin. In this study we analyze temporal trends in warfarin use and stroke rates in HD pts with AF over two decades.

Methods: We searched the USRDS database to identify prevalent HD pts in 1992-2009 with non-valvular AF. We used Medicare claims to count ischemic and hemorrhagic strokes after the AF diagnosis. We searched for Prothrombin-Time/INR claims (≥ 3 as a previously validated surrogate for warfarin use) to identify warfarin-treated pts. For each year's cohort, we calculated the rate of stroke events (per time at risk) and warfarin use within the cohort.

Results: The prevalence of non-valvular AF in the U.S. HD population grew from 5% in 1992 (n= 3,130; 53% male, 68% white, 30% black, mean age 67.6 yrs, primary cause of renal failure: 26% Diabetes, 39% HTN) to 11% in 2009 (n= 13,730; 58% male, 66% white, 30% black, mean age 68.6 yrs, primary cause of renal failure: 45% DM, 31% HTN). Ischemic stroke rates declined from 45.4 (per 1,000 pt-yrs) in 1992 to 22.7 in 2009, while hemorrhagic stroke rates remained stable (3.32 per 1,000 pt-yrs in 1992 vs. 3.37 in 2009). Warfarin use progressively increased from 12% to 43%.

Conclusions: Despite uncertainty regarding AC in HD pts with AF, progressively more HD AF pts have received warfarin therapy from 1992 to 2009 (mirroring trends in non-ESRD pts, but with lower utilization rates). The decline in ischemic stroke (with no change in hemorrhagic stroke) is temporally co-incident with increasing warfarin use. Clinical trials testing the safety and efficacy of AC for prevention of stroke in HD pts with AF are warranted.



Funding: NIDDK Support

FR-OR024

Anticoagulation, Anti-Platelet Therapy or No Treatment for Hemodialysis Patients with Atrial Fibrillation: A Decision Analysis Melanie Wyld,[1] Philip A. Clayton,[1,2] Rachael L. Morton,[1] Steven J. Chadban.[1,2] *[1]Sydney Medical School, University of Sydney, Sydney, NSW, Australia; [2]Department of Transplantation, Royal Prince Alfred Hospital, Sydney, NSW, Australia.*

Background: Optimal treatment of AF in the hemodialysis population is uncertain due to the exclusion of this group from randomized trials. The risk-benefit profile for anticoagulation and anti-platelet therapy in hemodialysis differs from the general population due to platelet dysfunction from uremia, altered pharmacokinetics and increased falls risk. We sought to determine whether anticoagulation or anti-platelet therapy is associated with improved outcomes for hemodialysis patients with atrial fibrillation (AF).

Methods: We developed a Markov model with a five-year time horizon to determine the preferred treatment (warfarin, aspirin, clopidogrel, no treatment) for hemodialysis patients with AF. Treatments were assessed by their expected utility (quality of life weight) and life years gained after factoring in the probability of stroke and bleeding events. Probability of events and utility data were obtained from published estimates. Outcomes were discounted at 5% per annum.

Results: In the base case (70 year-old-male on hemodialysis), the total health outcomes in life years and quality adjusted life years (QALYs) were 2.30 and 1.41 respectively for warfarin, 2.37 and 1.60 respectively for aspirin, 2.38 and 1.61 respectively for clopidogrel and 2.39 and 1.62 respectively for no treatment. The finding that warfarin had the lowest expected utility was robust to multiple one-way sensitivity analyses including the probability of bleeding, probability of stroke and relative risks of bleeding and stroke.

Conclusions: Our results suggest that hemodialysis patients should not be anticoagulated for AF as the risk of bleeding outweighs the potential benefit of stroke reduction. Further research is required to delineate the role, if any, of anticoagulation and anti-platelet agents in this patient population.

Funding: Government Support - Non-U.S.

FR-OR025

Spironolactone Reduces Cardio- and Cerebrovascular Morbidity and Mortality in Hemodialysis Patients Yoshihiro Matsumoto,[1] Shinji Kageyama,[2] Yasuo Mori,[3] Toru Yakushigawa,[3] Kazuo Arihara.[4] *[1]Shizuoka City Hospital; [2]Kageyama Clinic; [3]Shibukawa Clinic; [4]Ohtemachi Clinic.*

Background: Aldosterone receptor blockers reduce cardiac-related morbidity and mortality. Recently, we demonstrated that long-term low-dose spironolactone is clinically safe in many hemodialysis (HD) patients. In the present study, we assessed whether low-dose spironolactone treatment reduces the high incidence of cardio- and cerebrovascular (CCV) morbidity and mortality in HD patients.

Methods: A 3-year randomized trial involving 5 Japanese clinics was performed. Oligoanuric HD patients (n=309) were enrolled and 157 patients were randomly assigned to receive 25 mg of spironolactone daily without any restriction in dietary potassium intake. The primary end point was CCV events, and secondary end point was death from all causes. CCV events included stroke, myocardial infarction, angina pectoris, exacerbation of heart failure, cardiovascular surgery, dissecting aneurysm of the aorta, and sudden death.

Results: At 3 years, spironolactone significantly reduced CCV events (7.1% vs. 17.8%; P=0.0105) and all-cause mortality (7.9% vs. 22.9%; P=0.0011). With Cox proportional hazards analysis, spironolactone was an independent determinant of CCV events (hazard ratio [HR]: 0.33; 95% confidence interval [CI]: 0.14 to 0.71; P=0.0038) and all-cause mortality (HR: 0.33; 95% CI: 014 to 0.71; P=0.0015). Gynecomastia or breast pain was reported in 15 patients (9.6%) in the spironolactone group. Serious hyperkalemia led to discontinuation of treatment in 3 patients (1.9%).

Conclusions: Aldosterone receptor blockade by spironolactone substantially reduces the risk of both CCV morbidity and death among HD patients.

FR-OR026

Extracellular Volume Control in Dialysis Patients to Reduce Hospitalizations Thomas F. Parker,[1] Raymond M. Hakim,[2] Allen R. Nissenson,[3] Mahesh Krishnan,[4] Franklin W. Maddux,[5] Kevin Chan.[5] *[1]Renal Ventures Management; [2]Vanderbilt University School of Medicine; [3]DaVita, Inc; [4]DaVita Clinical Research; [5]Fresenius Medical Care.*

Background: Extracellular volume (ECV) overload is a leading cause of hospitalizations in chronic hemodialysis (HD) patients. Congestive heart failure, pulmonary edema and left ventricular failure hospitalizations far exceed other causes; yet objective measurement of ECV is not standard in HD care. Renal Ventures Management, DaVita, Inc and Fresenius Medical Care combined resources in a self funded quality initiative (QI) to determine if objective measurement of ECV removal and attainment of normalized ECV could reduce all cause and ECV related hospitalizations.

Methods: 14 facilities were randomly selected for either Education (E) or education + ECV monitoring (EM). In the EM facilities, ultrafiltration monitoring and assessment of normalized ECV - "dry weight" - was accomplished with a monitoring device (Critline). In the E group, clinical assessment used ultrafiltration algorithm to achieve "dry weight".

Results:
Hospitalization in facilities with Fluid Management Education or ECV monitoring devices

	Before ECV monitoring device	After ECV monitoring device	Before education	After education
N (patients)	571	587	625	644
hospital admits (per patient year)	2.03	1.55	1.83	1.60
hospital days (per patient year)	11.18	9.38	10.18	9.94
Fluid admits (per patient year)	0.18	0.09	0.14	0.31
Fluid days (per patient year)	0.75	0.16	0.68	0.82

before intervention: 9/4/10-9/4/11; after intervention: 9/4/11-3/4/12

Table 1 summarizes outcomes. Before E and EM implementation, all-cause and ECV related hospital rates were similar between the two groups. After E implementation, there was an actual increase in ECV hospitalizations and days, whereas, after EM implementation, there was a 50% decrease in ECV hospitalizations and and 78% decrease in ECV hospital days.

Conclusions: In this QI, among 3 major providers, objective measurement of ECV during dialysis is associated with significant decrease in ECV related hospitalizations, substantially more than education alone. Such processes hold promise for patient and provider outcomes.

Funding: Pharmaceutical Company Support - Renal Ventures Management, DaVita, Fresenius Medical Care

FR-OR027

The Impact of a Low Glucose Peritoneal Dialysis Solution Regimen on Serum Lipids and Lipoproteins in Diabetic Patients: The IMPENDIA and EDEN Randomized, Controlled Clinical Trials

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Background: The use of glucose-containing peritoneal dialysis (PD) solutions may exacerbate lipid and lipoprotein abnormalities in diabetic PD patients. We hypothesized that a low glucose PD regimen incorporating icodextrin and amino acids would lead to improvements in serum lipids and lipoproteins.

Methods: Two randomized controlled clinical trials were completed in 11 countries across 4 continents. In both studies, prevalent diabetic PD patients were randomized in a 1:1 manner to the intervention group (a low glucose PD regimen consisting of either the combination of Physioneal, Extraneal, and Nutrineal in the IMPENDIA trial, or Dianeal, Extraneal, and Nutrineal in the EDEN trial) or the control group (Dianeal only) and followed for up to 6 months after randomization. We previously reported the results of the primary efficacy and safety analyses. We now report the comparison of change from baseline between groups for serum lipids and lipoproteins (secondary endpoints).

Results: 251 PD patients were allocated to the intervention (n=124) or control groups (n=127). The difference between the intervention and control groups for change from baseline for the measured lipids and lipoproteins is shown in the table.

	Difference Between Groups (Change in Control - Change in Intervention)			
Parameter	Estimate	Lower 95% CI	Upper 95% CI	P-Value
Total cholesterol, mmol/L	0.3	0.0	0.7	0.07
LDL cholesterol, mmol/L	0.1	-0.2	0.4	0.59
HDL cholesterol, mmol/L	0.0	-0.1	0.0	0.30
VLDL cholesterol, mmol/L	0.3	0.1	0.5	0.003
Serum triglycerides, mmol/L	0.7	0.3	1.1	0.002
Apolipoprotein B, mg/dl	8.4	0.8	15.9	0.03
Lipoprotein (a), mg/dl	-1.9	-8.2	4.4	0.56
Apolipoprotein A1, mg/dl	5.0	-1.5	11.5	0.13

Conclusions: In diabetic PD patients, a low glucose PD solution regimen significantly improves serum triglycerides, VLDL-cholesterol, and apolipoprotein B.

Funding: Pharmaceutical Company Support - Baxter Healthcare

FR-OR028

A Randomized, Controlled Trial of Colestilan versus Simvastatin for Treatment of Dyslipidemia in Hemodialysis Patients

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Background: To demonstrate superiority of the new phosphate binder, colestilan (COL) over placebo and non-inferiority with simvastatin (SIM) in reducing serum LDL-cholesterol (LDL-C) in CKD 5D subjects.

Methods: Global, multi-centre, double-blind, double-dummy, randomised, flexible-dose, comparative study. Comprising: 2 to 6-week wash-out from lipid-modifying agents; subjects with LDL-C ≥100 mg/dL randomised 1:1 to COL (3, 6, 9 or 12g/day) or SIM (10, 20, 30 or 40 mg/day) with double-dummy design and matching placebos; titrated up (if LDL-C ≥70mg/dL) or down (if tolerability was an issue) as needed to control LDL-C, every 4 weeks for 16 weeks, followed by re-randomisation to active (COL or SIM) or placebo and a 4-week placebo-withdrawal phase.

Results: 260 subjects were randomised (127 to COL and 133 to SIM). At end of double-blind, double-dummy titration phase both COL and SIM reduced LDL-C by similar amounts (-29.5 and -28.9% respectively). There was no real change in HDL-C levels. At end of the placebo-withdrawal phase, COL and SIM both reduced LDL-C significantly versus placebo by 30.7 mg/dL and 36.1 mg/dL, respectively (percentage reductions versus placebo of 37.4 and 45.8% respectively; P<0.001 for both groups) whilst HDL-C was not essentially altered. Triglyceride levels were not significantly changed by COL but lowered by SIM (P<0.001). Both SIM and COL similarly reduced serum TC, oxidised LDL and apoprotein B levels. Also, COL reduced serum phosphate levels during the 16-week titration period, whilst SIM did not (P<0.01 between groups). The safety profile of COL was comparable to SIM, with discontinuations due to AEs mainly due to GI-related issues.

Conclusions: In subjects with CKD Stage 5D, COL effectively reduces LDL-C levels versus placebo and was non-inferior to SIM in reducing LDL-C during the active comparison phase thus achieving co-primary endpoints of the study.

Funding: Pharmaceutical Company Support - Mitsubishi Tanabe Pharmaceutical Corporation

FR-OR029

Systolic Blood Pressure (SBP), Mortality and Cardiovascular Disease (CVD) Outcomes in Incident Hemodialysis (HD) Patients: Role of Troponin I and NT-proBNP

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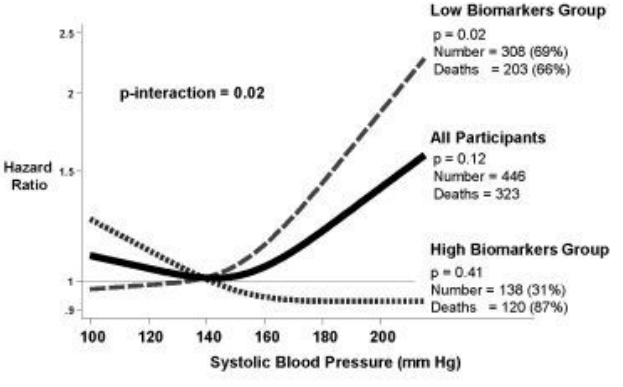
Background: There is uncertainty regarding treatment of hypertension in HD patients due to the observed "U" shaped association between blood pressure (BP) and death. This association likely reflects confounding due to CVD and its associated short-term mortality. We hypothesized that elevated levels of serum troponin I (TNI) and NT-proBNP may identify HD patients with higher risk of death and therefore less long-term benefit from BP reduction.

Methods: We measured TNI and NT-proBNP in baseline samples from 446 incident HD participants of the CHOICE Study, a national prospective cohort study. Presence of elevated cardiac biomarkers was defined as TNI ≥ 0.1 ng/mL or NT-proBNP ≥ 9,252 pg/mL, based on published cutoffs. Primary exposure was baseline predialysis SBP. Outcomes were mortality (overall and CVD) and first CVD event analyzed using Cox regression adjusted for age, sex, race and serum albumin.

Results: Mean age was 58 years; 64% white; 55% male; and mean SBP was 153±25 mmHg. Of the 446 participants, 138 (31%) were in the high biomarker group. There were 323 deaths during follow-up (median 3.6 yrs). High biomarker levels were independently associated with risk of death (HR, 1.84; p<0.001). Overall, SBP had a "U" shaped association with death. There was no association between SBP and death in the high biomarker group (p=0.41), but in the low biomarker group, SBP ≥160 mmHg was associated with an increased risk of death (HR per 10 mmHg increase, 1.15; p=0.03). Similar trends were seen with CVD deaths and events.

Figure: Adjusted Association between Predialysis Systolic Blood Pressure and All-Cause Mortality Stratified by Biomarker Levels



Conclusions: A stratification approach based on TNI and NT-proBNP has the potential to inform BP treatment in HD patients, distinguishing which patients are at increased risk of outcomes with uncontrolled SBP.

Funding: NIDDK Support

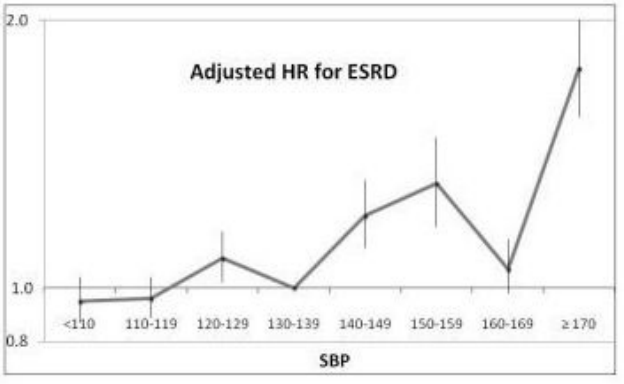
FR-OR030

Evaluating Ranges of Blood Pressure (BP) and Risk of End Stage Renal Disease (ESRD) in a Large Ethnically Diverse Hypertensive (HTN) Population Simran K. Bhandari,[1] Jiaxiao Shi,[1] Kristi Reynolds,[1] Dean A. Kujubu,[1] Kamyar Kalantar-Zadeh,[2] John J. Sim.[1] *[1]Nephrology & Hypertension, Kaiser Permanente Los Angeles Medical Center, Los Angeles, CA; [2]Nephrology & Hypertension, Harbor UCLA Medical Center, Torrance, CA.*

Background: HTN is a risk for ESRD particularly in those with CKD. The differential impact of blood pressure (BP) and ESRD risk are not well known. We sought to evaluate the association of BP ranges and ESRD incidence in a large HTN population.

Methods: Kaiser Permanente Southern California members age≥18 yrs (1/1/06-12/31/07) with HTN and min 4 months continuous follow-up evaluated. Demographics, medications, comorbidities, and outcomes data were retrieved from electronic medical records. Primary outcome was incident ESRD defined as need for dialysis or renal transplant. Cox proportional regression models used to calculate hazard ratios (HR) for different BP indexes in 10mmHg increments (SBP reference 130-139 and DBP 80-89) adjusted for age, gender, race, eGFR, diabetes, and cardiovascular disease.

Results: 470,988 subjects were identified (mean age, 65 yrs; mean BP, 133/75; 54% female; 21% Hispanic, 12% black). Mean eGFR was 71 ml/min and 8% had eGFR < 60. Over follow-up of 3.0 yrs, 13,034 (2.8%) progressed to ESRD. The lowest rates occurred in SBP 130-139 and DBP 90-100. Compared to SBP 130-139 multivariable HR (95% CI) for ESRD were 0.95 (0.87-1.04), 0.96 (0.89-1.04), 1.11 (1.02-1.21), 1.27 (1.15-1.40), 1.39 (1.23-1.56), 1.07 (0.98-1.18), and 1.82 (1.64-2.02) for SBP ranges <110, 110-119, 120-129, 140-149, 150-159, 160-169, and ≥170 respectively. DBP was not associated with ESRD risk.



Conclusions: In a large ethnically diverse HTN population primarily without CKD, we found a strong, graded linear association between SBP>140 and risk for ESRD. Whether aggressive BP treatment similarly protects against ESRD outcomes is yet to be determined.

FR-OR031

Impact of Cardiac Valvular Calcification at Beginning Hemodialysis Therapy on Cardiovascular Outcome in Patients with End-Stage Renal Disease Hirotake Kasuga,[1] Ryo Takahashi,[1] Chieko Matsubara,[1] Keiko Kimura,[1] Kyoko Kikuchi,[1] Yasuhiko Ito.[2] *[1]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Nephrology, Nagoya University Graduated School of Medicine, Nagoya, Japan.*

Background: Cardiac valvular calcification is highly prevalent in patients with end-stage renal disease (ESRD), and its presence may be potentially linked with the increasing risk of systemic atherosclerotic events. We investigated whether the presence of calcified valve at just beginning of hemodialysis (HD) therapy could predict individual cardiovascular (CV) events in ESRD patients.

Methods: A total of 1785 consecutive ESRD patients were screened by echocardiography at beginning of HD therapy. Calcification was defined as bright echoes >1mm on one or more cusps of the cardiac valve. They were divided into three groups; those without valve calcification (group 0, n=783), those with calcification in a single (aortic or mitral) valve (group 1, n=651) and those with calcification in both valves (group 2, n=351), and were followed up for up to 10 years.

Results: During follow-up period (median 55months),687 CV events [358 cardiac events (20.1%), 153 cerebrovascular events (8.6%) and 176 peripheral arterial events (9.9%)] occurred, and 536 patients (30.0%) died including 234 CV death (13.1%). In the group 0, 1 and 2, 10-year event-free rates were 67.6%, 58.6% and 40.9% for cardiac events (p<0.0001), 85.0%, 84.9% and 72.4% for cerebrovascular events (p=0.0008), 83.4%, 82.9% and 70.5% for peripheral arterial events (p<0.0001), and 56.6%, 48.0% and 29.1% for composite endpoint of CV events (p<0.0001), respectively. Similarly, survival rates were 82.8%, 77.1% and 62.6% for CV mortality, and 66.3%, 54.5% and 39.8% for all-cause mortality, respectively (p<0.0001 in both). Even after adjustment, valvular calcification was an independent predictor for all endpoints.

Conclusions: The presence of calcified valves at beginning of HD therapy could predict not only cardiac events but also cerebrovascular and peripheral artery events, and might be associated with systemic atherosclerotic events in ESRD patients.

FR-OR032

Increased Nox5 Activity Induces Podocyte Damage and Filtration Barrier Dysfunction Chet E. Holterman,[3] Chelsea Towaij,[3] Mark E. Cooper,[1] Rhian Touyz,[2] Chris R. Kennedy.[3] *[1]Baker IDI Heart & Diabetes Institute, Australia; [2]University of Glasgow, United Kingdom; [3]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Canada.*

Background: Reactive oxygen species (ROS) play a key role in glomerular filtration barrier damage. As part of this barrier podocytes are particularly sensitive to ROS. NADPH oxidase (Nox) enzymes are a major source of ROS and their role in the kidney has been well studied. However a paucity of information regarding the role of Nox5 in the kidney remains partly due to its absence from mouse and rat genomes. Here we establish a previously unappreciated role for Nox5 in podocyte damage and diabetic kidney diease in both humans and transgenic mouse models.

Methods: Human diabetic and control kidney biopsies were examined for Nox5 by immunofluorescence. Immortalized human podocytes (hPOD) were stimulated with TGFb, AngII, stretch, or high glucose and Nox5 expression and activity were examined. The effect of Nox5 knockdown via siRNA was also studied. Similar experiments were performed on mouse podocytes (mPOD) infected with Nox5 adenovirus. Transgenic mice ($Nox5^{Pod+}$) expressing Nox5 specifically in podocytes were generated and characterized.

Results: Biopsies from diabetic individuals had higher immunodetectable Nox5 expression in glomerular structures. Stimulation of podocytes with TGFb or AngII induced Nox5 expression and activity. Inhibition of Nox5 via siRNA blunted ROS production in response to these stimuli. Increased Nox5 ROS production altered podocyte actin cytoskeleton, resulted in a more motile phenotype, and increased expression of markers of epithelial to mesenchymal transition. $Nox5^{Pod+}$ mice displayed increased albumin to creatinine ratios as early as 4 weeks following birth. Kidney weight to body weight was increased in $Nox5^{Pod+}$ compared to non-transgenic littermates indicative of kidney hypertrophy. QPCR on mRNA from kidney cortex demonstrated elevated Cox2 expression and decreased nephrin expression in $Nox5^{Pod+}$ compared to non-transgenic littermates.

Conclusions: Upregulation of Nox5 in diabetic kidney occurs in response to classic diabetic stimuli and contributes to ROS–induced podocyte damage and filtration barrier dysfunction.

FR-OR033

Heat Shock Protein 70/CHIP/Nox4 NAD(P)H Oxidase Interaction in the Antioxidative Effect of Losartan on Proximal Tubule Cells from Spontaneously Hypertensive Rats (SHR) Patricia G. Vallés,[1] Andrea Gil Lorenzo,[2] Victoria Bocanegra.[2] *[1]Notti Pediatric Hospital; [2]CONICET.*

Background: Full expression of Angiotensin II signaling is dependent on the reactive oxygen species derived from NAD(P)H oxidase and the dynamic association of the Angiotensin II Type I receptor (AT_1R) with caveolae/lipid rafts.The chaperone Hsp70 regulates a diverse set of signaling pathways.CHIP (Carboxyl terminus of the Hsc70-Interacting Protein) is a E3 ubiquitin ligase that targets proteins for polyubiquitination and degradation.

We investigated Hsp70/CHIP contribution to the regulation of Nox4 after AT_1R receptor blockade,in primary culture of proximal tubule epithelial cells (PTCs) from SHR.

Methods: PTCs from 8 week SHR and WKY rats were stimulated with Angiotensin II (100 nmol/L) for15 min (AII),pretreated with Losartan (100 μmol/L) 90 min (L) and with Losartan75 min plus Angiotensin15 min(L+AII). Interaction and localization of Nox4, Hsp70 and CHIP were determined by immunoprecipitation and immunofluorescence confocal microscopy. PTCs transfection with p-SIREN RetroQ shHsp72 plasmid vector to knockdown Hsp72 or control empty vector was performed.

Results: Whereas SHR PTCs exposure to Angiotensin II downregulated membrane Caveolin-1 expression and overexpressed Nox4 NAD(P)H-oxidase,Losartan increased Caveolin-1expression, increased Hsp70 and decreased Nox4 protein levels in SHR (L) PTC membranes. Decreased Hsp70 in SHR(L) vs SHR(AII) in cytosolic fraction confirm Hsp70 translocation to PTC membranes.No differences were shown in Nox4 gene expression among groups. Increased levels ofHsp7/CHIP contrasts with the decreased immunoprecipitation of Nox4 in membrane from PTCs SHR(L)vs SHR(AII).To validate these results, knockdown of Hsp72 in PTCs was associated with higher Nox4 expression and increased NAD(P)H oxidase activity in SHR (L+AII) related to SHR (L+AII) without transfection.After Hsp72 silencing of PTCs from SHR (AII), Losartan could not prevent Angiotensin II-enhanced Nox 4 expression and NAD(P)H oxidase activity.

Conclusions: Membrane interaction of Hsp70/CHIP may induce Nox4 protein degradation, which could be involved in the cytoprotective effect of Losartan in PCTs from SHR.

FR-OR034

Unique Role of NADPH Oxidase 5 in Oxidative Stress in Renal Proximal Tubule Cells from Humans with Essential Hypertension Peiying Yu,[1] Van Anthony M. Villar,[2] Yu Yang,[3] Robin Allen Felder,[4] Pedro A. Jose.[5] *[1]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD; [2]Clinical Chemistry, University of Virginia, Charlottesville, VA.*

Background: NADPH oxidases (Nox) are the major sources of reactive oxygen species in the kidney. Nox1, Nox2, and Nox4, but not Nox3, are expressed in renal proximal tubule (RPT) cells from rodents and human kidneys. However, NADPH oxidase 5 (*NOX5)* gene is present in humans but not rodents. We tested the hypothesis that *NOX5* is expressed in human RPT cells but the expression is different between normotensive (NT) and hypertensive subjects (HT).

Methods: In addition to cell culture, cell treatment, Biotinylation, immunoprecipitation and immunoblotting, we have used four methods to measure NADPH oxidase activity and/or ROS production in RPT cells.

Results: *NOX5* mRNA expression (quantitative real-time RT-PCR) is 4.2±0.68-fold greater in HT than NT ($P<0.01$, n=4). Total protein expression of Nox5, but not Nox1, Nox2, or Nox4, quantified by immunoblotting, is 5.2 ± 0.65-fold greater in HT than NT. Apical membrane Nox5 is also 2.75 ± 0.46-fold greater in HT than NT. Basal total Nox activity is 4.13±0.26-fold greater in HT (n=4) than NT (n=3). Basal Nox5 activity is also 2.23±0.079-fold greater in HT than NT ($P<0.05$, n=6). Silencing of *NOX5* using *NOX5*-specific siRNA decreases Nox activity (54.87±3.21%, vs. control-siRNA, n=6-8) in HT but not NT. D_1 receptor stimulation with fenoldopam decreases Nox activity to a greater extent in NT (-32.4±1.6) than HT (-14.7±2.1). We demonstrate for the first time that of NOX5 is expressed to a greater extent in RPT cells from HT than NT and may be responsible for the increased oxidative stress in RPT cells from HT.

Conclusions: In conclusion, we report for the first time that Nox5 is the predominant Nox isoform expressed in human RPT cells and its expression and activity are increased in humans with essential hypertension. Thus, increased Nox5 expression and activity may contribute to the increased oxidative stress in human essential hypertension.

Funding: NIDDK Support, Other NIH Support - HL023081, HL074940, DK039308, HL092196, HL068686, and RR020185

FR-OR035

Essential Roles of Ghrelin in the Maintenance of Oxidative Stress Levels in the Renal Tubular Tissues by Mitochondria-Dependent Mechanisms

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Background: Recent study revealed that growth hormone secretagogue Ghrelin(Ghr) exerts renal protective effects. In this study we explored its renal protective effetcs by mitochondria(Mit)-related mechanism.

Methods: Renal tissue damages were induced by infusion of AngII in mice with osmotic mini-pump. Ghr was administered by the daily intraperitoneal injection. 8 weeks after the treatment, mice were sacrificed. In *in vitro* experiment, renal tubular HK-2 cells expressing the receptors for both Ghr and AngII, were used. Growth hormone secretagogue receptor(GHSR) null mice were utilized for the evaluation of the role of endogenous Ghr.

Results: AngII infusion raised blood pressure, which was lowered by Ghr. AngII infusion also induced renal damages as assayed by the urinary excretion of renal tubular marker, which were ameliorated by Ghr but not by blood pressure lowering by hydraladine. AngII also increased urinary protein excretion, which was attenuated both by Ghr and by hydraladine. AngII induces tissue oxidative stress, senescence,and interstitial fibrosis. These changes were attenuated by Ghr independently by its blood pressure-lowering effects. In the kidney of Ghr-treated mice, the expressions of Mit uncoupling protein UCP2 and PPAR-γ coactivator(PGC-1α) increased and Mit number increased. In HK-2 cells, Ghr increased UCP2 expression, reduced Mit membrane potential and Mit-derived ROS and increased Mit number.Transfectionn of siRNA for UCP2 negated the anti-oxidative effects by Ghr. Finally, GHSR null mice were presented with higher serum creatinine levels, renal protein excretion,more severe renal tubular damage, renal ROS levels, SA β-Gal staining levels as compared with those of wild-type littermates. Mit in proximal tubulus of GHSR null mice were elongated and less in number as compared with those of WT mice.

Conclusions: Our data indicated that Ghr suppressed AngII-induced renal damages by reducing the Mit-derived ROS production and Mit number through UCP2-dependent mechanisms. Endogenous Ghr/GHSR systems were essential for the maintenance ROS levels of renal tubular cells and these functions.

Funding: Government Support - Non-U.S.

FR-OR036

HVCN1 Is Required for Superoxide Production in Medullary Thick Ascending Limb in Response to H^+ Efflux

Paul O'Connor, Jingping Sun. *Experimental Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: We have previously reported that H^+ efflux stimulates superoxide (O_2^-) production in the medullary thick ascending limb of the loop of Henle (mTAL) in rats by activation of an as yet unidentified H^+ transport pathway. As HVCN1, a voltage gated H^+ channel associated with NADPH oxidase in immune cells, is also expressed in mTAL, we hypothesized that HVCN1 is responsible for H^+ efflux induced production of O_2^- in mTAL.

Methods: In order to test this hypothesis we utilized HVCN1$^{-/-}$ mice and wild type litter mates obtained from KOMP. To confirm that HVCN1 was functionally knocked out in HVCN1$^{-/-}$ mice we stimulated the respiratory burst using PMA (100mM) in peritoneal macrophages extracted from HVCN1$^{-/-}$ and WT mice and determined maximal O_2^- production using L-012 luminescence. mTAL were isolated from the inner stripe of the outer-medulla by micro-dissection and loaded for 60 min with the O_2^- sensitive dye dihydroethidium (DHE). Live mTAL were then imaged on a heated chamber attached to a fluorescent microscope and the ratio of ethidium (Eth) to DHE quantified using metafluor imaging software as an index of O_2^- production. O_2^- producing H^+ currents were isolated in 0 Na^+, 100mM $BaCl_2$ media and cellular H^+ efflux stimulated using an NH_4Cl (20mM) prepulse.

Results: Maximal O_2^- production during the respiratory burst was significantly lower in macrophages from HVCN1$^{-/-}$ mice (23±4AU; n=6) compared to WT mice (92±3AU; n=5; $p<0.05$), confirming loss of HVCN1 function. Following removal of NH_4Cl from the bath there was a significant increase in O_2^- production in mTAL from WT mice (Delta slope Eth/DHE 15.9±3.6 AU/sec; $p<0.02$ (n=5)) but not HVCN1$^{-/-}$ mice (Delta slope4.7±9.6; P=0.64 (n=6)). The response of WT mice was significantly greater than that of HVCN1$^{-/-}$ mice ($P<0.001$).

Conclusions: We conclude that HVCN1 is required for maximal O_2^- production in mTAL in response to H^+ efflux. These data suggest that HVCN1 may be a novel target to prevent renal oxidative stress.

Funding: Other NIH Support - American Heart Association.

FR-OR038

High Glucose Induces eNOS Internalization and Dysfunction in Glomerular Endothelial Cells

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Background: Glomerular endothelial cells (GEnCs) play a crucial role in the pathogenesis of diabetic nephropathy, and endothelial nitric oxide synthase (eNOS) is an important modulator of their function. We have previously shown that high glucose impairs eNOS activity, but the underlying molecular mechanisms have not been completely elucidated.

Methods: To mimic the *in vivo* environment, we utilized conditionally immortalized GEnCs cultured in either normal or high glucose (HG) medium.

Results: HG (30mM) medium progressively increased GEnC permeability to BSA and induced apoptosis (0.7±0.2% in normal medium vs. 5.1±0.7% in HG, n=4, $P<0.05$ at 48 hrs) as well as stimulating GEnC proliferation at early stages(24-48 hrs). HG significantly decreased eNOS dimerization (measured by the ratio of dimer/monomer) and decreased phosphorylation at Ser 1179 (an essential step in eNOS activation), while the levels of eNOS monomers and phosphorylation at Thr 497 remained unchanged. In normal growth medium, eNOS was predominantly localized to the membrane of GEnCs, and caveolin-1 (Cav-1) was also abundant at the cell surface. Immunofluorescence indicated that HG induced eNOS translocation to the cytoplasm, with co-localization with Cav-1. Immunoblotting confirmed decreased plasma membrane and increased cytosolic eNOS. In addition, co-immunoprecipitation studies indicated decreases in eNOS associated with Cav-1 at the plasma membrane and increases in cytosol. Taking together, these results suggested that HG stimulates eNOS translocation into the cytoplasm without significant dissociation from Cav-1. HG markedly reduced eNOS activity in GEnCs, measured by nitrate/nitrite production (0.53±0.07 fold control, n=4, $P<0.05$) and also reduced production of the downstream second messenger, cGMP (from 4.5±0.6 to 0.5±0.2 pmol/mg pro., n=4, $p<0.05$).

Conclusions: HG induced eNOS dysfunction in GEnCs via multiple mechanisms: eNOS uncoupling, defective phosphorylation of at Ser 1179 and promoting its translocation from plasma membrane to cytoplasm.

Funding: NIDDK Support

FR-OR039

Renal Tubular-Specific LKB1 Deletion Results in Severe Kidney Damage

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Background: The Lkb1 (Stk11) gene encodes a serine/threonine kinase. Mice with targeted Lkb1 mutations have shown the in vivo role of Lkb1 in the regulation of metabolism, cell growth, polarity and the cytoskeleton. The kidney contains highly differentiated and polarized cells, and we hypothesize that LKB1 must play a role in the development and maintenance of the structure of these different cell types as well as the regulation of cellular metabolism via undetermined downstream pathways. The aim of our study is to describe the effect of LKB1 deletion in renal tubular cells and the signaling pathways involved in the development of the LKB1 deficient phenotype.

Methods: Tubule-specific LKB1 knock out mice were developed using the Cre/LoxP system of gene splicing. The $Ksp^{Cre}/Stk^{Flox/Flox}$ mice (using the Cadherin 16 cre/Ksp cre mice) lack the Lkb1 gene in the collecting ducts and thick ascending limbs of Henle's loops. The $Pax8^{rtTA}/TRE^{Cre}/Stk^{Flox/Flox}$ mice are inducible transgenic mice that exhibit deletion of Lkb1 in the entire renal tubular system when exposed to doxycycline. We have assessed the renal phenotype of these mice at 5, 14 and 27 weeks and compared it to age-matched controls, using microscopy, RT-PCR, WB and immunohistochemistry.

Results: Kidneys from mice with tubule-specific LKB1 deletion show a strong phenotype at 27 weeks of age. They are grossly enlarged. On light microscopy, the normal renal architecture is lost and the kidney had a fibrotic appearance. The renal tubules in the medulla and inner cortex are largely dilated, while the glomeruli remain intact. Earlier lesions, at 14 weeks of age were consistent with unusual cuboidal appearance of the renal epithelial cells. These changes are not observed in the 5 week-old mice, and are very subtle in the 14 week-old mice, indicating that the damage occurs over time.

Conclusions: Renal tubular-specific LKB1 deletion results in severe damage. Experiments are ongoing to determine the mechanism underlying this damage and pathways involved in LKB1 signaling in the mice kidney.

FR-OR040

A Signal Relay from Transforming Growth Factor beta (TGFb): Stimulated Smad 3 to mTOR Involves Deptor Suppression to Force Mesangial Cell (MC) Hypertrophy Falguni Das,[1] Nandini Ghosh-choudhury,[2] Nirmalya Dey,[1] Hanna E. Abboud,[1] Balakuntalam S. Kasinath,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

Background: The ominous features of TGFb action in kidney consist of altered glomerular hemodynamics, whole kidney hypertrophy including glomerular hypertrophy and matrix synthesis. TGFb receptor activates Smad 3 and mTOR to force these effects. mTOR forms two kinase complexes, mTORC1 and mTORC2 that contain common and distinct proteins. One such common protein, deptor, is an endogenous inhibitor of both kinases. The role of deptor in TGFb-induced signal transduction is not known.

Methods: Human glomerular MCs were used.

Results: Incubation of MCs with TGFb rapidly increased mTORC1 and mTORC2 activities. However, extended incubation of MCs with TGFb significantly reduced the levels of deptor, resulting in increased activity of both kinase complexes. Prolonged incubation with TGFb was necessary for dissociation of deptor from mTOR. Using ATP competitive mTOR inhibitor PP242, we showed that both rapid and sustained activation of mTORC1/2 were required for deptor downregulation and 4EBP-1 translation repressor phosphorylation. Furthermore, PP242-induced reversal of deptor expression was associated with significant inhibition of TGFb-induced MC protein synthesis and hypertrophy. Interestingly, expression of Smad 7, an endogenous inhibitor of Smad 3 signaling, blocked TGFb-induced suppression of deptor and mTORC1/2 activities. Moreover, over-expression of Smad 3 was sufficient to reduce deptor expression and increase in mTORC1/2 activities, similar to TGFb stimulation. Finally, shRNA-mediated knockdown of deptor significantly reversed Smad 7-induced suppression of TGFb-stimulated protein synthesis and hypertrophy of MC.

Conclusions: Together our data provide the first evidence for the requirement of both early and late activation of mTOR complexes for TGFb-induced protein synthesis. These data reveal the presence of a Smad 3-dependent feed forward loop between deptor suppression and mTORC1/2 activation to drive MC hypertrophy.

Funding: NIDDK Support, Veterans Administration Support

FR-OR042

Podocyte Structural Parameters Do Not Predict Progression to Diabetic Nephropathy (DN) in Normoalbuminuric (NA) Type 1 Diabetic (T1D) Patients (pts) Tasma Harindhanavudhi,[1] Alicia Parks,[1] Michael Mauer,[2] Maria Luiza A. Caramori.[1] *[1]Medicine, University of Minnesota, Minneapolis, MN; [2]Pediatrics, University of Minnesota, Mineapolis, MN.*

Background: Although microalbuminuria has been used as a predictor of DN, it is well clear that substantial glomerular structural changes can occur before clinical DN is present. Podocyte injury has been implicated in DN pathogenesis. Increased foot processes (FP) width was reported in NA T1D pts. This study aimed to determine whether podocyte/endothelial structural parameters predict progression to proteinuria and/or ESRD (progressors;P) in initially NA T1D pts.

Methods: We performed kidney biopsies in 94 NA T1D pts with≥8 yrs T1D duration, and followed for≥5 yrs (11±7yrs). At follow-up,12 pts were P and 59 were non-progressors (NP). In a nested case-control study,podocyte parameters were studied in each P, in sex and T1D duration matched NP, and in sex and age-matched non-diabetic controls (C). Two P with inadequate tissue for studies were excluded. Glomerular, podocyte parameters and %endothelial fenestration (EF) were estimated by electron microscopy. Glomerular volume was measured on light microscopy.

Results: $HbA1_c$(11±2*vs*.8±1%;p=0.001) and DBP(74±8*vs*.67±7mmHg;p=0.04) were higher in P *vs*.NP. Podocyte number per glomerulus, numerical density of podocyte per glomerulus, FP width, %EF, and the fraction of peripheral glomerular basement membrane (PGBM) and mesangial GBM (MGBM) covered by intact FP were not different among groups. However, the fraction of PGBM(3.2±3.3*vs*.0.5±1.0%) and MGBM(3.5±4.0*vs*.0.5±1.0%) with FP detachment was greater in NP *vs*.C(p=0.014 and p=0.016,respectively),but not different from P. Mirroring the larger cohort, GBM width was greater in P(522.8±89.2nm) *vs*.NP(448.1±85.2nm;p<0.041) or C (361.6±43.1;p<0.001), and greater in NP than C(p=0.027). Mesangial fractional volume was greater in P(0.23±0.06;p=0.006) or NP(0.26±0.07;p=0.001) *vs*.C(0.15±0.04),with no differences between P and NP.

Conclusions: This study does not support the hypothesis that podocyte structural changes are a necessary precondition for DN progression in NA T1D pts. However, this does not preclude an important role for podocytes at later DN stages.

Funding: NIDDK Support, Private Foundation Support

FR-OR043

Renal Prognosis of Patients Diagnosed with Diabetic Nephropathy 10 Years after Renal Biopsy Koki Mise, Yoshifumi Ubara, Junichi Hoshino, Keiichi Sumida, Masayuki Yamanouchi, Tatsuya Suwabe, Kenmei Takaichi. *Nephrology, Toranomon Hospital, Japan.*

Background: Recently, a classification of diabetic nephropathy was reported by Tervaert et al., but the association between pathological findings and the clinical outcome is unknown.

Methods: Among 250 patients with diabetes mellitus who underwent renal biopsy from 1985 to 2002 and were confirmed to have diabetic nephropathy according to the classification of Tervaert et al., 150 patients (followed for longer than 10 years) were enrolled in this study. Cox proportional hazard regression analysis was used to calculate the hazard ratio (HR) and 95% confidence interval (CI) for death-censored renal death. In each regression analysis, two levels of multivariate adjustment were examined.

Results: The hazard ratio (HR) for death-censored renal death of each pathological category is shown below.

		model 1			model 2		
	n	HR	95%CI	p-value	HR	95%CI	p-value
glomerular-class I	12	0.23	0.03-1.79	0.16	0.27	0.03-2.18	0.22
IIA	48	reference			reference		
IIB	34	2.60	1.21-5.59	0.01	2.72	1.12-6.60	0.03
III	26	5.11	2.30-11.36	0.00	5.17	2.03-13.16	0.00
IV	30	2.69	1.23-5.89	0.01	2.78	1.07-7.23	0.04
IFTA 0	16	0.22	0.03-1.74	0.15	0.24	0.03-1.92	0.18
1	52	reference			reference		
2	35	4.25	1.99-9.10	0.00	3.80	1.67-8.63	0.00
3	47	5.79	2.51-13.37	0.00	4.76	1.90-11.88	0.00
Interstitial inflammation 0	19	0.19	0.04-0.80	0.02	0.22	0.05-0.96	0.04
1	119	reference			reference		
2	12	1.02	0.48-2.16	0.96	1.04	0.48-2.25	0.92
Arteriolar hyalinosis 0	15	0.26	0.08-0.86	0.03	0.33	0.10-1.13	0.08
1	24	0.36	0.15-0.86	0.02	0.43	0.17-1.06	0.07
2	111	reference			reference		
Arteriosclerosis 0	13	0.29	0.07-1.28	0.10	0.31	0.07-1.38	0.12
1	69	reference			reference		
2	54	1.32	0.75-2.33	0.34	1.09	0.62-1.94	0.76

In model 1, compared with glomerular class IIA, the HRs for classes IIB, III, and IV were respectively 2.60 (95% CI: 1.21-5.58), 5.11 (2.30-11.36), and 2.69 (1.23-5.89). Also, compared with an IFTA score of 1, HRs for scores of 2 and 3 were 4.25 (1.99-9.10) and 5.79 (2.51-13.37), respectively.

Conclusions: The progression of glomerular, interstitial, and vascular lesions was associated with higher HRs for renal death. These results suggest the clinical utility of Tervaert's pathological classification.

FR-OR044

Urinary MCP-1 and Progression to ESRD in Subjects withType 1 Diabetes Monika A. Niewczas, Jan Skupien, Jung Eun Lee, Tomohito Gohda, William Walker, Adam Smiles, Rita R. Holak, Kevin Patrick Mcdonnell, Jackson Jeong, James Warram, Andrzej S. Krolewski. *Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.*

Background: Macrophage accumulation is a hallmark of diabetic nephropathy and MCP1 is one of the most potent chemoattractant for those cells. MCP1-based interventions are under investigation; nevertheless the predictive value of MCP-1 as a marker for diabetic nephropathy progression in subjects with proteinuria has not been studied in depth.

Methods: Our cohort included 140 Joslin Kidney Study subjects with type 1 diabetes, proteinuria, and eGFR between 30-60 ml/min/173m^2 (chronic kidney disease stage 3) at baseline. Patients were followed for up to 12 years and incidence of end-stage renal disease (ESRD) and deaths were ascertained. At the study entry, serum Tumor Necrosis Factor receptor 1 (TNFR1) and urinary markers of filtration barrier damage (albuminuria, ACR, proteinuria, PCR, and IgG excretion) and of local inflammation (monocyte chemoattractant protein – 1, MCP-1) were measured.

Results: 56% (n=76) developed ESRD during the follow-up. Serum TNFR1 and urinary excretion of albumin, total protein, IgG and of MCP-1 were higher at baseline in subjects who subsequently developed ESRD. After adjustment for the relevant clinical covariates sTNFR1, ACR and MCP1 emerged as independent significant predictors for the progression to ESRD, whereas the effect of urinary excretion of total protein and IgG was weak. Effect of ACR was confounded by MCP1 (HR for ACR: 2.45, p<0.0001; HR for ACR adjusted by MCP1 1.69, p<0.0001).

Conclusions: In subjects with type 1 diabetes and proteinuria, circulating sTNFR1 and urinary excretion of albumin and MCP1, but not of IgG or total protein, are important predictors of progression to ESRD. Our data suggest also that mechanisms behind albuminuria-driven injury of diabetic kidney are partially mediated via MCP1-involved inflammation.

Funding: NIDDK Support

FR-OR045

Osteoprotegerin (OPG) Is Increased in Patients with Type 1 Diabetes and Diabetic Nephropathy and Predicts Mortality Aino Soro-Paavonen,[1] Daniel Gordin,[1] Carol Forsblom,[1] Niina Sandholm,[1] Merlin C. Thomas,[2] Per-Henrik Groop.[1] *[1]Department of Nephrology, Helsinki University Central Hospital, Helsinki, Finland; [2]Diabetic Complications, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia.*

Background: The bone-related peptide osteoprotegerin (OPG) is produced by vascular cells and is involved in vascular calcification. We investigated the predictive effect of serum OPG on the progression of diabetic nephropathy (DN), cardiovascular disease (CVD) events, and all-cause mortality in patients with type 1 diabetes (T1D).

Methods: Serum OPG was measured by a Time-Resolved Immunofluorometric Assay in 2,116 patients with T1D and in 212 healthy control subjects in the Finnish Diabetic Nephropathy (FinnDiane) study. The patients were followed for 11.1 (0.1-15.9) years (mean +/- range) and data was collected on DN, incidence of CVD and mortality.

Results: At baseline, serum OPG was increased in the patients with DN [in patients with macroalbuminuria; 2.07±0.08 ng/l or end-stage renal disease (ESRD); 2.70±0.14 ng/l] than in patients without DN (1.61±0.04 ng/l) or healthy controls (1.63±0.06 ng/l). During the follow-up, 130 (6%) patients progressed to a higher level of albuminuria or to ESRD; 31 to microalbuminuria, 46 to macroalbuminuria, and 53 to ESRD. OPG was increased in patients who progressed to ESRD as compared to those who did not. In the Cox-regression model, the highest (fourth) OPG quartile was a significant risk factor for an incident CVD event independently of clinical CVD risk factors [HR 1.02 (1.00-1.02); *P*=0.007]. OPG predicted progression to ESRD [1.20 (1.03-1.39); *P*=0.03 with adjustment with age, gender, SBP, total cholesterol, HbA_{1c}, smoking, and previous CVD], but this association was borderline after the inclusion of eGFR in the model [1.19 (0.82-1.73); *P*=0.36]. OPG independently predicted all-cause mortality in patients with T1D (covariate-adjusted HR 1.01 [1.01-1.01]; *P*<0.001).

Conclusions: In a large cohort of individuals with T1D, OPG was not only predictive of CVD events and progression to ESRD but also of all-cause mortality.

Funding: Private Foundation Support, Clinical Revenue Support

FR-OR046

A Urinary Proteomic Set of Biomarkers Predicts Albuminuria Progression in Type 2 Diabetes

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Background: In patients with diabetes, development of microalbuminuria is the first clinical sign of diabetic nephropathy and is associated with adverse renal outcomes. Detection of patients at risk to develop micro- and macroalbuminuria may allow "early" therapeutic intervention. We assessed the value of a urinary proteomic risk score (classifier) in predicting development of micro- or macroalbuminuria.

Methods: We conducted a prospective case-control study by using data from the PREVEND study and the Steno Diabetes Centre. Cases (n=44) represented patients with diabetes who progressed one albuminuria stage (from normo- to micro- or from micro- to macroalbuminuria). Controls (n=44) with diabetes but no albuminuria transitions were matched for age, gender, and albuminuria status. A model for the progression of albuminuria was built using a previously defined classifier based on the abundance of 273 urinary peptides.

Results: Conditional logistic regression revealed that the proteomic classifier was independently associated with albuminuria transition (OR=1.32, 95%CI=1.06-1.64, P=0.014). The classifier predicted onset of micro- and macroalbuminuria on top of baseline albuminuria and eGFR.

Area Under the Receiver Operating Characteristic curve and Integrated Discrimination Index (IDI) for albuminuria progression by using the proteomic classifier

	AUC	95% CI	P	IDI	95% CI	P
Control model*	0.91	[0.85-0.98]	-	-	-	-
+ Classifier	0.94	[0.89-0.99]	0.002	0.105	[0.038-0.172]	0.002

*Includes baseline albuminuria and eGFR

Among the biomarkers, fragments of collagens, glycoproteins, and uromodulin showed statistically significantly different levels between cases and controls.

Conclusions: Our findings suggest the use of urinary proteomics in renal risk assessment in diabetic patients.

FR-OR047

Metabolite Profiles and the Risk of Worsening of Albuminuria in Type 2 Diabetes

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Background: Microalbuminuria is an early sign of diabetic nephropathy and is associated with adverse renal outcomes. Early detection of patients with type 2 diabetes (T2D) at risk for micro- or macroalbuminuria may be a key strategy for early intervention. We assessed the predictive value of metabolomic biomarkers for development of micro- and macroalbuminuria in T2D.

Methods: T2D patients were selected from a general population cohort (the PREVEND study) and the Steno Diabetes Center. Cases (n=45) represented subjects who transitioned to a higher albuminuria stage (i.e. from normo- to micro- or from micro- to macroalbuminuria), whereas controls (n=45) had stable albuminuria during follow-up. Cases and controls were matched for age, gender, and baseline albuminuria status. Metabolite profiles were measured with flow injection analysis (FIA) and LC-MS/MS. The predictive performance of plasma and urine metabolites for albuminuria progression was assessed by the area under the ROC-curve (AUC) and integrated discrimination index (IDI).

Results: In plasma, histidine was decreased and acylcarnitine was increased, and in urine, tyrosine and glutamine were decreased, in cases compared to controls (fold change 0.30, p=0.03; fold change 0.32, p<0.01; fold change 0.87, p<0.01; and fold change 1.17, p=0.03, respectively). In plasma and urine, the metabolites improved risk prediction for albuminuria progression irrespective of other renal risk markers including blood pressure, glucose, albuminuria and eGFR.

Conclusions: Plasma and urine metabolites can predict albuminuria progression in T2D patients on top of traditional markers.

AUC-ROC and IDI for predicting albuminuria transition

	AUC	95% CI	P value	IDI	95% CI	P value
Control model*	0.89	0.83 - 0.96	-	-		
+plasma metabolites	0.93	0.88 - 0.98	0.06	0.07	0.01 - 0.13	0.02
+urine metabolites	0.92	0.86 - 0.98	0.14	0.08	0.02 - 0.13	<0.01
+all metabolites	0.94	0.89 - 0.99	0.04	0.11	0.04 - 0.17	<0.01

*Albuminuria and eGFR

FR-OR048

Urinary Proteomics Predict Onset of Microalbminuria in a Cohort of Normoalbuminuric Type 1 Diabetic Patients in the DIRECT 1 Study

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Background: Early prevention of diabetic nephropathy is not successful as early interventions have shown diverging results. Urinary proteomics has shown promise as an early indicator of future development of diabetic nephropathy.

Methods: In a post-hoc study of the DIRECT-Protect 1 study, a randomized, controlled clinical trial of candesartan for slowing the progression of diabetic retinopathy, we studied patients with type 1 diabetes and normoalbuminuria (n=782), followed for a mean of 4.7 years. We determined a previously defined CKD risk score based on proteomic measurement of 273 urinary peptides (CE-MS), selected from previous cross sectional case-control studies. A Cox regression model for progression of albuminuria was built. The primary endpoint was development of persistent microalbuminuria (MA) (3 out of 4 samples).

Results: Persistent MA developed in 45 patients (5,7%). At baseline the CKD risk score was able to predict development of MA during follow-up, independent of treatment (candesartan/placebo), age, gender, baseline systolic BP, baseline UAER, baseline eGFR, baseline HbA_{1c} and diabetes duration (HR 3.7 (95% CI 1.4-9.3), p=0.006). In the placebo treated group the HR was 7.7 (2.1 to 29.1) compared to 1.8 (0.4 to 7.0) in the candesartan group.

Conclusions: In this cohort of patients with type 1 diabetes and normoalbuminuria from a large intervention study, the urinary proteome-based CKD classifier was an independent predictor of MA. This may provide a better opportunity to select normoalbuminuric patients for early prevention of diabetic nephropathy as treatment with candesartan seems to mitigate this risk.

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FR-OR050

***APOL1* Variant-Associated FSGS Is More Aggressive but Manifests Similar Response to Cyclosporine and Mycophenolate Compared to Other Forms of Primary FSGS**

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Background: *APOL1* kidney risk variants, which are present in some individuals of recent African descent, are strongly associated with primary focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy, and hypertensive nephrosclerosis. Prior work has shown that individuals with and without *APOL1*-associated FSGS respond at similar rates to glucocorticoids.

Methods: The FSGS Controlled Trial randomized children and young adults with glucocorticoid-resistant FSGS to one year of therapy with cyclosporine and low dose daily prednisone (CSA, N=72) versus mycophenolate, pulse oral dexamethasone, and low dose daily prednisone (MMF/DEX, N=66). DNA was available from 94 subjects, including 32 African Americans, 59 European Americans, and 3 others. Subjects were genotyped for *APOL1* risk variants (G1, G2). Primary outcome was complete or partial remission by week 52 (treatment end).

Results: Two *APOL1* risk alleles were present in 23/32 African Americans (72%) and in 4/59 (7%) European Americans, of whom 2 homozygous risk subjects were of Hispanic ancestry. Baseline eGFR was lower among 2 risk-allele subjects, 96±39 vs 143±70 ml/min/1.73m2, p<0.001, but proteinuria levels were similar. Collapsing FSGS was more common among 2 risk-allele subjects (8/27 vs 3/67, p<0.002), and cortical atrophy/ fibrosis (p<0.001) and arteriosclerosis (p<0.05) were more severe among 2 risk-allele subjects. Remission rates were similar (p=0.22) and the test for interaction between genotype and intervention (CSA vs MMF/DEX) was non-significant (p=0.45). The risk for progression to ESKD during follow-up was three-fold higher among 2 risk-allele subjects, 10/27 vs 8/67, p<0.01.

Conclusions: Racial self-identification is not a reliable means to exclude the presence of *APOL1* risk alleles. FSGS patients with 2 *APOL1* risk-alleles have more aggressive kidney disease but comparable response to CSA and MMF/DEX compared to other individuals.

Funding: NIDDK Support

FR-OR051

Genetic Locus on Chromosome 2q31.1 Associated with End Stage Renal Disease in Women with Type 1 Diabetes Per-Henrik Groop,[1] Niina Sandholm,[2] A.J. McKnight,[3] Rany M. Salem,[4] Eoin P. Brennan,[5] On Behalf of the GENIE Consortium.[6] *[1]Division of Nephrology, Helsinki University Central Hospital, Finland; [2]Folkhälsan Research Center, Helsinki, Finland; [3]Queen's University of Belfast, United Kingdom; [4]Broad Institute, Cambridge; [5]University College Dublin, Ireland; [6]GENIE Consortium.*

Background: Diabetes is the leading cause of end stage renal disease (ESRD). Notably, male gender is a major risk factor for diabetic nephropathy and ESRD. As men are more likely to develop ESRD, we performed a genome-wide association study on Finnish subjects with type 1 diabetes (T1D) to elucidate if genetic variants explain this discrepancy between men and women.

Methods: We genotyped 3652 subjects with T1D from the FinnDiane study on the Illumina 610 Quad array, and used HapMap II CEU as the reference panel to impute 2.4 million single nucleotide polymorphisms (SNPs). After quality control, cases included 258 women and 387 men with ESRD, and controls consisted of 936 women and 655 men with T1D but no evidence of kidney disease. Association was evaluated separately in men and woman using logistic regression adjusted for T1D duration, age and ten first principal components. Supporting replication evidence was gained from additional T1D cohorts from the GENIE collaboration: 1830 UK-ROI, 1792 GoKinD US and 397 Italian T1D subjects.

Results: We identified a common variant on chromosome 2q31.1 associated with ESRD at genome-wide level of statistical significance in women (odds ratio (OR) 2.4, $P=3\times10^{-8}$) but not in men (P=0.77). This signal was replicated ($P=0.02$) and the combined meta-analysis resulted in a P-value of 3.8×10^{-8} and OR 1.8 in women. This intergenic variant is located between the *SP3* and *CDCA7* genes. SP3 encodes a transcription factor that regulates expression of *CD2AP*, a protein essential for the glomerular filtration barrier. *CDCA7* encodes a DNA-dependent transcription factor that participates in the regulation of cell proliferation.

Conclusions: This signal represents the first genetic factor robustly associated with diabetic ESRD in women, but not in men, and points to a plausible biological pathway that may contribute to observed gender differences in risk of ESRD.

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FR-OR052

Common Variant in Uromodulin Promoter: Pleiotropic Consequences for Uric Acid, Diastolic Blood Pressure and Renal Function Decline in Community Based Population Yu-qing Chen,[1] Jia Han,[1] Ying Liu,[1] Xingyu Wang,[2] Yu Liang,[1] Fang Wang,[1] Luxia Zhang,[1] Hong Zhang,[1] Haiyan Wang.[1] *[1]Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China; [2]Beijing Hypertension League Institute China, Beijing, China.*

Background: Uromodulin (UMOD) is the most abundant protein in human urine. Here we asked whether UMOD genetic variants influence plasma uric acid, blood pressure and CKD progression in a community based population.

Methods: We took advantage of a community based population from Beijing, including 1000 individuals, 48% males and 52% females, mean age 63.7±9.0 years. 7 UMOD SNPs across the gene were genotyped in this population. Urinary uric acid, sodium, uromodulin and creatinine were measured in 898 cases. Transcriptional activity of promoter variants was estimated in luciferase reporter plasmids transfected into human kidney cells.

Results: The most common haplotype ATTGGCA (5'-3') was associated with a higher urinary uromodulin levels (p=0.016). UMOD promoter variants (rs13333226, rs6497476 and rs4293393] were associated with plasma uric acid, diastolic blood pressure and GFR decline as well. Rs13333226G allele carriers (n=133) have higher diastolic blood pressure, higher plasma uric acid level compared with A homozygotes (n=777) (p=0.035, p=0.009). Rs6497476 C carriers (n=112) have higher diastolic blood pressure and plasma uric acid level compared with T homozygotes (n=767) (p=0.025, p=0.005). At the same time variants rs13333226 (OR 1.687, p=0.036) and rs6497476 (OR 1.875, p=0.016) were associated with renal function decline in the cohort during 4 years follow up. rs4293393 C/T associated with plasma uric acid level only, and C carriers have higher uric acid level than T homozygotes (p=0.032). Site-directed mutagenesis at two trait-associated UMOD promoter variants, rs13333226 (A to G) and rs6497476 (C to T) altered promoter activity in transfected luciferase reporter plasmids.

Conclusions: Our study indicated that uromodulin gene variant predicted plasma uric acid, diastolic blood pressure and renal function decline in a community based population, suggested important roles of UMOD in the regulation of renal function.

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FR-OR053

Prevalence of Combined Complement Gene Mutations in aHUS and Their Impact on Clinical Phenotype Marina Noris,[1] Elena Bresin,[1] Erica Rurali,[1] Jessica Caprioli,[1] Pilar Sánchez-corral,[2,3] Veronique Fremeaux-bacchi,[4] Santiago Rodriguez de Cordoba,[5,3] Sheila Pinto,[5,3] Tim Goodship,[6] Marta Alberti,[1] David Ribes,[7] Elisabetta Valoti,[1] Giuseppe Remuzzi.[1,8] *[1]IRFMN, Bergamo; [2]IdiPAZ, Madrid; [3]CIBERER, Madrid; [4]HEGP, Paris; [5]CSIC, Madrid; [6]IGM, Newcastle upon Tyne; [7]CHU, Toulouse; [8]OORR, Bergamo.*

Background: aHUS is characterized by hemolytic anemia, thrombocytopenia, renal impairment and is associated with complement gene mutations (mut). Multiple genetic factors have been proposed to contribute to full-blown disease.

Methods: By screening 795 aHUS patients from 4 European cohorts (I, S, F, UK) we evaluated the prevalence of combined complement gene mut and their impact on clinical phenotype.

Results: 27 out of 795 aHUS patients (3.4%) carried combined mut in at least two complement genes, while 323 patients (40.6%) had single mut. Combined mut were more frequently found in patients with CFI mut (27% of patients), followed by MCP (23%), CFB (10%), CFH (9%) and C3 (8%), indicating that CFI and MCP mut more frequently need a second mut to manifest aHUS. Extending genetic screening to available relatives of combined mutated patients, we found that 29, 26 and 5 subjects carried 1, 2 or 3 mut. HUS penetrance increased across subjects with 1 (10% affected), 2 (50%) or 3 (100%) complement gene mut. Concomitant presence of CFH (c.1-332T, c.2808T) and MCP*ggaac* risk haplotypes significantly increased penetrance in affected carriers of 2 mut (71% vs 25%, P<0.05). Long-term prognosis in patients with combined CFH, CFI or C3 mut (ESRF at 3yrs: 36%, 40%, 50%) was similar to that in patients with single CFH, CFI or C3 mut (ESRF at 3yrs: 23%, 40%, 33%). Graft outcome at 3yrs was similar in the combined and single mutated subgroups (50% of graft lost). At variance, 50% of patients with MCP-combined mut developed ESRF at 3yrs from onset vs 6% of patients with single MCP mut (P<0.05). Moreover, patients with MCP combined mut had worse kidney transplant outcome than patients with single MCP mut (50% vs 7% graft lost at 3yrs).

Conclusions: We recommend screening of all known disease-associated genes in aHUS patients, particularly before taking a decision about transplantation.

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FR-OR054

DNA Methylation Profile Associated with Rapid Progression of Kidney Disease: Findings from the CRIC Study Maria R. Wing,[1] Joseph M. Devaney,[2] Marshall M. Joffe,[3] Dawei Xie,[3] Harold I. Feldman,[3] Kevin A. Sterling,[1] Katalin Susztak,[4] Nicolas Jose Guzman,[1] James G. Herman,[5] Leslie Cope,[5] Brennan Harmon,[2] Alan S. Go,[6] Jiang He,[7] James P. Lash,[8] Eric Hoffman,[2] John W. Kusek,[9] Dominic S. Raj.[1] *[1]Division of Renal Disease and Hypertension, George Washington University; [2]Children's National Medical Center; [3]Clinical Epidemiology Unit, University of Pennsylvania School of Medicine; [4]Division of Nephrology, Albert Einstein College of Medicine, Bronx, NY; [5]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine; [6]Division of Research, Kaiser Permanente Northern California; [7]Tulane University School of Public Health and Tropical Medicine; [8]Division of Nephrology, University of Illinois; [9]DKUHD, NIDDK.*

Background: Recent evidence indicates that epigenetic mechanisms may be important in progression of chronic kidney disease (CKD).

Methods: The genome-wide DNA methylation pattern associated with rapid loss of kidney function was studied using the Infinium Human Methylation 450K BeadChip in 40 Chronic Renal Insufficiency Cohort (CRIC) Study participants (n=3,939) with the highest and lowest rates of decline in eGFR. The data was analyzed using an ANOVA with response (rapid loss of kidney function vs. stable kidney function), sex, race, and diabetes status.

Results: The mean eGFR slope was 3.6(1.4) and -3.7(1.2) ml/min/1.73 m^2 in the stable kidney function group and rapid progression group respectively. The *TPBG*, *ZFYVE21*, and *FKBP5* genes were hypomethylated in subjects with rapid decline in eGFR (P-values: 9.6E-06 to 5.7E-05). A CpG island within the *NOS3* gene was hypermethylated (P-value 1.6E-03) in diabetics. Other CKD-related genes with differential methylation included *NFKBIA*, *NFKBIZ*, *TGFRB3*, *TGFRB2*, *SOD1*, and *SOD2* (P-values: 3.8E-04 to 8.8E-03). Gene set enrichment analysis identified *CLU*, *TGFB2*, *NFkB* family genes, and DNMT3A to be differentially methylated. Pathway analysis showed that gene networks related to gene expression, cellular growth and cellular proliferation were epigenetically regulated.

Conclusions: These results indicate that epigenetic modifications could be important in determining the rate of loss of kidney function.

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FR-OR055

Genome-Wide Association Study (GWAS) of Renal Function Decline: The CKDGen Consortium Carsten A. Böger. *Nephrology, on Behalf of the CKDGen Progression Writing Group, University Hospital Regensburg, Regensburg, Germany.*

Background: Multiple loci associated with cross-sectional eGFR in population-based cohorts have recently been identified by GWAS, but a systematic genetic analysis of longitudinal kidney traits is outstanding. We thus performed GWAS meta-analysis of several definitions of kidney function decline.

Methods: 45409 participants from 16 CKDGen studies with follow-up data (AGES, AMISH, ARIC, ASPS, CHS, CoLaus, FHS, GENOA, HABC, JUPITER, KORA S3, KORA S4, MESA, Rotterdam Study, SHIP, 3C) participated. GFR was estimated from serum creatinine calibrated to NHANES standards. Median follow-up was 5.7 years. We derived four traits from serial eGFR: annual eGFR decline (eGFR-decline, in ml/min/1.73m^2 per year), incident CKD (new eGFR<60ml/min/1.73m^2,n=3187 cases), incident CKD with a 25% eGFR decline (CKDi25, n=1685 cases), rapid eGFR-decline (annual eGFR-decline^{3}3ml/min/1.73m^2, n=7748 cases). Study-specific GWAS for each trait was adjusted for age, sex, and baseline eGFR, and additionally for study site, principal components and family relatedness where applicable. Analyses stratified by presence of baseline CKD for rapid eGFR-decline and eGFR-decline were also performed. Meta-analysis was performed using an inverse-variance weighted fixed effects model.

Results: Seven loci were associated with at least one of the analyzed traits at p<1.0 x 10^{-6}. The previously identified *UMOD* locus showed genome-wide association with eGFR-decline (p=2.6x10^{-14}). The next highest ranking SNPs, both associated with rapid eGFR-decline, were located in the *MEOX2* (p=6.8x10^{-8}) and the *PRKAG2* loci (p= 3.0x10^{-7}). In those without CKD, one SNP was associated with rapid eGFR-decline in the *IL1RAP* region (p=4.1x10^{-7}). Other loci identified were the *MTHFR*/*NPPA/NPPB* and *CDH23* (in eGFR-decline in those with CKD), and *C2orf48* (in the CKDi25 analysis) regions.

Conclusions: Several novel loci and the previously identified *UMOD* locus were associated with clinically relevant kidney function decline traits. If confirmed, these findings suggest an additional genetic component to kidney function deterioration when compared to cross-sectional eGFR. Validation in independent cohorts and by functional studies is ongoing.

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FR-OR056

Sat1 Anion Transporter (SLC26A1) Is Linked to Calcium Oxalate Urolithiasis Daniel Markovich, Sarah C. Mcleay, Soohyun Lee. *School of Biomedical Sciences, University of Queensland, St Lucia, QLD, Australia.*

Background: Urolithiasis is a common disorder leading to significant health care costs. The most prevalent form of kidney stones in humans is calcium oxalate urolithiasis, which is linked to perturbations in oxalate homeostasis. Sat1 (Slc26a1) is an anion transporter that mediates oxalate transport across epithelial cells of the kidney, liver and intestine. In this study, we aimed to determine the role of Sat1 in oxalate homeostasis and calcium oxalate urolithiasis.

Methods: Blood and urine profiling, X-ray spectrometry, membrane transport assays and histological analysis was used to characterise abnormal physiological features in Sat1 (Slc26a1) null (Sat1$^{-/-}$) mice (generated in our laboratory) and compared to age- and gender-matched wild-type littermates.

Results: Sat1$^{-/-}$ mice have calcium oxalate urolithiasis, hyperoxaluria with hyperoxalemia, elevated urine oxalate/creatinine ratios and reduced oxalate transport in intestinal basolateral membrane vescicles (BLMVs). Sat1$^{-/-}$ mice also exhibit leukocyte infiltration around renal cortical vessels, suggestive of ureteral obstruction or obstructive uropathy.

Conclusions: Our data demonstrates that Sat1 is crucial for oxalate homeostasis and is linked to the development of calcium oxalate kidney stones. This animal model provides the foundation for studying the roles of SAT1 (SLC26A1) in human kidney stone formation.

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FR-OR057

Activation of the Ca^{2+}-Sensing Receptor Increases Renal Claudin-14 Expression and Urinary Ca^{2+} Excretion Henrik Dimke, Prajakta Desai, Wanling Pan, Jelena Borovac, R. Todd Alexander. *Pediatrics & Physiology, University of Alberta, Edmonton, AB, Canada.*

Background: Kidney stones are a prevalent clinical condition imposing a large economic burden to the healthcare system. Hypercalciuria remains the major risk factor for development of a Ca^{2+}-containing stone. The kidneys ability to alter Ca^{2+} excretion in response to changes in serum Ca^{2+} is in part mediated by the Ca^{2+}-sensing receptor (CaSR). A recent GWAS study linked claudin-14 (Cldn14) to kidneys stones. Moreover, recent expression studies have localized cldn14 to the thick ascending limb (TAL) and revealed that expression is regulated by the CaSR.

Methods: To interrogate the role of claudin-14 expression in calcium homeostasis we manipulated Ca^{2+}homeostasis *in vivo* by placing mice on altered calcium diets, administering 1,25-dihydroxyvitamin D_3 or the calcimimetic, Cinacalcet. We also over expressed Cldn14 in a renal epithelial cell culture model.

Results: We find that Cldn14 expression is increased by high dietary Ca^{2+} intake and by elevated serum Ca^{2+} levels induced by prolonged 1,25-dihydroxyvitamin D_3 administration. Consistent with this, activation of the CaSR *in vivo* via administration of the calcimimetic Cinacalcet, led to a 40-fold increase in Cldn14 mRNA abundance. Moreover, overexpression of Cldn14 in a cell culture model decreased paracellular Ca^{2+} flux by preferentially decreasing cation permeability and consequently increasing transepithelial resistance.

Conclusions: These data support the existence of a mechanism whereby activation of the CaSR in the TAL increases Cldn14 expression, thus blocking the paracellular reabsorption of Ca^{2+}. This molecular mechanism likely facilitates renal Ca^{2+} losses in response to elevated serum Ca^{2+}. Moreover, dysregulation of the newly described CaSR-Cldn14 axis likely contributes to the development of hypercalciuria and kidney stones.

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FR-OR058

Genetic Hypercalciuric Stone-Forming Rats Have a Decrese in Paracellular Permeability to Calcium in the Cortical Thick Ascending Limb Suresh K. Ramakrishnan,[1] Alexandre Loupy,[2] Aurelie Edwards,[1] David A. Bushinsky,[3] Pascal Houillier.[2] *[1]CNRS, P. et . Curie Univ., Paris, France; [2]INSERM, Paris Descartes Univ., Paris, France; [3]University of Rochester Medical Center, Rochester, NY.*

Background: Idiopathic hypercalciuria (IH) is the principal risk factor for calcium (Ca) nephrolithiasis. Patients with IH generally have increased intestinal Ca absorption, increased bone resorption and a decreased renal tubular Ca reabsorption. The Genetic Hypercalciuric Stone-forming (GHS) rats have been shown to have each of these pathophysiologic abnormalities resulting in hypercalciuria and stone formation. Previous clearance studies in GHS rats suggested deficient Ca transport in the thick ascending limb (TAL).

Methods: To test this hypothesis, we measured net transepithelial Ca transport (J_{Ca}) in in vitro microperfused cortical TAL from male GHS and control Sprague-Dawley (SD) rats.

Results: Under baseline conditions, J_{Ca} was significantly lower in cTAL from GHS than from SD rats (1.7±0.7 vs 4.9±1.2 pmol.min^{-1}.mm^{-1} (m±sd), p <0.001). In contrast, J_{Na}, J_{Cl} and the transepithelial voltage were similar in GHS and SD rats. The passive transepithelial permeability to Ca (P_{Ca}) was measured under conditions of transepithelial voltage clamp obtained by imposing a dilution potential. P_{Ca} was significantly lower in GHS than in SD rats (4.7±0.4 vs 8.4±0.6 x 10^{-5} cm.s^{-1}, p=0.0006). We then determined whether the defect in Ca absorption in the cTAL could be reversed by inhibition of the calcium-sensing receptor CaSR. When added in the bath, the calcilytic NPS2143 (1 μM) increased Ca absorption by 2.2±0.6 pmol.min^{-1}.mm^{-1} in the SD rats and by 1.8±0.6 pmol.min^{-1}.mm^{-1} in the GHS rats. J_{Ca} remained lower in GHS than in SD rats. Similarly, peritubular PTH (300 pM) increased J_{Ca} both in SD (by 2.1±0.7 pmol.min^{-1}.mm^{-1}) and GHS rats (by 2.3±0.6 pmol.min^{-1}.mm^{-1}) but J_{Ca} remained lower in GHS than in SD rats.

Conclusions: Thus GHS rats display a severe decrease in Ca absorption in the cTAL due to a decrease in paracellular pathway permeability, which cannot be reversed by inhibiting the CaSR or activating the PTH receptor. The molecular basis of the cTAL defect in Ca permeability remains to be determined.

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FR-OR059

Defective SLC26A6-Mediated Oxalate Secretion in *CFTR*-Null Mice Felix Knauf,[1] Robert Brent Thomson,[1] Marie E. Egan,[2] John R. Asplin,[3] Peter S. Aronson.[1] *[1]Medicine/Nephrology, Yale School of Medicine, New Haven, CT; [2]Pediatrics/Pulmonology, Yale School of Medicine, New Haven, CT; [3]Litholink Corporation, Chicago, IL.*

Background: Patients with cystic fibrosis have an increased incidence of hyperoxaluria and calcium-oxalate nephrolithiasis. Net intestinal absorption of dietary oxalate results from passive paracellular oxalate absorption as modified by SLC26A6-mediated back-secretion of oxalate. It has been postulated that fat malabsorption observed in patients with cystic fibrosis leads to an increased oxalate absorption by reducing precipitation of oxalate with calcium in the lumen of the intestine, thereby increasing soluble oxalate available for absorption. We used *CFTR*-null mice to test the additional hypothesis that SLC26A6-mediated oxalate secretion is defective in cystic fibrosis.

Methods: We mounted isolated intestinal tissue from wild-type and *CFTR*-null mice in Ussing chambers and measured active secretion of [14C]-oxalate. To determine the specificity of any observed defect in active oxalate secretion, we also assayed active [14C]-glucose absorption. Duodenum was harvested and probed for SLC26A6 protein expression normalized to actin by Western blot analysis. Plasma and urine oxalate levels were measured.

Results: Active oxalate secretion was completely abolished in intestinal tissue isolated from *CFTR*-null mice as compared to wild-type mice. Glucose absorption was not changed between wild-type and *CFTR*-null mice, indicating that the effect of CFTR deletion to reduce oxalate secretion is not the result of a general downregulation of intestinal transport processes. Intestinal SLC26A6 protein abundance was reduced by 50% in *CFTR*-null mice compared with wild-type mice. Serum and urine oxalate levels were 3-fold increased in *CFTR*-null mice compared to wild-type mice.

Conclusions: We demonstrate that SLC26A6-mediated oxalate secretion in the intestine is impaired in a mouse model of cystic fibrosis. Downregulation of SLC26A6 may contribute to the increased net absorption of dietary oxalate contributing to hyperoxalemia, hyperoxaluria and increased risk for calcium-oxalate stone formation observed in patients with cystic fibrosis.

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FR-OR060

Calcium-Induced Acid Secretion in the Cortical Collecting Duct Involves GPRC6A Marie-lucile Figuères,[1] Alexandre Loupy,[1] Suresh K. Ramakrishnan,[2] Patrick Bruneval,[1] Chantal Mandet,[1] Sanela Smajilovic,[3] Hans Bräuner-osborne,[3] Pascal Houillier.[1] *[1]INSERM, ParisDescartes Univ., Paris, France; [2]CNRS, P.&M. Curie Univ., Paris, France; [3]Copenhagen Univ., Copenhagen, Denmark.*

Background: Hypercalciuria stimulates urinary acidification that might protect against the formation of calcium(Ca) stones. However, the mechanism of Ca-sensing in the collecting duct (CD) remains elusive. We hypothesized that GPRC6A, a G protein-coupled receptor related to CaSR, may be involved in the urinary acidification elicited by hypercalciuria. This study aimed at localizing GPRC6A in the kidney and at assessing its role in the Ca-dependent urine acidification.

Methods: GPRC6A and CaSR proteins were localized by immunohistochemistry (IHC) on serial sections of mouse kidney, using anti-GPRC6A and anti-CaSR antibodies (from MBL and ABR, respectively). The activity of H^+ transporters was assessed by measuring the initial rate of alkalinisation of type A intercalated cells after intracellular acidification by the NH_3/NH_4^+ prepulse method in *in vitro* microperfused cortical CD.

Results: IHC experiments with the anti-GPRC6A antibody revealed a predominant staining of intercalated cells of cortical and medullary CD. No staining was present in $Gprc6a^{-/-}$ mice. No staining of CD was observed with the anti-CaSR antibody. In $Gprc6a^{+/+}$ mice, the initial rate of cell alkalinisation was 0.17±0.02 and 0.38±0.05 UpH/min in the presence of 1 and 3 mM luminal Ca, respectively ($p = 0.002$). In $Gprc6a^{-/-}$ littermate mice, the initial rate of cell alkalinisation was 0.29±0.07 and 0.23±0.06 UpH/min in the presence of 1 and 3 mM Ca, respectively ($p = 0.5$).

Conclusions: In the mouse CD, GPRC6A is expressed in intercalated cells of CD. Increasing luminal Ca concentration stimulates H^+ secretion by intercalated cells, an effect involving GPRC6A.

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FR-OR061

Effect of Parathyroid Hormone and FGF23 on Fractional Excretion of Phosphorus in Stone Formers and Controls

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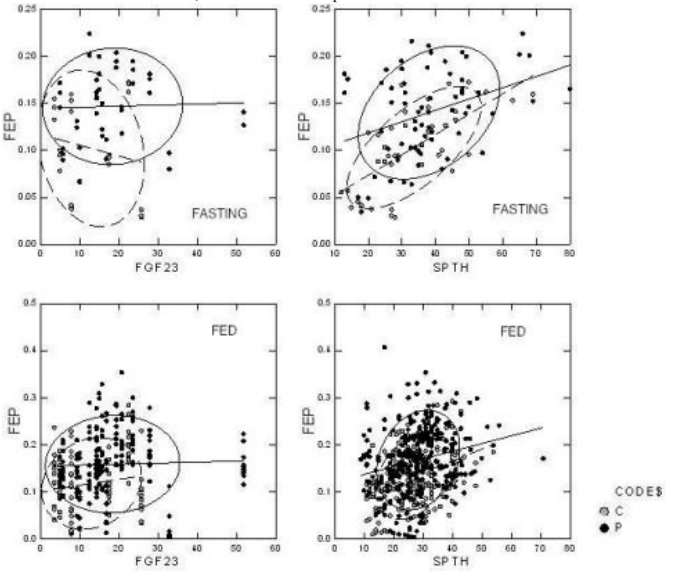
Background: The average serum phosphorus (P) of calcium stone formers (SF) with idiopathic hypercalciuria (IH) is lower than among normal people; their hypophosphatemia is associated with an increase in overall fractional excretion of filtered P (FEP).

Methods: We have measured FGF23 (using the Kainos assay), PTH, P, and FEP in 18 SF with IH and 7 controls (C) studied on standard diets in the CRC. Fasting and fed time periods were analyzed separately. To determine the independent correlation with FEP we used stepwise multivariate analysis with FEP as the dependent variable.

Results: FGF23 correlated directly with P in SF but not C. PTH correlated with FEP in SF and C. In the stepwise multivariate analysis with FEP in the fasting state as the dependent variable, PTH and subject group (Code, SF vs. C) entered the model with significant F values (PTH F=26, Code F=6, $p<0.01$ for both), $R^2 = 0.3$. In model of FEP in the fed state, PTH and Code (F=33 and 26, respectively; $R^2 = 0.11$) entered the model. FGF did not enter either model, nor did the cross product of Code with either PTH or FGF23.



Conclusions: Although FGF23 was strongly related to P in SF the relationship was a direct one, whereas it must be inverse if FGF23 is lowering P via increase of FEP. On the other hand PTH was strongly correlated with FEP as one expects if it is the cause of reduced P. FGF23 did not enter as an independent term in the multivariable model indicating a lack of effect. Analysis of TmP/GFR gave similar results (not shown). We conclude that the increased FEP of SF is not due to FGF23.

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FR-OR062

WNK Signaling Can Control Renal Oxalate Transport and Calcium Oxalate Crystal Formation in Drosophila

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Background: Kidney stones (nephrolithiasis) are a painful and expensive health care issue with complex etiology. More than 70% of stones are calcium oxalate (CaOx). Some Slc26 genes encode proteins that transport oxalate in the gut and kidney. Slc26a6 knockout mice have hyperoxaluria and CaOx stones. Yet, the details of ox^{2-} transport in the gut and kidney, e.g., regulation or signaling factors, are poorly understood or not investigated. To overcome this issue, our group established *Drosophila* kidney stone model using dSlc26a5/6 (dPrestin), *Drosophila* orthologue of Slc26a6, for a simple, fast and straight forward analysis. Additionally, we and others found that WNK signaling controls Slc26 transporter activities.

Methods: (*in vitro*) Using *Xenopus* oocytes, the effect of WNK signaling kinases (WNK or SPAK/OSR1) on mSlc26a6 and dPrestin transport activities were examined. (*in vivo*) To evaluate *in vivo* effect on stone formation in fly, we dissected Malpighian tubules (MT) from SPAK/OSR1 mutant (*frayed*) and wild-type and observed stone formation by soaking them in a Na-oxalate solution.

Results: mSlc26a6 and dPrestin transport activities are increased in the presence of active mWNK3 and dSPAK/OSR1, respectively and suppressed by dominant-negative clones of these kinases. OSR1 fly mutants showed decreased CaOx crystal formation in MT when compared to wild-type flies.

Conclusions: Our observations indicate that WNK/OSR1 signaling controls CaOx crystal formation by regulating oxalate secretion via dPrestin at MT, thus, we hypothesize that a similar regulation system exists in humans. This is the first report to examine *in vivo* function and intercellular signaling relating oxalate absorption and secretion by renal tubules.

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FR-OR063

Increased Intestinal Barrier Permeability Contributes to Hyperoxaluria in Obese Mice

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Background: The majority of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect stone risk. Obesity is a risk factor for kidney stones and obese patients often have mild hyperoxaluria. A positive correlation between increased body size and elevated urine oxalate excretion was reported. However, the mechanism(s) underlying this positive correlation remain(s) unknown. To explore the pathogenesis of obesity-associated hyperoxaluria, urine oxalate levels were measured in Ob (an animal model of obesity) mice and their lean controls (C). Ob mice were found to have significantly higher urine oxalate (adjusted for Cr) compared with C (μM: C=19.43±1.43; Ob=48.27±5.09). Significant hyperoxaluria was also seen in Db (another obesity model) mice compared with C (> 2-fold). Since the Ob mice are hyperphagic, the mice were pair-fed (4 g/mouse/day, based on our finding a control and an Ob mouse consume 4.03±0.22 and 7.58±0.59 g/day, respectively) and significant hyperoxaluria was still observed in Ob mice (μM: C=13.84±1.08; Ob=30.94±5.43), indicating that it is not due to overeating. Ob mice have increased intestinal paracellular permeability and intestinal oxalate absorption was shown to be passive through the paracellualr pathway. We thus used the mouse ex vivo intestinal loop model to assess intestinal paracellular permeability, and observed significantly higher jejunal (> 46%) and ileal (> 30%) ^{14}C-oxalate and ^{3}H-mannitol (a paracellular marker) fluxes (from lumen to outside medium bathing the loops) in Ob mice compared with C. Obesity is characterized by increased systemic inflammation (e.g. IFN-γ {I}, TNF-α {T}) and oxidative stress (e.g. H_2O_2). H_2O_2 and cytokines (I+T), which are known to disrupt intestinal barrier function, caused > 5 - and 2-fold increase in apical to basolateral ^{14}C-oxalate and ^{3}H-mannitol fluxes in human intestinal Caco2 cells compared with controls, respectively. We conclude that increased intestinal barrier permeability contributes to hyperoxaluria in obese mice by increasing passive oxalate absorptive flux.

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FR-OR064

Should Pediatric Idiopathic Hypercalciuria Be Treated with Hypocalciuric Agents?

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Background: The issues related to the need for hypocalciuric therapy, its nature and duration, in children with Idiopathic Hypercalciuria (IH) remains to be determined. This treatment may be required not only to protect against stone formation but also against low bone density, documented in many of these patients. The aim of this study was to examine whether thiazides have a beneficial effect on bone mass in children with IH.

Methods: We have followed 40 children with IH (62.5% male) aged 10.5±3.5 yr, for median time of 6.0 yr (4.5–8.3). Initial approach consisted of dietary modifications for 4 months. In case of no normalization of calcium excretion with dietary measures, potassium citrate was added (0.5–1.0 mEq/kg/day), to the dietary measures for 2 months. Treatment with thiazide was initiated (0.5–1.0 mg/kg/day) in association with K-citrate if there was no improvement of calciuria levels after 2 months on alkali treatment. Patients took K-citrate alone or combined with thiazides for at least 1 year. Initial bone densitometry (BMD) was performed before patients started treatment with K-citrate, and the final BMD was conducted at the end of follow-up. Nine patients took K-citrate alone (Group G1) and 31 received K-citrate and thiazides (Group G2).

Results: There were no differences in age, BMI Z-scores, biochemical and mineral parameters between G1 and G2 before and after treatment. BMD Z-score of the lumbar spine (L1–L4) increased significantly with treatment in G2 from -1.7 to -1.4 (p=0.04; U-Test Mann-Whitney) but there was no improvement in G1 (from -1.3 to -1.6; p=0.16).

Conclusions: Our results point to a beneficial effect of thiazides on BMD in children with IH. We speculate that thiazide may have a role in acheivement of positive calcium balance and consequently optimal peak bone mass, and suggest further prospective randomized studies on the effect of thiazide therapy in children with IH.

FR-OR065

Molecular Genetic Characterization of Primary Hyperoxaluria Pedigrees: Update of the International Primary Hyperoxaluria Registry

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Background: Primary Hyperoxaluria (PH) is an autosomal recessive condition characterized by the development of kidney stones and end stage renal failure. PH is caused by mutations at the *AGXT* (PH1), *GRHPR* (PH2) and *HOGA1* (PH3) genes.

Methods: The International PH Registry collected a cohort of 280 unrelated pedigrees with a clinical diagnosis compatible with primary hyperoxaluria where DNA from at least one proband was available for genetic analysis. We sequenced the 3 PH disease genes in this PH cohort by bidirectional sequencing of all the coding exons using M13-tailed primers. Mutations were segregated in all available family members.

Results: Of the 280 pedigrees, 219 (78%) provided sufficient mutation data for disease classification as PH1 (167, 76%), PH2 (22, 10%) or PH3 (30, 14%) disease, while 61 (22%) remained mutation negative. Of these 219 pedigrees, 208 (95%) were fully resolved with 2 recessively inherited mutations (160 *AGXT* pedigrees-77%, 20 *GRHPR*-10% and 28 *HOGA1*-13%); in 11 pedigrees (5%) only one mutation was identified (7 *AGXT* pedigrees, 2 *GRHPR* pedigrees and 2 *HOGA1* pedigrees). In total, we identified 327 *AGXT* disease alleles (110 missense-33%, 87 truncating-27%, 3 in frame indels-1%, and 127 mis-targeting-39%); 44 *GRHPR* disease alleles (17 missense-39%, 27 truncating-61%); and 60 *HOGA1* disease allele (16 missense-27%, 1 truncating-1%, 43 in-frame indels-72%). While the largest group of *AGXT* disease alleles is missense, truncating and in-frame indels are the largest group for *GRHPR* and *HOGA1*.

Conclusions: We characterized the genetics of a large PH population, ideal for genotype-phenotype correlations and disease natural history analysis. PH3 represents a considerable proportion of cases in this cohort, second only to PH1. Atypical mutations may be present in the 11 partially resolved PH pedigrees, suitable to further analysis by deep sequencing the entire genomic structure of the PH genes. The presence of a sizable group of mutation-negative PH pedigrees, suitable to whole exome analysis, suggests the involvement of additional, yet to be identified disease genes.

Funding: NIDDK Support

FR-OR066

Endogenous Klotho Regulates Proliferative Effects of FGF23 in Human Endothelial Cells, In Vitro

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Background: Rising FGF-23 levels have been associated with cardiovascular disease and mortality in CKD. We previously showed that this may in part, be due to progressive resistance to FGF-23 caused by Klotho deficiency in cells of the arterial wall. Here, we hypothesize site-specific differential expression of Klotho in human endothelial cells and that FGF-23 effects are dependent on this expression pattern.

Methods: *In vitro* models: Human aorta endothelial cells (HAECs) +/- Klotho siRNA; brain microvascular endothelial cells (HBMECs) +/- 0.2 nM recombinant human Klotho. Assessment of cell proliferation: XTT assay.

Results: We show for the first time Klotho is expressed in HAECs at both the mRNA and protein level. In addition, FGFR1 is also expressed in HAECs. Previous studies showed that Klotho-FGFR1 is the principal receptor-site for FGF-23 signaling, here we show that FGF-23 stimulated significant proliferation of HAECs at 50ng/ml and 100ng/ml in our dose dependent studies, however these effects were mitigated by Klotho knockdown. In contrast, we found that HBMECs expresses FGFR1 but not Klotho. And FGF-23 did not stimulate HBMEC proliferation in dose dependent studies (0 – 100 ng/ml). We next investigated whether circulating Klotho could render HBMECs responsive to FGF-23. Our results showed that treatment with FGF-23 in the presence of recombinant soluble human Klotho did not stimulate HBMEC proliferation.

Conclusions: Our study shows for the first time, site-specific Klotho expression within the human vascular endothelium where its expression confers FGF-23 responsive properties. Specifically, expression of Klotho in HAEC confers this cell-type responsive to FGF-23 proliferative effects whilst its absence in HBMECs renders these cells resistant to this FGF-23-related effect.

FR-OR067

Increased Plasma Fibroblast Growth Factor 23 Is Associated with an Impaired Response to Intensified Antiproteinuric Therapy

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Background: Fibroblast growth factor 23 (FGF23) is an independent risk factor for chronic kidney disease (CKD) progression and cardiovascular disease. High serum phosphate has been associated with impaired efficacy of RAAS-blockade, but the role of FGF23 is unknown. We hypothesized that high FGF23 levels impair the response to intensification of antiproteinuric therapy.

Methods: Post-hoc analysis of a 2x2 crossover RCT aimed at maximizing the effect of RAAS-blockade. Non-diabetic CKD patients (n=51) on background ACEi (lisinopril 40 mg/day) underwent four 6-week periods with add-on valsartan 320 mg/day (ACEi+ARB) or placebo, with either regular (RS, 186[150-216] mmol/d) or low (LS, 97[68-130] mmol/d) sodium diet. Plasma C-terminal FGF23 levels were measured by ELISA.

Results: At baseline (ACEi+RS) median UP was 1.9[IQR 0.9-3.4] g/d, creatinine clearance (CrCl) 69[50-108] ml/min and FGF23 146[119-244] U/mL. During maximal therapy (ACEi+ARB+LS) UP decreased to 0.7[0.4-1.4] g/d ($p<0.001$) and CrCl to 59[42-77] mL/min ($p<0.001$). At baseline, UP was similar in tertiles of FGF23. Patients in the lowest baseline FGF23 tertile responded stronger to therapy intensification (72%[46%-90%] UP reduction (UPR) by ACEi+ARB+LS vs baseline) compared to those in the highest tertile (40%[25%-65%] UPR, $p<0.05$). In multivariate analysis, baseline FGF23 predicted UPR (st. β -0.65, $p<0.05$) independent of CrCl (St. β 0.16, NS), BMI (St. β 0.46, $p<0.05$), UP (St. β 0.29, NS), serum phosphate (St. β 0.19, NS), phosphate excretion (St. β -0.10, NS), age and gender. FGF23 was also the only determinant (St. β 0.79, $p<0.05$) for UPR during ACEi+LS (47%[30%-66%]) but not during ACEi+ARB+RS (20%[-12%-41%]). Neither FGF23 nor other variables predicted SBP reduction.

Conclusions: Higher baseline FGF23 levels are independently associated with an impaired response to intensification of antiproteinuric therapy by add-on salt restriction with or without ARB. Future studies should confirm whether reduction of FGF23 could enhance RAAS-blockade efficacy.

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FR-OR068

Effects of Dietary Phosphate Restriction and Lanthanum Carbonate on Fibroblast Growth Factor 23 in Chronic Kidney Disease

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Background: FGF23 excess is the earliest and most common mineral metabolism abnormality in CKD patients, in whom it is associated with left ventricular hypertrophy and with increased risks of death and end stage renal disease. Though prior studies suggested that interventions targeting dietary phosphate intake or absorption may decrease FGF23, it remains unknown if combining phosphate binders with dietary counseling based phosphate restriction will have synergistic effects.

Methods: In this single-blinded, placebo-controlled 3-month study, we randomly assigned 39 patients with CKD stage 3–4 and normal serum phosphate levels to four groups: double placebo (ad lib diet plus lanthanum carbonate placebo, 10 patients), diet alone (900-mg phosphate diet plus lanthanum carbonate placebo, 10), lanthanum carbonate alone (ad lib diet plus lanthanum carbonate, 11), and diet-lanthanum carbonate (900-mg phosphate diet plus lanthanum carbonate, 8). The dose of lanthanum carbonate was 1000 mg three times daily with meals. Dietary restriction was accomplished with dietary counseling.

Results: At baseline, mean eGFR was 38±11 ml/min/1.73 m^2, mean serum phosphate was 3.6±0.7 mg/dl, and median FGF23 was 128 (interquartile range 89 – 155) RU/ml. Diet and lanthanum carbonate alone each significantly lowered FGF23: 22% and 19% decline, respectively ($P=0.01$ for both). However, the diet-lanthanum carbonate group resulted in a 35% decrease in FGF23 (P for interaction 0.009), suggesting synergy between diet and lanthanum carbonate.

Conclusions: Over the course of three months, lanthanum carbonate and dietary phosphate restriction synergistically decrease FGF23 levels in normophosphatemic CKD patients.

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FR-OR069

Vitamin D-Induced Antibacterial Activity in Cells from Peritoneal Dialysate Effluent Justine Bacchetta, Rene Chun, Barbara Gales, Joshua Zaritsky, Sandrine Leroy, Katherine Wesseling-Perry, Isidro B. Salusky, Martin Hewison. *UCLA, Los Angeles, CA.*

Background: Vitamin D has potent immunomodulatory properties, notably the induction of antibacterial innate immunity. The aim of this study was to assess potential antibacterial benefits of vitamin D in patients undergoing peritoneal dialysis (PD), hypothesizing that peritoneal monocytes may be targets for vitamin D.

Methods: PD cells were isolated from 24 hr PD effluent in 27 non-infected patients and studied by FACS (common leukocyte marker CD45 and monocyte marker CD14), and RT-PCR to assess expression of vitamin D-related genes (VDR, CYP27B1, CYP24A1, LL37 encoding antibacterial cathelicidin) at baseline and after in vitro exposure to vitamin D. We also performed a clinical prospective trial in 12 PD patients (median age 20.8 yrs) to study the impact of an oral supplementation with vitasmin D2 (100,000 IU/wk for 4 wks).

Results: In the 27 samples obtained at baseline, PD cells were mainly monocytic (38±18% CD14/CD45 double positive), while 25±15% were only CD45 positive and 32±20% were double negative. There was a strong association between expression of CYP27B1 and LL37 ($r= 0.637$, $p<0.001$). In vitro treatment with 25D (100 nM, 6hrs) or 1,25D (5 nM) induced expression of LL37 in PD cells, demonstrating the ability of these cells to utilize different forms of vitamin D for antibacterial responses. After one month of vitamin D2 supplementation, patient serum concentrations of 25D rose from 19±8 ng/ml to 40±15 ($p=0.002$) but this had no effect on: 1) associated circulating markers such as PTH; 2) expression of CD14/CD45 by PD cells; 3) concentrations of LL37 in dialysis effluent. However, after adjustment for the proportion of CD14 negative/CD45 positive cells, PD cells showed significantly higher levels of LL37 expression following vitamin D supplementation (1.85-fold, $p=0.01$).

Conclusions: These data show for the first time that PD cells have a functional intracrine system for vitamin D-induced innate immunity. This response is enhanced following vitamin D supplementation in vivo, highlighting an important new function for vitamin D in preventing infectious related complications in PD patients.

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FR-OR070

Furosemide-Induced Increase of Plasma Parathyroid Hormone Is Mediated by the Calcium-Sensing Receptor in Humans Valentina Forni, Marie-Eve Muller, Carole Zweiacker, Marc P. Maillard, Michel Burnier, Olivier Bonny. *Néphrologie/Hypertension, Centre Hospitalier Universitaire Vaudois, Lausanne, Vaud, Switzerland.*

Background: Chronic oral furosemide administration has been reported to increase intact plasma parathormone (iPTH) levels in humans, alike acute furosemide injections in the rat, but the mechanisms of this interaction are still unknown. Experiments on rats suggested that acute administration of a calcimimetic could blunt this effect. We designed a prospective randomized placebo-controlled crossover study addressing the role of the calcium sensing receptor in the iPTH response to furosemide.

Methods: 12 Caucasian, non-smoker healthy males were enrolled. After 3 days of a fixed salt diet, they received either a single dose of 60 mg cinacalcet or placebo with at least one week interval. Three hours after cinacalcet, 20 mg furosemide were given iv. Plasma levels of iPTH and plasma and urinary levels of calcium, sodium and potassium were measured at baseline (before cinacalcet), before the furosemide injection and at regular time intervals (every 15 minutes for the first hour, then each hour for the next ten hours) after the administration of furosemide.

Results: Plasma iPTH levels were suppressed (38.0±12.0 ng/l vs 2.4±1.7 ng/l, $p<0.05$), and calciuria was increased 3 h after administration of cinacalcet and before furosemide injection. Under placebo, a sharp increase in plasma iPTH levels was observed as soon as15 min after furosemide injection (from 20.9±6.6ng/l before to 33.2±10.7 ng/l), while subjects under cinacalcet showed only blunted iPTH response (from 2.4± 1.7 ng/l to 3.2±2.9 ng/ml). Furosemide induced a significant decrease in plasma ionized calcium in cinacalcet-treated subjects, an effect which was absent in subjects under placebo. The changes in plasma sodium and potassium after furosemide were comparable in both cinacalcet and placebo groups.

Conclusions: These data show in humans that furosemide acutely stimulates iPTH an effect which is blunted by the administration of a calcimimetic despite a decrease in plasma ionized calcium. Changes in sodium and potassium levels do probably not play any role in the iPTH response to furosemide.

Funding: Private Foundation Support

FR-OR071

microRNAs Are Essential to the Response of the Parathyroids to Hypocalcemia but Not to a Calcimimetic or Experimental Uremia in Mice Vitali Shilo, Chofit Chai, Justin Silver, Tally Naveh-Many. *Nephrology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.*

Background: microRNAs (miRNAs) are short non-coding RNA molecules that affect protein levels by sequence-specific repression of translation and mRNA degradation.The final step in miRNA maturation is mediated by Dicer, a RNase III-like enzyme expressed in all cell types that is essential for life.

Methods: To study the role of miRNAs in the parathyroid we have generated parathyroid specific *dicer* knock-out (PT-*Dicer*$^{-/-}$) mice.

Results: Remarkably, the PT-*Dicer*$^{-/-}$ mice failed to increase serum PTH after a short-term decrease in serum Ca^{2+} by EGTA as compared to the 3-fold increase in serum PTH in the control mice. Furthermore, the PT-*Dicer*$^{-/-}$ mice failed to develop secondary hyperparathyroidism (SHPT) due to a prolonged calcium depleted diet as compared to the 10-fold increase in serum PTH levels in the control mice. Chronic hypocalcemia not only failed to increase serum in the PT-*Dicer*$^{-/-}$ mice but also PTH mRNA levels. These results show that parathyroid miRNAs are necessary for the response of the parathyroid to both acute and chronic hypocalcemia. In contrast, activation of the calcium receptor (CaR) by a calcimimetic decreased serum PTH in both PT-*Dicer*$^{-/-}$ and control mice, suggesting that activation of the CaR acts through a different pathway from hypocalcemia that does not involve miRNAs. Our results imply that the increased PTH secretion of hypocalcemia is not just due to relaxation of the parathyroid CaR but is an active process dependent upon miRNAs. Experimental uremia was induced in control and PT-*Dicer*$^{-/-}$ mice by aristolochic acid and a high phosphorus diet. Surprisingly, both PT-*Dicer*$^{-/-}$ and control mice developed SHPT indicating a different mechanism for the SHPT of CKD from that of chronic hypocalcemia.

Conclusions: Therefore, the PT-*Dicer*$^{-/-}$ mice demonstrate that miRNAs in the parathyroid are essential for the response of the parathyroids to acute and chronic hypocalemia but not for the SHPT of CKD and activation of the CaR by a calcimimetic.

FR-OR072

The HNF1β Transcription Factor Regulates Human PTH Gene Transcription Silvia Ferrè,[2] Ernie M.H.F. Bongers,[3] Elisabeth Cornelissen,[4] Kazushige Sakaguchi,[5] Yasumasa Iwasaki,[6] Gerben A. Boekel Van,[1] Jack F. Wetzels,[1] Joost G. Hoenderop,[2] René J. Bindels,[2] Tom Nijenhuis.[1] *[1]Nephrology, Radboud University Nijmegen Medical Centre (RUNMC), Netherlands; [2]Physiology, RUNMC, Netherlands; [3]Genetics, RUNMC, Netherlands; [4]Pediatrics, RUNMC, Netherlands; [5]Molecular Cell Biology, Wakayama University, Japan; [6]Endocrinology, Kochi Medical School, Japan.*

Background: Parathyroid hormone (PTH) plays a key role in Ca^{2+} and PO_4^{3-} homeostasis. Heterozygous mutations or deletions of the transcription factor HNF1β result in a heterogeneous syndrome characterized by renal cysts and diabetes (RCAD), together with a variety of other extra-renal and renal manifestations. Since we observed hyperparathyroidism in several of these patients, we tested the hypothesis of a direct role of HNF1β in the transcriptional regulation of the human PTH gene in the parathyroid gland.

Methods: We assessed 11 patients, 9 with heterozygous HNF1β whole gene deletions and 2 with frameshift mutations (c.18delG and c.883C>T). HNF1β expression in human parathyroid tissue and in a rat parathyroid cell line (PT-r) was evaluated. The HNF1β-responsiveness of the human PTH promoter and localization of putative HNF1β-responsive elements were investigated by luciferase-reporter assays and serially deleted promoter analyses.

Results: Eight out of 11 patients showed hyperparathyroidism. In one of these, the hyperparathyroidism was clearly appropriate for the level of renal function, in the others it could be discrepant. We demonstrated HNF1β expression in nuclei of Ca^{2+}-sensing receptor-positive chief cells in human parathyroid gland, and in the PT-r cell line. Co-transfection of a PTH promoter-luciferase construct with a wild-type HNF1β construct resulted in a maximal reduction of 30% of PTH promoter activity, while HNF1β mutants lacked this inhibitory property. Serial deletions in the PTH promoter construct revealed that the inhibitory effect of HNF1β resides -200/-70 bp from the transcription initiation site.

Conclusions: Our data demonstrate that HNF1β is a novel repressor of human PTH gene transcription, which could explain the apparent early development of hyperparathyroidism in patients with HNF1β mutations or deletions.

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FR-OR074

FGF23 Regulates Renal Sodium Handling and Blood Pressure Olena Andrukhova,[1] Svetlana Slavic,[1] Ute Zeitz,[1] Victoria Shalhoub,[2] Beate Lanske,[3] Reinhold Erben.[1] *[1]University of Veterinary Medicine, Vienna, Austria; [2]Amgen Inc, Thousand Oaks, CA; [3]Harvard School of Dental Medicine, Boston, MA.*

Background: Fibroblast growth factor-23 (FGF23) is a bone-derived endocrine signal regulating renal phosphate reabsorption and vitamin D synthesis. In patients with chronic kidney disease (CKD), FGF23 serum levels are associated with cardiovascular risk and mortality for unknown reasons. In earlier studies, we found that FGF23 signaling in distal renal tubules involves serum/glucocorticoid-regulated kinase 1 (SGK1) and with-no lysine kinase-4 (WNK4) which are both involved in renal sodium (Na) handling.

Methods: Here, we examined the role of FGF23 in the regulation of renal Na reabsorption in gain- and loss-of-function models.

Results: Nine-month-old compound mutant mice deficient in both *Fgf23* and vitamin D receptor (VDR) function revealed increased urinary loss of Na, relative to wild-type (WT) and VDR mutant mice. Serum aldosterone was elevated in compound mutants. Compound mutants showed reduced membrane expression of the Na-chloride cotransporter NCC, but upregulated expression of the epithelial Na channel ENaC, as measured by immunoblotting of renal membrane preparations. Treatment of WT mice with recombinant FGF23 (rFGF23) profoundly upregulated NCC membrane expression, and decreased ENaC membrane abundance as well as serum aldosterone, 8 hours post-injection. After 5 days of treatment, urinary Na excretion and urine volume was decreased, whereas systolic and diastolic blood pressure, and heart-to-body weight ratio was increased in rFGF23-treated animals. *In vitro* experiments with isolated distal tubular segments showed that rFGF23 increased NCC and decreased ENaC protein expression in an ERK1/2-SGK1 dependent fashion.

Conclusions: Taken together, our data have uncovered a previously unknown function of FGF23 in distal renal tubular Na reabsorption. Gain of FGF23 function results in renal Na retention and volume overload due to increased NCC membrane abundance, leading to hypertension and heart hypertrophy. Our data may explain why serum FGF23 is associated with cardiovascular risk and mortality in patients with CKD.

FR-OR075

FGF23 Is More Strongly Associated with Death and Recurrent Cardiovascular Events in Patients with Low Urine Phosphorus Excretion: The Heart and Soul Study Julie R. Dominguez,[1] Michael Shlipak,[2] Kenneth J. Mukamal,[3] Mary Whooley,[4] Joachim H. Ix.[1] *[1]UCSD, San Diego, CA; [2]UCSF, San Francisco, CA; [3]Beth Israel Deaconess Medical Center, Boston, MA; [4]UCSF, San Francisco, CA.*

Background: Higher fibroblast growth factor-23 (FGF23) levels are associated with cardiovascular disease (CVD) events. We explored whether this association differed in persons with more or less phosphaturia in response to FGF23.

Methods: In 896 outpatients with stable CVD, we measured serum FGF23 and 24-hour urine fractional excretion of phosphorus (FePi). Cox regression evaluated associations between FGF23, FePi, and their interaction with CVD events (MI, stroke, CVD death) and death.

Results: Mean age was 67±11 years and 82% were men. Mean baseline eGFR was 71±22 mL/min/1.73m^2. There were 183 CVD events and 295 deaths during 6.7 years (mean) follow-up. The association of FGF23 with each outcome was modified by urinary FePi (P-interactions with either outcome <0.008). In models adjusted for CVD risk factors, kidney function, and PTH, those with high FGF23 but low FePi had the highest risk of recurrent CVD events and death.

Association of FGF23 and Urine FePi Above or Below the Median with CVD Events and All-Cause Mortality

	# Events	# at Risk	HR (95% CI)
CVD Events (MI, stroke, CVD death)			
Low FGF23/Low FePi	33	249	1.00 (ref)
Low FGF23/High FePi	36	199	1.16 (0.72-1.88)
High FGF23/High FePi	61	249	1.26 (0.79-2.02)
High FGF23/Low FePi	53	199	2.09 (1.33-3.29)
All-Cause Mortality			
Low FGF23/Low FePi	56	249	1.00 (ref)
Low FGF23/High FePi	55	199	1.07 (0.73-1.57)
High FGF23/High FePi	106	249	1.54 (1.07-2.20)
High FGF23/Low FePi	78	199	2.19 (1.52-3.15)

Adjusted for age, sex, HRT use (women), diabetes, SBP, BMI, smoking, total cholesterol, HDL cholesterol, CRP, eGFR, urine ACR, PTH

Conclusions: The association of high FGF23 with CVD events and death is stronger in persons who also have low urine phosphorus excretion, independent of PTH. In such individuals, the renal tubular response to FGF23 may not be optimal. Future studies should determine whether integrated evaluation of FGF23 and urine FePi could serve as an indicator of renal tubular dysfunction.

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FR-OR076

Intβ1 Is Required for Cystogenesis in Autosomal Dominant Polycystic Kidney Disease Kim Lee,[1] Sylvia Boctor,[1] Laura M.C. Barisoni,[2] G. Luca Gusella.[1] *[1]Medicine/Div of Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Pathology, University of Miami, Miami, FL.*

Background: Dysregulation of *PKD1* leads to ADPKD, a disorder characterized by the formation and expansion of multiple bilateral cysts that ultimately result in ESRD. Increased accumulation of extracellular matrix (ECM) and the development of tubulointerstitial fibrosis parallel the progression of ADPKD. Correspondingly, cystic epithelia express higher levels of integrins, a class of heterodimeric ECM receptors. Signal transduction *via* integrins mediates various cellular responses including proliferation, migration and survival. Since these cellular processes are also altered in cystic cells, we asked whether integrins are involved in the cystic development in ADPKD.

Methods: In the kidney, integrin β1 is the major β subunit that participates in most of the integrin heterodimers. We therefore targeted *Itgb1* to ascertain the role of integrins in the context of ADPKD. A double conditional knockout mouse model was generated in which both *Pkd1* and *Itgb1* are specifically ablated in collecting ducts expressing Aqp2-Cre transgene.

Results: The single *Pkd1* knockout (KO) animals present kidneys with multiple microcysts that become overtly polycystic by week 3, leading to renal failure and death between several weeks to one year. In contrast, cystic development in the double knockout (DKO) animals is dramatically reduced with significantly fewer and smaller renal cysts present at any tested time. Cell proliferation, as measured by BrdU incorporation, is readily detectable in the *Pkd1* KO kidneys, but rarely observed in the kidneys of DKO and control wild type animals. Concomitantly, the accumulation of ECM and interstitial fibrosis apparent in the *Pkd1* KO kidneys are undetectable in the DKO kidneys. Importantly, in accordance with the phenotypic data, the renal function of the DKO animals is maintained at normal levels, allowing them to reach a longer life span as control animals.

Conclusions: Overall, these data indicate that integrin β1 is a required component of renal cystogenic process in ADPKD and that targeting the integrin signaling pathway may be an effective approach against the progression of the disease.

Funding: Other U.S. Government Support

FR-OR077

Role of NEDD9 as a Regulator of Cystogenesis in Pkd1-Conditional Knockout Model Anna Nikonova,[1] Olga Plotnikova,[1] Gregory G. Germino,[2] Erica A. Golemis.[1] *[1]Fox Chase Cancer Center, Philadelphia, PA; [2]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive cyst formation and ultimate loss of renal function, caused by mutational inactivation of polycystins 1 and 2 (PC1 and PC2, encoded by *PKD1* and *PKD2*). PC1 and PC2 function at cilia; a growing number of mutations in genes regulating ciliary function are now known to impact cystogenesis. We have recently shown that the oncogenes NEDD9/HEF1 and Aurora-A (AURKA) have unexpected actions relevant to PKD. NEDD9 activation of AURKA at the ciliary basal body controls ciliary disassembly. NEDD9 and AURKA also bind and regulate the activity of the PC2 calcium channel, influencing cytoplasmic Ca^{2+}. NEDD9 and AURKA are abundant and periodically active in normal kidneys, with AURKA expression and activity elevated in early PKD-associated renal cysts.

Methods: We have now used a tamoxifen-Cre, Pkd1-floxed mouse model (>*Piontek et al, J Am Soc Nephrol 2004, 15:3035-43*) crossed to *Nedd9*$^{-/-}$ mice to investigate the effect of eliminating NEDD9 expression on renal cyst formation.

Results: Analysis of the progression in cyst development using a combination of immunohistochemical analysis and magnetic resonance imaging (MRI) revealed that cystic burden and kidney volume increased more rapidly, and kidneys attained a greater ratio to body weight, in *Pkd1*$^{-/-}$*Nedd9*$^{-/-}$ mice in comparison with *Pkd1*$^{-/-}$ mice, although *Nedd9* ablation per se had no impact on cyst formation. Analysis of proliferation in primary kidney cells obtained from P10 mice revealed accelerated rate of growth associated with a *Nedd9*$^{-/-}$ genotype. Cilia in primary kidney and kidney cells from *Pkd1*$^{-/-}$*Nedd9*$^{-/-}$ mice were lengthened and deformed, and centrosomal defects were observed. Interestingly, endogenous Nedd9 protein levels are upregulated in kidney lysates from *Pkd1*$^{-/-}$ mice in comparison to wild type kidneys; analysis of related signaling is in progress.

Conclusions: These and other findings newly identify NEDD9 as a physiological regulator of cystogenesis, and suggest use of NEDD9 as a biomarker for prognosis.

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FR-OR078

BBSome Regulates Ciliary Trafficking of Polycystin-1 Xuefeng Su, Kaitlin Driscoll, Gang Yao, Jing Zhou. *Harvard Center for Polycystic Kidney Disease Research, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Background: The Bardet-Biedl Syndrome (BBS) is a pleiotropic disorder characterized by six primary features including renal dysfunction and cysts. Although sixteen genes have been identified, the mechanism by which mutation in these genes causes BBS remains largely unknown, particularly for the renal phenotype. Polycystin-1 (PC1) is an atypical G-protein coupled receptor whose deficiency causes autosomal dominant polycystic kidney disease (ADPKD).

Methods: Yeast two-hybrid screening was performed to identify potential interacting partners of PC1. GST pull-down studies were used to identify interaction of PC1 with different BBSome subunits. N-terminal YFP-tagged full-length PC1 construct (YFP-PC1) and a set of related constructs were generated to examine whether PC1 trafficking to the primary cilia is controlled by the BBSome. YFP-PC1 trafficking in kidney epithelial cells was evaluated by immunostaining. To evaluate the role of individual BBSome components in the ciliary trafficking of PC1, stable BBS components depleted kidney epithelial cell lines were developed by expressing respective small hairpin RNAs in lentiviral vectors.

Results: Through a yeast two-hybrid screening, we identified BBS8, a component of the BBSome, as an interacting partner of polycystin-1. GST pull-down studies revealed that PC1 interacts with four out of seven components of the BBSome. Lack of one but not other BBSome components inhibits ciliary trafficking of PC1. Further experiments using a dominant-negative ADP ribosylation factor-like small GTPase suggests a role of holo-BBSome complex in this process. Deletion analyses of PC1 defined a critical motif in the C–terminal cytoplasmic tail for the ciliary but not the plasma membrane targeting of PC1. Notably, deletion of the most distal part of PC1 C-tail containing the VxPx motif, a previously reported ciliary targeting sequence, had no detectable effect.

Conclusions: Taken together, we provide the first evidence of a biochemical association between critical players of BBS and ADPKD. We propose that distinct components of BB. Some are required for the ciliary trafficking of PC1.

Funding: NIDDK Support

FR-OR079

Polycystin-2 Ciliary Targeting Requires Polycystin-1 in a GPS-Cleavage Dependent Manner in Distal Nephrons of Developing Kidneys Hyunho Kim, Hangxue Xu, Qiong Huang, Valeriu Cebotaru, Feng Qian. *Nephrology, Johns Hopkins University SOM, Baltimore, MD.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the formation of renal cysts that arise from dysfunction of the primary cilium. It is caused by mutation in *PKD1* or *PKD2*, which encode polycystin-1 (PC1) or polycystin-2 (PC2) respectively. PC1 is regulated in part by post-transcriptional modifications *via* cleavage at the GPS domain that results in heterodimer consisting of the N-terminal fragment (NTF) and C-terminal fragment (CTF). PC1 interacts physically with PC2, a calcium-permeable cation channel. They both have been found in renal cilia and are thought to function as a ciliary mechano-sensor that is important for maintaining proper renal tubular diameter. We have previously shown that GPS cleavage of PC1 is essential

for its full biological function and is frequently disrupted in ADPKD patients.Significantly, $Pkd1^{V/V}$ knockin mice with expression of a non-cleavable PC1 (PC1V) develop distal tubular cystic kidneys postnatally and die by ~1month of age.

Results: In this study, we found that PC2 is localized at the ER and cilia in Pkd1 WT MEF cells, whereas it is present only at the ER and is absent in the cilia of $Pkd1^{V/V}$ and Pkd1 KO MEF cells. Importantly, PC2 is not stained in the cilia of precystic and cystic distal tubules of $Pkd1^{V/V}$ and $Pkd1^{cko/cko}$ postnatal kidneys, while it is detectable in the cilia of the matching WT controls. This result indicates that PC2 ciliary targeting requires PC1 in a GPS cleavage dependent manner in the distal tubules of developing kidneys. Using an inducible expression system in MDCK cells, we showed that endogenous PC2 is targeted to cilia upon expression of WT PC1, while expression of PC1V or a mutant PC1 lacking the C-terminal PC2 interaction domain does not result in PC2 ciliary targeting. Biochemically, Endo H resistant form of NTF and PC2 were found in the cilia of MDCK cells expressing WT PC1, but were absent in the cilia of cells expressing the mutant PC1.

Conclusions: We conclude PC2 ciliary trafficking requires interaction with PC1. the PC1/2 complex traffics to cilia *via* trans-Golgi in a GPS cleavage dependent manner.

Funding: NIDDK Support, Pharmaceutical Company Support - Maryland NKF

FR-OR080

Polycystin-1 Regulates Polycystin-2 Dephosphorylation at a Critical Residue Important during Development and Disease Andrew J. Streets,[1] Oliver Wessely,[3] Dorien J.M. Peters,[2] Albert C. Ong.[1] *[1]Kidney Genetics Group, Academic Unit of Nephrology, Sheffield Kidney Institute, University of Sheffield, Sheffield, United Kingdom; [2]Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; [3]Department of Cell Biology, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Mutations in *PKD1* (85%) or *PKD2* (15%) account for almost all cases of autosomal dominant polycystic kidney disease (ADPKD). The ADPKD proteins, termed polycystin-1 (PC1) and polycystin-2 (PC2), interact via their C-termini to form a receptor-ion channel complex which regulates kidney tubulogenesis. PC2 is a member of the TRP superfamily of channel proteins whose function is regulated by phosphorylation. The study aimed to determine whether PC2 phosphorylation is regulated by PC1, which if pathologically increased might represent a therapeutic target in PKD1 patients.

Methods: LC MS/MS Mass Spec, phosphoserine antibody mapping, calcium imaging and Xenopus rescue experiments.

Results: LC MS/MS Mass Spec identified a PC2-Ser^{829} phosphorylation site. A new phosphospecific antibody to this site demonstrated that Ser^{829} is phosphorylated by two different protein kinases, Protein kinase A (PKA) or Aurora A (AurA), and dephosphorylated by Protein phosphatase 1 (PP1). Ser^{829} phosphorylation by PKA or AurA showed distinct cell cycle and subcellular organisation, however it was constitutively phosphorylated in cells and tissues lacking PC1. We show that Ser^{829} dephosphorylation is regulated by PC1 binding and the the recruitment of PP1. Ser^{829} phosphorylation was essential for Xenopus pronephric development but when constitutively overexpressed, was associated with enhanced ATP-dependent ER Ca^{2+} release.

Conclusions: These results reveal a new functional dynamic link between PC1 and PC2 which is critically dependent on their interaction. Targeting PC2 phosphorylation could represent a promising new therapeutic approach in the treatment of PKD1 patients.

Funding: Government Support - Non-U.S.

FR-OR081

miR-17~92 miRNA Cluster Promotes Kidney Cyst Growth through Repression of Cystic Kidney Disease Genes Vishal Patel, Darren Williams, Sachin S. Hajarnis, Peter Igarashi. *Internal Medicine, UT Southwestern, Dallas, TX.*

Background: microRNAs (miRNAs) are short, non-coding RNAs that negatively regulate post-transcriptional gene expression. Dysregulation of miRNA expression has been observed in animal models of PKD, but the roles of miRNAs in the pathogenesis of PKD remain unclear.

Methods: miRNA microarrays and qRT-PCR analysis were performed on kidneys from Ksp/Cre;$Kif3a^{F/F}$ (*Kif3a*-KO) mice, an animal model of PKD. Transgenic mice that either overexpress miR-17~92 or lack miR-17~92 in renal tubules were produced by Cre/loxP recombination. TargetScan was used to identify candidate miR-17~92 targets, which were validated by mRNA microarrays, qRT-PCR, and luciferase reporter assays.

Results: Microarray analysis showed increased expression of the miR-17~92 miRNA cluster in cystic kidneys from *Kif3a*-KO mice. Upregulation of miR-17~92 was also observed in three other mouse models of PKD. Transgenic overexpression of miR-17~92 in the kidney promotes proliferation of tubular epithelial cells, reduces the abundance of mRNAs encoded by *Pkd1*, *Pkd2*, and *Hnf-1*β, and produces kidney cysts. Conversely, deletion of miR-17~92 in the renal tubules of *Kif3a*-KO mice decreases proliferation, retards kidney cyst growth, improves renal function, and prolongs survival. Members of the miR-17~92 cluster bind to the 3'UTRs of mRNA transcripts encoded by *Pkd1*, *Pkd2*, and *Hnf-1*β and inhibit the expression of a linked reporter gene. Genome-wide expression profiling combined with bioinformatics analysis identified 258 additional miR-17~92 targets that may have relevance to PKD pathogenesis.

Conclusions: The miR-17~92 miRNA cluster is overexpressed in PKD. Overexpression of miR-17~92 is sufficient to produce kidney cysts, whereas inhibition of miR-17~92 retards cyst growth. miR-17~92 mediates these effects through repression of cystic disease genes. These findings demonstrate that miR-17~92 plays a key role in the progression of PKD in vivo and provide a strong rationale for the development of inhibitors of miR-17~92 as therapeutic agents in PKD.

Funding: NIDDK Support

FR-OR082

Crosstalk between Bicaudal-C and miRNAs in the Formation of Polycystic Kidney Disease Oliver Wessely, Debora M. Cerqueira, Uyen Tran. *Lerner Research Institute/Cell Biology, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Polycystic Kidney Diseases are the leading cause of end-stage renal failure and require extensive treatments, such as dialysis and kidney transplantation. Among the many animal models to study the pathogenesis of these diseases mice carrying mutations in the RNA-binding molecule Bicaudal-C (Bicc1) have been very valuable to understand multiple aspects of the disease. Indeed, Bicc1 has turned out to be an evolutionary conserved key player in polycystic kidney disease as loss-of-function causes cyst formation in humans, mice and even the amphibian *Xenopus*. In the kidney, Bicc1 modulates the expression of Polycystin-2 (Pkd2). Bicc1 acts as a post-transcriptional and regulates the stability of *Pkd2* mRNA. Moreover, molecular analyses demonstrate that the *miR-17* microRNA family represses Pkd2 and that Bicc1 antagonizes this activity.

Results: Here we provide additional data on the *in vivo* importance of this antagonism. Assaying the expression levels of the *miR-17* miRNA family members by small RNA sequencing demonstrated that only the miRNAs encoded in the *miR-17-92* and the *miR-106b-25* cluster were expressed in the kidney. Moreover, their levels were not altered in Bicc1 mutant mice. This implied that the antagonism between Bicc1 and the *miR-17* family was at the target gene level. It also suggested that lowering miRNA levels rescues the effects of loosing Bicc1. This hypothesis was addressed using mouse compound mutants. We initially focused on reducing the *miR-17* miRNA dose by analyzing *Bicc1*/*miR-17-92* compound mutant mice. Interestingly, removing only one copy of *miR-17-92* was already sufficient to dramatically reduce cystogenesis. The overall kidney volumes were reduced to almost wild type levels at E18.5. Transcriptional profiling by mRNA sequencing validated this effect. The kidney expression levels of several of the mRNAs altered in Bicc1 mutant mice were restored to wild-type levels in the compound mutant mice.

Conclusions: Together these data suggest miRNAs are a crucial component of the epithelial cell program disturbed in Polycystic Kidney Disease and that this program is disrupted in Bicc1 mutant mice.

Funding: NIDDK Support

FR-OR083

MCP-1 Is the Primary Macrophage Recruitment Factor Produced by Human and Mouse Polycystic Kidney Cells and Promotes Disease Progression in *cpk* Mice Katherine Swenson-Fields, Carolyn J. Vivian, Darren P. Wallace, Timothy A. Fields. *The Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Background: High concentrations of infiltrating macrophages (MΦs) are present within the kidneys of patients with autosomal dominant polycystic kidney disease (ADPKD) and mice with PKD. Studies using PKD mouse models have suggested that these MΦs, which in the context of kidney injury are known to promote tissue repair, act in the cystic kidney to promote cyst expansion and disease progression. Monocyte chemotactic protein 1 (MCP-1) has been found in high concentrations in the cyst fluids and urine of ADPKD patients, but it is not clear what role this factor plays in PKD pathophysiology. In this study, we assessed the contribution of MCP-1 to human ADPKD cyst cell- and mouse congenital polycystic kidney *(cpk)* cell-stimulated macrophage migration. We also used knockout mice to assess the contribution of MCP-1 to disease progression in the *cpk* mouse model.

Methods: Conditioned media (CM) from primary ADPKD cyst and *cpk* mouse kidney cells was collected and tested for monocyte chemoattractive activity (MCA) using a transwell migration assay of THP-1 cells in the presence or absence of neutralizing antibody against MCP-1. Heterozygous +/*cpk* mice were bred with MCP-1(*Ccl2*)$^{-/-}$ mice to generate +/*cpk*:*Ccl2*$^{-/-}$ mice. These were interbred to generate *cpk*/*cpk*:*Ccl2*$^{-/-}$ mice. Blood urea nitrogen (BUN) was measured on P10.

Results: Both primary ADPKD cyst cells and *cpk* kidney cells produced robust MCA. The majority of this MCA was blocked by the presence of maximally effective concentrations of neutralizing antibody to MCP-1 but not by equivalent concentrations of control Ig. To determine the contribution of MCP-1 to disease progression in *cpk* mice, homozygous *cpk* strains also deficient for MCP-1 were generated. MCP-1 knockout significantly improved both renal function (BUN of 51 ± 6 mg/dL vs 69 ± 4 mg/dL; p=0.038) and survival (21 ± 0.9 d vs 18 ± 0.3 d; p=0.04). for *cpk*/*cpk*:*Ccl2*$^{-/-}$ vs *cpk*/*cpk*:*Ccl2*$^{+/+}$ mice.

Conclusions: Therapeutic treatments that block MCP-1 production or neutralize its activity may be viable strategies to block disease progression in PKD.

Funding: NIDDK Support

FR-OR084

A Single Amino Acid Deletion in the Polycystin-1 (PC1) C-Tail Affects G-Protein Signaling and Causes PKD in Mice Stephen C. Parnell,[1] Brenda S. Magenheimer,[1] Robin L. Maser,[1] Mallory A. Havens,[2] Lynn Magenheimer,[1] Michelle Hastings,[2] James P. Calvet.[1] *[1]Kidney Inst, Univ of Kansas Med Ctr, Kansas City, KS; [2]Dept of Cell Biol and Anatomy, Rosalind Franklin Univ, Chicago, IL.*

Background: An ADPKD-associated mutation causing deletion of a single leucine residue within the PKD1 gene has been reported (L4131Δ, Afzal et al Hum Genet 1999 105:648), but is not known to be pathogenic. L4131 is located within a highly conserved region in the C-tail of PC1 adjacent to the G-protein activation sequence (Parnell et al J Biol Chem 2002 277:19566). To determine if L4131Δ is pathogenic, a knock-in mouse was made.

Methods: The corresponding mouse mutation (L4122Δ) was introduced by targeted insertion of a construct containing a floxed NEO cassette in Pkd1 intron 45 (Pkd1NEO). Mice carrying Pkd1NEO were bred with the EIIA-Cre mouse to generate the Pkd1ΔL allele. Matings with Pkd1NEO/+, Pkd1m1Bei/+ and Pkd1ΔL/+ mice were examined for cystic offspring and were used for metanephric organ culture to assay cAMP-mediated cyst formation. Splicing of intron 45 was analyzed by RT-PCR.

Results: While Pkd1NEO/+ and Pkd1ΔL/+ mice were normal, no Pkd1NEO/NEO, Pkd1NEO/m1Bei or Pkd1ΔL/ΔL pups were born, suggesting that the Pkd1NEO and ΔL alleles were embryonic lethal. RT-PCR demonstrated that the floxed NEO cassette prevented splicing of intron 45, consistent with the generation of a functionally null allele. However, RT-PCR showed that the ΔL allele was properly spliced. E15.5 embryos from Pkd1ΔL/+ crosses were generated for metanephric organ culture. Pkd1ΔL/ΔL embryos were edematous and had pancreatic cysts. Kidneys from ΔL/ΔL embryos showed massive cyst-like dilations with cAMP treatment as previously shown for Pkd1m1Bei/m1Bei kidneys. PC1ΔL protein expression was detected by immunofluorescence. Transient transfection assays showed that a membrane-anchored PC1 L4122Δ C-tail construct was significantly impaired in PC1-induced signaling to AP1 and NFAT promoter-reporter constructs, but retained the ability to interact with co-immunoprecipitated polycystin-2.

Conclusions: These results demonstrate that the human L4131Δ mutation is pathogenic, and suggest that disruption of G-protein signaling is sufficient to cause PKD.

Funding: NIDDK Support

FR-OR085

PKD1 Mutation Type, but Not the Mutation Location, Influences Renal Outcome in Autosomal Dominant Polycystic Kidney Disease

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Background: Age of end stage renal disease (ESRD) is highly variable in Autosomal Dominant Polycystic Kidney Disease (ADPKD). The aim of this work was to study the influence of the identified mutations on the renal outcome.

Methods: Genkyst is a registry including ADPKD patients from Brittany in France. Clinical and genetic data were collected in a population of 609 patients from 436 pedigrees. Mutation analysis was performed by direct sequencing of *PKD1* and *PKD2* coding sequences, and Quantitative Fluorescent Multiplex PCR and Array-CGH were performed to detect large rearrangements. Renal survival and median age at ESRD were studied for each mutational group, using Kaplan-Meier method. The influence of the position of the mutation in *PKD1* gene was also studied.

Results: A mutation was identified in 90.4% of the pedigrees, which is the highest mutation detection rate ever reported. 248 of the 609 patients of our cohort had reached ESRD, at the median age of 60.6 yrs. No gender effect was observed. Median age of ESRD was 57.5 yrs [55.7-59.2] when a *PKD1* mutation was implied vs 78.0 yrs [65.9-90.0] for *PKD2* mutations (p<0.0001). Among *PKD1* mutations, truncating ones (65.5%) were associated with poor renal survival, with a median age of ESRD at 55.4 yrs [53.2-57.6]. Conversely, mutations with no truncating effect (33.5%) led to ESRD 10 years later (median age 65.5 yrs [56.7-74.3], p<0.0001). The location of the mutation, in relation to the median nucleotide position, was not associated with the age at onset of ESRD (p=0.87).

Conclusions: We report here one of the largest cohorts of ADPKD patients with genetic data, and for the first time, a high influence of *PKD1* mutation type on the severity of the renal disease. This is an important finding, as targeted therapies are on the way, and make the identification of such prognostic factors crucial.

Funding: Government Support - Non-U.S.

FR-OR086

Ablation of Healthy Adult Kidney Pericytes and Fibroblasts In Vivo Leads to Acute Kidney Injury with Features of Early Acute Tubular Necrosis

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Background: Kidney pericytes and perivascular (PV) fibroblasts (Foxd1-derived, PDGFRb+, CD73+ Col1a1+ cells) are progenitors of scar-forming myofibroblasts. Pericytes are attached to endothelial cells & play important roles in vascular stabilization. After kidney injury, pericytes detach, migrate & differentiate into myofibroblasts, leaving an unstable endothelium, prone to rarefaction. Pericytes are derived from embryonic metanephric mesenchyme that activates a lineage restricted Fork-head transcription factor Foxd1.

Methods: We used a Cre-LoxP system to activate permanent diphtheria toxin receptor (DTR) expression in cells (& their daughters) which had expressed Foxd1 in nephrogenesis. Healthy adult mice expressing the transgene or controls were treated with IP injections of Diphtheria Toxin (DT). Tissues, blood, urine harvested at timepoints after injection. Mice with kidney injury and fibrosis were also subjected to ablation with DT at timepoints after injury.

Results: In healthy adult kidney, DT ablated >95% PDGFRb+ cells without effect on podocyte number at 48h & 72h in mice with the DTR. Controls unaffected. 60% of mice with ablation died at 72h, the remainder recovered. At 48h there was an increase in plasma Creatinine from 0.15 to 0.6mg/dL and a small decrease in BP in mice with ablation. Histologically, proximal tubules exhibited loss of polarization & severe vacuolization particularly basolaterally. Kidney microvasculature remained largely intact. In mice with kidney injury & fibrosis (UUO or unilateral IRI models), ablation of myofibroblasts DT on d8 resulted in 80% loss of aSMA myofibroblasts & >85% reduction in interstitial fibrosis on d10.

Conclusions: Acute ablation of Foxd1 derived pericytes & (PV) fibroblasts leads to loss of nephron function & acute proximal tubule injury. However, ablation of pericyte/fibroblast-derived myofibroblasts is potently anti-fibrotic.

Funding: NIDDK Support, Pharmaceutical Company Support - Genzyme GRIP Award

FR-OR087

TRPM2 Channels Activate Rac1 and Contribute to Ischemic AKI

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Background: TRPM2 (Transient receptor potential melastatin 2) is a non-selective cation channel activated by oxidative stress. Using TRPM2 deficient mice and TRPM2 inhibitors, we recently demonstrated a role for TRPM2 in mediating renal ischemic AKI. The present studies examined possible mechanisms whereby TRPM2 contributes to AKI. Oxidant stress plays a role in ischemia/reperfusion injury. Rac1, a small GTP-binding protein, is a subunit of the NADPH oxidase complex and is involved in its activation. The possible role of Rac1 in ischemic AKI and the interactions between Rac1 and TRPM2 are unknown.

Methods: For *in vitro* studies, primary cultures of proximal tubule epithelial cells (PTC) were prepared from wild type WT) and TRPM2 deficient (KO) mice. Cells were stressed with either H_2O_2 or hypoxia-reoxygenation. Cell viability was determined using the MTS assay and Rac1 activity using a pull-down assay. For *in vivo* studies, mice were subjected to 28 minutes of bilateral renal ischemia. BUN and creatinine were determined 24 hrs later.

Results: Rac1 was strongly activated by H_2O_2 in WT PTCs but not in TRPM2 KO PTCs. In addition, KO cells were resistant to hypoxic and oxidant-induced cell death. Likewise, WT kidneys subjected to IRI *in vivo* demonstrated greater activation of Rac1 than kidneys of KO mice. To investigate the role of Rac1 in IRI-induced AKI, we treated mice with a Rac1 inhibitor, NSC23766, prior to IRI. Mice treated with NSC23766 had better preservation of kidney function than vehicle-treated mice (BUN=53±13 vs 147±23 mg/dl, p<0.02; cr=0.37±0.08 vs 1.49±0.16 mg/dl, p<0.002).

Conclusions: These results indicate that Rac1 is activated during ischemic and oxidant stress to renal epithelial cells and that the TRPM2 channel modulates the activation of Rac1. These results also provide the first evidence for a role of Rac1 in mediating ischemic AKI *in vivo*. We also show that activation of Rac1 may account for some of the pathogenic actions of TRPM2 in ischemic AKI. Inhibition of TRPM2 or Rac1 may have an important impact in reducing kidney injury and preserving kidney function in AKI.

Funding: Pharmaceutical Company Support - Genzyme Corporation

FR-OR088

Regulation of Autophagy in Renal Ischemia-Reperfusion Injury: Orchestration of Multiple Signaling Pathways

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Background: Autophagy is induced during acute kidney injury (AKI), but its role in AKI remains debated and the regulatory signaling pathways are poorly understood.

Methods: We determined the role of autophagy in AKI using a conditional knockout mouse model, in which Atg7 was specifically deleted from proximal tubules. We further investigated the signaling mechanisms that regulate autophagy during ischemic AKI in vivo and hypoxia-reoxygenation in vitro in cultured renal tubular cells.

Results: Conditional knockout of Atg7 resulted in impaired autophagy and aggravated AKI following renal ischemia-reperfusion or cisplatin treatment, supporting a renoprotective role of tubular cell autophagy. In cultured rat proximal tubular cells (RPTC) during hypoxia-reoxygenation. Hypoxia-reoxygenation induced autophagy as indicated by LC3-II accumulation and GFP-LC3 puncta formation. AMPK, JNK, p38 and ULK1 were activated during hypoxia, whereas mTOR was inactivated, as shown by their phosphorylation status. Blockade of ULK1, JNK or AMPK by shRNA or pharmacological inhibitors partially suppressed LC3-II accumulation during hypoxia, while inhibition of p38 was not effective. After reoxygenation, hypoxia-induced changes of AMPK, mTOR and ULK1 were mostly abolished, but JNK and p38 remained active and ERK became activated. Under this condition, inhibition of either p38 or JNK, but not ERK, attenuated autophagy. The changes of these signaling pathways were further observed in kidney tissues after renal ischemia-reperfusion in mice.

Conclusions: Together, these results suggest that AMPK, mTOR, JNK and ULK1 may initiate autophagy during ischemia/hypoxia, while the induction and maintenance of autophagy following reperfusion/reoxygenation may depend on JNK and p38.

Funding: NIDDK Support, Veterans Administration Support

FR-OR089

Loss of Acid Sphingomyelinase Causes Defective Autophagy-Induction *In Vivo* and Sensitizes for Renal Ischemia/Reperfusion Injury Andreas Linkermann,[1] Signe Diness Vindelov,[2] Elisabeth Corcelle-Termeau,[2] Jan H. Bräsen,[3] Ulrich Kunzendorf,[1] Stefan Krautwald,[1] Marja Jaattela.[2] *[1]Clinic for Nephrology and Hypertension, Christian-Albrechts University, Kiel, Germany; [2]Unit of Cell Death and Metabolism and Centre for Genotoxic Stress Research, Danish Cancer Society Research Center, Copenhagen, Denmark; [3]Department of Pathology, Christian-Albrechts University, Kiel.*

Background: Niemann-Pick disease (NPD) is characterized by reduced activity of acid sphingomyelinase (ASMase). Autophagy is a renoprotective mechanism in acute kidney injury (AKI). In this study, we define ASMase as a critical component for the induction of autophagy. ASMase is inhibited by diverse commonly used drugs, but nothing is known about ASMase-inhibition in AKI.

Methods: *In vitro*, we investigated cells from NPD patients and from cancer cell lines for their capacity to initiate autophagy using electron microscopy and monitored autophagic flux by p62 and LC3 western blots. *In vivo*, we analysed basal levels of autophagy in ASMase-deficient mice by IHC and western blotting and challanged these mice in a model of renal ischemia/reperfusion injury (IRI). IRI was also performed in wt mice that were pre-treated with the ASMase-inhibitor trimipramin (Stangyl®).

Results: We identified inhibitors of ASMase activity as indirect inhibitors of autophagy and demonstrate ASMase activity to be prerequisite for the closure of the autophagophore. In ASMasedeficient mice, we detected markedly reduced basal levels of autophagy in all organs examined, resulting in severe sensitivity for IRI in both AKI and survival readouts. Similar effects were seen in wt mice that were pretreated with timipramine.

Conclusions: We identified ASMase as a component that is required for the progression of autophagic flux on the level of autophagophore closure. Desipramine and trimipramine inhibited ASMase activity in vitro and in vivo, respectively, the latter of which sensitises mice to AKI comparable to the sensitization of ASMase-ko mice. The autophagy inhibitory capacity of tricyclic antidepressants opens new possibilities for cancer treatment but opposes the use of these drugs in patients at risk for cardiovascular events.

Funding: Private Foundation Support

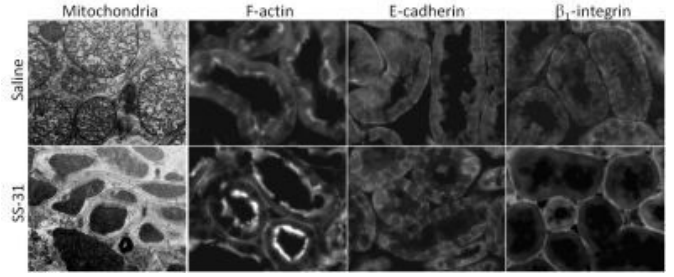
FR-OR090

Mitochondria-Targeting Peptide (SS-31) Promotes Rapid Repair of Actin Cytoskeleton Following Ischemia and Protects Tubular Epithelial Cell Architecture Hazel H. Szeto,[1] Shaoyi Liu,[1] Yi Soong,[1] Surya V. Seshan.[2] *[1]Pharmacology, Weill Cornell Medical College, New York, NY; [2]Pathology, Weill Cornell Medical College, New York, NY.*

Background: Actin cytoskeletal disruption is a hallmark of ischemic injury. Rapid restoration of ATP is essential for the maintenance of the tubular epithelial cell structure after ischemic insult. SS-31 is a mitochondria-targeting tetrapeptide that has been shown to accelerate ATP recovery, reduce oxidative stress, and minimize ischemic kidney injury (Szeto et al., JASN 22:1041-1052, 2011). Here we show that SS-31 preserves mitochondrial cristae during ischemia and protects epithelial cell architecture upon early reperfusion.

Methods: Sprague-Dawley ratswere assigned to sham, IR+saline and IR+SS-31 (n=4). Rats were subjected to bilateral renal ischemia for 30 min followed by 0, 5, 20 or 60 min reperfusion. SS-31 (2.0 mg/kg, sc) was administered 30 min before ischemia and immediately before reperfusion. Kidneys were harvested for histopathology.

Results: 30 min ischemia caused mitochondrial swelling with loss of cristae in the saline group, while SS-31-treated kidneys showed preservation of mitochondrial cristae. F-actin, E-cadherin and b_1-integrin were internalized into the cytosol in both saline and SS-31 groups after 30 min ischemia. However, rapid redistribution of F-actin, E-cadherin and b_1-integrin were observed in the SS-31 group as early as 5 min after reperfusion while they remained internalized in the saline group. Electron microscopy also revealed significant protection of the capillary endothelium in the SS-31 group.



Conclusions: SS-31 protects mitochondrial cristae during ischemia and accelerates recovery of ATP for maintenance of cell structure and viability.

Funding: Pharmaceutical Company Support - Stealth Peptides Inc

FR-OR091

Identification of Proton Pump Inhibitors as Novel Therapy for the Prevention of Acute Kidney Injury Almut Grenz, Eunyoung Tak, Douglas Ridyard, Holger Eltzschig. *Anesthesiology, UC Denver, Denver, CO.*

Background: During renal ischemia, shifts in the metabolic supply to demand ratio – particularly for oxygen – result in severe tissue hypoxia. Cellular responses to hypoxia are regulated by enzymes that sense cellular oxygen levels and coordinate transcriptional responses to hypoxia or ischemia. Central among these enzymes are three oxygen-sensing prolyl hydroxylases (PHD1-3). Limited oxygen availability results in inhibition of PHDs with subsequent stabilization of hypoxia-inducible factors (HIFs). Activation of HIF results in a transcriptionally regulated response that re-programs cellular metabolism towards hypoxia adaptation. Systematic studies in mice with genetic deletion of PHD's pointed us towards a very surprising role of the renal proton pump ATP4A.

Methods: Gene targeted mice for PHD1, 2 and 3 ($PHD1^{-/-}$, $PHD2^{+/-}$, $PHD3^{-/-}$) were studied in an ischemic model of acute kidney inury (AKI) by utilizing a hanging weight system. Renal function was determined by FITC-labeled inulin clearance, serum creatinine, renal cytokine levels, renal myeloperoxidase (MPO ELISA) and histology.

Results: Here, we exposed gene-targeted mice for *Phd1*, *Phd2* or *Phd3* to AKI and assessed renal function by measuring glomerular filtration rate (GFR), or renal histology. Indeed, we found a selective phenotype in *Phd1-/-* mice with remarkable protection from ischemic AKI. To gain mechanistic insight into how *Phd1* deletion protects the kidneys from ischemia, we performed microarray studies. The most profound difference in gene expression was an over 10 fold repression of Atp4a, when comparing ischemic kidneys from *Phd1-/-* mice with controls. Subsequent studies with pharmacologic ATP4A inhibitors (esomeprazole, SCH28080) mimicked the kidney protection from ischemia seen in *Phd1-/-* mice. Moreover, *Atp4a-/-* mice were remarkably protected from renal ischemia.

Conclusions: Based on these findings we hypothesize that proton pump inhibitors can be used to prevent or treat ischemic AKI as occurs in perioperative patients.

FR-OR092

TRAPing Cell Type-Specific Signatures in Acute Kidney Injury A. Michaela Krautzberger,[1,2] Jing Liu,[1,2] Shannan J. Ho Sui,[3] Oliver Hofmann,[3] Winston Hide,[3] Andrew P. McMahon.[1,2] *[1]Dept. of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; [2]Broad CIRM Center, University of Southern California, Los Angeles, CA; [3]Dept. of Biostatistics, Harvard School of Public Health, Boston, MA.*

Background: Acute kidney injury (AKI) can be triggered by multiple insults including hypoxia, sepsis and nephrotoxic drugs. Identifying novel biomarkers and therapeutic targets requires a better understanding of the complex molecular and cellular interactions underlying the pathogenesis of AKI.

Methods: We have generated a new mouse strain enabling Cre-dependent expression of an eGFP-tagged ribosomal protein (eGFP-L10a) that allows extraction of mRNA by *t*ranslating *r*ibosome *a*ffinity *p*urification (TRAP) for subsequent profiling by microarray or RNA-seq. To determine cell type-specific signatures during early damage and later regenerative phases of AKI, we combine the TRAP methodology with an ischemia-reperfusion injury (IRI)-generated AKI model in mice.

Results: Six2-, Foxd1-, Cdh5-, and Lyz2-Cre driver strains were used to activate eGFP-L10a expression in nephron epithelial, interstitial, endothelial, and macrophages/dendritic cells, respectively. Immunofluorescent analysis confirmed transgene expression in the expected cell population, and an initial proof-of-principle experiment using TRAP-extracted RNA from untreated kidneys demonstrated significant population-specific GO term enrichment. We next examined cell type-specific signatures 24 hours after bilateral IRI. Bioinformatic analysis revealed a large number of genes deregulated in specific target cell populations. In addition, responses shared in two or more cell compartments were identified. Expression changes of top candidates were confirmed by real-time qPCR and section *in situ* hybridization at the RNA level, and by immunostaining at the protein level.

Conclusions: We have generated and validated a novel system for defining cell type-specific translational profiles during AKI. This approach will help to elucidate the complex mechanisms involved in AKI, and thus facilitate the discovery of new biomarkers and potential therapeutic strategies.

Funding: Other NIH Support - R37DK054364

FR-OR093

MicroRNA-668 Is Induced during Renal Ischemia-Reperfusion to Protect against Acute Kidney Injury Qingqing Wei, Zheng Dong. *Department of Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center.*

Background: MicroRNAs are important regulators of gene expression in a variety of physiological as well as pathological conditions.

Methods: Here we investigated the role of specific microRNAs in ischemic acute kidney injury (AKI) using both in vivo animal and in vitro proximal tubular cell models.

Results: We showed that conditional knockout of Dicer (a microRNA biogenesis enzyme) from proximal tubular cells resulted in microRNA depletion and protected mice from ischemic AKI, supporting a critical pathogenic role of microRNAs. Further microarray analysis identified a dozen of microRNAs that were significantly changed during ischemic AKI in mice. mir-668 was one of the microRNAs that were significantly up-regulated in ischemic AKI. The induction of mir-668 in ischemically injured kidney tissues was further confirmed by real-time PCR mir-668 was also induced by hypoxia in cultured rat renal proximal tubular cells (RPTCs). Hypoxic induction of mir-668 was diminished in

HIF-1-deficient cells, suggesting a role of HIF-1 in the inductive response. Functionally, transfection of anti-mir-668 in RPTC cells induced apoptosis. In vivo in C57BL/6 mice, anti-mir-668 treatment increased AKI after ischemia-reperfusion as indicated by renal function and histological examination.

Conclusions: Together, the results suggest that mir-668 may be induced via HIF-1 to protect against ischemic AKI.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-OR094

MMP-2 Activation Perpetuates Injury in AKI Timothy A. Sutton, Henry Mang, Nicci Knipe. *Department of Medicine/Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

Background: Ischemic acute kidney injury (AKI) is characterized by a complex interplay of tubular injury, inflammation and microvascular alterations. We have previously shown that in a rodent model of ischemic AKI, gelatinases {matrix metalloproteinases (MMP)-2 and -9} are activated and this activation is associated with increased microvascular permeability. However, genetic deletion of MMP-9 alone did not confer protection of renal function as determined by serum creatinine.

Methods: To further investigate the selective role of MMP-2 in ischemic AKI we utilized a 19 minute bilateral artery clamp model of AKI (RAC) to examine the effect of pharmacologic MMP-2/9 inhibition in the MMP-9 knockout (KO) mouse on kidney and microvascular function.

Results: MMP-9 KO mice administered the inhibitor had a significantly lower mean creatinine (0.74 mg/dL) as compared to the MMP-9 KO mice (2.1 mg/dL, p=0.02) 24 hours following injury. In addition, altered microvascular permeability was significantly improved in the MMP-9 KO mice administered the inhibitor compared to the MMP-9 KO mice as determined by an Evans blue dye assay (314 vs. 448 mcg EBD/g dried tissue, p=0.04). Similar protection was observed in FVB-NJ background (WT) mice administered the inhibitor in the RAC model. These findings support the importance of MMP-2 activation in the early phase of ischemic AKI. To further delineate the cellular source of gelatinase activity in ischemic AKI, we utilized bone marrow transplantation to generate chimeric mice. Surprisingly, we found that chimeric mice generated by transplanting MMP-9 null bone marrow into irradiated WT mice (MMP-9 KO into WT) demonstrated functional protection following acute ischemic injury (serum creatinine 1.37 mg/dL, $p<0.05$) as compared to WT into WT (2.15 mg/dL) and WT into KO (2.13 mg/dL) chimeric mice. Furthermore, altered microvascular permeability was improved in the KO into WT as compared to the WT into WT (268 vs. 488 mcg EBD/g dried tissue, $p> 0.1$).

Conclusions: Together these data suggest that MMP-2 activation is an important pathophysiologic mechanism in ischemic AKI that can be partially mitigated by selective MMP-9 inhibition in radiosensitive bone marrow cells.

Funding: NIDDK Support, Private Foundation Support

FR-OR095

Tubular Kidney Injury Molecule-1 Mediated Phagocytosis Downregulates the Kidney's Innate Immune Response after Injury Li Yang,[1,2] Craig R. Brooks,[1] Sheng Xiao,[3] Venkata Sabbisetti,[1] Takaharu Ichimura,[1] Vijay K. Kuchroo,[3] Joseph V. Bonventre.[1] *[1]Renal Division, Brigham and Women's Hospital and Harvard Medical School; [2]Renal Division, Peking University First Hospital, Beijing, China; [3]Center for Neurologic Disease, Brigham and Women's Hospital, Harvard Medical School.*

Background: Kidney Injury Molecule-1 (Kim-1) is upregulated more than any other protein in the renal proximal tubule in acute kidney injury (AKI), and is a phosphatidylserine receptor, which mediates phagocytosis of apoptotic cells. This study aimed to investigate whether Kim-1 facilitated phagocytosis of apoptotic cells is protective against AKI.

Methods: Wild-type (WT) and Kim-1$^{\Delta mucin}$ mice were exposed to bilateral ischemia/reperfusion(IRI) or cisplatin injection. The Kim-1$^{\Delta mucin}$ mouse expresses a mutant protein lacking the mucin domain. Serum creatinine and tubular necrosis were analyzed after injury. Bone marrow (BM) transplantation was performed to determine the relative importance of tubular vs BM-derived Kim-1 in the pathogenesis of kidney IRI. Proximal tubular cells derived from the WT and Kim-1$^{\Delta mucin}$ mice were exposed to LPS or TNF-α with or without apoptotic cells pre-feeding. Inflammatory cytokines, toll-like receptors (TLR), anti-inflammatory growth factors were evaluated.

Results: Proximal tubular cells expressing Kim-1$^{\Delta mucin}$ had markedly decreased ability to phagocytose apoptotic cells compared to WT Kim-1 expressing cells. Kim-1$^{\Delta mucin}$ mice had greater functional impairment, inflammation and mortality in response to IRI and cisplatin. The differential susceptibility to IRI between WT and Kim-1$^{\Delta mucin}$ mice was attributed to the kidney parenchymal cells as determined with BM chimeric mice. In cell culture, Kim-1-mediated phagocytosis of apoptotic cells resulted in decreased proximal tubular cell inflammatory cytokines and TLR4 production, and increased anti-inflammatory growth factor secretion which together decrease the inflammatory activity of macrophages. By contrast the Kim-1$^{\Delta mucin}$ cells had markedly impaired anti-inflammatory function.

Conclusions: Kim-1-mediated epithelial cell phagocytosis protects the kidney from acute injury by downregulating innate immunity.

Funding: NIDDK Support

FR-OR096

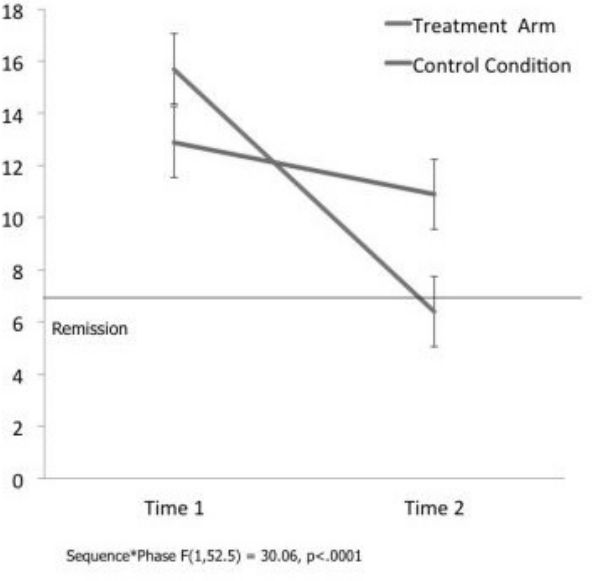
Depression, Quality of Life and Fluid Adherence Are Improved by Psychosocial Intervention: Results of an RCT in Two Urban Hemodialysis Centers Daniel Cukor,[1] Nisha Ver Halen,[1] Subodh J. Saggi,[2] Paul L. Kimmel.[3] *[1]Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY; [2]Medicine, SUNY Downstate Medical Center, Brooklyn, NY; [3]Medicine, George Washington Uninversity, Washington, DC.*

Background: Depression is the primary mental health concern of ESRD patients, and is associated with lower QOL, decreased adherence, and decreased survival. Antidepressants have not been well tested in ESRD patients.

Methods: A 10 session individual cognitive behavioral intervention (CBT) aimed at reducing depression was compared to a wait-list control. Secondary goals were to determine if the CBT had positive effects on quality of life (KDQOL) and fluid compliance (IDWG).

Results: 63 depressed subjects on HD for at least 6 months enrolled. 59 completed all assessments. The sample was 94% Black, 72% female, 30% diabetic and been on dialysis 4.1 ± 2.8 years. Baseline depression was 15.2 ± 8.9 (moderate) on a clinician-administered measure (Hamilton Depression). There was a significant effect for the intervention on depression (62% reduction) with the mean dropping to 6.2 ± 3.7, below clinical cut-off. There was no significant change in the untreated group. The difference between groups was highly significant ($p<0.001$).

Hamilton-D ratings pre and post treatment for the treatment (n=30) and control (n=29) groups



Sequence*Phase F(1,52.5) = 30.06, p<.0001

There was significant improvement in QOL (18%) and fluid adherence (30%) compared to controls. Assessments were done blind to condition.

Conclusions: Depression is associated with negative health outcomes in HD samples. These results highlight a powerful effect of a behavioral intervention, especially, given the (1) ease of administration (2) acceptability of the intervention (no side effects), and (3) complex population studied. The intervention was also successful at improving the secondary outcomes of quality of life and fluid adherence. This intervention has the promise of reducing morbidity associated with depression in ESRD patients.

Funding: NIDDK Support

FR-OR097

Physicians' Communication during Decision-Making to Start Dialysis: Patients' Perspectives Mi-Kyung Song,[1] Robert M. Arnold,[3] Constance Gilet.[2] *[1]School of Nursing, University of North Carolina-Chapel Hill; [2]UNC Kidney Center, University of North Carolina-Chapel Hill; [3]Internal Medicine, Univ. of Pittsburgh, Pittsburgh, PA.*

Background: Because of significant burdens associated with dialysis and its limited survival benefits, clinicians have a moral obligation to fully explain the need for dialysis and its impact on the patient's quality of life and survival. However, there are few data on how decisions to start dialysis are made in practice and how patients perceive the decision-making process.

Methods: We report preliminary results of a qualitative interview study to explore patients' perspectives of how decisions to start dialysis were made. Thirty patients on in-center hemodialysis (HD) completed a semi-structured interview (age, M±SD=60.2±11.4). Years on dialysis were 4.4 (M).

Results: Although 70% (n=21) of the patients knew what conditions led to their kidney failure at that time, 60% (n=18) said that the need for dialysis was unexpected. Sixteen patients were told about the imminent need for dialysis during an acute hospitalization. Mortality without dialysis was presented clearly, but no patient recalled any information about prognosis with dialysis, given by their nephrologists. While 90% said that the life-long need of dialysis was presented, only 19 patients said dialysis options other than HD were presented (even when the decision was made during an office visit). Only one patient reported that information about side effects of dialysis or complications was given. Twenty-three (76.7%) recalled that their doctors were trying to make sure the patient understood information, but only 5 perceived that the doctor tried to understand their values and what's important in their life. Eighteen felt that they had no choice about dialysis. The most difficult part of decision making was accepting their life would be confined and not knowing what to expect once the decision to start dialysis is made.

Conclusions: Patients perceived that the decision to start dialysis was made with little information. Although preliminary, our data suggest that there is a significant room for improvement in decision-making process surrounding dialysis initiation.

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FR-OR098

Relationship between Prognostic Expectations of Hemodialysis Patients and Their Nephrologists Melissa Wachterman,[1] Edward Marcantonio,[1] Roger B. Davis,[1] Robert A. Cohen,[1] Sushrut S. Waikar,[2] Russell Phillips,[1] Ellen P. McCarthy.[1] *[1]Beth Israel Deaconess Medical Center; [2]Brigham & Women's Hospital.*

Background: The average 1-year mortality rate for hemodialysis (HD) patients is >20%. Perceptions of prognosis and expectations for transplant may influence goals of care (GOC). We compared HD patients' and their nephrologists' perceptions of prognosis and transplant candidacy, and explored the relationship between patients' expectations and GOC.

Methods: We conducted interviews with seriously-ill HD patients and their nephrologists. 2 prognostic models were used to identify patients with a ≥20% chance of dying in the next year. Of 207 patients treated at 2 HD units, 151 (73%) had a ≥20% mortality risk. 81 patients were eligible after exclusions including cognitive impairment, with 63 participating (78% response). We assessed prognostic expectations by asking patients and their nephrologists what they thought the chances were of 1-year survival (≥90%, 61-89%, 40-60%, 11-39%, ≤10%) and whether they thought kidney transplant was a possibility. Patients were also asked about their GOC (prefer mainly life-extending or symptom-directed care). McNemar's test was used to examine whether patients' and nephrologists' beliefs differed; Fisher's exact tests explored the association between GOC and expectations.

Results: Patients (mean age 68±10 yrs; 57% female; 51% African American) were significantly more likely than their nephrologists to report a ≥90% chance of being alive at 1 year (81% vs. 25%, P<.0001). In 60% of patient-nephrologist pairs, patients were more optimistic than their doctor, whereas doctors were more optimistic in 10% of pairs. Patients were also significantly more likely than their doctor to report they were transplant candidates (66% vs. 39%, P<.01). Of patients reporting a ≥90% chance of being alive at 1 year, 44% preferred care focused on extending life, compared to 9% of patients reporting a lower chance of survival (P=0.045).

Conclusions: HD patients appear more optimistic about prognosis and transplant than their nephrologists. Patients' potentially unrealistic expectations may influence their treatment choices. This suggests a need for improved prognostic communication.

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FR-OR099

How Robust Is the Surprise Question in Identifying Increased Mortality Risk in Haemodialysis Patients Maria Da Silva-gane,[1] David Wellsted,[2] Ken Farrington.[1] *[1]Renal, Lister Hospital, Stevenage, Hertfordshire, United Kingdom; [2]Centre for Lifespan & Chronic Illness Research, University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.*

Background: Mortality on haemodialysis (HD) is high. Many patients are elderly and frail. Their end-of-life care needs are often unrecognised. The Surprise Question (SQ) "would I be surprised if this patient were to die in the next 12 months" may aid timely identification of patients with such needs. In a cohort of HD patients, we assessed the prognostic value of SQ and the variability of responses among individual clinicians.

Methods: In a private interview undertaken by a Research Fellow, clinicians in each of our 3 HD units were asked to answer the SQ about all patients dialysing in their unit. There were 29 nurses of varying seniority and 6 nephrologists. There were 344 patients, 116 in Unit-1, 132 in Unit-2 and 96 in Unit-3. Patients were subsequently followed-up for 12 months.

Results: A negative SQ response – "I would not be surprised if this patient were to die in the next 12 months", was reported for between 6 and 43% of patients (mean 24 ± 9%). Doctors responded negatively for significantly more patients, than nurses. 52 patients (15%) died during 12 months follow-up. There were wide variations between clinicians in the predictive power of SQ responses. Sensitivity and Relative Risk Ratio (RR) were significantly higher for doctors than for nurses. SQ responses of 67% of doctors and senior nurses, 50% of nurses of intermediate seniority, and 36% of less senior nurses, significantly improved baseline models of 12 month mortality. SQ responses of nurses in Unit-3 had significantly lower Positive Predictive Value (PPV) and RR than those in other units. Agreements between clinicians on SQ responses improved PPV.

Conclusions: The SQ provides a unique contribution to prediction of short term prognosis in HD patients. The predictive power of the SQ responses of individual clinicians varied with clinical discipline, seniority and clinical setting.

FR-OR100

Outcomes, Prognostication, and Clinician Comfort with In-Patient Dialysis Initiation Jennifer S. Scherer, Vaughn W. Folkert. *Division of Nephrology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY.*

Background: Acute kidney injury (AKI) requiring hemodialysis (HD) increases patient mortality, yet there is no prognostic model for these patients. This prognostic ambiguity often leads to discordance between caregivers concerning the appropriate level of care. The objectives of this study were to obtain demographic and outcome data on patients who initiated HD in an urban medical center, to determine the degree of clinician agreement concerning this decision, and to estimate caregivers' prognostic abilities.

Methods: An observational study of 45 patients that initiated HD during hospitalization. Outcomes measured: survival, disposition, and renal function at discharge. Objective data was coupled with data from a survey, completed at the time of HD initiation that asked clinicians if they believed HD would change patient mortality, if they were comfortable with HD initiation, and if they thought HD was prolonging death. Clinicians were also asked to prognosticate patient survival.

Results: Survival was 64%. 52% of survivors were discharged home, 17% to nursing homes, and 31% to rehabilitation facilities; 48% were HD dependent. 98 surveys for 32 patients were obtained from clinicians representing seven hospital departments. There was 80% concordance between the nephrology fellows' and their attendings' prognostic opinions that HD would change the patient's mortality. The nephrology team had an 82% correct prognostication rate of survival. There was 44% prognostic discordance between nephrologists and non-nephrologists, with the nephrology team being correct 75% of the time. 24% of responders felt that HD was prolonging the patient's death. In 22% of cases there was at least one clinician who was uncomfortable with HD initiation.

Conclusions: AKI requiring HD has a high mortality rate. Overall, the nephrology team had good agreement concerning the initiation of HD, as well as good prognostic abilities. In comparison, there was high prognostic discordance between nephrologists and non-nephrologists, with the nephrology team often predicting the correct prognosis. In many cases there was at least one caregiver who was uncomfortable with HD initiation.

FR-OR101

Use of the Distress Thermometer in Low Clearance Populations Helen Alston, Aine Burns. *Centre for Nephrology, University College London, London, United Kingdom.*

Background: Patients with advanced CKD and multiple comorbidities have high symptom and depression burdens. Quality of life is comparable with advanced cancer patients. Commonly-used depression and symptom scores are not practical for routine clinic use, being long and unwieldy. We found the distress thermometer (DT), a self-scoring visual analogue scale for recording distress, easy to use in our multi-ethnic CKD population. We piloted its use to enhance communication and ensure a patient-centred approach at outpatient visits.

Methods: We reviewed 45 DTs completed by patients immediately prior to outpatient consultation. We compared distress scores with the clinical assessment. The distribution of scores was compared with standardised distributions from other UK patient populations. We characterised our sample using information from the electronic patient record (age, gender, eGFR, haemoglobin, Charlson comorbidity index), and looked for correlation between these factors and DT scores.

Results: 100% of patients with DT scores 8-10 were assessed as requiring clinical intervention during subsequent consultation, compared with 36% of those scoring 4-7 and 10% of those scoring 0-3. Patients ≥70yrs had higher DT scores than younger patients (fig 1). These distributions are significantly higher than standardised distributions for other UK populations such as cancer patients. Charlson score correlated with DT score, and patients ≥70yrs also had higher mean Charlson scores than those <70. There was no correlation between eGFR and DT score, neither was there a gender difference.

Conclusions: DT scores correlate with Charlson scores and age but not with eGFR, suggesting distress in CKD patients is due to factors other than renal function. The distribution of DT scores was higher in renal patients than in other populations. This may represent higher incident levels of distress within our population (renal patients have high levels of psychological morbidity), or may indicate that the DT requires recalibration before it can be used as a screening tool in nephrology. We suggest formal validation of this tool in renal populations to clarify this. The DT can continue to be used to stimulate communication in the clinical consultation.

FR-OR102

Clinical Outcomes of Suboptimal and Crash Starts to Dialysis Kenrry Chiu, Sameena Z. Iqbal, Ahsan Alam. *Division of Nephrology, McGill University Health Centre, Montreal, QC, Canada.*

Background: Many patients start dialysis suboptimally with a catheter despite having prior nephrology care, while others "crash" onto dialysis never having seen a nephrologist before. The aim of this study was to examine the impact of suboptimal and crash dialysis starts on mortality and kidney transplantation.

Methods: We retrospectively reviewed 377 incident dialysis patients from January 2006-April 2011 at two adult tertiary care hospitals with dedicated pre-dialysis clinics. Dialysis start type was categorized as: 1) optimal, 2) suboptimal with permanent access attempted, 3) suboptimal without permanent access attempt, and 4) crash start. Cox proportional hazard models were used to examine the association of start type with all-cause mortality or kidney transplantation, adjusting for age, gender, eGFR, serum phosphate, and Charlson comorbidity index.

Results: There were 377 patients (mean age 66 years, 57% male, 63% Caucasian, 56% diabetic, mean eGFR at start 8 ml/min/1.73 m^2) consisting of 27% optimal, 59% suboptimal, of which 45% had a permanent access attempt, and 14% crash starts. During a median follow up of 1.6 yrs, there were 104 deaths and 44 kidney transplants. As compared to an optimal start, adjusted mortality was increased for those with crash starts (HR 2.28; 95% CI 1.14-4.53) or suboptimal starts (HR 2.09; 95% CI, 1.21-3.60). However, suboptimal starts with a permanent access attempt had outcomes not significantly different than optimal starts. Compared to optimal starts, crash starts were less likely to have a kidney transplant (HR 0.08; 95% CI 0.01-0.61). Suboptimal starts did exhibit a decreased risk for kidney transplant (HR 0.57; 95% CI 0.30-1.07), but this was non-significant and not modified by prior permanent access attempt.

Conclusions: In our study, non-optimal starts represent the largest proportion of those starting dialysis. Suboptimal starts are a heterogeneous risk group, with a prior permanent access attempt conferring a favourable risk profile. Crash starts exhibit the highest risk for mortality and are less likely to receive a kidney transplant. Strategies to improve the rates of optimal dialysis starts should be identified, as this may improve patient outcomes on dialysis.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR103

Impact of End Stage Renal Disease in Children on Their Parents

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Background: End stage renal disease (ESRD) in children is very problematic condition to their families as well as patients themselves. We performed the cross-sectional investigation of impact of ESRD on their families, especially parents with children undergoing renal replacement therapies.

Methods: We used the Korean version of PedsQL Family Impact Module (FIM) and compared the results of Health-related Quality of Life (HRQOL) estimated from PedsQL 4.0 Generic Core Scales and PedsQL 3.0 ESRD module after adjustment for child age, child gender, treatment method, cause of disease, co-morbidity, parent's age, education level, parent's marital status, Hemoglobin, and Kt/V. Ninety two pediatric patients with ESRD aged 2-18 year old were enrolled in four Korean university hospitals. PedsQL Family Impact Module was completed by their parents.

Results: Male: Female ratio was 44:48 and most common cause of ESRD was chronic glomerulonephritis. Fifth five children have been treated by dialysis and thirty seven children received renal transplantation. Treatment modality and co-morbidity were significant variables to the impact of ESRD on their parents. Hemodialysis produced worse impact on their parents than other modalities including peritoneal dialysis and transplantation, especially in the three domains and total score: Emotional function, Social functioning and Cognitive functioning. Co-morbidity also produced worse impact on their parents in the five domains and total score: Social functioning, Cognitive functioning, Communication, Daily activities and Family relationship. Moreover, HRQOL of patients and family impact score showed significant positive correlation.

Conclusions: Treatment modality, co-morbidity and HRQOL of patients are very important factors to decide the impact of ESRD on their parents with children undergoing dialysis or renal transplantation. Larger, longitudinal prospective study is needed.

FR-OR104

The Impact of Real-Time Dosage Guidance for CKD: A Cluster Randomized Controlled Trial

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Background: Patients with CKD are at risk for frequent adverse medical events due to improper medication dosing. We aimed to design and implement computerized decision support tool to improve the rate of appropriate drug prescriptions in CKD.

Methods: A decision support tool was developed using best practice alert (BPA) functionality within the EPIC electronic health record. Alerts on 30 study medications for CKD patients (stage 3-5) were displayed in inpatient and outpatient settings during the process for ordering new medications and prior to the provider signing. Additionally, our tool featured "look backs" which provided alerts on existing medications when kidney function changed. We randomized all physicians at our institution to receive either standard of care (pharmacist review after order signing) or alerts in addition to standard of care. In both groups, we prospectively reviewed these opportunities for potential prescription adjustment to determine the proportion of study prescriptions that were appropriately adjusted for kidney function prior to pharmacist review. We created a multivariate logistic regression model that adjusted for GFR, gender, age, weight, hospitalized status and clustering within the prescribing provider.

Results: During the first 16 days of this study, a total of 346 opportunities for potential drug dosage adjustment or discontinuation occurred in 144 unique patients; 154 in the intervention arm and 192 in the control arm. Patient demographics were well matched with respect to age, gender, kidney function and length of stay. The primary outcome, drug discontinuation or dosage adjustment was 23.4% vs. 5.2% in the intervention and control arm, respectively (OR 3.9 [95%CI 1.8-8.8], p <0.001).

Conclusions: The rate of appropriate drug prescribing in CKD is low and remains a patient safety concern. Preliminary results suggest that our decision support tool improves drug prescribing in CKD.

FR-OR105

Improving In-Patient Detection of Acute Kidney Injury Stage 3 (AKI-3)

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Background: AKI affects 20% of hospital admissions. Retrospective studies have demonstrated a linear relationship between mortality and AKI grade. AKI-3 represents a powerful predictor of inpatient mortality and is a risk factor for chronic kidney disease.

In 2009 the UK National Confidential Enquiry into Patient Outcome and Death report 'Adding Insult to Injury' showed serious deficiencies in AKI care in UK hospitals. As a result in North Central London an AKI network has been set up to try and improve care.

In 2010, a retrospective audit of 40 patients with AKI-3 admitted in 2009/10, identified through coding and the pathology services, showed significant shortcomings in our own practice.

In 2011 we introduced a creatinine alert system and re-audited to identify impact of earlier intervention in AKI-3 care.

Methods: Nephrologists were e-mailed daily the Identifiers of in-patients with a Creatinine >300μmol/l who were unknown to their team. We studied management and outcome of patients generated by this method who fulfilled criteria for AKI-3 for each day in 2011.

Results: 123/282 patients generated by the alert system, fulfilled criteria for AKI-3 (see table).

Mortality of 35.8% in this study shows no improvement compared to 2010 audit figure of 33%. However, compared to our previous audit, we have shown 50.4% made a full renal recovery compared to 35% previously.

Patient demographics and Outcome

1. Patient Demographics		
	n=	% of total
Total Alerts	282	
AKI-3	123	43.6
Male	80	65.0
Female	43	35.0
Average Age	70.2	
Age Range	21-98	
2. Patient outcomes		
No recovery	31	25.2
Partial recovery	30	24.4
Full recovery	62	50.4
Died	44	35.8

Conclusions: A creatinine alert system identifies more patients with AKI-3 at an earlier stage and improves chances of full renal recovery. Abnormal renal function may represent end stage of an underlying disease process, rather than reversible renal causes per se (accounting for unchanged mortality). Other measures which may improve detection of an at risk population may include structured clerking pro-formas at point of care or a lower threshold for alerts (i.e. >200μmol/l).

FR-OR106

Peritoneal Dialysis Reduces the Number of Hospitalization Days in Heart Failure Patients without End-Stage Renal Disease

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Background: Heart failure is the most common reason for hospitalization in patients older than 65, and represents a significant burden on healthcare expenditure. Previous small-sized studies have reported favorable results for peritoneal dialysis (PD) in the setting of chronic refractory heart failure (CRHF). This study was designed to evaluate the impact of PD in a larger cohort of patients who did not have end-stage renal disease (ESRD).

Methods: All non-ESRD patients who received PD therapy for CRHF between January 1995 and December 2010 in two medical centers in France were included. Baseline characteristics were compared with a number of clinical parameters over the first year after initiation of PD (e.g. left ventricular ejection fraction [LVEF]). Safety and sustainability of PD, as well as mortality rate were analyzed.

Results: A total of 126 patients were included, with a mean age of 72 ± 11 years and an estimated glomerular filtration rate of 33.5 ± 15.1 ml/min/1.73m^2. Mean time on PD therapy was 16 ± 16.6 months. There was a significant reduction in the number of days of hospitalization for acute decompensated heart failure compared to the year preceding PD therapy (3.3 ± 2.6 vs. 0.3 ± 0.5 days/patient/month; $p < 0.0001$). Patients with LVEF ≤ 30% experienced improvement in cardiac function during the first year (30% ± 10 vs. 20% ± 6, $p< 0.0001$). There was a reduction in body weight at 3 months (74 ± 15 vs. 72 ± 15 kg, p=0.04). One-year mortality was 42% and cardiovascular events were the most common cause of death.

Conclusions: This study, the largest in this field, shows that PD can significantly reduce the number of days that patients with CRHF are hospitalized for heart failure-related complications. PD is also associated with significant improvement in LVEF, especially in patients with more compromised cardiac function. Whether these beneficial effects will translate into a beneficial impact on mortality and financial aspects of heart failure needs to be tested in future randomized studies.

FR-OR107

Stimulation of Soluble Guanylate Cyclase Attenuates Epithelial-Mesenchymal Transition of the Peritoneal Membrane and Prevent the Development of Peritoneal Fibrosis Hiroyuki Kadoya, Minoru Satoh, Hajime Nagasu, Kengo Kidokoro, Yuko Nishi, Chieko Ihoriya, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: The decrease in ultrafiltration capacity after prolonged peritoneal dialysis (PD) is the major reason for its discontinuation. The peritoneal membrane is continuously exposed to the high glucose, leading to peritoneal fibrosis and functional deterioration of the peritoneum. However, the mechanisms underlying this process have not yet been elucidated. The epithelial-mesenchymal transition (EMT) of peritoneal mesothelial cells has been regarded as an important mechanism of peritoneal fibrosis. Recently, nitric oxide (NO) has been reported to attenuate EMT in tissue fibrosis of various organs. We hypothesized that NO signaling inhibits EMT and thereby prevents the development of peritoneal fibrosis. The aims of this study are to evaluate the role of eNOS-NO signaling pathway and to elucidate the mechanisms of peritoneal fibrosis.

Methods: We used eNOS-deficient (eNOSKO) and wild type (WT) mice. A peritoneal fibrosis model was created by mechanical scraping. Some eNOSKO were treated with the soluble sGC stimulator Bay41-2272 (10 mg/kg/day, i.p). The mice were divided into the following groups: WT-sham, WT-peritoneal fibrosis(PF), eNOSKO-sham, eNOSKO-PF, and eNOSKO-PF-Bay. Two weeks after the operation, the mice were sacrificed. Peritoneal fibrosis was evaluated by Masson's trichrome staining. EMT was evaluated with α-smooth muscle actin (SMA) and vimentin expressions.

Results: Peritoneal fibrosis in eNOSKO-PF was more aggravated than in WT-PF. The expressions of α-SMA and vimentin were increased in eNOSKO-PF compared to WT-PF. The fibrosis and EMT of the peritoneal membrane were significantly attenuated in eNOSKO-PF-Bay. Furthermore, peritoneal function, assessed by a peritoneal equilibration test, was significantly improved in eNOSKO-PF-Bay.

Conclusions: The eNOS-NO-sGC signaling pathway was identified as potential therapeutic target to prevent peritoneal fibrosis. Thus the drugs that activate sGC possess therapeutic efficacy for protection of peritoneum in PD therapy.

FR-OR108

First Year Mortality in Incident Hemodialysis versus Peritoneal Dialysis Patients Joseph P. Pulliam,[1] Nien-chen Li,[1] Raymond M. Hakim,[2] J. Michael Lazarus,[3] Franklin W. Maddux,[1] Eduardo K. Lacson.[1] *[1]Fresenius Medical Care, North America, Waltham, MA; [2]Vanderbilt University, Nashville, TN; [3]University of North Carolina, Chapel Hill, NC.*

Background: The survival advantage observed in Canadian incident peritoneal dialysis (PD) patients was attributed largely to high incident hemodialysis (HD) catheter use. We sought to confirm these findings in a US cohort of incident patients.

Methods: Survival of incident patients starting dialysis in Fresenius Medical Care North America facilities in 2009 were compared by modality for one year using an intention to treat methodology. Cox models were adjusted for case-mix and the first available results for albumin, hemoglobin, & phosphorus. HD patients were also stratified by catheter vs. non-catheter (fistula/graft) access.

Results: PD patients were younger (57 vs. 64 years), admitted later (admission vintage 22 vs. 11 days), had fewer black patients (21% vs. 31%), larger BMI (32 vs. 31 kg/m^2) and had fewer comorbidities (DM: 53% vs. 62%, CHF: 3% vs. 4%, PVD: 6% vs. 10%, & Amputations: 3% vs. 5%) than HD patients (all p<0.001), although both cohorts had 56% males. Mortality rates were 0.10 and 0.25 per patient-year for PD and HD patients, respectively (p<0.0001). Among HD patients initiated with fistula/graft and catheter, mortality rates were 0.13 vs. 0.30 per patient-year (p < 0.0001). Kaplan-Meier 1st year mortality rates were 9.3% for PD vs. 21.5% for HD patients. The adjusted hazard ratio for death was 0.47 (0.40-0.56) favoring PD. The corresponding adjusted hazard ratios for non-catheter patients was 1.40 (95% CI 1.16-1.68) and for catheter patients was 2.31 (1.95-2.75), all p<0.0001.

Conclusions: In this national US cohort, incident PD patients had lower death risk than HD patients in the first year, despite adjusting for favorable case-mix. Unlike in the Canadian study, access type did not abrogate the difference in death risk for non-catheter access patients, although the associated risk in HD catheter patients was much higher. Further study is needed to determine if predialysis management practices and modality selection processes differ between cohorts (and countries) that may explain these findings.

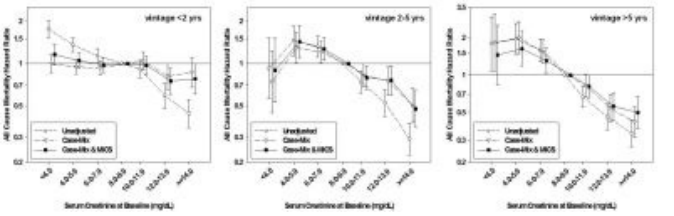
FR-OR109

Serum Creatinine and Mortality in Peritoneal Dialysis: Impact of Dialysis Vintage Jongha Park,[1,2] Rajnish Mehrotra,[3] Miklos Zsolt Molnar,[1] Lilia R. Lukowsky,[1,4] Sapna Singh Patel,[3] Joel D. Kopple,[3,4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3,4] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Ulsan University Hospital, Ulsan, Republic of Korea; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]David Geffen School of Medicine and Fielding School of Public Health at UCLA, Los Angeles, CA; [5]University of Tennessee, Memphis, TN.*

Background: Serum creatinine (Cr) levels are affected by body muscle mass and protein (meat) intake, both of which are associated with mortality in end-stage renal disease. However, serum Cr may be influenced dialysis vintage due to the effects of residual renal function.

Methods: Association of baseline serum Cr with all-cause mortality was explored in three different PD vintage groups (<2 yrs, 2-5 yrs, >5yrs) in 10,840 patients treated from July 2001 to June 2006 in 580 units owned by a large dialysis organization in the US.

Results: The patients were 55±15 yrs old and included 52% women, 24% Blacks and 48% diabetics. In patients with vintage <2yrs (n=7,175), there was an inverse association between serum Cr and mortality in the unadjusted model, but the association was no longer significant after adjustment for confounders. However, in patients with vintage 2-5 yrs (n=1,995), the relationship remained significant even after full adjustment except in patients with serum Cr <4.0 mg/dl. Patients with vintage >5 yrs (n=1,670) showed a robust association. Compared to patients with serum Cr range of 8.0-9.9 mg/ml as reference, hazard ratios were 1.63, 1.31, 0.81 and 0.60 in patients with serum Cr of 4.0-5.9, 6.0-7.9, 10.0-11.9 and 12.0-13.9 mg/dl, respectively (all p < 0.05).



Conclusions: The longer the PD vintage, the more closely serum Cr was associated with mortality as the confounding influence of residual renal function is mitigated.

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FR-OR110

Standardising Data Definitions for Peritoneal Dialysis(PD): Progress towards P-DOPPS Mark Lambie,[1] Simon J. Davies,[1] Jeffrey Perl,[4] David W. Johnson,[2] Martin E. Wilkie,[5] Ronald L. Pisoni,[3] Friedrich K. Port,[3] Bruce M. Robinson.[3] *[1]U. Hosp. of North Staffordshire; [2]Princess Alexandra Hosp.; [3]Arbor Research; [4]U. of Toronto; [5]Sheffield Teaching Hosp.*

Background: Standardizing definitions across data sources is vital to understanding differences in PD care and outcomes and to identify barriers to PD utilization. Within and between national renal registries, the study of fundamental issues (e.g. rates and causes of PD technique failure [TF]) is challenged by variability in the population covered, data completeness, definitions of PD start/end dates, and definitions of causes of TF. This project seeks to identify variation across registries in the definition, reporting, and documentation of PD TF and its causes.

Methods: We surveyed 11 national and international registries. Available information regarding data collection procedures, data completeness, comorbidities and definitions, definitions of PD start/end dates, and reasons for PD TF were systematically evaluated. Selected preliminary results are shown for national registries in Australia/New Zealand (ANZDATA), the United Kingdom (UKRR), Canada(CORR) and the United States(USRDS).

Results: See table.

Table: National and International PD Registry Characteristics

Registry	Data Collection Technique	Calculating Rate of Technique Failure: Specific Definition of PD Start Date	Calculating Rate of Technique Failure: Specific Definition of PD End Date (Duration off PD)	Causes of Technique Failure: # of Available Causes of TF	Causes of Technique Failure: > 1 cause permitted	Other Complications: Peritonitis Episodes Recorded	Other Complications: Exit Site Infections Recorded
ANZDATA	Form (paper or web-based) completed by physician	No	Yes	31	No	Yes	No
CORR	Form (paper) completed by nurse	No	No	12	No	No	No
UKRR	Electronic transfer and ad hoc audits	No	No	14, not routinely collected	No	No	No
USRDS	Principally administrative data	No	No	No PD TF specific causes recorded	No	No	No

Conclusions: We identified differences in data collection methods and the scope of data, as well as weaknesses across registries. As a result, comparisons of rates and causes of PD TF remain limited, both across facilities and between countries. The Peritoneal Dialysis Outcomes and Practice Patterns Study(P-DOPPS), led by Arbor Research Collaborative for Health along with the International Society of PD(ISPD), will address these limitations by development of a set of consensus data definitions to allow for valid comparisons of differences in PD practice and outcomes across facilities and between countries. These definitions will be published by ISPD as a resource for the PD community.

FR-OR111

A Random Glucose in Non-Diabetic Prevalent Peritoneal Dialysis Patients Is Strongly Predictive of Death: Results from the GLOBAL Fluid Study

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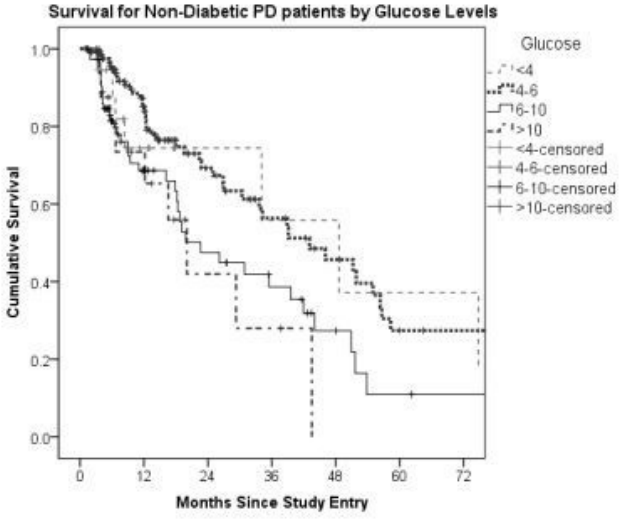
Background: Glucose control is a significant predictor of death in diabetic patients. In peritoneal dialysis (PD), the local toxic effects of peritoneal glucose are well recognized, but despite large amounts being absorbed, the systemic effects are not clear.

Methods: We analysed the Global Fluid Study, an observational cohort study of over 1,200 PD patients from 16 centres in 6 countries. 10 centres from 3 countries with high data quality were selected, with survival analysis by forward selection of a stratified Cox regression model after multiple imputation. Multivariate multilevel analysis was used for log transformed random glucose levels.

Results: By multivariate Cox models, random glucose levels were highly significant predictors of death in non-diabetic prevalent PD patients, (HR 1.05 per 1mmol/l increase, p=0.002), but not in incident patients. A Kaplan-Meier plot is shown.



On multivariate analysis random glucose levels were significantly associated with only daily dialysate glucose (0.018mmol/l increase per 1 litre 1.5% equivalent, p=0.001) and serum albumin levels (0.014mmol/l decrease for 1g/l increase, p<0.0001). Peritoneal Solute transport was positively associated until albumin was included.

Conclusions: Random glucose levels are strongly predictive of survival in non-diabetic prevalent PD patients, and these levels are partly determined by dialysate glucose.

Funding: Pharmaceutical Company Support - Baxter, Private Foundation Support

FR-OR112

Characterization of Mesothelial Cell VEGF Release during Peritonitis: The Role of c-Fos-Mediated Stimulation by TGF-β1 and TNFα

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Background: TGF-β1 is thought to contribute to adverse peritoneal vasculopathy that develops during peritoneal dialysis (PD) by inducing VEGF expression. The mesothelium has been identified as a major source of VEGF in the peritoneum. Mechanism of VEGF induction is strictly cell type- and context-dependent and the regulatory circuits dependent on other pathophysiology relevant cytokines remain to be clarified. We have hypothesized direct involvement of TGF-β1 and TNFα in regulation of VEGF expression in human peritoneal mesothelial cells (HPMC).

Methods: HPMC were isolated from normal omentum obtained during elective surgery. Dialysate effluent was obtained from stable PD patients and during peritonitis. VEGF mRNA and protein levels were measured by RT-qPCR and ELISA respectively. The involvement of transcriptional factors was assessed by EMSA and transient HPMC transfection with VEGF promoter constructs.

Results: Quiescent HPMC secreted only trace amounts of VEGF. Stimulation with TGF-β1 resulted in a time- and dose-dependent increase in VEGF mRNA expression and protein release. These effects were further synergistically amplified by TNFα, which alone exerted only minimal effect. Stimulation of HPMC with TGF-β1 ± TNFα led to the induction of the transcription factor c-Fos and activation of the VEGF promoter region that contained high affinity binding sites for c-Fos. Exposure of HPMC to dialysate effluent obtained during acute peritonitis and containing increased levels of TGF-β1 and TNFα resulted in a dose-dependent VEGF induction. This effect was significantly attenuated in cells treated simultaneously with an inhibitor of the activator protein-1 that is formed by c-Fos subunits.

Conclusions: Dialysate TGF-β1 and TNFα act through c-Fos to synergistically up-regulate VEGF production in the peritoneal mesothelium, thus pointing at an important link between inflammation, fibrosis and angiogenesis in the peritoneal membrane.

FR-OR113

Ceramides, Cardiac Structure and Function in Children with Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is associated with an increased incidence of cardiac dysfunction, even in subjects with an early decline of kidney function. The mechanism of accelerated cardiomyopathy is not well-understood. Recent animal studies determined that elevated sphingolipid, ceramide, causes dilated cardiomyopathy. Ceramide has not been studied in patients with CKD. We hypothesized that ceramide levels are elevated in children with CKD and that ceramide is associated with known cardiovascular risks and abnormal cardiac structure and function.

Methods: Multiple species of ceramide (C16, C18, C20, C22, C24) were determined as a part of sphingolipid analysis by LC/ESI/MS/MS using a TSQ Quantum Ultra-triple quadrupole mass spectrometer. Pilot data from 64 children aged 1-16 years enrolled in the CKiD study, median (IQR) iohexol GFR 49 (38, 64) ml/min/1.73m^2, with complete demographic, clinical and laboratory information and sphingolipid measures were analyzed cross-sectionally. Echocardiography was performed to determine LVM index, systolic (shortening fraction, SF) and diastolic (E/A) function. Ceramide levels were compared to 24 age-matched healthy controls.

Results: Serum ceramides were significantly higher in the CKD children, with especially pronounced difference in C24:0 lactosyl (L) ceramide (p<0.0001). In univariate analysis, logC24:0L was inversely associated with SF, (%) (r=-0.28, p=0.01) and E/A ratio (r=-0.24, p=0.04). In multivariate analysis adjusted for age, BMI Z score, LVM index, SBP Z score, DBP Z score, GFR and hemoglobin, higher logC24:0L was the only independent predictor of lower SF (β= - 20.480, p=0.01). Similar analysis for diastolic function determined that higher LVM index (β= -0.26, p=0.04) and C24:0L (β= -0.31, p=0.01) were only independent predictors of lower E/A ratio.

Conclusions: We concluded that ceramide levels are increased in children with CKD. The study identified C24:0L as independent predictor of lower diastolic and systolic function in these children. Larger confirmatory studies are needed to evaluate ceramide as a possible biomarker or a cause of abnormal cardiac function in CKD.

Funding: NIDDK Support

FR-OR114

Protective Role of Latent TGF-β1 in Diabetic Kidney Disease

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Background: Transforming growth factor-β1 (TGF-β1) has diverse roles in renal fibrosis and inflammation. While activation of TGF-β1 induces renal fibrosis in diabetes, the role of latency-associated peptide-TGF-β1 (LAP-TGF-β1) in diabetic kidney disease (DKD) remains unexplored.

Methods: A type-1 diabetic model was induced by strepotozotocin in TGF-β1 transgenic (Tg) mice that overexpress a human LAP-TGF-β1 in skin. Diabetic kidney injury including fibrosis and inflammation pathways were examined after 16 weeks.

Results: While wild type mice developed progressive DKD as demonstrated by a significant increase in microalbuminuria, renal fibrosis, and inflammation (all p<0.05), unexpectedly, LAP-TGF-β1 Tg mice were protected against DKD as evidenced by the findings that LAP-TGF-β1 Tg mice were able to prevent the development of microalbuminuria (60%↓, p<0.05), renal fibrosis including collagen I, and IV expression, and renal inflammation such as T cell and macrophage accumulation (50-60%↓, p<0.01) and upregulation of TNF-α, IL-1β and ICAM-1 (all p<0.01). Further studies revealed that these inhibitory effects in Tg mice were associated with elevated levels of LAP-TGF-β1, but not active TGF-β1 in the plasma and diseased kidneys (p<0.01), which was associated with upregulation of renal Smad7 (p<0.01), thereby blocking both TGF-β/Smad3 and NF-κB/p65 signaling pathways (all p<0.01). Moreover, we also found that blockade of Th-17-mediated renal injury by inhibiting expression of renal IL-6, Stat3, and IL-17A was another mechanism by which LAP-TGF-β1 Tg mice were protected from diabetic renal injury. Interestingly, all these protective effects of LAP-TGF-β1 on DKD were reversed by blocking LAP-TGF-β with a neutralizing antibody, validating a protective role of LAP-TGF-β1 in DKD.

Conclusions: Mice overexpressing LAP-TGF-β1 in skin are able to protect against DKD. Upregulation of renal Smad7, thereby inhibiting TGF-β/Smad3-mediated renal fibrosis and NF-κB-dependent renal inflammation, and blockade of Th-17 immune response, may be mechanisms whereby LAP-TGF-β1 protects DKD.

FR-OR115

Prospective Randomized Trial of Effect of Direct Renin Inhibition in Non-Diabetic Chronic Kidney Disease (DRINK): An Interim Analysis
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Background: Aliskiren reduces proteinuria among type 2 diabetics in the AVOID study. However, the ALTITUDE trial has been prematurely terminated on account of increased adverse events at 18-24 months in the aliskiren arm. We report the interim results of the DRINK study initiated in 2009 to investigate the renoprotective potential and safety of aliskiren added to maximum-dose angiotensin receptor blocker (ARB) in non-diabetic CKD stage 3-4 (eGFR 15-59ml/min/1.73 m^2) patients, with a planned follow-up time of 3 years.

Methods: Eligible patients receiving an ARB were randomly assigned aliskiren (150 mg titrated up to 300 mg daily) or conventional antihypertensive treatment to achieve blood pressure under 130/80 mmHg. The primary outcome was the composite of a doubling of baseline serum creatinine (sCr), ESRD or death. Analysis was by intention to treat.

Results: Seventy-six patients were randomized: 37 to aliskiren (27 male, mean age 55.0±11.1 y), and 39 to control (27 male, mean age 55.1±9.4 y). There was no difference in baseline demographics, sCr (194±61 *vs.* 214±65 mmol/l, P=0.15), eGFR (31.9±9.0 *vs.* 28.0±9.0 ml/min/1.73m^2, P=0.06), serum K^+ (4.3±0.5 *vs.* 4.4±0.6 mmol/l, P=0.23) and urine protein-to-creatinine ratio (98.8±19.0 *vs.* 74.4±10.9 mg/mmol, P=0.26) between the treatment *vs*. control group. After a median follow-up of 96 weeks (IQR: 64-112), two patients in each group reached the composite endpoint of doubling of sCr or ESRD (5.4% *vs.* 5.1%, P=0.769). The number of cardiovascular events was 4 (10.8%) *vs.* 1 (2.5%), P=0.217. Hyperkalemia (serum K^+ >5.5 mmol/l) was encountered in 7 (18.9%) *vs.* 2 (5.1%) patients (P=0.045), which was controlled with resin therapy without withdrawing the study medications. There was no difference in the change in proteinuria between the 2 groups.

Conclusions: Our interim results demonstrated no significant increase in adverse events except for more hyperkalemia in aliskiren-treated non-diabetic CKD stage 3-4 patients. The DRINK study will continue as planned. Funding: partially from the Yu Professorship in Nephrology Endowment Fund.

FR-OR116

AKB-6548, a New Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor, Increases Hemoglobin in Chronic Kidney Disease Patients without Increasing Basal Erythropoietin Levels
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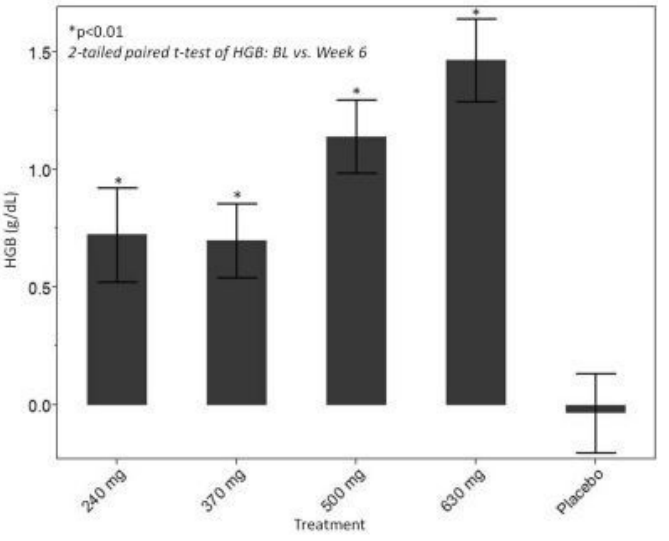
Background: Treatment of anemia associated with chronic kidney disease (CKD) using currently available erythropoiesis-stimulating agents often results in prolonged, supraphysiologic circulating erythropoietin (EPO) levels, which have been implicated in increased thromboembolic events. Therefore, a drug that increases hemoglobin (HGB), while only moderately increasing EPO levels, is highly desirable. AKB-6548, a new, short-acting hypoxia-inducible factor prolyl hydroxylase inhibitor, has been shown to induce such daily rises in EPO levels in patients with CKD in a manner that mimics the physiologic diurnal response in healthy individuals.

Methods: In a double-blind, placebo-controlled Phase 2a trial, 93 patients with CKD stage 3, 4, or 5, not on dialysis, were randomized to receive either placebo or AKB-6548 240 mg, 370 mg, 500 mg, or 630 mg once daily for 6 weeks.

Results: By Week 6, AKB-6548 significantly increased HGB (Figure 1) compared to baseline in all dose groups and compared to placebo (ANOVA, p <0.0001). The increase in HGB occurred without increasing basal (pre-dose measurement) EPO levels. Results at Week 6 also revealed a significant dose-related increase in total iron binding capacity and a decrease in hepcidin, suggesting improved iron mobilization. No drug-related serious adverse events were reported, and dosing was generally well-tolerated.

Conclusions: Thus, AKB-6548 significantly increases HGB in anemic CKD patients by inducing only moderate daily increases in EPO levels in a manner similar to the physiologic diurnal response and by enhancing iron mobilization. Figure 1: HGB: Change from Baseline to Week 6 in the Intent-to-Treat Population.



Funding: Pharmaceutical Company Support - Akebia Therapeutics

FR-OR117

Fruits and Vegetables or Oral $NaHCO_3$ Preserve GFR and Reduce Urine Angiotensinogen, a Marker of Kidney Angiotensin II Activity, in Stage 3 CKD
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Background: KDOQI directs Na^+-based alkali therapy of metabolic acidosis (MA) in CKD patients with plasma total CO_2 ($PTCO_2$) <22 mM and such treatment preserves eGFR. Because MA increases kidney angiotensin II (AII) activity, dietary alkali might preserve eGFR through reduced kidney AII and so treating less severe MA in CKD might also preserve eGFR. Fruits and vegetables (F+V) are also dietary alkali so we explored if added F+V or $NaHCO_3$ reduce kidney AII activity and preserve eGFR in CKD patients with MA and $PTCO_2$ 22-24 mM.

Methods: We randomized 108 subjects with CKD stage 3 eGFR (30-59 ml/min) and $PTCO_2$ >22 but <24 mM as follows: F+V (n=36) added to reduce dietary potential renal acid load (PRAL) 50%, oral $NaHCO_3$ (HCO_3, n=36) to reduce PRAL 50%, or no alkali (Control, n=36). All were treated toward systolic blood pressure (SBP) <130 mmHg with regimens including ACE inhibition and followed 3 years with yearly cystatin C calculated eGFR, $PTCO_2$, and urine angiotensinogen, factored by g creatinine (AGT/cr).

Results: Entry SBP (p=0.12), PRAL (p=0.60), $PTCO_2$ (p=0.62), eFGR (p=0.99), and AGT/cr (p=0.99) were not different among groups. At 3 years, $PTCO_2$ decreased in Control (23.0 to 22.4 mM, p<0.0001) but increased in F+V (23.0 to 23.9 mM, p<0.0001) and HCO_3 (23.1 to 24.0, p<0.0001). At 3 years, AGT/cr increased in Control but decreased in F+V and HCO_3 (all p<0.0001), was lower (p<0.0001) in F+V (32.1 ug/cr) and HCO_3 (32.0 ug/cr) than Control (38.8 ug/cr), but not different between F+V and HCO_3 (p=0.92). At 3 years, Control eGFR (26.6 ml/min) was lower (p<0.0001) than F+V (34.3 ml/min) and HCO_3 (32.7 ml/min) but was not different between F+V and HCO_3 (p=0.34).

Conclusions: F+V or $NaHCO_3$ each reduced a marker of kidney AII activity and preserved eGFR in stage 3 CKD patients with entry $PTCO_2$ 22-24 mM, supporting alkali therapy for CKD patients with MA less severe than that for which KDOQI directs therapy.

Funding: Private Foundation Support, Clinical Revenue Support

FR-OR118

A Crossover Trial of Lowering Dietary Sodium (Na) in Chronic Kidney Disease (CKD)
Rajiv Saran,[1] Robin L. Sands,[1] Brenda W. Gillespie,[1] Michael Heung,[1] Scott L. Hummel,[1] Vimal K. Derebail,[4] Bertram Pitt,[1] Nathan W. Levin,[2] Fansan Zhu,[2] Peter Kotanko,[2] Philip J. Klemmer.[4] *[1]Univ. of MI; [2]RRI; [3]Univ. of NC.*

Background: CKD patients are often volume expanded and hypertensive (HTN). Despite guideline recommendations, few prior controlled studies have assessed the effects of dietary Na restriction on these two factors. We conducted a randomized crossover trial of dietary Na restriction in CKD to assess hydration status measured by bioelectrical impedance spectroscopy (BIS) and 24-hr ambulatory blood pressure (ABP).

Methods: Stable adults with Stage 3-4 CKD were randomized to low Na diet (<2g Na/day) or 'usual diet' for 4 weeks, followed by a 2-week wash-out before the second 4-week phase. Weekly dietary advice using a motivational interviewing technique (MIT) was provided by study dieticians. Dietary Na intake was measured by 24-hour urine Na. BIS measurements included calf, whole body (WB) and segmental readings using a Hydra 4200. T-tests for crossover studies analyzed treatment and period effects.

Results: 58 subjects were enrolled (mean age 61±13, 60% male, 71% white, 43% diabetic, 93% HTN, mean BMI 32kg/m^2, eGFR 39ml/min/1.73m^2, 24-hr urine Na 171mmol/d and 24-hr systolic ABP 138mmHg). Baseline BIS measurements were not significantly different across CKD stage suggesting equivalent fluid status. WB extracellular volume decreased significantly on average by 0.9L in the low Na phase and calf normalized resistivity increased by 0.01 Ω m^3/kg, both implying lowered water content ($p<0.01$). On average, there were significant reductions in 24-hr urinary Na (52mmol/d), weight (2.2kg), and 24-hr systolic ABP (11.3mmHg) during the low Na diet phase (all $p<0.005$). Albuminuria was not statistically lower in the low Na group (mean change=0.07mmol). Mean serum creatinine rose by 0.13mg/dL ($p=0.04$) in the low Na group. No period effects were observed.

Conclusions: Low Na dietary intervention with MIT significantly reduced ABP and measures of volume expansion in CKD Stage 3-4 patients in this short-term crossover trial. This simple intervention may be similar in effect to a single antihypertensive agent and merits more formal study on a larger scale to assess its effect on outcomes.

Funding: Private Foundation Support

FR-OR119

Specialised Renal Care in Patients with CKD Stage IIIb-IV: Does It Impact on Mortality and Emergency Hospitalisation? Patrick Saudan, Nicola Marangon, Sophie M. De Seigneux, Belen Ponte, Pierre-Yves F. Martin. *Nephrology, Geneva University Hospitals, Geneva, Switzerland.*

Background: Long-term prognosis of patients with CKD is poor and there is a need for the development and implementation of new strategies through collaboration between primary care physicians (PCP) and nephrologists. We undertook a prospective randomised trial to determine the impact of regular renal care compared to guidelines-directed management by primary care physicians (PCP) on prognosis, planning of RRT and patient satisfaction in CKD patients.

Methods: Single center prospective randomised study. Inclusion criteria: CKD patients with an eGFR< 45 ml/min and > 15 ml/mn, aged 18-80 years old and enrolled during a hospitalization. Exclusion criteria: AKI or ESRD, estimated life expectancy < 2 yrs, refusal or inability to sign writing consent and patients previously known by nephrologists. The primary composite endpoint is death and/or hospitalisation during the 24 months after inclusion. The secondary endpoints are urgent RRT, decline of renal function and quality of life. Study design: Patients are randomised in two arms: -Combined management PCP and nephrologists (4 nephrology visits/year) -Management by PCPs with the help of written instructions and consultations being provided by our unit if requested by PCPs.

Results: The study began in November 2009. At the end of April 2012, 194 patients have been randomised, of whom 68% are male and 40% diabetics type II. Mean age is 69 ± 9 yrs, mean baseline eGFR is 33 ± 9 ml/min, mean 24-hr proteinuria is 1.24 ±1.9 g/24h. At the end of April 2012 (mean follow-up is 18 ± 9 months), 60% of the patients in the combined management group have reached the primary endpoint vs 55% in the PCP management group. Mean follow-up is 18 ± 9 months. Mortality rose to 11 % in the combined management group vs 15 % in the PCP management group. Free time interval until next hospitalisation is 313 ±280 days in the combined management group vs 304 ±256 in the PCP management group.

Conclusions: These short-term preliminary results do not demonstrate a benefit of regular renal care in terms of survival or rehospitalisation rate in patients with CKD stage IIIb-IV.ClinicalTrials.gov**:** NCT00929760.

FR-OR120

Is Time-Updated Blood Pressure Related to End-Stage Renal Disease in the Setting of Chronic Kidney Disease after Accounting for Kidney Function over Time? Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study Amanda Hyre Anderson,[1,2] Wei (Peter) Yang,[1,2] Raymond R. Townsend,[1,2] Glenn M. Chertow,[1] Jiang He,[1] Radhakrishna Reddy Kallem,[1,2] John W. Kusek,[1] James P. Lash,[1] Edgar R. Miller,[1] Mahboob Rahman,[1] Susan P. Steigerwalt,[1] Matthew R. Weir,[1] Jackson T. Wright,[1] Harold I. Feldman.[1,2] *[1]Chronic Renal Insufficiency Cohort (CRIC) Study; [2]University of Pennsylvania Perelman School of Medicine.*

Background: Blood pressure (BP) is frequently inadequately controlled in chronic kidney disease (CKD) patients. Previous reports of the longitudinal relationship between BP and end-stage renal disease (ESRD) often relied solely on baseline measures of BP and kidney function, and those with updated information may have inadvertently adjusted for pathway effects.

Methods: Data from CRIC Study participants (N=3,817, follow-up: 4 yrs) were used in a marginal structural model to assess time-updated BP exposure on the risk of ESRD with proper adjustment for time-dependent confounding. The time-updated BP exposure was characterized as mean systolic BP (SBP), % time with BP uncontrolled (≥130 and ≥120 mmHg), and mean SBP variability (SBPVar).

Results: In separate models, mean SBP, % time with BP ≥130 or ≥120 mmHg, and mean SBPVar each significantly increased risk for ESRD after adjustment for demographics, co-morbidities, and time-updated kidney function and antihypertensive medication use. In a model including both SBP and SBPVar, mean SBP retained significance (HR, 95% CI: 1.40, 1.27-1.55) but SBPVar did not (1.12, 0.97-1.30). The interaction between mean SBP and urine protein excretion was of borderline significance (P=0.07) with the BP association attenuated among those with higher levels of proteinuria.

	Adjusted HR (95% CI)
Mean SBP, per 10mmHg increase	1.38 (1.28-1.49)
% time SBP ≥130 mmHg, per 10% increase	1.11 (1.06-1.16)
% time SBP ≥120 mmHg, per 10% increase	1.12 (1.06-1.19)
Mean SBPVar, per 10 mmHg change	1.33 (1.21-1.47)

Conclusions: After accounting for time-updated kidney function and antihypertensive medication use, the relationship between BP and ESRD was robust and consistent with prior studies. Modification of this relationship by proteinuria requires further study.

Funding: NIDDK Support, Other NIH Support - CTSA and GCRC Awards

FR-OR121

Risk of Hypoglycemic Episodes among Diabetic Patients with Chronic Kidney Disease (CKD) Bessie A. Young,[1,2,3] Luis A. Bent-Shaw,[2,3] Margaret K. Yu.[2,3] *[1]Veterans Affairs Puget Sound Health Care System, Seattle Epidemiology Research and Information Center, Seattle, WA; [2]Kidney Research Institute, University of Washington, Seattle, WA; [3]Division of Nephrology, University of Washington, Seattle, WA.*

Background: Intense glycemic control is one of the cornerstones of diabetes care and has been shown to improve both microvascular and macrovascular events. Hypoglycemia is a known complication of more intensive glycemic control in patients and has been associated with increased risk of mortality Less is known regarding risk of severe hypoglycemic reactions requiring hospitalizations. We hypothesize that patients with diabetes and CKD will haveincreased risk of severe hypoglycemic episodes requiring hospitalization over time, which will increase depending on stage of kidney function.

Methods: We analyzed the incidence of severe hypoglycemic reactions requiring hospitalization among a primary care cohort with diabetes followed longitudinally as part of the Pathways to Health Study. All patients who completed baseline surveys and had laboratory tests available to assess eGFR were analyzed. The primary outcomes of interest was severe hypoglycemic reactions requiring emergency room visit or hospitalization identified by ICD-9 code over 5 years of follow up.

Results: 3943 subjects were identified at baseline with stage 0-5 CKD, of whom 1227 were found to have an eGFR<60ml/min/1.73m^2. The mean age was 70.7, 83.8% were white, 7.2% African American and 6.5% Asian. Over a 5 year period, 240 episodes of severe hypoglycemia were identified. Cox proportional hazards models found that CKD stages 3 (Hr=2.2, 95% CI1.22-4.10), stage 4 (HR=3.45, 95% CI=1.65-7.23), and stage 5 (HR=4.34, 95% CI=2.07-9.00)were associated with greater risk of severe hypoglycemic reaction compared to stage 0 CKD after adjusting for age, gender, race, type of diabetes, insulin use, oral hypoglycemic agent, and ace-inhibitor. Other risk factors included depression, insulin use, and type 1 diabetes.

Conclusions: Patients with diabetes are at greatly increased risk of severe hypoglycemic episodes. Patients with latter stage CKD and diabetes should be closely managed to avoid severe hypoglycemic reactions.

Funding: NIDDK Support, Veterans Administration Support

FR-OR122

Do the Associations of eGFR and Albuminuria with Mortality and Renal Failure Differ by Gender? A Meta-Analysis from a Global Consortium (for the CKD-PC Collaborators) Dorothea Nitsch, M. Grams, Yingying Sang, Corri Black, Massimo Cirillo, Ognjenka Djurdjev, Kunitoshi Iseki, Simerjot K. Jassal, Heejin Kimm, Florian Kronenberg, Cecilia Montgomery Øien, Andrew S. Levey, Adeera Levin, Mark Woodward, Brenda Hemmelgarn. *CKD Prognosis Consortium.*

Background: There is substantial clinical uncertainty whether the associations of chronic kidney disease (CKD) with mortality and end-stage renal disease (ESRD) differ between men and women.

Methods: We assessed the presence of a gender interaction in the associations of estimated glomerular filtration rate (eGFR) and albuminuria with all-cause mortality, cardiovascular mortality and ESRD in 2,051,158 participants (54% women) from general population (n=1,861,052), cardiovascular high-risk (n=151,494), and CKD (n=38,612) cohorts. We used random-effects meta-analysis using pooled individual participant data.

Results: All-cause and cardiovascular mortality risk was higher in men at all levels of eGFR and ACR. While higher risk was associated with lower eGFR and higher ACR in both genders, the risk relationship for all-cause and cardiovascular mortality was steeper in women than in men. Compared with eGFR 95, the adjusted hazard ratio (HR) for all-cause mortality at eGFR 45 was 1.32 (95% CI, 1.08 to 1.61) in women and 1.22 (CI, 1.00 to 1.48) in men (p for interaction<0.001). Compared with ACR 5, the HR for all-cause mortality at ACR 30 was 1.69 (CI, 1.54 to 1.84) in women and 1.43 (CI, 1.31 to 1.57) in men (p for interaction=0.005). Conversely, there was no evidence of a gender difference in associations of eGFR and ACR with ESRD risk.

Conclusions: While both genders face increased risk of all-cause and cardiovascular mortality and ESRD with lower eGFR and higher albuminuria, the relative mortality risk in women increases more steeply with diminished kidney function. These findings were robust across a large global consortium.

Funding: Private Foundation Support

FR-OR123

VALIGA: Preliminary Clinical Results from the European Collaborative Database of 1178 IgA Patients Designed to Validate the Oxford Classification

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Background: The independent prognostic value of renal biopsy features in patients with IgA Nephropathy (IgAN) was established by the Oxford Classification from 265 patients and deserves validation.

Methods: The ERA-EDTA Immunonephrology Working Group sponsored in 2009 the European Validation Study (VALIGA), which enrolled 1178 IgAN patients from 55 centers of 13 countries. The clinical data collection at renal biopsy and during follow-up has been completed and dual review of biopsies according to the Oxford criteria will be available by August 2012. We report here the VALIGA clinical risk factors for progression.

Results: 1178 IgAN patients aged 36±16 years, 73% M, eGFR 75±34ml/min, proteinuria (UP) 1.27 (IQ 0.57-2.6) g/day, mean BP (MAP) 98±13mmHg. Mean follow-up 5.8 years ± 4.5, loss of e-GFR (slope) of -2.0±8.1 ml/min/year. ESRD developed in 141 cases (12%), 50% loss of e-GFR in 171 (15%) and combined end point in 195 (16.5%). Initial UP, e-GFR and MAP and follow-up UP and MAP were strongly correlated by multivariate analysis with 50% loss of eGFR or combined end points, and slope of e-GFR (all P<0.0001). The predictive value of follow-up proteinuria was the strongest predictor. Assuming the end point of 50% reduction in eGFR, ROC analysis indicated the best cut-off value for UP was 0.96 g/day (sensitivity 91%, specificity 64%, AUC 0.85) versus for follow-up BP the best MAP was 98 mmHg (sensibility 67%, sensitivity 67%, AUC 0.74).

Conclusions: The clinical data from the 1178 IgAN European cohort (VALIGA) has validated the relevance of the definition of partial remission (persistent proteinuria < 1 g/day) as the major risk factor for preventing progression.

FR-OR124

Impacts of Tonsillectomy Plus Steroid Pulse Therapy on IgA Nephropathy Depending on Histological Classification: A Multicenter Cohort Study

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Background: Besides corticosteroids and RAS inhibitors, tonsillectomy may have a beneficial impact on the clinical course of IgA nephropathy (IgAN). However, much uncertainty remains about the indications for and protocols of therapeutic options for IgAN.

Methods: We enrolled 334 patients with biopsy-proven IgAN diagnosed between 2000 and 2010 at four centers. Baseline characteristics at biopsy were analyzed in relation to clinical remission. The impact of treatment was evaluated after a median follow up of 4.1(1.5-8.6) years according to histological grade of the lesions and in line with the guidelines of the Study Group on Progressive Glomerular Disease in Japan. Clinical remission was defined as disappearance of both proteinuria and hematuria.

Results: During the follow-up period, 151 patients(46%) achieved clinical remission with a median time-to-remission of 382 days. Patients reaching remission had younger age(p=0.008), lower incidence of hypertension(p=0.01), higher levels of albumin(p=0.04) and eGFR(p=0.003), and lower levels of triglyceride(p=0.002). Multivariate logistic regression analysis found that tonsillectomy plus steroid pulse therapy independently contributed to achieving remission only in the histological grade 3 group with glomerulosclerosis, crescent formation or adhesion to Bowman's capsule in 10–30% of all biopsied glomeruli, or mild cellular infiltration in the interstitium(OR 2.4, 95%CI 1.2–5.0, p=0.02). In patients reaching remission, the patients with tonsillectomy had shorter time-to-remission compared to patients without tonsillectomy[365(62–1447) days vs 579(22–2304) days, p=0.03].

Conclusions: The combination therapy of tonsillectomy and steroid pulse for IgAN may have a beneficial impact on the clinical course for patients with mild to moderate glomerular and interstitial lesions.

FR-OR125

A Pilot Study to Determine Dose, Effectiveness and Depletion of Anti-PLA2R Antibodies of Adrenocorticotrophic Hormone (ACTH Acthar® Gel) in Subjects with Nephrotic Syndrome and Idiopathic Membranous Nephropathy (iMN)

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Background: H.P. Acthar® Gel is obtained from porcine pituitary. Active ingredients include structurally related peptides such as ACTH and the melanocyte stimulating hormones, which bind to melanocortin receptors (MCRs) expressed in glomerular podocytes reducing oxidative stress, diminishing podocyte apoptosis, and podocyte loss in kidney injury models. We hypothesized that H.P. Acthar® Gel would induce remission of proteinuria in patients with iMN.

Methods: Twenty patients were randomized to receive either 40 or 80 IU SQ twice weekly for 120 day with a total follow-up period of one year (final follow-up still pending in 2 patients). Proteinuria, albumin, cholesterol profile, changes in eGFR and anti-PLA2R antibodies along with tolerance and safety were assessed.

Results: Baseline characteristics were median proteinuria of 7802 (5665-10092) mg/24h, albumin of 2.7±8 g/dL, total cholesterol of 7.9±3.5 mmol/L and eGFR of 81±25 ml/min. The use of Acthar® Gel resulted in a significant reduction in median proteinuria to 2025(951-5140) mg/24h (p<0.001) along with improvements in both albumin and total cholesterol levels. At last follow-up, 60% of the patients had > 50% decrease in proteinuria. The majority of the patients did not respond to the 40 IU dose. No significant change in eGFR was noted over the period of follow-up nor were then any significant adverse side effects. Anti-APLA2R antibodies were present in 15/20 patients at baseline with decreased or increased levels noted prior to improvements or worsening of proteinuria, respectively.

Conclusions: H.P. Acthar® Gel is a safe and well-tolerated potential therapy to induce remission of proteinuria in patients with iMN. A dose of at least 80 IU units SQ twice weekly appears necessary for maximal effect. Anti-APLA2R levels may assist with prediction of response to therapy.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals, Inc.

FR-OR126

Long Term Outcomes of a Restrictive Treatment Regimen in Idiopathic Membranous Nephropathy

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Background: We present the long term disease course and outcomes of patients with biopsy proven membranous nephropathy (MN) who were treated according to a restrictive treatment regimen that was initiated in 1995.

Methods: We prospectively evaluated 269 patients. Progression risk was estimated using low-molecular weight protein excretion. Subsequently, intensive ACEi/ARB and statin treatment was advised for low risk patients with well preserved kidney function, whereas immunosuppressive therapy, mostly with alkylating agents, was given to patients at high risk for disease progression as soon as kidney function deteriorated. We gathered follow-up data on treatment, outcomes and adverse events from medical records, and used Kaplan-Meier estimates to calculate five and ten year cumulative incidence rates of outcomes.

Results: The table shows characteristics and disease course for the cohort, which was followed for a median 6.1 (IQR 3.3 to 10.3) years.

Biopsy variables	**total cohort**
n (% male)	269 (70%)
age	52 (14.9)
year of biopsy	2000 (7.0)
eGFR	68 (24)
proteinuria	7.0 (4.6)
Therapies	
none	142 (53%)
cyclophophamide	116 (43%)
other	11 (4%)
Outcomes	
remission of proteinuria	147 / 203 (72%)
relapse of proteinuria	47 / 147 (32%)
doubling of serum creatinine	37 / 235 (16%)
end stage renal disease	12 (4%)
Adverse events	
death	9 (3%)
malignancy	20 (7%)
cardiovascular and thrombotic events	27 (10%)
leucopenia	48 (18%)
infection	53 (20%)

data are presented as mean (SD) or percentages

The restrictive treatment strategy resulted in 127 (46%) patients receiving immunosuppressive therapy. Five year cumulative incidence rates of remission, end stage renal disease and death were 68%, 3.2% and 1.8%, respectively. In addition, respective ten year incidence rates were 75%, 4.5% and 3.6%.

Conclusions: A restrictive therapeutic regimen leads to favorable long term outcomes in iMN, whilst limiting exposure to potentially hazardous immunosuppressive drugs. The high incidence of adverse events such as cardiovascular complications and infections necessitates a search for less harmfull therapies.

Funding: Private Foundation Support

FR-OR127

Prophylactic Anticoagulation in Membranous Nephropathy: A Decision Analysis

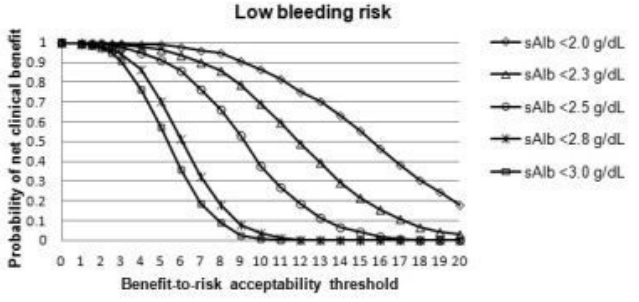
Taewoo Lee,[1] Andrea K. Biddle,[3] Sean Barbour,[2] Sophia Lionaki,[1] Yichun Hu,[1] Vimal K. Derebail,[1] Michelle A. Hladunewich,[2] Caroline Jennette Poulton,[1] Shannon L. Mahoney,[1] Susan L. Hogan,[1] Shannon L. Mahoney,[1] Ronald J. Falk,[1] Daniel C. Cattran,[2] Heather N. Reich,[2] Patrick H. Nachman.[1] *[1]UNC Kidney Center, University of North Carolina, Chapel Hill, NC; [2]Division of Nephrology, University of Toronto, Toronto, ON, Canada; [3]Health Policy and Management, School of Public Health, University of North Carolina, Chapel Hill, NC.*

Background: In membranous nephropathy, the risk of venous thromboembolic events (VTE) is inversely correlated to serum albumin level (sAlb). This study estimates the benefit of prophylactic anticoagulation (VTEs prevented) relative to the risk (bleeds incurred) by sAlb level.

Methods: A hybrid Markov-decision tree model was constructed to compare anticoagulation to observation. The risk of VTE was obtained from a cohort of 539 patients. The categorized (low, intermediate, high) risk estimates of bleeding were obtained from the ATRIA study (J Am Col Cardiol 58:395). The benefit-to-risk ratios were calculated by bleeding risk category. We used probabilistic sensitivity analyses to estimate effects of parameter uncertainty.

Results: For patients at low bleeding risk, the base-case model predicts a benefit-to-risk ratio of 4.5:1 and 13:1 for sAlb <3.0 g/dL and <2.0 g/dL respectively. With intermediate or high bleeding risk, the ratios are 3.9:1 and 1.8:1 for sAlb < 2.0 g/dL. In probabilistic sensitivity analysis, patients at intermediate or high risk are unlikely to benefit from anticoagulation whereas those at low risk are likely to benefit with a benefit-to-risk ratio of 5:1 and 10:1 for sAlb <2.5 g/dL and <2.0g/dL.

Conclusions: The decision for anticoagulation should begin with assessing a patient's bleeding risk. We provide a tool to estimate the likelihood of benefit based on sAlb level and acceptable benefit-to-risk ratio.



FR-OR128

An Effective Regime for Prevention of Venous Thromboembolism in Nephrotic Syndrome Nicholas R. Medjeral-Thomas, Stela Ziaj, Jack Galliford, Jeremy B. Levy, Tom Cairns, Megan Griffith. *West London Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Venous thromboembolism (VTE) occurs in 7-40% of nephrotic patients. The severity and underlying cause of nephrotic syndrome (NS) influence the VTE risk. This study investigates the use of low dose prophylactic anticoagulation to prevent VTE in patients (pts) with NS caused by primary glomerulonephritis (GN).

Methods: Pts presenting with NS secondary to primary membranous GN (MGN), minimal change GN (MinGN) and focal segmental glomerulosclerosis (FSGS) from September 2006 to September 2011 were considered for the study. Pts already taking anticoagulation for a pre-existing condition were excluded. Pts with serum albumin <20g/L received prophylactic dose low molecular weight heparin or low dose coumarin; pts with albumins 20–30g/L received aspirin or clopidogrel 75mg once daily. All thrombotic events and bleeding complications were recorded.

Results: 162 pts were identified (65 MGN, 49 Min GN, 48 FSGS). Mean follow-up was 168 weeks (range 38-307). Initial mean serum albumin was 14.5 g/L (range 5-29) and UPCR was 1365 units (range 255-14000). 11/162 pts were diagnosed with VTE at presentation and were treated following local VTE guidelines. 151 pts received prophylactic anticoagulation. 4/151 pts subsequently developed VTE. Only 1/4 was taking the prophylaxis at the time of VTE (6 days after diagnosis and commencement of prophylaxis). 1 pt had VTE when the prophylaxis was stopped pending biopsy. 2 pts had stopped prophylaxis as their NS was in remission; 1 relapsed with NS and presented with VTE prior to anticoagulation being restarted; 1 developed VTE when not nephrotic, but had a prior history of DVT 3 years before. 3/151 pts on prophylactic anticoagulation were admitted with a gastrointestinal bleed requiring transfusion. 1/3 was also taking steroids for the underlying GN at the time of the bleed.

Conclusions: This regime of prophylactic anticoagulation appears relatively safe and effective in preventing VTE in NS. Care must be taken when stopping the anticoagulation peri-procedure, and in patients in remission likely to relapse or with a history of DVT.

FR-OR129

Role for miR-30 in Podocytes and Its Clinical Relevance Junnan Wu, Chun-xia Zheng, Wanfen Zhang, Changming Zhang, Zhao-hong Chen, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Background: miRNAs are essential for glomerular podocyte homeostasis as shown by selective Dicer deletion in mouse podocytes, and miR-30 family has been suggested to be a player as evidenced by its anti-apoptotic effect in podocytes in vitro. Here, we explored the clinical relevance of miR-30, and further define its actions in podocytes.

Methods: Glomeruli were microdissected from renal biopsies, and subjected to RNA preparation and qRT-PCR of miR-30. miR-30 levels were correlated with proteinuria remission of the patients. Conditionally-immortalized human podocyte cell line was used for in vitro studies. Podocyte injury was evaluated by apoptosis, cytoskeletal alteration and injury markers. The expressions of miR-30 targets were examined by western blotting.

Results: miR-30 members were abundantly expressed in podocytes of glomeruli, but all downregulated in patients of FSGS and MN. Glomerular miR-30 levels of FSGS patients positively correlated with proteinuria remission. In vitro, human podocytes were treated with TGF-β, LPS and PAN, and miR-30 members were found all downregulated. When exogenous miR-30 was expressed to sustain miR-30 level of the podocytes in the treatment, both apoptosis and cytoskeletal damage were significantly ameliorated. We further show that miR-30 exerted its protective role, at least partly, through inhibition of its targets, Notch1 and p53. Lastly, we show glucocorticosteroid prevented TGF-β and LPS-induced miR-30 downregulation in podocytes, accompanied with prevention of upregulation of proapoptotic p53, Bax and Notch1, and restoration of anti-apoptotic Bcl2 and CD2AP expression.

Conclusions: miR-30 protects podocytes, at least partly, through inhibiting its targets, Notch1 and p53, two known culprits in podocyte injury. Downregulation of miR-30 causes podocytes vunerable to injury. miR-30 expression appears to have a signficant clinical relevance as shown by its downregulation in podocytes of patients, its correlation with proteinuria, its restoration by glucocorticosteroid in vitro. Thus, miR-30 family is a promising therapeutic target for glomerular disease.

Funding: Government Support - Non-U.S.

FR-OR130

Plasma Soluble Urokinase Receptor Levels Were Elevated in Patients with Primary Focal Segmental Glomerulosclerosis and Were Associated with Renal Damage Gang Liu, Jing Huang, Ming Hui Zhao. *Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Background: To detect the plasma soluble urokinase receptor (suPAR) levels of patients with primary focal segmental glomerulosclerosis (FSGS) and investigate their associations with clinical and pathological data.

Methods: Sixty-eightpatients with primary FSGS, diagnosed between Jan 2006 and Aug 2011, with complete clinical and pathologic data were enrolled. Healthy donors and patients with minimal change disease (MCD), membranous nephropathy (MN), secondary FSGS (s-FSGS) were used as controls. Plasma suPAR was measured using commercial ELISA kits.

Results: The plasma suPAR levels of patients with primary FSGS (3676.4± 2241.3 pg/ml) were significantly higher than that of patients with MCD (2024.7± 378.4 pg/ml, $P<0.001$), MN (2092.5± 874.7 pg/ml, $P<0.001$), and normal subjects (1787.2± 455.9 pg/ml, $P<0.001$) respectively. The plasma suPAR levels were also higher than that of patients with s-FSGS (2597.9± 695.4 pg/ml, $P=0.181$), though it did not have statistical differences. The mean plasma suPAR levels increased in the order of tip variant to not otherwise specified variant to cellular variant (2925.5± 1704.4 pg/ml, 3838.7± 2099.8 pg/ml, and 4179.9± 2475.2 pg/ml, respectively).The plasma suPAR level was negatively correlated with creatinine clearance at presentation (r=-0.454, $P=0.001$) and positively correlated with the percentage of glomerular crescents (r=0.303, $P=0.012$). During follow-up, we collected plasma samples of 16 patients with therapeutic responses. The plasma suPAR level of 7 patients with complete remission decreased substantially (4778.9± 3326.7 pg/ml vs. 2845.3± 935.4 pg/ml, $P=0.128$), for 4 patients with partial remission, it was comparable (2340.3± 239.3 pg/ml vs. 2055.2± 385.9 pg/ml, $P=0.273$), however, for 5 patients with treatment failure, it was even increased (3180.9± 757.4 pg/ml vs. 4360.6± 1573.2 pg/ml, $P=0.080$).

Conclusions: Plasma suPAR levels were specifically elevated in patients with primary FSGS and might be associated with severe renal damage. The plasma suPAR might be helpful to differentiate primary FSGS from MCD and certain s-FSGS.

Funding: Government Support - Non-U.S.

FR-OR131

Urinary Excretion of Vitamin D Binding Protein Is Markedly Increased in Focal Segmental Glomerulosclerosis versus Minimal Change Nephrotic Syndrome Michael R. Bennett, Hermine Brunner, Prasad Devarajan. *Cincinnati Children's.*

Background: Idiopathic nephrotic syndrome (NS) is the most common glomerular disorder of childhood. Pathology on invasive biopsy, including minimal change disease and focal segmental glomerulosclerosis (FSGS), remains the diagnostic method of choice for NS. Vitamin D deficiency is a common complication of chronic kidney disease. This is thought to occur due to loss of functioning 25-OH-D-1a-hydroxylase in the proximal tubules as well as through urinary loss of vitamin D binding protein (VDBP), the main carrier of vitamin D in the blood. Due to the prolonged and persistent proteinuria in FSGS, it is likely that vitamin D binding protein is excreted to a greater degree than in MCD. If VDBP is higher in FSGS, this could serve both as a non-invasive biomarker for diagnosis of NS as well as a possible therapeutic target. We set out to determine if urinary VDBP levels were elevated in FSGS vs MCD.

Methods: Urine and clinical data were collected from patients at Cincinnati Children's Hospital recently diagnosed with active nephrotic syndrome as well as healthy controls. Patients with a history of gross hematuria, active/recurrent UTI or nephrotic syndrome secondary to systemic disease were excluded from the study. Urinary VDBP measurements were performed with a commercially available ELISA kit and normalized to urine creatinine.

Results: This study included subjects in three groups: biopsy-proven FSGS (n=15), presumed MCD (n=12), and normal controls (n=5). Median VDBP levels were calculated and subjected to Mann-Whitney Rank Sum analysis. VDBP was significantly higher ($p<0.001$) in FSGS (14526 ng/ml, IQR 976-45541) than both MCD (85 ng/ml, IQR 15-274) and healthy controls (23 ng/ml, IQR 22-99, $p=0.002$). Results did not change with NGAL corrected for urine creatinine.

Conclusions: Urinary excretion of VDBP was markedly elevated in FSGS vs. both MCD and healthy controls. The massive loss of VDBP in FSGS could potentially result in greater vitamin D deficiency in patients with FSGS vs MCD. VDBP could serve as both

a target for therapy in FSGS as well as a non-invasive biomarker that could distinguish FSGS, which is associated with poor prognosis, from the more benign MCD.

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FR-OR132

Clinicopathological Features and Predictors of Outcome in C3 Glomerulopathy Michelle M. O'Shaughnessy,[1] John O'Regan,[1] Limy Wong,[1] Carol A. Traynor,[1] Michael James Flanagan,[1] Anthony M. Dorman,[1] Chia Wei Teoh,[2] Nicholas R. Medjeral-Thomas,[3] Matthew C. Pickering,[3] H. Terence Cook,[3] Peter J. Conlon.[1] *[1]Nephrology, Beaumont Hospital, Dublin, Ireland; [2]Nephrology, Children's University Hospital, Temple Street, Dublin, Ireland; [3]Centre for Complement and Inflammation Research, Imperial College London, London, United Kingdom.*

Background: Dysregulation of the alternative complement pathway can result in a variety of forms of glomerular injury, collectively termed C3 glomerulopathy (C3G). Light microscopic (LM) and electron microscopic (EM) appearances are heterogeneous. Diagnosis relies upon identification of isolated deposits of C3 without immunoglobulin on immunofluorescence (IF). Differences in pathophysiology, clinical phenotype and renal outcomes between patients with dense deposit disease (DDD) and other forms of C3G are largely unknown.

Methods: All native renal biopsies (n=4,554) referred to a single pathology service from 1995 to 2011 were retrospectively reviewed. Cases satisfying pathological criteria for a diagnosis of C3G, as determined by two independent renal pathologists, were included. Demographic, clinical and laboratory data were obtained. Patients with and without DDD were compared.

Results: Sixty-five patients were identified (13 DDD, 52 non-DDD): 36 male, 29 children. At presentation, 46% had a creatinine >1.5mg/dL, 44% had nephrotic syndrome, 93% had hematuria. Membranoproliferative glomerulonephritis (GN) (n=26) and diffuse proliferative GN (n=22) were prevalent on LM. DDD and non-DDD groups had similar baseline clinical characteristics. Low serum C3 was more common in the DDD group (100% vs. 55%, p=0.017). At last follow-up, 67% of DDD and 24% of non-DDD patients had reached end-stage kidney disease (ESKD). By multivariate Cox regression analysis, DDD was the only independent predictor of ESKD (HR 8.34, 95% CI 1.98 to 35.20, p=0.004).

Conclusions: We have described the clinical phenotype and renal outcomes of 65 patients with C3G. We have demonstrated that DDD on EM may portend a particularly poor prognosis in this population cohort. We plan to investigate these patients for acquired and genetic defects in alternative complement pathway regulation.

FR-OR133

SYK Inhibition Reduces Autoantibody Production and Abrogates Crescentic Glomerulonephritis (cGN) and Lung Haemorrhage (LH) in Experimental Autoimmune Glomerulonephritis (EAG) Stephen Paul McAdoo,[1] John Reynolds,[1] Jennifer Smith,[1] Gurjeet Bhangal,[1] Esteban S. Masuda,[2] H. Terence Cook,[1] Charles D. Pusey,[1] Frederick W.K. Tam.[1] *[1]Imperial College London, United Kingdom; [2]Rigel Pharmaceuticals.*

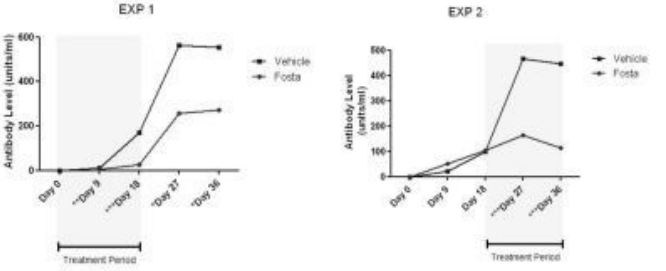
Background: SYK is a critical component of FcR and BCR signaling pathways. SYK inhibition with Fostamatinib (Fosta) prevents immune-mediated injury in several animal models, including rat nephrotoxic nephritis. The effect of SYK inhibition on other models of GN is not known, nor is the effect of SYK inhibition on autoantibody (autoAb) production in any experimental model clearly defined. This study aimed to address these questions in EAG.

Methods: In EAG, rats immunized with rat GBM antigen (α3) at day 0 develop autoAb to α3 and cGN with proteinuria by day 18, and have LH at day 36. In study 1, animals (n=8/group) received either Fosta 40mg/kg or Vehicle (Veh) by twice daily gavage from day 0-18, in order to examine the effects of SYK inhibition on induction of autoimmunity. In study 2, animals received Fosta or Veh from day 18-36, to study the effects of treatment on established disease. In both studies, animals were monitored until Day 36.

Results: Expressed as mean/group at Day 36:

	EXP 1			EXP 2		
	Veh	Fosta	Reduction	Veh	Fosta	Reduction
Proteinuria (mg/day)	172	96	41%*	118	2	98%†
Haematuria (cell/ul)	139	34	75%*	200	0	100%†
Crescentic Glom (%)	74	41	45%†	70.5	1.5	98%†
Macrophage/ Glom (Score)	10.5	12.6	NS	9.8	0.2	99%†
LH Score	2	0.85	58%*	1.75	0	100%†

*=p<0.05;† =p<0.01; NS=not significant



Conclusions: SYK inhibition reverses cGN and prevents LH in EAG. This effect appears to be mediated by inhibiting both the induction of humoral autoimmunity and the effector phase of the immune response. We believe this is the first report that SYK inhibition reduces autoAb production in a genuine model of autoimmunity. The results augur well for use of SYK inhibition in human renal disease.

Funding: Pharmaceutical Company Support - Rigel Pharmaceuticals; AstraZeneca, Government Support - Non-U.S.

FR-OR134

MicroRNA-155 Drives Th17 Immune Response and Tissue Injury in Experimental Crescentic Glomerulonephritis Christian Krebs, Sonja Kapffer, Hans-Joachim Paust, Tilman Schmidt, Sabrina Bianca Bennstein, Anett Peters, Gesa Stege, Silke R. Brix, Oliver M. Steinmetz, Rolf A. Stahl, Ulf Panzer. *Nephrology, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: IL-17-producing $CD4^+$ Th17 cells play a pivotal role in the pathogenesis of autoimmune diseases, including human crescentic glomerulonephritis and its murine model, nephrotoxic nephritis. MicroRNAs (miRNAs) have emerged as important regulators of immune cell function, however, the impact of miRNAs in the regulation of Th17 immunity remains to be fully elucidated.

Methods: MicroRNA expression was quantified in the course of NTN by qRT-PCR in mice. NTN was induced in Mir-155-deficient and in wildtype mice. Th17 responses were quantified by intracellular cytokine staining and FACS analysis as well as by renal IL-17 expression and IL-17 ELISA of splenocyte cultures. Mir-155-deficient and wildtype $CD4^+$ cells were transferred into Rag1-deficient mice. MiRNA expression was quantified in renal tissue of patients with crescentic GN by array analysis (Affimetix) and by qPCR.

Results: Here we report that upregulation of microRNA-155 (miR-155) in the spleen preceded renal Th17 cell infiltration and that Th17 immune response in the kidney and secondary lymphoid organs was markedly reduced in nephritic Mir-$155^{-/-}$ mice. Moreover, Mir-155-deficient mice developed less severe nephritis, with reduced histologic and functional injury. To examine the function of miR-155 specifically in $CD4^+$ T cells we transferred wild-type and Mir-$155^{-/-}$ $CD4^+$ T cells into nephritic Rag$1^{-/-}$ mice. Recipients of Mir-$155^{-/-}$ cells had reduced Th17 immune response and developed less severe GN than wild-type recipients. Finally we show that miR-155 expression is selectively up-regulated in the kidneys of patients with crescentic GN.

Conclusions: These findings indicate that the ameliorated glomerulonephritis in Mir-155-deficient mice is due to impaired renal and systemic Th17 immune response and identify miR-155 as a potential therapeutic target in Th17-mediated human crescentic glomerulonephritis.

FR-OR135

ANCA Epitope Specificity Determines Pathogenicity Aleeza J. Roth,[1] Jacob Hess,[1] Mirjan M. Van Timmeren,[2] Elisabeth Berg,[1] Caroline Jennette Poulton,[1] Madelyn Burkart,[1] Susan L. Hogan,[1] Yichun Hu,[1] Patrick H. Nachman,[1] Coen A. Stegeman,[2] Peter Heeringa,[2] J. Charles Jennette,[1] Gloria A. Preston,[1] Ronald J. Falk.[1] *[1]UNC Kidney Center, Department of Medicine, Univeristy of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands.*

Background: Anti-neutrophil cytoplasmic autoantibodies (ANCA) specific for myeloperoxidase (MPO) or proteinase 3 (PR3) are detectable in >90% of patients with ANCA-associated vasculitis (AAV). ANCA titers do not correlate well with disease activity. *In vivo* and *in vitro* studies demonstrate that ANCA are pathogenic. Herein we report an analysis of MPO-ANCA epitopes that elucidates the poor correlation between disease activity and ANCA titer, and failure to detect ANCA by conventional assays in some AAV patients.

Methods: Epitope mapping entailed binding MPO-ANCA to MPO, which protects epitopes from enzymatic digestion. Epitope peptides were eluted and identified by matrix-assisted laser desorption mass spectrometry (MALDI-MS). ^{16}O-to-^{18}O exchange was used to detect peptide targets of low level autoantibodies. Samples were from 52 active or 35 remission AAV patients, and 10 healthy controls. Reactivity with epitope peptide was assayed by ELISA. Pathogenic potential of ANCA was tested using *in vitro* neutrophil action.

Results: 25 unique anti-MPO epitopes were detected. Pathogenic epitopes (12/25) reacted only with ANCA from patients with active disease. Nonpathogenic epitopes (6/25) reacted with ANCA from patients in remission. Natural epitopes (7/25) reacted at very low levels with immunoglobulin from healthy controls. Antibodies to one pathogenic epitope were detectable when an epitope-masking fragment of ceruloplasmin was removed in 8/10 AAV patients who were negative by conventional assays.

Conclusions: ANCA titers in clinical assays reflect a combination of both pathogenic and nonpathogenic ANCA. Pathogenic ANCA alone correlate better with disease activity. IgG from patients with ANCA-negative AAV reacts with a restricted pathogenic anti-MPO ANCA whose target epitope is masked in serum by ceruloplasmin.

Funding: NIDDK Support

FR-OR136

Increased Recruitment of Dendritic Cells Is Associated with Tubular Atrophy in Human Kidney Disease

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Background: Dendritic cells (DC) have been defined as key players in immune-mediated kidney diseases. However, the identification and enumeration of human DC subsets in diseased kidney tissue has been restricted to limited pathologies and performed using only immunohistochemistry, demonstrating that DC are primarily localised to the tubulointerstitium.

Methods: We developed novel protocols for obtaining single cell suspensions from biopsies from a wide range of human kidney diseases to allow the detection and quantitation of DC subsets via flow cytometry. DC were defined as $CD45^+$ $lineage^-$ $HLA\text{-}DR^+$ cells and were classified into $CD11c^-CD123^+$ plasmacytoid DC (pDC) and $CD11c^+$ myeloid DC (mDC), which were subdivided into $CD1c^+$, $CD141^+$ and $CD16^+$ subsets, and enumerated with Flow-Count Fluorospheres.

Results: We observed significantly elevated numbers of total DC, $CD1c^+$ and $CD141^+$ mDC subsets in diseased kidney biopsies with interstitial fibrosis/tubular atrophy (IF/TA) compared to both diseased biopsies without IF/TA and healthy kidney tissue. Numbers of $CD16^+$ mDC were similar between groups, whilst pDC numbers were significantly higher in the IF/TA cohort compared to healthy tissue only. The expression of HLA-DR on DC in diseased biopsies with IF/TA was significantly reduced compared to healthy kidney tissue, suggestive of an altered DC phenotype. The majority of $CD1c^+$ mDC lacked expression of both CD1a and DC-SIGN, confirming these cells as infiltrating blood DC and not resident or monocyte-derived DC. Notably, higher levels of chemokine IL-8, inflammatory cytokine IL-1β and Th1-inducing IL-12p70 were detected in the dissociation supernatants of diseased kidney biopsies than healthy kidney tissue.

Conclusions: This study provides novel tools for the phenotyping and enumeration of renal DC that will allow us to better understand their functional roles in the kidney. Our data suggest that DC may play a critical role in the development of IF/TA and thus, progression to chronic kidney disease.

Funding: Government Support - Non-U.S.

FR-OR137

Reduced CD5+ B Cells in Active Anti-Neutrophil Cytoplasmic Autoantibody Vasculitis and Relapse after Rituximab

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Background: B cell significance in ANCA disease pathogenesis is underscored by the effectiveness of rituximab as therapy in ANCA-small vessel vasculitis (ANCA-SVV). To avoid infections and adverse events from therapy, clinicians require improved markers of disease activity and impending relapse to guide immunosuppression strategies post-rituximab. We hypothesize that CD5, a surrogate marker of B regulatory cells, may serve this purpose.

Methods: We investigated B cell phenotype in patients with active ANCA-SVV and in remission by flow cytometry. 54 patients were followed longitudinally for 4 to 99 m and compared to 68 healthy controls (HC). Whole blood or PBMCs were stained with antibodies to CD19, CD20, CD24, CD38, CD45, and CD5. We examined B cell phenotype in 19 patients after rituximab. Additional $CD5^+$ B cell data was acquired from clinical Rituxan panels for 29 patients.

Results: Patients with active ANCA-SVV had lower $\%CD5^+$ B cells (21±13; p=0.003) than those in remission and were similar to HC (30±14 and 30±13 respectively). After rituximab, median time to relapse was 31 months in patients maintaining normalized $\%CD5^+$ B cells, with or without maintenance immunosuppression (IS). Patients whose B cells repopulated with low $\%CD5^+$ B cells and were on low or no maintenance IS had a shorter time to relapse (17m) than patients who repopulated with low $\%CD5^+$ B cells maintained on high levels of oral maintenance IS (29m, p= 0.002). Preliminary analysis of Rituxan panel data indicates patients who had ≤30% CD5+ B cells at the time of B cell repopulation (≥1% B cells) relapsed sooner (16m) than patients who repopulated with >30% CD5+ B cells (22m; p=0.05).

Conclusions: The $\%CD5^+$ B cells, as a component of the human B regulatory cell phenotype, is a useful indicator of disease activity, remission and future relapse, and therefore, may guide remission maintenance therapy following rituximab.

Funding: NIDDK Support, Private Foundation Support

FR-OR138

A Novel Murine Model of B Cell-Mediated Proteinuria Suggests Cytokines Mediate Podocyte Injury

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Background: B cell depletion therapies have been efficacious in several glomerulopathies. The contributions of B cells to proteinuria and foot process effacement remain unknown. The development of a murine model of B-cell induced proteinuria would enhance our understanding of immune-based glomerular diseases.

Methods: The B cell model antigen model hen egg lysozyme (HEL) was biotinylated and complexed to avidin. Following intravenous (IV) injection in mice, purified naïve HEL-specific B cells were adoptively transferred and proteinuria assessed using PAGE. Kidneys were processed for immunofluorescence (IF), H&E and PAS staining, and scanning electron microscopy (SEM). Cultured podocyte membrane ruffling was assessed with DIC videomicroscopy.

Results: HEL embedded within the glomerular basement membrane (GBM) following IV injection. Proteinuria occurred after the transfer of HEL-specific B cells and was associated with foot process effacement. No antibody or complement deposition was observed in the GBM. 2-photon microscopy of live mice demonstrated that HEL-specific B cells arrested trafficking within glomeruli in the presence of HEL. The rapid kinetics of proteinuria induction suggested cytokines secreted by activated intraglomerular B cells may be responsible. We hypothesize that activation of the small Rho GTPase Rac is important in podocyte injury by virtue of its ability to regulate the actin cytoskeleton. Using murine cultured podocytes, we measured membrane ruffling in the presence of cytokines as a surrogate for Rac activation. IL-4 significantly increased cultured podocyte membrane ruffling and induced foot process retractions on ex vivo fragments of renal cortex. Hydrodynamic DNA immunization of wild-type 129 mice with plasmid encoding IL-4 lead to proteinuria.

Conclusions: We have developed a novel model of B cell-induced proteinuria with foot process effacement. Furthermore, these data demonstrate that cytokines can induce alterations in foot process morphology, leading to proteinuria. This has important implications in therapies preserving podocyte function in glomerular disease.

Funding: Private Foundation Support

FR-OR139

CD25+CD45RA+CD4+ T-Cell Subset as a Predictive Marker of Responsiveness in Pediatric Nephrotic Patients Treated with Rituximab

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Background: Rituximab, an anti-CD20 chimeric monoclonal antibody, is an increasingly popular modality of therapy in steroid-resistant nephrotic syndrome (SRsNS) patients who have failed therapy with conventional immunosuppressants. This study aimed to identify markers predicting responsiveness in pediatric nephrotic patients treated with rituximab.

Methods: Ten patients with SRsNS or cyclosporine-dependent nephrotic syndrome were given 2-weekly doses of rituximab (375 mg/m^2) for 4 doses. Both urinary and blood biochemical parameters were monitored throughout the study. Quantitation of B cells (CD19) and T cells (including CD4+CD25+CD45RA+ (naïve regulatory T-cells), CD4+CD25+CD45RO+ (memory regulatory T-cells), CD4+CD45RA+ (naïve CD4+ cells), CD4+CD45RO+ (memory CD4+ cells), CD4+CD25+ (activated CD4+ cells), CD4+ICOS+ (stimulated ICOS+CD4+ cells), CD8+ICOS+ (stimulated ICOS+ CD8+ cells), CD8+CD25+ (activated CD8+ cells), CD8+CD45RO+ (memory CD8+ cells), CD8+CD45RA+ (naïve CD8+ cells), CD8+CD25+CD45RA+ (activated naïve CD8+ cells), CD8+CD25+CD45RO+ (activated memory CD8+ cells)) was performed using multi-color flow cytometry.

Results: Six (60%) patients achieved complete remission defined as urine protein:creatinine (PCR) ratio of <0.02 g/mmol, while the rest only had partial remission (urine PCR 0.02-0.2 g/mmol). Analysis of the cellular subsets revealed a statistically significant decrease in percentage of CD19+ cells in all patients post-rituximab infusion (14.53±6.23% (pre) vs 0.46±0.58% (post), p<0.001) as anticipated. Of the various immunological subsets analyzed, CD25+CD45RA+CD4+ cells was a potential marker for complete responsiveness towards rituximab therapy (8.40±2.06 (partial responders) vs 3.11±0.69 (complete responders), p= 0.021). Using ROC curve analysis for CD25+CD45RA+CD4+, the area under the curve is 0.917 (95% CI: 0.73-1.00).

Conclusions: Our study illustrated the potential utilization of CD25+CD45RA+CD4+ cellular subset as a marker of complete responsiveness. Further studies are, however, required to affirm the above findings.

Funding: Government Support - Non-U.S.

FR-OR140

Alternative Complement Pathway Is Essential for Glomerular C3 Deposition and Progressive Proteinuria in Murine Membranous Nephropathy

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Background: Membranous nephropathy (MN) is a major cause of nephrotic syndrome in adults. Pathology is driven by subepithelial immune complexes activating complement, leading to C5b-9-mediated podocyte injury and proteinuria. Which complement pathways are implicated is not known. The alternative pathway may be involved because human IgG4 autoAbs, which are prevalent in idiopathic MN, do not activate complement via the classic pathway.

Methods: We evaluated how genetic and pharmacologic targeting of factor B (a key component of the alternative pathway) alters disease course in an active mouse model of MN that faithfully recapitulates human disease, including the prevalence of non-complement fixing IgG subclasses.

Results: Glomerular deposition of C3 and development of proteinuria after the induction of experimental MN in C57Bl/6 mice were completely abolished in congenic factor B-/- mice. After inducing experimental MN in more susceptible DBA/1 mice, treatment with a blocking anti-factor B mAb was initiated when ACR increased over 10-fold over normal values. Inhibition of factor B prevented the glomerular C3 deposition and blunted further exacerbation of albuminuria by >80%, compared to untreated control mice. Ablation of factor B activity did not affect serum titers of autoAbs or glomerular deposition of IgG and antigen.

Conclusions: Our results show that the amplification of complement activation by the alternative pathway is necessary for glomerular C3 deposition and progression of proteinuria in experimental MN. Thus, the alternative pathway has a pivotal role in the MN pathomechanism. Inhibiting factor B uncouples subepithelial immune complexes from pathogenic complement activation, providing proof-of-concept about the feasibility of this novel therapeutic strategy for treating MN.

Funding: NIDDK Support, Private Foundation Support

FR-OR141

Autoimmunity to the Alpha 3 Chain of Type IV Collagen Is Triggered by 'Autoantigen Complementarity'

John Reynolds,[1,4] Gloria A. Preston,[2] Barrak M. Pressler,[3] Peter Hewins,[2] Michael C. Brown,[2] Aleeza J. Roth,[2] Elizabeth A. Alderman,[2] Donna O. Bunch,[2] J. Charles Jennette,[2] H. Terence Cook,[1] Ronald J. Falk,[2] Charles D. Pusey.[1] *[1]Renal Section, Department of Medicine, Imperial College, London, United Kingdom; [2]UNC Kidney Center, Department of Medicine, University of Noth Carolina, Chapel Hill, NC; [3]Department of Pathology, University of North Carolina, Chapel Hill, NC; [4]Institute of Biomedical and Environmental Science and Technologies, University of Bedfordshire, Luton, United Kingdom.*

Background: 'Autoantigen complementarity' is a theory proposing that the initiator of an autoimmune response is not necessarily the autoantigen or its molecular mimic, but may instead be a peptide that is 'antisense/complementary' to the autoantigen. We investigated whether such complementary proteins play a role in the immunopathogenesis of autoimmune glomerulonephritis. Experimental autoimmune glomerulonephritis, a model of anti-glomerular basement membrane (GBM) disease, can be induced in Wistar Kyoto (WKY) rats by immunization with the α3 chain of type IV collagen.

Methods: In this study, WKY rats were immunized with a complementary α3 peptide (c-α3-Gly) comprised of amino acids that 'complement' the well characterized epitope on α3(IV)NC1, pCol(24-38).

Results: Within 8 weeks post-immunization, these animals developed cresentic glomerulonephritis, similar to pCol(24-38)-immunized rats, while animals immunized with scrambled peptide were normal. Anti-idiotypic antibodies to epitopes from c-α3-Gly-immunized animals were shown to be specific for α3 protein, binding in a region containing sense pCol(24-38) sequence. Interestingly, anti-complementary α3 antibodies were identified in sera from patients with anti-GBM disease, suggesting a role for 'autoantigen complementarity' in immunopathogenesis of the human disease.

Conclusions: This work supports the idea that autoimmune glomerulonephritis can be initiated through an immune response against a peptide that is anti-sense or complementary to the autoantigen. The implications of this discovery may be far reaching, and other autoimmune diseases could be due to responses to these once unsuspected 'complementary' antigens.

Funding: Government Support - Non-U.S.

FR-OR142

Costimulation Blockade with Systemic SiRNA Anti CD40 Ameliorates Experimental Lupus Nephritis in a Dose Dependent Manner

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Background: In a murine model of lupus nephritis we assayed the potential therapeutic benefit of mice-specific-siRNA-CD40 with different doses, taking into the pathogenic role of CD40-CD40L pathway in this autoimmune nephritis.

Methods: Six month old NZB/NZWF1 mice were divided into the following groups:Cyclophosphamide CYPgroup:mice were treated with intraperitoneal Cyclophosphamide,50mg/kg/10d;CTLA4 group:mice were treated with 50µg of intraperitoneal CTLA4 three times/week;si1w group:mice were treated with 50µg of intraperitoneal siRNA once/week, si2w group:mice were treated with 50µg of intraperitoneal siRNA twice/week, and LES (Control-group):without therapy.Mice were treated for 12 weeks.

Results: Mice from CYP and siRNA-CD40 groups had lower proteinuria and albuminuria versus control group.CYP was able to interrupt the progression of the antibody production, a dose dependent scale effects with the treatment of siRNA-anti-CD40 was seen. All treatments prolonged cumulative survival. Costimulatory blockade clearly reduced both the mean score and elementary lesions of lupus nephritis.It was more effective in reducing the deposition of IgG in the kidney than CYP treatment.siRNA treatment induced down regulation of renal mRNA for CD40, modulated innate immunity gene expression, chemokine and cytokines, and the expression of inflammasome components.CYP treatment reduced B cell population and did not affect the activation process; of note, the main feature of costimulation blockade with siRNA was a reduction in early B/T cell activation.Plasma cell presence in kidney was reduced in all treatment groups, those groups with costimulatory blockade treatment, shows less plasma cell presence than CYPgroup.

Conclusions: From our results we can conclude that our specific siRNA anti CD40 is highly effective to slow down the course of experimental lupus nephritis. Thus, the blockade of costimulatory signal CD40 becomes as a potential therapeutic tool in this renal autoimmune disease.

FR-OR143

Cellular Infiltrates and NFKB Subunit c-Rel Signaling in Kidney Allografts of Patients with Clinical Operational Tolerance

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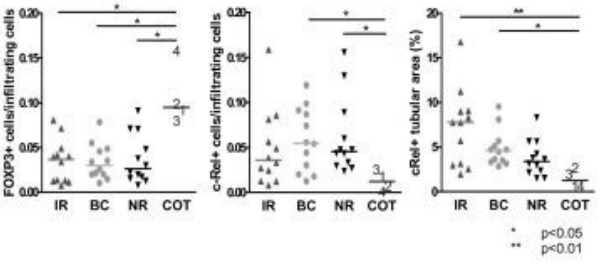
Background: NFKB plays a potential role in tolerance by orchestrating onset and resolution of inflammation and T-reg differentiation through subunit c-Rel. We characterized cellular infiltrates and expression of NFKB1, c-Rel and its upstream regulators phosphatidylinositol 3-kinase (PI3K)/RAC-alpha serine-threonine kinase (Akt1) in allograft biopsies from patients with spontaneous clinical operational tolerance (COT).

Methods: Paraffin-fixed kidney allograft biopsies from 40 patients with COT (n=4), interstitial rejection (IR; n=12), borderline changes (BC; n=12) and long-term allograft function without rejection (NR; n=12) were studied. Cellular infiltrates and immunohistochemical expression of key proteins of the NFKB pathway were evaluated in the cortical tubulointerstitium using digital image analysis. Results were given as mean±SEM.

Results: Biopsies from patients with COT exhibited a comparable amount of cellular infiltrate to IR, BC and NR (COT: 191±81; IR: 291±62; BC: 178±45; NR: 210±42 cells/mm^2), but a significantly higher proportion of FOXP3 positive cells (COT: 11±1.7%; IR: 3.5±0.70%; BC: 3.4±0.57%, NR: 3.7±0.78% of infiltrating cells, p=0.02). C-Rel expression in cellular infiltrates was significantly elevated in IR, BC, and NR when analyzing the number of positive cells/mm^2 (p=0.02) and positive cells/infiltrating cells (p=0.04). In contrast, tubular PI3K and c-Rel expression were significantly higher in IR and BC, but not in NR compared to COT (p=0.03 and p=0.006, respectively). With Akt1, similar tendencies were seen.



Conclusions: Allografts from COT patients show significant cellular infiltrates but a distinct expression of proteins involved in the NFKB pathway and a higher proportion of FOXP3 positve cells.

FR-OR144

Increased Cellular Immune Response Related Gene Expresion in Both DSA+ and DSA- Transplant Glomerulopathy

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Background: Transplant Glomerulopathy (TGP) with donor-specific anti-HLA antibodies (DSA) and positive C4d staining is a criterion of chronic antibody-mediated rejection (AMR). The etiology of DSA-/C4d-TGP is unclear. We investigated the mechanisms of DSA-/C4d- TGP using microarray analysis of allografts.

Methods: We studied 255 clinically indicated kidney transplant biopsies. The biopsy gene expression profiles were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: There were 15 (6%) DSA+ and 24 (9%) DSA– TGP patients. 48 (19%) patients with non-specific interstitial fibrosis/tubular atrophy (IFTA) without DSA were used as controls. DSA+ TGP patients had higher peritubular capillary C4d+ staining (20% vs. 0% vs. 2%), microcirculation inflammation scores as seen by higher glomerulitis (0.7 vs. 0.4 vs. 0.1; p=0.02) and peritubular capillaritis (1, 0.3, 0.2; p=0.02) compared to DSA- TGP and IFTA. Microarray analyses were performed in 10 DSA+, 8 DSA-/C4d- TGP, 10 IF/TA, and 9 pre-implantation living donor biopsies. Both DSA+ and DSA- TGP biopsies showed upregulation of genes related to activation of the cellular immune response including T cells, mast cells, T cell receptor, interferon gamma and transforming growth factor beta receptor signaling pathways, T cell co-stimulation, innate immunity, and chemotaxis. Using Pathogenesis Based Transcripts, upregulation of Endothelial Cell Associated Transcripts (ENDAT) was seen in all transplant kidney biopsies. There was a trend towards significant upregulation of interferon gamma and rejection induced and cytotoxic T cell associated transcripts in both DSA+ (p=0.06) and DSA– TGP (p=0.07). The only significant difference between DSA+ and DSA- TGP was upregulation of transcripts differentially expressed between rejection-classified biopsies from DSA+ patients.

Conclusions: Microarray analysis revealed significant activation of cellular immune-response related genes in both DSA+ and DSA- TGP biopsies. The upregulation of ENDAT in all transplant biopsies questions its promise as a molecular biomarker for AMR.

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Reproducibility for C4d and BK Immunohistochemistry in Renal Allografts: Results from the Banff Initiative for Quality Assurance in Transplantation (BIFQUIT) Michael Mengel,[1] Robert B. Colvin,[2] Heinz Regele,[3] Parmjeet S. Randhawa.[4] *[1]Laboratory Medicine and Pathology, Univeristy of Alberta; [2]MGH; [3]University of Insbruck; [4]University of Pittsburg.*

Background: Detection of C4d and BK related proteins is crucial for diagnosing antibody mediated rejection and polyoma virus nephropathy (PVN), respectively. Yet formal reproducibility studies are limited.

Methods: We conducted an international multi-center trial (78 participants at 60 institutions) to assess the reproducibility for C4d and BK immunohistochemistry on paraffin-sections. Tissue microarrays (TMA) were constructed comprising kidney allograft specimens representing the whole analytical spectrum for C4d and PVN, respectively. Participants stained the TMA slides using local protocols, evaluated their stains following the current Banff grading schemas and entered their scores online. Stained slides were returned for centralized panel scoring. Weighted kappa statistics were used to determine reproducibility.

Results: Inter-observer reproducibility (comparing local participant scoring to panel scores) was fair (kappa 0.45) for C4d and substantial for BK (kappa 0.64). Inter-laboratory reproducibility was moderate (kappa 0.46) for C4d but was inacceptable for BK (kappa -0.22). Inter-observer reproducibility for C4d could be significantly improved by omitting the Banff C4d grading schema and only considering +/- calls (kappa 0.60). Scoring only C4d+/- inter-laboratory reproducibility also improved considerably (kappa 0.78). Collapsing the proposed BK scoring schema into a simple positive/negative call improved the BK inter-laboratory reproducibility to 0.77.

Conclusions: These results indicated that C4d and BK (SV40 large-T antigen) results reported from paraffin section are highly variable between institutions. For C4d this is mainly due to complexity of the current Banff grading schema. But also for BK, the proposed grading schema combining staining intensity and % stained cells was the major source of variability between laboratories. Respective simplification of the grading schemas would significantly improve reproducibility for C4d and BK immunohistochemistry on paraffin sections.

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FR-OR146

Interstitial Fibrosis and Tubular Atrophy on Biopsy for Cause in Recipients of Kidney Transplants Is Not Always due to Calcineurin Inhibitor Exposure Venkata R. Reddivari,[1] Ravinder K. Wali,[2] Matthew R. Weir.[1] *[1]UMMC; [2]Inova Transplant Center.*

Background: Intersitial fibrosis and tubular atrophy(IF-TA) is often attributed to cumulative exposure to calcineurin inhibitor.Can replacement of CNI with m-TOR inhibitors prevent progression in IF-TA and graft failure?

Methods: Patients with IF-TA on for cause biopsy and on maintenance CNI and MMF without steroids were treated with sirolimus (SRL) as the exposure group and continuation of CNI as the comparator group between Jan 2003 to Apr 2006,prospectively followed for 3 yrs.The primary outcome was a composite of graft loss and death with functioning graft (DWF).Secondary out comes included e-GFR and Cr at 1,2,and 3yrs.

Results: A total of 497 recipients of kidney transplants with index biopsy had IF-TA. As routine care CNI was discontinued in 221(45%) patients.There were no differences in the baseline characteristics between the two groups (table 1). There was a difference in the primary and secondary outcomes in the two groups.

TAB1			
Recipient data	Sirolimus (N=221)	Tacrolimus (N=276)	P-value
Age ,years (Mean±SD)	46.2 ±18.4	48.6 ±18.4	0.152
Gender, female	83 (38%)	99 (36%)	0.324
Race, blacks	108 (47%)	125 (53%)	0.324
Type of transplant,Deceased	147 (67%)	182 (55%)	0.782
Time to index biopsy (months), Mean ±SD (95% CI)	10.1 ±15.4 (8.14-12.24)	10.2 ±16.6 (8.19-12.3)	0.96
Serum Cr at index biopsy, Mean ± SD (95% CI)	3.1 ±1.9 (2.3-3.3)	3.3 ± 2.5 (3.0-3.6)	0.282
e-GFR at index biopsy, Mean ± SD (95% CI)	31.2± 22.6 (28.18-34.33)	32.0 ±19.8 (29.52-34.49)	0.245
TAB 2			
Primary out come,n (%) a	76 (35%)	53 (19%)	0.001
Graft loss,n (%)	28 (12.6%)	31 (11.2%)	0.08
Death with functioning graft, n (%) b	54 (25%)	22 (8%)	0.001

Unless otherwise noted, data are represented as numbers (percentage) of patient, Primary outcome(graft loss and DFW,Tab 2 a and b Kaplan-Meier log-rank p value is <0.05

After adjusting for baseline variables,none of these had a impact on primary outcome and graft function on Cox analysis.

Conclusions: Discontinuation of CNI does not abrogate IF-TA. SRL use increased the risk of graft loss, DWF, and death after graft failure. Routine practice of changing CNI to SRL in patients with IF-TA needs to be studied.

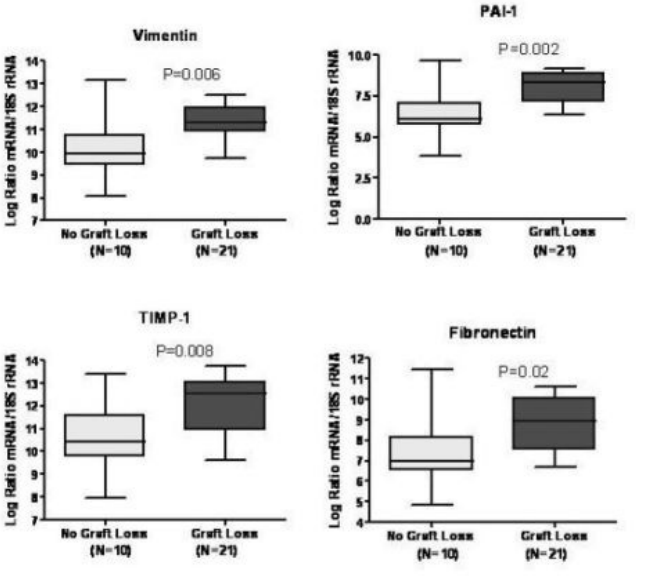
FR-OR147

Prediction of Graft Loss Following BKV Nephropathy (BKVN) Non-Invasively Darshana Dadhania, Catherine Snopkowski, Ruchuang Ding, Thangamani Muthukumar, Jun B. Lee, Vijay K. Sharma, Surya Seshan, Manikkam Suthanthiran. *Nephrology, Weill Cornell Med. Center, NY, NY.*

Background: BKVN associated graft loss is common among renal allograft recipients. Development of prognostic biomarkers may aide in the management & development of novel treatment strategies. We studied whether biomarkers associated with fibrosis measured in urinary cells at the time of initial BKVN diagnosis would predict subsequent graft loss.

Methods: We isolated total RNA from urinary cells from 31 kidney recipients with BKVN (10 with & 21 with out graft loss) and measured mRNA levels associated with inflammation (granzyme-B, perforin, CD25, FoxP-3, CTLA4, CD103), tubular cells (E-cadherin, NKCC2, USAG), fibrosis (TGFb1, Vimentin, BMP, FGF, HGF, PAI-1, TIMP-1, FIbronectin, a-smooth muscle actin, collagen 1A1), BKV VP1 and house keeping gene 18S rRNA using pre-amplification enhanced real-time quantitative PCR assays. We compared log transformed 18S-normalized mRNA levels to identify any differences between the two groups & then performed multivariate logistic regression analysis using backward stepwise elimination method to indentify independent biomarkers for graft loss.

Results: Univariate analysis of mRNA levels demonstrated that vimentin, PAI-1, TIMP-1, and fibronectin at the time of initial BKVN diagnosis were elevated in individuals with graft loss compared to those without (Figure). Serum creatinine at time of biopsy in those with graft loss was 2.9±1.3 vs. and 2±0.7 (P=0.01). Multivariate logistic regression analysis identified PAI-1 as an independent predictor of graft loss (P=0.007).



P-value was calculated by comparing the two groups using Mann Whitney Test.

Conclusions: We identified PAI-1 as an independent predictor of graft loss following BKVN diagnosis. Measurement of PAI-1 at the time of BKVN diagnosis will allow identification of high-risk patients and personalization of therapy.

FR-OR148

Transcriptomic Profile of Peripheral Blood Mononuclear Cells (PBMC) in Renal Chronic Antibody-Mediated Rejection (CAMR) Paola Pontrelli,[1] F. Rascio,[1] Matteo Accetturo,[1] Margherita Gigante,[2] Giovanni Stallone,[2] Anna Zito,[1] Maddalena Gigante,[2] G. Castellano,[1] Loreto Gesualdo,[1] Giuseppe Grandaliano.[2] *[1]Dept. of Emergency and Organ Transplantation, University of Bari, Italy; [2]Dept. of Medical and Surgical Sciences, University of Foggia, Italy.*

Background: CAMR represents one of the main causes of chronic graft injury, but the molecular mechanisms underlying it are still unclear. Aim of the study was to define a transcriptomic profile of CAMR using genome-wide expression analysis.

Methods: We enrolled 10 patients with biopsy-proven CAMR and 10 stable transplant recipients with normal graft function (control group). mRNA expression profile of PBMC isolated from both groups was assessed by Agilent microarray.

Results: The statistical analysis (unpaired t tests, Benjamini-Hochberg correction) revealed 67 probe sets (53 genes) differentially expressed in the two groups ($p<.05$; $FC\geq2$). In 97% of the cases (65/67) gene expression was increased in CAMR. PCA showed the ability of these genes to distinguish the two groups. The biological functions concerned immune ($p<.01$) and inflammatory response ($p<.01$).The main canonical pathways were complement system ($p=8.49*10^{-7}$) and IFNα ($p=6.0*10^{-5}$). The functional networks, generated by Ingenuity Pathway Analysis, showed that more than 41% of the differentially-expressed genes (22/53) regulate or are regulated by IFNα (IPA score=52, $p\leq.05$). Real time PCR confirmed an increase of two genes included in IFNα a network, GBP1 and CXCL9, in CAMR when compared to control group (GBP1: FC=4.8, p=.0001; CXCL9: FC=1.6, p=.0004) and patients with chronic cell-mediate rejection (GBP1: FC=2.9, p=.001; CXCL9: FC=1.3, p=.04). Moreover, we observed by flow cytometry an increase of circulating $BDCA1^+$ myeloid dendritic cells (DC) (p=.03) and a reduction of circulating $BDCA2^+$plasmacytoid DC (p=.03), the main producers of IFNα, in CAMR group compared to the other two groups. The $BDCA2^+$DC were increased in the kidney graft biopsies of CAMR (p=.04) patients along with MXA, a protein induced by IFNα (p=.02), as shown by confocal microscopy.

Conclusions: Our data suggest a key role of IFNα during CAMR and open new perspectives for identification of therapeutic targets.

Funding: Government Support - Non-U.S.

FR-OR149

Non-C1q-Fixing Antibodies Predominate in C4d-Negative Chronic Antibody Mediated Rejection Evan A. Farkash, Peng Zhang, Zeeshan Khawaja, Susan Saidman, Rex Neal Smith, Winfred W. Williams, A. Bernard Collins, Alessandro Alessandrini, Robert B. Colvin. *Massachusetts General Hospital, Boston, MA.*

Background: Chronic active antibody mediated rejection (CAMR) is a major cause of late kidney allograft dysfunction and loss. CAMR diagnosis requires circulating donor specific antibody (DSA), C4d deposition in peritubular capillaries (PTC), and histologic evidence of chronic injury. However, molecular and longitudinal studies suggest that some cases of CAMR lack C4d staining. Either the C4d stain is insensitive, or some alloantibodies act via a complement-independent pathway. We hypothesize that some cases of CAMR are caused by antibodies unable to fix complement.

Methods: All for-cause biopsies on transplants of >1 year duration from 2006 to 2011 with saved serum from within 3 weeks of the biopsy were identified. PTC C4d staining was evaluated by indirect immunofluorescence. Serum samples were tested for HLA class I and II DSA using single lot Luminex single antigen beads (LabScreen, OneLambda). To determine whether DSA fixed complement, positive sera were heat-treated, incubated with C1q, and evaluated using beads and an anti-C1q reporter (C1qScreen, OneLambda).

Results: Of 129 specimens, 56 were DSA+C4d+, 40 were DSA-C4d-, 28 were DSA+C4d-, and 5 were DSA-C4d+. DSA in the absence of tissue C4d portended a rapid progression to graft failure. 19/28 cases of C4d-negative CAMR (DSA+C4d-) lacked detectible C1q-fixing antibody. In contrast, 48/56 cases of C4d-positive CAMR (DSA+C4d+) had detectible C1q-fixing antibody ($p<5x10-6$, Fisher's exact test). Tissue C4d positivity and C1q-fixation both show a strong correlation with increasing DSA MFI. Interestingly, several patients have stronger or weaker C1q fixation than predicted by their DSA MFI.

Conclusions: We successfully identify cases of C4d-negative late antibody mediated rejection. These cases have poor 2 and 5 year graft survival. Direct endothelial injury or leukocyte-recruitment via Fc receptor ligation may predominate in cases of C4d-negative CAMR. Chronic rejection via these pathways may cause rapid progression to graft failure. Future studies will also evaluate whether DSA C1q-fixation correlates with antibody subclass.

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FR-OR150

Antigen Specific ELISA to Improve Anti-PLA2R1 Antibody Monitoring after Renal Transplantation for Membranous Nephropathy Barbara Seitz-Polski,[1] Christine Payre,[2] Laetitia Albano,[1] Elisabeth Cassuto,[1] Sylvia Benzaken,[3] Ghislaine Bernard,[3] Vincent L.M. Esnault,[1] Gerard J. Lambeau.[2] *[1]Nephrology, Nice University Hospital, Nice, France; [2]Institute of Molecular and Cellular Pharmacology, CNRS and University of Nice, Valbonne, France; [3]Immunology, Nice University Hospital, Nice, France.*

Background: Membranous Nephropathy (MN) recur after renal transplantation (RT) in 30 to 42% of cases, decreasing graft survival. Autoantibodies (Anti-PLA2R1 Ab) directed to phospholipase A2 receptor type 1 (PLA2R1) are found in 65 to 82% of patients with MN. The pathogenic role and the interest of anti-PLA2R1 Ab to predict recurrence of MN in kidney recipiens remain controversial.

Methods: An antigen specific ELISA measuring levels of anti-PLA2R1 Ab in patients'sera was established and validated in a cohort of 120 MN patients, and compared with Western blot (WB) and Indirect ImmunoFluorescence (IIF) on HEK cells transfected with PLA2R1.

Results: ELISA had higher sensibility and accuracy than WB and IIF. Twelve kidney recipiens with MN had serial sera available for follow-up studies. Out of 8 patients with anti-PLA2R1 Ab at the time of RT, 1 patient relapsed with persistent high anti-PLA2R1 Ab titers and was successfully treated with rituximab, 1 patient had a histological relapse with persistently high anti-PLA2R1 Ab titers but no proteinuria under maximal renin angiotensin system blockadge, and 6 patients with a decrease in their anti-PLA2R1 Ab titers after RT did not relapse, including 1 with an increase of proteinuria induced by a BK virus nephropathy. One out of 4 patients relapsed with no anti-PLA2R1 Ab at the time of RT, suggesting the presence of another antigen target.

Conclusions: The established ELISA appeared to be a suitable assay to accurately monitor anti-PLA2R1 Ab and predict MN recurrence after RT.

Funding: Government Support - Non-U.S.

FR-OR151

Do Anti-Phospholipase A2 Receptor Antibodies Predict Recurrence of Membranous Nephropathy after Transplantation? Rivka Ayalon,[1] Laurence H. Beck,[1] Fernando C. Fervenza,[2] Fernando G. Cosio,[2] Daniel C. Brennan,[3] David J. Salant.[1] *[1]Boston University Medical Center; [2]Mayo Clinic; [3]Washington University.*

Background: Primary Membranous Nephropathy (MN) recurs in 10-45% of cases after transplantation. Early diagnosis of recurrent MN should prompt treatment as graft loss occurs in a high proportion of cases. Previous studies have not disclosed any pre-transplant variables that differentiate patients that will recur from those that will not. We analyzed if the presence of circulating Anti-Phospholipase A2 Receptor Antibodies (anti-PLA2R) at the time of transplantation predicts recurrence and assessed the prevalence of anti-PLA2R soon after recurrence.

Methods: We conducted a retrospective analysis of anti-PLA2R by western blotting of sera obtained at or before transplantation (pre-TP), when available, and within 4 months of recurrence or non-recurrence as diagnosed by protocol biopsy or proteinuria. Anti-PLA2R level was semi-quantitated by band intensity.

Results: Pre-TP sera were available from 34 patients. MN has recurred in 17 of the 23 patients that were anti-PLA2R positive (74%); however 8 (32%) of the patients that recurred were negative for pre-TP anti-PLA2R. Of 16 patients in which serum was available at the time of recurrence, anti-PLA2R was detected in 11 (69%) and was negative in 5 (31%). The median time for recurrence in 9 patients that were strongly positive for anti-PLA2R pre-TP was significantly shorter than in 7 patients with undetected pre-TP anti-PLA2R [11 (1- 45) vs 51 (12 – 270) weeks, p=0.0127].

Conclusions: We found that positive anti-PLA2R pre-TP predicts a high risk for recurrence but not all seropositive cases have recurred to date. The absence of pre-TP anti-PLA2R does not rule out future recurrence, which might be explained by post-TP reappearance of anti-PLA2R or other as yet unidentified antibodies. The prevalence of anti-PLA2R at the time of recurrence is similar to that seen in primary MN. Our results also suggest that high pre-TP anti-PLA2R levels predict early recurrence. Thus we propose that the presence of anti-PLA2R before or after transplantation merits close monitoring for recurrent MN.

FR-OR152

Soluble Urokinase Receptor (suPAR) in the Serum and Urine of Patients with Focal Segmental Glomerulosclerosis (FSGS) and IgA Nephropathy Carlos R. Franco-Palacios,[1] John C. Lieske,[1] Hani Wadei,[2] Andrew D. Rule,[1] Nick Voskoboev,[1] Mark D. Stegall,[1] Fernando G. Cosio,[1] Hatem Amer.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic, Jacksonville, FL.*

Background: Elevated serum suPAR levels have recently been identified in a subset of patients with FSGS.

Methods: Serum and urine suPAR were measured in bio-banked pre-kidney transplant (KTx) samples using a commercial ELISA kit. Four groups were studied (n=10 each): FSGS with recurrence in the first year post (R-FSGS); FSGS patients without recurrence post KTx (NR-FSGS); IgA nephropathy (IgA) and healthy kidney donors (Do).

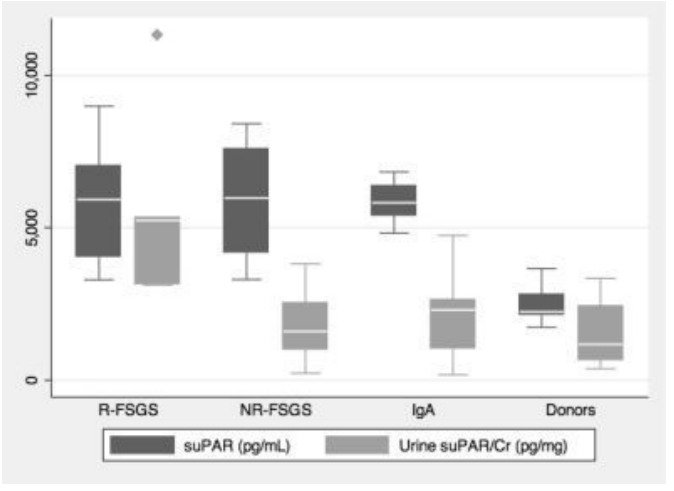
Urine samples were not available for 2 NR-FSGS and 5 R-FSGS patients. Samples were collected before any plasmapheresis or anti CD20 therapy.

Results: Results are summarized in Table 1 and Figure 1.

Table 1

	R-FSGS	NR-FSGS	IgA	Do	P
Age at KTx, yrs*	47.1 (17.4)	53.1 (14.1)	42.7 (10.8)	42.4 (9.3)	>0.05
Male**	6 (60)	8 (80)	7 (70)	4 (40)	>0.05
Cystatin C eGFR (ml/min/1.73m2)*	9 (3)	12 (6)	8 (4)	100 (15)	<0.001 vs Do
Urinary albumin mg/g creat***	7576 [4120, 9896]	1752 [820, 2894]	1237 [726, 3889]	6 [2, 11]	<0.001
Serum suPAR pg/ml ***	5922 [4027, 7313]	5974 [4061, 7706]	5819 [5297, 6362]	2246 [2048, 2879]	<0.001 vs Do
Urinary suPAR pg/mg***	5239 [3131, 8321]	1598 [754, 2815]	2304 [964, 2889]	1175 [662, 2492]	0.01

* mean (SD), ** N (%), *** median [25, 75th percentile]



In KTx patients, serum suPAR did not correlate with pretransplant albuminuria or proteinuria, Spearman's rho=0.26 and 0.16, P>0.05. Urinary suPAR did correlate with albuminuria and proteinuria, Spearman's rho=0.69 (both), P=<0.001.

Conclusions: Serum suPAR was elevated in both recurrent and non-recurrent FSGS, as well as IgA nephropathy. Urinary suPAR appears to be elevated in R-FSGS. Further studies regarding the use of serum and urine suPAR to predict renal histology and risk of disease recurrence after KTx are needed.

Funding: Private Foundation Support

SA-OR001

Circulating Klotho Levels Are Independently Associated with eGFR in Patients with Chronic Kidney Disease

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Background: Klotho is expressed in the renal distal tubules and exerts coreceptor function for FGF-23. It can also be released into the circulation by proteinase-induced cleavage or alternative splicing, and acts like an anti-oxidant. However, it is unknown whether circulating klotho levels are associated with estimated glomerular filtration rate (eGFR) in patients with chronic kidney disease (CKD).

Methods: We analyzed 243subjects who were diagnosed as CKD between January 2006 and December 2011. Demographic and clinical data were reviewed based on medical records. We measured serum concentrations of klotho, FGF-23, high-sensitivity C-reactive protein (hsCRP), and intact parathyroid hormone (iPTH).

Results: The mean age of the subjects was 45.7 years. The median klotho and FGF-23 levels were 396.3 (306.3-558.4) and 68.2 (52.2-99.1) pg/ml, respectively. When the subjects were categorized into 5 groups according to eGFR, circulating klotho levels gradually decreased as the stages of CKD increased (P for trend <0.01). Log-transformed klotho (log α-klotho) was positively correlated with eGFR (γ=0.503, P<0.001), serum albumin (γ=0.274, P<0.001) and calcium concentrations (γ=0.257, P <0.001), whereas it was negatively correlated with age (γ=-0.395, P<0.001), mean arterial pressure (γ=-0.161, P<0.001), phosphorus levels (γ=-0.169, P = 0.009), log FGF-23 (γ=-0.245, P<0.001), log iPTH (γ=-0.196, P=0.003), log hsCRP (γ=-0.285, P<0.001), and log proteinuria (γ=-0.377, P<0.001). In a multivariate linear regression, log α-klotho was independently associated with eGFR (β=0.179, P<0.001).

Conclusions: Circulating klotho levels decreased as renal function declined and were independently associated eGFR in CKD patients, suggesting that klotho may play a protective role in disease progression.

SA-OR002

Dietary Habits, Poverty, and Chronic Kidney Disease in an Urban Population

Deidra C. Crews,[1] Marie Kuczmarski,[2] Edgar R. Miller,[1] Alan B. Zonderman,[3] Michele Kim Evans,[3] Neil R. Powe.[4] *[1]Johns Hopkins U.; [2]U. of Delaware; [3]National Institute on Aging, NIH; [4]San Francisco General Hospital.*

Background: Poverty is associated with chronic kidney disease (CKD) in the US and worldwide. Poor dietary habits may contribute to this disparity.

Methods: We examined the relationship between adherence to the Dietary Approaches to Stop Hypertension (DASH) diet and CKD, by poverty status in the Healthy Aging in Neighborhoods of Diversity across the LifeSpan (HANDLS) study (Baltimore, MD). DASH scoring was based on 9 target nutrients (total fat, saturated fat, protein, fiber, cholesterol, calcium, magnesium, sodium, and potassium), with adherence defined as a score ≥4.5. CKD was defined as eGFR <60 ml/min/1.73m² (CKD-EPI). Multivariable logistic regression assessed the relationship of DASH score tertile and CKD, stratified by poverty status (income below or above 125% of poverty guideline).

Results: Among 2,058 participants (mean age 48 years; 57% black; 44% male; 42% with poverty), median DASH score was 1.5 (IQR, 1-2.5). Only 5.4% were adherent. Evaluation of DASH score tertiles revealed male sex, black race, poverty and smoking to be more prevalent among the lower tertiles, while higher education and regular health care were prevalent among the highest tertile (P<0.05 for all). Nutrient analysis by poverty status revealed fiber, calcium, magnesium and potassium intake to be lower, and cholesterol higher, among the poverty as compared to non-poverty group (P<0.05 for all), with no difference in sodium intake. CKD was present among 5.6% of the poverty, and 3.8% of the non-poverty group (P=0.05). DASH tertile was associated with CKD only among the poverty group (final model P interaction 0.02).

Logistic Regression Models of DASH Score Tertiles and CKD, by Poverty Status

		Poverty (N=869)			Non Poverty (N=1189)		
Model	Variables Included	DASH Tertile	OR (95%CI)	p-value for trend	DASH Tertile	OR (95%CI)	p-value for trend
1	DASH tertile only	Lowest	3.32 (1.26-8.73)	0.02	Lowest	0.77 (0.38-1.56)	0.5
		Middle	2.93 (1.06-8.13)		Middle	0.81 (0.38-1.71)	
		Highest	reference		Highest	reference	
2	+age, sex, race	Lowest	3.20 (1.19-8.62)	0.03	Lowest	0.91 (0.44-1.89)	0.8
		Middle	2.85 (1.00-8.10)		Middle	0.98 (0.45-2.10)	
		Highest	reference		Highest	reference	
3	+ years of education, regular health care	Lowest	3.12 (1.16-8.41)	0.03	Lowest	0.90 (0.43-1.88)	0.8
		Middle	2.73 (0.96-7.76)		Middle	0.91 (0.42-1.97)	
		Highest	reference		Highest	reference	
4	+ diabetes, hypertension, smoking status	Lowest	2.91 (1.06-7.96)	0.06	Lowest	0.77 (0.36-1.67)	0.5
		Middle	2.71 (0.94-7.82)		Middle	0.95 (0.44-2.07)	
		Highest	reference		Highest	reference	

Conclusions: Poor dietary habits are strongly associated with CKD among the urban poor and may represent a target for interventions aimed at reducing disparities in CKD.

Funding: Other NIH Support - National Institute on Aging

SA-OR003

Educational Attainment or Income Inequality, Which Is the Stronger Determinant of Chronic Kidney Disease in a Western European and a US Population?

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Background: Higher income inequalities and income dependent access to health care are potential explanations for more consistent associations of CKD with income-based measures of socio-economic status (SES) than educational level in US populations. In Western Europe access to health care is mostly income independent, and education as SES measure might therefore be a stronger determinant of CKD. Therefore, we investigated the relative strengths of the associations of educational and income levels with CKD in a Western European and a US population.

Methods: Baseline data were used from PREVEND (N=7,367) and NHANES 1999-2002 (N=4,563), general population-based cohort studies in the Netherlands and USA, respectively. Education and income were divided in five levels of nearly equal size. CKD was defined as eGFR <60 mL/min/1.73m2 (using creatinine and cystatin C) and/or albuminuria ≥30 mg/24h or ACR≥30mg/g.

Results: In logistic regression models, including age, gender, race, education (for income) and income (for education), only educational level in PREVEND showed a significant association with CKD [OR=1.43, 95% CI (1.02–1.99), p=0.037 and 1.36, 95% CI (0.95–1.97), p=0.096 for lowest vs. highest category of education and income, resp.] and only income level in NHANES [OR = 2.89, 95% CI (1.56–5.85), p=0.001 and 0.78, 95% CI 0.43–1.40), p=0.392 for lowest vs. highest category of income and education, resp.]. For PREVEND, adding education to the model with income improved fit of the model significantly (p=0.002), while for NHANES adding income to the model with education did so (p<0.001). Sensitivity analyses with other CKD defining variables (eGFR CKD-EPI <60 and/or albumin: creatinine ratio>30 mg/g) and other acknowledged SES associated outcomes like smoking and obesity resulted in similar findings.

Conclusions: Educational level is a stronger determinant of CKD in a Western European population, while income inequality is in the US population. Preventive strategies addressing social inequalities in CKD prevalence should therefore employ a region based approach.

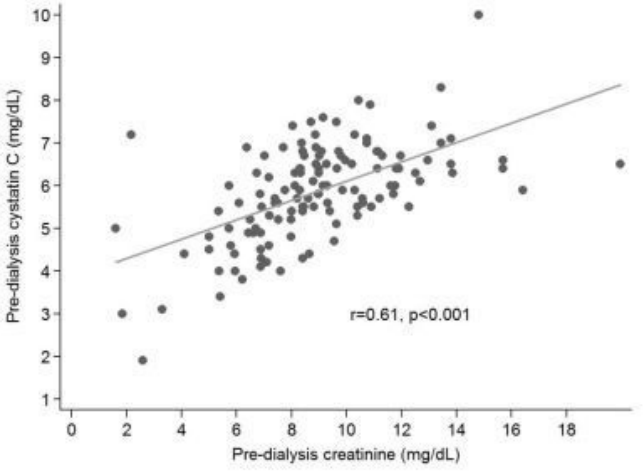
SA-OR004

Body Composition Affects Serum Creatinine but Not Serum Cystatin C Levels Nestor E. Almeida,[1] T. Alp Ikizler,[3] Y. Zhang,[1] R. Filipowicz,[1] G. Morrell,[1] G. Wei,[1] J. Abraham,[1] A. N. Habib,[1] T. S. Bjordahl,[1] Tom Greene,[1] Alfred K. Cheung,[1,2] S. Beddhu.[1,2] *[1]Univ of Utah, SLC, UT; [2]VA, SLC, UT; [3]Vanderbilt Univ, Nashville, TN.*

Background: Muscle mass is known to affect serum creatinine (SCr), it is unclear whether body composition (BC) affects serum cystatin C (SCy). Non-renal factors affecting SCy could be better studied when the kidney function is known. Therefore, in hemodialysis (HD) pts with measured residual urine volume, we examined the associations of BC with SCy and SCr.

Methods: 93 pts in the ongoing Protein Intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) study with MRI data on mid-thigh muscle area (MTMA) and intra-abdominal fat area (IAFA) and preHD SCy and SCr were included. Cross-sectional multiple linear regression analyses were used to relate preHD SCy and SCr to MTMA and IAFA.

Results: Mean age was 52±16 yrs, 59% men, 84% Caucasians and 46% had DM. PreHD mean SCr was 8.8±2.8 mg/dL and mean SCy was 5.8±1.2 mg/dL. PreHD SCr and SCy were correlated (Figure 1)



The associations of SCy and SCr with BC measures are presented in Table 1.

Multiple Linear Regression Model of SCr and SCy

	SCr (mg/dL), β, (95%, CI), p value	SCy (mg/dL), β, (95%,CI), p value
For each 10 yrs ↑ in age	-0.63 (-0.94 to -0.33), <0.001*	-0.18 (-0.33 to -0.03), 0.02*
Male	0.19 (-0.98 to 1.36), 0.7	0.023 (-0.55 to 0.59), 0.9
Black	1.75 (0.47 to 3.04), 0.008*	0.58 (-0.04 to 1.21), 0.09
Smoking	-0.14 (-1.04 to 0.77), 0.8	-0.42 (-0.86 to 0.02), 0.06
For each 10 cm² ↑ in MTMA	0.34 (0.11 to 0.58), 0.004*	-0.02 (-0.14 to 0.09), 0.7
For each 10 cm² ↑ in IAFA	0.008 (-0.063 to 0.08), 0.82	0.008 (-0.025 to 0.043), 0.62
For each doubling of CRP	0.01 (-0.23 to 0.26), 0.92	-0.08 (-0.2 to 0.04), 0.18

Adjusted by URR and residual urine volume

Conclusions: Muscle mass is strongly associated with pre-dialysis SCr. Neither muscle nor fat mass appear to substantially affect SCy. In people in extremes of nutritional status SCy may be better than SCr in estimating kidney function.

Funding: NIDDK Support, Private Foundation Support

SA-OR005

Prediction of Renal Function Trajectories in Early Autosomal Dominant Polycystic Kidney Disease Michal Mrug,[1] Sylvie Mrug,[1] Lisa M. Guay-Woodford,[2] Vicente E. Torres,[3] Kyong Tae Bae,[4] Peter C. Harris,[3] Doug Landsittel,[4] Michael F. Flessner,[5] William M. Bennett,[6] Jared J. Grantham,[7] Arlene B. Chapman.[8] *[1]U Alabama Birmingham; [2]George Washington U; [3]Mayo Clinic; [4]U Pittsburgh; [5]NIDDK; [6]Legacy Good Samaritan Medical Center; [7]U Kansas; [8]Emory U.*

Background: There are no reliable formulas for predicting renal function in early autosomal dominant polycystic kidney disease (ADPKD).

Methods: We have developed longitudinal mixed statistical models of GFR trajectories in ADPKD and tested their utility to predict GFR decline over time using data collected by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP), a prospective, observational, longitudinal, multicenter study of 241 ADPKD adults with preserved renal function.

Results: After a mean follow-up of 8.7 years, mean iothalamate clearance (GFR) decreased from 97.8 to 74.0 ml/min/1.73m². Baseline GFR alone was a poor predictor of true GFR trajectories over time; the correlation of predicted vs. true individual GFR slopes was 0.01 (p=0.86). Addition of thirteen demographic and routine laboratory data improved this correlation to 0.49 (p<0.0001). However, addition of MR based height adjusted total kidney volume (htTKV), cyst number and renal blood flow (RBF) data further improved this correlation to 0.76 (p<0.0001; both htTKV and RBF were significant predictors of slope of GFR decline). Predicting the development of CKD within 8 yrs using ROC analyses demonstrated an area under the curve (AUC) of 0.83 for a simplified model utilizing baseline GFR, age, hypertension, and 24 hr urinary excretion of albumin, calcium and sodium for CKD stage 3b. Addition of the MR based imaging data (htTKV, cyst number and RBF) improved the AUC to 0.93. Similar AUCs were obtained using 10-fold cross-validation. The GFR based modeling was repeated with MDRD-GFR and obtained similar outcomes.

Conclusions: Longitudinal mixed models using routine clinical data predicted GFR decline in patients with early ADPKD. Improvement in their accuracy by adding MR based data (htTKV, cyst number and RBF) points to critical role of imaging in predicting the development of CKD in these patients.

Funding: NIDDK Support

SA-OR006

The Urea to Creatinine Ratio Is Highly Predictive of the Onset and Progression of the Cardiorenal Syndrome in Ambulatory Heart Failure Patients Manish M. Sood,[1] Mahwash Fatima Saeed,[2] Leigh Anne Shafer,[1] Navdeep Tangri,[1] Claudio Rigatto,[1] Paul Komenda,[1] Shelley Zieroth.[2] *[1]Medicine, Section of Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, Section of Cardiology, University of Manitoba, Winnipeg, MB, Canada.*

Background: Development and progression of the cardiorenal syndrome often follow changes in renal perfusion and solute clearance. We investigated the prognostic value of serial measures of the fractional excretion of urea(FEur), the fractional excretion of sodium (FEna) and the urea to creatinine ratio (UCr) in determining subsequent declines in renal function in an ambulatory cohort of heart failure patients.

Methods: A total of 140 patients (83% male, mean EF 24.8±7.0%) were prospectively followed for up to 24 months (median 333 days (IQR 192-457). Laboratory values and clinical outcomes were assessed every 3 months. Renal function was determined by the eGFR as calculated by the MDRD equation. Mixed and ordinary linear regression models with a sandwich estimator were employed to determine the relationship between changes in FEur, FEna and UCr and subsequent changes in renal function. Models were adjusted for renal function, NYHA class, age, sex, diabetes, and proteinuria.

Results: The mean baseline eGFR was 69.6±24.2 ml/min/m² with a mean decline of 0.3 per month over the course of follow up.Mean values for the UCr (N=805), FEur (N=758 measurements), and FEna (N=768) were 0.09 ± 0.03, 35.5 ± 13.0% and 1.44 ± 2.1%, respectively. An increase in the UCr was highly predictive of a subsequent monthly decline in renal function (adjusted eGFR decline 3.2 (95% CI 1.9-4.6)ml/min/m², p=0.001 per 0.1 unit increase in UCr) whereas FEur and FEna were not (FEurea p=0.6, FEna p=0.8).

Conclusions: The UCr is a simple and cheap biomarker that is strongly predictive of the onset or progression of the cardiorenal syndrome in ambulatory heart failure patients and may be useful in guiding therapeutic decision making.

SA-OR007

Selected Biomarkers Have Differential Prognostic Value in Specific Primary Kidney Diseases Adeera Levin,[1] Ognjenka Djurdjev,[2] Claudio Rigatto,[3] Francois Madore,[4] Brendan J. Barrett,[5] Norman Muirhead.[6] *[1]UBC; [2]BC PRA; [3]UofM; [4]UdeM; [5]MUN; [6]UWO.*

Background: Patients with chronic kidney disease (CKD) experience variable progression of kidney disease (KD) and heart disease. Better prediction models are needed.

Objective: To determine if inclusion of selected novel biomarkers (NBM) improves prediction of renal replacement therapy (RRT) at one year in specific primary KD.

Methods: Pan-Canadian prospective cohort study of 2546 referred CKD patients, from 25 rural, urban, academic and non-academic nephrology centres. NBM tests at baseline included asymmetric dimethylarginine (ADMA), high sensitivity C-reactive protein (CRP), interleukin 6 (IL6), pro-brain natriuretic peptide (proBNP), troponin I, transforming growth factor beta-1 (TGβ1) and cystatin C .

Main Outcome: Dialysis or transplantation (RRT) within one year. We used proportional hazards modeling to evaluate impact of NBM to base models for specific KD.

Results: Mean age of the cohort is 68yrs; median eGFR was 28 ml/min/1.73m² (20% < 20ml/min, 38% 20-29ml/min and 41% 30-45mil/min); 62% were male. Base models for each KD included age, sex, eGFR and uACR. Base models, extended (base + NBM) models and improvement in prediction with the extended models are presented in the following table.

	Diabetic Nephropathy	Hypertensive Nephropathy	Glomerular Nephritis	Other
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age (5 years)	0.91 (0.80-1.03)	0.99 (0.86-1.14)	0.99 (0.86-1.15)	0.94 (0.77-1.15)
Sex (Male vs. Female)	2.75 (1.39-5.43)	2.06 (0.89-4.77)	2.53 (0.85-7.52)	0.78 (0.22-2.73)
GFR mL/min	0.88 (0.83-0.93)	0.85 (0.79-0.91)	0.78 (0.70-0.88)	0.88 (0.79-0.98)
Log uACR	1.33 (1.10-1.62)	1.34 (1.07-1.69)	1.56 (1.07-2.29)	1.48 (1.07-2.06)
Log ProBNP	1.43 (1.14-1.78)	1.37 (1.02-1.85)	n.s.	n.s.
Log IL6	n.s.	0.65 (0.45-0.94)	n.s.	n.s.
Log CRP	n.s.	n.s.	n.s.	1.67 (1.03-1.70)
C Statistic Difference	0.01 (-0.00-0.03)	0.02 (0.01-0.05)	-0.00 (-0.00-0.02)	0.02 (-0.00-0.08)
NRI	0.4% (-8.4%-19.5%)	3.0% (0.8%-33.2%)		-4.0% (-12.1%-27.6%)

Conclusions: Different NBMs are independently associated with RRT progression in specific KDs after adjustment for age, sex and kidney markers (eGFR and uACR). Addition of NBMs to base models results in differential and modest improvement of RRT risk prediction.

Funding: Pharmaceutical Company Support - Ortho Biotech Canada

SA-OR008

Long Term Outcomes of Severe AKI: Results of the Post-Renal Study Martin P. Gallagher,[1] Rinaldo Bellomo,[2] Alan Cass,[1] David Gattas,[3] Joanne Y. Lee,[1] Serigne N. Lo,[1] Shay Mcguinness,[4] John A. Myburgh,[1] Rachael L. Parke.[4] *[1]The George Institute for Global Health, Australia; [2]The Austin Hospital, Australia; [3]Royal Prince Alfed Hospital, Australia; [4]Auckland City Hospital, New Zealand.*

Background: The literature on long term outcomes from AKI is derived from retrospective cohorts that often use administrative data and exclude early patient death from the analyses. Recent literature has emphasised the increased risk of end stage kidney disease in survivors of an episode of AKI with few prospectively derived reports of the markers of chronic kidney disease in such survivors. We report the 3.5 year outcomes of a large prospective cohort of patients with acute kidney injury (AKI) enrolled in the Randomised Evaluation of Normal vs Augmented Level of Renal Replacement (RENAL) Study.

Methods: The RENAL Study randomised patients with AKI in intensive care to standard dose (25 ml/kg/hr) or higher dose (40 ml/kg/hr) continuous renal replacement, with no mortality difference at 90 days. Ethics approval was granted at 35 centres to ascertain mortality, dialysis and clinical outcomes on all patients surviving beyond day 90. Extended follow up was derived by using a combination of data linkage to mortality registers and the ANZDATA Registry, along with clinical visits in consenting survivors.

Results: Outcomes were ascertained on 98% (1464/1508) of the randomised RENAL Study participants at a median of 43.9 months following randomisation. During follow up 258 patients (32% of those alive at day 90) died, giving an overall mortality from randomisation of 62%. Survival was not different between the two study interventions. A total of 44 patients (5.4% of patients alive at day 90) entered a maintenance dialysis program during follow up. Albuminuria was present in 42% of survivors and mean eGFR was 58 ml/min/1.73m^2.

Conclusions: Mortality risk over the 3.5 years following an episode of severe AKI in ICU is greater than the requirement for maintenance dialysis. Dialysis dose intensity does not influence mortality nor requirement for long term dialysis. Survivors have a high prevalence of risk factors for chronic kidney disease and mortality, most notably albuminuria.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR009

Polymorphisms in the Myeloperoxidase Gene Locus and Acute Kidney Injury: A Two-Step Genetic Association Study Mary Celine R. Perianayagam,[1] Hocine Tighiouart,[2] Orfeas Liangos,[3] Diana Kouznetsov,[4] Ron Wald,[5] Fangwen Rao,[6] Daniel T. O'Connor,[6] Bertrand L. Jaber.[1] *[1]Department of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Biostatistics Research Center, Tufts Medical Center, Boston, MA; [3]III. Medizinische Klinik, Klinikum Coburg, Coburg, Germany; [4]University of Massachusetts Memorial Medical Center, Worcester, MA; [5]Division of Nephrology, St. Michael's Hospital and University of Toronto, Toronto, Canada; [6]Department of Medicine, Center for Human Genetics and Genomics, La Jolla, CA.*

Background: Myeloperoxidase (MPO) is a lysosomal enzyme that may be involved in oxidative stress-mediated kidney injury.

Methods: Using a 2-step approach, we examined the association of 4 polymorphisms (rs2243828, rs7208693, rs2071409, and rs2759) acrossthe MPO gene locus with systemic markers of oxidative stress and adverse outcomes in 262 hospitalized adults with AKI *primary cohort*), and 277 patients undergoing cardiac surgery with cardiopulmonary bypass at-risk for post-operative AKI *secondary cohort*). Dominant and haplotype multivariable logistic regression analyses were performed.

Results: In the primary cohort, a genotype-phenotype association was observed between all 4 MPO polymorphisms and 2 markers of oxidative stress, plasma MPO and urinary 15-F_{2t}-isoprostane levels. In adjusted analyses, all 4 polymorphic allele groups had 2-3-fold higher odds for the composite of dialysis requirement or in-hospital death, as well as the composite of dialysis requirement, assisted mechanical ventilation or in-hospital death. The MPO T-G-A-T haplotype copy-number was associated with lower plasma MPO levels and lower adjusted odds for the composite outcomes. Significant but less consistent associations were observed in the *secondary cohort*.

Conclusions: This 2-step genetic association study identifies several polymorphisms spanning the entire MPO gene locus and a common haplotype as risk markers for artificial organ support or in-hospital death in patients with or at-risk for AKI. Additional studies are needed to replicate these findings, and establish the mechanisms underlying the influence of genotypes on disease traits.

Funding: NIDDK Support

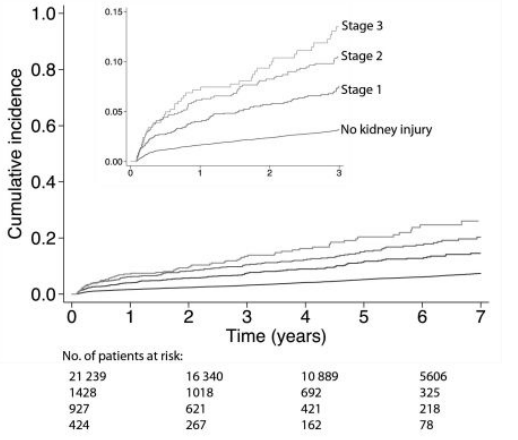
SA-OR010

Acute Kidney Injury Following Coronary Artery Bypass Surgery and Long-Term Risk of Incident Heart Failure Daniel Olsson, Ulrik Sartipy, Martin Holzmann. *Karolinska Institute, Stockholm, Sweden.*

Background: Acute kidney injury (AKI) after coronary artery bypass grafting (CABG) is common and increases the risk of post-operative complications and mortality. There is little information on the association between AKI after CABG and long-term risk of incident heart failure.

Methods: All patients (n=24 018) undergoing primary, isolated CABG between 2000 and 2008 with complete information on pre- and post-operative serum creatinine values and no prior hospitalization for heart failure were included. Acute kidney injury was divided into three stages based on absolute increase in post-operative serum creatinine; Stage 1. 0.3-0.5 mg/dL; Stage 2. 0.5-1 mg/dL; Stage 3. >1 mg/dL. Hazard ratios with 95% confidence intervals for first hospitalization for heart failure were calculated for different stages of AKI using Cox proportional hazards regression.

Results: Mean age was 67 years, 21% were women, 23% had diabetes mellitus and 19% chronic kidney disease. Twelve percent of the study population developed AKI and during a mean follow-up of 4.1 years there were 1325 (5.5%) cases of new-onset heart failure. Among patients with AKI 12% developed heart failure during follow-up compared to 5% among patients without AKI. Hazard ratios for heart failure in AKI stage 1, 2 and 3 were 1.60 (1.34-1.92), 1.87 (1.54-2.27), 1.98 (1.53-2.57) respectively, after adjustment for age, sex, diabetes mellitus, estimated glomerular filtration rate, left ventricular ejection fraction, myocardial infarction before or during follow-up. The cumulative incidence of first hospitalization for heart failure according to AKI is shown in Figure 1.



Conclusions: Acute kidney injury is associated with increased long-term risk of developing heart failure after CABG. Patients undergoing CABG should be assessed for development of post-operative AKI and followed more closely in order to detect early changes in cardiac function.

SA-OR011

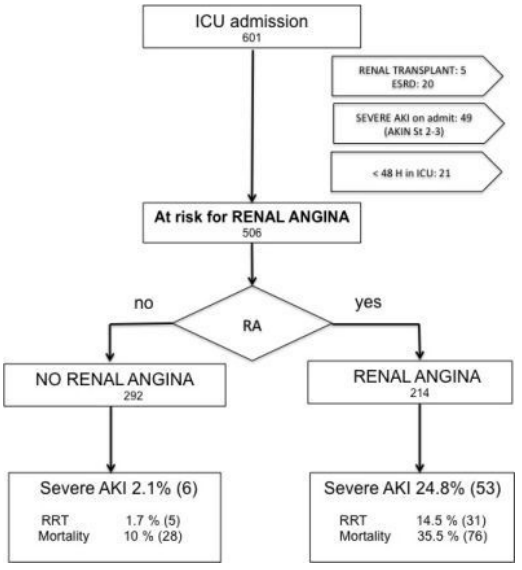
Renal Angina Syndrome (RA) Predicts Severe Acute Kidney Injury (AKI) in Critically Ill Patients Asunción Ferrer-Nadal,[1] Silvia Gramaticopolo,[2] Pasquale Piccinni,[2] Nicola Brienza,[3] Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]IRRIV, Dept. of Nephrology, Dialysis and Transplantation, S. Bortolo Hospital, Vicenza, Italy; [2]Dept. of Intensive Care and Anesthesiology, S. Bortolo Hospital, Vicenza, Italy; [3]Univ. Bari.*

Background: Identifying patients at risk for AKI in ICUs could allow physicians to start earlier preventitive strategies. This could also serve as an "enriched" population for AKI biomarker testing. Recently, a definition for a renal anginal syndrome equivalent has been proposed. Our aim was to evaluate if RA is a good predictor of severe AKI in the ICU.

Methods: We performed a secondary analysis of data from a prospective multicenter ICU cohort (NEFROINT study). We excluded ESRD, severe AKI (defined as AKIN St 2-3) on ICU admission, and ICU stay<48h, leaving 506 patients at risk for developing RA. We classified patients as very high, high and low risk (VHR, HR, LR) for RA based on published acute, major and minor risk factors (Chawla 2005). RA was defined as ↑sCr 0.1, 0.3 and 0.4mg/dL over baseline in VHR, HR and LR patients, respectively. These were then labeled as RA1, RA2 and RA3. Main outcome was development of severe AKI after RA.

Results: Of 506 patients (mean age 62 yrs, 60% M), 214 (42%) had RA. Among them, RA1 occurred in 90 (42%), RA2 in 80 (37%) and RA3 in 44 (21%) patients. RA subjects were older, had more CVD, CKD and sepsis, and higher APACHE II (p<0.001 for all). Severe AKI (AKIN St 2-3) developed in 53 (25%) subjects in the RA group vs 6 (2%) in patients without RA (Fig 1). They also had higher need for RRT, mortality (Fig 1) and longer ICU stay (13±15 vs 8±9 days [no RA], p<0.001).

Conclusions: The proposed definition for RA syndrome can identify patients at high risk to develop AKI and adverse outcomes in the ICU. This may be useful for informing clinical decision making and trial design. RA patients can be a target population for AKI biomarker testing.



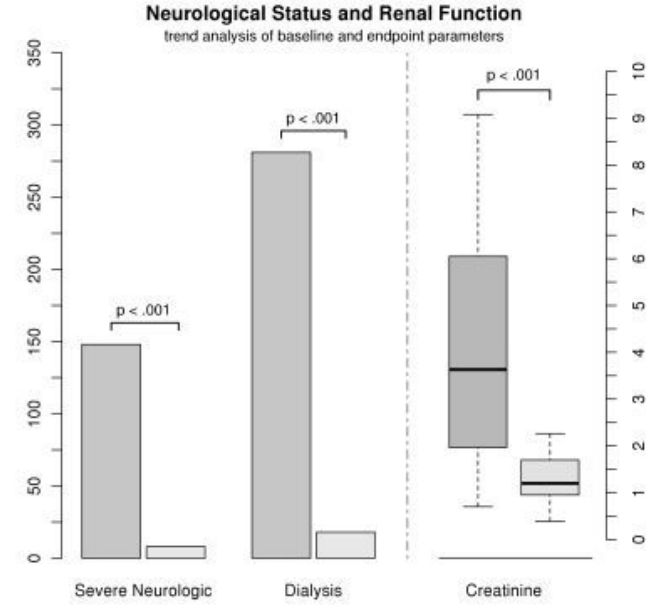
SA-OR012

Comparison of Therapeutic Plasma Exchange Alone or in Combination with Eculizumab for the Treatment of Shiga-Toxin-Producing E. Coli O104:H4 Induced Hemolytic Uremic Syndrome: An Analysis of the German STEC-HUS Registry Jan T. Kielstein, Gernot Beutel, Tobias N. Meyer, Andreas Kribben, Sylvia Stracke, Eiske Dorresteijn, Ola G. Samuelsson, reinhard Brunkhorst Richard Brunkhorst. *STEC-HUS Registry of the German Society of Nephrology.*

Background: In 2011 an outbreak of Shiga-toxin-producing E. coli (STEC) O104:H4 in Northern Germany claimed 52 deaths in 2,987 STEC and 855 HUS cases.

Methods: Using a web-based registry, established 5 days after the start of the outbreak, we aimed to describe short term effectiveness of best supportive care (BSC), therapeutic plasma exchange (TPE), and TPE with eculizumab (TPE-Ecu) using propensity score analysis.

Results: Of 621 entries from 84 hospitals (Germany, Sweden, Netherlands) 491 fulfilled the definition of HUS (median age 46 years; 71% females). Median [IQR] hospital stay was 22 [14-31] d. 281 (57%) patients underwent dialysis, 114 (23%) mechanical ventilation. 57 patients received BSC, 241 TPE and 193 TPE-Ecu. Disease severity (hemolysis, need for dialysis, frequency of seizures) were lower in BSC than in TPE and TPE-Ecu patients. At study endpoint (hospital discharge or death), median creatinine was lower in BSC (1.1 mg/dL [0.9-1.3]) than in TPE (1.2 mg/dL [1.0-1.5], p<0.05) and TPE-Ecu (1.4 mg/dl [1.0-2.2], p<0.001). Need for dialysis was not different between BSC (0.0%, n=0), TPE (3.7 %; n=9) and TPE-Ecu (4.7%, n=9). Severe neurolgical symptoms were markedly reduced at endpoiunt.



Total hospital mortality in HUS patients was 4.1% (n=20) and did not differ between TPE and TPE-Ecu.

Conclusions: Despite frequent renal impairment, advanced neurological disorders, and severe respiratory failure short term outcome was better than expected as compared to previous reports. Within the limitations of a retrospective registry analysis, our data do not prove marked differences in treatment efficiency between the groups.

SA-OR013

Quality of Communication about Acute Kidney Injury (AKI) at Hospital Discharge to Patients and Their Outpatient Providers Raquel Greer, Deidra C. Crews, Hamid Rabb, Bernard G. Jaar, L. Ebony Boulware. *Medicine, Johns Hopkins Medical Institutions, Baltimore, MD.*

Background: Given the long-term consequences associated with AKI (i.e. chronic kidney disease, cardiovascular disease, and death), communication between hospital-based and outpatient health care providers about the occurrence and care plan for patients hospitalized with AKI is important to ensure patients' adequate follow-up care.

Methods: We characterized the prevalence and quality of written discharge communications about the occurrence of AKI during hospitalizations for 284 adult patients in Baltimore, MD during 2009. We reviewed inpatients' medical records, discharge summaries, and documentation of patient's discharge instructions. Using a standardized data collection form, we assessed hospital-based providers' documentation of AKI in patients' discharge summaries, including their documentation of the cause of AKI, peak and discharge creatinine and instructions for follow-up appointments. We also assessed mention of AKI in discharge instructions provided to patients.

Results: Of 284 patients with AKI (stages 1-3 defined by AKIN criteria) during their hospitalization, only 168 (59%) discharge summaries documented the occurrence of AKI. While the discharge summaries mentioned the cause of AKI in all documented cases, many did not include a discharge creatinine (61%) or peak creatinine (41%). Most (77%) included instructions for patients to follow-up with an outpatient provider (12% with a nephrologist and 76% with a primary care or other provider). However, follow-up medical plans for treatment or observation of patients were not provided in 20% of summaries. Of the patients whose creatinine did not return to baseline prior to discharge (n=177), only 27% included clear plans for follow-up assessment. Among 88 patients whose patient discharge instructions were available, few received information about the diagnosis (31%) or cause (12%) of AKI.

Conclusions: Communication of the occurrence of AKI and follow up care plans after hospital discharge to outpatient providers and patients is suboptimal. Interventions to improve transitions of care in hospitalized patients with AKI are needed.

Funding: Other NIH Support - National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and the NIH Roadmap for Medical Research (5KL2RR025006)

SA-OR014

Association of Kallikrein-1 Gene Polymorphisms and Acute Kidney Injury: A Case-Control and Cohort Study Paweena Susantitaphong,[1,2] Mary Celine R. Perianayagam,[1] Sunwoo Kang,[3] Wenyi Zhang,[3] Fangwen Rao,[3] Daniel T. O'Connor,[3] Bertrand L. Jaber.[1] *[1]Medicine, St.Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn University, Bangkok, Thailand; [3]Medicine, University of California, La Jolla, CA.*

Background: Kallikrein-1 (KLK1) is a highly conserved serine protease that is expressed in the kidney, and involved in blood pressure regulation. The activity of this enzyme is diminished in experimental and human AKI. We evaluated the potential role of functional multiallelic *KLK1* promoter gene polymorphisms in human AKI.

Methods: We first performed a case-control study of 481 subjects (214 patients with AKI and 267 healthy controls). The complex, multiallelic G/C-rich repeat region of the proximal *KLK1* promoter was determined in each subject by direct Sanger/capillary re-sequencing. Association between *KLK1* promoter polymorphisms and development of AKI, and the composite of doubling of serum creatinine, oliguria, or dialysis requirement were explored by logistic regression analyses.

Results: Sixteen alleles were identified in a complex, polymorphic G/C-rich region of the *KLK1* proximal promoter; frequencies for 5 of these alleles (F, G, H, I, and K) were associated with development of AKI. Alleles I and G were classified as risk-alleles (unadjusted OR 1.86; 95% CI 1.23, 2.81; P=0.003), whereas alleles F, H, and K were classified as protective-alleles (unadjusted OR 0.32; 95% CI 0.22, 0.46; P<0.001), according to their directional association with development of AKI. After adjustment for sex, race, or APACHE II score, the *KLK1* risk-allele carrier state was associated with the composite of doubling of serum creatinine, oliguria, or dialysis requirement (adjusted odds ratio 2.62; 95% CI 1.11, 6.20, P=0.03). The *KLK1* risk-allele carrier state was also marginally associated with the composite of doubling of serum creatinine, oliguria, dialysis requirement, or in-hospital death (adjusted OR 2.26; 95% CI 0.96, 5.33; P=0.06).

Conclusions: *KLK1* promoter polymorphisms are associated with development of AKI, and adverse kidney-related outcomes. Further studies are needed to validate these findings.

Funding: NIDDK Support

SA-OR015

Nutritional Supplement Use and Mortality in Hemodialysis

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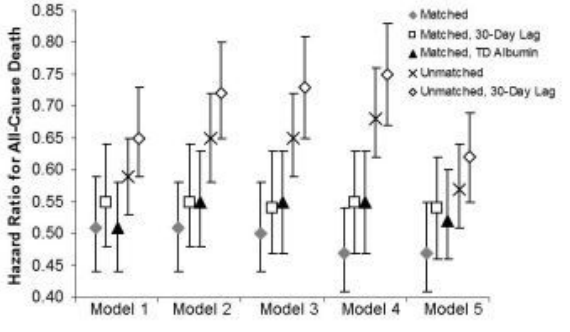
Background: Hemodialysis patients have high mortality rates, and malnutrition may be an important factor contributing to poor outcomes. DCI widely implemented a nutritional supplement protocol (NSP), such that, when serum albumin was ≤3.5 g/dl (BCG), patients would receive 15g of oral protein at each dialysis session until albumin reached 4 g/dl. We evaluated the impact of this program on mortality.

Methods: Patients in facilities that largely adopted the NSP in 9-10/2010 were paired with patients from facilities that did not adopt the NSP using a greedy matching algorithm based on a propensity score for physician ordering of the NSP. The propensity score was built using extensive data on individual patient and facility characteristics. We then used Cox models to assess the patient-level association between the NSP and mortality through March 2012, with secondary analyses incorporating time dependent albumin measures, evaluating a 30-day lag, and looking at unmatched data with multivariable adjustment.

Results: Of 7568 eligible patients, 2702 were ordered the NSP and 1571 of these were matched to controls; only blood pressure had standardized difference >10% between groups. In the matched cohort, there were 331 deaths in the NSP group vs 504 in controls. Albumin increased over time only in the NSP group, and, in all analyses, the NSP was associated with significantly lower mortality (figure). This effect was most prominent at lower baseline albumin levels.

Conclusions: NSP protocol use is associated with a significant reduction in mortality for in-center hemodialysis patients.



Model 1: Univariate +/- time-dependent albumin
Model 2: Model 1 + age, sex, race
Model 3: Model 2 + ESRD cause, access, vintage
Model 4: Model 3 + patient clinical characteristics and labs
Model 5: Model 4 + facility characteristics, including prior SMR

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SA-OR016

The DOPPS Practice Monitor: Patient Hemoglobin (Hgb) Levels and Changes in Anemia Management Practice in US Hemodialysis Patients from 2010 to 2011

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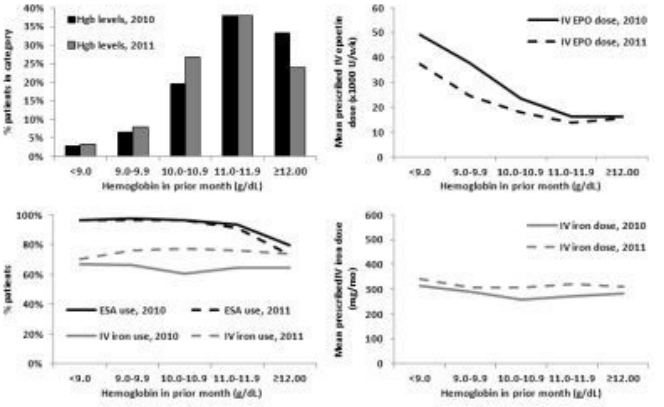
Background: In response to changes in erythropoietin-stimulating agent (ESA) labeling, reimbursement policy, and quality incentive payment (QIP) targets for US dialysis patients (pts), it has been reported that Hgb levels declined from 2010 to 2011 and rates of red blood cell transfusions increased. Here we identify changes in anemia management practices (AMPs) over this time period.

Methods: Using cross-sectional samples from the US Dialysis Outcomes and Practice Patterns Study (DOPPS) Practice Monitor (DPM), we describe Hgb levels in Sep 2010 and Sep 2011 (n=6,958), as well as anemia management practices (AMPs) in response to these Hgb levels (in Oct 2010 and Oct 2011; n=6,899).

Results: From 2010 to 2011, Hgb ≥12 g/dL declined by 9% and Hgb 11-11.9 declined by 5%, while Hgb 10-10.9 rose by 9% and Hgb <10g/dL rose by 5%.



% ESA use was stable in most Hgb groups but declined in pts with Hgb ≥12 g/dL. Mean prescribed IV Epo dose declined 31% for Hgb <10 g/dL, 24% for Hgb 10-11 g/dL, and 12% for Hgb ≥11 g/dL. However, the decline in mean delivered IV Epo dose was similar for all Hgb groups (28%) because of an increase in Epo dose holds at high Hgb levels (not shown). % IV iron use rose modestly for all Hgb levels except Hgb <9. Prescribed IV iron dose increased 7-10% for Hgb <10 g/dL, and 18% for Hgb 10-12 g/dL.

Conclusions: There was a large shift toward lower Hgb levels from 2010 to 2011. Although the largest change in distribution was from ≥12 to 10-10.9 g/dL, 14% of patients had Hgb <10g/dL in Sep 2011. Pts with Hgb <10 g/dL had the largest decline in subsequent prescribed EPO doses, but similar IV iron prescription patterns as other pts. To limit transfusions, consideration to raising EPO dose and IV iron use in pts with Hgb <10 g/dL is warranted.

Funding: Pharmaceutical Company Support - The DOPPS Is Administered by Arbor Research Collaborative for Health and Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), Abbott (Since 2009), Baxter (Since 2011), and Vifor Fresenius Renal Pharma (Since 2011), without Restrictions on Publications

SA-OR017

Variation in Observed Infection Control Practices in the NOTICE Project

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Background: The National Opportunity to Improve Infection Control in ESRD (NOTICE) project is designed to assess recommended infection control (IC) practices at US dialysis facilities with the aim of identifying potential areas for quality improvement.

Methods: Trained IC Evaluators observed 73 distinct IC practices at 34 randomly selected hemodialysis facilities. Facility selection was stratified on large dialysis organization (LDO) affiliation, size, socioeconomic status, and urban/rural status from 4 ESRD Networks. Observations were made using an IC Worksheet (ICWS) developed under contract with AHRQ in collaboration with CMS and the CDC.

Results: There was considerable variation in IC practices across enrolled facilities. Overall adherence was 68% (range=53% to 92%). Overall adherence to expected hand hygiene (HH) practice was 72% (range=30% to 95%). Use of chlorhexidine was 19% overall but varied from 35% in independent facilities to 0% in LDO facilities. Overall HH and medication preparation procedures (cleaning the injection port, proper needle insertion, and proper assembly of supplies) were significant predictors of ICD-9 based infection rate (p=0.02).

Mean % Adherence to ICWS items selected for low adherence

Selected ICWS Items	Mean %
Overall Adherance	68
Adherance to Hand Hygiene Items	72
Use Antimicrobial Ointment	17
Transfer of Non-Disposable Items to Common Areas	18
Use Chlorhexidine	19
Vacate Dialysis Chair Prior to Disinfecting	26
Scrub External CVC Hub at Termination	29
Disinfect Non-Disposable Items	31
Scrub Internal CVC Hub at Initiation	34
Scrub Internal CVC Hub at Termination	36
Disinfect Surfaces per Manufacturer DFU	41
Scrub External CVC Hub at Initiation	45
Wash Skin Over CVC Access	53

Conclusions: Our findings suggest that there is room for improvement in HH and other IC practices. These NOTICE project findings will help to inform the development of a larger quality improvement initiative at dialysis facilities.

Funding: Other U.S. Government Support

SA-OR018

Geographic Variation in the Timing of Dialysis Initiation in the US
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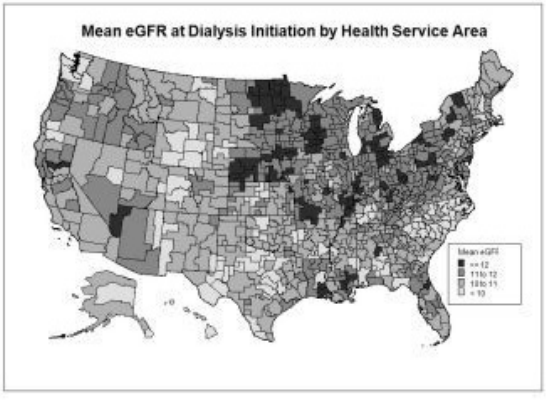
Background: There is an ongoing trend of earlier dialysis initiation worldwide despite no evidence of clinical benefit. Regional differences in the timing of dialysis initiation in the US have not been examined.

Methods: Using the US Renal Data System ESRD database, we identified 310,962 adult patients initiating dialysis between 2006-2008 within the 805 health service areas (HSAs) in the US. We obtained demographics, comorbidity and serum creatinine from CMS Form 2728 for each participant and estimated GFR at initiation using the 4-variable MDRD equation. We plotted the HSA-level mean eGFR at dialysis initiation using a Bayesian hierarchical model to smooth the eGFR means and delineate patterns. A random effects model was used to calculate adjusted and unadjusted mean eGFR for each HSA and assess geographic variation based on those estimates.

Results: Mean eGFR at dialysis initiation showed a regional pattern.



The mean HSA-level eGFR at dialysis initiation was 10.92 (SD 0.92) ml/min/1.73m^2. The variability in mean eGFR at dialysis initiation across HSAs was decreased by 11% after adjusting for age, sex, race, ethnicity and income (mean eGFR 10.92; SD 0.82 ml/min/1.73m^2). Variability did not decrease any further after adjustment for comorbidity index, diabetes and congestive heart failure (mean eGFR 10.92; SD 0.85 ml/min/1.73m^2). With full adjustment, 92% of the variation across HSAs was not accounted for by patient-level characteristics.

Conclusions: The timing of dialysis initiation varies across the US. Differences in patient characteristics only account for a small portion of the variability, suggesting that patient/provider preferences and/or health system factors may account for this variation.

Funding: Other U.S. Government Support

SA-OR019

Early Referral to Nephrologist Improves Risk Stratification of Mortality in Patients at Dialysis Initiation but Exerts Limited Survival Advantage
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Background: Impact of early referral (ER) on predialysis risk stratification of mortality and on long-term survival are closely interacted, however they have been assessed separately. Thus, the present study was aimed to examine whether ER improves risk stratification of mortality at dialysis initiation and exerts long-term survival advantage.

Methods: Using Cox proportional hazards models, the effect of referral timing on long-term survival stratified by eGFR at dialysis initiation was analyzed in 603 consecutive patients between 2001 and 2008 in Senshu district in Osaka prefecture, Japan.

Results: The median eGFR at dialysis initiation was 5.26 ml/min/1.73m^2 and the 5-year-mortality rate was 56%. In ER group (referred to nephrologist ≥ 6 months before dialysis initiation), unadjusted analysis revealed that the hazard ratio (HR) of mortality was increased with increased eGFR at dialysis initiation, but after adjustment for age, gender, diabetes, and other clinical characteristics at dialysis initiation, HR was identical among the eGFR categories. Contrary, in late referral (LR) group, mortality risk was independent on the eGFR categories.

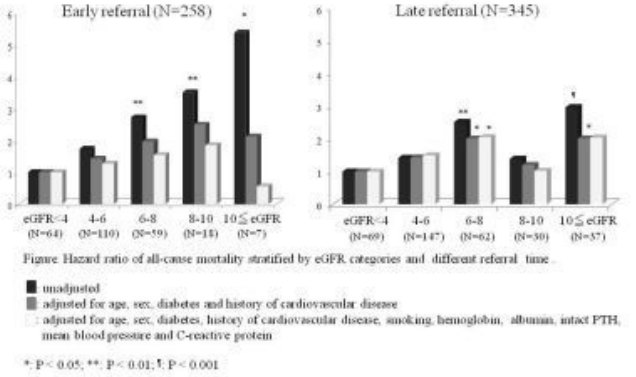

Figure Hazard ratio of all-cause mortality stratified by eGFR categories and different referral time.

unadjusted
adjusted for age, sex, diabetes and history of cardiovascular disease
adjusted for age, sex, diabetes, history of cardiovascular disease, smoking, hemoglobin, albumin, intact PTH, mean blood pressure and C-reactive protein

*: P < 0.05; **: P < 0.01; ¶: P < 0.001

Furthermore, the detrimental effect of ER on survival was limited to the first 6 months. The effect of referral timing on survival was not significant (HR 1.124, 95%CI 0.828-1.527). Of note, HR of LR for early death was 2.615 (95%CI 1.440-4.750) and after extensive adjustment, the HR became marginal (HR 1.878, 95%CI 0.968-3.644).

Conclusions: Although our results suggest that predialysis care improve patients survival by stratifying the mortality risk at dialysis initiation, the effect is limited to the first few month. The strategy to prevent early death on dialysis for LR patients needs to be established.

SA-OR020

Factors Associated with Low Kidney Transplant Rates among United States Dialysis Facilities
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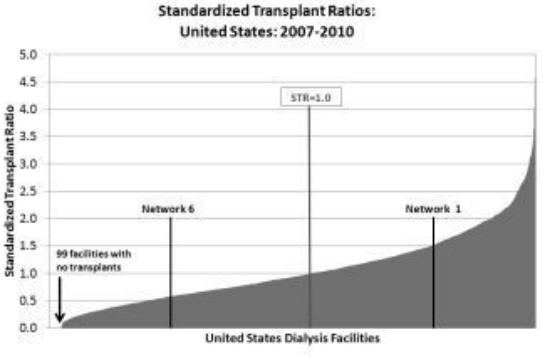
Background: Variability in transplant rates between different dialysis units has been noted, yet little is known about facility-level factors associated with low standardized transplant ratios (STRs) across facilities in the 18 End Stage Renal Disease (ESRD) Networks within the United States (US).

Methods: We analyzed Centers for Medicare and Medicaid Services Dialysis Facility Report data for facilities open from 2007 to 2010. Multivariable-adjusted linear regression models were used to identify the facility characteristics associated with low four-year average STR and to examine regional differences across ESRD Networks.

Results: Among 4,098 dialysis facilities treating 243,038 patients, there was variability in facility-level STRs across the 18 ESRD networks. Median 4-year facility-level STRs ranged from 0.61 [Interquartile Range (IQR): 0.36, 0.94] in Network 6 (Southeastern Kidney Council) to 1.52 (IQR: 0.95, 2.18) in Network 1 (New England). 99 (2.4%) of dialysis facilities reported an STR of zero for all four years. The majority of dialysis facilities reporting STRs of zero were in Network 6 (34.3%), followed by ESRD Network 13 (11.1%). In multivariable analyses, factors significantly associated with a lower STR (p<0.0001) included a higher percentage of African Americans, older patient age, diabetes, cardiovascular disease, a higher proportion of patients on ESRD therapy for > five years, lower number of facility staff, lack of pre-ESRD nephrology care, for-profit facility status, and ESRD network region.

Conclusions: There is significant variability in dialysis facility-level STRs in the US. Understanding the modifiable factors associated with low transplant rates can be used to develop interventions to improve access to kidney transplantation among low-performing dialysis facilities.



SA-OR021

Segregation, Income Disparity and Survival in US HD Patients

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Background: Social determinants of health and ecologic analyses have become increasingly important in analyses of population health. Residential segregation has been highlighted as an important correlate of health in the US. Socioeconomic status (SES) is associated with improved survival in diverse conditions. US states with greater income maldistribution have increased mortality. Black US end-stage renal disease (ESRD) HD patient survival, however, is improved compared to Whites, with higher income. The underlying mechanisms are unknown. We examined associations of income inequality and residence as social determinants of health with Black and White ESRD patients' survival.

Methods: US Renal Data System information, 2000-2008, regarding patients starting HD for ESRD was merged with Census Bureau Black and White race-specific median household income to assess SES, and Gini coefficients to assess income inequality. Residential segregation was determined by the Dissimilarity Index (DI). Survival was compared using Cox proportional hazard models, controlling for known risk factors.

Results: 589,036 patients were included. Mean median area household income for Black and White patients was $26,742 and $41,922 ($p<0.001$). Residence in areas with higher median household income was associated with improved survival. In White patients only, income inequality was associated with mortality (hazard ratio (HR) for the highest relative to lowest quartile of Gini coefficient was 1.06, $p<0.001$). In Black patients exclusively, residence in highly segregated areas was associated with increased mortality (HR for the highest relative to lowest quartile of DI was 1.13, $p<0.001$).

Conclusions: Black US ESRD HD patients are particularly susceptible to gradients in income and residential segregation. Unknown biological, psychosocial or cultural factors associated with SES and neighborhood characteristics may mediate differential survival for Black ESRD patients. Interventions will be challenging, involving multiple organizations at varying levels, but those directed at highly segregated Black neighborhoods might favorably affect HD patient outcomes.

Funding: NIDDK Support

SA-OR022

Generation and Characterization of a Novel Pericyte-Specific Conditional Cre Mouse Line

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Background: Cre drivers are now indispensable tools for studying the biology of specific cell populations *in vivo*. The ability to control the time point and frequency of Cre mediated recombination is desirable in many situations, and there is a lack of such conditional Cre driver lines to study pericytes - a main source of myofibroblast recruitment in renal fibrosis.

Methods: We utilized the collagen 1α1 promoter/enhancer to drive expression of a triple fusion protein consisting of GFP, Cre recombinase and the mutated estrogen receptor ERT2 and created a novel conditional Cre mouse line with tamoxifen-inducible *cre* activity in pericytes.

Results: We validated this Coll1α1GCE mouse by crossing it to three different reporter lines (R26LacZ, mT/mG and R26Tomato) and treating bigenic offspring with tamoxifen. Genetically labeled cells were found in kidney tubulointerstitium of all three bigenic lines. These cells stained positive for pericyte marker PDGFRβ but not for CD31, F4/80, CD3, αSMA or S100A4 in healthy kidneys thus verifying their pericyte identity. Morphological analysis revealed a typically spindle- or triangle-like cell shape with prominent projections and a close association with endothelial cells. Some pericyte processes projected within the capillary basement membrane. One submaximal dose of tamoxifen at P7 labeled 1.5 ± 0.4% and three consecutive tamoxifen pulses (P7-P9) labeled 25.8 ± 5.1% of all PDGFRβ+ pericytes. During UUO, fate labeled pericytes acquired αSMA positivity reflecting their transformation into myofibroblasts. Morphometrical analysis of single fate-labeled pericytes showed that while there was no significant difference in cell size between kidney pericytes of P9 pups (92.5 ± 3.4μm^2) and adult mice (101.8 ± 4.3 μm^2), there was a notable increase in size (128.8 ± 3.3μm^2; $P<0.0001$) and complexity after myofibroblast differentiation in UUO kidneys.

Conclusions: In conclusion, we have developed a new pericyte-specific conditional Cre driver mouse as a versatile new tool to study the biology of pericytes in health and disease.

Funding: NIDDK Support

SA-OR023

Dysfunction of Fibroblasts of Extra-Renal Origin Underlies Renal Fibrosis and Renal Anemia

Masayuki Takase,[1] Nariaki Asada,[1] Jin Nakamura,[1] Akiko Oguchi,[1] Misako Asada,[1] Norio Suzuki,[2] Narihito Nagoshi,[3] Shinsuke Shibata,[3] Nageswara Rao Tata,[4] Hans Jörg Fehling,[4] Atsushi Fukatsu,[5] Naoko Minegishi,[2] Hideyuki Okano,[3] Masayuki Yamamoto,[2] Motoko Yanagita.[1] *[1]Kyoto University, Japan; [2]Tohoku University, Japan; [3]Keio University, Japan; [4]University of Clinics Ulm, Germany; [5]Yachiyo Hospital, Japan.*

Background: Renal anemia and renal fibrosis are common complications during chronic kidney disease (CKD), which is caused by the dysfunction of renal fibroblasts. Renal fibrosis is mediated by the accumulation of myofibroblasts, whereas renal anemia is mediated by the reduced production of erythropoietin (EPO) in some fibroblasts. Despite of the essential role of fibroblasts in the kidney, their characteristics and developmental origin remain unknown.

Methods: To explore the origin of the fibroblast, we trace the fate of neural crest-derived cells, utilizing transgenic mice expressing *cre* recombinase under the control of myelin protein zero (*P0-Cre* mice), a marker of migrating neural crest cells. In *P0-Cre:indicator* mice, the cells arising from *P0-Cre*–expressing precursors were tagged with the expression of indicator genes.

Results: We demonstrated that most of the fibroblasts in the kidney cortex and outer medulla are *P0-Cre* lineage-labeled cells (P0 cells), and that some of them are EPO producing cells. P0 cells migrate into the embryonic kidney at E13.5 and differentiate into fibroblasts. In the diseased kidney, P0 cells transdifferentiate into myofibroblasts and predominantly contribute to fibrosis, with concomitant loss of EPO production. We further demonstrated that attenuated EPO production in transdifferentiated myofibroblasts is recovered by the administration of neuroprotective agents. Moreover, the in vivo administration of tamoxifen, a selective estrogen receptor modulator, restores reduced production of EPO and attenuates renal fibrosis.

Conclusions: These findings clarify the direct link between renal fibrosis and renal anemia. Therapeutic trials targeting *P0-Cre* lineage-labeled fibroblasts might be effective for both renal fibrosis and renal anemia.

SA-OR024

Exploring the Function of Renal Fibroblasts of Extra-Renal Origin

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Background: We previously reported that most of the fibroblasts in the kidney cortex and outer medulla are myelin protein zero-Cre (*P0-Cre*) lineage-labeled cells, and that some of them are EPO producing cells in the healthy kidney. In the diseased kidney, *P0-Cre* lineage-labeled cells transdifferentiate into myofibroblasts and predominantly contribute to fibrosis, with concomitant loss of EPO production. In the present study, we further investigated the pathophysiological function of *P0-Cre* linage-labeled fibroblasts (P0 cells) and the crosstalk between these cells and tubuloepithelial cells.

Methods: To analyze the function of P0 cells in the kidney, we utilized *P0-Cre* inducible simian diphtheria toxin receptor (DTR) transgenic mice (*P0-Cre:iDTR* mice) in which Cre-mediated excision of a STOP cassette renders P0 cells sensitive to diphtheria toxin (DT). The binding of DT to DTR halts protein synthesis within the cells.

Results: First we confirmed that DTR is expressed in almost all fibroblasts in the cortex and outer medulla in the kidney of *P0-Cre:iDTR* mice. After the administration of DT to *P0-Cre:iDTR* mice, the expression of DTR and fibroblast markers in the kidney was significantly decreased, indicating the effective cessation of protein synthesis in P0 cells. Simultaneously, the expression of EPO was also significantly reduced, and did not increase even after the induction of severe anemia. The administration of DT to *P0-Cre:iDTR* mice with unilateral ureteral obstruction (UUO) decreased the expression of PDGFRb, collagen1a1, and aSMA. Futhermore, we demonstrated that the administration of DT to *P0-Cre:iDTR* mice causes the expression of proximal tubule injury marker Kim1, and the proliferation of proximal tubule cells in the cortical-medullary junction.

Conclusions: Cessation of protein synthesis in P0 cells reduced the expression of EPO in healthy kidney and the fibrosis markers in UUO-operated kidney, supporting our previous findings. Cessation of protein synthesis in P0 cells also induced proliferation and injury of proximal tubule cells, suggesting the possible interactions between P0 cells and tubular epithelial cells. We are currently searching for the molecules responsible for the interactions.

SA-OR025

Lineage Tracing Reveals an Endothelial Origin for the Crim1$^{KST264/KST264}$ Renal Interstitial Fibrosis but Not Periglomerular Fibrosis

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Background: Tubulointerstitial fibrosis is a detrimental pathological feature which exists in virtually all cases of progressive renal disease. The main characteristics include *de novo* appearance of myofibroblasts and collagen deposition within the interstitial space. The Crim1$^{KST264/KST264}$ hypomorphic mice develop progressive renal disease, identified by a series of renal abnormalities such as glomerular cysts, leaky peritubular capillaries and compromised endothelial integrity, eventually progressing to the development of peritubular interstitial and periglomerular fibrosis.

Methods: To delineate whether the endothelium may in part contribute to the fibrogenic process, endothelial gene expression profiling, transmission electron microscopy imaging of the endothelium as well as Tie2Cre-DsRed endothelial lineage tracing was undertaken in the Crim1$^{KST264/KST264}$ mice.

Results: In-depth ultrastructural analysis of the Crim1$^{KST264/KST264}$ renal endothelium revealed several endothelium abnormalities which include cellular detachment and abnormal collagen accumulation within the basement membrane. To further investigate this endothelium dysfunction, gene expression profiling of CD31$^+$ endothelial cell was performed which showed differentially expressed transcripts associated with renal fibrosis (*Col3a1, Loxl1, Serpina10*), endothelial dysfunction (*Abp1, Dcn, Lcn2*), biomarkers of renal damage (*Lcn2, Havcr1*) as well as evidence for a TGFβ1/TNF associated inflammatory process. Our lineage tracing of the Crim1$^{KST264/KST264}$ mice showed that approximately 31% of αSMA$^+$ myofibroblasts present within the interstitium were derived from the endothelium via Endothelial to Mesenchymal Transition. Conversely, a majority (88%) of αSMA$^+$ myofibroblasts surrounding glomerular cysts were not of an endothelial origin.

Conclusions: These results confirm the contribution of Endothelial to Mesenchymal Transition towards interstitial fibrosis, but also indicate differences in the origin of fibrosis within the same kidney in this model of renal damage.

Funding: Government Support - Non-U.S.

SA-OR026

Adiponectin Promotes Macrophage Polarization and Monocyte-to-Fibroblast Transition in Renal Fibrosis

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Background: Progressive renal fibrosis is the final common manifestation of chronic kidney disease resulting in irreversible loss of kidney parenchyma and renal function. We have recently demonstrated that bone marrow-derived fibroblasts contribute significantly to the pathogenesis of renal fibrosis. However, the signaling mechanisms underlying the recruitment and maturation of bone marrow-derived fibroblast precursors into the kidney in response to injury are incompletely understood. We found that adiponectin was upregulated in the kidney in a murine model of renal fibrosis. Therefore, we examined if adiponectin can regulate the maturation of bone marrow-derived fibroblasts in the kidney and development of renal fibrosis.

Methods: We performed unilateral ureteral obstruction to induce renal fibrosis in wild-type and adiponectin-knockout mice and utilized mouse splenocytes to examine the effect of adiponectin on monocyte to fibroblast transition.

Results: Compared with wild-type mice, adiponectin-knockout mice displayed significantly fewer bone marrow-derived fibroblasts in obstructed kidneys. Furthermore, we demonstrated that $CD206^+$ M2 macrophages produced procollagen I, which is associated with induction of Th2 cytokines - IL-4 and IL-13. Adiponectin knockout mice exhibited a significant reduction CD206 and procollagen I dual positive fibroblasts in obstructed kidneys, which was associated with a reduction of Th2 cytokines - IL-4 and IL-13. Consistent with these findings, targeted deletion of adiponectin inhibited myofibroblast activation, reduced total collagen deposition, and suppressed expression of collagen I and fibronectin. In vitro studies showed that adiponectin promoted macrophage polarization and monocyte to fibroblast transition and increased extracellular matrix protein expression.

Conclusions: Our study identifies adiponectin as a critical regulator of macrophage polarization and monocyte-to-fibroblast transition. Therefore, inhibition of adiponectin signaling may represent a novel therapeutic target for fibrotic kidney disease.

Funding: Other NIH Support - NHLBI, Private Foundation Support

SA-OR027

Increased Proximal Tubule PPARα Reduces Renal Interstitial Fibrosis by Reducing miR21 Expression and by Increasing Autophagy

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Background: MicroRNA21(miR-21) expression is significantly increased in mice subjected to unilateral ischemia and unilateral ureteral obstruction (UUO). Reducing miR21 expression in kidney tissue by using oligos or miR21 knockout mice results in increased expression of PPARα in kidney tissue and amelioration of renal fibrosis (Sci Transl Med 2012 Feb). In the present study we examined mechanisms by which increased expression of proximal tubule PPARα reduces kidney fibrosis.

Methods: C57BL wild type mice and proximal tubule PPARα transgenic mice were subjected to 5 days of UUO. We compared the development of interstitial fibrosis detected by Sirius red staining of the kidneys, and mRNA expression of pathological matrix proteins Col1a1, Col3a1. We also measured expression of miR21, PPARα and fatty acid oxidation(FAO) genes MCAD and CPT1. In *in vitro* studies we examined the fibrotic response of proximal tubular epithelial cells in culture (TKPTS) to aristolochic acid (AA), a compound that induces progressive interstitial fibrosis. AA-mediated fibrosis was measured by changes in mRNA levels of Col1a1, TGFβ and miR21. Proximal tubular cell autophagy was measured by western blot as increased expression of LC3-II protein.

Results: Interstitial fibrosis induced by UUO was significantly reduced in PPARα Tg mice when compared to wild type mice. UUO-mediated reduction of FAO in wild type mice was recovered to normal levels in PPARα Tg mice. miR 21 expression was increased in wild type mice but significantly attenuated in PPARα Tg mice. Exposure of TKPTS cells to 10 uM AA for 18 hrs resulted in increased expression of TGFβ, Coll1a1 and miR21 mRNA levels. Increased expresion of PPARα by adenovirus infection attenuated AA-mediated increased expression of fibrotic genes and increased the expression of LC3II protein levels.

Conclusions: Increased expression of proximal tubule PPARα reduces UUO-mediated interstitial fibrosis by increasing FAO via reduced expression of miR21. PPARα reduces AA-mediated fibrotic response in TKPTS cells by increasing intracellular autophagy.

Funding: NIDDK Support, Veterans Administration Support

SA-OR028

Renal Fibrosis Is Attenuated by Targeted Disruption of miR-22

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Background: Progressive renal fibrosis is the final common manifestation of various chronic kidney diseases resulting in irreversible loss of kidney parenchyma. MicroRNAs are small noncoding RNAs that negatively regulate gene expression by base pairing to partially complementary sites in the 3'-untranslated regions of specific target mRNAs. An emerging body of evidence suggests that microRNAs serve as important therapeutic targets in a wide range of complex human diseases, including kidney diseases. miR-22 is a microRNA with predicted involvement in several pathways central to the development and progression of fibrosis.

Methods: We generated a miR-22 null mouse using a targeted strategy to replace and delete the genomic region containing the primary miR-22 sequence. A targeting vector with homology arms flanking this genomic locus was used to replace the pre-miR-22 hairpin. Renal fibrosis was induced by unilateral ureteral obstruction (UUO) in mice. Changes in mRNA and miRNA expression were assessed by SYBR Green qPCR (Bio-Rad, Hercules, CA) on RNA extracted from total kidney lysates.

Results: We used a classical knockout approach using miR-22 deficient mice that were recently generated in our laboratory. Age and sex-matched knock out ($miR\text{-}22^{-/-}$) and wild type ($miR\text{-}22^{+/+}$) mice were subjected to UUO maneuver and killed after 10 days. We found that kidney gene expression levels of established fibrotic markers were considerably lower in UUO kidneys from $miR\text{-}22^{-/-}$ mice compared with $miR\text{-}22^{+/+}$ mice. Specifically, we identified ~20% decrease in mRNA expression levels in Type I and III collagens and a reduction (~25%) in a-Smooth Muscle Actin (a-SMA). Of note, no appreciable differences in fibronectin and Type IV collagen mRNA expression levels were observed between the two groups. Histopathological analyses of UUO kidneys revealed a significant attenuation of chronic tubulointerestitial damage and reduced collagen deposition in miR-22 deficient mice.

Conclusions: Our data suggest that miR-22 serves as an important regulator of renal fibrosis. Targeted disruption of miR-22 may provide a therapeutic rationale for combating progressive renal fibrosis.

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SA-OR029

Blockade of miR-433 Inhibits Renal Fibrosis In Vitro and In Vivo

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Background: We have previously shown that TGF-β/Smad3-dependent microRNAs are key mediators in renal fibrosis. Here we report that miR-433 is a new fibrogenic miRNA regulated by TGF-b/Smad3 and is a therapeutic target for renal fibrosis in vitro and in vivo.

Methods: Expression of miR-433 was examined in tubular epithelial cells (TECs) after TGF-b treatment and in unilateral ureteral obstructive nephropathy (UUO). The functional role and therapeutic potential of miR-433 in fibrosis were examined in vitro in a rat NRK52E tubular epithelial cell (TEC) line by overexpressing or down-regulating of miR-433 and in vivo a mouse model of UUO by an ultrasound- microbubble- mediated anti-miR-433 gene transfer.

Results: Renal miR-433 was significantly increased in NRK52E cells after TGF-b1 stimulation and in the UUO kidney. Upregulation of miR-433 was associated with activation of Smad3 signaling. Findings that knockdown of miR-433 suppressed, but overexpression of miR-433 enhanced, TGF-β1-induced collagen I and fibronectin expression revealed a functional importance of miR-433 in renal fibrosis. More importantly, ultrasound-microbubble- mediated gene transfer of miR-433 knockdown plasmid into the mouse kidney before or after UUO (an established UUO) was capable of preventing or blocking renal fibrosis, particularly in the established obstructive nephropathy ($p<0.01$), demonstrating a therapeutic potential for renal fibrosis by targeting miR-433. Furthermore, we also found that antizyme inhibitor 1 (Azin1) was a potential target of miR-433. Overexpression of miR-433 suppressed Azin1 expression, whereas Azin1 overexpression blocked the fibrotic effect of TGF-β1.

Conclusions: MiR-433 is a key mediator of TGF-β/Smad3-induced renal fibrosis and is a therapeutic target for chronic kidney disease associated with progressive fibrosis.

SA-OR031

Recombinant Fusion Protein of Albumin-Retinol Binding Protein Attenuates Renal Fibrosis in Unilateral Obstructive Nephropathy

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Background: In previous study, Oh et al introduced a novel recombinant fusion protein of albumin-retinol binding protein (albumin domain I/III coupled to RBP, R-III), which induced phenotypic reversal of pancreatic stellate cells from the myofibroblast to the fat storing cells. Considering that the importance of pathologic changes into myofibroblasts in renal fibrosis and the increased uptake of R-III in the kidney.

Methods: We investigated the potential of R-III to reduce fibrogenic and inflammatory responses in an established interstitial fibrosis model-unilateral ureteric obstruction (UUO). After inducing UUO, R-III was injected every day for 7days through a tail vein.

Results: Compared to UUO group, R-III treated group showed decreased urinary protein excretion ($p<0.05$) and lower level of urinary 8-isoprostane ($p<0.05$). R-III treatment also suppressed the increase of renal levels of MCP-1, TGF-β1, CTGF, type I collagen ($p<0.05$) and phosphorylation of Smad3. It also attenuated NF-kB activation in kidney. Sirius red staining also showed decreased renal fibrosis by fusion protein treatment.

Myofibroblast cells were increased in the tubulointerstitial tissues in UUO kidney confirmed by aSMA staining, and RBP-peptide was co-localized in the aSMA positive myofibroblast cells. Interestingly, most fusion protein was localized in the tubule and myofibroblast cells of the obstructed kidney by IHC. Additionally, macrophage infiltration measured by F4/80 staining was markedly decreased in fusion protein treatment. In cultured cell lines, the uptake of fusion protein was increased in podocyte, HK2 proximal tubule cells and mouse mesangial cells (MMC). The expression of STRA6, RBP specific receptor, was increased in podocytes and MMC.

Conclusions: Our results suggest that the recombinant fusion protein (albumin-RBP) has anti-fibrotic effects in the experimental UUO murine model, probably via RBP receptor (STRA6) mediated uptake to the kidney.

SA-OR032

Intra-Abdominal Pressure and Bioimpedance-Based Volume Estimation in Septic Shock Patients Treated by Continuous Veno-Venous Hemofiltration

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Background: Intra-abdominal pressure (IAP) is an important risk-factor for the outcome of septic shock and acute kidney injury (AKI). It was the aim to analyze to which degree continuous veno-venous hemomofiltration (CVVH) affected IAP as well as abdominal perfusion pressure (APP), and how these variables were related to fluid volume as measured by bioimpedance analysis and to patient outcome.

Methods: Adult septic shock patients were studied before, 6, 12, 24, 48, 72 and 96 h after the beginning of CVVH because of AKI. The Kron method was used to measure IAP (in mm Hg). APP was determined as mean arterial pressure (MAP) minus IAP. Fluid volume excess (VE), total body water (TBW), extracellular body water (ECW), and intracellular body water (ICW) were derived from whole-body bioimpedance measurements (BCM, Fresenius Medical Care, Germany). Based on clinical outcome, the data were analyzed for survivors (S) and non-survivors (nS).

Results: 30 patients were studied. 24 patients survived CVVH and 6 died during CVVH but after the observational period. VE, TBW, ECW, ICW, and IAP were successfully decreased in S whereas these variables remained essentially unchanged in nS. APP slowly increased in S during CVVH but remained unchanged in nS. IAP strongly correlated with VE in S.

Tab. 1

	APP baseline (mmHg)	APP 96h (mmHg)	ECW baseline (L)	ECW 96h (L)
S	54 [49.75-69]	73 [69.25-79.83]***	26.2 [26.2-32.7]	20.1 [14.9-20.5]***
nS	52 [49-63.25]	60.5 [57.5-67.5]	26.6 [20.7-3-.25]	21.6 [18.1-24.86]
ID	P=0.69	P<0.05	P=0.55	P<0.01

Median [quartile 1 and 3]; S – survivors; nS – non-survivors; ID – intergroup difference; *** P<0.001 – significant difference relative to baseline (Wilcoxon test)

Conclusions: 1. CVVH successfully reduced IAP and TBW in critically ill patients who survived, 2. APP increased during CVVH in survivors, 3. Failure to increase APP predicted fatal outcome, 4. IAP was a sensitive indicator of fluid volume excess measured by whole-body bioimpedance analysis, and 5.) Bioimpedance analysis was useful to monitor fluid volume indicating positive patient outcome.

Funding: Clinical Revenue Support

SA-OR033

Inflammatory Cytokines and Markers of Volume Excess Assessed by Bioimpedance Analysis in Patients with Acute Kidney Failure

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Background: Increased microvascular permeability mediated by cytokines such as vascular endothelial growth factor (VEGF), tumour necrosis factor α (TNFα), E-selectine, and histamine leads to an expansion of extracellular volume (ECV) and to an increase in intra-abdominal pressure (IAP). It was the aim to analyze the relationship between IAP and fluid volumes using bioimpedance analysis and cytokines in critically ill patients treated by continuous veno-venous haemofiltration (CVVH) or furosemide infusion.

Methods: Adult critically ill patients with acute kidney failure (AKF) of different origin were studied. Variables were analyzed at the time of ICU admission (t=1) as well as 24 (t=2) and 48 h (t=3) after ICU admission. Patients were divided into CVVH and furosemide treatment groups. IAP was measured using the Kron technique. Whole-body bioimpedance (BCM, Fresenius Medical Care, Germany) was used for the measurement of intra- and extracellular volumes (ICV, ECV). Cytokines were measured by immunochemistry.

Results: 40 patients aged 53±15 years were enrolled, 17 were treated using CVVH, and 23 using furosemide, respectively. In all patients ECV and VEGF were highly and positively correlated with IAP (Tab. 1). IAP, VEGF, and ECV were higher in CVVH compared to furosemide treated patients.

Tab. 1

Groups	Time	IAP (mmHg)	VEGF (pg/mL)	ECV (L)
CVVH	t=1	13 [10-15]**	64.4 [25.8-247.9]**	26.0 [25-32.4]***
	t=2	11.5 [10-12]*	35.3 [15.2-94.5]**	25.4 [25.1-32.4]**
	t=3	12 [11-12]***	32.3 [20.7-60.4]**	25.2 [24.7-32.4]*
IAP correlation			P<0.0000; r=0.81	P<0.0001; r=0.51
Furosemide	t=1	7 [6-12]	14.3 [12.6-34.3]	21.3 [17.9-23.7]
	t=2	9 [6.5-11.5]	17.9 [14.0-25.7]	21.2 [19.1-25.2]
	t=3	7 [6.5-10]	15.2 [14.0-25.4]	20.5 [19.2-26.75]
IAP correlation			P<0.0000; r=0.51	P<0.0000; r=0.78

Median [quartile 1 and 3]; * P<0.05, ** P<0.01, *** P<0.001 – significant difference relative to Furosemide group

Conclusions: 1. IAP strongly depends on plasma VEGF concentration. 2. ECV depends on plasma VEGF and TNFα concentrations. 3. IAP is a sensitive marker of ECV expansion.

Funding: Clinical Revenue Support

SA-OR034

Urine Output as a Determinant to Initiate Continuous Renal Replacement Therapy for Acute Kidney Injury

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Background: Although some studies have found that early initiation of continuous renal replacement therapy (CRRT) is associated with better prognosis, no consensus exists on the best timing to start CRRT. We investigated whether the timing of CRRT initiation was relevant to overall mortality and explored which factors at the time of CRRT initiation were associated with better outcomes in critically ill patients with acute kidney injury (AKI).

Methods: A total of 361 patients, who received CRRT for AKI between 2009 and 2011, were collected and divided into two groups based on the median BUN levels or 6-hour urine output immediately before CRRT start. The impact of the timing of CRRT initiation stratified by BUN concentration or urine output on 28-day all-cause mortality was compared between the groups. Cox proportional hazards analysis was also performed to determine whether early CRRT initiation was an independent prognostic factor.

Results: When the timing of CRRT initiation was stratified by 6-hour urine output, 28-day all-cause mortality rates were significantly lower in the early CRRT group compared to the late CRRT group (P = 0.02). In contrast, clinical outcomes were not different between early and late CRRT groups classified by BUN levels (P = 0.30). Cox regression analysis revealed that 28-day all-cause mortality risk was significantly lower in the early CRRT group stratified by 6-hour urine output, even after adjusting for age, gender, mean arterial pressure, serum biomarkers, and APACHE II and SOFA scores (Hazards ratio: 0.85, 95% confidence interval: 0.65-0.99, P = 0.04).

Conclusions: Urine output might be more useful than BUN concentrations in making decision to initiate CRRT in critically ill patients with AKI.

SA-OR035

Survival with Self-Care Conventional versus In-Centre Nocturnal Hemodialysis: A Prospective Cohort Study

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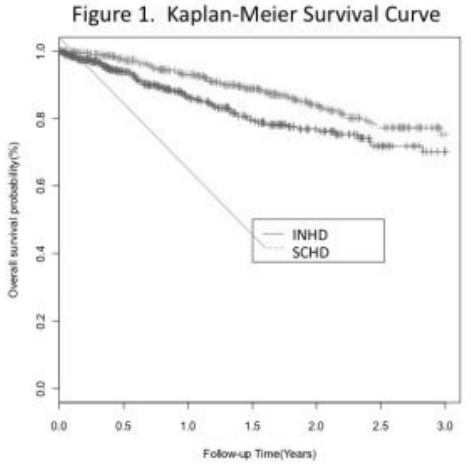
Background: In-centre nocturnal HD (INHD) associates with better survival than conventional full-care in-centre HD. However, unmeasured confounders including motivation, health literacy, functional and cognitive ability may account for this finding. We used self-care status as a proxy variable for these factors to test the hypothesis that INHD and self-care conventional HD (SCHD) have similar survival.

Methods: Adults on INHD (>6 hrs, 3/wk) were identified in the Fresenius US database (Jan2006-Jun2010), and matched (1:1) by age, vintage, and propensity score (PS) to US patients on SCHD (<6 hrs, 3/wk) in DOPPS(Feb2002-Apr2009). PS included:age, sex, race, vintage, dry weight, albumin, Hb, phos, DM, cancer, CVA, HTN, PVD, and psychiatric illness. We used Cox regression, attributing deaths within 90 days of a modality switch to baseline exposure.

Results: We matched 691 pairs from eligible pools of 1244 (INHD) and 1524 (SCHD) patients. Prognostic variables were balanced with std. difference <10%. 174 deaths occurred in 1688 patient-yrs; 240 (35%) of INHD patients switched to conventional HD. Median follow-up (SD) was 0.6(0.98)vs.1.42(0.87) yr, mean HD time 466(37) vs. 232(34) minutes and transplant rates 3.8(2.5-5.7) vs. 5.3(4.1-6.9)/100pt-yr in INHD and SCHD (p=0.13). In the primary as-treated model, the HR for death was 1.63 (1.20-2.20;p=0.002), favoring SCHD. Sensitivity analyses with an intent-to-treat model, 60-day attribution rule, and analysis of the entire sample with PS adjustment yielded similar results.

Conclusions: Results were unexpected and require further study. Self-care ability may be a more potent prognostic factor than length of HD session and warrants consideration for adjustment in future observational modality comparisons.



SA-OR036

The Feasibility of Caregiver Assisted Home Nocturnal Hemodialysis Karthik K. Tennankore, Joseph Kim, Christopher T. Chan. *Medicine, Division of Nephrology, Toronto General Hospital, Toronto, ON, Canada.*

Background: Home nocturnal hemodialysis (NHD) has several clinical advantages. However, NHD patients that require caregiver-assistance for dialysis may be at-risk for poor outcomes. In an effort to see if NHD can be extended to this patient subgroup, an analysis of their comparative outcomes is important.

Methods: We examined a single center cohort of all patients that started and completed NHD training between 01 Jan 2003 to 31 Dec 2010 (last follow up 01 Jul 2011). Patients were classified as "dependent" if they relied on a caregiver to perform some or all of their NHD therapy. The primary outcome of this study was to compare time to first hospitalization, technique failure or death for dependent versus independent patients, censored at transplantation. Secondary outcomes included hospitalization rate and hospital days.

Results: 47 (31%) and 105 (69%) dependent and independent NHD patients were included in this study. Dependent patients were older (51 vs. 42 years), were more likely to have diabetic ESRD (26% vs. 8%) and had higher Charlson Comorbidity Index Scores (4 vs. 3) compared to independent patients. Unadjusted, there was a significant difference in the time to first event for dependent versus independent patients (HR 1.71 [95% CI, 1.10 to 2.66]). Adjusting for comorbidity, age, dialysis catheter access, diabetic ESRD, race, gender and renal replacement therapy vintage, this difference was no longer statistically significant (HR 1.25 [95% CI, 0.76 to 2.04], figure 1). Incidence rate ratios for hospitalizations (IRR 1.58 [95% CI, 0.95 to 2.65]) and hospital days (1.84 [95% CI, 0.78 to 4.34]) were not significantly different for dependent versus independent patients.

Conclusions: The outcomes of caregiver-assisted versus independent NHD patients are driven by comparative differences in case-mix. The need for caregiver assistance alone should not be a deterrent to NHD.

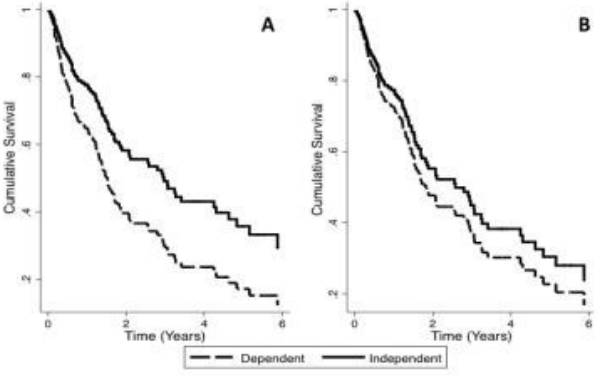


Figure 1. Unadjusted (A) and adjusted (B) Cox survival curves for time to first hospitalization, technique failure or death

SA-OR037

Effect of Frequent Dialysis on Residual Kidney Function in the Frequent Hemodialysis Network (FHN) Nocturnal and Daily Trials John T. Daugirdas,[1] Tom Greene,[2] Michael V. Rocco,[3] George A. Kaysen,[4] Thomas A. Depner,[4] Nathan W. Levin,[5] Glenn M. Chertow,[6] Daniel B. Ornt,[7] Jochen G. Raimann,[5] Brett Larive,[8] Alan S. Kliger,[9] The FHN Trial Group.[10] *[1]Univ of IL at Chicago; [2]Univ of Utah; [3]Wake Forest Univ; [4]Univ CA Davis; [5]Renal Research Institute; [6]Stanford Univ; [7]Rochester Inst Technol; [8]Cleveland Clinic; [9]Yale Univ; [10]NIDDK, NIH.*

Background: Frequent hemodialysis can alter levels of extracellular fluid volume and blood pressure and the concentration of osmotically active solutes, each of which might have an effect on residual kidney function (RKF).

Methods: In the Frequent Hemodialysis Network (FHN) Nocturnal and Daily Trials, we examined the effects of 6 versus 3 times per week hemodialysis on follow-up levels of RKF as measured by daily urine volume (UVol), and kidney urea (Kru) and creatinine (Krcreat) clearances. RKF inclusion criteria were Kru <10 mL/min/35L body water for the Nocturnal Trial, and Kru <3.0 for the Daily Trial.

Results: In both trials, baseline RKF was inversely correlated with years since onset of ESRD. In the Nocturnal Trial, 63/87 participants had nonzero RKF at baseline (mean urine volume 0.76 L/d, Kru 2.3 mL/min, Krcreat 4.7, mL/min). At months 4 and 12, UVol, Kru, and Krcreat were all lower in the group assigned to 6 times per week hemodialysis compared to the group assigned to 3 times per week. Of those with nonzero RKF at baseline, at month 12, 70% in the 6/week group had lost all RKF compared to 40% of controls. In the Daily Trial, 83/245 subjects had nonzero RKF at baseline (mean urine volume 0.43 L/d, Kru 1.2 mL/min, Krcreat 2.7 mL/min). Treatment assignment did not significantly influence level of RKF (as Uvol, Kru or Krcreat) at 4 or 12 months. Of those with nonzero RKF at baseline, at month 12, 48% in the 6/week group had lost all RKF compared to 53% of controls.

Conclusions: Frequent nocturnal hemodialysis is associated with more rapid loss of RKF, the mechanism of which remains to be determined. Whether this also occurs with frequent daily treatments could not be determined from the FHN Daily Trial design, which excluded subjects with higher levels of RKF.

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SA-OR038

Effects of Daily Hemodialysis on Heart Rate Variability: Results from the Frequent Hemodialysis Network (FHN) Daily Trial Christopher T. Chan,[1] Glenn M. Chertow,[2] John T. Daugirdas,[3] Tom Greene,[4] Peter Kotanko,[5] Brett Larive,[6] Andreas Pierratos,[1] John B. Stokes,[7] The FHN Trial Group.[8] *[1]U of Toronto; [2]Stanford Univ; [3]Univ of IL; [4]Univ of Utah; [5]Renal Research Institute; [6]Cleveland Clinic Foundation; [7]Univ of Iowa; [8]NIDDK.*

Background: End-stage renal disease is associated with reduced heart rate variability (HRV), components of which generally are associated with increased age, diabetes, and left ventricular mass (LVM). We reported previously, that daily in-center hemodialysis (DHD) resulted in reductions in LVM. We hypothesized that DHD would increase HRV globally and that changes in those components of HRV linked to LVM might be associated with changes in LVM.

Methods: The FHN Daily Trial randomized 245 patients to receive 12 months of 6 versus 3 times per week in-center hemodialysis (conventional HD). In 131 patients, HRV measures were calculated from 24-hour Holter monitoring. HRV measures included low frequency power (LF, a measure of sympathetic modulation), high frequency power (HF, a measure of parasympathetic modulation), and standard deviation of the R-R interval (SDRR, a measure of beat to beat variation). We tested for an effect of frequent dialysis on components of HRV, and for associations between change in LVM and changes in HRV components.

Results: Baseline to month 12 change in LF was 49% greater (95% CI 7.3%, 107%), p=0.018) and LF+HF was augmented by 38% (4.2%, 84.0%) in patients assigned to DHD compared to conventional HD. In contrast to LF, changes in HF and SDRR were similar between the DHD and conventional HD groups. Older age and diabetes (predefined subgroups) each were associated with weaker effects of DHD on LF. Changes in LVM were inversely associated with changes in HF (r = -0.20, p =0.02) and SDRR (r = -0.18, p = 0.04).

Conclusions: DHD increased the LF component of heart rate variability. Reduction of LVM by DHD was associated with increased vagal modulation of heart rate (HF) and with increased beat to beat heart rate variation (SDRR), suggesting an important functional correlate to the structural effects of DHD on the heart in uremia.

Funding: NIDDK Support

SA-OR039

FGF9 and FGF20 Maintain the Stemness of Nephron Progenitors in Mice and Man In Vivo and In Vitro Raphael Kopan,[1] Hila Barak,[1] Sung-ho Huh,[1] Shuang Chen,[1] Cecile Jeanpierre,[2] Mélanie Parisot,[3] Christine Bole-feysot,[4] Patrick Nitschke,[5] Remi Salomon,[2] Corinne Antignac,[2] David M. Ornitz.[1] *[1]Developmental Biology, Washington University, St. Louis, MO; [2]Universite´ Paris Descartes, Sorbonne, Paris, France; [3]Institut Imagine, Paris, France; [4]Genomic Plateform, Fondation Imagine, Paris, France; [5]6 Bioinformatic Plateform, Universite´ Paris Descartes, Sorbonne, Paris, France.*

Background: The identity of niche signals necessary to maintain embryonic nephron progenitors is unclear. FGF ligands are candidates for such signal because inactivation of FGF receptors in the progenitors results in arrested kidney development (Poladia et

al., 2006; Sims-Lucas et al., 2011) and because supplementing isolated metanephric mesenchyme (MM) *in vitro* with FGF2 (which is not present in the niche and its removal does not have a delaterious effect) and Bmp7 (which is in the niche, is required *in vivo* but is not sufficient *in vitro*) can maintain MM competent to differentiate for 48 hours in culture (Dudley et al., 1999).

Methods: We investigated the role of the *Fgf9* sub-family in renal development using mouse genetics, tissue culture, and human genetics.

Results: We discovered that Fgf9 acts redundantly with Fgf20 to promote the proliferation, survival and stemness of mouse MM progenitors *in vivo*. Moreover, we identified a human missense mutation in *FGF20* that results in bilateral renal agenesis. We show that Fgf9 or Fgf20 are sufficient to maintain MM with nephron progenitors *in vitro* for 5 days, whereas sorted Six2+ cells retained competence to form epithelia for at least 48h in culture when grown in serum-free media containing Fgf9 or Fgf9+BMP7. Differentiation-competent Six2+ cells slowly disappear over 7 days *in vitro,* and optimizing conditions for Six2+ self-renewal seems incompatible with maintaining competence to differentiate.

Conclusions: Our studies identify Fgf20/9 as integral parts of the MM niche where they act in autocrine and paracrine loop to maintain a self-renewing progenitor population competent to respond to Wnt signals. Though this lays the foundation for creating an artificial niche, much remains to be done to substentailly extend progenitor life *in vitro*.

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SA-OR041

Development of Efficient Induction Methods from Human iPSCs/ESCs into Intermediate Mesoderm by Using Low-Molecular Weight Compounds

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Background: The increased prevalence of chronic kidney disease has caused a rise in the number of dialysis patients, and is associated with elevated morbidity and mortality. Development of kidney regeneration therapy is required because most patients with chronic kidney disease never recover renal function. Kidney is derived from one of early embryonic germ layers, intermediate mesoderm (IM), and directing pluripotent stem cells, such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), into IM lineage is a crucial step for kidney regeneration.

Methods: To identify small molecules that can efficiently induce the differentiation from human iPSCs/ESCs into IM lineage cells, we screened a chemical library of about 1,800 low-molecular weight compounds using human iPSC lines that contain an allele of an IM-specific marker gene OSR1 into which a green fluorescence protein (GFP) gene was knocked-in by homologous recombination.

Results: We identified two chemical compounds with the inducing ability and established a differentiation protocol using the combinational treatment of these compounds that direct differentiation of human iPSCs/ESCs into IM lineage cells. The combinational treatment with these compounds induces nearly 90% of OSR1(+) cells from human iPSCs, in the same way as that achieved by our combinational treatment of growth factors. Moreover, the generated OSR1(+) cells produced the substantial expression of other IM markers, and had the ability to differentiate into the cells expressing the specific markers for renal cells constituting the adult kidney *in vitro* and *in vivo*. We also elucidated a part of the mechanisms involved in the differentiation of human iPSCs/ESCs into IM lineage cells.

Conclusions: Our differentiation methods using chemicals alone can produce more consistent and lower-cost generation of IM cells than growth factor-based protocols, which may contribute to understanding the mechanisms of kidney development and supplying the cell sources for the regenerative medicine strategies for chronic kidney disease.

SA-OR042

Parietal Podocytes: Transdifferentiation from Parietal Epithelial Cells or Migration of Podocytes?

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Background: In adults, it is still unresolved whether parietal epithelial cells (PECs) can potentially act as progenitor cells by differentiating into podocytes. "Parietal podocytes", i.e. fully differentiated podocytes residing on the inner aspect of Bowman's capsule, are an interesting model in this context. Parietal podocytes have been observed in multiple human kidney diseases, in particular in atubular glomeruli.

Methods: First, a mouse model to generate atubular glomeruli by electrocoagulation was established and characterized in different genetic backgrounds.

Results: Parietal podocytes were observed exclusively in atubular glomeruli. In addition, glomerular cysts were formed particularly in young mice of the Sv129 genetic background. Next, PECs or podocytes were traced in the above-described model for detubularization by irreversible genetic tagging in triple transgenic mice (PEC- or Pod-rtTA/LC1/R26R). Our results showed conclusively that PECs undergo apoptosis after detubularisation and that visceral podocytes migrate onto Bowman's capsule. No direct transdifferentiation from PECs towards podocytes was observed. This finding was confirmed in the unilateral ureter obstruction (UUO) model. Immunohistochemical stainings of human kidney biopsies always showed a sharp-edged border between PECs and podocytes on Bowman's capsule of atubular glomeruli. No gradual differentiation of PECs into podocytes could be observed, supporting that no transdifferentiation of PECs into parietal podocytes occurrs also in humans.

Conclusions: In summary, there are two surprising findings of this study: 1. Detubularisation leads to acute ablation of PECs and 2. visceral podocytes migrate onto Bowman's capsule in atubular glomeruli. Transdifferentiation from PECs to parietal podocytes did not occur in this model.

Funding: Government Support - Non-U.S.

SA-OR043

The Directed Differentiation of Podocyte Progenitors Following Kidney Reprogramming

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Background: The loss of podocytes is a key event in the progression of chronic kidney disease. Therefore, the reprogramming of adult cells to generate induced pluripotent stem (iPS) cells with a high proliferative ability and progenitor potential represents a major advance in the field. We have previously generated iPS cells from human kidney cells (JASN 2011), and now provide the first report of directed differentiation of iPS cells to podocyte progenitors.

Methods: iPS cells were differentiated into podocytes with the addition of BMP-7, retinoic acid and activin A. Morphology was assessed by phase contrast and scanning electron microscopy (SEM). Immunofluorescence microscopy and qPCR determined protein and mRNA expression of podocyte markers compared to primary human podocytes. iPS podocytes were transduced with RFP-talin/actin and visualized with confocal and live cell imaging providing functional assays of the contractile response to angiotensin II (AII), and uptake of albumin and integration into developing glomeruli.

Results: The iPS-derived podocytes shared a cell morphology similar to human podocytes including tertiary cytoplasmic processes by SEM. Using immunofluorescence microscopy the iPS podocytes showed protein localisation of and podocyte markers. qPCR confirmed that iPS podocytes had an upregulated expression of Wilm's tumor protein (WT1), nephrin, podocin and synaptopodin and down-regulation of the pluripotency gene OCT3/4. Using a kidney reaggregation explant assay the iPS-derived podocytes integrated into WT1+ developing glomeruli and had functional characteristics of podocytes in their contractile response of RFP-talin to AII and endocytosis of albumin.

Conclusions: This study establishes the derivation of iPS-derived podocytes that will be useful for screening new treatments, understanding podocyte pathogenesis, and offering possibilities for regenerative medicine.

SA-OR044

Incorporation of the Endogenous Renal Mesenchymal Stem Cells into the Elongating Collecting Ducts

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Background: Endogenous mesenchymal stem cells (MSC) have been identified in many solid organs where their specific role and phenotype is unclear. Previously we described endogenous renal MSCs (ER-MSCs) present in the adult mouse kidney[1].

Methods: Colony forming efficiency and clonogenicity showed that ER-MSCs were enriched in the papilla region of the kidney when isolated based on regional dissection. While the immunophenotype and differentiation capacity of these cells was similar to bone marrow MSCs (Bm-MSCs), gene expression profiling revealed differential expression of >2400 genes, including the collecting duct gene *Hoxb7*. To investigate the renal epithelial capacity of these cells we microinjected GFP^+ ER-MSC into the neonatal kidney (P1) under ultrasound guidance.

Results: The ER-MSCs integrated into $Aqp2^+$ collecting duct within the medulla and papilla region 3 days after injection. There was no integration into any other tubular structures. Epithelial integration was never observed from Bm-MSCs. Given the differential *Hoxb7* expression of ER-MSCs, we isolated the GFP^+ fraction from adult Hoxb7GFP kidneys. This fraction also gave rise to MSCs culture with classical MSC immunophenotype. When cultured in 3-D culture, these ER-MSCs showed a capacity to convert to an epithelial phenotype *in vitro.* Temporal analysis of the location of *Hoxb7* showed evidence for an initial neonatal $GFP^{+/lo}$ interstitial population that was absent in the adult kidney.

Conclusions: We have identified an endogenous MSC population in the adult kidney capable of selective integration into the neonatal collecting duct. This population may initially exist in the interstitium and epithelializes shortly after birth. This challenges our understanding of the origin of the collecting duct and the presence of stem/progenitor cells within this compartment. Further *in vivo* experiments will examine whether these cells play a role in normal tissue turnover as well as in renal damage or disease.

1. Pelekanos R et al, Stem Cell Research, 2012.

Funding: Government Support - Non-U.S.

SA-OR045

Infusion of Autologous Bone Marrow Mononuclear Cells Leads to Transient Reduction in Proteinuria in Treatment Refractory Patients with Idiopathic Membranous Nephropathy Upal Sengupta, Vinod Sharma, Ashok Kumar Yadav, Harbir Singh Kohli, Neelam Marwaha, Vinay Sakhuja, Vivekanand Jha. *Nephrology, Postgraduate Medical Institute, Chandigarh, India.*

Background: The current treatment options for idiopathic membranous nephropathy (IMN) carry significant toxicity. Stem cells have been used for immunomodulation in many diseases. In this open label, prospective, observational pilot study, we used one-time infusion of autologous bone marrow derived mononuclear cells (MNCs) in adults with treatment refractory IMN.

Methods: Twelve patients of biopsy proven IMN who had failed a cyclical 6-month regimen of steroid and cyclophosphamide were enrolled. Four had also been unresponsive to tacrolimus. Bone-marrow was harvested from the iliac crest and MNCs isolated. Cells were counted and subjected to viability testing before being infused in a dose of 2-4x10^6/kg through a peripheral vein. Subjects were followed up monthly for the next six months. Supportive treatment including angiotensin antagonists and stains was continued throughout the study period.

Results: All the 12 subjects exhibited full-blown nephrotic syndrome despite being on on ACE inhibitors, statins and diuretics. The proteinuria, protein-creatinine ratio (PCR), serum albumin and creatinine values at entry were 3.27± 0.41 gm/d, 3.37±0.6 g/g, 2.38 ± 0.6 gm/l and 0.9 ± 0.2 mg/dl respectively. The average number of MNCs injected was 293.58±96.14 X 10^6. There was a reduction in proteinuria ($p<0.001$) and PCR ($p=.0024$) and increase in serum albumin ($p=0.026$) at 1 month, with 64% of the subjects showing > 50% reduction in proteinuria. However, the proteinuria again increased to almost or more than the baseline value in majority of the cases. At 6 months, only 2 patients had >50% reduction. No infusion related side effects were noted. Serum creatinine was stable during the study period.

Conclusions: Autologous MNC infusion in peripheral vein appears can lead to transitory reduction in proteinuria and improvement in serum albumin in treatment refractory IMN patients. This effect, however, is transient. Whether this can be overcome by infusion of cultured MSCs or directed delivery to the kidneys needs to be investigated.

Funding: Government Support - Non-U.S.

SA-OR046

The Renal Ciliopathy Gene Product Regulates DNA-Damage Induced Cell Cycle Progression Amiya K. Ghosh,[1] Rannar Airik,[1] Moumita Chaki,[1] Rachel H. Giles,[2] Gisela G. Slaats,[2] Edgar Otto,[1] Friedhelm Hildebrandt.[1,3] [1]*Department of Pediatrics, University of Michigan, Ann Arbor, MI;* [2]*Department of Nephrology and Hypertension, University Medical Center, Utrecht, Netherlands;* [3]*Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.*

Background: Nephronophthisis-related ciliopathies (NPHP-RC) are recessive disorders featuring dysplasia or degeneration of retina, kidney and cerebellum. By virtue of localization to the cilia/centrosome complex, diseases associated with NPHP gene products are termed as 'ciliopathies', although the disease mechanism remains elusive. We have recently identified recessive loss-of-function mutations of centrosomal protein CEP164 as a novel cause of NPHP-RC (Chaki & Airik, *Cell, in press*).

Methods: To assess the effects of Cep164 loss of function on cell cycle, we generated inducible-gfp-hCEP164 IMCD3 cells for both wild type and disease associated mutant constructs. Stable knockdown of Cep164 in IMCD3 cells were also generated using retroviral transduction system. To address the role of Cep164 in NPHP-RC, cell lines were studied as described below.

Results: We observe:

a. Wild type but not its disease-associated mutant Cep164 localizes to the mother centriole in an inducible N-gfp-cep164 expression system in IMCD3 cells.

b. Lack of endogenous *Cep164* leads to reduced cell proliferation and cilia formation, which was rescued by expression of Human wild type *CEP164* but not by disease-associated mutant in cell culture models.

c. Endogenous *Cep164* knockdown leads to cell cycle block in S-phase under thymidine induced replication stress. This is rescued wild type h*CEP164* but not by disease-associated mutants.

d. Under replicative stress Cep164 additionally localizes to TIP60 positive nuclear foci. (TIP60 is known to activate the DNA damage response protein ATM at sites of DNA damage).

e. Upon irradiation, stable knockdown of *Cep164* in IMCD3 cells resulted in elevated g-H2AX levels, a marker of increased DDR.

Conclusions: Our observations point towards a novel disease mechanism that implicates DNA damage response (DDR) signaling and retarded cell cycle progression in the pathogenesis of NPHP-RC.

Funding: NIDDK Support

SA-OR047

***Gli2* Dosage Modulates Renal Cystogenesis in the *Thm1* Mouse Model of Ciliopathy** Pamela Vivian Tran,[1] George Talbott,[2] Michael P. Schonfeld,[1] David Beier.[2] [1]*Anatomy and Cell Biology, Kidney Institute, University of Kansas Medical Center, Kansas City, KS;* [2]*Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Cystic kidney disease (CKD) is a leading cause of renal failure. Mutations associated with CKD affect genes encoding proteins that localize to primary cilia, though the precise molecular mechanisms connecting ciliogenesis to renal cystogenesis remain unclear. The ciliary gene *Thm1* (also *Ttc21b*) negatively regulates the Hedgehog (Hh) pathway (Tran et al. 2008) and has been identified as the most commonly mutated gene in ciliopathies (Davis et al, 2011), which manifest renal cysts as a major clinical feature.

Methods: We examined the role of *Thm1* in renal cystogenesis in the mouse using *Thm1*-null and *Thm1* conditional knock-out (cko) alleles. To explore a causative role for enhanced Hh activity in *Thm1* renal cystogenesis, we utilized a *Gli2*-null and a *Gli2* cko allele to create different *Gli2* doses on the *Thm1* cko background.

Results: We report that loss of murine *Thm1* indeed leads to renal cysts, both in the perinatal lethal *alien* (*aln*; $Thm1^{aln/aln}$) mouse mutant and postnatally in *Thm1* cko mice following *Thm1* ablation at E17.5. In *Thm1* cko mice, renal cysts are present at P20 and progress to severe CKD at 6 weeks of age. Although Hh signaling has not been commonly explored as a causal factor for CKD, we hypothesize that *Thm1* renal cystogenesis is mediated by increased Hh signaling. In support of our hypothesis, $Thm1^{aln/aln}$ embryonic renal cysts were prevented by genetically reducing *Gli2*, which encodes the main transcriptional activator of the Hh pathway. Further, preliminary results indicate that the reduction of *Gli2* expression by 50% in $Thm1^{fl/aln}$, $Gli2^{+/-}$ mutants markedly ameliorated the *Thm1* cko renal cystic disease, suggesting Hh signaling also mediates *Thm1* renal cystogenesis postnatally. We have generated $Thm1^{fl/aln}$, $Gli2^{fl/-}$ mutant mice in which *Gli2* expression can be completely ablated in postnatal development; the analysis of these mice is in progress.

Conclusions: Our results establish a role for *Thm1* in renal cystogenesis and suggest that GLI2 activity may present a valuable target in the treatment of CKD.

Funding: NIDDK Support

SA-OR048

A Transition Zone Complex of Ciliopathy Proteins Regulates Ciliary Composition Jeremy Reiter. *Biochemistry and Biophysics, University of California, San Francisco, CA.*

Background: Pkd1, Pkd2, and Fibrocystin, the products of genes mutated in polycystic kidney disease localize to the primary cilia of kidney epithelial cells. Defects in ciliary function can result in polycystic kidney disease, suggesting that these proteins regulate epithelial proliferation at the cilium.

Methods: Through a combination of mass spectrometry, biochemistry, and mouse genetics, we have identified a complex of proteins that form part of the transition zone, a region at the base of the cilium.

Results: This transition zone complex includes the three members of the Tectonic family (Tctn1, Tctn2 and Tctn3), extracytosolic proteins that interact with transmembrane components of the transition zone such as Tmem67. These transmembrane proteins connect to a intracellular transition zone complex comprised of many known Joubert- and Meckel-associated proteins including Cc2d2a and Mks1. Joubert syndrome is a defect in cerebellar vermis formation resulting in ataxia, and can be associated with kidney cysts. Meckel syndrome is a postnatal lethal disorder characterized by heterotaxia, polydactyly and cystic kidneys.

Loss of components of this transition zone complex in mice compromises ciliogenesis in some tissues, and deregulates ciliary protein composition in others. In particular, the ciliary localization of Smoothened (Smo), a central component of the Hedgehog pathway, and Pkd2, mutated in autosomal dominant PKD, depends on this complex. As Smo functions at the cilium, many mouse transition zone mutants show deregulation of Hh signaling. Mouse transition zone mutations also develop kidney cysts, consistent with a critical ciliary function for Pkd2.

Defining the components of the transition zone has led to the identification of additional genes underlying Joubert and Meckel syndromes including Tctn1, Tctn2 and B9d2. We hypothesize that Joubert and Meckel syndromes are caused by transition zone dysfunction that disrupts intercellular signaling, leading to developmental defects including kidney cysts.

Conclusions: The signaling functions of kidney cilia require regulation of ciliary composition, which depends on the control of protein traffic into and out of cilia by the transition zone.

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SA-OR049

TRPV4 Functional Status Is Impaired in the Collecting Duct-Derived Cysts of ARPKD Mykola Mamenko, Oleg L. Zaika, Roger G. O'Neil, Oleh Pochynyuk. *Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX.*

Background: Autosomal recessive polycystic kidney disease (ARPKD) is a rapidly progressing congenital pathology, characterized by the development of fluid-filled cysts in the collecting ducts (CD). At the cellular level, ARPKD is associated with loss of mechano-sensitivity and compromised Ca^{2+} homeostasis. Ca^{2+}-permeable TRPV4 channel was recently shown to have a pivotal role in flow-sensitivity of CD cells.

Methods: To investigate TRPV4 functional status during in ARPKD progression, we used Fura-2 Ca^{2+} imaging with IHC in freshly isolated split-opened CDs of S/D rats, non-dilated CDs and monolayer cyst fragments from PCK453 rats (rat model of ARPKD).

Results: We found that elevated flow induces comparable TRPV4-mediated elevations of $[Ca^{2+}]_i$ in individual cells of the CDs from S/D rats and non-dilated CDs from PCK453 rats. However, Ca2+ responses to flow were abolished in cyst cells from PCK453 rats. Consistently, TRPV4 function and basal $[Ca^{2+}]_i$ levels were drastically diminished in ARPKD cyst cells, but not in non-dilated CDs. ATP-mediated activation of TRPV4 channel was also virtually absent in freshly isolated cyst segments from PCK453 rats. Surprisingly, TRPV4 expression was preserved during ARPKD progression, while TRPV4 subcellular localization was markedly shifted towards the luminal plasma membrane. Potentiation of TRPV4 activity with a selective activator GSK1016790A, rescues Ca^{2+} homeostasis by restoring flow-mediated Ca^{2+} responses, increasing basal $[Ca^{2+}]_i$ levels and recovering purinergic signaling. At the systemic level, GSK1016790A attenuates renal ARPKD progression by decreasing cyst area and numbers.

Conclusions: Overall, during development of ARPKD, functional activity of TRPV4 channels is significantly impaired, contributing to reduced $[Ca^{2+}]_i$ levels and disrupted mechano-sensitivity in the CD-derived cysts. Treatment with a TRPV4 activator significantly attenuates morbific manifestations of ARPKD on both systemic and cellular levels.

Funding: Private Foundation Support

SA-OR050

Characterization of a New ARPKD Mouse Model: An "in Vivo" Model to Study the Function of the C-Terminal Region of Fibrocystin/Polyductin

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Background: Autosomal Recessive Polycystic Kidney Disease affects 1/20,000 and results from mutations of PKHD1. The gene exhibits a complex splicing pattern and its longest mRNA encodes a >400kDa polypeptide (FPC) that undergoes Notch-like post-translational processing. Its C-terminus is thought to translocate to the nucleus in response to cleavage. In humans, renal disease is associated with mutations scattered along the length of the gene, but in rodent models renal disease is mild, if it develops at all. Hepatobiliary disease is a universal feature in both humans and rodents. All of the reported rodent mutations are 5' of exon 49, raising the possibility that an intact C-terminus expressed by alternative splicing modulates severity.

Methods: To test this hypothesis, we generated a novel floxed allele of *Pkhd1* in the C57Bl6 strain with lox P sites flanking its last coding exon and a triple-HA epitope tag inserted in-frame to the carboxyl terminus of FPC.

Results: We find that homozygotes for the floxed allele are healthy and that anti-HA antibodies can be used to detect full length FPC in lysates and to affinity purify native protein. The predominant species appears to be full-length protein, though a minor cleaved product is detected as well. Using anti-HA sera, we can detect endogenous tagged FPC by IF in collecting ducts and in epithelial cells of the biliary tract. At a subcellular level, HA-tagged FPC was found in ciliated cholangiocytes and cells lining pancreatic ducts. After deleting the LoxP cassette, homozygous Pkhd1Δ67/67 mice were born at the expected frequency. Remarkably, mutant mice lacked typical hepatobiliary abnormalities at up to 4m of age.

Conclusions: In conclusion, we have developed a novel, HA-tagged Pkhd1 allele that can be used to track the endogenous protein by standard methods. This will be a useful tool complementary to prior 5'tagged models. We show that the C-terminus, which was hypothesized to be an essential domain, is not necessary for normal hepatobiliary development. Our findings challenge the current model of FPC function.

SA-OR051

Missense Mutation of the Transcriptional Regulator *FSGS8* Is a Cause of Autosomal Dominant FSGS

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Background: FSGS is a clinical disorder characterized by focal scarring of the glomerular capillary tuft, podocyte injury and loss, and nephrosis. While idiopathic forms of this heterogeneous disorder constitute the majority of cases, recent insights into the molecular and genetic causes of inherited forms of FSGS have greatly enhanced our understanding of the pathogenic mechanisms of FSGS. Here we report the discovery of a novel mutation of the transcriptional regulator *FSGS8* which caused an early-onset form of autosomal dominant (AD) FSGS in a 88 family member kindred (Family 6524) from Lumberton, North Carolina.

Methods: In this study, we performed genome-wide linkage analysis using the Illumina Infinum II HumanLinkage-24 beadchip genotyping assay and whole-exome sequencing. All identified novel variants were confirmed by Sanger sequencing. Complimentary molecular genetic analyses in HEK 293 cells and zebrafish were also peformed.

Results: We identified a missense mutation R458Q within a c-terminal zinc finger domain of *FSGS8* as a new cause of AD FSGS. The mutation segregates with the disease in each of the seven affected family members within our study cohort and the mutation was not found in 1600 control chromosomes. The change is evolutionarily conserved and is considered damaging by *in silico* simulation. In complimentary *in vitro* studies in HEK 293 cells, overexpression of FSGS8 significantly downregulated nephrin and synaptopodocin mRNA expression, increased apoptosis, and altered the subcellular distribution of nephrin protein expression. Additionally, zebrafish morphants lacking *FSGS8* exhibited marked cardiac and yolk sac edema as well as retinovascular and trunk vascular network dextran leak consistent with nephrosis.

Conclusions: In summary, we report the identification of a novel mutation of the transcriptional regulator *FSGS8* which causes a particularly aggressive form of AD FSGS in a large US kindred. The FSGS8 R458Q mutation adversely affects the transcriptional regulation of key slit diaphragm proteins, causes mislocalization of nephrin, and promotes cellular apoptosis.

Funding: NIDDK Support

SA-OR052

APOL1 Genotype and Kidney Microanatomy: Insights into the Predisposition of African Americans to Kidney Disease

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Background: APOL1 variants associate with excess kidney disease in African Americans (AA). We investigate their association with kidney parameters in autopsies.

Methods: 149 adult AA and 111 Whites from Mississippi were studied at autopsy. People with clinical histories, anatomic kidney abnormalities or histologic findings of renal disease were excluded. Glomerular number (N_{glom}) and mean glomerular volume (V_{glom}) were estimated using the disector/fractionator method. DNA from kidney blocks was typed for APOL1 renal risk alleles.

Results: Leading causes of death were misadventure and cardiovascular (CV) disease (29% each). 64% of AA (vs 2% of whites) were APOL1 risk allele positive: 44% and 20% had one and two alleles respectively. In aggregate, there were no differences in BMI, kidney weight, N_{glom} or V_{glom} in AA by APOL1 status, but APOL1 positive subjects, unlike APOL1 negative subjects, showed striking increases in V_{glom} ($p=0.02$) and kidney weight ($p\leq0.001$) with increasing BMI, tending to smaller values below, and higher values above the BMI median of 29 kg/m^2. On this cross-sectional study, APOL1 positive AA had 9480 fewer glomeruli per kidney per yr between ages 18 and 65 yr ($p=0.004$), vs 1724 per yr in APOL1 negative AA ($p=0.66$). All these phenomena were more marked in AA with two than with one APOL1 risk allele. In APOL1 positive AA, the adjusted OR (95%CI) for hypertension was 2.9 (1.1-7.3), and for CV death was 2.8 (1.6-6.8) compared with APOL1 negative AA.

Conclusions: APOL1 risk alleles were dominantly associated with excessive glomerular and kidney hypertrophy in relation to increasing body size, and accelerated nephron loss with age, all with a gene dose effect, and with hypertension and CVD. We speculate that the more extreme forms of APOL1-associated glomerular hypertrophy and nephron loss predispose to glomerulosclerosis, subclinical and clinical kidney disease and associated hypertension.

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SA-OR053

Negative Selection of Deleterious Variants in Genes Associated with Mendelian Forms of Nephrotic Syndrome

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Background: Genes associated with Mendelian diseases tend to undergo negative selection of deleterious single nucleotide variants (SNV). Annotating known and/or candidate nephrotic syndrome (NS) genes with measures of purifying selection may help to identify disease-associated loci. Leveraging genome-wide SNV data from the 1000 Genomes Project (1000G) coupled with functional prediction software, we developed and implemented both novel and known methods to quantify the degree of negative selection in twelve genes of known association with NS and compared these results with 17,494 other genes.

Methods: Using 1000G data, we measured the frequency of non-synonymous SNV present in coding regions across the genome and used functional prediction software to stratify them as deleterious or benign. We then created two metrics of selection: 1) number of SNV/kilobase ("Suppressed Mutation") & 2) percentage of SNV that are private ("Enriched Rare Variants"). Using a Wilcoxon rank sum test, we contrasted the difference of frequency spectrum between benign and silent mutations for 17,506 genes across the genome and established the quantile in which the 12 NS-associated genes were located.

Results: *APOL1* (.008 quantile of all genes tested), *LAMB2* (.016 quantile), and *TRPC6* (.036 quantile) demonstrated the most enrichment of rare deleterious variants. The NS genes with the strongest suppression of deleterious SNV were *INF2* (.008 quantile), *MYH9* (.011 quantile), and *ACTN4* (.045 quantile). *INF2* had a 66x lower rate of deleterious vs benign SNV.

Conclusions: Multiple NS genes exhibit strong negative selection, suggesting that, when present, deleterious SNV in these genes are likely functional and could have large effects. The prevalence of these rare, deleterious SNV can be further investigated by sequencing these genes in large NS cohorts. Additionally, utilizing these metrics of negative selection to functionally annotate genes may help in prioritizing particular NS candidate genes for further study.

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SA-OR054

FAN1 Mutations Cause Karyomegalic Interstitial Nephritis, Linking DNA Damage Response Signaling to Renal Fibrosis Friedhelm Hildebrandt,[1,2] Weibin Zhou,[1] Edgar Otto,[1] Andrew Cluckey,[1] Katrina A. Diaz,[1] Heon Yung Gee,[1] Rannar Airik,[1] Moumita Chaki,[1] Toby W. Hurd,[1] Amiya K. Ghosh,[1] Jaap A. Joles,[3] Rachel H. Giles,[3] Agata Smogorzewska.[4] *[1]Pediatrics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; [3]Nephrology and Hypertension, University Medical Center, Utrecht, Netherlands; [4]Laboratory of Genome Maintenance, Rockefeller University, New York, NY.*

Background: Chronic kidney disease (CKD) remains one of the largest health problems in developed countries. Its prevalence increases for unknown reasons, and its central phenotypic feature, renal fibrosis, is not well understood.

Methods: In order to identify single-gene causes of renal fibrosis/CKD we performed homozygosity mapping and whole exome resequencing in a model disorder for renal fibrosis known as 'karyomegalic interstitial nephritis (KIN)' (Mihatsch et al. *Clin Nephrol 12:54, 1979).*

Results: We identified recessive mutations of Fanconi anemia-associated nuclease 1 (FAN1) as causing karyomegalic interstitial nephritis (KIN) in 9 of 10 families ascertained. KIN causes CKD with renal histology indistinguishable from NPHP, except for the presence of karyomegaly. FAN1 has nuclease activity and acts in DNA interstrand crosslinking (ICL) repair within the Fanconi anemia (FA) pathway of DNA damage response (DDR). Interestingly, ICL-causing genotoxins generate a KIN-like phenotype. We demonstrate that cells from individuals with FAN1 mutations exhibit sensitivity to the ICL agent mitomycin C. However, they do not exhibit chromosome breakage or cell cycle arrest after diepoxybutane treatment, unlike cells from patients with Fanconi anemia. We complement ICL sensitivity with wild type FAN1 but not mutant cDNA from individuals with KIN. The FAN1 defect was not epistatic with the Fanconi anemia pathway. By depletion of fan1 in zebrafish we recapitulated increased DDR, apoptosis, and kidney cysts akin to NPHP.

Conclusions: Our findings implicate susceptibility to environmental genotoxins and inadequate DNA repair as novel mechanisms of renal fibrosis and CKD.

Funding: NIDDK Support, Private Foundation Support

SA-OR055

Identification of Homozygous Mutations in Two alpha Integrin Encoding Genes in Fetuses with Severe Kidney Development Defects Cecile Jeanpierre,[1,2] Mélanie Parisot,[1,3] Flora Silbermann,[1,2] Stephanie Reichen,[1] Christine Bole-feysot,[3] Patrick Nitschke,[2] Laurence Heidet,[4,5] Patricia Blanchet,[6] Remi Salomon,[1,2,5] Corinne Antignac,[1,2] Sophie Saunier.[1,2] *[1]Inserm U983, Paris, France; [2]Université Paris Descartes, Paris, France; [3]Institut Imagine, Paris, France; [4]MARHEA Reference Center, AP-HP, Necker Hospital, Paris, France; [5]Pediatric Nephrology Department, AP-HP, Necker Hospital, Paris, France; [6]Genetic Department, Hôpital Arnaud de Villeneuve, Montpellier, France.*

Background: Renal hypodysplasia (RHD) is an heterogeneous condition encompassing a spectrum of kidney development defects responsible for pediatric end-stage renal failure and mortality. Dominant mutations in *PAX2*, *EYA1*, *SIX1* and *HNF1B* have been identified in syndromic forms of RHD. However, other genes involved in isolated and syndromic RHD remain to be identified. Moreover, examination of families highly suggests that not only dominant but also recessive forms of RHD exist.

Methods: In order to identify genes involved in recessive RHD, we analysed fetuses belonging to independent consanguineous families by whole exome sequencing [50 Mb SureSelect assay (Agilent) and SOLiD4 (Life Technologies)].

Results: We identified homozygous mutations in two genes encoding alpha integrin subunits. A splicing mutation in *ITGA8*, leading to skipping of exon 28, was identified in two fetuses from a family with bilateral renal agenesis. This mutation was absent from dbSNP, 1000genome and our in-house databases. A missense variation in *ITGA3* was detected in two fetuses with multicystic renal dysplasia from a family originating from Turkey. This mutation, L518P, was predicted as damaging by Polyphen2 and Sift softwares. It was absent from databases as well as from 30 Turkish DNA controls.

Conclusions: Integrins play a crucial role during development of epithelial tissues, notably in the kidney. Integrin a8- and integrin a3-deficient mice display abnormal kidney development, with renal agenesis and cystic formation respectively. This is the first report of human mutations in these genes associated with RHD. Functional studies based on 3D-cultures and adhesion assays are in progress to formally demonstrate the causative effect of these mutations.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR056

Regulation of Sclerostin Expression and Bone Volume by Phosphate in a Model of Adynamic Bone Disease Juliana C. Ferreira,[1] Guaraciaba O. Ferrari,[1] Raquel T. Cavallari,[1] Katia R. Neves,[1] Wagner V. Dominguez,[1] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Elizabeth M. Oliveira,[1] Shiguang Liu,[2] Yves Sabbagh,[2] Vanda Jorgetti,[1] Susan Schiavi,[2] Rosa M.A. Moyses.[1] *[1]Nephrology Division, Universidade de São Paulo, Brazil; [2]Genzyme, A Sanofi Company.*

Background: Recent studies have linked the protein Sclerostin (SOST), a Wnt pathway inhibitor, to the pathogenesis of CKD-MBD, where a negative association between PTH and SOST was found. In this study, we investigated the role of phosphate (P) in SOST regulation by testing it in a experimental model of CKD adynamic bone disease mantained on diets with different P contents.

Methods: Rats were divided into 3 groups and subjected to nephrectomy (Nx) and parathyroidectomy (PTx) with different P content in the diet: CKD 1.2% (P = 1.2%), CKD 0.6% or Control (sham NX/PTX + 0.6%). After 8 wks, biochemical, histomorphometric and bone gene expression (TLDA) analyses were performed.

Results: CKD rats showed higher Creat and P, but lower iCa and FGF-23 than Controls. Comparing uremic rats, P overload was associated with higher FeP, lower bone volume (BV/TV), as well as higher serum SOST, elevated bone expression of SOST and RANK, and apoptotic rate of osteoblasts (1.3 vs 0.4%) and osteocytes (0.4 vs. 0.2%; $p<0.05$ for all), when compared to 0.6% P rats.

	CKD 1.2%	CKD 0.6%	Control
Creat(mg/dl)	1.3±0.2	1.4±0.9	0.6±0.1[a]
P(mg/dl)	11.7±1.9	12.2±1.9	5.5±0.6[a]
iCa(mmol/L)	0.5±0.1	0.5±0.1	1.2±0.1[a]
FeP(%)	42(32-54)[a]	1.0(0.3-9.8)	7.6(3.7-10)
PTH(pg/ml)	16(5-224)	26(19-117)	124(88-199)
FGF-23(pg/ml)	138±118	192±42	286±92[a]
serum SOST(ng/ml)	1.10(0.51-0.70)	0.15(0.07-0.43)[a]	1.71(0.71-3.35)
BV/TV(%)	26.8±5.5	33.6±4.4[a]	24.2±5.0
SOST(fold change)	4.1±0.3[a]	0.5±0.2	1.2±0.4
RANK(fold change)	1.1±0.04	0.6±0.2[a]	1.0±0.2

a = $p< 0.05$ vs. all

Therapy with sevelamer, but not with calcium, decreased bone expression of SOST in animals fed with 1.2%P.

Conclusions: We demonstrate that in CKD, P can regulate bone SOST mRNA expression and serum protein levels independent of PTH. P was also shown to increase the expression of RANK and the apoptosis of osteoblasts, leading to a lower bone volume. Our findings underscore the role of P in CKD-MBD, emphasizing the value of its control in this disease.

Funding: Pharmaceutical Company Support - Genzyme Co., Government Support - Non-U.S.

SA-OR057

Secreted Klotho Protects against Stress-Induced Cardiac Hypertrophy by Down-Regulating TRPC6 Channel Jian Xie, Sung Wan An, Makoto Kuroo, Chou-Long Huang. *UT Southwestern Medical Center, Dallas, TX.*

Background: Klotho is an anti-aging membrane protein predominantly produced in the kidney, which sheds its extracellular N-terminus into the systemic circulation. It was recently reported that increased serum FGF23 contributes to cardiac hypertrophy of CKD via a Klotho-independent mechanism. Klotho expression is decreased in CKD and aging, two conditions associated with increased risk for heart failure. Whether Klotho is cardioprotective is unknown.

Methods: Mice overexpressing Klotho (KL-Tg) and wild-type littermates were studied at baseline and in response to isoproterenol (ISO) overstimulation (sc x 10 days). Hypertrophy was assessed by measuring heart size, heart weight/body weight (HW/BW) ratio, and expression of cardiac fetal genes (*BNP*, *ANP*, and β-*MHC*). Serum phosphate and FGF23 levels were measured.

Results: Heart size and HW/BW ratio were not different between WT and KL-Tg mice at baseline. ISO treatment induced cardiac hypertrophy, and Klotho overexpression blunted the ISO-induced cardiac hypertrophy. Consistently, Klotho overexpression did not alter cardiac fetal gene expression at baseline, but attenuated ISO-induced increases in expression. Klotho overexpression in mice did not alter serum phosphate or FGF23 levels. It was reported that increased TRPC6 induces cardiac hypertrophy. Indeed, mice with heart-specific overexpression of TRPC6 (C6-Tg) developed spontaneous cardiac hypertrophy and had increased fetal gene expression and decreased long-term survival relative to WT. Transgenic Klotho overexpression ameliorated hypertrophic responses and improved long-term survival of C6-Tg mice. Direct addition of secreted Klotho inhibits TRPC6 currents recorded by patch-clamp in isolated cardiomyocytes.

Conclusions: Klotho has no effect on hearts at baseline, but protects against stress-induced pathological cardiac hypertrophy. Cardioprotection by Klotho is mediated by down regulation of cardiac TRPC6. As FGF23 appears to induce cardiac hypertrophy independently of stress factors, increased FGF23 and Klotho deficiency may synergistically contribute to cardiac hypertrophy in CKD by participating at different stages of pathogenesis.

Funding: NIDDK Support

SA-OR058

Inhibition of Systemic Factors Produced by Kidney Injury Decreases Cardiovascular Risk in the CKD-MBD Charles Ginsberg, Yifu Fang, Toshifumi Sugatani, Keith A. Hruska. *Pediatric Nephrology, Washington University School of Medicine, St. Louis, MO.*

Background: Kidney injury causes recapitulation of developmental programs in attempted repair leading to increased production of factors that circulate, some of which are inhibitory to skeletal remodeling. The early CKD-MBD begins in stage 2 CKD and is characterized by the inhibitors of skeletal remodeling, stimulation of VC, increased secretion of FGF23 and normal mineral metabolism. In CKD, osteoblastic differentiation of cells in the neointima causes calcification of atherosclerotic plaques and stimulates medial vascular calcification (VC). VC is an important cause of CV morbidity in CKD and is a CV risk factor associated with CKD. Here we tested the hypothesis and examined the mechanisms that neutralization of the inhibitors of skeletal remodeling would decrease CV risk in CKD.

Methods: We used neutralizing monoclonal antibodies to specific circulating skeletal inhibitory factors produced by renal injury in early CKD. *Ldlr-/-* mice fed high fat diets

were subjected to renal cortical electrocautery and contralateral nephrectomy at 12-14 weeks of age to produce the early CKD–MBD, euthanasia was at 22 or 28 weeks. Treatment with vehicle, DKK1 mab (30 mg/Kg tiw IP), or CaAc (3% w/w mixed in diet) was begun at 14 or 22 wks.

Results: Dickkopf-1 (Dkk1) levels were increased from normal wt 1868±772 or sham 1650±882 to 3132±1590 (pg/ml, p<0.01). Glomerular filtration rate was reduced to 76% of normal (stage 2 CKD). BUN, Ca, Pi and PTH levels were normal. CKD stimulated and DKK1 mab inhibited accumulation of aortic Ca levels (0.52±0.19 (CKD) vs. 0.26±0.14 (wt), 0.32±0.23 (sham) and 0.4±0.1 (DKK1 mab) mg/g dry weight, p<0.05). CaAc did not improve CKD stimulated VC. Serum levels and aortic expression of FGF-23 were also increased in CKD, but were not decreased by the DKK1 mab (554±263 (CKD) vs. 242±56 (wt), 309±64 (sham) and 590±185 (DDK1 mab).

Conclusions: Factors, such as Dkk1, produced by kidney injury and inhibitory to the skeleton stimulate VC without evidence of direct action on the vasculature suggesting that inhibition of bone formation may be a contributory mechanism of VC early CKD.

SA-OR059

FTI-277 Inhibits Calcification by Promoting Akt Signalling and Regulating Osteogenic Differentiation of VSMC Arvind Ponnusamy,[1] Smeeta Sinha,[1,2] Gareth D. Hyde,[2] Heather Jane Eyre,[2] Nick Ashton,[2] Andrew Gilmore,[1] Philip A. Kalra,[1] Ann E. Canfield.[2] *[1]Renal Department, Salford Royal Hospital, Salford, United Kingdom; [2]University of Manchester, United Kingdom.*

Background: Patients with chronic kidney disease have poor cardiovascular outcomes due to increased vascular calcification and atherosclerosis. Vascular calcification is a highly-regulated process involving the osteogenic differentiation of vascular smooth muscle cells (VSMC) and VSMC apoptosis. This study aims to indentify new therapeutic agents for vascular calcification.

Methods: Bovine and human VSMC, FTI-277 (farnesyl transferase inhibitor), wortmannin (PI3K inhibitor) and SH6 (Akt inhibitor) were used in mineralisation and apoptosis experiments. Rat aortic rings from 5/6 nephrectomy models were used in *ex vivo* experiments.

Results: FTI-277 significantly inhibits calcification of VSMC (p<0.001). Pre-incubation of VSMCs with FTI-277 (10 μM) inhibits Ras activation and markedly enhances serum-induced Akt phosphorylation. Calcification of VSMC is associated with decreased Akt phosphorylation. Mineralisation was detected in cells incubated with FTI-277 and wortmannin (100 nM) or FTI-277 and SH6 (10μM), demonstrating that the effects of FTI-277 can be partially negated by preventing PI3K/Akt signalling. FTI-277 significantly inhibits phosphate-induced VSMC apoptosis (p<0.05) and this effect is negated by SH6 (p<0.05). FTI-277 also inhibits Runx2, Msx 2 and alkaline phosphatase mRNA expression (p<0.05), promotes matrix Gla protein mRNA expression (P<0.05), and maintains α-smooth muscle actin mRNA expression. These effects are negated by co-incubation of VSMC with FTI-277 with SH6.Rat aortic rings from short-term (CKD stage1-3) and long-term (CKD stage 4-5) 5/6 nephrectomy models were induced to undergo mineralisation with elevated phosphate +/- FTI-277. Calcium assays and histology demonstrate that FTI-277 inhibits mineralisation in rat aortic rings from both short-term and long-term 5/6 nephrectomy models.

Conclusions: These studies demonstrate that FTI-277 inhibits VSMC osteogenic differentiation, apoptosis and mineralisation by increasing Akt signalling. FTI-277 also inhibits mineralisation in CKD rat aortic rings *ex vivo*.

SA-OR060

Deletion of the Proton Receptor OGR1 in Mice Leads to Increased Bone Density Nancy Krieger,[1] Zhenqiang Yao,[2] Kelly Kyker-snowman,[1] Brendan F. Boyce,[2] David A. Bushinsky.[1] *[1]Medicine, Univ. of Rochester, Rochester, NY; [2]Pathology, Univ. of Rochester, Rochester, NY.*

Background: Chronic metabolic acidosis (MET) stimulates net calcium (Ca) efflux from bone mediated primarily by increased prostaglandin E_2-induced stimulation of RANKL and increased osteoclastic bone resorption. Osteoblasts express the H^+-sensing G-protein coupled receptor, OGR1, which signals through IP_3-mediated intracellular Ca release. We found that H^+-induced Ca_i signaling in osteoblasts requires OGR1, which suggests OGR1 is the H^+ sensor activated by MET to initiate bone resorption. Mice with a genetic null mutation in OGR1 (KO, provided by K. Seuwen, Novartis) were utilized to test the hypothesis that lack of OGR1 would lead to increased bone mass in actively growing mice, a time when large amounts of metabolic acid are generated.

Methods: Bones from 8 wk old KO and wild type C57/Bl6 mice (WT) were dissected, fixed and decalcified as necessary and analyzed by μCt and histomorphometry. All indicated comparisons are p<0.05.

Results: Although the KO mice have no gross phenotype when compared to WT, there are μCT and histologic abnormalities in KO bones. By μCT, KO vertebrae have increased bone volume (BV/TV=0.38±0.02 vs 0.28±0.01), trabecular number (7.3±0.2 vs 6.2±0.1) and trabecular thickness (0.054±0.001 vs 0.047±0.001) and decreased trabecular spacing (0.127±0.003 vs 0.151±0.004) compared to WT. Tibiae from KO mice also have increased trabecular bone volume (0.23±0.01 vs 0.15±0.01) and trabecular number (7.2±0.1 vs 5.2±0.1) and decreased trabecular spacing (0.128±0.003 vs 0.185±0.005) compared to WT. Histomorphometric analysis of tibial metaphysis sections demonstrates increased bone volume (11.2±1.0% vs 7.5±0.7%) and decreased trabecular spacing (45.0±2.6 vs 67.0±4.9/mm^2) in KO vs WT. However, there was an increase in tibial trabecular osteoclast numbers/mm bone surface (17.8±3.2 vs 13.5±2.8) and increased osteoclast surface/bone surface (28.1±4.5% vs19.7±6.7%) in KO vs WT.

Conclusions: These data demonstrate that in rapidly growing mice which generate large amounts of H^+, lack of the H^+ receptor OGR1 leads to increased trabecular bone mass and an increased number of osteoclasts.

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SA-OR061

Bone Biopsy Findings, Biochemical Markers and Aortic Calcification in Waitlisted Dialysis Patients Satu Keronen,[1] Leena Martola,[1] Patrik Finne,[1] Inari S. Tamminen,[4] Leena Kauppila,[2] Heikki Kroger,[3] Eero Honkanen.[1] *[1]Helsinki University Central Hospital; [2]Terveystalo Healthcare; [3]Kuopio University Hospital; [4]University of Eastern Finland.*

Background: It has been shown that histomorphometric parameters of bone volume and turnover are correlated with arterial calcifications and arterial stiffness in CKD patients. The aim with this prospective, observational trial was to study whether the bone histomorphometric findings have correlations with aortic calcification score or parameters of calcium homeostasis and bone turnover in CKD5d patients. The present study is an interim cross sectional analysis of baseline data.

Methods: After double tetracycline labelling transiliac bone biopsy was performed and analyzed using the TVM (turnover -volume- mineralization) classification on 50 consecutive dialysis patients as routine procedure. 72% were men and HD/PD ratio was 52%/48%. All patients were waitlisted for the kidney transplantation. Blood samples were obtained for parameters of calcium homeostasis and bone turnover. Also the lateral lumbar radiography of the abdominal aorta was taken to determine the abdominal aortic calcification score (AAC).

Results: TVM was classified high in 31 (62%) pts., low in 14 (28%) pts. and 5 (10%) pts.had normal turnover. Demographic and biochemical data in high, normal and low bone turnover states are shown in Table 1. Only 3 pts. (6%) had entirely normal bone histology according to TMV classification.

Table 1

	TVM		
	Low	**Normal**	**High**
	n=14	n=5	n=31
Age (years±SD)	54±11	49±9	49±13
M:F	11:3	3:2	22:9
Dial duration (mo)	32±35	18±5	30±32
AAC score(0-24)	8±9	3±3	8±8
PTH (ng/l)	344±230	181±155	438±239
Bone ALP (ug/l)	15±11	10±2	17±13

Conclusions: In this cross sectional analysis almost all patients had abnormal bone histology. Low turnover bone was less common than previously reported. Both high and low turnovers states were associated with elevated bone ALP. Repeated analyses (including bone biopsy) in the ongoing study will probably elucidate relationships between dynamic changes in bone histology and aortic calcification.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

SA-OR062

MicroRNA Expression Is Altered in Chronic Kidney Disease Neal X. Chen,[1] Kraiwiporn Kiattisunthorn,[2] Kalisha O'Neill,[1] Xianming Chen,[1] Vincent H. Gattone,[3] Sharon M. Moe.[1,4] *[1]Medicine, Indiana University School of Medicine; [2]Faculty of Siriraj Medical School, Mahidol University, Bangkok, Thailand; [3]Anatomy and Cell Biology, Indiana University School of Medicine; [4]Roudebush Veterans Affairs Medical Center, Indianapolis, IN.*

Background: Several studies have shown that specific microRNA (miRNA) may lead to altered gene expression resulting in downstream signaling pathways that contribute to vascular disease. Specifically, miRNA 145 is decreased in patients with cardiovascular disease and diabetes, miRNA 155 is associated with inflammation, and miRNA125b is involved in calcification of vascular smooth muscle cells (VSMC) and osteoblast differentiation. The purpose of the present study was to determine if these miRNA are altered in CKD.

Methods: Sera were collected from 10 stage 3-4 CKD, 10 hemodialysis patients and 8 healthy volunteers. VSMC were isolated from aorta from the Cy/+ rat, a model of progressive CKD, and normal littermates. Total RNA were isolated from serum or VSMC and miRNA concentration determined by Agilent Bioanalyzer. The expression of miRNAs, miRNAs processing enzymes and target genes was determined by real-time PCR.

Results: The results demonstrated that circulating levels of miR125b, miR145, miR155 were all significantly decreased with declining eGFR. These same miRNA were lower in CKD and hemodialysis patients compared to healthy volunteers despite a greater percentage of miRNA in the RNA samples. VSMC from CKD rats also showed decreased expression of miR-125b, miR-145 and miR-155 compared to VSMC from normal rats. Downstream target genes of miR145 (myocardin) and miR125b (RUNX2) were decreased and increased, respectively, coinciding with the known effects of these miRNA. There was no difference in the expression of the miRNA processing proteins DROSHA and DICER.

Conclusions: Circulating levels of vascular miRNA 125b, 145, 155, are decreased in CKD. The observed decrease in miRNAs is specific, results in downstream gene changes, and not due to altered cellular processing or degradation in sera. Thus, decreased miRNA expression in VSMC and/or sera of patients with CKD may contribute to cardiovascular disease and vascular calcification.

Funding: Veterans Administration Support

SA-OR063

Decreased Serum Soluble αKlotho Levels Are Significantly Associated with Cardiovascular Dysfunction in Patients with Chronic Kidney Disease
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Background: Klotho was originally identified in a mutant mouse strain that was unable to express the gene and consequently showed shortened life spans. In humans, low serum αKlotho levels are related to a prevalence of cardiovascular (CV) diseases in community-dwelling adults; however, it is unclear whether the serum αKlotho levels are associated with CV dysfunction in patients with chronic kidney disease (CKD).

Methods: We assessed the relationships between the serum soluble αKlotho levels measured by ELISA and parameters of CV dysfunction, including flow-mediated dilatation (FMD), intima-media thickness (IMT), ankle-brachial pulse wave velocity (baPWV) and aortic calcification index (ACI), in 114 CKD patients. Early peak diastolic annular velocity (E') and left ventricular mass index (LVMI) were evaluated using echocardiography to detect left ventricular diastolic dysfunction (LVDD).

Results: The serum αKlotho levels positively correlated with FMD values ($r = 0.3174$; $p = 0.0016$) and inversely correlated with both baPWV and mean IMT values; however, no correlation was observed with ACI values. We also found a significant correlation between the serum αKlotho levels and LVDD assessed by LVMI and E'. Comparing patients with fibroblast growth factor 23 (FGF23) levels < 44.4 pg/mL (median), FMD values in patients with serum αKlotho ≥ 616.3 pg/mL (median) were higher than those in patients with the serum αKlotho < 616.3 pg/mL, thus suggesting that, even at low FGF23 levels, the lower serum αKlotho levels correlate with endothelial dysfunction.

Conclusions: Decreases in the serum αKlotho levels are significantly associated with CV dysfunction in CKD patients. Moreover, the combined use of the serum αKlotho levels and FGF23 levels delineates the degree of CV dysfunction. Serum αKlotho measurements may be sensitive and early biomarkers of CV dysfunction, and endothelial dysfunction in particular, in CKD patients.

SA-OR064

Effects of Calcimimetics on Bone in a Rodent Parathyroidectomy Model
Mariano Rodriguez,[3] Ignacio Lopez,[1] Victor Lorenzo,[2] Escolastico Aguilera-tejero,[1] Eduardo C. Salido,[2] Fatima Guerrero,[1] Addy Rosa Montes de Oca Gonzalez,[1] Carmen Pineda.[1] *[1]Medicina y Cirugia Animal, Universidad de Cordoba, Spain; [2]Hospital Universitario, Servicio Canario de Salud, Tenerife, Spain; [3]Servicio Nefrologia, Hospital Reina Sofia, Córdoba, Spain.*

Background: We have shown that calcimimetics (CM) prevent vascular calcification (VC) and accelerate regression of extraosseous calcification and we have proposed the increase in urinary Ca excretion induced by CM as a potential factor in the protective effect on VC. CM have been reported to have a deleterious effect on bone which may be related to changes in PTH. In the present study we evaluate the impact of CM on bone independently of PTH.

Methods: Wistar rats were parathyroidectomized (PTX) and randomly allocated to 4 groups of 5 rats each. Sham: underwent a sham surgery; PTX: received constant infusion of vehicle using miniosmotic Alzet Pump; PTX+PTH: received a physiological dose of PTH, and PTX+PTH+CM also received PTH through the pump and 1.5 mg/kg/48 h of CM AMG 641 sc. Rats were sacrificed on day 28 and the ileum was dissected and processed to study bone histomorphometry.

	Sham	PTX	PTX+PTH	PTX+PTH+CM
TBV (%)	12.3±1.7	9.8±1.4	12.4±1.8	11.6±2.1
OV (%)	0.53±0.24	0.23±0.07	0.51±0.16	0.80±0.18b
BS (%)	5.4±2.3	2.5±0.6	4.5±1.2	7.2±1.3b
BSO (%)	9.5±0.9	2.1±0.5a	7.1±1.4b	10.3±2.1b
RS (%)	1.98±0.43	2.17±0.62	2.19±0.55	3.65±1.03
RSOcl (%)	2.68±0.49	0.65±0.24a	1.31±0.42	1.58±0.52

TBV: trabecular bone volume; OV: osteoid volume, BS: bone surface; BSO: bone surface cover by osteblasts, RS: resorptive surface and RSOcl: resorptive surface cover by osteoclasts. Values are means ± SE, n=5. ap<0.05 vs Sham, bp<0.05 vs PTX

Results: PTX rats showed a decrease in bone turnover, bone formation and bone resorption. The addition of PTH increased bone turnover to normal values. The addition of CM to this model induced minimal changes in bone. In contrast to previous published data, we have not found detrimental effects on bone by CM, in fact AMG 641 improved bone turnover.

Conclusions: These data suggest that in a rodent PTX model, inclusion of CM in the therapeutic regimen do not induce important changes in bone turnover, and the changes observed show a tendency to increased bone formation.

Funding: Other U.S. Government Support, Pharmaceutical Company Support - AMGEN

SA-OR065

Pit-1 and BMP-2 Expression in the Radial Arteries from Uremic Patients and Their Relationship with Histological Changes
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Background: To investigate sodium-phosphate cotransporter (Pit-1) and bone morphogenetic protein-2 (BMP-2) expressions in the radial arteries from uremic patients.

Methods: Pieces of radial arteries were removed from 45 uremic patients during arterial venous fistula operation. Ten patients with subtotal gastrectomy and normal renal function were chosen as controls. Segments of radial arteries were evaluated by HE, Masson and von Kossa staining. The expressions of Pit-1 and BMP-2 were detected by immunohistochemistry.

Results: Non-calcified radial arteries presented uniformly normal media. Calcified radial arteries presented thickened media and intima, in which most calcification occurred in medial layer and most of smooth muscle cells were in disorder. The thickened media decreased in elastin, increased in collagen fibers and matrix. 24/45 (53.33%) had no evidence of vessel calcification, 11/45 (24.44%) had mild/moderate calcification and 10/45 (22.22%) had severe calcification, while calcification was not found in controls. Staining for Pit-1 was marked in the media from uremic patients and controls. The expression of Pit-1 in the calcified arteries was more marked compared with non-calcified arteries and controls ($P<0.01$). Staining for BMP-2 was more intense in calcified versus non-calcified arteries and controls ($P<0.01$). And there were 12 specimens of the non-calcified arteries that presented positive staining of BMP-2, while not in controls. Staining for Pit-1 and BMP-2 was more marked in severe calcified arteries compared with from mild/moderate calcified arteries ($P<0.05$). The calcification score was positively correlated with serum phosphorus and C-reactive protein (CRP) levels ($P<0.05$). The staining for Pit-1 and BMP-2 significantly correlated with serum phosphorus and CRP ($P<0.01$), and also the staining for Pit-1 correlated with the staining for BMP-2 ($P<0.01$).

Conclusions: Uremic radial artery calcification is mainly located in medial 1ayer and positively correlates with serum phosphorus and CRP. The increased expression of Pit-1 and BMP closely correlates with vascular calcification. The expression of BMP-2 is upregulated before marked vascular calcification.

Funding: Government Support - Non-U.S.

SA-OR066

Mitochondrial Permeability Transition Pore Inhibitor Improves Renal Outcomes after Revascularization in Experimental Atherosclerotic Renal Artery Stenosis
Alfonso Eirin, Zilun Li, Xin Zhang, James Krier, John R. Woollard, Xiang-Yang Zhu, Sandra Herrmann, Amir Lerman, Stephen C. Textor, Lilach O. Lerman. *Mayo Clinic, MN.*

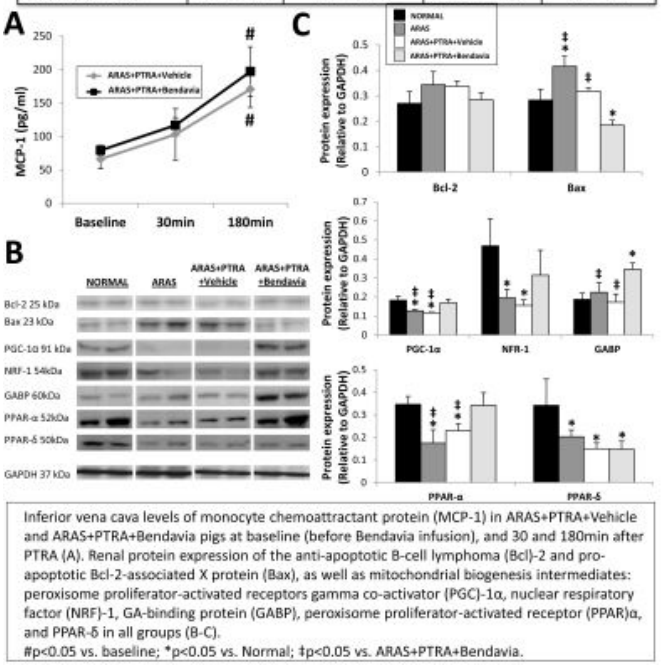
Background: Percutaneous transluminal renal angioplasty (PTRA) fails to improve renal function for most patients with atherosclerotic renal artery stenosis (ARAS), possibly due to injury incurred during reperfusion. Bendavia is a novel tetrapeptide that prevents mitochondrial permeability transition pore (mPTP) opening and ischemia reperfusion injury, but its potential for improving renal response to PTRA is unknown. We hypothesized that adjunct Bendavia would improve renal structure and function during PTRA.

Methods: Pigs with 6 wks of ARAS or control were treated with PTRA (or sham), with adjunct systemic 4-hr infusion of Bendavia (0.05 mg/kg IV) or Vehicle during PTRA (n=7 each). Single-kidney hemodynamics and function were studied in vivo 4 wks later and renal mitochondrial biogenesis, microvascular architecture, and injurious pathways ex-vivo.

Results: Monocyte chemoattractant protein (MCP-1) levels rose over 3 hours after PTRA, consistent with inflammatory injury in all PTRA-treated pigs. Bendavia infusion did not affect MCP-1 levels, yet 4 weeks later, stenotic-kidney GFR and RBF that remained decreased in PTRA+Vehicle were restored in PTRA+Bendavia pigs. Renal mitochondrial biogenesis and microvascular density were restored after PTRA+Bendavia, and fibrosis and apoptosis decreased only in Bendavia-treated pigs.

Conclusions: Infusion of Bendavia during PTRA preserved mitochondrial biogenesis, restored renal function, and attenuated tissue injury in swine ARAS. Functional mitochondrial injury during renal reperfusion sustains inflammatory injury and limits recovery after PTRA. Inhibition of mPTP opening with Bendavia may provide a novel therapeutic potential for improving kidney function and outcomes following PTRA in ARAS.

	NORMAL	ARAS	ARAS+PTRA +Vehicle	ARAS+PTRA +Bendavia
Blood pressure (mmHg)	85.9±2.8	166.6±2.4*‡	90.6±2.8	106.8±2.9
Degree of stenosis (%)	0	88.7±3.9*‡	0	0
GFR (ml/min)	82.9±8.8	51.4±3.8*‡	49.7±4.9*‡	67.8±4.4
RBF (ml/min)	652.1±23.7	394.0±30.7*‡	425.7±48.2*‡	613.2±48.3
Microvessels (/cm²)	309.6±101.2	160.1±45.7*‡	189.0±48.6*‡	279.9±67.3
Renal fibrosis (%)	2.35±0.32	6.33±1.09*‡	5.36±0.98*‡	4.16±1.57*



Inferior vena cava levels of monocyte chemoattractant protein (MCP-1) in ARAS+PTRA+Vehicle and ARAS+PTRA+Bendavia pigs at baseline (before Bendavia infusion), and 30 and 180min after PTRA (A). Renal protein expression of the anti-apoptotic B-cell lymphoma (Bcl)-2 and pro-apoptotic Bcl-2-associated X protein (Bax), as well as mitochondrial biogenesis intermediates: peroxisome proliferator-activated receptors gamma co-activator (PGC)-1α, nuclear respiratory factor (NRF)-1, GA-binding protein (GABP), peroxisome proliferator-activated receptor (PPAR)α, and PPAR-δ in all groups (B-C).
#p<0.05 vs. baseline; *p<0.05 vs. Normal; ‡p<0.05 vs. ARAS+PTRA+Bendavia.

Funding: Pharmaceutical Company Support - This Study Was Supported by a Grant from Stealth Peptides

SA-OR067

Deletion of NOS1 in the Macula Densa Induces Salt-Sensitive Hypertension

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Background: Nitric oxide (NO) released from NO synthase 1 (NOS1), which is highly expressed in the macula densa (MD), blunts tubuloglomerular feedback (TGF). However, the role of MD NOS1 and TGF in regulation of salt-water balance and blood pressure is unknown.We developed a tissue specific NOS1 knockout strain to examine the significance of NOS1 in the MD.

Methods: We developed a strain of MD Cre mice using the sodium, potassium, 2 chloride cotransporter (NKCC2) promoter. To characterize this new Cre strain, we crossed the NKCC2-Cre mice with an EGFP reporter strain (Rosa). Cre activity as measured by EGFP fluorescence was found in MD and was further confirmed *via immunofluorescent staining in isolated perfused MD and found to be limited to the MD* and not in the distal tubule.

Results: Next, we generated tissue specific NOS1 knockout mice by crossing NKCC2-Cre mice with $NOS1^{flox/flox}$, which targets at exon-6 of NOS1 and deletes all splice variants of NOS1. We confirmed NOS1 KO by immunohistochemistry and found no staining of NOS1 in the MD in KO mice. To measure NO generation in the MD, we perfused isolated MD and loaded with fluorescent dye DAF-2. When we switch tubular NaCl from 10 to 80 mM, NO generation in the MD was from 114±12 to 165±11 units/min in WT mice, while in KO mice NO production were absent at both 10 and 80 mM NaCl. We measured maximal change of stop flow pressure as an indicator of TGF using micropuncture. In WT mice, TGF was 4.5±0.3 mmHg; while in KO mice, TGF enhanced to 8.4±0.7 mmHg. (n=5; p<0.01)

To determine the effect of MD NOS1 deletion on the blood pressure response to changes in sodium intake, we measured mean arterial pressure (MAP) by telemetry in mice fed a low salt diet (0.01% NaCl) for 10 days, followed by a high salt diet (8%NaCl) for 14 days. MAP of KO mice increased by 10.5±2.4 *versus* 3.1±2.7 mmHg in WT mice (p <0.01, n=5).

Conclusions: These results indicate that NOS1 in the MD and TGF play important roles in the regulation of blood pressure in response to changes in sodium intake.

Funding: Other NIH Support - National Heart, Lung, and Blood Institute

SA-OR068

Alaninie-Glyoxylate Aminotransferase-2 Metabolises Endogenous Methylarginines and Controls Blood Pressure in Mice and Humans

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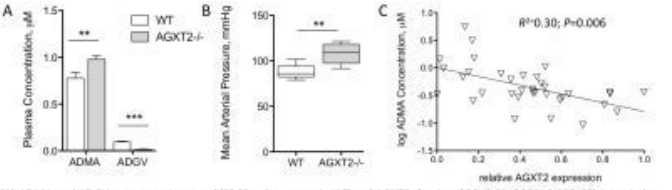
Background: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthesis that may mediate cardiovascular disease. Alanine-glyoxylate aminotransferase-2 (AGXT2) has been reported to degrade ADMA. We investigated the significance of AGXT2 in ADMA metabolism *in vivo* and examined the impact of this enzyme on blood pressure (BP).

Methods: We measured ADMA and asymmetric dimethylguanidino valeric acid (ADGV; the product of AGXT2 metabolism of ADMA) and invasive terminal BP in AGXT2 knockout (AGXT2-/-) mice. To investigate the effects of AGXT2 in humans we quantified the association between renal allograft protocol biopsy gene expression and both ADMA and ADGV. Finally, taking a hypothesis-driven approach, we examined the association between the intermediate-frequency AGXT2 coding variant rs37369 (which leads to a valine to isolecucine substitution and increased substrate levels) and BP in data from subjects of European origin in a meta-analysis of genome-wide studies.

Results: AGXT2-/- mice demonstrate reduced ADGV, high ADMA and hypertension (Fig. A and B). In transplant patients allograft AGXT2 expression was correlated with urinary ADGV and strongly inversely associated with plasma ADMA (Fig. C). The association between AGXT2 and ADMA remained after adjustment for potential confounders including renal function and DDAH1 expression (partial R^2=0.20; P=0.009). In data from the meta-analysis (n=69,000) each T allele at rs37369 was associated with a 0.31±0.11mmHg increase in diastolic BP (P=0.0052).



(A) ADMA and ADGV concentrations, and (B) Blood pressure in WT and AGXT2-/- mice; **P<0.01; ***P<0.005. (C) Association between plasma ADMA and allograft AGXT2 gene expression in transplant patients.

Conclusions: Although the impact of variation at rs37369 needs further study these findings imply AGXT2 is an important regulator of ADMA and represents a novel mechanism through which the kidney influences BP.

Funding: Private Foundation Support

SA-OR069

An Orally Active Epoxyeicosatrienoic Acid (EET) Analog Decreases Renal Injury in the Dahl Salt-Sensitive Hypertensive Rat

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Background: Renal injury is associated with salt-sensitive hypertension. The Dahl salt-sensitive hypertensive rat (Dahl SS) is a model of salt-sensitive hypertension and the related kidney injury. The kidney injury in Dahl SS hypertension is associated with oxidative stress, inflammation and ER stress. We have recently developed orally active EET analogs that provide organ protection in hypertension and other pathologies through multiple signaling pathways. We hypothesized that these EET analogs with their anti-inflammatory, anti-oxidant and anti-ER stress effects will protect the kidney in Dahl SS hypertension.

Methods: Dahl SS rats received high salt diet and treated either with an EET analog (10 mg/kg/d) or vehicle in drinking water for 14 days. Systolic blood pressure (SBP) was measured and urine, plasma and tissue samples were collected at the end of the treatment protocol. Urinary creatinine, albumin, nephrin, monocyte chemoattractant protein-1 (MCP-1) and renal TBARS content were measured using ELISA or colorimetric assays. Renal expression of oxidative, inflammatory and ER stress marker genes were examined using RT-PCR. Histological analysis was done to assess renal injury.

Results: In Dahl SS rats, EET analog treatment did not affect the SBP compared to vehicle (183±6 vs. 185±12 mmHg). Interestingly, the EET analog treatment in Dahl SS reduced albumin-creatinine ratio (49±17 vs. 12±3) and nephrinuria (11±1 vs. 4±1 mg/d). EET analog treatment also reduced glomerular injury, intra-tubular cast formation and kidney fibrosis in Dahl SS rats compared to vehicle. In Dahl SS rats, EET analog treatment caused a 40% reduction in kidney TBARS content compared to vehicle. Moreover, the EET analog treatment In Dahl SS resulted 2-10 fold attenuation in the renal expression of oxidative (*p47PHOX*), inflammation (*IL-6, TGF*-β), and ER stress (*CHOP, GADD34*) genes.

Conclusions: These data, demonstrate that a novel orally active EET analog provides kidney protection in Dahl SS hypertension by reducing oxidative stress, inflammation and ER stress, and this kidney protection was independent of blood pressure reduction.

Funding: NIDDK Support

SA-OR070

Intercalated Cell Nedd4-2 Modulates Blood Pressure and Cl^- Absorption in the Mouse Cortical Collecting Duct Masayoshi Nanami,[1] Vladimir Pech,[1] Diana Agazatian,[1] Yoskaly Lazo Fernandez,[1] John B. Stokes,[2] Baoli Yang,[2] R. Lance Miller,[3] Jill W. Verlander,[4] Susan M. Wall.[1] *[1]Department of Medicine, Emory University School of Medicine, Atlanta, GA; [2]Department of Medicine, University of Iowa School of Medicine, Iowa City, IA; [3]Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT; [4]Department of Medicine, University of Florida School of Medicine, Gainesville, FL.*

Background: The expression and subcellular localization of many renal Na^+ and Cl^- transporters, such as ENaC and NCC, are highly regulated by the ubiquitin ligase, NEDD4-2. NEDD4-2 binds to proteins, such as ENaC, which results in ubiquitination, and then internalization and degradation. With global NEDD4-2 gene ablation, salt-sensitive hypertension is observed, in part, from the increased renal NaCl transporter expression. While principal cell NEDD4-2 function has been studied, virtually nothing is known about NEDD4-2 within intercalated cells (ICs). Thus, the purpose of this study was to determine the effect of IC Nedd4-2 ablation on blood pressure and on Cl^- absorption in the cortical collecting duct (CCD).

Methods: IC-specific NEDD4-2 null mice were generated using Cre-loxP technology and fed a high NaCl diet for 1 week (1.4 meq NaCl/day). Mean arterial pressure was measured by telemetry. Transepitherial net Cl^- transport and voltage were measured in CCDs perfused in vitro.

Results: Mean arterial pressure was ~10 mM Hg higher in the IC NEDD4-2 null than in wild type mice ($P < 0.05$). Moreover, CCDs from wild type mice secreted Cl^- (-9.0 ± 3.3 pmol/mm/min, n=8) whereas CCDs from IC-specific NEDD4-2 null mice absorbed Cl^- (+9.3 ± 1.7 pmol/mm/min, n=6, $P < 0.001$). However, there was no significant difference in transepithelial voltage and no difference in either the benzamil-sensitive or the thiazide-sensitive components of Cl^- absorption.

Conclusions: IC Nedd4-2 gene ablation contributes to the pathophysiology of salt-sensitive hypertension, at least in part, by increasing Cl^- absorption by ICs through a mechanism independent of the benzamil- or thiazide-sensitive components of Cl^- absorption.

SA-OR071

Regulation of Renal Sodium Chloride Cotransporter by D_3 Dopamine Receptor Xiaoyan Wang, Laureano D. Asico, Crisanto Escano, Ines Armando, John Edward Jones, Pedro A. Jose. *Medicine, Unversity of Maryland, Baltimore, MD.*

Background: The regulation of salt and water reabsorption by renal D_3R may be involved in the pathogenesis of hypertension since disruption of the D_3R gene (*Drd3*) in mice is associated with a decreased ability to excrete an acute salt load and chronic dietary salt load.

Methods: The role of the D_3 dopamine receptor (D_3R) on renal function and blood pressure was studied by measuring renal protein abundance of sodium chloride cotransporter (NCC), sodium excretion, and salt sensitivity in D_3 knockout (D_3-/-, D_3+/-) mice and wild-type (D_3+/+) littermates.

Results: D_3R was found in proximal and distal convoluted tubules, macula densa, and thick ascending limb and co-stained with NCC in distal convoluted tubule in D_3+/+ mice. NCC abundance (immunoblotting) was increased in D_3-/-(whole kidney homogenates, 596±43 vs 100±23%, n=5/group) and D_3+/-(17k and 200k membrane fractions, 161±8 vs 100±8% and 140±6 vs 100±4% respectively, n=5-6/group) relative to D_3+/+ mice. Its mRNA (real-time PCR) was similar in D_3+/+ and D_3-/- mice. The NCC inhibitor, hydrocholorothiazide (IP, 30 mg/kg, qd), increased sodium excretion (6 hrs) to a greater extent in D_3+/- than D_3+/+ mice and diminished the difference in blood pressures (3 days) between the two strains. High NaCl diet increased blood pressure (telemetry) of D_3-/- mice which was associated with a reduced ability to excrete the sodium load relative to D_3+/+ mice (n=4/group). Membranous NCC abundance was decreased by high NaCl diet in both strains, but remained higher in D_3-/- than D_3+/+ mice (183±19 vs 100±15%, n=4/group). NCC co-immunoprecipitated and co-localized with D_3R in mouse distal convoluted tubule cells and its activity inhibited by a D_3R agonist (PD128907, 1µm, 1hr).

Conclusions: D_3R directly regulates NCC expression and function and increased NCC abundance and function are associated with salt sensitivity and hypertension in D_3 deficient mice.

Funding: NIDDK Support, Other NIH Support - NHLBI Support

SA-OR072

MYH9 E1841K Mutation Enhances Susceptibility to Angiotensin-II Induced Proteinuria and Podocyte Injury that Are Ameliorated by AT_1 Receptor Inhibition Thu H. Le,[1] Michael Kelly,[2] Sylvia Cechova.[1] *[1]Medicine, University of Virginia, Charlottesville, VA; [2]Medicine, Duke University Medical Center.*

Background: The *MYH9* gene encodes the nonmuscle myosin heavy chain IIA. Recent GWAS suggested that intronic variants of *MYH9* are associated with non-diabetic kidney disease and hypertension-attributed ESRD in African-Americans. However, this has been challenged by the discovery of stronger kidney disease associations of the functional variants of the *APOL1* gene that are in linkage disequilibrium with the *MYH9* risk variants. Rare autosomal-dominant missense mutations in *MYH9* cause macrothrombocytopenia, and are occasionally associated with nephropathy. The E1841K mutation is the most common *MYH9* missense mutation and is associated with nephropathy in some carriers.

Methods: To determine the role of the *MYH9* E1841K mutation in the development of kidney disease, we studied a mouse line carrying the E1841K mutation.

Results: On a mixed genetic background (129S6 and C57BL/6), there was no difference in systolic blood pressure (SBP) nor albumin/creatinine ratio (A/C) between wild-type (WT) and mice with the E1841K mutation (E1841K (+): +/- and/or +/+) at baseline or after high salt diet (HSD,6 % NaCl). We assessed the effects of the E1841K mutation in response to angiotensin II (Ang II) infusion @1000 ng/kg/min for 28 days by mini-osmotic pump. Despite similar increases in SBP (WT 197 ± 4 mm Hg, n=15; E1841K +/- 197 ± 3 mm Hg, n=9; E1841K +/+ 199 ± 5 mm Hg; n=6), E1841K(+) mice displayed severe proteinuria (A/C (ug/mg): WT 806±94, E1841K +/- 1341±293, E1841K +/+ 2209± 366, p=0.001, ANOVA). WT mice displayed mild mesangial expansion and intact podocytes, whereas E1841K +/+ mice displayed focal segmental glomerulosclerosis and severe podocyte foot process effacement/fusion. Treatment of E1841K (+) mice with candesartan, an angiotensin type 1 receptor (AT_1R) inhibitor, significantly prevented Ang II-induced hypertension and proteinuria (E1841K (+) (+/- (n=4) and +/+ (n=2)): SBP = 138 ± 4 mm Hg, A/C ratio = 111 ± 47 ug/mg; $p \leq 0.0001$ vs. untreated), and ameliorated podocyte foot process effacement in E1841K +/+ mice.

Conclusions: The *MYH9* E1841K mutation predisposes to AT_1R mediated podocyte injury.

Funding: Clinical Revenue Support

SA-OR073

Uncovering the Surprising and Complex Roles of Collecting Duct Adenylyl Cyclases Donald E. Kohan,[1] Vladislav V. Bugay,[2] Elena V. Mironova,[2] James D. Stockand,[2] Nirupama Ramkumar,[1] Karl P. Roos.[1] *[1]Division of Nephrology, University of Utah, Salt Lake City, UT; [2]Department of Physiology, University of Texas San Antonio, San Antonio, TX.*

Background: Hormone-stimulated cAMP is a fundamental mechanism for regulating collecting duct (CD) biology. Since the CD expresses several adenylyl cyclase (AC) isoforms, we initiated studies to delineate AC isoform-specific actions.

Methods: Mice were generated with CD-specific knockout (KO) of AC3 or AC6 using loxP-flanked *Adcy3* or *Adcy6* genes and a mouse transgenic for the aquaporin-2 promoter diving Cre recombinase expression.

Results: CD-specific KO of AC6 causes a mild urinary concentrating defect, characterized by reduced urine osmolarity (Uosm) and decreased AVP-stimulated cAMP accumulation in acutely isolated IMCD. In contrast, CD AC3 KO surprisingly caused increased Uosm and enhanced AVP-induced cAMP levels in acutely isolated IMCD. CD AC6 KO did not detectably alter BP (as assessed by telemetry) or urinary Na excretion (UNaV) regardless of Na intake. However, plasma renin concentration was increased in these animals, suggesting a compensatory response. CD AC6 KO mice had decreased renal mRNA content of all three ENaC isoforms as well as reduced ENaC-alpha and -gamma protein expression. Patch clamp analysis of split-open cortical CD revealed that CD AC6 KO mice had abolished AVP-stimulated ENaC Po and N. In contrast, CD AC3 KO mice had a 30-35% decrease in UNaV as compared to control mice that was evident on a low, normal or high Na diet.

Conclusions: In summary, these studies have uncovered previously unsuspected and potentially opposing roles for AC3 and AC6 in the CD. AC6 mediates, at least in part, AVP stimulation of CD water reabsorption and may fully account for AVP stimulation of ENaC activity. In contrast, AC3 activation may actually oppose the effects of AC6, exerting a net inhibitory effect on CD Na and water transport. These findings point to previously unsuspected and complex roles for individual AC isoforms in the CD.

Funding: Veterans Administration Support

SA-OR074

Phosphorylation in Ligand Binding Domain of Mineralocorticoid Receptor Regulates Ligand Binding and Activation of Electrolyte Flux Shigeru Shibata,[1] Jesse Rinehart,[1] Gilbert W. Moeckel,[2] Richard P. Lifton.[1] *[1]Department of Genetics, Yale University, New Haven, CT; [2]Department of Pathology, Yale University.*

Background: Mineralocorticoid receptor (MR) is a ligand-activated nuclear receptor that regulates fluid and electrolyte homeostasis. Aldosterone is produced in two distinct physiological states, hypovolemia and hyperkalemia; the mechanisms by which the kidney responds differently to these states are poorly understood.

Methods: Phosphorylation sites in purified MR were identified by TiO_2 - affinity coupled to tandem mass spectrometry. The consequence of specific phosphorylation sites was evaluated by assay of transcriptional activation of luciferase reporters in mammalian cells and in vitro binding assays. The in vivo significance of phosphorylation was analyzed using antibodies specific for phospho-sites in MR and assaying the effect of MR signaling on target proteins by Western blotting.

Results: We identified 16 phosphorylation sites in MR in COS-7 cells. One phosphorylation site at serine 843 in the ligand binding domain (S843-P), prevented aldosterone and cortisol binding to MR, inhibiting transcriptional activation. S843-P occurs exclusively in α and β intercalated cells in vivo in mice and humans. S843-P was reduced by NaCl restriction or in mice lacking NCC (*Slc12a3*$^{-/-}$) ($P<0.01$), whereas it was increased by a high-K^+ diet ($P<0.002$). NaCl restriction did not alter S843-P in mice lacking AT1a receptor. Angiotensin II (AII) infusion on high salt diet reduced S843-P in wild-type mice, confirming the role of AII. Intercalated cell proteins pendrin and B1 H^+ ATPase were increased in *Slc12a3*$^{-/-}$ and this was reversed by spironolactone, identifying downstream MR-dependent effectors. The intercalaeted cell sodium-dependent Cl/HCO3 exchanger was also upregulated by AII.

Conclusions: Our data provide the first example in which ligand binding to a nuclear receptor is regulated by phosphorylation. This modification contributes to the distinct effects of MR in hypovolemia and hyperkalemia by promoting the intercalated electroneutral salt reabsorption pathway in volume depletion, maximizing Na-Cl reabsorption and minimizing K^+ loss.

SA-OR075

sgk1 Regulation by miR-466g Paru P. Kathpalia,[1] Sheela V. Thomas,[1] Emily J. Noonan,[1] Alan C. Pao.[1,2] *[1]Department of Medicine, Stanford University School of Medicine, Palo Alto, CA; [2]Veterans Affairs Palo Alto Health Care System, Palo Alto, CA.*

Background: In the cortical collecting duct (CCD), aldosterone stimulates the expression of genes that increase activity of the epithelial sodium channel (ENaC); in the early phase of aldosterone induction, one such gene is serum and glucocorticoid regulated kinase 1 (sgk1). MicroRNAs (miRNAs) are a class of small non-coding RNAs that bind target mRNA transcripts and silence gene expression. We hypothesized that aldosterone regulates the expression of miRNAs in the early phase of induction to control expression of target genes that stimulate ENaC activity.

Methods: We treated $mpkCCD_{c14}$ cells with aldosterone or vehicle for 1 hour and used a miRNA microarray to analyze differential miRNA expression. We identified 4 miRNAs to be downregulated, including miR-466g. We fed C57/Bl6 mice either high (8%) or low (0.01%) sodium chloride (NaCl) diet for 1 week to suppress or stimulate endogenous aldosterone and found that renal expression of miR-466g was decreased in mice fed a low NaCl diet. Next, we identified a putative miR-466g binding site in the 3'UTR of sgk1. To test functional interaction between miR-466g and sgk1, we constructed an sgk1 3'-UTR luciferase reporter and found that co-transfected miR-466g suppressed luciferase activity in HEK293 cells in a dose dependent manner. Deletion or introduction of point mutations that disrupt the miR-466g binding site in the sgk1 3'-UTR reporter resulted in attenuation of miR-466g directed suppression of luciferase activity. To determine whether miR-466g regulates sgk1 and/or ENaC activity, we heterologously expressed miR-466g in $mpkCCD_{c14}$ cells and found that both sgk1 mRNA and equivalent current were decreased compared to cells expressing scrambled miRNAs.

Results: See above.

Conclusions: Our findings implicate miR-466g as an aldosterone responsive miRNA that regulates sgk1 and equivalent current in CCD cells. We propose that, at baseline, miR-466g suppresses sgk1 expression and ENaC activity in CCD cells; with aldosterone stimulation, expression of miR-466g is decreased, relieving inhibition of sgk1 expression and leading to increased ENaC activity.

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SA-OR076

Pore-Forming Sites within the Epithelial Sodium Channel γ Subunit Contribute to Channel Gating and Amiloride Block Shujie Shi,[1] Thomas R. Kleyman.[1,2] *[1]Medicine, University of Pittsburgh, Pittsburgh, PA; [2]Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA.*

Background: The epithelial sodium channel (ENaC) is a key regulator of extracellular fluid volume and blood pressure, and channel's activity is tightly regulated a variety of endogenous and external factors. Functional channels are comprised of three homologous subunits, namely α, β, and γ. Previous studies suggested that the transmembrane (TM2) pore-forming regions have critical roles in channel gating, ion selectivity and amiloride binding. Channels composed solely of α and β subunits (αβ channels) exhibit a very high open probability (Po) and a reduced efficacy of amiloride block, in contrast to channels that include all three subunits (αβγ channels). These observations suggest that site(s) within the γ subunit dampen channel activity and enhance amiloride efficacy.

Methods: In order to identify these sites, we generated a chimeric γ subunit construct (γ-βTM2) where the region immediately preceding (β12 and wrist) and encompassing TM2 (γI522-γF564) was replaced with the corresponding region of the β subunit (βI505-βI546).

Results: We observed that αβγ-βTM2 channels displayed characteristics reminiscent of αβ channels, a reduced efficacy of amiloride block (IC_{50} of ~ 7 μM) as well as a loss of Na^+ self-inhibition (reflecting a high Po). Additional mutations identified a key residue (γE531) in γTM2 that dampened channel activity. Na^+ self-inhibition response was largely reduced in αβγE531V channels, whereas the high Po phenotype of γ-βTM2 could be partially rescued by the swapping back the γ residues in the γ-βTM2 construct (γ-βTM2-VW531/2EM). Analyses of a panel of γ subunit mutants suggested that multiple sites within γTM2 were required to confer the high amiloride efficacy of αβγ channels.

Conclusions: Our data suggest that the pore-lining residues of the γ subunit are important for maintaining proper channel gating and its interaction with amiloride.

Funding: NIDDK Support, Private Foundation Support

SA-OR077

Acute Renal Homeostatic Effect of a Single Potassium Load Involves Rapid Dephosphorylation of the NCC Transporter Mads V. Sorensen,[1] Solveig Grossmann,[1] Marian Andreas Roesinger,[1] Dominique Loffing-Cueni,[1] Gery Barmettler,[2] Urs Ziegler,[2] Alex Odermatt,[3] Johannes Loffing.[1] *[1]Institute of Anatomy, University of Zürich, Zürich, Switzerland; [2]Center for Microscopy and Image Analysis, University of Zürich, Zürich, Switzerland; [3]Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland.*

Background: A dietary K^+ load induces a rapid kaliuretic and natriuretic response, which may occur even before plasma K^+ and aldosterone (aldo) levels increase.

Methods: To get insights into underlying molecular mechanisms, we analyzed in mice the time course (15', 30', 2h, and 6h) of the effect of a gastric K^+ load on plasma ion concentrations, aldo levels, urinary ion excretion, and expression and/or phosphorylation of renal ion transport proteins.

Results: Following a gastric gavage of 2% KCl, plasma K^+ concentrations rose rapidly (at 15'), followed by a significant rise of plasma aldo (at 30'). Enhanced urinary K^+ and Na^+ excretion was detectable as early as spot urines could be collected (~30'). The functional changes were accompanied by a rapid and sustained dephosphorylation of the NaCl cotransporter (NCC) (15'-6h) and a later up-regulation of proteolytic activated epithelial sodium channels (ENaC) (6h). The rapid effect on NCC and the late effects on ENaC were independent from the co-administered anion (same effect with $KHCO_3$; no effect with NaCl). In contrast to the proteolytic ENaC regulation, NCC dephosphorylation was independent of plasma aldo as indicated by experiments in aldo-deficient mice. The observed urinary Na^+ loss was likely related to NCC, as it was not seen in NCC-deficient mice.

Conclusions: Thus, rapid aldo-independent down-regulation of NCC is a part of the acute homeostatic adaptation of the kidney to an increased K^+ intake. The increased Na^+ delivery to the ENaC-positive renal collecting system (CS) likely improves the electrochemical gradient for K^+ secretion in the CS.

SA-OR078

SPAK Is Critical for Sodium Reabsorption in Thick Ascending Limb Chih-Jen Cheng, Joonho Yoon, Michel G. Baum, Chou-Long Huang. *Medicine, University of Texas Southwestern Medical Center, Dallas, TX.*

Background: SPAK and OSR1 are Ste20-related protein kinases that activate SLC12 transporters, NCC and NKCCs. In vitro studies have provided ample support for activation of NCC and NKCCs by SPAK and OSR1. While regulation of NCC by SPAK in DCT is well accepted, the role of SPAK in regulating NKCC2 in TAL is controversial. Also, it is recently reported that kinase-deficient kidney-specific (KS-) SPAK is the predominant isoform in renal medulla and functions as an antagonist of full-length (FL-) SPAK. Here, we examined the role of SPAK in TAL using in vitro microperfusion .

Methods: mTAL and cTAL dissected from 4-month old SPAK-KO and age-and gender-matched WT mice were microperfused in vitro under no transepithelial osmotic gradients. $[Na^+]$ in perfusate and collected fluids were analyzed using ion selective electrodes. Transepithelial potential difference (PD_{TE}) was measured. mRNA levels of FL- and KS-SPAK in mTAL and cTAL dissected from 8-week old WT mice were determined using real-time PCR (qRT-PCR).

Results: Compared to WT, Na^+ reabsorption ($pmol.min^{-1}.mm^{-1}$) in both mTAL and cTAL of SPAK- KO mice were much reduced (mTAL: 176 ± 15 & 22 ± 2, WT vs KO, $p < 0.01$; cTAL: 162 ± 18 & 48 ± 5, WT vs KO, $p < 0.01$). Lumen-positive PD_{TE} (+mV) was similarly decreased in KO mice (mTAL: 9.5 ± 1.1 & 4.9 ± 0.8, WT vs KO, $p < 0.05$; cTAL: 6.5 ± 1.2 & 2.6 ± 1.0, WT vs KO, $p < 0.05$). Vasopressin (DDAVP, 100 pM in bath) stimulated Na^+ reabsorption in mTAL and cTAL of WT as well as KO mice (after 30-min incubation, mTAL: 297 ± 21 & 88 ± 7 for WT & KO, respectively; cTAL: 296 ± 19 & 137 ± 16 for WT & KO, respectively; $p < 0.05$ vs before DDAVP for each). qRT-PCR showed that FL-SPAK mRNA was most abundant in mTAL, followed by cTAL and DCT. mRNA for KS-SPAK in TAL and DCT were higher than for FL-SPAK.

Conclusions: SPAK is abundantly expressed in TAL and regulates NKCC2-mediated Na^+ reabsorption. While mRNA of KS- SPAK is more abundant than FL-SPAK, the net effect of FL-SPAK + KS-SPAK on Na^+ reabsorption in TAL is stimulatory. SPAK is not essential for vasopressin stimulation of NKCC2-mediated Na^+ reabsorption.

Funding: NIDDK Support, Government Support - Non-U.S.

SA-OR079

SPAK Deficiency Corrects WNK4 Mutation-Causing Pseudohypoaldosteronism II Pei-yi Chu,[1] Sung-Sen Yang,[1,2] Shinichi Uchida,[3] Sei Sasaki,[3] Shih-Hua P. Lin.[1,2] *[1]National Defense Medical Center, Graduate Institute of Medical Sciences, Taipei, Taiwan; [2]Department of Medicine, Tri-Service General Hospital, Division of Nephrology, Taipei, Taiwan; [3]Graduate School of Medicine, Tokyo Medical and Dental University, Department of Nephrology, Tokyo, Japan.*

Background: An enhanced Osr1 (Oxidative stress-responsive kinase-1)/Spak [STE20 (sterile 20)/SPS1-related proline/alanine-rich kinase]-Ncc (Na^+-Cl^- cotransproter) phosphorylation signaling plays a major role in Wnk [With-No-Lysine (K)] kinase 4 D561A knock-in (KI) mice as a model of pseudohypoaldosteronism type II (PHA II) with salt-sensitive hypertension and hyperkalemia. The aim of this study was to investigate the respective role of OSR1 and SPAKin the pathogenesis of PHA II.

Methods: Wnk4 D561A KI mice were crossed with kidney tubule-specific (KSP-) Osr1 knockout (KO) and Spak KO mice. Blood pressure, blood and urine electrolytes and biochemistries, and relevant protein expression in the kidneys were examined.

Results: Compared with wild-type (WT), Wnk4 KI, Spak KO, and KSP-Osr1 KO mice recapitulated the phenotype of PHA II, Bartter-like syndrome (BS) and Gitelman syndrome (GS), respectively. Wnk4$^{D561A/+}$KSP->Osr1$^{-/-}$ still exhibited PHA II phenotype but Wnk4$^{D561A/+}$Spak$^{-/-}$mice became normotensive without serum and urine electrolytes abnormalities. The abundance of p-Spak and p-Ncc was significantly increased in Wnk4$^{D561A/+}$KSP-Osr1$^{-/-}$mice but p-Ncc expression was relatively normal despite the increased OSR1 expression in Wnk4$^{D561A/+}$Spak$^{-/-}$ mice. The immunofluorescence results of Ncc, p-Ncc, Nkcc2, and p-Nkcc2 expression in the kidney tissue of WT, Wnk4$^{D561A/+}$KSP-Osr1$^{-/-}$,and Wnk4$^{D561A/+}$Spak$^{-/-}$ mice were consistent with those observed by immunoblotting. Furthermore, Wnk4$^{D561A/+}$KSP-Osr1$^{-/-}$mice had anexaggerated salt excretionbut Wnk4$^{D561A/+}$Spak$^{-/-}$ mice exhibited normal in response to thiazide.

Conclusions: An activation of SPAK-NCC might play a more dominant role than OSR1-NCC in the pathogenesis of PAH II. Inhibition of SPAK may be a promising target for the disorders with salt-sensitive hypertension related to WNK4 activation.

Funding: Government Support - Non-U.S.

SA-OR080

An Eye-Catching View of the Glomerulus: Non-Invasive Imaging of Glomeruli Transplanted into the Anterior Chamber of the Mouse Eye

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Background: Advanced in vivo imaging technologies allow to study complex physiological and pathological processes. Here, we utilized the anterior chamber of the mouse eye as a natural body window to image transplanted glomeruli engrafted on top of the highly vascularized iris.

Methods: Mouse glomeruli were isolated by sequential sieving, handpicked and microinjected into the anterior chamber of recipient mice. For allogeneic transplantation, immune deficient recipient mice were used.

Results: Within 3 weeks after transplantation 10% of transplanted glomeruli gained access to the iris vasculature, and became perfused as demonstrated by intravenous injection of fluorescently labeled dextrans. Laser-scanning confocal microscopy allowed repetitive visualization of transplanted glomeruli. Survival of podocytes could be demonstrated for more than 5 months in transplanted glomeruli isolated from transgenic mice expressing CFP under control of the nephrin promoter. Use of recipient mice expressing GFP under an endothelial-specific promoter revealed the contribution of recipient endothelial cells to the glomerular filtration barrier (GFB). While 10kD dextran was filtered across the GFB of transplanted glomeruli, 70kD dextran did only leak through the GFB after induction of glomerular disease. Filtration of 10kD dextran revealed the presence of a presumed subpodocyte space between CFP-expressing podocytes and GFP-labeled endothelium that restricted further passage of the dye-labeled dextran. Electron microscopical analysis confirmed preservation of interdigitating podocyte foot processes in transplanted glomeruli.

Conclusions: We established a novel technique that allows repetitive non-invasive *in vivo* imaging to study glomerular physiology in the absence of parietal epithelial cells. In addition to convenient high resolution visualization, this novel technique allows for easy delivery of pharmacological compounds as well as local injection of intravital dyes.

Funding: Government Support - Non-U.S.

SA-OR081

Evidence for the Presence of Mesangial Cells within the Zebrafish Glomerulus

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Background: Pericytes are specialized vascular smooth muscle cells that are associated with microvessels and, through cell-cell interactions with neighboring endothelia, perform important functions in blood vessel formation, stabilization and permeability. In the kidney glomerulus, the mesangial pericytes are responsible for the organization of endothelial capillary loops and the regulation of glomerular filtration rate. While the zebrafish has served as an excellent genetic and bio-imaging system for studying hematopoiesis, vasculogenesis, and sprouting angiogenesis, the presence and function of a pericyte cell population is unknown.

Results: We report a descriptive, ultrastructural, and functional study of pericytes in zebrafish with a special focus on glomerular pericytes, the mesangial cells. Using pdgfrb mRNA and protein expression as molecular markers of pericytes, we have documented for the first time the presence of a vascular associated pericyte population within the zebrafish. We identify vascular associated pdgfrb positive cells within the eye, brain, and kidney glomerulus. Confocal microscopy reveals how individual pericytes associate with micro-vessels and capillaries. By electron microscopy, we identify the glomerular mesangial cells and pericytes associated with brain microcapillaries.

Functional inactivation of pdgfrb using antisense morpholinos results in loss of capillary loop structure phenocopying those results seen in Pdgf-b -/- and Pdgfrb -/- knock out mice. The phenotypes are reproducible with additional morpholinos and can be rescued by co-injection of human Pdgfrb mRNA. We have analyzed the glomerular structure by electron microscopy. In addition, brain vasculature in pdgfrb morphants sprouts normally but displays increased leakiness as evidenced by dextran dye filtrations studies suggesting a role for pericytes in regulating permeability.

Conclusions: We conclude that pericytes exist in zebrafish and that they have a conserved function within the vasculature and kidney glomerulus. The results advance the zebrafish as a system for studying mesangial cell biology in vivo.

Funding: Government Support - Non-U.S.

SA-OR082

Cell Fate Tracking with Serial Multiphoton Imaging of a New Podocyte Confetti Mouse In Vivo Reveals Podocyte Proliferation and Migration

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Background: Podocytes are critical in the maintenance of a healthy glomerular filter and in the development of glomerular disease. However, their ability to proliferate, migrate and regenerate is highly debated. We aimed to develop a direct visual approach to track single podocytes over time in vivo.

Methods: Podocyte confetti mice featuring cell-specific expression of 4 different fluorescent proteins (membrane-bound CFP, nuclear GFP, cytosolic YFP and RFP) were developed using podocin-Cre and floxed confetti reporter mice. C57BL6 mice (3 to 5 weeks of age) were subjected to unilateral ureteral ligation (UUO, a model of tubulointerstitial fibrosis) since podocytes seem to be protected from injury in this condition. Between 2-4 weeks after UUO mice were anaesthetized, the left kidney was exteriorized and the kidney was imaged *in vivo* on a multiphoton microscope. Multiple surviving surgeries were performed every 24 hours to exteriorize the same region of the left kidney and to image the same glomeruli.

Results: In heterozygous podocyte confetti mice only a few podocytes per glomerulus were labeled in one of the 4 different colors. This allowed an unobstructed view of the podocyte primary, secondary and foot processes. The number of FP expressing cells was increasing continuously over time migrating from the visceral to the parietal layer of the Bowman's capsule. These multi-cellular podocyte projections developed in the same color suggesting that the newly formed cells were daughter cells of the same parent podocyte. Podocyte proliferation was confirmed using BrdU labeling. The serial *in vivo* imaging revealed a dynamic regulation of these projections.

Conclusions: In summary, the robust proliferation/migration of podocytes after UUO suggests that under specific conditions podocytes are able to proliferate and migrate to other glomerular regions. This function may be consistent with glomerulus and nephron regeneration, particularly in disease conditions.

Funding: NIDDK Support, Private Foundation Support, Government Support - Non-U.S.

SA-OR083

Tracing Podocyte Injury in Confetti Mice with a Stochastic Multicolor Cre-Reporter

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Background: Podocyte injury is a critical step in the pathogenesis of proteinuria. However, we have limited tools to examine podocyte injury in vivo, mostly still limited to fixed tissue and electron microscopy. The purpose of this study is to utilize a stochastic multicolor Cre-reporter, encoding four fluorescent proteins, to label and image individual podocytes and foot processes.

Methods: A new double-transgenic mouse was developed (NPHS2^{Cre} /R26R-Confetti$^{TG/TG}$ or NPHS2^{Cre} /R26R-Confetti$^{TG/WT}$) in C57/B6 mice. Adriamycin nephropathy was induced by a one-time injection of (25mg/kg) adriamycin. Seven days later, animals were sacrificed and kidneys were imaged without any fixation using a Leica SP5 AOBS Laser Scanning Confocal microscope.

Results: In the normal glomerulus, podocytes were randomly labeled as red (cytoplasmic), yellow (cytoplasmic), blue (cell membrane) and green (nucleus). Individual podocytes and their foot processes can be easily identified. In control saline injected mice, there was no change in the urinuria and the foot process interaction between neighboring podocytes can be clearly demonstrated and images were comparable to SEM images. In mice injected by ADR, urine albumin increased 500-fold and remarkable protein casts in the proximal tubule were identified. The foot processes (secondary and tertiary) were significantly widened, consistent with the foot process effacement also observed on SEM. There was a significant decrease in labeled podocyte number in this model also. Podocyte cell body measurements based on confocal Z-stack series, showed an increase in podocyte size from 727±344μm^3 in normal saline injected mice to 1108±336μm^3 in ADR injected mice, indicating that cell hypertrophy is early feature of podocyte injury.

Conclusions: By the virtue of confetti, a multicolor Cre-reporter, podocyte/injury can be viewed in real time by confocal microscopy, at almost similar resolution as with the SEM. It will serve as a useful and practical method in podocyte research.

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SA-OR084

Live In Vivo Imaging of Zebrafish Podocytes: An Approach to Understanding Podocyte Biology and Disease Ritu Tomar, Aleksandr Vasilyev, Iain A. Drummond. *Nephrology Division, Massachusetts General Hospital, Charlestown, MA.*

Background: Glomerular disease is caused by podocyte foot process effacement and disruption of filtration slits, resulting in proteinuria and nephrotic syndrome. Using electron microscopy to visualize podocyte foot process effacement in pathological conditions is labor-intensive and does not provide information on cellular dynamics that occurs in vivo. The transparency of zebrafish larva offers the possibility to study organ development and function by *in vivo* imaging using transgenic zebrafish expressing fluorescent reporters. The zebrafish pronephric glomerulus consists of cell types common to all vertebrates, making it a relevant model to understand podocyte biology and glomerular diseases.

Methods: We have employed a bipartite Gal4-UAS technology to image podocytes in living zebrafish larve. We generated a zebrafish podocin:Gal4 transgenic line and crossed it to UAS:fluorescent protein lines to image live podocytes by confocal microscopy.

Results: Live podocytes in three day old larvae imaged at high resolution clearly showed dynamic primary processes enveloping glomerular blood vessels. Time-lapse imaging of podocytes under normal and nephrotoxic conditions will shed light on foot process development and dynamics after injury.

Conclusions: The versatility of the Gal4-UAS system will enable us to express constitutively active and dominant-negative forms of signaling proteins that regulate formation of filtration apparatus. This approach will be useful to model glomerular diseases in zebrafish and enable generation of a high-throughput screening platform for therapies for nephrotic syndrome.

Funding: NIDDK Support

SA-OR085

Podocyte Calcium Waves Visualized In Vivo in Podocin: GCaMP3 Mice Using Multiphoton Microscopy James L. Burford, Lisa Lam, Matthias Hackl, Janos Peti-Peterdi. *Dept. of Physiology & Biophysics, University of Southern California.*

Background: Mutations in calcium channels (including TRPC6) highlight the role of podocyte cytosolic calcium [$(Ca^{2+})_i$] in the regulation of glomerular filtration rate, development of albuminuria and glomerulosclerosis. However, due to technical constraints, the physiological dynamics and role of podocyte $(Ca^{2+})_i$ is incompletely understood. We aimed to directly visualize podocyte $(Ca^{2+})_i$ in the intact kidney and study its temporal correlations with changes in cell shape and glomerular capillary diameter.

Methods: Podocin:GCaMP3 mice featuring cell-specific expression of the fluorescent calcium indicator protein GCaMP3 were developed using podocin-Cre and floxed GCaMP3 mice. Mice were anaesthetized, the left kidney was exteriorized and the kidney was imaged *in vivo* on a multiphoton microscope. Alexa594-albumin was injected to label the plasma.

Results: Podocytes showed two-component regular $(Ca^{2+})_i$ oscillations with a faster (0.12 Hz, cycle time about 10 seconds) and a slower (0.023 Hz, cycle time about 45 seconds) frequencies, suggesting that podocyte $(Ca^{2+})_i$ is controlled by the afferent arteriole myogenic and tubuloglomerular feedback (TGF) mechanisms. Laser-induced focal podocyte injury triggered a robust podocyte $(Ca^{2+})_i$ wave propagating to the entire glomerulus with a speed of 5μm/s. Increased podocyte $(Ca^{2+})_i$ correlated with cell flattening and reductions in glomerular capillary diameter. In addition, Munich-Wistar-Fromter rats, a model of FSGS were anesthetized and surgically prepared for renal micropuncture and microperfusion delivery of the calcium fluorophore Rhod-2AM (10 μM) directly into the Bowman's space for 15 min. High podocyte $(Ca^{2+})_i$ was observed in focal adhesions and heterogeneously throughout the glomerulus. Flufenamic acid (FFA, 10mg/kg iv) increased while the ACE inhibitor ramipril (5mg/kg iv) decreased $(Ca^{2+})_i$ suggesting that TRPC6 and angiotensin II regulate podocyte $(Ca^{2+})_i$.

Conclusions: This study visually demonstrated in vivo for the first time the high functional integration of the intraglomerular podocyte network (syntitium) and the importance of podocyte $(Ca^{2+})_i$ in the control of glomerular functions in health and disease.

Funding: NIDDK Support

SA-OR086

Podocyte-Specific NFAT Activation in Mice Results in Foot Process Effacement and Profound Proteinuria, but Does Not Cause Progressive Glomerular Injury Alexis J. Sloan,[1] Britta Sylvia Walter,[2] Ansel P. Amaral,[1] Saurav Singh,[1] Alessia Fornoni,[2] Christian Faul.[2] *[1]Department of Cell Biology & Anatomy, University of Miami Miller School of Medicine, Miami, FL; [2]Department of Medicine, Division of Nephrology & Hypertension, University of Miami Miller School of Medicine, Miami, FL.*

Background: Gain-of-function mutations in the TRCP6 calcium channel can cause alterations in proper podocyte morphology and glomerular filter function leading to proteinuria. The protein phosphatase calcineurin is activated when cytoplasmic calcium levels rise, and our group has shown that constitutive activation of calcineurin in podocytes *in vivo* leads to proteinuria. The role of calcineurin's canonical downstream target, the transcription factor nuclear factor of activated T-cells (NFAT), in the kidney and podocytes in particular has not been well characterized.

Methods: We have generated a transgenic mouse model where NFAT is constitutively activated in podocytes (NFATc1nuc) in the presence of doxycycline (Dox).

Results: These mice develop profound proteinuria within 4 days of Dox exposure and display partial podocyte foot process (FP) effacement. Prolonged NFATc1nuc expression for up to 12 months leads to an increase in FP effacement, but does not result in histological changes in the glomerulus or tubular system and does not cause renal failure. The level of FP effacement accompanied by chronic proteinuria is reminiscent of the pathology of Minimal Change Disease and challenges the concept that proteinuria alone is sufficient to induce tubular damage and to drive progressive kidney disease. Our preliminary data show that mice expressing NFATc1nuc for 2 months are unable to revert to normal podocyte morphology and glomerular filter function once Dox is removed and normal chow is re-introduced, indicating the induction of epigenetic changes that are non-reversible, but also non-progressive.

Conclusions: Future studies aim to identify direct NFAT target genes in podocytes that are involved in the development of FP effacement and epigenetic changes that occur as a result of persistent NFAT activation and force the podocyte to remain in an effaced state.

Funding: NIDDK Support

SA-OR087

The Pharmacokinetics and Safety of a Single Dose of Sotatercept (ACE-011), in Subjects on Hemodialysis and the Effects of Its Murine Analog (RAP-011) on Anemia and in Preventing Bone Loss in C57BL/6 Mice with 5/6 Nephrectomy Thomas D. Wooldridge,[1] Mark Kaplan,[2] Harry W. Alcorn,[2] Aaron W. Mulivor,[3] William T. Smith.[4] *[1]Nephrology & Hypertension Associates, Tupelo, MS; [2]DaVita Clinical Research, Minneapolis, MN; [3]Acceleron Pharma, Cambridge, MA; [4]Celgene Corporation, Summit, NJ.*

Background: Sotatercept is a recombinant protein composed of the extracellular domain of ActRIIA linked to the Fc domain of human IgG1 that acts as a soluble TGF-β superfamily ligand trap, primarily for activins, BMPs, and GDF-11 but not for TGF-β. Animal models using ACE-011, and its murine analog (RAP-011), showed dual anabolic-antiresorptive effects on bone and increased RBC parameters. Phase 1 studies in healthy postmenopausal women dose-dependently increased hemoglobin, biomarkers of osteoblast activity, and bone mineral density (BMD). Some subjects also had blood pressure increases.

Methods: A double-blind, placebo-controlled, single-dose study (ACE-011-REN-001) evaluating pharmacokinetics (PK) and safety enrolled hemodialysis subjects with proven responsiveness to epogen. Subjects were washed out of epogen to hemoglobin <10 g/dL and randomized to a single dose of ACE-011 0.1 mg/kg or placebo. Studies in female C57BL/6 mice undergoing 5/6 nephrectomy surgery or corresponding sham surgery were also conducted. Mice received vehicle control or RAP-011 continuously starting at 4 weeks postsurgery in a preventive model and 8 weeks postsurgery in a treatment model. Mice were DEXA scanned and cheek bled monthly, and bones and blood were analyzed at study end.

Results: Seven hemodialysis subjects enrolled (ACE-011=6, placebo=1). PK parameters were similar to phase 1 results (slow absorption/elimination) but with higher variability. ACE-011 was not dialyzed. ACE-011 was safe and well tolerated and did not induce blood pressure response. RAP-011 prevented anemia and bone loss in both mouse models and significantly increased cortical bone thickness (femur), trabecular bone volume and thickness, BMD, and RBC parameters.

Conclusions: ACE-011 was safe and well tolerated in hemodialysis subjects. RAP-011 prevented anemia and bone loss in mice.

Funding: Pharmaceutical Company Support - Celgene Corporation

SA-OR088

Potential of Therapeutic Drug Monitoring of Ertapenem (E) and Daptomycin (D) Using Effluent in Continuous Renal Replacement Therapy (CRRT) Bruce A. Mueller,[1] Rachel F. Eyler,[2] A. Mary Vilay,[3] Michael Heung,[1] Kevin M. Sowinski.[4] *[1]Univ. Michigan, Ann Arbor, MI; [2]College of Pharmacy, Univ. Connecticut, Storrs, CT; [3]College of Pharmacy, Univ. New Mexico, Albuquerque, NM; [4]College of Pharmacy, Purdue Univ, Indianapolis, IN.*

Background: Use of spent effluent in therapeutic drug monitoring of piperacillin/tazobactam has recently been proposed as an alternative to plasma concentration monitoring in an effort to minimize blood sampling. Measurement of effluent simplifies the analytical procedures required for quantitating drug concentrations. The purpose of this study was to evaluate the ability of effluent concentrations of E & D in predicting free drug concentrations of these agents.

Methods: Sixteen subjects receiving CRRT were enrolled in this study, 8 received D and 8 received E. E study subjects received a single 1000 mg dose and serial blood and effluent samples were collected over the next 24 hours. D study subjects received a single 8 mg/Kg dose and serial blood and effluent samples were collected over the next 48 hours. D & E total plasma, free plasma and effluent concs were determined using HPLC. The CV% for all assays was <10%. Predictive performance analysis using the method of Altman and Bland was utilized to assess the relationship between the free and effluent concs.

Results: A total of 55 (E arm) and 14(D arm) free & effluent concentrations were collected. The free and effluent concentrations were collected simultaneously. The mean ± SD effluent flow rates were 38±10 ml/Kg/hr in the E arm & 33±5 ml/Kg/hr in the D arm.

Comparison of Ertapenem/Daptomycin Effluent & Free Concentrations

	Concentration Range (mg/dL)		Mean difference ± SD of the difference (SDd) (mg/dL)	Limits of Agreement (mg/dL)
	Free	Effluent	Free-Effluent	Free-Effluent
Ertapenem (n=55 pairs)	0.68-58.5	0.66-46.4	-1.23±3.38	-7.99 to 5.53
Daptomycin (n=14 pairs)	0.72-21.85	0.40-12.64	-3.31±8.19	-19.67 to 13.06

Conclusions: Effluent concentrations were lower than free plasma concentrations for both D & E. Further, substantial variability was observed for both D & E. These data suggest that neither effluent concentrations of D nor E are useful for therapeutic drug monitoring.

Funding: Pharmaceutical Company Support - Cubist, Merck

SA-OR089

Gradual versus Rapid Vitamin D Repletion in Stage 3 & 4 CKD Joel Z. Melnick,[1] Martin P. Petkovich,[1,2] Jay A. White,[1] Charles W. Bishop,[1] Eric Messner,[1] Samir P. Tabash.[1] *[1]Cytochroma Inc., Markham, ON, Canada; [2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.*

Background: CYP24, the vitamin D-specific catabolic enzyme, is upregulated in CKD patients and contributes to vitamin D insufficiency. Rapidly administered vitamin D therapies such as intravenous (IV) or Immediate-release (IR) forms, further upregulate CYP24 and, thereby, limit the effectiveness of such repletion strategies. CTAP101 Capsules is a modified-release (MR) formulation of calcifediol ($25D_3$) that raises serum $25D_3$ levels gradually and which in animal models has been shown to minimize CYP24 induction. The present study in patients with stage 3 & 4 CKD examined the effectiveness of gradual vs. rapid $25D_3$ in reducing elevated plasma iPTH.

Methods: Data from two similar studies in patients with stage 3 and 4 CKD, secondary hyperparathyroidism (SHPT) and vitamin D insufficiency were combined to compare $25D_3$ exposures and resultant reductions in iPTH following MR or IV $25D_3$ administration. Study CL-2008 examined the iPTH lowering effects of 3 daily doses (30, 60 or 90 μg) of MR $25D_3$ after 6 weeks. Study CL-2004 compared the iPTH effects of a single dose (450 or 900 μg) of MR vs. 450 μg IV $25D_3$.

Results: Mean exposures (AUC in ng*d/mL) over 6 weeks were 58, 188, 817, 1531 and 2134 for the 450, 900, 30, 60 and 90 μg MR groups, respectively, with mean % iPTH changes (from pre-treatment baseline) of 3.4, -16.8, -20.2, -32.0 and -37.9. Exposure for the 450 μg IV group was 709 with a mean % iPTH change of -2.1.

Conclusions: Rapid administration of $25D_3$ to correct vitamin D insufficiency in stage 3 and 4 CKD can limit or prevent the effective treatment of the associated SHPT, possibly by inducing CYP24. CTAP101 Capsules provides a means to gradually raise serum total 25D and effectively lower elevated plasma iPTH in CKD patients.

Funding: Pharmaceutical Company Support - Cytochroma

SA-OR090

Experimental Kidney Failure Leads to Increased Exposure to Warfarin Metabolites Thomas D. Nolin,[1] Emilie Robidoux,[2] François A. Leblond,[2] Josée Michaud,[2] Judith Naud,[2] Desiree Kosmisky,[1] Vincent Pichette.[2] *[1]Pharmacy and Therapeutics, University of Pittsburgh School of Pharmacy, Pittsburgh, PA; [2]Centre de Recherche Guy-Bernier, Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Background: The anticoagulant warfarin undergoes hepatic elimination via CYP2C9. Use in end-stage renal disease patients is associated with lower required doses independent of CYP2C9 genotype, poorer anticoagulation control, a doubled risk of major hemorrhage, and a 27% increase in mortality. We hypothesized that uremia leads to altered pharmacokinetics (PK) of warfarin, thereby leading to increased pharmacologic effects. Therefore, the aim of this project was to explore the effect of experimental kidney failure (KF) on the PK of warfarin and its hydroxy-metabolites.

Methods: KF was induced in Sprague–Dawley rats by performing two-staged, five-sixths nephrectomies. Concentrations of warfarin and its metabolites were determined using a validated LC-MS/MS method. KF rats and control rats were administered a single-dose of warfarin 2 mg/kg IV bolus, and serial plasma samples were collected up to 144 hours after administration. PK analysis was performed by standard non-compartmental methods.

Results: The PK of warfarin was not affected by KF. However, systemic exposure (area under the plasma concentration-time curves, AUC) to 6-, 7-, and 8-OH warfarin was increased by 112% (p=0.09), 198% (p=0.01), and 352% (p=0.16), respectively, in KF vs control rats. The AUC of 10-OH warfarin was reduced by 83% (p=0.12) in KF vs control.

Conclusions: These findings suggest that the PK of warfarin metabolites, but not warfarin itself, are significantly altered in the presence of KF. Possible mechanisms include decreased renal clearance of 6-, 7-, and 8-OH warfarin, and decreased metabolic formation of 10-OH warfarin. Further study is warranted to investigate the pharmacological effects of individual hydroxywarfarin metabolites.

Funding: Government Support - Non-U.S.

SA-OR091

Systemic Evaluation of Treatment with Vitamine B6 in Patients with Primary Hyperoxaluria Type I: A Pilot Trial Bernd Hoppe,[1] Heike Katharina Hoyer-Kuhn,[1] Bodo B. Beck.[2] *[1]University Hospital, Department of Pediatrics and Adolescents Medicine, Division of Pediatric Nephrology, University of Cologne; [2]Institute of Human Genetics, University of Cologne.*

Background: Primary hyperoxaluria type I (PH I) as an hereditary autosomal recessive disease is caused by a deficiency of liver specific peroxisomal enzyme alanine-glyoxylate:aminotransferase that requires vitamine B6 as a cofactor. Treatment with vitamine B6 is based on an agreement of experts due to retrospective analyses and case reports. It should, however, be administered in all patients with PH I to evaluate its effect on the reduction of urinary oxalate (UOX) excretion. Primary objective of this pilot trial was to investigate the relative reduction of UOX excretion under increasing dosages of vitamine B6 at week 24 compared to baseline in a prospective setting.

Methods: 12 patients (7 males; age 13.5 ± 3.3 years; eGFR 139 ± 37 ml/1.73m^2/min) were included with confirmed PH I by mutation analyses: c.508G>A homozygous (n=3), c.508G>A heterozygous (n=5), c.508G>A negative (n=4). Medication was interrupted for 4 weeks or until serum levels were normal in the 8/12 patients treated with vitamine B6 at study entry. Vitamine B6 dosage at baseline was 5 mg/kg/day for 6 weeks and increased by 5 mg/kg steps to a final dosage of 20 mg/kg/day every six weeks until week 24. Two 24 h urines were sampled at baseline and each step of dosage adjustment.

Results: Preliminary data of an intention-to-treat analysis showed a mean relative reduction of UOX excretion of 25.0%. UOX excretion declined from 2.14 ± 0.54 mmol/1.73m^2/day at baseline to 1.57 ± 0.58 mmol/1.73m^2/day ($p<0.02$) at week 24 and vitamine B6 levels increased from 24 ± 10 ng/ml to 1228 ± 813 ng/ml ($p<0.001$), respectively. Five patients showed a relative reduction of UOX ≥ 30%.

Conclusions: UOX excretion could be reduced by vitamine B6 treatment in a prospective setting comparable to retrospective data. These preliminary data underline the need for further evaluation in a multicenter trial to analyze a genotype/phenotype related response to vitamine B6 treatment.

Funding: Clinical Revenue Support

SA-OR092

Personalised Azathioprine Therapy in Renal Transplantation: Are We Missing a Trick? Jennifer R. Joslin, Paul A. Blaker, Anthony Marinaki, Jeremy D. Sanderson, David Goldsmith. *Guy's & St Thomas' Hospital, London.*

Background: Immunosuppression for renal transplantation (RTx) has shifted from azathioprine- (AZA) to mycophenolate mofetil (MMF)-containing regimens with improved five-year patient and graft survival rates. However, data to support this strategy did not include AZA optimisation by measurement of thiopurine-*S*-methyltransferase (TPMT) activity, or AZA metabolites, thioguanine nucleotides (TGN) and methyl-mercaptopurine (MMP). In other disciplines these are used to optimise the dose of AZA, suggesting that AZA use in RTx may be sub-optimal. We hypothesized that these biomarkers would identify patients at risk of AZA toxicity, under-dosing and non-adherence.

Methods: EDTA blood samples were collected from 71 AZA long-term RTx patients and tested for TPMT activity and AZA metabolite profiles. TPMT activity, TGN and MMP levels were correlated with mean white cell counts (WBC), lymphocyte counts, alanine transaminase (ALT) and haemoglobin (Hb) concentrations.

Results: One of 4 patients with intermediate TPMT activity was prescribed a higher than recommended dose of AZA (1.5mg/kg). Of patients with normal TPMT activity (n=67, mean 36.9pmol/gHb/hr, range 27-59), the mean AZA dose was 1.06mg/kg. Mean TGN and MMP levels were 239pmol/8x10^{8}RBC (range 0-831) and 743pmol/8x10^{8}RBC (range 0-8787) respectively. 43/71 patients had TGN levels ≤ 240pmol/8x10^{8}RBC. 1 of 2 patients with TGN and MMP levels of zero, suggesting non-adherence, showed chronic rejection. The dose of AZA correlated with both TGN ($p=0.001$) and MMP ($p=0.007$) levels. There was no correlation between TGN or MMP levels and the mean WBC, lymphocyte count, ALT or Hb.

Conclusions: Pre-treatment TPMT phenotyping identifies patients with low or intermediate activities at risk of AZA toxicity. In our cohort the mean AZA dose was at the lower limit of the recommended dose, and more than 50% of patients had a TGN level less than the range considered therapeutic in a number of chronic inflammatory conditions (240-400pmol/8x10^8 RBC). Prospective studies are needed to determine the ideal therapeutic range of AZA metabolites in RTx, since switching AZA to MMF without optimising the dose of AZA is potentially a missed opportunity.

SA-OR093

An Inosine 5'-Monophosphate Dehydrogenase Gene Variant in Mycophenolic Acid Efficacy Wolfgang Winnicki, Gerald Cohen, Florina Regele, Michael Baier, Gurkan Sengoelge. *Department of Medicine III, Division of Nephrology and Dialysis, Medical University of Vienna.*

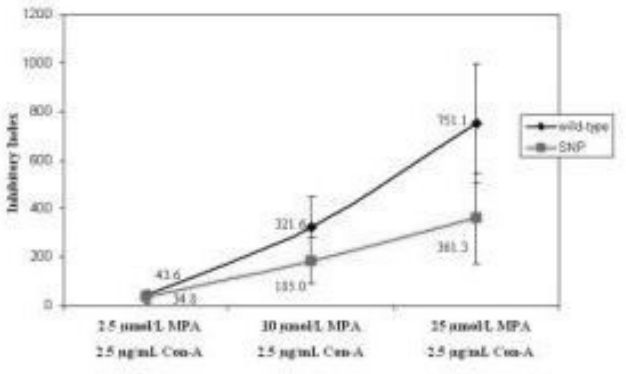
Background: Mycophenolic acid (MPA) is a selective inhibitor of inosine 5'-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme of purine metabolism. The isoenzyme IMPDH2 predominates in activated lymphocytes, and its inhibition by MPA is part of standard immunosuppressive regimens.

Significant unexplained differences in MPA efficacy and tolerability among patients are known, whether variants in the IMPDH2 gene lead to changes in IMPDH activity and to differences in responsiveness to MPA therapy is tested.

Methods: All 14 exons and intron–exon boundary regions of IMPDH2 are sequenced from genomic DNA probes from 100 healthy individuals by bi-directional PCR-based dideoxy chain termination sequencing.

After lymphocyte isolation and incubation at different MPA concentrations a 3H-thymidine proliferation assay is performed and lymphocytic IMPDH activity is determined by means of high-performance liquid chromatography.

Results: One intronic polymorphism (rs11706052) is identified in 19% of the study population. Lymphocyte IMPDH activity and proliferation under three therapeutic relevant MPA concentrations are compared in rs11706052 carriers and wild-type individuals. The presence of the rs11706052 polymorphism reduces the antiproliferative effect of MPA on lymphocytes by approximately 50% compared with the IMPDH2 wild-type form.



Conclusions: Renal transplant recipients with the rs11706052 polymorphism may suffer from higher rejection rates due to poorer response to MPA therapy.

To confirm these findings a prospective clinical study is underway at our center that combines MPA pharmacokinetic, IMPDH pharmacodynamic and rs11706052 pharmacogenetic approaches in 150 renal transplant recipients with a follow-up of 1 year. Preliminary data will be shown.

Funding: Government Support - Non-U.S.

SA-OR094

Long Term Effects of Migalastat HCl on Fabry Nephropathy

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Background: Fabry disease (FD) is caused by mutations affecting α-galactosidase A. Accumulation of globotriaosylceramide (GL-3) leads to multiorgan dysfunction, including progressive kidney disease.

Methods: This analysis is on the effects of oral migalastat (AT1001/GR181413A) on kidney histology and function in FD, as evaluated in ongoing FAB-CL-205/NCT00526071 (cut-off April 2012). This open-label study was in 23 patients who completed a preceding phase 2 study. Kidney biopsy was performed and the average number of GL-3 inclusions per peritubular capillary (PTC) is compared with results from the baseline (B) biopsy. eGFR was calculated and 24-hour urine protein was measured.

Results: Median treatment duration, including the preceding study, was 5.0 years (range: 1.9/6.2 years). By an in-vitro assay, 11/14 males and 5/9 females had mutations considered amenable to migalastat. Eight patients had paired biopsies: 5 amenable (2 males) and 3 non-amenable (3 females). Median treatment duration in FAB-CL-205 at the time of biopsy was 60 weeks (44 weeks on a 500 mg 3 days on 4 days off regimen). Median GL-3 in PTCs decreased by 78% in patients with amenable mutations and increased by 114% in patients with non-amenable mutations. Mean decline in eGFR was 0.48 ml/min/1.73m^2/year in all patients (0.87 in amenable and +0.41 in non-amenable; 0.99 in males and +0.3 in females). 24-hour urine protein decreased in patients with amenable mutations, and was associated with use of renal protective therapies.

Conclusions: All patients with amenable mutations had a reduction of GL-3 in kidney PTCs. Mean decline in eGFR was similar to the decline seen in the normal population.

Funding: Pharmaceutical Company Support - Amicus Therapeutics

SA-OR095

Genetic Profile of ACE-Inhibitor Responder Patients in Essential Hypertension

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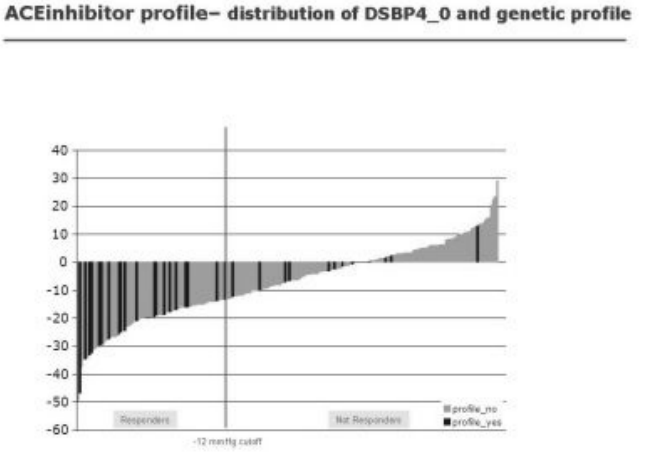
Background: Less than 25% (about 1 million) of patients currently under antihypertensive agents have target blood pressure values, despite numerous antihypertensive drugs available. The enormous variation in the individual response to antihypertensive treatment could be solved by pharmacogenomic studies. ACE-inhibitor (ACEi) therapy is recommended by guidelines, however BP variability is under unknown genetic control. Our aim was to built the genetic profile of hypertensive patients responder to ACEi mono-therapy.

Methods: 175 essential hypertensive patients (114 naïve) from the hypertension clinic of our hospital were treated for 4 weeks with Perindopril 4 mg. The outcome was the variation in systolic and diastolic BP (ΔSBP and ΔBP at 1st month).We performed genetic study using both candidate gene and Genome Wide Analysis.

Results: As expected a great variability of blood pressure response is present, with approximately one third of ACEi responders (ΔSBP 1st month <-12.6 mmHg, ΔDBP 1st month <-9 mmHg). Patients carrying specific combination of NKAIN2 and APBA2 or MYO16 and PRKG1 or NKAIN2 + PRKG1 genes (15% of sample) showed a greater decrease of SBP compared to not carrying (-15,8 mmHg vs -6 mmHg; GLM p<0.00001 adjusted for sex, age, BMI, basal SBP and Plasma renin activity). The OR to be responder was 4.0 (IC 95% 1.69-9.60 p-model <0.001) in the patients carrying the genetic profile. The specificity of ACEi genetic profile was 92%, whereas positive predictive value was 70% and negative predictive values was 62%.



Conclusions: Our results identified, for the first time, a genetic combination able to predict the response to ACEi therapy in essential hypertension. The same approach could be applied in kidney disease to personalize treatment and select those patients that will reverse renal function decay.

SA-OR096

Genetic and Epigenetic Variants Important in Susceptibility to Clofarabine Cytotoxicity: Implications for Application to Renal Cell Death

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Background: Clofarabine, a purine nucleoside analog, was associated with acute kidney injury (AKI) in up to 50% of cases in clinical trials. The use of clofarabine is increasing as indications now include stem cell transplant induction and treatment of adult leukemias. The etiology of AKI is often multifactorial in these patients and it is presently unclear the extent of nephrotoxicity due to clofarabine. This novel investigation aims to determine whether genetic variation mediates changes in susceptibility to clofarabine cytotoxicity in lymphoblastoid cell lines (LCLs) and whether genes identified in our genome wide association study (GWAS) impact susceptibility to clofarabine in renal cells.

Methods: Dose-response clofarabine cytotoxicity curves were generated for HEK293 cells and LCLs. Genetic factors were identified using an unbiased whole-genome approach using LCLs derived from persons of European (CEU) or African (YRI) ancestry. Over 2 million single nucleotide polymorphisms (SNPs) were interrogated for association with susceptibility to clofarabine. Epigenetic modification due to DNA methylation was interrogated for association with cytotoxicity. Strength of association between cytotoxicity and expression of 450 transcription factors and 13,000 target genes guided prioritization for validation by siRNA knockdown.

Results: HEK293 cells and LCLs demonstrate dose related cytotoxicity to clofarabine at similar drug concentrations. Associations between cytotoxicity and expression of 230 genes as well as methylation status of 157 CpG sites reached genome wide significance. From GWAS, 11 SNPs were associated at p <5x10^{-6}. Seven of the 11 SNPs were expression quantitative trait loci with target genes correlating with one or more of the 230 genes identified above.

Conclusions: Genetic and epigenetic variation are important determinants in susceptibility to clofarabine. The LCL model is appropriate to generate hypotheses for future functional studies and mechanistic inquiry in renal cell lines.

Funding: NIDDK Support

SA-OR097

Multi-Target Therapy of Lupus Nephritis: A Prospective Randomized Control Multi-Center Trial Zhi-Hong Liu, Hai-tao Zhang, Wei-Xin Hu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Background: To evaluate the efficacy and safety of multi-target regimen in the induction therapy of lupus nephritis (LN).

Methods: A 24-week randomized, open-label, superiority trial was conducted to compare multi-target therapy (MT group, oral mycophenolate mofetil 1.0 g/d, tacrolimus 4 mg/d and corticosteroids) with monthly intravenous cyclophosphamide (IVCY group, CTX, 0.5-1.0 g/m2 BSA and corticosteroids) as induction therapy for active LN. The study protocol specified adjunctive care and the use of corticosteroids (intravenous methylprednisolone, 0.5 g/d for 3 days followed by oral prednisolone 0.6 mg/kg/d and tapering). The primary end point was complete remission (CR) at 24 weeks. The secondary end point was partial remission (PR) and adverse effects.

Results: 362 patients (329 females and 33 males, average age 32.7±10.2 years) were enrolled in 26 Chinese centers. The renal pathology was classed according to ISN/RPA 2003 classification, including class III (n=20), IV (n=150), V (n=68), V+III (n=26) and V+IV(n=98).These patients were randomly assigned to MT group (n=181) and IVCY group (n=181). 310 patients completed the 24 weeks treatment. The accumulative rate of CR at 24-week in MT group (43.65%) was significantly higher than that in IVCY group (23.2%) (P<0.01). This difference was statistically significant in class V+IV(42.22% vs 20.75%, P<0.05)and class V (37.5% vs 5.56%, P<0.01). The PR rate was 49.03% and 41.18% respectively in two groups. There was no significant difference of adverse events between two groups (51.11% vs 54.29%). However, MT group had markedly lower incidences of gastrointestinal symptoms, GPT/GOT elevation and leucopenia, but higher incidences of tremor and new onset hypertension comparing IVCY group. No significant difference was found in infection and hyperglycemia.

Conclusions: The multi-target regimen was more effective than intravenous CTX in inducing remission of LN and had a more favorable safety profile.

Funding: Government Support - Non-U.S.

SA-OR098

Myeloperoxidase Antibodies Associate with the Future Diagnosis of Proliferative Lupus Nephritis Mark D. Poirier, Christina M. Yuan, Kevin C. Abbott, Stephen W. Olson. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Systemic Lupus Erythematosus (SLE) is a systemic disease with significant morbidity to include Lupus Nephritis (LN). In addition to anti-double stranded DNA antibodies, anti-neutrophil cytoplasmic antibodies (ANCA) have been shown to associate with LN at the time of diagnosis. There have been no previous studies to evaluate ANCA levels prior to the diagnosis of LN and specifically proliferative lupus nephritis (PLN), which carries the worst renal prognosis.

Methods: We utilized the Department of Defense Serum Repository (DoDSR) to compare longitudinal pre-diagnostic ANCA levels in 23 biopsy confirmed proliferative lupus nephritis cases to 23 age, race, gender, and age of serum matched SLE without LN disease controls identified by the DoDSR.

Results: A greater percent of PLN cases versus disease controls had a single elevated myeloperoxidase (MPO) antibody level (>5 U/mL) at any time prior to diagnosis (57% vs. 5%, p<0.001). An MPO antibody rate of rise greater than 0.5 U/mL was 100% specific for future disease (45% vs. 0%, p=0.001). A greater percent of PLN cases versus disease controls had an absolute rise (> 0 U/mL) in MPO antibody over time (70% vs. 16%, p=0.001).

Conclusions: An elevated and rising MPO antibody level is associated with the future diagnosis of PLN. Our data set better defines the role of MPO antibody in the pathogenesis of PLN. MPO antibody could contribute to a future autoantibody profile that better describes the risk for future PLN in patients with SLE.

SA-OR099

The Efficacy of Rituximab versus Cyclophosphamide for Treatment of Renal Disease in ANCA-Associated Vasculitis: The RAVE TRIAL Duvuru Geetha, Fernando C. Fervenza. *RAVE-ITN Group.*

Background: Rituximab (RTX) is non-inferior to cyclophosphamide (CYC) followed by azathioprine (AZA) for remission-induction in severe ANCA associated vasculitis (AAV) but details of outcomes among patients with renal involvement have not been reported. We present the long-term outcomes of patients who had renal involvement at baseline in the RAVE trial.

Methods: Patients with renal involvement defined by a Birmingham Vasculitis Activity Score/Wegener's Granulomatosis (BVAS/WG) renal item score ≥ 3 at baseline were included. Glomerular filtration rate was estimated (e-GFR) by Cockcroft-Gault formula. Complete remission (CR) was defined by BVAS/WG = 0, off prednisone; Renal flare by renal BVAS/WG ≥ 3. Remission rates, slopes of eGFR and renal flares were compared between treatment groups.

Results: 102 of the 197 (52%) patients had renal involvement at entry (GPA: 68; MPA: 34; PR3-ANCA: 58; MPO-ANCA: 44; new diagnosis: 58; relapsing disease: 44). The mean age was 55 years, 52% were males. 51 patients each received RTX or CYC/AZA. Except for lower mean baseline e-GFR in the RTX group (53 ml/min vs 69ml/min p=0.01), there were no clinical differences between the treatment groups. 60.8 % patients treated with RTX and 62.7 % patients treated with CYC/AZA achieved CR by 6 months, and 74.5 % and 76.5% at any time on the originally assigned treatment, respectively. Median times to CR were similar in both groups. The mean e-GFR increased in parallel in the two groups during the 18 months. The number of renal flares did not differ between the two groups at 6, 12, or 18 months. When stratified by ANCA or AAV type or new vs relapsing disease, there were no differences in remission rates or slopes of e-GFR increase at 18 months. Four MPA patients treated with RTX without maintenance therapy had a total of 5 renal flares by month 18 vs none treated with CYC/AZA (p=0.04).

Conclusions: A single course of RTX is as effective as 18 months of therapy with CYC/AZA for induction and maintenance of remission in patients with AAV with renal disease. MPA patients treated with RTX and no maintenance therapy may be more likely to experience a renal flare following B cell reconstitution.

Funding: Other NIH Support - National Institute of Allergy and Infectious Diseases, Pharmaceutical Company Support - Genentech; Biogen

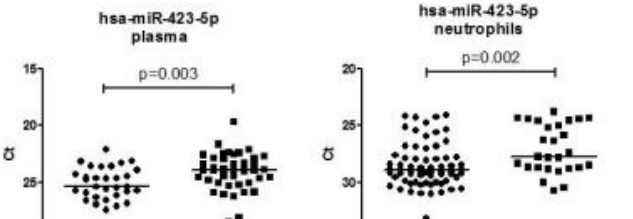
SA-OR100

Neutrophil and Plasma microRNA Expression during Remission in Patients with Systemic Vasculitis Marten Segelmark,[1] Camilla Skoglund,[1] Tino Kurz,[1] Anting Liu Carlsen,[2] Thomas Hellmark,[3] Per Eriksson,[1] Niels H. Heegaard.[2] *[1]Medical and Health Sciences, Linköping University, Linköping, Sweden; [2]Autoimmunity, Statens Serum Institut, Copenhagen, Denmark; [3]Clinical Sciences, Lund University, Lund, Sweden.*

Background: Patients with ANCA-associated vasculitis (AVV) in remission live under a constant threat of relapse. Levels of ANCA in plasma have limited capacity to predict relapses, new biomarkers are needed. MicroRNAs (miRNA) are small non-coding oligonucleotides present in cells as well as in microparticles in plasma. Microparticle miRNA has been proven to be stable during storage.

Methods: Candidate miRNAs were chosen using a PCR based mini-array on plasma samples (39 patients and 106 controls) and an Affymetrix 2.0 miRNA microarray Chip on neutrophil RNA (4 patients and 4 controls). All candidate miRNA were tested on a cohort consisting of 68 patients with AAV in remission and 28 controls using quantitative real-time PCR (qPCR) and specific Taqman probes.

Results: Out of about 1000 human miRNAs present on the chip 500 could be detected in neutrophils, and out of 45 miRNAs present on the mini-array 26 could be detected in all plasma samples. All miR detected in neutrophils could also be detected in plasma, indicating that a proportion of plasma miRNA is of neutrophil origin. After a selection process 20 miRNA were subjected to qPCR on neutrophil RNA. Significant differences between AAV patients and controls were detected for 4 miRNAs. MiR-20a*, miR-339-5p and miR-423-5p were found to down-regulated while miR-638 was up-regulated. MiR-423-5p was also down-regulated in AAV plasma.



Conclusions: Patients with vasculitis in remission exhibit significant differences in their miRNA expression, compared to healthy controls, both intracellulary in neutrophil and extracellulary in plasma microvesicles. These differences have a potential to be exploited as biomarkers.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR101

Eculizumab (ECU) in Atypical Hemolytic Uremic Syndrome (aHUS) Patients (pts) with Progressing TMA: Continued Improvements at 2-Year Follow-Up Christophe M. Legendre,[1] Laurence A. Greenbaum,[2] Sunil Babu,[3] Richard Furman,[4] Neil Sheerin,[5] David J. Cohen,[6] A. Osama Gaber,[7] Frank Eitner,[8] Yahsou Delmas,[9] Camille Bedrosian,[10] Chantal Loirat.[11] *[1]Hôpital Necker, France; [2]Emory Univ, GA; [3]Fort Wayne Med, IN; [4]Weill Cornell Med Coll, NY; [5]Newcastle Univ, United Kingdom; [6]Columbia Univ Med Center, NY; [7]Methodist Hosp, TX; [8]Univ Aachen, Germany; [9]CHU Pellegrin, Bordeaux, France; [10]Alexion Pharmaceuticals Inc.; [11]Hôpital Robert Debré, Paris, France.*

Background: aHUS is a genetic disease of chronic uncontrolled complement activation and systemic thrombotic microangiopathy (TMA). Despite plasma exchange/infusion (PE/PI), 33-40% of aHUS pts progress to ESRD/die during the first clinical manifestation[1,2]. We report longer follow-up with ECU, a terminal complement inhibitor, in aHUS pts with progressing TMA.

Methods: aHUS pts ≥12 yrs with progressing TMA (platelets <150x10^9/L at screening) despite ≥4 PE/PI sessions 1 wk before were enrolled in an phase II trial and continued in a long-term extension. Primary endpt: platelet count change. We report data for median 100 wks (range, 2-145) duration.

Results: 17 pts enrolled (2 discontinued at 1 and 6 wks; protocol violation, an adverse event (AE) unrelated to ECU, respectively). Median time from diagnosis to screening=10 mo (0.3-236). 13 pts continued in the extension study.

Chronic ECU continued to increase platelets and more pts achieved key renal endpoints with time (Table). ECU was generally well tolerated (1 serious AE [severe hypertension] possibly drug related).

Conclusions: Long-term ECU therapy inhibited complement-mediated TMA and resulted in significant and continuous improvements in renal function. At a median duration of almost 2 yrs, all pts receiving chronic ECU therapy remain alive.

Key Outcomes	Week 26 ECU (N =17)*	Median duration of 100 wks ECU (N=17)†
Mean change in platelet count, $x10^9$/L (95% CI) (primary endpoint)	73 (40, 105) P=0.0001	88 (63, 112) P<0.0001 (wk 96)
TMA-event-free status, n (%)	15 (88)	15 (88)
eGFR increase of ≥15 mL/min/1.73m^2, n (%)	8 (47)	10 (59)
CKD improvement of ≥1 stage, n (%)	10 (59)	12 (71)
Serum creatinine decrease of ≥25%, n (%)	11 (65)	13 (76)
Mean change in eGFR‡, mL/min/1.73m^2 (95% CI)	31 (17, 45) P=0.0001	32 (15, 49) P<0.0008 (wk 96)
Decrease in proteinuria ≥1 grade, n/N (evaluable patients)	12/15	7/9 (wk 96)
Mean change in health-related quality of life§, EQ-5D score (95% CI)	0.32 (0.27, 0.38) P<0.0001	0.33 (0.30, 0.36) P=0.0001 (wk 96)

ECU dosage: 900mg/wk for 4 wks, 1200mg at wk 5, 1200mg q2 wks thereafter.
Significance was tested with a repeated measures model.
*Two pts discontinued at wks 1 and 6.
†Two pts discontinued at wks 1 and 6, two pts discontinued at week 26 and two during the extension phase (one pt discontinued at wk 68 and another at wk 83).
‡ECU eliminated the need for dialysis in 4/5 pts receiving dialysis at baseline. An additional pt (eGFR, 19 mL/min/1.73m^2 at screening) initiated dialysis at wk 64.
§Clinically meaningful threshold ≥0.6.

1.Caprioli et al. Blood 2006;108:1267-79. 2. Noris et al. CJASN 2010;5:1844-59.

Funding: Pharmaceutical Company Support - This Study Was Sponsored by Alexion Pharmaceutical Inc.

SA-OR102

Open-Label, Multi-Center Trial of Eculizumab in Patients with Shiga-Toxin-Producing *E. coli* Hemolytic Uremic Syndrome (STEC-HUS)

Rolf A. Stahl, The STEC-HUS Study Group.

Background: Patients (pts) with Shiga-toxin–producing *E.coli* infection can develop hemolytic uremic syndrome (STEC-HUS), a rare, systemic, life-threatening disease in which shiga-toxin–mediated uncontrolled complement activation leads to systemic inflammation and thrombotic microangiopathy (TMA), resulting in immediate, severe organ damage and also severe, long-term and/or delayed-onset sequelae. While protracted, the exact duration of excessive complement activation is unknown. Supportive pt management, including plasmapheresis, is generally considered ineffective. Use of eculizumab (ecu), a humanized monoclonal anti-C5 antibody that inhibits terminal complement activation, is associated with positive STEC-HUS clinical outcomes in case reports. From May-July 2011, Germany experienced one of the largest STEC outbreaks and 855 STEC-HUS cases. In response to physician requests, an ecu compassionate access program was initiated and controlled trial started to ensure pt monitoring and to assess safety/efficacy of ecu treatment for STEC-HUS.

Methods: This 28wk, open-label trial enrolled STEC-HUS pts with TMA/systemic organ complications. In adults, IV ecu 900mg was administered on Days 0,7,14,21, then 1200mg on Days 28,42,56; pediatric pts received weight-based dosing. Ecu was permitted for an additional 8wks (1200mg every 2wks). Follow-up continued to a total of 28wks. Pts received meningococcal vaccination per protocol before ecu and antibiotic prophylaxis for ≥14d post-vaccination. Primary/key secondary study endpoints are listed (Table). The study was approved by the Paul-Ehrlich-Institute and each site's Independent Ethics Committee.

Results: 198 pts were enrolled; baseline characteristics are summarized (Table). Previously presented interim (8wk) results showed that ecu treatment substantially improved TMA/systemic organ complications in STEC-HUS. Final (28wk) results will be presented.

Primary and Key Secondary Study Endpoints	
Primary endpoint: • Global assessment of efficacy (improvement in markers of systemic TMA and vital organ involvement) at 8 weeks	
Key secondary endpoints: • Platelet normalization • Dialysis use • New organ involvement • PE use • Renal and neurologic function • Safety	
Baseline Demographic and Disease Characteristics of ITT Study Population* (N=198)	
Female, n (%)	142 (72)
Age Median (range), years ≥18 years, n (%)	 39 (8, 85) 189 (96)
Caucasian, n (%)	198 (100)
Systemic organ involvement, n (%) ≥1 Organ Kidney Brain Kidney and brain	 198 (100) 190 (96) 166 (84) 158 (80)
Seizure,† n/N (%)	43/166 (26)
Modified Rankin Score (n=150),† median (range)	4 (0, 5)
Laboratory values, median (range) All patients (N=198) WBC, $x10^9$/L Platelet count (n=197), $x10^9$/L LDH (n=196), U/L Hemoglobin (n=149), g/L No dialysis required (n=47) Serum creatinine,‡ µmol/L eGFR,‡ mL/min/1.73m^2	 12 (5, 44) 56 (10, 364) 783 (162, 2472) 83 (52, 127) 202 (80, 634) 33 (9, 77)
Platelet count <150x10^9/L, n (%)	174 (88)
Supportive patient management measures Hospitalization, n (%) PE, n (%) Dialysis,‡ n/N (%) Ventilator, n (%) Therapeutic coma,† n/N (%)	 197 (99.5) 181 (91) 137/190 (72) 47 (24) 35/166 (21)
Time intervals, median (range), days First onset of diarrhea to first PE session First onset of diarrhea to eculizumab treatment initiation First PE session to eculizumab treatment initiation	 7 (2, 26) 11 (4, 121) 5 (-2, 113)

ITT, intent-to-treat; **LDH**, lactate dehydrogenase; **PE**, plasma exchange; **WBC**, white blood cell count.
*ITT population includes patients receiving ≥1 eculizumab dose.
†Patients with brain involvement at baseline.
‡Patients with kidney involvement at baseline.

Funding: Pharmaceutical Company Support - Alexion Pharmaceuticals, Inc.

SA-OR103

Eculizumab (ECU) Is Effective in Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts) with a Long Disease Duration and CKD: 2-Year Data

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Background: Chronic uncontrolled complement activation causes systemic thrombotic microangiopathy (TMA) in aHUS. We report longer follow-up from the 26-wk, phase II trial with the complement inhibitor ECU in aHUS pts with a long disease duration and CKD previously undergoing plasma exchange/infusion (PE/PI).

Methods: aHUS pts ≥12 yrs receiving chronic PE/PI (unchanged regimen) were enrolled in a phase II trial. After 8 wks observation, pts stopped PE/PI and initiated ECU. Primary endpt: TMA event-free status (≥12 wks: no platelet change >25% + no PE/PI + no new dialysis). Median ECU duration at data cut-off=114 wks (26-129).

Results: 20 pts received ECU through wk 26; 19 continued ECU. Median time from diagnosis to screening=48 months (0.66-286). Chronic ECU therapy (>2 yrs) resulted in 19 pts achieving TMA event-free status. As compared to chronic PE/PI before ECU, 8 pts achieved eGFR increase ≥15 mL/min/1.73m^2(Table). No pt required PE/PI or progressed to ESRD/dialysis. ECU was well tolerated; 3 SAEs related to ECU (severe, all resolved). No meningococcal infection. 1 death (GI bleed unrelated to drug). 18 pts treated >2 yrs are alive.

Conclusions: Long-term ECU sustained suppression of TMA, was associated with no new ESRD, continued to markedly improve renal function and was well-tolerated.

Parameter	ECU Treatment 26 wks (N=20)	Median Duration of ECU Treatment 114 wks* (N=20)
TMA event-free status, n (%)	16 (80)	19 (95)
eGFR increase of ≥15 mL/min/1.73m^2, n (%)	1 (5)	8 (40)
Improvement in ≥1 CKD, n (%)	7 (35)	12 (60)
≥25% reduction in serum creatinine, n (%)	3 (15)	11 (55)
Mean change in eGFR, mL/min/1.73m^2, (95% CI)	6.1 (3.3, 8.7) P<0.0001	7.1 (-0.03, 14)[†] P=0.05 (wk 96)
Decrease in proteinuria ≥1 grade, n/N	8/16[‡]	12/15[‡] (wk 96)
Mean change in health-related quality of life (EQ-5D score) (95% CI) (clinically meaningful threshold ≥0.06)	0.12 (0.07, 0.17) P<0.0001	0.14 (0.10, 0.18) P<0.0001 (wk 96)

ECU dosage: 900mg/week for 4 wks, 1200mg at wk 5, then 1200mg q2 wks thereafter.
Significance was tested with a repeated measures model.
*Median duration of 114 weeks unless otherwise indicated.
[†]One pt on dialysis received a transplant on day 217, renal data from this pt were censored on day 217 and during continued ECU treatment.
[‡]Evaluable pts.

Funding: Pharmaceutical Company Support - Study Sponsored by Alexion Pharmaceuticals Inc.

SA-OR104

A 4 Plus 2 Infusion Protocol of Rituximab Provides Long-Term Beneficial Effects in Patients with HCV-Associated Mixed Cryoglobulinemia with Diffuse Membranoproliferative Glomerulonephritis and Severe Polyneuropathy

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Background: B cells expansion triggered by HCV infection plays a central role in the pathogenesis of Mixed cryoglobulinemia syndrome (MCs).The long term effects of B-cells depletion in MCs are still on debate.

Methods: 27patients,(mean age 60.2[range35-78]years,HCV infection in96%of cases) with symptomatic type-II MCs with systemic manifestations,including renal involvement(diffuse membranoproliferative glomerulonephritis in 15cases),peripheral neuropathy (26cases)and large skin ulcers (9 case,in7necrotizing)were considered eligible for Rituximab(RTX)therapy.RTX (375mg/m^2) was administered on days1,8,15and22.Two more doses were administered 1 and 2 months later.No other immunosuppressive drugs were added.Response was evaluated by assessing the changes in clinical signs, symptoms, laboratory parameters and electromyographic indices for a very long term follow-up(mean 54.3months[12-96]).

Results: Complete remission of pre-treatment active manifestations was observed in all the cases of skin purpuric lesions and non-healing vasculitic leg ulcers, and in80% of the peripheral neuropathy. A significant improvement($p>0.02$) in the clinical neuropathy disability score was observed. Electromyography examination revealed that the amplitude of compound motor action potential had increased($p<0.04$).Improvement of glomerulonephritis in 15affected patients, starting from the second month after RTX, was documented by the decrease of serum creatinine from 2.2±1.9SD to1.6±1.2SD mg/dl, $p<0.05$; 24-hour proteinuria levels from 2.3±2.1SDto0.9±1.9SD g/24h,$p<0.05$.Improvement of cryoglobulinemic serological hallmarks, such as cryocrit and low complement C4, was also observed($p<0.05$).The safety of RTX was confirmed by the absence of severe side effects recorded during the mean 54-month follow-up. Re-induction was performed in 9 relapsed cases (after a mean of 31.1 months, range 12-54) with definitely beneficial effects.

Conclusions: In this open prospective study, RTX appeared to be effective and safe in the treatment of patients with MCs-associated neuropathy and membranoproliferative glomerulonephritis.

SA-OR105

Renal Involvement in Non-Infectious Mixed Cryoglobulinemia

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Background: Mixed cryoglobulinemia (MC) has been primarily characterized in patients with hepatitis C virus infection while non infectious MC has been poorly investigated. Our aim was to study the spectrum of renal disease in non-infectious MC.

Methods: Retrospective analysis of patients with non infectious MC and biopsy-proven renal involvement, identified from a collaborative French multicentric survey (the "CryoVas" study).

Results: 80 patients were included (50F/30M, mean age 63±14 years). MC was related to Sjögren syndrome (pSS) alone (18 patients, 22%), lymphoproliferative disorders (23 patients, 29%), 7 of whom had a history of pSS, or finally classified as essential (39 patients, 49%). Renal features included hematuria, proteinuria >1g/day, hypertension and renal failure (mean eGFR 40±20 ml/min/1.73m²) in 97%, 85%, 85% and 82% of cases, respectively. Extrarenal manifestations included skin (71%), neurological (43%) and joint (35%) involvement. 73 patients (91%) had type 1 membranoproliferative glomerulonephritis while 7 had mesangioproliferative glomerulonephritis. Renal interstitium was infiltrated by CD3+ and CD20+ lymphocytes in >50% of cases with a nodular organization in 40% of cases. Mild to severe fibrosis was observed in 20% of cases. Vascular lesions included arteriosclerosis and more rarely necrotizing arteritis. First line treatment consisted of steroids (88%) and/or immunosuppressive agents including cyclophosphamide (40%) and/or rituximab (30%). 18 patients had plasma exchanges. Initial renal remission was observed in 86% of cases. However, relapses occurred in half of patients. At last follow-up, mean eGFR was 49±26 ml/min/1,73m² including 5% of ESRD. Severe infections and new onset of B-cell lymphoma occurred in 30% and 8% of cases. 19 (25%) patients died, 9 of whom within the 6 months after biopsy.

Conclusions: Non infectious MC is characterized by a high rate of pSS and B-cell lymphoma at diagnosis and during disease course. 25% of patients died underscoring the importance to identify prognostic factors of renal non infectious MC.

SA-OR106

Abundant APRIL-Producing Macrophages in IgG4-Related Kidney Disease

Takahiro Kawakami,[1] Kazunori Yamada,[1] Ichiro Mizushima,[1] Hiroshi Fujii,[1] Kiyoaki Ito,[2] Shozo Izui,[2] Bertrand Huard,[2] Mitsuhiro Kawano.[1] *[1]Division of Rheumatology, Kanazawa University Hospital, Kanazawa, Japan; [2]Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland.*

Background: IgG4-related disease (IgG4-RD) is a systemic disease characterized by histopathologic features of marked infiltration of IgG4-positive plasma cells in association with fibrosis. Kidney is one of the representative organs affected by this disease, and plasma cell-rich tubulointerstitial nephritis (TIN) is a typical manifestation. However, cellular and molecular mechanisms responsible for plasma cell accumulation in IgG4-related TIN is unknown.

Methods: We performed immunohistochemical staining for a proliferation inducing ligand (APRIL), a member of the TNF superfamily implicated in plasma-cell survival, in IgG4-related TIN with two different antibodies specifically recognizing APRIL-producing cells (Stalk-1) and secreted APRIL (Aprily-8). We also analyzed lacrimal and submandibular glands of patients with IgG4-RD and Sjögren's syndrome, and compared the staining pattern between these organs.

Results: Numerous Stalk-1-positive APRIL-producing cells were detectable in lesions of IgG4-related TIN. Using double immunofluorescence staining method, CD68-positive macrophages were proved to be the major cell type for APRIL production. The secreted form of APRIL, stained with Aprily-8, was distributed close to infiltrating plasma cells. Essentially identical results were obtained in lacrimal or submandibular glands of IgG4-RD. In contrast, APRIL-producing cells were rarely detectable in salivary glands with Sjögren's syndrome. After successful corticosteroid treatment, infiltration of APRIL-producing cells and plasma cells markedly decreased in IgG4-RD.

Conclusions: Abundant infiltration of APRIL-producing macrophages and localization of secreted APRIL close to plasma cells in IgG4-related TIN suggest a role of APRIL in the pathogenesis of plasma cell-rich TIN in IgG4 RD.

SA-OR107

C5 Inhibition in Mice Prevents Fatal C3 Glomerulopathy

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Background: Improper control of the alternative pathway (AP) of complement has been implicated in the pathogenesis of several kidney diseases including C3 glomerulopathy and atypical hemolytic uremic syndrome (aHUS). Although Eculizumab, a humanized anti-C5 mAb, has been approved for the successful treatment of aHUS, current therapy for C3 glomerulopathy remains nonspecific and ineffective, and many patients eventually progress to end-stage renal failure. We developed a murine model of fatal C3 glomerulopathy and performed experimental therapeutics of C5 inhibition in this study.

Methods: We used gene targeting to create a mouse model of lethal C3 glomerulopathy resembling human dense deposit disease. The mutant mice ($fH^{m/m}/fP^{-/-}$) carried a C-terminal mutation in the negative AP complement regulator factor H (fH) and were also deficient in the positive complement regulator properdin (fP). We treated $fH^{m/m}/fP^{-/-}$ mice with an anti-mouse C5 mAb (BB5.1) at 4 or 6 weeks of age and monitored mouse survival, proteinuria and renal pathology.

Results: $fH^{m/m}/fP^{-/-}$ mice developed severe C3 glomerulonephritis (GN) characterized by heavy proteinuria by 6-8 weeks and all died from rapidly progressive, crescentic GN by 14 weeks. Treatment with anti-C5 mAb starting at 4 weeks of age rescued them from death and prevented proteinuria with marked improvement in renal pathology. Mice appeared healthy throughout a 4-month treatment period, but proteinuria and lethal GN rapidly returned once anti-C5 therapy stopped. When anti-C5 mAb was given to 6-week old $fH^{m/m}/fP^{-/-}$ mice which had already developed high levels of proteinuria, 6 of 10 treated mice also survived with diminished proteinuria and significant improvement in renal pathology.

Conclusions: These results suggest that C5 activation plays a key role in the pathogenesis of C3 glomerulopathy and treatment of human patients with Eculizumab may be effective and should be investigated in clinical studies. This conclusion is in agreement with recent case reports describing anecdotal successes of Eculizumab use in human C3 glomerulopathy patients (see NEJM, 366(12): 1161-1166, 2012).

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SA-OR108

Recombinant Murine Proteinase 3 Induces Crescentic Glomerulonephritisin NOD Mice Peiqi Hu, Hong Xiao, Ronald J. Falk, J. Charles Jennette. *Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Background: Proteinase 3 (PR3) is a major target antigen of antineutrophil cytoplasmic autoantibody (PR3-ANCA) that are closely associated with pauci-immune crescentic glomerulonephritis (CGN) and small vessel vasculitis (SVV). However, a direct pathogenic role for PR3-ANCA remains unclear. Here we report the induction of immune-complex CGN in autoimmune-prone NOD mice by immunization with recombinant mouse PR3 (rPR3).

Methods: 8-12 wk-old NOD/LtJ mice were immunized i.p. with 100ug of affinity-purified enzymatically inactive rPR3 (n=11) or BSA (n=5)as control in CFA and boosted i.p. 4 times at 10-20 day intervals with the same doses of antigens in IFA. Autoimmune responses were confirmed by measurement of circulating anti-mouse PR3 by ELISA. Mice were sacrificed at day 75 post-immunization.

Results: NOD mice immunized with rPR3 developed high levels of anti-PR3 antibodies. No anti-PR3 was detected in BSA immunized control mice. The rPR3 recipient mice also developed urine abnormalities with average scores (0-4+) of proteinuria (4.0), leukocytes (3.0) and hematuria (0.36), whereas none of BSA control mice had urine abnormalities. All rPR3-immunized mice had crescentic glomerulonephritis (average 25.6% glomerular crescents and 3.7% glomerular necrosis). Glomeruli had well defined, diffuse, global, finely granular capillary wall staining by immunofluorescence microscopy for IgG, C3 and PR3. There were no glomerular lesions and no immune deposits in control animals.

Conclusions: Immunization of NOD mice with rPR3 can break tolerance toward self-PR3 and result in a high level of circulating anti-PR3. The development of CGN in all rPR3-immunized mice suggests that anti-PR3 antibodies are pathogenic. The presence of well-defined granular capillary wall immunostainging for IgG and PR3 is not typically present in pauci-immune ANCA CGN in patients. Thus, injection of rPR3 induces CGN in NOD mice, but it is not pauci-immune. This phenotypic complexity in NOD mice requires further investigation to understand the role of anti-PR3 in pathogenesis.

Funding: NIDDK Support

SA-OR109

All ANCA Are Not Equally Pathogenic Hong Xiao, Peiqi Hu, Donna O. Bunch, Ronald J. Falk, J. Charles Jennette. *Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Background: Immunizing MPO-/- mice with murine MPO produces anti-MPO IgG and anti-MPO splenocytes that cause necrotizing and crescentic glomerulonephritis (NCGN). Despite 85% identity between human MPO (hMPO) and mouse MPO (mMPO), immunizing MPO-/- mice with hMPO does not produce pathogenic anti-MPO. Thus, regions of heterogeneity between mMPO and hMPO apparently contain pathogenic epitopes. To test this, we immunized MPO-/- mice with chimeric m/hMPO to identify mMPO regions responsible for inducing pathogenic antibodies.

Methods: MPO was divided into four heavy chain segments (a, b, c, d for mMPO; and A, B, C, D for hMPO) in addition to the light chain (l or L). Recombinant full-length mMPO (labcd) and hMPO (LABCD), and 2 chimeric m/h MPO (LAbcd and LABcD) were expressed in HEK293 cells, affinity purified, and used to immunize MPO-/- mice. 1x10^8 anti-MPO splenocytes from these mice (and control anti-BSA splenocytes) were transferred into Rag2-/- mice. Mice were sacrificed on day 13 and blood and tissues collected for analysis.

Results: All Rag2-/- mice that received splenocytes developed similar levels of antibodies to the corresponding antigens. Rag2-/- mice receiving splenocytes against full-length hMPO or BAS did not develop NCGN. Rag2-/- mice receiving splenocytes against MPO containing murine segments b, c, and d developed severe NCGN (avg. 89.5% crescents) similar to the Rag2-/- mice that received splenocytes against full-length mMPO (avg. 90.7% crescents). Rag2-/- mice that received splenocytes against MPO molecules only containing murine segments c developed mild NCGN (avg. 16.3% crescents).

Conclusions: Not all anti-MPO antibodies are equally pathogenic. Anti-MPO to epitopes on full-length hMPO are not pathogenic in mice. Anti-MPO to epitopes on mMPO segments b and/or d induced robust nephrotogenic antibodies. Epitopes on murine segment c induced weakly nephrotogenic antibodies. These data indicate that all anti-MPO antibodies are not equally pathogenic. These observations agree with recent observations that all MPO-ANCA are not equally pathogenic in patients with ANCA-associated vasculitis (Roth et al, ASN 2012 abstract).

Funding: NIDDK Support

SA-OR110

The Protective Role of NADPH Oxidase in ANCA-Induced Vasculitis Adrian Schreiber,[1,2] Sylvia Krueger,[1] Friedrich C. Luft,[1] Ralph Kettritz.[1,2] *[1]Experimental and Clinical Research Center (ECRC) at the Max-Delbrueck Center for Molecular Medicine at the Charité, Berlin, Germany; [2]Department of Nephrology, Campus Virchow Clinic, Medical Faculty of the Charité, Berlin, Germany.*

Background: ANCA-activated neutrophils and monocytes cause necrotizing crescentic glomerulonephritis (NCGN) and vasculitis. *In vitro* studies suggest that both reactive oxygen species (ROS) and serine proteases mediate the disease, but their *in vivo* role is unclear. Generation of ROS involves activation of the NADPH oxidase in myeloid cells. We tested the hypothesis that ROS generation is essential to induce NCGN in ANCA disease.

Methods: To induce NCGN, we immunized MPO-deficient mice with murine MPO followed by bone marrow (BM) transplantation from either wild-type (WT) or gp91^{phox}-deficient (gp91$^{phox-/-}$) mice.

Results: WT BM-transplanted mice developed NCGN, whereas the gp91$^{phox-/-}$ BM-transplanted mice developed a significant stronger renal phenotype (13.3±2.5% vs. 60.7±8.9% glomerular crescents). The aggravated NCGN was confirmed in a second independent set of experiments using a different NADPH-knock out (p47$^{phox-/-}$). In addition to necrosis and crescents, gp91$^{phox-/-}$ mice showed markedly stronger glomerular inflammatory cell influx and higher kidney levels of IL-1β (757±120 vs. 1708±360 pg/mg). We hypothesized that superoxide generated by ANCA-stimulated NADPH oxidase down-regulates IL-1β generation by inhibition of the NLRP3-inflammasome and caspase-1, the classical pathway of IL-1β generation. Stimulation of WT murine monocytes with murine anti-MPO IgG resulted in IL-1β generation that was significantly accelerated in both gp91$^{phox-/-}$ und p47$^{phox-/-}$ monocytes. This increase was reduced by pretreatment with a specific caspase-1 inhibitor. Finally, we treated p47$^{phox-/-}$BM transplanted mice with the specific IL-1-receptor antagonist Anakinra. Untreated mice developed NCGN with 45.5±10.1% crescents, whereas Anakinra-treated mice were rescued from the aggravated renal phenotype (14.8±6.3% crescents).

Conclusions: Our data strongly suggest that ROS generated by ANCA-stimulated NADPH oxidase are important for down-regulating the inflammatory cascade by blocking caspase-1- and NLRP3-dependent IL-1β generation.

Funding: Government Support - Non-U.S.

SA-OR111

Mineralocorticoid Receptor Activation in Macrophages Promotes Renal Injury in Experimental Glomerulonephritis David J. Nikolic-Paterson,[1] Louis L. Huang,[1] Yingjie Han,[1] Morag Young,[2] Gregory H. Tesch.[1] *[1]Nephrology and Medicine, Monash University and Monash Medical Centre, Clayton, Victoria, Australia; [2]Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia.*

Background: Clinical and experimental studies have identified that MR antagonists substantially reduce kidney injury; however, the side effect of hyperkalemia, particularly in the context of renal impairment, is a major barrier to the use of this therapy. Thus, it would be beneficial to determine the specific cellular targets and mechanisms by which MR antagonists protect against kidney injury. This studied aimed to establish the contribution of macrophage mineralocorticoid receptor (MR) signaling to kidney inflammation and injury in experimental glomerulonephritis.

Methods: A 15 day model of accelerated anti-GBM glomerulonephritis was examined in groups (n=8) of: (i) wild type (WT) mice; (ii) MR$^{flox/flox}$/LysM^{Cre} mice lacking MR signaling in macrophages; and (iii) WT mice receiving the MR antagonist eplerenone (100mg/kg/bid).

Results: WT mice with anti-GBM glomerulonephritis developed glomerular crescents (37±5%), severe proteinuria and a 6-fold increase in serum cystatin-C. In comparison to these disease controls, MR$^{flox/flox}$/LysM^{Cre} mice and eplerenone-treated WT mice with anti-GBM glomerulonephritis displayed similar proteinuria; however, both groups had a 35% reduction in serum cystatin-C levels ($p<0.05$ vs WT) and had reduced crescent numbers (MR$^{flox/flox}$/LysM^{Cre} = 26±4%, eplerenone-treated = 11±5%, both $p<0.001$ vs WT). The protection seen in MR$^{flox/flox}$/LysM^{Cre} mice was associated with a 50% reduction in macrophages and a marked reduction in gene expression of M1 proinflammatory markers (TNF-α, iNOS, MMP-12). In contrast, the gene expression of profibrotic molecules (TGF-β1, PAI-1, collagen IV) was not affected.

Conclusions: MR signaling in macrophages contributes to the proinflammatory response, glomerular damage and loss of renal function in mouse anti-GBM nephritis. Our comparison with eplerenone treatment shows that MR signaling in macrophages accounts for a substantial degree of the protection achieved with systemic MR blockade in this disease model.

Funding: Government Support - Non-U.S.

SA-OR112

Mineralocorticoid Receptor Depletion in Macrophage Protects against Lipopolysaccharide-Induced Acute Kidney Injury and Inflammation Kenichi Ishizawa,[1] Shigetaka Yoshida,[1] Nobuhiro Ayuzawa,[1] Kohei Ueda,[1] Morag Young,[2] Toshiro Fujita,[1] Masaomi Nangaku,[1] Miki Nagase.[1] *[1]Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine, Tokyo, Japan; [2]Prince Henry's Institute, Clayton, Australia.*

Background: Mineralocorticoid receptor (MR) overactivation is known to promote renal damage via induction of inflammation and fibrosis. In the present study, we examined the role of macrophage MR in acute kidney injury and inflammation, and mechanisms of MR activation in macrophage.

Methods: MR was selectively deleted in macrophage using Cre/loxP system (MΦMRKO). Acute kidney injury model was created by intraperitoneal injection of lipopolysaccharide (LPS) in flox control and MΦMRKO mice. Macrophage M1 or M2 phenotype was evaluated by qPCR and flow cytometric analysis.

Results: LPS provoked acute kidney injury, as revealed by increased serum creatinine and BUN, in control mice. Serum creatinine and BUN was markedly suppressed in LPS-injected MΦMRKO mice. LPS promoted renal infiltration of F4/80-positive macrophages both in control and MΦMRKO mice. However, induction of M1 cytokine by LPS, including TNFα and IL-6, was reduced, whereas expression of M2 markers, such as Arg1 and Fizz1, was enhanced in the kidney of MΦMRKO. Consistently, flow cytometric analysis showed that macrophages in LPS-treated MΦMRKO kidney were polarized to M2 phenotype compared with those in LPS-injected control kidney. LPS-induced M1 cytokine

production was diminished by MR blocker spironolactone or MR knockdown in primary cultured peritoneal macrophages. As for mechanisms of MR activation, LPS augmented MR-dependent luciferase activity, and Rac inhibitor EHT1864 suppressed this activity, suggesting the involvement of Rac1. Indeed, LPS increased active Rac1 in macrophage, and EHT1864 diminished LPS-induced M1 cytokine production. EHT1864 as well as MR blocker eplerenone ameliorated LPS-evoked renal inflammation.

Conclusions: We demonstrate that MR in macrophage plays a pivotal role in the pathophysiology of acute kidney injury, possibly by controlling macrophage M1/M2 polarization. Our data also suggest that Rac1 mediates LPS-induced MR activation and M1 cytokine production in macrophage.

SA-OR113

Increases in Glomerular Permeability due to Free Fetal Hemoglobin (HbF), Released in Early Preeclampsia (PE): Reversal with Scavengers of Reactive Oxygen Species (ROS) Kristinn Sverrisson,[1] Josefin Axelsson,[1] Anna Rippe,[1] Magnus Olsson,[2] Bo Akerstrom,[2] Stefan R. Hansson,[3] Bengt Rippe.[1] *[1]Department of Nephrology, Lund University, Lund, Sweden; [2]Division of Infection Medicine, Lund University, Lund, Sweden; [3]Division of Obstetrics and Gynecology, Lund University, Lund, Sweden.*

Background: The pathogenesis of PE is not fully understood, but increased plasma levels of free HbF have been reported to occur early in PE. Free HbF, as well as adult Hb (HbA), are pro-inflammatory and will release ROS. Alpha-1-microglobulin (A1M) is a heme- and ROS scavenger that counteracts vascular permeability increases induced by HbA in perfused placenta. This study was performed to investigate whether HbF and HbA will increase glomerular permeability and to test whether the ROS scavengers, A1M and Tempol, can prevent their effects.

Methods: In anaesthetized Wistar rats blood access was achieved and the left ureter was cannulated for urine collection. Rats were continuously infused i.v. with either HbA, HbF or cyano-inactivated (CN)-HbF, together with polydisperse fluorescein isothiocyanate (FITC)-Ficoll-70/400, Inulin and ^{51}Cr-EDTA for 2h. Plasma and urine samples were taken simultaneously at different time points and analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients (θ) for Ficoll. Glomerular filtration rate was assessed with ^{51}Cr-EDTA. In separate experiments A1M or Tempol was given before and together with the HbF infusion.

Results: Free HbF caused rapid, partly reversible increases in glomerular permeability to large Ficoll molecules ($Ficoll_{50\text{-}80A}$), contrary to the effects of HbA and CN-HbF. For HbF $\theta\text{-}Ficoll_{70A}$ thus increased from $3.69 \times 10^{-5} \pm 1.11 \times 10^{-5}$ to $2.41 \times 10^{-4} \pm 9.57 \times 10^{-5}$ at 15 min. Treatment with A1M or Tempol reduced the increase in glomerular permeability caused by free HbF.

Conclusions: Free HbF causes marked, rapid and partly reversible increases in glomerular permeability, which is reduced by rescue treatment with ROS scavengers, such as endogenous A1M or by Tempol. Our data strongly suggest that free HbF contributes to the increases in glomerular permeability and proteinuria occurring in PE.

Funding: Government Support - Non-U.S.

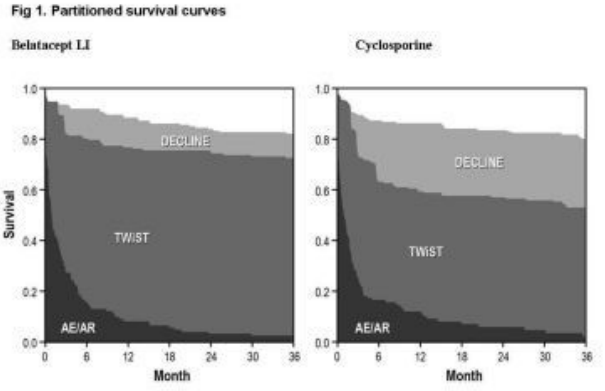
SA-OR114

A Q-TWiST Analysis of Belatacept Compared with Cyclosporine in Kidney Transplant Recipients Beth Sherrill,[1] Jianmin Wang,[1] Diana Hall,[1] Anupama Kalsekar.[2] *[1]RTI Health Solutions, Research Triangle Park, NC; [2]Bristol-Myers Squibb, Princeton, NJ.*

Background: The objective of this analysis is to compare quality adjusted survival in patients treated with belatacept (approved regimen) vs cyclosporine (CsA) in BENEFIT-EXT clinical trial using a novel methodology: quality-adjusted time without symptoms or toxicity (Q-TWiST).

Methods: The time period from transplant until graft loss or death or end of the trial was partitioned into 3 health states. AE/AR (toxicity) included time spent with adverse events or acute rejection prior to renal function decline, which was defined as the earliest date at which the calculated GFR decreased to ≤ 30 mL/min/1.73m^2 after excluding the first 56 days post-transplant. DECLINE included time post-renal function decline until death/graft loss/end of trial and TWiST was the remaining time in trial. Kaplan-Meier analyses were run to determine time spent in AE/AR, TWiST and DECLINE. Q-TWiST was defined as a weighted average of the 3 health state durations, weights accounting for quality of differences between the health states.

Results: Overall time to all-cause graft loss and time with adverse events/acute rejection were comparable between the belatacept (N=175) and CsA (N=184) groups.



Survival analyses of time to renal function decline, demonstrated separation of groups beginning at month 3 and increasing over time (p =0.0002). Mean time spent without toxicity or renal function decline (TWiST) in belatacept group was 6 months longer than cyclosporine group (p=0.0001). Q-TWiST differences between groups favored the belatacept group across an entire matrix of weights and were statistically significant for most plausible values.

Conclusions: The renal-sparing effects of Belatacept may allow patients more quality-adjusted survival time than cyclosporine.

Funding: Pharmaceutical Company Support - Bristol-Myers Squibb

SA-OR115

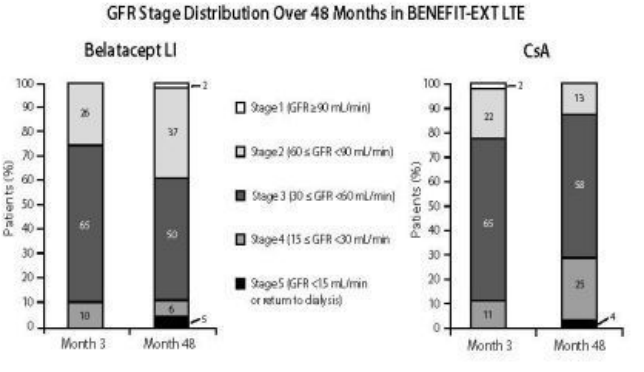
Likelihood of Improving or Sustaining Renal Function over Four Years with Belatacept or CSA: Insights from the BENEFIT-EXT Long-Term Extension Study J. Grinyo,[1] B. Charpentier,[2] R. Zhang,[3] M. Abouljoud,[4] F. Lehner,[5] M. Rial,[6] J. Morales,[7] M.B. Harler,[8] J. Medina-Pestana.[9] *[1]Univ Hosp Bellvitge; [2]Bicêtre Hosp; [3]Tulane Univ; [4]Henry Ford Hosp; [5]Medizinische Hochschule Hannover; [6]Instituto de Nefrologia; [7]Hosp Universitario 12 de Octubre; [8]Bristol-Myers Squibb; [9]Hosp do Rim e Hipertensão.*

Background: Sustained renal function improvement and a consistent safety profile were observed at 4 yrs in belatacept-treated recipients of extended criteria donor (ECD) kidneys in the long-term extension (LTE) of BENEFIT-EXT. Changes in cGFR stage of pts in the LTE cohort are reported.

Methods: In BENEFIT-EXT, recipients of ECD kidneys were randomized to a more or less intensive (LI) belatacept regimen, or CsA. Pts remaining on assigned therapy through Yr 3 were eligible to enter the LTE. This posthoc analysis used cGFR (MDRD) and assessed shifts in cGFR staging between Months (Mos) 3 and 48 in LTE pts. cGFR stages were defined per the KDOQI classification of CKD stages. cGFR was imputed as 0 for death or graft loss. Only pts who had a GFR value at Mo 3 and Mo 48 are included in the analysis (99/113 LI and 74/87 CsA pts had data available at both Mos 3 and 48).

Results: GFR stage at Mo 3 and 48 in LI and CsA pts of the LTE are shown in the Figure. Among Stage 2 pts at Mo 3, GFR stage was sustained or improved through Mo 48 in 17 (68%) LI and 3 (18%) CsA pts. In Stage 3 pts, GFR stage from Mo 3–48 was sustained or improved in 59 (94%) LI and 33 (67%) CsA pts. At Mo 3, 11 LI and 6 CsA pts were in Stage 4; 10 (91%) LI and 5 (83%) CsA pts sustained or improved their GFR stage through Mo 48. Trends from Mo 12–48 were similar to those reported above.

Conclusions: Belatacept LI-treated pts receiving ECD kidneys were more likely than CsA pts to experience sustained or improved renal function over 4 yrs in BENEFIT-EXT LTE cohort.



Funding: Pharmaceutical Company Support - Bristol-Myers Squibb

SA-OR116

Pre-Transplant Polyoma BK-Virus Replication in Urine or Blood of Donor or Recipient Is a Risk Factor for Post-Transplant BK Virus Infection of the Recipient Anke Schwarz,[1] Sylvia Linnenweber,[1] Hermann G. Haller,[1] Albert Heim,[2] Lubna Raggub,[2] Corinna Schmitt.[2] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Virology, Hannover Medical School, Hannover, Germany.*

Background: Spontaneous polyoma BKV replication in donor and/ or recipient urine or blood before renal transplantation (tx) may be a risk factor for post-tx recipient infection. This was tested in donor-recipient pairs before and after living transplantation.

Methods: Since 2008, we test blood and urine of donor-recipient pairs before living kidney tx by quantitative PCR (qPCR) (Cepheid-Affigene); the recipient then is followed by post-tx qPCR controls. In post-tx viremic and/ or viruric patients, the pre-tx and post-tx blood and/ or urine specimens are compared by sequencing of the BK VP1 typing region and additionally of the complete VP1 gene.

Results: 203 donor-recipient pairs had blood and urine tests before and recipient tests after tx; 57 of them showed pre-tx donor (n=54) or recipient (n=38) urine or blood viral replication. 68/180 recipients (37%) with a post-tx follow-up of at least 3 mos developed either BKV nephropathy (n=19, 11%) or BKV replication in blood or urine (n=49, 27%) after tx; in 32 of these 68 recipients (47%) donor or recipient had pre-tx viral replication in blood or urine compared to 25 out of 112 patients (22%) without post-tx BKV-infection (p=0.001). In 28/50 donors (56%) and 15/35 recipients (43%) with pre-tx BKV replication in urine genotyping of the VP1 region was possible. In 14/14 donor-recipient pairs with positive pre-tx donor replication and later recipient BKV-infection, the BKV VP1-sequence was identical (100%); however, in only 3/6 pairs with pre-tx recipient replication the VP1-sequence was identical (50%, p=0.001), while in 2 other pairs (17%), it was not identical. In 1 pair with positive pre-tx donor as well as positive recipient urine replication (different subtypes), later BKV-infection of the recipient showed the subtype of the donor.

Conclusions: Pre-tx BKV replication in donor or recipient urine or blood is a risk for later BKV-infection of the recipient. According to genotyping of the BK VP1 typing region, the donor seems to carry the higher risk.

SA-OR117

Outcomes of Simultaneous Liver and Kidney Transplantation Using Donation after Brain Death Compared to Donation after Cardiac Death Donors Umar Farooq, Christin M. Spatz, Erik Lehman, Tarek Alhamad, Nasrollah Ghahramani, Tadahiro Uemura. *Penn State University Hershey Medical Center, Hershey, PA.*

Background: The demand for organs for transplantation exceeds the supply of available donation after brain death (DBD) donors. In order to expand the donor pool, use of donation after cardiac death (DCD) donors has become more common. In renal and liver single organ transplant, DCD organs are frequently utilized and have similar outcomes to DBD organs. Outcomes for recipients of DCD organs for simultaneous liver and kidney transplant (SLKT) are not well established.

Methods: We used the UNOS (United Network for Organ Sharing) database to compare recipient outcomes of DBD and DCD donation for SLKT in regard to graft survival. Multivariate survival analysis was performed using a Cox proportional hazards model to evaluate graft survival controlling recipient and donor factors.

Results: A total of 4132 SLK recipients were identified from which 4028 (97.5%) received DBD organs and 104 (2.5%) received DCD organs. The DBD recipients had a higher MELD score (29.3 vs. 26.8) and older donors (35.4 vs. 32 years) compared to DCD. The mean graft survival in years (y) was lower for DCD liver (6.8y vs. 10y p=0.015) and kidney (6.6y vs. 9.3y p=0.027) compared to DBD recipients. There was improved graft survival in DBD compared to DCD with the unadjusted hazard ratio (HR) for liver and kidney graft survival 0.68 (p =0.015) and 0.71 (p = 0.027), respectively. When the model was adjusted for recipient age, donor age, sex, dialysis status, MELD score, and UNOS region the allograft survival benefit of DBD over DCD remained positive with respective HRs of 0.56 and 0.58.

Conclusions: In SLKT, DBD recipients have a statistically significant improved graft survival when compared to DCD recipients even after adjustment for common co-variables. Although outcomes with DCD recipients were inferior to DBD, use of these organs has previously been shown to offer a survival advantage compared to no transplant. Further studies are needed to determine which patients may benefit most from DCD donation.

Funding: Clinical Revenue Support

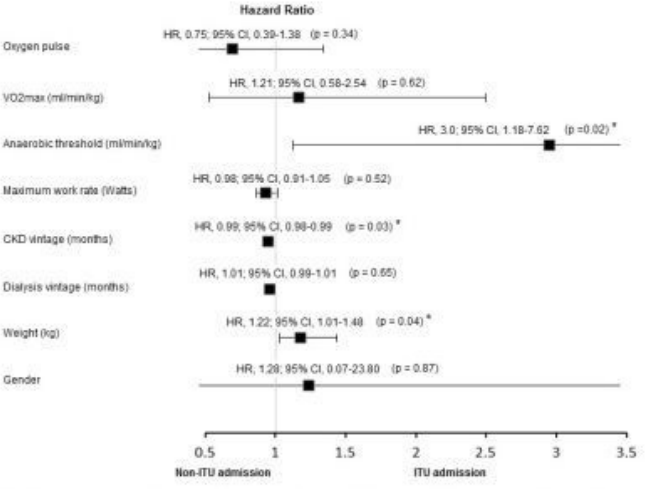
SA-OR118

Reduced Functional Measure of Cardiovascular Reserve Predicts Admission to Intensive Care Unit for Patients Undergoing Kidney Transplantation Stephen M. Ting,[1] Hasan Iqbal,[1] Prithwish Banerjee,[1] Robert Higgins,[1] Chris H.E. Imray,[1] Nicolas Aldridge,[1] Rosemary Bland,[2] Daniel Zehnder.[2] *[1]University Hospitals Coventry & Warwickshire NHS Trust, UK; [2]Warwick Medical School, University of Warwick.*

Background: There is no effective preoperative risk stratification assessment for patients undergoing kidney transplantation that is able to identify those at risk of cardiopulmonary complication needing intensive care (ICU) support. We sought to determine if objective measures of cardiovascular reserve could identify these high risk patients.

Methods: 68 kidney transplant recipients were prospectively assessed between 2010 and 2012. Cardiopulmonary exercise testing (CPX), TD echo and aortic pulse wave velocity were performed within 4 weeks prior to surgery.

Results: 13 patients (19%) required admission to ICU. There were no deaths. In univariate analysis, significant findings in the ITU vs non-ITU attendees – female (69.2 vs 30.8%, p=0.01), weight [60.6 (56.5-75.2) vs 72.7 (65.6-78.5) kg, p=0.02], CKD vintage (p=0.04), dialysis vintage (p=0.001), VO2max [18.1 (15.3-19.5) vs 21.3 (17.7-25.7) ml/min/kg, p=0.02], anaerobic threshold (AT) [10.7 (9.2-11.2) vs 12.4 (10.4-13.3) ml/min/kg, p=0.002], oxygen pulse (p=0.02), maximal work rate (p=0.001). Echo and aortic compliance measures were not significant. In multivariate logistic regression analysis, reduced AT is the most significant independent predictor of ICU admission (p= 0.02). Shorter duration of CKD is associated with a lower risk (p= 0.03) and lower body weight is associated with a higher risk of ICU admission (p= 0.04).



Independent predictors of ITU admission following kidney transplantation

Conclusions: Measures of cardiovascular reserve, especially the AT, strongly predict post-operative morbidity in kidney transplant recipients. This is the first prospective study to demonstrate the usefulness of CPX testing as a preoperative risk stratification tool for kidney transplantation.

Funding: Clinical Revenue Support

SA-OR119

Racial Variation in Medical Outcomes among Older Living Kidney Donors: (From the WHOLE-DONOR Disparities Collaborative) Krista L. Lentine,[1] Dorry L. Segev,[2] David Axelrod,[3] Daniel C. Brennan,[4] Amit X. Garg,[5] Connie L. Davis,[6] Thomas E. Burroughs,[1] Janet E. Tuttle-newhall,[1] Mark Schnitzler.[1] *[1]Saint Louis Univ; [2]Johns Hopkins; [3]Dartmouth; [4]Washington Univ; [5]Univ Western Ontario; [6]Univ Washington.*

Background: We constructed a novel database that links Organ Procurement and Transplant Network (OPTN) identifiers of living kidney donors (LKDs) to Medicare billing claims to examine correlates of post-nephrectomy (NTX) medical diagnoses in a sample of generally older prior LKDs.

Methods: Eligible persons had OPTN records of serving as a LKD in 1987-2008 and post-NTX billing claims within the available claims data (2004-2008). Post-NTX medical diagnoses of hypertension (HTN), chronic kidney disease (CKD), and diabetes mellitus (DM) were ascertained by ICD-9-CM diagnosis codes on claims. We examined relative risks (adjusted hazard ratio, aHR) of post-donation diagnoses by race using multivariate Cox regression with left-censoring for time from NTX to start of captured Medicare. Diagnosis rates within observed Medicare data were also examined as rates per 100-person years (PY).

Results: We identified 4007 prior LKDs, of whom 40% were men. Median ages at NTX and at the start of captured Medicare claims were 57 & 65 yrs, respectively. Racial composition included: 8.1% black, 83.4% white, and 5.7% Hispanic. After adjustment for age and gender, black LKDs, as compared with white LKDs, had an increased risk of HTN (aHR 1.41, P=0.0003), DM (aHR 1.50, P=0.009) and CKD (aHR 1.85, P<0.0001) diagnoses. Diagnosis rates per 100 PY enrollment were higher in black versus white LKDs (Table).

ADJUSTED RELATIVE RISKS FOR POST-DONATION MEDICAL DIAGNOSES AMONG MEDICARE-INSURED PRIOR LIVING KIDNEY DONORS

	Hypertension Diagnosis aHR (95% CI)	Diabetes Diagnosis aHR (95% CI)	CKD Diagnosis aHR (95% CI)
Age (per yr)	**1.02 (1.01–1.02)†**	1.00 (1.00–1.01)	**1.03 (1.02–1.04)†**
Male	**1.12 (1.041–1.23)***	**1.38 (1.16–1.65)†**	**2.33 (1.95–2.78)†**
Race			
Black	**1.41 (1.17–1.70)***	**1.50 (1.11–2.04)†**	**1.85 (1.34–2.46)†**
White	Reference	Reference	Reference
Hispanic	1.17 (0.95–1.45)	**2.11 (1.53–2.89)†**	1.23 (0.75–1.69)
Other	1.03 (0.76–1.39)	1.6 (1.00–2.57)	1.18 (0.71–1.98)

aHR, adjusted hazards ratio * P<0.04-0.0001; † P <0.0001

POST-DONATION MEDICAL DIAGNOSES RATES DURING CAPTURED MEDICARE BENEFITS PERIOD

	Rates per 100 Person-Years of Medicare Enrollment		
	Hypertension Diagnosis	Diabetes Diagnosis	CKD Diagnosis
Black	**9.12**	**3.30**	**3.08**
White	**7.41**	**2.20**	**2.14**
Hispanic	**7.56**	**3.56**	**2.19**

Conclusions: Among older prior LKDs enrolled in Medicare, black LKDs experience higher relative risks of HTN, DM and CKD compared with white LKDs. These data support a need for increased monitoring and evaluation of health outcomes among African American living kidney donors.

SA-OR120

Systolic Blood Pressure and Risk of Cardiovascular-Related Death in the FAVORIT Trial Daniel E. Weiner,[2] Myra A. Carpenter,[1] John W. Kusek,[3] Lawrence G. Hunsicker,[4] Andrew S. Levey.[2] *[1]Biostatistics, University of North Carolina, Chapel Hill, NC; [2]Tufts Medical Center, Boston, MA; [3]NIDDK, NIH, Bethesda, MD; [4]University of Iowa, Iowa City, IA.*

Background: The 2009 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines recommend a systolic blood pressure (SBP) target of 130 mm Hg or lower in kidney transplant recipients (KTRs). Some studies have shown increased risks of cardiovascular events and deaths in patients with diabetes when targeting low SBP levels (i.e., a J-shaped survival curve). The impact of specific SBP targets has not been studied in KTRs.

Methods: FAVORIT was a randomized controlled trial to evaluate homocysteine-lowering therapy on CVD and kidney outcomes in 4,110 KTRs in the US (n=3,000), Brazil (n=612) and Canada (n=498) with elevated homocysteine levels and stable graft function.

Results: Among 3,673 participants with complete baseline data, mean ± SD age was 52 ± 9.4 years; 37% were women, 41% had a history of diabetes, and 64% had eGFR between 30 and 60 ml/min/1.73m^2. Mean follow-up time was 4.1 ± 1.5 years. Most participants (89%) were being treated for HTN. The mean SBP at baseline was 136 ± 20 (interquartile range, 122 – 147.5) mm Hg. CV-related death occurred in 172 (5%) of KTRs. After adjusting for age, sex, race, randomized group, country, diastolic blood pressure, use of antihypertensive medications, history of CVD, diabetes, smoking, lipids, BMI, eGFR, graft vintage and donor type, each 20 mm Hg increase in SBP was associated with a 44% increase in risk of CV-related death (HR: 1.44, 95% CI: 1.22 – 1.70), with no suggestion of a 'J'-shape. In the subgroup of 1,695 KTRs with eGFR ≤45 ml/min/1.73m^2, there were 104 CV-related deaths (HR: 1.31, 95% CI: 1.06 – 1.61). In this subgroup, those with SBP <120 mmHg had the lowest risk of CVD-death (HR: 0.84, 95% CI: 0.39 – 1.84, vs reference of 120-129 mm Hg).

Conclusions: These results suggest that among KTRs lower systolic blood pressure is associated with lower risk of CV-related death throughout the entire range measured. Subgroup analysis of the risk of CV-related death among KTRs with lower eGFR is consistent with this finding.

Funding: NIDDK Support

SA-OR121

Associations between Metabolic Syndrome, Renal Function, and Histology in Living Kidney Donors Yasushi Ohashi, George Thomas, Sankar D. Navaneethan, Emilio D. Poggio. *Nephrology and Hypertension, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH.*

Background: Prospective living kidney donors are now older and have higher BMI along with other co-morbidities that are known risk factors for chronic kidney disease. We evaluated the association of metabolic syndrome (MetS) with renal function and histology in living kidney donors.

Methods: We identified 376 donors between 2005 to 2011 in whom we assessed metabolic risk factors, kidney size, function, and histology of implant biopsy at donation, 158 of whom had post-donation follow-up [median (10–90th percentile) of 13 months (6–26 months)]. MetS was defined using modified NCEP-ATP III criteria, and histological abnormalities were defined as any one or more of: a) >5% global glomerulosclerosis, b) >5% interstitial fibrosis with any tubular atrophy, or c) any arteriosclerosis.

Results: MetS was present in 47 (12.5%) donors, who were more likely to be male (53.2% vs. 38.9%, p<0.05), older (45.3 ± 9.9 vs. 40.7 ± 10.6 years, p<0.01) and Caucasian (97.9% vs. 89.4%, p<0.05). There was no difference in kidney volumes by CT scan between MetS and non-MetS, but donors with MetS were more likely to have abnormal histology (65.7% vs. 42.7%, p<0.01) and a significantly lower iothalamate GFR (iGFR) (90.6 ± 14.3 vs. 99.3 ± 20.2 ml/min/1.73m^2, p<0.01). Rise of serum Creatinine (SCr) post-nephrectomy was significantly associated with male gender, African-American (AA) race, and higher kidney volume; whereas recovery of SCr was associated with younger age and AA race, but not with MetS nor with abnormal histology. Donors with lower HDL and elevated uric acid were more likely to develop MetS after donation, manifesting with a significantly higher BMI, diastolic blood pressure, and elevated triglyceride post-nephrectomy.

Conclusions: MetS is associated with histological abnormalities and lower iGFR in living kidney donors, but nephrectomy in these donors was not associated with subsequent changes in kidney function. There is a predisposition for development of MetS in donors who had lower HDL and elevated uric acid levels pre-donation. These findings emphasize the importance of life style modification in living kidney donors.

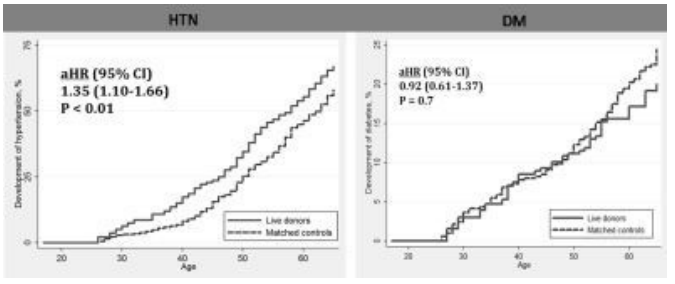
SA-OR123

Hypertension and Diabetes after Living Kidney Donation: Racial Differences and Comparison to Healthy Matched Controls Brian Boyarsky, Mara Mcadams, Allan Massie, Kyle Van Arendonk, M. Grams, Po-han Chen, Dorry L. Segev. *Johns Hopkins.*

Background: Though living donation is generally considered safe, long-term morbidity is not well characterized. Recent studies suggest a higher rate of hypertension (HTN) and diabetes in black versus white donors, but no comparisons have been made to healthy non-donors, so the risk attributable to donation remains unknown.

Methods: We surveyed 1,046 donors between 1970-2011. Participants reported new onset HTN and diabetes since donation, as well as all current medications; interviews were augmented by detailed review of medical records since donation. Healthy non-donors were drawn from a large cohort study where contraindications to donation were captured, and matched controls who would have been eligible for donation at the time of matching were selected.

Results:



19% of donors (26% black, 18% non-black) developed HTN. Compared to healthy non-donors, donors had an increased risk of post-donation hypertension (aHR 1.35 95%CI 1.10-1.66, p <0.01); this risk was seen in non-black donors (aHR 1.44 95%CI 1.15-1.83, p <0.01) but not in black donors (aHR 1.01 95%CI 0.62-1.63, P=0.9). 4% of donors (8% black, 3% non-black) developed diabetes. Compared to healthy non-donors, donors had a similar rate of developing diabetes (aHR 0.92 95%CI 0.61-1.37, P=0.7), with no differences by race.

Conclusions: Living donors are at a greater risk for developing HTN than healthy controls. Interestingly, while black donors had a higher rate of HTN compared with non-black donors, a risk attributable to donation was only seen in non-black donors. Donors did not have an increased risk of developing diabetes compared to healthy controls, contrary to some recent reports. It is critical to compare donors to healthy non-donors in studies of post-donation morbidity, so that risks attributable to the actual donation can be understood.

TH-PO001

Angiopoietin-2 Contributes to the Pathophysiology of Septic Acute Kidney Injury Sascha David,[1] Chandra C. Ghosh,[2] Aditi Mukherjee,[2] Eliyahu V. Khankin,[2] S. Ananth Karumanchi,[2] Samir M. Parikh.[2] *[1]Nephrology and Hypertension, Medical School Hannover, Germany; [2]Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: Intensive basic research into sepsis and associated multi organ dysfunction has yielded numerous putative molecular targets, but many have not translated into successful therapies. We hypothesized that Angiopoietin-2 (Angpt-2), a partial antagonist of the endothelium-stabilizing receptor Tie-2 secreted by endothelium, contributes to acute kidney injury (AKI) and adverse outcomes in this disease. We determined its mechanistic effects (i.e. vascular permeability and inflammation) by depletion experiments in murine and cellular models.

Methods: Murine sepsis was induced by endotoxin (LPS) and cecal ligation and puncture (CLP) in Angpt-2 heterozygotes (+/-) or wildtype littermates (+/+). Vascular leakage was assessed by bronchoalveolar lavage and consecutive protein measurements. Fluorescent immunohistochemistry (IF) from snap frozen kidneys and lungs was used to visualize changes in adhesion molecule expression (ICAM-1, VCAM-1) and tissue inflammation (Cd11b). Immunofluorescence for cytoskeletal (F-actin) and junctional (VE-cadherin) components from human micro-vascular endothelial cells (HMVEC) challenged with septic serum +/- Angpt-2 Antibody was performed.

Results: Angpt-2 +/- mice compared to their wildtype littermates had less adhesion molecule expression (VCAM-1) in different organ beds that consequently led to less tissue inflammation (Cd11b), and less vascular leakage. As a result heterozygotes developed milder AKI (crea: WT 0.6 mg/dl vs. het 0.2 mg/dl, p=0.02). Moreover, Angpt-2 +/- mice experienced over 40% survival advantage in both models (p = 0.004 and 0.018) without treating sepsis per se. Next, we studied the effect of human septic serum on endothelial cell architecture. Compared to control serum, septic serum disrupted the barrier function of HMVECs, an effect fully neutralized by a monoclonal Angpt-2 antibody.

Conclusions: We conclude that sepsis induced Angpt-2 release contributes to AKI and adverse outcomes in sepsis, opening a new avenue for therapeutic investigation.

Funding: Government Support - Non-U.S.

TH-PO002

Hypoxic Preconditioning Enhances the Benefit of Bone Marrow Mesenchymal Stem Cell Therapy for Treatment of Acute Kidney Injure by Improving Cell Homing Xiaofang Yu,[1] Chunlai Lu,[2] Hong Liu,[1] Yi Fang,[1] Xiaoqiang Ding.[1] *[1]Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China; [2]Thoracic Surgery, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: Hypoxic preconditioning (HP) of bone marrow mesenchymal stem cell (MSC) has been tested for enhancing the benefit of cell therapy for cardiovascular disease after transplantation. The present study was to test whether hypoxic preconditioned MSC transplantation could enhance the efficacy of cell therapy on AKI by stimulating MSC homing to the injured kidney using magnetic resonance imaging (MRI).

Methods: Passage 3 MSC was subjected to HP by culture for 24 h in 200μmol/L cobalt.

Results: Compared with normoxia preconditioning (NP), HP significantly increased the mRNA and protein expressions of HIF-1α and CXCR4 in MSC. HP enhanced the migration of MSC in vitro, as assessed by scratch wound healing assay and Transwell assay, and this effect was removed by siRNA HIF-1α or AMD3100 incubation. Intracarotid administration of SPIO labeled HP-MSC (3×10^6/animal) immediately to anesthetized rats with ischemia-reperfusion-induced AKI (40-min bilateral renal pedicle clamping) resulted in more cells homing to the ischemia kidney and longer retaining time than NP-MSC, as demonstrated by MR imaging. Histologically, HP increased the number of MSC located in glomerular capillaries than NP, as evidenced by Prussion bun staining. Importantly, occasional tubules showed iron labeling in HP group, while no tubules had iron labeling in NP group, indicating the possibility of tubular transdifferentiation after HP. Furthermore, the increased recruitment of HP-MSC was associated with reduced kidney injure and enhancedfunctional recovery.

Conclusions: HP could improved the migration of MSC in vitro through the activation of HIF-1α and up-regulation of CXCR4, and HP could be a useful method of enhancing the homing and prolonging the retaining of MSC for AKI cell therapy.

TH-PO003

Redox-Sensitive Glycogen Synthase Kinase 3β Directed Control of Mitochondrial Permeability Transition: Rheostatic Regulation of Acute Kidney Injury Zhen Wang,[1,2] Yan Ge,[1] Rujun Gong.[1] *[1]Nephrology, Brown Medical School, Providence, RI; [2]Department of Nephrology, Tongji University School of Medicine, Shanghai, China.*

Background: Mitochondria dysfunction plays a pivotal role in the development of acute kidney injury (AKI) and necroapoptotic death of tubular epithelial cells. Evidence suggests that glycogen synthase kinase (GSK) 3β is a novel modulator of AKI.

Methods: The regulatory effect of GSK3β on mitochondrial permeability transition (MPT) was examined in cultured tubular epithelial cells and in a murine model of AKI induced by the pro-oxidant herbicide praquat.

Results: In tubular epithelial cells, a specific subcellular distribution of GSK3β was detected in mitochondria. Co-immunoprecipitation studies confirmed that GSK3β physically interacts with cyclophilin F, a key component of the MPT pore complex that possesses multiple GSK3β phosphorylation consensus motifs as demonstrated by *in silico* analysis, suggesting that GSK3β have a direct control of MPT pore activity. Upon a strong burst of reactive oxygen species elicited by praquat, the activity of the redox-sensitive GSK3β was drastically enhanced. This was accompanied with elevated phosphorylation of cyclophilin F and associated with MPT and cell death. TDZD-8, a highly selective small molecule inhibitor of GSK3β abrogated praquat-induced phosphorylation of cyclophilin F, markedly improved cellular viability and prevented MPT and cell death. Conversely, ectopic expression of a constitutively active GSK3β amplified the effect of praquat on cyclophilin F phosphorylation and sensitized cells to praquat induced MPT and death. *In vivo*, praquat injection elicited marked oxidant injury in the kidney and resulted in acute kidney dysfunction and massive tubular apoptosis and necrosis. Consistent with *in vitro* findings, the activity of GSK3β was enhanced in the injured kidney, associated with increased phosphorylation of cyclophilin F and MPT. Concomitant treatment with TDZD-8 blocked GSK3β activity in the kidney, blunted cyclophilin F phosphorylation, prevented MPT, attenuated tubular damage and improved praquat-induced AKI.

Conclusions: Our data suggest that redox-sensitive GSK3β regulates AKI via controlling the activity of MPT pore.

Funding: NIDDK Support

TH-PO004

Tamm-Horsfall Protein (Uromodulin) Is Uniquely Trafficked during Acute Kidney Injury and Its Presence Promotes Faster Recovery Tarek M. El-Achkar,[1] Monique R. Heitmeier,[1] Yan Liu,[2] Ruth A. Mccracken,[1] Xue-Ru Wu.[2] *[1]Nephrology, St. Louis University and St. Louis VA Medical Center; [2]Urology, New York University and Manhattan VAMC.*

Background: Tamm-Horsfall protein (THP) is a glycoprotein uniquely expressed in the thick ascending loops (TAL) of Henle. We recently showed that THP has a protective role in acute kidney injury (AKI). We also showed that its expression is increased during kidney recovery without an increase in urinary excretion. The significance of this expression pattern and its relationship to kidney recovery is unclear.

Methods: Using a murine model of ischemia–reperfusion (IRI), we studied the changes in THP expression using immunogold electron microscopy. We also measured changes in THP serum levels and its effect on tubular inflammatory signaling and renal recovery.

Results: THP was predominantly expressed at the apical domain of the TAL in sham sections. 48 hours after IRI, THP gold labeling was increased in the cellular and basolateral aspect of TAL, along with a marked increase in the interstitium. Occasionally, THP was also identified in the basolateral aspect of S3 segments. To investigate if THP is retained within the kidney or released systemically, we measured its serum levels by ELISA. During recovery from AKI, the serum level of THP in mice subjected to IRI was significantly higher compared to sham, supporting a significant uptake of THP by the peritubular vascular network. This trafficking pattern is unique to THP, because the expression of NKCC2 was unaltered by ischemia. THP also inhibited the expression of the macrophage chemo-attractant MCP-1 in HK-2 proximal cells subjected to ischemia. In addition, THP deficiency was associated with delayed recovery from AKI In THP-/- mice compared to wild type mice subjected to an equivalent degree of injury.

Conclusions: Our findings suggest that THP is specifically directed towards the interstitial and intravascular space in the recovery phase of AKI, where it down-regulates tubular inflammation and possibly promotes repair. Because of its increased systemic levels, we propose that serum THP could serve as a biomarker predicting renal recovery and possibly mediating a protective distant effect after kidney injury.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO005

Transcriptomic Analysis of Proximal Straight Tubules (PST) and Medullary Thick Ascending Limbs (mTAL) during Cisplatin (CP) Acute Kidney Injury (AKI) Jonathan Michael Starkey,[2] Wen Liu,[1] Beth Zavilowitz,[1] Bruce A. Luxon,[2] Lisa M. Satlin,[1] Robert L. Safirstein.[3] *[1]Pediatrics, Mount Sinai School of Medicine, New York, NY; [2]Biochemistry & Molecular Biology, UTMB Health, Galveston, TX; [3]Internal Medicine, UAMS, Little Rock, AR.*

Background: CP AKI is characterized by PST necrosis while the mTAL remain intact. Regenerating cells reline the PST accompanied by a robust transcriptional response.

Methods: We performed microarrays of PST and TAL during CP (20mg/kg or saline) AKI in mice to reveal the cell source of these changes. At 24 and 72 h after injection (encompassing early and peak events in CP AKI), PST and mTAL were individually micro-dissected and total RNA prepared from pooled samples of each segment. Affymetrix 3' IVT Mouse Expression Set 430 v 2.0 microarrays were used and results analyzed using Bioconductor packages in the R programming environment. Differentially expressed mRNA transcripts were analyzed by IPA.

Results: In the PST at 24 h expression of oxidative response genes, most notably those induced by CEBPA (Regulation z-score 2.5, p = 0.01), was observed. In the PST at 72 h, expression of genes relevant to lipid metabolism and molecular transport were reduced indicative of mitochondrial dysfunction. Pathway analysis supports a key role of mitochondrial dysfunction and network analysis reveals an NFE2L2-driven signaling hub. By contrast in the TAL at 72 h, gene ontology analysis reveals prominent changes in genes regulating cellular movement, immune cell trafficking (z-score 2.175, p = 0.003) and genes developmentally regulated, including the Hedgehog induced GLI1 transcription factor (Regulation z-score -2.5, p=0.002) recently connected to fibrotic reactions in the kidney. Network analysis reveals hubs of CEBPB, NFkB, p53 and ERK in the TAL at 72 h.

Conclusions: The data suggest that the response to CP AKI includes an increase in oxidative stress and early inflammation in the PST and an inhibition of cell cycle events in the TAL along with delayed inflammatory activity. The results reveal a distinct pattern of gene expression in the two segments in response to CP and suggest an interaction among the segments to direct repair of the injury.

Funding: NIDDK Support, Veterans Administration Support

TH-PO006

Downregulation of Matrix Metalloproteinase-8 Is Associated with Worsened Effects of Ischemic Acute Kidney Injury

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Background: After ischemic acute kidney injury (AKI), the role of the matrix metalloproteinase family of proteases (MMPs) is poorly understood. While knock-out models of the gelatinases MMP-2 and MMP-9 offer protection from ischemic AKI, little is known about the role of neutrophil collagenase (MMP-8), presumed to be anti-fibrotic. We recently reported the derivation and validation of serum MMP-8, using microarray technology, as a putative biomarker of severe sepsis associated AKI (SSAKI) in critically ill children.

Methods: Here we report an association between MMP-8 and ischemic AKI using a mouse model of ischemic AKI (bilateral renal pedicle clamping for 30 minutes) in wild type and MMP-8 null mice.

Results: Mice subjected to ischemia-reperfusion had significantly higher serum MMP-8 levels than sham operated controls after 24 hours (61 [50-100] vs. 50 [40-52], p=0.03). However, tissue MMP-8 expression and activity were significantly decreased in ischemic AKI mice at 6, 12, 24, and 48 hours (6h, etc.) after ischemia. (Decrease in activity: 6h – 52%, 12h – 37%, 24h – 25%, 48h – 52%; decrease in expression: 6h – 78%, 12h – 27%; $p < 0.05$ for all experiments). Use of an MMP-8 inhibitor resulted in higher serum creatinine after ischemia versus untreated mice (23% increase, $p = 0.05$). Additionally, the fold increase in serum creatinine after ischemia versus sham was higher in MMP-8 null mice than wild type controls (9.6 vs. 2.5). Finally, immunofluorescent staining 24 hours after ischemia demonstrates decreased tubular epithelial MMP-8 staining in mice after AKI versus sham controls.

Conclusions: Taken together, our data suggest that while serum MMP-8 levels may increase in response to ischemia, mirroring a neutrophilic inflammatory response, MMP-8 is down-regulated at the level of kidney. This may reflect suppression of the homeostatic role of MMP-8 after AKI – required to balance the potential deleterious effects of other MMPs (2 and 9). Further work is required to understand the precise role of MMP-8 after ischemic AKI.

TH-PO007

Exposure of Cultured Proximal Tubular and Mesenchymal Stem Cells to Serum from Rats with Acute Kidney Injury Induces Oxidative and Mitochondrial Stress

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Background: Acute kidney injury (AKI) is a common and largely treatment-resistant syndrome in which complex mechanisms mediate injury and repair. "Uremic" changes in the internal milieu caused by Ischemia/Reperfusion (I/R)-induced AKI result in pathological manifestations in virtually all major organs.

Methods: To further investigate the distant organ effects of of acute azotemia, either induced in rats by bilateral IR-AKI or bilateral nephrectomy (NPHX), we exposed cultured normal rat kidney cells (NRK, proximal tubular) and rat mesenchymal stem cells (MSC), highly renoprotective in AKI (AJP Renal 2005), to 10% serum obtained from rats 24 hrs post 50 min IR-AKI, NPHX or from shams.

Results: Both AKI and NPHX resulted at 24 hrs in similar increases in serum creatinine levels (AKI: 4.9, NPHX: 4.8 mg/dL). Exposure of both cell types to 10% AKI serum for 48 hours caused, compared to incubation with NPHX or sham animal sera, up regulation of anti-oxidant genes (catalase, HO-1), increased reactive oxygen species activity (CM-H_2DCFDA**),** higher mitochondrial membrane potential, increased mitochondrial complex I activity, and reduced mitochondrial reserve capacity (XF Cell Mito Stress Test). Summary: At comparable levels of azotemia (AKI vs. NPHX), AKI induced distinct changes in the internal milieu that adversely and similarly affected NRK and MSC functions.

Conclusions: These data identify yet inadequately defined pathogenic signals that are generated by the injured kidney and that likely target renal tissue, distant organs, and administered MSCs. identification of the exact nature of these noxious signals is expected to further advance our understanding of the acutely uremic state and how it affects renal and systemic organ functions. In addition, these initial observations provide the impetus for the development of therapeutic interventions that are both renoprotective in AKI and protective for therapeutic MSCs.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO008

Proximal Tubule H-Ferritin Mediates Renal Iron Trafficking and Confers Protection in Acute Kidney Injury

Abolfazl Zarjou,[1] Subhashini Bolisetty,[1] Reny Joseph,[1] Amie Traylor,[1] Jozsef Balla,[2] Anupam Agarwal.[1] *[1]Medicine, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, University of Debrecen, Debrecen, Hungary.*

Background: Ferritin plays a central role in iron metabolism and is made of 24 subunits of two types (heavy [H] and light [L] chain). The H-chain has ferroxidase activity that is important not only for iron incorporation but also in controlling toxicity of iron. The purpose of this study was to investigate the role of H-ferritin in acute kidney injury (AKI) and renal iron handling. Proximal tubule specific H-ferritin knockout mice (H-ferritin$^{PT-/-}$) were generated by breeding floxed H-ferritin mice with PEPCK-Cre mice to test the hypothesis that H-ferritin expression in the proximal tubules is essential for protection in AKI.

Methods: Proximal tubule specific H-ferritin knockout mice (H-ferritin$^{PT-/-}$) were generated by breeding floxed H-ferritin mice with PEPCK-Cre mice to test the hypothesis that H-ferritin expression in the proximal tubules is essential for protection in AKI after rhabdomyolysis and cisplatin nephrotoxicity.

Results: Deletion of H-ferritin in the proximal tubules of H-ferritin$^{PT-/-}$ mice was confirmed by standard and real time-PCR, western blot and immunohistochemistry. In wild-type mice, H-ferritin was markedly upregulated in proximal tubules following AKI and was not detectable in the H-ferritin$^{PT-/-}$ mice. H-ferritin$^{PT-/-}$ mice demonstrated significantly higher mortality, worse structural and functional evidence of renal injury and higher levels of cleaved caspase-3 expression in glycerol-induced rhabdomyolysis and cisplatin-induced AKI, despite significantly higher expression of heme oxygenase-1 (HO-1), an anti-oxidant enzyme. Expression of divalent metal transporter-1 (required for normal export of iron from phagosomes) was not affected by deletion of H-ferritin. However, ferroportin expression was significantly lower under both basal and rhabdomyolysis induced-AKI in H-ferritin$^{PT-/-}$ mice.

Conclusions: These studies demonstrate a protective role of H-ferritin in AKI and reveal that proximal tubule H-ferritin is actively involved in regulation of iron trafficking that may have important consequences in AKI and subsequent outcomes.

Funding: NIDDK Support

TH-PO009

Serum microRNAs Are Predictive Biomarkers of Acute Kidney Injury after Cardiac Surgery

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Background: Acute Kidney Injury is one of the most frequent complications after cardiac surgery (CS) and its severity is determinant for patient outcome. miRNAs are small non-coding RNAs which regulate gene expression. Recently, it has been demonstrated that miRNAs are present in body fluids, including serum. Moreover, serum microRNAs have been unveiled as biomarkers for diagnosis and monitoring of several pathologies.

Our aim is to identify and validate serum miRNAs as early biomarkers for AKI development after CS.

Methods: Total RNA was extracted from serum samples. Firstly, a screening experiment was performed and a panel of 10 miRNAs modulated in patients with ischemic AKI was selected. Next, we studied if these miRNAs could predict AKI development in a cohort of patients which underwent CS with cardiopulmonary bypass. We analyzed 50 patients organized in the following groups: IA, adults with 0-2 points in Thakar5 system; IB: Paediatric patients firstly operated; II: adults with altered basal renal function and > 5 points in Thakar5 system; III: adults with normal renal function and > 5 points in Thakar5 system. For each patient, we have analyzed serum samples at different time-points: Basal (before surgery), Immediate post-surgery, 24, 48, 72 hours and 7 days of evolution.

Results: Our results demonstrate that miR-26b, miR-27a and miR-127-3p shows different levels in basal samples between patients which develop ARF and those which do not develop ARF after surgery. Moreover, ROC analysis demonstrates that these miRNAs present AUC values higher than 0.8. Similar results were found at immediate post-surgery time.

Conclusions: In summary, serum levels of miR-26b, miR-27a and miR-127-3p could be early biomarkers of AKI development after CS with predictive value. These new biomarkers could improve patient management and outcome after cardiac surgery by allowing the application of early therapeutic strategies.

Funding: Government Support - Non-U.S.

TH-PO010

Protein SUMOylation in Experimental Models of Acute Kidney Injury

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Background: SUMOylation is a central regulatory post-translational modification whereby Small Ubiquitin-like Modifier proteins (SUMO) are covalently attached to target proteins to regulate their function. SUMOylation has been demonstrated during hypoxia, oxidative, genotoxic and metabolic stresses, suggesting an important role of this modification in cellular stress response. However, it is largely unclear if SUMOylation contributes to the pathogenesis of kidney diseases, such as acute kidney injury (AKI).

Methods: Here we examined protein SUMOylation in two experimental models of AKI: cisplatin nephrotoxicity and renal ischemia-reperfusion. We further examined the regulation of SUMOylation during cisplatin treatment of cultured rat kidney proximal tubular cells.

Results: It was shown that N-acetylcysteine and dimethyurea (two ROS scavengers) could attenuate cisplatin induced SUMOylation. In addition, SUMOylation by SUMO-2/3, but not SUMO-1, was partially suppressed by pifithrin-alpha, a pharmacological inhibitor of p53.

Conclusions: Together, the results demonstrate the first evidence of SUMOylation in AKI, which may regulate tubular cell response to injury. SUMOylation in AKI may result from oxidative stress and p53 signaling.

Funding: NIDDK Support, Veterans Administration Support

TH-PO011

Tubular Cell Necroptosis Is Specifically Triggered by Fas Ligand through a Receptor-Interacting Protein Kinase 1 and 3-Dependent Pathway

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Background: Receptor-interacting protein kinase 1 (RIP1)-mediated programmed necrosis (necroptosis) was demonstrated to significantly contribute to acute tubular necrosis and AKI in an ischemia/reperfusion model. The renal necroptosis signaling pathway downstream of RIP1 and the activating stimulus of necroptosis in vivo have not been identified.

Methods: *Ex vivo*, we freshly isolated primary tubules from RIP3-deficient mice and cell death was analyzed in various readout systems after cisplatin-incubation and hypoxia/reoxygenation. RIP3-deficient mice and caspase-8/RIP3-double deficient mice were investigated in cisplatin-induced acute renal failure and a renal ischemia/reperfusion model in the presence and absence of Nec-1 and the Fas Ligand-inactivating monoclonal antibody MFL3 and inhibitors of other TNFR-family members.

Results: RIP3-deficient Renal tubules were protected from hypoxia/reoxygenation and cisplatin-induced cell death. In vivo, application of MFL3 was similarly effective in protecting mice from renal IRI as was genetic deficiency for RIP3. No further protection was achieved by application of MFL3 in RIP3-deficient mice or in combined extrinsic apoptosis/necroptosis-deficient caspase-8/RIP3-dko mice.

Conclusions: We demonstrate that AKI-induced necroptosis in the kidney depends on a RIPK1 - RIPK3 -dependent pathway. In the first renal caspase-8-deficient *in vivo* model we further demonstrate that blockade of extrinsic apoptosis did not provide any further protection from AKI. Finally, we identified Fas Ligand as the specific activating receptor of this pathway that can be clinically interfered with by either MFL3 or a Fas-Fc fusion protein.

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TH-PO012

Hydrogen Sulfide Donor GYY4137 Prevents Acute Renal Failure

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Background: Ischemia/reperfusion (I/R) and medicine toxicity are the most common causes of acute renal failure (ARF) in hospitalized patients. Recent pharmacologic intervention strategies seem to be incapable of reducing the mortality and morbidity. Hydrogen sulfide (H_2S), recently identified as an endogenous gaseous messenger, was protective for ischemia/reperfusion injury of various organs. However, most commonly used H_2S-releasing "drug" releases H_2S instantaneously, which is different from its biological production. Here, the therapeutic potential of GYY4137, an ideal molecule releasing H_2S over a long period both in vivo and in vitro, was examined in acute renal failure mice.

Methods: In this study, acute renal failure was induced by I/R injury and cisplatin treatment, respectively. GYY4137 was daily administered intraperitoneally. Renal function and morphology were evaluated.

Results: In both ARF mice models, treatment with GYY4137 reduced renal function impairment measured by blood urine nitrogen and serum creatinine. Renal lipid oxidative stress was also decreased in GYY4137-treated groups. Moreover, GYY4137 treatment improved ATP recovery after I/R. Pro-inflammatory NF-κB signaling, as well as chemokines and cytokines was all markedly reduced after GYY4137 treatment. The tubular morphologic changes, including brush boarder loss, tubule dilation, tubular cells necrosisand detachment was greatly attenuated by GYY4137. TUNEL staining also revealed a minimized tubular cell apoptosis in GYY4137-treated groups. Furthermore, the reduced tubular cells apoptosis and necrosis were probably attributed to the preservation of pro-survival Akt and Erk signaling induced by GYY4137.

Conclusions: These results supported GYY4137 as a pharmacologic maneuver to prevent tubule apoptosis and necrosis, recover mitochondrial function, reduce oxidative stress and limit inflammation. GYY4137 might be a novel therapeutic strategy for treatment of ARF.

Funding: Government Support - Non-U.S.

TH-PO013

Overexpression of Protein Kinase G Protects Proximal Tubular Cells from Hypoxia/Reoxygenation Induced Apoptosis

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Background: Inflammation and cell apoptosis are two major pathological responses of acute kidney injury. Our previously published studies demonstrated that overexpression of cGMP dependent protein kinase G-I (PKG-I) attenuates ischemia/reperfusion induced kidney injury (IRI) through inhibiting inflammatory cells infiltrating into the kidney and inhibiting tubular cell apoptosis. In the current studies, we further investigated the role of PKG-I in regulating apoptosis of mouse and human proximal tubular cells in an in vitro IRI model.

Methods: Mouse primary proximal tubular cells were isolated from wild type (WT) mice or transgenic mice (Tg) with overexpression of the constitutively active PKG-I in the kidney. Mouse primary proximal tubular cells were cultured and exposed to hypoxia (1% oxygen) for 24 hours and then returned to normal oxygen condition (21 % Oxygen) for additional 24 hours.

Results: We found that this hypoxia/reoxygenation model induced wild type PTC apoptosis with increased caspase 3 activity, increased TUNEL positive cells, and increased cytochrome C levels in the cell lysates. However, proximal tubular cells from transgenic mice exhibited reduced hypoxia/reoxygenation (H/R) induced apoptosis. To determine the effect of PKG-I on H/R induced apoptosis in human proximal tubular cells, HK-2 cells (purchased from ATCC) were used. HK-2 cells were undergone apoptosis after H/R exposure, associated with reduced PKG-I levels in these cells. Overexpression of the constitutively active PKG-I protected HK-2 cells from H/R induced apoptosis; while knockdown of PKG-I by siRNA promoted HK-2 cells to undergo apoptosis.

Conclusions: Taken together, these data indicate that overexpression of PKG-I protects both mouse and human proximal tubular cells from hypoxia/reoxygenation induced apoptosis.

Funding: NIDDK Support, Veterans Administration Support

TH-PO014

Autophagy Protects against Necrotic Renal Epithelial Cells-Induced Death of Renal Interstitial Fibroblasts

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Background: Necrotic renal proximal tubular cells (RPTC) can induce the death of renal interstitial fibroblasts, the underlying mechanism remains unclear.

Methods: Since autophagy plays either cytoprotective or cytodestructive roles depending on the experimental condition, the present study was carried out to investigate whether necrotic RPTC would induce autophagy of renal interstitial fibroblasts and if so, whether autophagy would contribute to cell death or exerts a protective effect.

Results: Exposure of necrotic RPTC supernatant (RPTC-Sup) induced autophagy in renal interstitial fibroblast cells (NRK-49F) in a time and dose dependent manner, and its induction was earlier than caspase 3 activation. Inhibition of autophagy with 3-methyladenine (3-MA) or knockdown of Beclin 1, a molecule involved in the initiation of autophagosome formation, with siRNA significantly enhanced necrotic RPTC-Sup induced cell death. Necrotic RPTC-Sup induced phosphorylation of extracellular signal-regulated kinases (ERK1/2), p38, c-Jun N-terminal kinases (JNKs) and AKT. Treatment with an ERK1/2 pathway inhibitor, but not by specific inhibitors for p38, JNKs or AKT pathways, blocked NRK-49F autophagy and cell death upon exposure to necrotic RPTC-Sup. Further, knockdown of MEK1 with siRNA also reduced autophagy along with cell death in NRK-49F exposed to necrotic RPTC-Sup. In contrast, overexpression of MEK1/2 increased RPTC-Sup induced fibroblast cell death without enhancing autophagy.

Conclusions: Collectively, this study demonstrates that necrotic RPTC induces both autophagy and cell death and that autophagy plays a cytoprotective or prosurvival role in renal fibroblasts. Further, necrotic RPTC induced autophagy and cell death in renal fibroblasts is mediated by the activation of the MEK1-ERK1/2 signaling pathway.

Funding: NIDDK Support

TH-PO015

AMPK/Autophagy Axis in the Kidney Ischemia Reperfusion Model of AKI

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Background: Kidney ischemia-reperfusion injury (IRI) leads to cellular damage and organ dysfunction. 5'AMP-activated protein kinase (AMPK) activity induces protective cell survival mechanisms, including autophagy. We have shown previously that renoprotective AMPK decreases during IR.

Methods: We examined the molecular effects of AMPK agonists AICAR (0.1g/kg) or metformin (0.3g/kg) applied i.p. 24 hrs prior to kidney ischemia in rats. Plasma creatinine and protein expressions were evaluated after 24 hrs of reperfusion. N=4-5 animals per group.

Results: In IR, accumulation of the linker protein p62, and increase in p70S6k, reflecting mTOR activity, indicate low autophagy (C, IR, AICAR, IR+AICAR for all data sets; p62: 5716±747, 10956±923, 8199±1029, 6445±757; p70S6K: 4623±816, 11281±1018, 4945±670, 4560±232, relative densitometric units for all immunoblots). Autophagy marker LC3-II is increased in IR+AICAR vs IR (LC3-II: 469±137, 1205±169, 885±172, 3276±474), accompanied by normalization of p62 and p70S6K. Caspase-3-dependent cleavage of beclin-1 inactivates beclin-1 induced autophagy and promotes apoptosis. We

observe an increase in caspase-3 and beclin-1 fragments with IR, responses attenuated in IR+AICAR (casp3: 2591±232, 5699±307, 3421±169, 4352±390; beclin1 frag: 774±51, 1541±146, 512±67, 1101±47). The increase in hemoxygenase-1 in IR is attenuated in IR+AICAR, implying less stressor stimulation (HO-1: 240±30, 5000±1135, 669±207, 3397±651). TGF-beta induces Wnt/beta-catenin to increase PAI-1 expression, which enhances fibrosis. Up regulation of beta-catenin and PAI-1 expressions in IR are reduced in IR+AICAR (b-cat: 2190±378, 5465±481, 2636±207, 3204±167; PAI-1: 515±40, 1107±81, 586±40, 771±54). IR+AICAR increases plasma creatinine beyond IR alone (0.061±0.006, 0.116±0.027, 0.052±0.002, 0.221±0.035, in mmol/l). The AICAR effect might reflect a stronger decrease in GFR due to AMPK-induced transport inhibition and tubuloglomerular feedback activation, which could be protective during this early phase of tubular injury. Results with metformin mirrored those for AICAR.

Conclusions: Our data indicate that pharmacological activation of AMPK offers a viable strategy for reducing cellular IRI.

Funding: NIDDK Support, Veterans Administration Support

TH-PO016

Programmed Cell Death 1 Ligands Promote Resistance to Renal Ischemia Reperfusion Injury Gilbert R. Kinsey, Katarzyna Jaworska, Liping Huang, Mark D. Okusa. *Medicine - Division of Nephrology, Center for Immunity, Inflammation and Regenerative Medicine, University of Virginia.*

Background: Ischemia reperfusion injury (IRI) is a major contributor to acute kidney injury. The inflammatory response to ischemic injury, involving innate immune cells such as neutrophils, compounds the renal cell death and loss of function induced by IRI. Recent studies have demonstrated that regulatory T cells (Tregs) suppress the immune response to kidney IRI and preserve renal function. Blockade of the co-inhibitory receptor programmed cell death 1 (PD-1) on the surface of Tregs negates their ability to protect from ischemic kidney injury. We hypothesized that one or both of the known PD-1 ligands must interact with PD-1 on Tregs and possibly other PD-1 expressing cells to mediate resistance to kidney IRI.

Methods: Naïve C57Bl/6 mice were treated with a B7-H1 (PD-L1) blocking antibody, B7-DC (PD-L2) blocking antibody or an isotype control antibody 18 hr prior to mild bilateral renal ischemia. Kidneys were allowed to reperfuse for 18 hr then renal function and histology were assessed and accumulation of leukocytes was measured by flow cytometry.

Results: Compared to isotype control antibody treated mice after IRI (plasma creatinine (PCr) level: 0.5±0.1, n=5; sham PCr: 0.3±0.1, n=3) blockade of either PD-L1 (PCr: 1.3±0.3, n=5) or PD-L2 (PCr: 1.4±0.2, n=5) significantly enhanced renal functional impairment. Likewise, acute tubular necrosis, assessed by H&E staining, was markedly increased in the PD-1 ligand blocking antibody treated mice. Flow cytometric analysis of kidney infiltrating leukocytes revealed that blockade of either PD-1 ligand significantly enhanced accumulation of neutrophils (CD45+Live/Dead cell stain-negativeCD11b+GR-1high) after ischemia and reperfusion.

Conclusions: These studies suggest that both PD-1 ligands participate in the natural defense against kidney IRI and that the PD-1 ligand interactions regulate innate immune responses to renal injury.

Funding: NIDDK Support

TH-PO017

Endothelial Sphingosine 1-Phosphate Receptor 1 Signaling Protects Kidneys from Acute Kidney Injury Amandeep Bajwa, Sangju Lee, Hong Ye, Liping Huang, Mark D. Okusa. *Medicine, CIIR, University of Virginia, Charlottesville, VA.*

Background: Sphingosine 1-phosphate (S1P), a sphingolipid that is the natural ligand for five G-protein coupled receptors (S1P1-5Rs) and S1PR agonists reduced kidney ischemia-reperfusion injury (IRI) in mice. S1P maintains endothelial cell barrier integrity (primarily via S1P1) and functions in the development of a stable and mature vascular system. We sought to determine the role of endothelial S1P1 in mediating tissue protection from IRI *in-vivo* and whether endothelial S1P1 are necessary and sufficient to mediate the protection of S1P1 agonists.

Methods: We used conditional endothelial $S1P1^{-/-}$ ($Tie2CreER^{T2}S1pr1^{fl/fl}$) and control ($Tie2CreER^{T2}$) mice to determine the role of endothelial S1P1, because germline S1P1 deficiency is embryonically lethal. Tamoxifen was given daily (1 mg/mouse; i.p., 5d) prior to mild kidney IRI. Renal injury was assessed by plasma creatinine (PCr; mg/dl), FACS, Evans blue dye to measure changes in vascular permeability and real time RT-PCR.

Results: Under normal conditions, there was no difference in vascular permeability in control and $S1P1^{-/-}$ mice after 5 days of tamoxifen treatment. Compared to control mice (PCr:0.34±0.02) IRI induced more injury in endothelial $S1P1^{-/-}$ mice (0.98±0.10, $p<0.01$). Pretreatment with the S1P agonist FTY720 partially protected $S1P1^{-/-}$ mice, and there were no differences in circulating lymphocyte in control and $S1P1^{-/-}$ mice after IRI with FTY720. Compared to control mice, $S1P1^{-/-}$ mice had higher levels of pro-inflammatory cytokines (CXCL1, MCP-1 and TNF-α) and infiltrating inflammatory cells in the kidney. FTY720-treated $S1P1^{-/-}$ mice had reduced infiltration of pro-inflammatory cytokines and infiltration of inflammatory leukocytes after IRI, similar to control mice.

Conclusions: In summary, endothelial S1P1 activation attenuates kidney IRI and S1P1 on endothelial cells serve as a target for FTY720. We conclude that activation of endothelial S1P1 is vital for kidney tissue protection in addition to known protective effects to induce lymphopenia and proximal tubule survival. This study and our prior studies demonstrate multiple targets of S1P1 activation leading to tissue protection.

Funding: NIDDK Support

TH-PO018

ABCG2 Protects Kidney Side Population Cells from Hypoxic/Ischemic Injurythroughactivating MEK/ERK Pathway Hongbao Liu. *Nephrology, Xijing Hospital, Xi'an, Shaanxi, China.*

Background: Breast cancer resistance protein 1 (BCRP1/ABCG2) is used to identify the side population (SP) within a population of cells, which is enriched for stem and progenitor cells in different tissues. Here, we investigated the role of extracellular-regulated kinase (ERK) 1/2 in the signaling mechanisms underlying ischemic/hypoxic conditions in kidney SP cells.

Methods: The kidney SP cells were isolated using Hoechst 33342 dye-mediated fluorescein-activated cell sorting and then incubated under hypoxia/reoxygenation (H/R) with or without verapamil, a selective BCRP1/ABCG2 inhibitor.ABCG2 expression, ERK activity, cell viability, metabolic activity and membrane damage were tested after H/R treatment. To evaluate the role of ERK 1/2 on the expression and function of ABCG2, the expression of mitogen-activated protein kinase/ERK kinase (MEK), which preferentially activates ERK, was upregulated bytransfection with the recombinant sense expression vector pcDNA3.1-MEK and downregulated by pretreatment with U0126, a specific MEK inhibitor.

Results: We found that hypoxia activated ERK activity in the kidney SP cells but not in non-SP cells both *in vitro* and *in vivo*. Overexpression of MEK mimicked hypoxia-induced ABCG2 expression. Contrarily, U0126 inhibited hypoxia- and MEK-upregulated ABCG2 expression. Furthermore, H/R induced significant increases in nuclear, metabolic and membrane damage in both SP cells and non-SP cells; however, this H/R-induced cytotoxicity was much more severe in non-SP cells than in SP cells. Importantly, the viability of kidney SP cells was enhanced by MEK overexpression and inhibited by U0126. Verapamil treatment reversed MEK-induced viability of kidney SP cells. When administered systemically into animals with renal ischemia/reperfusion injury, the SP cells significantly improved renal function, accelerated mitogenic response, and reduced cell apoptosis. However, this improved therapeutic potential of SP cells was significantly reduced by pretreatment with verapamil.

Conclusions: Collectively, these findings provide evidence for a crucial role for the MEK/ERK-ABCG2 pathway in protecting kidney SP cells from ischemic/hypoxic injury.

TH-PO019

Monitoring Live Actin Cytoskeleton Alterations in the Setting of Ischemia-Reperfusion Injuries Peter R. Corridon,[1] George Rhodes,[1] Robert L. Bacallao,[1,2] Simon J. Atkinson.[1,3] *[1]Department of Medicine, Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN; [2]Richard, L. Roudebush VA Medical Center, Indianapolis, IN; [3]Department of Biology, School of Science, Indiana University - Purdue University Indianapolis (IUPUI), Indianapolis, IN.*

Background: Our present understanding of the kidney in various disease settings is limited. As a result, renal dysfunction is both a common and progressive problem threatening the lives of millions worldwide. Ischemia-reperfuson injury is a major cause of such dysfunction. Gene delivery has been proposed as a novel approach to investigate underlying causes of this complex disorder. Recent work conducted in our laboratory has developed hydrodynamic gene delivery as a robust method of providing significant transgene expression in all nephron segments.

Our present understanding of the kidney in various disease settings is limited. As a result, renal dysfunction is both a common and progressive problem threatening the lives of millions worldwide. Ischemia-reperfuson injury is a major cause of such dysfunction.

Gene delivery has been proposed as a novel approach to investigate the underlying causes of this complex disorder. Recent work conducted in our laboratory has developed hydrodynamic gene delivery as a viable and robust method of gene delivery by providing efficient transgene expression.

Methods: Utilizing this technique, we introduced fluorescent actin plasmid expression vectors in rat kidneys, subjected to ischemia-reperfusion injury. We then tracked subsequent structural and functional changes in actin cytoskeleton renal injury initiated by renal pedicle cross clamp using intravital multiphoton microscopy.

Results: We were able to monitor the loss and remodeling of actin components in live proximal tubule epithelial cells, which is the major site of ischemia-reperfusion injury.

Conclusions: These results provide a novel approach to study in detail cellular and subcellular events associated with renal ischemic injury. This work paves the way for evaluating protective interventions in ischemia reperfusion and extends the use of reporter constructs for use in intravital imaging studies.

Funding: NIDDK Support, Veterans Administration Support

TH-PO020

Novel Mouse Models for Studying the Role of Renal Cytochrome P450 Enzymes in Chemically Induced Kidney Injury Senyan Liu,[1,2] Yunyi Yao,[2] Shijun Lu,[2] Xinxin Ding,[2] Changlin Mei,[1] Jun Gu.[2] *[1]Division of Nephrology, Changzheng Hospital, Shanghai, China; [2]Wadsworth Center, New York State Department of Health, Albany, NY.*

Background: The kidney is a primary target for numerous toxic compounds. Cytochrome P450 enzymes (P450s), responsible for metabolic activation of chemical compounds, are predominantly expressed in proximal tubules in the kidney. The in vivo role of renal P450s in chemically induced renal injury is not clear due to lack of appropriate animal models.In this study, we have developed one mouse model having proximal tubule-specific deletion of cytochrome P450 reductase (Cpr) gene (the enzyme required for all microsomal P450 activities), and another mouse model with proximal tubule-specific rescue of CPR activity (extra-renal Cpr-low) bred from an existing mouse with global suppression

of CPR activity (Cpr-low). Kidney injury induced by chloroform was studied in the two new mouse models along with Cpr-low and wild-type (WT) mice.

Methods: The mice were treated with a single oral dose of chloroform at 200 mg/kg. The tissue samples were collected at 24 h after the treatment. The kidney injury was examined by serum BUN and creatinine and pathology, and the levels of chloroform in the blood and tissues were analyzed by gas chromatography.

Results: The Cpr gene deletion was observed in 84% of the proximal tubules in kidney-Cpr-null mouse, and the CPR activity rescue was achieved in 76% of the proximal tubules in extrarenal-Cpr low mouse. Chloroform induced tubular lesions were less severe in kidney-Cpr-null mice and Cpr-low mice, compared to WT and extra-renal-Cpr low mice, respectively, which is in agreement with BUN and creatinine levels in the four strains. There is no significant difference in chloroform levels in the blood, liver, and kidney between kidney-Cpr-null and WT mice, and between extra-renal-Cpr low and Cpr-low mice.

Conclusions: The novel mouse models are useful for studying the in vivo roles of proximal tubule P450 enzymes in renal function and injury and local P450 dependent metabolic activation plays an important role in kidney injury induced by chemical compounds such as chloroform.

Funding: Government Support - Non-U.S.

TH-PO021

Outcome of Acute Renal Injury in Diabetic Mice with Experimental Endotoxemia: Role of Hypoxia Inducible Factor-1α

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Background: The role of diabetes in the outcome of acute renal injury (AKI) is not well defined. Herein we evaluate the outcome of lipopolysacharide (LPS)-induced AKI in experimental diabetes, as well as the potential role of Hypoxia inducible factor (HIF-1α) in this condition.

Methods: Diabetes was induced by streptozotocin (65mg/Kg) 6 weeks before LPS (10mg/Kg) injection (n=104). Renal HIF-1α expression was assessed by Western blot and immunohistochemistry. The effect of glycated albumin (GA) and LPS on HIF-1α expression as well as the production of the vascular endothelium growth factor (VEGF) in proximal tubular HK-2 cells was also analyzed by Western blot and ELISA respectively.

Results: Although 6h after LPS injection diabetic and non-diabetic mice developed a similar decrease in renal function as assessed by endogenous creatinine clearance, the diabetic animal showed a better recovery of renal function throughout the study (72h). HIF-1α was found to be upregulated in diabetic mice. After LPS injection, all animals showed an upregulation of HIF-1α, although it was statistically higher in the diabetic group. GA was found to upregulate HIF-1α in HK-2 cells, which resulted in increased production of the putative renal protector VEGF. Interestingly, LPS cooperated with AG to induce an over-increase in the expression of HIF-1a.

Conclusions: Diabetic mice display a better recovery of renal function after experimental endotoxemia. Interestingly, diabetic animals showed an increased expression of HIF-1α that was reproduced by incubating renal cells with GA. Since GA determined an HIF-1a-dependent increase in VEGF, a survival factor for tubular cells, our findings suggest that the diabetic condition displays renal upregulation of HIF-1α that might function as a "precondition state" offering protection from endotoxic AKI.

Funding: Government Support - Non-U.S.

TH-PO022

Role of Megalin in Cisplatin Induced Acute Kidney Injury

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Background: Megalin, a proximal tubule, multiligand, endocytic receptor, is involved in the nephrotoxic effect of a number of substances mediating the tubular uptake of these. Cisplatin, a nephrotoxic chemotherapy drug, affects proximal tubule endocytic function and megalin gene polymorphisms are associated with increased sensitivity to cisplatin ototoxicity suggesting involvement of megalin in cisplatin toxicity. We examined the role of megalin in cisplatin induced acute kidney injury.

Methods: Megalin knockout (MKO) and wild type (WT) mice were injected with 15mg/kg of cisplatin i.p. Blood, urine, and renal tissue were collected after 4 days. Cisplatin uptake was quantified by ICP-MS. Renal tissue damage was examined histologically. We further analysed plasma creatinine (HPLC), renal expression of damage markers (Q-PCR), and urinary excretion of KIM1 and NGAL (ELISA).

Results: Following cisplatin treatment a significant increase in plasma creatinine and histological evidence of tubular necrosis was observed, but there was no difference between MKO and WT and no difference in renal uptake of cisplatin. Urinary KIM1 was higher at baseline in MKO compared to WT. After cisplatin treatment urinary NGAL and KIM1 were increased in both groups and the excretion of both markers was higher in MKO (Figure 1A). Furthermore, KIM1 expression after cisplatin was higher in WT than MKO (Figure 1B).

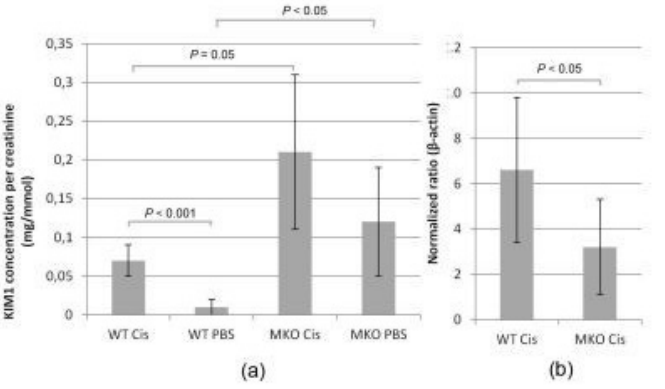

Figure 1: (a) KIM1 Excretion in urine. (b) Renal expression of KIM1 mRNA

Conclusions: Megalin knock out does not protect against cisplatin induced acute kidney injury nor does it affect renal cisplatin-uptake; however, a differential expression and urinary excretion of the renal damage markers NGAL and KIM1 were observed. Urinary KIM1 was higher in MKO despite lower renal expression suggesting a role of megalin for the expression and clearance of KIM1.

Funding: Pharmaceutical Company Support - Lundbeck Foundation, FSS, Aarhus University

TH-PO023

A Potential Acute Renal Failure Marker, microRNA494, Modulates Expression of the Inducible Transcription Factor ATF3 and Affects Kidney Function

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Background: Previous reports have shown ATF3 plays a protective role in acute renal failure, ischemia/reperfusion (I/R) injury. The aim of this study was to analyze the potential regulation of ATF3 expression by microRNAs.

Methods: Lenti-miRNA overexpression, mouse renal ischemia reperfusion.

Results: In this study, we demonstrated that miR-494 target 3' untranslated region of ATF3 reporter and decreased ATF3 mRNA expression by reporter assay and molecular analysis. Overexpression miR-494 in mice kidney was significantly attenuate ATF3 mRNA and protein, accompany with I/R induced inflammatory related factor include IL-6, MCP-1, P-selectin resulting cells apoptosis and renal function decreased. Importantly, the mice urinary miR-494 was increased significantly earlier than serum creatinine after renal I/R. In clinical, the urinary miR-494 expression was higher in acute kidney injury (AKI) patients than patients with no AKI and normal.

Conclusions: These data suggest that up-regulation of miR-494 contributes to inflammatory factor or adhesion molecule induced kidney injury after I/R via inhibition ATF3 expression and miR-494 may act as an early specific and non-invasive biomarker in AKI.

TH-PO024

Magnesium Controls Cisplatin-Induced Acute Kidney Injury

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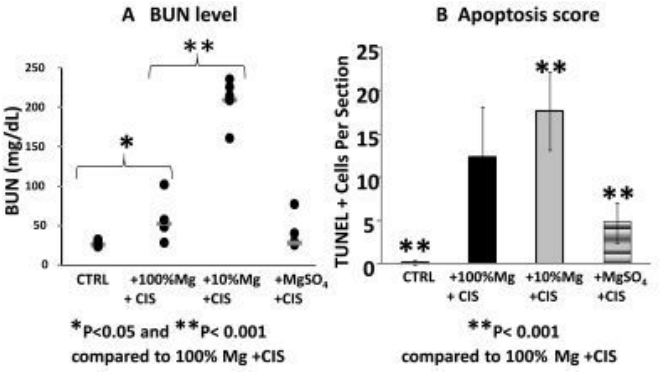
Background: Cisplatin-induced acute kidney injury (AKI) limits the use of this chemotherapeutic agent. Renal handling of magnesium (Mg) is severely impaired by cisplatin. Because Mg deficiency is associated with enhanced inflammation, we examined the effects of Mg deficiency and Mg supplementation on cisplatin-induced renal epithelial cell death and cisplatin-induced AKI.

Methods: ***In vitro:*** Renal epithelial (HK-2) cells were grown in 0%, 100% and 200% Mg ($MgSO_4$) media and treated with cisplatin; cell death was measured 48hrs later. ***In vivo:*** C57BL/6 mice (n=4-5 per group, males) were maintained on normal (100% Mg) or 10% Mg deficient diets for 2wks; cisplatin (20mg/kg, ip) was injected on day 14. One group of mice received $MgSO_4$ (200mg/kg, ip daily) on days 14-16. Mice were euthanized 48hrs post cisplatin. Kidney function was measured by blood urea nitrogen (BUN) levels; renal cell apoptosis was analyzed by TUNEL and inflammatory and apoptotic markers were measured by Q-PCR.

Results: ***In vitro*****:** Mg deficiency significantly increased cisplatin-induced HK-2 cell death; $MgSO_4$ significantly reduced cisplatin-cell death. ***In vivo*****:** Mg deficiency significantly elevated BUN levels and $MgSO_4$ decreased BUN levels in cisplatin-mice treated compared to cisplatin controls (Fig.1A). Mg-deficient mice had significantly more cisplatin-induced renal apoptosis compared to cisplatin controls; $MgSO_4$ significantly reduced cisplatin-renal apoptosis (Fig. 1B).



Renal markers of inflammation (CCL2, CXCL2, CXCL10) and apoptosis (BAK) were increased in Mg-deficient mice and decreased in $MgSO_4$ mice compared to cisplatin controls.

Conclusions: Mg deficiency worsens cisplatin-AKI (as determined by BUN levels) and promotes renal inflammation and apoptosis. $MgSO_4$ improves cisplatin-induced AKI as evidenced by lower BUN levels, apoptosis, and inflammation.

Funding: Private Foundation Support

TH-PO025

Deletion of Ace2 Gene Exacerbates Kidney Injury in Mice after Renal Ischemia Reperfusion Fei Fang,[1] James W. Scholey,[1] George Chu Liu,[1] Xiaohua Zhou,[1] Amanda Hu,[1] Vanessa R. Williams,[1] Gavin Oudit,[2] Rohan John.[1] *[1]Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada; [2]Department of Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: Activation of the renin-angiotensin system has been implicated in several forms of kidney injury, including ischemia/reperfusion (I/R). ACE2 is a homologue of ACE and functions to counterbalance the effect of ACE by metabolizing angiotensin II (AngII) to angiotensin-(1-7) (Ang-(1-7)). Previous reports suggest that ACE2 is beneficial to kidney in models of chronic kidney disease and diabetic nephropathy. However the role of ACE2 in I/R-induced kidney damage has not been fully elucidated. We hypothesized that ACE2 is protective against acute kidney I/R injury, and we tested this hypothesis by studying I/R in wild-type control mice and mice with a deletion in the gene for *Ace2*.

Methods: 8-week old male control and ACE2 knock-out (ACE2 KO) mice were subjected to sham operation or left kidney ischemia for 45 min. Kidneys were harvested after 48 hours of reperfusion for measurement of AngII levels, histologic kidney injury, inflammatory gene expression (IL-1β, IL-6 and TNFα) and immunohistochemical analysis of infiltrating cells, oxidative stress, and apoptosis.

Results: I/R increased wet kidney weight and resulted in severe histologic kidney injury compared to kidneys subjected to sham operation. Deletion of the *Ace2* gene did not significantly affect the injury score. AngII levels were higher in kidneys of ACE2 KO mice than in wild type mice following I/R. ACE2 KO mice had higher IL-1β, IL-6 and TNFα mRNA levels compared to wild-type mice after IR, though the differences were significant only for IL-1β. T cell and neutrophil infiltration was increased in kidneys of both ACE2 KO and wild-type mice after I/R. Deletion of the *Ace2* gene further increased T cell and neutrophil infiltration in association with enhanced oxidative stress (nitrotyrosine staining) and apoptosis (activation of caspase-3).

Conclusions: Deletion of the *Ace2* gene increases AngII levels and oxidative stress in kidneys subjected to I/R and promotes I/R-induced inflammation and apoptosis.

TH-PO026

BNIP3 Mediates Mitophagy (Mitochondrial Autophagy) of Renal Tubular Cells in Acute Kidney Injury Masayuki Ishihara,[1] Madoka Urushido,[1] Nazuki Okada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Toru Kagawa,[1] Toshihiro Takao,[2] Yoshio Terada.[1] *[1]Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan; [2]Division of Community Medicine, Department of Community Nursing, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan.*

Background: Autophagy is one of the systems, which protects renal tubular cells from acute kidney injury (AKI). Mitophagy is one of the forms of autophagy, and may play damaged mitochondria recycling system. However, little is known about mechanism and roles of mitophagy in AKI. Bcl-2/adenovirus E1B 19kDa-interacting protein 3 (BNIP3) is one of the target proteins of hypoxia inducible factor -1a (HIF-1a). We also report that autophagy in renal tubules in AKI is induced by at least two independent pathways, p53-sestrin2 pathway and HIF-1a-BNIP3 pathway The aim of this study is to reveal the roles of BNIP3 in mitophagic pathway in AKI.

Methods: We used rat ischemia/reperfusion (I/R) AKI model in vivo and cultured renal tubular cells as an in vitro AKI model. We established NRK cells which stably transfected with a fusion protein of green fluorescent protein and light chain 3 (LC3-GFP) as a marker of autophagy. Overexpression of BNIP3 induced autophagy in NRK-LC3-GFP cells and induced LC3 protein expression. We used Mitochondria-red plasmid to detect mitochondria in confocal microscopy and also used electron-microscope for detection of mitophagy.

Results: The expression of BNIP3 is up-regulated after I/R in proximal tubules in immunostaining and immunoblotting. BNIP3 is up-regulated in cytoplasm under hypoxia condition in NRK cells. Mitophagy detected as LC3 and Mitochondria-Red double positive particles are significantly increasing under hypoxia condition. Overexpression of BNIP3 induced mitophagy in NRK cells even in normal oxygen condition. We also confirm mitophagy in electron microscope in hypoxia and BNIP3 overexpression.

Conclusions: Although there will be several pathway of mitophagy, in this study, we revealed BNIP3 induce mitophagy in renal tubular cells. We firstly demonstrate that mitophagy are observed in proximal tubules in hypoxic condition and BNIP3 regulates mitophagy in renal cells.

TH-PO027

Meprin Metalloproteases Cleave OS-9 In Vitro and In Vivo Elimelda Moige Ongeri. *Biology, North Carolina A&T State University, Greensboro, NC.*

Background: Meprins are metalloproteinases that are abundantly expressed in the brush border membranes (BBM) of proximal kidney tubules, and have been implicated in the pathology of ischemia-reperfusion (IR) induced renal injury. Osteosarcoma-9 (OS-9), a cytoplasmic protein, has been shown to interact with the carboxyl-terminal tail of meprin β. However, it has not been determined if OS-9 is a meprin substrate, and whether OS-9 interaction with meprin β is significant *in vivo.* More importantly, OS-9 has been shown to interact with the hypoxia-inducible factor 1α (HIF-1α) and prolyl hydroxylase, a protein which mediates the cell's response to changes in oxygen concentration. The interaction of OS-9 with HIF1α promotes oxygen-dependent degradation/hydroxylation of HIF1α, Von Hippel–Lindau tumor suppressor(VHL) binding, and inhibition of HIF-1-mediated transcription. OS-9 is associated with the endoplasmic reticulum (ER) and is present in both nuclear and cytoplasmic protein extracts, suggesting that it could traffic HIF proteins between the nucleus and the ER during the hypoxic response.

Methods: To determine whether meprin β cleaves OS-9, kidney proteins from meprin αβ double knockout (KO) mice were incubated with the activated form of purified recombinant meprin β. Control reactions were incubated with (i) tris buffer, and (ii) latent meprin β. Products were electrophoretically separated, and Western blot analysis used to detect OS-9 protein bands. To determine whether meprin β cleaves OS-9 *in vivo*, wild-type (WT) and meprin KO mice were subjected to renal IR, and kidney tissue harvested at 3h- and 6h-post renal artery clamping. Western blot analysis was used to evaluate OS-9 fragments in kidney proteins.

Results: Degradation of OS-9 was observed in proteins from meprin αβ KO kidneys incubated with activated meprin β, but not in control reactions. Fragmentation of OS-9 was observed in kidney proteins from WT mice subjected to IR, with a unique 60 KDa OS-9 fragment being detected. This fragmentation was not observed in proteins from meprin β KO kidneys subjected to IR, or sham operated WT controls.

Conclusions: These data suggest that meprin β plays a role in the hydrolysis of OS-9 during hypoxia/ATP-depletion *in vivo.*

TH-PO028

Cdk2-Dependent Bax Activation Is Necessary for Cisplatin-Induced Renal Cell Death *In Vitro* and *In Vivo* Peter M. Price,[1,2] Adel Tarcsafalvi,[1] Rawad Hodeify,[1] Nang San Hti Lar Seng,[1] Judit Megyesi.[1] *[1]UAMS; [2]VA Med Ctr.*

Background: Expression of p21 *in vitro* and *in vivo* protected kidney cells from cisplatin cytotoxicity. The protective effect of p21 *in vitro* was by its inhibition of Cdk2 activity, and Cdk2 inhibitory drugs also protected from cisplatin cytotoxicity *in vitro* and *in vivo*. Similarly, induction of DN-Cdk2 in kidney proximal tubules protected from cisplatin-induced AKI. We now show that the mechanism of this protection is by prevention of Bax activation. This structural change in Bax is required for Bax oligomerization in the mitochondrial membrane and subsequent permeabilization of the outer mitochondrial membrane (MOMP), release of intermembrane proteins, caspase activation, and cell death.

Methods: Cultured mouse proximal tubule cells were used as an *in vitro* model; cells were exposed to cisplatin at 25 μM. Mice were administered cisplatin at 20 mg/kg body weight. Bax activation was assessed by a conformation-specific antibody; cell fractionation into cytoplasmic, nuclear and mitochondrial fractions was by differential centrifugation. Proteins were detected by Western blot after PAGE.

Results: Bax was reported to shuttle between the cytoplasm and the mitochondrial membrane, where it interacts with anti-apoptotic proteins, such as Bcl-xL, and is retrotranslocated back to the cytoplasm. Accumulation of Bax on mitochondrial membrane causes auto-activation. Previous studies by other investigators showed that Bcl-xL phosphorylation caused dissociation of Bax/Bcl-xL heterodimers, in which Bax release was responsible for induction of apoptosis. We provide preliminary evidence that Bcl-xL is phosphorylated by Cdk2 *in vitro* and *in vivo* after cisplatin exposure. Also, we show that cytoplasmic Bax accumulated on mitochondrial membranes after cisplatin exposure, supporting the idea that Bax/Bcl-xL interactions were affected.

Conclusions: We propose the following hypothesis for the mechanism of Cdk2-dependence of cisplatin induced cytotoxicity in vitro and nephrotoxicity in vivo. Cdk2 phosphorylation of Bcl-xL interferes with Bax retrotranslocation back into the cytoplasm, resulting in Bax accumulation on mitochondrial membranes, Bax activation, MOMP, and subsequent cell death.

Funding: NIDDK Support, Veterans Administration Support

TH-PO029

Pathomechanistic Relevance of the Extracellular (ec) Superoxid-Dismutase SOD in Ischemic Acute Kidney Injury (iAKI) Boris Betz,[1] Kerstin Möller-Ehrlich,[2] Christoph Wanner,[1] Reinhard Schneider.[1] *[1]Dept. Nephrology, University Hospital Wuerzburg; [2]Center of Experimental Moleculare Medicine (ZEMM), Wuerzburg.*

Background: Generation of reactive oxygen species is an important pathomechanism in iAKI. Here, superoxide radicals (O2-*) are responsible for e.g. inactivation of nitric oxide (NO) and generation of further oxygen derived radicals with detrimental effects on cellular or protein levels. Superoxide dismutase (SOD) initiates the first step of O2-*-radical detoxification. Here, we analyzed the influence of the extracellular SOD (ecSOD) in iAKI with regard to renal function, inflammation and tubular epithelial transport in an ecSOD deficient mouse model.

Methods: iAKI was induced in ecSOD -/- and ecSOD +/+ mice (c57bl6) by ligation of renal arteries for 45 min followed by reperfusion. 24h and 72h after ischemic injury the clearance of inulin (CIN, resp. GFR) and para-aminohippuric acid (CPAH, resp. RPF) were measured. PAH net secretion (NSPAH) was determined to elucidate tubular epithelial transporter capacities and respective expressions (OAT1/OAT3). In parallel, markers of inflammation which include COX1/COX2, HO1/HO2, HSP70, MCP1, IL1b, CX3CL1/CX3CR1 and NO-synthases (iNOS/eNOS) were characterized in homogenate of renal cortex by qPCR and western blot.

Results: Nonischemic ecSOD -/- and ecSOD +/+ demonstrated neither difference regarding CIN, CPAH and NSPAH nor expression of OATs, but eNOS and markers of inflammation were already induced (e.g. COX1, HO1, HSP70). In iAKI, ecSOD deficiency caused pronounced functional deterioration of CIN, CPAH and NSPAH but no additional down regulation of OATs. Following I/R injury, ecSOD deficient mice exhibit a differential regulted inflammatory pattern (COX1/COX2, HO1/HO2, HSP70, MCP1, IL1b, CX3CL1/CX3CR1), whereas and iNOS induction was delayed.

Conclusions: These results emphasize the detrimental pathophysiologic impact of the extracellular O2-* radical in iAKI with regard to renal function in an ecSOD deficient mouse modell. Tubular epithelial transport might be additionally regulated by primary or secondary O2-* depenent mechanisms in iAKI.

Funding: Government Support - Non-U.S.

TH-PO030

Small Heat Shock Protein beta-1 (HSPB1 or HSP27) Is Up-Regulated and Regulates Autophagy and Apoptosis of Renal Tubular Cells in the Acute Kidney Injury In Vitro and In Vivo Kosuke Inoue,[1] Koji Ogata,[1] Masayuki Ishihara,[1] Madoka Urushido,[1] Nazuki Okada,[1] Yoshiko Shimamura,[1] Kazu Hamada,[1] Toru Kagawa,[1] Toshihiro Takao,[2] Yoshio Terada.[1] *[1]Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Oko-cho, Nankoku, Kochi, Japan; [2]Department of Community Nursing, Kochi Medical School, Kochi University, Oko-cho, Nankoku, Kochi, Japan.*

Background: HSPB1 is a member of small heat shock proteins and participates in several processes in cells. HSPB1 was reported to protect cells against oxidative stress. Autophagy is one of the systems which protects life from many kinds of stresses. Autophagy is thought to play an important role for preventing AKI from stresses. However, little is known about the role of HSPB1 in autophagy and apoptosis of AKI pathogenesis.

Methods: We used a rat ischemia/reperfusion AKI model in vivo and cultured renal tubular cells as an in vitro model. We observed up-regulation of HSPB1 mRNA and protein expression in 6-24 h and 12-72 h after ischemia/reperfusion by RT-PCR and western blot analysis. In immunohistological examination, HSPB1 expressed in the proximal tubule cells and co-localized with light chain 3 (LC3). To elucidate the regulation of HSPB1, we evaluated the promoter activity and expression of HSPB1 in NRK-52E cells in the presence of H_2O_2.

Results: HSPB1 promoter activity, mRNA, and protein expression were induced by H_2O_2 dose-dependently. To examine HSPB1 regulates autophagy or not, we established NRK cells which stably transfected with a fusion protein between GFP and LC3 as a marker of autophagy. Overexpression of HSPB1 induced autophagy in NRK cells in normal oxygen condition by confirmed LC3 positive granules in confocal microscopy and electron microscope. Furthermore autophagy induced by H_2O_2 (400μM) was inhibited by transfection of siRNA for HSBP1. Overexpression of HSPB1 reduced H_2O_2 (600μM) induced-apoptosis measuring by caspase3 activity.

Conclusions: We showed that HSPB1 expression increased in oxidative stress in the AKI. Increments of HSPB1 expression caused autophagy and inhibited apoptosis in renal tubular cells. These results indicate the up-regulated HSPB1 play a role in the pathophysiology of AKI.

TH-PO031

Deletion of NOS1 in the Macula Densa Exacerbates Ischemia-Induced Acute Kidney Injury Yan Lu, Kiran B. Chandrashekar, Ying Ge, Yiling Fu, Robert Kampen, Istvan Arany, Luis A. Juncos, Richard J. Roman, Ruisheng Liu. *University of Mississippi Medical Center.*

Background: Nitric oxide synthase 1 (NOS1) is highly expressed in the macula densa (MD) and generates NO that dilates the afferent arteriole (Af-Art) by blunting tubuloglomerular feedback (TGF). Increased tone of the Af-Art contributes to the fall in GFR following acute kidney injury (AKI), but the role of MD NOS1 and alterations in TGF in this process is unknown.

Methods: In the present study we generated a new MD specific NOS1 knockout mouse strain to study the role of MD derived NO in ischemia/reperfusion (I/R) injury. The MD specific NOS-1 KO mice were generated by crossing NKCC2 Cre mice with $NOS1^{flox/flox}$ mice in which exon-6 of NOS1 is targeted to inactivate all splice variants of NOS1. We confirmed that NOS1 is expressed in the MD in wild type (WT) mice and verified the deletion of NOS1 in the MD in the KO mice by immunohistochemistry. NO generation in the MD of KO and WT mice was measured in isolated perfused MD preparation using DAF-2.

Results: When we switch tubular NaCl from 10 to 80 mM, NO generation in the MD increased from 114±12 to 165±11 units/min in WT mice, NO generation in the MD was absent in the KO mice (n=5; $p<0.01$). TGF was measured *in vivo* using micropuncture when flow to the MD was increased from 0-40 nl/min. TGF was 4.5±0.3 mmHg in WT and enhanced to 8.4±0.7 mmHg in the KO animals (n=5; $p<0.01$). The effect of MD NOS1 deletion on AKI was compared in WT and KO animals subjected to bilateral renal ischemia for 18 min at 37°C. Twenty four hours post I/R injury, serum creatinine concentration (mg/dl) rose to 0.89±0.03 in KO mice *versus* 0.56±0.02 in WT animals and 0.35±0.02 in the sham controls. NGAL excretion (pg/ml) increased from 349±18 to 2975±42 after I/R in KO mice, while it increased from 283±21 to 2327±37 in WT controls. Concentration of nitrotyrosine in kidney homogenates (μmol/L) was 2.4±0.4 in KO mice and 1.5±0.5 in WT controls ($p<0.05$).

Conclusions: These data indicated that KO of NOS1 in the MD which augments TGF response worsens ischemia-induced AKI. Maintaining NO levels in the kidney to attenuate TGF may provide a new therapeutic strategy for the treatment for AKI.

Funding: Other NIH Support - National Heart, Lung, and Blood Institute

TH-PO032

Roles of Endoplasmic Reticulum Stress in the Radiocontrast-Induced Nephropathy: Emphasize in Caspase 12 Activation Zong-yu Li,[2] Cheng-tien Wu,[3] Kuan-Yu Hung,[2] Chih-Kang Chiang,[123] Shing-Hwa Liu.[3] *[1]Department of Integrated Diagnostics & Therapeutics, National Taiwan University Hospital, Taipei, Taiwan; [2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [3]Institute of Toxicology, College of Medicine; National Taiwan University, Taipei, Taiwan.*

Background: Contrast medium (CM)-induced nephropathy played a significant role in acute kidney Injury. We previously demonstrated activation of GRP78 /EIF2α-phosphorylation played a protective role in urografin-induced tubular injury. In this study, we comprehensively examined endoplasmic reticulum (ER)-related proapoptotic activators in urografin-induced tubular cells injury.

Methods: Male Wistart rats were intravenously injected with indomethacin and L-NAME, and followed by Urografin (10 ml/kg) administration to ensure the induction of CM-induced nephropathy. Rats were sacrificed for pathology and cellular signals studies 24 and 48 hours later. Urografin-treated normal rat kidney epithelial cells (NRK-52E) were pretreated with specific ER-stress inhibitors or siRNA, and then analyzed the tubular apoptosis.

Results: Treatment with urografin significantly induced CM-induced nephropathy, which presented as tubular dilation, tubular cells detachment and increasing in serum creatinine level in rats' sample. Tubular apoptosis, ER stress-related proapoptotic activators, and kidney injury molecule-1 were induced in CM-treated kidney. We found urografin activated ER stress-related signals, including ATF6/CHOP, Bax/caspase 12 and IRE/caspase 12 pathways. The findings suggested ER stress implicated in the urografin-induced tubular apoptosis. We further explored the roles of ER signals by administered specific blockers or siRNA of ER pathways in NRK-52E cells. We found that ATF6 inhibitor (AEBSF), JNK phosphorylaed inhibitor (SP600125), and CHOP siRNA failed to reverse the CM-induced tubular apoptosis, but Caspase 12 inhibitor (zATAD), at least partially, attenuated Urografin-induced renal tubular apoptosis.

Conclusions: In addition to GRP78 /EIF2α-phosphorylation, ER stress-dependent caspase12 pathway might be a novel therapeutic target in the CM-induced kidney injury.

Funding: Government Support - Non-U.S.

TH-PO033

ERK ½ Activity Mediates Hydrogen Peroxide-Induced Renal Epithelial Cell Injury that Is Inversely Affected by Occludin Protein Content Danielle Janosevic, Victoria V. Rohring, Josephine Axis, Kurt Amsler. *Biomedical Sciences, New York College of Osteopathic Medicine of NYIT, Old Westbury, NY.*

Background: Oxidative stress has been implicated in the disruption of renal epithelial tight junctions and consequent increased paracellular permeability in several pathologic processes including ischemia/reperfusion injury, radiocontrast-induced nephropathy, and exposure to calcium oxalate.

Methods: In order to study this process in more detail, we examined the effect of H_2O_2 treatment, as a model of oxidative stress, on paracellular permeability of two renal epithelial cell lines, LLC-PK_1/Cl4 (proximal tubule-like) and MDCK II (distal tubule-like). Permeability of the two components of paracellular permeability, the pore pathway (small solutes and ions) and the leak pathway (large solutes), was monitored by TransEpithelial Resistance (TER) and calcein flux, respectively. Cell damage was monitored with Trypan Blue staining.

Results: At sublethal concentrations, H_2O_2 produced a concentration-dependent increase in calcein flux in both renal cell lines. In contrast, TER only decreased at H_2O_2 concentrations that also increased the number of Trypan Blue-staining cells. ERK ½ inhibitors blocked both the increased calcein flux produced at sublethal H_2O_2 concentrations and the increased calcein flux, TER, and cell damage produced at higher H_2O_2 concentrations. H_2O_2 produced

a decrease in basal F-actin stress fibers and a modest change in occludin protein localization. Occludin knockdown in MDCK cells produced an enhanced responsiveness to sublethal H_2O_2 treatment, whereas, occludin overexpressing MDCK cells were resistant to H_2O_2 at similar concentrations. ERK ½ inhibition attenuated the H_2O_2-induced increase in calcein flux in both occludin knockdown and occludin overexpressing cells.

Conclusions: Our results indicate that ERK ½ mediates both the H_2O_2-induced increase in leak pathway at sublethal concentrations and increased cell damage at lethal concentrations. Occludin protein protects against both the sublethal and lethal effects of H_2O_2.

Funding: Private Foundation Support

TH-PO034

A Model of Chronic Cisplatin-Induced Kidney Injury in Mice with Cancer

Hyun-Jung Kim, Raphael A. Nemenoff, Zhibin He, Danica Ljubanovic, Charles L. Edelstein. *Univ. of Col. Denver.*

Background: Patients with cancer may require chronic administration of cisplatin (Cis). However, most previous in vivo studies of Cis-induced AKI (CIA) have been in models of acute (3 days), high dose (25mg/kg) Cis administration in normal mice. Aim of study was to develop a model of 4 week, low dose (5 mg/kg) Cis administration in mice with cancer, that resembles the Cis dosing regimen for 4 cycles that is used in humans with cancer.

Methods: Mice were injected with 10^5 lung cancer cells into the flank at day 0. The first dose of Cis 5 mg/kg or vehicle (Veh) was given at day 9. Cis 5 mg/kg or Veh was then given on days 16, 20, 23 and 27 for a total of 5 doses. Mice were sacrificed on day 29. Tumors were measured on day 16, 20 and 29. IL-33 and CXCL1 (IL-8) are pro-inflammatory cytokines. IL-33 signals via the ST2 receptor on CD4 T cells. We have previously described the role of the IL-33/ST2/CD4 T cell/CXCL1 axis in a 3 day model of CIA.

Results: AKI model: BUN (mg/dL) was 23 in Veh and 114 in Cis (P<0.001). SCr (mg/dL) was 0.2 in Veh and 0.5 in Cis (P<0.001). ATN score was 0 in Veh and 3.5 in Cis (P<0.001). Apoptosis in tubular cells (/HPF) was 0 in Veh and 1.2 in Cis (P<0.001). Cancer model: Tumor volume (mm^3) in Veh was 308 on day 16 and 1791 on day 29 (P<0.001). Tumor volume in Cis was 604 on day 16 and 702 on day 29 (NS). At the end of the study, tumor weight (g) was 1.23 in Veh and 0.46 in Cis (P<0.001). IL-33/ST2/CD4 T cell/CXCL1 signaling: IL-33 (pg/mg) was 70 in Veh and 198 in Cis (P<0.001). CXCL1 (pg/mg) was 1.9 in Veh and 58 in Cis (P<0.001). CD4 T cell subset (% of live cells) on flow cytometry was 13 in Veh and 35 in Cis (P<0.001). In time course experiments, the increase in IL-33, CD4 T cells and CXCL1 at week 1 preceded the increase in BUN and SCr at week 2 after Cis administration.

Conclusions: We have developed a model of chronic Cis-administration that resembles the human condition in mice with cancer. There are increases in BUN, SCr, ATN, apoptosis, IL-33, CD4 T cells and CXCL1 and also a significant chemotherapeutic effect of Cis on the tumor. The effect of IL-33, ST2 or CXCL1 inhibition on the CIA and the chemotherapeutic effect of Cis will be interesting in future experiments.

TH-PO035

Shiga Toxin Triggered Renal Apoptosis Is Caused by Imbalance between Bax and Bcl-xl Expression and Rescued by Ouabain/Na,K-ATPase Signaling

Ievgeniia Burlaka,[1] Johan Rebetz,[2] Ida Arvidsson,[2] Diana Karpman,[2] Xiao Liu,[1] Anita Aperia.[1] *[1]Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden; [2]Pediatrics, Lund University, Lund, Sweden.*

Background: Hemolytic uremic syndrome (HUS), a life threatening disease with renal failure as a prominent symptom, generally occurs after gastrointestinal infection with Shiga toxin2 (Stx2) producing *E coli*. Stx2 binds to Gb3 receptors and triggers apoptosis by activating the apoptotic factor Bax. Since we recently found that signaling via the ouabain/Na,K-ATPase/IP3R complex increases expression of Bcl-xL, an inhibitor of Bax, we have tested if ouabain can protect from Stx2 triggered apoptosis.

Methods: In vitro studies were performed on primary cultures of rat proximal tubule cells (RPTC) expressing Gb3 receptors. Other methods include TUNEL staining and FACS analysis, using early and late markers of apoptosis; Western blot; immunostaining; p65 DNA binding assays; In vivo studies were performed on mice given ip injection of Stx2 285 ng/kg and receiving either ouabain 15µg/kg/d or saline via subcutaneous pumps. Mice were sacrificed after 4 days. Kidneys were serially sectioned and TUNEL stained for quantitative analysis of tubule cell apoptosis.

Results: Exposure of RPTC to Stx2 resulted in massive apoptosis. Co-incubation of Stx2 with 10nM ouabain almost completely rescued from apoptosis. Stx2 caused 40% up-regulation of Bax, 50% increase in cleaved caspase (downstream effect of Bax) and 20% down-regulation of Bcl-xL. All effects are significant. Ouabain co-incubation significantly attenuated Bax up-regulation, reduced caspase cleavage and increased Bcl-xL expression. Bcl-xL up-regulation was mediated via transcription factor p65, member of the NF-kB family. Ouabain translocated p65 to nucleus. Inhibition of p65 DNA binding abolished anti-apoptotic effect of ouabain. Preliminary results from in vivo studies provide proof of principle that ouabain protects from apoptotic effects of Stx2.

Conclusions: Non-toxic concentrations of the cardiotonic steroid ouabain can rescue from Stx2 triggered renal apoptosis, a main cause of permanent renal damage following *E coli*-associated HUS.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO036

The Role of Mannose Receptor in Folate Nephropathy

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Background: Folic acid (FA) administration induces dose-dependent acute and chronic tubular injury which is associated with heavy macrophage infiltration. FA nephritis is therefore a good model of acute and progressive renal failure secondary to tubular damage. We have previously demonstrated deficiency of mannose receptor (MR) protects mice from glomerulonephritis via altered cytokine production and FcR mediated signalling, and we have now evaluated the role of MR deficiency in FA mediated acute kidney injury.

Methods: MR -/- homozygote and heterozygote mice and control WT C57BL6 mice were used at 8-12 weeks age. FA 240mg/kg was injected intraperitoneally with 0.2ml sodium bicarbonate as vehicle. Mice were sacrificed at 7 or 14 days after injection. We assessed renal function, histological damage using an acute tubular injury (ATI) score and immunohisto-chemistry staining. Real-time quantitative polymerase chain reaction (qPCR) was used to evaluate gene regulation in our model.

Results: At day 7 following injury, serum urea and creatinine were similar between WT and MR-/- mice; however, the ATI score was higher in WT and MR+/- than MR -/- mice (60.20±16.56, 42.60±10.56, & 10.67±1.78 respectively) with decreased CD68 staining in MR-/- mice. Two weeks following injury, the ATI score showed significant reduction according to MR genotype (WT:40.67±4.30, MR+/-:25.33±2.00, MR-/-:12.09±3.56; p<0.01). Using qPCR analysis at day 14, we found Arg1 (WT 241.24±161.11 vs MR-/- 5.91±3.04, p=0.020), Ym1(WT 10.24±4.23 vs MR-/- 4.23±2.17, p=0.039), and Mrs1 (WT 19.84±12.77 vs MR-/- 2.07±0.79, p=0.025) were significantly down-regulated in the MR-/- mice, whilst the expression of IL1b and iNOS were also reduced but to a lesser extent (8.98±5.96 vs 3.99±2.05 & 4.72±2.38 vs 3.57±1.28 respectively).

Conclusions: Mannose receptor deficient mice are protected from acute FA-induced macrophage mediated kidney damage, with evidence of reduced infiltration and attenuated alternative macrophage activation. MR antagonism may be a novel therapeutic strategy for various forms of acute kidney injury mediating an important effect on macrophage activation.

TH-PO037

Genetic Deficiency of Adiponectin Protects against Acute Kidney Injury

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Background: Acute kidney injury (AKI) is a common clinical condition that is associated with high morbidity and mortality. Ischemia-reperfusion injury (IRI) is a common cause of AKI. There is no effective therapy for this devastating clinical condition except renal replacement. Therefore, a better understanding of the pathogenic mechanisms underlying IRI is essential for ultimately developing effective therapy. Inflammation plays a critical role in the pathogenesis of acute kidney injury. Adiponectin is a cytokine that regulates inflammation, but its role in acute kidney injury is not known.

Methods: To determine if adiponectin contributes to acute kidney injury, we subjected wild-type and adiponectin knockout mice to 30 minutes of ischemia followed by 24 hours of reperfusion.

Results: Compared with wild-type mice, adiponectin knockout mice demonstrated lower serum creatinine and less tubular damage. Tubular cell apoptosis induced by ischemia-reperfusion injury was decreased in the kidney of adiponectin knockout mice, which was associated with a decrease in Bax, a proapoptotic protein, and reduced activation of p53 and caspase-3. Furthermore, targeted disruption of adiponectin inhibited the infiltration of neutrophils, macrophages, and T cells into the injured kidneys, which was associated with a decrease in the expression of the proinflammatory molecules – IL-6, TNF-α, MCP-1, and MIP-2. Moreover, targeted disruption of adiponectin inhibited NF-κB activation in the kidney after ischemia-reperfusion injury.

Conclusions: Our results demonstrate that adiponectin plays a pivotal role in the pathogenesis of acute renal ischemia-reperfusion injury, suggesting that inhibition of adiponectin may be a potential therapeutic strategy in acute kidney injury.

Funding: Other NIH Support - NHLBI, Private Foundation Support

TH-PO038

Preconditioning Mouse Proximal Tubular Cells with A-769662, a Pharmacologic Activator of AMP-Activated Protein Kinase (AMPK), Ameliorates Apoptosis Induced by Subsequent Metabolic Stress

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Background: We have previously demonstrated that acute metabolic stress activates AMPK in mouse proximal tubular cells (MPTs) and that, once activated, AMPK ameliorates the stress-induced apoptosis (Lieberthal et. al.: AJP, 301:F1177, 2011). We now test the hypothesis that preconditioning of MPT cells with A-769662, a pharmacologic activator of AMPK, can reduce apoptosis induced by a subsequent period of metabolic stress.

Methods: MPT cells were preconditioned with the AMPK activator A-769662, or its vehicle, and then subjected to metabolic stress using a mitochondrial inhibitor (antimycin A) in the presence of reduced concentrations of dextrose. Control cells were incubated in medium containing 5 mM dextrose without antimycin A.

Results: Apoptosis during metabolic stress was substantially reduced when MPT cells were subjected to "long-term" (24 hr) preconditioning with A-769662. Long-term preconditioning also significantly ameliorated the decrease in ATP induced by metabolic stress. These effects of long-term preconditioning were associated with increases in expression of the Glut1 transporter, in glucose influx, and in the rate of glycolysis (assessed by lactic acid production). By contrast, "short-term" (2 hrs) preconditioning with A-679662 had no effects on apoptosis, ATP levels, or glucose metabolism.

Conclusions: Long term preconditioning with A-769662 substantially reduces apoptosis of MPT cells subjected to metabolic stress, an effect likely due to amelioration of ATP depletion. Preservation of energy stores is probably mediated, in part, by preconditioning-induced increases of glucose uptake and glycolytic activity. The difference in the effects of long- vs. short-term preconditioning suggests a requirement for AMPK-regulated new gene expression.

Funding: Veterans Administration Support

TH-PO039

Role of beta-Actin in DNase I-Mediated Death of Tubular Epithelial Cells during Kidney Ischemia-Reperfusion Injury Tariq Fahmi,[1] Xiaoying Wang,[1] Eugene Apostolov,[1] Alena Savenka,[1] Intisar Islam,[1] Sudhir V. Shah,[1,2] Alexei G. Basnakian.[1,2] *[1]University of Arkansas for Medical Sciences, Little Rock, AR; [2]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: DNase I is the most active endonuclease in the kidney which is responsible for DNA fragmentation leading to tubular epithelial cell death. The only known endogenous inhibitor of DNase I is monomeric (beta) actin. However relationship between DNase I and beta-actin are not understood. This study was aimed to determine mechanistic relationships between the two proteins during ischemia-reperfusion (IR) in rat kidney.

Methods: Real-time RT-PCR and immunocytochemistry (IHC) was used to assess expression of DNase I and beta-actin, and TUNEL assay was applied to measure DNA fragmentation.

Results: We showed that kidney IR injury in rats is associated with DNA fragmentation located mainly in cortico-medullary junction of the kidney and peaking at 16 hrs of reperfusion. According to IHC, both DNase I and beta-actin were elevated and partially colocalized at the same time point. At low levels of TUNEL, DNase I was linearly and positively associated with TUNEL and beta-actin, but later beta-actin induction decreased, which created an excess of DNase I. To study the mechanism of beta-actin induction by DNase I, two DNase I mutants were produced: one with inactive DNA binding/cleavage site and another with defective actin-binding site. Transfection of rat kidney tubular epithelial NRK-52E cells with wild-type (WT) DNase I gene or the mutants followed by real-time RT-PCR and IHC showed that beta-actin is induced by DNase I, but not by the mutants, suggesting that DNase I induces beta-actin through DNA breaks and actin binding. Transfection of the cells with the actin-binding mutant resulted in more cytotoxicity than with WT DNase I as determined by LDH assay suggesting that beta-actin is important regulator of DNase I cytotoxicity.

Conclusions: Overall our results show that during IR, DNase I induces beta-actin through DNA breaks and actin-binding, but induced beta-actin cannot inhibit DNase I completely, which continues raising, inducing DNA breaks and causing cytotoxicity.

Funding: NIDDK Support, Veterans Administration Support

TH-PO040

Positive and Negative Regulation of Endonucleases by EndoG in Kidney Tubular Epithelial Cells Alexei G. Basnakian,[1,2] Dmitry D. Zhdanov,[1] Tariq Fahmi,[1] Xiaoying Wang,[1] Alena Savenka.[1] *[1]University of Arkansas for Medical Sciences, Little Rock, AR; [2]Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Cisplatin toxicity to kidney was shown to be mediated by cytotoxic/apoptotic endonucleases, of which deoxyribonuclease I (DNase I) is the most active, and endonuclease G (EndoG) is the second active. We have previously obtained evidence of an endonuclease network, in which EndoG and other endonucleases are induced by DNase I through its DNA-degrading activity. Because DNA breaks can be produced by any endonuclease, our next question was whether another endonuclease could have the same effect.

Methods: we transfected rat kidney tubular epithelial NRK-52E cells with EndoG gene cloned in pECFP vector. The expression of EndoG-CFP fusion protein was monitored for up to 24 hours by using quantitative fluorescent microscopy, while CFP expression was used as control. At 24 hours post-transfection, mRNA expressions of eight other endonucleases, including DNase I, DNase X, DNase gamma, DNase 1L2, DNase IIalpha, DNase IIbeta, L-DNase II and caspase-activated DNase (CAD) were measured using real-time RT-PCR.

Results: Our data showed that within first several hours, EndoG overexpression induced only DNase I-like endonucleases (DNase I, DNase X, DNase gamma, DNase 1L2). Unexpectedly, we observed that DNase I was downregulated to almost zero level by 20 hours post-transfection. Agarose electrophoresis of DNase I gene amplification product showed that DNase I pre-mRNA is alternatively spliced lacking 84 bases directly corresponding to exon 4. The alternatively-spliced DNase I without exon 4 was dominant-negative against wild-type DNase I.

Conclusions: This is the first evidence that EndoG can activate DNase I-like endonucleases and downregulate DNase I by inducing alternative splicing of its pre-mRNA.

Funding: NIDDK Support, Veterans Administration Support

TH-PO041

A Modified Ultrasound Regimen Reduces Kidney Ischemia Reperfusion Injury in Mice via Splenic Cholinergic Modulation Joseph C. Gigliotti,[1] Kryt Chattrabhuti,[1] Liping Huang,[1] Amandeep Bajwa,[1] Hong Ye,[1] Sangju Lee,[1] Diane L. Rosin,[2] Alexander L. Klibanov,[3] Kambiz Kalantari,[1] Mark D. Okusa.[1] *[1]Med, CIIR, Univ of Virginia, Charlottesville, VA; [2]Pharmacology, CIIR, Univ of Virginia, Charlottesville, VA; [3]Med, Cardiology, Univ of Virginia, Charlottesville, VA.*

Background: Activation of innate immunity is key in the pathogenesis of ischemia reperfusion injury (IRI). Previous research suggests that ultrasound (US) exposure modulates the immune response. Our hypothesis is that prior exposure to US will reduce the severity of kidney injury in a mouse model of IRI.

Methods: Anesthetized male C57BL/6 mice were exposed to US (frequency: 7MHz; MI: 1.2). Mice underwent bilateral renal IRI 24 hr after US exposure. After reperfusion, tissue samples were collected for plasma creatinine (PCr), renal tissue histology, and flow cytometry. In subsequent studies, TNFα was quantified in LPS treated splenocytes isolated 24 hr after US exposure. In others, mice underwent splenectomy 7 d prior to US and IRI. To determine the role of cholinergic signaling in US prevention of IRI, the α-7 nicotinic antagonist α-bungarotoxin (BT) was administered i.v. 30 min prior to US.

Results: Following IRI, US treated mice had reduced PCR ($P<0.001$), tubular damage ($P<0.001$), and kidney leukocyte infiltration ($P=0.003$) compared to mice receiving IRI alone. Protection from IRI ($P<0.005$; PCr) lasted up to 2-3 d following US exposure. Splenocytes from US treated mice produce 75% less TNFα in response to ex vivo LPS stimulation compared to non-US treated animals. Furthermore, prior splenectomy ($P=0.01$) removed the protective effect of US. BT administration also abolished US protection from IRI.

Conclusions: Our data is consistent with the hypothesis that US mediated kidney tissue protection occurs via activation of the splenic cholinergic anti-inflammatory pathway. We believe that this finding could have an important impact in attenuating human acute kidney injury given the routine noninvasive nature of US, which is a safe, portable and an inexpensive method of immune modulation.

Funding: NIDDK Support, Other NIH Support - NIH T32 Institutional Training Grant

TH-PO042

ER Stress-Induced Autophagy Provides Cytoprotection from Chemical Hypoxia and Oxidant Injury and *Ameliorates Ischemia-Reperfusion-Induced AKI* Bhavya Balan Chandrika,[1] Cheng Yang,[1] Alexandrea Holmes,[2] Sue Theus,[2] Sarika Deshmukh,[1] Didier Portilla,[1,2] Gur P. Kaushal.[1,2] *[1]Division of Nephrology, UAMS, Little Rock, AR; [2]Division of Nephrology, CAVHS, Little Rock, AR.*

Background: The role of ER stress and accompanied UPR is not completely understood in renal pathophysiology. ER stress afforded protection in Renal Tubular Epithelial cells (RTEC) *in vitro* against oxidants as well as against renal ischemia-reperfusion (IR) injury *in vivo*. However, the mechanism of protection by ER stress-induced UPR in acute kidney injury (AKI) is not known.

Methods: The study encompassed different techniques for assaying apoptotic and necrotic cell death such as activity assays for caspase3/7, PI staining by FACS, LDH release and western blot analysis. Autophagy was scored by LC3 puncta formation by imaging and by western blot for major autophagy proteins. Implemented Transfection and siRNA mediated silencing of specific genes for the study. AKI was induced in mouse models via Ischemia Reperfusion surgery.

Results: We demonstrated that the ER stress inducer Tunicamycin activated autophagy via AMPK-mTOR pathway in RTEC as a survival mechanism without impairing autophagic flux. ER stress-induced autophagy markedly provided cyto-protection in renal cells from the oxidants H_2O_2 and tert-Butyl hydro peroxide as well as chemical hypoxia induced by antimycin A. Inhibition of ER stress-induced autophagy both by pharmacological and genetic approaches, significantly enhanced cell death in response to oxidative and chemical hypoxic stress. ER stress-induced autophagy attenuated renal IR injury as evident from significant improvement in renal function and histology. Inhibition of ER stress-induced autophagy worsened renal function in IR injury and supported a protective role of ER stress-induced autophagy in IR-induced AKI.

Conclusions: ER stress mediated cyto-protection for renal cells *in vitro* and *in vivo* from oxidative/hypoxic stress is conferred by the activation of autophagy mechanism suggesting that ER stress initiated autophagy as a pro survival mechanism and further insights may offer new avenues for devising therapeutic strategies against AKI.

Funding: NIDDK Support, Veterans Administration Support

TH-PO043

Natural IgM Can Induce Tolerance in Bone Marrow Dendritic Cells Kailo H. Schlegel, Li Li, Amandeep Bajwa, Mark D. Okusa, Peter I. Lobo. *Medicine/CIIR, University of Virginia, Charlottesville, VA.*

Background: Prior clinical observations have shown an association between high levels of natural IgM autoantibodies in transplantation recipients, especially IgM with anti-leucocyte reactivity (IgM-ALA), and protection from rejection of renal and cardiac allografts. We have shown that natural IgM inhibits inflammation in mouse kidneys induced by ischemia- reperfusion injury (IRI) and by rejection in cardiac allografts. The current studies were aimed at determining if natural IgM mediates these anti-inflammatory effects by inducing tolerance in dendritic cells (DCs), which normally activate effector natural

killer cells and T cells by presenting antigen, providing cytokines (IL-12, TNF-α) and co-stimulatory signals via receptors (CD86 and CD40).

Methods: Bone marrow DCs (BMDCs), obtained from femurs of C57BL/6 mice and cultured with GMCSF, were activated for 18 hr in-vitro with LPS and vehicle, IgM (50 μg/ml), IgG or isotype IgM lacking IgM-ALA activity, and cells were analyzed for expression of CD40, and TNF-α. Secondly, 18 hr pretreated BMDCs were washed and added to a 1-way mixed lymphocyte reaction (MLR) with CFSE-labeled splenic responders from C57BL/6 mice and Balb/c irradiated splenic stimulators. Lastly, BMDC were pulsed with glycolipid (α-GalCer) to determine if IgM inhibits DC glycolipid presentation via the CD1d receptor. We used an antibody that detects the glycolipid/CD1d complex by flow cytometry.

Results: Pretreatment with IgM, but not IgG or isotype IgM, inhibited LPS-induced up-regulation of CD40 (MFI: 6200 (IgG) vs 3000 (IgM)) and enhancement of TNF-α production (pg/ml: 6300 (IgG) vs 3900 (IgM) by BMDCs. Additionally, IgM pre-treated BMDCs significantly inhibited alloantigen activated T cells from proliferating (CFSE (%): 61.7% (IgG) vs 19.5% (IgM), and differentiating into Th-1 cells. Finally, we show that IgM pretreated (18 hr) DCs blocked cell surface expression of the glycolipid/CD1d complex.

Conclusions: The mechanism by which natural IgM attenuates inflammation may in part be mediated by the effects of IgM directly on DCs, through induction of DC tolerance. These in-vitro findings, prompted by clinical observations, may have therapeutic relevance.

Funding: NIDDK Support

TH-PO044

The Purified Micronized Flavonoid Fraction Effect in the Sepsis-Associated Acute Kidney Injury (AKI) Maria De Fatima Vattimo, Carolina Ferreira Pinto, Mirian Watanabe, Cassiane Dezoti Fonseca. *School of Nursing, University of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: The concept of renal vasoconstriction and kidney ischemia as a key pathogenic factor is still valid for sepsis-associated AKI. This may result from global renal hypoperfusion, mediated by the upregulation of pro-inflammatory cytokines (TNF-α and IL-6), with tubular cell apoptosis. Ischemia and reoxygenation in the reperfusion cause oxygen cell damage and capillary stoppage, leading to the renal injury mainly in inflammatory enviromnent as sepsis. The aim of this study was to evaluate the effect of the purified micronized flavonoid fraction (Diosmin), that reduces microvascular permeability and leukocyte adhesion, with antioxidant role, on the creatinine clearance, urinary peroxides and the kidney histology in sepsis associated AKI .

Methods: Adult male Wistar rats (250–300g) were used. Renal function (RF) (creatinine clearance, crCl), urinary peroxides (UP, FOX-2), kidney, lung and intestinal tract levels of TNFα and IL-6 (ELISA) and kidney histology were evaluated. Sepsis was induced by cecal ligature and puncture (CLP). Groups: Sham (without CLP); Sepsis (CLP); Sepsis+Diosmin (Diosmin 3mg/Kg 30 minutes before CLP).

Results:

Physiological paramethers, creatinine clearance and UP

Groups	MAP (mmHg)	BT (°C)	crCl (ml/min)	UP (mmmol/mg creat)
SHAM (n=6)	77 ±5	36.4±0.3	0.60±0.11	3.4±0.6
Sepsis (n=5)	53±6♣	35.7±0.2♣	0.27±0.06♣	13.9±5.1♣
Sepsis+Diosmin (n=5)	72±13♦	36.6±0.6♦	0.34±0.03♣♦	7.4±3.1♦

♣ p<0.05 vs SHAM; ♦ vs Sepsis. Being MAP: mean arterial pressure and BT: body temperature

A similar quantitative inflammatory response (TNFα and IL-6) in distant organs and in the kidneys was observed in CLP animals. CLP induced a decrease in mean arterial pressure, body temperature and creatinine clearance, accompanied by a prominent increase in UP. These paramethers were significantly changed in the pretreated Diosmin group. In the kidney histology, loss of brush border was evident as was a severe dilation of the tubular lumen after CLP.

Conclusions: Remote organs can mediate sepsis-associated AKI. These data provide a new insight about Diosmin attenuating the Sepsis-associated AKI action by decreasing oxidant damage and endothelial swelling.

Funding: Government Support - Non-U.S.

TH-PO045

Ectodomain Shedding by TACE/ADAM17 Regulates Kidney Injury Molecule-1 (KIM-1) Mediated Phagocytosis of Apoptotic Cells Rushi Gandhi,[1,3] James Yi,[1,3] Hang Shi,[1,3] Sahra Nathoo,[1,3] Ola Ismail,[1,3] Xizhong Zhang,[2,3] Lakshman Gunaratnam.[1,2,3] *[1]Dept of Microbiology and Immunology, Western Univ., London, ON, Canada; [2]Division of Nephrology, Schulich School of Medicine and Dentistry, London, Canada; [3]Matthew Mailing Centre for Translational Transplant Studies, Lawson Health Research Institute, London, Canada.*

Background: Apoptosis of tubular epithelial cells is a hallmark of ischemic and toxic acute kidney injury. Phagocytic clearance of apoptotic cells protects multicellular organisms from chronic inflammation and autoimmunity. KIM-1 is a receptor that enables proximal tubule epithelial cells (PTECs) to engulf and clear apoptotic cells. KIM-1 is rapidly upregulated on PTECs following cellular injury. It undergoes constitutive membrane-proximal cleavage resulting in release of a soluble (90 kDa) protein into the urine, or into culture medium of KIM-1-expressing cells. Although KIM-1 is a biomarker of acute kidney injury, the enzyme responsible for KIM-1 shedding and the biological significance of this phenomenon are unknown.

Methods: In addition to studying constitutive shedding, we used phorbol-12-myristate-13-acetate to accelerate KIM-1 shedding. TACE activity was repressed with an inhibitor (TAPI-O) and by shRNA knockdown. We used Western blot to detect KIM-1 cleavage products. Conditioned medium containing shed KIM-1 was used to competitively inhibit the phagocytic uptake of apoptotic cells by PTECs.

Results: We report for the first time that TACE/ADAM17 regulates both constitutive and accelerated shedding of KIM-1.

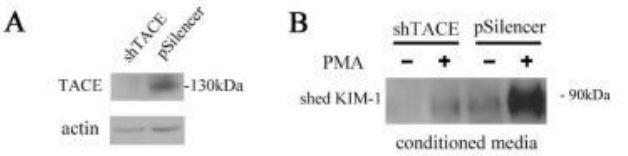

Figure 1. (A) shRNA was used to stably knockdown TACE (shTACE) in 786-O cells expressing endogenous KIM-1. (B) Constitutive and accelerated KIM-1 shedding was studied in the above cells.

The accelerated shedding of KIM-1 inhibited its phagocytic function while silencing TACE expression had the opposite effect.

Conclusions: We conclude that KIM-1-dependent phagocytosis of apoptotic cells is regulated by TACE-mediated shedding. We postulate that the biological significance of this regulation may have significant implications to acute kidney injury.

Funding: Government Support - Non-U.S.

TH-PO046

mTORC1 Influences Tubular Transport and Ischemic Stress Response by Regulating Autophagy and Mitochondrial Energy Provision Florian Grahammer, Tobias B. Huber. *Renal Division, Department of Medicine, University Hospital, Freiburg, Germany.*

Background: mTORC1 is a known master regulator of mitochondrial biogenesis and autophagy. Despite widespread use of mTOR inhibitors in clinical practice hardly anything is known on its physiological und pathophysiological role within renal tubules.

Methods: Cell specific deletion of conditional mTORC 1 (*Raptor*) allels was achieved by using *Ksp* or inducible *Pax8* Promotor driven Cre lines. These mice were analyzed further using functional assays, light and electron microscopy, immunofluorescence, western blotting and MRI.

Results: Both *Raptor fl/fl*Ksp*Cre and induced *Raptor fl/fl*Pax8*TetOCre* mice were viable. *Raptor fl/fl*Ksp*Cre mice presented with a urinary concentration defect due to reduced transport capacity in the TAL. Kidney morphology of these mice was altered macroscopically with MRI showing an enlarged medulla. Histologically an increased interstitial proliferation, increased fibrous tissue and loss of tubular cells were evident. Ultrastructurally prominent, fragmented mitochondria could be detected, biochemically markers of mitochondrial biogenesis were reduced as were TAL specific transport proteins.

*Raptor fl/fl*Pax8*TetOCre* mice did not show any gross macroscopic nor histologic phenotype. Ultrastructurally aberrant formation of multi-layered autophagosomes were present in proximal tubular cells. When exposed to ischemia – reperfusion these mice showed an increased proximal tubular damage, increased apoptosis and reduced recovery as evidenced by diminished proliferation after injury.

Conclusions: Lack of mTORC1 alters renal function and stress response depending on the tubular segment.

Funding: Government Support - Non-U.S.

TH-PO047

Testosterone (TST) Augments Renal Heme Oxygenase-1 (HO-1) Expression Independent of Androgen Receptor (AR) or Estrogen Conversion and Improves Ischemia Reperfusion Induced Acute Kidney Injury (IR-AKI) Andrea P. Soljancic,[1] Arnaldo F. Lopez-Ruiz,[1] Kiran B. Chandrashekar,[1] Istvan Arany,[2] Ruisheng Liu,[3] Luis A. Juncos.[1,3] *[1]Medicine/Nephrology, University of Mississippi Medical Center, Jackson, MS; [2]Pediatrics, University of Mississippi Medical Center, Jackson, MS; [3]Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS.*

Background: Sex hormones modulate renal function during IR-AKI. We reported that AKI reduces plasma TST, and that TST replacement improves AKI. However, the mechanism involved is unclear. We tested if TST-induced renal cytoprotection is via AR activation or conversion to estradiol.

Methods: Male Sprague-Dawley rats treated with TST and/or either flutamide (Flut; AR antagonist), anastrazole (AN; an aromatase inhibitor) or both, were subjected to I/R-AKI (40 mins of bilateral renal pedicle clamping). At 48 hours, we determined renal outer medullary RBF (OM-RBF), function (Pl Creat), injury (KIM-1), inflammation (TNFα), and HO-1 levels. We also examined the *in vitro* effect of TST on proximal tubular cells (LLC-PK1) exposed to oxidative stress.

Results:

	PlCreat mg/dl	KIM-1 pg/ml	OM-RBF (TPU)	TNFα pg/ug	HO-1 ng/ug
Sham	0.5±0.06	712±21	19±2	1.6±0.2	0.05±0.01
AKI	2.1±0.05*	4576±75*	11±1*	5.7±0.5*	0.5±0.1*
AKI-TST	1.4±0.07#	1720±30#	20±3#	2.8±0.1#	1.1±0.1#
AKI-Flut	2.4±0.3	4465±100	10±1	8.4±0.2*	0.5±0.05
AKI-Flut-TST	1.6±0.1	1750±90	15±1	3±0.2	0.9±0.1
AKI-AN	1±0.03#	1375±10#	14#	2.2±0.1#	0.6±0.02#
AKI-AN-TST	1±0.07 Y	1040±20 Y	20±3 Y	1.2±0.2 Y	1.3±0.1 Y

Data: Mean±SEM *p<0.05 vs S #p<0.05 vs AKI Y p<0.05 vs AKI-T

TST ameliorated I/R-AKI and increased renal HO-1. independenlyof the androgen receptor or of conversion of TST to estradiol by aromatase. In fact, blocking endogenous estrogen generation with AN ameliorated IR-AKI and potentiated the effect of TST on I/R-AKI and HO-1. TST blunted LDH release in LLC-PK1 cells exposed to H2O2 by inducing HO-1 and antioxidant

response element (ARE) in an ERK1/2-dependent manner.

Conclusions: TST-mediated protection against I/R-AKI is associated with ERK1/2-dependent induction of HO-1 and ARE. This effect is independent of the androgen receptor and does not due to the conversion of TST to estrogen.

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TH-PO048

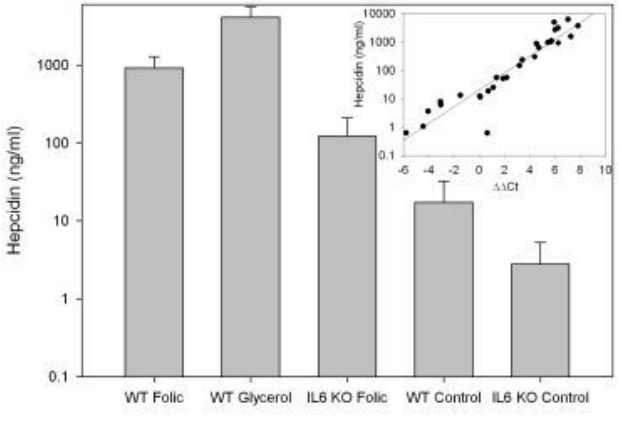
Increased Hepcidin May Contribute to the Anemia of AKI Joshua Zaritsky,[1] Erika Valore,[2] Victoria Rivka Gabayan,[2] Elizabeta Nemeth,[2] Tomas Ganz,[2] Isidro B. Salusky.[1] *[1]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Anemia is a frequent complication in patients with acute kidney injury (AKI) however disordered iron regulation as a potential cause remains unexplored. Therefore we sought to examine the expression of hepcidin, the key regulator of iron homeostasis, in two well described mouse models of AKI.

Methods: AKI was produced by either 1) IP injection of folic acid (200mg/kg) or 2) IM injection of 50% glycerol (7.5ml/kg) in 12 week old male wild-type (WT) BL6 or IL6 KO mice. Mouse serum hepcidin was measured by ELISA. Hepcidin, liver serum amyloid A1 (SAA1), and NGAL mRNAs were measured by real-time PCR amplification and reported as ΔΔCt.

Results: AKI at 48 hours was confirmed by elevated serum BUN and increased kidney NGAL mRNA ($p<0.01$ vs. control) (table). Serum hepcidin levels were highly elevated in AKI models ($p<0.01$ vs. control) (figure) which highly correlated with increased liver hepcidin mRNA ($r= 0.958$; $p<0.01$) (figure inset). SAA1 mRNA, a marker of inflammation, was also very elevated in AKI animals ($p<0.01$ vs. control). Although the absolute levels of serum hepcidin and liver hepcidin and SAA1 mRNA were lower in IL6 KO animals their relative increase in AKI versus control were similar to WT. There were no differences seen in kidney hepcidin mRNA.

	n	BUN	Liver Transcript (ΔΔCt)		Kidney Transcript (ΔΔCt)	
		(mg/dl)	Hepcidin	SAA1	NGAL	Hepcidin
WT Folic	16	93±4	5.5±1	10±1	8.2±1.2	0.6±1.5
WT Glycerol	6	88±9	6.6±0.8	8.5±0.5	7.2±0.9	0.7±3
IL6 KO Folic	6	89±12	2.5±0.9	6.4±0.5	-	-
WT Control	16	16±2	0±1.5	0±1.3	0±2	0±1.1
IL6 KO Control	6	26±5	-4.3±1	-1.4±1	-	-



Conclusions: In both mouse models, AKI results in very high serum hepcidin levels which appear to be mediated by increased liver mRNA via an IL6-independent inflammatory pathway. Future studies are needed to determine if these inflammatory driven acute elevations in hepcidin contribute to disordered iron regulation and anemia in the setting of AKI.

Funding: NIDDK Support

TH-PO049

Production of Mitochondrial Reactive Oxygen Species in Proximal Tubules after Hypoxia/Reoxygenation Anja H. Bienholz,[1,2] Ahmad Al-Taweel,[1] Thorsten Feldkamp,[2] Joel M. Weinberg.[1] *[1]Department of Internal Medicine, Division of Nephrology, University of Ann Arbor, Ann Arbor, MI; [2]Nephrology, University of Duisburg-Essen, Essen, Germany.*

Background: Reactive oxygen species (ROS) figure prominently in both acute and chronic kidney injury and mitochondria are major intracellular sources. We have investigated approaches to best assess mitochondrial ROS (mtROS) production in isolated mouse and rabbit proximal tubules (PT) and the effects of hypoxia/reoxygenation (H/R) on the process.

Methods: Among widely used methods, dichlorofluorescein was unsuitable because of active secretion of the probe by the tubules and the signal from MitoSOX had strong nuclear localization and was increased in the absence of tubules by nonesterified fatty acids (NEFA), precluding its use to test effects of H/R-induced NEFA increases in PT. Measurement of H2O2 by Amplex Red was not subject to artifact and detected appropriate responses based on known mtROS biology.

Results: Oxygen consumption from succinate was 2.9x higher in mouse than in rabbit and concomitant mtROS production was 9.5x higher (238 ± 15 vs. 25 ± 3 nmol H2O2/mg protein/min, $P<0.001$). In both species, supraphysiological succinate (4 mM) produced high rates of mtROS production by reverse electron transport in complex I that were sensitive to decreases of proton motive force (PMF) by NEFA, chemical uncouplers, and H/R. Antimycin-induced mtROS production in complex III by both species was increased 3x by lowering succinate concentrations to 0.03-0.125 mM, and was not inhibited by uncoupling or H/R. mtROS production from complex I-dependent substrates was increased 3.3x by rotenone in mouse PT, but was not affected in the rabbit and was not altered by H/R in either species.

Conclusions: High rates of mtROS production in mice are not necessarily shared with other species and could impact generalizability of data obtained with them. Under conditions in which mitochondria develop a NEFA-mediated energetic deficit that strongly impacts on cellular recovery, H/R has a relatively small effect on mtROS production except for mtROS produced by reverse electron transport from succinate, which is inhibited by the PMF-lowering effect of accumulated NEFA.

Funding: NIDDK Support, Private Foundation Support

TH-PO050

Paricalcitol Supresses Renal Inflammation during Ischemia, Reperfusion Induced Acute Kidney Injury Jae-Won Lee, Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho. *Division of Nephrology, Department of Internal Medicine, Korea University Anam Hospital, Seongbuk-Gu, Seoul, Republic of Korea.*

Background: The pathophysiologic mechanisms of ischemic acute kidney injury (AKI) are thought to include a complex interplay among vascular endothelial cell dysfunction, inflammation and tubular cell damage. In this study, we investigated the effect of the synthetic vitamin D analogue paricalcitol on renal inflammation in a mouse model of ischemia/reperfusion (I/R)-induced AKI.

Methods: Paricalcitol(500ng) or vehicle was administered via intraperitoneal injection 24 hour before ischemia, and then mice underwent ischemia through bilateral clamping of renal pedicles. At 24hour after I/R injury, mice were sacrificed for measurement of several parameters. To examine the direct effect of paricalcitol in tubular cellls, we also performed *in vitro* study using tubular epithelial cell line, HK-2 cells.

Results: Pretreatment with paricalcitol attenuated I/R induced AKI and reduced neutrophil and macrophage infiltration in kidney. It also reduced pro-inflammatory cytokine, interleukin (IL)-6, and chemokine, monocytechemotactic protein (MCP)-1 in kidney tissue. Tubular cell apotosis, determined by the number of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive nuclei, was decreased by paricalcitol pretreatment. Bcl-2–associated X protein (Bax) in kidney tissue was also decreased by paricalcitol. Increased expression of toll-like receptor (TLR) 4 in tubular cells after I/R injury was attenuated by paricalcitol pretreatment. Paricalcitol pretreatment suppressed I/R-induced nuclear translocation of P65, suggesting that it blocked the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) mediated inflammatory pathway following I/R. In *in vitro* experiment, paricalcitol pretreatment also suppressed the depletion of cytosolic inhibitor of κB (IκB) induced by tumor necrosis factor (TNF)-α in HK-2 cells.

Conclusions: These results suggest that beneficial effect of paricalcitol might be mediated by suppressing the innate immune response or inflammation including the activation of TLR4 and NF-kB pathway following I/R injury.

Funding: Private Foundation Support

TH-PO051

Neutophil Fate in Ischemia/Reperfusion Induced Acute Kidney Injury Sang-Kyung Jo,[1] Eunjung Cho,[1] So-young Lee,[2] Won-Yong Cho,[1] Hyoung-kyu Kim.[1] *[1]Internal Medicine, Korea University Medical College, Seoul, Korea; [2]Eulji Medical School.*

Background: Neutrophils rapidly infiltrate kidney and play an important role in I/R injury. For injured kidneys to repair and restore normal function, effective removal of neutrophils and other inflammatory cells seems to be prerequisite and inappropriate delay in neutrophil clearance might lead to chronic inflammation. Here we show an in-vivo evidence that neutrophil apoptosis is a critical step in resolution of inflammation in I/R induced AKI.

Methods: Annexin V positive apoptotic neutrophils were identified during the recovery phase and the role of neutrophil apoptosis on repair process was assessed in chimeric mice in which bone marrow was ablated by sublethal irradiation and then reconstituted with bone marrow from apoptosis resistant Bax KO mice (Bax KO->WT). WT bone marrow transplanted to WT mice (WT->WT) served as control.

Results: Initial neutrophil influx and kidney injury were comparable between the two groups. However, compared to control, neutrophils persisted in Bax KO-> WT chimeric mice throughout the recovery phase and this impaired neutrophil clearance due to resistance to apoptosis was associated with delayed recovery of kidney function and histologic kidney injury, persistently higher tissue proinflammatory cytokine level and also impaired regeneration (decreased PCNA positive cells). In addition to neutrophil apoptosis, we also identified other possible mechanisms involved in resolution of neutrophilic inflammation; reverse transendothelial migration and urinary expulsion. Following I/R injury, ICAM-1^{high}, $CXCR1^{low}$ long-lived neutrophils that are phenotypically distinct from circulating

ICAM- neutrophils that have been demonstrated in a model of reverse transendothelial migration appeared in peripheral blood during the recovery phase. In addition, Gr-1 positive neutrophils were also identified by flow cytometric analysis of urine obtained in recovery phase.

Conclusions: We suggest that neutrophil apoptosis, reverse transendothelial migration and urinary expulsion are involved in resolution mechanisms of neutrophilic inflammation and kidney repair in I/R induced AKI and might serve as new targets for facilitating recovery.

TH-PO052

Pituitary Adenylate Cyclase-Activating Polypeptide38 Ameliorates Renal Ischemia/Reperfusion Injury in Mice through TLR Mediated NF-κB Pathways Altaf-M. Khan,[1] Min Li,[1] Kristine E. Gullo,[1] Anna Wei Cai,[1] Solange Abdulnour-Nakhoul,[2] Jerome L. Maderdrut,[3] Eric E. Simon,[1,4] Vecihi Batuman.[1,4] *[1]Medicine, Nephrology & Hypertension, Tulane Medical University; [2]Gastroenterology, Tulane Medical University; [3]Peptide Research Laboratory, Tulane Medical University; [4]Department of Veterans Affairs, SLVHCS, New Orleans, LA.*

Background: We investigated whether pituitary adenylate cyclase-activating polypeptide38 (PACAP38) ameliorates kidney injury after ischemia/reperfusion (IR) through modulation of TLR-associated signaling pathways.

Methods: Male C57BL/6 mice (6-8 weeks-old, n = 4) were subjected to bilateral renal ischemia for 45 min followed by 3 d of reperfusion. PACAP38, 20 µg in 100 µl saline, was administered i.p. either 1 hr before IR with two additional doses at 24 and 48 hr or only at 24 and 48 hr after IR, and mice were euthanized at 72 hr. Sham-operated mice received the same amount of saline i.p. on the same schedule.

Results: In IR mice, PACAP38 maintained serum creatinine near control levels, (0.81 ± 0.08 vs. 0.69 ± 0.17 mg/dl in controls, p = NS, vs. 1.8 ± 0.03 in saline-treated IR mice, $p <$ 0.01) and significantly reduced expressions of kidney injury biomarkers KIM-1, Nogo-B1 and netrin-1. PACAP38 significantly reduced levels of apoptosis and neutrophil infiltration, demonstrated by immunohistology and by RT-PCR (Aifm-1 and CD11b) and protected against tubular damage assessed histologically. In PCR array, 59 of 83 TLR-related genes significantly changed their expression after IR. TLR2 increased 162-fold, followed by Fadd (37-fold) and TLR6 (24-fold), while Ube2v1 decreased 55-fold. PACAP38 given 24 hr after IR injury significantly reversed these changes in 56 genes, including TLR2, TLR4 and TLR6 with the largest effect on TLR6 (62-fold decrease) and also genes in the NF-κB pathways. The alterations in TLR2, TLR3, TLR6, and Ube2v1 were confirmed by RT-PCR. After IR, PACAP38 also suppressed protein levels of TLR-associated cytokines (TNF-α, MCP-1, IL2, IL6, IL1-β, and IFN-γ).

Conclusions: Acute kidney injury due to IR stimulates TLRs and associated NF-κB signaling pathways. PACAP38 reversed these IR-activated signaling pathways even when treatment was started 24 hr after IR.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO053

Pituitary Adenylate Cyclase-Activating Polypeptide38 Is a Potential Therapeutic Agent for Contrast-Induced Nephropathy in Human Kidney Cells and eNOS-Deficient Mice Altaf-M. Khan,[1] Min Li,[1] Herman L. Toliver,[1] Kristine E. Gullo,[1] Anna Wei Cai,[1] Jerome L. Maderdrut,[2] David H. Coy,[2] Eric E. Simon,[1,3] Vecihi Batuman.[1,3] *[1]Department of Medicine, Section of Nephrology & Hypertension, Tulane Medical University; [2]Department of Medicine, Peptide Research Laboratory, Tulane Medical University; [3]Department of Veterans Affairs, SLVHCS, New Orleans, LA.*

Background: We investigated whether pituitary adenylate cyclase-activating polypeptide38 (PACAP38) ameliorates contrast–induced nephropathy (CIN) in human renal proximal tubule epithelial (HK-2) cells and in a novel model of CIN using homozygous endothelial nitric oxide synthase-deficient ($eNOS^{-/-}$) mice.

Methods: Cultured HK-2 cells, pretreated with 10^{-9}-10^{-6} M PACAP or vasoactive intestinal peptide (VIP) for 1 hr, were exposed to ionic (Urografin) or non-ionic (iohexol) contrast media at 50 mg iodine/ml for 24 hr. Male $eNOS^{-/-}$ mice (22-26 g, n = 5-8) received Urografin iv (1.85 g iodine/kg) after 24-hr water deprivation, and PACAP38 (10 µg) i.p. 1 to 2 hr before and 12 hr after Urografin injection; control mice received an equal volume of saline. All mice were euthanized at 72 hr.

Results: Urografin and iohexol increased the release of LDH and KIM-1 into culture medium, induced apoptosis and inhibited cell proliferation in HK-2 cells ($p < 0.01$). PACAP38 and VIP reversed these changes in a dose-dependent manner. PACAP38 was more potent than VIP. In $eNOS^{-/-}$ mice, Urografin raised serum creatinine and cystatin C levels, caused renal tubule damage, apoptosis (Aifm-1 and Fas-1), neutrophil influx (CD11b), and increased protein levels of TNF-α, TGF-β1, MCP-1 and IFN-γ,and mRNA levels of kidney injury biomarkers KIM-1, Nogo-B1, and netrin-1 as well as Nox-4, Nox-2 and iNOS. PACAP38 significantly reversed these changes and restored towards baseline the mRNA levels of selected immune modulatory genes.

Conclusions: Both contrast media are toxic to HK-2 cells but Urografin was more toxic than iohexol. Urografin caused acute kidney injury in $eNOS^{-/-}$ mice. PACAP38 protected HK-2 cells and mouse kidney from CIN and is a potential therapeutic agent for CIN.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO054

Crucial Role of Calcium-Binding Protein S100A8/A9 in Controlling Macrophage-Mediated Renal Repair Following Ischemia/Reperfusion Mark Dessing, Gwendoline J.D. Teske, Loes Butter, Nike Claessen, Sandrine Florquin. *Department of Pathology, Academic Medical Center, Amsterdam, Netherlands.*

Background: Upon ischemia/reperfusion (I/R)-induced injury, several damage associated molecular patterns (DAMPs) are released including calcium-binding protein S100A8/A9. S100A8/A9 can be recognized by Toll-like receptor (TLR)-4 and activation of this TLR is known to deleteriously contribute to renal I/R induced injury. We hypothesized that S100A8/A9 contributes to renal I/R-induced injury.

Methods: Wild-type (WT) and S100A9 knockout (KO) mice (deficient for S100A8/A9 complex) were subjected to renal I/R and sacrificed after 1, 5 or 10 days.

Results: Although S100A8/A9 expression was significantly increased, 1 day after I/R, S100A9 KO mice displayed similar renal dysfunction, damage and neutrophil-influx compared to WT mice. Interestingly, S100A9 KO mice showed an impaired repair mechanism 5 days post I/R as reflected by increased renal damage, sustained inflammation, induction of fibrosis and increased expression of collagen. This coincided with enhanced expression of alternatively-activated macrophage (M2) markers while the expression of classically-activated macrophage (M1) markers was comparable. Similar, S100A8/A9 deficiency favored M2-macrophage activation *in vitro*, whereas M1-polarization was similar between the two strains.

Conclusions: We showed for the first time that S100A8/A9 inhibits M2-polarization of macrophages, thereby preventing the induction of renal fibrosis and damage after acute kidney injury. We conclude that S100A8/A9 plays a crucial part in controlling macrophage-mediated renal repair following I/R.

TH-PO055

Lcn-2 Over-Expressing Bone Marrow-Derived Macrophages Promote Renal Regeneration Anna Sola,[1] Marina Ventayol,[2] Georgina Hotter.[2] *[1]CIBER-BBN; [2]IIBB-CSIC.*

Background: Recent studies revealed a reparative role of macrophage therapy against different types of human pathologies. Specifically, re-injection of macrophages ex vivo genetically modified towards an anti-inflammatory phenotype has been described to protect against nephrotoxic nephritis.

Methods: Lipocalin-2 (Lcn-2) is a 25 kDa protein of the lipocalin superfamily that is synthesized from macrophages and that exerts a positive modulation of acute inflammation. In the kidney, it is known that Lcn-2 is produced at sites of injury and may modulate renal repair. In this study, we found that ex vivo genetically modified bone marrow derived macrophages towards lipocalin- 2 (Lcn-2) over-expression were capable of modulating injury and inflammation outcome in ischemic kidneys, promoting kidney repair and tissue regeneration.

Results: Renal function markers BUN and creatinine were decreased upon adoptive transfer of these macrophages and the expression of pro-inflammatory mediators was attenuated. Results indicated that macrophage-derived Lcn-2 promotes the proliferation of tubular epithelial cells. Immunostaining for the regeneration markers stathmin and PCNA showed markedly positive expression in the kidney sections with Lcn-2-macrophage treatment. Real-Time RT-PCR of the proliferation markers Ki-67 and PCNA further confirmed these effects.

Conclusions: In conclusion, we provide a novel role for macrophage-derived Lcn-2 on kidney regeneration and protection from experimental renal ischemia/reperfusion injury. Our results indicate a possible target for further therapeutic use in disease, since Lcn-2 not only modulates the macrophage phenotype, but also its pro-repair properties.

Funding: Government Support - Non-U.S.

TH-PO056

Blockade of TNFa Prevents Kidneys from Atrophy after Ischemia-Reperfusion Injury Takaomi Adachi,[1,2] Noriyuki Sugiyama,[2] Yasukiyo Mori,[1] Takahiko Yokoyama.[2] *[1]Department of Nephrology, Kyoto Prefecture University of Medicine, Kyoto, Japan; [2]Department of Anatomy and Developmental Biology, Kyoto Prefecture University of Medicine, Kyoto, Japan.*

Background: Recent studies have reported that a significant population of patients with AKI develops a predisposition toward chronic kidney disease (CKD). However, the mechanism of AKI progression is not known well, and the available therapies are not found yet. We aim to reveal the mechanism of AKI progression and propose a new therapeutic approach for AKI.

Methods: We made two ischemia-reperfusion injury (IRI) mice models (repaired kidney model and atrophic kidney model) and compared them about phenotype and cellular response. In addition, we compared IRI kidneys treated with TNFa neutralizing antibodies with IRI kidneys treated with isotype IgG.

Results: Kidneys exposed to 45min IRI repaired completely, but kidneys exposed to 60 min IRI fell into atrophy at 28 days after IRI. We regarded kidneys exposed to 45 min IRI as repaired model, whereas we regarded kidneys exposed to 60 min IRI as atrophic model.

Kidney weight and nephron number were significantly decreased in atrophic model in comparison with repaired model. To find difference in cellular response that may cause different outcomes between two models, we examined cell proliferation and apoptosis after IRI. Interestingly, there was a high peak of apoptosis at 14 days after IRI only in atrophic model. From these results, we thought that renal atrophy on IRI kidney was due to apoptosis on later phase after IRI.

We found that TNFa was upregulated at 1 and 14 days after IRI only in atrophic model. To elucidate if suppression of TNFa prevent renal atrophy in atrophic model, we performed a blockade of TNFa before IRI using neutralizing antibody. As a result, blockade of TNFa before IRI repressed apoptosis at 14 days and prevented kidney from atrophy after IRI. Next, we performed a blockade of TNFa after IRI. As a result, blockade of TNFa after IRI also repressed apoptosis at 14 days and prevented kidney from atrophy after IRI.

Conclusions: We propose that TNFa-associated apoptosis might play a major role in AKI progression and blockade of TNFa might be a new therapeutic approach for AKI.

Funding: Government Support - Non-U.S.

TH-PO057

Systematic Screening Identifies Interleukin-22, Produced by Intrarenal Mononuclear Phagocytes, to Drive Kidney Regeneration In-Vitro and In-Vivo Onkar Kulkarni, Shrikant R. Mulay, Jan H. Hagemann, Murthy Darisipudi, Ingo Hartter, Mi Ryu, Santhosh Kumar V, Dana Thomasova, Hans J. Anders. *Nephrology Center, Medizinische Klinik and Poliklinik IV, Munich, Bavaria, Germany.*

Background: Growth factors are essential for wound healing or tissue repair. Here we speculate that the intrarenal mononuclear phagocytes (rMoPh), besides mediating kidney inflammation, secrete interleukins that have a selected capacity to drive kidney regeneration upon acute kidney injury (AKI).

Results: We used the isolation of primary tubular epithelial cells (TECs) as an in-vitro assay to evaluate the potential of 25 interleukins (IL) to enhance tubular cell survival as well as regenerative outgrowth from surviving TECs. Only IL22 showed significant improvement in TEC survival and regeneration. Pharmacological blockade of JAK/STAT & ERK pathway of IL22 signalling abrogates the regeneration potential of IL22. Supernatant from necrotic TECs (NS), Ahr ligand-, and ROS-induced IL22 release in bone marrow-derived mononuclear cells.

IL22 was expressed in the ischemic kidney and in the serum upon murine unilateral ischemia with unilateral renal pedicle clamping for 45 min. To understand its functional role, we injected anti-IL22 or control IgG on day 2-4 and analysed kidneys on day 5. IL22 blockade aggravated tubular injury, defined by more necrotic tubules (PAS stain), reduced living distal and proximal tubules (THP-1 and Lectin stain) and increased expression of injury markers such as πGTP, Kim-1, TGF-β, MCP-1 and TNF-α. We observed similar aggravation of tubular injury with depletion of rMoPh by clodronate liposome injection which reduced intra-renal $CD11b^{+}CD103^{-}IL22^{+}$, $CD11b^{+}CD103^{+}IL\text{-}22^{+}$, $CD11c^{+}IL\text{-}22^{+}$ cells (FACS) and reduced IL22 expression in ischemic kidneys (IHC). Reconstitution of IL22 by injection of rm-IL22 (20µg) on day 3 in clodronate injected mice reverted the tubular injury and improved the percentage of repaired THP-1+ or lectin+ tubules and reduced tubular necrosis (PAS stain).

Conclusions: We conclude that the tubular injury induced resident renal rMoPh to secrete IL22 which induces proliferation of TECs through STAT3/ERK signaling and thereby promotes the kidney regeneration during AKI.

TH-PO058

CD26/DPP4 Deficiency Impairs Kidney Function in the Ischemia/Reperfusion Model in the Rat Christoph Daniel,[1] Christina Grigo,[1] Stephan Von Hoersten,[2] Kerstin U. Amann.[1] *[1]Nephropathology, University Erlangen-Nuremberg, Erlangen, Germany; [2]Experimental Biomedicine, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Dipeptidyl peptidase 4 (DPP4) is an exopeptidase inactivating incretins that promote insulin secretion and were therefore used in the treatment of type II diabetes. Beside its ability to cleave incretins, several chemokines and neuropeptide Y were also regulated by DPP4. In lung ischemia/reperfusion injury (IRI) treatment with DPP-4 inhibitors were protective, but the effect of DPP-4 inhibition in renal IRI is unknown.

Methods: Wildtype Dark Agouti (DA) rats (n=8) and congenic rats who did not express CD26/DPP4 (DPP4-/-) (n=8) were used for induction of renal IRI. For analysis of renal function blood was collected 24h and 3 days after reperfusion. Rats were sacrificed on day 3 after IRI and kidney was collected for analysis of morphologic changes, capillary rarefaction, proliferation and inflammation.

Results: Twenty four hours after induction of IRI DPP4-/- rats showed significantly reduced kidney function compared to similar treated DA rats, as assessed by serum creatinine (3.1±0.5 mg/dl vs. 1.7±0.3 mg/dl) and serum urea (237±29.7 mg/dl vs. 173±29.6 mg/dl). In addition, the high serum creatinine and urea levels persisted in DPP4-/- while these parameters declined in DA rats from day 1 to 3. Impairment of kidney function in DPP4-/- rats was accompanied by increased renal morphologic damage, as reflected by tubular injury and a higher proliferative activity. Furthermore, capillary rarefaction, as detected by CD31 positive capillaries, was significantly increased in DPP4-/- rats after IRI (29.8±2,7% vs. 21.2±5.2%). At the inner stripe, ED-1 positive macrophages were significantly increased in DPP4-/- compared to DA rats. In contrast, CD161a positive NK-cells could be detected in significantly fewer numbers in DPP4-/- rat compared to DA IRI controls (23.2±9.3 vs. 64.9±10.3 CD161a pos. cells/mm^2).

Conclusions: CD26/DPP4 deficiency impairs IRI as shown by decreased renal function and capillary density as well as increased tubular damage and macrophage infiltration. Further studies are needed to determine the complete mechanism of this CD26/DPP4 dependent impairment.

Funding: Private Foundation Support

TH-PO059

Role of Spleen in B Cell Trafficking during Repair from Ischemic Acute Kidney Injury in Mice Hye Ryoun Jang,[1] Minsu Kim,[1] Jung Eun Lee,[1] Wooseong Huh,[1] Yoon-Goo Kim,[1] Dae Joong Kim,[1] Ha Young Oh,[1] Hamid Rabb.[2] *[1]Medicine, Sunkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; [2]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: B cells are a newly identified mediator of kidney repair after ischemia reperfusion injury (IRI), and trafficking of B cells into kidneys underlies this response. *The spleen contains B cells and splenectomy can modify systemic B cell function. We therefore hypothesized that B cell changes during repair from kidney IRI are, in part, mediated by the spleen.*

Methods: C57BL/6 mice were randomly allocated into IRI group and IRI-splenectomy (SPX) group. IRI surgery was performed on the left renal pedicle and splenectomy was performed prior to reperfusion. Splenic B cells were analyzed in the kidney IRI group. B cells were compared in the post-ischemic kidneys of IRI and IRI-splenectomy groups on day 1, 3, and 10 after IRI with flow cytometry.

Results: Total B cells expressing CD 19 increased in the spleen after kidney IRI [Day 0: 29.2±4.33, Day 1: 36.8±2.54, Day 3: 52.1±2.33, Day 10: 47.7±3.3%]. Activated mature B cells expressing CD 69 and CD21/35, and plasma cells expressing CD126 and CD138 also increased in the spleen after IRI. The total number of mononuclear cells decreased in the post-ischemic kidney on day 3 after splenectomy, but was comparable on day 10 [IRI vs. IRI-SPX,Day 3: 10.1±0.51 vs. 2.0±0.21, Day10: 4.7±0.38 vs. 3.3±0.9 ($X10^5$)]. Total B cells decreased in the post-ischemic kidney on day 3 after splenectomy, but were similar on day 10 [IRI vs. IRI-SPX, Day 3: 33.4±1.54 vs. 27.9±2.31, Day 10: 15.1±0.98 vs. 14.1±6.91%]. Activated mature B cells were comparable between IRI and IRI-SPX groups [IRI vs. IRI-SPX, Day 3: 8.1±1.11 vs. 10.1±1.66, Day 10: 15.7±2.84 vs. 18.1±2.09%]. Plasma cells were decreased in the post-ischemic kidney by splenectomy [IRI vs. IRI-SPX, Day 3: 9.1±1.20 vs. 4.5±1.33, Day 10: 5.5±0.65 vs. 3.6±1.35%].

Conclusions: B cells in spleen became activated and differentiated after ischemic AKI. Splenectomy decreased B cell infiltration into the post-ischemic kidney and suppressed the final differentiation of B cells to plasma cells during the early repair phase of AKI.

Funding: Private Foundation Support

TH-PO060

Renoprotective Effect of Human Umbilical Cord-Derived Mesenchymal Stem Cells in Immunodeficient MiceSuffering from Acute Kidney Injury Te-Chao Fang,[1,2,3] Cheng-yoong Pang.[3] *[1]Department of Internal Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [2]Department of Medicine, Medical College, Tzu Chi University, Hualien, Taiwan; [3]Institute of Medical Sciences, Medical College, Tzu Chi University, Hualien, Taiwan.*

Background: It is unknown whether human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) can improve the renal function of patients suffering from acute kidney injury (AKI). Moreover, before beginning clinical trials, it is necessary to investigate this renoprotective effect of hUC-MSCs in a xenogeneic model of AKI.However, no previous studies have examined the application of hUC-MSCs to immunodeficient mice suffering from AKI. The objectives of this study were to examine whether hUC-MSCs could improve renal function in nonobese diabetic-severe combined immune deficiency (NOD-SCID) mice suffering from AKI, and to investigate the mechanism(s) for hUC-MSCs to improve renal function in this xenogeneic model.

Methods: Administration of hUC-MSCs (10^6 cells) was performed via the external jugular vein into NOD-SCID mice treated with either vehicle only or folic acid (FA) (250 mg/kg body weight) for resulting inAKI.

Results: hUC-MSCs improved renal function of mice suffering from FA-induced AKI, as evidenced by decreased serum urea nitrogen and serum creatinine levels, as well as a reduced tubular injury score. The beneficial effects of hUC-MSCs were through promoting proliferation of renal tubular cells, reducing caspase -3 and -9, and the rise of Akt phosphorylation.However, these benefits were independent of inflammatory cytokine effects and transdifferentiation.

Conclusions: hUC-MSCs improved renal function in NOD-SCID mice suffering from FA-induced AKI. The beneficial effects of hUC-MSCs in this xenogeneic model were through reducing apoptosis and promoting proliferation of renal tubular cells, and these benefits were independent of inflammatory cytokine effects and transdifferentiation.

Funding: Government Support - Non-U.S.

TH-PO061

Effective Treatment of IRI AKI in Rats with Human Alkaline Phosphatase Transgenic Rat Marrow Stromal Cells, Human Marrow Stromal Cells or Human Adipose-Derived Stem Cells Elicits an Immune Response Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Elizabeth Phillips,[1] Dmitry O. Traktuev,[3] Stephanie Merfeld-clauss,[3] Keith L. March,[3] Christof Westenfelder.[2] *[1]Medicine, U. of Utah and VAMC., Salt Lake City, UT; [2]Physiology, U. of Utah, Salt Lake City, UT; [3]Vascular and Cardiac Center for Adult Stem Cell Therapy, IU SOM and VAMC, Indianapolis, IN.*

Background: We showed that allogeneic rat and human Marrow Stromal Cells (MSCs) effectively protect renal function post experimental IRI AKI (AJP 2005), and appear to be renoprotective in on-pump cardiac surgery patients at risk for post-op AKI (Nat Rev Neph 2010). Such paracrine effects in allogeneic settings are obtained without the elicitation of an antibody response, confirming MSCs' immune privileged properties.

Methods: The present study tested whether (1) the expression of human antigens by MSCs from Fischer344 rats transgenic for human Alkaline Phosphatase (hPAP-MSC; a gift of Dr. Eric Sandgren, U. of WI), by human MSCs (hMSC) or human Adipose-derived Stem Cells (hASC) alters the ability of these cells to protect renal function in female Fischer344 rats (F344; 150-200 g) with IRI-induced AKI (40' bilateral pedicle clamp), and (2) the administration of these cells elicits an antibody response in the recipients. IRI AKI was induced in 4 groups of F344s (n=6 each). Post reflow, groups were infused (suprarenal aorta) with $1x10^6$ hPAPMSC or hASCs /animal, or $0.5x10^6$ hMSC/kg bw, or vehicle.

Results: Compared to controls, all three cell therapies significantly protected renal function and accelerated recovery from AKI, as assessed by serum Cr levels and tissue injury scores. Although robust organ-protective and repair-stimulating actions were observed by d 1-3, within 14 days, each of these cell treatments elicited a significant IgG antibody response (57-99%) directed at the infused cell type.

Conclusions: Although the long-term significance of this immune response is unknown, it essentially rules out the single and particularly the repeated use of transgenic and xenogeneic MSCs or ASCs. The impact these cell-specific antibodies have on the treatment of AKI in rats with these sensitizing cell types is under investigation.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO062

ECFC Represent the Major Subtype of Endothelial Progenitor Cells Recruited to the Kidney after Endothelial Injury in Mice Jan Sradnick, Bernd Hohenstein, Christian Hugo. *Division of Nephrology, Department of Internal Medicine III, University Hospital CGC, Technical University Dresden, Dresden, Germany.*

Background: We have previously shown that CD34+/Flk-1+ EPCs were recruited to the kidney after selective endothelial injury in mice (Am J Physiol Renal Physiol 298:1504-1514, 2010). Two types of endothelial progenitor cells (EPCs), the (early) colony forming units (CFU-EC) and (late) endothelial outgrowth (EOC, ECFC) were described in vitro recently. However, whether and to what extent these types might contribute to the cells recruited to the kidney is unclear. Therefore, the present study investigated these EPC subtypes after selective endothelial injury in the mouse kidney.

Methods: Selective endothelial injury was induced in left kidneys of 25 C57/Bl6 mice. Kidneys and spleens were harvested at days 1, 3, 5 and 7. Five mice served as healthy controls (ctrl). After digestion, non-viable cells were excluded and CD34+/Flk-1+/CD133-/CD45- ECFC, CD34+/Flk-1+/CD133+ CFU and also CD150+/CD117+ HSC were measured using multicolour FACS analysis.

Results: CD34+/Flk-1+ cells were increased in kidney on days 3-7 after endothelial injury as demonstrated before (ctrl=0.2%, d1=0.19%; d3=0.58%; d5=0.59%; d7=0.69%all $P<0.01$). Among these cells, ECFC represented the major subtype at d1-d7 (d1=73%; d3=94%; d5=93%; d=93%) and in controls (98%). CFU represented a minor population in diseased kidneys at any time point. Hematopoietic stem cells increased significantly on d5 (0.8% vs. 0,3% ;$p<0.01$). In contrast to our former study, neither ECFCs nor CFUs were found in significant amount in the spleen, while HSC were increased at d7 ($p<0.05$).

Conclusions: The present study verified that CD34+/Flk-1+ cells are recruited to kidneys subsequent to endothelial injury. We now demonstrate that ECFC represent the major EPC subtype recruited to the kidney and could not detect these cells in the spleen, while HSC were found in the kidney and the spleen.

TH-PO063

Magnification Effect for CXCR4 Gene Transfection on Repairing Acute Kidney Injury for Mice by Bone Marrow Mesenchymal Stem Cells Transplantation Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: To construct CXCR4 gene transinfected bone marrow mesenchymal stem cells(BM-MSCs), take cell transplantation for acute kidney injury(AKI) mice via tail vein, observe whether there is magnification effect on AKI repairing.

Methods: Lentivirus that carry CXCR4 target gene had been constructed, then transinfecting BM-MSCs; Transinfection efficiency,CXCR4 expression, differentiation and multiplication capacity were all tested. 100 C57BL/6 mice were divided into normal group, model group (AKI group), BM-MSCs transplantation group, null-BM-MSCs transplantation group and CXCR4-BM-MSCs transplantation group; the transplanted BM-MSCs were marked by BrdU for every group. Some mice were sacrificed after the model been constructed 1d, 3d, 7d, and 14d; testing BUN and Scr level; pathological changes for nephridial tissue were observed and the injury degree for renal tubular had been scored; BM-MSCs distributed were observed with IHC and stromal cell derived factor-1 (SDF-1) level in injured nephridial tissue was tested by Western Blot.

Results: Target lentivirus carrying CXCR4 gene together with null lentivirus both successfully transinfected BM-MSCs. Alizarin red staining, alkaline phosphatase staining and oil red staining results for the transinfected BM-MSCs all reflected positive; Cell multiplication capacity was not changed by MTT. CXCR4 expression in CXCR4-BM-MSCs group was enhanced for mRNA and protein level. Contrasted with BM-MSCs transplantation group and null-BM-MSCs transplantation group, BUN, Scr and ATN scores in CXCR4-BM-MSCs transplantation group were declined sharply. SDF-1 level for nephridial tissue after AKI model constructed were rising. IHC showed that $BrdU^+$ cell distribution could be tested in mouse kidney in BM-MSCs transplantation group, positive cell proportion for null-BM-MSCs transplantation group was similar. While in CXCR4-BM-MSCs transplantation group, the $BrdU^+$ positive cells were increased significantly.

Conclusions: CXCR4 gene transinfection could increase the number of BM-MSCs homing to injured kidney after BM-MSCs transplantation for AKI mice, so that, the AKI repairing effect for transplanted stem cells was distinctly enhanced.

Funding: Government Support - Non-U.S.

TH-PO064

Lineage-Negative Cord Blood (CB) Cells Protect against Ischemia/Reperfusion-Acute Kidney Injury (IR-AKI) in Rats Camila Eleuterio Rodrigues,[1] Ana C. de Bragança,[1] Mauro Shigueharu Oide Junior,[1] Enio Jose Bassi,[2] Niels O.S. Camara,[2] Antonio C. Seguro,[1] Lucia Andrade.[1] *[1]Nephrology, University of Sao Paulo Medical School, Sao Paulo, SP, Brazil; [2]Immunology, University of Sao Paulo Biomedical Institute, Sao Paulo, SP, Brazil.*

Background: IR-AKI induces cell-cycle inhibitors, including the cyclin-dependent kinase inhibitor p21(WAF1/CIP1), which is protective against AKI. However, it is known that critically short telomeres cause increased p21 expression, which increases the number of apoptotic renal cells. In telomerase-deficient mice, IR-AKI-induced impairment of renal function is more severe than in normal mice. Young stem cells can alleviate renal aging in mice and have less effect on p21 expression than do aged stem cells. The telomeres of CB stem cells are longer than are those of other stem cells. Although many stem cell types show potential as therapies for kidney diseases, uncultured lineage-negative CB cells (CBLin-) cells have never been tested in IR-AKI.

Methods: Blood was collected from healthy women and babies, and the mononuclear fraction was used for depletion of lineage-committed cells. Cell fluorescence was evaluated using a flow cytometer. Male Wistar rats were induced to IR by 45-min clamping of both renal arteries, and some rats received 10^6 CBLin- cells (via tail vein) 6h after surgery. We studied three groups: control (C, n=8); IR (n=9); and IRLin- (n=6). After 48h of IR, we measured inulin clearance (Cin) and urinary volume. Immunoblotting for p21, eNOS and Klotho was performed in renal tissue. Data are mean±SEM.

Results: Selected cells were negative for CD2, CD3, CD14, CD16, CD19, CD24, CD34, CD56, CD66b, CD73, CD90, CD 105, HLA-DR and glycophorin A.

Group	Cin (ml/min/100 g BW)	Urinary output (ml/h)	p21 expression (%)	eNOS expression (%)
C	0.94±0.05	0.56±0,13	97±7	87±8
IR	0.40±0.04[a,b]	1.04±0.22	179±16[a,b]	69±6[b]
IRLin-	0.63±0.08[a]	0.67±0.11	99±1	101±4

[a]$p<0.05$ vs. C; [b]$p<0.05$ vs. IRLin-

Renal blood flow and mean arterial pressure did not differ among the groups. Klotho expression was dramatically decreased in IR rats and restored in IRLin- rats.

Conclusions: CBLin- cells could be used in treating IR-AKI. Further studies could identify the exact mechanism of their action.

FAPESP/CAPES/CNPq.

Funding: Government Support - Non-U.S.

TH-PO065

Human Renal Stem/Progenitor Cells Repair Tubular Epithelial Cell Injury through TLR2-Driven Inhibin-A and Microvesicle-Shuttled Decorin Fabio Sallustio, Vincenzo Costantino, Sharon N. Cox, C. Divella, Francesco Paolo Schena. *Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy.*

Background: Acute renal failure (ARF) is emerging as a public health problem worldwide and many studies recently focused their attention on the possibility of using human adult renal stem/progenitor cells (ARPCs) to improve regeneration in ARF. We studied the influence of ARPCs on the regenerative process of cisplatin-injured renal proximal tubular epithelial cells (RPTECs) and the mechanisms underlying the ARPC regenerative processes. Moreover, we investigated the nature of the regenerative factors secreted by ARPCs.

Methods: We set up an in vitro model of cisplatin-induced toxicity, in which RPTECs were co-coltured with ARPCs. We studied apoptosis and proliferation by immunofluorescence experiments. By bioinformatic analyses on microarray data and by multiplex cytokine assays, we identified some specific cytokines and growth factors secreted specifically by ARPCs following the damage perception.

Results: We found that tubular ARPCs (tARPCs), but not glomerular ARPCs, provided a protective effect by promoting tubular cell proliferation of survived cells and inhibiting cisplatin-induced apoptosis. The regenerative effect was specific of tARPCs, occurred only following the sensing of the damage and was completely cancelled blocking the TLR2, present on tARPCs. Moreover, we found that tARPCs, but not gARPCs, were resistant to the apoptotic effect of the drug. We showed that tARPCs operated mainly through the TLR2 engagement and the secretion of inhibin-A protein and of microvesicle-shuttled decorin, inhibin-A and cyclin D1 mRNA. These factors worked synergistically and were essential in the regenerative process. The involvement of tARPC-secreted inhibin-A and decorin mRNA in the pathological process of ARF was confirmed also *in vivo* on transplant patients affected by delayed graft function.

Conclusions: We demonstrated that TARPCs could have a regenerative effect on damaged RPTECs by both preventing apoptosis and enhancing proliferation of surviving cells through the TLR2 engagement, and identified factors that were indispensable for the regenerative processes.

Funding: Government Support - Non-U.S.

TH-PO066

Proximal Tubuli Contain a Phenotypically Distinct, Scattered Cell Population Involved in Tubular Regeneration Bart Smeets,[1,4] Peter Boor,[2] Henry Dijkman,[3] Katja Berger,[1] Shagun V. Sharma,[4] Jürgen Floege,[1] Johan Van der Vlag,[4] Jack F. Wetzels,[4] Marcus J. Moeller.[1] *[1]Nephrology, University Hospital Aachen, RWTH, Aachen, Germany; [2]Pathology, University Hospital Aachen, RWTH, Aachen, Germany; [3]Pathology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [4]Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

Background: Regeneration of injured tubular cells occurs after acute tubular necrosis primarily from intrinsic renal cells. This may occur from a fixed intratubular stem/progenitor cell population or from any surviving proximal tubular cell.

Methods: In this study, we characterize a CD24, CD133, and vimentin positive subpopulation of cells scattered throughout the proximal tubule in normal human kidney.

Results: Compared to adjacent "normal" proximal tubular cells, these CD24+ cells contained less cytoplasm, less mitochondria and no brush border. In addition, 49 marker proteins are described that also identify this distinct cell population. In human biopsies of patients with acute tubular necrosis (ATN), the number of CD24+ tubular cells was increased. In both normal human kidneys and in the ATN biopsies, around 85% of proliferating cells were CD24 positive – indicating that this cell population participates in tubular regeneration. In healthy rat kidneys, the novel cell subpopulation was absent. However, upon unilateral ureteral obstruction (UUO), the novel cell population was detected in significant amounts in the injured kidney.

Conclusions: In summary, in human renal biopsies, the CD24+ cells represent tubular cells with a deviant phenotype, characterized by a distinct morphology and marker expression. After acute tubular injury, these cells become more numerous. In healthy rat kidneys, these cells are not detectable, whereas after UUO, they appeared *de novo* – arguing against the notion that these cells represent a fixed progenitor cell population. Our data indicate rather that these cells represent transiently dedifferentiated tubular cells involved in regeneration.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO067

Kidney Stem Cell Responses in Acute Kidney Injury Repair Gilbert W. Moeckel, Dong Chen. *Pathology, Yale University School of Medicine, New Haven, CT.*

Background: The renal medulla is a proposed stem cell niche in the kidney. This study focuses on isolating and characterizing an endogenous kidney stem cell population from primary mouse medullary interstitial cells and assessing their role in the repair of tubular cell injury.

Methods: Mouse medullary interstitial cell primary cultures were grown in Knockout Serum Replacement (KSR) buffer. Some cells were also cultured in Dulbecco's modified Eagle Medium/F12 (DMEM/F12) with N-2(1x) Supplement. Cells were grown under both hyperosmotic and hypoxic conditions. Selectively grown kidney progenitor cells were stained with fluorescence labeled antibodies against CXCR4, CXCR7, CD24, CD34, Rat-CD44, Rabbit CD-44, Nestin and Pax-7. Cell survival and proliferation was evaluated using MTT assay and PCNA western blot. In wound healing experiments different tubular epithelial cell lines were grown on fibronectin coated plates, and the repair of a wound following treatment with progenitor cell supernatant recorded after 6 and 26 hours.

Results: We saw an enrichment of stem cell marker positive cells by up to 865%, compared to cell cultures without selective growth conditions. Immunofluorescent staining revealed, that the enriched cells stain positive for CXCR4 (95%), positive for CXCR7 (56%), positive for CD24 (68%), positive for Nestin (43%), positive for Pax 7 (77%) and positive for several other kidney stem cell markers. Wound healing assays showed significant differences between the control and the supernatant treated cells regarding number of cells migrating into the wound area.

Conclusions: We have developed a new method to enrich potential stem cells from the kidney medulla. After enrichment, these cells were characterized and expressed positivity for stem cell markers: CD24, CXCR4, CXCR7, Nestin, CD44 and Pax7. Furthermore, these stem cells exhibit phenotypic traits of renal stem cells and have biological effects that benefit kidney injury repair as demonstrated with PCNA proliferation assay, MTT cell viability assay and wound healing assay. Our studies confirm the hypothesis that the renal medulla is indeed a reservoir for endogenous kidney stem cells.

Funding: NIDDK Support

TH-PO068

Human Endothelial Colony Forming Cells Attenuate Ischemic Acute Kidney Injury in Mice Dylan Burger, David Allan, Yuan Chung, Alex Gutsol, Anthony Carter, Rhian Touyz, Kevin D. Burns. *Kidney Research Centre, Dept. of Medicine, Ottawa Hospital Research Centre, University of Ottawa, Ottawa, ON, Canada.*

Background: Microvascular injury is a key feature of ischemic acute kidney injury (AKI), suggesting that strategies targeting endothelial health could promote recovery of renal function. While the administration of certain progenitor cell populations improves renal recovery following AKI, we recently reported that a population of human cord blood-derived $CD133^+$ progenitor cells exacerbated ischemic AKI in mice, associated with enhanced inflammation and apoptosis (Burger D. et al. Nephrol Dial Transplant 2012). Accordingly, the choice of progenitor cell populations may be important in cell-based therapy in human AKI.

Methods: The purpose of this study was to examine the effects of human endothelial colony forming cells (ECFCs) in a mouse model of AKI, induced by bilateral ischemia-reperfusion (I/R). ECFCs were isolated from human umbilical cord blood, expanded in culture, and injected (10^6/mouse) via the jugular vein into non-obese diabetic severe combined immunodeficient (NOD-SCID) mice (male, 8 weeks) at the time of reperfusion. Renal functional and structural outcomes were evaluated.

Results: Analysis of human ECFCs by flow cytometry revealed expression of CD31 but not CD14, CD45, or CD133, consistent with an endothelial, but not a hematopoietic phenotype. Administration of ECFCs attenuated I/R-induced renal injury at 24 hours as revealed by reductions in plasma urea (control: 78±2 mM vs. ECFCs: 65±5 mM, $P<0.05$, n=5-6) and creatinine (control: 174±6 μM vs. ECFCs: 106±22 μM, $P<0.05$, n=5-6) and reduced hyperphosphatemia ($P<0.05$, n=5-6). At 24 hours post I/R, mice receiving ECFCs also displayed significantly less tubular injury (anuclear tubules: control: 64±1% vs. ECFCs: 57±2%, $P<0.001$, n=5-6).

Conclusions: These data indicate that human ECFCs attenuate renal injury in a mouse model of ischemic AKI. The results suggest that these effects may be achieved by improvement in post-ischemic endothelial function, and imply a potential therapeutic role for ECFCs in human AKI.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

TH-PO069

Mesenchymal Stem Cells Promote Renal Regeneration via Alteration of Macrophage Phenotype Andrea F. Wise,[1] Timothy M. Williams,[1] Junli Zhuang,[1] Chrishan S. Samuel,[2] Sharon D. Ricardo.[1] *[1]Monash Immunology and Stem Cell Laboratories, Monash University, Melbourne, Victoria, Australia; [2]Department of Pharmocology, Monash University, Melbourne, Victoria, Australia.*

Background: Mesenchymal stem cells (MSCs) have cytoprotective and immunomodulatory properties that aid in tissue regeneration. This study investigated the ability of human mesenchymal stems (hMSCs) to alter macrophage phenotype and provide renoprotection following acute tubulointersitial injury.

Methods: C57BL6/J mice (20-25g; n=5/group) with unilateral IR injury were injected with $1x10^6$ hMSCs or PBS (i.v.). At 7 days post-hMSC treatment, kidney histology was examined, total collagen concentration quantified by hydroxyproline assay and macrophage phenotype analyzed via FACS and qPCR. In addition, hMSCs and bone marrow-derived macrophages were co-cultured directly/indirectly for 48 hours and macrophage phenotype assessed using qPCR.

Results: hMSC treatment promoted structural repair with increased tissue matirx remodeling and decreased collagen accumulation at 7 days post-treament compared to vehicle treatment. *In vitro,* hMSCs further increased the gene expression of the anti-inflammatory M2 marcophage markers *Ccl2* (unstimulated and M2 stimulated macrophages), *Arg1* (unstimulated, M1 and M2 stimulated macrophages), *Chi313* (M2 stimulated macrophages) and *Fizz1* (M2 stimulated macrophages). This alteration in macrophage phenoytpe is translatable to an *in vivo* hMSC promotion of tissue repair.

Conclusions: The administration of hMSCs to mice with IR injury promoted renal regeneration and repair 7 days post-treatment. The promotion of this structural repair may be due to the ability of the hMSCs to alter macrophage phenotype to an M2 polarization state resulting in downstream tissue remodeling and accelerated wound healing within the damaged kidney.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO070

Activation of DNA Injury-Associated Histone H2AX-ATM Complex and Expression of Development-Associated Regulator β-Catenin in Pro-Fibrotic Kidney Injury Models Yumin Liu, Takaharu Ichimura, Li Yang, Joseph V. Bonventre. *Renal Division, Brigham & Women's Hospital/Harvard Med. School, Boston, MA.*

Background: We have proposed that renal epithelial cell cycle arrest after acute kidney injury (AKI) contributes to chronic kidney disease. Cell cycle arrest results in a secretory profibrotic phenotype in part via DNA damage response regulator ATM (ataxia telangiectasia mutated). β-catenin has previously been proposed to play an important role in renal fibrosis. We now report an association between β-catenin and ATM activation in kidney epithelial cells.

Methods: We evaluated the temporal and spatial patterns of the aristolochic acid (AA)-induced activation of phosphorylated-ATM (pATM) and phospho-Histone (pH2AX), which is a substrate of ATM and together form a DNA damage complex. AA induces AKI with accelerated chronic kidney disease in humans and rodents. We also characterized the acivation of β-catenin in proximal tubular epithelial cells after AA.

Results: AA resulted in activation of ATM and was associated with formation of DNA damage complexes. On days 11-14 after AA exposure p-ATM co-localized with pH2AX, often in BrdU positive, TUNEL-negative nuclei of epithelial cells. Nuclear pH2AX-positive cells were often positive for Kidney injury molecule-1 (Kim-1). At these stages of injury, upregulated β-catenin was commonly detected in the p-ATM positive proximal tubule epithelial cells. This upregulated β-catenin was also found at later stages after renal ischemia/reperfusion in mouse kidneys (days 5-7), a model also associated with fibrosis. AA-treatment induced β-catenin translocation to the nucleus in primary epithelial cells isolated from the mouse kidney. In AA-treated LLC-PK1 renal epithelial cells, the p-ATM-H2AX complex was co-activated and transcription of pro-fibrotic TGF-β and CTGF genes was upregulated.

Conclusions: These data suggest that activation of ATM and H2AX with co-activation of β-catenin may be important regulatory steps in Kim-1 positive injured tubular epithelial cells to cause a maladaptive fibrotic response resulting in tubulo-interstitial fibrosis. This activation pathway may be a potential therapeutic target in chronic kidney disease.

Funding: NIDDK Support

TH-PO071

Trps1 Plays a Critical Role in Mesenchymal-Epithelial Transition of Epithelial Cells Response to Acute Ischemic Kidney Injury Kun Huang, Jurong Yang, Yani He. *Department of Nephrology, DaPing Hospital, Third Military Medical University, ChongQing, China.*

Background: Incomplete kidney repair, induced by acute ischemic reperfusion injury, is a major cause of chronic kidney disease(CKD). The process of AKI repair has been considered to be the reappearance of embryonic metanephron development. Trps1 has been shown to activate mesenchymal-epithelial transition (MET) in kidney development.This study explored the role of Trps1 in reparation of acute ischemic reperfusion kidney injury.

Methods: Rat renal I/R injury (45 and 60 minutes)model was established. The serum creatinine, BUN, changes of histology, Na^+-K^+-ATPase were tested. The expression of Trps1, α-Catenin and vimentin was detected by immunohistochemistry and western blotting. The over-expression of Trps1 was used to investigate the effect of Trps1 by transfecting GFP-Trps1 adenovirus into kidney.

Results: Compared with I/R 45min group, the renal function of serious ischemic-reperfusion kidney injury (I/R 60min group) was delayed recovery. Pathological examination showed the repair of renal tissues in serious I/R injury group was delayed and incomplete. Trps1 expression decreased during the phase of injury, then elevated during the phase of repair. The Trps1 expression in serious kidney injury group was lower than I/R 45min group. In the serious injury group, the Trps1 expression was decreased in impaired areas of tissues, while the Trps1 expressed high in well repaired areas. The increased expression of Trps1 correlated with epithelial re-differentiation rather than de-differentiation, leading to increased expression of epithelial marker(α-catenin) and decreased expression of mesenchymal marker(vimentin). Compared with serious injury group, Trps1 over-expression group showed an earlier decrease of serum creatinine and BUN, alleviated chronic lesions, especially about tubular atrophy and tubulointerstitial fibrosis.

Conclusions: Trps1 promotes the repair of acute ischemic reperfusion kidney injury by mediating the MET of injured renal tubular epithelial cells.

Funding: Government Support - Non-U.S.

TH-PO072

The SIRT1 Activator SRT1720 Improves Renal Cortical Mitochondrial and Tubular Function Following Ischemia-Reperfusion Injury Jason A. Funk, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Ischemia-reperfusion (IR) injury in the kidney initiates lethal and sub-lethal injury in the proximal tubule. Mitochondrial dysfunction is a pathological consequence of I/R injury, and promoting mitochondrial biogenesis (MB) as a repair mechanism after injury may offer unique benefits to restore mitochondrial and organ function. Since we previously showed that the SIRT1 activator SRT1720 stimulates MB in renal cells, we examined its efficacy in IR injury.

Methods: Rats were subjected to bilateral renal pedicle ligation for 22m or sham surgery and then treated once daily with SRT1720 (5 mg/kg, i.p.) or vehicle starting 24h after reperfusion until 72h or 144h. Mitochondrial proteins, as well as Kim-1, Na^+,K^+-ATPase, and vimentin were examined after injury. State 2 and uncoupled respiration of isolated mitochondria was determined using a Clark-type oxygen electrode.

Results: Mitochondrial proteins ATP synthase β, COX I, and NDUFB8 were diminished at 24h, 72h and 144h in rats subjected to IR. Rats treated with SRT1720 after IR (IR+SRT1720) had improved mitochondrial protein expression by 144h and restoration of state 2 and uncoupled mitochondrial respiration. PGC-1α was elevated at 72h and 144h in both IR and IR+SRT1720 rats; however, SRT1720 treatment increased de-acetylated PGC-1α, the more active form. Renal Kim-1, a sensitive marker of tubular injury, was persistently expressed in the renal cortex of IR rats but was attenuated in IR+SRT1720 rats. Additionally, sustained loss of Na^+,K^+-ATPase expression and elevated vimentin in IR rats was normalized in IR+SRT1720 rats, suggesting SRT1720 treatment was associated with restoration of a differentiated, polarized proximal tubule epithelium.

Conclusions: Overall, these results reveal that treatment with SRT1720 increased de-acetylated PGC1a and stimulated MB, enhancing recovery of mitochondrial protein expression and function. Rescue of mitochondrial function was associated with faster recovery of proximal tubule integrity. Targeting mitochondrial biogenesis may offer unique therapeutic benefits as a strategy to improve tubule repair following ischemic injury.

Funding: Other NIH Support - NIH Grant GM084147, Veterans Administration Support

TH-PO073

Phenylthio Butanoate Class HDAC Inhibitors Enhance Recovery from Acute Kidney Injury (AKI) Nataliya Skrypnyk,[1] Tatiana Novitskaya,[1] Neil A. Hukriede,[2] Mark P. De Caestecker.[1] *[1]Division of Nephrology, Vanderbilt University, Nashville, TN; [2]Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA.*

Background: We previously identified a new class of HDAC inhibitors (HDACi), phenylthio butanoates (PTBA), which promote renal progenitor cell expansion in zebrafish embryos. Due to conservation of signaling pathways in kidney organogenesis and regeneration, we hypothesized these compounds would also enhance renal regenerative responses in mouse models of AKI.

Methods: PTBA analogues given in 20% cyclodextrin (vehicle control) IP. Renal histone H4 acetylation (H4Ac) evaluated by fluorescence immunoblot; EC50 by CNT assay with Tg (*Cdh17:GFP*) zebrafish larvae; serum creatinine by enzymatic assay after moderate IR-induced AKI (BALB/c, 26 mins ischemia), or severe IR-induced AKI (BALB/c, 30 mins unilateral ischemia, contra-lateral nephrectomy day 8).

Results: We first evaluated effect of 8 PTBA analogues on renal H4Ac (marker of HDACi activity) after IP injection in mice: 5 increase H4Ac: 4 with carboxylic acid (CA) and 1 with hydroxamic acid (HA) "warheads". 3 analogues with highest biologic activity in zebrafish (EC50s 500-900nM) were tested in IR-induced AKI: #29 (HA derivative) and #22 (CA derivative) induce 1.5X increases in renal histone acetylation 12 hours after IP injection; #25 (CA derivative) induces 2-3X increase after 2 hours, and also has the lowest EC50 (470nM). Daily treatment with #22 or #29 started 24 hours after injury reduce serum creatinine at 2 and 3 days post injury, respectively, but effects are not sustained. In contrast, daily treatment with #25 gives a sustained decrease in serum creatinine from day 3-7 post injury. Daily treatment with #25 for 7 days in severe IR-induced AKI also reduces serum creatinine 1-7 days post nephrectomy.

Conclusions: PTBA analogues ameliorate IR-induced AKI if administered 24 hours after injury; the PTBA analogue #25 is shortest acting, has greatest effect on H4Ac and has highest biological activity in zebrafish assays, also most effectively ameliorates AKI. These studies are the first evidence that compounds identified from renal progenitor cell expansion screen in zebrafish also ameliorate AKI in mice.

Funding: NIDDK Support

TH-PO074

Phenylthio Butanoate Class HDAC Inhibitors Ameliorate Chronic Kidney Injury by Improving Functional Recovery after Different Forms of Acute Kidney Injury in Mice Nataliya Skrypnyk,[1] Tatiana Novitskaya,[1] Neil A. Hukriede,[2] Mark P. De Caestecker.[1] *[1]Division of Nephrology, Vanderbilt University, Nashville, TN; [2]Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA.*

Background: We previously identified a new class of HDAC inhibitors (HDACi), phenylthio butanoates (PTBA), which promote expansion of renal progenitor cells in zebrafish embryos. We hypothesized that PTBA analogues would also enhance renal regenerative responses in mouse models of kidney injury, and have shown that the PTBA analogue, #25, accelerates recovery after ischemia reperfusion (IR)-AKI in mice when given daily starting 24 hours after injury. Since impaired recovery following AKI is associated with development of CKI, we now hypothesize that #25 would reduce CKI post AKI.

Methods: The PTBA analogue #25 was administered IP at different time points following: a) severe IR-induced renal fibrosis (BALB/c, 30 mins unilateral ischemia and contra-lateral nephrectomy at day 8); b) aristolochic acid (AA) induced CKI (BALB/c, 4.7mg/kg); and c) unilateral ureteric obstruction (UUO, C57Bl/6). Serum creatinine, renal fibrosis and QRT-PCR were performed using standard techniques.

Results: In severe IR, daily treatment with #25 for 7 days starting 24 hours after injury reduces serum creatinine 1-7 days post nephrectomy and reduces renal fibrosis at day 28. Daily treatment with #25 starting 5 days after AA decreases serum creatinine from day 7-42 after injury. Short term treatment with #25 from days 5-10 and from days 5-7 after AA also reduce serum creatinine, but there is no reduction in renal fibrosis in #25 treated vs. controls. In UUO #25 was administered as a single dose 3 days after surgery. There is reduced renal *Kim1* (injury marker) and *Collagen1* mRNA in #25 treated mice, but no reduction in renal fibrosis at 5 days post surgery.

Conclusions: The PTBA analogue #25 ameliorates CKI by accelerating recovery after different forms of renal injury. Decreased fibrosis after severe IR-induced AKI suggests PTBA analogues ameliorate AKI induced CKI but do not reverse progressive renal fibrosis.

Funding: NIDDK Support

TH-PO075

Tubule-Specific Hepatocyte Growth Factor Signaling Is Essential in Renal Repair and Regeneration after Acute Kidney Injury Dong Zhou,[1] Roderick J. Tan,[2] Youhua Liu.[1] *[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; [2]Department of Medicine, University of Pittsburgh, Pittsburgh, PA.*

Background: Hepatocyte growth factor (HGF) is a pleiotrophic factor that plays an essential role in promoting injury repair and regeneration in multiple organs. After acute kidney injury (AKI), HGF receptor, c-met, was induced predominantly in renal tubular epithelium; but the potential role of tubule-specific induction of c-met in injury repair after AKI was elusive.

Methods: To address this issue, we generated conditional knockout mice, designated as Ksp-met-/-, in which the c-met gene was specifically disrupted in renal tubules.

Results: The Ksp-met-/- mice were phenotypically normal and displayed no appreciable defect in kidney morphology and function. However, in AKI induced by either cisplatin or ischemia/reperfusion injury (IRI), loss of tubular c-met substantially aggravated renal dysfunction and lesions. Compared with controls, Ksp-met-/- mice displayed higher serum creatinine and more severe morphologic injury. More apoptosis was detected in Ksp-met-/- kidneys, accompanied by an increased expression of Bax and Fas ligand and decreased phosphorylation/activation of Akt. In addition, tubule-specific ablation of c-met also augmented pro-inflammatory cytokine expression and promoted renal infiltration of inflammatory cells after AKI. Consistently, ectopic expression of exogenous HGF in vivo inhibited tubular cell apoptosis, decreased renal inflammation and repressed TNF-α and Bax expression after AKI.

Conclusions: Collectively, these results suggest that tubule-specific HGF/c-met signaling is crucial in conferring renal protection after AKI, primarily via its anti-apoptotic and anti-inflammatory mechanisms.

Funding: NIDDK Support, Government Support - Non-U.S.

TH-PO076

Characterization of Kidney Injury Molecule (Kim) Family of Proteins in Zebrafish Wenqing Yin, Said Movahedi Naini, Dirk M. Hentschel, Joseph V. Bonventre. *Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: The Kim/Tim (T-cell immunoglobulin mucin) family of genes encodes an IgV-like and a mucin-like domain, with three members in human and eight members in mice. (Kim-1/Tim-1), was the first identified member of this family and shown to be an important marker of proximal tubule injury in all mammalian species analyzed to date. KIM-1 also serves as a highly sensitive and specific urinary biomarker, and is markedly upregulated on dedifferentiated proximal tubular epithelium after ischemic or toxic kidney injury. Use of a kidney injury model and a wide array of genetic methods in zebrafish would facilitate an understanding of the role of Kim proteins in the development and recovery from acute kidney injury.

Methods: Kim family members were identified in zebrafish and their expression pattern determined in different stages of development and in adult TU/AB zebrafish, by performing PCR, western blotting, in situ hybridization (ISH) and immunostaining, We also used Tol2-mediated transgenesis to identify zkim-1 tissue specific regulatory elements.

Results: We identified Kim1, Kim3 and Kim4 in zebrafish, on chromosome 21. We cloned the zebrafish orthologs of Kim-1 with three splice forms, which we called long zKim-1, short zKim-1 and immunoglobulin zKim-1. zKim-1 encoded a type-1 cell transmembrane glycoprotein, including a six-cysteine immunoglobulin-like domain and threonine-serine-proline (T/SP) rich domain in the extracellular domain, and a relatively short intracellular domain. zKim3 and zKim4 are expressed in zebrafish larvae during development as detected by PCR and ISH. zKim-1 is markedly up-regulated on the brush borders of the injured tubular cells after injury in a model of gentamicin toxicity in the pronephros as well as mesonephros. We identified 25 conserved regions as potentialregulatory elements and created stable transgenic zebrafish lines harboring each conserved region to elucidate zkim-1 regulatory elements.

Conclusions: Zebrafish express Kim1, Kim3 and Kim4. zKim-1 expression recapitulates post-injury expression of mammalian Kim1. Highly structural and functional similarities exist between human KIM-1 and zebrafish. The structural similarities also allow for identification of tissue specific regulatory elements for human Kim-1 expression.

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TH-PO077

HIF-1α Promotes Renal Tissue Repair after Ischemic Damage Controlling Proximal Tubule Cell Proliferation, Cell Death and Inflammatory Response Elisa Conde, Edurne Ramos, Ignacio Blanco Sanchez, Elia Aguado Fraile, Esperanza Macarena Rodriguez Serrano, Laura Garcia-Bermejo. *Anatomia Patologica, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain.*

Background: Acute tubular necrosis (ATN) caused by ischemia/reperfusion (I/R) during renal transplantation delays allograft function. Identification of factors and mechanisms that mediate epithelium recovery could help to improve graft outcome. We previously reported an induction of Hypoxia Inducible Factor (HIF-1α)during reperfusion which is critical for tissue regeneration after renal I/R in a rat model. Here, we unveil several mechanisms triggered by HIF-1αinvolved in this regeneration.

Methods: For that purpose we have silenced HIF-1α in the rat I/R model by specific siRNAs. We estimate cell proliferation by BrdU staining, cell death by TUNEL, protein expression by western blot, proinflammatory mediators expression by qRT-PCR and cell infiltration by H&E staining.

Results: *In vivo* interference of HIF-1αexacerbated proximal tubule cell proliferation but not cell death, leading to an abnormal tissue repair. Moreover, HIF-1α inhibition altered p21 and p27 expression as well as bcl2/bax ratio.We also demonstrate that HIF-1α regulates the inflammatory response associated to ischemic renal damage. Its inhibition leads to an increase of proinflammatory mediators expression such as MCP-1, IL-1β e INFγ an increase of VCAM-1 expression and, consequently, an increase in inflammatory cells infiltration. Altered renal inflammatory response resolution will lead also to abnormal tissue repair.

Conclusions: In summary, we have identified several mechanisms triggered by HIF-1α, including the control of cell proliferation vs cell death and the inflammatory response, involved in the renal tissue repair after I/R, pointing out the critical role of HIF-1α in the kidney response to I/R. Modulation of this transcription factor could be considered a therapeutic option to reduce ATN and improve allograft outcome after renal transplantation.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO078

MG53 Attenuates Ischemia Reperfusion (I/R) Caused Acute Kidney Injury Pu Duann,[1] Ling-Mei Chiang,[2] Shing Li.[1] *[1]Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Paediatric, Chang Gung Memorial Medical Center, Keelung, Taiwan.*

Background: Ischemia is a central pathophysiological factor in developing acute kidney injury (AKI). In rat models of AKI. The tubular ischemic necrosis involving the proximal tubule and the thick ascending limb of Henle's loop. Reactive oxygen species was generally consided as the core factor in AKI pathophysiology. But several other factors like acute membrane damage of tubule epithelial cells was also suggested. Dysferin plays an importasnt role in maintenance of sacrolemmal membrane integrity. It was proposed that dysferin can function as a fusogen to allow vesicles to form a membrane repair patch. Immunostaining observed that dysferin concentrated at injury site at patch. However, dyferin itself can not translocate vesicles associated with acute membrane damage. Mitsuguimin 53 (MG53), a muscle-specific TRIM-family protein, acts as a sensor of oxidation to nucleate recruitment of intracellular vesicles to the injured plasma membrane. MG53 interact with dysferin form a repair machinery and repair membrane in acute injury. In this study we investigated the protective effect of MG53 in ischemia AKI.

Methods: Ischemic AKI was induced by clamping unilateral renal artery for 35 min with 2 days of reperfusion afterwards. Recombinant MG53 (6 mg/Kg BW) was intravanously given 5 min before or 35 minutes after ischemia induction. Kidney tissues and serum samples were collected 48 hours after I/R from three groups rats: sham treated group, I/R groups with or without MG53 treatment.

Results: Renal morphology and functional parameters confirmed clamping of the renal pedicles led to profound ischemic injury in the outer medulla. Compared to wildtype rat, MG53 treatment functionally (creatinine: AKI with MG53 treated, n=6, 0.48±0.17 vs. AKI no treatment, n=6, 1.42±0.42 mg/dl, p=0.007; p= 0.006; Ualb/Uc AKI with MG53 treated, 0.026±0.011, AKI no treatment, 0.057±0.032, p=0.002) and morphologically protected rat from ischemic AKI.

Conclusions: Our findings underscore the role of the acute membrane injury, most likely tubule epithelialcells, in the pathophysiology of AKI. The MG53 point to new therapeutic options for AKI.

TH-PO079

Association with Active Protein Kinase C-α Is Required for Recovery of F_0F_1-ATPase Activity Following Injury in Renal Proximal Tubules Grazyna Nowak, Diana Bakajsova. *Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: We have shown that F_0F_1-ATPase activity and ATP content are decreased by oxidant exposure in primary cultures of renal proximal tubular cells (RPTC) and that these changes are preceded by reduction of protein levels of phosphorylated (active) PKC-α in mitochondria of oxidant-injured RPTC. Recovery of active PKC-α levels in mitochondria precedes the recovery of F_0F_1-ATPase activity and ATP content. The goal of this study was to determine whether PKC-α interacts with F_0F_1-ATPase and if this association plays a role in the recovery of F_0F_1-ATPase activity following oxidant injury.

Methods: Wild-type PKC-α (wtPKC-α) and inactive PKC-α (dnPKC-α) were overexpressed in primary cultures of RPTC to increase mitochondrial levels of active and inactive PKC-α. PKC-α was immunoprecipitated from mitochondria after RPTC injury induced by the model oxidant, *tert*-butylhydroperoxide (TBHP), and proteins associated with mitochondrial PKC-α were identified. In addition, F_0F_1-ATPase activity and ATP content were assessed following injury.

Results: Overexpressing wtPKC-α prevented TBHP-induced decreases in active PKC-α levels and F_0F_1-ATPase activity in RPTC mitochondria. Overexpressing dnPKC-α blocked recovery of F_0F_1-ATPase activity and ATP content after oxidant injury. Protein levels of the catalytic (F_1) subunit in isolated mitochondria decreased at 24 h but not at 4 h after TBHP injury demonstrating that the activity of F_0F_1-ATPase declines prior to decreases in its protein levels. Proteomic analysis demonstrated association of both subunits (F_0 and F_1) of F_0F_1-ATPase with PKC-α in non-injured and oxidant-injured RPTC. The association of these subunits was predominantly with the active PKC-α. Overexpressing dnPKC-α markedly decreased the association of F_0 and F_1 subunits with PKC-α at 4 h following TBHP injury. Protein levels of both subunits associated with PKC-α were greater in injured RPTC overexpressing wtPKC-α than RPTC overexpressing dnPKC-α.

Conclusions: We conclude that F_0F_1-ATPase associates with active PKC-α and that this association promotes recovery of F_0F_1-ATPase activity and ATP content following oxidant injury in RPTC.

Funding: NIDDK Support

TH-PO080

BMP-7 Serves as a Prognostic Biomarker for Evaluating the Regenerative Capacity of the Kidney after Renal Injury Scott R. Manson, Paul F. Austin. *Department of Surgery, Washington University School of Medicine, St. Louis, MO.*

Background: Although obstructive uropathies are correctable through surgery, these conditions remain a leading cause of renal insufficiency because reliable prognostic indicators for surgical intervention are critically lacking. This study examines the mechanisms that contribute to the repair of obstruction-induced renal injuries and their clinical utility in the evaluation of obstructive uropathies.

Methods: A reversible murine model of unilateral ureteral obstruction (UUO) was used to characterize kidney repair following the correction of short-term obstructions (2d) that lead to reversible renal injury and prolonged obstructions (7d) that lead to irreversible renal injury. The role of BMP-7 was examined by treatment with BMP-7-neutralizing antibodies or recombinant BMP-7. The clinical relevance of our findings was assessed by examining BMP-7 levels in renal biopsies and urine samples from patients with obstructive uropathies.

Results: While renal injuries resulting from short-term UUO are effectively repaired following the correction of obstruction, treatment with BMP-7-neutralizing antibodies results in a 74.1 decrease* in collagen remodeling and a 84.9 % decrease* in tubular restoration in the kidney. In contrast, prolonged UUO results in irreversible renal injury and a 67.6% decrease* in BMP-7 levels. The restoration of BMP-7 activity with recombinant BMP-7 enhances kidney repair following prolonged UUO by promoting a 4.0-fold increase in collagen remodeling and a 3.8-fold increase* in tubular restoration in the kidney. Finally, injured kidneys from patients with obstructive uropathies exhibit a 64.1% decrease* in BMP-7 levels, a change mirrored by urinary BMP-7 levels.

* $p<0.01$.

Conclusions: These findings demonstrate that, while BMP-7 plays a critical role in the repair of renal injuries, the potential for renal recovery following chronic injury is diminished due to the loss of BMP-7. Together, these findings suggest that assessing the regenerative capacity of the kidney by measuring BMP-7 levels is an effective diagnostic approach to evaluate the likelihood of renal dysfunction and the necessity for surgery in patients with obstructive uropathies.

Funding: Private Foundation Support

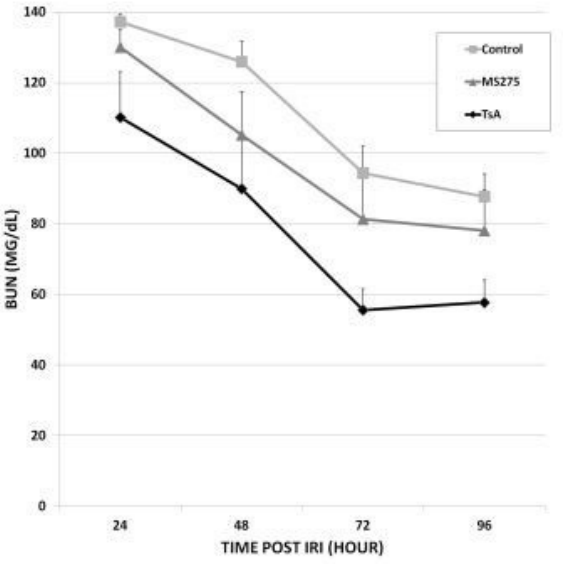
TH-PO081

Pan Histone-Protein Deacetylase Inhibition Protects Early Renal Function and Inhibits Fibrosis in Renal Ischemia Reperfusion Injury Matthew H. Levine,[1] Tricia Bhatti,[2] Yanfeng Wang,[1] Zhonglin Wang,[1] Wayne W. Hancock.[2] *[1]Surgery, University of Pennsylvania, Philadelphia, PA; [2]Pathology, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: Histone Protein Deacetylases (HDACs) remove acetyl groups from histone and non-histone proteins. Class I HDACs alter histone acetylation and gene accessibility. Class II HDACs deacteylate a variety of non-histone proteins and regulate heat shock factor 1 (HSF1) trafficking and downstream heat shock protein (HSP) expression. We wished to determine if HDAC inhibitors (HDACi) could improve renal ischemia reperfusion injury (IRI) tolerance and determine HDAC class-specificity.

Methods: Mice were treated with pan-HDACi Trichostatin (TSA), class-I specific HDACi (MS-275), or DMSO contol and then subjected to unilateral renal IRI with contralateral nephrectomy. Class II member HDAC6-/- mice were similarly tested. Early renal function after IRI was assessed by BUN daily for the first 4 days after IRI and computerized Sirius Red scoring for renal fibrosis was done at 28 days after IRI.

Results: Mice treated with TSA had improved tolerance of IRI compared to control mice over all early time points by BUN ($p = 0.001$). This correlated with diminished fibrosis (8 vs 16%) at 28 days by Sirius Red percentage ($p = 0.002$). MS-275 treated mice had a trend toward improved BUN but this did not reach significance ($p = 0.18$). HDAC-6-/- mice had no improvement in BUN relative to wildtype controls. HSP70 expression was lower in HDACi treated mice.



Conclusions: Pan-HDAC inhibition improved tolerance to renal IRI with less peak renal injury, more rapid recovery, and diminished fibrosis. Class I HDAC partially reproduced these effects. HDAC6-/- mice did not have altered IRI tolerance compared to control and HSP70 expression was lower in HDACi treated mice, demonstrating that HSP70 induction was not responsible for renal protection.

Funding: NIDDK Support

TH-PO082

Modeling Acute Kidney Injury in Zebrafish Larvae Lauren Brilli,[1,2] Chiara Cianciolo Cosentino,[1] Neil A. Hukriede.[1] *[1]Developmental Biology, University of Pittsburgh, Pittsburgh, PA; [2]Medical Scientist Training Program, University of Pittsburgh, Pittsburgh, PA.*

Background: Acute kidney injury (AKI) is associated with significant morbidity and mortality. Currently, there are no therapies available that can enhance human kidney repair. Several vertebrate species, including zebrafish, have maintained a robust ability to regenerate the kidney following injury; therefore, elucidating the mechanisms of organ regeneration in zebrafish may help us improve human kidney regeneration. Our lab has developed a model of AKI in zebrafish larvae that enables us not only to study the regeneration process but also to screen compounds that have the potential to enhance regeneration. Using this method, we have identified methyl 4-(phenylthio)butanoate (m4PTB), a novel histone deacetylase inhibitor (HDACi) that expands the population of renal progenitor cells during organogenesis. HDACis are known to improve renal injury in rodents; therefore, we hypothesized that m4PTB might enhance renal recovery following nephrotoxic damage in zebrafish larvae.

Methods: To induce AKI, we injected gentamicin into the circulatory system of zebrafish larvae. We performed H&E to evaluate tubular damage and TUNEL assay to visualize apoptotic cells. We examined effects on epithelial polarity by staining for Na^+/K^+ ATPase. To visualize proliferating cells in the proximal tubule, we performed double immunofluorescence using anti-EdU and anti-3G8. For drug treatment studies, we treated AKI-induced larvae with m4PTB and determined survival rate.

Results: Following gentamicin injection, larvae developed edema in a dose-dependent manner, reflecting loss of kidney water balance function. In AKI-induced larvae, we observed tubular cell apoptosis, cell sloughing, and loss of epithelial polarity. Treatment with m4PTB resulted in enhanced proliferation in the proximal tubular epithelium and improved survival.

Conclusions: These results suggest that the zebrafish larval pronephric kidney provides a suitable model for the study of kidney regeneration and point to the potential value of HDACi for improving post-AKI recovery. Whether m4PTB is a promising therapeutic agent to promote renal regeneration requires further study.

Funding: NIDDK Support, Other NIH Support - NICHD, Government Support - Non-U.S.

TH-PO083

Renal Functional Decline and Glomerulotubular Injury Are Arrested but Not Restored by Release of Unilateral Ureteral Obstruction (UUO) Françoise Praddaude,[1] Wassim Chaabane,[1] Marie Buleon,[1] Acil Jaafar,[1] Marion Vallet,[1] Carolina I. Galarreta,[2] Robert L. Chevalier,[2] Ivan A. Tack.[1] *[1]INSERM U1048, CHU Toulouse, Toulouse, France; [2]Dpt of Pediatric, University of Virginia, Charlottesville, VA.*

Background: Murine UUO is a classical model of progressive chronic kidney disease. Release of UUO permits the study of recovery even if most models do not permit measurement of renal function. Loss of proximal tubular mass and injury to the glomerulotubular junction (formation of atubular glomeruli) are major pathways for progression in this model.

Methods: Adult C57BL/6 mice underwent sham-operation or reversible UUO. In one group, kidneys were harvested after 7 days. In a second group, the obstruction was released after 7 days and animals allowed to recover. Physiologic study of left and right kidneys was performed 30 days after. Renal blood flow (RBF, PAH clearance), glomerular filtration rate (GFR, inulin clearance), urine protein and albumin excretion were measured after ligation of either the left or right ureter. Glomerular volume (PAS), glomerulotubular integrity and fractional proximal tubular mass (*Lotus tetragonolobus lectin) and interstitial collagen (Sirius red) were measured.*

Results: Obstructed kidney weight was reduced by 15% at 7d, but was not different from sham after 30d. Glomerular volume and fractional proximal tubular area of the obstructed kidney were reduced by 55% at 7d, but normalized after 30d. Interstitial collagen deposition increased 2.4-fold after 7 days of UUO, and normalized after release. However, GFR and RBF were reduced by 40% and urine albumin/protein ratio was increased 2.8-fold after 30d ($p<0.05$ for all data). This was associated with ~50% reduction in glomerulotubular integrity following UUO release, which was not improved compared to the value after 7d of UUO.

Conclusions: We conclude that release of 7 days UUO can arrest, but does not restore, normal function of the postobstructed kidney. Although remaining intact nephrons have hypertrophied, glomerular injury is revealed by albuminuria. These results suggest that glomerulotubular injury, rather than interstitial fibrosis, should become the primary target of slowing progressive kidney disease.

TH-PO084

Effect of Carbonic Anhidrase II (CAII) on Cell Regeneration and Damage in the Renal Lesion Induced by Ischemia-Reperfusion Marina Ventayol,[1] Anna Sola,[2] Georgina Hotter.[1] *[1]IIBB-CSIC; [2]CIBER-BBN.*

Background: Carbonic anhydrase is a metalloenzyme that is widely distributed throughout the entire body (renal cortex, glial tissues, the eye, erythrocytes, etc.) and reversibly catalyses the conversion of carbonic anhydride and water into carbonic acid. This enzyme presents four isoforms. Amongst them, carbonic anhydrase II (CAII) is the most active isoenzyme. The aim of this work is to demonstrate the capacity of CAII to induce regeneration and inhibit apoptosis in an in vivo experimental model of renal ischemia-reperfusion in rats.

Methods: The following groups were performed: I/R.- Animals subjected to 45 minutes of ischemia and 24 hours of reperfusion; I/R + CAII.- Animals subjected to I/R, but with injection of CAII i.v., CONTROL.- Control animals; CONTROL + CAII.-control group, but with intravenous injection of CAII ; I/R + Act: I/R, but with intraperitoneal injection of the CAII inhibitor, Acetazolamide.

Results: Regeneration, evaluated by the overexpression of the regenerative genes stathmine and PCNA, and erythropoietin synthesis evaluated by Western blot, were increased after administration of CAII to ischemic rats. Administration of the CAII inhibitor acetazolamide reversed the increases, indicating that CAII is a regenerative promoter and an inductor of EPO synthesis.

On the other hand, CAII showed a regenerative effect only on the damaged tissue, since the administration thereof to controls (control + CAII) did not induce regeneration.

Apoptosis, evaluated by the activity of caspase 3 was drastically reduced following treatment with CAII. The administration of CAII to control animals shows that CAII does not affect the apoptosis that is naturally present in healthy tissue (control group + CAII).

Conclusions: CAII promotes regeneration and prevents apoptosis after renal ischemia/reperfusion.

Funding: Government Support - Non-U.S.

TH-PO085

p66ShcA Deficiency Restores Redox-Sensitive Stress Response Program in Cisplatin-Induced Acute Kidney Injury Rungwasee Rattanavich,[1] Andrei Plagov,[1] Partab Rai,[1] Dileep Kumar,[1] Himanshu Vashistha,[2] Mohammad Husain,[1] Ashwani Malhotra,[1] Leonard G. Meggs,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Ochsner Clinic, New Orleans, LA.*

Background: Overwhelming oxidative stress and compromised tubular cell antioxidant response have been incriminated for cisplatin (Cis) -induced acute kidney injury (AKI). We hypothesized that Cis-induced AKI was the outcome of the deactivated redox-sensitive stress response program (RSSRP).

Methods: Wild (WT) and p66ShcA$^{+/-}$ mice (n=6) were administered either saline (WT) or Cis (12.5 mg/kg, intraperitoneal, Cis/WT). Renal biomarkers were collected and kidneys were harvested for renal histology. Severity of tubular injury was scored on coded samples. In addition, renal cortical sections were stained for TUNEL positive cells. Renal tissue lysates were immunoelectrophoresed and probed for the expression of phospho-p66ShcA, phospho-Foxo3A, MnSOD, and catalase. The same blots were stripped and reprobed for total p66, total Foxo3A and actin. In *in vitro* studies, mouse proximal tubular cells (MPTC) were treated with either vehicle or Cis. Immunoblots were probed for phospho-p66, phospho-p53, Bax, and MnSOD. Control and p66-silenced MPTCs were treated with Cis and evaluated for apoptosis.

Results: Cis/WT showed increased ($P<0.01$) BUN levels and enhanced tubular cell apoptosis and necrosis when compared to Cis/p66$^{+/-}$. Cis/p66$^{+/-}$ developed a clinically occult AKI (normal BUN and only microscopic alterations). Immunoblots from the lysates of renal tissues of Cis/WT displayed enhanced expression of phospho-p66ShcA, and phospho-Foxo3A but attenuated expression of MnSOD and catalase; conversely, p66 deficit prevented these alterations in Cis milieu. In *in vitro* studies, Cis treated mouse proximal tubular cells (MPTCs) displayed enhanced phosphorylation of p66ShcA and no increase in MnSOD expression. Renal tissues of Cis/WT and Cis-treated MPTCs also displayed enhanced phosphorylation of p53 and Bax expression.

Conclusions: Cis-induced AKI was caused by the deactivated RSSRP (attenuated anti-oxidant response) and activation of pro-apoptotic (p53-induced Bax expression) pathway.

Funding: NIDDK Support

TH-PO086

NADPH Oxidase Activation in Renal Ischemia-Reperfusion (I/R) Injury: The Role of Complement System Activation S. Simone,[1] F. Rascio,[1] G. Castellano,[1] P. Ditonno,[1] M. Battaglia,[1] Antonio Crovace,[1] F. Staffieri,[1] Beatrijs D. Oortwijn,[3] Francesco Paolo Schena,[1] Loreto Gesualdo,[1] Giovanni Pertosa,[1] Giuseppe Grandaliano.[2] *[1]Dept. of Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]Dept. of Medical and Surgical Sciences, Univ of Foggia, Foggia, Italy; [3]Pharming BV, Leiden, Netherlands.*

Background: Renal I/R plays a key role in the pathogenesis of delayed graft function after renal transplantation and is characterized by increased ROS generation. We demonstrated the activation of complement (C) system in a swine model of renal I/R damage. Aim of this study was to investigate the activation of NADPH oxidase in a swine model of renal I/R injury.

Methods: Renal I/R was induced in 10 pigs by arterial clamping; 5 of them were treated with a recombinant form of human recombinant C1-inhibitor (C1-INH). We collected biopsies before ischemia (T0), after 30' (T30') and 60' of reperfusion (T60').

Results: I/R injury was able to induce protein expression of NADPH oxidase subunits, gp91phox/NOX-2 and p22phox, with a peak at T60' ($p=.04$) in infiltrating monocytes-macrophages (CD163^{+}) and myeloid dendritic cells (SWC3a^{+}). A colocalization of gp91phox/NOX-2 and RAC was already observed in these cells at T30', supporting an early activation of the enzyme. NADPH oxidase renal isoform, NOX-4, was also studied by immunohistochemistry. We observed an increase in tubular NOX-4 expression with a peak at T60' ($p=.03$) that was completely inhibited by C1INH infusion. The NADPH oxidase activity was assessed (chemiluminescence) on renal tissue taken from both control and treated animals at T0, T30' and T60'. Enzyme activity significantly increased at 60' (220.2±18.8 vs baseline 91.6±12.0 ΔURL/Dt, $p=.01$) and was drastically reduced in C1INH-treated animals at the same time point (128.4±33 ΔURL/Dt, $p=.02$). In cultured tubular epithelial cells C3a induced NOX-4 expression in a time-dependent manner with a peak at T15'($p=.03$).

Conclusions: Data suggest that NOX-2 and NOX-4 are activated during I/R in a C-dependent manner and play a key role in ROS generation in renal I/R. Therefore, NADPH oxidases and C system may represent pharmacological targets to prevent oxidative damage during I/R injury.

TH-PO087

Surgically Injured Collecting Ducts in the Adult Rat Kidney Exhibit Capacity for *De Novo* Tubulogenesis Natalia O. Litbarg,[1,2] Snezana Vujicic,[1,2] Suman Setty,[1] Jose A.L. Arruda.[1] *[1]Medicine, UIC, Chicago, IL; [2]Medicine, JBVA Medical Center, Chicago, IL.*

Background: We have previously described a novel model of surgical wound regeneration, in which the polectomized kidney of adult rats was wrapped in an inert plastic pouch to prevent extrarenal tissue adhesions. During healing, we observed formation of branching tubular epithelial outgrowths (TEOs) that extended from the wounded edge of the kidney into the surrounding granulation tissue. Here, we sought to determine the nephronic origin of these TEOs.

Methods: Adult male rats were subjected to right nephrectomy and left renal polectomy followed by plastic pouch enclosure. Tissues were obtained between 1 and 12 wks after injury and subjected to serial sectioning and immunohistological profiling.

Results: Branching TEOs were anatomically connected to tubules at the kidney's wound edge. They were not involuting tubular remnants, since they showed increased expression of several proliferative and mitotic markers (pHH3, Ki67, and PCNA). TEOs and their tubules of origin expressed the following markers characteristic of collecting ducts and ureteric buds: *Dolichos biflorus* agglutinin, E-cadherin, pancytokeratin, Pax2, HoxB7, and calbindin. TEOs, however, lacked Aquaporin 2 (a marker of terminal differentiation first expressed by ureteric buds at embryonic day 18), suggestive of a dedifferentiated state. As the granulation tissue surrounding TEOs fails to express a number of markers of metanephric mesenchyme (Cited1, Pax2, Six2), the mechanism of tubulogenesis in our adult kidney injury model differs from embryonic tubulogenesis.

Conclusions: Together, our results suggest that surgically disrupted adult collecting ducts are capable of dedifferentiation and propagation into adjacent granulation tissue in the form of branching tubular epithelial outgrowths (TEOs). Our model offers evidence of a novel and previously unrecognized potential for regeneration by the adult mammalian kidney.

Funding: Veterans Administration Support

TH-PO088

Gβγ Signaling Plays a Critical Role in Recovery from Renal Ischemia-Reperfusion Injury in Rats Emily C. Haines, Sarah M. White, Frank Park, Kevin R. Regner. *Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Renal tubular epithelial cell (RTEC) proliferation is vital to tubular regeneration following ischemia-reperfusion injury (IRI). The mechanisms that regulate RTEC proliferation following kidney injury remain poorly understood. Heterotrimeric G proteins are pivotal molecular switches that can regulate epithelial cell function. We previously demonstrated that pharmacologic and genetic inhibition of Gβγ subunit activity

decreased renal epithelial cell proliferation in vitro. We therefore hypothesized that inhibition of Gβγ subunit activity would impair recovery from renal IRI in vivo.

Methods: Male Sprague-Dawley rats underwent 30 min bilateral renal ischemia (or sham) and reperfusion for 24, 72, or 168 hours. Rats were treated with a small molecule inhibitor of Gβγ (gallein, 100 mg/kg s.c.) or an equivalent volume of vehicle (phosphate buffer saline) daily for 4 days beginning one hour after reperfusion.

Results: At 24 hours, plasma creatinine (measured by LC-MS/MS) was 3.8 ± 0.6 mg/dl in vehicle treated rats and 3.6 ± 0.3 mg/dl in gallein treated rats (p=0.8). By 72 hours, plasma creatinine was significantly higher in gallein treated rats in comparison to vehicle treated rats (3.1 ± 2.3 mg/dl vs 0.90 ± 0.5 mg/dl, p=0.03) This was associated with a significant increase in the degree of tubular injury (tubular cell necrosis, tubular cast formation, and dilated tubules) in the outer stripe of the outer medulla and persistent expression of neutrophil gelatinase-associated lipocalin in gallein treated kidneys in comparison to vehicle treated kidneys. Gβγ subunit inhibition significantly increased mortality following IRI in a 7 day survival study. Median survival in gallein treated rats was 3 days in comparison to 100% survival in vehicle treated rats (p=0.03).

Conclusions: Taken together, these data suggest that Gβγ subunit signaling modulates kidney repair following IRI.

Funding: NIDDK Support

TH-PO089

α-Ketoglutarate-Related Inhibitors of HIF Prolyl Hydroxylases Are Substrates of the Renal Organic Anion Transporter 1 (OAT1) Birgitta C. Burckhardt,[1] Yohannes Hagos,[1] Gunnar Schley,[2] Johannes Schoedel,[2] Carsten Willam,[2] Gerhard Burckhardt.[1] *[1]Department of Systemic Physiology and Pathophysiology, University Medical Center Göttingen, Göttingen, Germany; [2]Department of Nephrology and Hypertension, University Medical Center Erlangen, Erlangen, Germany.*

Background: α-Ketoglutarate (αKG) is a substrate of HIF prolyl hydroxylases 1-3 that decrease cellular levels of the hypoxia inducible factor 1α (HIF-1α) in the presence of oxygen. αKG analogues are applied to stabilize HIF-1α even in the presence of oxygen and thus provide a novel therapeutic option in treating kidney diseases. In the kidneys, the organic anion transporters 1 and 3 (OAT1, OAT3) in cooperation with the sodium-dependent dicarboxylate transporter 3 (NaDC3) might be responsible for the uptake of αKG analogues across the basolateral membrane into proximal tubular cells.

Methods: Using the radiolabelled substrates p-aminohippurate (PAH; OAT1), estrone-3-sulfate (ES; OAT3), and succinate (NaDC3), N-oxalylglycine (NOG), dimethyloxalyl glycine (DMOG), 2,4-diethylpyridine dicarboxylate (2,4-DPD), and pyridine-2,4-dicarboxylic acid (PDCA) were tested in cis-inhibition and trans-stimulation experiments on HEK293 cells stably transfected with either human NaDC3, OAT1 or OAT3.

Results: None of the αKG analogues interacted with hNaDC3. At a concentration of 0.1 mM, 2,4-DPD and PDCA inhibited ES uptake in HEK293 cells stably transfected with OAT3 by 31.3 ± 8.3 and 29.7 ± 5.7%, respectively. NOG, 2,4-DPD and PDCA, but not DMOG, inhibited PAH uptake by OAT1 exhibiting IC50 values <0.1 mM. In trans-stimulation experiments, uptake of PAH increased upon pre-incubation of OAT1-transfected HEK293 cells for 2 h with NOG and PDCA. NOG and PDCA are substrates of OAT1, because of OAT1-mediated stabilization of HIF-1α by NOG and PDCA. Stabilization of HIF-1α was prevented by application of probenecid, an inhibitor of OAT1.

Conclusions: Therefore, OAT1 can be used for targeting αKG-related PHD inhibitors to proximal tubule cells to avoid hypoxic kidney injury.

Funding: Government Support - Non-U.S.

TH-PO090

IRAK-M Prevents Chronic Injury after Acute Kidney Injury through Suppression of Renal Inflammation Maciej Lech, Regina Groebmayr, Mi Ryu, Hans J. Anders. *Nephrologisches Zentrum, Klinische Biochemie, Medizinische Klinik und Poliklinik IV der LMU, Munich, Germany.*

Background: Acute kidney injury (AKI) is defined as a rapid decrease in the glomerular filtration rate with an accumulation of serum urea and creatinine. If functional parameters of kidneys return to normal levels within 30 days of dialysis the AKI is considered as reversible. Nevertheless, patients that recovered from AKI often develop chronic kidney disease (CKD), probably due to incomplete regeneration and persistent renal inflammation. IRAK-M is an inhibitor of TLR- and IL-1R-signaling, that prevents dissociation of IRAK-1 and IRAK-4 from MyD88 and inhibits proinflammatory signals in macrophages. As macrophages are important regulators of renal inflammation and tubular healing, we hypothesize, that genetic factors which influence the activation state of these cells have influence on inflammatory processes and regeneration after AKI.

Methods: Kidneys from wild type and IRAK-M-/- mice were investigated 3, 5 and 10 weeks after ischemia/reperfusion injury.

Results: Post-ischemic wild type kidneys regenerate successfully after 5 weeks and show no significant changes in size and structure compared to sham operated kidney. In contrast, IRAK-M-/- kidneys shrank to 1/3 of their original size due to loss of proximal tubules and formation of atubular glomeruli. Remarkably, macrophages infiltrates were massively increased in IRAK-M-/- mice. Lack of IRAK-M aggravated post-ischemic chronic renal failure evidenced by increased fibrosis and loss of tubular compartment as well as increased mRNA expression of TNF-a and the kidney damage markers. Finally, the TNF-a signaling pathway inhibitor Etanercept prevented loss of renal tissue in post-ischemic kidneys of IRAK-M -/- mice and reduced chronic inflammation.

Conclusions: Thus IRAK-M suppresses the persistent activation of renal macrophages and renal chronic inflammation upon transient renal ischemia by blocking expression of the proinflammatory and proapoptotic cytokine TNF. This process promotes tubular repair and recovery from post-ischemic tubular injury. IRAK-M loss-of-function mutations may predispose to chronic kidney injuries caused by acute triggers.

TH-PO091

Changes in Notch Signaling Contribute to Defective Regeneration and Increased Senescence in the Aging Kidney Inga Soerensen, Song Rong, Nathan D. Susnik, Hermann G. Haller, Roland Schmitt. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: Notch signaling is involvedin determining cell fate during development, tissue maintenance,and repair. The aging kidney has a diminished regenerative potential and an increased tendency to develop fibrosis. In this study, we wanted to test the impact of Notch signaling on the aging kidney in the context of acute damage and repair.

Methods: Notch activation in young (10 weeks) and old (18 months) mice was compared after renal ischemia/reperfusion (IR). Mice with conditional overexpression of constitutively active Notch1 in proximal tubules were created by mating GGT::Cre-ERT2 mice with floxed RosaNICD mice (NICD mice). Post-ischemic kidneys were analyzed by quantifying damage, apoptosis, inflammation, cell proliferation and senescence markers. In vitro, tubular epithelial cells were analyzed after overexpression of Notch signaling components.

Results: After IR injury, we observed a rapid activation of Notch1 in renal tubular cells in both young and old mice. This Notch activation and the expression of the downstream targets, Hes1 and Hey1, decreased quickly in young kidneys but old kidneys showed significantly prolonged activation. To analyse the consequences of sustained Notch activation, NICD mice were observed after IR. NICD overexpression was associated with higher expression of tubular damage markers Kim1 and NGAL and increased signs of fibrosis. In parallel, NICD kidneys showed higher expression of the cellular senescence marker $p16^{INK4A}$, but no differences were found in proliferation of tubular cells or inflammation. In cell culture, we used primary tubular epithelial cells of NICD mice or stimulated tubular epithelial cells with Notch ligand, Dll4, and observed a pro-senescent effect of Notch signaling.

Conclusions: Our data indicate that Notch activation is sustained in old versus young kidneys after IR. This mechanism might contribute to the overall worse outcome of old kidneys since transgenic activation of Notch is associated with defective regeneration, enhanced fibrosis and with a higher risk of developing cellular senescence.

Funding: Government Support - Non-U.S.

TH-PO092

Kidney Proximal Tubular Epithelial Cell-Specific Overexpression of Netrin-1 Suppresses Acute Kidney Injury Induced Interstitial Fibrosis and Glomerular Sclerosis Punithavathi Vilapakkam Ranganathan, Calpurnia Jayakumar, Ganesan Ramesh. *Medicine/Vascular Biology Center, Georgia Health Sciences University, Augusta, GA.*

Background: Acute kidney injury (AKI) is recognized as a major risk factor for the development chronic kidney disease (CKD), which remains one of the leading causes of death in the developed world. Fibrosis is responsible for chronic progressive kidney failure. However, knowledge on molecules that may suppress fibrogenic response after injury is lacking. In ischemic models of AKI, we demonstrate a new function of netrin-1 in regulating interstitial fibrosis.

Methods: Acute kidney injury was induced by clamping renal pedicle for a period of 26 minutes and then reperfused for a period of 1, 2 and 3 weeks. Kidney function was determined by measuring serum creatinine. Fibrosis, macrophage infiltration and vascular density were quantified by Western blot analysis, immunostaining and RT-PCR.

Results: Acute injury was promptly followed by rise in serum creatinine in both wild type and netrin-1 transgenic animals (1.2± 0.1 mg/dl vs. 1.1±0.1mg/dl). However, wild type showed a slow recovery of the kidney function as compared to netrin-1 transgenic animals (0.65±0.1 mg/dl vs. 0.37±0.1 mg/dl on day 7) and reached baseline by three weeks. Histological examination showed increased infiltration of interstitial macrophages, extensive fibrosis, reduction of capillary density and glomerular sclerosis. Collagen IV and α-smooth muscle actin expression was absent in sham operated kidney, however, expression was significantly increased at 2 weeks and peaked at 3 weeks after reperfusion. These changes were minimal in transgenic mice kidney which overexpresses netrin-1 in the proximal tubular epithelial cells.

Conclusions: Our data suggests that proximal tubular epithelial cells may play an important role in interstitial fibrosis and netrin-1 could be a useful therapeutic agent for treating kidney fibrosis.

Funding: NIDDK Support

TH-PO093

COMT LL Genotype Predisposes to Postoperative Acute Kidney Injury in Australian and German Cardiac Surgery Cohorts Annemarie Dittrich,[1] Johanna Kube,[1] Anja Haase-Fielitz,[1] Michael Plass,[2] Martin Zenker,[3] Denny Schanze,[3] Rinaldo Bellomo,[4] Peter R. Mertens,[1] Michael Haase.[1] *[1]Nephrology, Otto von Guericke University, Magdeburg, Germany; [2]Anaesthesiology, German Heart Center, Berlin, Germany; [3]Human Genetics, Otto von Guericke University, Magdeburg, Germany; [4]Intensive Care, Austin Health, Melbourne, Australia.*

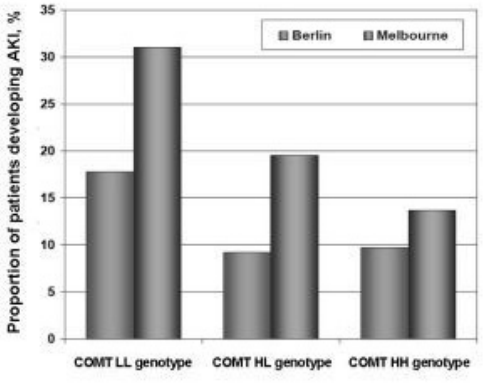
Background: Cardiocirculatory shock and acute kidney injury (AKI) are severe complications after cardiac surgery. Catecholamin-O-methyltransferase (COMT) is a ubiquitous enzyme, involved in systemic and renal degradation of catecholamines. In

a pilot study enrolling 260 cardiac surgical patients in Melbourne, Australia, 24.6% of patients were homozygous for the COMT "L"-allele, coding for thermolabile COMT variant Val158Met with low enzyme activity. COMT LL carriers more frequently developed AKI with or without acute dialysis compared to patients with HL (intermediate) or HH (high) enzyme activity [JASN 2009;20:1393-03].

Methods: In the present study, we wished to validate the results of the Australian study in an independent cohort prospectively enrolling 200 patients at the German Heart Center, Berlin. AKI was defined according to the RIFLE criteria.

Results: COMT genotype distribution (LL 23.1%; HL 55.9% HH 21.0%) was similar to the Australian cohort. There was no difference in age, comorbidities and operation. In the Berlin cohort, 17.8% of patients with LL, 9.2% with HL and 9.7% with HH developed AKI. *(Fig. 1)*. In 9.1% of LL, 5.6% of HL and 7.3% of HH patients acute dialysis was initiated. After pooling of both cohorts, COMT LL carriers showed higher AKI and dialysis rates (P=0.002, P=0.027) compared to HL and HH carriers.

Conclusions: Patients with COMT LL genotype are predisposed to worse renal outcomes after cardiac surgery. For preoperative risk stratification, COMT genotyping appears to be useful in patients who present with classical renal risk factors.



Funding: Private Foundation Support

TH-PO094

Risk of Acute Kidney Injury from Oral Acyclovir: A Population-Based Study Ngan Lam,[1] Matthew A. Weir,[1] Zhan Yao,[2] Peter G. Blake,[1] Michael Matthew Beyea,[1,3] Tara Gomes,[2] Sonja Gandhi,[1] Muhammad Mamdani,[2,3] Ron Wald,[3] Chirag R. Parikh,[4] Daniel G. Hackam,[1] Amit X. Garg.[1,3] *[1]Nephrology, University of Western Ontario, London, ON, Canada; [2]Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; [3]Nephrology, University of Toronto, Toronto, ON, Canada; [4]Nephrology, Yale University School of Medicine, New Haven, CT.*

Background: Intravenous acyclovir-induced acute kidney injury (AKI) from drug crystallization in the renal tubules is described in case reports, review articles and drug prescribing manuals. Similarly, AKI from oral acyclovir is described in case reports but the risk in routine practice is unknown.

Methods: We studied a large cohort of older patients in Ontario, Canada receiving new outpatient prescriptions between 1997 and 2011 for oral acyclovir or valacyclovir (which is metabolized to acyclovir). The primary outcome was hospital admission with AKI in the 30 days following the initial prescription. The comparison group was older patients receiving new prescriptions for famciclovir, an antiviral used for similar indications as acyclovir but with no known renal toxicity.

Results: A total of 76,269 patients received acyclovir or valacyclovir and 84,646 received famciclovir. On average, patients were 76 years old (interquartile range 71 to 81 years) and the prescription duration was 7 days. Acyclovir or valacyclovir use was not associated with a higher risk of hospital admission with AKI (209 [0.27%] events with acyclovir or valacyclovir versus 238 [0.28%] events with famciclovir, relative risk 0.97, 95% confidence interval 0.81 to 1.17). Results were consistent in adjusted analyses, in all subgroups and in the subpopulation with laboratory measurements.

Conclusions: In this population-based study, it is reassuring that routine use of oral acyclovir in older adults was not associated with serious AKI.

Funding: Private Foundation Support

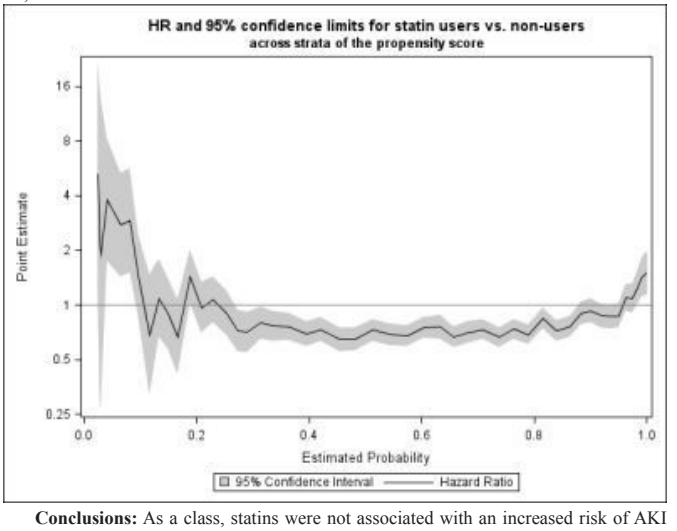
TH-PO095

The Risk of Acute Kidney Injury among Statin Initiators J. Bradley Layton,[1,2] M. Alan Brookhart,[1] Michele Jonsson Funk,[1] Ross J. Simpson,[2] Virginia Pate,[1] Til Stürmer,[1] Abhijit V. Kshirsagar.[2] *[1]Epidemiology, Univeristy of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Statins are widely used medications for preventing acute cardiovascular events, but recent reports have suggested they may increase risk of renal injury. We investigated the risk of acute kidney injury (AKI) in a large, claims-based observational cohort based in the United States during 2000-2010.

Methods: We identified individuals with new statin use following a six month washout period among employer-insured individualxs in a large, US, administrative claims database. A comparison group of non-users were identified at outpatient physician visits. Individuals were followed for one-year, and diagnosis codes in claims were searched for evidence of AKI or renal failure. Rates of AKI were compared in statin users vs. non-users with Cox-proportional hazard models to calculate hazard ratios (HR) and 95% confidence intervals (CI). We employed standard adjustment and propensity score (PS) methods.

Results: We identified 4,146,506 eligible statin users and 4,033, 800 non-users. Users had a 1-year AKI incidence of 0.9%, and non-users' was 0.3%. Adjusted models for statin users vs. non-users yielded HR=0.97 (95% CI: 0.94-0.99), while PS matching yielded HR=0.79 (95% CI: 0.76-0.81). Heterogeneity was observed over the distribution of the PS, with elevated risks observed at the tails of the PS distribution.



Conclusions: As a class, statins were not associated with an increased risk of AKI in the majority of users. Yet, substantial heterogeneity was observed in the baseline characteristics of statin initiators.

Funding: NIDDK Support

TH-PO096

Renal Tubular Injury Biomarkers in Non-Critically Ill Children Treated with Aminoglycosides Michael Zappitelli,[1] Melissa Piccioni,[1] Michael Pizzi,[1] Ang Gao,[3] Zubaida Al-Ismaili,[1] Michael R. Bennett,[2] Prasad Devarajan,[2] Julie Ho.[3] *[1]Pediatrics, McGill, Montreal, QC, Canada; [2]Pediatrics, Cin Child Hosp Med Cent, Cincinnati, OH; [3]Nephrology, U Manitoba, Winnipeg, MB, Canada.*

Background: Amionoglycosides (AG) are nephrotoxins commonly used in children. Little data exist on acute kidney injury (AKI) biomarkers in nephrotoxicity other than contrast-AKI. We evaluated previously studied urine biomarkers alpha-glutathione S-transferase (a-GST, µg/L) and pi-GST (µg/L) for reflecting AG-related tubular injury and performed a pilot study of hepcidin-25 (HEP-25, ng/ml) and liver-type fatty acid-binding protein (L-FABP, ng/ml) for AG-AKI diagnosis.

Methods: We prospectively studied 103 children receiving AG treatment [Rx](no renal disease, Rx≥3 days, available baseline serum creatinine[SCr]) on non-critical care units. Daily SCr and urine were collected for biomarker measurement. AKI was:SCr rise ≥50% from baseline (≤3 days pre-Rx). We calculated biomarker area under the curve (AUC) from a)2 days pre to 1 day post-AKI diagnosis and b) the 1st 2 Rx days to diagnose AKI.

Results: Mean[SD] age was 8.4[4.6] yrs (19% AKI;52% boys;73% tobramycin[Tobra]). All AUC's are shown in the table. Pi-GST performed better at AKI diagnosis when only Tobra patients were studied (max AUC 0.72 at 2 days after 1st AKI evidence). Pre-AKI a- nor pi-GST predicted AKI. Early Rx a- and pi-GST: only pi-GST predicted AKI development (AUC 0.77), especially on Tobra. Early Rx L-FABP was also diagnostic of AKI; HEP-25 was diagnostic of NON-AKI status. We confirmed higher early Rx HEP-25 levels in non-AKI patients ($p<0.05$).

Conclusions: Pi-GST is more discriminatory than a-GST for AG-AKI and more so with Tobra. We confirmed the HEP-25 association with non-AKI status. Together, these biomarkers may help identify early on the presence of AG-renal injury and ultimately lead to fewer AG renal complications.

AUC's (95% CI) for various biomarkers to diagnose AKI when obtained at different timepoints.

	DAYS FROM DAY OF 1ST AKI DIAGNOSIS (DAY 0 = 1ST AKI DAY)				EARLY AG RX URINE SPECIMEN (≤3 DAYS OF RX)
Biomarker	**-2**	**-1**	**0**	**1**	
Pi-GST					
All pts	0.49 (0.22-0.76)	0.54 (0.21-0.87)	0.52 (0.35-0.70)	0.64 (0.47-0.81)	**0.66 (0.51-0.81)**
Tobra only	0.59 (0.29-0.89)	0.65 (0.27-0.98)	0.62 (0.43-0.82)	**0.72 (0.55-0.88)**	**0.77 (0.61-0.93)**
Alpha-GST					
All pts	0.46 (0.21-0.72)	0.57 (0.33-0.80)	0.40 (0.25-0.51)	0.47 (0.31-0.64)	0.53 (0.28-0.77)
Tobra only	0.48 (0.22-0.74	0.60 (0.35-0.86)	0.40 (0.25-0.56)	0.49 (0.32-0.66)	0.54 (0.29-0.79)
L-FABP	N/A	N/A	N/A	N/A	**0.66 (0.50-0.82)**
HEP-25	N/A	N/A	N/A	N/A	0.63 (0.41-0.85)***

**** Note: Hepcidin-25 predicted NON-AKI status.*

Funding: Pharmaceutical Company Support - Argutus Medical Limited

TH-PO097

Post-Operative Proteinuria as a Biomarker of Acute Kidney Injury in Pediatric Cardiac Surgery: A TRIBE-AKI Consortium Study
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Background: We determined if early post-operative (post-op) cardiac surgery (CS) urine albumin to creatinine ratio (ACR) predicts post-op AKI in children.

Methods: We performed a 3-center (Montreal, Cincinnati, Yale-New Haven), prospective study of 294 children having CS (excluded:end stage renal disease;<1 month old). Post-op ACR was measured 0-6 hrs post-intensive care unit (ICU) entry. AKI definition: Stage 1(≥50% or 0.3 mg/dl rise from baseline); Stage 2 (≥SCr doubling or dialysis) AKI. We calculated adjusted relative risk (aRR) of post-op ACR to predict AKI. We used the C-statistic (AUC) and continuous net reclassification improvement (NRI) to determine if post-op ACR enhanced a clinical+biomarker (serum Cystatin C, urine neutrophil gelatinase-associated lipocalin, interleukin-18) AKI prediction model. We used multiple regression to determine if post-op ACR predicted longer hospital and ICU stay (LOS). We conducted analyses in children < *vs.* ≥2 years old (y.o.).

Results: 145/294 (49.3%) were <2 y.o. 78/145(54%) and 43/149(29%) of <2 *vs.* ≥2 y.o. developed AKI. In the ≥2 y.o. group, children in the highest tertile post-op ACR had an aRR=2.1 (95% CI 1.1-4.1) for AKI. Only in the ≥2 y.o. group, post-op ACR improved AKI prediction when added to a clinical-biomarker model (Table, NRI p≤0.03). Higher post-op ACR predicted longer hospital and ICU LOS (adjusted p = 0.02 and 0.01, respectively) in the ≥2 y.o. group but only hospital LOS (adjusted p = 0.02) in the <2 y.o. group.

Conclusions: Post-op ACR is a readily available early test for AKI development after CS in children ≥2 y.o.

Table. Performance of early post-operative albumin to creatinine ratio (ACR) to predict post-operative AKI (≥50% serum creatinine rise or receipt of dialysis). All Area under the curve (AUC) values with associated standard errors. All net reclassification improvement (NRI) values with associated upper and lower 95% CI.

	AUC for AKI: ACR only	**AUC for AKI:** Clinical factors + other biomarkers	**AUC for AKI:** Clinical factors + other biomarkers + **ACR** (p-value: difference with previous AUC)	NRI after adding ACR to Clinical+biomarker model (p-value)
1 month-2 y.o.	0.57 (0.05)	0.79 (0.04)	0.80 (0.04) p=0.46	0.22 (0.17) p=0.21
≥2 y.o.	0.63 (0.05)	0.86 (0.03)	0.88 (0.03) p=0.46	0.39 (0.13) p=0.03

Clinical factors: age, gender, site, cardiopulmonary bypass time, surgical severity score, pre-operative renal function.
Other early post-op biomarkers: urine neutrophil gelatinase- associated lipocalin and interleukin-18; serum cystatin C.

Funding: NIDDK Support

TH-PO098

Vancomycin-Induced Acute Kidney Injury (Van-AKI): An Underreconized Issue on the Rise with Incomplete Recovery but Preventable by Mandatory Drug & Serum Creatinine Monitoring
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Background: Although the literature suggests Van-AKI, conclusive evidence has been elusive. Recently the incidence seems to rise due to MRSA & MRSE epidemics, escalating use for health care associated pneumonia & osteomyelitis, & organisms sensitive only to levels >15-20 mg/L. We here present evidence from 17 cases to argue for Van-AKI, analyze risk factors & forewarning signs, provide a functional profile & guidelines for prevention.

Methods: These cases were seen in 3-mon renal consults & diagnosed by toxic levels & vigorously excluding all other known causes.

Results: The mean therapy duration was 21 d & mean dose was 2.3 g /d. On day 16, 3 d before the highest vancomycin level, drug level was already elevated at 27 mg/L. On this day, serum creatinine (Screat) was 1.6 mg% & estimated creatinine clearance (eCrCl) fell by 33 % vs baseline 110 ml/min. Maximal vancomycin level (66 ± 5 mg/L) was noted 18 d after the 1st dose & 8.4 h after the last dose. On this day, Screat had risen to 4.1 vs baseline 0.97 mg% & CrCl fell by 79 %. Screat peaked at 5.8 mg%, ~5.4 d after maximal level or 57 h after the last dose. At this point, eCrCl fell to a nadir of 22 ml/min, an 80 % drop. After stopping vancomycin, Screat fell to a nadir of 1.34 mg%, ~ 36 d after the last & 57 d after the first dose. Recovery was incomplete even 36 d after the last dose since the best eCrCl of 61± 7 ml/min indicated 45% loss. 4.6 d before maximal level, elevated level of 27.3 +/-5.3 mg/L already existed, which should be a warning signal for toxicity. 8.4 h before the last dose, toxic level (66 mg/L) was already reported, which should prompt omission of the last dose, given the markedly increased Screat of 4.1mg/dl.

Conclusions: Van-AKI is a real & costly financially & clinically since significant irreversible loss ensues. Prescribers must document the last trough level & creatinine before orders. We suggest dose reduction if level ≥ 20-25 mg/L &/or Screat doubles the baseline.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO099

Peritoneal Dialysis for Acute Kidney Injury in Developing Counties: Decreasing Maternal and Childhood Mortality Rates
Mary Carter,[1,2] Fredric O. Finkelstein,[3] Karen E. Yeates,[4] Peter Kotanko,[1,2] Nathan W. Levin,[1,2] John Callegari.[1,2] *[1]Renal Research Institute, NY, NY; [2]Sustainable Kidney Care Foundation, NY, NY; [3]Hospital of St Raphael, Yale Univ., New Haven, CT; [4]Queens Univ., Kingston, ON, Canada.*

Background: There is an increasing need for treatment of AKI in developing countries. Sustainable Kidney Care Foundation (SKCF), working with industry and professional societies starts PD programs for AKI with a focus on the UN Millennium Goals, namely saving the lives of children and women of childbearing age. An shortage of clinicians with nephrology training exists.

Methods: SKCF model focuses on sustainability and capacity building. The hospitals need to have a reliable medical laboratory and trained clinical staff who can perform PD catheter placement. PD consumables are donated for 2 years during which payment from insurance and/or patients is collected, when possible, and then saved and used for acquisition of supplies for subsequent years. PD was selected as the modality of choice as it can be provided in low technology settings vs. HD, which requires major capital expenditures and technical sophistication. Training has been provided by ISN, ISPD and IPNA.

Results: Successful programs are ongoing in Tanzania and Ghana, with new programs starting in Benin, Cameroon, Ethiopia and Uganda. 85% of treated AKI patients have had a full recovery of their kidney function after an average of 10 days of PD. One of the challenges continues to be educating healthcare workers in remote areas to recognize AKI for early referral.

Conclusions: PD for AKI programs can be organized in developing countries. In addition to saving lives, they can become important catalysts for starting a national discussion about kidney disease, prevention and treatment; result in the inclusion of nephrology in the medical school curriculum and outreach to rural communities. While kidney disease was not specifically mentioned in the WHO September 2011 meeting calling for the reduction of 4 major non-communicable diseases (heart attacks, strokes, cancer and respiratory disease), a rising number of renal patients can be expected and saving lives with adequate dialysis is feasible.

Funding: Private Foundation Support

TH-PO100

Hypophosphatemia during Continuous Veno-Venous Hemofiltration Associated with Mortality in Critically Ill Patients with Acute Kidney Injury
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Background: To determine whether continuous veno-venous hemofiltration (CVVH) associated hypophosphatemia is conferring effect on the global outcome of critically ill patients with acute kidney injury (AKI).

Methods: We studied 622 patients who were diagnosed as AKI, receiving CVVH therapy more than 72 hours. The primary outcome was death during the 28-day period or survival at 28 days after initiation of CVVH. Demographic and clinical data including serum phosphorus levels were recorded along with clinical outcome.

Results: The multivariate analysis indicated that the incidence of CVVH associated hypophosphatemia was an independent risk factor of outcome, conferring a 1.513-fold (95% CI 1.160–1.972, p = 0.002) increased mortality rate at 28 days compared with those without developing hypophosphatemia. Analysis with the subcohort of patients who hadhypophosphatemia episodes during the CVVH therapy period indicated that the frequency of hypophosphatemia (which defined as the hypophosphatemia days in the ratio of total CVVH therapy time) but not the incidence of severe hypophosphatemia (p = 0.138) was an independent risk factor of outcome. Compared with the patients having low hypophosphatemia frequency (< 0.49), those with high hypophosphatemia frequency (≥ 0.49) conferring a 2.302-fold increased mortality rate (95% CI 1.617–3.275, p<0.001).

Conclusions: CVVH associated hypophosphatemia was an independent risk factor of the global clinical outcome of critically ill patents with AKI. During the CVVH therapy period, the frequency of hypophosphatemia but not the incidence of severe hypophosphatemia served as an independent risk factor of outcome, and high hypophosphatemia frequency suggested increased mortality rate.

Funding: Clinical Revenue Support

TH-PO101

Acute Kidney Injury Requiring Renal Replacement Therapy in Patients without Pre-Existing Disease
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Background: Acute kidney injury (AKI) necessitating renal replacement therapy (RRT) in the critically ill is associated with an excessively high mortality. After hospital discharge, these patients have an increased risk for progression towards end stage renal disease (ESRD) and increased mortality. However, critically ill patients are often known with pre-existing diseases which may significantly confound the association of AKI with in-hospital mortality and its long-term consequences.

Methods: We performed a retrospective cohort study in a large academic hospital, selecting for critically ill patients with AKI necessitating RRT without pre-existing disease. The associations with mortality and ESRD requiring dialysis in the short and long term were evaluated.

Results: Of the 1221 critically ill patients recruited, only 94 (7.7%) had no pre-existing disease. In-hospital mortality was 43.6%, which was equally high as in a group of patients matched for age and sex with pre-existing disease. Those who survived hospital admission had a mean follow-up of 8.6 years (range = 0 - 17) and 49.1% left the hospital with an estimated glomerular filtration rate (eGFR) <90 ml/min/1.73m² of which two were dialysis dependent. The 1-year, 5-year and 10-year cumulative survival rates for patients that survived hospital admission were, 96.2%, 91.4% and 85.6%, respectively. Cumulative survival rates for the matched patients with pre-existing disease were 89.8%, 65.7% and 57.0%, respectively. Compared to the predictive survival rate in the average Dutch population, the 10-year survival rate in this study was 7% lower. Besides the two survivors that progressed towards ESRD during hospital admission, one additional patient reached ESRD during follow-up after 7.5 years, which constituted a 10-year renal survival of 93%.

Conclusions: Critically ill patients with RRT-requiring AKI have an excessively high in-hospital mortality risk, even in the absence of pre-existing disease. However, those without pre-existing disease have a good long-term prognosis.

TH-PO102

Acute Renal Failure in the Pediatric Intensive Care Unit: Etiology and Outcome Rainer Büscher, Janet Atinga, Anja K. Büscher, Peter F. Hoyer. *University of Duisburg-Essen, Pediatrics 2, Pediatric Nephrology, Essen, Germany.*

Background: Acute renal failure (ARF) is common in critically ill children and characterized by a sudden but reversible increase of serum creatinine, nitrogenous waste products and disturbances of the electrolyte and fluid balance. The etiology of ARF has shifted from primary renal disease to multifactorial causes. Therapeutic strategies and prognosis depend on the underlying disease. The aim of this retrospective study was to define etiology and clinical features of ARF in 147 children and to evaluate prognostic factors and the outcome after renal replacement therapy.

Methods: Between 2001 and 2011, 147 pediatric patients (66 females, 81 males), were admitted to our hospital for ARF. Out of those, 26 (17.6 %) were newborns (median age 4 days, range 1-22 days) and 121 patients (82.4%) were children older than 1 month (median 3.21 years, range 1 month–18 years). Causes of ARF, accompanying medical conditions, pediatric-modified RIFLE criteria, treatment, indications and mode of dialysis as well as patient outcome were retrospectively analyzed.

Results: While haemolytic uremic syndrome (n=42; 35%), sepsis (n=36; 30%) and dehydration (n=34; 28%) were the most common causes of ARF in children older than 1 month, renal vein thrombosis (n=11; 42%) as well as shock and asphyxia (n=10; 38%) were predominant reasons in newborns. Dialysis was performed in 12.5% (n=3, all CVVHD) of newborns and 55% (n=66, 37 CVVHD, 29 CVVHF) of children older than 1 month. Overall mortality was 23% (n=34) and was predominantly observed in the group of septic children following bone marrow transplantation (BMT; n=30, 88%). All BMT patients underwent dialysis treatment. Out of 66 dialysed patients restitution could not be achieved in 15 cases (22.7%) and chronic dialysis treatment became necessary.

Conclusions: Our overall results suggest a favourable outcome of ARF in children regardless the necessity of dialysis. In contrast, ARF in children following BMT and sepsis is associated with a 100% mortality rate. Improved understanding of the pathophysiology, early biomarkers of AKI, and better classification are required to optimize successful therapeutic efforts.

TH-PO103

Hyperglycemia and Acute Kidney Injury in Critically Ill Children Roberto Gordillo,[1] Robert Woroniecki.[2] *[1]Pediatric Nephrology, University of Illinois Medical College at Peoria, Peoria, IL; [2]Pediatric Nephrology, Columbia University College of Physicians and Surgeons, New York, NY.*

Background: Hyperglycemia has been associated with increased mortality and morbidity in critically ill adults and children. However, it is still not clear if hyperglycemia is associated with acute kidney injury (AKI) in critically ill children.

Methods: We conducted a retrospective review of charts of children admitted to the pediatric intensive care unit (PICU) of the University of South Alabama Children's and Women's Hospital (n= 37) followed by ongoing prospective subject enrollment (n=6)at the Children's Hospital of Illinois. Critically ill subjects were defined as subjects on mechanical ventilation within 36 hours of admission.

We excluded infants and children with known kidney disease. Pediatric RIFLE criteria and the revised Schwartz formula were used to determine degree of AKI. Peak glycemia was correlated with the degree of AKI and with the length (days) of PICU stay.

Results: Out of 43 subjects 19 had AKI.

	No-AKI (n=24)	AKI (n=19)	p
Age (years)	5.98 ± 1.18	6.2 ± 1.07	0.3
Female (%)	45	42	0.99
Caucasian (%)	38	42	0.52
Peak glucose mg/dl	194.54 ± 13.12	240.52± 18.15	0.03
Length of hospital stay (days	24.29± 3.84	29.42± 4.74	0.4
Length of PICU stay (days	14.79± 1.94	19.1± 3.81	0.57

± standard error of the mean

We found a statistical difference in peak glycemia between the no-AKI group and the AKI group and a significant positive correlation between length of hospital ($p<0.05$) and intensive care unit stay ($p<0.05$) and peak glycemia. No difference in serum glycemia was found between the Risk and the Injury/Failure groups ($p=0.72$). 1 subject died in the in the AKI group, none died in the no-AKI group.

Conclusions: Serum glucose was significantly higher in the AKI group. A positive correlation between length of hospital and intensive care unit and serum glucose was found. The intensity of AKI did not correlate with serum glucose.

Funding: Private Foundation Support

TH-PO104

Predictors of Dialysis and Death in Hospitalized Patients with Severe Acute Kidney Injury Francis P. Wilson, Wei (Peter) Yang, Harold I. Feldman. *Medicine, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA.*

Background: Acute kidney injury (AKI) carries a substantial risk of mortality, even after adjustment for comorbidities. Effective risk stratification may lead to more effective therapeutic interventions for high risk subgroups.

Methods: We identified adults who suffered severe in-hospital AKI from 1/1/2004 – 8/31/2010 at three hospitals in the University of Pennsylvania Health System. Patients were included if baseline creatinine was ≤1.4mg/dl for men or ≤1.2 mg/dl for women, and serum creatinine doubled during the hospital admission. Cox proportional hazards models predicting death, dialysis, or both were fit using data from patients admitted to the Hospital of the University of Pennsylvania (n=4,263), and validated at the two other UPHS facilities (n=758, n=1098). Models were evaluated with C-statistics.

Results: 6119 patients were included; 602 (9.8%) received dialysis therapy. 14-day mortality was 44% in the RRT group and 19% in the non-RRT group ($p<0.001$). In adjusted analyses, strong predictors of the combined endpoint included ICU location (vs. floor), medical service, liver disease, higher creatinine, greater rate of change in creatinine, and greater number of pressor medications. Higher absolute creatinine concentration was associated with greater use of dialysis, but lower overall mortality in adjusted analyses. Harrell's C-index for the model predicting the combined endpoint was 0.85 (0.84-0.86) in the derivation cohort, and 0.83 (0.80 – 0.86) and 0.84 (0.82 – 0.86) in the validation cohorts. Early predictors (measured on day 1 of AKI) of the combined endpoint included medical service, lower admission eGFR, lower bicarbonate, ICU location, and liver disease. C-index for the prediction of death or dialysis using only day 1 metrics was 0.78 (0.77 – 0.79) in the derivation cohort and 0.77 (0.72 – 0.81) and 0.75 (0.72 – 0.78) in the validation cohorts.

Conclusions: A small group of easily measured clinical factors has good ability to predict mortality and dialysis in severe AKI. The predictive power increases as AKI progresses, but early prediction of death or dialysis is feasible.

Funding: NIDDK Support

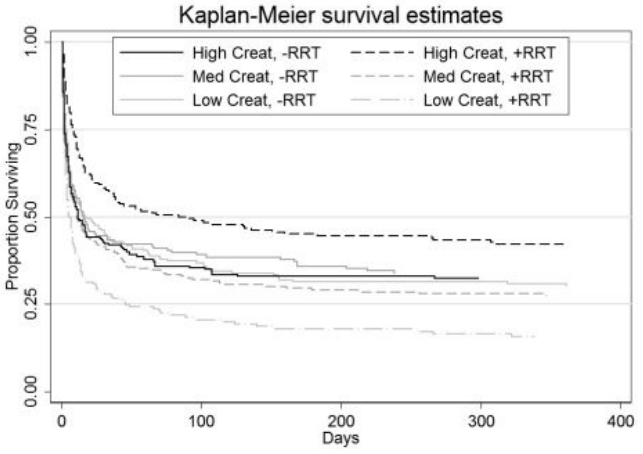
TH-PO105

Dialysis at a Higher Creatinine Is Protective in Acute Kidney Injury: A Propensity-Matched Cohort Study Francis P. Wilson, Wei (Peter) Yang, Harold I. Feldman. *Medicine, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA.*

Background: Currently, no guidelines regarding the provision of dialysis based upon the severity of acute kidney injury exist. We designed a propensity-matched cohort study to assess the benefit of dialytic therapy for the treatment of AKI.

Methods: We identified adults who suffered severe in-hospital AKI from 1/1/2004 – 8/31/2010 at three hospitals in the University of Pennsylvania Health System. Patients were included if baseline creatinine was ≤1.4mg/dl for men or ≤1.2 mg/dl for women, and serum creatinine doubled during the hospital admission. Logistic regression relating a variety of demographic, clinical, and laboratory variables to the initiation of dialysis was used to generate a daily propensity score to receive dialytic therapy for each of the 14 days following the onset of AKI (defined by AKIN 1 criteria). AKI patients who received dialysis were then matched to non-dialyzed patients based on the propensity score for dialysis and days since AKI onset. The primary analysis was implemented to define laboratory parameters that would identify patients in whom dialysis was most beneficial.

Results: 6119 patient hospitalizations met entry criteria, representing 143,891 patient-days of follow-up. 602 patients received dialytic therapy. Compared to patients who were not dialyzed, those receiving dialysis were younger, more often male, more often on a surgical service and less often black, (all $p<0.001$). After propensity score matching, covariates were well-balanced between the groups, and the overall hazard ratio for death in dialyzed vs. non-dialyzed patients was not significantly different than 1.0. Serum creatinine concentration strongly modified the association between dialysis and survival, with a 16% greater benefit from dialysis on survival for each mg/dL increase in serum creatinine concentration ($p<0.001$), with net benefit occurring at a creatinine greater than 3.9 mg/dL.



Conclusions: Our results suggest that dialysis may be particularly beneficial for AKI patients with higher serum creatinine concentration. This may reflect benefit due to AKI severity, but could be due to a protective effect of increased muscle mass.

Funding: NIDDK Support

TH-PO106

TTP in Australia: Initial Data from a New National Registry Shlomo J. Cohney,[1] Simon Wilkins,[1] James Sloane,[1] Louise E. Phillips,[1] Paul K. Cannell,[3] Sunelle Engelbrecht,[2] Danny Hsu,[4] Zoe Mcquilten,[1] Stephen Opat,[1] David J. Roxby,[5] Erica M. Wood.[1] *[1]Department of Epidemiology, Monash University, Victoria, Australia; [2]ARCBS, Victoria, Australia; [3]Hematology, Royal Perth Hospital, Western Australia, Australia; [4]Liverpool Hospital, NSW, Australia; [5]Flinders Medical Centre, South Australia, Australia.*

Background: Thrombotic Thrombocytopenic Purpura (TTP) and other TMA are rare, but cause significant morbidity & mortality with uncertainty around incidence, diagnosis and optimal management. Recent insights into pathophysiology, new diagnostic tests and therapies, have made the need for clinical data collection increasingly important to determine optimal therapy.

Methods: A registry was established to determine the incidence, clinical characteristics, & natural history of patients with TTP, & recently expanded to encompass atypical hemolytic syndrome (aHUS) & other thrombotic microangiopathies (TMA).

Results: Data have been received from 14 hospitals on 67 episodes in 60 patients (16-83y, 59% female). Presenting features included neurological (55%), gastrointestinal or genitourinary (51%), hemorrhagic (35%), fever (30%) & thrombotic (17%). Precipitants included infection (31%), autoimmune (21%), malignancy (16%), medication (15%), allograft (1%) or postpartum (1%). ADAMTS13 levels were requested in 70%; 40% had <10% levels, 43% had >10% & in 16% results were unknown. 70% of patients with <10% ADAMTS13 were in complete remission (CR) without impairment compared with only 38% of those >10%. Most patients initially received plasma exchange (PEx), at least daily. 42% had PEx tapered. 65% received additional therapy, including corticosteroids (73%) or rituximab (30%), to augment PEx (41%), because of suboptimal response (11%) or other (26%). 21% are receiving ongoing therapy, 41% patients are in CR without impairment, 8% in CR with persisting impairment and 16% died.

Conclusions: TTP continues to cause significant morbidity and mortality, with continued variation in management. Increased understanding of pathophysiology and new therapies makes collection and analysis of patient data critical to direct future treatment appropriately.

Funding: Government Support - Non-U.S.

TH-PO107

Pathognomonic Constellation of Ultrasound Findings in EHEC-Associated Hemolytic-Uremic Syndrome and Their Clinical Relevance Ansgar Reising,[1] Gunilla Einecke,[1] Marcus Hiss,[1] Jan T. Kielstein,[1] Carsten Hafer.[2] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Nephrology, Nephrologisches Zentrum Niedersachsen, Hann. Münden, Germany.*

Background: During the 2011 German epidemic of hemolytic-uremic syndrome caused by EHEC O104:H4 we performed abdominal ultrasound at time of hospital admission in 44 patients and repeatet it after recovery (6 ± 2 weeks later).

Methods: We screened the patients for pleural effusion, ascites, kidney size and morphology, renal resistance indices (RI) and size of the liver and spleen. For quantification of parenchymal density we performed a computer-based image analysis of the kidney parenchyma and the liver parenchyma grey intensity values in 16 patients. For each patient we obtained 10 measurements each from two separate ultrasound images. For standardization we calculated the ratio between average kidney and liver intensity values for each image.

Results: Upon initial presentation, we observed moderate hepatomegaly in 43% of patients, splenomegaly in 16 patients, ascites and pleural effusions in 87% of patients. There was no significant relationship between the presence of pleural effusion and the necessity for dialysis treatment (p = 0.246). The kidneys appeared swollen, with an average intrarenal RI of 0.79 ± 0.09. RI-values were higher in patients with dialysis-dependent renal failure (0.82 ± 0.07) than in patients who did not require dialysis (0.75 ± 0.10, p = 0,006). The kidney/liver intensity ratio was 1.49 ± 0.39 at the time of diagnosis but was significantly reduced after recovery (0.99 ± 0.31 (p < 0.001). The kidney/liver intensity ratio at time of diagnosis correlated with the number of dialysis treatments before recovery (r = 0.44). Patients who required dialysis treatment had higher values than those who did not (1.58 ± 0.41 vs 1.23 ± 0.20).

Conclusions: Patients with EHEC-HUS had a characteristic constellation of morphologic abnormalities on ultrasound examination. Surprisingly, the majority of patients had pleural effusions, ascites, and striking changes in kidney morphology. The parameters reflecting renal perfusion correlated with the necessity for renal replacement therapy and thus may have prognostic value in future patient evaluation.

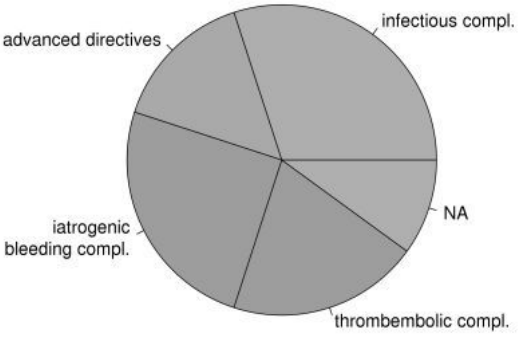
TH-PO108

Detailed Analysis of Causes of Death from HUS during the 2011 STEC-HUS Outbreak in Germany Gernot Beutel,[1] Jan T. Kielstein,[1] Tobias N. Meyer,[3] Reinhard Brunkhorst Richard Brunkhorst.[2] *[1]Department of Internal Medicine, Medical School Hannover, Hannover, Germany; [2]Krankenhaus Region Hannover, Department of Nephrology and Hypertension, Hannover, Germany; [3]Department of Nephrology, Asklepios Klinik Barmbeck, Hamburg, Germany.*

Background: The 2011 Shiga-toxin-producing E. coli (STEC) O104:H4 outbreak in Germany claimed 52 deaths among 2,987 STEC and 855 confirmed HUS cases. Survival data in adult patients with STEC- HUS are limited. We aimed to provide an in depth analysis of causes of death of the 621 HUS patients in the STEC-HUS registry, established by the Germany Society of Nephrology in response to the outbreak.

Methods: To identify main risk factors for death 100 parameters including demographic data, clinical symptoms, time course illness, microbiological information and laboratory parameters were collected. Firther, analysis by treatment options best supportive care (BSC), therapeutic plasma exchange (TPE), and TPE with eculizumab (TPE-Ecu) was performed.

Results: Age was the main risk factor for predicting death rate. While survivors had a median age of 45 years (IQR 31-60) non-survivors were 70 years old (IQR 61-76, p<0.001). Mortality rate was higher in patients receiving BSC (10.6%, p<0.05) than in TPE (3.7%) or TPE-Ecu (2.6%) patients. However deaths were frequently found not HUS related: Six (30%) patients suffered from infectious complications, five (25%) from iatrogenic bleedings, and four (20%) from thrombembolic complications. Three (15%) patients had advanced directives.



Those patients increased mortality rate for an intend-to-treat analysis from 4 to 6 patients in the BSC group.

Conclusions: Mortality rate in adult patients during the 2011 outbreak of O104:H4 in 2011 was lower than during previous outbreaks with O157:H7. More than half of the patients died due to limitation of care and iatrogenic bleeding complications. Age was the main risk factor for death.

TH-PO109

Epidemiology of Acute Kidney Injury from the O'Brien Center Registry Josee Bouchard,[1] Anjali Acharya,[2] Jorge Cerda,[3] Elizabeth R. Maccariello,[4] Rajasekara Chakravarthi Madarasu,[5] Ashita J. Tolwani,[6] Xinling Liang,[7] Ping Fu,[8] Zhi-Hong Liu,[9] Ravindra L. Mehta.[10] *[1]U de Montreal; [2]Jacobi Medical Center; [3]Albany Medical College, Albany, NY; [4]Rede d'Or; [5]CARE Hospitals; [6]UAB; [7]Guangdong General Hospital; [8]Sichuan Univ; [9]Nanjing Univ; [10]UCSD.*

Background: AKI is frequent and is associated with poor outcomes. However, there is lack of multicenter large databases on the epidemiology of mild to severe AKI throughout the world.

Methods: We conducted a prospective observational study in ICU patients in 14 centers to determine the incidence of AKI (AKIN criteria) and to characterize clinical factors and processes of care associated with patient outcomes. Patients were screened at ICU admission, and daily for 7 days.

Results: Over the last 4 years, 1275 of 6647 patients had AKI (19%) and 1007 (79%) were enrolled in our Registry. Data was available for 745 patients. Mean age was 61±18

years, 62% were male, 57% non-Caucasian, 52% had CKD. At ICU admission, 37% were on ventilator, 24% on pressors, 39% on diuretics, 30% were oliguric and 44% had sepsis. Mean APACHE 3 scores were 56±27. 24% required renal replacement therapy (RRT): CRRT, IHD, SLED and PD in 76%, 30%, 34% and 2% of patients, respectively. Hospital mortality was 22% (32% in RRT vs. 19% in non-RRT patients; p<0.001) and 15% were dialysis-dependent at hospital discharge. Among AKI patients, independent risk factors associated with hospital mortality included age (OR 1.02; 95%CI 1.01-1.04;p=0.006), use of pressors (OR 2.53; 95%CI 1.44-4.47;p=0.001), APACHE 3 score (OR 1.03; 95%CI 1.01-1.04;p<0.001) and cumulative fluid balance (L) (OR 1.05; 95%CI 1.02-1.09;p=0.003). After controlling for fluid balance, diuretic use had no protective effect on mortality. Mortality was higher as AKIN stage progressed (stage 1 18%; 2 31%; 3 32%;p<0.001) and in the absence of renal function recovery (30% vs. 14%;p<0.001).

Conclusions: This multinational registry provides a contemporary view of clinical factors, management and outcomes of AKI in the ICU with an incidence of 19% and mortality of 22%. These data highlight the changing characteristics of AKI and can inform the design and conduct of future trials.

TH-PO110

Acute Kidney Injury Is a Major Determinant of Mortality in Burn Patients

Meryem Tuncel-Kara,[1] Sharmila Dissanaike,[2] Melvin E. Laski.[1] *[1]Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX; [2]Surgery, Texas Tech University Health Sciences Center, Lubbock, TX.*

Background: The data available regarding renal injury in patients with burns are not extensive. Established predictors of burn survival are % burn, patient age, and concomittent respiratory injury. We investigated the impact of AKI on survival and AKI interactions with established risk factors.

Methods: Data were abstracted from EMRs of 1,142 burn patients admitted to University Medical Center, Lubbock between 1/1/2000 and 6/31/2007. Age, gender, race, % burn, initial, highest, lowest, and final serum creatinine, length of stay, survival/non-survival, co-morbidities includiing DM, CAD, COPD, cancer, CHF, CKD with stage, use of antibiotics, and use of pressor agents were recorded. Since admissions of less than two days represent small burn areas or non-survivable injuries, we limited analysis to patients who stayed over two days (n = 728). A model was established and odds ratios were generated by multivariant analysis.

Results: Data from 728 patients were analyzed; 77 died and 651 survived. Most (83.7%) had an initial creatinine consistent with an eGFR (by MDRD) over 60 ml/min/1.73M^2. Using the difference between low and high serum creatinine we found that 21.8% had modified RIFLE scores (precise urine output could not be determined) of R, I, or F, and 28.6% had AKIN scores of one or higher. In the final model, the odds ratio for mortality for AKIN score was 5.88 for AKIN 1, 12.49 for AKIN 2, and 10.43 for AKIN 3 as compared to AKIN 0 (all p= or < 0.001). For RIFLE scores, the OR was 3.0 (p = 0.25) for R, 7.51 (p<0.001) for I, and 8.13 for F (p=0.001). The OR was 1.29 per 10 years (0.03) for age, and 1.42 per 10% burn area (<0.001) when controlled for RIFLE score, and 1.26 (0.05) per 10 years, and 1.44 (0<0.001) per 10% burn when controlled for AKIN score.

Conclusions: AKI as defined by either RIFLE or AKIN classification had a major effect on burn mortality. Increased creatinine during the burn hospitalization was associated with greater mortality independent of age and burn extent.

Funding: Clinical Revenue Support

TH-PO111

Risk of Mortality and Re-Hospitalization among Patients with Acute Myocardial Infarction and Acute Kidney Injury

Kenn B. Daratha,[1,3] Katherine R. Tuttle,[1] Jonathan Himmelfarb.[2] *[1]Providence Medical Research Center, Spokane, WA; [2]Kidney Research Institute, University of Washington, Seattle, WA; [3]Washington State University, Spokane, WA.*

Background: The primary objective of this study was to determine risks of death and re-hospitalization in patients with acute myocardial infarction (AMI) with and without acute kidney injury (AKI).

Methods: This longitudinal cohort study included persons hospitalized in Washington State 2004-2008 followed through 2009 (WA-CHARS). Patients with a primary diagnosis code of AMI (N=28,050) were classified into an AKI cohort (n=1,651) based on co-occurring diagnosis code for AKI during the index hospitalization and a reference cohort (n=26,399) for patients without AKI during index hospitalization. Fully-adjusted statistical models controlling for demographic and clinical and procedural characteristics were used to examine increased risks of in-hospital death and subsequent hospitalization by cohort.

Results: Compared to the reference cohort, patients in the AKI cohort were older and more likely to be female, Medicare insured, and hospitalized for longer lengths of stay. They were also more likely to have coronary artery bypass graft surgery, cardiogenic shock and acute respiratory failure. The AKI cohort was less likely to have a percutaneous coronary intervention or cardiac catheterization. Fully-adjusted risks for in-hospital death were significantly increased among patients in the AKI cohort (HR=2.70; 95%CI=2.29-3.18; p<0.001). Unadjusted increased risks were also observed for all-cause re-hospitalization, re-hospitalization for AKI and fatal re-hospitalization. Fully-adjusted risks for re-hospitalization for AKI were significantly increased among patients in the AKI cohort (HR=1.67; 95%CI=1.32-2.05; p<0.001). Patients were followed an average of 43 months in which 9.5% of the reference cohort and 37.4% of the AKI cohort died.

Conclusions: Patients admitted with AMI who experience AKI are at substantially higher risk for death and subsequent re-hospitalization for AKI. Measures to minimize AKI in AMI patients as well as closely monitoring of those who experience AKI should be a major focus to improve patient outcomes.

TH-PO112

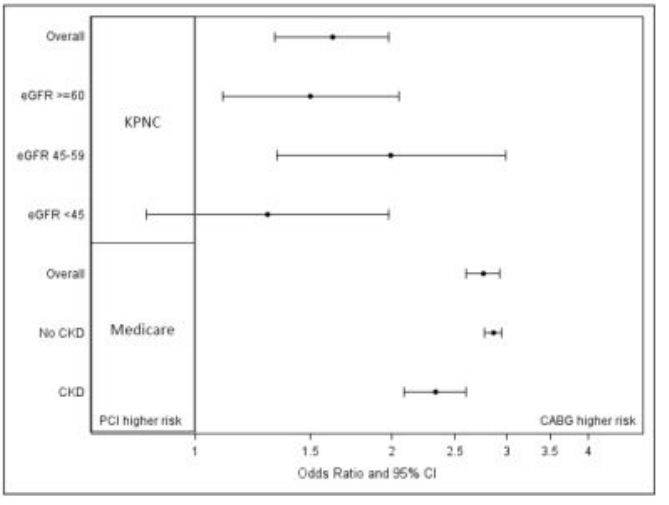
Risk of Acute Kidney Injury after Coronary Artery Bypass Surgery versus Percutaneous Coronary Intervention for Multivessel Coronary Disease

Tara I. Chang,[1] Thomas Leong,[2] Derek Boothroyd,[1] Mark Hlatky,[1] Alan S. Go.[2] *[1]Stanford; [2]Kaiser Permanente Northern California.*

Background: Relatively little is known about the risk of acute kidney injury (AKI) after coronary artery bypass grafting (CABG) compared with percutaneous coronary intervention (PCI).

Methods: We examined patients receiving incident CABG or PCI for multivessel coronary disease in two complementary cohorts. First, we used data from Kaiser Permanente Northern California (KPNC), which has detailed laboratory data, defining AKI as an increase in serum creatinine ≥0.3 mg/dL and/or ≥150% above baseline during the index hospitalization (1996-2008). Second, we used data from Medicare beneficiaries (1992-2008), defining AKI as an ICD-9 584.x primary or secondary discharge code during the index hospitalization and AKI requiring dialysis (AKI-D) as an 584.x code plus a dialysis procedure code. In both cohorts, patients on dialysis pre-revascularization were excluded. We used logistic regression to determine the odds ratio of AKI with CABG versus PCI.

Results: In 7295 patients in the KPNC cohort, 20% of CABG patients and 15% of PCI patients had AKI. The incidence of AKI increased as baseline kidney function decreased. In 105,216 Medicare patients, 9% of CABG patients and 4% of PCI patients had AKI. In both cohorts, CABG was associated with higher odds of AKI compared to PCI. Only 0.3% and 0.1% of Medicare patients undergoing CABG or PCI had AKI-D, respectively.



Conclusions: In two separate cohorts, we found that AKI is common after coronary revascularization, and that the incidence of AKI is higher in patients with lower kidney function. CABG was associated with higher odds of AKI compared with PCI across levels of kidney function. When weighing revascularization options, the risk of AKI should be considered and minimized where possible, particularly in patients with underlying kidney disease.

Funding: Private Foundation Support

TH-PO113

Incidence and Risk Factors for the Development of Acute Kidney Injury in Trauma Patients: Development of a Risk Prediction Score

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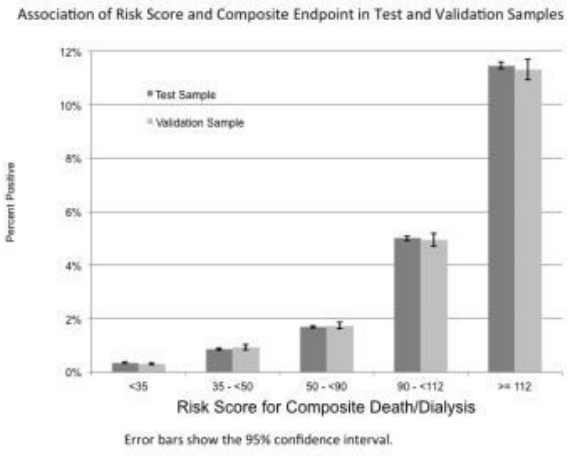
Background: Trauma related injuries are the leading cause of death for persons < 44 years of age. Incidence of AKI in this population is not well characterized. We sought to determine the incidence and risk factors for AKI in subjects with a trauma related injury.

Methods: We analyzed the National Trauma Data Bank, a nationally representative dataset of over 400 US trauma centers. We assessed demographic and relevant clinical variables for hospital admissions from 2002-2008. Patients under the age of 18 years, those with burns, and baseline CKD were excluded. AKI was defined as the need for dialysis during the index hospitalization. The primary outcome variable was the composite of inpatient death/dialysis.

Results: 2,014,803 admissions were examined, of which 1,205,307 had complete data. The incidence of death was 2.5%, and the incidence of AKI was 0.32%. We conducted a multivariable analysis that included age, gender, race, ISS, trauma type, comorbidities, and insurance type. All predictors were independently associated with the composite outcome variable except for baseline DM and chronic lung disease. Using a 90% data sample, we developed a risk score to predict the composite of death/dialysis. We then validated the score in the remaining 10%. The test and validation model were significant (p<.0001 for both) and good prediction accuracy (ROC AUC = .85, and .83, respectively).



Conclusions: Trauma is an important cause of AKI. By comparison, there are @ 300K cardiac surgeries/year with a severe AKI incidence of @ 1-2%. There are over 2.5 million trauma admissions per year, and we found an incidence of AKI of 0.32%, which suggests that trauma contributes more annual cases of AKI than cardiac surgery. We also developed and validated a risk prediction score.

TH-PO114

Stroke Volume Variation (SVV) and Oxigenation Index(OI) Are Risk Factors for Acute Kidney Injury (AKI) in Abdominal Aortic Aneurysm (AAA) Surgery Paolo Lentini,[1] Luca Zanoli,[2] Valentina Pellanda,[1] Andrea Contestabile,[1] Anna Basso,[1] Massimo de Cal,[3] Claudio Ronco,[3] Graziella Berlingò,[1] Antonio Granata,[4] Roberto Dell'Aquila.[1] *[1]Nephrology, San Bassiano Hospital, Bassano del Grappa, Italy; [2]University of Catania, Catania, Italy; [3]Nephrology, S.Bortolo Hospital, Vicenza, Italy; [4]Nephrology, S. G. Di Dio Hospital, Agrigento, Italy.*

Background: AAA surgery patients are at high risk for AKI. Hemodynamic instability, hypovolemia, haemorrhage and reduced cardiac output may play a key role in AKI. SVV predicts volume responsiveness in mechanically ventilated patients; elevated levels of Oxigenation Index (OI) are linked to fluid overload and associate to poor outcome in critical ill patients but little is known about their role in AKI. We aimed to assess if patients with wide changes of SVV or OI at clamping and declamping time of the aorta are associated with high risk of AKI development.

Methods: We enrolled 21 consecutive hypertensive patients undergoing elective AAA surgery with supra-renal clamping. Patients were all on volume-control mechanical ventilation (8 ml/kg) and positive end expiratory pressure of 4 cmH_2O. Patients with arrhythmias, spontaneous ventilations, and those extubated before 12h post-operative were excluded. SVV was measured with the FlowTrack/Vigileo (Edwards Lifesciences®) device every 3 minutes during the procedure and for 24h after surgery. OI was calculated as =mean airway pressure/(PaO2/FiO2)x100. Patients received 70ml/kg/h of crystalloids; fluid boluses and transfusions were given if needed. Data were compared with ANOVA.

Results: 9 patients(43%) developed AKI, defined as RIFLE Risk category. SVV and OI were significantly higher at aortic clamping ($p<0.0001$) and declamping time ($p< 0.0001$ and $p< 0.01$ respectively).

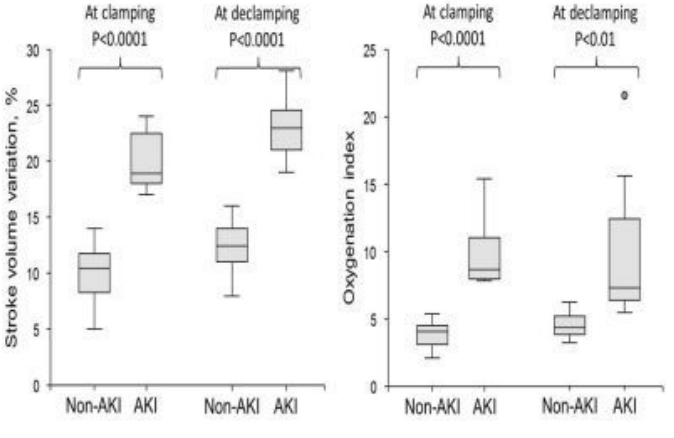


Fig.1 SVV and OI at Clamping and Declamping of the aorta

Conclusions: High SVV and OI during and after suprarenal AAA surgery are associated with high risk of AKI.

TH-PO115

Urinary Basigin/CD147 as a Biomarker of Acute Kidney Injury Hiroshi Kojima, Tomoki Kosugi, Waichi Sato, Hibiki Shinjo, Yuka Sato, Kayaho Maeda, Hiroshi Nagaya, Mayuko Maeda, Seiichi Matsuo, Shoichi Maruyama. *Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

Background: Acute kidney injury (AKI) develops due to complex interactions between acute insults, including ischemia and infection, and subsequent activation of the immune system such as inflammation and coagulation. The mortality and morbidity associated with AKI remains high, despite advances in interventions. Basigin (Bsg)/CD147 is a transmembrane glycoprotein, and contributes to cell survival, invasion and metastasis. Recently, we documented that Bsg is involved in the pathogenesis of renal inflammation caused by ischemia and renal fibrosis. We therefore investigated whether urinary Bsg could serve as an early and sensitive biomarker of AKI.

Methods: 397 patients, who entered the surgical intensive care units (SICU) in Nagoya university hospital, were enrolled. Spot urine samples were collected three times at the baseline before surgery, immediately after SICU admission, and 12 hours after SICU admission. Urinary Bsg and neutrophil gelatinase-associated lipocalin (NGAL) values were measured by ELISA method and normalized to those of urinary creatinine. AKI was defined by the Acute Kidney Injury Network criteria.

Results: While no obvious differences in both groups were found in urinary Bsg values and serum creatinine before operation, mean Bsg level in patients with AKI was significantly higher than those with non-AKI immediately after SICU admission. Regardless of the involvement of carcinoma metastasis, these were not affected in the presence or absence of aging and malignant diseases. Interestingly, the time course of Bsg was similar to that of NGAL, one of reliable biomarkers for AKI after cardiac surgery, during the experimental periods. In addition, Bsg is mainly distributed in tubular epithelial cells of healthy kidneys and its expression was extremely lower in injured and atrophic tubules. Indeed, there was the strong relationship between urinary Bsg and serum creatinine in patients with AKI.

Conclusions: Urinary Bsg may be a prime candidate for developing a new procedure for the evaluation of AKI.

TH-PO116

Does Sitagliptin Modifies Risk and Outcome of Acute Kidney Injury in Diabetes Patients with Septic Shock? Kadapalakere Reddy, Lori M. Rose, Arash Rashidi. *Internal Medicine, Fairview Hospital A Cleveland Clinic Hospital, Cleveland, OH.*

Background: Sitagliptin, a DPP4 inhibitor, used for treatment of type 2 diabetes mellitus has been shown to be protective against renal injury in rats. It was suggested that the anti-inflamatory effect and increased levels of various antioxidants, as well as decreased necrosis and apoptosis might mediate this effect. We therefore hypothesized that sitagliptin could be protective against acute kidney injury (AKI) in humans.

Methods: We conducted a retrospective cohort study of diabetes patients admitted to our institution between January 2010 and September 2011 with the diagnosis of sepsis and/or septic shock. Of 257 patients studied, 29 were taking sitagliptin and 228 were not. Baseline variables including demographics, co-morbidities, severity of illness with respect to vasopressors and lowest blood pressure, medications at the time of admission, exposure to aminoglycosides and contrast agents and levels of serum creatinine, blood urea nitrogen and glomerular filtration rate were collected. We then determined the incidence and severity of AKI, need for renal replacement therapy (RRT), length of stay and time to return to a stable creatinine. Twenty three patients who had end stage renal disease or did not have a baseline serum creatinine level were excluded. Both univariate and multivariate analyses were conducted.

Results: There was no difference in regard to baseline characteristics between patients who were and were not on sitagliptin in univariate analysis. In the multivariate regression analysis no difference was found between the two groups in regard to the incidence or severity of AKI (Peak creatinine 3.0 vs 2.10 mg/dl, p=0.2), RRT (17/1 vs 80/7 p=0.99), LOS (12.1 vs 11.9 days, p=0.91), and days to return to a stable creatinine (14.6 vs 9.70 p=0.21).

Conclusions: In contrast to experimental models in rats, in our study on patients, pre-exposure to sitagliptin did not appear to be protective against acute kidney injury in patients with T2DM admitted to an intensive care setting with multiple complex medical issues.

TH-PO117

Urinary Levels of Prostanoid Metabolites Predict for Acute Kidney Injury in Adult Japanese Heterogenous ICU Patients Haruyo Ujike,[1] Yohei Maeshima,[1] Masaru Kinomura,[1] Daisuke Saito,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Norikazu Hinamoto,[1] Hitoshi Sugiyama,[1] Hiroshi Morimatsu,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama University, Okayama, Japan; [2]Anesthesiology, Okayama University, Okayama, Japan.*

Background: Acute kidney injury (AKI) is associated with increased risk of morbidity and mortality in critically ill patients. Prostanoids regulate numerous biological functions including hemodynamics and tubular transport process. Here, we investigated the ability of urinary prostanoid metabolites to predict the onset of AKI in adult ICU patients.

Methods: We prospectively collected data of patients admitted to the ICU in the Okayama University Hospital (Nov. 2010-July 2011). Patients already in end-stage renal disease and receiving renal replacement therapy (RRT) were excluded. Urine was collected within 24 h of ICU admission (Day 1). Urinary levels of prostaglandin E_2 (PGE_2), PGI_2 metabolite (2,3-dinor-6-OXO-$PGF_{1\alpha}$) and thromboxane A_2 metabolite (11-dehydro-TXB_2), NAG and albumin were determined and were normalized to creatinine levels.

Results: Of the 93 patients, 24 developed AKI (AKIN criteria). The mean age (66, 60; non-AKI, AKI, respectively) and the mean baseline eGFR (72, 73) were equivalent. Surgical operation (93%, 75%) was the leading cause of ICU admission. In the AKI group, 2 patients (8.3%) required RRT and a patient died. Overall, the ratio of serum Cr at Day1 after admission to ICU to baseline positively correlated with urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratio (R=0.57, P<0.0001) or 11-dehydro-TXB_2/Cr ratio (R=0.47, P<0.0001). The levels of urinary prostanoid metabolites except for PGE_2, also positively correlated with urinary NAG/Cr level. In 16 cases developing de novo AKI, the levels of urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr were significantly elevated compared with non-AKI group (18477 vs. 8277 ng/gCr, P=0.002; 18637 vs. 7926 ng/gCr, P<0.002), but urinary levels of PGE_2/Cr were not.Urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr showed the most excellent diagnostic and predictive performance among them in the ROC analysis (ROC-AUC 0.75).

Conclusions: Urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr associate with subsequent onset of AKI.

TH-PO118

Serial Measurement of Urinary L-FABP and NAG Can Predict Progression of Acute Kidney Injury in ICU Population Maki Tsukamoto,[1] Kent Doi,[1,2] Daisuke Katagiri,[1] Yoshifumi Hamasaki,[1] Masaomi Nangaku,[1] Takehiro Matsubara,[2] Naoki Yahagi,[2] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Emergency and Critical Care Medicine, University of Tokyo, Tokyo, Japan.*

Background: Reportedly, combination of urinary L-type fatty acid-binding protein (L-FABP) and N-acethyl-β-D-glucosaminidase (NAG) can detect acute kidney injury (AKI) before serum creatinine elevation in adult post-cardiac surgery AKI [Ann Thorac Surg. 2012;93:577-83]. However, critically ill patients treated in ICU frequently suffer from multiple and transient/persistent insults. This study was aimed to evaluate whether serial measurement of urinary L-FABP and NAG enables us to predict AKI progression in these patients.

Methods: We prospectively studied 274 adult critically ill patients who were treated in mixed ICU of the University of Tokyo Hospital. Urinary L-FABP and NAG were measured at ICU admission (day 1) and 24 hr after (day 2). Patients who showed anuria or died within 24 hr were excluded. Diagnosis and severity of AKI was determined by the RIFLE criteria with one exception; the patients who needed RRT were categorized as Failure, as suggested by the AKIN criteria (i.e. stage 3).

Results: Of 159 AKI patients, 64 (40%) patients showed worsening kidney function. Urinary L-FABP and NAG of day 1, day 2, their average, maximum and minimum values were all significantly associated with worsening kidney function. Neither absolute change nor increase/reduction rate contributed to prediction of worsening kidney function. Among these factors, the highest area under the curve of receiver operating curve (AUC-ROC) was observed with minimum L-FABP [0.76, 95%CI (0.69–0.83)] and NAG at day 2 [0.71, 95%CI (0.63–0.78)]. Combination of minimum values of L-FABP and NAG, and serum creatinine at day 1 significantly increased AUC-ROC [0.85, 95%CI (0.79–0.90)] compared with any single measurement of these three markers.

Conclusions: In a heterogeneous cohort of adult mixed ICU, serial measurement of urinary L-FABP and NAG could contribute to prediction of worsening AKI by combining their minimal values during 24 hours after ICU admission.

Funding: Government Support - Non-U.S.

TH-PO119

Predictor of Snakebite Mediated Acute Kidney Injury: A Prospective Study Pinaki Mukhopadhyay, Raghwendra Mishra, Debarati Mukherjee, Monoj Kar, Gautam Mukherjee, Piyali Banerjee. *[1]Dept of Neph, NRSMC, Kol, WB, India; [2]Dept of Biochem, NRSMC, Kol, WB, India; [3]Dept of Gynaec, NBMC, Siliguri, WB, India; [4]Dept of Gynaec, BRH, Howrah, WB, India.*

Background: To determine the prognostic predictors of snakebite induced AKI (SAKI) required renal replacement therapy. To measure the oxidative and carbonyl stress level in SAKI patient and correlate with adverse outcome.

Methods: All SAKI patients admitted from Apr-10 to Dec-11 and received HD were included. Demographical, clinical & biochemical data were analyzed and they are followed from hospitalization to discharge or death. Oxidative & carbonyl stress markers [Advanced oxidation protein product (AOPP), Advanced Glycation End product (AGE), Pentosidine, Dityrosine, Thioberbituric acid reactive substance (TBARS) and Methyl glyoxal (MG)] were measured consecutively in 60 SAKI patient according to standard protocol. All data were analyzed with appropriate statistical methods.

Results: Among 205 SAKI patients received HD. Male: Female was 2.5:1. The mean age was 37.2 years (4-75 years). Commonest site of bite was lower limb (89.5%).About 76.2% received primary treatment. Oliguria & bleeding manifestation were the common presentation. Hypotension was found in 35.5% cases, cellulites & inflammation was found in about 66% patients. About 27.8 % had disseminated intravascular coagulation (DIC). Antisnake venom (ASV) was used 22.5±1.85 vial. Median HD was required 3 session .Mean hospital stay was 12 days (2-34 days). Bite to HD initiation time was 3.3 days. Out of 28.5%) patients died 34.48% were <18 years. About 78.2% had cellulites, 52.5% had shock/hypotension at initial presentation (p<0.05), bleeding manifestation was found in 80.4% & 46.8% had DIC (p<0.05).DIC & shock/hypotension at initial presentation was came out as independent predictor of death at multivariate level. Among the biochemical markers measured for oxidative and carbonyl stress (n=60) AOPP and MG came out as independent predictor (p<0.05) of adverse outcome.

Conclusions: Shock/hypotension, DIC, advanced oxidation protein product (AOPP) and methyl glyoxal (MG) was surrogate prognostic marker in SAKI patients requiring dialysis leading to death.

TH-PO120

NGAL, KIM-1 Do Not Predict Recovery from Rise in Serum Creatinine in Heart Failure Exacerbation Meyeon Park, Eric Vittinghoff, Kathleen D. Liu, Michael Shlipak, Chi-yuan Hsu. *University of California, San Francisco, CA.*

Background: Patients with acute heart failure (HF) exacerbation commonly have deterioration in renal function (i.e. serum creatinine rise), often ascribed to prerenal azotemia. We evaluated whether subtle ischemic tubular injury is detectable using sensitive biomarkers neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), and whether elevations of these markers are associated with recovery of kidney function.

Methods: We recruited consecutive patients admitted for HF exacerbation from March 2-May 31, 2011 at one medical center, regardless of renal function. We collected daily urine samples (up to 5 days) and measured urine NGAL (AntibodyShop) and KIM-1 (R&D Systems). Acute kidney injury (AKI) was defined by AKIN criteria. Outcome was recovery from AKI, defined as return to within 10% of the original creatinine (cr). We also assessed recovery in patients whose cr rise had occurred prior to admission, i.e. those admitted above baseline cr (ABL). We defined baseline cr as the most recent to the index hospitalization (excluding values within 7 days or older than 1 year).

Results: 53 eligible patients were recruited. 20 experienced AKI (9 recovered). Mean age was 65; 30 were men; mean baseline cr was 1.2 mg/dl. Mean NGAL and KIM-1 were similar in those with and without AKI (Table). 9 patients presented ABL (6 recovered) and 24 had no change in renal function; mean biomarkers were not significantly different between ABL and at baseline groups. In pooled logistic regression models, NGAL and KIM-1 were not associated with recovery from serum cr rise. In contrast to NGAL, KIM-1 levels rose over the first 5 days of hospitalization in individuals with no AKI and stayed static in those who experienced AKI.

Mean (SD) Biomarkers

	AKI (n=20)	No AKI (n=33)	p
NGAL/cr (ng/mg)	70.0 (27.4)	65.9 (11.7)	0.56
KIM-1/cr (ng/mg)	1.41 (0.21)	1.61 (0.13)	0.26

Conclusions: In this pilot study, levels of urine NGAL and KIM-1 were not associated with recovery from serum cr rise in patients with HF exacerbation. Patterns of NGAL and KIM-1 may differ in this clinical setting.

Funding: NIDDK Support, Other NIH Support - UCSF CTSI

TH-PO121

Acute Renal Tubular Injury Biomarkers in Non-Cardiac Critically Ill Children Ana Palijan,[1] Prasad Devarajan,[2] Qing Ma,[2] Michael R. Bennett,[2] Michael Zappitelli.[1] *[1]Nephrology, McGill University Health Centre, Montreal, QC, Canada; [2]Nephrology and Hypertension, Cincinnati Children's Hospital Medical Centre, Cincinnati, OH.*

Background: Acute kidney injury (AKI) in non-cardiac pediatric intensive care unit (ICU) patients (pts) is common, associated with poor outcome and earlier AKI diagnosis is needed. Little AKI biomarker research exists in this population. We evaluated alpha-glutathione S-transferase (a-GST; proximal), pi-glutathione S-transferase (pi-GST; distal) and liver fatty-acid-binding protein (LFABP- proximal) for AKI diagnosis in an attempt to validate findings in other study populations.

Methods: We prospectively followed 74 ICU children admitted ≥2days and collected SCr, clinical data and urine daily. AKI was defined as ≥50% rise from pre-ICU baseline; severe AKI was doubling of SCr. In all pts, we measured urine a-GST and pi-GST (EIA; Argutus Medical Ltd); in a subset we measured LFABP (ELISA). We compared AKI *vs.* non-AKI peak ICU biomarker levels (Mann-Whitney test). We calculated area under the curve [AUC] to evaluate the biomarkers for discriminating presence of AKI and severe AKI.

Results: Mean (±SD) age and ICU length of stay were 5.2(±5.9) yrs and 9.9(±9.6) days, respectively; 66% male, 78% ventilated, 46% vasopressors, 74% ≥ mild AKI, 36% ≥ severe AKI.**1st 3 ICU days urine:** early ICU pi-GST (not a-GST) was higher in AKI *vs.* non-AKI pts (p<0.05); AUC for AKI diagnosis was 0.69 (95%CI 0.51-0.86). **2 pre-AKI days urine**: pre-AKI a-GST (not pi-GST) was higher in AKI *vs.* non-AKI pts (p=0.01, AKI diagnostic AUC=0.72 95%CI=0.51-0.93). **Day of AKI event urine:** pi-GST was higher in pts with *vs.* without AKI (p=0.05) and those with severe *vs.* non-severe AKI (p<0.003) (diagnostic AUC=0.69, 95%CI 0.51-0.87; AUC=0.85, 95%CI of 0.70-1.00, respectively). LFABP, but not a-GST or pi-GST, was higher in AKI *vs.* non-AKI pts (p<0.04) on the day after AKI diagnosis.

Conclusions: We confirmed an association between a-GST, pi-GST and LFABP and AKI in non-cardiac ICU children for the first time to our knowledge. a-GST appeared to rise before AKI while pi-GST and LFABP were later AKI biomarkers. Future larger studies will further determine their clinical and research utilities.

Funding: Government Support - Non-U.S.

TH-PO122

Prognosis after Acute Kidney Injury in the Pediatric Intensive Care Unit Marina Ferreira,[1] Isac De Castro,[2] Emmanuel A. Burdmann,[2] Emerson Quintino Lima.[1] *[1]Nephrology Division, FAMERP, Sao Jose Rio Preto, Sao Paulo, Brazil; [2]Nephrology Division, University of Sao Paulo, Sao Paulo, Brazil.*

Background: Acute kidney injury (AKI) is associated with increased morbidity in critically ill children. We identified hospital and long term mortality risk factors after admission to pediatric intensive care unit (PICU).

Methods: Data from children admitted to PICU between 2004 to 2008 was retrospectively collected. AKI was identified according to pediatric RIFLE. Long term

survival (up to 7 years) after PICU admission was evaluated by phone call or hospital data bank verification. Bivariate and logistic regression analyses were used to identify hospital and long term mortality risk factors.

Results: 434 children (49 ± 47 months, 246 (57%) boys) were admitted to PICU. AKI occurred in 279 (64%) children (Risk 106 [24%], Injury 113 [26%], Failure 60 [14%]) during their PICU stay and pRIFLE was associated with hospital mortality (no AKI 4%, R 33%, I 26%, F 38%; p=0,001). Long term survival was not affected by AKI occurrence (no AKI 10% vs AKI 16%; p=0,14). After logistic regression, the identified hospital mortality risk factors were: AKI (OR 3.3; 1.09-13.43), mechanical ventilation (OR 19.1; 2.14-17.05), dopamine (OR 4.85; 1.59-14.77), volume overload (OR 2.3; 1.2-3.01) and weight (OR 1.2; 1.02-2.1). Long term death risk factors were: mechanical ventilation (OR 4.61; 2.36-9.02), diuresis (OR 1.2; 1.2-4.07), PRISM score (OR 1.11; 1.04-1.18) and systolic blood pressure (OR 1.09, 1.04-1. 14).

Conclusions: AKI was highly prevalent and associated with increased hospital mortality after PICU admission. Unlike what occurs in adults, AKI in pediatric patients was not related to long term survival. Studies are needed to evaluate the consequences of AKI in adulthood.

TH-PO123

Biomarkers of Acute Kidney Injury in Urine Exosomes from Care Units Patients Carlos E. Irarrazabal,[1] Guillermo Eugenio Villamizar,[2] Sergio Alvarez,[2] Antonio Vukusich,[2] Margarita Hurtado,[2] Sandra Villanueva,[1] Guilio Innocenti,[2] David Carvajal,[2] Rogelio Altuzarra,[1] Andres Boltansky.[2] *[1]Universidad de Los Andes, Santiago, Chile; [2]Clinica Davila, Santiago, Chile.*

Background: The acute kidney injury (AKI) is an important marker of morbidity and mortality in Intensive Care Unit (ICU). Despite of experience in the use of hemodialysis and other renal replacement therapies in the management of AKI, the mortality remains high and many problems remain unresolved. Recently, it was showed that AKI incidence in patients admitted in the Critical Units (tertiary health center in Santiago-Chile) was 27.69%. The severe AKI (AKIN criteria) increased the hospital length of stay and the mortality. There are now several molecules with potential for diagnosis of AKI in its early stages. However, more clinical evidence is required to validate the sensitivity and specificity in both plasma and urine. Exosomes are vesicles released into urine from the renal epithelium and have been proposed as a source of exploration of biomarkers of renal injury.

Methods: Urine were collected at ICU admission. Protease inhibitor was added to the urine and the cell fraction was discarded by centrifugation (4000g x 10 minutes, 4 ° C). The supernatant was ultracentrifuged (38000g x 1hr, 4 ° C), resulting in the supernatant (S) and pellet rich en exosome (E). The NGAL, KIM-1 and HIF-1α was determined in 100 micrograms of total protein by Western blot and the results were expressed as E/S ratio.

Results: We prospectively analyzed 52 patients from ICU. The average age was 57 years (21-95) and 58% were male. The average cretininemia on the day of ICU admissions was 1.47 ± 1.5 mg/dl, and 37% were classified as AKI using AKIN criteria. The E/S NGAL expression in AKI (4,41±2,239) was higher than without AKI (0,80 ± 0,46) patients. The E/S KIM-1 expression in AKI (6,18 ± 3,814) was also higher than without AKI (0,80 ± 0,559) patients. The E/S HIF-1α expression in AKI (0,888 ± 0,520) was equally to no AKI (0.988±0,764) patients.

Conclusions: Our data establish that NGAL and KIM-1 in the exosomal fraction are better associated with AKI, but not HIF-1α.

Funding: Other NIH Support - FONDECYT 1100885-Chile, Government Support - Non-U.S.

TH-PO124

Biomarker Performance at the Time of ICU Admission Jay L. Koyner,[1] Dana Hoffmann,[2] Eoin J. Cotter,[3] Patrick T. Murray,[3] Vishal S. Vaidya.[2] *[1]University of Chicago; [2]Brigham and Women's Hospital; [3]University College Dublin.*

Background: Internationally accepted diagnostic criteria for acute kidney injury (AKI) are well validated and define AKI via changes in either serum creatinine (SCr) or urine output (UOP). The majority of AKI biomarker studies report AKI based on changes in SCr and little has been published about biomarker's ability to predict UOP based AKI.

Methods: We prospectively collected urine samples on ICU admission from patients in a Medical ICU (Chicago) and compared the ability of biomarkers to detect AKI Network (AKIN) Stage 1 via SCr and UOP. Additionally we assessed the ability to detect severe AKI defined as the future need for renal replacement therapy (RRT).

Results: We enrolled 59 patients, 40 (68%) of whom developed AKIN Stage 1 or higher AKI during the first 5 days of their ICU stay. 38 subjects developed SCr based AKI while 21 developed AKI based on UOP. 8 subjects (13.6%) required RRT. Table 1 demonstrates the area under the curve (AUC) and p values for SCr and blood urea nitrogen (BUN) and several urinary biomarkers to predict AKIN Stage 1 (SCr and UOP) and RRT at the time of ICU admission.

Biomarker Performance at ICU Admission

	AKIN-Stage 1 SCr -(n=38) AUC (p value)	AKIN Stage 1 UOP (n=21) - AUC (p value)	RRT AUC (n=8) (p value)
Serum Creatinine mg/dl	0.79 (0.0001)*	0.69 (0.02) *	0.67 (0.13)
BUN mg/dl	0.82 (0.0001)*	0.63 (0.11)	0.57 (0.52)
Urine Albumin mg/L	0.68 (0.03) *	0.66 (0.04) *	0.63 (0.26)
Urine NGAL ng/ml	0.81 (0.0002) *	0.75 (0.003) *	0.77 (0.02) *
Urine Cystatin C ng/ml	0.67 (0.04) *	0.61 (0.20)	0.77 (0.02)*
Clusterin ng/ml	0.63 (0.11)	0.74 (0.003)*	0.68 (0.11)
KIM-1 pg/ml	0.60 (0.23)	0.80 (0.0003)*	0.59 (0.42)
Fibrinogen ng/ml	0.56 (0.49)	0.46 (0.59)	0.48 (0.89)
α-Glutathione-S-transferase μg/L	0.42 (0.33)	0.53 (0.67)	0.44 (0.62)
π Glutathione-S-transferase μg/L	0.60 (0.22)	0.68 (0.04) *	0.79 (0.01) *

* p value < 0.05

Conclusions: Biomarkers can detect AKIN Stage 1 (SCr and UOP) and the need for RRT at the time of ICU admission. Each biomarker has its own specific prognostic fingerprint and the ability to detect SCr based AKI does not necessarily equate to UOP-based AKI. Future studies are needed to better understand which biomarkers can detect AKIN stage 1 and the need for RRT early on in the setting of critical illness.

Funding: NIDDK Support, Pharmaceutical Company Support - Alpha and Pi GST Assays Provided by Argutus Medical Inc

TH-PO125

Urine Cystatin C and Acute Kidney Injury after Cardiac Surgery Jay L. Koyner, Amit X. Garg, Michael Shlipak, Uptal D. Patel, Kyaw Sint, Prasad Devarajan, Charles L. Edelstein, Michael Zappitelli, Chirag R. Parikh. *TRIBE AKI Consortium.*

Background: Acute Kidney Injury (AKI) is common following cardiac surgery and is associated with adverse patient outcomes. Urine Cystatin C (UCysC), is a biomarker of proximal tubule function and may rise earlier in settings of injury than serum creatinine.

Methods: We conducted the prospective multicenter Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) study, a cohort involving 1219 adults and 311 children. We evaluated the performance of UCysC in the peri-operative period and defined AKI as a doubling of serum creatinine from baseline or need for acute renal replacement therapy (RRT). The cohort was divided into quintiles using the first post-op value of UCysC (0-6 hour timepoint).

Results: AKI occurred in 73 (4.9%) adults and 53 (17%) of children after surgery; with 18 adults (1.5%) receiving RRT. There was no statistical difference between the pre-operative UCysC values for those with and without AKI in the adult (p=0.76) or pediatric (p=0.92) cohorts. UCysC measured in at the 0-6 hour timepoint did not correlate with the future development of AKI in either the adult or pediatric cohort. The area under the curve (AUC) for the receiver operator characteristics (ROC) curve for the first post-operative UCysC to predict AKI was (AUC(SE)) 0.60 (0.04) in adults and 0.62 (0.04) in children (p=NS for both). Further, when defining AKI as AKIN Stage 1 (0.3 mg/dl or 50% increase), UCysC did not improve risk stratification in adults or children. However in adults, the highest quintile of the first post-operative UCysC was associated with a 7-fold increased odds for the receipt of RRT compared to the lowest two quintiles.

Risk of RRT Startified by UCysC (mg/L) in Adults in the Early Post-op Period

Quintile (cutpoints)	N(%)	Unadjusted OR (95%CI)
Q1&Q2 (<0.12) n=485	2(0.8)	1 (referent)
Q3 (0.13,0.20) n=242	4(1.6)	4.0 (0.7-22)
Q4 (0.21, 0.28) n=231	3 (1.3)	3.2 (0.5, 19)
Q5 (>0.28) n=245	7 (2.9)	7.1 (1.4, 34)

Conclusions: UCysC values do not associate with the development of AKI following cardiac surgery. However, there may potentially be a role in predicting the most severe forms of AKI treated with RRT.

Funding: NIDDK Support, Other NIH Support - R01HL-085757

TH-PO126

Urinary NGAL, KIM-1, Hsp-72 and Interleukin 18: Prognostic Biomarkers of Acute Graft Dysfunction in Kidney Transplant Recipients Juan Carlos Ramirez-Sandoval,[1] Jonatan Barrera-Chimal,[2] Alejandro Rojas Montaño,[1] Perla E. Simancas,[1] Ricardo Correa-Rotter,[1] Norma Bobadilla,[2] Luis E. Morales-Buenrostro.[1] *[1]Nephrology, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico DF, Mexico; [2]Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico DF, Mexico.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule (KIM-1), interleukin 18 (IL-18) and heat shock protein 72 (Hsp72) are novel biomarkers of renal tubular damage which have been used as prognostic markers in acute kidney injury (AKI). However these biomarkers have not been tested in kidney transplant recipients (KTR) with AKI. The aim of this study was to establish the prognostic value of NGAL, KIM-1, IL-18 and Hsp-72 in KTR.

Methods: We included 71 KTR from out-patient and hospital scenarios presenting AKI (according to AKIN criteria) with baseline glomerular rate function (GRF) >30 mL/min/1.73m2. NGAL, KIM-1, IL-18 and HSP-72 were measured at AKI diagnosis with ELISA, in a single urinary specimen. Patients were followed for one year. Three groups were identified related to kidney function evolution: return to baseline kidney function and GRF >30 mL/min/1.73m2 (group A n=47, 66%), persistent GRF between 15 and 30 mL/min/1.73m2 (Group B n=16, 22%) and GRF <15 mL/min/1.73m2 or graft loss during follow-up related to AKI episode (Group C n=5, 7%). Three patients did not complete the follow up. All measurements were blinded to the investigators.

Results: Median NGAL urinary biomarkers levels were 92, 89 and 244 mcg/dL for groups A, B and C respectively (P: 0.001). KIM-1, levels were 99.2, 108.4 and 244 ng/dL for groups A, B and C respectively (P: 0.004). There were no statistical differences between groups for IL-18 and Hsp-72. A NGAL level of 217 mcg/dL had a sensitivity of 100% and a specificity of 86% (ROC AUC: 0.89, 95% IC: 0.81-0.97, P: 0.004) for graft failure during one year follow up.

Conclusions: NGAL and KIM-1 may be useful for prognostic markers in KTR with AKI.

Funding: Government Support - Non-U.S.

TH-PO127

Novel Biomarkers for the Prediction of Acute Renal Replacement Therapy: A Systematic Review

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Background: Novel biomarkers (BM) have been reported to aid in early diagnosis of acute kidney injury (AKI) and its severity. BM may have potential to identify AKI patients who will need renal replacement therapy (RRT). Our aim was to evaluate the performance of BM for prediction of RRT.

Methods: We performed a systematic review to identify studies which reported on novel BM and RRT as an outcome, whether separate or part of a composite. Studies which reported area under the receiver operating curve and/or sensitivity/specificity for any outcome which included RRT were eligible. We excluded studies in transplant patients. Two authors independently extracted data on study characteristics and measures of diagnostic accuracy.

Results: 34 studies were identified (31 prospective, 2 retrospective, 1 RCT), representing total 10431 patients. 782/3897 AKI patients needed RRT. Clinical settings included cardiac surgery (CS, n=8), critical illness (ICU, n=9), CS&ICU (n=2), hospital AKI (n=9) and others (n=6). Both urine and blood BM were studied (Fig1). The BM most frequently studied were Neutrophil Gelatinase Associated Lipocalin (n=22), CystatinC (n=12), Kidney Injury Molecule-1 (n=6) and Interleukin-18 (n=7). Various assays were used. The reported cut-off values for selected urine BM were: NGAL 16-680 ng/ml and 41 ng/ml/mmol/l Cr and 130 mcg/g Cr; KIM-1, 2.8 ng/ml and 210 pg/ml/mmol/l Cr and >15 ng/mg Cr; IL-18, 17-69.8 pg/ml and 36 pg/ml/mmol/l Cr. Fig 2 shows the corresponding sensitivity and specificity.

Conclusions: There was a wide range of cut-off values; most had fairly low sensitivity/specificty (i.e <0.85). Additional research must clarify the optimal BM values for categorizing high PPV/NPV for specific clinical management decisions. This will be in part also be determined by platform standardization and inter-laboratory calibration.

Fig 1: Study distribution by specimen type, BM number and evaluation time-points

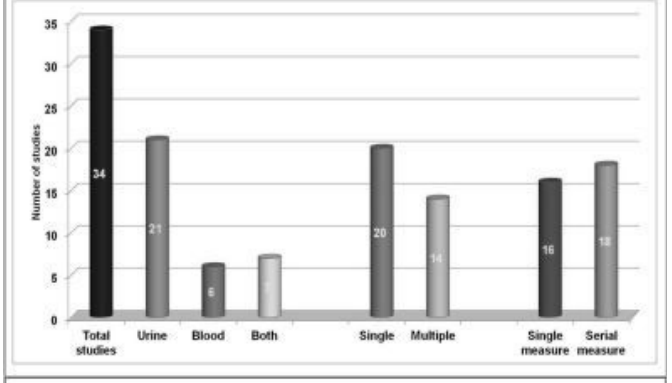
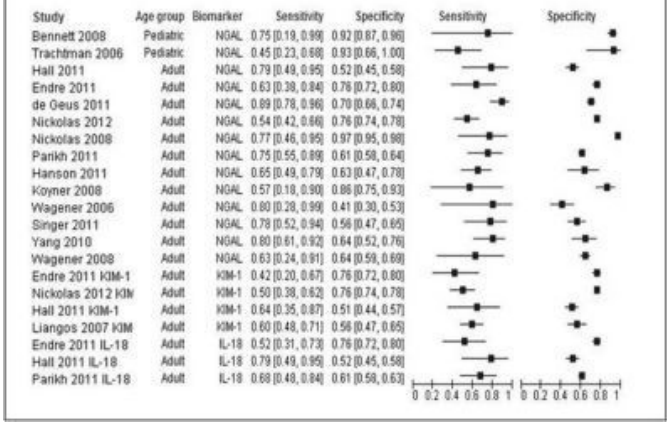


Fig 2: Sensitivity/Specificity of selected urinary BM for RRT prediction



TH-PO128

Determination of Biomarker Cut-Offs in Acute Kidney Injury

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Background: Current definitions of acute kidney injury (AKI) utilise arbitrary cut-offs for defining change in serum or plasma creatinine and urine output. Biomarkers of renal injury have been validated against these definitions. There is an urgent need for an independent outcome against which to judge both functional change and structural markers of injury. We propose using sensitivity for the combined outcome of mortality and need for dialysis as a better alternative for both parameters of change in renal status.

Methods: We retrospectively analysed data in 507 intensive care patients in whom urinary neutrophil-gelatinase associated lipocalin (NGAL) was measured on admission, at 12 and 24h, and plasma creatinine was measured daily for 7 days. The sensitivity of AKI (defined using KDIGO definition; Functional-AKI: plasma creatinine increase ≥0.3mg/dl in 48h or ≥50% in 7d) for dialysis or death within 30 days (the outcome) was determined in a randomly selected cohort of 254 patients (development cohort). Structural-AKI was subsequently defined using as >NGAL cut-off concentration with the same sensitivity as that of Functional-AKI for the same outcome. The relative risk (RR) of the outcome was then assessed in a validation cohort of 253 patients using the same Functional-AKI and Structural-AKI cut-offs.

Results: In the development cohort the sensitivity of Functional-AKI for the outcome was 62%. The threshold for urinary NGAL with a 62% sensitivity for this outcome was 140ng/ml. Sixty-five (25.6%) patients had both Functional-AKI and Structural-AKI, 38 (15%) Structural-AKI only, and 45 (17.7%) only Functional-AKI. Compared to the referent group (no-AKI) the RR of Functional-AKI and Structural-AKI was 3.62, of Structural-AKI only was 2.48 and of Functional-AKI only 2.09. The validation cohort was not different from the development cohort (p=0.87).

Conclusions: Sensitivity to need for dialysis and death can be used to link and give equal weight to functional or biomarker-based definitions of AKI. This hypothesis awaits validation in large multicentre datasets.

Funding: Government Support - Non-U.S.

TH-PO129

Urinary Angiotensinogen: A Novel Prognostic Biomarker of Acute Kidney Injury

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Background: Prognostic biomarkers that discriminate between patients who will develop severe acute kidney injury (AKI) and those who will not at the time of diagnosis with mild AKI could facilitate timely intervention in populations at risk of adverse outcomes.

Methods: Liquid chromatography-tandem mass spectrometry was used to discover potential prognostic biomarkers of severe AKI in the urine of 12 patients with AKI after cardiac surgery. Angiotensinogen was the best candidate biomarker. Subsequently, urinary angiotensinogen was measured by ELISA in patients who developed AKI after cardiac surgery (n=97) and evaluated for its prognostic potential using the area under the ROC curve (AUC).

Results: There were no differences among outcome groups with respect to demographics, type of surgery, bypass time, or pre-op serum creatinine. The urine angiotensinogen-to-creatinine ratio (uAnCR) was discriminative of the following outcomes: worsening of AKI (AUC=0.7), AKIN stage 3 AKI (AUC=0.71), the need for renal replacement therapy (RRT) (AUC=0.71), discharge ≤7 days after sample collection (AUC=0.74), as well as the composite outcomes AKIN stage 2 or 3 (AUC=0.64), AKIN stage 3 or death (AUC=0.75), and RRT or death (AUC=0.71). Furthermore, its prognostic predictive power was improved when only patients who were classified as AKIN stage 1 at the time of sample collection (n=79) were analyzed, having an AUC=0.81 for AKIN stage 3 or death. The predictive power of uAnCR was also augmented in patients who developed AKI after off-pump cardiac surgery (n=24), among whom it was an excellent predictor of AKIN stage 3 AKI and RRT (AUC=0.93 and 0.86, respectively).

Conclusions: Urinary angiotensinogen is a useful prognostic biomarker of AKI after cardiac surgery and can potentially be used alone or in combination with other biomarkers to predict adverse outcomes early in the course of the disease.

Funding: NIDDK Support, Veterans Administration Support

TH-PO130

Urinary π-Glutathione-s-Transferase Predicts Developing (Septic) Acute Kidney Injury at Least as Well as Urinary Neutrophil Gelatinase Associated Lipocalin

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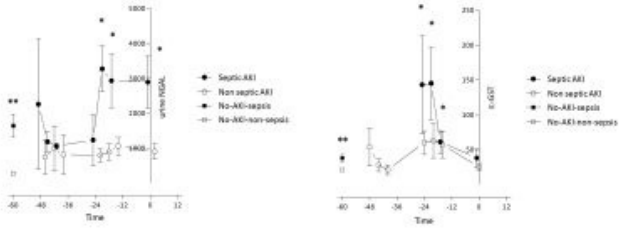
Background: Urinary Neutrophil gelatinase associated lipocalin (NGAL) is a biomarker for acute kidney injury (AKI) and considered as a reference marker for other candidates such as urinary π-Glutathione-s-transferase (π-GST). The aim of this study was

to assess the ability of π-GST to predict for AKI in adult critically ill patients with and without sepsis and to compare its performance with urinary NGAL.

Methods: 700 adult ICU patients were prospectively included for urine measurements at four time-points (T=0, 4, 8 and 24 h) straight after entry. Samples were analysed after study completion for biomarker expression. Patients were stratified according to the presence of sepsis at entry. Receiver operating characteristic (ROC) curves were generated using peak biomarker values and the area under the curve (AUC) was calculated. The primary study endpoint was the development of AKI (stage 1, 2 or 3 according to AKIN classification).

Results: Individual data of 508 patients entered the final analysis. Of these 57 developed the primary outcome. Thirty patients had sepsis at ICU entry (19 without AKI [4%] and 11 with AKI [19%]). The predictive value for any AKI of NGAL and π-GST was 0.75 and 0.70 (P=0.33). In the subgroup of septic patients both NGAL and π-GST concentrations were higher at all time points preceding AKI compared to non-septic patients (P≤0.05) (Figure 1). Sepsis did not affect the predictive properties of the biomarkers for AKI, except for π-GST; the AUC for the latter increased to 0.91 (P=0.005).

Conclusions: Peak urinary NGAL and π-GST concentrations preceding AKI after ICU admission express a comparable predictive power. In septic AKI, the prediction of π-GST further improves.



TH-PO131

Urine NGAL, L-FABP, and Cystatin C for the Early Detection of AKI and Prognosis of Critically Ill Adults with Preserved Kidney Function

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Background: Early detection of acute kidney injury(AKI) in the critically ill with urinary biomarkers is hindered by the confounding effects of pre-existing injury and injury diversity.

Methods: We examined the utility of urine NGAL, L-FABP, and Cystatin C for the early detection of AKI and predicting dialysis/death in a nested case-control study of 380 critically ill adults (127 cases:245 controls) with eGFR≥60 ml/min/1.73 m^2 at measurement.

Results: The AUC-ROCs for AKI discrimination over 48-hours for uNGAL, uL-FABP, and uCyst were 0.58(95%CI:0.52-0.64), 0.59(95%CI:0.52-0.65), and 0.51(95%CI:0.48-0.57), respectively. Combining uNGAL and uL-FABP resulted in an AUC of 0.59(95%CI:0.56-0.69). Discrimination for AKIN stages II and III improved to 0.69(95%CI:0.59-0.77), 0.65(95%CI:0.55-0.74) and 0.69(95%:0.65-0.82) for uNGAL,uL-FABP, and the combination, respectively. Neither uNGAL nor uL-FABP independently associated with the composite outcome of death or dialysis [uNGAL HR 1.35 (95%CI:0.93-1.96), uL-FABP HR 1.15 (95%CI:0.82-1.62)] though both independently predicted dialysis after adjusting for APACHE II [uNGAL HR 3.17 (95%CI:1.58-6.34), uL-FABP HR 2.12(95%CI:1.18-3.84)].

Cox Model for Clinical Outcomes

Outcome	uNGAL HR	95% CI	uL-FABP HR	95% CI
Dialysis (N=10)[a]	3.17	1.58-6.34	2.12	1.18-3.84
Mortality 28d (N=38)[b]	1.11	0.73-1.68	0.95	0.65-1.40
Dialysis or Mortality(n=48)[c]	1.35	0.93-1.96	1.15	0.82-1.62

Biomarkers were log10 transformed. Separate cox proportional hazards regression models for uNGAL, and uL-FABP were adjusted for: a: APACHE II b: APACHE II, sepsis c: modified APACHE II, sepsis, creatinine

Conclusions: Urine NGAL and L-FABP performed modestly, alone or combined, for the early detection of AKI in critically ill adults with preserved kidney function. Both markers provide useful prognostic information but require further study in early established injury.

Funding: NIDDK Support

TH-PO132

Prognostic Value of Urine Neutrophil Gelatinase Associated Lipocain in Critical Patients at the Admission to an Intensive Care Unit

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Background: NGAL is a promising biomarker for early AKI detection. However the performance of Urine NGAL (uNGAL) at admission to ICU has not been studied. Our objective is to estimate the diagnostic accuracy of uNGAL at the admission to an adult general ICU for early detection of AKI, need for Renal Replacement Therapy (RRT) and prediction of thirty days mortality.

Methods: A prospective observational study of 415 consecutive adult patients admitted to a general ICU was approved by the institutional review board. AKI was defined by AKIN criteria. Patients (APACHE, SAPS index, RRT) were follow up 30 days until their discharge from Hospital or their death. Samples: uNGAL at the admission in the ICU, Creatinine daily till 96 h, weekly and discharge creatinine. NGAL was done by Standardized Clinical Platform ARCHITECT assay, provided by Abbott Diagnostics. Diagnostic characteristic of uNGAL were evaluated with receiver –operating characteristic (ROC) curves for AKI diagnosis, need of RRT and thirty days mortality. Yeuden test was used to find best sensitivity, specificity, predictive positive value (PPV) and predictive negative value (PNV). SPSS17 was used.

Results: 99 patients (23, 3%) developed AKI, 46 (11%) need RRT and 71 (17, 1%) died. The ROC curve for uNGAL at admission and the occurrence of AKI was 0.845 (IC 0.80 a 0.89) p<0.001. We found for NGAL values >60ng/ml 78% sensitivity, 78%specificity, 53% PPV and 92% PNV. (Yeuden test) The ROC curve for uNGAL at admission and the need of RRT was 0.80 (IC 0.74 a 0.87) p<0.001. Yeuden test found for values >156 of u NGAL 71% sensitivity, 81% specificity, 32% PPV and 96% PNV. The ROC curve for uNGAL at admission and 30 days mortality was 0.66 (IC 0.59 a 0.74) p<0.001. Yeuden test found for values >100 of uNGAL 57% sensitivity, 73% specificity, 30% PPV and 89 % predictive negative value PNV.

Conclusions: Urine NGAL at admission in ICU predicts AKI, need for RRT and 30 days survival.

TH-PO133

Tubular Damage Biomarkers Linked to Inflammation or Iron Metabolism Predict Acute Kidney Injury

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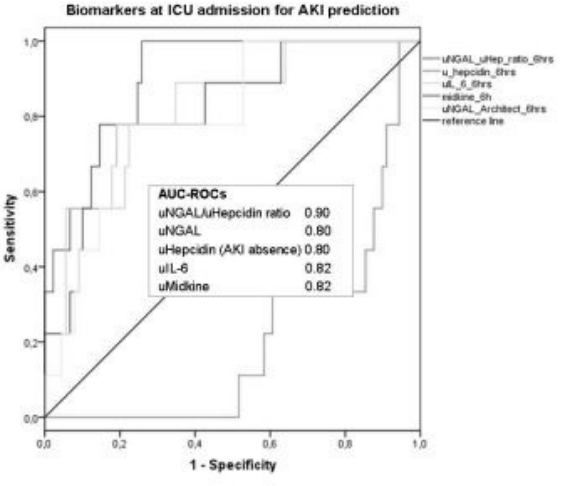
Background: Novel tubular damage biomarkers have been suggested to be included in acute kidney injury (AKI) definition to complement excretory renal functional criteria.

We compared the value of tubular damage markers measured early after cardiac surgery for prediction of postoperative AKI compared to conventional renal risk markers.

Methods: 100 adult patients undergoing cardiac surgery were analyzed. Preoperatively and at ICU admission, we quantified *in urine*: NGAL (ARCHITECT), hepcidin, midkine, IL-6, alpha1-microglobulin (all ELISA); *in plasma*: hepcidin, haptoglobin, CK, CKMB, CRP, leukocytes, lactate, urea, creatinine. Endpoint was RIFLE-AKI.

Results: Preoperatively, no biomarker predicted AKI (N=9). At ICU arrival, four urinary biomarkers showed good discriminatory ability for subsequent development of or absence of AKI (Fig 1). An excellent predictive value was found for uNGAL/uhepcidin ratio (Fig 1). Contrary, at ICU admission, none of the plasma biomarkers, including creatinine, was an early AKI predictor.

Conclusions: Several urinary markers of tubular damage predict AKI after cardiac surgery and the combination of NGAL and hepcidin provides excellent AKI prediction. This ratio combines an AKI prediction marker (NGAL) and a marker of AKI absence (hepcidin), potentiating their individual discriminatory values. Both markers may have unique roles in AKI pathophysiology, thereby multiple functions within inflammatory processes and in iron metabolism may be assessed. Validation in larger studies is desirable.



TH-PO134

Retooling the Creatinine Clearance Equation to Estimate Kidney Function when the Serum Creatinine Is Changing Acutely: Kinetic eGFR Formula

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Background: It is desirable to estimate the GFR at the bedside to better assess acute kidney injury (AKI) or renal recovery. Current eGFR formulas can estimate a kidney function when the serum creatinine is stable but do not work if the creatinine is changing rapidly. To analyze renal function outside of the steady-state, a novel formula is proposed.

Methods: The formula is $CrCl_{between2creats.}$=SteadyStateP_{Cr}×CorrespondCrCl×{Time(h)/24–[P_{Cr}(2)–P_{Cr}(1)]/nadirP_{Cr}}×mean$P_{Cr}$$^{-1}$×Time(h)$^{-1}$×24. It builds upon the creatinine clearance equation. Urinary creatinine excretion, U_{Cr}×V, is solved indirectly as the P_{Cr}×CrCl. CrCl is available from any eGFR formula. The {Time(h)/24–deltaP$_{Cr}$/nadirP_{Cr}}×Time(h)$^{-1}$×24 takes on one of three values: =1 in steady-state, <1 when the serum creatinine is rising, and >1 when the creatinine is declining. This adjusted urinary creatinine excretion gets divided by the serum creatinine, really the mean of two consecutive creatinines, to yield the eGFR a patient must have in order to transition from one creatinine to the next.

Results: The power of the kinetic eGFR formula is best seen in an example of AKI. A 40-year-old man with a baseline and nadir creatinine of 1.0 develops AKI and in the next 24 hours increases his creatinine to 1.9 mg/dl. If his P_{Cr} of 1.0 corresponds to an eGFR of 100 ml/min, the formula gives an answer of 1.0×100×{24/24-[1.9-1.0]/1.0}×1.45^{-1}×24^{-1}×24=6.90 ml/min. Consistent with our sense of severe AKI, the GFR has plummeted to <10 ml/min. This is immediately and quantitatively revealed by the kinetic formula, whereas a misapplied clearance equation wrongly informs that the GFR at a P_{Cr} of 1.9 is 1.0×100/1.9=52.6 ml/min. The clearance equation and other eGFR formulas will not be applicable until the serum creatinine stabilizes. But the kinetic formula works at all times and in fact reduces to the clearance equation in steady-state, attesting to its intrinsic validity.

Conclusions: Deduced from first principles of creatinine balance and mathematically proved for all cases, the kinetic eGFR formula upgrades the clearance equation with the ability to ascertain kidney function in AKI or renal recovery, which is of clinical and educational value.

TH-PO135

Kidney Injury Molecule-1 Staining Is a Specific Marker of Acute Tubular Injury in Pediatric Renal Biopsies

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Background: Kidney injury molecule-1 (KIM-1) has proven very useful in identifying acute tubular injury (ATI) present in proximal tubules of adult renal biopsies, but its role in pediatric biopsies has not been fully characterized. In this study we compared the KIM-1 staining patterns in children and adults and correlated these findings with clinical indices.

Methods: 77 adults (mean age of 52 years, ranging from 21 to 81 years old) and 60 children (mean age of 12 years, ranging from 2 to 20 years old) were included in the study. Their renal biopsies showed a large range of glomerular, tubulointerstitial and renal vascular diseases, and were all stained for KIM-1 (AKG monoclonal antibody). KIM-1 staining scores in proximal tubules were graded from 0 to 3+ in all biopsies and correlated with serum creatinine levels (sCr).

Results: In adults, positive KIM-1 staining was seen in 73 % (56/77) of adult biopsies. Nine (9) of 21 KIM-1 negative cases had elevated sCr ranging from 1.4 to 3 mg/dl and included 2 "acute kidney injury", 2 minimal change disease, 1 membranoproliferative glomerulonephritis, 2 focal segmental glomerulosclerosis, 1 IgM nephropathy and 1 IgA nephropathy. In pediatric biopsies, 82% (49/60) had positive staining for KIM-1. Only one pediatric KIM-1 negative case (IgA nephropathy) had elevated sCr (2.8 mg/dl). By linear regression analysis, in both adults and children there were significant correlations between respective KIM-1 staining scores and sCr (adult: r = 0.353, p = 0.0013; pediatric r = 0.483 and p = 0.0001). In adults, plotting sCr vs KIM-1 score showed a slope of 1.019 with intercept of 1.831. In pediatric slope was 0.760 with intercept of 0.310.

Conclusions: KIM-1 staining is a specific measure of ATI in both adult and pediatric groups with similar linear regression slopes. A lower intercept in the pediatric group would be expected from the lower baseline sCr values in children. Few negative KIM-1 staining cases with elevated sCr may result from a prerenal cause for the creatinine accumulation or stable chronic kidney disease with low levels of ongoing injury.

Funding: NIDDK Support

TH-PO136

Urinary α-Glutathione S-Transferase (GST) and π-GST for Prediction of Severe Acute Kidney Injury (AKI) Following Cardiopulmonary Bypass (CPB)

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Background: Urinary α-GST and π-GST are proximal and distal tubular leakage markers that have been proposed for early detection of AKI. We examined the performance characteristics of these 2 markers for detection of post-CPB AKI (>50% or >0.3 mg/dl ↑sCr), including severe AKI (>2-fold ↑ sCr or dialysis) and the composite of severe AKI or in-hospital death.

Methods: These hypotheses were tested in a prospective cohort of 252 adults undergoing CPB at 3 hospitals. Pre-CPB and 2-hr post-CPB urinary samples were obtained and treated with protease inhibitors. α-GST and π-GST were measured by ELISA. Receiver-operating- characteristic (ROC) curves were generated to explore the diagnostic performance of urinary α-GST and π-GST (modeled in tertiles).

Results: AKI developed in 72 (28.6%) patients. The area under-the-ROC curves (AUCs, with 95%CI) examining the performance of α-GST and π-GST are summarized in the Table. Overall, urinary π-GST measured 2-hr post-CPB performed better than α-GST for predicting severe AKI. Urinary α-GST alone performed poorly. However, combining urinary α-GST and π-GST 2-hr post-CPB improved discrimination for predicting severe AKI.

Conclusions: In adults undergoing CPB, urinary π-GST, measured 2-hr post-CPB, performed better than α-GST for predicting severe AKI, but neither marker displayed good discrimination.

Urinary marker	>0.3 mg/dl or >50% ↑sCr (n=72)	>2-fold ↑sCr or dialysis (n=9)	>2-fold ↑sCr, dialysis, or in-hospital death (n=16)
α-GST			
Pre-CPB	0.53 (0.46, 0.61)	0.57 (0.39, 0.76)	0.58 (0.44, 0.73)
2-hr post-CPB	0.54 (0.46, 0.62)	0.60 (0.38, 0.83)	0.53 (0.37, 0.69)
π-GST			
Pre-CPB	0.53 (0.45, 0.60)	0.73 (0.62, 0.84)	0.61 (0.48, 0.75)
2-hr post-CPB	0.58 (0.51, 0.65)	0.70 (0.56, 0.83)	0.60 (0.50, 0.70)
α-GST + π-GST			
Pre-CPB	0.55 (0.47, 0.62)	0.76 (0.67, 0.85)	0.69 (0.56, 0.81)
2-hr post-CPB	0.62 (0.54, 0.70)	0.74 (0.59, 0.90)	0.61 (0.46, 0.71)

TH-PO137

Biomarkers for Adult Cardiac Surgery Related Acute Kidney Injury:a Systematic Review and Meta Analysis

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Background: Cardiac Surgery related AKI (CSA-AKI) represents an important complication of heart surgery. Although several new biomarkers of CSA-AKI have been proposed, the clinical utility of these new tests in intraoperative or early postoperative diagnosis remains unclear. We conducted a systematic review and meta-analysis of the diagnostic performance of early biomarkers of CSA-AKI.

Methods: We searched EMBASE, CINAHL, Cochrane, SCOPUS and PUBMED using MeSH terms for AKI, cardiac surgery, and biomarkers. We reviewed all cohort studies in adult cardiac surgery patients having an N of at least 100 and an event rate of at least 30. All studies had to have a clearly defined AKI outcome (AKIN/RIFLE), and report the timing of the biomarker assessment (intraoperative or postoperative (≤24 h)). We excluded studies in whom the timing of biomarker assessment was unknown or more than 24 hours after surgery. We extracted and summarized the AUROC for each biomarker.

Results: Of the 2745 studies identified in the initial search, 17 studies examining 2 plasma and 7 urine biomarkers met the search criteria after full text review. Table 1

Table 1

Postoperative Biomarkers of CSA-AKI	Summary AUROC [*95 CI*]	N Studies
Urinary NGAL	0.73 [*0.69, 0.76*]	10
Plasma NGAL	0.69 [*0.65, 0.73*]	6
Plasma Cystatin C	0.71 [*0.65, 0.77*]	5
Urinary Cystatin C	0.67 [*0.60, 0.74*]	4
Urinary NAG	0.70 [*0.63, 0.76*]	4
Urinary KIM-1	0.79 [*0.75, 0.84*]	4
Urinary IL-18	0.68 [*0.63, 0.73*]	5
Intraoperative biomarkers of CSA-AKI		
Urinary NGAL	0.62 [*0.55, 0.69*]	3

14 studies reported diagnostic performance in the early postoperative period, whereas 3 studies reported intraoperative data. All biomarkers studied post operatively, including NGAL, showed at best moderate discrimination (summary AUROC for each biomarker ranging from 0.68-0.79,. Only urinary NGAL was studied in the intraoperative period, and showed poor diagnostic performance (summary AUROC 0.6, Table 1).

Conclusions: Current biomarkers exhibit modest discrimination for CSA-AKI in the early postoperative period. Intraoperatively, only NGAL has been studied to any extent, and its performance is poor. Efforts on development of new biomarkers for CSA-AKI are still needed.

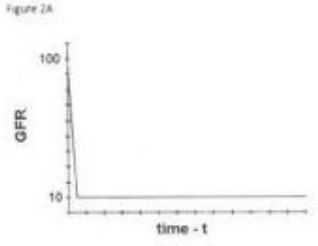
TH-PO138

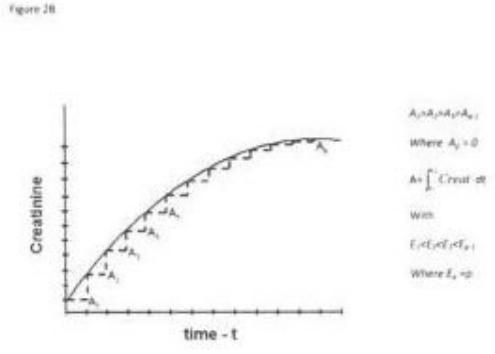
The Description of a New and Unique Method for Measuring Glomerular Filtration Rate in Acute Kidney Injury John Mellas. *Nephrology, St. Mary's Health Center, St. Louis, MO.*

Background: Acute kidney injury is defined by severity according to the AKIN and RIFLE criteria. KDIGO guidelines for AKI suggest that GFR should be measured when possible. The AKIN and RIFLE criteria do not measure GFR directly nor is there a method generally availble to measure GFR in AKI.

Methods: Herein is descibed a method to measure GFR in AKI using the following variables; Urine creatinine excretion (E); measured as urine volume times urine creatinine concentration, creatinine production rate (P); equal to [29-(.203xage)]x weight in kg, and basleine GFR which is the estimated creatinine clearance at time t1. By this method actual GFR=estimated GFR(at time t1)x E divided by P (E/P).

Results: In a patient with AKI the baseline GFR (at time t)x E/P is a constant number and thus measures actual GFR at any time course of AKI in a particular patient.





Patient with ARF. Baseline C creat is 100 ml/min and drops to 10 ml/min abruptly.
Assume P= 1440 mg/day (production)
At C creat 100 = U x V/P = 1440/p x 1440 x 100, p=1 (Creatinine)
At C creat 10 = U xV/P = 1440/p x 1440 x 100, p=10

S creat	GFR by MDRD	Actual C creat	U x V = E	E/P	MDRD X E/P
1	100	10	144	0.1	100x.1=10
2	50	10	288	0.2	50x.2=10
3	33.3	10	432	0.3	33.3x.3=10
4	25	10	576	0.4	25x.4=10
5	20	10	720	0.5	20x.5=10
6	16.7	10	864	0.6	16.7x.6=10
7	14.3	10	1008	0.7	14.3x.7=10
8	12.5	10	1152	0.8	12.5x8=10
9	11.1	10	1296	0.9	11.1x.9=10
10	10	10	1440	1	10x1=10

Conclusions: In AKI actual GFR = GFR estimated at time t1 x E/P measured over time intervals t1 to t2. This method provides the most accurate estimate of GFR in AKI thus far described.This method avoids the pitfalls of serum creatinine concentration where the distribution volume of creatinine would be required to measure serum creatinine accumulation and hence GFR, with the serum volume of distribution of creatinine diiffcult if not impossible to measure with accuracy.

TH-PO139

Novel Urinary Biomarkers Remain Elevated Following AKI in Children Donna J. Claes, Michael R. Bennett, Lori Brunner, Qing Ma, Stuart Goldstein. *Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Novel urinary biomarkers have been studied to diagnose AKI & CKD. There are no human studies that have longitudinally followed novel urinary biomarkers post-AKI.

Methods: We prospectively enrolled 18 pediatric pts (mean age 14.6 +/- 4.8 y) who developed either pRIFLE-I (n=6) or -F (n=12) any-cause AKI ≥ 48 h during admission. Serum creatinine (SCr) and novel urinary biomarkers were studied at AKI onset, then 2 wk, 1 mo, & 3 mo post-AKI.

Results: 44% (10/18) of pts had no underlying medical history; other PMHx included cardiac disease (16.7%), ESLD (11%), s/p liver Tx (5.6%), and cerebral palsy (5.6%). Baseline SCr was known in 58%. Mean pre-AKI Schwartz eGFR was 131 ml/min/1.73 m2 (SD 37). No pts had underlying hypertension; 3 pts (20%) were on anti-HTN meds for non-HTN causes. Pts had more than one etiology for AKI; causes ranged from nephrotoxic meds (67%), pre-renal (44%), sepsis/shock (39%), acute GN (22%), tumor lysis (5.6%), & rhabdomyolysis (5.6%). Median AKI duration was 14 d (IQR 10-22). 67% were admitted to the ICU for 6.5 d (IQR 3, 16.5). 6 pt required RRT (n=3 CRRT & IHD, n=3 IHD only) for 8 d (IQR 6-17).

18 pts represent 31 encounters. Markers of renal function/injury (eGFR and urinary biomarkers) from onset of AKI over time show the following (median, IQR provided):

Time post-AKI	Days from AKI	n	Schwartz eGFR (ml/min/1.73 m2)	NGAL (ng/ml)	IL-18 (pg/ml)	KIM-1 (pg/ml)	L-FABP (ng/ml)
Baseline	5 (4, 7)	9	25.8 (15, 40.6); n=18	164 (67, 679)	71 (20, 131)	1912.2 (1589, 2815)	17.4 (12, 49)
2 wk	20 (17, 24)	7	55.2 (33, 95)	25 (21, 58)	27.3 (18, 113)	1980 (1032, 2297)	6.7 (6, 63)
1 mo	33 (32, 37)	6	104 (81, 115)	19 (7, 143)	52 (18, 215)	1206 (616, 3469)	11.4 (4, 48)
3 mo	64.5 (56, 80)	9	116 (85, 119)	21 (3, 55)	30 (8, 93)	439 (376, 2221)	6 (4, 8)

Conclusions: In this small pediatric cohort, urinary KIM-1 appears to remain elevated up to 3 months post-AKI while other urinary biomarkers decrease to normal reference levels. We suggest that screening for future CKD risk at 3 months using urinary KIM-1 may identify pts at greater CKD risk.

TH-PO140

Renal Angina Prediction of Acute Kidney Injury Improves with Inclusion of Serum Biomarkers in Critically Ill Children Rajit K. Basu,[1] Lori Brunner,[1] Derek Wheeler,[1] Hector R. Wong,[2] Stuart Goldstein.[1] [1]*Center for Acute Care Nephrology, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH;* [2]*Critical Care, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH.*

Background: Earlier detection of acute kidney injury (AKI) with biomarkers may expedite treatment and improve outcomes. Our empiric concept of *Renal Angina* (ANG) identifies children (pts) at-risk for AKI in the pediatric intensive care unit (PICU) by combining known AKI risk factors with graded thresholds of early clinical AKI signs creating an ANG index (RAI). We suggest only pts who fulfill ANG should undergo biomarker assessment.

Methods: We studied 215 PICU pts with sepsis to determine if day of admission 1) ANG predicts severe sepsis AKI (SSAKI; (50% reduction in creatinine clearance)) on PICU day 7 and 2) serum biomarkers (matrix metalloproteinase-8 (MMP-8) and neutrophil elastase-2 (Ela-2)) improved upon ANG prediction of SSAKI. Based on derivation methodology, RAI ≥8 (range: 1-40) fulfilled ANG. We previously derived and validated MMP-8 and Ela-2 cutoffs for the prediction of SSAKI (≥11 ng/ml and ≥235 ng/ml, respectively).

Results: The area under the curve – receiver operating characteristic (AUC-ROC) for ANG was greater than the AUCs for both MMP-8 and Ela-2. Biomarker incorporation improved the AUC for ANG. Notably, the combination of the biomarkers with ANG increased the **specificity** of Day 0 ANG for Day 7 SSAKI (Table). Finally, while negative predictive value (NPV) remained high, positive predictive value (PPV) of ANG increased when combined with either biomarker.

	Sensitivity	Specificity	PPV	NPV	AUC-ROC
RAI ≥ 8	93 (76-99)	36 (33-37)	18 (15-19)	97 (90-99)	0.846
Ela-2 ≥ 235	89 (73-97)	45 (42-46)	19 (16-21)	97 (91-99)	0.725
MMP-8 ≥ 11	86 (69-95)	33 (30-34)	16 (13-18)	94 (87-98)	0.693
RAI-8 ≥ 8, Ela-2 ≥ 235	83 (65-93)	65 (62-67)	27 (21-30)	96 (92-99)	0.862
RAI ≥ 8, MMP-8 ≥ 11	89 (72-97)	57 (54-58)	24 (19-26)	97 (93-99)	0.841

Conclusions: In summary, we suggest that stratification using the renal angina prodrome allows a practitioner identify pts at-risk vs. not at-risk for AKI, focusing subsequent biomarker measurement to "rule out" AKI only in pts with ANG to increase predictive precision.

TH-PO141

Fluid Balance in ICU Setting: The More May Not Always Be the Better: Relationship with Mortality and Acute Renal Failure Pedro V.A. Aguiar,[1] Pedro Francisco Azevedo,[1] Hugo Mário Silva,[1] Anibal Marinho.[1] *[1]Centro Hospitalar do Porto - Hospital Santo Antonio, Porto, Portugal.*

Background: Fluid resuscitation from the Early Goal Directed Therapy led to an increased incidence of fluid overload in intensive care units (ICU). Though, recent observational studies revealed an increased 60-day mortality in the overhydrated patients, particularly if associated to acute renal failure (ARF) and the need to renal replacement therapy.

Methods: We conducted a prospective observational study in an ICU from a single center. All patients over 18 years old were included. Demographic data, fluid balance, SOFA score, serum creatinine, and diuretic therapy from day 1 to day 7 were recorded.

Results: A total of 47 patients (66% male, age 68,2±16,3, mean time on ICU 9,3 ± 6,3 days) were included. Most were admitted due to medical complications (62%). Six (13%) patients died. Positive fluid balances were found from day 1-3 (712,6 vs 362,3 vs 538,6mL) followed by negative tendency (-29,9 vs -248 vs -301,1mL). Patients with renal failure (serum creatinine > 2,5mg/mL) presented higher fluid balances (389,5 vs 1189,9mL), as opposed to those on furosemide therapy (249,9 vs 3536,1mL). Deceased patients presented higher fluid balances from day 3 onwards (day 3 4181,8 vs 1238,3mL; day 5 4363 vs 940ml, day 6 1563,8 vs -135,2mL), higher catecholamine doses, and higher SOFA score (8,5 vs 6,4%). Overall, patients with need for vasopressor support presented higher fluid balances (min: 183,1-max:1354,2mL vs min:-559,0- max: 379,0mL). No fluid balance differences were found between deceased patients under catecholamine therapy and the rest from days 1-4.

Conclusions: Increased hydration was found in patients presenting acute renal failure. In non-oliguric patients furosemide may prevent fluid overload. More, positive fluid balance was increased in deceased patients in the ICU setting.

TH-PO142

The Accuracy of Acute Kidney Injury Diagnosis Depends on Fluid Balance in ICU Patients Pedro Fidalgo,[1] Silvia Coelho,[1] Bruno Rodrigues,[1] Luis Inchaustegui,[1] Ana Luisa Papoila,[3] Fernando Liano,[2] Karina Soto.[1] *[1]Nephrology, Hospital Fernando Fonseca, Portugal; [2]Nephrology, Hospital Ramon y Cajal, Portugal; [3]Biostatistic, Faculty of Medical Sciences of Lisbon, Portugal.*

Background: Fluid management is an integral element of the care of critically ill patients. However, fluid overload may modify the volume distribution of serum creatinine (SCr). The aim of this study was to determine whether fluid balance changes the accuracy of Acute Kidney Injury (AKI) diagnosis and staging.

Methods: In a prospective study of 128 consecutive ICU patients AKI was classified by RIFLE criteria. SCr and serum Cystatin C (SCysC) were measured daily. Fluid balance (FB) was calculated with daily input-output fluids. Sum of daily FB was defined as Cumulative fluid balance (CFB). The Percentage of CFB (PCFB) was calculated according to the formula:[∑ daily FB/admission body weight (kg)]*100. SCr and SCysC were adjusted with the formula: biomarker x ((admission body weight x 0.6) + (Σ daily FB))/(admission weight x 0.6). A new adjusted RIFLE was calculated with adjusted SCr and were distributed into: maintaining RIFLE stage (Eq); worsening (Wr) and improved (Ip). Mann-Whitney test was used. Generalized linear models and linear mixed-effects models were applied. p value <0.05 was considered significant.

Results: AKI was diagnosed in 35.2 % of patients. A significant difference was detected between measured and adjusted SCr and SCysC, incrementing with the length of stay in ICU and increasing with CFB. Comparison between RIFLEs showed that, 79.8% were Eq most of them corresponding to the RISK class; 12.5% were Wr and had a significantly higher median of PCFB, 22.9% (12-33) compared to the other groups. Additionally, 7.8% were Ip, corresponding to lower CFB.The worst performance of unadjusted SCr was in "Failure" stage of RIFLE, diagnosing only 53.7% of cases correctly. Finally, the threshold of PCFB above which the unadjusted RIFLE classification was inaccurate, was determined to be 6.7% (2.3-13.0).

Conclusions: Unadjusted SCr influences the RIFLE classification, particularly in patients with higher volume overload. Patients in "Failure" stage have a higher probability of being misclassified.

TH-PO143

The Role of Depletion of Renal Progenitors in the Development of Renal Fibrosis in a Rat Remnant Kidney Model Xinzhou Zhang,[1] Liu Junmin.[2] *[1]Department of Nephrology, The Second Clinical College of Jinan University, Shenzhen, Guangdong, China; [2]Department of Nephrology, The Second Clinical College of Jinan University, Shenzhen, Guangdong, China.*

Background: Recent studies demonstrate renal stem/progenitor cells exist in mature mammalian kidney and participate in regeneration of podocytes and tubular epithelia after kidney injury. Subtotal nephrectomy cause development of renal fibrosis, involving glomerulosclerosis and tubulointersticial fibrosis, which were characterized by podocyte damage and tubule atrophy respectively. This study hypothesized that depletion of renal progenitor exist during renal fibrosis in the remnant kidney model.

Methods: Kidney specimens were obtained from the rats underwent 5/6nephrectomy. Morphologic analysis was based on HE-staining. The analysis of CD133,Pax-2,Podocin and Ki67 expression on/in renal cortex cells was performed by flow cytometry. The level of TGF-β1,VEGF and AngiotensinII(AngII) in renal cortex were measured by western blotting and enzyme linked immunosorbent assay (ELISA) respectively. The expression of AngII type1 receptor (AT1R), AT2R and Pax-2 mRNA on/in $CD133^+$cells was evaluated by RT-PCR.

Results: The level of AngII and TGF-β1 were upregulated in cortex of remnant kidneys compared with normal kidneys, as well as increased sign of AT1R mRNA expression on $CD133^+$cells. Going with gradual downregulation of Pax-2 gene expression in $CD133^+$ cells, the proportions of $CD133^+Pax\text{-}2^+$cells and $Podocin^+Ki67^+$cells in cortex cells of remnant kidneys underwent progressive decrease with development of glomerulosclerosis.

Conclusions: Subtotal nephrectomy induces nephrogenous AT1R-mediated activation of RAS and upregulation of TGF-β1 level, which cause downregulation of Pax-2 gene expression, thus lead to reduction of renal progenitors and deficiency of podocyte regeneration, suggesting that depletion of renal progenitors exist in the procession of remnant kidney and maybe play a pivotal role in the pathogenesis of renal fibrosis.

Funding: Government Support - Non-U.S.

TH-PO144

TIMAP Is Critical for Glomerular Endothelial Cell (EC) Angiogenesis Marya Obeidat, Barbara J. Ballermann. *Dept. of Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: Glomerular capillaries develop through vasculogenesis and angiogenesis. Angiogenesis involves migration of tip EC towards the angiogenic stimulus, proliferation of stalk EC to elongate the sprout, and controlled apoptosis to remove superfluous cells. We showed that TGFβ1 regulates glomerular capillary formation *in vitro* and *in vivo,* in part by enhancing EC apoptosis. TIMAP is an EC- predominant inhibitory protein phosphatase 1 (PP1c) regulatory subunit strongly repressed by TGFβ1. This study sought to define the role of TIMAP in glomerular EC angiogenesis.

Methods: Human glomerular EC were transfected with TIMAP-specific siRNAs or control siRNA (siRNAScr). The rate of change of electrical impedance by non-confluent EC was used to monitor EC proliferation. DNA synthesis was quantified by flow-cytometric analysis of EdU incorporation. To quantify 3D-angiogenesis, collagen-coated beads containing confluent glomerular EC were suspended in fibrin gels and for each transfection, sprouts per bead were counted for 40 consecutive beads. Protein phosphorylation was assessed by Western blot analysis. Caspase 3 activity was quantified as a measure of apoptosis. All experiments, except Caspase 3 activity, were repeated at least 3 times and are presented as the mean ± SEM.

Results: After 72 hours of transfection with siRNA, TIMAP protein was reduced by 76 ± 8% compared to siRNAScr. TIMAP knockdown reduced the rate of electrical impedance development by 93 ± 2% compared to siRNAScr ($p<0.05$). TIMAP siRNA reduced EdU incorporation by 38 ± 2% vs. siRNAScr ($p<0.05$). Angiogenic sprouts decreased by 43 ± 1% in EC transfected with TIMAP siRNA compared to siRNAScr ($p<0.05$). TIMAP siRNA reduced phosphorylation of AKT on Ser473 by 83 ± 1% ($p<0.01$), without effect on total AKT. ERK1/2 MAPK phosphorylation was unaffected. In two experiments so far, TIMAP knockdown augmented staurosporine-induced Caspase 3 activity, 1.6 and 2.1 fold, compared to siRNAScr.

Conclusions: AKT phosphorylation on Ser473 is critical for EC survival and proliferation. Our data indicate that TIMAP promotes the Ser473 phosphorylation of AKT, potentially by inhibiting its dephosphorylation. In turn, TIMAP enhances EC proliferation and protects EC from apoptosis.

Funding: Government Support - Non-U.S.

TH-PO145

MMP-9 Contributes to TGF-β-Induced Endothelial-Mesenchymal Transition through Notch Signalling in Human Renal Glomerular Endothelial Cells Ye Zhao,[1] Thian Kui Tan,[1] Jianlin Zhang,[2] Changqi Wang,[1] Yiping Wang,[1] Ya Wang,[1] Yuan Min Wang,[3] Vincent W.S. Lee,[1] Stephen Alexander,[3] Tania Tsatralis,[1] Guoping Zheng,[1] David C. Harris.[1] *[1]Centre for Transplant and Renal Research, Westmead Millennium Institute, Sydney, NSW, Australia; [2]Dept. of Biochemistry and Molecular Biology, Shanxi Medical University, Taiyuan, Shanxi, China; [3]Centre for Kidney Research, Children's Hospital at Westmead, Sydney, NSW, Australia.*

Background: Endothelial-mesenchymal transition (EndoMT) has been shown to be a major source of myofibroblast formation in kidney fibrosis. Our previous study showed a profibrotic role for MMP-9 in kidney fibrosis by inducing epithelial-mesenchymal transition (EMT). Inhibition of MMP-9 activity reduced kidney fibrosis in murine unilateral ureteral obstruction. This study investigated whether MMP-9 also plays a role in EndoMT.

Methods: Human renal glomerular endothelial cells (HRGEC) were treated with TGF-β1 to induce EndoMT. EndoMT was assessed by morphological changes, immunofluorescence staining (IF) and western blot (WB) of endothelial (CD31 and VE-cadherin) and mesenchymal markers (α-SMA, vimentin and N-cadherin). Snail expression and Notch signaling were examined by RT-PCR and WB. MMP-9 and MMP-2 expression were examined by zymography.

Results: TGF-β1 (10 or 20 ng/ml) induced EndoMT in HRGEC as evidenced by morphological changes, and by significant downregulation of VE-cadherin & CD31 and upregulation of α-SMA, vimentin and N-cadherin. RT-PCR showed an upregulation of Snail, a known inducer of EMT. The TGF-β1-induced EndoMT was abrogated by MMP inhibitor GM6001. Zymography showed that MMP-9 was unregulated in the TGF-β1 treated HRGECs.Recombinant MMP-9(2 μg/ml) also induced EndoMT in HRGECs with an upregulation of Notch signaling evidenced by an increase of Notch intracellular domain (NICD) accompanied by a decrease of Notch 1. Inhibition of MMP-9 activity by its inhibitor demonstrated a dose-dependent response in preventing TGF-β1-induced α-SMA and NICD in HRGECs.

Conclusions: MMP-9 has an important role in TGF-β1-induced EndoMT in HRGECs, potentially through upregulation of Notch signaling.

Funding: Government Support - Non-U.S.

TH-PO146

MicroRNA-155 Functions as a Negative Regulator of RhoA Signaling in TGF-β-Induced Endothelial to Mesenchymal Transition Ruben de Bruin,[1] Roel Bijkerk,[1] Coen van Solingen,[1] Jacques Duijs,[1] Eric P. Van der Veer,[1] Peter Dijke,[2] Ton J. Rabelink,[1] Marie-jose Goumans,[2] Anton Jan Van Zonneveld.[1] *[1]Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Molecular Cell Biology and Centre for Biomedical Genetics, Leiden University Medical Center, Leiden, Netherlands.*

Background: Endothelial to mesenchymal transition (EndoMT) has been proposed to be involved in the loss of microvascular capillaries in the pathophysiology of renal fibrosis and renal failure. In EndoMT, endothelial cells (ECs) undergo a mesenchymal transition associated with the loss of cell-cell contacts and the acquisition of a synthetic phenotype that can contribute to organ fibrosis.

Methods: Here, we sought to identify microRNAs (miRNAs) that could play a central role in regulating EndoMT. In a TGF-β dependent *in vitro* model for EndoMT, we identified miRNAs that were differentially expressed in normoxic and hypoxic conditions. Furthermore, cellular characteristics such as morphological transition, stress fiber formation, endothelial cell markers (e.g. CD31) and fibrotic markers (e.g. a-SMA, collagen) were investigated.

Results: This study identifies a variety of miRNAs to be differentially expressed in endoMT. Especially miR-155 is significantly upregulated upon TGF-betainduced EndoMT. Silencing of miR-155 directly increased RhoA expression and activity in endothelial cells and affected phosphorylation of downstream LIMK. In contrast, overexpression of miR-155 counteracted RhoA function. Using a selective Rho kinase inhibitor, we could partly suppress EndoMT, strengthening the notion that RhoA plays a central role in EndoMT. Moreover, forced overexpression of miR-155 completely suppressed EndoMT, as evidenced by the maintenance of EC characteristics and blocking the acquisition of a mesenchymal phenotype.

Conclusions: Our data demonstrate that several miRNAs and in particular miR-155, are differentially expressed upon TGF-β induced EndoMT. We show that miR-155 functions as a negative regulator of RhoA signaling and hereby modulates the capability of endothelial cells to aquire a synthetic phenotype.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO147

Dapper1, a Wnt-Pathway Inhibitor, Mediates Human Mesangial Cell, TGF-β1-Induced, Apoptosis Daniele Pereira Jardim,[1,2] Alexandre Holthausen Campos.[1] *[1]Experimental Research Center, Albert Einstein Hospital, Sao Paulo, SP, Brazil; [2]Medicine - Nephrology Division, UNIFESP, Sao Paulo, SP, Brazil.*

Background: TGF-β1 promotes extracellular matrix deposition, cell hypertrophy and death, main components of glomerular damage in chronic nephropathies. We detected the upregulation of Dapper1 (Dpr1) in human immortalized mesangial cells (hiMC) in response to TGF-β1.It is already known that Dpr1 negatively modulates the pro-survival Wnt pathway. In addition, Wnt reduces apoptosis of mesangial cells cultured in high ambient glucose. This study aims to investigate the role of Dpr1 in mesangial cell apoptosis promoted by TGF-β1.

Methods: hiMC were treated with TGF-β1 (0.2-2ng/ml) or vehicle for various periods of time (1-24h). Dpr1 overexpression was achieved by means of adenoviral infection. Dpr1 constitutive expression was blocked by siRNA transfection. Apoptosis was evaluated through chromatin morphology (Hoeschst 33342 staining) and caspase-3 activity analysis. For *in vivo* experiments, male Wistar rats (150-200g) underwent subtotal nephrectomy (Nx 5/6), according to methods largely described. After four weeks, remaining renal mass was used for TGF-β1 and Dpr1mRNA expression level analysis by qPCR.

Results: TGF-β1 increases Dpr1 expression in a time- and concentration-dependent manner (peak response at 3h: 3.7x; p=0.00007). Similar TGF-β1 concentrations lead to marked hiMC apoptosis (+2.4x, N=4, $p<0.01$ in the absence of serum). Dpr1 forced expression potentiated serum withdrawal-induced apoptosis (+2.1x, N=6, $p<0.01$). In fact, Dpr1 caused hiMC death even in the presence of 10% serum (+1.5x N=6, $p<0.01$). Dpr1 knock-down significantly decreased apoptosis induced by TGF-β1 (- 1.9x, N=4, $p<0.01$). Dpr1 expression was shown to be increased in samples from Nx5/6 animals (2.7x, N=12, $p<0.02$) and Dpr1 mRNA levels positively correlated to TGF-β1 expression in diseased kidneys ($r^2=0.88$, $p<0.01$).

Conclusions: The collective analysis of our data indicates that Dpr1 is an essential mediator of TGF-β1-induced apoptosis in mesangial cells. Additional studies are underway to better define the influence of Wnt pathway inhibition by TGF- β1 in the pathophysiology of chronic renal diseases.

Funding: Government Support - Non-U.S.

TH-PO148

Gene Expression Profiling of Wild-Type and Progeroid Mice Reveals Pathways Involved in Glomerular Aging Valerie Bartels,[1] Bernhard Schermer,[1] Peter Frommolt,[1] Bianca H. Habermann,[1] Marianne Roodbergen,[2] Jan H.j. Hoeijmakers,[3] Peter Nuernberg,[1] Martijn E. Dollé,[2] Thomas Benzing,[1] Roman-ulrich Mueller,[1] Christine E. Kurschat.[1] *[1]Cologne University, Germany; [2]RIVM Bilthoven, Netherlands; [3]Rotterdam University, Netherlands.*

Background: Due to the worldwide increase in the elderly population prevention and therapy of aging-related diseases such as chronic kidney disease have become important medical issues. One of the factors contributing to renal aging is DNA damage. The Ercc1 protein is a major component of nucleotide excision repair which detects and repairs DNA damage. $Ercc1^{-/\Delta 7}$ mice carry one hypomorphic variant of Ercc1 leading to a progeroid phenotype. To elucidate molecular mechanisms involved in glomerular aging we analyzed gene expression levels of glomeruli from young and old wild-type (wt) and $Ercc1^{-/\Delta 7}$ mice.

Methods: Wt mice were sacrificed at 14 and 96 wks, $Ercc1^{-/\Delta 7}$ mice at 4 and 14 wks. Glomeruli were isolated by a magnetic particle concentrator after kidney perfusion with magnetic beads. Glomerular RNA was reverse transcribed and cDNA was hybridized to Affymetrix microarrays. Statistical, GO enrichment and network analyses were conducted using R/ Bioconductor, DAVID server and NetBox software.

Results: Hierarchical clustering of 521 genes differentially regulated between young and old wt and $Ercc1^{-/\Delta 7}$ mice showed cluster formation between young wt and $Ercc1^{-/\Delta 7}$ and old wt and $Ercc1^{-/\Delta 7}$ samples. There was a significant overlap of 77 genes differentially regulated between both young and old wt and young and old $Ercc1^{-/\Delta 7}$ mice ($p<0.0001$). GO enrichment revealed these genes to be involved in biological functions such as immune and inflammatory response, cell death, and chemotaxis. A network analysis showed that these genes were part of insulin signalling, chemokine and cytokine signalling, and extracellular matrix pathways.

Conclusions: Our findings demonstrate that beyond insulin signalling immune and inflammatory signalling as well as extracellular matrix pathways play major roles in the aging glomerulus. These molecular components can also be found in the progeroid $Ercc1^{-/\Delta 7}$ mouse model which might be a helpful tool for exploring glomerular aging in the future.

TH-PO149

Notch2 Pathway Reactivation Ameliorates Urinary Protein and Glomerular Sclerosis in Adriamycin Nephropathy Mice Eriko Tanaka,[1,2] Katsuhiko Asanuma,[1] Kanae Nonaka,[1] Juan Alejandro Oliva Trejo,[1] Takuto Seki,[1] Rin Asao,[1] Yoshiko Hosoe,[1] Eun-hee Kim,[1] Miyuki Takagi,[1] Hideo Yagita,[3] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Juntendo University, Tokyo, Japan; [2]Department of Pediatrics and Developmental Biology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Immunology, Juntendo University, Tokyo, Japan.*

Background: Notch signaling pathway is an evolutionarily conserved intracellular signaling pathway that regulates cell fate. Recently, it has been reported that Notch1 and Notch2 pathways are reactivated in glomerular disease and that Notch1 pathway reactivation correlates with glomerulosclerosis. However, the role of Notch2 pathway reactivation remains unclear.

Methods: We used adriamycin (ADR) nephropathy mice as nephrotic syndrome and focal segmental glomerulosclerosis model. Anti-Jagged1 antagonistic antibody, anti-Notch1 receptor agonistic antibody or anti-Notch2 receptor agonistic antibody was injected intraperitoneally in ADR nephropathy mice simultaneously with ADR. The levels of urinary protein and the ratio of sclerotic glomeruli were evaluated in each group. Next, to elucidate the Notch2 signaling pathway in podocytes, we treated cultured podocytes injured by ADR with anti-Notch2 agonistic antibody. The effect for cytotoxic reduction and the pathways induced by anti-Notch2 agonistic antibody were examined.

Results: Injection of anti-Notch2 agonistic antibody reduced the levels of urinary protein. The ratio of sclerotic glomeruli in ADR nephropathy mice treated with anti-Notch2 agonistic antibody was dramatically decreased. Anti-Notch2 agonistic antibody reduced cell toxicity in cultured podocytes injured by ADR. Phosphorylated Akt was significantly increased by anti-Notch2 agonistic antibody in cultured podocytes injured by ADR.

Conclusions: This is the first report demonstrating that Notch2 reactivation has a role for podocyte recovery and that Notch2 acceleration by Notch2 agonistic antibody ameliorates urinary protein and glomerular sclerosis in focal segmental glomerulosclerosis mouse model, possibly through the Akt pathway. The Notch2 signaling pathway could be a new target for the treatment of glomerular diseases.

TH-PO150

hVps34 Deficiency Reveals the Importance of Endosomal Pathways for Podocyte Homoeostasis Wibke Bechtel,[1] Martin Helmstädter,[1] Bjorn Hartleben,[1] Shuya Liu,[1] Jan Balica,[1] Fatima Hrnjic,[1] Dontscho Kerjaschki,[2] Tobias B. Huber.[1] *[1]Renal Division, University Medical Center Freiburg, Freiburg, Germany; [2]Department of Pathology, Medical University of Vienna, Vienna, Austria.*

Background: Podocytes are critical components of the glomerular filtration barrier and their injury often leads to progressive glomerulosclerosis and proteinuria. Recent functional genetic studies identified the pathological relevance of cytoskeletal-, metabolic- and receptor signalling pathways for the integrity of podocytes. However, the role of endosomal pathways for podocyte function and maintenance remained largely unknown.

Methods: We generated podocyte-specific hVps34-deficient mice to functionally analyse the importance of endosomal pathways for podocytes homeostasis using a Nphs2-cre line. Further analysis on primary podocyte cell cultures was performed after isolation and FACS sorting of GFP-labelled primary podocytes. D. melanogaster virgins of UAS-VPS34RNAi were crossed to prospero-Gal4 for further analysis of a Garland Cell Nephrocyte (the podocytelike cells of Drosophila) specific knockdown of Vps34.

Results: Here we demonstrate that the class III phosphoinositide 3-kinase hVps34 plays a critical role in regulating endocytic pathways maintaining podocyte homeostasis. Podocyte-specific hVps34-conditional knockout mice develop early proteinuria and die within 3 to 9 weeks of age. hVps34 deficient podocytes develop substantial vacuolization and foot process effacement progressing to glomerular scarring and severe proteinuria. Rab5-positive endosomal compartments and autophagosome formation are disrupted at early stages. In addition, endo-lysosomal fusion is inhibited in conditional hVps34 knockout mice. Strikingly, these hVps34 dependent pathways are highly conserved in nephrocytes, the podocyte-like cells of Drosophila.

Conclusions: In summary, these data identify hVps34 as a major regulator of enodolysosomal pathways in podocytes and underline the fundamental role of endosomal trafficking for the maintenance of the glomerular filtration barrier.

Funding: Government Support - Non-U.S.

TH-PO151

PGC-1α Activation Protects against Aldosterone-Induced Podocytes Damage via Regulating Mitochondrial Antioxidant Genes Aihua Zhang, Yanggang Yuan, Songming Huang, Guixia Ding, Min Su. *Nephrology, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Podocytes are specialized visceral epithelial cells that reside on the glomerular basement membrane (GBM) outside of the glomerular capillaries. It therefore forms the final barrier to protein loss, which explains why podocyte damage is typically associated with marked proteinuria. Previous study has showed that reactive oxygen species (ROS) play a crucial role in the pathogenesis of proteinuria and podocyte damage. This study investigated whether the transcriptional coactivator, peroxisome proliferator activated receptor-g coactivator 1α (PGC-1α), a major regulator of oxidative metabolism and mitochondrial function, prevented podocyte damage by regulating mitochondrial antioxidant genes.

Methods: Podocyte cell line MPC-5 was infected with PGC-1α adenoviral vector and cultured in the presence or absence of Aldo. The expression of mitochondrial antioxidant genes, including superoxide dismutase 2 (SOD2), peroxiredoxin 3 (Prx3) and peroxiredoxin 5 (Prx5) were detected by real time RT-PCR and immunoblotting. Mitochondrial ROS production was examined by mitochondrial specific probe MitoSOX staining.

Results: Aldosterone (Aldo) induced mitochondrial ROS production and decreased PGC-1α expression in a dose dependent manner. Increased PGC-1α levels in podocytes by infection with PGC-1α adenoviral vector prevented Aldo-induced mitochondrial ROS production and podocyte damage. Meanwhile, PGC-1α overexpression increased the levels of mitochondrial antioxidant genes SOD2, Prx3, and Prx5. Finally, suppression of endogenous PGC-1α expression results in the down-regulation of the mitochondrial detoxification machinery.

Conclusions: These results unveiled that PGC-1α was important in regulating mitochondrial antioxidant genes. PGC-1α activation enhanced the expression of mitochondrial antioxidant genes, which then inhibited Aldo-induced mitochondrial ROS production and finally protected against Aldo-induced podocyte damage.

Funding: Government Support - Non-U.S.

TH-PO152

Mitochondrial Dysfunction Is an Early Event in Aldosterone-Induced Podocyte Injury Aihua Zhang, Songming Huang, Yanggang Yuan, Guixia Ding. *Nephrology, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: We previously showed that mitochondrial dysfunction is involved in an aldosterone (Aldo)-induced podocyte injury. Here, the potential role of mitochondrial dysfunction in the initiation of podocyte damage was investigated.

Methods: The dynamic changes of urinary protein, urinary F_2-isoprostane and renal malondialdehyde levels, kidney ultrastructure morphology, mitochondrial DNA (mtDNA) copy number, mitochondrial membrane potential ($\Delta\Psi_m$), mitochondrial complex I activity, and nephrin and podocin expression were detected in Aldo-infused mice and Aldo-stimulated podocyte. Mitochondrial transcription factor A (TFAM) shRNA or adenoviral vectors (Ad-TFAM) was transfected into podocyte to suppression or overexpression of TFAM, respectively.

Results: Aldosterone-infusion first induced renal oxidative stress, as evidenced by increased levels of urinary F_2-isoprostane and renal malondialdehyde, and mitochondrial dysfunction, as demonstrated by reduced mtDNA, $\Delta\Psi_m$, ATP production, and complex I activity. Later, at 5 days after aldosterone-infusion, proteinuria and the decreases of nephrin and podocin began to appear. In cultured podocytes, Aldosterone induced mitochondrial dysfunction after 2–8 h of treatment, whereas the decreased nephrin and podocin expression occurred later after 12 h of treatment. Thus, Aldo treatment both in vitro and in vivo indicated that mitochondrial dysfunction occurred prior to the decreases of nephrin and podocin. Additionally, MtDNA depletion by mitochondrial transcription factor A (TFAM) RNAi induced mitochondrial dysfunction, further promoting podocyte damage. TFAM expression was found to be reduced in aldosterone-infused mice and aldosterone-treated podocytes. Adenoviral vector-mediated overexpression of TFAM prevented aldosterone-induced mitochondrial dysfunction and protected against podocyte injury.

Conclusions: These findings support mitochondrial dysfunction as an early event in podocyte injury, and manipulation of TFAMmay be a novel strategy for treatment of glomerular diseases such as podocytopathy.

Funding: Government Support - Non-U.S.

TH-PO153

All-Trans Retinoic Acid Inhibits the Fibrotic Effect of TGF-β1 on Renal Tubular Epithelial Cells by Activating Pea3/BMP-7 Signaling Aihua Zhang, Songming Huang, Min Su, Yanggang Yuan. *Nephrology, Nanjing Children's Hospital, Nanjing, Jiangsu, China.*

Background: Renal interstitial fibrosis is the final common pathway that determines the long-term prognosis of chronic kidney diseases. Phenotypic changes in the proximal tubular epithelial cells driven by maladaptive transforming growth factor-β1 (TGF-β1) signaling are crucial to this process. Based on current evidence, all-trans retinoic acid (ATRA),which are natural derivatives of vitamin A, play an important role in renal diseases. Retinoic acids act mainly through nuclear receptors such as retinoic acid receptors (RARs) and rexinoid receptors (RXRs). In this study, we investigated the molecular mechanism of ATRA in the phenotypic changes induced by TGF-β1 in renal tubular epithelial cells.

Methods: Human renal proximal tubular epithelial cell line HK-2 was cultured in the presence or absence of ATRA. **TGF-β1-induced** fibrogenic responses were assessed by the expression of α-SMA, collagen I, CTGF, E-cadherin, and ZO-1. Chromatin immunoprecipitation assays were conducted to detect the interaction of PEA3, RARs, and RXRs with BMP-7 promoter.

Results: ATRA suppressed TGF-β1-induced α-SMA, collagen I, and CTGF expression and blocked TGF-β1-reduced E-cadherin and ZO-1 expression. Interestingly, ATRA regulated and activated the BMP-7 signaling pathway through RARα and RXRα. BMP-7 knockdown abrogated the ATRA-induced inhibition of TGF-β1. Moreover, as with RARα and RXRα, Pea3 mediated ATRA-induced BMP-7 activation, and Pea3 knockdown increased the TGF-β1-induced fibrogenic responses. Conversely, overexpression of Pea3 blocked the effects of TGF-β1 by targeting the BMP-7/Smad1/5/8 signaling pathway. BMP-7 knockdown in Pea3-overexpressing cells also abrogated Pea3-induced inhibition of TGF-β1. Finally, we demonstrated the interaction of RARα and Pea3 with the BMP-7 promoter.

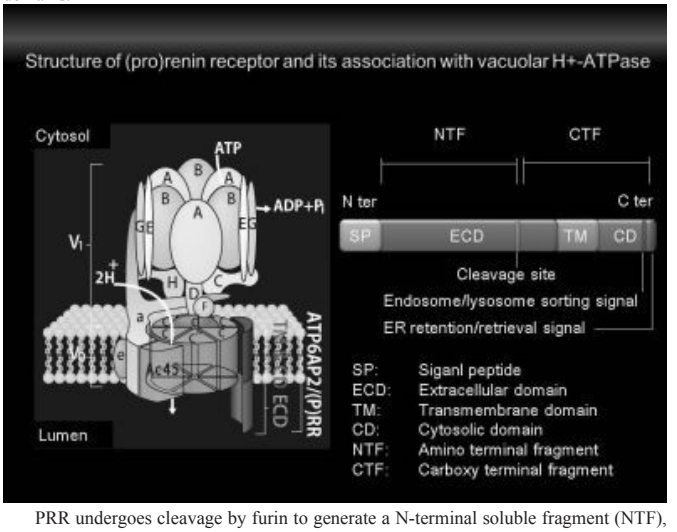
Conclusions: These results suggest that ATRA may directly inhibit TGF-β1-induced fibrogenic responses in HK-2 cells by affecting the RARα/RXRα/Pea3/BMP-7 signaling pathway, which may represent a novel therapeutic target for the treatment of renal tubulointerstitial fibrosis.

Funding: Government Support - Non-U.S.

TH-PO154

Structure of (Pro)renin Receptor and Its Association with Vacuolar H^+-ATPase Kenichiro Kinouchi,[1] Atsuhiro Ichihara,[2] Kanako Bokuda,[1] Hideaki Kurosawa,[1] Hiroshi Itoh.[1] *[1]Division of Endocrinology, Metabolism, and Nephrology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan; [2]Department of Endocrinology and Hypertension, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Podocyte specific ablation of (pro)renin receptor (PRR) results in nephrotic syndrome and renal failure due to dysfunction of vacuolar H^+-ATPase (V-ATPase) which maintains intracellular vesicular acidification. The PRR contains four different domains.



PRR undergoes cleavage by furin to generate a N-terminal soluble fragment (NTF), along with a C-terminal residue (CTF). While PRR is essential for assembly of V-ATPase, exact domains that need for V-ATPase assembly remain determined.

Methods: We performed rescue experiments, where several different constructs of PRR were overexpressed in PRR-null mouse embryonic fibroblasts (MEF). Protein expressions of V-ATPase subunits were analyzed in Western Blot analysis. Acidification of intracellular compartments was evaluated by LysoTracker staining.

Results: While PRR-null MEF demonstrated reduced expression of membrane-bound Vo subunits of V-ATPase and decreased staining of LysoTracker, full-length PRR overexpression in PRR-null MEF completely rescued expressions of V-ATPase subunits and LysoTracker staining. Extracellular domain (ECD) and transmembrane domain (TM) overexpression similarly abrogated the reduction of V-ATPase subunits expression and LysoTracker staining, while CTF overexpression failed to cancel these effects. Interestingly, mutant PRR that cannot be cleaved by furin also successfully recovered expressions of V-ATPase subunits and LysoTracker staining.

Conclusions: ECD and TM of PRR are essential for assembly of V-ATPase. Since prorenin binds to ECD of PRR, ECD is not only an important domain as a receptor, but also as an accessory protein of V-ATPase.

TH-PO155

IQGAP1 Mediates Angiotensin II-Induced Apoptosis of Podocyte via ERK1/2 MAPK Signaling Pathway Yipeng Liu,[1] Wei Liang,[1] Qian Yang,[1] Xinghua Chen,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, China; [2]Medicine, North Shore LIJ Hofstra Medical School.*

Background: Podocyte is an important component of glomerular filtration barrier and plays a pivotal role in proteinuria onset and progression of glomerular diseases. Our previous studies have demonstrated that Ang II is capable to directly promote podocyte apoptosis. However the precise mechanisms of Ang II- induced apoptosis of podocyte deserve further investigation. IQGAP1, as a scaffold protein of the mitogen activated protein kinase signaling pathway, plays a significant role in the process of apoptosis. In the present study, we evaluated the role of IQGAP1 in Ang II-induced podocyte apoptosis.

Methods: Thirty-six male Wistar rats were randomly assigned to receive either normal saline or AngII by osmotic mini-pump, or be used as normal control. The podocyte apoptosis was detected by TUNEL assay at day 14, 28 respectively. In vitro, differentiated mouse podocytes were exposed to AngII at different concentrations for 6h or at 10-8M for variable incubation times. Podocyte apoptosis was assessed by nucleus staining with Hochest-33258 and flow cytometry. The expression of IQGAP1 and phosphorylation of ERK1/2 were analyzed by western blotting. Co-immunoprecipitation was used to evaluate the interaction between ERK1/2 and IQGAP1. IQGAP1 siRNA and U0126 (inhibitor of ERK1/2) were further introduced to investigate the role of IQGAP1 in the process.

Results: Ang II promoted podocyte apoptosis in vivo and in vitro. Under control conditions, IQGAP1 was distributed linearly along the capillary loops in glomeruli, and was located in celluar membrane and cytoplasm in cultured podocyte. Exposure to Ang II stimulated the expression of IQGAP1 and elevated phosphorylation of ERK1/2 in vivo and in vitro. Furthermore, knockdown of IQGAP1 with siRNA obviously prevented Ang II-induced apoptosis of podocyte in vitro and reduced Ang II-induced phosphorylation of ERK1/2, which was accompanied with a lower interaction between ERK1/2 and IQGAP1.

Conclusions: IQGAP1, as an adaptor protein, contributes to AngII-induced apoptosis of podocyte via ERK1/2 signaling pathway.

TH-PO156

Block of Par1 Signaling Induces Podocyte Apoptosis In Vitro Mary Gomez,[1] Karen Jorge,[1] Zhongfang Du,[1] Anne Muesch,[2] David Cohen,[2] Katalin Susztak,[3] Kimberly J. Reidy.[1] *[1]Pediatrics/ Nephrology, Albert Einstein College of Medicine, Bronx, NY; [2]Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY; [3]Internal Medicine/ Nephrology, University of Pennsylvania, Philadelphia, PA.*

Background: Partioning defective (Par1) is a serine threonine kinase that interacts with the complex of Par3/Par6/aPKC to specify cell membrane domains and estabish cell polarity. It has been shown that the apical polarity protein Par3 co-localizes with nephrin at podocyte slit diaphrams and that conditional deletion of aPKC in podocytes resulted in foot process effacement, proteinuria, and focal glomerulosclerosis (FSGS) lesions in mice, suggesting there is a polarity proteins are required to maintain podocyte structure. We have previously identified altered expression of Par1 homologues Par1a and 1b in glomeruli of patients with minimal change disease and focal glomerulosclerosis. We hypothesized the Par1a/b contribute to maintenance of podocytes, and sought to examine their effect on podocytes in vitro.

Methods: Western blot was used to examine for Par1a/1b expression in immortalized murine podocytes. To examine Par1a/1b function in podocytes, differentiated murine podocytes were infected with adenoviral constructs to block Par1 function with a adenovirus expressing a GFP tagged dominant negative Par1 virus. Controls were infected with Adenovirus expressing GFP.

Results: Expression of both Par1a and 1b was identified in immortalized murine podocyes. Block of Par1 by Adeno-DNPar1 led to decreased cell number. Cell quantification at 24 hours revealed a 40% decreased of DNPar 1 cell counts vs. GFP-only infected cells. Increased cleaved caspase 3 was identified in Adeno-DNPar1 infected podocytes as compared to Adeno-GFP controls, suggesting apoptosis contributed to decreased cell survival.

Conclusions: Our results suggest Par1 signaling may contribute to podocyte cell survival.

Funding: NIDDK Support, Private Foundation Support

TH-PO157

Inhibition of a Novel p66ShcA Signaling Pathway in Podocytes Prevents Hyperglycemia-Induced Danger Signals and Apoptosis Himanshu Vashistha,[1,3] Pravin C. Singhal,[2] Ashwani Malhotra,[2] Krzysztof Reiss,[3] Leonard G. Meggs.[1,3] *[1]Nephrology, Ochsner Clinic Foundation, New Orleans, LA; [2]Nephrology, Feinstein Research Institute, New York, NY; [3]Neurological Cancer Research, SSSCC, LSUHSC, New Orleans, LA.*

Background: Podocytes are terminally differentiated epithelial cells, that are key target cells in the pathobiology of diabetic glomerulosclerosis (DG), the leading cause of ESRD. A major limitation in the development of innovative strategies to arrest or prevent DG, is the absence of podocyte regeneration, following podocyte loss or apoptosis. Here, we demonstrate cell based gene interventions, targeting the p66 longevity gene, confer a survival advantage in podocytes maintained under hyperglycemic conditions, by preventing the generation of danger signals that trigger apoptosis.

Methods: To inhibit p66, conditionally immortalized differentiated human podocytes (CIDHP) were transfected with p66shRNA or dominant negative p66 expression vector. To simulate hyperglycemia, CIDHP were maintained in serum free media at 40 mM glucose (HG) for 48 h.

Results: In contrast with CIDHP/p66shRNA at HG, empty vector (EV)-CIDHP exhibit a striking increase in apoptosis, in association with robust generation of reactive oxygen species (ROS) and increased levels of angiotensin II (ANG II) in conditioned media. Moreover, signals for p66 and ROS were increased in mitochondria, as was the % of EV-CIDHP undergoing mitochondrial depolarization and cytochrome c release, indicative of the intrinsic apoptosis program. A key role for the prolyl isomerase Pin1, in both HG-induced p66 translocation and p66 redox function in mitochondria, was identified by Pin1 siRNA experiments. Finally, we show p66 is indispensable for the generation of ANG II by podocytes maintained under hyperglycemic conditions, where HG-induced p66 redox signals, activate transcription of p53 dependent genes, including the renin substrate, angiotensinogen.

Conclusions: The activated p66 redox enzyme targets the mitochondrial compartment via Pin1, to increase ROS production and destabilize mitochondria and the nuclear compartment via p53, leading to ANG II generation and podocyte apoptosis.

Funding: NIDDK Support

TH-PO158

Upregulation of Autophagy and Autophagy-Related Proteins in Podocytes in Patients with Minimal Change Nephrotic Syndrome Ayu Ogawa, Hitoshi Sugiyama, Keiichi Takiue, Toshio Yamanari, Masashi Kitagawa, Hiroshi Morinaga, Yoko Kikumoto, Tatsuyuki Inoue, Shinji Kitamura, Yohei Maeshima, Hirofumi Makino. *Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan.*

Background: Autophagy is a cellular process for the bulk degradation of proteins and organelle turnover. The autophagy-related genes (ATG) encode various proteins that are important for the initiation and maturation in autophagosomes. Recent studies have shown the significance of autophagy of tubular epithelium in several renal tubulointerstitial disorders by using transgenic or knockout mice models. However, the role of autophagy in regulating human glomerular diseases is undetermined. This study aimed to elucidate the morphological evidence of autophagy, the expression of autophagy-related proteins, and their association with the clinical parameters in human glomerular diseases.

Methods: The total study population included 41 patients with human glomerular diseases (minimal change nephritic syndrome 12, IgA nephropathy 13, others 16), who underwent renal biopsy. The study investigated autophagy by electron microscopy and the expression of LC3, a mammalian homologue of ATG8, by immunohistochemistry. The relationship between the expression of ATG8 and the clinical parameters was also determined.

Results: The number of autophagosomes and autophagic vacuoles in glomerular cells, especially, in podocytes, was significantly correlated with the degree of proteinuria ($r = 0.35$, $p = 0.03$), and tended to increase along with the decrease in serum albumin levels. The LC3-positive cells colocalized with WT1, a podocyte marker and their number significantly increased in glomeruli in patients with minimal change nephritic syndrome in comparison to those in IgA nephropathy ($p = 0.03$).

Conclusions: The data indicated that autophagy of podocytes may be associated with proteinuria in patients with glomerular diseases. Activation of the mechanism of autophagy, as detected by LC3, may underlie the pathophysiology of minimal change nephrotic syndrome.

Funding: Government Support - Non-U.S.

TH-PO159

Deletion of Scavenger Receptor A Enhances the Autophagy-Specific Lipids Degradation in Podocytes and Renal Tubular Cells via Rho Kinase Pathway Wenjian Wang, Wei Shi, Xinling Liang, Shuangxin Liu, Jianchao Ma, Zhiming Ye. *Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guanzhou, Guangdong, China.*

Background: Autophagy has been identified as a major pathway that delivers damaged proteins, and organelles to lysosomes in order to maintain cellular homeostasis. Our previous study found that scavenger recepor A (SR-A) accelerated progressive nephropathy in high fat diet (HDF) mice by increasing the lipid vacuole formation and decreasing autophagy. However, the role and the mechanism of SR-A involved in the autophagy in the lipid-induced

nephropathy remains unclear. In this study, we hypothesized that SR-A plays a critical role in the progressive nephropathy induced by HFD via its involvement in ROCK-modulating autophagy and autophagy-specific lipid decomposition.

Methods: In vitro, signaling proteins involved in the lipid vacuolar formation and the autophagy in lipid-stimulated HK-2 cells and podocytes were analyzed in the condition of forced-expression or knock-down of SR-A-ROCK. In vivo, SR-A diffiency mice treated with fasudil were used to evaluated the the effects of SR-A/ROCK1 double-blocking on the progression of nephropathy induced by HFD.

Results: Here we demonstrated for the first time that SR-A modulated ROCK activity in oxidative low-density-lipoprotein(ox-LDL)-treated HK-2 cells and podocytes. Furthermore, our data showed that at the cellular level, SR-A-ROCK signal pathway modulateds the lipid vacuolar formation and the autophagy in lipid-stimulated HK-2 cells and podocytes via regulating the phosphorylation of dynamic protein (MLC, Arp-2 and CRMP-2), activation of PIK3/Akt signaling pathway, and the expression of autophgy molecules. Importantly, with a experimental model of lipid-induced kidney disease, we found that double block of SR-A/ROCK markedly meliorates the progression of nephropathy by inhibiting ROCK-mediated lipid vacuolar degeneration and increased autophagy-specific lipid decomposition.

Conclusions: Our data provides a new insight into the pathogenesis of the lipid-induced nephropathy. It hleps to development of novel therapeutic strategies in the prevention of progression of chronic kidney disease.

Funding: Government Support - Non-U.S.

TH-PO160

MiR-21 Upregulation Promotes Podocyte and Tubular Cell Injuries in IgA Nephropathy Hao Bao, Shaolin Shi, Changming Zhang, Yaojun Liang, Weisong Qin, Cai-hong Zeng, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing.*

Background: IgA nephropathy (IgAN) is a common cause of end-stage renal disease with a molecular pathogenesis largely unknown. We found miR-21 is one of the miRNAs that are significantly upregulated in kidney of IgAN patients. We explored the clinical significance of miR-21 upregulation and the role for miR-21 in renal cell injury.

Methods: Glomerular and tubular fractions of renal biopsies were isolated and subjected to qRT-PCR for miR-21. In situ hybridization was employed for miR-21 localization. Conditioned media from mesangial cells stimulated with IgA of IgAN patients and controls were used to treat immortalized human podocyte and HK2 tubular cells. qRT-PCR and western blot were used to for gene expression quantification.

Results: Twenty patients and ten normal controls were enrolled, and miR-21 level was found to be higher in both glomeruli and tubuli of IgAN patients than that of controls, and positively correlated with the levels of urinary protein and serum creatinine, as well as the degrees of glomerulosclerosis and tubulointerstitial fibrosis. Enhanced expression of miR-21 occurred mainly in podocytes and tubular cells. IgAN conditioned media (but not the healthy control) upregulated miR-21 and downregulated nephrin, CD2AP and synaptopodin of the podocytes. IgAN conditioned media also upregulated miR-21 in HK2 cells, accompanied with upregulation of collagen I and fibronectin, and reduced expression of E-cadherin. Overexpression of miR-21 in podocytes and HK2 cells exacerbated the damage of the cells in the treatment of IgAN conditioned media as shown by loss of epithelial morphology, decreased expression of podocyte specific proteins and fibrogenic activation of HK2 cells. While inhibition of miR-21 prevented the decrease of nephrin, CD2AP and synaptopodin in podocytes, attenuated the synthesis of collagen I and fibronectin and preserved E-cadherin in HK2 cells.

Conclusions: miR-21 upregulation may promote both podocyte and tubular cell injuries in IgAN. Inhibition of miR-21 may represent a therapeutic approach for IgAN.

Funding: Government Support - Non-U.S.

TH-PO161

Identification and Characterization of a Novel HIF-1 Target that Regulates Cytokinesis as a Defensive Mechanism against Polyploidy Kumi Shoji,[1] Imari Mimura,[1] Takashi Murayama,[2] Takehiko Wada,[1] Tetsuhiro Tanaka,[1] Takamoto Ohse,[1] Reiko Inagi,[1] Hiroyuki Aburatani,[3] Tatsuhiko Kodama,[3] Masaomi Nangaku.[1] *[1]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan; [2]Department of Pharmacology, Juntendo University School of Medicine, Tokyo, Japan; [3]Research Center for Advanced Science and Technology, University of Tokyo, Tokyo, Japan.*

Background: Hypoxia plays a crucial role in many physiological and pathophysiological phenomena such as chronic kidney disease, acute kidney injury and cancer biology. Hypoxia inducible factor-1 (HIF-1) is a key transcription factor that regulates adaptive responses against hypoxia.

Methods: We performed genome-wide analysis of HIF-1 targets utilizing microarray analysis in combination with chromatin immunoprecipitation with deep sequencing. We focused one of the new targets, sperm associated antigen 4 (SPAG4). We performed cell biological studies utilizing live cell imaging, flow cytometry, immunocytochemistry, siRNA technique and deletion mutants to investigate SPAG4 function.

Results: We identified 37 new targets of HIF-1, and SPAG4 was selected for a further analysis, of which function remains unknown. We demonstrated that SPAG4 was up-regulated under hypoxia via the hypoxia response element motif in its enhancer. We generated stable transfectants of HeLa cells expressing SPAG4-EGFP, and live cell imaging showed that SPAG4 was localized in nuclear envelop and ER in interphase, and also at the intercellular bridge during telophase. We also examined the localization of truncated forms of SPAG4. The localization suggested SPAG4 involvement in cytokinesis. Flow cytometric analysis showed that hypoxia increased the number of tetraploid cells, and furthermore, knockdown of SPAG4 by siRNA increased the number of tetraploid cells. Microarray analysis using SPAG4 siRNA confirmed the significant involvement of SPAG4-regulated genes in cytokinesis.

Conclusions: In conclusion, we identified SPAG4 as a novel HIF-1 target that plays a crucial role in cytokinesis. It is likely that SPAG4 serves a defensive role against hypoxia-induced polyploidy via regulation of cytokinesis.

Funding: Government Support - Non-U.S.

TH-PO162

New Autophagy Reporter Mice Reveal a Dynamic Autophagy Process in Post-Ischemic Renal Tubules Ling Li,[1] Megan G. Carmody,[1] Zhao Wang,[2] Joseph A. Hill,[2] Fangming Lin.[1] *[1]Pediatrics, Columbia University College of Physicians and Surgeons, New York, NY; [2]Internal Medicine (Cardiology), University of Texas Southwestern Medical Center, Dallas, TX.*

Background: Autophagy is induced in post-ischemic kidneys.

Methods: To study autophagy flux and examine the cell fate of autophagic cells, we used our new CAG-RFP-GFP-LC3 mice, in which RFP and a pH-sensitive GFP were expressed ubiquitously in cells undergoing autophagy. The quench of GFP but persistence of RFP signals in low pH after fusion of autophagosome with lysosome would allow visualization of newly formed autophagic vesicles and identification of autophagic cells.

Results: In control kidneys, no GFP and few RFP signals were detected in the nephron. At 1 day post ischemia-reperfusion injury (IRI), 21% of proximal tubular cells showed both GFP and RFP puncta. However, at 3 days, GFP puncta were detected in 0.9% while RFP puncta were visualized in 18% of proximal tubular cells, suggesting little new autophagosome formation and/or increased fusion of autophagosome with lysosome. Injection with hydroxychloroquine to raise the lysosomal pH led to an increase in GFP puncta that were co-localized with a lysosomal marker LAMP-1, supporting the pH sensitivity of GFP. At 14 days, fluorescent signals in the kidneys were similar to that of controls and were in an agreement with LC3II levels. Furthermore, the presence of RFP correlated with the expression of Atg5, validating the mice as autophagy reporters. Our results also suggest that the persistence of RFP could be used to track cells that had recent autophagy. In proximal tubules, Ki67 expression was significantly lower in RFP^+ cells compared to RFP^- cells (13% vs. 38%, $p<0.05$) at 3 days post IRI, suggesting reduced proliferation in autophagic cells. To understand molecular events that lead to proliferation, we examined mTOR pathways and found increased levels of p-Akt, p-S6 and p-4E-BP1 proteins. Immunostaining showed increased p-S6 expression in proliferating RFP^+ cells.

Conclusions: Our studies indicate that autophagy may render cell cycle arrest and renal repair is accompanied by mTOR activation and resolution of autophagy.

Funding: NIDDK Support

TH-PO163

Autophagy Protects Kidney from Metabolic Stress through the Maintenance of Metabolism in Mitochondria Tomonori Kimura,[1] Yoshitsugu Takabatake,[1] Atsushi Takahashi,[1] Tomoko Namba,[1] Takeshi Yamamoto,[1] Jun-Ya Kaimori,[2] Isao Matsui,[1] Harumi Kitamura,[1] Fumio Niimura,[3] Taiji Matsusaka,[4] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Pediatrics, Tokai University, Isehara, Kanagawa, Japan; [4]Institute of Medical Science, Tokai University, Isehara, Kanagawa, Japan.*

Background: Mitochondria are master of energy metabolism, and generate ATP through TCA cycle. Damaged mitochondria are linked to pathogenesis. On the other hand, autophagy degrades damaged mitochondria (this process is specifically called 'Mitophagy'), which involves the sequestrationof mitochondria in a double-membraned autophagosome, followed by fusion with lysosome. We aimed to analyze the role of autophagy in the quality control of mitochondria from the metabolic points of view.

Methods: Metabolic stress was induced in proximal tubule-specific autophagy-deficient mice by treating with cyclosporine A, and histological changes and biological profiles were examined. We also constructed cell lines of immortalized autophagy-deficient proximal tubular cells and their revertants, and examined the effect of autophagy against metabolic stress.

Results: Autophagy-deficiency exaggerates cyclosporine A-induced kidney injury with significant increase in apoptotic cells. Metabolome analysis demonstrated that cyclosporine A induced metabolic stress with abnormal metabolism of TCA cycle in proximal tubular cells, and this stress is further enhanced in autophagy-deficient proximal tubular cells when compared with autophagy-retrieved control cells.

Conclusions: These observations proved our hypothesis that autophagy protects kidney tubular cells against metabolic stress through the maintenance of TCA cycle in mitochondria. Further understanding of autophagy will provide a novel therapeutic approach for kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO164

Albumin Endocytosis Leads to Dysfunctional Autophagy Raymond Yan, Jonathan M. Gall, Steven C. Borkan, John H. Schwartz, Andrea Havasi. *Renal Department, Boston University Medical Center, Boston, MA.*

Background: Proteinuria is a risk factor for chronic kidney disease progression. In addition, exposure of proximal tubular epithelial (PTEC) cells to excess albumin promotes tubular atrophy and fibrosis, key predictors of progressive organ dysfunction. We hypothesize that exposing PTEC to albumin inhibits autophagy, a function responsible for effective turnover of cellular macromolecules and organelles, including dysfunctional mitochondria.

Methods: Autophagy was measured both in suspension of intact tubules and cultured mouse tubular cells exposed to albumin at baseline and during starvation, an autophagy stimulus. To assess autophagy, steady state LC3-II, an autophagy marker was quantified by immunoblot and autophagosomes (AP) were visualized in cells that expressed an LC3-GFP fusion protein. Autophagic flux (accumulation of LC3-II) was measured in the presence of bafilomycin, an H^+-ATPase inhibitor that prevents lysosomal LC3-II degradation.

Results: We show that albumin overload, designed to mimic nephrotic glomerular filtrate, is associated with autophagic and lysosomal dysfunction. Exposure to either recombinant human albumin or fatty acid free bovine albumin decreased LC3-I to LC3-II conversion in a concentration-dependent manner. In the presence of bafilomycin, increased albumin exposure caused a progressive decrease in autophagic flux. Albumin treatment decreased the number of GFP positive AP both in normal media (*basal* autophagy) as well as in starved cells (*induced* autophagy). During 48hr exposure to albumin, large GFP positive structures were observed. In contrast, prolonged albumin exposure (3-5 days) markedly reduced the number of APs. Albumin exposure increased lysosomal pH and decreased lysosomal enzyme activity in PTEC.

Conclusions: These data show that at shorter time points albumin inhibits fusion between AP and lysosomes without impairing the maturation of small autophagosomes into larger structures. Prolonged albumin exposure blocks AP formation and causes lysosomal dysfunction. This inhibition of autophagy and lysosomal function might be a major contributor to renal epithelial cell toxicity caused by albumin overload.

Funding: NIDDK Support, Private Foundation Support

TH-PO165

Autophagy Protects Kidney from Diseases through the Reduction of Mitochondrial Reactive Oxygen Species Yoshitsugu Takabatake,[1] Tomonori Kimura,[1] Atsushi Takahashi,[1] Tomoko Namba,[1] Takeshi Yamamoto,[1] Jun-Ya Kaimori,[2] Isao Matsui,[1] Harumi Kitamura,[1] Fumio Niimura,[3] Taiji Matsusaka,[4] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Department of Pediatrics, Tokai University School of Medicine, Isehara, Kanagawa, Japan; [4]Institute of Medical Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: Mitochondria can produce intracellular reactive oxygen species (ROS), which sometimes lead to an oxidative damage. Thus, damaged mitochondria are supposed to be degraded by autophagy (or specifically called 'Mitophagy'), which involves the sequestrationof cytoplasmic contents in a double-membraned autophagosome, followed by fusion with lysosome. We aimed to analyze the role of autophagy in the clearance of damaged mitochondria using mitochondria-related kidney disease models.

Methods: Proximal tubule-specific autophagy-deficient mice were treated with mitochondria-damaging drugs (cisplatin and cyclosporine A), and histological changes and biological profiles were examined. We also constructed cell lines of immortalized autophagy-deficient proximal tubular cells and their revertants, and examined the effect of autophagy against damaged mitochondria by assessing mitochondrial ROS.

Results: Autophagy-deficiency exaggerates cisplatin-induced kidney injury with significant increase in apoptosis and DNA damage. This damage is associated with increase in mitochondria ROS in immortalized autophagy-deficient proximal tubular cells when compared with autophagy-retrieved control cells. Cyclosporine A treatment also increased ROS-producing damaged mitochondria in proximal tubule-specific autophagy-deficient mice, and this damage is associated with increase in mitochondria ballooning *in vivo*.

Conclusions: These observations proved our hypothesis that autophagy protects kidney tubular cells from kidney diseases through quality control of mitochondria. Further understanding of autophagy may provide a novel therapeutic approach for kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO166

Autophagy Upregulation Reduces Progressive Renal Fibrosis during Unilateral Ureteral Obstruction Cheng Yang,[1] Alexandrea Holmes,[2] Christian Herzog,[1] Sue Theus,[1,2] Judit Megyesi,[1,2] Gur P. Kaushal.[1,2] *[1]Internal Medicine/Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR; [2]Internal Medicine/Nephrology, Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Autophagy is an evolutionarily-conserved and dynamic process of degradation of intracellular organelles and long-lived proteins by lysosomes. The specific role of autophagy in the development of progressive renal fibrosis during chronic kidney disease (CKD) is poorly understood. We used both genetic and pharmacologic approaches to upregulate autophagy and examined the effect of autophagy in accumulation of fibrosis in a unilateral ureteral obstruction (UUO) model.

Methods: We produced proximal tubule-specific beclin-1 transgenic (beclin-I Tg) mice under control of the androgen-inducible KAP2 (kidney androgen-regulated protein) promoter and obtained overexpression of beclin-1 in the proximal tubules.

Results: In wild-type mice, during the progression of UUO injury at 1d, 3d, 5d, 7d, and 9d, a significant increase in the conversion of LC3-I to LC3-II and expression of beclin-1, Atg5, Atg7, and Atg12 as well as production of matrix proteins including collagen type-1, fibronectin, and α-SMA was observed. In beclin-1 Tg mice, there was a corresponding marked reduction in matrix accumulation in the UUO model, indicating beclin-1 overexpression promoted a reduction in matrix proteins. The autophagic-enhancing drug Torin 1, a selective and better inducer of autophagy than rapamycin, was quite effective in reducing renal fibrosis. Inhibition of autophagic flux by chloroquine was unable to reduce UUO-induced accumulation of matrix proteins. Moreover, we observed that autophagic substrates p62 and LC3-II were accumulated during the progression of UUO injury, indicating impaired autophagic flux.

Conclusions: In conclusion, our studies demonstrated that overexpression of the key autophagic protein beclin-1 in the proximal tubule and upregulation of the autophagic pathway markedly reduced renal fibrosis in the UUO model. In contrast, inhibition of the autophagic pathway enhanced matrix protein accumulation. These findings suggest that autophagy is an important therapeutic target in CKD.

Funding: NIDDK Support, Veterans Administration Support

TH-PO167

Gene Targeting in Renal Collecting Duct Cells Reveals mVPS34 as a Key Determinant of Nephron Number Bo Hu, Jianchun Chen, Jian-Kang Chen. *Nephrology/Medicine, Vanderbilt University, Nashville, TN.*

Background: Nephron number plays an important role in regulation of blood pressure and kidney function. mVPS34 (the mammalian homologue of yeast vacuolar protein sorting defective 34) is indispensable in glomerular podocytes, but its role in renal collecting duct system has not previously been reported.

Methods: We generated an mVPS34-floxed mouse ($mVPS34^{flox/flox}$) and crossed with Hoxb7-Cre mice to produce renal collecting duct cell-specific mVPS34 knockout mice ($mVPS34^{cdKO}$) and compared them with their $mVPS34^{flox/flox}$;Hoxb7-$Cre^{(-)}$ wild type littermates (*WT*).

Results: $mVPS34^{cdKO}$ mice exhibited decreased kidney-to-body weight ratios (0.39±0.17% vs. *WT*: 0.66±0.10%, N=4; P<0.001), without difference in body weight or glomerular size at birth. By 4 weeks of age when nephrogenesis is complete, $mVPS34^{cdKO}$ mice had a strikingly lower number of glomeruli: 24±2 vs. *WT*: 53±5, N=6, P<0.0001 (the totalnumber of glomeruli in 5 randomly chosen fields/kidney section), with drastically thinner renal medulla, and developed glomerular and tubular hypertrophy, proteinuria (Alb/Cr in μg/mg: $mVPS34^{cdKO}$: 278.20±67.27 vs. *WT*: 20.49±2.36, N=6, P<0.01), and elevated BUN (mg/dl: 53.33±8.43 vs. *WT*: 23.75±7.60, N=6; P<0.001). Dilated renal tubules and protein casts were also seen. Immunofluorescence staining with nephron segment-specific markers indicated that tubular enlargement and dilation occurred in the proximal tubule, thick ascending limb, distal tubule as well as collecting duct. Masson's trichrome staining revealed glomerulosclerosis and interstitial fibrosis. Immunohistochemistry confirmed increases in fibronectin and fibroblast-specific protein-1 (FSP1). TUNEL-positive tubular epithelial cells were markedly increased in the collecting ducts, suggesting a potential mechanism underlying the phenotype of low nephron number, defective renal medulla, dilated upstream renal tubules and the subsequent glomerulosclerosis and tubulointerstitial fibrosis, leading to impaired renal function.

Conclusions: This study indicates an essential role for mVPS34 in dictating nephron number and maintaining normal renal structure and function through a mechanism of antagonizing programmed cell death.

Funding: NIDDK Support

TH-PO168

Bif-1 Interacts with PHB2 to Regulate OPA-1 Processing and Mitochondrial Fragmentation during Apoptosis Sunggyu Cho, Zheng Dong. *Department of Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center.*

Background: Mitochondria are highly dynamic, constantly undergoing fission and fusion. During cell stress, mitochondrial dynamics is shifted to fission, resulting in mitochondrial fragmentation that contributes to mitochondrial membrane permeabilization and subsequent cell death. Mitochondrial fragmentation involves the cleavage of both outer and inner membranes. While the outer membrane cleavage involves Drp-1, Fis-1 and mitofusins, cleavage of the inner membrane may involve the regulation of OPA-1. However, the regulation of OPA-1 during mitochondrial fragmentation and apoptotic cell death is largely unknown.

Methods: Here we show that Bif-1 regulates OPA-1 processing via the binding of PHB2 at mitochondria.

Results: Bif-1, originally identified as a Bax-interacting protein, translocated to mitochondria during apoptosis. By yeast-two hybrid and immunoprecipitation pull-down assays, we detected the interaction of Bif-1 with PHB2, a mitochondrial protein implicated in OPA-1 regulation. Functionally, Bif-1-deficient cells were resistant to mitochondrial fragmentation, release of cytochrome c and apoptosis. Interestingly, Bif-1 deficiency did not affect Bax translocation to mitochondria, but inhibited Bax insertion and oligomerization in mitochondrial membrane. Moreover, the proteolytic processing of OPA1 was prevented in Bif-1-deficient cells.

Conclusions: It is suggested that upon cell stress, Bif-1 translocates to mitochondria to interact with PHB2 to induce OPA-1 processing, resulting in inner membrane cleavage, mitochondrial fragmentation, sensitization to Bax insertion and apoptosis.

Funding: NIDDK Support, Veterans Administration Support

TH-PO169

Mechanical Deformation Induces Apoptotic Induction in Response to Na^+/H^+ Exchanger NHE1 Isoform Downregulation Following ERK Activation and p38 Inhibition Victoria Bocanegra,[1] Andrea Gil Lorenzo,[1] Patricia G. Vallés.[2] *[1]CONICET; [2]Notti Pediatric Hospital-School of Medicine.*

Background: Mechanical deformation following congenital ureteral obstruction is traduced into biochemical signals that represent a key step in the understanding of the mechanisms that leads to the tubular atrophy during obstructive nephropathy.The study included the analysis of Na^+/H^+ isoform 1 exchanger, NHE1, on apoptosis regulation and the signaling pathways associated with NHE1 regulation.

Methods: For cell mechanical deformation,HK-2 (Human Kidney-2) cells were subjected to mechanical stretch which included 15, 60, 90 and 180 minutes of cyclic exposure. Apoptosis was examined with flow cytometry (Annexin V) and protein expression with western blot that included Caspase 3,Bcl2,NHE1,RhoA,ERK 1/2 and p38.NHE1 expression inhibition was assessed with NHE1-siRNA.

Results: when mechanical stretch was applied to HK-2 cells, we observed an apoptosis induction in a time dependent manner with an increase in Annexin V^+ cells, a decrease in Bcl2 and an increase in caspase 3 expression.Apoptotic response to mechanical stretch was associated with a progressive decrease of NHE1.NHE1 expression inhibition with siRNA lead to a subsequent decreased Bcl2 expression and increased Caspase 3 activation. HK-2 cells subjected to mechanical stretch showed progressive RhoA activation,with early activation of ERK 1/2 associated to an opposite p38 expression effect.Treatment with specific MAPKinasas signaling pathway inhibitors increased NHE1 expression by inhibiting p38 pathway with absence of caspase 3 expression,whereas persistent lower NHE1 protein levels and increased Caspase 3 expression were shown after ERK 1/2 inhibition.

Conclusions: Renal tubular apoptosis following mechanical deformation is associated with decreased NHE1 protein expression. Different signaling pathways participate in NHE1 regulation including both RhoA GTPase activation and the opposing actions of the ERK and -p38 MAP kinase signaling pathways as events involved in tubular cell apoptosis induction in response to mechanical injury.

TH-PO170

Metformin Inhibits mTOR Pathway Independent of AMPK in HIV-Infected Tubular Cells Partab Rai, Tejinder Singh, Gautam Kishore Valecha, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Patients with HIV infection are living an almost normal life and are increasingly prone to develop obesity and diabetes. Mammalian target of rapamycin (mTOR) pathway has been reported to play an important role in the development of renal lesions in patients with diabetes as well HIV infection. Since metformin is known to alter glucose metabolism as well as known to inhibit mTOR pathway, we hypothesized that metfomin may be an ideal therapeutic agent in HIVAN patients with diabetes mellitus (DM). In the present study, we evaluated the effect of metformin on HIV-induced activation of mTOR pathway in mouse tubular cells.

Methods: Mouse proximal tubular epithelial cells (MPTECs) were transduced with either gag/pol-deleted NL4-3 (HIV/MPTEC) or empty vector (EV/MPTEC). EV/MPTEC and HIV/MPTECs were incubated in media for 48 hours. Immunoblots were probed for phospho-AMPK, phospho-mTOR, S70S6 kinase, phospho-eEF2, eIF4B, and 4EBP-1. To evaluate the effect of metformin on AMPK and mTOR pathway, EV/MPTECs and HIV/MPTECs were incubated in media containing either buffer or metformin (0.5 mM) for 24 hours. Subsequently, cellular lysates were immunoelectrophoresed and probed for phospho-AMPK, phospho-mTOR, S70S6 kinase, phosphor-eEF2, eIF4B, and 4EBP-1. The same blots were stripped and reprobed for actin.

Results: Analysis of mTOR revealed two-fold increase in phospho-mTOR expression in HIV/MPTECs when compared to vector/MPTECs. Further downstream analysis of mTOR pathway revealed enhanced (2.5-fold) phosphorylation of p70S6 kinase and associated diminished phosphorylation of eEF2 in HIV/MPTECs; moreover, HIV/MPTECs displayed enhanced (two-fold) phosphorylation of eIF4B and 4EBP-1. However, HIV/MPTEC did not show any alteration in phospho-AMPK expression. Metformin inhibited HIV-induced mTOR phosphorylation and associated down stream signaling. However, metforimin did not alter phosphorylaion of AMPK in HIV/MPTECs.

Conclusions: Metformin attenuates HIV-induced activation of mTOR pathway independent of AMPK. These observations provide a basis to confirm these findings in experimental animal and human studies.

Funding: NIDDK Support

TH-PO171

Epithelial-Mesenchymal Transition of Aging Kidney Were Alleviated by Short-Term Caloric Restriction and Caloric Restriction Mimetics via AMPK/mTOR Signaling Guangyan Cai, Dan Dong, Yichun Ning, Yang Lu, Quan Hong, Shaoyuan Cui, Bo Fu, Xiang-Mei Chen. *Department of Nephrology, State Key Laboratory of Kidney Diseases, Chinese PLA General Hospital, Beijing, China.*

Background: Epithelial-mesenchymal transition (EMT) in aging kidney plays an import role during renal fibrosis, which contributes to declining renal function in the elderly. This study is to investigate whether 8-week short-term caloric restriction (CR) and caloric restriction mimetics (CRMs) alleviates EMT process during kidney aging.

Methods: 25-month-old male Sprague-Dawley rats were divided as control group (fed ad libitum, OAL), AL group (40% food intake restriction, OCR) and metformin treatment group (metformin 300mg/kg/d, OMET). 3-month-old rats were fed ad libitum as young age control (YAL). High glucose (HG) was used to induce premature senescence and EMT in human primary proximal tubular cells (PTC). The effects of CRMs, including resverotrol or metformin, were explored. siRNA technology was used to deplete AMPK to test the role of AMPK-mTOR signaling. Senescence-associated markers were analyzed. P16, P21, E-cadherin, alpha-SMA, ZEB1, AMPK, P-AMPK, mTOR and P-mTOR were detected by western blot or immunofluorescence.

Results: Expression of E-cadherin was decreased, while expression of alpha-SMA and ZEB1 were increased significantly in old kidneys. After treatment for 8 weeks, age related EMT in old kidney was alleviated in aged rats which were subjected to OCR and OMET. Alleviation of EMT in old kidney was associated with up regulation of AMPK and down regulation of mTOR. In vitro study demonstrated high glucose induced both premature senescence and EMT in cultured PTC. CRMs including both resverotrol and metformin decreased the EMT process accompanying with activation of AMPK-mTOR signaling. Silenced AMPK was associated with mTOR over-expression and promoted EMT of senescent PTC. However, protective effect of CRMs on EMT in senescent PTC diminished significantly when AMPK was silenced.

Conclusions: Short-term CR and CRMs alleviated age-related EMT of PTC via AMPK-mTOR signaling, which serves as potential interventions for protecion of renal fibrosis during kidney aging.

TH-PO172

Mannan-Binding Lectin Mediates Mitochondrial Injury, Endoplasmic Reticulum Stress, and Autophagy in Tubular Epithelial Cells Pieter van der Pol,[1] Nicole Schlagwein,[1] Danielle Van Gijlswijk,[1] Hetty C. de Boer,[1] Ingeborg M. Bajema,[2] Cees van Kooten.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Ischemia/reperfusion (I/R) injury is a key event in kidney transplantation. Recently, we demonstrated a crucial role for Mannan-binding lectin (MBL), the initiator of the lectin pathway of complement, in the pathophysiology of renal I/R (*Van der Pol et al. AJT 2012*). In the present study we explored the underlying mechanism of this MBL-mediated tubular injury.

Methods: *In vivo*, rats were subjected to 45 min of unilateral renal ischemia. After 2, 5 or 24h of reperfusion, renal tissue was collected and analyzed for histology, deposition of MBL and expression of ER-stress genes sXBP-1 and CHOP. *In vitro*, human tubular epithelial cells (TEC) were incubated with purified human MBL for 24h and analyzed for sXBP-1 and CHOP expression and conversion of LC-II using Western blot.

Results: Exposure of human TEC to purified MBL *in vitro* resulted in binding and internalization of MBL followed by epithelial cell death. Internalized MBL partially co-localized with mitochondria and affected the mitochondrial membrane potential. Moreover, co-localization of MBL and GRP78, a molecular chaperone involved in the unfolded protein response following endoplasmic reticulum (ER) stress was observed. Therefore, sXBP-1 and CHOP, markers for ER-stress were assessed, demonstrating a twenty- and tenfold induction within two hours of MBL exposure. ER stress is known to induce autophagy, an intracellular self-degradation system, characterized by conversion of LC3-II from LC3-I. Using Western blot, we demonstrated an extensive LC-II conversion within six hours of MBL exposure.

Assessment of rat sXBP-1 and CHOP following I/R *in vivo* revealed an induction of ER-stress within 2 hours following reperfusion, which was accompanied by intra-epithelial presence of MBL, formation of autophagic membrane structures followed by tubular cell death within 24 hours following reperfusion.

Conclusions: These findings demonstrated that MBL affects mitochondrial homeostasis in tubular cells, induces ER-stress and autophagy leading to tubular injury.

TH-PO173

Vitamin D Receptor Plays a Critical Role in p53-Induced Tubular Cell Epithelial Mesenchymal Transition Gautam Kishore Valecha, Nirupama Chandel, Tejinder Singh, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Both p53 and vitamin D receptor (VDR) play important roles in cellular proliferation and apoptosis. We hypothesize that p53 may be modulating tubular cellular epithelial mesenchymal transition (EMT) by modulating VDR expression, renin angiotensin system (RAS) and generation of reactive oxygen species (ROS).

Methods: Human renal proximal tubular cells (HRPTC) were treated with normal glucose (5 mM, NG) or high glucose (30 mM, HG) for 48 hours. Similarly, HRPTC were

transduced with empty vector (EV/HRPTC) or HIV (NL4-3, HIV/HRPTC) constructs. In parallel experiments, HRPTCs were silenced for p53 and VDR by transfecting with respective siRNAs and scrambled (Scr) siRNA. Subsequently, immunoblots were probed for VDR, phospho-p53, markers of epithelial mesenchymal transition (Snail, proliferating nuclear antigen [PCNA], α-SMA, vimentin, fibroblsast specific factor [FSP]-1), and renin. The same blots were reprobed for actin. To confirm the role of p53 and VDR in cross talk, stable colonies of HRPTCs over-expressing p53 and activated VDR were evaluated for above mentioned molecular markers. Tubular cell ROS generation was determined in all the above mentioned conditions.

Results: Both HG/HRPTC and HIV/HRPTC demonstrated enhanced expression of phospho-p53 and down regulation of VDR. Silencing of p53 stimulated tubular cell VDR expression under basal as well as HG/HIV stimulated states. On the other hand, silencing of VDR enhanced expression of phospho-p53 both under basal and HG/HIV stimulated states. HRPTC silenced for p53 displayed normal renin expression but diminished ROS generation; whereas, HRPTC silenced for VDR displayed enhanced renin expression as well as ROS generation. Nonetheless, HRPTC silenced for either p53 or VDR displayed enhanced expression of EMT markers.

Conclusions: These findings suggest that down regulation of p53 and upregulation of VDR contribute to down regulation of ROS generation and enhanced expression of EMT markers; whereas, down regulation of VDR enhanced ROS generation and might have initiated EMT as compensatory feed back to ROS-induced tubular cell injury.

Funding: NIDDK Support

TH-PO174

Nek1 Regulates G1: S Transition and DNA Replication in Cell Cycle Mallikarjun Patil, Zheng Dong. *Department of Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center.*

Background: Never in Mitosis Gene A (NIMA) was originally identified in *A Nidulans*. Fungal NIMA regulates G2M phase progression, spindle organization and cytokinesis in cell division. In mammals, there is a family of NIMA-related kinases (NEK1 to Nek11). While several Neks (Nek6, Nek7 and Nek9) regulate mitosis in mammalian cells; Nek1 has been suggested to regulate DNA damage response, centrosome duplication and primary cilium formation. The role of Nek1 in cell cycle regulation is unknown. Nek1 mutant mice develop polycystic kidney disease (PKD) similar to human autosomal dominant PKD, although the underlying molecular mechanism is unclear.

Methods: Here we report that Nek1 is required in G1 phase to S phase transition and DNA replication in cell cycle.

Results: Knockdown of Nek 1 inhibited cell proliferation. Cell cycle analysis revealed more Nek1 knock down cells in S phase. Nek1 knock down cells failed to progress into the S and G2M phase following cell cycle synchronization with thymidine block and release. Renal proximal epithelial cells derived from Kat2J (Nek1 mutant) mice also showed defective S phase. Moreover, chromatin localization of DNA replication factors was compromised in these cells resulting in replication stress. Interestingly, in Nek1 knock down cells the centrosome was organized but failed to duplicate.

Conclusions: These results reveal a new role of Nek1 in G1-S transition and DNA replication in cell cycle.

Funding: NIDDK Support, Veterans Administration Support

TH-PO175

Atg5-Dependent Autophagy Activation Promotes Aristolochic Acid-Induced Renal Apotposis Ching-chin Yang,[1] Cheng-tien Wu,[1] Chih-Kang Chiang,[1,2] Shing-Hwa Liu.[1] *[1]Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan; [2]Department of Internal Medicine, University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan.*

Background: Autophagy involves a bulk degradation pathway to maintain cellular homeostasis, and is highly conserved during the evolution. Ingestion of aristolochic acid (AA) causes AA nephropathy by inducing apoptosis. AA-induced aristolactam-DNA adducts lead to tubular epithelial cells death via activation of cell cycle arrest. However, the roles of autophagy in the acute phase of AA nephropathy are still unclear. Here we provided evidences that Atg5-dependent autophagy activation is involved in the AA nephropathy both *in vivo* and *in vitro*.

Methods: Male Wistart rats were subcutaneously injected with aristolochic sodium (AANa, 10 mg/kg body weight) or with the solvent (H_2O) for 7 days to ensure the induction of AANa-induced kidney injury. Normal rat kidney epithelial cells (NRK-52E) were pretreated with AANa, and then evaluate the effect of autophagy in AANa-induced tubular apoptosis by western blotting and immunofluorescence (IF).

Results: Renal cell autophagy was proven by the induction of Atg5 and LC3-II expression in the AANa-treated kidney. Punctuated LC3-GFP dots were detected by IF staining, and autophagosome formation was demonstrated by transmission electron. Treatment of AANa (100uM) suppressed the viability of NRK52E. It accompanied by the cleavage of PARP and the condensation and fragmentation of nuclei. In addition, AANa also induced the Atg5 and LC3-II expression, and punctuated LC3-GFP dots IF staining in the NRK52E cells. Pretreated 3-methyl adenine (3-MA), an autophagy inhibitor, attenuated AANa-induced apoptosis as shown by lower expression levels of cleavaged PARP, fewer condensation of nuclei, and lesser negative acridine orange/ethidium bromide staining cells. Furthermore, knockdown of Atg5 by using the short hairpin RNA attenuated the expression LC3-II and PARP cleavage in the NRK52E cells.

Conclusions: In conclusion, we demonstrated autophagy involved in the acute phase of AAN *in vivo*, and Atg5-dependent autophagy activation prompted renal tubular apoptosis.

Funding: Government Support - Non-U.S.

TH-PO176

A Novel In-Vivo Model Displaying Accelerated Senescence in the Mutant AS/AGU Rat Kidney Marc Gingell-littlejohn, Paul G. Shiels, Marc J. Clancy. *University of Glasgow.*

Background: The mutant rat sub-strain (AS/AGU) arose spontaneously as a result of a specific single gene mutation (PKCγ) in a colony of Albino Swiss (AS) rats. PKCγ is a member of an important family of cell signalling molecules with a wide range of functions in various cell types. This knowledge enhances the importance of this strain, as it provides a defined molecular change from which all subsequent physiological and pathological changes derive. The strain was demonstrated to display accelerated bio-ageing in the kidney (Wright et al: unpublished data) and we have subsequently performed glomerular filtration (GFR) and biochemical studies to phenotypically characterise this model.

Methods: 0.2% w/v FITC-Inulin was used for GFR experiments by constant infusion under general anaesthesia. Measurements of fluorescence in the urine of each kidney and plasma at equilibrium provided quantitative data on the filtration process across the glomerulus, which was then calculated according to the equation: *GFR = Urine FITC fluorescence x urine flow rate / Plasma FITC fluorescence* and standardised to 100 grams body weight. Biochemical analysis was performed on separated plasma and urinary samples.

Results: A significant difference was observed in both sodium and urea concentrations between the strains (n=61), with mutant AS/AGU having higher mean urea concentrations (8.67mmol/L vs 7.23mmol/L, p=0.009) and lower mean sodium concentrations (144.7mmol/L vs 146.9mmol/L, P=0.023). There was a proportional increase in GFR with increasing weight of the animal (n=24, p=<0.001) and a significant difference in GFR/100gr body weight between AS and AS/AGU rats in female rats (n=11, p=0.028). The GFR difference between AS and AS/AGU in the total experimental cohort approached significance (n=24, p=0.065).

Conclusions: It is postulated that the PKCγ mutation impairs the sodium-urea counter transporter in the rat inner-medullary collecting duct and in conjunction with the difference in GFR between both strains, confirms the premature senescent genotype of the mutant AS/AGU strain. This strain is a unique and useful model of human diseases of ageing and organ dysfunction in particular, for renal dysfunction and transplant related pathologies.

Funding: Government Support - Non-U.S.

TH-PO177

microRNA:21:dependent Positive Feedback Loop Involving NFkappaB (NFkB) Regulates Renal Cancer Cell Proliferation Amit Bera,[1] Nirmalya Dey,[1] Falguni Das,[1] Balakuntalam S. Kasinath,[1] Hanna E. Abboud,[1] Nandini Ghosh-choudhury,[2] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

Background: Renal cell carcinoma is often resistant to chemotherapy due to upregulation of kinase cascades that promote proliferation and survival of tumor cells. MicroRNAs (miRs) are well suited for regulation of tumor growth because of their capacity to repress mRNA translation. miR-21 is upregulated in renal cancer. Neither the underlying mechanism nor the identification of its downstream targets that force proliferation are known.

Methods: ACHN human renal carcinoma cells were used.

Results: Here we report that the transcription factor NFkB increases the expression of miR-21 in ACHN renal cancer cells (RCCs). Expression of phosphorylation-deficient mutant of p65 NFkB subunit (p65 S536A) inhibited transcription of miR-21. Expression of miR-21 Sponge (Sponge), which quenches endogenous miR-21 level, abrogated phosphorylation of p65, IkB kinase (IKK) and IkB concomitant with attenuation of Akt phosphorylation. Sponge inhibited expression of NFkB target cyclin D1 mRNA and protein. We have previously shown that miR-21 targets and downregulates the tumor suppressor PTEN in renal proximal tubular epithelial cells. siRNA-directed downregulation of PTEN (siPTEN) reversed Sponge-induced inhibition of phosphorylation of these proteins and suppression of cyclin D1, suggesting miR-21 utilizes PTEN for its action. Expression of constitutively active (CA) Akt along with Sponge reversed the Sponge-induced inhibition of phosphorylation of p65, IKK and IkB. Additionally, Sponge increased cyclin kinase inhibitor p27 phosphorylation/inactivation, which was blocked by expression of siPTEN or CA Akt. Moreover, p65 S536A, dominant negative IKK, mutant IkBA3 and Sponge inhibited RCC DNA synthesis. Expression of wild type p65, IKK or CDK4 reversed the Sponge-induced inhibition of DNA synthesis in RCC.

Conclusions: We uncover a positive feed back loop involving miR-21-PTEN-Akt-NFkB, which act in concert with miR-21-PTEN-Akt-p27 to regulate cyclin D1-CDK4 axis driving RCC proliferation.

Funding: NIDDK Support, Veterans Administration Support

TH-PO178

Anthracycline Inhibits Recruitment of Hypoxia-Inducible Transcription Factors, Blunts the Induction of Lysyl Oxidase and Suppresses Migration of Renal Cell Carcinoma Tetsuhiro Tanaka,[1] Junna Yamaguchi,[2] Kumi Shoji,[2] Masaomi Nangaku.[2] *[1]Division for Health Service Promotion, University of Tokyo, Tokyo, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan.*

Background: Anthracycline chemotherapeutic agents of the topoisomerase inhibitor family are widely used for the treatment of various tumors. While targeted tumor tissues are generally situated in a hypoxic environment, the connection between efficacy of anthracycline agents and cellular hypoxia response has not been investigated in depth.

Here, we report that doxorubicin (DXR) impairs the transcriptional response of the hypoxia-inducible factor-1 (HIF-1) in human proximal tubular cells and renal cell carcinoma (RCC) cell lines.

Methods: The hypoxic induction by HIF was measured by luciferase reporter assays. The expression of HIF-1α, HIF-target gene mRNA and protein was quantified by real-time PCR and immunoblotting. Recruitment of HIF-1α to the enhancer was evaluated by chromatin immunoprecipitation (ChIP). Migration of RCC was evaluated by scratch assays.

Results: DXR treatment significantly suppressed the hypoxic induction of HIF-1-target genes, such as glucose transporter 1 and vascular-endothelial growth factor. While there was no quantitative difference in HIF-1α protein between the DXR(-) and DXR(+) groups, ChIP assays revealed inhibition of HIF-1-recruitment by DXR. Notably, this pleiotropic effect was also observed in the HIF-2-mediated hypoxia response and retarded migration of von Hippel-Lindau (VHL)-defective RCC and that of VHL-competent RCC in hypoxia, which was accompanied by a co-ordinated downregulation of HIF target lysyl-oxidase (LOX) family members, such as LOX and LOX-like2. The functional involvement of LOX in RCC migration was confirmed by inhibiting LOX catalytic activity using β-aminopropionitrile.

Conclusions: These findings highlight the impaired hypoxia response by anthracycline agents which counteract the invasive phenotype of RCC, and offer a promising opportunity to develop HIF-inhibitors using DXR as a chemical template.

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TH-PO179

bcl-7, Caenorhabditis elegans Homologue of Human *BCL7B* Gene, Located at Williams-Beuren Syndrome Locus, Regulates Both Somatic and Germ Cell Division

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Background: *BCL7B* gene is expressed in kidney and located on chromosome 7q11.23, which is the microdeletion region in Williams-Beuren syndrome (WBS). Approximately 50% of patients with WBS have kidney or urinary tract dysfunction including developmental anomaly. It is unclear how loss of function of *BCL7B* causes these anomalies. We found that *bcl-7* (*Caenorhabditis elegans BCL7* gene homologue) deletion mutants had sterile (Ste) and protruding vulva (Pvl) phenotypes. Here, we show *bcl-7* is a regulator of cell division in both somatic and germ cells.

Methods: Firstly, we monitored cell cycle of gonads by immunostaining with an anti-PH3 antibody as a mitotic marker. Secondly, we examined patterns of cell division of somatic stem-like cells, seam cells, with the integrant transgenic worms that expressed green fluorescent protein (GFP) in seam cell nuclei. Thirdly, we searched for suppressor genes of somatic phenotypes of mutants with RNA interference (RNAi) screening.

Results: In germ cells, the mitotic region was maintained about distal 10~15 cell diameters in the adult hermaphrodite gonad of wild type *C. elegans*. In contrast, *bcl-7* deletion mutants have expanded mitotic region in gonads as shown by the anti-PH3 immunoreactivity. As somatic stem-like cells, seam cells divide asymmetrically during each larval stage and have self-renewal property. This characteristic pattern of cell division in seam cells was also seriously damaged in mutants. RNAi screening showed that *bcl-7* as a regulator of somatic cell division has genetic interactions with genes in the Wnt pathway.

Conclusions: We analyzed the functions of *BCL7B* gene homologue, *bcl-7*, and found that it works as the regulator of cell division in both somatic and germ cell line. In addition, as a regulator of somatic cell division, *bcl-7* works possibly through the Wnt pathway. One of the next issues to be solved is whether *BCL7B* gene has the same function and works in the same pathway in human.

TH-PO180

Candidate Action Mechanisms of Peginesatide-Induced Erythropoiesis

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Background: Peginesatide is a pegylated, peptide-based erythropoiesis stimulating agent that stimulates the erythropoietin receptor (EPOR) that has recently been approved for the treatment of anemia due to chronic kidney disease in adult patients on dialysis. Peginesatide has been shown to stimulate sustained erythropoiesis in vivo, allowing for once monthly dosing. As a dimeric peptide compound conjugated to a 40 kDa PEG moiety, peginesatide exhibits an increased in vivo half-life (47.9 h) compared to recombinant human erythropoietin (rHuEPO, 7 h). Beyond the extended in vivo persistence, additional unique properties may contribute to peginesatide's durable erythropoietic action.

Methods: To investigate these candidate properties, multi-parametric flow cytometry and Western blotting were utilized to evaluate the effects of peginesatide vs. rHuEPO treatment on erythroid precursor cells.

Results: First, it was found that peginesatide up-modulates cell surface EPOR expression. Specifically, when UT-7/EPO erythroid progenitor cells were cultured in peginesatide vs. rHuEPO, EPOR levels among cells expanded in peginesatide were discovered to be elevated approximately 6 fold. This was observed at varied, functionally matched-doses with ligand replacement. Possible mechanisms for increased EPOR expression include extended peginesatide residence time on the EPOR (1300 min vs. 77 min for rHuEPO), and/or diminished peginesatide-EPOR complex turnover. A second unexpected property was revealed in studies of primary murine bone marrow erythroid progenitor development ex vivo. When used to support erythroid cell expansion, peginesatide promoted an increased expansion of early $cKIT^{pos}CD71^{low}$ erythroid progenitors when compared to rHuEPO. Via multi-parametric flow cytometry, this unique population was identified as $cKIT^{pos}CD71^{low}CD36^{pos}CD13^{pos}$ progenitors.

Conclusions: Peginesatide therefore appears to more effectively recruit early $cKIT^{pos}CD71^{low}$ erythroid progenitors from a myeloerythroid compartment than rHuEPO, possibly due to peginesatide's effect on increased EPOR expression.

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TH-PO181

Modulation of Epigenetic Factors by HIV-1 in Podocytes Causes Downregulation of P-Cadherin and Nephrin through Upregulation of Snail

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Background: Podocytes in HIV-associated nephropathy have been demonstrated to display enhanced expression of Snail, down regulation of p-cadherin, and nephrin. However, the involved mechanism needs to be clearly definced. We hypothesized that HIV-induced epigenetic factors might be contributing to upregulation of Snail and downregulation of p-cadherin and nephrin.

Methods: Immortalized and differentiated human podocytes (IDHPs) were transduced with either empty vector (EV) or HIV (NL4-3) constructs. Both EV/IDHPs and HIV/CIDHPs were de-differentiated (at 33°C). Proteins and RNAs were extracted and probed for Snail, p-cadherin, and nephrin. To determine the role of epigenetic factors, EV/IDHPs and HIV/IDHPs were incubated in media containing buffer, 5-azacytidine (AZAC, a demethylating agent, 5 μM), or suberoylanilide hydroxamic acid (SAHA, a HDAC blocker, 20 μM) for 48 hours. Immunoblots were probed for Snail, p-cadherin, and nephrin. To determine the role of Snail in downregulation of p-cadherin and nephrin, HIV/IDHPs were silenced for Snail as well as stable colonies of IDHPs over-expressing Snail were developed. Renal tissues of four weeks old, control and Tg26 (HIV transgenic mice) in groups of six were evaluated for expression (both protein and mRNA) of Snail, p-cadherin, and nephrin. Renal cortical sections of control and Tg26 mice were labeled for Snail, p-cadherin, and nephrin and examined under an immunofluorescence microscope.

Results: HIV/IDHPs displayed 3-fold enhanced expression of Snail, and 2-3-folds attenuated expression of p-cadherin, and nephrin. HIV/IHDPs silenced for Snail displayed normalization of p-cadherin and nehrin expression. HIV/IDHPs displayed 3-fold increase in DNA methyl transferase (Dnmt)-3 expression; whereas, AZAC inhibited HIV-induced podocyte up regulation of Snail and down regulation of p-cadherin and nephrin. Renal sections of Tg26 mice also revealed upregulation of Snail and downregulation of p-cadherin and nephrin by podocytes.

Conclusions: These findings suggest that HIV-induced epigenetic factors contributie to podocyte damage in HIVAN.

Funding: NIDDK Support

TH-PO182

Susceptibility of Podocytes to Palmitic Acid Is Regulated by LXR-Dependent Stearoyl-CoA Desaturases 1 and 2

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Background: Type 2 diabetes mellitus is associated with elevated free fatty acid (FFA) levels. We reported the antagonistic effects of saturated FFAs (SFAs) and monounsaturated FFAs (MUFAs) for podocyte survival, with SFAs being deleterious and MUFAs protective. Here, we elucidate whether fatty acid metabolism is altered in diabetic nephropathy and whether induction of stearoyl-CoA desaturases (SCDs) can prevent palmitic acid-induced podocyte death.

Methods: Human glomerular gene expression was studied by microarray analysis in patients with diabetic nephropathy and healthy controls. Murine podocytes were used. mRNA levels were quantified by RT-PCR and apoptosis and necrosis by staining with annexin V and PI. Incorporation studies were performed using [^{3}H]palmitic acid and TLC.

Results: Gene expression analysis revealed a significant induction of SCD-1 in diabetic nephropathy. Similarly, palmitic acid upregulated Scd-1 and Scd-2 mRNA in podocytes. The effect of SCDs was investigated by the LXR-agonist TO901317 (TO), a known SCD-inducer. TO induced Scd-1 (2.5x) and Scd-2 (5.0x) mRNA and reduced palmitic acid-induced apoptosis and necrosis by 40% and 30%. Only combined gene silencing of Scd-1 and Scd-2 reverted the TO-effect, indicating a causative role for both SCD-isoforms. In addition, Scd-1-overexpression ameliorated survival of palmitic acid-treated podocytes. Finally, TO shifted palmitic acid-derived FFAs into biologically inactive triglycerides.

Conclusions: These results indicate a protective effect of SCDs on palmitic acid-induced podocyte death. The observed induction of SCD-1 in glomerular extracts of patients with diabetic nephropathy may be part of a protective mechanism against SFAs.

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TH-PO183

PDK1 Regulates Apoptosis in Podocytes Pauliina H. Saurus,[1] Mervi Ristola,[1] Moin Saleem,[2] Harry B. Holthofer,[1] Sanna H. Lehtonen.[1] *[1]Haartman Institute, University of Helsinki, Helsinki, Finland; [2]University of Bristol, Southmead Hospital, Bristol, United Kingdom.*

Background: Podocyte injury or loss caused e.g. by cell detachment or apoptosis leads to proteinuria, a hallmark of most glomerular diseases, including diabetic nephropathy. 3-phosphoinositide-dependent kinase-1 (PDK1) activates protein kinase B (PKB)/Akt and many other soluble guanylyl cyclase kinases. Since Akt is known to inhibit apoptosis in other cell types by phosphorylating the Bcl-2 family member Bad or by activating IKKα, PDK1 might promote also podocyte survival by increasing the phosphorylation level of Akt.

Methods: To study the role of PDK1 in podocyte apoptosis, PDK1 was knocked down in cultured human podocytes using lentiviral small hairpin RNA (shRNA) constructs. The apoptotic rate of podocytes was measured by Annexin V staining and flow cytometry. The involvement of phosphatidylinositol 3-kinase/Akt (P13-K/Akt) and p38 mitogen-activated protein kinase (p38MAPK) signaling cascades and the expression levels of proapoptotic BAX and antiapoptotic BCL-2 after knockdown of PDK1 were analyzed by quantitative Western blotting. Further, the expression level of PDK1 in glomeruli of lean and obese Zucker rats was studied.

Results: PDK1 is expressed in rat glomeruli and tubules and in cultured human podocytes. Knockdown of PDK1 increased apoptosis in cultured human podocytes, and inhibited the PI3-K/Akt and activated the p38MAPK pathways. BCL-2 level was decreased and BAX increased in PKD1 knockdown cells. Further, the level of PDK1 was lower in glomeruli of obese Zucker rats compared to lean littermates.

Conclusions: Our data show that PDK1 may protect podocytes against apoptosis. Downregulation of PDK1 in Zucker rat glomeruli might suggest a protective role for PDK1 in the development of podocyte injury.

TH-PO184

Apoptotic Cells Inhibit Proximal Tubular Cell Growth via Activation of AMP-Activated Protein Kinase (AMPK) Vimal Patel,[1] Donald Massenburg,[1] Lanfei Feng,[1] Angelika Antoni,[4] Natalia O. Litbarg,[1] Snezana Vujicic,[1] Joyce Rauch,[2] Wilfred Lieberthal,[3] Jerrold S. Levine.[1] *[1]Medicine, University of Illinois at Chicago, Chicago, IL; [2]Medicine, McGill University, Montreal, QC, Canada; [3]Medicine, SUNY at Stony Brook, Stony Brook, NY; [4]Medicine, Kutztown University, Kutztown, PA.*

Background: Cells undergoing apoptosis acquire new activities that modulate the fate and function of adjacent live cells. We have shown that apoptotic (Apo) target cells inhibit the viability and proliferation of kidney proximal tubular cell (PTC) responders. Here, we examine the effect of Apo targets on PTC responder cell growth (cell size during G1 of cell cycle).

Methods: We used BU.MPT cells, a conditionally immortalized PTC line, as responder cells. BU.MPT cells, induced to undergo apoptosis in several ways, were used as Apo targets.

Results: Apo targets induced dose-dependent inhibition of mTOR (assessed by inhibition of phosphorylation of p70S6K, a direct target of mTOR), as well as concomitant inhibition of cell growth (~30% reduction of cell volume during G1) in BU.MPT responders. Apo targets also activated AMPK in responders, whether in the presence or absence of EGF stimulation. In contrast, necrotic targets had no effect on responder cell growth. Compound C, a pharmacologic inhibitor of AMPK, prevented both inhibition of p70S6K phosphorylation and reduction in cell volume, indicating that Apo target modulation of mTOR and cell growth occurs via AMPK. Notably, AMPK activation occurred via an energy-independent mechanism, as responder cell ATP levels were unchanged. Infection of BU.MPT responders with a constitutively active construct of Akt, which lies upstream of mTOR and is itself inhibited by Apo targets, did not prevent inhibition of cell growth, implying that inhibition of BU.MPT growth is largely AMPK-dependent and induced via a novel signaling pathway, independent of changes in ATP.

Conclusions: These data reveal that Apo targets inhibit cell growth in nearby PTC responders. By acting as sentinels of environmental change, Apo targets may allow nearby viable cells, especially non-migratory epithelial cells, to monitor and adapt to local stresses.

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TH-PO185

Dose-Dependent Expression of ApoL1 Protein Sensitizes Cell Death Pathways Zhonghai Chen,[1] Barry I. Freedman,[1] Wayne D. Graham,[1] Nicole A. Ross,[1] Martin R. Pollak,[2] Michael D. Ross,[2] Mariana Murea,[1] Lijun Ma,[1] Peter A. Antinozzi.[1] *[1]Wake Forest School of Medicine, Winston-Salem, NC; [2]Beth Israel Deaconess Med Ctr, Boston, MA.*

Background: Two apolipoprotein L1 gene (*APOL1*) variants associate with non-diabetic kidney disease in African Americans. These non-synonymous variants confer resistance to trypanosomiasis. Whereas ApoL1 has a known role as the trypanolytic factor of human serum, the cellular function of ApoL1 and how specific variants contribute to kidney disease are unknown. Dose-dependent expression of ApoL1 was evaluated to better understand its cellular role.

Methods: Four eukaryotic expression vectors were constructed for the transfection of HEK293, HepaC1C7, and HepG2 cells: (1) control vector (no ApoL1); (2) ApoL1 (reference sequence); (3) ApoL1 G1 (S342G/I384M); and (4) ApoL1 G2 (DN388/Y389). Cell-by-cell analysis of ApoL1 expressing cells was enabled by the co-expression of GFP and an automated high-content cell imaging system. Cell fates were scored by tracking GFP positive cells, propidium iodide (PI) staining and localization of autophagy markers.

Results: Dose-dependent expression of each ApoL1 variant was verified by western blot and immunofluorescence. Time-lapse microscopy was initiated 24h post-transfection and monitored for an additional 24h. Cell survival was assessed by semi-automated scoring of loss of GFP and gain of PI staining. Consistent with ApoL1 alteration of lipid membrane permeability, cell death was increased in a dose-dependent manner with ApoL1 expression (reference sequence). To directly compare the potency of G1 and G2 vs the reference variant, matched levels of protein expression were attained. G1 was the most potent cell death sensitizer (61%), followed by G2 and the reference variant, 52% and 34%, respectively. Confirmation of this order of potencies was re-demonstrated with the fluorescent autophagy marker LC3-RFP.

Conclusions: These data suggest that ApoL1 sensitizes cell death pathways and this may underlie its functional role in kidney disease. G1 and G2 *APOL1* variants have co-evolved with the parasite to protect against human trypanosomal infection. These variants also sensitize cell death pathways and may contribute to kidney disease.

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TH-PO186

Endothelial-to-Mesenchymal Transition (EndMT) Is a Source for Cancer-Associated Fibroblasts in Renal Cell Carcinoma (RCC) Margherita Gigante,[1] G. Castellano,[2] Paola Pontrelli,[2] Alessandra Stasi,[2] C. Divella,[2] Matteo Accetturo,[2] Giuseppe Grandaliano,[1] Loreto Gesualdo,[2] E. Ranieri.[1] *[1]University of Foggia; [2]University of Bari.*

Background: The progression of tumors towards a malignant phenotype does not depend exclusively on the cell-autonomous properties of cancer cells themselves but it is also deeply influenced by tumor stroma reactivity. In tumors, EndMT is an important source of cancer-associated fibroblasts (CAFs), which are known to facilitate tumor progression. The aim of our study was to investigate whether tumor-derived endothelial cells (TECs) isolated from RCC may be assimilated to CAF phenotype with enhanced migratory capacity and invasiveness.

Methods: We evaluated differences between TEC andendothelial cells (HUVEC)in genome wide gene expression by using a microarray approach. Genes with an FDR≤5% and a fold-change≥2 were considered to be differently expressed. Furthermore, we evaluated the changes of TECs phenotype *in vitro* by using confocal microscopy and flow cytometry. Finally, we analyzed tissue specimens from RCC patients.

Results: The comparison between TEC vs HUVEC, revealed that differently regulated genes were involved in EndMT in TEC. Among these genes, we found FSP1 significantly up-regulatedand CD31 and cadherin-associated proteins significantly down-regulated. Various genes belonging to the TGF-b pathway were modulated (BMP2, NFAT, Wnt/b-catenin). The surface phenotypic analyses confirmed a statistically significant ($p<0.01$) down-regulation of endothelial markers CD31(10%vs98%),VE-cadherin(3.1%vs40,4%), CD105(41%vs96%), CD146(60%vs98%) and up-regulation of mesenchymal markers such as N-cadherin (80%vs18%). We also found a slight but significant ($p<0.03$) increase of CD34(2,1%vs0,3%) and CD133(1,8%vs0,2%) in TEC vs HUVEC. Interestingly, we identified a substantial proportion of CAF in RCC tissue, as a unique population of cells that co-express CD31 along with FSP1 and a-SMA, indicating the occurrence of EndMT in vivo.

Conclusions: These data suggest that EndMT is a relevant mechanism for CAF recruitment to the tumor stroma, probably mediated by TGF-β. These CAFs may have a unique role in RCC progression and invasiveness.

TH-PO187

Sphingosine 1-Phosphate/Sphingosine 1-Phosphate Receptor-3, Potential Mediators of Renal Fibrosis, Are Assessed by siRNA and Scratch Assay Shunji Shiohira, Takumi Yoshida, Junko Kohei, Hidekazu Sugiura, Kosaku Nitta, Ken Tsuchiya. *Department of Medicine Four, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

Background: Sphingosine 1-phosphate (S1P), a bioactive lipid mediator, has been suggested to be involved in the mechanism of renal fibrosis. Previously, we have shown the direct effects of S1P on the fibrotic process in the UUO model using nude mice which were characterized by deficit of immune response. To get more insight into roles for S1P and receptor subtype effects *in vivo*, we performed siRNA knockdown of receptor subtype and wound healing scratch assay.

Methods: NRK-49F (normal rat kidney interstitial fibroblast cells) were stimulated with exogenous S1P and the expressions (mRNA/Western blotting) of a-SMA, E-cadherin, collagen type 1 (COL1), collagen type 4 (COL4), TIMP1 and PAI1 were examined. To specify the kidney specific signal pathway, siRNAs targeted to S1P receptor subtype were generated. Then, the morphological changes of the NRK-49F after stimulation by S1P were examined (3 days, 7 days). The growth and migration of cultured cells was quantified by using CL-Quant software to analyze time-lapse images in a Nikon BioStation CT. The real-time images of cell migration were monitored for 2 days.

Results: S1P stimulated fibrosis of NRK-49F in a dose- and time-dependent manner as previously observed, and induced morphological changes (elongation of the cell shape with spindle-like extension, increased migration) of the NRK-49F. Migration of NRK49F was accelerated and increase in a-SMA, COL1, COL4, TIMP1 and PAI1 expressions and decrease in E-cadherin expression were observed by addition of S1P. In preliminary data, S1PR3(S1Precptor-3) siRNA transfection to NRK-49F attenuated cell growth and migration, in addition, the expression of fibrotic markers was also diminished by S1P stimulation.

Conclusions: These results suggest that activation of S1P signaling mediated by S1PR3 results in chronic pathological fibrosis, such as in chronic kidney disease (CKD).

TH-PO188

Podocyte Derived Expression of Fibronectin Tarunkumar H. Madne,[1] Rachel Lennon,[2] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]University of Manchester, Manchester, United Kingdom.*

Background: Cell phenotype and survival are regulated by extracellular matrix proteins (ECM), particularly those of basement membranes. In disease there are changes to the quantity and quality of matrix and basement membrane proteins contributing to the progression of disease. The glomerular basement membrane (GBM) is dynamic structure made up of protein secreted by endothelial cells (EC) and podocytes (Pod); fibronectin (Fn) is an ECM protein in fibrosis but its expression in the GBM and its potential to regulate Pod function is unknown. The interaction of Pod with GBM specifically with Fn could be critical in regulating Pod phenotype & survival. We are investigating the potential of human Pod to produce Fn and regulate its alternative splicing and how, subsequently Fn may alter Pod responses and survival.

Methods: LC –MS/MS was performed on glomerular matrix enriched fraction derived from normal human kidney. Experiments were conducted on conditionally immortalised human Pod incubated with TGFβ1 (2.5ng/ml) for 24-72 h. Fn expression was assessed by RT-PCR, Western blotting (WB) and Immunofluorescence (IF).Cells were also grown on different matrices; including collagen IV (Col IV) and Fn with and without the EDA Exon (EDA+ Fn, EDA –Fn).

Results: Analysis of glomerular ECM by MS shows the presence of Fn potentially derived from both EC and Pod. In culture Pod express Fn and TGFβ1 further induced expression as determined by IF. To quantify the expression, cell lysates were subjected to WB and an increase in both EDA+Fn and EDA-Fn was observed. TGFβ1 regulated the alternative splicing of Fn as evinced by increase in the ratio EDA+Fn to EDA-Fn. On investigating signalling Fn expression was shown to be P38 MAPK dependent. TGFβ1 also induced PI3K activity. Cell grown on Fn had altered morphology and TGFβ1 responsiveness compared to cells grown on plastic or col IV.

Conclusions: Our evidence suggests that human Pod in vivo and in vitro can express Fn and TGFβ1 can induce alternative splicing to produce the pathological isoform EDA+. Pod phenotype appears to be altered by the presence of Fn in culture.

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TH-PO189

Phosphorylation of Nephrin Regulates Adhesion of Podocyte by PINCH-1-ILK-Parvin Complex Dongqing Zha,[1] Cheng Chen,[1] Wei Liang,[1] Xinhua Shu,[1] Tean Ma,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Nephrin is a signal molecule which transduces signal with its phosphorylation. The downregulation of phosphorylation of nephrin has been demonstrated to correlate with podocyte injury. PINCH-1-ILK-a-parvin (PIP) complex, as an important molecule of focal adhesion, play a crucial role in cell adhesion and cytoskeleton. In this study, we evaluated whether phosphorylation of nephrin influence cytoskeleton and cell adhesion in podocyte via regulation of PIP complex.

Methods: Wistar rats were received either puromycin aminonucleoside (15 mg/100 g) or normal saline via one-time intraperitoneal injection. 24-hour urinary albumin and serum biochemical profile were measured on 0, 1, 4, 7, 14, 28 days after injection. The glomerular morphology and podocyte ultrastructure were observed under the light and transmission election microscopies respectively. In vitro, cultured murine podocytes were exposed to puromycin aminonucleoside pretreated with or without PINCH-1 siRNA or nephrin siRNA or protamin for variable concentrations and time periods. Nephrin and phosphorylation of nephrin expression were analyzed by Western-blotting and immunofluorescence. PIP complex.

Results: Rats injected with puromycin aminonucleoside displayed diminished phosphorylation of nephrin, PIP complex, F-actin gradually, then increased in consistent with glomerular/podocyte injury and proteinuria when compared to control rats. Knockdown of PIP complex protein(using siRNA) resulted in reorganization of cytoskeleton and decreasing of cell adhesion and spreading, but nephrin and phosphorylation of nephrin kept no change. Furthermore, knockdown or overexpression of phosphorylation of nephrin protein(using siRNA or protamin) inhibited or enhanced PIP complex expression, disorganized cytoskeleton of podocyte and decreased cell adhesion and spreading.

Conclusions: These findings indicate that alteration of nephrin phosphorylation disorganized cytoskeleton of podocyte and discreased cell adhesion through a PIP complex-dependent mechanism.

TH-PO190

Elucidating the Role of a Renal Proximal Tubule-Specific Olfactory Receptor Blythe D. Shepard,[1] Lydie Cheval,[2] Alain Doucet,[2] Jennifer L. Pluznick.[1] *[1]Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Centre de Recherche des Cordeliers, Paris, France.*

Background: Olfactory receptors (ORs) are seven transmembrane domain G protein-coupled chemosensors that detect odorants in the nose. Recently, studies have found that ORs are also expressed and play important functions outside of their native tissue. We previously reported that OR signaling plays a role in the kidney, and that 9 ORs, including Olfr1393, are present in the kidney (PNAS 2009).

Methods: In order to elucidate the role that Olfr1393 plays in the kidney, we examined its localization and ligand profile. To determine its localization, we performed RT-PCR from microdissected renal segments. We determined potential ligands for Olfr1393 using a luciferase reporter assay, as well as a calcium signaling assay.

Results: In microdissected renal segments, we found that Olfr1393 is exclusively expressed in all three proximal tubule segments (S1, S2 and S3, n=3). The majority of ORs, including Olfr1393, are "orphan receptors" with no known ligand. To screen for ligands, Olfr1393 must be expressed on the plasma membrane (PM). However, most ORs are retained in the ER when expressed heterologously. We cloned Olfr1393 and found that it can traffic to the PM in HEK293T when coexpressed with Receptor Transporting Protein (RTP). Using a comprehensive screening approach, we found that Olfr1393 detects cyclic molecules containing either carbonyl or alcohol groups. These ligands were confirmed by Fura-2 calcium imaging. We also found that coexpression of Olfr1393 with RTP allows Olfr1393 to traffic to the surface in in MDCK cells, where it localizes to the apical, *not the basolateral, PM.*

Conclusions: These data suggest that Olfr1393 is expressed on the apical membrane of the proximal tubule where it comes in contact with the newly forming urine to detect molecules containing cyclic carbonyls and/or alcohols. There are a vast array of physiological chemicals that are derived from this basic structure including bile acids, vitamins and steroid hormones. We are currently focusing on defining the full complement of ligands for Olfr1393, and understanding the physiological role that this OR plays in the kidney.

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TH-PO191

Protein Kinase G and Calcineurin Antagonistically Regulate TRPC6 Activity in Podocytes Gentzon Hall,[1] Janelle Rowell,[2] Rasheed A. Gbadegesin,[1] Peter J. Lavin,[1] Guanghong Wu,[1] David A. Kass,[2] Michelle P. Winn.[1] *[1]Duke University; [2]Johns Hopkins University.*

Background: The emerging role of TRPC6 as a central contributor to various pathologic processes affecting podocytes has generated considerable interest in the development of therapeutic strategies to modulate its function. The development of selective pharmacologic inhibitors of TRPC6 has been hindered by the high degree of sequence homology between TRPC family members. Protein Kinase G (PKG) is now recognized as a potent negative modulator of TRPC6 activity via phosphorylation of 2 highly conserved amino acid residues, threonine 69 (Thr 69) and serine 321 (Ser321). In an effort to characterize the role of PKG in the modulation of TRPC6 activity in podocytes, we evaluated the regulation of Thr69 phosphorylation and dephosphorylation in conditionally immortalized podocytes.

Methods: We examined the role of PKG-mediated TRPC6 phosphorylation at Thr69 using a combination of immunoblotting, immunofluorescence, adenoviral gene expression, and scratch wound healing assays in immortalized mouse podocytes.

Results: In unstimulated podocytes, TRPC6 is heavily phosphorylated at Thr69. This basal phosphorylation is reduced by Ang II, overexpression of a constitutively active calcineurin (Cn), and pharmacologic inhibitors of PKG. Pretreatment of podocytes with the PKG signaling agonists SNAP, 8br-cGMP, Bay 41-2772, and a selective phosphodiesterase 5 inhibitor (PDE5*i*) attenuate Ang II-induced decrease in Thr69 phosphorylation and inhibits TRPC6-dependent podocyte motility by 30-70%. In Ang II-stimulated podocytes overexpressing a phosphomimetic mutant TRPC6, TRPC6-dependent podocyte migration was inhibited by greater than 30%. Finally, the increased basal and Ang II-induced podocyte motility observed in $TRPC6_{P112Q}$ overexpressing podocytes was significantly inhibited by 25% and 55% respectively after pretreatment with 8br-cGMP and PDE5*i*.

Conclusions: In conclusion, PKG and Cn antagonize one another in the regulation of TRPC6 activity in podocytes. These findings further elucidate the complex regulation of TRPC6 activity and highlight the potential value of phosphodiestrase inhibition in the modulation of aberrant TRPC6 activity in podocytes.

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TH-PO192

p66ScA Deficiency Retards Development of HIV-Associated Nephropathy Andrei Plagov,[1] Partab Rai,[1] Rivka Lederman,[1] Himanshu Vashistha,[1] Mohammad Husain,[2] Ashwani Malhotra,[1] Leonard G. Meggs,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Ochsner Clinic, New Orleans, LA.*

Background: p66ShcA is considered an atypical signal transducer that converts redox reaction into pro-apoptotic signals. On that account, intracellular levels of ROS are decreased in p66ShcAdeficient cells. Therefore, p66ShcA deficient mice have diminished levels of both systemic and intracellular oxidative stress. Recently we demonstrated that tubular cells and podocytes silenced for p66ShcA displayed resistance to HIV-induced injury. We hypothesized that p66ShcA deficit status will provide protection against the development of overt renal lesions in HIVAN mice. in the present study, we evaluated the development of renal lesions in genetically engineered p66ShcA deficient HIV transgenic mice.

Methods: HIV transgenic mice (Tg26) were bred with $p66ShcA^{+/-}$ mice to develop colonies of Tg26 with partial deficit status of p66ShcA. Three groups of animals: FVB/N mice (serving as negative controls of HIVAN), Tg26 mice (serving as positive controls of HIVAN), and $Tg26;p66^{+/-}$ mice (n=4) were studied. Mice were sacrificed at 8 weeks. Blood pressure was measured. Renal biomarkers (blood and urine) were collected; kidneys were harvested for renal histology, immunohistochemistry, immunoblotting and real time PCR studies. Severity of renal lesions was scored on the coded slides.

Results: Tg26 mice displayed elevated BUN levels (FVBN, 38 mg/dl; Tg26, 100 mg/dl, and $p66^{+/-}$;Tg26, 37 mg/dl) and increased proteinuria (albumin: creatinine ratio, FVBN, 51 mg/g creatinine; Tg26, 807.4 mg/g creatinine; and $Tg26;p66^{+/-}$, 109 mg/g creatinine). FVB/N mice did not display any sclerosed glomeruli; Tg26 mice displayed focal segmental sclerosis (FSGS) in 33% of glomeruli and collapsing glomerulopathy in

29% of glomeruli; whereas, Tg26;p66$^{+/-}$ mice showed FSGS in only in 1% of glomeruli and collapsing glomerulopathy only in 6% of glomeruli. These findings indicated that p66 deficient Tg26 mice were resistant to HIV-induced injury.

Conclusions: p66 deficient Tg26 mice displayed resistant to develop overt HIVAN.

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TH-PO193

Dickkopf Related Protein-1 Inhibits Fibrogenesis by Blocking Multiple LRP-6-Regulated Signaling Pathways in Pericytes and Myofibroblasts

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Background: Fibrosis of vital organs is a major public health problem with limited therapeutic options. Recent studies indicate that mesenchyme-derived pericytes and fibroblasts are the progenitors of scar-forming cells in kidney and other organs.

Methods: - Col1a1-GFP mice
- TCF/LEF:H2B-GFP WNT reporter mice
- Axin2+/lacz WNT reporter mice
- UUO (unilateral ureteric obstruction)
- Unilateral IRI (ischemia reperfusion injury)

Purification of pericyte/myofibroblast from kidneys and study the signaling in vitro.

Results: We observed canonical WNT signaling responses in normal kidney pericytes which were markedly upregulated following kidney injury, during pericyte transition to myofibroblasts. Here we show Dickkopf related protein-1 (DKK-1), a ligand for canonical WNT receptors, Low Density Lipoprotein Receptor-related proteins-5 and -6 (LRP-5, LRP-6), effectively inhibits pericyte activation, detachment and transition to myofibroblasts *in vivo* in response to kidney injury. The consequence is attenuated fibrogenesis, capillary rarefaction and inflammation. DKK-1 also reverses evolving fibrogenesis. DKK-1 blocks activation and proliferation of established myofibroblasts *in vitro*, triggering G1 cell-cycle arrest. DKK-1 blocks pericyte proliferation to platelet-derived growth factor (PDGF), and pericyte migration, gene activation, and cytoskeletal reorganization in response to transforming growth factor-b (TGF-b) or connective tissue growth factor (CTGF). Although DKK-1 affects these responses partly by canonical WNT signaling, it acts dominantly by inhibiting PDGF-, TGFb-, and CTGF-activated MAPK and JNK signaling cascades. LRP-6 is necessary for these responses independently of the canonical WNT pathway, suggesting LRP-6 is a co-receptor for pathways other than WNT. DKK-1 therefore blocks all of the changes in pericytes required for myofibroblast transition, attenuates established myofibroblast proliferation/activation by novel mechanisms dependent on LRP-6, but not the WNT signaling cascade.

Conclusions: DKK-1 is a candidate therapy for evolving fibrogenesis in kidney, and potentially elsewhere.

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TH-PO194

Transforming Growth Factor-β (TGF-β)-Activated Kinase 1 (TAK1) Plays a Crucial Role in Podocytes

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Background: Transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1) is a serine/threonine kinase belonging to the mitogen-activated kinase (MAPK) kinase kinase family. TAK1 is a key intermediate in signal transduction induced by TGF-β or inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1). Although it is well known that these cytokines mediate podocyte injury responses that lead to proteinuria and glomerulosclerosis, the role of TAK1 in podocyte is not yet known. We show that the inhibition of TAK1 in human podocyte abrogated the activation of p38 and JNK MAPK induced by TGF-β1 or lipopolysaccharide, indicating that TAK1 mediates the signal transduction induced by TGF-β as well as by inflammatory cytokines in podocytes.

Methods: To examine the *in vivo role of TAK1, we generated podocyte-specific Tak1 knockout mice (Nphs2-Cre^{+}:Tak1$^{fx/fx}$ herein referred to as Tak1$^{\Delta/\Delta}$).* Effect of *Tak1* deletion in podocytes was analyzed by immunohistochemical and immunofluorescence staining of kidney tissues.

Results: Targeted deletion of *Tak1* in podocytes resulted in approximately 50% perinatal lethality of new born *Tak1*$^{\Delta/\Delta}$ mice and 90% of them died within one week after birth. *Tak1*$^{\Delta/\Delta}$ mice developed proteinuria from postnatal day 1 (P1), and exhibited delayed glomerulogenesis and reduced expression of Wilms' tumor 1 (WT1) and nephrin in podocytes. In addition, nephrin was abnormally distributed in podocytes of *Tak1*$^{\Delta/\Delta}$ mice, compared to *Tak1*$^{fx/fx}$ mice. Intriguingly, *Tak1*$^{\Delta/\Delta}$ mice showed increased expression of vascular endothelial cell growth factor (VEGF) in podocytes and abnormal glomerular microvasculature. Furthermore, 4-week and 7-week old *Tak1*$^{\Delta/\Delta}$ mice also developed increased proteinuria and renal fibrosis as evidenced by enhanced collagen deposition in the mesangium and tubular interstitial area.

Conclusions: Taken together, our data demonstrate that TAK1 plays a crucial role in podocyte differentiation and glomerular microvasculature during kidney development and kidney function through the regulation of the expression of WT1, nephrin and VEGF.

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TH-PO195

Akt Mediates Albumin Endocytosis via Phosphorylation of Dab2

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Background: Protein kinase B (Akt) is involved in proliferation, cell survival and insulin signaling. We demonstrated that Akt mediates albumin endocytosis in the proximal tubule through its interaction with disabled-2 (Dab2), an endocytic adaptor that binds to megalin. We hypothesized that Dab2 may be a novel substrate of Akt. Our aims were to evaluate phosphorylation of Dab2 by Akt and to examine the physiological relevance of this event by functional studies. Furthermore we aimed to identify the isoform and domain of Akt involved in albumin endocytosis.

Methods: We utilized human kidney proximal tubule cells (HKC-8) to study albumin endocytosis by fluorometry and confocal microscopy. Phosphorylation of Dab2 was examined by in-vitro phosphorylation assays. Dab2 phosphorylation sites Akt were identified and mutated by site-directed mutagenesis. The Akt isoform that is involved in albumin endocytosis was examined by silencing RNA technique. The interacting domains of Akt and Dab2 were investigated by yeast two hybrid (Y2H) method and GST pull-down experiments.

Results: Both Akt1 and 2 were expressed in the proximal tubule where as Akt3 expression was negligible. Silencing experiments revealed redundancy between Akt 1 and Akt2 in albumin endocytosis. Our results showed a diversity in the interaction and phosphorylation sites of Dab2 and Akt. Akt phosphorylated Dab2 at Ser-448-449 residues in the M15 portion (335-610). Dab2 Ser to Ala mutants at these phosphorylation sites reduced albumin endocytosis ($p<0.001$). Y2H and GST-PD experiments revealed that pleckstrin homology (PH) domain of Akt interacts with proline rich domain (PRD) of Dab2 and the minimally sufficient region in Dab2 for interaction with Akt is Dab2-PRD(600-619).

Conclusions: Akt is an important regulator of albumin endocytosis in the proximal tubule. Disabled-2 is a novel substrate of Akt and phosphorylation of Dab2 by Akt is physiologically relevant in Akt mediated albumin endocytosis. We showed a functional redundancy between Akt 1 and Akt2 in albumin endocytosis. We postulate that PH domain of Akt serves as a hub in albumin endocytosis connecting membrane lipids, Dab2 and megalin.

Funding: NIDDK Support

TH-PO196

Resveratrol Has Anti-Fibrotic Effect on the Kidney of CKD Rodent via Deacetylating Smad3

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Background: TGF-β/Smad3 signaling plays an important role in the pathogenesis of chronic kidney disease (CKD). Sirt1, an NAD^{+} deacetylase, has been shown to deacetylase transcriptional factors or co-factors. The present study examines the effect of SIRT1 on Samd3 signaling and its role in the progression of CKD.

Methods: Immortalized murine glomerular mesangial cells were used to examine the effect of SIRT1 on TGFß/Smad signaling. 5/6 nephrectomy was used to generate an animal model of CKD. The binding of SIRT1 with Smad3 was assessed with co-immunoprecipitation.

Results: In cultured mesangial cells, TGF-β increased fibronectin and type I collagen expression. Resveratrol (RSV) dose-dependently attenuated TGF-β induced fibronectin and type I collagen expression. Knocking-down Sirt1 using a RNAi attenuated these effects of RSV. Further studies showed that TGF-β increased acetylation levels of Smad3, and RSV significantly down-regulated TGF-β induced acetylation of Smad3. Down-regulation of Sirt1 increased TGF-β-induced Smad3 acetylation, consistent with the deacetylation effect of Sirt1 on Smad3. RSV failed to reduce TGF-β induced phosphorylation of Smad3. Co-immunoprecipitation showed the binding of SIRT1 with Smad3 and this binging was associated with reduced Smad3 acetylation. To examine whether SIRT1 alters Smad3's transcription activity, cells were transfected with a Smad3 transcription reporter construct. TGF-β significantly increased Smad3 transcriptional reporter activity. Sirt1 deletion substantially enhanced TGF-β induced-Smad3 reporter activity (330%). Sirt1 deletion also attenuated the effect of RSV on Smad3 reporter activity. In subtotal nephrectomized rats, RSV significantly reduced urinary protein (79.87±34.27 vs 152.14±30.48 mg/day) and serum creatinine (83±14.69 vs 111.6±21.5 µmol/L) at 12 weeks after the surgery. RSV reduced the acetylation levels of Smad3 in the remnant kidney. Finally, loss of one allele of Sirt1 (Sirt1$^{+/-}$) in mice caused higher proteinuira and higher BUN levels compared with wild type mice following 5/6 nephrectomy.

Conclusions: These results suggest that Sirt1 deacetylases Smad3 and attenuates TGF- β signaling, protecting the kidney from damage in CKD.

TH-PO197

Renally-Targeted Activation of Epac1 Reduces Oxidative Stress in Ischemia-Reperfusion Injury

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Background: Activation of Epac1, a cAMP-dependent exchange factor for Rap1, by the cAMP analogue 8-pCPT-2'-O-Me-cAMP (8-pCPT) reduced cell detachment during renal ischemia-reperfusion (IR) injury and promoted survival of proximal tubular epithelial cells (PTEC) during exposure to cisplatin. Here we studied whether activation of Epac by 8-pCPT protects against ROS-mediated oxidative stress during renal IR injury.

Methods: Effects of forskolin and 8-pCPT on ROS levels were measured using 5,6-carboxy-DCF-DA conversion and MitoSOX Red labeling. Morphology changes were studied in a 3D cell culture model during diethyl maleate (DEM) exposure. Lysozyme-conjugated 8-pCPT (LZM-8-pCPT) or vehicle was injected intravenously in mice. Renal Rap1 activity was determined by pull down assay. Bilateral renal IR injury was induced for 35 minutes and animals were sacrificed after 1, 24 or 72 hours. Plasma urea and creatinine levels were measured and sections were stained for clusterin-α, active caspase-3, Nrf2, Keap1, heme oxygenase-1 and neutrophils.

Results: Epac activation by 8-pCPT or forskolin decreased ROS production in conditionally immortalized PTEC during in vitro IR injury. Epac activation prevented tubular network disruption caused by DEM in 3D culture. Renal targeting of 8-pCPT by LZM conjugation led to prolonged activation of Rap1 compared to non-conjugated 8-pCPT. LZM-8-pCPT was specifically taken up by proximal tubules and reduced renal failure during IR injury. Epac activation reduced tubular injury, apoptosis, oxidative stress and inflammation during IR injury.

Conclusions: Pharmacological activation of Epac1 reduced oxidative stress in proximal tubules during IR injury. Our data suggest that Epac1 decreases ROS production by preventing mitochondrial dysfunction during IR injury.

Funding: Government Support - Non-U.S.

TH-PO198

Dynamic Change of the Chromatin Conformation in Response to Hypoxia Enhances the Expression of *GLUT3* (SLC2A3) by Cooperative Interaction of HIF1 and KDM3A Imari Mimura,[1,2] Tsuyoshi Inoue,[1,2] Yasuharu Kanki,[2] Hiroyuki Aburatani,[2] Youichiro Wada,[2] Tatsuhiko Kodama,[2] Masaomi Nangaku.[1] *[1]Division of Nephrology and Endocrinology, Univ. of Tokyo, Japan; [2]Laboratory for Systems Biology and Medicine, Research Center for Advanced Science and Technology, Univ. of Tokyo, Japan.*

Background: Hypoxia has a crucial role in chronic kidney disease. We aimed at clarifying a new epigenetic regulation induced by hypoxia.

Methods: Chromatin immunoprecipitation with deep sequencing (ChIP-Seq) is one of the powerful tools to identify the genome-wide binding sites of hypoxia inducible factor (HIF) 1. We performed ChIP-seq and clarified the histone modifications in human umbilical vein endothelial cells. In order to identify downstream targets of HIF1 and KDM (lysine-specific demethylase)3A, a well-known histone demethylase, we performed DNA microarrays using siRNA. Additionally, we performed chromatin conformation capture assay to identify the chromatin structural change under hypoxia.

Results: HIF1 ChIP-seq clarified that HIF1 mainly binds to the intergenic regions distal from transcriptional starting sites under both normoxia and hypoxia. The temporal profiles of gene expression under hypoxia demonstrated that early hypoxia responsive genes are functionally associated with glycolysis including *GLUT3* (glucose transporter 3). HIF1 binds to the promoter and distal enhancers of *GLUT3* and we clarified that the distal HIF1 binding region enhances *GLUT3* expression by changing chromatin conformational structure via HIF1. *GLUT3* expression is reduced under hypoxia when HIF1 or KDM3A is knocked down, suggesting that *GLUT3* is regulated by both HIF1 and KDM3A. We performed ChIP using KDM3A antibody and confirmed that KDM3A is recruited to the enhancer regions of *GLUT3* in a HIF1-dependent manner. On the loci of *GLUT3* enhancers KDM3A demethylates dimethyl-H3K9, repressive mark of histone modifications, in order to up-regulate the expression of *GLUT3* under hypoxia. We also confirmed the endogenous interaction of HIF1 and KDM3A under hypoxia.

Conclusions: These findings provide novel insights into the epigenetic regulation of HIF1 via chromatin conformational change and interaction with KDM3A.

Funding: Government Support - Non-U.S.

TH-PO199

Silencing of the Insulin Receptor Attenuates Cellular Accumulation of Fibronectin in Renal Mesangial Cells Naohiro Yano,[1] Daisuke Suzuki,[2] Masayuki Endoh.[2] *[1]Pediatrics, Women & Infants Hospital, Providence, RI; [2]Nephrology and Metabolism, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: Inslin receptor and insulin signaling proteins are widely distributed throughout the kidney cortex. The insulin signaling can act in the kidney in multiple ways, some of which may be totally independent of its primary role of the maintenance of whole-body glucose homeostasis. However, descriptions of the insulin signaling in the mesangial cells (MCs) are quite limited and the roles of insulin signaling in MC functions have not been sufficiently elucidated.

Methods: Stable trasfection of gene specific single hairpin (sh)-RNA was used to silence InsR expression in MCs. Activities of kinases and expression of related proteins were evaluated by western blotting, in vitro PI3K assay, Ras pull-down assay, immunoprecipitation and RT-PCR. Genetic and pharmacological manipulations were employed to modify signaling pathways.

Results: InsR silencing induced a unique phenotype of reduced fibronectin (FN) accumulation in renal glomerular MCs. Transcription level of FN showed no significant changes in the InsR silenced cells, suggesting the phenotype switching was caused by post-transcriptional modification. The decreased expression of InsR was associated with enhanced activity of insulin-like growth factor-1 receptor (IGF-1R)/PI3K/Akt signaling pathway which contributed in part to the attenuation of the cellular FN accumulation. Formation of IGF-1R homodimer was increased in InsR silenced cells. InsR silenced cells also showed increased sensitivity to exogenous IGF-1, and increased PI3K activity was reversed significantly by incubating cells with IGF-1R specific antagonist, AG538. PI3K/Akt dependent activation of cAMP response element-binding protein (CREB)-1 induced expression of matrix metalloproteinase (MMP)-9 and suppressing MMP activity by doxycycline partially reversed FN accumulation in InsR silenced cells.

Conclusions: Effects of InsR silencing on cellular FN accumulation are, at least partially, mediated by increased degradation of FN by MMPs which is induced by enhanced signaling sequence of IGF-1R/PI3K/Akt/CREB-1.

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TH-PO200

TonEBP Stimulates NF-κB via Direct Interaction and Promotes Inflammatory Action of TLR2 and 4 Satoru Sanada, H. Moo Kwon. *Division of Nephrology, University of Maryland, Baltimore, MD.*

Background: TonEBP (Tonicity-responsive enhancer binding protein) enables cellular adaptation to hypertonic stress by stimulating transcription of its target genes. Under isotonic conditions, however, TonEBP is involved in the pro-inflammatory gene expression following TLR2 and 4 (Toll-like receptors 2 and 4) ligation. We hypothesize that TonEBP stimulates nuclear factor-κB (NF-κB) by direct interaction and recruitment of its powerful transactivation domains. Stimulation of NF-κB contributes to the pro-inflammatory action of TonEBP.

Methods: Protein interaction was analyzed by co-immunoprecipitation assays. NF-κB activity was assessed by reporter assays and quantitative PCR analyses of its target gene mRNAs.

Results: TonEBP and p65 co-immunoprecipitated in both directions demonstrating their interaction, which was maintained when they were expressed from cDNA constructs. Analyses of various recombinant constructs revealed that the interaction was between RHDs (Rel-homology domain = DNA binding domain) of TonEBP and p65. TonEBP lacking RHD (TonEBPdRHD) did not stimulate NF-κB. On the other hand, TonEBP lacking C-terminal quarter of RHD (TonEBPdIPT) retained the ability to stimulate NF-κB along with its ability to interact with p65, even though it lost TonEBP activity. Fusion of the TonEBP transactivation domains to p65 resulted in a dramatically higher transcriptional activity suggesting that recruitment of the TonEBP transcriptional domains led to stimulation of NF-κB. MEF cells established from mice homozygous for a deletion mutant in which C-terminal third of TonEBP was deleted in-frame displayed dramatically reduced NF-κB activity in association with reduced interaction between the mutant TonEBP and p65. Taken together, the data provide a strong support that TonEBP directly stimulates NF-κB by bringing its powerful transactivation domains.

Conclusions: TonEBP stimulates the transcriptional activity of NF-κB through direct interaction. Since TonEBP is a critical regulator in inflammatory diseases such as rheumatoid arthritis and atherosclerosis, we propose that TonEBP is a central regulator in inflammation due to its action on NF-κB.

Funding: NIDDK Support

TH-PO201

In Vivo Knockdown of TRPV4 Decreases Flow-Induced NO Production in the Thick Ascending Limb Pablo D. Cabral, Jeffrey L. Garvin. *Internal Medicine-Hypertension and Vascular Research Division, Henry Ford Hospital, Detroit, MI.*

Background: Nitric oxide (NO) is a potent regulator of blood pressure and renal function. In the thick ascending limb it inhibits sodium chloride transport. Transient receptor potential vanilloid 4 (TRPV4), a member of the TRPV family of cation channels, is activated by luminal flow in different types of cells. We have previously shown that luminal flow stimulates NO production in this nephron segment. Thus, we hypothesized that TRPV4 mediates flow-induced NO production in the thick ascending limb.

Methods: To test this hypothesis we examined the effect of reducing and enhancing TRPV4 activity by using: 1) the selective TRPV4 antagonist RN 1734; 2) an adenovirus-delivered TRPV4 small hairpin (sh) RNA; and 3) the selective TRPV4 agonist GSK1016790A on NO production in the absence and/or presence of luminal flow. The NO-sensitive dye DAF FM was used to measured NO production in isolated and perfused thick ascending limbs from Sprague Dawley rats.

Results: Increasing luminal flow from 0 to 20 nL/min stimulated NO production from 8 ± 3 to 45 ± 12 arbitrary units (AU)/min ($p<0.05$, $n=5$). In the presence of the TRPV4 selective antagonist RN 1734 (10 μM) flow did not increase NO production (from 11 ± 7 to 9 ± 2 AU/min, $n=4$). In the absence of luminal flow, activating TRPV4 using the TRPV4 selective agonist GSK1016790A (10 ηM) increased NO production 118 ± 52 % ($n=5$). We next tested whether decreasing TRPV4 expression blocks flow-induced NO production. Three days after in *vivo* delivery of an adenovirus expressing TRPV4 shRNA to the outer medulla, TRPV4 protein expression was decreased by 50 ± 5 % in thick ascending limbs lysates as measured by Western blot. Flow did not increase NO production in thick ascending limbs from TRPV4 shRNA transduced kidneys (26 ± 29 %, $n=5$).

Conclusions: From these data we conclude that TRPV4 activation mediates flow-induced NO production in the thick ascending limb.

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TH-PO202

ANGPTL4, a Mediator of Nephrotic Syndrome, Is Induced through a HIF1 alpha-PPAR beta/delta Axis under Hypoxia Tsuyoshi Inoue,[1,2] Imari Mimura,[1] Takahide Kohro,[2] Hiroyuki Aburatani,[2] Tatsuhiko Kodama,[2] Youichiro Wada,[2] Masaomi Nangaku.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Laboratory for Systems Biology and Medicine, University of Tokyo, Tokyo, Japan.*

Background: Angiopoietin-[zwj]like 4 (*ANGPTL4)* has drawn attention as a cause of minimal change nephrotic syndrome recently, but the mechanism of this gene induction has not been elucidated yet. ANGPTL4 regulates vascular permeability, lipid metabolism, and glucose metabolism.

Methods: We used epigenetic methods including Chromatin Immunoprecipitation (ChIP) assay with deep sequencing (ChIP-seq) analysis of HIF1α, PPARβ/δ, PolII and H3K27ac, and Chromosome Conformation Capture (3C)-PCR assay to dissect the molecular mechanism of HIF1α dependent gene expression.

Results: *ANGPTL4* is the most markedly induced gene extracted by microarray analysis (20.1-fold change) of human umbilical vein endothelial cells (HUVEC) under hypoxia (1%O_2 for 24h) vs normoxia. As *ANGPTL4* is also known to be induced by peroxisome proliferator-activated receptors (PPARs), we investigated the presence of crosstalk between PPARβ/δ and HIF signaling axes. Synergistic activation of *ANGPTL4* by PPARβ/δ ligand (GW501516) plus hypoxia was observed with HUVEC by quantitative RT-PCR (124 % higher than the additive) and this was also confirmed by reporter assay. The combination of ChIP-seq and reporter assay identified the functional hypoxia responsive element (HRE) and PPAR responsive element (PPRE) in the promoter and intron regions of *ANGPTL4*. In addition, 3C-PCR assay showed that spatial proximity of two responsive elements induced by two stimuli could be the molecular background of additive transcriptional induction, supported by temporal profile of acetylation of H3K27, the active enhancer marker in *ANGPTL4.*

Conclusions: These results imply that hypoxic and PPARβ/δ stimulations are important for activation of *ANGPTL4* transcription, and the synergistic activation is achieved by spatial proximity formation in *ANGPTL4 loci.*

Funding: Government Support - Non-U.S.

TH-PO203

Endoplasmic Reticulum Stress Induces Fibrogenesis by Activating C/EBP Homologous Protein (CHOP) Gang Li, Yordanka Ivanova, Meghan Clements, Steven R. Ledbetter, Anna Zuk. *Tissue Protection and Repair, Genzyme-Sanofi R&D, Framingham, MA.*

Background: Tubulo-interstitial fibrosis is a prominent feature of chronic kidney disease and is closely associated with progression to end-stage renal disease. Recent studies implicate tubular injury in facilitating fibrogenesis, yet the cellular mechanisms are incompletely understood. Using ischemic and nephrotoxic mouse models of acute kidney injury, we first show that the unfolded protein response (UPR), including IRE1α, ATF6 and CHOP, are markedly induced with tubular damage and renal dysfunction. However, CHOP induction, but not the other two UPR branches, correlates with progression of kidney fibrosis. Therefore, we examined how epithelial injury contributes to fibrogenesis and the effect of ER stress in mediating this process. Using in-vitro ATP depletion-repletion and toxin epithelial models to recapitulate tubular injury in vivo, we observed that epithelial injury induces ER stress, which causally leads to a fibrogenic response. Induction of pro-fibrotic and pro-inflammatory cytokines is significantly suppressed when CHOP expression is inhibited by siRNA, providing direct causal evidence that CHOP is a mediator of this response. Moreover, these cytokines are induced in a calcium- and IP3R-dependent manner in injured epithelial cells, indicating the role of CHOP-mediated ER calcium release in fibrogenesis. In support of the role of ER stress in mediating fibrosis, we also show that conditioned medium from the stressed epithelial cell promotes fibroblast activation, and this effect is markedly inhibited when epithelial CHOP expression is suppressed by siRNA. These data provide new insight into how ER stress in general, and the CHOP branch in particular, contributes to fibrogenesis. These findings suggest that interventions targeting CHOP and its subsequent downstream signaling may provide new opportunities to prevent kidney fibrosis.

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TH-PO204

A Protective Role for Extracellular Superoxide Dismutase in Adriamycin Nephropathy Roderick J. Tan,[1] Ying Li,[2] Li Li Zhou,[2] Tim D. Oury,[2] Youhua Liu.[2] *[1]Dept of Medicine, University of Pittsburgh, Pittsburgh, PA; [2]Dept of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Development of an effective therapy for chronic kidney disease (CKD) remains elusive, emphasizing the need for a greater understanding of its molecular pathogenesis. Current evidence suggests a role for oxidative stress (the imbalance between reactive oxygen species and antioxidants) in the progression of CKD. Extracellular superoxide dismutase (EC-SOD) is an antioxidant enzyme abundant in normal kidney whose role is to scavenge the superoxide free radical. Based on its unique extracellular localization, we hypothesized that EC-SOD plays a protective role against CKD and that EC-SOD is depleted from the kidney in disease states, ultimately leading to increased oxidative stress and renal damage.

Methods: To study CKD, we utilized a mouse model of adriamycin nephropathy, which recapitulates focal segmental glomerulosclerosis. After exposure to adriamycin via a single tail vein injection, mice were euthanized at various timepoints and kidneys recovered for RNA, protein, and histologic analysis. Serum and urine were collected for creatinine and albuminuria analysis, respectively. Homozygous EC-SOD null mice were similarly exposed to adriamycin and samples recovered for disease severity analysis.

Results: During the development of adriamycin nephropathy, we found that levels of EC-SOD were significantly decreased as early as one week after exposure to adriamycin. Depletion of this antioxidant was associated with development of disease as well as with increases in oxidative stress markers. To definitively assess EC-SOD's role in adriamycin nephropathy, we exposed homozygous EC-SOD null mice to injury. Null mice had significantly greater levels of albuminuria, serum creatinine, and histologic injury compared to wild type controls.

Conclusions: Depletion of EC-SOD from the kidney appears to be a pathologic mechanism underlying adriamycin nephropathy. Loss of EC-SOD is associated with progression of disease and measurable increases in oxidative stress. Complete absence of EC-SOD leads to increased disease severity, indicating that it plays a critical role in the pathogenesis of adriamycin nephropathy.

Funding: NIDDK Support

TH-PO205

Involvement of NADPH-Derived Reactive Oxygen Species in the Down-Regulation of Na/K-ATPase in Chronic Kidney Disease Yu Wang, Liqiang Meng, Lei Qu, Jiawei Tang, Xiaomei Li. *Renal Division, Dept. of Medicine, Institute of Nephrology, Beijing, China.*

Background: The Na/K-ATPase contributes to sodium reabsorption of kidneys by regulating transcellular Na transport. The purpose of this study was to observe the changes of Na/K-ATPase expression under CKD status and the possible involvement of NADPH-derived ROS in this particular regulation.

Methods: Renal biopsy specimens of CGN patients were studied. UUO model was made in male Wistar rats and apocynin 100ug/kg was administered by oral gavage once daily. The expression of NOX2, NOX4, p22-phox and a1 Na/K-ATPase in obstructed kidneys were detected by Western blot. LLC-PK1 cells were treated with glucose oxidase.

Results: 28 CGN patients with different eGFRs were studied. Compared to normal kidneys, the tubular expression of a1 Na/K-ATPase (percentage of positive area) in CKD patients was significantly decreased [2.1% (0.5%-6.2%) vs. 5.6% (3.5%~10.8%), P=0.003]. Consecutive staining and double immunofluorescence staining of both a1 Na/K-ATPase and aquaporin 1 suggested that the decrease of a1 Na/K-ATPase was mostly derived from proximal tubules. Furthermore, semi-quantification of a1 Na/K-ATPase staining was positively correlated with the urinary sodium excretion of those patients (r=0.551, p=0.002). There was also decreased staining of a1 Na/K-ATPase in UUO rats. Western blot confirmed the early down-regulation of a1 Na/K-ATPase at 24 hours after surgery ($p<0.01$). Meanwhile, there was increased expression of NOX2 (1.00±0.20 vs 1.45±0.42, $p<0.05$), NOX4 (1.00±0.31 vs 1.99±0.54, $p<0.01$) and p22-phox (1.00±0.17 vs 1.29±0.11, $p<0.05$), which was inhibited by 33%, 40% and 30%, separately, with the treatment of apocynin. Simultaneously, the decrease of the Na/K-ATPase expression was significantly prevented, as compared to UUO group (0.96±0.07 vs 0.69±0.05,$p<0.01$). Glucose oxidase treatment induced endocytosis of cell surface Na/K-ATPase and down regulated the Na/K-ATPase protein levels in cultured LLC-PK1 cells (1.00±0.00 vs 0.85±0.06,$p<0.01$).

Conclusions: Na/K-ATPase is down-regulated in CKD. ROS, at least partly derived from NADPH, may participate this regulation.

Funding: Government Support - Non-U.S.

TH-PO206

Renal GABA Prduction, Release and Locally-Functional GABA Signaling in Rodents Junichi Yatabe,[1] Midori Sasaki Yatabe,[1] Kozue Takano,[1] Tsuyoshi Watanabe,[1] Robin Allen Felder,[2] Pedro A. Jose,[3] Hironobu Sanada,[4] Junko Kimura.[1] *[1]Fukushima Med. Univ., Japan; [2]Univ. of Virginia; [3]Univ. of Maryland; [4]Fukushima Welfare Federation of Agricultural Cooperatives, Japan.*

Background: Gamma-aminobutyric acid (GABA) administration induces natriuresis and lowers blood pressure. However, the mechanism has not been fully elucidated. Therefore, the GABAergic system and a possible local GABA signaling in the kidney were examined.

Methods: Distribution of GABA-producing enzyme (GAD67) was observed using GAD67-EGFP hetero knock-in mice. Urinary and plasma GABA was measured by HPLC. GABA release from kidney slices were assayed using immobilized enzyme-linked photoanalysis. GABA receptor expressions were detected by RT-PCR, immunoblotting and immunostaining. GABA(B) signaling was assayed by phospho-MAPK detection.

Results: GAD67-EGFP fluorescence was observed in the kidney cortex, with stronger signal in young (4-days old) compared to old (6-months old) mice. GABA concentration in rat urine was approximately 100 times that of plasma. Real-time GABA imaging revealed GABA release from kidney slices in responses to Ang II and dopamine. These suggest GABA production and regulated secretion in the kidney. As for GABA receptors, GABA(A) α1, β3 and π subunits, GABA(B) R1, R2 subtypes and GABA(C) ρ1, ρ2 subunits were found in rat renal cortex. In cultured rat mesangial and proximal tubule cells, GABA(A) α1 expression was approximately 1000 times higher in proximal tubule cells, and GABA(A) α3 expression was approximately 16 times higher in mesangial cells. Both types of cells also expressed GABA(B) R1 and R2. Response downstream of GABA(B) receptor was confirmed in proximal tubule cells, as baclofen, a GABA(B) receptor agonist, phosphorylated p42/44 MAPK in a time- and dose-dependent manner.

Conclusions: The results show the existence of a unique set of GABAergic system components in the kidney with characteristic expression patterns in different renal cell types. Locally-produced GABA may regulate ion transport or renal hemodynamics to modulate blood pressure in a paracrine/autocrine fashion.

Funding: Government Support - Non-U.S.

TH-PO207

Alterations of Oxygen Metabolism in Pathophysiology Prabhleen Singh,[1,2] Hai Pham,[2] Susanna Petrosyan,[1] Guy Perkins,[1] Weylin Wagnon,[1] Joseph Satriano,[1,2] Joseph Cameron Finley,[1,2] Sameh Ali.[1,2] *[1]UCSD; [2]VASDHS.*

Background: The kidney exhibits a high rate of oxygen consumption (QO2) and oxidative metabolism. Early in remnant kidneys, compensatory adaptations like hyperfiltration and hypertrophy can increase QO2. We examined the the alterations in QO2 and mitochondrial function in early subtotal nephrectomy (STN) and the impact of hypoxia inducible factor (HIF-1α) activation in STN.

Methods: In 1-week STN rats, renal blood flow (RBF), GFR, whole kidney QO2 along with mitochondrial QO2, reactive oxygen species (ROS) generation by electron paramagnetic resonance (EPR) and morphology by electron microscopy was examined. AMPK activation in the STN kidney and markers of cellular hypertrophy, autophagy and apoptosis were also examined. Finally, effects of HIF-1 α induction by dimethyloxaloylglycine (DMOG) treatment was examined. Data presented as mean±sem.

Results: Whole kidney QO2 factored for GFR was elevated in STN (0.08±0 vs. 0.05±0 ml/min, p=0.03), despite lower RBF and GFR. Mitochondrial QO2 in STN showed elevated State3ADP, State4oligomycin, and State3u rates (p<0.01). EPR revealed high ROS production in STN in state 3 (212±10 vs. 148±4 a.u., p=0.001) and state 4 (232±12 vs. 173±4, a.u., p=0.003). Morphological analyses revealed no differences in mitochondrial lengths, but fewer mitochondria (0.34±01. vs. 0.72±0.1 sq.microns, p=0.001) and mitochondrial volume density (7.4±1.1 vs. 18±2 %, p<0.001) in STN. However, cristae were more abundant in STN (1.4±0.1 vs. 1±0.1, p=0.008). In STN, high total AMPK, but low phosphorylated AMPK (pAMPK) levels were observed. Markers for hypertophy (p70S6k) and apoptosis (cleaved caspase-3) were elevated, while autophagy marker (LC3-II/LC3-1 ratio) was lower in STN. HIF induction with significantly improved several parameters including GFR, RBF, QO2/GFR, pAMPK levels and reduced markers for hypertrophy and apoptosis.

Conclusions: In the STN kidney, significant increases in QO2 were observed at the whole kidney and mitochondrial levels. ROS production was elevated and significant morphological changes in mitochondria were observed even in the very early stages. Several of these abnormalities were corrected by HIF induction.

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TH-PO208

Plasma Membrane (PM) Is a Biomechanical Sensor for Fluid Shear Stress (FSS) Mediated Signaling in Collecting Duct (CD) Cells Daniel Armando Flores,[1,2] Yu Liu,[2] Rajeev Rohatgi.[1,2] *[1]Medicine, James J. Peters VAMC, Bronx, NY; [2]Medicine and Pediatrics, Mount Sinai School of Medicine, New York, NY.*

Background: The PM is an inhomogenous mixture of phospholipids, glycolipids, and steroids with distinct regions of high viscosity/low fluidity (e.g., lipid rafts). Whereas cholesterol (chol) raises PM viscosity in localized regions to form lipid rafts, FSS reduces PM viscosity and stimulates signaling complexes embedded locally in the PM. We hypothesize that chol, through changes in spatial PM viscosity, differentially regulates FSS-induced signaling in CD cells.

Methods: Immortalized inner medullary CD3 (IMCD3) cells were exposed to a physiologic level of FSS. ATP and prostaglandin E2 (PGE2) were measured in the media bathing the cells before and after treatments that decreased (methyl-β-cyclodextrin[MβC], filipin) or increased (chol) PM viscosity.

Results: Compared to static controls (n=4), FSS (n=6-8) activates a robust increase in PGE2 (3.9±1.9 vs. 34.1±1.4 pg/mL/ug protein; p<0.05) and ATP(0.14±0.02 vs. 1.5±0.1 nM/ug protein; p<0.05) concentration in the media bathing IMCD3 cells. Pre-treatment of cells with MβC, a compound that chelates chol and decreases PM viscosity, increased the FSS-mediated PGE2 concentration (101.1±26.9 pg/mL/ug protein; n=4) compared to shear untreated cells in a concentration- and time-dependent manner. In contrast, MβC reduced medium ATP concentrations (0.47±0.01 nM/ug protein; n=3) in shear exposed IMCD3 cells. Incubation of cells with chol, to increase PM chol and viscosity, stimulated FSS-mediated ATP release by 86±3% (n=3) vs. that detected in untreated sheared cells, but did not alter FSS-mediated PGE2 secretion. The effect of filipin, which binds and disrupts chol, inhibited FSS-mediated ATP release, but did not affect FSS-induced PGE2 secretion. DCVJ, a viscosity sensitive flourophore, revealed that FSS decreased PM viscosity .

Conclusions: Specificity of FSS-activated signaling may be due to local variations in PM viscosity, which is related to PM chol content, and the signaling complexes embedded within the PM. We speculate that PM chol may regulate flow-induced PGE2 and ATP in the CD and, in turn, regulate Na excretion.

Funding: Veterans Administration Support

TH-PO209

Endoplasmic Reticulum (ER) Stress Signal Impairs Erythropoietin Production: A Role for ATF4 Chih-Kang Chiang,[1,2] Masaomi Nangaku,[1] Tetsuhiro Tanaka,[1] Reiko Inagi.[1] *[1]Div of Nephrol and Endocrinol, Univ of Tokyo Sch of Med, Tokyo, Japan; [2]National Taiwan University Hospital, Taipei, Taiwan.*

Background: Hypoxia induces hypoxia inducible factor (HIF) pathway and ER stress signal, unfolded protein response (UPR). The crosstalk of both signals orchestrates the cellular responses to hypoxia, suggesting the alteration of HIF target gene expression by UPR.

Methods: To assess the effect of UPR in transcription of erythropoietin (EPO), a representative HIF target gene, HepG2 were treated with ER stress inducers (tunicamycin or thapsigargin) and/or an ER stress modulator (salubrinal) under hypoxic conditions ($CoCl_2$ or 0.1% O_2). UPR state was estimated by expression of UPR regulator GRP78. EPO transcriptional level of HepG2 overexpressing the wild or mutated (loss of function) UPR transcription factor ATF4 was also assessed. The effect of ATF4 on EPO 3' enhancer activity, which is mainly regulated by HIF, was evaluated by the reporter and ChIP analyses.

Results: UPR activation by ER stress inhibitors suppressed hypoxia-induced EPO transcription in HepG2 and it was restored by normalization of the UPR state. Importantly, the decreased EPO expression was also observed in HepG2 overexpressing ATF4. Overexpression of mutated ATF4, which lacks transcriptional activity, did not alter EPO transcriptional regulation. Transcriptional activity of EPO 3' enhancer region was abolished by both ER stress inhibitors and ATF4 overexpression, while nuclear HIF accumulation or expression of other HIF target genes was not suppressed by ER stress. ChIP analysis identified a novel ATF4 binding site (TGACCTCT) within the EPO 3' enhancer region, suggesting a distinct role for ATF4 in UPR-dependent suppression of the enhancer. Induction of ER stress in rat liver and kidney by tunicamycin decreased the hepatic and renal mRNA, respectively, and plasma level of EPO.

Conclusions: ER stress, namely pathogenic UPR activation, selectively impaired the transcriptional activity of EPO but not of other HIF target genes. This effect was mediated by suppression of EPO 3' enhancer activity via ATF4 without any direct effect on HIF, indicating that UPR contributes to oxygen-sensing regulation of EPO.

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TH-PO210

Complement-Mediated Activation of Calcium-Independent Phospholipase $A_2\gamma$: Role of Protein Kinases and Phosphorylation Hanan Elimam, Tomoko Takano, Joan Papillon, Andrey V. Cybulsky. *Medicine, McGill University, Montreal, QC, Canada.*

Background: In experimental membranous nephropathy, complement C5b-9-induces glomerular epithelial cell (GEC) injury and proteinuria. The effects of C5b-9 are mediated via signaling pathways, including calcium-independent phospholipase $A_2\gamma$ (iPLA$_2\gamma$), and mitogen-activated protein kinases (MAPKs), that is extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. The iPLA$_2\gamma$ pathway is cytoprotective. We address the mechanisms of iPLA$_2\gamma$ activation.

Methods: Cultured GEC were stably transfected with iPLA$_2\gamma$ cDNA. GEC were incubated with antibody and normal serum (to assemble C5b-9) or heat-inactivated serum (control). To study MAPKs in iPLA$_2\gamma$ activation, COS-1 cells were transfected with iPLA$_2\gamma$ and cyclooxygenase-1 (COX-1). PLA$_2\gamma$ activity was monitored by quantifying prostaglandin E_2 (PGE_2) release.

Results: In GEC, GFP-iPLA$_2\gamma$ localized at the endoplasmic reticulum and mitochondria. Complement-mediated production of PGE_2 was amplified in GEC that overexpress iPLA$_2\gamma$, compared with control cells, and stimulated iPLA$_2\gamma$ activity was blocked by the iPLA$_2\gamma$ inhibitor, bromoenol lactone (BEL) in both control and iPLA$_2\gamma$-overexpressing GEC. In GEC that overexpress iPLA$_2\gamma$, complement-mediated PGE_2 production was reduced significantly by chemical inhibitors of MAPK/ERK kinase-1 (MEK1) and p38, but not JNK. In COS-1 cells that overexpress iPLA$_2\gamma$, PGE_2 production and ERK activation were induced by expression of constitutively active MEK1 or by stimulation with epidermal growth factor (EGF)+ionomycin, and the effect of EGF+ionomycin was inhibited by BEL. Stimulated iPLA$_2\gamma$ activity was not affected by mutations in iPLA$_2\gamma$ putative ERK phosphorylation sites (S168A, S271A and S168A/S271A), but was markedly attenuated by the S511A/S515A double mutation. Moreover, EGF+ionomycin induced S511 phosphorylation.

Conclusions: Complement-mediated activation of iPLA$_2\gamma$ is mediated via ERK and p38 pathways. Activation is dependent on phosphorylation of S511 and/or S515 by a kinase that remains to be identified. Defining the mechanisms by which complement stimulates iPLA$_2\gamma$ provides opportunities for development of novel therapeutic approaches to GEC injury and proteinuria.

Funding: Government Support - Non-U.S.

TH-PO211

HIV-Induced Epigenetic Factors Down Regulate Vitamin D Receptor (VDR) and Promote Tubular Cell DNA Damage Divya Salhan, Nirupama Chandel, Tejinder Singh, Gautam Kishore Valecha, Ashaan Subrati, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Epigenetic factors have been demonstrated to play a key role in the alteration of gene expression. We hypothesize that HIV-induced epigenetic factors will cause down regulation of tubular cell VDR and associated DNA damage in HIV-associated nephropathy (HIVAN).

Methods: To have a model of tubular cell HIV infection, primary human renal proximal tubular cells (HRPTC) were transduced with either empty vector (EV) or HIV (NL4-3) constructs. Measurement of VDR methylation status, cytosine phosphate guanosine (CpG) DNA methylation qPCR (Epitect Restriction) and methylation qPCR assay with VDR specific primers was carried out in EV/HRPTCs and HIV/HRPTCs. Immunoblotting and real time PCR studies were conducted for protein and mRNA expression for VDR and renin. Ang II ELISA was carried out on cells prepared under similar conditions. To determine DNA damage and repair EV/HRPTCs an d HIV/HRPTCs were co-labeled for H2AX and KU80. To confirm relationship between DNA methylation, VDR and DNA and tubular cell DNA damage EV/HRPTCs and HIV/HRPTCs were treated with either vehicle, a vitamin D receptor agonist (VDA, EB1089, 10 nM), or a demethylating agent (DMA; azacytidine, AZAC, 5 μM) for 24 hours and evaluated for DNA damage and repair.

Results: HIV/HRPTCs displayed two fold enhanced CpG methylation of VDR, 2.5-fold increase in DNA methyl transferase (Dmnt)-1 expression, 3-fold down regulation of VDR and activation of the RAS. Similarly, siRNA-VDR/HRPTCs displayed activation of the RAS. Both DMA as well as VDA inhibited the effect of HIV on tubular cell VDR expression. HIV/HRPTC not only displayed enhanced double strand breaks but also showed diminished DNA repair when compared to EV/HRPTCs. On the other hand, Both DMA and VDA not only enhanced DNA repair but also attenuated DNA damage in HIV/HRPTECs.

Conclusions: These findings indicated HIV-induced VDR downregulation was mediated by HIV-induced DNA methylation. HIV-induced DNA damage was mediated through down regulation VDR. Both DMA and VDA provided protection against HIV-induced tubular cell DNA damage.

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TH-PO212

Hyperglycemia Accelerates HIV-Associated Nephropathy through Downregulation of Vitamin D Receptor Partab Rai, Dileep Kumar, Andrei Plagov, Rivka Lederman, Guohua Ding, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Patients with HIV infection are living almost a normal life and are thus prone to develop diabetes mellitus (DM) and its associated complications. We hypothesized that development of hyperglycemia in HIVAN (HIV-associated nephropathy) mice would exacerbate the progression of HIVAN through down regulation of renal cell vitamin D receptors (VDR) and associated activation of the renin-angiotensin system (RAS).

Methods: Four weeks old control (C) and Tg26 (HIV) mice (n=4) were either administered vehicle or streptozotocin (STZ, 150 mg/Kg, intraperitoneal, single dose). At the end of two weeks, mice were sacrificed. Blood and urine were collected and kidneys were harvested for renal histology, imunohistochemical, and immunoblotting studies for VDR, renin and angiotensinogen (Agt) expression. In addition, renal tissue Ang II content was measured by ELISA. In *in vitro* studies, podocytes and tubular cells were incubated in media containing either 5 mM or 30 mM glucose for 24 hours. Immunoblots were probed VDR, renin, Agt and reprobed for actin. Renal tissues and cells were also probed for occurrence of oxidative stress.

Results: STZ-treated mice (C-STZ) developed higher proteinuria vs. control (C) mice (C, 0.3 ± 0.08 vs. C-STZ, 3.1 ± 1.0 mg/g creatinine; P<0.01); STZ-treated Tg26 mice (HIV-STZ) displayed greater proteinuria vs. vehicle-treated Tg26 mice (C-HIV) (HIV-STZ, 24.0 ± 0.8 vs. C-HIV, 15.7 ± 1.1 mg/g creatinine; P<0.01). HIV-STZ displayed higher BUN levels when compared to C-HIV (C-HIV, 48 ± 2 vs. HIV-STZ, 59 ± 5 mg/dl; P<0.05). Renal tissues of C-HIV displayed down regulation of VDR, activation of the RAS (including higher renal tissue Ang II levels) and increased oxidative DNA damage vs control mice; however HIV-STZ displayed increased (two-fold) downregulation of VDR and higher activation of the RAS (including renal tissue Ang II levels) when compared to C-HIV mice.

Conclusions: Development of hyperglycemia in HIVAN mice accelerated the progression of HIVAN by renal tissue down regulation of VDR and enhanced activation of the RAS.

Funding: NIDDK Support

TH-PO213

p66ShcA Deficiency State Downregulates HIV-Induced Tubular Cell Activated mTOR Pathway Partab Rai,[1] Andrei Plagov,[1] Mohammad Husain,[1] Ashwani Malhotra,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Background: Both mammalian target of rapamycin (mTOR) and p66ShcA pathways have been demonstrated to play an important role in the developmen of HIVAN. We asked whether there was any relationship between mTOR and p66ShcA pathways.

Methods: Four weeks old control and Tg26 mice were bred with $p66^{+/-}$ mice and a colony heterozygous p66; Tg26 mice was developed. Renal function and histology was evaluated in 8 weeks old control, Tg26, $p66^{+/-}$ and Tg26;$p66^{+/-}$. Immunoblots of protein lysates from renal tissues of control, Tg26, $p66^{+/-}$ and Tg26;$p66^{+/-}$ were probed for phospho-mTOR, S706 Kinase, phospho-eEF2, phospho- eIF4B, and phospho-4EBP-1. The same blots were stripped and reprobed for total mTOR and actin. Renal cortical sections of all mice were labeled for 8-Oxo-dG. Immortalized differentiated human podocytes (IDHPS) were transduced with either empty vector or HIV (NL4-3) constructs. Subsequently, EV/IDHPs and HIV/IHDPs were transfected with siRNA- p66 the role of reaactive oxygen species (ROS), HIV/IHDPs were incubated in media containing either buffer or superxoxide dismutase (SOD, 500 U) for 48 hours. Immunoblots were probed for mTOR pathway.

Results: Renal tissues of Tg26 mice displayed activation of mTOR pathway and associated down stream signaling when compared to control mice; on the other hand Tg26;$p66^{+/-}$mice as well as $p66^{+/-}$ mice displayed attenuated expression of phoshpho-mTOR and associated down stream molecules. Renal cortical sections of Tg26 mice displayed enhanced number of 8-Oxo-dG +ve nuclei when compared to Tg26;$p66^{+/-}$ mice as well as $p66^{+/-}$ mice. siRNA-p66/IDHP and siRNA-p66-HIV/IDHPs displayed attenuated expression of phospho-mTOR and associated down stream molecules. SOD also attenuated the activation of mTOR pathway in HIV/IDHPs.

Conclusions: These findings indicate that p66ShcA-induced ROS generation contributed to HIV- induced mTOR pathway activation in HIVAN. Conversely, deficit of p66ShcA-induced not only attenuated oxidative stress but also inhibited activation of mTOR pathway.

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TH-PO214

Caspase-3 Acts Downstream of Wnt11 and Stimulates Branching Morphogenesis by Activating Rho-Associated Protein Kinase 1 and Inhibiting β Catenin Signaling Midori Awazu,[1] Yoshifumi Yamaguchi,[2] Michio Nagata,[3] Masayuki Miura,[2] Mariko Hida.[1] *[1]Department of Pediatrics, School of Medicine, Keio University, Tokyo, Japan; [2]Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; [3]Renal and Vascular Pathology, University of Tsukuba, Tsukuba, Japan.*

Background: Inhibition of caspase-3 leads to reduced ureteric bud branching and kidney size in organ culture. Caspase-3 is known to activate Rho-associated protein kinase 1 (ROCK-1) and inactivate ß catenin, critical molecules in ureteric branching, by cleavage. The metanephroi cultured with caspase-3 inhibitors closely resemble those of mice deficient in Wnt11, a noncanonical Wnt ligand. We thus investigated the role of caspase-3 in Wnt11 signaling and in branching morphogenesis.

Methods: Phosphorylation of myosin phosphatase targeting subunit 1 (P-MYPT1), a regulator of actin cytoskeleton organization, at threonine 696 by ROCK1 and ß catenin were detected by immunoblot. Cell migration was assessed by wound healing assay. Caspase-3 activity was detected by carboxyfluorescein caspase-3 detection kit. Glomerular number of caspase-3 knockout mice (Casp3-/-) was determined by acid maceration at 3 weeks.

Results: Activation of caspase-3 was observed at the wound-edge UB cells at 4 h after scratching, which disappeared by 24 h when the wound was almost closed. A caspase-3 inhibitor Ac-DNLD-CHO (DNLD) inhibited wound healing dose-dependently. Recombinant Wnt11 increased caspase-3 activity in UB cells with no signs of apoptosis. P-MYPT1 was increased and stabilized ß catenin was decreased by Wnt11. These changes were completely reversed by DNLD. Wnt11 enhanced UB cell wound healing, which was inhibited by DNLD, lithium chloride, and a ROCK inhibitor. Casp3-/- had 30% fewer glomeruli than heterozygotes (Casp3+/-). At embryonic day 13, ureteric bud tip numbers (10±3 vs 36±6) and kidney surface area (60%) were reduced in Casp3-/- compared with Casp3+/-. In kidney lysates from embryonic day 15 Casp3-/-, P-MYPT1 was markedly reduced and ß catenin was increased compared with Casp3+/-.

Conclusions: Caspase-3 acts downstream of Wnt11 and mediates ureteric branching *via* modulation of ROCK1 and ß catenin.

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TH-PO215

Hypoxia and Aldosterone Stimulate Erythropoietin Production in Intercalated Cells of the Collecting Ducts Takanori Nagai,[1] Kahori Hori,[1] Yuichiro Izumi,[2] Yushi Nakayama,[3] Yukiko Hasuike,[1] Takahiro Kuragano,[1] Masayoshi Nanami,[4] Akito Tanoue,[5] Katsumasa Kawahara,[6] Kimio Tomita,[3] Takeshi Nakanishi,[1] Hiroshi Nonoguchi.[7] *[1]Kidney and Dialysis, Hyogo Col Med, Japan; [2]Systems Biology Center, NHLBI, NIH; [3]Nephrol, Kumamoto Univ, Japan; [4]Renal Div, Emory Univ; [5]Pharmacol, Nat Res Inst for Child Health, Japan; [6]Physiol, Kitasato Univ, Japan; [7]Int Med, Kitasato Univ Kitasato Inst Med Cent Hosp, Japan.*

Background: Erythropoeietin (Epo) production has been suggested to occur in the peritubular interstitial cells and/or in the nephron. The role of vasopressin and aldosterone for Epo production is not known. The purpose of our study is to examine the effect of vasopressin and aldosterone on Epo production and to identify the Epo production in intercalated cells of the collecting ducts.

Methods: Vasopressin V1a receptor knockout (V1aR-KO), wild type (WT) mice, and rat intercalated cell line (IN-IC cells) were used for Western blot, ELISA, real time PCR and immunohistochmistry of Epo after stimulation by hypoxia or aldosterone. HepG2 cells were also examined as a positive control to confirm the effect of hypoxia on Epo production. Rat kidney slices were also used for immunohistochemistry of Epo.

Results: Plasma hemoglobin levels were not different between WT and V1aR-KO mice. However, the administration of fludrocortisone increased plasma Epo (ELISA) in WT by 3 folds but slightly in V1aR-KO mice. Epo and Epo-receptor mRNAs were detected in rat kidney and IN-IC cells. Hypoxia (1% O2) increased Epo production (ELISA) by 50-60% and Epo abundance in cytoplasm (immunostaining) in IN-IC cells. Western blot showd a band at 42kDa and it was increased by hypoxia. Aldosterone dose-dependently increased Epo mRNA and protein in IN-IC cells. Aldosterone increased Epo abundance (immunostaining) in IN-IC cells. Knockdown of V1aR gene abolished aldosterone-induced Epo production. Immunohistochemistry of Epo showed a staining of the collecting ducts, especially in the deeper portion, as well as peritubular interstitial cells in rat.

Conclusions: Epo production is regulated by aldosterone and vasopressin in intercalated cells of the collecting duct.

Funding: Government Support - Non-U.S.

TH-PO216

Endothelin Converting Enzyme (ECE)-1: A Plausible Target Gene for Hypoxia Inducible Factor (HIF) Mogher Khamaisi,[6] Imari Mimura,[2] Hala Toukan,[6] Christian Rosenberger,[3] Jonathan H. Axelrod,[1] Ahuva Shina,[1] Galia Skarzinsky,[1] Robert Koesters,[7] Gail Walkinshaw,[4] Seymour Rosen,[5] Masaomi Nangaku,[2] Samuel N. Heyman.[1] *[1]Hadassah Hebrew University Hospitals, Jerusalem, Israel; [2]University of Tokyo School of Medicine, Japan; [3]Carite, Berlin, Germany; [4]FibroGen, CA; [5]BIDMC Harvard, Boston, MA; [6]Rambam Medical Center - Technion, Haifa, Israel; [7]Tenon Hospital, University of Paris 6, France.*

Background: Renal endothelin converting enzyme (ECE)-1, a key regulator of endothelin synthesis, is induced in experimental diabetes and following radiocontrast administration (Khamaisi, KI 2008), conditions characterized by renal hypoxia, HIF stabilization and enhanced endothelin synthesis. We have also reported cross-stimulation of HIF and p-STAT3 in vivo (ASN 2009). We now explored the possibility that ECE-1 might be a HIF/STAT3-target gene by their selective induction.

Methods: HIF stimulation was induced in rats subjected to the HIF prolyl-hydroxylase inhibitors mimosine or FG-4497, or in conditional Von Hippel Lindau knock-out (VHL KO) mice. Renal tubular p-STAT3 was induced in mice transfected by or injected with a chimeric IL-6/IL6-receptor protein (HIL-6, Nechemia-Arbely, JASN 2008). ChIP seqencing was performed in normoxic and hypoxic cultured human umbilical vascular endothelial cells (HUVEC).

Results: Mimosine and FG-4498, as well as HIL-6 in rats and mice, respectively, led to renal HIF-1α and p-STAT3 co-expression, principally in distal nephron segments. HIF-1α and p-STAT3 co-stimulation was associated with markedly enhanced ECE-1 protein expression, predominantly in the inner and outer medulla. Immunostaining confirmed enhanced ECE-1 expression principally in distal nephron segments following mimosine and in conditional VHL KO mice, which develop intense HIF-1α and p-STAT3 expression over time. Finally, ChIP-seq of HIF-1 binding sites, performed in cultured HUVEC subjected to hypoxia, revealed HIF attachment to the promoter and intron regions of the ECE-1 gene, supporting the possibility that it triggers ECE-1 under hypoxia.

Conclusions: Collectively, these findings suggest that ECE-1 expression might be induced by HIF and p-STAT3 co-stimulation.

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TH-PO217

TGF-β/Smad3 Activates Mammalian Target of Rapamycin Complexes to Promote Kidney Fibrogenesis Benaya Rozen-zvi, H. William Schnaper. *Pediatrics, Northwestern Univ. Feinberg School of Medicine, Chicago, IL.*

Background: The transforming growth factor (TGF)-β pathway activates mammalian target of Rapamycin (mTOR) in human mesangial cells (HMC) and fibroblasts. mTOR inhibition is antifibrotic in some animal models of kidney disease. We investigated the pathways by which TGF-β activates mTOR and the role of mTOR activity in TGF-β-induced fibrogenesis.

Methods: mTOR activity was evaluated in TGF-β-treated HMC by Western blot for phosphorylated S6 kinase-1 and S6 ribosomal protein. Collagen expression was evaluated by reporter assay and western blot.

Results: TGF-β stimulated HMC mTOR activity within 10 minutes. The PI3K inhibitor wortmannin blocked both basal and TGF-β-induced mTOR activation, whereas blockade of TGFβ signaling by TβRI kinase inhibition abolished the TGF-β induced mTOR activation but did not reduce basal activity. Surprisingly, in Smad3-null mouse embryonic fibroblasts (MEF) basal mTOR activity was increased compared to wild type (WT) MEF but, unlike the WT MEF, there was no increase after stimulation with TGF-β. In human embryonic kidney cells (HEK), overexpressing WT Smad3 decreased basal mTOR activity but increased TGF-β-stimulated activity. On the other hand, overexpressing the dominant-negative Smad3A reduced both basal and TGF-β-stimulated mTOR activity. Rapamycin, a relatively selective mTOR complex 1 (TORC1) inhibitor, or shRNA for the TORC1 protein RAPTOR, reduced collagen-1 promoter activity and protein expression in HMC. This inhibition was fully reversible by expressing non-degradable (ND) hypoxia-inducible factor (HIF1)-1α or the hypoxia mimetic, desferoxamine mesylate (DFO). However, ND HIF-1α or DFO did not overcome the reduction in collagen1 promoter activity caused by either the non-selective mTOR kinase inhibitor, PP242, or shRNA for the TORC2 protein RICTOR .

Conclusions: mTOR signaling contributes to TGF-β-stimulated collagen expression. Inactive Smad3 may reduce basal mTOR activity, whereas active Smad3 appears essential for TGF-β-stimulated TORC1 and TORC2 actions. Activation of TORC1 promotes kidney fibrogenesis, probably by facilitating normoxic HIF activity, while TORC2 stimulates fibrogenesis in a separate pathway.

TH-PO218

Dual RAS Blockade Normalizes Renal Tubular Atrial Natriuretic Peptide and Angiotensin-Converting Enzyme-2 Expression in Angiotensinogen-Transgenic Mice Chao-Sheng Lo,[1] Shyi-jang Shin,[2] Shiao-ying Chang,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[3] Julie R. Ingelfinger,[4] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hospital, Montreal, QC, Canada; [2]Endorinol & Metab, Kaohsiung Med Univ Hosp, Kaohsiung, Taiwan; [3]Res. Ctr., Maisonneuve-Rosemont Hosp, Montreal, QC, Canada; [4]Pediatr Nephrol Unit, Mass Gen Hosp, Boston, MA.*

Background: Atrial natriuretic peptide (ANP) is an endogenous angiotensin II (Ang II) antagonist that acts both systemically and locally. In the present study, we investigated the interaction of Ang II, ANP and angiotensin-converting enzyme-2 (Ace-2) in renal proximal tubular cells (RPTCs), and the effect of dual RAS blockade on renal ANP and Ace-2 expression in transgenic (Tg) mice with specific overexpression of angiotensinogen (Agt) in their RPTCs.

Methods: Adult male Agt-Tg mice (age 13 weeks) were treated ± RAS blockers (losartan, 30 $mg.Kg^{-1}.day^{-1}$ and perindopril, 4 $mg.Kg^{-1}.day^{-1}$ in drinking water). Untreated non-Tg littermates served as controls. Systolic blood pressure (SBP) and albuminuria were monitored bi-weekly up to age 20 weeks. Kidneys were processed for immunostaining of Agt, ANP and Ace-2. Renal proximal tubular (RPT) Agt, ANP and Ace-2 mRNA were evaluated by real-time quantitative polymerase chain reaction (RT-qPCR). Urinary Ang II and Ang 1-7 levels were quantified by ELISA. We also examined ANP and Ace-2 mRNA expression in immortalized rat cultured RPTCs that were stably transfected with the control plasmid pRC/RSV or the plasmid containing the rat Agt cDNA (pRSV/Agt).

Results: SBP was significantly higher at 20 weeks in male Agt-Tg as compared to non-Tg and was normalized with RAS blockade. Furthermore, both ANP and Ace-2 mRNA and protein expression were decreased in RPTs of Agt-Tg and normalized by dual RAS blockade. Urinary Ang II and Ang 1-7 levels were respectively increased and decreased in Agt-Tg mice, and were normalized with RAS blockade. *In vitro*, ANP and ACE2 mRNA expression was reduced in RPTCs stably transfected with pRSV/Agt as compared to control RPTCs.

Conclusions: These data demonstrate that overexpression of Agt inhibits renal ANP expression via Ang II-induced down-regulation of Ace-2 and Ang 1-7 expression.

Funding: Government Support - Non-U.S.

TH-PO219

GPR40 Serves as a Functional Receptor for EETs Seong Kwon Ma,[1,2] Jianchun Chen,[1] Raymond C. Harris,[1] Jian-Kang Chen.[1] *[1]Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN; [2]Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: The cytochrome P450 epoxygenase-dependent arachidonic acid metabolites, epoxyeicosatrienoic acids (EETs), are potent mitogens for renal tubular epithelial cells, but the potential functional receptor(s) mediating the biological effect of EETs has remained elusive.

Methods: siRNA was used to knock down GPR40 expression and assess its effect on EET-induced phosphorylation of extracellular signal-regulated kinase (ERK) in the renal proximal tubular epithelial-like cell line, LLCPKcl4. A human GPR40 cDNA construct was stably transfected into the human embryonic kidney cell line, HEK293.

Results: Knocking down GPR40 with siRNA inhibited EET-induced ERK phosphorylation in LLCPKcl4 cells. GPR40 overexpression in HEK293 cells significantly enhanced EET-induced cell proliferation and markedly augmented epidermal growth factor receptor (EGFR) phosphorylation and ERK activation, which were inhibited by either the EGFR tyrosine kinase inhibitor, AG1478, or the HB-EGF inhibitor, CRM197. In hGPR40-transfected HEK293 cells, EETs significantly enhanced HB-EGF release into the culture medium. RT-PCR indicated GPR40 mRNA expression in murine kidney. Immunoblotting revealed that GPR40 is primarily expressed in the renal cortex and outer stripe of outer medulla (OSOM), with minimal expression in the inner medulla and papilla. Immunohistochemistry indicated high expression in the OSOM and a subset of cortical tubules, and immunofluorescence staining with nephron segment specific markers confirmed that GPR40 is highly expressed in S2 and S3 segments of the renal proximal tubules, with moderate expression in cortical collecting ducts.

Conclusions: This study provides the first demonstration that GPR40 serves as a functional receptor to mediate the mitogenic signaling of EETs. GPR40 is highly expressed in S2 and S3 segments of the renal proximal tubule, the nephron segments previously reported to express the highest level of EET synthase (cytochrome P450 epoxygenase) in the kidney and in cortical collecting duct, which has previously been shown to be a target for EET regulation of sodium reabsorption.

Funding: NIDDK Support

TH-PO220

Imbalance of CCN2/CCN3: A Novel Mechanism for Glomerular Matrix Accumulation in Experimental Glomerulonephritis Long Chen, Dan Liu, Linli Lv, Haifeng Ni, Bi-Cheng Liu, Jiandong Zhang. *Institute of Nephrology, Southeast University, Nanjing, China.*

Background: Members of CCN family proteins such as CCN2 and CCN3 play important but controversial role in remodeling extracellular matrix. Our recent work suggested that divergent alteration in glomerular expression of CCN2/CCN3 associated with rapid expansion of mesangial matrix in experimental models. Furthermore, the imbalance

of CCN2/CCN3 correlated with glomerular histological changes in patients with chronic kidney diseases. Therefore, we hypothesize that imbalance of CCN2/CCN3 contributes to the glomerular matrix accumulation in experimental glomerulonephritis.

Methods: To test this, we first administered maximal dose of Enalapril (100mg/L) to nephritic rats between day 4 and day 6 after injection of OX-7 Ab.

Results: Enalapril treatment substantially normalized the imbalance of CCN2/CCN3 in experimental glomerulonephritis(reducing CCN2(by 37.7% in mRNA, by 63.8% in protein); increasing CCN3(by 165.6% in mRNA, by 191.9% in protein), accompanying with the repression of glomerular matrix expansion (43.2±3.12 %vs. 65.2±3.12%, p<0.05), and proteinuria by 54.5% (66.0±8.1 vs. 30±8.3 mg, p<0.05). Next, we decided to directly test the individual contribution of CCN2/3 in matrix accumulation by using cultured glomeruli. Depressing CCN2 expression in glomeruli by specific siRNA by 85% in cultured nephritic glomeruli reduced mRNA expression of fibrotic markers PAI-1(49.2%), TGFß1 (23.6%),FN(24.5%), comparably to recombinant CCN3 protein(10ng/ml). Additionally, combination therapy led to a further reduction in disease markers such as production of TGFß1 protein by additional 40.3% to CCN2 siRNA alone (217.7±14.8 vs. 128.9±6.2 pg TGFß1/5000 glomeruli, p<0.05). Furthermore, recombinant CCN3,but not CCN2 siRNA gained "add-on" effects to maximal dose of enalapril treatment in cultured glomeruli(273.3±6.9 vs. 185.6±2.2 pg TGFß1/5000 glomeruli, p<0.05).

Conclusions: Collectively, these results demonstrate that imbalance of CCN2/CCN3 plays critical role in the accumulation of glomerular matrix in experimental glomerulonephritis, and posit CCN3 as a novel therapeutic means at least supplementing to Ang II inhibitor.

Funding: Government Support - Non-U.S.

TH-PO221

A Pro-Inflammatory Role of C5L2 in C5a-Primed Neutrophils for ANCA-Induced Activation Min Chen, Jian Hao, Ming Hui Zhao. *Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Background: The complement system is crucial for the development of ANCA-associated vasculitis (AAV). In particular, C5a and its receptor on neutrophils, CD88, play a central role. The function role of the second receptor of C5a, C5L2, remains unclear. In the current study, we investigated the role of C5L2 in C5a-primed neutrophils for ANCA-induced activation.

Methods: The effect of blocking C5L2 were tested on respiratory burst and degranulation of C5a-primed neutrophils activated with ANCA, as well as on membrane-bound proteinase 3 (PR3) and concentration of myeloperoxidase (MPO) in supernatant of C5a-primed neutrophils.

Results: Blocking C5L2 resulted in a decreased MPO concentration in C5a-primed neutrophils supernatant.mPR3 expression increased from 223.0±36.1 in untreated cells to 423±40.7 after C5a treatment (P<0.001) and decreased to 348.0±52.0, 323.4±57.9 and 313.8±49.7 by pre-incubating blocking C5L2 antibody at 2.5μg/ml, 5μg/ml or 10μg/ml (compared with that C5a-priming group, P<0.001, P<0.001, and P<0.001), respectively. In C5a-primed neutrophils, subsequently activating with MPO-ANCA-positive IgG, the MFI value was 348.1±198.2, which decreased to 306.7±193.7, 297.7±163.4 and 291.5±163.3 upon pre-incubation with mouse anti-human C5L2 blocking antibody at 2.5μg/ml, 5μg/ml or 10μg/ml (compared with that C5a-primed neutrophils, for MPO-ANCA-positive IgG induced activation, P<0.05, P<0.05, and P<0.05), respectively. Blocking C5L2 also resulted in a decreased C5a-primed neutrophils for PR3-ANCA-positive IgG induced activation. Moreover, the lactoferrin concentration decreased in pre-incubation with anti-human C5L2 blocking antibody, compared with that C5a-primed neutrophils induced by PR3- or MPO ANCA-positive IgG supernatant.

Conclusions: C5L2 may implicate in the pro-inflammatory role in C5a-primed neutrophils for ANCA-induced activation.

Funding: Government Support - Non-U.S.

TH-PO222

Transforming Growth Factor β1-Induced Vascular Endothelial Cell Growth Factor-D from Renal Proximal Tubule Cells and Macrophages Is Associated with Lymphangiogenesis in a Unilateral Ureteral Obstruction Won Kim, Aesin Lee, Dal Kim, Yujin Jung, Young Min Hong, Kyung Pyo Kang, Sik Lee, Sung Kwang Park, Jung Eun Lee. *Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Jeonbuk, Korea.*

Background: Lymphatic endothelial proliferation has been detected in tubulointerstitial fibrotic regions in rat remnant kidney and human kidney transplant. Vascular endothelial cell growth factor (VEGF)-D is a lymphangiogenic factor. However, there is few data about the role of VEGF-D in lymphangiogenesis in renal fibrosis.

Methods: We performed quantitative real-time RT-PCR and ELISA of VEGF-D, immunohistochemistry in a unilateral ureteral obstruction (UUO) model. We also evaluated role of VEGF-D in inhibitory effect of transforming growth factor β1 on VEGF-C-induced in vitro lymphangiogenesis.

Results: The number of LYVE-1-positive lymphatic vessels was increased after ureteral obstruction. VEGF-D mRNA and protein expression in the UUO kidney was significantly increased to sham-operated mice. VEGF-D mRNA expression was increased in macrophages (Raw 264.7 cells) or renal proximal tubule cells (HK-2 cells) by stimulation with TGF-β1. Treatment of Raw 264.7 cells or proximal tubule cells with TGF type 1 receptor inhibitors (SD208) and an inhibitor of TGF-β superfamily type I activin receptor-like kinase (ALK) receptors ALK4, 5, and 7 (SB431542) decreased TGF-β1-induced VEGF-D mRNA expression. Depletion of macrophages with clodronate decreased lymphangiogenesis in UUO kidney. Blockade of VEGF-D signaling after direct injection into the kidney with adenovirus encoding the soluble extracellular domain of VEGFR-3 (Ad-sVEGFR3) decreased the UUO-induced lymphangiogenesis. As TGF-β1 is a negative regulator in VEGF-C-induced lymphangiogenesis and migration, capillary-like tube formation and proliferation are important component in *in vitro* lymphangiogenesis, we evaluated the effect of VEGF-D. VEGF-D reversed the inhibitory effect of TGF-β1 on VEGF-C-induced migration, capillary-like tube formation and proliferation of human lymphatic endothelial cells.

Conclusions: These results indicate that VEGF-D is associated with lymphangiogenesis in fibrotic kidney in UUO mouse model.

Funding: Government Support - Non-U.S.

TH-PO223

Colony Stimulating Factors Regulates the Expressions of Interleukin 6, Tumor Necrosis Factor-alfa and Plasminogen Activator Inhibitor-1 Induced by Thrombin in Human Proximal Tubular Epithelial Cells in Culture Michiko Shimada, Yoshiko Shutto, Ikuyo Narita, Yuko Shimaya, Takeshi Fujita, Reiichi Murakami, Norio Nakamura, Hideaki Yamabe, Ken Okumura. *Nephrology, Hirosaki University, Hirosaki, Japan.*

Background: In the tubulointerstitial injury, fibrin deposition is commonly seen, therefore, the local activation of thrombin is suggested. Colony-stimulating factors (CSFs) are well-known hematopoietic growth factors. However, recent studies revealed that CSFs are involved in many inflammatory conditions. In the experimental anti-glomerular membrane glomerulonephritis, both glomerular lesions and tublointerstitial lesions were attenuated in the granulocyte-macrophage CSF (GM-CSF) knockout mice. We have recently reported that thrombin increased the production of M-CSF, GM-CSF and G-CSF in human proximal tubular epithelial cells (PTEC). In this study, we examined the role of CSFs in the pathorogic effects of thrombin in PTEC utilizing siRNA.

Methods: Primary human PTEC were purchased and used between passages 4-6. The cells were transfected with M-CSF, GM-CSF and G-CSF siRNA or non-coding control siRNA for the transient knockdown of the gene expression. 48hours later, cells were incubated with or without thrombin (5U/ml) for 6 hours and total RNA was harvested. The expressions of CSFs,Interleukin-6 (IL-6) and Tumor Necrosis Factor-alfa (TNF-alfa), Plasminogen Activator Inhibitor-1 (PAI-1) and Metalloproteinase-9 (MMP-9) were examined by quantitative RT-PCR.

Results: In PTEC, 6-hour incubation with thrombin (5U/ml) significantly increased mRNA expression of IL-6, (4.1±0.1fold, P<0.0001), TNF-alfa (3.3±0.3fold, P<0.001), PAI-1(2.74±0.2fold, P<0.001) and MMP-9 (3.3±0.9 fold, P<0.05) when transfected with non-coding siRNA. The augumented expression of IL-6, TNF-alfa, PAI-1 was significantly reduced by the knockdown of CSFs, whereas MMP-9 expression was not affected.

Conclusions: Locally increased CSFs play a significant role in regulating IL-6, TNF-alfa and PAI-1 which are all important factors for the tissue remodeling in the tubulointerstitium in the kidney.

Funding: Government Support - Non-U.S.

TH-PO224

Inducible CTGF (CCN2) Knockout Mice Attenuates Anti-Glomerular Basement Membrane Nephritis Naohiro Toda, Hideki Yokoi, Masato Kasahara, Kiyoshi Mori, Takashige Kuwabara, Hirotaka Imamaki, Kenichi Koga, Akira Ishii, Yukiko Kato, Keita P. Mori, Shoko Ohno, Akira Sugawara, Masashi Mukoyama, Kazuwa Nakao. *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: Connective tissue growth factor (CTGF/CCN2) is a matricellular protein that regulates signaling of other growth factors and promotes fibrosis. We previously showed that CTGF overexpression in podocytes aggravates diabetic nephropathy in mice and that CTGF is upregulated in glomeruli in anti-glomerular basement membrane (GBM) nephritis in rats. It is interesting to examine the phenotypes of CTGF knockout mice with renal diseases, however, perinatal death of the mice hampers invesitgation of CTGF in adult mice.

Methods: To address this problem, we generated inducible CTGF knockout mice. CTGF floxed mice were crossed with RosaCreER^{T2} mice, which ubiquitously express a tamoxifen-inducible Cre recombinase. We administered 4-hydroxytamoxifen (4-OHT) to 3-week old mice and then examined CTGF mRNA expression and histological changes in the kidney at 8 weeks of age. Next, we evoked anti-GBM nephritis in inducible CTGF knockout mice and investigated the progression of renal injury for 28 days.

Results: CTGF mRNA expression in the kidney was decreased by 77% with the treatment of 4-OHT. Inducible CTGF knockout mice showed normal renal appearance. After induction of anti-GBM nephritis, severe proteinuria and renal injury including mesangial expansion and crescents, were developed in fl/fl mice. In contrast, inducible CTGF knockout mice exhibited reduced proteinuria by 47% at day 7 with ameliorated histological changes at day 28. Analyses on glomerular expression revealed that upregulated expressions of TGF-β1, fibronectin, col1a1 and col4a3 in fl/fl mice were significantly reduced in inducible CTGF knockout mice.

Conclusions: These results indicate that the deficiency of CTGF can reduce proteinuria and ameliorate glomerular injuries in anti-GBM nephritis, suggesting that CTGF/CCN2 plays an important role in the progression of anti-GBM nephritis.

Funding: Government Support - Non-U.S.

TH-PO225

CCR2 Inhibition Ameliorates Renal Damage in Mice with 2-Kidney, 1-Clip Hypertension Stella Hartono, Bruce Knudsen, Joseph P. Grande. *Mayo Clinic, Rochester, MN.*

Background: Renal artery stenosis (RAS) affects 7% of individuals over 65 and up to 40% of individuals previously diagnosed with coronary or peripheral vascular disease. We have previously shown that induction of CCL2 precedes arrival of inflammatory cells in 129SV mice subjected to RAS. In this study, we further examined the contribution of CCL2/CCR2 signaling to the pathogenesis of renal artery stenosis in C57/BLKS mice. We hypothesize that abrogation of CCL2/CCR2 signaling prevents chronic renal damage in the stenotic kidney of mice with RAS.

Methods: RAS was established by placing a 0.2mm (internal diameter) polytetrafluoroethylene cuff on the right renal artery of C57/BLKS mice. Mice were either given a CCR2 antagonist or vehicle orally. After 2 weeks, kidneys were harvested and processed for histology or PCR.

Results: RAS induced the expression of CCL2 mRNA and protein in stenotic kidneys, which was followed by infiltration of macrophages and CD3+ T cells. Inhibition of CCR2 was associated with significant reduction of renal atrophy in the stenotic kidney. The expression of CCL2 was increased in mice treated with the CCR2 antagonist compared with vehicle treated mice. However, the number of infiltrating macrophages was markedly decreased in the RAS mice treated with CCR2 antagonist (0.4 vs. 1.4 cells/10HPF, $p<0.05$). CCR2-antagonist treatment did not affect the number of CD3+ T cells, and did not significantly reduce blood pressure (systolic BP 151±8 vs. 152±3mmHg, p = ns).

Conclusions: We conclude that CCL2/CCR2 signaling plays a key role in the pathogenesis of renal artery stenosis by inducing infiltration and activation of macrophages, and offers a potential therapeutic target for treatment of renal artery stenosis.

Funding: Other NIH Support - NHLBI Support

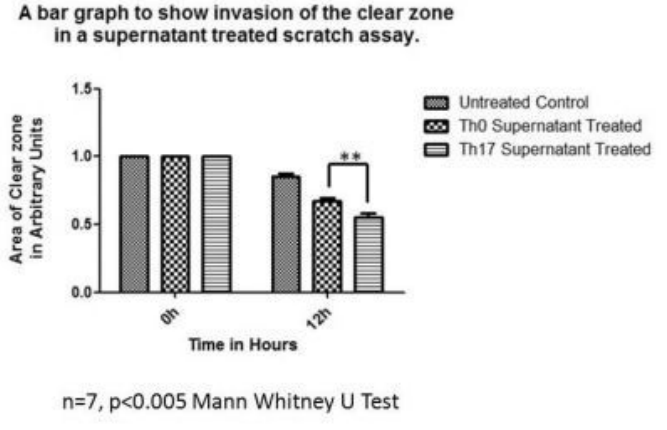
TH-PO226

Effect of Th17 Supernatant on the Motility of the Podocyte Carl James May,[1] Gavin Iain Welsh,[1] Richard Lee,[2] Moin Saleem.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]Academic Unit of Ophthalmology, University of Bristol, Bristol, United Kingdom.*

Background: Steroid Resistant Nephrotic Syndrome (SRNS) is a common renal disease whose pathophysiology is poorly understood. The 'circulating factor hypothesis' in the pathogenesis of the disease suggests that an abnormality of T-cell function leads to the secretion of a toxic 'SRNS factor' that has deleterious effects on the podocyte. Schewitz *et al* (IOVS 2009) showed that there is a discrete population of CD4+ cells that survive steroid treatment in steroid resistant patients. Due to a lower IL-10/IL-17 ratio in the steroid resistant patients this CD4+ population is thought to be of the Th17 subtype. We tested the hypothesis that these cells release soluble factors causing podocyte effacement.

Methods: Th17 cell culture supernatants were collected and applied to differentiated podocytes in vitro. Th0 cell culture supernatants were also collected and used as control. A scratch assay system was used to measure the motility of the podocyte. Podocytes also had their protein extracted after treatment with supernatant for use in Western blot experiments.

Results:



Th0 and TH17 supernatant was applied to wild type podocytes. Th17 supernatant significantly increased their motility compared to those treated with Th0 supernatant. Th17 supernatant treatment also stimulated p38MAPK and Jnk signalling in the podocyte while Th0 supernatant did not.

Conclusions: Treatment of the podocytes with Th17 supernatant significantly increased their motility. The effector of this is specifically released by the Th17 cells. *In vivo* this could lead to increased foot process effacement and concomitant nephrotic syndrome. This work provides evidence for a link between T-cell function and nephrotic syndrome.

TH-PO227

Gremlin via VEGFR2 Increases Profibrotic Evens in Renal Cells Sergio A. Mezzano,[1] Raquel Rodrigues-Diez,[2] Carolina Lavoz,[2] Alberto Ortiz,[3] Jesus Egido,[3] Marta Ruiz-Ortega.[2] *[1]Nephrology, Universidad Austral, Valdivia, Chile; [2]Cellular Biology in Renal Diseases Laboratory, Universidad Autónoma, Madrid, Spain; [3]Nephrology, Instituto de Investigacion Sanitaria Fundacion Jimenez Diaz, Madrid, Spain.*

Background: Gremlin is a developmental gene upregulated in chronic renal diseases associated to extracellular matrix (ECM) accumulation but its direct role in the regulation of renal fibrosis is still unclear. In endothelial cells gremlin induces angiogenesis via vascular endothelial growth factor receptor-2 (VEGFR2) pathway. Our aim was to investigate the receptor and mechanisms involved in gremlin-mediated fibrogenic events in renal cells.

Methods: Human tubular epithelial cells (HK2) and renal fibroblasts were studied. The effect of renal fibrosis was determined by evaluation of key profibrotic factors, ECM and EMT markers, by western blot/confocal microscopy or Real-time PCR.

Results: In tubular epithelial cells and fibroblasts gremlin binds and phosphorylates VEGFR2. As gremlin is a BMP antagonist, we evaluated the involvement of BMPs in the activation of VEGFR2. In the presence of BMPs (BMP-2, 4 or 7) gremlin-induced VEGFR2 activation was not modify, showing BMPs-independent effect. In renal fibroblasts Gremlin increases gene expression of profibrotic factors and matrix proteins (including collagens), which were markedly downregulated by VEGFR2 inhibition. Similar results were found in tubular epithelial cells. Moreover, in these cells gremlin induced phenotypic changes to myofibroblast-like morphology, including loss of epithelial markers and induction of mesenchymal markers. Among the intracellular signaling systems involved in renal fibrosis Smad pathway is one of the most relevant. Gremlin increases Smad2/3 phosphorylation and nuclear translocation after 20 min. This early Smad activation was TGF-β-independent. Smad7 overexpression, which blocks Smad2/3 activation, diminished Smad-dependent gene transcription and epithelial mesenchymal changes in gremlin-transfected tubuloepithelial cells.

Conclusions: We propose that gremlin, by VEGFR2 and activation of Smad signaling, increased profibrotic events and therefore could contribute to renal fibrosis.

Funding: Government Support - Non-U.S.

TH-PO228

Deacetylase Inhibition Attenuates Renal Fibrosis by Downregulation of Multiple Profibrotic Growth Factor Receptors Shougang Zhuang.[1,2] *[1]Department of Medicine, Rhode Island Hospital and Alpert Medical School of Brown University, Providence, RI; [2]Department of Nephrology, Shanghai East Hospital, Tongji University, Shanghai.*

Background: Inhibition of histone/protein deacetylases (HDACs) has been shown to attenuate the development and progression of renal fibrosis, however, the underlying mechanism remains poorly understood.

Methods: In this study, we studied the effect of class I HDAC inhibition on the expression of mutiple profibrotic growth factor receptors in the kidney after unilateral ureteral obstruction and in the cultured renal interstitial fibroblasts.

Results: We demonstrated that development of renal fibrosisafter unilateral ureteral obstruction (UUO) is concurrent with a sustained increase in the expression of three profibrotic receptors: transforming growth factor-beta (TGF-beta) receptor I, platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR). Inhibition of HDACs by MS-275 blocked expression of all those receptors and attenuated activation of renal fibroblasts and deposition of extracellular matrix proteins in the obstructed kidney. MS-275 treatment also abrogated UUO-induced PDGFR and EGFR phosphorylation, as well as activation of Smad-3, STAT3 (signal transducer and activator of transcription 3) and ERK½ (extracellular signal-regulated kinase½)pathways. Furthermore, MS-275 suppressed TGF-beta1 and serum-induced activation and proliferation of renal interstitial fibroblasts. However, MS-275 treatment did not affect expression/phosphorylation of c-MET, a receptor that antagonizes renal fibrosis.

Conclusions: Taken together, these findings provide a novel mechanism by which HDAC inhibition attenuates renal fibrogenesis through selective downregulation of multiple profibrotic growth factor receptors.

Funding: NIDDK Support

TH-PO229

Expression of Gas6 and Axl in Human IgA Nephropathy: A Possible Involvement of Gas6 in Podocyte Damage Masashi Miyoshi,[1] Kojiro Nagai,[1] Takei Kake,[2] Naoshi Fukushima,[2] Motokazu Matsuura,[1] Eriko Shibata,[1] Satoshi Yamada,[1] Kazuhiro Yoshikawa,[1] Akira Mima,[1] Fumi Kishi,[1] Seiji Kishi,[1] Tatsuya Tominaga,[1] Hideharu Abe,[1] Toshio Doi.[1] *[1]Department of Nephrology, University of Tokushima, Tokushima, Japan; [2]Chugai Research Institute for Medical Science Inc., Gotenba, Shizuoka, Japan.*

Background: Gas6 is a growth factor which causes proliferation of mesangial cells in the development of glomerulonephritis. The purpose of this study is to clarify Gas6 involvement in the progression of human IgA nephropathy (hIgAN).

Methods: Thirty-one biopsy proven primary hIgAN cases were enrolled. They were aged 16 to 63 years (mean ± SD, 35.9 ± 14.6 years). There were 16 women (51.6%). Immunohistochemistry of Gas6/Axl was performed and stained area in glomeruli was measured. We compared the expression level with histological severity, clinical data, or p27

that is expressed in podocytes in normal glomeruli and decrease in proliferating glomeruli, which was inversely correlated with mesangial proliferation in hIgAN in the previous report.

Results: In 28 of 31 cases, Gas6 was upregulated mainly in podocytes. In the other 3 cases, Gas6 expression was induced in endothelial and mesangial cells, which was similar as that in rat nephritis model. Among three kinds of Gas6 receptors named Axl, Dtk, and Mer, Axl was mainly expressed in endothelial cells. Among 28 podocyte pattern cases, the expression level of Gas6 was correlated with mesangial hypercellularity score of hIgAN Oxford classification and urine protein excretion. It was also inversely correlated with p27 expression in glomeruli. In vitro, cultured podocytes express Dtk and Mer. TGFβ could increase Gas6 mRNA and decrease p27 in podocytes. Gas6 itself could reduce the expression of p27. Moreover, Gas6 urine excretion increased in IgA nephropathy patients compared as normal subjects.

Conclusions: Gas6 upregulation in hIgAN was observed in either endothelial/mesangial cells or podocytes. The expression pattern can be a marker to classify hIgAN. Gas6 has a possibility to be involved in podocyte damage via Dtk or Mer in hIgAN.

Funding: Government Support - Non-U.S.

TH-PO230

Prostaglandin E2 Modulates Cell Cycle Regulatory Genes in Restoring Function and Pathology in Nephrotoxic Nephritis Nino Kvirkvelia, Maggie McMenamin, Michael P. Madaio. *Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: Though PGE_2 has been studied for its regulatory properties in: maintaining tissue homeostasis, controlling inflammation and fibrosis, and reshaping immunity, its role in tissue regeneration has been less evident. We observed that PGE_2 promoted recovery from NTN, and enhanced cellular regeneration contributed to the improvement. The present aim was to define the pathways involved in recovery.

Methods: Microarray gene expression and pathway analysis was carried out. Total RNA (trizol extraction) was isolated from kidneys of NTS, NTS/PGE_2 treated, and control mice, and processed. Affymetrix Gene Chip: Mouse gene 1.0 array was used for gene expression profiling. Further pathway and statistical analysis were generated through the use of iReport (Ingenuiy Systems).

Results: "Proliferation of cells", "tissue development" and "growth of the cells" were ranked highest based on p_value enrichment score and the number of differentially expressed genes (DEG: 272, 232, 195 respectively). In particular, genes specific for cell cycle/growth promoting pathways were identified as responsive to PGE_2 treatment and upregulated in PGE_2 treated kidneys. Among them: CDK1 (5.568 fold), FOXM1 (3.882fold), S100A6 (8.484 fold), CCN2 (2.003 fold), CCNF (2.014 fold), ANLN (8.698 fold) were of particular relevance.

Conclusions: Beneficial effects of PGE_2 in acute nephritis was mediated at least in part, by eliciting in growth promoting genes, regulating cell cycle progression and recovery processes within the kidney. Further definition of functional relevance of identified genes /pathways are under investigation.

Funding: NIDDK Support

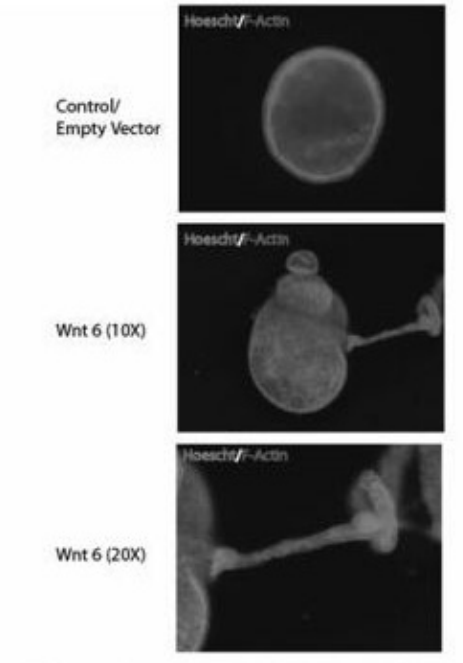
TH-PO231

Wnt 6 Is Increased in Diabetic Nephropathy and Modulates De Novo Tubulogenesis and Opposes TGFβ1 Mediated Epithelial Cell Differentiation In Vitro John Crean, Andrew Gaffney, Noel Faherty, Catherine Godson, Debra F. Higgins. *UCD Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Dublin, Co. Dublin, Ireland.*

Background: Studies from our group and others have identified a role for Wnt signalling in the pathogenesis of diabetic nephropathy. In this context, Wnt pathways are increasingly seen as targets for therapeutic intervention.

Methods: Microarray analyses of renal biopsis from patients with diabetic nephropathy were performed identifying genes whose expression was significantly increased. One of these genes, Wnt6, was cloned and expressed in renal epithelial cells. Tubulogenesis was analysed in MDCK cells. The effect of Wnt 6 on signaling and proliferation were analysed in HKC8 cells. Fluorescent microscopy was used to assess the effect of Wnt6 on TGFβ1 mediated epithelial to mesenchymal differentiation.

Results: Wnt6 expression is significantly increased in nephropathy correlating with disease severity. *In vitro* Wnt6 expression leads to de novo tubulogenesis(Figure 1) associated with enhanced cell proliferation, phosphorylation and activation of the EGF receptor.



Wnt 6 induces tubulogenesis in MDCK 3-dimensional culture. MDCK Cells were grown in 3D culture in Matrigel for 10 days. Cells were then transfected with a expression vector encoding Wnt 6 or a control/empty vector. Cultures were grown for a further 72 hours, fixed and stained with Alexa 488 Phalloidin and Hoescht 33342. Control cells maintained a cyst-like spherical structure while cells expressing Wnt 6 underwent cellular proliferation and differentiation resulting in the production of *de novo* tubular process, analagous to tubulogenesis

The canonical Wnt pathway was activated in cells treated with Wnt6 conditioned media as evidenced by increased phosphorylation of GSK3β (Ser9), nuclear accumulation of β-catenin and TCF/LEF activity. Overexpression of Wnt6 increased e-cadherin, suggesting a role in maintenance of epithelial phenotype. Importantly, overexpression of Wnt6 rescued epithelial to mesenchymal cell transdifferentiation in response to TGFβ.

Conclusions: We propose that Wnt 6 is involved in determining cell fate specification and as such its increased expression activates transcriptional programmes that determine phenotypic transition. These studies provide a valuable insight into the pathogenesis of diabetic nephropathy and raise the possibility that manipulation of Wnt6 may be of therapeutic benefit.

Funding: Government Support - Non-U.S.

TH-PO232

Angiotensin II Regulated Proteome of Human Kidney Cells Uncovers Nrf2 Target Proteins Ana Konvalinka,[1] Eleftherios P. Diamandis,[2] James W. Scholey.[1] [1]*Medicine, Division of Nephrology, University of Toronto, Toronto, ON, Canada;* [2]*Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.*

Background: Angiotensin II (ANGII), an effector of the renin angiotensin system leads to kidney disease progression, but there are no measures of renal ANGII activity. Accordingly, we sought to define an ANGII-stimulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of ANGII activity in the kidney.

Methods: We utilized stable isotope labeling with amino acids (SILAC) in PTECs to compare proteomes of ANGII treated to Control cells. PTECs treated with ANGII were also compared to ANGII + Angiotensin-type1 receptor (AT-1R) blocker stimulated PTECs. Experimental PTEC pairs of lysates were processed together. Desalted peptide fractions were analyzed using LTQ-Orbitrap. Peptide/Protein identification and calculation of ANGII:Control ratios was performed by MaxQuant on the human IPI database. Ingenuity Pathway Analysis (IPA) and Cytoscape identified protein networks and enriched Gene Ontology (GO) terms.

Results: Of 5010 identified proteins, 4968 were quantified in 5 replicates. Eighty four proteins were differentially regulated in more than one replicate with $p<0.01$. IPA uncovered differential regulation of proteins in antioxidant response (Nrf2) pathway by ANGII. The most consistently upregulated protein in response to ANGII, heme oxygenase-1 (HO-1), is downstream of Nrf2. HO-1 protects from oxidative stress and limits fibrosis. Enriched GO term network of differentially expressed proteins was overlaid with gene expression set from an analogous study in mice. "Response to stress" was an enriched GO term in both sets and contained five proteins from Nrf2 network, including HO-1. In the presence of AT-1R blocker, HO-1 was upregulated 2.3 fold, compared to 1.6 fold with ANGII alone. The same pattern of HO-1 gene expression was identified by RT-PCR.

Conclusions: SILAC and bioinformatic analyses have identified Nrf2 related proteins, which may serve as markers of ANGII activity in the kidney, and represent potential therapeutic targets. Pattern of HO-1 upregulation suggests novel mechanism of action of AT-1R blockers.

TH-PO233

Impact of Prophylactic Antibiotic Lock on Healthcare Utilization and Costs
Carol Moore, Anatole Besarab, Vivek Soi, Edward Peterson, Beth Adams, Jerry Yee, Lois Lamerato. *Henry Ford Health System, Detroit, MI.*

Background: Catheter-related bacteremia (CRB) is associated with significant morbidity and healthcare utilization in hemodialysis patients. Prophylactic antibiotic lock (ABL) can reduce CRB, its impact on healthcare utilization has not been described.

Methods: We conducted a prospective, multicenter, observational cohort study comparing heparin (HEP) and prophylactic ABL containing gentamicin 0.32mg/ml with trisodium citrate (4%) over 33 months in pts dialyzing with tunneled caths. Primary outcome compared infection-related hospitalization (IR-HOSP) rates between grps. Other outcomes were CRB, overall HOSP and vascular access procedures (VASC). Charges from billing records were available for 83% of pts and used as a proxy for cost. Since patients could have CRB, HOSP, VASC and charge accrual in both time periods we adjusted for correlation between measurements using a ratio estimator from sampling theory to test the various rates.

Results: The population (n=555) had a median age of 62, 50% male and 71% black. 427 subjects were followed for 90,627 days in HEP period compared to 304 and 73,169 during ABL period.

Demographics and Outcomes Comparisons

	HEP (n=427)	ABL (n=304)	P
Age	62 +/- 15	62 +/- 15	0.752
Sex	213 (50%)	157 (52%)	0.523
Black	299 (70%)	227 (75%)	0.090
CRB/1000 days	1.6	0.4	0.001
IR-HOSP/pt yr	1.3	0.9	0.004
Overall HOSP/pt yr	3.6	3.0	0.146
VASC/pt yr	2.8	2.5	0.274
HOSP Charges ($)/pt yr	128,050	91,120	0.015
VASC Charges ($)/pt yr	18,555	16,134	0.194
Total Charges ($)/pt yr	146,605	107,253	0.015

CRB declined 75% from 1.6 to 0.4/1000 days, p=0.001. 328 IR-HOSP occurred in HEP period and 176 in ABL period (1.3 vs 0.9/pt yr, p=0.004). Extrapolating this reduction, we estimate 85 IR-HOSP were averted due to use of ABL. Overall HOSP and VASC were similar. HOSP costs were significantly lower by $36,930/pt yr, p=0.015, in ABL period and VASC costs were similar. Overall healthcare costs were significantly lower by $39,352/pt yr, p=0.015, in ABL period. Extrapolating this reduction, we estimate $7,903,275 in costs averted due to use of ABL over study period.

Conclusions: The use of prophylactic ABL was associated with a significant reduction in CRB, healthcare utilization and costs.

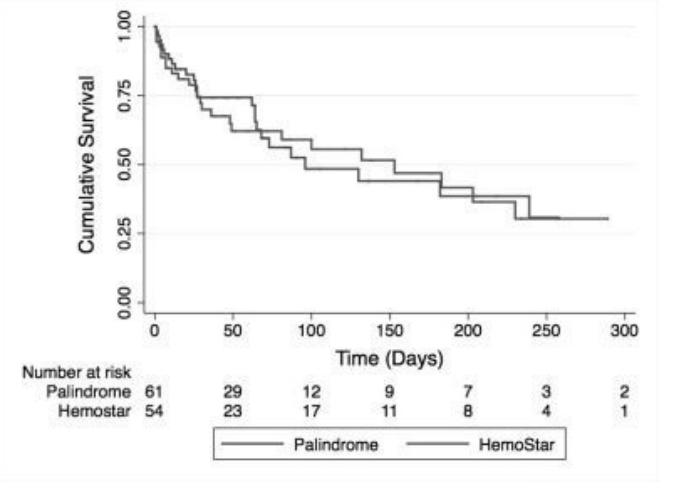
TH-PO234

Comparison of Heparin and Silver Coated and Standard Central Venous Catheters: Randomized Controlled Trial
Meteb M. AlBugami, Karthik K. Tennankore, Paula Mossop, Bryce A. Kiberd, Steven D. Soroka. *Department of Medicine, Division of Nephrology, Dalhousie University, Halifax, NS, Canada.*

Background: The use of central venous catheters (CVC) in hemodialysis has been growing, thereby more infections and thromboses are encountered on daily basis. Certain enhancements have been applied to the CVCs to minimize these complications, however, these come at a cost. Using Enhanced CVCs would be cost effective if they require less fibrinolysis and have longer patency.

Methods: A randomized controlled trial comparing the Enhanced, heparin and silver coated (*Sapphire Palindrome*) to Standard CVCs (*Hemostar*), with the hypothesis that using Enhanced CVCs will result in decreased tPA use and prolonged catheter patency. Those with new CVC incretion or replacement, using 19 or 23 cm placed over the right or left IJ were included. The primary outcome was the difference in tPA use. The secondary outcomes were the time to the first tPA use, and the times to CVC replacement for poor flow or bacteremia.

Results: 61 Enhanced and 58 Standard CVCs in 92 patients were included in the final analysis. Patients characteristics were the same. The mean tPA doses were 15.6 mg and 17.1 mg for the Enhanced and Standard groups, respectively (*P*=0.59). The cumulative time to first tPA use was 162 (95% CI 116 to 209) and 156 (95% CI 113 to 200) days for both groups respectively (*P*=0.54).



12 Enhanced and 8 Standard CVCs were changed due to poor flow, respectively (*P*=0.47), also 7 and 11 were changed due bacteremia, respectively (*P*=0.31). There were no differences on cumulative time to CVC removal due to poor flow or bacteremia. More cuff migrations were seen in the Enhanced group.

Conclusions: Using heparin and silver coated CVCs does not seem to be cost effective, as there was no difference in catheter survival. Given the small numbers of bacteremia, significant difference might have been missed.

TH-PO235

A Comparison of Costs of Using Once Weekly rt-PA or Heparin as a Locking Solution for Hemodialysis Catheters: Findings from the Prevention of Catheter Lumen Occlusion with rt-PA versus Heparin(Pre-CLOT) Randomized Trial
Braden J. Manns,[1] Nairne William Scott-Douglas,[1] Scott Klarenbach,[2] Louise M. Moist,[3] Marcello Tonelli,[2] Pietro Ravani,[1] Flora Au,[1] Marc Dorval,[5] Charmaine E. Lok,[4] Betty K. Chui,[2] Natasha Wiebe,[2] Brenda Hemmelgarn.[1] *[1]Univ Calgary, Calgary, AB, Canada; [2]Univ Alberta, Edmonton, AB, Canada; [3]Univ W ON, London, ON, Canada; [4]Univ Toronto, Toronto, ON, Canada; [5]Moncton Univ, Moncton, NB, Canada.*

Background: In a recent RCT, weekly rt-PA, 1 mg per lumen, once per week and twice weekly heparin as a locking solution, resulted in lower risks of hemodialysis catheter malfunction and catheter-related bacteremia compared to thrice weekly heparin. We conducted an extensive costing study using data collected alongside the RCT to determine how using rt-PA, rather than heparin, would affect overall health care costs.

Methods: The primary objective was to determine the cost associated with using rt-PA rather than heparin, considering the cost of locking solutions and all other relevant medical costs (including the cost of managing catheter dysfunction, and catheter-related bacteremia) during the 6 month trial. As the data for costs from the trial were skewed, non-parametric bootstrap estimates were used to derive 95% CIs and mean cost differences.

Results: The cost of the locking solution was higher in patients receiving rt-PA, but this was offset by lower costs for managing complications. Overall, the difference in unadjusted mean cost for managing patients with rt-PA vs heparin was CAN -$118 (95% CI -$1,480 to $1,244; p = 0.865).

	rt-PA (n=110)	Heparin (n=115)
Mean rt-PA and heparin costs for locking solution (SD)	$1206 (598)	$130 (85)
Mean cost of managing patients with catheter malfunction (SD)	$47 (249)	$149 (499)
Mean cost managing patients with catheter-related bacteremia (SD)	$466 (2785)	$1550 (7038)
Total mean cost (SD)	$1718 (2724)	$1830 (7013)
Mean cost difference (rt-PA minus heparin) (95% CI; p-value)	-$118 (-1,480 to +1,244; p=0.865))	

Conclusions: Using patient-specific data on all relevant health care costs collected alongside a RCT, we observed no significant difference in the mean cost of using once-weekly rt-PA as a locking solution for catheters, compared to heparin.

Funding: Pharmaceutical Company Support - Roche Canada

TH-PO236

Crossing Infections in Patients Undergoing Hemodialysis through a Temporary Dual Lumen Catheter: A Prospective Study with Bacterial Genotyping Methods
Jose A.C. Costa, Miguel Moyses-Neto, Viviane Ferreira, Juliana P. Falcão, Roberto Martinez. *Faculty of Medicine-USP, Ribeirao Preto, SP, Brazil.*

Background: Patients undergoing hemodialysis (HD), through a temporary dual lumen catheter (TC), have a high incidence of infection. The aim of this study was to compare isolated bacteria, by genotyping methods, collected at different sites in the same patient as well as strains isolated in the same sites, but in different patients.

Methods: Patients with acute kidney injury at an Intensive Care Unit (ICU) were studied with microbiological analysis of samples collected at different sites: TC insertion site, blood cultures (BC) of TC lumen, peripheral vein BC, and BC of other central venous accesses before the first session of HD; at TC removal, we analyzed the cultures of TC catheter tip and all BC previously mentioned. The bacterial isolation was carried out in conventional culture media. Genotyping diversity was performed by Pulsed-Field Gel Electrophoresis with enzyme XbaI for *Klebsiella pneumoniae* and speI for *Pseudomonas aeruginosa*. Data were analyzed by Bionumerics 5.1 program (Applied Maths) and the similarity of fragment length patterns between two strains was scored by the Dice coefficient.

Results: Although 96 patients were evaluated, our results are related to 6 patients who presented 14 strains of *P.aeruginosa* and 6 patients who presented 14 strains of *K.pneumoniae* at different sites. From 6 patients infected with *P.aeruginosa*, 3 of them were infected by similar strains in at least 3 different sites at TC removal (BC of TC lumen, culture of catheter tips, BC of a peripheral vein, and BC of any other central venous accesses), and at least at one site of other 3 patients. From 6 patients infected with *K.pneumoniae* 5 of them were infected by similar strain in at least 3 sites, and at least at one site of other patient at TC removal.

Conclusions: The same strain of *P.aeruginosa* and *K.pneumoniae* has infected the same patient at different sites suggesting that these patients should have better assistance to avoid spreading of the bacteria. Also, the isolation of the same strain with high genotypical similarity in different patients suggest that crossing infections are occurring in the ICU.

Funding: Government Support - Non-U.S.

TH-PO237

Microbiological Screening for Detection of Central Venous Catheter Related Bloodstream Infections in Patients Undergoing Hemodialysis

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Background: Catheter colonization with a cut off level of 1000 organisms/ml catheter blood is considered to be a forerunner of central venous catheter related bloodstream infections (CRBSI). This prospective trial was undertaken to evaluate whether the PNA FISH test could serve as a screening tool to detect CRBSI in a subclinical stage in hemodialysis patients with central venous catheters (CVCs) used with non antimicrobial locking anticoagulants (heparin, citrat 4%).

Methods: Fiftyfive hemodialysis patients with 60 catheter episodes were prospectively investigated. Screening for CRBSI was performed by PNA FISH test with universal hybridisation probes (for detection of all relevant bacteria and fungi) three times a week using EDTA blood from both lumina of CVCs obtained prior to hemodialysis. In addition blood samples were quantitatively cultured on chocolate agar. If CRBSI was clinically suspected, routine investigation was performed by the Gram stain/acridine orange leucocyte cytospin (AOLC) test, differential time to positivity (DTP) and Brun Buisson method.

Results: 2972 blood samples were investigated. With routinely performed investigations 2 CRBSIs were detected in 3691 catheter days resulting in a CRBSI rate of 0,5/1000 catheter days. Both of these 2 CRBSIs were detected by PNA FISH screening before the diagnosis was established by routine measures. In 58 catheter episodes no CRBSI could be detected by routinely performed tests. In 3 of these catheter episodes PNA FISH screening was false positive. The sensitivity and specificity of PNA FISH screening were 100% and 95%, the PPV and NPV were 40% and 100%.

Conclusions: Screening of blood drawn from CVCs prior to hemodialysis by PNA FISH helps to detect patients at risk for clinically evident CRBSI. Thus, patients with positive screening should be thoroughly observed with regard to development of clinically evident CRBSI. Patients with negative PNA FISH screening tests are unlikely to develop CRBSI.

TH-PO238

Impact of Enhanced Infection Control Practices on Access Infections in an Acute Dialysis Unit

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Background: Infections are a major contributor to mortality and morbidity. Parkland Health and Hospital System (PHHS), a large public academic health system in Dallas, TX, provides emergent dialysis to numerous unfunded individuals. We performed this quality improvement study to determine if changes in the acute dialysis unit's (ADU) infection control policies improved rates of access-related infections in this population.

Methods: We conducted a retrospective review of all patients who underwent dialysis treatments in the ADU during a 3 month period before (period 1) and after (period 2) infection control policy changes were implemented in August 2011, including increased hand hygeine, use of personal protective equipment with gowns/gloves by all hospital staff and no food/drinks in the unit. We assessed catheter-related bloodstream infection (CRBSI) and complication (death, ICU transfer, endocarditis, bone/joint or other) rates per 100 patient-weeks of hemodialysis.

Results: Overall, 264 patients underwent 1379 patient-weeks of dialysis in period 1, and 256 patients underwent 1340 patient-weeks of dialysis in period 2. Forty percent were unfunded. There was no significant difference in rates of CRBSI (1.54 v 1.53) or complications (0.87 v 0.57) among the unfunded patients. The unfunded patients were significantly more likely to have a tunneled catheter for access and to have CRBSI of gram-negative or polymicrobial etiology when compared to funded patients.

Comparison by funding status

	Unfunded	Funded	*P-value*
Tunneled catheter (%)			
Period 1	82	46	*<0.000*
Period 2	80	44	*<0.000*
Gram-negative or polymicrobial infections (%)			
Period 1	69	18	*0.026*
Period 2	56	10	*0.021*

Conclusions: CRBSI rates did not change after implementation of enhanced infection control practices. Unfunded patients who receive emergent dialysis differed from the overall dialysis population, with a greater susceptibility to CRBSI of gram-negative or polymicrobial etiology.

TH-PO239

Cost Analysis of the Hemodialysis Infection Prevention with Polysporin Ointment Study

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Background: In a multicenter RCT, application of polysporin triple ointment (PTO) at the catheter (CVC) entry site significantly decreased hemodialysis (HD) CVC-related bacteremia, hospitalizations and mortality (Lok, 2003). We performed an economic analysis of the use of PTO over standard HD catheter care without PTO (SOC) in Ontario, Canada.

Methods: Health care utilization data comparing PTO to SOC was calculated from a third party payer perspective, including opportunity costs of dialysis nurses. Resource use associated with CVC-related bacteremia, all visit data for all HD patients with CVC from April 1, 2008 to March 31, 2011 was extracted from the University Health Network's Case Costing Database.

Results: The cost of PTO was $240.36 (per patient) and $1,449,615.36 (all HD patients in Ontario with CVC) for one year from a third party payer perspective. The cost reduction with PTO over SOC due to the treatment of CVC-related bacteremia in Ontario is $22,893,676.84. The total savings from PTO use after accounting for the cost of PTO implementation in Ontario and considering the infection related costs of PTO instead of SOC is approximately $21,444,061.48 (Table 1).

Table 1

Type of Cost	**Unit Cost**	**Total Cost per Year**
Polysporin Triple Ointment	$43.20/pt year	$259,891.20
Hemodialysis Nurse Application of PTO	$197.76/pt year	$1,189,724.16
Cost of PTO Program for Hemodialysis Patients in Ontario		**$1,449,615.36**
Catheter Removal and Insertion	$1,121.17/event	-$1,146,642.98
Outpatient treatment for Bacteremia	$262.19/event	-$630,934.02
Hospitalization for Bacteremia	$22,842.81/event	-$21,116,099.84
Infection Outcome Related Costs of PTO versus SOC*		**-$22,893,676.84**

* Using catheter removal, bacteremia, and hospilitization rates from the HIPPO trial data, the expected number of patients with event in one year in Ontario using the PTO program of care - the expected number of patients with event in one year in Ontario using SOC

Conclusions: This cost analysis demonstrates that PTO has marginally more upfront costs than SOC, but has significant long-term cost savings associated with reduced rates of bacteremia.

TH-PO240

Identifying and Targeting the Causes of Central Venous Catheters in Chronic Dialysis Patients

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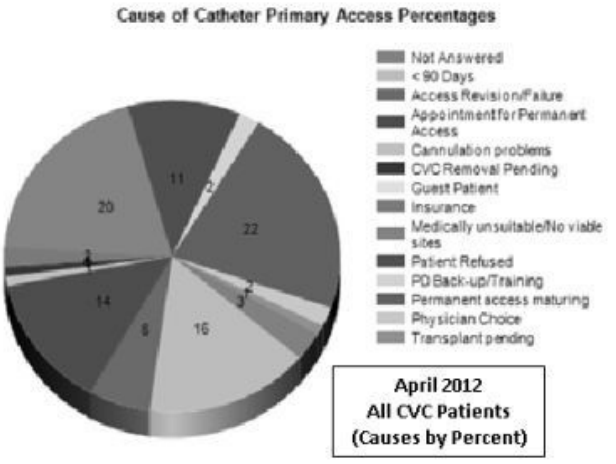
Background: Central venous catheters (CVCs) in patients on hemodialysis are associated with increased morbidity and mortality. The 2012 performance year QIP target for CVC utilization is ≤14% of hemodialysis patients. This study summarizes an initiative to identify and target the reasons that patients have CVCs.

Methods: Clinical staffs at the system of 155 centers in the US (11,000 ESRD patients) self-reported the primary cause for CVCs in patients who were not using AV fistula or graft accesses. Causes were selected from a list of 'expected causes' prepared prior to the study, and revised during the study.



Results: For April 2012, 59.7% of incident patients used CVC accesses and 17.8% prevalent patients had CVC accesses. Twenty percent of prevalent patients with a catheter were identified as "medically unsuitable or no viable access site"; 20% of prevalent patients were identified as "permanent access maturing."

A regression analysis demonstrated that incident CVC rate is an important predictor of prevalent CVC rate (R^2=0.2109, 95% CI slope=0.1287 to 1.1382).

Incident Patient CVC Rate (ICVC) as a Predictor of Prevalent Patient CVC Rate (PCVC)

Variable	DF	Parameter Estimate	Std Error	t Value	Pr > |t|	95% CL - Lower	95% CL - Upper
Intercept	1	-0.1989	0.14112	-1.41	0.1711	-0.4896	0.0918
ICVC	1	0.6334	0.2451	2.58	0.0160	0.1287	1.1382

Conclusions: Understanding patient-specific causes for CVCs are important to systematic interventions to achieving access targets. CVCs are increasingly common in incident patients, and incident CVCs are a predictor of prevalent CVCs. Clinicians must focus efforts to reduce CVCs in the incident population. Developing consistent criteria to evaluate patients who are "medical unsuitability" for permanent access placement will be important to reduce the number of patients with CVCs.

TH-PO241

A Novel Mouse Model to Assess Soluble Epoxide Hydrolase and Pro-Thrombotic Factors in Catheter-Induced Vascular Occlusion Yuxia He,[1] Huan Li,[1] Ilya S. Zhuplatov,[1] Donald Blumenthal,[2] Christi M. Terry,[1] Alfred K. Cheung.[1,3] *[1]Medicine, Univ of UT, Salt Lake City, UT; [2]Pharmacology, Univ of UT, Salt Lake City, UT; [3]Medicine, VASLCHCS, Salt Lake City, UT.*

Background: Indwelling central catheters predispose veins to neointimal hyperplasia (NH) and thrombosis. Pro-inflammatory (soluble epoxide hydrolase or sEH) and pro-thrombotic (common coagulation cascade) pathways that may participate in these events were examined. Vasculo-protective P450 epoxygenase metabolites are catabolized by sEH while factor Xa is central in coagulation.

Methods: A 0.5-cm long section of polyurethane catheter (0.6 mm i.d.) was inserted into the jugular vein of either wildtype (WT) or sEH knockout (KO) mice and left in place for 7 days. Vehicle or rivaroxiban (ROX) (10 ng/kg), a direct factor Xa inhibitor, were administered by daily gastric gavage in a blinded fashion to WT and KO mice. Ultrasound was performed in vivo to assess patency and the animals were euthanized, blood samples obtained and histomorphometry performed on the explanted vein containing the catheter to assess NH. Cell proliferation in the vein wall was assessed by Ki67 immunostaining. Plasma levels of the prothrombotic plasminogen activator inhibitor-1 (PAI1) were assessed by western blotting.

Results: Vein patency and vein wall cell proliferation rates at day 7 are shown in the Table.

Effect of sEH genetic background and rivaroxiban on patency and cell proliferation.

	Vehicle (% patent, % Ki67-positive cells)	Rivaroxiban (% patent, % Ki67-positive cells)
WT	40% (n=5), 72%	80% (n=5), 24% ±11
KO	67% (n=3), 35%±20	100% (n=6), 32% ±6

Higher patency rates and less cell proliferation were observed in sEH-KO mice compared to WT. ROX also increased patency rates and decreased cell proliferation. ROX decreased plasma PAI1 levels in mice of either genetic background. There were no untoward bleeding events in any group.

Conclusions: These preliminary results suggest that sEH may contribute to catheter-induced central venous stenosis and that an inhibitor of sEH may be useful in this and other vascular stenosis settings. ROX was associated with increased patency in both WT and sEH backgrounds and may also be useful as a preventative therapy.

Funding: NIDDK Support, Veterans Administration Support

TH-PO242

Vascular Access Type and the Trajectory of the Inflammatory Markers in Hemodialysis (HD) Patients Tanushree Banerjee,[1] Joseph Kim,[2] Brad C. Astor,[3] Tariq Shafi,[4] Josef Coresh,[4] Neil R. Powe.[1] *[1]University of California, San Francisco; [2]University Health Network, Toronto; [3]University of Wisconsin; [4]Johns Hopkins University.*

Background: The contribution of vascular access (VA) type to inflammation in HD patients is unclear. We investigated the role of VA type on serial inflammatory markers in the CHOICE cohort.

Methods: In a national, prospective cohort study of incident HD patients we measured serum C-reactive protein (CRP) and Interleukin-6 (IL-6) after the insertion of the VA type and at subsequent time points after access placement over first 3 years of HD. We limited the analyses to patients with little comorbidity (ICED score=0 or 1) to minimize confounding by comorbid disease status. Type of VA was categorized as central venous catheter (CVC), arteriovenous graft (AVG) and arteriovenous fistula (AVF). We accounted for the changes in VA type over time and corresponding VA type was determined for each available period of cytokine measurement. Multivariate analyses for repeated measures were performed using mixed effects models with adjustment for age, sex, race, BMI, diabetes, CVD, VA type and days after access placement, with CRP and IL-6 as log-transformed dependent variables.

Results: 423 incident HD patients were included (CVC=174, AVG=144, AVF=105). Baseline characteristics did not differ across the 3 types of access except for mean participant age was greater in those with AVG (p=0.01). In multivariate analyses, in comparison to AVFs, the presence of CVC was associated with nearly 40% increase in CRP (p=0.02). IL-6 levels also positively correlated with CVC showing 4% increase in the IL-6 levels, although this did not reach statistical significance (p=0.07). The presence of AVG was associated with 80% increase in CRP (p=0.01) and with IL-6 the change associated was 2% (p=0.06). The levels of CRP decreased over time, with the highest inflammatory state of 30 days after access placement when compared with markers over 180 days (p=0.02). Similar results were obtained for IL-6 levels, though they did not reach statistical significance.

Conclusions: CVCs and AVGs in comparison to AVFs are associated with a greater state of inflammation in incident HD patients.

TH-PO243

An Observational Study of the Effect of Anticoagulation with Warfarin on Femoral Tunnelled Dialysis Catheter Associated Deep Venous Thrombosis and Catheter Patency William G. Herrington,[1] Helen Nye,[2] Richard Haynes,[1] Christopher G. Winearls,[1] Emma C. Vaux.[2] *[1]Oxford Kidney Unit, Oxford University Hospitals NHS Trust, Oxford, United Kingdom; [2]Royal Berkshire Hospital Renal Department, Royal Berkshire NHS Foundation Trust, Reading, United Kingdom.*

Background: To determine the incidence and predictors of femoral tunnelled dialysis catheter (TDC)-associated complications and whether prophylactic warfarin is associated with reduced catheter-associated deep vein thrombosis (DVT) or prolonged catheter patency.

Methods: A retrospective review of femoral TDCs inserted for maintenance hemodialysis, comparing patients from two dialysis units using different strategies to reduce thrombotic complications. One centre routinely considered all femoral TDCS for prophylactic anticoagulation, whilst the other restricted warfarin use to catheters that had required repeated treatment with urokinase locks to maintain patency. Survival analyses were performed to establish complication rates, identify predictors of complications and assess the effect of a policy of prophylactic warfarin use.

Results: Of the 194 identified femoral TDCs, 178 (92%) were associated with at least one complication. Approximately three quarters did not provide adequate small solute clearance; one half were not in use by 3 months; one quarter were associated with at least one proven infection (2.3 per 1,000 catheter days); and one in ten developed a catheter-associated DVT (1.1 per 1,000 catheter days). Prophylactic warfarin use was not associated with significant improvements in rates of catheter blockage (hazard ratio [HR] 1.4 [95% confidence interval 0.7-2.9]; p = 0.33), DVT (HR 0.7 [95% CI 0.3-1.7]; p = 0.39), infection or with improved dialysis adequacy. A previous ipsilateral femoral TDC was identified as a significant predictor of a catheter-associated DVT (HR 3.6 [95% CI 1.4-9.3]; p = 0.01).

Conclusions: Femoral TDCs are associated with poor patency and high complication rates, re-using femoral veins for TDCs should be avoided where possible, and there is no proven benefit of prophylactic anticoagulation with warfarin in patients with femoral TDCs.

TH-PO244

Vascular Access Use and Access Infections from Dialysis Claims Data Craig Solid,[1] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Starting in July of 2010, Medicare claims billed by outpatient (OP) dialysis facilities include information on the type of vascular access (VA) used for each dialysis session and whether there was a VA infection. This represents the first time that the use of types of VA can be tracked using claims data.

Methods: We used the USRDS database to identify hemodialysis (HD) patients who were either prevalent as of July 1, 2010 or incident during 2010 such that Medicare coverage (day 91) began during July through September of 2010. Outpatient dialysis claims and HCPCS modifier codes were used to identify the type of VA patients used during the remainder of 2010. Patients were grouped based on the type of VA(s) used during the entire study period. A VA infection represents the presence of a HCPCs modifier code indicating such on at least one OP dialysis claim during the study period.

Results: A total of 204,372 prevalent patients were identified, 177,875 (87%) of whom had dialysis claims in all 6 months of July through December of 2010. For these patients, 10% used only a catheter, 56% only an AVF, and 21% only an AVG. Those using only a catheter more often had an indication of a VA infection (7%) compared to those with internal accesses (<1%). Within the 11,290 incident patients having at least 4 months of claims in 2010, 38% used a catheter only,18% an AVF only, 5% an AVG only = 5%, and 38% used catheter and internal access. VA infections occurred in 6% of catheter users and 1.6% of those only using an internal access.

Table 1

	Prevalent Pts		Incident Pts	
Total	117,875	VA Inf	11,290	VA Inf
Catheter Only	10.3%	7.1%	37.6%	5.9%
AVF Only	56.2%	0.9%	18.4%	1.6%
AVG Only	21.3%	0.9%	4.9%	1.6%
Cath+Internal	9.8%	6.3%	38.4%	6.2%

Conclusions: For the first time, vascular access use for each dialysis session used is available from claims. Initial investigations demonstrate that most prevalent patients consistently use one type of access, while for incident patients it is more common to use multiple types. VA infections reported on the dialysis claims are more common for those using catheters than those using internal accesses.

Funding: NIDDK Support

TH-PO245

Procoagulant Antibodies Do Not Predict Catheter Dysfunction Albert J. Power, Janet Lee, Peter Hill, Damien Ashby, David Taube, Neill D. Duncan. *Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Thrombotic mechanical dysfunction is the prime reason for inadequate hemodialysis [HD] in patients dependent on central venous catheters [CVC]. The role of procoagulant profiles in access thrombosis has been studied in arteriovenous grafts and fistulae with conflicting results and is very poorly characterized in CVCs. We therefore examined this association in the setting of a prospective trial.

Methods: The VyTes study was a randomized controlled trial comparing CVC performance in adults incident to HD. Patients were randomized to receive either a TesioCath [MedComp] or LifeCath Twin [Vygon] CVC, dialyzed to target blood flow rate [BFR] of 450ml/min and followed up for 12 months. Catheter dysfunction was defined by a BFR ≤250ml/min. Available serum was tested for anticardiolipin [ACA] IgG, IgM & IgA antibodies and lupus anticoagulant [LA]. In addition anti-β2-glycoprotein-1 [b2-GP1] antibodies were tested for in a subset of patients with prior arteriothrombotic disease [e.g. stroke, myocardial infarction, peripheral arterial disease].

Results: 80 patients were enrolled [mean age 61±16 yrs]. There was no significant difference in dialysis adequacy between the TesioCath and LifeCath groups [mean spKt/V 1.81±0.29 vs. 1.85±0.36,p=0.07] or BFR [mean 413±46 vs. 411±43ml/min,p=0.5]. The overall rate of CVC dysfunction was 2.8/1000 catheter days [95% CI, 2.1-3.5]. In total 7/49 [14%] patients assayed were ACA IgM +ve, 2% IgG and 7% IgA +ve. Only 1/36 patients tested was anti-b2-GP1 IgG +ve. No patients tested positive for anti-b2-GP1 IgM or lupus anticoagulant [n=41]. None of the patients testing positive for any antibody subclass experienced CVC dysfunction or a systemic thrombotic event during the period of follow-up.

Conclusions: In the largest prospective study of its kind to date to our knowledge we found no association between ACA, LA and anti-b2-GP1 antibody status and thrombotic CVC dysfunction. Despite the relatively small sample size and low event rate this finding is consistent with prior data in arteriovenous accesses and suggests that screening for procoagulant antibodies has no role in risk stratification for CVC dysfunction.

TH-PO246

Comparison of Tesio and LifeCath Twin Permanent Dialysis Catheters (VyTes): A Randomized Controlled Trial Albert J. Power, Peter Hill, Seema Singh, Damien Ashby, David Taube, Neill D. Duncan. *Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Central venous catheters [CVC] for hemodialysis [HD] are designed to deliver high blood flow rates [BFRs] to achieve dialysis adequacy. Despite its good long-term performance the TesioCath [TC] is reported to function poorly immediately post-insertion. We prospectively studied the performance of the TC and a similar twin-catheter CVC, the LifeCath Twin [LC].

Methods: This single-center randomized controlled trial [NCT 01022359] allocated adult incident patients 1:1 to receive either a TesioCath™ [MedComp] or LifeCath Twin™ [Vygon]. All patients dialyzed to target spKt/V≥1.6 and target BFR 450ml/min and followed up for 12 months or until change of dialysis access or modality, death or transfer out. The primary outcome was achievement of the target BFR during the 1st HD post-insertion. Secondary outcomes included thrombotic dysfunction, displacement and CVC-related infection. Dysfunction was defined by a BFR ≤250ml/min and treated with urokinase [UK].

Results: 80 patients were randomized [mean age 61.0±16.1 yrs, 48% diabetic] with 24179 total catheter days follow-up. More LCs reached the primary endpoint compared to TCs [44% vs. 10%,p=0.001] delivering a higher BFR [mean 383±82 vs. 277±79ml/min, p<0.001]. Significant differences in BFR persisted until the 4th session after which both CVCs delivered equivalent BFRs [mean 411±43 vs. 413±46ml/min, p=0.5] and adequacy [mean spKt/V 1.85±0.36 vs. 1.81±0.29,p=0.07]. Rates of CVC-related bacteremia [0.40 vs. 0.51/1000 catheter days,p=0.7] and exit site infection were similar between groups [p=0.4]. The overall rate of catheter dysfunction was 2.8/1000 catheter days with no difference in rates of UK lock use between groups [p=0.3] although the LC group required more UK infusions [6 vs. 0,p=0.01].

Conclusions: The LifeCath CVC can deliver greater BFRs in the first 3 HD sessions following insertion although this did not translate into differences in performance, dialysis adequacy or complication rates with long term use. This data confirms that both CVC types can consistently deliver high BFRs and dialysis adequacy over an extended period of time.

TH-PO247

Catheter Flow Monitoring Can Predict Dysfunction in Maintenance Hemodialysis Albert J. Power, Claire Edwards, Seema Singh, Damien Ashby, David Taube, Neill D. Duncan. *Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Access surveillance is advocated for arteriovenous fistulae and grafts with criteria for dysfunction [NKF K/DOQI 2006]. Despite the enduring prevalence of central venous catheter [CVC] use with associated high rates of mechanical dysfunction there are no equivalent studies with this access form. We therefore studied the role of flow monitoring as a predictor of CVC dysfunction.

Methods: We retrospectively studied all patients with CVCs established [>90d] on hemodialysis [HD] receiving protocolised care at 1 dialysis unit at our center [Oct 2008-Feb 2011]. CVC function was assessed by the ratio of blood flow rate:outflow access pressure [BFR:AP]. CathRisk, an indicator variable, was positive when BFR:AP<0.9. Target BFRs were ≥350ml/min with dysfunction defined by consistent BFR<250ml/min and/or declining spKt/V [target ≥1.6]. CVCs were locked with heparin and 2-hour urokinase [UK] dwells used for dysfunction with infusions given in the event of treatment failure.

Results: A total of 40333 HD sessions with 224 CVCs were studied [164 patients, mean age 62.7±15.1yrs, 45% diabetic] spanning 108114 catheter days. 132/224 [59%] CVCs were the first access form. Mean session length was 4.3±0.5hrs, BFR 402±53ml/min and ultrafiltration [UF] volume 1.9±1.1l. 87% HD sessions achieved target BFRs with rates of CVC dysfunction 5.5/1000 catheter days [95% CI 5.1-6.0]. UK locks increased BFR≥40ml/min in 78/254[31%] cases with greater improvement when used in more advanced dysfunction [mean 4.7±0.6ml/min per 10ml.min^{-1} BFR decline,p<0.001]. On multivariate logistic regression the risk of CVC dysfunction was associated with greater dry weight [4%/kg,p=0.009], CVC vintage [2%/month,p=0.003], UF rate [70%/l.hr^{-1},p=0.05] and most significantly CathRisk +ve [OR 7.83,p<0.001].

Conclusions: In the first study of its kind to date to our knowledge we characterized the natural history of CVC function and correlated it to a dynamic performance parameter. This can provide a novel framework for flow monitoring in CVCs with potential to transform care through pre-emptive thrombolysis for incipient dysfunction as opposed to current strategies.

TH-PO248

Infective Endocarditis in Hemodialysis Patients: Association with Increased Utilization of Cardiac Rhythm Devices Brinda Desiraju,[1] Priyanka Singh,[1] Douglas Sepkowitz,[2] Neal Mittman.[1] *[1]Avram Division of Nephrology, SUNY Downstate at Long Island College Hospital, Brooklyn, NY; [2]Division of Infectious Diseases, SUNY Downstate at Long Island College Hospital, Brooklyn, NY.*

Background: Infective endocarditis (IE) is a serious complication of bacteremia in patients (pts) undergoing (HD), especially in those utilizing central venous catheter (CVC) hemoaccess. Transvenous cardiac rhythm devices (CRD) have recently become more common in HD pts, leading to greater recognition of device-related complications, including systemic infection.

Methods: We compared the association of CVC and CRD in HD pts with documented infective endocarditis (positive blood cultures and echocardiographic findings) diagnosed in our urban hospital during the past year to those occurring during the period of 2002-2008.

Results: There were 40 cases of documented IE in HD pts during 2002-2008, of whom 26 had CVC (65%). CRD were present in 3 pts (8%), all of whom were dialyzed with CVC. Mean age was 57.3 yr. There were 21 women. Nine pts expired during the course of IE, six with CVC. During the past year, 9 cases of IE were diagnosed. Six pts were dialyzed with CVC (66%) and 3 pts (33%) with arteriovenous grafts (AVG). Of note, six pts (66%) had CRD, including all three pts with AVG. The mean age was 56.1 yr. There were four women. Only one pt expired during the course, she with CRD and AVG.

Conclusions: CRD have been utilized increasingly in the HD population. Current guidelines recommend the use of CRD in survivors of cardiac arrest, in structural heart disease with malignant dysrhythmias, and as primary prevention in pts with severe cardiomyopathy. However, there have been no studies documenting outcome benefit which included HD pts. The compelling risks of CRD in this population, including central vein stenosis/occlusion and device-related bacteremia, and the common use of CVC during dialysis initiation, require prospective, randomized trials to provide definitive indications for CRD use. Until this is available, alternative treatment paradigms should be explored, including the use of non-endovascular CRD placement or a greater impetus to modality change to peritoneal dialysis.

TH-PO249

Improving Veterans Outcomes through Shared Knowledge: VA Hemodialysis Infection Surveillance Collaborative Susan T. Crowley,[1,2] Karen Sovern,[3] Wissam M. Kourany.[4] *[1]Yale University School of Medicine, New Haven, CT; [2]VA Connecticut HCS, West Haven, CT; [3]IPEC, VHA Office of Analytics, Cincinnati, OH; [4]VA Durham HCS, Durham, NC.*

Background: As part of a national antimicrobial stewardship plan the VA established a partnership amongst its out-patient(pt) hemodialysis(HD) units, initiating a HD vascular access associated infection (HD-VAI) surveillance program in FY'12 to reduce HD-VAI.

Methods: Collaborating with the VA Inpatient Evaluation Center (IPEC), a representative sample of VA out-pt HD units engaged in the HD-VAI surveillance pilot. The Collaborative's goals were to measure outcomes related to HD-VAI and develop/implement tracking systems to support evidence based HD-VAI mitigation strategies. Ten sites were mentored through monthly teleconferences. During calls nomenclature was defined, guidelines established, VA and similar federal initiatives aligned, and a data management infrastructure developed including a SharePoint tool kit and website for data entry/reporting. Shared knowledge also included a facilitated data collection tool ensuring reporting consistency. Infection rates per pt month(mo) were defined as #HD out-pts with a positive blood culture (of uncertain etiology or believed related to vascular access) or a local access site infection (without bacteremia) as numerator, and #HD pts with any access device on the first 2 working days of mo as denominator.

Results: After 8 mo, 22 VAIs occurred in 3441 pt-mo for a national aggregate HD-VAI rate of 0.6/100 pt-mo. Sixteen (73%) VAIs occurred among pts using a tunneled catheter of which 12 (75%) were blood stream infections (BSI). In contrast, only 2 BSI occurred in pts with AVFs.

Conclusions: Through shared knowledge the VA HD-VAI surveillance collaborative developed a transparent data reporting system, aligned a strategic focus for VA Infection Control, Public Health & Specialty Care, and demonstrated the feasibility of creating a data driven national quality assessment plan to reduce HD-VAI in a large integrated healthcare organization. Roll out of the program to all VA out-pt HD units is set for FY12.

Funding: Veterans Administration Support

TH-PO250

Low Dose Alteplase for Central Venous Catheter Declotting: Impact on CVC Exchanges and Cost Analysis Henry H. Chen, Andre F. Charest, Bharat Nathoo, Grace Leung, Emmelia Lau. *York Central Hospital, Richmond Hill, ON, Canada.*

Background: Hemodialysis (HD) central venous catheter (CVC) occlusions are a common problem in all hemodialysis centers. Usual treatment involves intraluminal Alteplase (tPA) dwell at a dose of 2mg/lumen. When tPA dwells fail, CVC exchanges are typically necessary. On Sep 30, 2010 our dialysis program implemented a new protocol of tPA 1mg/lumen dosing for occluded CVCs. Objective: To determine the impact on the number of CVC exchanges and cost implications when changing from 2mg to 1mg/lumen of tPA for CVC declotting.

Methods: A cohort study was done to look at the number of CVC exchanges and amount of tPA used between Apr 2010–Apr 2012. The cohort was retrospective from Apr 2010-Sep 30 2010, then data was collected prospectively. Study subjects included all patients with CVCs receiving HD. Prevalent subjects with CVCs ranged from 153-192 during the study duration. The number of CVC exchanges was expressed as percentage of prevalent lines changed in the 6 months before, 0-6 months after, and 0-19 months after intervention.The cost of tPA was expressed as Canadian (CDN) dollars spent on tPA (averaged)/prevalent CVC/month.

Results: The total number of CVC exchanges was: 33 at 6 months preceding intervention, 24 at 0-6 months, and 80 at 0-19 months. Expressed as the percentage of prevalent CVCs changed: 3.29% at 6 months preceding intervention, 2.45% (p=0.24) at 0-6 months, and 2.44% (p=0.20) at 0-19 months. Although not statistically significant, the percentage of CVC exchanges trended lower after the intervention of changing from 2mg to 1mg/lumen of tPA. The pre-protocol average cost of tPA/prevalent CVC/month was $52.80. The post-protocol average cost of tPA/prevalent CVC/month was $31.01 at 0-6 months and $23.74 at 0-19 months. This represents an average cost savings of $21.70 (p=0.0054) at 0-6 months and $29.06 (p< 0.0001) at 0-19 months per prevalent CVC/month. As compared to pre-intervention, approximately $23,000 was saved at 6 months and $95,000 was saved at 19 months post-intervention.

Conclusions: Implementing a low dose tPA (1mg/lumen) CVC occlusion treatment protocol did not increase the number of CVC exchanges and resulted in significant cost savings.

TH-PO251

Anticoagulants for the Prevention of Intravascular Catheter Malfunction in Haemodialysis: A Systematic Review and Meta-Analysis Ying Wang,[1] Jessica Ivany,[2] Vlado Perkovic,[1] Martin P. Gallagher,[1] Meg J. Jardine.[1] *[1]Renal and Metabolic Division, The George Institute for Global Health, Camperdown, NSW, Australia; [2]Sydney Children's Hospital, Randwick, NSW, Australia.*

Background: Catheter malfunction (CM), including thrombosis, is associated with reduced dialysis adequacy, as well as an increased risk of catheter-related bacteraemia (CRB) and mortality. The role of anticoagulants in the prevention of CM remains uncertain.

Methods: A systematic review and meta-analysis were performed examining all randomized controlled trials (RCTs) assessing anticoagulants for the prevention of CM in adult patients receiving haemodialysis for end stage kidney disease. Medline, EMBASE and the Cochrane Library database were searched to January 2012. The primary outcome was CM, and secondary outcomes were CRB, all-cause mortality and bleeding events. Relative risks with 95% confidence intervals for individual trials were pooled using random effects models.

Results: The search yielded 27 trials that included 3025 patients and assessed citrate locking solutions, warfarin, low concentration heparin locking solutions, antibiotic locking solutions, tissue plasminogen activator (t-PA) and low molecular weight heparin. The only intervention type that reduced the rate of CM was t-PA (RR 0.52, 95% CI 0.32-0.86), with no effect observed for citrate (RR 0.83, CI 0.69-1.01), warfarin (RR 0.59, CI 0.28-1.22), heparin (RR 0.92, CI 0.54-1.57), or antibiotics (RR 1.48, CI 0.79-2.74). Similarly, no effect on the rate of CRB was observed in trials of citrate (RR 0.64, CI 0.32-1.30), warfarin (RR 2.40, CI 0.88-6.52), heparin (RR 0.77, CI 0.31-1.95), antibiotics (RR 0.26, CI 0.03-2.07), or t-PA (RR 0.31, CI 0.01-10.40). All-cause mortality was not affected by any treatment type (citrate RR 0.81, CI 0.53-1.23; warfarin RR 0.78, CI 0.37-1.65; t-PA RR 0.63, CI 0.15-2.56). Only 6 trials reported bleeding events with no clear effect demonstrated. Trials were mainly of low quality (Jadad score ≤2 in 19 trials).

Conclusions: The net benefit of different anticoagulants for prevention of CM remains uncertain. Further higher quality randomised trials, including safety outcomes, are needed.

TH-PO252

Antimicrobial Activity of Hypertonic Citrate Lock Solution in Hemodialysis Catheters: A New Concept Regarding the Theory of Lock Spillage Gernot Schilcher,[1] Werner Ribitsch,[1] Joerg H. Horina,[1] Alexander R. Rosenkranz,[1] Daniel Schneditz,[3] Christina Strempfl,[2] Bernadette Neuhold,[2] Martin Hoenigl,[2] Thomas Valentin,[2] Jasmin Wagner,[2] Robert Krause.[2] *[1]Division of Nephrology, Medical University of Graz, Austria; [2]Section of Infectious Diseases, Medical University of Graz, Austria; [3]Institute of Physiology, Medical University of Graz, Austria.*

Background: Hypertonic citrate (46.7%) instilled into catheters inevitably spills into the systemic circulation in exchange for blood entering the catheter and diluting the concentration of the lock solution. This process is driven by different densities of whole blood and lock solution. Hence, in a vertical catheter, intra-luminal citrate concentration ranges from 0 (at the tip in catheters with sideholes), 3% (between the sideholes and the relativ highest point of the catheter) to 46,7% (at the luer end) with potential differences in antimicrobial effects. We in vitro investigated the antimicrobial efficacy of pure citrate 46,7%, citrate 46,7% diluted with whole blood (=citrate 3%) and of whole blood, mimicking in vivo conditions in different catheter compartiments.

Methods: Time kill studies (in duplicate) measuring the antimicrobial effect up to 24 h exposure to citrate 46,7%, citrate 3% and whole blood without citrate on overnight cultures ($5x10^8$ cfu/ml) of E. coli and Staph. aureus were performed with each test solution.

Results: Citrate 46,7% reduced E. coli (2 log units) but after 24 h 10^6 cfu/ml were still present. Citrate 3% and whole blood had no antimicrobial effect. Citrate 46,7%, citrate 3% and whole blood had no antimicrobial effect on Staph. aureus within 24 h.

Conclusions: Spillage of catheter lock solution replaced by whole blood leading to reduced intra-luminal citrate concentration considerabely reduces the antimicrobial effect of citrate 46,7% on E. coli. There was no effect on Staph. aureus with all solutions tested. Therefore, the antimicrobial efficacy of hypertonic citrate lock solution has to be seriously questioned. Furthermore, as dilution with whole blood alters antimicrobial activity in general, antimicrobial activity of lock solutions needs to be tested in presence of whole blood.

TH-PO253

Ethanol Lock Technique Causes Protein Precipitation in Vascular Access Devices: An Explanatory Model for Observed Catheter Occlusion Gernot Schilcher,[1] Hubert Scharnagl,[2] Tatjana Stojakovic,[2] Werner Ribitsch,[1] Daniel Schneditz,[3] Robert Krause,[4] Alexander R. Rosenkranz,[1] Joerg H. Horina.[1] *[1]Division of Nephrology, Medical University of Graz, Austria; [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria; [3]Institute of Physiology, Medical University of Graz, Austria; [4]Section of Infectious Diseases, Medical University of Graz, Austria.*

Background: Ethanol lock technique has been proposed as a potential mechanism to eradicate organisms in biofilms and hence, treat or prevent catheter related infections. Following instillation of ethanol lock solution into the catheter the density difference between whole blood and ethanol causes gravity induced seepage of ethanol paralleled by a reverse whole blood influx into the catheter. Therefore, whole blood is exposed to highly concentrated ethanol, which is a common agent for protein precipitation. We aimed to investigate protein precipitation in ethanol locked catheters as a possible cause of catheter occlusion.

Methods: In vitro, blood plasma and ethanol (concentrations ranging from 7 to 70%) mixtures in a ratio of 1:4, representing the intraluminal catheter conditions, were centrifuged at room temperature and 4000 r.p.m. for 10 min. to visibly assess protein precipitation.

Results: Optically detectable protein precipitation was observed in test solutions containing ethanol lock solutions above a concentration of 30%.

Conclusions: Our in vitro data suggest that observed occlusion of central venous catheters locked with ethanol might be due to intraluminal protein precipitation following exposure of plasma proteins to ethanol. Furthermore, injection or gravity induced leakage of precipitated protein particles into the pulmonary circulation may cause pulmonary embolism. We suggest that ethanol lock solution up to the concentration of 30% can be used safely.

TH-PO254

Effect of Catheter Locking on Catheter Related Bacteremia and Catheter Thrombosis Peter B. De Oreo. *Medical Affairs and Quality Assurance, Centers for Dialysis Care, Cleveland, OH.*

Background: Infections cause morbidity and mortality in ESRD patients. Catheter related bacteremias (CRB) are a significant cause of blood stream infections. We implemented 2 published methods of catheter closure to compare the impact on CRB and the use of tissue plasminogen activator (TPA).

Methods: We chose 2 facilities of similar demographics, comorbidities and all type catheter prevalence. In one facility (320 patients) we locked the catheter to its fill volume with 320 µg/ml gentamicin in 4% sodium citrate (GCL) by a published procedure (Moran, AJKD, 2012). In the other facility (110 patients) we used a needle free heparin free catheter cap (NFHF) according the manufacturer's directions. We compared the incidence of CRB in events per 100 catheter months for the same months of 2011 as the observation months in 2012. We compared the cost of TPA averaged over all treatments in the month. We sent blood cultures to the same lab. Cultures separated by fewer than 15 days were considered the same event. GCL costs $2 per treatment. The NFHF cap costs $2 per week. RNs administers GCL. A patient care technician applies the NHFH cap.

Results: The data show a 72% reduction in CRB for both methods. The monthly CRB range in the GLC facility (0 to 9.6) and NFHF facility (7.4 to 12) fell to 0 to 1.6 and 0 to 9.7 respectively. In the NFHF facility 2 of 3 months had 0 CRB. In the GCL facility there was no difference in TPA use ($2 vs $1.95) per treatment. In the NFHF facility the TPA use fell 69% ($5.33 to $1.69) per treatment. None of the organisms in the GCL facility was resistant to gentamicin.

Table 1. Results

	gent/citrate lock		needle free / heparin free	
	CRB *	TPA^	CRB *	TPA^
Ave 2011	3.7	$1.49	6.7	$3.53
BL 2011	5.0	$2.00	9.2	$5.33
Obs 2012	1.1	$1.95	3.2	$1.69
% Change BL v Obs	- 78%	- 2.5%	- 65%	- 69%

per 100 cath-mos* dollars/treatment^

Conclusions: Both methods were effective in reducing CRB. The GCL had no effect on catheter thrombosis as measured by TPA usage. The NFHF system appears to reduce both CRB and catheter thrombosis. The NFHF eliminates the risk and cost of heparin and exposure to gentamicin. Considering RN time and reagent costs, the NFHF cap appears to be a more cost effective method for reducing CRB.

Funding: Clinical Revenue Support

TH-PO255

Risk Factors Associated with Hemodialysis Catheter Malfunction: Results from a Randomized Trial David Ward,[1] Brenda Hemmelgarn,[1] Jennifer M. MacRae,[1] Braden J. Manns,[1] Nairne William Scott-Douglas,[1] Marcello Tonelli,[2] Adeera Levin,[3] Charmaine E. Lok,[4] Louise M. Moist.[5] *[1]University of Calgary, Calgary, AB, Canada; [2]University of British Columbia, Vancouver, BC, Canada; [3]University of Toronto, Toronto, ON, Canada; [4]University of Western Ontario, London, ON, Canada.*

Background: Catheter-locking solutions (CLS) aim to prevent complications such as malfunction and bacteremia in hemodialysis (HD) patients using central venous catheters (CVC). We found a two-fold reduction in CVC malfunction when once-weekly substituting heparin CLS with recombinant tissue-plasminogen activator (rt-PA), compared with thrice-weekly heparin. Determining those who will benefit from prophylactic rt-PA will inform future use and control costs. Using RCT data we sought to determine risk factors associated with CVC malfunction.

Methods: HD patients with newly placed CVC were randomized to rt-PA mid-week and heparin (5000u/ml) on the other HD runs (n=110) or thrice-weekly heparin (5000u/ml) CLS (n=115). We defined CVC malfunction; peak blood flow < 200 mL/min for 30 mins; mean blood flow < 250 mL/min for 2 consecutive HD runs; or inability to initiate HD. Cox regression determined association between patient demographics and HD variables (blood flow, pump speed, CVC reversal) in the 6 runs prior to CVC malfunction (those with primary outcome) or in the 6 runs prior to study end or censoring (those without primary outcome), and CVC malfunction risk.

Results: Baseline patient characteristics were similar between groups. Risks for CVC malfunction were line reversal at the prior run (HR 12.51; 95% CI 7.10 – 22.04), or at least once in the prior 6 runs (HR 9.30; 95% CI 4.36 – 19.84). Malfunction risk increased for each run with a line reversal (HR 1.75; 95% CI 1.55 – 1.98). Reduction in liters of blood processed or blood flow rate were associated with increased malfunction risk.

Conclusions: HD characteristics are associated with CVC malfunction; line reversals demonstrate the highest risk. This study may identify those at increased risk of CVC malfunction who may benefit from once-weekly prophylactic rt-PA. The effectiveness of routine prophylactic rt-PA CLS use in these patients is unknown.

Funding: Pharmaceutical Company Support - Roche Canada

TH-PO256

Comparative Effectiveness of Hemodialysis Catheter Care Protocols to Prevent Bacteremia Alex J. Rosenblum, Jill A. Hall, Weiling Wang, Franklin W. Maddux, Eduardo K. Lacson. *Fresenius Medical Care North America, Waltham, MA.*

Background: The CDC published revised recommendations for central venous catheter care in 2011. We streamlined our hemodialysis catheter care procedure and added an alcohol pad "scrub the hub" and cleansing of the exit site using 2% chlorhexidine swab with 70% alcohol.

Methods: In 2011, 422 Fresenius Medical Care North America facilities were matched 1:1 based on access type, size, and location. We randomly assigned 211 facilities to the revised protocol (RP) and the corresponding matched pair (N=211) into usual care (UC). Training and implementation was in July, 2011 with a 3-month baseline (4/1-6/31) compared to a 3-month follow-up (8/1 -10/30) period. The primary outcome was the rate of positive blood cultures ("bacteremia"). Rates of new IV antibiotic starts, hospitalization for sepsis and adverse reactions to chlorhexidine were also tracked. Historically, seasonal increases in infections occurred over the summer, thus the need for concurrent controls.

Results: There were 364,115 (RP) vs. 361,963 (UC) catheter-days at baseline and 351,357 (RP) vs. 352,538 (UC) catheter days during follow-up. The bacteremia rate was 0.86/1000 cath-days each at baseline, but upon follow-up was 0.80 vs. 1.04/1000 cath-days in favor of the RP group (p=0.02). Baseline antibiotic starts were 1.78 (RP) vs. 1.93 (UC) per 1000 cath-days (p=n.s). Follow-up revealed 2.01 (RP) vs. 2.41 (UC) new IV antibiotic starts per 1000 cath-days (p=0.03). Changes in hospitalizations for sepsis were not significant although documentation was poor - RP: 0.14 to 0.16/1000 cath-days vs. UC: 0.21 to 0.22/1000 cath-days – remaining lower in the RP group. Minor skin reactions were reported in up to 2% of patients using chlorhexidine, some alleviated by decreasing 'rubbing' pressure on the swab and allowing more time to dry before dressing.

Conclusions: Over three months, bacteremia rates declined significantly after initiating a revised catheter care protocol that incorporated CDC recommendations, accompanied by fewer antibiotic starts. Limited data did not show a decline in hospitalizations due to sepsis.

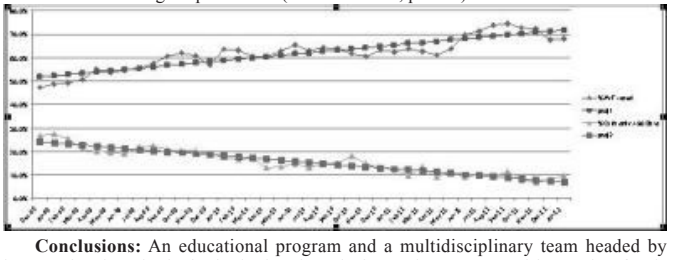
TH-PO257

Catheter Reduction in Hemodialysis Patients: A Multidisciplinary Team Approach Headed by Interventional Nephrology Fawad Qureshi,[1] Tim Deaconson,[2] Stephen S. Cha.[3] *[1]Nephrology & Hypertension, Mayo Clinic Health System, Mankato, MN; [2]Surgery, Mayo Clinic Health System, Mankato, MN; [3]Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN.*

Background: Tunneled hemodialysis catheters continue to be a major contributor to morbidity and mortality. In this analysis, we report the results of our approach to minimizing the use of tunneled hemodialysis catheters.

Methods: Rather than focusing on interventions itself a team based approach (nephrologists, vascular surgeons and dialysis nurses) was implemented to minimize the use of catheters and increase the prevalence of fistulae. Vascular access education delivered on a monthly basis formed the backbone of the approach. Catheter patients who refused to be evaluated to receive an arteriovenous fistula were educated on a weekly basis. Data was collected for all patients dialyzing in our Mankato unit from 2008 to 2012. Regression analyses were performed to check the trend. A positive estimate for slope, or calendar time, indicated an increasing trend, or vice versa. A P-value less than 0.05 were considered statistically significant.

Results: The catheter rates declined from 26.9% to 9.7% (p=0.0001) while the arteriovenous fistula rates increased from 47.4% to 68.1% (p<0.0001). There was no change in the arteriovenous graft prevalence (10.3% to 6.9%; p=0.44).



Conclusions: An educational program and a multidisciplinary team headed by interventional nephrologist is the key in reducing catheter rates and improving fistula prevalence.

TH-PO258

Prevalence, Risk Factors, and Sequelae of Central Venous Stenosis in Hemodialysis Patients at a Hospital-Based Dialysis Center Elaine Ku, Delphine S. Tuot, Anitha B. Toke. *Nephrology, UCSF, San Francisco, CA.*

Background: Subclavian catheters are a known risk factor for central venous stenosis (CVS). Few studies have examined CVS risk factors since the shift towards use of internal jugular tunneled catheters (TCs). USRDS data suggest a recent trend towards increased catheter use, which may increase CVS rates. We examined CVS risk factors and its sequelae in a hospital-based dialysis center.

Methods: A chart review was conducted on prevalent HD patients at an urban hospital-based dialysis center with a large underserved population as part of a quality initiative. CVS was defined by documentation of "central stenosis" at the superior vena cava,

subclavian, or innominate veins in radiology reports. Age, gender, comorbid conditions (diabetes, cardiovascular and peripheral vascular disease), radiology and operative reports were reviewed. HD vintage and race/ethnicity were based on 2728 forms. Multivariable logistic regression adjusting for demographics, comorbid conditions, and HD vintage was used to determine risk factors for CVS and the impact of CVS on arteriovenous fistula and graft (AVF/AVG) failures.

Results: In our cohort, 30% (42/142) have CVS. Odds of CVS are higher with receipt of > 1 TC (vs. 0) [adjusted odds ratio (AOR) =4.51, 95%CI 0.91-22.3)] with each additional catheter conferring a 13% greater odds of CVS (0.94-1.37). In patients with a history of TC, HD vintage was the only significant predictor of CVS (p=0.004), although age 36-65 (vs. 18-35) and diabetes conferred higher odds of CVS (AOR=5.7, 0.98-33.2 and AOR=2.4, 0.97-5.8, respectively). Length of catheter use > 1 year, but not 6 months-1 year, was significantly associated with CVS in our adjusted model (AOR 3.73, 1.13-12.3). In patients with CVS, unadjusted odds of having one or more failed AVF/AVG was 2.63 (1.21-5.69), which was attenuated in the adjusted model (AOR=1.18, 0.46-3.05).

Conclusions: CVS prevalence remains high even with shift to use of internal jugular TCs. Number of lifetime TCs place HD patients at risk for CVS, as do HD vintage, diabetes, age, and length of catheter use, among patients who have a history of TC. CVS may lead to increased risk of failed AVF/AVG.

TH-PO259

Association between Type of Hemodialysis Access and Clinical Outcomes: Meta-Analysis of Cohort Studies Pietro Ravani,[1] Suetonia Palmer,[2] Matthew J. Oliver,[3] Robert R. Quinn,[1] Jennifer M. MacRae,[1] Davina J. Tai,[1] Chandra Mary Thomas,[1] Neesh I. Pannu,[4] Brenda Hemmelgarn,[1] Jonathan C. Craig,[2] Braden J. Manns,[1] Marcello Tonelli,[4] Giovanni F.M. Strippoli,[2] Matthew T. James.[1] *[1]University of Calgary, Canada; [2]Cochrane Renal Group, Australia; [3]University of Toronto, Canada; [4]University of Alberta, Canada.*

Background: Based on observational studies, clinical practice guidelines recommend an arteriovenous fistula as the preferred form of hemodialysis access. However an increasing proportion of individuals treated with hemodialysis never achieve a functioning fistula and use catheters. We sought to examine the association between type of access (fistula, graft and catheter) and patient outcomes.

Methods: We searched MEDLINE (1950 to April 2012) and article reference lists without language restriction. Electronic searching identified 2,309 citations, of which 65 articles (60 cohort studies; n=577,373 participants) met the inclusion criteria. We extracted data on study design, participants, exposure (access type), outcomes (all-cause mortality, infection, and cardiovascular event), and risk of bias. We summarized data across study populations using random-effects meta-analysis.

Results: Patients using catheters experienced increased all-cause mortality (risk ratio, 1.53; 95% confidence interval 1.40–1.67), fatal infections (2.12; 1.79–2.52), and cardiovascular events (1.38; 1.24–1.54), compared to those with fistulas; and increased mortality (1.38; 1.25–1.52), fatal infections (1.49; 1.15-1.93), and cardiovascular events (1.26; 1.11-1.43), compared to patients using grafts. Compared to patients with fistulas, patients using grafts experienced increased all-cause mortality (1.18; 1.09-1.27), and fatal infection (1.36; 1.17-1.58), but not cardiovascular events (1.07; 0.95-1.21). Risk of bias was generally high; all studies except two compared outcomes according to the access achieved, as opposed to that intended.

Conclusions: Patients using catheters for hemodialysis had the highest risk of death, infections, and cardiovascular events, and patients who used fistulas had the lowest risk. There is high risk of selection bias in existing studies.

Funding: Government Support - Non-U.S.

TH-PO260

Is It Possible to Improve the Management of the Central Venous Catheters (CVC) for Hemodialysis (HD)? Emanuele Mambelli, Elena Mancini, Annalisa Zucchelli, Cinzia Elia, Vincenza Guadagno, Antonio Santoro. *Nefrology, Dialysis and Hypertension, Policlinico S. Orsola-Malpighi, Bologna, Italy.*

Background: The management of the CVC at the beginning of the HD session represents a fundamental point both for the administration of the correct HD dose and for the risk of CVC-related infection. However, the procedure is not standardized and generally poorly considered.

We compared the management protocol for the preparation of the CVC presently in use at our Center with the use of a new device (Hemocatch ®) having a specific indication for the management of the HD-CVC.

The number of syringe connections to the CVC with each procedure was the primary end point. The amount of blood wasted during the manoeuvres and the prediction of the blood flow through the CVC were the secondary end points.

Methods: We studied 12 patients with jugular, permanent or temporary, double lumen CVC, over two months. Each patient underwent the management of the CVC with both the conventional process (C) and with Haemocatch (H), over one week each.

Results: Out of 150 HD sessions (75 C, 75 H) the number of the direct connections to the CVC proved lower with Haemocatch (2.19 ± 0.59 with H vs. 4.23 ± 0.78 with C, $p < 0.05$). The same was for the volume of blood wasted (5.97 ± 2.77 ml for H vs. 14:57 ± 6.3 ml for C, $p < 0.05$).

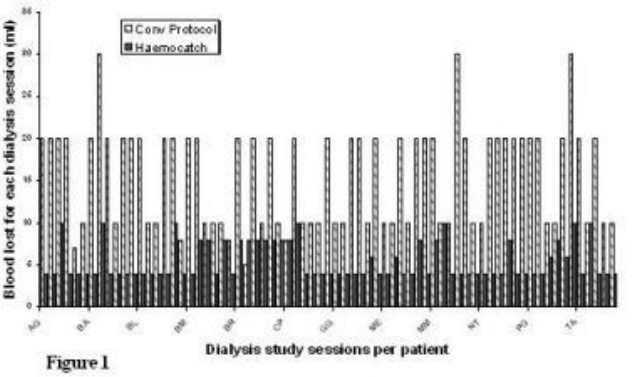


Figure 1

The test for the blood flow forecasting did not show any statistically significant difference between the two procedures.

Conclusions: The use of a device with a specific indication for the CVC management in HD could help in standardizing the procedure and improving the quality of the manoeuvre itself by reducing both the blood loss and the number of syringe connections needed, with a reduction in the infective risk.

TH-PO261

High-Protein Diet Exacerbates Decrease in Muscle Mitochondria and Exercise Endurance after 5/6 Nephrectomy in Mice Masanori Tamaki, Kazutoshi Miyashita, Shu Wakino, Masanori Mitsuishi, Kumiko Tanaka, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio University School of Medicine, Tokyo, Japan.*

Background: Chronic kidney disease decreases physical performance, although the mechanism is unclear. High-protein diet has been recommended for maintenance of muscle mass and power, although the effect in chronic kidney disease patients is not elucidated. We investigated physical performance of 5/6 nephrectomy mice, a model of mild chronic kidney disease and examined the effect of dietary protein on physical performance under chronic kidney disease.

Methods: C57bl/6 mice which had undergone 5/6 nephrectomy at 6-7 weeks were examined for physical performance in young (up to 20 weeks) and aged (up to 52 weeks old) groups. Protein adjusted diets were fed from 8 weeks old.

Results: A decrease in muscle mitochondria and running distance was identified in young 5/6 nephrectomy mice, although muscle mass and power were preserved. A decrease in grip power and muscle mass became apparent in aged 5/6 nephrectomy mice. High-protein diet further decreased running distance of 5/6 nephrectomy mice, associated with a decrease in pyruvate dehydrogenase activity and an increase in lactate production, although muscle mass was increased. Pyruvate dehydrogenase stimulator dichroloacetate recovered pyruvate dehydrogenase activity in skeletal muscle and exercise endurance.

Conclusions: Muscle mitochondria and exercise endurance were decreased by 5/6 nephrectomy from an early age. Thereafter, muscle mass and strength were decreased in aged 5/6 nephrectomy mice. Decreases in muscle mitochondria and ATP production were identified as potential new mechanisms for a reduction in exercise endurance of chronic kidney disease. Decreased pyruvate dehydrogenase activity would explain why a high-protein diet failed to improve exercise endurance. The findings clarified "Kidney-muscle axis" and suggested a new strategies to improve physical performance of patients with chronic kidney disease.

TH-PO262

Dysregulation of Hepatic Fatty Acid Metabolism in Chronic Kidney Disease Kyu-Bok Jin,[1,2] Keith C. Norris,[3] Nosratola D. Vaziri.[1] *[1]Nephrology and Hypertension, University of California, Irvine, Irvine, CA; [2]Internal Medicine, Inje University Haeundae Paik Hospital, Busan, Republic of Korea; [3]Internal Medicine, Charles Drew University, Los Angeles, CA.*

Background: Chronic kidney disease (CKD) results in hypertriglyceridemia which is largely due to impaired clearance of triglyceride-rich lipoproteins occasioned by down-regulation of lipoprotein lipase and VLDL receptor in the skeletal muscle and adipose tissue and of hepatic lipase and LDL receptor-related protein (LRP) in the liver. However data on the effect of CKD on fatty acid metabolism in the liver is limited and was investigated here.

Methods: Male Sprague-Dawley rats were randomized to undergo 5/6 nephrectomy (CRF) or sham operation (control) and observed for 12 weeks. The animals were then euthanized and their liver tissue tested for nuclear translocation (activation) of carbohydrate-responsive element binding protein (ChREBP) and sterol responsive element binding protein-1 (SREBP-1) which independently regulate expression of key enzyme in fatty acid synthesis i.e. fatty acid synthase (FAS) and acyl-CoA carboxylase (ACC) as well as nuclear PPARα which regulates expression of enzymes involved in fatty acid oxidation and transport i.e. L-FABP and CPT1A. In addition expression of ATP synthase α, ATP synthase β, glycogen synthase, and diglyceride acyltransferase 1 (DGAT1) and DGAT2 were determined.

Results: Compared with the controls the CKD rats exhibited hypertriglyceridemia, elevated plasma and liver tissue free fatty acids, increased nuclear ChREBP and reduced nuclear SREBP-1 and PPARα, upregulation of ACC and FAS and downregulation of L-FABP, CPT1A, ATP synthase α, glycogen synthase, and DGAT in the liver tissue.

Conclusions: Liver in animals with advanced CKD exhibits ChREBP-mediated upregulation of enzymes involved in fatty acid synthesis, down-regulation of PPARα-regulated fatty acid oxidation system, and reduction of DGAT resulting in reduced fatty acid incorporation in triglyceride.

Funding: NIDDK Support

TH-PO263

Calcineurin-NFAT Signaling Regulates Atrogin-1 and MuRF1 via microRNA-23a during CKD or Glucocorticoid-Induced Muscle Atrophy

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Background: Skeletal muscle atrophy is prevalent in CKD and microRNAs (miR) may play a regulatory role in the process. miR-23a has been reported to negatively regulate the expression of two atrophy-related ubiquitin ligases, atrogin-1 and MuRF1, as well as the atrophy-inducing FoxO transcription factors. In cardiomyocytes, miR-23a expression is positively regulated by the calcineurin (Cn) substrate NFATc3. The objective of this study is to investigate whether Cn/NFATc signaling and miR-23a expression is altered in skeletal muscle during CKD-related atrophy.

Methods: MicroRNA microarray analysis was performed with CKD and control mouse hindlimb muscle. To induce atrophy in C2C12 or L6 muscle cells, myotubes were treated with 100 nM dexamethasone (DEX). Atrogin-1 and MuRF1 mRNAs and miR-23a were measured by qRT-PCR. Cn/NFATc signaling was evaluated by measuring the mRNA of MCIP1.4, a Cn-regulated gene target. NFATc3 was measured by western blot analysis of nuclear extracts from cultured muscle cells.

Results: Microarray analysis of CKD mouse muscle revealed that miR-23a was reduced 74% vs control ($P<0.05$). Treatment of C2C12 myotubes with DEX for 48 h mimicked CKD by reducing miR-23a 68% ($P<0.05$); MCIP1.4 mRNA was decreased 40% ($P<0.05$), indicating that Cn/NFATc signaling was reduced. In contrast, FOXO3a protein was significantly increased 830% ($P<0.05$) as were the levels of atrogin-1 and MuRF1 mRNAs (1110% and 281%, respectively; $P<0.05$). Nuclear NFATc3 was decreased within 1 h of treatment with DEX, indicating that DEX rapidly suppresses Cn/NFATc signaling; miR-23a was also decreased 79% ($P<0.05$) within1 h of treatment.

Conclusions: Collectively, these findings indicate that Cn-NFAT signaling plays an important regulatory role in the wasting process by suppressing miR23a during CKD and glucocorticoid-induced muscle atrophy.

Funding: NIDDK Support, Private Foundation Support

TH-PO264

Ghrelin Attenuates Cachexia, Muscle Wasting, Cardiovascular Complications, Inflammation and Mortality in Chronic Kidney Disease

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Background: Cachexia, muscle wasting, cardiovascular complications and inflammation are important risk factors associated with high mortality in chronic kidney disease (CKD). Ghrelin is an appetite-regulating hormone with additional effects in the modulation of systemic inflammation and the cardiovascular system. CKD patients with low serum ghrelin have the highest mortality risk and may benefit from ghrelin therapy.

Methods: C57BL/6J mice underwent 5/6 nephrectomy (CKD) or sham operation (S). Ghrelin (150 nmole/kg/day) or saline as vehicle (V) was given to CKD or S mice via osmotic pump subcutaneously.

Results: CKD mice had higher BUN and creatinine levels than S mice ($p<0.001$). CKD+ghrelin, CKD+V and S+V mice were all fed *ad libitum*. Food intake of CKD+ghrelin mice was significantly increased than CKD mice ($p<0.01$). CKD+ghrelin mice gained more weight than CKD mice ($p<0.01$). CKD+ghrelin mice gained lean and fat mass while CKD mice lost lean and fat mass ($p<0.01$). 24 hr metabolic rate was increased and efficiency of food consumption (weight gain/food consumption) was decreased in CKD mice versus S mice ($p<0.001$). Ghrelin normalized these abnormalities in CKD mice ($p<0.01$). Ghrelin corrected systolic hypertension in CKD mice (116.2±2.6 vs 147.8±8.2 mm Hg, $p<0.01$). Left ventricular hypertrophy (LVH, left ventricular mass/body weight) was ameliorated in CKD+ghrelin mice (5.4±0.4 mg/g) versus CKD mice (6.0±0.2 mg/g, $p<0.01$). TNF-α and IL-6 mRNA levels were significantly increased in gastrocnemius and cardiac muscles of CKD mice versus S mice ($p<0.001$). Ghrelin significantly decreased the expression of these inflammatory cytokines in skeletal and heart muscle of CKD mice ($p<0.01$). Over 90-days, mortality rates of 9 month old ghrelin-treated CKD mice (15%) and S mice (0%) were significantly better than those of CKD mice (57.5%).

Conclusions: Ghrelin ameliorates cachexia, muscle wasting, systemic hypertension and LVH with associated improvement in inflammation and mortality in CKD.

Funding: Private Foundation Support

TH-PO265

Lipidomic Analysis of Kidney Tissue in a High-Fat Diet Model: A Key Role of AMPK in Lipid Content and Storage

Anne-Emilie Decleves,[1,2] Joseph Satriano,[1] Zarazuela Zolkipli Cunningham,[1] Alex Thomas,[1] Joelle L. Nortier,[2] Marilyn G. Farquhar,[1] Dorothy D. Sears,[1] Oswald Quehenberger,[1] Edward A. Dennis,[1] Robert K. Naviaux,[1] Kumar Sharma.[1] *[1]University of California, San Diego, San Diego, CA; [2]Université Libre de Bruxelles, Belgium.*

Background: Lipids may contribute to renal cell toxicity. AMP-activated protein kinase (AMPK) is an important energy sensor that may play a critical role in regulating the chronic cellular response to lipid excess.

Methods: To evaluate the role of AMPK, male C57BL/6J mice were randomized to a standard diet, a High Fat Diet (HFD) or a HFD + AICAR (AMPK activator) for 14 weeks. A comprehensive lipidomic approach was combined with renal functional and structural studies along with electron microscopy (EM).

Results: Mice given the HFD showed renal hypertrophy and impaired renal function. Evidence of proximal tubule injury was observed with the presence of enlarged clear vacuoles, and multilaminar inclusions concurrently with an increase of tissue lipid content and a decrease of autophagy. The margins of the clear vacuoles were positive for the endolysosomal marker, LAMP1, suggesting lysosome dysfunction. Characterization of multilaminar inclusions by EM revealed that these contained onion skin-like accumulations of phospholipids. AMPK activation reversed the clinical and structural effects of HFD. To further determine the role of AMPK, we performed an analysis of lipid species by quantitative mass spectrometry to explore the connections between lipid metabolism and biochemical pathways. Lipidomic analysis revealed that HFD significantly affected eicosanoid metabolism, involving arachidonic acid, eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA), an effect that was modulated by AICAR treatment.

Conclusions: These findings reveal that HFD is a potent source of renal damage. There is likely a contribution of lysosomal dysfunction and lipid species alteration that results in the changes observed. A novel role of AMPK was demonstrated to normalize the changes in renal lipid content despite chronic exposure to lipid challenge. The activation of AMPK may be a potential strategy to improve altered lipid metabolism in HFD-induced chronic kidney disease.

TH-PO266

Mild Chronic Kidney Disease Alters Tissue Vitamin K Status in a Rat Model

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Background: Patients with chronic kidney disease (CKD) develop vascular calcification which leads to an increased risk of cardiovascular disease. Matrix-Gla protein is a vitamin-K dependent protein that inhibits calcification. Phylloquinone (K1), the major form of vitamin K consumed in the diet, is preferentially found in the liver. Menaquinone-4 (MK-4), present in the diet in small quantities, is preferentially found in extra-hepatic tissues. In the present study we assessed tissue concentrations of K1 and MK-4 in the presence of CKD.

Methods: Sprague Dawley rats were fed a diet containing 0.25% adenine (CKD; n=6) or no adenine (control; n=6), both diets containing low vitamin K1 (0.2 mg/kg) for 3 weeks. Kidney function was assessed by measuring serum creatinine. The concentrations of K1 and MK-4 in tissue and serum were determined by reversed phase HPLC and expressed relative to wet weight.

Results: Serum creatinine was elevated in the CKD group (142 ± 27 μM) compared to controls (69 ± 11 μM, $p<0.001$). CKD animals had significantly higher levels of MK-4 in the kidney medulla (73 ± 14 vs 44 ± 21 pmol/g, $p=0.02$), kidney cortex (31 ± 17 vs 9.5 ± 3 pmol/g, $p=0.01$), and the testes (184 ± 11 vs 166 ± 15 pmol/g, $p=0.03$), and lower levels of K1 in the heart (24 ± 7 vs 34 ± 9 pmol/g, $p=0.04$), liver (22 ± 5.5 vs 44 ± 14 pmol/g, $p<0.01$), and spleen (8.4 ± 1.8 vs 19 ± 4.5 pmol/g $p<0.01$).

Conclusions: These results indicate that CKD modifies tissue concentrations of K1 and MK-4 under low dietary conditions. CKD animals had significantly higher MK-4 levels and lower K1 levels in various tissues suggesting two possibilities: (1) there are changes that enhance conversion in CKD of dietary K1 to MK-4 and/or (2) that utilization of MK-4 may be impaired in CKD. UbiA prenyltransferase domain containing 1(UBIAD1), a recently identified MK-4 biosynthetic enzyme, is widely expressed and may be responsible for the regulation of MK-4 production. To confirm this, further studies targeting UBIAD1 expression and activity in CKD are warranted.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO267

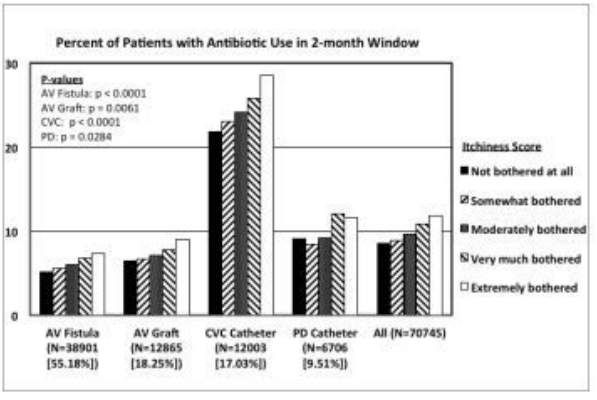
Patient-Reported Pruritus Rates and Indicators of Infection Co-Vary Directly among Patients Receiving Dialysis

T. Christopher Bond, Gilbert Marlowe, Helen M. Wilfehrt, Amy Young, Mahesh Krishnan, Tracy Jack Mayne. *DaVita Clinical Research, Minneapolis, MN.*

Background: Patients on dialysis often report pruritus; scratching may result, disrupting skin integrity and increasing infection risk. The Kidney Disease Quality of Life 36 (KDQOL) questionnaire, administered yearly at a large dialysis organization, assesses itch. We hypothesized increased patient-reported itching would be associated with IV antibiotic use and infection-related inflammation would be associated with increased epoetin alfa (EPO) use (NDT 2009;24(3):919).

Methods: We examined health records for patients undergoing dialysis (1/2009-5/2012) for various characteristics, including IV antibiotic use and mean monthly EPO dose: the former was tracked within a 2-month window of KDQOL administration and other measures were obtained for the month of the questionnaire. Tests for trend in antibiotic use were performed via the Cochran-Armitage test.

Results: On the KDQOL 60% of patients reported itch. IV antibiotics use typically varied directly with increased itch, across access types (Figure). Among patients receiving EPO, mean monthly dose rose with increased itchiness (Table). Other patient characteristics positively associated with degree of itch (not shown).



Pruritus Among Hemodialysis Patients Receiving EPO

During the past 4 weeks, to what extent were you bothered by itchy skin?	Patient-reported itchiness - n (%)	Mean Monthly EPO Dose - U
Not bothered at all	22,255 (39.88%)	55,638.43
Somewhat bothered	16,798 (30.10%)	57,348.48
Moderately bothered	8,545 (15.31%)	58,736.90
Very much bothered	5,210 (9.34%)	62,930.04
Extremely bothered	2,992 (5.36%)	66,133.66

Conclusions: Increased itch varied directly with IV antibiotic usage and EPO dose. These data suggest that increasing pruritus may be directly correlated with increased rates of infection. Research funded by Mitsubishi Tanabe Pharma Corporation.

Funding: Pharmaceutical Company Support - Mitsubishi Tanabe Pharma Corporation

TH-PO268

Circulating Levels of Hepatocyte Growth Factor (HGF) Are Associated with Fat Mass in Chronic Kidney Disease (CKD) Stage 5 Patients

Jiangzi Yuan,[1] Makoto Watanabe,[1,2] Abdul Rashid Tony Qureshi,[1] Jonas Axelsson,[1] Peter F. Barany,[1] Olof Heimburger,[1] Peter Stenvinkel,[1] Bengt Lindholm.[1] *[1]Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden; [2]Nephrology, Showa University School of Medicine, Tokyo, Japan.*

Background: CKD patients have increased serum levels of hepatocyte growth factor (HGF), a multifunctional pleiotropic growth factor implicated in obesity, metabolic syndrome and cardiovascular disease in the general population. However, the association between HGF and body composition in CKD patients remains unknown. Here we investigated determinants of HGF in CKD stage 5 patients and evaluated possible links between body composition, including body fat compartments, and HGF in CKD patients.

Methods: In 224 CKD stage 5 patients (159 males, mean age 53 years, median GFR 6.3 ml/min/1.73m^2) starting on dialysis, fasting blood samples were obtained for analyses of HGF, high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), leukocytes and intercellular adhesion molecule-1 (ICAM-1; a marker of endothelial dysfunction), insulin and blood lipids. Body mass index (BMI) was recorded and total fat mass (FMI), truncal fat mass (TFMI) and LBM (LBMI) indices, expressed as kg/m^2, were calculated based on body composition assessment by dual-energy X-ray absorptiometry. Nutritional status was assessed by subjective global assessment, SGA.

Results: Higher levels of HGF (median 2.94; interquartile range 2.17–4.3 ng/ml) were associated with high age, cardiovascular disease, malnutrition, BMI, FMI, TFMI, serum albumin, insulin, hsCRP, IL-6, leukocytes, ICAM-1, and low density lipoprotein (all rho<0.05). Multivariate linear regression models adjusting for age, gender, CVD, nutritional status, insulin, blood lipids and inflammation showed that HGF was associated with FMI and TFMI.

Conclusions: In this the first study investigating the relationship between HGF and body composition in CKD stage 5 patients, a high HGF level was associated with increased total fat mass and truncal fat mass, but not lean body mass, as well as with inflammation, insulin and blood lipids. These results support a role of HGF in the metabolic syndrome of CKD patients.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

TH-PO269

The Glutathione/Glutathione Disulfide (GSH/GSSG) Redox Potential E_h) Is Low in Erythrocyte (RBCs) of Maintenance Hemodialysis (MHD) Patients (Pts) and It Associates with Parameters of Dietary Intake

Khaled Khazim,[1,4] Daniela Giustarini,[5] Ranieri Rossi,[5] Darlene Verkaik,[3] Sue Cunningham,[1] Franco Folli,[2] Shweta Bansal,[1,3] Paolo Fanti.[1,3] *[1]Nephrology, Univ. Texas HSC, San Antonio, TX; [2]Diabetes, Univ. Texas HSC, San Antonio, TX; [3]ALM VA Hospital, San Antonio, TX; [4]Nephrology, W. G. Hospital, Nahariya, Israel; [5]Dept. Evol. Biology, Univ. of Siena, Siena, Italy.*

Background: GSH, a low molecular mass thiol, protects cells from oxidative damage and regulates cell function. Levels of GSH, its disulfide (GSSG) and the GSH/GSSG ratio are abnormal in MHD, but their relationship with clinical outcomes remains elusive. Using an accurate and precise method to analyze GSH and GSSG, we tested the hypothesis that the intracellular GSH/GSSG and GSH/GSSG $E_h$are lower in MHD Pts than in healthy controls (HCs), and that the GSH/GSSG $E_h$correlates with standard clinical parameters in Pts.

Methods: Blood samples were collected from 33 stable Pts and 10 HCs. Samples for GSH analysis were immediately spiked with *N-ethylmaleimide (NEM)* to form stable -SH-NEM adducts and prevent ex vivo artifactual oxidation of -SH groups, and processed in 6 minutes from phlebotomy to frozen storage. GSH and GSSG were measured by HPLC and GSH recycling method respectively.

Results: GSSG levels were higher in the Pts vs. HCs (28.6 ± 15.6 vs. 15.2 ± 4.6 pmol/mg Hb, p<0.01), while GSH levels were virtually identical between groups. Both GSH/GSSG (353.7± 151.1 vs. 602.1 ± 179.6, p<0.001) and GSH/GSSG E_h(-233.9 ± 5.5 vs. -244.7 ± 5.2 mV, p=0.002) were shifted to oxidation in Pts vs. HCs indicating a net loss of reducing capacity. In the Pts, the GSH/GSSG E_h correlated with BUN (r = 0.48, p = 0.004), nPCR (r = 0.39, p = 0.02) and PO_4 (r = 0.36, p = 0.04). The correlations were similar when using GSH/GSSG instead of GSH/GSSG E_h.

Conclusions: The robust correlation of GSH/GSSG E_h with BUN, PO_4 and nPCR demonstrates a previously unrecognized interaction between cell redox potential and dietary protein and PO_4 intake. This could point to a double-edged sword role of foods containing protein and/or PO_4, i.e. nutritionally necessary but also GSH-consuming pro-oxidants.

Funding: Other NIH Support - NCCAM, Veterans Administration Support, Clinical Revenue Support

TH-PO270

Obesity, Anthropometric Measures and Chronic Kidney Disease Complications

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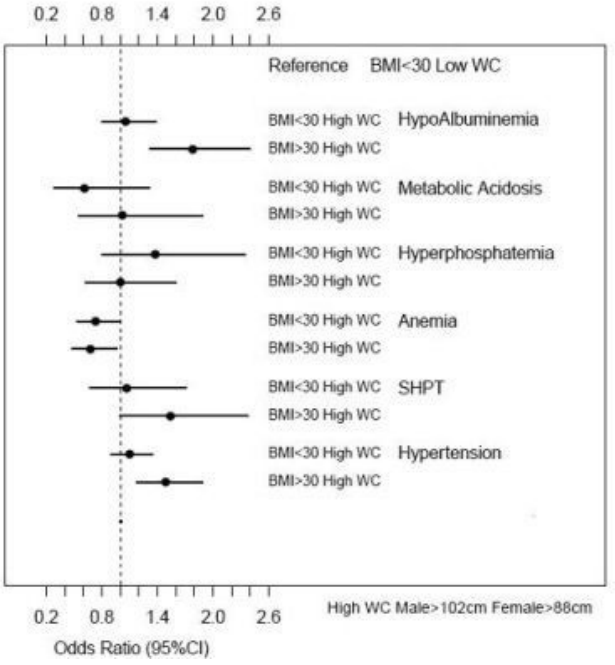
Background: Anthropometric measures such as body mass index (BMI) and waist circumference (WC) have differential associations with incident chronic kidney disease (CKD) and mortality. We examined the associations of BMI and WC with various CKD complications.

Methods: We conducted a cross-sectional analysis of 2853 adult participants with CKD in the National Health and Nutrition Examination Surveys 1999-2006. The associations of BMI and WC (as continuous and categorical variables) with CKD complications such as anemia, secondary hyperparathyroidism (SHPT), hyperphosphatemia, metabolic acidosis, hypoalbuminemia, and hypertension were examined using logistic regression models while adjusting for relevant confounding variables.

Results: CKD participants with a BMI ≥30 kg/m^2 have higher odds of SHPT, hypoalbuminemia, and hypertension than CKD participants with a BMI <30 kg/m^2. Participants with a high WC (>102 cm in men and >88 cm in women) have higher odds of hypoalbuminemia and hypertension than participants with a low WC. When BMI and WC were examined as a continuous variable (2kg/m^2 increase in BMI and 5 cm increase in WC), similar associations were noted. Participants with BMI ≥30 kg/m^2 and high WC (vs. BMI <30 kg/m^2 and low WC) have higher odds of SHPT, hypoalbuminemia, and hypertension while participants with BMI <30 kg/m^2 and high WC (vs. BMI <30 kg/m^2 and low WC) had no increased odds of CKD complications.



Conclusions: Anthropometric measures such as BMI and WC are associated with SHPT, hypoalbuminemia, and hypertension among those with CKD. These findings suggest the need for closely monitoring the development of these CKD complications among those who are obese.

Funding: NIDDK Support

TH-PO271

Is Conventionally Measured Body Mass Index Accurate in African American Populations? Sandra Williams,[2] Dulcie Kermah,[1] Keith C. Norris.[1,3] *[1]Charles R Drew University of Medicine & Science; [2]Department of Endocrinology, Cleveland Clinic; [3]David Geffen School of Medicine at UCLA.*

Background: Racial differences are being increasingly recognized within calculated indices utilized for health assessment. Muscle mass and bone mass are commonly reported to be higher in African Americans compared to their non-African American counterparts. Therefore calculated indices which are dependent on these factors likely require modification for improved accuracy. The now validated and widely utilized "correction coefficient" for estimated glomerular filtration rate (eGFR) in African Americans, represents one example.

Methods: Given the increasing role of increased body mass index (BMI) on cardio-renal outcomes we explored the relationship between BMI and both % body fat and total lean body mass (as assessed by DEXA) for 2,557 blacks and 6,570 non Hispanic whites enrolled in the NHANES 1999-2004.

Results: The estimated correction factor for % body fat for a given BMI for Blacks was 1.082 and for total lean body mass (excluding bone mineral content [g]) was 1.016. The % body fat * total lean body mass correction ratio was 1.099.

Conclusions: Thus a BMI of 25 for the white population is similar to a BMI of 27.48 in regards to the equilibration for % body fat & total lean body mass, the two most metabolically active elements BMI represents. Prospective studies should consider this correction when comparing cardio-renal outcomes across races.

Funding: Other NIH Support - NIH-NIMHD Grant U54MD007598 (formerly U54RR026138); P20MD00182; U54RR022762

TH-PO272

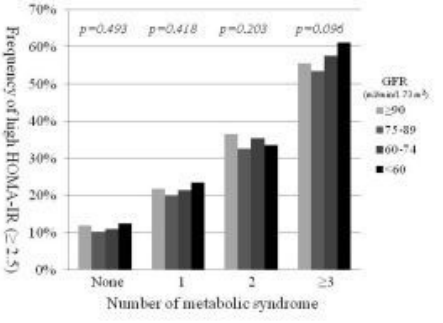
Mild to Moderate Decrease of Glomerular Filtration Rate Is Not Directly Related to Increase of Insulin Resistance Seung Min Lee,[1] Ho Jun Chin,[2] Suhnggwon Kim.[1] *[1]Internal Medicine, Seoul National University Hospital, Korea; [2]Internal Medicine, Seoul National University Bundang Hospital, Korea.*

Background: Insulin resistance is associated with type 2 diabetes, metaolic syndrome, and other cardio-renal risk factors. Decreased glomerular filtration rate (GFR) is assumed to be related to increase of insulin resistance (IR) but the causal relationship between them is not convincing. We tried to confirm whether mild to moderate decline of GFR is directly related to IR without the influence of cardio-renal risk factors.

Methods: We enrolled 17,157 subjects with routine health checkup in SNUBH who were tested fasting blood glucose, insulin, serum creatinine, and components of metabolic syndrome. We calculated HOMA-IR index as a IR marker and GFR by MDRD equation using IDMS traceable creatinine. We estimated the adjusted HOMA-IR index of GFR groups (GFR 1; ≥ 90, GFR2; 75-89, GFR3; 60-74, and GFR 4; <60 ml/min/1.73 m^2) by ANCOVA test adjusted with factors related to HOMA-IR.

Results: The mean GFR in group 1 was 85±6, in group 2, 74±4, in group 3 64±4, and, in group 4, 53±6 ml/min/1.73 m^2. The factors associated to HOMA-IR index were well known cardio-renal riks factors such as, age, gender, number of metabolic syndrome component, hypertension, and etc. The estimated and adjusted HOMA-IR index was highest in GFR group 1 and lowest in GFR group 2. The adjusted HOMA-IR index in GFR group 4 was not different from that of GFR group 2 and 3 (GFR group 1: 2: 3: 4=2.240±0.022: 2.15±0.014 :2.197±0.016 : 2.231±0.045). When we stratified the subjects with number of components of metabolic syndrome, the frequency of high HOMA-IR index (≥2.5) was not different among GFR groups.

Figure 1



(All-p values >0.05)

Conclusions: Mild to moderate decline of GFR was not linearly related to increase of HOMA-IR index, especailly, with controlling the effect of component of metabolic syndrome.

Funding: Private Foundation Support

TH-PO273

Plasma Obestatin Levels Are Inversely Correlated with Renal Function in Patients Undergoing Conservative Treatment Denise Mafra,[1] Natalia Alvarenga Borges,[2] Amanda Barros,[1] Cristiane Moraes.[1] *[1]Graduation Programme in Cardiovascular Sciences, Federal University Fluminense, Rio de Janeiro, Brazil; [2]Antonio Pedro University Hospital, Federal University Fluminense, Rio de Janeiro, Brazil.*

Background: Patients with chronic kidney disease (CKD) frequently experience loss of appetite, which increases during disease progression. Obestatin hormone, an appetite suppressant, can be altered in these patients due to decreased renal function. The aim of this study was to analyze obestatin plasma levels in CKD patients undergoing conservative treatment and its correlation with creatinine clearance (CrCl), appetite and body composition.

Methods: Thirty-three CKD patients (27.3% men, 48.6% diabetics, 65.5±9.4 yrs, BMI 27.6±4.7kg/m^2, CrCl 38.3 [15.0-84.4mL/min], 10% in stage 2, 10% in stage 3a, 60% in stage 3b and 20% in stage 4 of CKD) were studied. The Cockcroft & Gault formula was used to estimate the creatinine clearance. Obestatin plasma levels were measured using the enzyme-linked immunoabsorbent assay kit (Missouri City, TX, USA). Body composition was assessed through Dual-Energy X-Ray Absorptiometry (DEXA) and appetite by Simplified Nutritional Appetite Questionnaire (SNAQ). Statistical analysis was performed using SPSS 17.0.

Results: The mean of SNAQ score was 15.8±1.8 and 31% of patients presented significant risk of weight loss, the % body fat was 33.9±10.6% for men and, 43.1±6.9% for women, the obestatin plasma levels were (0.9[0.3-3.7ng/mL]) and did not correlate with SNAQ or body composition. However, obestatin plasma levels were correlated inversely with creatinine clearance (rho= - 0.55, p= 0.02). There was no difference between obestatin levels in diabetic and non-diabetic patients.

Conclusions: In CKD patients with low CrCl, undergoing conservative treatment, the obestatin levels are increased, suggesting a role of the kidneys in the clearance of this hormone. Therefore, the appetite in these patients can be decreased with the disease progression, providing worse prognosis.

Funding: Government Support - Non-U.S.

TH-PO274

Daily Activity Levels Are Associated with Estimated Kidney Function in Stage 3-5 Chronic Kidney Disease Sarah L. West,[1] Maryum Chaudhry,[2] Tanya D. Dahonick,[2] Charmaine E. Lok.[1,2] *[1]University of Toronto, Toronto, ON, Canada; [2]Toronto General Hospital, Toronto, ON, Canada.*

Background: Chronic kidney disease (CKD) is associated with a decrease in functional ability and fatigue. Only one study has characterized daily activity levels in patients with stage 1-2 CKD and reported that activity level was negatively associated with worsening kidney function. The purpose of our study was to assess the association between daily activity and kidney function in patients with stage 3-5 CKD.

Methods: In patients age ≥18 years with stages 3-5 CKD (using NKF criteria by MDRD), we measured daily activity by triaxial accelerometry (StayHealthy RT3), worn for 7 consecutive days on the hip, and categorized activity as sedentary, light, or moderate/vigorous. Statistical analyses were performed using SAS.

Results: Overall, 59 men and 44 women completed accelerometry assessments, with a mean age of 64 years. The primary cause of CKD was diabetes (25%) and the mean eGFR was 26±15 ml/min/1.73m^2. On average, subjects expended 397±263 kcal/day performing daily activities (i.e., kcal expended above resting energy expenditure). Subjects were primarily sedentary: 1145±114 minutes/day were spent sedentary, while only 288±114 and 7±10 minutes/day were spent completing light and moderate/vigorous activity, respectively. Increasing eGFR was positively correlated with the number of kcals expended/day (r=0.357, p=0.0002), the amount of light activity performed/day (r=0.312, p=0.001), the amount of moderate/vigorous activity performed/day (r=0.237, p=0.02) and was negatively correlated with sedentary activity (r= -0.193, p=0.05). Results were similar when kidney function was estimated by the Cockcroft-Gault and the CKD-EPI equations.

Conclusions: In our patients with stage 3-5 CKD, those with increased eGFR (better kidney function) were more active. A reduction in energy expenditure and physical activity with progressive CKD may have clinical implications, such as risk of functional decline and its complications (falls and fractures) or dietary advice of caloric needs. Future physical activity intervention studies aimed at improving daily activity levels in those with worsening kidney function may provide further insights.

TH-PO275

Cellular Mechanism of Insulin Resistance in CKD: Putative Role of Lipotoxicity in Liver Laetitia Koppe,[1,2] Caroline Pelletier,[1,2] Vella Roxane,[2] Croze Marine,[2] Denis Fouque,[1,2] Soulage Christophe,[2] Fitsum Guebre-egziabher.[1,2] *[1]Department of Nephrology, Hospices Civils de Lyon, Lyon, France; [2]INSA, CarMeN, INSERM U1060, Université de Lyon, Lyon, France.*

Background: Chronic kidney disease (CKD) is associated with insulin resistance (IR). However the molecular mechanisms are unknown. Growing evidence suggest that hepatic IR is related to multiple causes including endoplasmic reticulum (ER) stress, inflammation and hepatic lipid accumulation. Excessive accumulation of fat metabolites may interfere with insulin signaling by activating novel protein kinase (PKCε). Recently, it has also been postulated that dysregulation of the energy sensor, AMPK pathway may in itself be a contributing factor. We hypothesize that IR in the context of CKD, may be related to liver fat accumulation and the subsequent disturbances of these metabolic pathways.

Methods: C57BL/6 mice underwent a 5/6 nephrectomy and were compared to pair fed sham-operated mice. Insulin sensitivity was estimated through intra-peritoneal insulin (ipITT) and glucose tolerance (ipGTT) tests. The phosphorylation of AMPK and the activation of PKCε (membrane translocation) and ER stress markers were studied by Western Blot in the liver. The intra-hepatic lipids were measured.

Results: The CKD mice exhibited a marked decrease in insulin sensitivity (-76%, p<0.01) and altered glucose tolerance (+24 %, p<0.001). Insulin resistance was associated with a significant decrease in white adipose tissue accretion (-57%, p<0.001) and increased liver (+38%, p<0.05) lipid content. Furthermore, we show that this increase in hepatocellular lipid content is associated with PKCε activation (+52 %, p<0.05) and decreased AMPK phosphorylation in the liver (-54%, p<0.01).In contrast, insulin resistance in these mice was not associated with increased ER stress.

Conclusions: Taken together, our data show that ectopic lipid accumulation in liver is associated with PKCε activation and decreased AMPK activation in CKD. This suggests the potential involvement of lipotoxicity in the development of IR and the increased risk of cardiovascular disease in CKD. Furthermore, ER stress might not be a likely contributor to the observed IR.

Funding: Government Support - Non-U.S.

TH-PO276

Effects of Sevelamer-HCl Treatment on Cardiovascular Abnormalities in CRF Mice Julien Maizel, Isabelle Six, Sebastien Dupont, Edouard Secq, Benedicte Dehedin, Fellype Carvalho Barreto, Joyce Benchitrit, Sabrina Poirot, Michel Slama, Christophe M. Tribouilloy, Gabriel Choukroun, Jean-claude A.R. Maziere, Tilman B. Drueke, Ziad Massy. *INSERM 1088, Amiens University Hospital (Univeristy of Picardie), Amiens, France.*

Background: Elevated serum phosphate and FGF23 levels are associated with cardiovascular disease in patients with chronic renal failure (CRF). The calcium-free phosphate binder sevelamer has been shown to decrease both serum phosphorus and FGF23. Limited data indicate that sevelamer may improve CRF-related cardiac and vascular conditions, such as diastolic dysfunction, left ventricular hypertrophy (LVH) and aortic stiffness. We investigated the effects of sevelamer-HCl on cardiovascular abnormalities in a murine model of CRF.

Methods: Groups of CRF and non-CRF mice received either sevelamer-HCl or placebo treatment for 14 weeks, starting 6 weeks after the initiation of CRF or sham operation. Animals underwent echocardiography and arterial blood pressure and pulse wave velocity (PWV) were measured.

Results: After 8 weeks of sevelamer-HCl treatment CRF mice had decreased serum phosphorus levels and improved aortic systolic expansion rate (ESAo), PWV and diastolic function, although LVH remained unchanged. However, following an additional 6-week course of sevelamer-HCl treatment, LVH also improved. In a multiple regression analysis, both serum phosphorus and FGF23 were independently correlated with LV mass. ESAo and LV diastolic parameters were correlated with serum phosphorus but not with FGF23.

Conclusions: Sevelamer-HCl improved aortic stiffness and cardiovascular abnormalities in CRF mice. Whether sevelamer may also improve cardiovascular disease in CRF patients remains to be established.

Funding: Pharmaceutical Company Support - Genzyme

TH-PO277

The Role of Local Bone Vascularization and Paracrine Factors in Chronic Kidney Disease Related Growth Retardation Yael Segev,[1] Ariel Troib,[1] Ralph Rabkin,[2] Daniel Landau.[3] *[1]Shraga Segal Dept. of Microbiology and Immunology, Ben Gurion University, Beer Sheva, Israel; [2]Medicine, Veterans Administration Health Care System, Palo Ato, CA; [3]Padiatrics, Soroka University Medical Center, Beer Sheva, Israel.*

Background: Growth retardation, a major problem in children with chronic kidney disease (CKD) is partly caused by GH resistance with impaired IGF-I bioavailability. Normal longitudinal growth requires chondrocyte maturation in the epiphyseal growth plate (EGP), mediated by numerous autocrine and paracrine factors. We have previously shown evidence for GH-R resistance at the bone level of young growth retarded CKD rats. We now investigated the role of vascularization and paracrine factors in developing bones in the same model.

Methods: Three week-old rats underwent a 2 step 5/6 nephrectomy (CKD) or sham operation (C). Both groups were pair-fed with standard chow and sacrificed after 2 weeks.

Results: As previously shown, there was a significant decrease in weight gain and longitudinal growth in CKD Vs C in spite of pair-feeding. Serum phosphate levels were unchanged but PTH increased 2-fold in CKD (p< 0.01). EGP morphometry showed a wider hypertrophic zone in CKD rats. The decrease in EGP GHR signaling was associated with a decrease in immunostainable IGF-I, mainly in the resting and proliferative zones. Histochemical staining with Movat's pentachrome and Aniline blue showed a decreased vascularization process at the primary ossification center distal to the hypertrophic chondrocytes, as well as a decrease in VEGF mRNA in the CKD group. In addition, indian hedgehog (IHH) protein, which normally synchronizes skeletal angiogenesis and perichondrial maturation with cartilage development, was decreased in the EGP of CKD animals.

Conclusions: Growth retardation in CKD juvenile rats is associated with decreased EGP GHR signaling and IGF-I expression, further explaining GH resistance in CKD. In addition, the widened hypertrophic EGP zone and decreased vasularization, mediated by a decreased VEGF and IHH expression suggesting a chondrocyte maturation arrest in CKD.

Funding: Government Support - Non-U.S.

TH-PO278

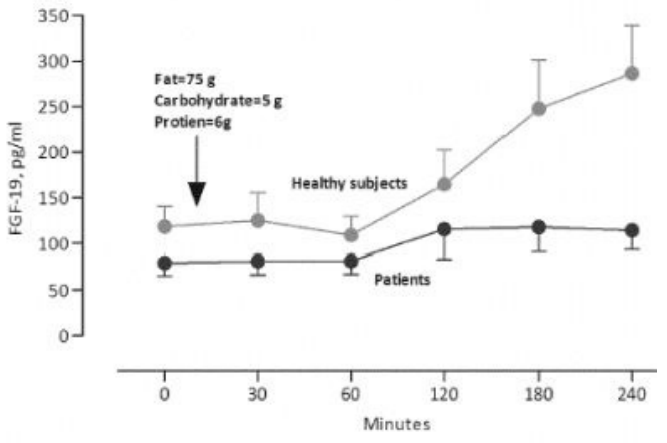
Impaired Postprandial Plasma Fibroblast Growth Factor (FGF)-19 Response in CKD Stage 5 Patients Meng Li,[1] Björn Anderstam,[2] Jonas Axelsson.[1] *[1]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [2]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden.*

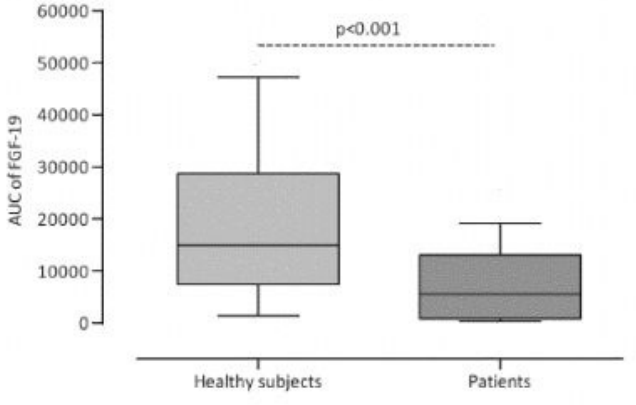
Background: Uraemia is associated with carbohydrate and fat dysmetabolism that predicts mortality. Fibroblast growth factor (FGF)-19 is a hormone-like protein that was recently reported to regulate carbohydrate, lipid and bile acid metabolism. The aim of this study was to evaluate FGF-19 response to a fat- and carbohydrate- rich meal provocation in CKD patients.

Methods: Six CKD patients on maintenance HD and nine matched healthy subjects were studied on a dialysis-free day. A standardized meal (75 g of milk fat, 5 g of carbohydrates and 6 g of proteins/m^2 of body surface area, corresponding to a total caloric intake of 700 kcal/m^2 of body surface area) was administered. Each participant was tested on 4 separate occasions (7 days in between each), and the results were combined for analysis. Blood was collected at baseline (following a 8 hrs fast) and 30, 60, 120, 180 and 240 min following the meal. FGF-19 was analyzed using a commercial ELISA kit (R&D Systems, U.K.).

Results: FGF-19 in plasma from healthy subjects rose following the meal to reach a peak 286.2±53.1 pg/mL at 240 min. However, in uremic patients FGF-19 did not rise significantly in response to the meal (peak 114±20.5 pg/mL at 120 min). We did not see significant differences in circulating glucose or TG concentrations between patients and controls; it is unlikely to explain the blunted FGF-19 response. $AUC_{0\text{-}240min}$ of FGF-19 was thus significantly higher in healthy than in CKD participants (*$P < 0.001$), Figure 1A and B.*

Conclusions: CKD stage 5 patients showed a markedly blunted postprandial FGF-19 response. While mechanisms were not investigated, the blunted FGF-19 response may be linked to uremic dysmetabolism, most likely involving the liver.





Funding: Government Support - Non-U.S.

TH-PO279

Rate of Change in Fibroblast Growth Factor 23 Level Predicts the Progression of Chronic Kidney Disease from Early-Stage in Elderly Patients Eiichiro Kanda,[1] Masayuki Yoshida,[2] Sei Sasaki.[2] *[1]Tokyo Kyosai Hospital; [2]Tokyo Medical and Dental University.*

Background: The level of fibroblast growth factor 23 (FGF23), which regulates phosphate and vitamin D metabolism, increased with the progression of chronic kidney disease (CKD). FGF23 as a risk factor for the progression of CKD in elderly CKD patients has not been fully established.

Methods: 65 elderly CKD patients were enrolled in this prospective cohort study and followed up for one year in Tokyo, Japan. We compared estimated glomerular filtration rate (eGFR) and the levels of CKD-mineral and bone disorder related markers, such as 1,25(OH)2 vitamin D (1,25-VD), intact PTH, and FGF23, at the start of the study as the baseline with those one year later, and identified which marker predicted the progression of CKD. The rate of change in log-FGF23 was determined using the following calculation: change in log-FGF23/baseline log-FGF23. Factors associated with the change in eGFR were assessed by regression models.

Results: 46 patients were male: average age(SD), 72.7(7.7) years; diabetics, 64.6%; eGFR, 51.1(7.7) ml/min/1.73m^2; calcium, 9.4(0.5) mg/dl; phosphate, 3.4(0.7) mg/dl; 1,25-VD, 48.8(21.2) pg/ml; intact PTH, 127.7(92.6) pg/ml; and FGF23, 72.8(83.4)pg/ml. A decreased eGFR was observed in 36.9% of the patients. The average change in eGFR was -0.305(1.41) ml/min/1.73m^2/year. Spearman's rank correlation showed that the change in eGFR significantly correlated with serum calcium level, 1,25-VD level, intact PTH level, log-FGF23, rate of change in log-FGF23 (P<0.0001). In stage-3 patients, adjusted multivariate analysis showed that change in eGFR was associated negatively with rate of change in log-FGF23 (P=0.0087). Adjusted multiple regression analysis showed that the high rate of change in log-FGF23 was associated with the decrease in GFR adjusted odds ratio (1.174; 95% confidence interval, 1.006-1.372). However, these relationships were not observed in stage-4 or -5 patients.

Conclusions: In elderly CKD patients, our findings suggest that the rate of change in log-FGF23 is associated with the progression of CKD, particularly in early stage. FGF23 level is a candidate marker for identifying patients in the state of CKD progression.

TH-PO280

A 16 Week Study on Impact of Vitamin D Supplementation on Endothelial Function in CKD Nihil Chitalia, Tuan F. Ismail, Juan C. Kaski, Debasish Banerjee. *Cardiovascular Research Centre, St Georges, University of London, United Kingdom.*

Background: Atherosclerosis in Chronic Kidney Disease (CKD) patients is the major cause of mortality. We previously demonstrated endothelial dysfunction and its association with atherosclerosis and Vitamin D deficiency. However the effect of Vitamin D supplementation on endothelial function in CKD is unknown.

Methods: This study aimed to investigate the effect of 25(OH) Vitamin D supplementation on endothelial function in patients with CKD stages 3/4 over 16 weeks. The study was approved by NHS Research Ethics Committee. The endothelial function was measured with post-ischaemic brachial artery flow mediated dilatation at a fasting state by trained staff using a method previously established in our laboratory. The central artery stiffness was measured with pulse wave velocity using SphygmoCor machine. Vitamin D was supplemented with 2 doses of 300,000 units of Cholecalciferol (Martindale) at baseline and 8 weeks.

Results: Twenty-one stable CKD patients with Vitamin D <75 nmol/L; and without diabetes, heart failure, infection, malignancy, autoimmune disease, CRP>20 mg/L, hypercalcaemia, recent myocardial infarction or stroke; were enrolled. At baseline the clinical characteristics were; age 52±14 (mean±SD) years, females 33%, Southeast Asian 24%, Afro Caribbean 24%, dyslipidaemia 43%, smokers 24%, BMI 29±5 kg/m^2, systolic BP 133±13 mmHg, diastolic BP 86±9 mmHg, eGFR 38±11 ml/min/1.73m^2, haemoglobin 13.7±1.4 g/dl, vitamin D 45±15 nmol/L, calcium 2.37±0.1 mmol/L, phosphate 1.1±0.2 mmol/L, parathyroid hormone 11.6±9.1 pmol/L. With treatment the vitamin D level increased (45±15 to 79±29 nmol/L; p<0.001), calcium increased (2.37±0.09 to 2.43±0.09 mmol/L; p=0.001), parathyroid hormone decreased (11.6±9.1 to 7.5±2.4 pmol/L;p=0.008). No patients developed hypercalcaemia. Serum phosphate, eGFR levels remained unchanged. Endothelial function improved (2.80±3.87 to 5.59±2.89 %; p<0.004). The pulse wave velocity remained the same (7.9±1.3 and 7.8±2.2 m/s; p=0.78).

Conclusions: This study demonstrated for the first time that a short course of 25(OH) Vitamin D therapy in patients with moderate CKD was safe and improved endothelial function.

TH-PO281

Metabolic Effects of Paricalcitol in Stage 3-4 Chronic Kidney Disease Ian H. de Boer, Michael Sachs, Andrew N. Hoofnagle, Bryan R. Kestenbaum, Kristina M. Utzschneider, Steven E. Kahn, Jonathan Himmelfarb. *University of Washington, Seattle, WA.*

Background: Patients with CKD are often insulin resistant and glucose intolerant, and these abnormalities appear to promote cardiovascular disease. Animal-experimental and human data suggest that vitamin D receptor agonists improve insulin sensitivity and glucose tolerance in CKD.

Methods: We conducted a clinical trial of 22 persons with estimated GFR 15-59 mL/min/1.73m^2 and fasting plasma glucose 100-125 mg/dL. In a cross-over design, each participant was allocated oral paricalcitol 2 µg daily for 8 weeks and matching placebo for 8 weeks, separated by an 8-week washout period. The order of interventions was randomly assigned and blinded to both participants and investigators. The primary study outcome was glucose tolerance measured using the oral glucose tolerance test.

Results: Paricalcitol reduced serum concentrations of parathyroid hormone (29.4 vs 70.9 pg/mL, p<0.001), 1,25-dihydroxyvitamin D (10.8 vs 26.4 pg/mL, p<0.001), and 25-hydroxyvitamin D (23.7 vs 30.4 ng/mL, p<0.001) and increased serum concentrations of fibroblast growth factor-23 (118.4 vs 68.2 pg/mL, p<0.001) and 24,25-dihydroxyvitamin D (3.8 vs 2.6 ng/mL, p<0.001). However, paricalcitol had no effect on glucose tolerance (difference in glucose area under the curve -1.5%, 95% confidence interval -5.9%, 3.2%, p=0.54), insulin sensitivity, beta-cell insulin response, plasma free fatty acid suppression, or urinary F2-isoprostane excretion.

Conclusions: In stage 3-4 CKD, paricalcitol has substantial effects on vitamin D metabolism beyond suppression of parathyroid hormone but does not improve glucose metabolism.

Funding: NIDDK Support, Other NIH Support - NHLBI, NCRR, Pharmaceutical Company Support - Abbott Laboratories

TH-PO282

Gland Sparing Parathyroidectomy with Target Parathyroid Gland Reduction Offers Safe Long-Term Metabolic Control: A Single-Center Experience in the Current Era Christopher Clark,[1] Karen T. Pitman,[1] Mihaly Tapolyai,[2] Mehrdad Hamrahian,[1] Eva Csongradi,[3] Tibor Fulop.[1] *[1]University of Mississippi Medical Center, Jackson, MS; [2]Fresenius Medical Care - Semmelweis University, Hungary; [3]Medical and Health Science Center - University of Debrecen, Hungary.*

Background: Long-term results of surgical parathyroidectomy (PTX) in end-stage renal disease patients with targeted parathyroid hormone (PTH) reduction are less well known in the modern era.

Methods: Retrospective chart review of ESRD patients, who underwent PTX during index period of 08/2006 - 08/2011 and, when records available, follow-up data up to 3 years. All PTX were performed by a single surgeon (KTP) with intra-operative PTH monitoring.

Results: Of the identified 37 patients 94.6% were African-American and 59.5% females. In this relatively young cohort (48.4 ±13.9 years), histology recovered predominantly hyperplasia (94.6%) rather than adenoma. 45.9% received cinacalcet (CNC) before PTX (66.43 ±20.9 mg). Preoperative calcium/phosphorus was 9.6 ±1.2/6.6 ±1.7 mg/dL with PTH of 1,589.1 ±827 pg/mL. Mean length of stay was 5.5 ±2.4 days. Stabilized PTH values (4-8 days after PTX) measured 145.4 ±119.2 pg/mL. Pre-operative PTH strongly correlated ($p<0.0001$) with both Alkaline Phosphatase (ALP) (r 0.596) and length of inpatient days (r 0.545), but not with CNC administration. Long-term follow-up results were available for only a part of the cohort, shown on Table 1.

Table1. Long-Term Biochemical Control in Local Follow-up Cohort

	6 Month	12 Month	24 Month	36 Month
Calcium, (mg/dL)	8.5 ±1.1	8.7 ±0.6	8.5 ±0.7	8.6 ±0.9
Phosphorus, (mg/dL)	6.1 ±2.1	6.8 ±1.8	6.2 ±1.2	5 ±1.6
PTH, Intact (pg/mL)	400 ±366	323 ±227	465 ±326	285 ±202
Alk. Phos, (U/L)	155 ±117	83 ±18	63.8 ±21.7	70.7 ±20.1
Total Bioactive Vitamin-D or Analog Dose, (mg/week)	6.9 ±9	3.7 ±3.60	6.17 ±3.70	4.8 ±4.4
N, (Participant)	12	12	8	6

At 24 months, 2 of the 8 subjects received CNC, 30 mg/day. No clinical fractures were observed.

Conclusions: Gland sparing PTX improved PTH control with less active vitamin-D utilized in our patients. While hyperphosphatemia may be a persisting problem in these patients, ALP and calcium levels remained normalized up to 3 years.

TH-PO283

Parathyroidectomy versus Medical Management for Secondary Hyperparathyroidism in ESRD: Patient Eligibility, Characteristics and Clinical Outcomes Girish N. Nadkarni,[2] Ioannis Konstantinidis,[1] Vijay Lapsia.[1] *[1]Medicine, Mount Sinai School of Medicine, New York, NY; [2]Medicine, St.Luke's Roosevelt Hospital Center, New York, NY.*

Background: Secondary hyperparathyroidism (SHPT) is a common complication of End Stage Renal Disease (ESRD). Parathyroidectomy (PTX) is indicated to manage SHPT refractory to medical management (MTX). We compared baseline characteristics and clinical outcomes of ESRD patients undergoing PTX with those receiving MTX at one year.

Methods: Data on ESRD patients treated between Aug 2007 and Dec 2011 at our institution were retrospectively reviewed. Patients with persistently elevated levels (>800 pg/mL) of intact parathyroid hormone (iPTH) for minimum of six months associated with hypercalcemia and/or hyperphosphatemia for at least 50% of that time period were included. Patients with less than 12 months of follow up were exlcuded. Baseline characteristics, mortality and change in iPTH, calcium, phosphorus, albumin and Alkaline phosphatase (ALP) at baseline and 12 months were compared using chi-square and paired t-tests.

Results: Sixty-one patients satisfied inclusion criteria (PTX=10, MTX=51). At baseline, PTX patients had been on dialysis longer, 12.5 years vs 5.5 years ($p=0.001$), with higher iPTH (2968 vs 1431, $p<0.001$), calcium ($p=0.008$), ALP ($p=0.001$) and were less likely to be African American ($p=0.007$). Seventy percent (n=7) of surgical patients were receiving cinacalcet at the time of inclusion compared to 27% (n=12) of patients in the MTX cohort ($p=0.023$). At 12 months, iPTH decreased 96% ($p<0.001$) and ALP by 67% ($p=0.0004$) in the PTX cohort, whereas iPTH decreased 23% ($p=0.031$) without any significant reduction of ALP in the MTX cohort. All PTX patients who complained of preoperative pruritis and bone pain experienced complete resolution after surgery.

Conclusions: A large proportion of patients did not receive PTX despite meeting criteria. Dialysis vintage, higher levels of iPTH and cinacalcet therapy were more likely to be associated with PTX. Patients in the PTX group had better reduction in PTH and ALP and achieved symptomatic control. Larger studies are required to identify barriers to PTX.

TH-PO284

Association between Plasma Levels of Fructose with Uric Acid and Body Mass Index in Chronic Kidney Disease (CKD) and Peritoneal Dialysis PD Patients (pts) Abdul Rashid Tony Qureshi, Ann-christin Bragfors Helin, Olof Heimburger, Peter F. Barany, Peter Stenvinkel, Bengt Lindholm, Björn Anderstam. *Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: Excessive intake and altered metabolism of fructose have been suggested to play a role in the development and progression of metabolic syndrome, possibly via links to increased formation of uric acid. However, there is a lack of data regarding fructose metabolism in CKD and PD pts. We studied the association between fructose levels, uric acid, and carbohydrate and lipid metabolites in healthy subjects (HS), CKD stage 3-4, non-dialyzed CKD stage 5 (CKD5-ND) pts and PD pts.

Methods: Plasma fructose and uric acid were analyzed in 80 HS [females 29%; median age 63 (range 22-80) yrs], 90 CKD-stage 3-4 [females 28%; median age 60 (range 23-80) yrs], 214 CKD5-ND [females 39%; median age 55 (range 19-70) yrs] pts and 83 PD [females 32%; median age 55 (range 25-85) yrs] pts. Estimated GFR (ml/min) was 85 (72-96) in HS, 23 (15-32) in CKD 3-4 and 6.5 (5-8) in CKD5-ND and 5 (4-7) in PD pts. Data on demographic nutritional and metabolic characteristics at baseline were recorded.

Results: The median (range) level of fructose (umol/L) was 86 (62-109) in HS, 86 (64-116,) in CKD 3-4, 64 (44-87, $p<0.01$) in CKD5-ND pts and 76 (54-112) in PD pts. Uric acid (mg/L) was 330 (range 273-391) in CKD 1-2, 478 (427-544, $p<0.01$) in CKD 3-4, 398 (316-481, $p<0.01$) mg/L in CKD5-ND and 309 (range 265-360, NS) in PD pts. Fructose and BMI were associated in HS (rho=0.41, $p<0.001$) and CKD 3-4 pts (rho=0.22, $p<0.05$), but not in PD (rho=0.21, $p=0.09$) or CKD5-ND (rho=0.11, NS) pts. Fructose and hsCRP were associated in HS (rho=0.56, $p<0.001$) and PD pts (rho=0.31, $p<0.001$). Uric acid and hsCRP were associated in HS (rho=0.37, $p<0.01$) in CKD 3-4 pts (rho=0.22, $p=0.09$) and in CKD5-ND pts (rho=0.22, $p<0.001$).

Conclusions: In comparison to HS, CKD 3-4 pts, PD-pts, fructose levels were lower in CKD5-ND pts. Fructose levels were associated with BMI in HS and CKD 3-4 pts and with inflammation in HS and PD pts.High levels of uric acid in CKD 3-4 and CKD5-ND pts may be associated with inflammation.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

TH-PO285

Effects of Febuxostat versus Allopurinol in Reducing Serum Urate in Subjects with Hyperuricemia and CKD Stage 3-5: A 12-Week, Randomized Trial Nobuhito Hirawa,[1] Mari Katsumata,[1] Keisuke Yatsu,[1] Yoshiyuki Toya,[2] Gen Yasuda,[1] Satoshi Umemura.[2] *[1]Nephrology and Hypertension, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan; [2]Nephrology and Hypertension, Yokohama City University Hospital, Yokohama, Kanagawa, Japan.*

Background: Febuxostat (Feb), an orally administered nonpurine selective inhibitor of xanthine oxidase, was developed recently. Compared to alloprinol (Allo), Feb directly inhibits xanthine oxidase and is metabolized by liver, partly. Thus, Feb may be easy to use in patients with renal impairment. In this study, to clarify the urate-lowering efficacy and safety of Feb, we compare the effectiveness of Feb and Allo in subjects with hyperuricemia and CKD stage 3-5.

Methods: Forty patients with CKD stage 3-5 and hyperuricemia (serum urate level > 8mg/dl) were randomized to receive once-daily Feb (10mg) or Allo (50mg) for 4 weeks. After that, Feb and Allo were permitted to increase the dose until 40mg/day (Feb) in all participants, and 100 mg (Allo) in CKD stage 3. The 1st endpoint was the UA values at 12 weeks. Furthermore, we examined the effects on renal function and investigated the safety of Feb and Allo treatments in CKD stage 3-5 patients.

Results: The average age and eGFR of the participants were 63 years old and 22 ml/min/1.73m2. There were no differences in BP, Cr, UA values (9.7 mg/dl) between Feb group and Allo group in the control period. Feb and Allo significantly decreased the serum UA levels at 2 weeks after treatments. The UA values of Feb and Allo were the same until 4 weeks of treatment. Meanwhile, the UA level of Feb group was significantly lower than Allo group in 8 and 12 weeks of treatments (6.8 vs 8.1 mg/dl, $p<0.025$). Serum Creatinine, eGFR were not changed with UA lowering treatments. The changes of the slope of 1/Cr were negatively correlated with the alterations of the serum UA. Using the multivariate analysis, the UA values of 12 weeks treatments were associated with Feb use, baseline UA value and urinary protein / Cr ratio. Obvious side effects were not recognized in Feb treatments.

Conclusions: Febuxostat seem to be effective and safe in patients with hyperuricemia and CKD stage 3-5.

TH-PO286

The Inhibition of NADPH Oxidase 4 Ameliorated the Fibrotic Responses In Vitro, and in Diabetic Mouse Kidneys Rachel Lingling Yong, Sonia Saad, Xinming Chen, Chunling Huang, Carol A. Pollock. *Renal Medicine, Kolling Institute, St Leonards, NSW, Australia.*

Background: NADPH oxidase family has been identified as a major source of superoxide and hydrogen peroxide generation in the cardiovasculature and kidney during health and disease. Nox4 is most accountable for ROS-induced renal hypertrophy and myofibroblasts activation induced by TGFβ1 which plays a central role in diabetic renal fibrosis. This study aimed to investigate the role of Nox4 inhibition on TGFβ1-induced fibrotic responses in vitro and in diabetic kidneys in a mouse model.

Methods: In vitro, immortalised human proximal tubular cells (HK2) were incubated with TGFβ1 (2ng/ml) +/- plumbagin (1.5μM/L), a selective inhibitor of Nox4 for 48 hours. Then the expression of mRNA and protein of collagen 4 and fibronectin were measured by qRT-PCR and Western blotting. In addition, the collagen 4 and fibronectin in Nox4 silenced HK2 cells exposed to TGFβ1 were examined. In vivo, C57BL mice with diabetes induced by Streptozotocin were administered with plumbagin (2 mg/kg/day) or vehicle (DMSO) alone for 3 weeks after induction. At 24 weeks, mice were culled and kidneys were collected for histological analysis.

Results: TGFβ1-induced expression of fibronectin and collagen 4 in HK2 cells was significantly reversed by plumbagin or in Nox4 gene silenced cells compared to control ($P<0.01$). In animal studies, diabetic mice have increased levels of fibronectin and collagen 4 in addition to increased extracellular matrix deposition. All were significantly reduced in the presence of plumbagin in diabetic kidneys compared to vehicle controls.

Conclusions: This study suggests that Nox4 regulates the expression of collagen 4 and fibronectin and Nox4 inhibition ameliorated diabetic kidney nephropathy.

TH-PO287

The Blocking of Renin-Angiotensin-Aldosterone System Decline Both EPO and EPO Receptor in CKD Rat Jae Won Yang,[1] Seung-Ok Choi,[1] Byoung Geun Han,[1] Minseob Eom.[2] *[1]Nephrology, Yonsei University Wonju College of Medicine, Wonju, Gangwon, Korea; [2]Pathology, Yonsei University Wonju College of Medicine, Wonju, Gangwon, Korea.*

Background: While many investigators observe reduced hemoglobin associated with angiotensin-converting enzyme (ACE) inhibitor, the basis for this effect is not well understood. The angiotensin II may be considered as one important physiological modulator of EPO production. We investigated that the ACE inhibitor can modulate the EPO in kidney and EPO receptor in bone marrow.

Methods: A 5/6 nephrectomy was performed in Sprague-Dawley rats (N= 24, male), and divided three groups after 8 weeks. We supplied general diet to the control (N=8), low salt diet to the LSD (N=8) group, and low salt diet with enalapril 50mg/L in drinking water to the ACEI group (N=8) for 2 weeks. After the collection of blood on day 14, the remaining kidney and femur was ressected surgically, the serum EPO and angiotensin II levels by ELISA, and tissues were investigated by IHC stain, and RT-PCR for EPO and EPO receptor.

Results: The Hb levels were increased in LSD group (17.30±1.21g/L) relative to control (12.55±0.86g/L), but decreased in ACEI group (16.40±1.30g/L) relative to LSD group (p=0.002). The serum angiotensin II levels were decreased in ACEI group (1,968.71±101.82pg/mL) compared to control (2,159.93±172.14 pg/mL) and LSD group (2,090.39±323.94pg/mL). The serum EPO levels were increased in LSD (16.02±28.95 mIU/mL) and ACEI group (32.05±30.12mIU/mL) compared to control group (4.67±8.70mIU/mL). Also, In IHC, the EPO in kidney was stained more strongly in ACEI group compared to control and LSD group, but the EPO receptor in bone marrow was stained less strongly in ACEI group relative to control and LSD group. In RT-PCR, the mRNA expression of EPO in kidney was decreased in ACEI group (1.86±0.41) relative to control (5.06±8.59) and LSD group (3.10±4.39) and the mRNA expression of EPO receptor in bone marrow was increased in ACEI group (4.80±2.34) relative to control (1.59±0.61) and LSD group (1.19±0.56).

Conclusions: The blocking renin-angiotensin-aldosterone system in CKD rats can cause anemia via both mechanism to decline erythropoietin in kidney and erythropoietin receptor in bone marrow.

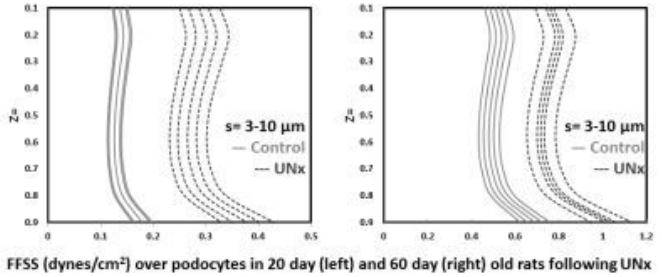
TH-PO288

Increased Fluid Flow Shear Stress on Podocytes Contributes to Glomerular Injury Tarak Srivastava,[1] Ellen T. McCarthy,[2] Alexander Kats,[1] Ram Sharma,[3] Gianni Celsi,[4] Mukut Sharma,[3] Virginia J. Savin.[3] *[1]Section of Nephrology, The Children's Mercy Hospital, Kansas City, MO; [2]Kidney Institute, KUMC, Kansas City, KS; [3]Research and Development, KCVA Medical Center, Kansas City, MO; [4]Uppsala University Hospital, Uppsala, Sweden.*

Background: Podocytes are exposed to mechanical forces arising from both glomerular capillary pressure (P_{GC}) and ultrafiltration. The flow of filtrate into Bowman's space (BS) generates fluid flow shear stress (FFSS) over podocyte. Adaptive hyperfiltration is characterized by increased single nephron GFR (SNGFR) and P_{GC}, and glomerular hypertrophy. Hyperfiltration associated increase in SNGFR increases FFSS. A better understanding of the mechanism underlying hyperfiltration mediated podocyte injury from FFSS is vital. The aim of the study was to determine the effect of uninephrectomy (UNx) on FFSS.

Methods: Sprague-Dawley rats underwent UNx at 5 days of age, and were sacrificed on days 20 and 60 for micropuncture studies. SNGFR, filtration fraction (f) and diameter ($2R_T$) were measured. FFSS to which podocyte is exposed *in vivo* was calculated as: $\tau = [3\eta.f.SNGFR/\prod. s^2(s+2R_T)]x[z/\sqrt{z}.(1-z)]$ [AJP(Renal)2006;F856], where FFSS (τ) is dependent on viscosity (η), f, SNGFR, $2R_T$, width of BS (s) and position between vascular and urinary pole (z). Since 's' is difficult to measure we modeled 's' across a range of values assuming 3 separate scenarios: (a) s=6 µm, (b) s=2 µm (at z=0) to 8 µm (at z=1), and (c) s=3 µm (at z=0) to 10 µm (at z=1).

Results: Calculated FFSS following UNx was increased ~2 fold in all 3 models of 's' on both Day 20 and 60.



FFSS (dynes/cm²) over podocytes in 20 day (left) and 60 day (right) old rats following UNx

Conclusions: The results provide a rationale for the contribution of increased SNGFR and fluid flow to podocyte injury in the constrained BS. We hypothesize that increased FFSS from reduced renal mass will contribute to podocyte injury and to progression of chronic kidney disease.

Funding: Private Foundation Support

TH-PO289

Identification of Four Major Subsets of Renal Mononuclear Phagocytes in Healthy and Diseased Kidneys Qi Cao,[1] Xin M. Wang,[2] Changqi Wang,[1] Vincent W.S. Lee,[1] Ya Wang,[1] Guoping Zheng,[1] Stephen Alexander,[3] Yiping Wang,[1] David C. Harris.[1] *[1]Centre for Transplantation and Renal Research, Westmead Millennium Institute, University of Sydney, Westmead, NSW, Australia; [2]Flow Cytometry Facility, Westmead Millennium Institute, University of Sydney, Westmead, NSW, Australia; [3]Centre for Kidney Research, Children's Hospital at Westmead, University of Sydney, Westmead, NSW, Australia.*

Background: Renal mononuclear phagocytes (rMP), conventionally comprising macrophages and dendritic cells, play a central role in health and disease of the kidney. However, the classification and functions of rMP remain unclear.

Methods: Multiple markers (MHC-II, CD11c, F4/80, CD103, CD11b) were used to identify four subsets of rMP in kidney of normal BALB/c mice and mice with Adriamycin nephrosis (AN). The distribution, phenotype and function of subsets of rMP were assessed in normal and AN mice. Adoptive transfer of four subsets of rMP was used to assess their *in vivo* functions.

Results: rMP subsets: F4/80+CD11c- (rMP1), F4/80+CD11c- (rMP2), CD11c+CD103+ (rMP3) and CD11c+CD103- (rMP4) accounted for more than 90% of rMP in normal and AN kidneys. rMP1 were found in cortex and medulla of normal and AN kidney, while rMP2, 3 and 4 were confined predominantly to cortex. rMP1 and rMP2 showed high expression of macrophage markers CD68, CD204 and CD206, and high phagocytotic ability. However, rMP3 and rMP4 displayed lower phagocytotic ability but greater antigen presenting ability than rMP1 and rMP2. In AN, rMP1 and rMP2 displayed a typical phenotype of M1 macrophages with high expression of iNOS, IL-1beta, IL-6, TNF-α and MCP-1. rMP2 were different from rMP1 in their slightly higher expression of IL-10. rMP3 and rMP4 expressed IL-6 highly, but not iNOS, IL-1beta, TNF-α and MCP-1in AN mice. Adoptive transfer of rMP1 and rMP2 aggravated the renal injury in AN mice, whereas that of rMP3 and rMP4 had no effect on renal injury.

Conclusions: These four major subsets of rMP displayed distinct properties, including distribution, phenotype and *in vitro* and *in vivo* function, suggesting a distinct role for each in normal and diseased kidneys.

Funding: Government Support - Non-U.S.

TH-PO290

Prophylactic Angiotensin II Receptor Antagonism Prevents Chronic Kidney Disease Induced by Acute Kidney Injury Roxana Rodriguez, Jonatan Barrera-Chimal, Rosalba Pérez-villalva, Gerardo Gamba, Norma Bobadilla. *Molecular Physiology Unit, Instituto de Investigaciones Biomédicas, UNAM and Instituto Nacional de Ciencias Médicas y Nutrición SZ, Mexico.*

Background: Acute Kidney Injury (AKI) is an important risk factor to develop Chronic Kidney Disease (CKD). Because angiotensin II (ANGII) has been proposed as a key mediator of renal injury and progression, this study was designed to determine if angiotensin I receptor (AT1R) blockade with losartan (L) before and after renal ischemia (I) may prevent or diminish the CKD development.

Methods: 27 rats were divided in: sham-operated (S), treated with L (50mg/kg) 72, 48 and 24h before S (L), underwent bilateral I for 45 min (I), receiving Lo before I (L+I) and receiving L 3 h after I (I+L) groups. All rats were followed throughout 270 days. Proteinuria (UProtV) and creatinine clearance (CrCl) were evaluated every 30 and 90 days, respectively. At the end of the study, renal blood flow (RBF), mean arterial pressure (MAP) was measured, as well as urinary Kim-1. Right kidney was used for IL6 mRNA levels and the left kidney for histopathological analysis. Glomerular diameter (GD) was measured and distributed by ranks.

Results: Rats underwent ischemia developed CKD characterized by a progressive increase in UProtV, together with a progressive decrease in CrC. The Table shows the results at the end of the study, no differences in MAP (data not shown).

Group (n=6)	**RBF** ml/min	**CrC** ml/min	**UProtV** mg/24h	**Kim-1** ng/24h	**GD >176µ** %	**IL6/18s**
S	8.3± 1.1	1.7 +0.1	32+7	27+9	8.5	1+0
L	8.3+0.6	2.2+0.2	25+6	28+4		1.3+0.7
I	6.5+0.9	0.9+0.2[a]	302+29[a]	78+12[a]	36.0	9.6+3.5[a]
L+I	8.9+1.3	1.9+0.1[b]	127+43[b]	24+10[b]	12.0	1.7+0.4[b]
I+L (n=3)	8.7+0.7	1.4+0.3	227+63	71+29	37.6	1.2+0.6

[a] $p < 0.05$ vs Sh, [b] $p < 0.05$ vs I

Conclusions: AT1R antagonism before ischemia slowed CKD progression, since no renal dysfunction, severe UProtV, glomerular hypertrophy, Kim-1 elevation or IL-6 over-expression were observed. In contrast, L administration post-ischemia was not able to prevent or to reduce CKD in spite of reduction of IL-6. These results suggest that ANGII plays an important role during ischemia, but do not play a significant role during reperfusion process.

This study was supported by CONACyT and PAPPIT.

Funding: Government Support - Non-U.S.

TH-PO291

Induction of Neutral Calponin Expression Suppresses the Development of Interstitial Fibrosis in Both Kidneys and Heart in Old Mice Kiyoko Inui,[1] Yoshihiko Inoue,[1] Shigeki Iwasaki,[2] Ashio Yoshimura.[1] *[1]Nephrology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan; [2]Nephrology, Seirei Yokohama Hospital, Yokohama, Kanagawa, Japan.*

Background: Onset of CVD is a serious problem in CKD patients. Demonstration of a common factor participating in both kidneys and heart injury may provide a new way to treat CKD. Therfore we studied the effect of neutral calponin (Ncal) overexpression on the interstitial injury of both kidneys and heart because Ncal showed anti-fibrotic property in UUO model mice.

Methods: Both kidneys and heart from Ncal transgenic mice (Ncal-Tg, n=4) or wild type mice (WT, n=4) were studied histologically. All of them were 20-26 months old. Ncal overexpression was induced by cadmium sulfate treatment (0.5mg/kg/day), that was started from six months old and continued to sacrifice (7-10 days/month). Immunohistochemistry for interstitial proliferaing cells (ki-67), macrophages (F4/80), collagen I and TGF-βwas performed for all kidney tissues. Fibrosis of heart was studied by Masson-Trichrom staining. All data (number for positive cells or staining score) were evaluated by computer-analysis system and were expressed as mean±SE.

Results: There was no significant difference in the weight of kidneys (weight(g) / body weight(g) x100, 0.79±0.04 in Ncal-Tg, vs 0.72±0.06 in WT) and heart (g/body weight(g) x 100, 0.22 0.03 vs 0.18 0.02). Interstitial macrophages infiltration (F4/80+ cells/high power field, hpf) was significantly suppressed in Ncal-Tg (15.9±4.4 vs 32.8±1.6 in WT, $p<0.05$), however there was no difference in the number of proliferating cells (ki-67+ cells/hpf) (22.8±4.3 in Ncal-Tg, vs 25.5±2.0 in WT). Staining score for collagen I was reduced in Ncal-Tg than WT (1.22±0.02 vs 1.43±0.05, $p<0.05$). There was no difference in TGF-β staining in kidneys. Masson-Trichrom staining showed interstitial fibrosis of heart was signicantly suppressed in Ncal-Tg (0.079±0.013 vs 0.104±0.004 in WT, $p<0.05$).

Conclusions: Intersittial inflammation and development of fibrosis were suppresed in Ncal- Tg than in WT. Fibrosis was also suppreed in heart in Ncal-Tg compared to WT. Thus, the induction of Ncal is expected as one of the therapeutic strategy for CVD in old CKD patients.

TH-PO292

Nephrogenic Systemic Fibrosis (NSF) and ASARM Induced Release of Gadolinium from Gd3+-Binding Contrast Agents Peter S.N. Rowe,[1] Lesya Zelenchuk,[1] Tarak Srivastava,[2] Ellen T. McCarthy.[1] *[1]Kidney Institute, University of Kansas Medical Center; [2]Childrens Mercy Hospital Kansas.*

Background: Gadolinium binding contrast agents (GBCAs) are used for high contrast magnetic resonance imaging (MRI). Subsets of chronic kidney disease (CKD) patients exposed to GBCAs develop NSF, a progressive disease with multiple organ failure and death. Release of toxic Gd3+ from GBCAs likely plays a role but the etiology of release is unknown. Our previous work showed circulating ASARM-peptides bind to GBCAs. These bone-derived peptides are linked with renal-handling defects and bone-mineralization abnormalities. We hypothesize increased levels of acidic ASARM-peptides also induce release of Gd3+ resulting in NSF pathology.

Methods: To test our hypothesis, we used Liquid Chromatography and Inductively-Coupled Mass-Spectrometry to measure release of Gd3+ from Gadodiamide. We also used a 4.2kDa synthetic peptide (SPR4) that binds specifically to ASARM-peptide to inhibit ASARM-mediated release of Gd3+. A murine model (HYP) with markedly increased circulating ASARM-peptides, mineralization and renal phosphate handling defects was used to measure the effects of GBCAs in the presence and absence of SPR4 peptides *in vivo*.

Results: ASARM induced a 30% increase in free to bound Gd3+ and co-incubation with SPR4 blocked release of Gd3+. GBCA treated HYP mice became normophosphatemic and hypercalcemic with increased renal Na+-phosphate-cotransporters (NPT2a/c) mRNA expression. Also, computed tomography (μCT) showed renal and dermal metastatic calcifications and bone defects in GBCA treated HYP mice. SPR4-peptide inhibited the ASARM-induced changes in tissue, bone and circulation.

Conclusions: In summary: 1. ASARM peptides induce release of toxic Gd3+; 2. SPR4 binds to ASARM and inhibits GD3+ release; 3. co-treatment with SPR4 prevents GBCA induced pathology in HYP mice. In conclusion, ASARM-peptides play a key etiological role in NSF and SPR4-peptide is an ideal candidate adjuvant. These findings have clinical implications for GBCA use in inherited or acquired renal bone-mineral loss disorders with increased circulating ASARM-peptides.

Funding: Other NIH Support - National Institutes of Health (NIH) Grant Number 5R01AR051598 to PSNR (National Institute of Arthritis and Musculoskeletal and Skin Diseases; NIAMS)

TH-PO293

Omniscan-Induced CD163$^+$ Ferroportin$^+$ Osteogenic Cells in Nephrogenic Systemic Fibrosis Sundararaman Swaminathan,[1] Chhanda X. Bose,[1] Sudhir V. Shah,[1] Kim M. Hiatt.[2] *[1]Division of Nephrology, University of Arkansas for Medical Sciences and Central Arkansas VA Health System, Little Rock, AR; [2]Division of Dermatology and Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: Gadolinium contrast is linked to nephrogenic systemic fibrosis (NSF) in patients with renal insufficiency. Its pathology is characterized by abnormal tissue repair—fibrosis and ectopic ossification. The mechanisms by which gadolinium could induce fibrosis and ossification are not known.

Methods: We examined *in vitro* the effect of gadolinium contrast (OmniscanTM) on human peripheral blood mononuclear cells (PBMC) for phenotype and function relevant to the pathology of NSF utilizing immunoflourescence, flow cytometry, RT-PCR, and osteogenic assays. We also examined peripheral blood and tissues of NSF patients with flow cytometry and immunohistochemistry to identify the presence of cells with phenotype induced gadolinium.

Results: Gadolinium contrast induced differentiation of human PBMC into an unique cellular phenotype- CD163$^+$ cells expressing proteins involved in fibrosis and bone formation. These cells express fibroblast growth factor 23, osteoblast transcription factors Runx2 and osterix, and exhibit an osteogenic phenotype in in vitro assays. We demonstrate the in vivo presence of CD163$^+$/procollagen-1$^+$/osteocalcin$^+$ cells in the fibrotic and calcified tissues of NSF patients. We also demonstrate increased circulating CD163$^+$/Runx2$^+$/osteocalcin$^+$/procollagen-1$^+$ cells with an osteogenic phenotype in patients with NSF.

Conclusions: Gadolinium contrast-induced CD163$^+$ ferroportin$^+$ FGF23$^+$ cells with osteogenic potential may play a role in systemic fibrosis and ectopic ossification in NSF.

Funding: Other NIH Support - University of Arkansas for Translational Research (Parent Grant 1 UL1R R029884) Pilot Study Award and KL2 Award, Veterans Administration Support

TH-PO294

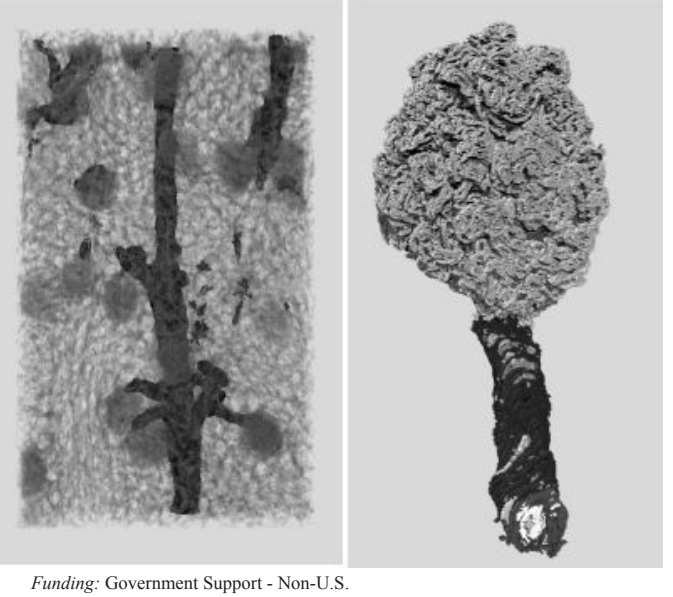
Three-Dimensional Visualization of Vasculo-Glomerular Alterations with Progression of Chronic Kidney Disease Noriko Uesugi,[1] Takaaki Aoba,[2] Yoshihito Shimazu,[2] Michio Nagata.[1] *[1]Pathology, Tsukuba University, Tsukuba, Ibaragi, Japan; [2]Pathology, Nippon Dental University, Chiyoda, Tokyo, Japan.*

Background: Impairment of intrarenal microvessels promotes chronic kidney disease(CKD). In order to visualize progression of vascular damages and glomerulosclerosis in CKD, we developed high-resolution three-dimensional(3D) tissue reconstruction that enables to inspect a wide-range vasculo-glomerular architecture in human kidney.

Methods: Paraffin embedded specimens were obtained from non-tumor parts of renal carcinoma of four Japanese male (72-78 y.o, with CKD stage 2-3 without proteinuria). Serially-cut 300 sections (3.5um thick) were processed for dual-immunostaining of CD34 and smooth muscle actin(SMA). The images were digitized by whole-slide imaging system (virtual slide). The 3D reconstructions were conducted by computer-assisted image registration and color segmentation using Image J and TRI-SRF2 software.

Results: The 3D image enabled to visualize the intricate vascular network of interlobular arteries/arterioles and glomeruli (Figure) to inspect the details of impaired vascular architecture at discrete stages of CKD. The 3D visualization showed that the regular run and branching of arteries and elliptic -shaped glomeruli were featured in CKD stage 2, while the tortuous run of arteries in CKD stage 3 without hypertension (HT) nor cardiovascular disease (CVD). Of particular interest was the decreased vasculature, the irregular vascular branching, the expanded glomerular SMA- positive area and the anomalous development of extra-afferent and efferent arteriolesin CKD stage 3 with HT or CVD.

Conclusions: High-resolution 3D reconstructions in combination with dual immunolabeling and whole-slide imaging significantly expand our scope to diagnose changes in vascular-glomerulus system with CKD.



Funding: Government Support - Non-U.S.

TH-PO295

Volume Status and Cardiac Autonomic Neuropathy Were Associated with Progressive Kidney Disease in Chronic Kidney Disease Patients Min Ji Shin, Sang Heon Song, Eun Young Seong, Harin Rhee, Ihm Soo Kwak, Byeong Yun Yang. *Department of Internal Medicine, Pusan National University School of Medicine, Busan, Republic of Korea.*

Background: We investigated the relationship between volume status, cardiac autonomic neuropathy(CAN) and atherosclerosis in predialysis chronic kidney disease(CKD) patients. In addition, we analyzed whether these parameters can predict deterioration of renal function in those patients.

Methods: 54 predialysis CKD patients were enrolled. Volume status was determined by extracellular water(ECW)/total body water(TBW) by bioimpedance analysis. Measurement of brachial-ankle pulse wave velocity(baPWV) was used to assess the vascular stiffness. Intima-media thickness of carotid artery and carotid plaque were measured for assessment of atherosclerosis. CAN was assessed by the Ewing's method and the standard deviation of the normal-to-normal interval(SDNN). The slope of monthly reduction of reciprocal serum creatinine was measured to assess the deterioration of renal function.

Results: 24(44.4%) had CAN. Compared to the patients without CAN, patients with CAN had higher systolic blood pressure(SBP), C-reactive protein(CRP), ECW/TBW, baPWV and lower hemoglobin and SDNN. Patients with edema(ECW/TBW ratio≥0.39) were older and had higher SBP,CRP,HbA1C,CAN score,baPWV than patients without edema. ECW/TBW was positively correlated with CAN score(r=0.450,p=0.001),baPWV (r=0.569,p<0.001) and negatively correlated with SDNN(r=-0.376,p=0.005). CAN score was positively correlated with baPWV(r=0.578,p<0.001) and negatively correlated with SDNN(r=-0.347,p=0.01). In univariate linear regression analysis,SBP,CAN score,ECW/TBW,baPWV,CRP,hemoglobin,albumin,proteinuria were significantly associated with the declining rate of kidney function. In multivariate regression analysis, the declining rate of kidney function was found to associate with CAN score,ECW/TBW and proteinuria.

Conclusions: We found the correlationship among edema, vascular stiffness and cardiac autonomic neuropathy in CKD patients and those variables were related with inflammatory status. Apart from classic risk factors, elevated ECW/TBW and CAN were associated with deterioration of renal function in CKD patients.

TH-PO296

Association of Serum Fibrinogen with Mortality among Subjects with Normal and Reduced Kidney Function in the General Population Urszula Donigiewicz,[1] Ahad Abdalla,[2] Liam F. Casserly,[2] Cornelius John Cronin,[2] Ailish Hannigan,[3] Hoang Thanh Nguyen,[3] Austin G. Stack.[2,3] *[1]Medicine, National University of Ireland Galway, Ireland; [2]Department of Nephrology, Department of Medicine, University Hospital Limerick, Ireland; [3]Department of Medicine, Graduate Entry Medical School, University of Limerick, Ireland.*

Background: It is postulated that novel risk factors contribute to cardiovascular (CV) risk in chronic kidney disease. We explored the association of serum fibrinogen, a thrombotic factor, with total and CV mortality in the setting of reduced kidney function in a population-based cohort.

Methods: A cohort of 8,494 subjects age >40 were identified from the Third National Health and Nutrition Examination Survey (1988-1994) and vital status was obtained through linkage with the National Death Index. Serum fibrinogen levels (mg/dL) were compared across categories of estimated glomerular filtration rate (eGFR) (<60, 60-90, >90 mL/min). Weighted multivariable Cox regression modelled relationships of fibrinogen in quartiles (Q1; 0-261 [referent], Q2; 261-305, Q3; 305-356, Q4 >356 mg/dL) with total and CV mortality.

Results: Mean (±standard error [SE]) fibrinogen levels were inversely correlated with eGFR at 298 (2.9), 305 (2.8) and 352 (6.3) in each lower GFR category. With 10 years of follow-up, total mortality was 17.1 % of which 8.0 % were cardiac. In the unadjusted analyses, the hazard ratios (HR) per 10 mg/dL increase in fibrinogen were 1.03 (95% CI 1.02-1.04) and each increasing quartile of fibrinogen was associated with total and CV mortality [HR=1.00, 1.36 (1.02-1.82), 1.94 (1.54-2.44), 3.78 (3.05-4.68). Adjusting for Framingham factors and eGFR, the relationships were attenuated but remained significant with all-cause [HR=1.00; 1.08 (0.89-1.31), 1.44 (1.20-1.72) and 1.68 (1.32-2.14)] and CV mortality [(HR1.00, 1.18 (0.88-1.59), 1.43 (1.08-1.91) and 1.89 (1.50-2.38) respectively. Further stratification by GFR category yielded virtually identical mortality estimates.

Conclusions: Serum fibrinogen levels are higher among individuals with kidney impairment than those with normal kidney function and contribute independently to increased total and CV mortality.

Funding: Government Support - Non-U.S.

TH-PO297

Renal Biopsy in Elderly Patients: Indication, Diagnosis, Safety and Clinical Use George Greenhall, Chris Jones, Sapna Shah, Satish Jayawardene. *Department of Renal Medicine, King's College Hospital, London, United Kingdom.*

Background: The incidence of renal failure is rising as the elderly population increases. Age is no longer considered a contra-indication to renal biopsy or subsequent treatment. Biopsy has been shown to yield treatable diagnoses in up to 70% of elderly patients.

Methods: We retrospectively examined the indications, complications and outcomes of native renal biopsies in patients aged over 70 years in our tertiary referral unit, between 2005-2010. Data were collected from laboratory reports and clinical notes.

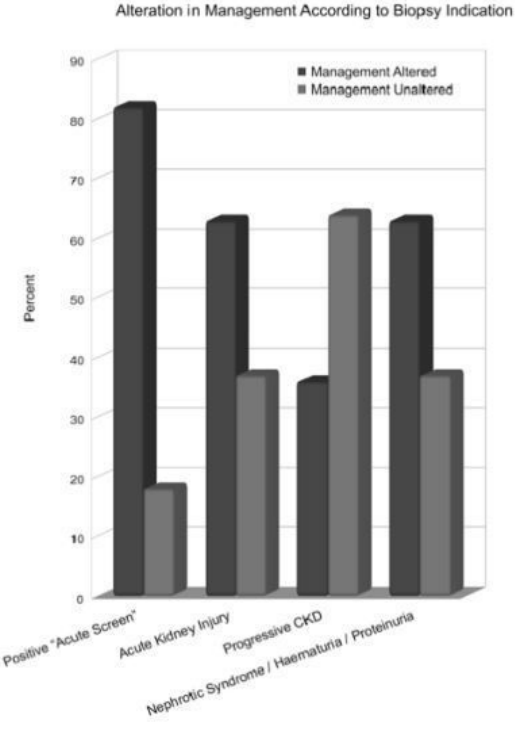
Results: 61 patients were included (51% male, mean age 77.5 years (70-94)). 31% had existing CKD; 18% were diabetic. Primary indications and diagnoses are shown in Table 1. One patient suffered a large peri-nephric haematoma, requiring embolization. Histological findings resulted in a change in the management of 67% of patients. Management alterations comprised: commencing immunosuppression (68%), chemotherapy (20%), ceasing immunosuppression (12%), antibiotics (5%), and plasma exchange (5%). At biopsy, 31% required RRT. Six months after biopsy, 22% required RRT and 7% had died. One year after biopsy, 16% required RRT and 14% had died. Absence of pre-existing CKD was positively associated with a change in management following biopsy (p=0.02).

Conclusions: Biopsy in our elderly population is safe, and yields clinically useful findings.

Primary Histological Diagnosis by Primary Biopsy Indication

	Pauci-immune Glomerulopathy	Other Glomerulopathy	Tubulo-Interstitial Nephritis / Acute Tubular Necrosis	Diabetic / Hypertensive Nephropathy	Renal Myeloma	Other / Inconclusive
Positive "Acute Screen"* (n=34)	13	7	5	1	5	3
Acute Kidney Injury (n=8)	0	2	1	1	2	2
Progressive CKD (n=11)	0	3	4	2	0	2
Nephrotic Syndrome / Haematuria / Proteinuria (n=8)	0	6	0	1	1	0

(*ANCA, anti-GBM or myeloma screen abnormal)



TH-PO298

Glomerular Volume and Density Associate with Risk Factors among Living Kidney Donors Hisham Elsherbiny, Lynn D. Cornell, Walter Park, Joseph Larson, Lilach O. Lerman, Mikel Prieto, John C. Lieske, Andrew D. Rule. *Mayo Clinic.*

Background: Glomerular hypertension is a characteristic of early kidney injury. In animal models, consequent glomerular and tubular hypertrophy and hyperplasia increases nephron size, which in turn decreases the density of glomeruli per unit cortex. We hypothesized that measures of increased nephron size would relate to chronic kidney disease (CKD) risk factors among relatively healthy adults.

Methods: Living kidney donors had a core needle biopsy of their donated kidney during the transplant surgery at Mayo Clinic Rochester from 2000 to 2009. Two adjacent 3 um thick sections of the biopsy were stained with periodic acid-Schiff and Masson's trichrome and scanned into high resolution images. The areas of cortex and mean glomerular cross-sections were measured. Mean glomerular volume (mm^3) and volumetric glomerular density (mm^{-3}) were estimated using stereological models (Weibel and Gomez), and regressed on kidney function and CKD risk factors ascertained just prior to donation. Multivariable (MV) models identified independent predictors.

Results: The 996 studied donors were 42% men, 6% hypertensive, and 57% with family history of end-stage renal disease (ESRD); age range: 18-77 years; mean±SD measure glomerular filtration rate (GFR): 103±18 ml/min/1.73 m^2, 24-h urine albumin (UAlb): 6±10 mg, body mass index (BMI): 28±5, and serum uric acid: 5.2±1.4. Glomerular volume was 0.0026±0.0010 mm^3 and density was 16±7 per mm^3. Glomerular volume correlated with glomerular density (r_s=-0.63, p<0.001) and both associated with CKD risk factors (table).

Glomerular volume and density regression on donor characteristics

	Glom. Volume		Glom. Density	
Predictor	Unadj. ß	MV-adj. ß	Unadj. ß	MV-adj. ß
Male	.00033*	.00010	-1.7*	-.56
Age per 10 y	-.00002	.00000	-.12	-.25
Family history of ESRD	.00021*	.00017*	-1.03*	-1.05*
GFR per SD	.00004	.00006	.10	-.07
Log 24-h UAlb per SD	.00011*	.00006	-.78*	-.49
BMI per SD	.00110*	.00024*	-1.83*	-1.61*
Uric acid per SD	.00024*	.00013*	-1.43*	-.82*
Hypertension	.00027*	.00015	-.83	.30

*p<.05

Conclusions: In a relatively healthy population, family history of ESRD, obesity, and hyperuricemia are independent predictors of morphological changes on renal biopsy indicative of increased nephron size.

Funding: NIDDK Support

TH-PO299

Increased Kidney Cortical Volume: A Potential Marker of Early Chronic Kidney Disease Xiangling Wang, Ramesh Avula, Walter K. Kremers, Harini A. Chakkera, Terri J. Vrtiska, Lilach O. Lerman, Andrew D. Rule. *Mayo Clinic.*

Background: The kidney cortex is known to atrophy in patients with advanced chronic kidney disease (CKD), however the determinants of cortical volume in normal adults are less clear. We aimed to determine whether cortex volume is reflective of kidney function or CKD risk factors among relatively healthy adults.

Methods: Kidney cortical volume was measured on contrast-enhanced abdominal CT images in 878 potential kidney donors between 2001 and 2008. Cortex volumes were summed between both kidneys. Glomerular filtration rate (GFR) was measured by iothalamate clearance. Cortex volumes were correlated with age, sex, body surface area (BSA), GFR, 24-h urine albumin, and CKD risk factors. Multivariable regression was used to determine independent predictors.

Results: There were 878 potential donors (59% female; 22% smokers; ages range 18 to 75 years; BSA 2.0±0.2 m^2, GFR 117±28 ml/min, urine albumin 8.1±21.8 mg/24 h). Men had larger cortex volumes than women (241 vs. 195 cc; p<0.01). Cortex volume correlated with BSA (r=0.60, p<0.01), age (r=-0.3, p<0.01), GFR (r=0.72, p=<0.01), systolic blood pressure (r=0.07, p<0.01), serum uric acid (r=0.3, p<0.01), HDL cholesterol (r=-0.37, p<0.01), glucose (r=0.15, p<0.01) and urine albumin excretion (r =0.1, p<0.01). Smokers had a cortex that was 18cc larger than nonsmokers (p<0.01). Only male gender, BSA, GFR, urine albumin excretion, and smoking, were independent predictors.

Multivariable predictors of kidney cortical volume (r2=0.64)

Predictor	Beta (change in cc)	P-value
Age per 10 y	-0.48	0.61
Female	-10.6	<0.0001
Smoker	+6.6	<0.0001
BSA per SD	+8.2	<0.0001
24-h urine albumin per doubling	+1.88	0.002
Uncorrected GFR per SD	+22.9	<0.0001
Systolic BP per SD	-0.51	0.67
Diastolic BP per SD	-0.40	0.73
Serum glucose per SD	+0.95	0.37
Serum uric acid per SD	-0.92	0.44
HDL cholesterol per SD	-1.3	0.22

Kidney cortex was 46 cc larger among the 7.8% of donors with a corrected GFR > 130ml/min/1.73m^2 (p<0.01) and 20 cc larger among the 3.7% of donors with a urine albumin excretion > 30 mg/24 h (p<0.01).

Conclusions: In a relatively healthy population, increased cortical volume associates with hyperfiltration and albuminuria and may be an indicator of early CKD.

Funding: NIDDK Support

TH-PO300

Malignancies after ANCA-Associated Vasculitis: A Large Single Centre Experience Chinar Rahmattulla,[1] Arda Goceroglu,[1] Marlies E.J. Reinders,[2] Ernst C. Hagen,[3] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden University Medical Center, Netherlands; [2]Nephrology, Leiden University Medical Center, Netherlands; [3]Internal Medicine, Meander Medical Center Amersfoort, Netherlands.*

Background: Recent studies indicate that patients with ANCA-associated vasculitis (AAV) have a significantly higher risk of developing malignancies, that the mortality in AAV patients is 2.6 times higher than that of the general population, and that malignancies are the second cause of death after the first year of diagnosis. Drawbacks from these studies are a relatively short follow-up, and possibly, under-reportage due to lack of access to registries.

Methods: We investigated the occurrence of malignancies in 187 histologically confirmed AAV patients after diagnosis at our center between 1982 and 2011 by performing a search in PALGA, a Dutch national pathology database which covers all the histologically confirmed malignancies diagnosed in The Netherlands.

Results: 136 patients with AAV had a follow-up of at least 1 year; 46 of those developed 93 malignancies during a mean follow-up of 12.3 years. There were 64 skin malignancies, including 63 non-melanoma skin cancers (NMSC). There were 13 multiply occurring malignancies: 4 of the bladder, 4 of the prostate, 3 of colon/rectum and 2 of the lung. There was a variety of one time occurring malignancies. The mean age of AAV patients developing a malignancy was similar to patients without a malignancy (58 years).

Conclusions: This study on the development of malignancies after AAV from a large single center experience shows a higher incidence of malignancies in AAV patients than was recently reported for a European study group. One explanation for this discrepancy could be the accurate data reporting through the Dutch PALGA system by which virtually no malignancy could have been missed. In our study there was no significant age difference between patients with and without malignancies. Notably, there was a high number of NMSCs in our study group which is most likely related to the immunosuppressive therapy these patients receive. In the management and treatment of patients with AAV, it is of major importance to monitor closely for developing malignancies.

TH-PO301

How Does Quality of Life Vary over a Six Month Period in High Risk CKD? Stephanie J. Stringer,[1,2] Mary Dutton,[2] Chantelle Waite,[2] Mark David Jesky,[1,2] Paul Cockwell.[1,2] *[1]Immunity and Infection, University of Birmingham, Birmingham, United Kingdom; [2]Nephrology, QEHB, Birmingham, United Kingdom.*

Background: The EQ5D questionnaire is used to assess quality of life and to formulate quality adjusted life years. The degree of interpatient variation in the absence of intervention is important. There has been little work in this area in the CKD population where patients often have reduced QoL and significant co-morbidity.

Methods: Patients recruited to the Renal Impairment In Secondary Care (RIISC) study undergo assessment at six study visits over a ten year period, assessment of quality of life using the EQ5D is performed at each visit. The EQ5D contains five domains relating to mobility, self care, usual activities, pain and anxiety or depression and a visual analogue scale from 0-100 (0 being the worst perceived health and 100 being the best).

Results: 165 patients have been assessed at baseline and six months, the mean age is 62 (+/-16) years, 70% were white. The majority of patients' scores in the five domains did not change between baseline and six months (78% in mobility, 82% in self care, 67% in usual activities, 65% in pain/discomfort and 75% in anxiety/depression). The baseline median visual analogue score was 60.1 (+/-21.5), the six month mean score was 65.4 (+/-19.5). 62% of patients' six month visual analogue scale score was within 25% of their baseline score, 11% had a six month score that was >25% lower than the baseline score and 27% had a >25% higher score at six months.

Conclusions: We report little interpatient variation in self reported quality of life using the EQ5D instrument in a structured interview. Scores in both the five domains and the visual analogue scale were comparable between visits over this short time period; this was confirmed when both the mean scores of the cohort and the scores obtained by individuals were compared at the two visits. These results suggest that the EQ5D instrument has acceptable degrees of interpatient variability in patients with CKD when administered in a structured interview.

Funding: Private Foundation Support

TH-PO302

Quality of Life (QoL) and Functional Status Are Maintained a Cohort of Patients with Progressive Chronic Kidney Disease Despite Significant Co-Morbidity Stephanie J. Stringer,[1,2] Mary Dutton,[2] Chantelle Waite,[2] Mark David Jesky,[1,2] Khai Ping Ng,[1,2] Paul Cockwell.[1,2] *[1]School of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom; [2]Department of Nephrology, University Hospital Birmingham, Birmingham, United Kingdom.*

Background: Impaired quality of life (QoL) and functional status has been described in patients with End stage Kidney Disease but there has been little evaluation of QoL in progressive Chronic Kidney Disease despite that fact that these patients may have significant co-morbidity. We assessed the relationship between QoL and functional status in a prospective cohort of patients with progressive CKD recruited to the Renal impairment In Secondary Care (RIISC) study.

Methods: Patients recruited to the RIISC study undergo a detailed bio-clinical, and socio-economic status (SES) assessment. QoL is ascertained using the EQ5D questionnaire and a visual analogue scale where patients rate their health from 0 (the worst) to 100 (the best).Co-morbidities are scored using the Charlson index.

Results: 277 individuals had been recruited at the time of this analysis, the mean age was 62.7 (+/- 16.7), 58% were male and 69% were of white ethnicity. 20% were in current employment, 30% were unemployed and 48% were retired from employment. 47%, 87% and 52% reported no problems with mobility, self care and usual activities. 52% reported no regular pain or discomfort and 30% reported feelings of anxiety or depression. The mean score on the visual analogue scale was 59.5(+/-21.3). By univariate analysis increasing age**, decreasing eGFR*, educational attainment** and increasing co-morbidity** were associated with lower scores (** p<0.01, *p<0.05); in a multivariate analysis only co-morbidity was associated with lower scores (p= 0.001). Both educational attainment and co-morbidity were associated with poorer functioning (p = 0.009 and p = 0.001 respectively).

Conclusions: In patients with progressive and high risk CKD, co-morbidity was an important determinant of QoL and reported functional status. Employment status, educational attainment and deprivation were not associated with QoL; however patients with lower educational attainment and more co-morbidity did have lower functional scores.

Funding: Private Foundation Support

TH-PO303

The Value of Questionnaire Investigation in Recognition of SAS in HD Patients Ilona Miskowiec-wisniewska,[1,2] Rafal Donderski,[1] Jacek Manitius,[1] Jacek Klaew.[2] *[1]Dept. of Nephrology, Hypertension and Internal Disease, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University of Torun, Bydgoszcz, Poland; [2]Dept of Hygiene and Epidemiology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University of Torun, Bydgoszcz, Poland.*

Background: The prevalence of sleep disorders in CKD is much higher than in general population and such disturbances are detected in almost 50% of pts. The clinical consequences of sleep disorders is increased cardiovascular morbidity and mortality related to higher incidence of HA, LVH,CAD, ventricular arrhythmias and strokes.

Methods: We evaluated 61 HD pts (24F,37M),age 58,2±12,2 y, mean dry weight: 68,16±13,57kg, mean dialysis duration: 73,49±55,33months. In each patients 3 HD a week were performed. HD session time was:4,43±0,43 hours. Epworth Sleepiness Scale (ESS) was used to assess severity of sleep disorders. The patients were asked 22 questions concerning :symptoms of sleep disorders, EDS, insomnia, snoring, narcolepsy,drug administration and others. In 17 male pts complete PSG evaluation was performed.

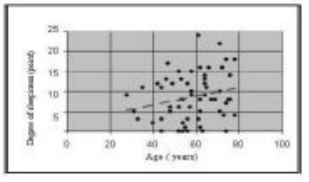
Results:

The comparison of questionnaire investigation and PSG in assessment of severity of sleep disorders.

Patient status	According to Index RDI TST*			According to questionnaire investigation		
	Characteristics	*n*	%	Characteristics	*n*	%
Healthy	AHI<5	2	11.8	up to 50 pt	14	82.4
Mild apnea	5≤AHI<15	6	35.3	51-52 pt	0	0.0
Intermediate apnea	15≤AHI<30	4	23.5	53-58 pt	3	17.6
Severe apnea	AHI≥30	5	29.4	over 58 pt	0	0.0
total:		17	100		17	100

RDI TST* - Respiratory disorders index total sleep time.

		Sleepiness			
		Without sleepiness	intermediate	severe	Total number of pts
Gender	F	18	2	4	24
	M	17	13	7	37
Total number of pts.		35 (57,4%)	15 (24,6%)	11 (18%)	61 (100%)
Test χ^2 (χ^2_{kr}=5.99)	χ^2	6.43			
	p	<0.05			



The correlation table of relationship between degree of daytime sleepiness and gender.

According to questionnaire investigation assessment there were 82,4% of healthy pts in compare to Index RDI TST assessement while we found only 11,8% of healthy subjects (u=4,59>1.96=$u$kr, p<0.0001). There is no correlation between questionnaire investigation and PSG assessment.

Conclusions: Questionnaire investigation may be valuable in preliminary assessment of sleep disorders. PSG is the best test to confirm sleep disorders. Both male gender and elderly age contribute to EDS in HD patients.

TH-PO304

Polysomnography Sleep Parameters in Children, Adolescents and Young Adults with and without Chronic Kidney Disease Ritu K. Soni,[1] Maria-Eleni Roumelioti,[2] Jonathan Yabes,[1] Mark L. Unruh.[1] *[1]Renal-Electrolyte Division, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Nefroiatriki Dialysis Unit, Athens, Greece.*

Background: There are only limited studies examining sleep disorders in the pediatric and adolescent chronic kidney disease (CKD) population despite their vulnerability to the negative impacts of these disorders. This is the first study using polysomnography (PSG) to evaluate sleep disorders in pediatric and adolescent CKD patients.

Methods: 11 patients with advanced CKD (estimated glomerular filtration rate <30 mL/min/1.73 m^2) or dialysis-dependent end stage renal disease were compared with 26 healthy controls. In-home PSG was performed for objective sleep assessment.

Results: The CKD group had significantly higher mean systolic and diastolic blood pressure. CKD patients were more likely than the non-CKD participants to have higher sleep latency and lower sleep efficiency but the differences did not reach statistical significance. Sleep apnea was significantly more frequent in the CKD group.

Baseline Characteristics and PSG Results

Variable	CKD Patients (N=11)	Controls (N=26)	*P*
Age (y)	18.0 ± 5.75	15.7 ± 3.88	0.122
Male	5 (45.5)	11 (42.3)	1.000
White	7 (63.6)	20 (76.9)	0.442
Body Mass Index (kg/m^2)	22.2 ± 6.39	21.8 ± 4.69	0.769
Systolic blood pressure (mmHg)	130.9 ± 31.52	113.7 ± 10.41	0.064
Diastolic blood pressure (mmHg)	86.3 ± 18.75	72.0 ± 14.16	0.043
Total Sleep time (min)	400.3 ± 146.85	384.9 ± 110.92	0.455
Sleep latency (min)	35.9 ± 32.2	30.2 ± 34.78	0.803
Sleep efficiency (%)	76.7 ± 15.5	80.4 ± 17.18	0.435
Stage 1 (%)	5.5 ± 3.61	3.6 ± 2.99	0.189
Stage 2 (%)	48.1 ± 11.91	48.4 ± 8.88	0.987
REM (%)	29.6 ± 17.23	27.9 ± 11.12	0.934
Apnea-hypopnea index	3.8 ± 4.14	0.9 ± 0.84	0.070
Periodic limb movement index	3.2 ± 1.98	5.4 ± 3.08	0.038
Apnea-hypopnea index >5	3 (27.3)	0 (0)	0.021

Note: Values expressed as mean ± SD or number (%).

Conclusions: This study demonstrates that sleep architecture was preserved in the CKD population. There was evidence for more sleep apnea in the CKD patients but larger studies are needed to define the magnitude of the associations between sleep disorders and CKD.

Funding: Private Foundation Support

TH-PO305

Neuropsychological Profile of Children and Young Adults with Moderate to Severe Chronic Kidney Disease Divya Moodalbail,[1] Jerilynn Radcliffe,[1] Abbas F. Jawad,[1] Kathryn Reiser,[1] Susan L. Furth,[1,3] Stephen R. Hooper.[2] *[1]Pediatrics, Children's Hospital of Philadelphia; [2]Psychiatry, University of North Carolina School of Medicine; [3]Epidemiology, University of Pennsylvania.*

Background: Few studies have examined the neuropsychological profile of children and young adults with moderate to severe chronic kidney disease (CKD).

Methods: Cross sectional observational study comprising 18 children and young adults with CKD (eGFR below 60 ml/min/1.73m2), ages 8 to 25 years. Demographics and neurocognitive data from the administration of a broad based neuropsychological test battery (including WASI Vocabulary, WASI Similarities, WASI Block Design, WASI Matrix Reasoning, Wechsler Memory Scales-III, WISC-IV Digit Span, Continuous Performance Test-II, and Delis-Kaplan Executive Function Scales) were collected at study visit. We evaluated multiple types of skills within the language, attention, and executive functioning domains in CKD subjects with reference to age based norms. Mean z-scores were computed for each domain. These means were based on results from all measures included within each domain of interest.

Results: Median age was 15 years (range 14 - 17.5 years) and median eGFR was 32 ml/min/1.73m2. The sample included 76% male and 81% Caucasian subjects. About 29%(6) had transplant, and 5%(1) were on dialysis after a failed transplant. Mean z-scores and standard deviations were reported for each functional domain. Overall, weakest performance was shown in the areas of attention (-0.48), verbal memory (-0.44), set shifting (-0.28), and visuo-spatial skills (-0.42). In contrast, language (-0.08), and inhibitory control (-0.05) were relatively stronger.

Conclusions: Neuropsychological findings indicate relative weaknesses in attention, verbal memory, visuo-spatial skills and set shifting, with near-normal performance in language and inhibitory control. However, wide variability in functioning was found within each domain. Ongoing recruitment of 90 CKD subjects and 90 controls and simultaneous evaluation of structural and functional brain imaging will further assess the presence of these deficits and structure/function correlations.

Funding: Other U.S. Government Support

TH-PO306

Carotid-Intima-Media Thickness in Children with Chronic Kidney Disease: Lack of Association with Markers of Oxidative Stress and Inflammation Antonio García-Bello, Rita A. Gómez-díaz, Alicia Contreras, Margarita Díaz-flores, Daniel Hernandez, Juan Manuel Gallardo, Rafael Mondragon, Adan Valladares, Lorena Sanchez, Juan Talavera, Niels Wacher, Alejandra Aguilar, Jesus Lagunas. *Instituto Mexicano del Seguro Social.*

Background: Non-traditional cardiovascular risk factors have been implicated in the pathogenesis of atherosclerosis in CKD. The aim of this study was to evaluate the relationshipbetween markers of oxidative stress, inflammation and carotid artery intima-media thickness (cIMT) and vascular calcifications in children.

Methods: The study included 85 children aged 6-16 with CKD of unknown etiology without acute infectious disease. Variables included time with CKD, dialysis modality, anthropometric and biochemical parameters including intact molecule parathyroid hormone (iPTH), C-reactive protein (hsCRP), IL-1β, IL-6, TNFα, superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO). cIMT was measured according to American Heart Association 2009 recommendations. Statistical analysis: Lineal regression was done to identify the variables associated with cIMT. To value the effect of the potential cardiovascular risk factors on cIMT, logistic regression model was built.

Results: Of 85 children 49.6% were female; 17.6% had no dialysis, 30.6% were on hemodialysis and 51.8% on peritoneal dialysis (PD). Mean values were: age 13.1±2.4 years, evolution time 38.6±37.3 months, Hb 10.7±2.4 g/dL, cholesterol 170.1±47.3, TGL 155±72.6, HDL-C 40.0±12.4 mg/dL, iPTH 664 (33-5572) pg/mL, GSH 417.4±134.7 µM, hsCRP 0.91 mg/L (0.07-186.9), IL-6, 0.15 pg/mL (0.00-596.2), IL-1β 1.71 (0-709.7) pg/mL, TNFα 5.27 pg/mL (0-2544.3), MDA 1.75±1.0 nM, NO 51.4±23.4 µM. A significant correlation ($p<0.05$) was found between cIMT and CaxP product, NO and SOD. Logistic regression showed that only cholesterol and PD modality were associated with a cIMT over 0.614 mm (75 Pc). Adding inflammatory and oxidative stress markers to model showed no significant effect.

Conclusions: No association was found between markers of oxidative stress or inflammation and cIMT in CKD pediatric cases.

Funding: Government Support - Non-U.S.

TH-PO307

Restless Legs Syndrome in Pediatric Chronic Kidney Disease: Is Iron to Blame? Sandeep K. Riar,[1] Roberta Leu,[2] Donald L. Bliwise,[3] Laurence A. Greenbaum.[1] *[1]Pediatrics, Nephrology Division, Emory University and Children's Healthcare of Atlanta, Atlanta, GA; [2]Division of Pulmonology, Allergy, Cystic Fibrosis and Sleep Medicine, Emory University and Children's Healthcare of Atlanta, Atlanta, GA; [3]Neurology, Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA.*

Background: Restless legs syndrome (RLS) can adversely affect sleep and is increased in chronic kidney disease (CKD) patients. Central nervous system iron deficiency is thought to be involved in RLS pathogenesis, with serum ferritin levels used to guide treatment. Children with CKD are at risk of iron deficiency, but are also in an inflammatory state, which affects both ferritin and transferrin saturation (TSAT) – standard measures of iron status. We studied the prevalence of RLS in children with CKD and compared serum ferritin and inflammatory status in those with and without RLS.

Methods: Cross-sectional study of RLS in CKD patients (non-transplant, non-dialysis [NT, ND] CKD patients with an estimated GFR < 60 mL/min/1.73 m2; renal transplant [Tx]; and dialysis patients). RLS was diagnosed using an NIH criteria based questionnaire. Serum ferritin <100 ng/mL or TSAT <20% defined iron deficiency. Serum high sensitivity C-reactive protein of ≥ 1 mg/L defined inflammation.

Results: Of 124 patients with CKD, 66 were Tx patients, 23 were on dialysis, and 35 were NT, ND. 15.3% of children with CKD had RLS. RLS prevalence was higher in patients *without* iron deficiency compared to those with iron deficiency (22.6% vs 12.5%, p= 0.15). Median ferritin levels were *higher* in RLS+ patients versus RLS- patients (51.2 vs 40.1 ng/mL; p= 0.08). CKD patients with inflammation had higher RLS rate than those without inflammation (21.6% vs 12.3%, p=0.18).

Conclusions: In pediatric CKD, iron deficiency is not associated with RLS. RLS subjects had higher ferritin values, contrary to non-CKD subjects with RLS. Inflammation may be responsible for this finding. This study suggests that the factors mediating pathogenesis of RLS in pediatric CKD are different from those known to increase risk in non-CKD populations. The role of inflammation in RLS in CKD deserves further study.

Funding: Private Foundation Support

TH-PO308

From Cultured Muscle Cells to Human Muscle Biopsies: Stat3 Inhibition Is a New Treatment for Muscle Wasting Jenny S. Pan,[1] Liping Zhang,[1] Giacomo Garibotto,[2] Yanlan Dong,[1] Yanjun Dong,[1] William E. Mitch.[1] *[1]Nephrology Division, Baylor College of Medicine, Houston, TX; [2]Nephrology Division, Genoa University, Genoa, Italy.*

Background: There is no effective therapy for the prevalent, costly and debilitating loss of muscle in chronic kidney disease (CKD) or other catabolic conditions. Epidemiologic evaluations implicate inflammatory cytokines (e.g., interleukin-6 (IL-6) and Tumor Necrosis Factor α (TNFα)) plus impaired insulin/IGF-1 signaling as mediators of muscle wasting. However, the molecular mechanisms that link inflammatory cytokines to impaired insulin/IGF-1 signaling and muscle wasting are unknown.

Methods: We examined biopsies of rectus abdominis muscles from CKD patients and control subjects. In C2C12 myotubes treated with a cytokine mixture (IL-6 plus TNFα) and mice with CKD (subtotal nephrectomy), we inhibited STAT3 with a small molecule inhibitor (C188-9).

Results: In muscle biopsies from CKD patients, levels of IL-6, TNFα and activated Stat3 (p-Stat3) were significantly increased. Stat3 activation was also increased in muscles of mice with CKD. To explore how Stat3 regulates the muscle wasting induced by CKD or other inflammatory conditions, we subjected mice to subtotal nephrectomy (CKD) and inhibited Stat3 with C188-9. Stat3 inhibition in CKD mice increased their body and muscle weights vs results from CKD mice treated with D5W (diluent). It also improved muscle metabolism and strength. To examine the pathway that accelerates muscle protein losses, we treated C2C12 myotubes with IL-6 and TNFα; Stat3 was activated at 15 minutes after exposure to IL-6/TNFα, followed by upregulation of Stat3 target genes SOCS3 at 30 minutes, C/EBPδ at 3 hours and myostatin at 24 hours. In cultured C2C12 myotubes or mice with CKD, Stat3 inhibition attenuated IL-6/TNFα and CKD-induced expression of SOCS3, C/EBPδ and myostatin. In muscle biopsies from CKD patients, increased expression of p-Stat3, C/EBPδ and myostatin confirmed activation of the pathway.

Conclusions: Inflammation stimulates muscle proteolysis by a pathway from Stat3 to C/EBPδ to myostatin. Inhibition of Stat3 is a therapeutic target for preventing muscle wasting.

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TH-PO309

Decreased Circulating Klotho Level Predicts Renal Outcome in Patients with Chronic Kidney Disease Seung Hyeok Han,[1] Dae-Suk Han,[1] Dong Ho Shin,[1] Mi Jung Lee,[1] Shin-Wook Kang,[1,2] Hye-young Kang,[2] Seong Hun Kim.[2] *[1]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei University, Seoul, Republic of Korea.*

Background: Klotho is mainly expressed in the renal distal tubules and exerts coreceptor function for FGF-23. It can also be released into the circulation and acts like a hormone that can have anti-oxidative and anti-senescence effects. However, it is unknown whether altered circulating klotho levels may predict renal outcome in patients with chronic kidney disease (CKD).

Methods: We conducted an observational cohort study in 243 subjects who were diagnosed as CKD between January 2006 and December 2011. Demographic and clinical data were reviewed based on medical records. We measured baseline serum concentrations of klotho and FGF-23 using enzyme-linked immunosorbent assay. Primary outcome was the composite of a doubling of the baseline serum creatinine levels and end-stage renal disease (ESRD).

Results: The mean age of the patients was 45.7 years and 52.7% were male. The mean eGFR and the median value of 24-hour urinary protein excretion were 52.2±34.7 ml/min/1.73m^2 and 1.5 (0.5-4.6) g/day, respectively. When patients were categorized into 2 groups according to the median serum klotho levels, 43 patients (35.2%) with α-klotho≤397 pg/ml reached the composite outcome of a doubling of the baseline serum creatinine and ESRD compared with 17 patients (14.0%) with α-klotho>397 pg/ml [hazard ratio (HR), 2.507; P=0.019]. Cox regression analysis revealed that α-klotho concentrations independently predicted the composite outcome after adjustment for age, gender, diabetes, blood pressure, eGFR, FGF-23, proteinuria, C-reactive protein, and parathyroid hormone (per 10 pg/ml increase; HR, 0.952; P<0.001).

Conclusions: Decreased circulating klotho levels may predict adverse renal outcome in patients with CKD.

TH-PO310

Serum Level of Soluble Secreted αKlotho Is a Novel Predictor for Renal Prognosis in CKD Patients Yoshiko Shimamura,[1] Kazu Hamada,[1] Koji Ogata,[1] Kosuke Inoue,[1] Toru Kagawa,[1] Masayuki Ishihara,[1] Kenji Yuasa,[2] Yoshio Terada.[1] *[1]Department of Endcrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Nankoku, Kochi, Japan; [2]Kochi-Takasu-Hospital, Kochi, Japan.*

Background: α-Klotho was first identified as an aging gene and was later shown to be a regulator of phosphate metabolism. We previously reported serum levels of soluble secreted α-Klotho in CKD patients relation with serum creatinine, age, hemoglobine, and log-FGF23 levels. Furthermore, we reported soluble secreted α-Klotho is a potential new biomarker for the diagnosis of CKD. This study was designed to investigate whether serum soluble αKlotho may become new predictor for prognosis of renal function in CKD patients.

Methods: We made a continuously survey of CKD patient characteristics and outcomes in Kochi prefecture (Western area of Japan) for 12 months. Patients of CKD (total N=395, Stage3-5: N=297) were enrolled. Serum sample were collected and measured soluble αKlotho by using an ELISA kits. In addition, serum creatinine, hemoglobin, albumin, calcium, phosphate and FGF23 were measured. This study was approved by Kochi Medical School review board. All patients provided written informed consent.

Results: During the study period of 12 months observation, estimated glomerular filtration rate (eGFR) was decreased (mean -5.10ml/min./1.73m^2 during 12 months) in 87 patients and eGFR was stable in 94 patients (CKD Stage3-5). We compared several parameters between the group of decreased eGFR and the group of stable eGFR. Interestingly, the levels of serum soluble α-Klotho were significantly lower in the group of decreased eGFR compared with stable eGFR group (p<0.05). The group of decreased eGFR was older (p<0.05), and also lower rate of the utilization of Angiotensin II receptor blocker (ARB) (p<0.05). In our study, serum FGF23 levels were not association with the change of eGFR during 12 months (p=0.19).

Conclusions: Our data indicate that soluble α-Klotho level is a novelpredictor for renal prognosis in CKD patients.

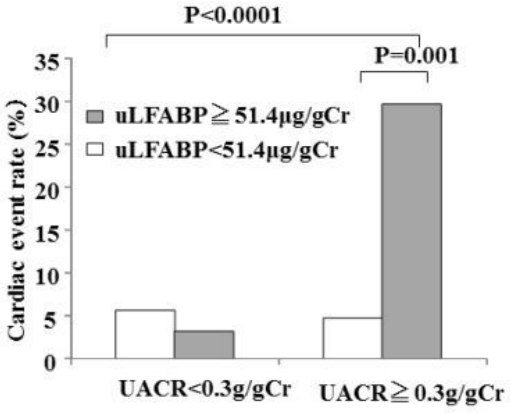
TH-PO311

Urinary Liver-Type Fatty Acid Protein as a Predictor of a Cardiac Event and Progressive Renal Dysfunction in Non-Diabetic Patients with Chronic Kidney Disease Midori Hasegawa, Kyoko Kanayama, Yukio Yuzawa. *Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan.*

Background: The aim of this study was to evaluate urinary liver-type fatty acid-binding protein (uL-FABP) as a predictor of a cardiac event and progressive renal dysfunction in non-diabetic patients with chronic kidney disease (CKD).

Methods: A total of 273 non-diabetic CKD patients whose eGFR was below 60 mL/min/1.73 m^2 and who were not receiving dialysis were enrolled in this study between March 2009 and September 2010. The median follow-up period was 22 months. The levels of uL-FABP were measured and its relationships with cardiac events and renal outcomes were assessed.

Results: (1) Cardiac events: There were 31 cardiac events. Kaplan-Meier incidence rates of cardiac events were 0.88%, 11.5%, 18.6% and 23.6% among quartiles of uL-FABP levels (p<0.0001). The best cut-off value for a cardiac event was 51.4μg/gCr of uL-FABP (sensitivity 74.2%, specificity 64.5%) according to the ROC curves (AUC0.713). There was a significantly greater tendency for cardiac events among patients with increased UACR and uL-FABP concentrations compared with patients with only increased UACR (P = 0.001) (Fig1).(2) Renal outcomes: The patients were classified as progressors when the eGFR value decreased by 5 ml/min/1.73 m^2/year or the patient received renal replacement therapy. At the end of follow-up, 74 patients were classified as progressors. The initial values of uL-FABP, UACR, eGFR, Hb, and albumin significantly differed between progressors and non-progressors. On logistic regression analysis, initial L-FABP (OR1.002, P value=0.015) and UACR (OR1.001, P value<0.0001) remained as independent variables associated with the progression of renal dysfunction.



Conclusions: Urinary L-FABP may be useful for predicting a cardiac event and the progression of renal dysfunction in non-diabetic patients with CKD.

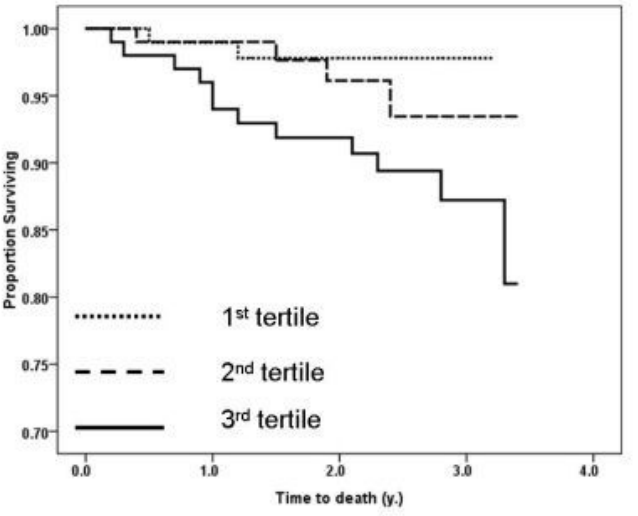
TH-PO312

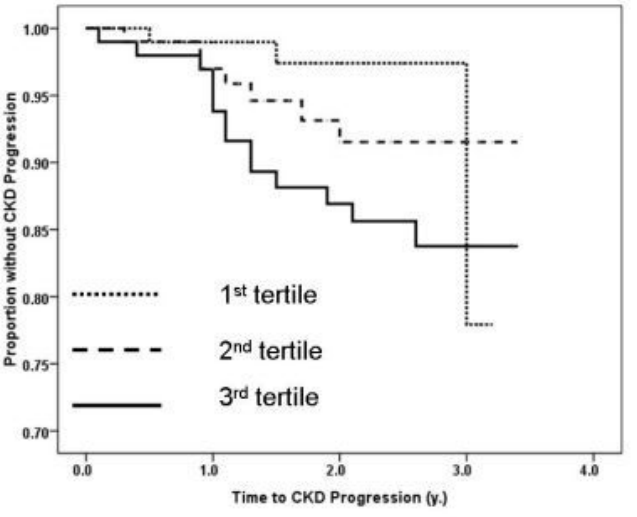
Association of Placental Growth Factor with Chronic Kidney Disease Progression and Mortality: CARE FOR HOMe Study Mehdi Rambod,[1] Sarah Seiler,[2] Kyrill S. Rogacev,[2] Danilo Fliser,[2] Samir S. Patel,[3] Rama Dwivedi,[3] Ali Ramezani,[3] Harry S. Gill,[3] Dominic S. Raj,[3] Gunnar H. Heine.[2] *[1]Texas Tech Health Sciences Center, El Paso, TX; [2]Saarland University Medical Center and Saarland University Faculty of Medicine, Germany; [3]The George Washington University.*

Background: Placental growth factor (PlGF) is a member of the human vascular endothelial growth factor family of signaling proteins, which are associated with inflammation and atherosclerotic disease in the general population. In this study, we examined the association of PlGF with the risk of progression of chronic kidney disease (CKD) and death.

Methods: Serum level of PlGF was measured in 301 CKD patients. Progression of CKD was defined as 50% decrease in glomerular filtration rate (GFR) or need for renal replacement therapy.

Results: Sixty-one percent of participants were male and 39% were diabetic. Mean(SD) age, body mass index (BMI), and GFR were 65.6(11.8) y., 30.3(5.5) kg/m^2, and 44(16) ml/min, respectively. Mean(SD) PlGF was 5.4(3.8) pg/mL ranging from 0.06 to 22.35 pg/mL. PlGF was inversely correlated with high-density lipoprotein (HDL) (r=-0.23, p<0.001), and positively correlated with log triglyceride (r=0.23, p<0.001) and leukocyte count (r=0.16, p=0.006). During 3.4 y. of follow up, 6% (n=18) died and CKD progressed in 8% (n=24). Compared to the first tertile of PlGF, 2nd and 3rd tertiles of PlGF were associated with increased death rate and CKD progression (Figure). After adjustment for age, sex, BMI, albumin, HDL, and diabetes, 2nd and 3rd tertiles of PlGF were associated with increased risk of composite outcome (death or CKD progression) (HR: 2.5, p=0.12; and 6.0, p=0.001, respectively).





Conclusions: In CKD patients not yet on dialysis, PlGF is associated with an atherogenic lipid profile, increased leukocyte count, and higher risk of CKD progression and mortality.

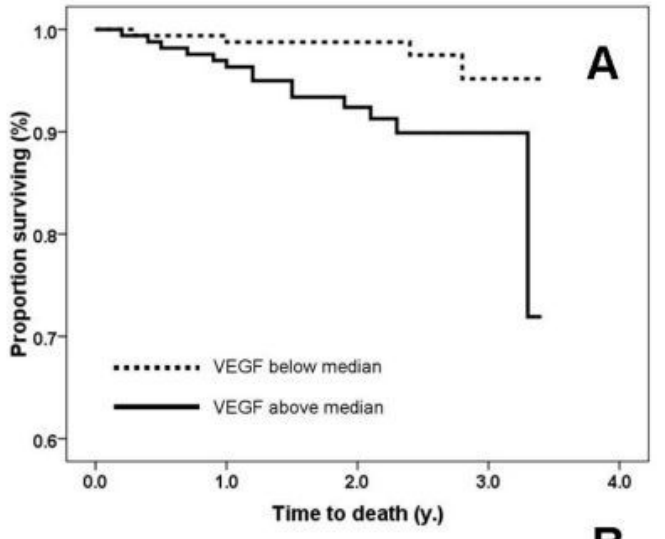
TH-PO313

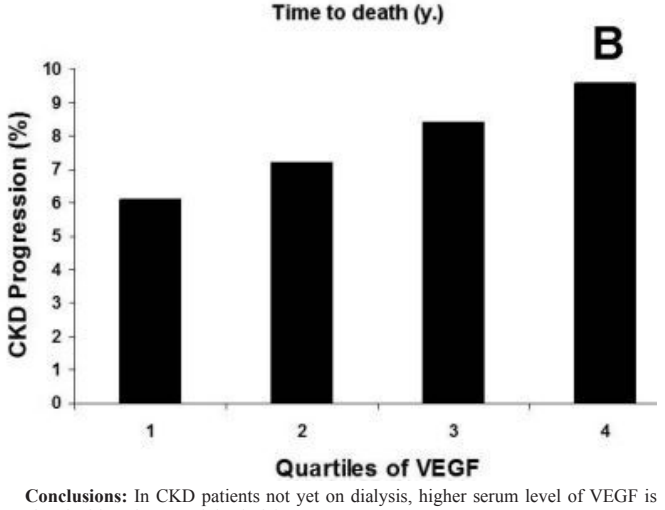
Vascular Endothelial Growth Factor Is Associated with Higher Mortality in Chronic Kidney Disease: CARE FOR HOMe Study Mehdi Rambod,[1] Sarah Seiler,[2] Kyrill S. Rogacev,[2] Danilo Fliser,[2] Samir S. Patel,[3] Rama Dwivedi,[3] Ali Ramezani,[3] Harry S. Gill,[3] Dominic S. Raj,[3] Gunnar H. Heine.[2] *[1]Texas Tech Health Sciences Center, El Paso, TX; [2]Saarland University Medical Center & Saarland University Faculty of Medicine, Hamburg, Germany; [3]The George Washington University, Washington, DC.*

Background: Vascular endothelial growth factor (VEGF), a key regulator of angiogenesis, generally considered renoprotective; conversely, VEGF is reported to be associated with a higher rate of end-stage renal disease, but less is known about association of VEGF and mortality in chronic kidney disease (CKD) patients.

Methods: Serum level of VEGF was measured in 331 CKD patients. Progression of CKD was defined as 50% decrease in glomerular filtration rate (GFR) or need for renal replacement therapy.

Results: Sixty-one percent of patients were male and 39% were diabetic. Mean (SD) age, body mass index (BMI), and GFR were 65 (12) y., 30.4 (5.5) kg/m^2, and 44 (16) ml/min, respectively. Mean (SD) VEGF was 69 (29) pg/mL. VEGF was inversely correlated with GFR (r=-0.12, p=0.028), albumin (r=-0.14, p=0.012) and BMI (r=-0.11, p=0.043) and positively correlated with serum endotoxin level (r= 0.18, p=0.008). During 3.4 y. of follow-up, 5.4% (n=18) died and CKD progressed in 8% (n=26). Compared to the patients with VEGF<median, patients with VEGF>=median had higher death risk [hazard ratio (HR)=3.99; 95%CI: 1.3–12.2, p=0.015] (Figure-A). This increased death risk remained significant after adjustment for age, sex, BMI, GFR, serum albumin and diabetes status (HR=3.4; 95%CI: 1.1–10.7, p=0.034). Moreover, a trend was seen towards increased risk of CKD progression across quartiles of VEGF, though the observed trend was not statistically significant (Figure-B).





Conclusions: In CKD patients not yet on dialysis, higher serum level of VEGF is associated with an increased death risk.

TH-PO314

Serum Alkaline Phosphatase and Risk for ESRD and Death in Stage 3 and Stage 4 CKD Sankar D. Navaneethan,[1] Jonathan J. Taliercio,[1] Anne S. Tang,[2] James F. Simon,[1] Stacey Jolly,[3] John W. Sharp,[3] Martin J. Schreiber,[1] Jesse D. Schold,[2] Joseph V. Nally.[1] *[1]Nephrology, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Medicine, Cleveland Clinic.*

Background: Higher serum alkaline phosphatase (SAP) increases vascular calcification and may contribute to the cardiovascular deaths in the general population. CKD patients have higher SAP due to the distrubances in the bone mineral metabolism which in turn may contribute to the higher cardiovascular burden. We examined the associations between SAP and end stage renal disease (ESRD) and all-cause mortality in those with stage 3 and stage 4 CKD.

Methods: 28398 patients with stage 3 and stage 4 CKD who had SAP measured one year before the diagnosis of CKD were included. Cox proportional hazards models were used to examine the associations between SAP and the composite end-point of death or ESRD while adjusting for relevant confounding variables including liver function tests.

Results: During a median follow-up of 2.3 years, 5295 participants died or reached ESRD.In the multivariate adjusted analyses, each standard deviation increase in SAP was associated with a 17% increased hazard for death or ESRD (Hazard ratio[HR] 1.17, 95% CI 1.14,1.19). Compared to the lowest quartile (SAP <66 U/L), there was a graded increase in the hazard for death or ESRD in the highest quartiles of SAP (Table 1).

Table 1. Associations between serum alkaline phosphatase levels and death and ESRD

	Adjusted HR (95%CI) Composite	Adjusted HR (95%CI) Death	Adjusted HR (95% CI) ESRD
Categorical			
Q2 vs. Q1	1.10(1.01, 1.20)	1.13(1.03, 1.24)	0.83(0.64,1.09)
Q3 vs. Q1	1.24(1.14, 1.35)	1.26(1.15, 1.38)	1.02(0.80, 1.30)
Q4 vs. Q1	1.68(1.55, 1.82)	1.71(1.56, 1.86)	1.36(1.09, 1.70)
Continuous			
Each SD increase in SAP	1.17(1.15, 1.19)	1.18(1.15, 1.20)	1.17(1.10, 1.23)

Q1: <66 U/L, Q2: 67-81 U/L, Q3: 82-101 U/L, Q4: >102 U/L, SD: Standard deviation, HR: Hazard ratio

When the analysis was restricted to those who had SAP levels within the normal range (<149 U/L),results were similar. In a subgroup analysis that included those who had serum phosphorus data (n=5789), each SD increase in SAP was associated with a 13% increased hazard for the composite end-point.

Conclusions: Higher SAP (even within the normal range) is associated with an increased risk of death and ESRD in those with non-dialysis dependent CKD. Studies examining the mechanisms that explain these associations are warranted.

Funding: Pharmaceutical Company Support - Development of the CKD Registry Was Supported by an Unrestricted Educational Fund to the Department of Nephrology and Hypertension

TH-PO315

Mineral Metabolites and Progression to End-Stage Renal Disease in AASK Julia J. Scialla,[1] Brad C. Astor,[2] Tamara Isakova,[1] Huiliang Xie,[1] Lawrence J. Appel,[3] Myles S. Wolf.[1] *[1]University of Miami; [2]University of Wisconsin; [3]Johns Hopkins University.*

Background: African Americans progress faster to ESRD than Caucasians. Disordered mineral metabolism is particularly severe among African Americans with CKD and may contribute to progression.

Methods: We measured fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), phosphate and 25-hydroxyvitamin D (25D) in 809 African Americans with hypertensive CKD enrolled in AASK. We analyzed the association of baseline levels (continuously and in quartiles) with risk of ESRD or death over a median of 8 years using Cox models.

Results: Mean GFR was 45 ± 18 ml/min/1.73m^2. 95% of participants were 25D insufficient (<30 ng/ml) and 33% severely deficient (<10). Higher quartiles of FGF23, PTH, and phosphate and lower quartiles of 25D were associated with greater risk of ESRD or death after adjustment for age, sex and randomized group in the trial (each p<0.05). Only FGF23 and PTH remained associated after full adjustment.

		Incidence rate (per 1000 person years)	Hazard ratio of ESRD or death (95% CI)*
FGF23 (pg/ml):	≤30.7	26.2	Ref
	30.8-44.2	47.4	1.47 (0.98-2.20)
	44.3-64.3	70.0	1.67 (1.04-2.69)
	≥64.4	141.3	2.24 (1.39-3.60)
	p-trend	--	<0.01
PTH (pg/ml):	≤24.0	35.6	Ref
	24.1-37.0	38.6	0.80 (0.57-1.13)
	37.1-60.7	71.0	1.01 (0.80-1.27)
	≥60.8	138.2	1.33 (1.01-1.75)
	p-trend	--	0.01

*adjusted for age, sex, income, prior cardiovascular disease, smoking status, body mass index, GFR, proteinuria, serum albumin, and randomized group in the trial

In adjusted continuous models log-FGF23 exhibited a linear dose-response relationship with outcomes (p<0.01), whereas higher log-PTH and phosphate were associated in their higher ranges (PTH >39 pg/ml, p<0.01; phosphate >3.5 mg/dl, p<0.01) and 25D was not associated (p=0.4). The rate of ESRD or death among those with concomitant elevations of FGF23 (>50 pg/ml), PTH (>65 pg/ml) and phosphate (>4.6 mg/dl) was 487/1000 person years, compared to 214/1000 person years in stage 4/5 CKD.

Conclusions: Among African Americans with hypertensive kidney disease, abnormalities of mineral metabolism are associated with greater risk of progression to ESRD and may be useful as adjunctive metrics of CKD severity. FGF23 was most consistently associated with CKD progression and may be a novel therapeutic target. 25D deficiency was prevalent, but not independently associated with outcomes.

Funding: NIDDK Support, Other NIH Support - Office of Research in Minority Health, Pharmaceutical Company Support - DiaSorin

TH-PO316

Serum 1,25 Dihydroxyvitamin D and the Development of Chronic Kidney Disease in a Japanese Community: The Hisayama Study Kensuke Izumaru,[1,2] Toshiharu Ninomiya,[1,2] Masaharu Nagata,[1,2] Tomoko Usui,[1,2] Kazuhiko Tsuruya,[2] Takanari Kitazono,[2] Yutaka Kiyohara.[1] *[1]Department of Environmental Medicine, Kyushu University, Fukuoka; [2]Department of Medicine and Clinical Science, Kyushu University, Fukuoka.*

Background: Lower serum 25 hydroxyvitamin D [25(OH)D] level has been reported as a risk factor for end-stage kidney disease. However, there are limited studies addressing whether serum 1,25 dihydroxyvitamin D [$1,25(OH)_2D$] affects the development of chronic kidney disease (CKD) in the general population.

Methods: We followed up 2,417 community-dwelling individuals without CKD aged ≥40 years for 5 years and examined the effects of baseline serum $1,25(OH)_2D$ levels on the development of CKD by using logistic regression model. CKD was defined as eGFR <60 ml/min/1.73m^2.

Results: During follow-up, 378 subjects experienced CKD. The age- and sex-adjusted cumulative incidence rates of CKD increased significantly with decreasing quartiles of serum $1,25(OH)_2D$ concentrations (≥79.3, 66.8-79.2, 56.5-66.7, and <56.5 pg/ml), being 12.0%, 14.0%, 17.9%, and 19.4%, respectively (p for trend <0.01). Compared with those with $1,25(OH)_2D$ of ≥79.3 pg/ml, the multivariate-adjusted odds ratio (OR) for the development of CKD in subjects with $1,25(OH)_2D$ of56.5-66.7 and <56.5 pg/ml were 1.71 (95% confidence interval [CI], 1.17-2.50) and 1.60 (95% CI, 1.08-2.34), respectively, after adjusting for potential confounding factors.Additionally, the rate of change in kidney function for 5 years decreased gradually with lower serum $1,25(OH)_2D$ levels (-1.8, -2.0, -2.2, and -2.4 ml/min/1.73m^2/year, respectively, p for trend <0.001).

Conclusions: Our findings suggest that lower serum $1,25(OH)_2D$ level is a significant risk factor for the development of CKD in the general population.

Funding: Government Support - Non-U.S.

TH-PO317

The Association of Glycated Hemoglobin with Mortality and ESRD among Persons with Diabetes and Chronic Kidney Disease Chutatip Limkunakul,[1] Ian H. de Boer,[2] Bryan R. Kestenbaum,[2] Cassianne Robinson-Cohen,[2] Michael Sachs,[2] Jonathan Himmelfarb,[2] T. Alp Ikizler.[1] *[1]Nephrology, Vanderbilt University, Nashville, TN; [2]Kidney Rsch Institute, University of Washington, Seattle, WA.*

Background: Glycated hemoglobin (HbA1c) is a measurement of chronic hyperglycemia in diabetes mellitus. While higher HbA1c is clearly associated with increased risk of developing kidney disease, its associations with progression of existing kidney disease, cardiovascular disease, and death are not well defined.

Methods: We performed a cohort study of 513 persons with chronic kidney disease in the Seattle Kidney Study, of whom 275 had diabetes. Subjects with diabetes were grouped by HbA1c (<6.5; 6.5 - 6.9; 7 - 7.9; 8 - 9; >9). The primary outcome was a composite of ESRD and death. Cox models were used to calculate the hazard ratio (HR) after adjustment for clinical and demographic variables.

Results: Compared to participants without diabetes, those with diabetes had higher unadjusted incidence rates of each outcome within any category of baseline HbA1c: range 11.3-14.1 vs 6.6 per 100 person-years for the primary composite outcome, range 5.8-7.3 vs 3.0 per 100 person-years for ESRD, and range 6.1-10.9 vs 3.8 per 100 person-years for death. Among participants with diabetes who were receiving insulin therapy, each 1% higher HbA1c was associated with a 42% lower adjusted increased risk of ESRD (HR 0.58; 95% CI, 0.32 – 1.06; p = 0.078), which was not observed for increased risk of death. Among participants with diabetes who were not receiving insulin therapy, each 1% higher HbA1c was associated with a 91% greater adjusted risk of death (HR 1.91; 95% CI, 1.17 – 3.12), which was not observed for increased risk of ESRD. Risk of ESRD was greatest among participants with diabetes and HbA1c <7%.

Conclusions: Among persons with diabetes and established chronic kidney disease, lower HbA1c was not associated with lower risk of ESRD and death in all patients. There is a differential risk profile according to the use of insulin in diabetic patients. In this population, control of HbA1c to currently recommended levels may be associated with harm in certain individuals.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO318

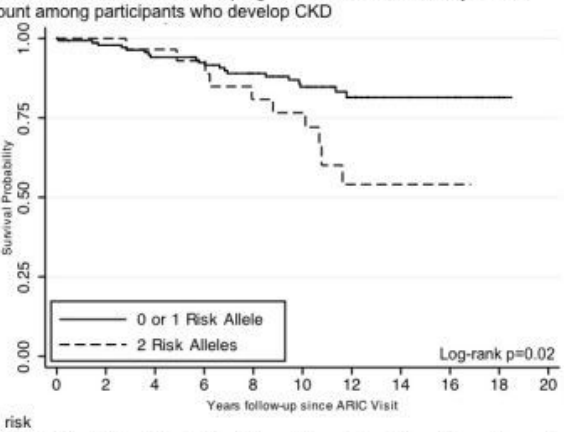
APOL1 and Risk of Incident and Progressive Chronic Kidney Disease (CKD) in African Americans: The Atherosclerosis Risk in Communities (ARIC) Study Meredith C. Foster,[1] Josef Coresh,[1] Myriam Fornage,[2] Brad C. Astor,[3] M. Grams,[1] Nora Franceschini,[4] Eric Boerwinkle,[2] Rulan S. Parekh,[1] Wen Hong Linda Kao.[1] *[1]Johns Hopkins University; [2]University of Texas; [3]University of Wisconsin; [4]University of North Carolina.*

Background: Common variants in the APOL1 gene are strongly associated with prevalent end stage renal disease (ESRD) in African Americans but prospective and general population data are lacking.

Methods: We analyzed data from 3,067 African Americans free of CKD at ARIC baseline to evaluate the association of APOL1 G1 & G2 risk alleles (2 vs. 0 or 1 allele) with incident CKD (eGFR<60mL/min/1.73m^2) and the subsequent progression to ESRD amongst the group with CKD using time-to-event analyses. Cox models were adjusted for age, sex, study center, and European ancestry.

Results: Overall 13.2% carried 2 APOL1 risk alleles, with 8.2% developing CKD compared to 5.9% among those with 0 or 1 risk allele over 8.6 years median follow-up(hazard ratio [HR] 1.5; 95% confidence interval [CI] 1.0-2.2). European ancestry adjustment did not significantly change results. Among those with incident CKD, those with 2 risk alleles were also more likely to progress to ESRD than their counterparts with 0 or 1 risk allele (Figure; HR 2.2; 95% CI 1.0-4.8), especially after about 6 years of follow up. With an interaction term between risk and time, the HR associated with genotype was 0.9 (95% CI 0.2-4.1) during the first 6 years of follow-up and 3.8 (95%CI 1.4-9.0) after the first 6 years.

Figure: Kaplan-Meier survival curves for progression to ESRD event by *APOL1* risk allele count among participants who develop CKD



Number at risk											
0 or 1 Risk Allele	148	134	120	112	95	76	41	19	16	4	0
2 Risk Alleles	32	31	27	23	20	17	8	2	1	0	0

Conclusions: In a large sample of African Americans from the general population, the APOL1 G1/G2 risk alleles increased the risk of developing CKD and progression to ESRD. These results show that the effect of APOL1 on ESRD risk may begin early and become stronger at later stages of CKD, suggesting that earlier intervention or treatment strategies need to be studied.

Funding: NIDDK Support, Other NIH Support - NIH/NHLBI T32HL007024; HHSN268201100009C

TH-PO319

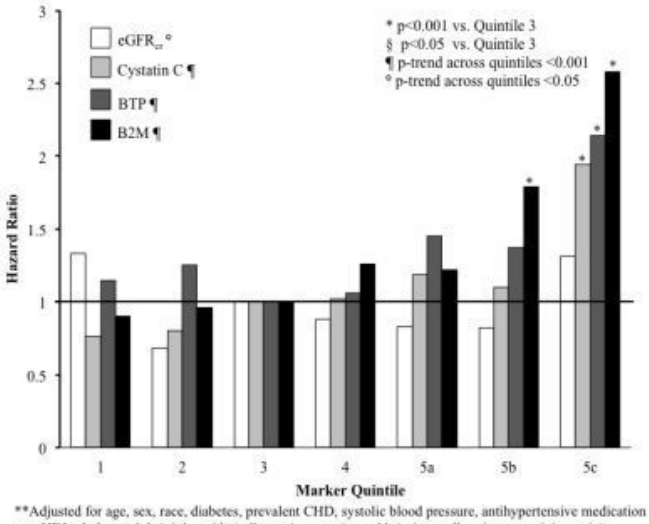
Novel Filtration Markers as Predictors of All-Cause and Cardiovascular Disease (CVD) Mortality in a Representative Sample of US Adults: CKD Biomarkers Consortium Meredith C. Foster,[1] Lesley Stevens Inker,[2] Andrew S. Levey,[2] Elizabeth Selvin,[1] John H. Eckfeldt,[3] Stephen P. Juraschek,[1] Josef Coresh.[1] *[1]Johns Hopkins University; [2]Tufts Medical Center; [3]University of Minnesota.*

Background: It is unclear whether serum concentrations of new filtration markers such as β-trace protein (BTP) and β2 microglobulin (B2M) are similar to cystatin C in having stronger associations with mortality than creatinine-based estimated glomerular filtration rate ($eGFR_{cr}$) in the general US population.

Methods: Adults age 20 and older (n=6,541) from NHANES III with mortality linkage through 2006. Creatinine, cystatin C, BTP and B2M were measured in stored serum samples; $eGFR_{cr}$ calculated with the CKD-EPI equation. Markers were categorized into quintiles, splitting the highest quintile (lowest $eGFR_{cr}$ quintile) into tertiles. Adjusted associations with all-cause, CVD and CHD mortality were evaluated using Cox models weighted for the sampling design.

Results: We observed 2,398 deaths (1,082 CVD & 607 CHD deaths) over a median follow-up of 14.4 years. All four markers were associated with mortality after adjusting for demographics (p-trend<0.02 for $eGFR_{cr}$ and <0.001 for others). Compared to the middle quintile, the adjusted hazard ratio (95% CI) for mortality of the highest sub-quintile was 1.9 (1.4-2.6) for cystatin C, 2.1 (1.6-2.9) for BTP and 2.6 (2.0-3.4) for B2M compared to 1.3 (0.8-2.0) for the lowest $eGFR_{cr}$ sub-quintile (Figure). Similar trends were observed for CVD and CHD mortality and for cystatin-C, BTP and B2M in adults with $eGFR_{cr}$≥60mL/min/1.73m^2 where the $eGFR_{cr}$ association was flat. Novel markers also added prediction beyond $eGFR_{cr}$ (p<0.01 for NRI).

Figure: Multivariable adjusted** hazard ratios of all-cause mortality by filtration marker category.



**Adjusted for age, sex, race, diabetes, prevalent CHD, systolic blood pressure, antihypertensive medication use, HDL-cholesterol, ln(triglycerides), C-reactive protein, and ln(urinary albumin to creatinine ratio)

Conclusions: BTP and B2M share the stronger, linear association of cystatin C with mortality compared to eGFR$_{cr}$ which may under-estimate the kidney function-mortality relationship due to confounding by muscle mass.

Funding: NIDDK Support, Other NIH Support - NIH/NHLBI T32HL007024, Pharmaceutical Company Support - Siemens Healthcare Diagnostics

TH-PO320

Association between Kidney Function and Telomere Length: The Heart and Soul Study Nisha Bansal,[1] Mary Whooley,[1] Mathilda Regan,[1] Charles E. McCulloch,[1] Joachim H. Ix,[2] Elissa Epel,[1] Elizabeth H. Blackburn,[1] Chi-yuan Hsu.[1] *[1]UCSF; [2]UCSD.*

Background: Telomere attrition has emerged as a novel cardiovascular risk factor in the general population, however studies of telomere length in relation to kidney function are limited. We explored the association of kidney function with telomere length and telomere shortening, which may be important cardiovascular risk factors in patients with chronic kidney disease.

Methods: The Heart and Soul study is a longitudinal study of participants with stable coronary heart disease (CHD). Measures of baseline kidney function included: creatinine-derived estimated glomerular filtrate rate (eGFR$_{CKD-EPI}$), 24-hour urine creatinine clearance, cystatin C eGFR (eGFRcys), and urine albumin to creatinine ratio (ACR). Telomere length was measured from peripheral blood leukocytes at baseline (N=954) and 5 years later (N=608). Linear regression models were used to test the association between kidney function and i) baseline telomere length and ii) change in telomere length over the next 5 years.

Results: At baseline, mean age was 66.7 (±11) years, 18.6% were female and 16.5% were Black. Mean eGFR$_{CKD-EPI}$ was 72.6 (± 21.5) ml/min/1.73 m^2, eGFRcys was 71.0 (± 23.1) ml/min/1.73 m^2 and ACR was 8.6 (±12.3) mg/gm. In cross-sectional analysis, lower baseline eGFR$_{CKD-EPI}$ (but not the other measures of kidney function) was associated with shorter baseline telomere length (9.1 [95% CI 1.2-16.9] shorter in base pairs for every 5 ml/min/1.73 m^2 lower eGFR$_{CKD-EPI}$). Adjustment for age rendered this association no longer significant. In longitudinal analysis, lower baseline eGFR$_{CKD-EPI}$ (and all other measures of kidney function) predicted more rapid telomere shortening (10.8 [95% CI 4.3-17.3] decrease in base pairs over 5 years for every 5 ml/min/1.73 m^2 lower eGFR$_{CKD-EPI}$). After adjustment for age, these associations were no longer statistically significant.

Conclusions: In patients with CHD, the associations between reduced kidney function and i) shorter baseline telomere length and ii) more rapid telomere shortening over 5 years are both entirely explained by older age. Telomere shortening and decrease in kidney function are likely parallel processes that occur with aging.

Funding: NIDDK Support

TH-PO321

Renal Biopsy mRNA Profiling Implicates Shared Molecular Pathways in Progression of Chronic Kidney Disease Wenjun Ju,[1] Viji Nair,[1] Felix H. Eichinger,[1] Celine C. Berthier,[1] Ann Randolph,[1] Sebastian Martini,[1] Shahaan Smith,[1] Jennifer Yi-Chun Lai,[1] Jennifer Joyce Hawkins,[1] Frank C. Brosius,[1] Kerby Shedden,[1] Clemens D. Cohen,[2] Crystal A. Gadegbeku,[3] Matthias Kretzler.[1] *[1]University of Michigan; [2]University of Zurich; [3]Temple University, for the ERCB and CPROBE Consortium.*

Background: Molecular markers identifying patients at risk for progression of chronic kidney disease (CKD) are currently lacking and will facilitate our understanding of pathogenesis and development of targeted interventions. The goal of this study is to test if intra-renal mRNA can predict kidney function and functional change over time in patients with CKD.

Methods: We performed array based genome-wide expression profiling followed by qRT-PCR validation on micro-dissected kidney biopsies of patients with a wide range of CKD causes and stages in two independent cohorts: 164 European patients out of ERCB and 42 American patients from Michigan O'Brian Renal Center observational cohort (CPROBE).

Results: We identified 68 genes to be highly correlated with renal function, as assessed by estimated glomerular filtration rate (MDRD eGFR), with significant differential expression between patients and controls (living donor biopsies). Using qRT-PCR the marker-panel was confirmed to predict eGFR in an independent cohort of 56 CKD patients (r^2=0.54, predicted vs. actual eGFR). The GFR prediction also remained robust in the diverse multi-center CPROBE cohort (n=42, r^2=0.46). In 18 CPROBE subjects, eGFR slope could be estimated by mixed effects model using at least 3 serial measurements over 4.2±1.8 years. Notably, 36 of the 68 markers correlated with loss of renal function over time (|r|≥0.48, p≤0.05, FDR<0.1). Functional analysis of predictive transcripts revealed altered signaling pathways in CKD, including beta1 integrin cell surface interactions, antigen processing and presentation, and D4-GDI signaling pathway.

Conclusions: An intra-renal mRNA marker set is able to predict renal functional impairment in CKD irrespective of patients' disease type, stage, race or environment. The marker set provides a foundation for non-invasive marker identification and mechanism-driven intervention.

Funding: NIDDK Support

TH-PO322

Association between Urine Osmolality and Progression of Chronic Renal Failure: A Cohort Study Max Plischke,[1] Maria Kohl,[2] Ammon Handisurya,[1] Martin Haas.[1] *[1]Department of Internal Medicine III, Division of Nephrology and Dialysis, Medical University Vienna, Vienna, Austria; [2]Section for Clinical Biometrics, Center of Medical Statistics, Informatics and Intelligent Systems, Medical University Vienna, Vienna, Austria.*

Background: Vasopressin (VP) has been associated with progression of chronic kidney disease (CKD). Precise measurement of VP is difficult; however, based on its antidiuretic action urine osmolality (Uosm) may be used as a substitute. In this retrospective cohort study the association between urine osmolality and progression of CKD to dialysis was investigated in 273 patients with CKD stage 1-4.

Methods: Mean Uosm values during a run-in phase of one year were used as baseline values. The association between Uosm and the risk of dialysis was assessed by a multivariate proportional sub-distribution hazards model for competing risk data according to Fine and Gray. Co-variables were selected via the purposeful selection algorithm.

Results: Dialysis was reached in 105 patients over a median follow-up period of 92 months. Non-adjusted competing risk analysis suggested a lower incidence of dialysis in patients with a urine osmolality greater than median (510 mOsmol/L). However, after adjustment, a higher risk was found in patients with a higher Uosm. The adjusted sub-distribution hazard ratio for dialysis was 2.04 (95% confidence interval, 1.06 to 3.92) for each doubling of urine osmolality. After 72 months, the estimated adjusted cumulative incidence probabilities of dialysis were 15%, 24%, and 34% in patients with a baseline urine osmolality of 315, 510, and 775 mOsmol/L, respectively.

Conclusions: We conclude that lower urine osmolality is associated with a decreased risk of progression to dialysis in CKD patients. Antagonizing the effect of vasopressin or increasing fluid intake might therefore improve the course of chronic renal failure.

TH-PO323

Urinary Procollagen Type III Aminoterminal Propeptide Predicts the Decline of Renal Function in Pre-Dialysis CKD Patients Akira Suzuki,[1] Kodo Tomida,[1] Tatsuya Shoji,[1] Noriyuki Okada,[2] Yoshiharu Tsubakihara,[3] Terumasa Hayashi.[1] *[1]Kidney Disease and Hypertention, Osaka General Medical Center, Osaka, Japan; [2]Clinical Examination, Osaka General Medical Center, Osaka, Japan; [3]Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Tubulointerstitial fibrosis is the final common pathway in all kidney disease falling into end stage kidney disease. The severity of fibrosis can predict the prognosis of the renal function. Recent studies had demonstrated that the urinary procollagen type III aminoternal propeptide (U-PIIINP) was correlated significantly with the extent of fibrosis in renal biopsy. We hypothesized that the U-PIIINP may be correlated with the decline of renal function in pre-dialysis CKD patients.

Methods: 76 patients with CKD were recruited whose Cr levels were available at 6 month before the recruitment. Each participant measured the PIIINP level and biochemistry tests in urine and serum. Six month later, serum biochemical tests were repeated. All liver diseases and lung fibrosis were excluded. The slope of 1/Cr was calculated using the Cr level during the 12 months.

Results: The population included 41 patients with CKD stage 3, 31 patients with CKD stage4, and 4 patients with CKD stage 5. The etiology of CKD was benign nephrosclerosis; 32 patients, chronic glomerulonephritis; 22 patients, diabetic nephropathy; 19 patients, polycystic kidney disease; 3 patients. Serum PIIINP (S-PIIINP) and U- PIIINP/Cr ratio revealed no significant correlation, suggesting U-PIIINP was derived mostly from kidney. Each of S-PIIINP and U-PIIINP/Cr was correlated with eGFR (R=-0.52434, P<0.0001 and R=-0.38628, P=0.0005, respectively). Slope of 1/Cr was correlated with each of U-PIIINP/Cr (R=-0.50318, P<0.0001), NAG(R=0.27975, P=0.0137) and Urinary protein/Cr ratio (R=-0.31761, P<0.0049), but not with S-PIIINP, Age, BP, BMI, and eGFR. Multiple regression analysis using the factors showing significant correlation revealed the U-PIIINP/Cr was the strongest factor significantly correlated with the Slope of 1/Cr.

Conclusions: The results suggest that U-PIIINP/Cr may predict the decline of renal function in pre-dialysis CKD patients.

TH-PO324

Urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) Level and Risk of Progressive Chronic Kidney Disease: Results from the CRIC Study Kathleen D. Liu, Wei (Peter) Yang, Amanda Hyre Anderson, Harold I. Feldman, Sevag Demirjian, Takayuki Hamano, Jiang He, James P. Lash, Eva Lustigova, Sylvia E. Rosas, Michael S. Simonson, Kelvin Tao, Chi-yuan Hsu. *For the CRIC Study.*

Background: Novel biomarkers may improve our ability to predict which CKD patients are at higher risk for progressive loss of renal function.

Methods: Baseline urine NGAL concentration was determined using the Abbott ARCHITECT platform among 3386 participants of the prospective Chronic Renal Insufficiency Cohort (CRIC) study. Cox models were used to examine the independent association between urine NGAL and risk of CKD progression, defined as halving of estimated GFR or incident ESRD.

Results: Baseline mean eGFR was 42.4 ml/min/1.73m^2; median 24-hr urine protein 0.2 gm/day; median urine NGAL concentration 17.2 (IQR 8.1-39.2) ng/mL. Over an average follow-up of 3.2 years, 689 episodes of CKD progression occurred. Even after accounting for baseline eGFR, proteinuria and other traditional CKD progression risk factors such as diabetes and BP level, urine NGAL was an independent risk factor in CKD progression (HR 1.70 highest vs. lowest quartile; p=0.01)(Table). The association between baseline urine NGAL and risk of CKD progression was strongest within the first 2 years of the biomarker measurement. Within this time frame, adding urine NGAL to a model which included eGFR, proteinuria and other CKD progression risk factors led to net reclassification improvement of 24.7% (95% CI 0.4%-38.5%); but the C-statistic remained nearly identical (0.879 vs. 0.880).

Conclusions: Urine NGAL was an independent predictor of progression among patients with established CKD of diverse etiology but did not substantially improve prediction of outcome events.

Association between urine NGAL concentration and risk of CKD progression (halving of eGFR or ESRD)

Quintiles of urine NGAL concentration (ng/ml)	Unadjusted Hazard Ratio	Adjusted for demographics, eGFR, 24-hr urine protein and other CKD progression risk factors
<= 6.9	Ref	Ref
> 6.9 - <= 12.9	1.75 (1.22-2.51)	1.37 (0.94-1.98)
> 12.9 - <= 22.6	2.52 (1.79-3.56)	1.24 (0.86-1.79)
> 22.6 - <= 49.5	4.30 (3.11-5.93)	1.39 (0.97-2.00)
> 49.5	9.34 (6.86-12.72)	1.70 (1.16-2.48)

Funding: NIDDK Support

TH-PO325

Predicting the Risk of End-Stage Renal Disease in Patients with Stage 3 Chronic Kidney Disease: A Pragmatic Risk Score Micah L. Thorp,[1] David Smith,[1] Eric S. Johnson,[1] Xiuhai Yang,[1] Amanda F. Petrik,[1] Robert Platt.[2] *[1]The Center for Health Research, Kaiser Permanente Northwest, Portland, OR; [2]Department of Epidemiology and Biostatistics, McGill University, Montreal, QC, Canada.*

Background: Chronic kidney disease (CKD) is common, but only progresses to end stage renal disease (ESRD) in a minority of patients. Developing a tool for early identification of patients likely to progress to ESRD would enable clinicians and medical systems to target resources to patients likely to benefit from interventions.

Methods: We conducted a cohort study by linking data among 38,483 patients with stage 3 CKD. We measured patient characteristics during the year before patients became eligible because of their poor kidney function. We followed patients for up to one year. Seven routinely measured patient characteristics predicted accurately the risk of ESRD. By combining those characteristics with numeric weights for their importance, the risk score stratified patients based on risk.

Results: We observed 461 patients who developed ESRD, a one-year risk of 1.6 per 100 patients. We judged the risk score's effectiveness by dividing the cohort into ten equal groups or deciles, which included approximately 3,849 patients. Patients at or above the 90th percentile of predicted risk (top decile) were 60 times more likely to suffer ESRD when compared with patients below the 10th percentile (bottom decile): 11.0 per 100 patients (top decile) versus 0.2 per 100 (bottom decile). The c-statistic was 0.84 and sensitivity 57%.

Conclusions: This pragmatic risk score appears to be the first of its kind for predicting end-stage renal disease in patients with stage 3 CKD. Our risk score can predict a patient's *absolute risk (e.g., 11 per 100 patients) and will allow clinicians to stratify patients with stage 3 CKD based on their 5 year risk of ESRD.*

Example risk score of a 50 year old man with eGFR 40 and microalbuminuria

Predictor	Points
50 years old	72
Man	30
Kidney function of 40 mL/min	47
Small (micro) amount of albumin in urine	36
Systolic blood pressure (130 mm Hg)	21
Diabetes with only one other complication	27
No anemia (hemoglobin in top quartile)	0
Total	233

233 points = 5% five year absolute risk of ESRD

Funding: Other U.S. Government Support

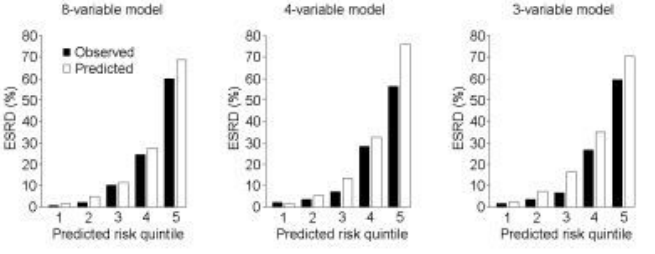
TH-PO326

Validation of the 2011 Kidney Failure Risk Equation in European CKD Patients Mieke J. Peeters,[1] Arjan D. Van Zuilen,[2] Jan A.J.G. van den Brand,[1] Michiel Bots,[2] Peter J. Blankestijn,[2] Jack F. Wetzels.[1] *[1]Radboud University Medical Center, Nijmegen; [2]University Medical Center Utrecht, Utrecht, Netherlands.*

Background: Patients with CKD are at risk of progression to ESRD. Using data of Canadian CKD patients, Tangri *et al* recently developed models to predict progression of CKD stages 3-5 to ESRD within 5 years [JAMA 2011;305:1553-9]. We validated the 2011 Kidney Failure Risk Equation in European CKD patients.

Methods: We selected patients with CKD stages 3-5 from the MASTERPLAN study, a randomized controlled trial in patients with CKD [NDT 2010;25:3647-54]. ESRD was defined as initiation of dialysis or kidney transplantation within 5 years. Mortality before ESRD was defined as no ESRD. Patients followed for less than 5 years, who did not develop ESRD and did not die, were excluded. 5 year ESRD risk was predicted by 3 different models developed by Tangri. MASTERPLAN follow-up data were used to determine actual ESRD rate. Model performance was evaluated using the area under the receiver operating characteristic curve (ROC-AUC) and Net Reclassification Improvement (NRI).

Results: 694 patients were included, 135 developed ESRD. The 8-variable model (including age, sex, eGFR, albuminuria, calcium, phosphate, bicarbonate, albumin) was slightly more accurate than the 4-variable model (including age, sex, eGFR, albuminuria) and the 3-variable model (including age, sex, eGFR, albuminuria); ROC-AUCs were 0.878 (95% CI 0.848-0.908), 0.868 (95% CI 0.834-0.901), and 0.867 (95% CI 0.834-0.900), respectively. The respective NRIs for the 8-variable model compared with the 4-variable and 3-variable model were 10% (p=0.01) and 3% (p=0.61). The Figure shows the mean observed and predicted 5 year ESRD risk.



Conclusions: The 2011 Kidney Failure Risk Equation accurately predicted progression to ESRD in European CKD patients. The benefits of the 8-variable model do not outweigh its added complexity.

TH-PO327

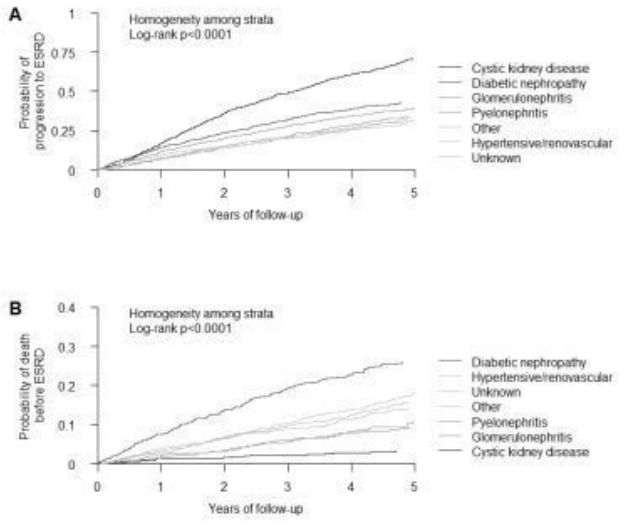
Relevance of Renal Diagnosis to Prognosis of Chronic Kidney Disease: Results from the Study of Heart and Renal Protection (SHARP) Richard Haynes. *On Behalf of the SHARP Collaborative Group, Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, Oxfordshire, United Kingdom.*

Background: Previous studies have found conflicting results on the relevance of renal diagnosis to prognosis in CKD, but have been small with short follow-up.

Methods: The SHARP study was a randomized controlled trial of LDL cholesterol lowering with ezetimibe/simvastatin in 9270 patients with CKD (including 6247 not on dialysis). Relative risks of end-stage renal disease (ESRD) and death over 4.9 years were estimated with Cox regression.

Results: Lower eGFR and higher urine albumin:creatinine ratio (ACR) were associated with higher risks of ESRD in all categories of renal diagnosis. The unadjusted rate of ESRD was highest among patients with polycystic kidney disease (PKD) despite lower baseline ACR. Compared with patients with hypertensive/vascular disease, pyelonephritis or other causes of renal disease (reference group), the unadjusted hazard ratio for ESRD in patients with PKD was 3.01 (95% CI 2.69–3.36). There was also an increased risk of ESRD for patients with diabetic nephropathy (DN) (HR 1.57 [1.38-1.78]) or glomerulonephritis (GN) (HR 1.32 [1.17-1.49]). After adjustment (including eGFR and ACR), there was an increased risk of ESRD among those with PKD (HR 3.51 [3.09-3.98]) and DN (HR 1.40 [1.14-1.72]) but not GN (HR 0.97 [0.85-1.10]). Conversely, patients with PKD were at much lower risk of death (and DN at much higher risk) than other diagnoses.

Figure 1. Life table plots of ESRD and death before ESRD by renal diagnosis



Conclusions: Patients with PKD had the highest risk of ESRD despite lower ACR. DN was associated with a 40% increased risk of ESRD but the increased risk associated with GN was fully explained by eGFR and ACR (compared to other renal diagnoses). Patients with PKD were at the lowest risk of death while those with DN were at the highest risk. Renal diagnosis provides useful prognostic information in addition to eGFR and ACR.

Funding: Pharmaceutical Company Support - Merck and Co., Inc.

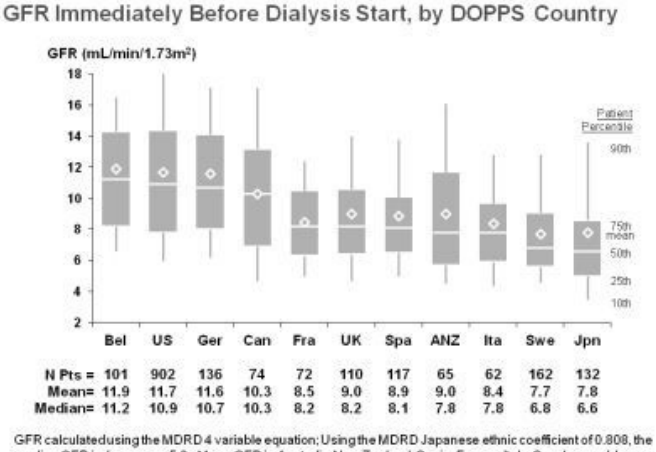
TH-PO328

International Differences in eGFR at Dialysis Start: Results from the Dialysis Outcomes and Practice Patterns Study Brian Bieber,[1] Yun Li,[1,2] David C. Mendelssohn,[5] Panduranga S. Rao,[2] Christian Combe,[3] Joan Fort,[4] Tadao Akizawa,[6] Friedrich K. Port,[1,2] Bruce M. Robinson.[1,2] *[1]Arbor Research; [2]U of Michigan; [3]CHU de Bordeaux; [4]U Hospital Val Hebron; [5]Humber River Regional Hospital; [6]Showa U.*

Background: Though earlier dialysis initiation is not associated with prolonged survival or improved quality of life in clinical trial or observational data, the estimated GFR (eGFR) at dialysis start has risen recently in the US and elsewhere. This study evaluated international differences in estimated GFR at dialysis initiation.

Methods: The DOPPS is a prospective cohort study of HD patients. Data were from 1933 patients in 12 countries with dialysis vintage ≤120 days at DOPPS 4 enrollment (2009-2011). Linear mixed models were used to estimate country differences in eGFR at dialysis start (mL/min/1.73m^2, by 4-var MDRD). Cox regression stratified by country estimated the association of eGFR at start with mortality. Models were adjusted for age, gender, catheter use, 13 summary comorbidities, and pre-dialysis nephrology care.

Results: The median eGFR at dialysis start was highest (>10) in Belgium, Canada, Germany, and US, and lowest (<7) in Sweden and Japan. Findings were similar after adjustment. In patients with 6 months of pre-ESRD data, the average decline in eGFR before dialysis start was 4.9 over 6 months. Higher eGFR at dialysis start was associated with elevated mortality (HR=1.11 per 2.5 mL/min/1.73m^2 [95% CI = 1.01-1.21]).



GFR calculated using the MDRD 4 variable equation; Using the MDRD Japanese ethnic coefficient of 0.808, the median GFR in Japan was 5.3. Mean GFR in Australia-New Zealand, Spain, France, Italy, Sweden, and Japan remained lower than the US even after adjustment for age, gender, 13 summary co-morbid conditions, and timing of pre-ESRD nephrology care

Conclusions: Country differences in average eGFR at dialysis start, of up to 4 mL/min/1.73m^2, may represent an additional six months or more off dialysis (extrapolating from our data and prior publications). Delaying dialysis when feasible may allow for better preparation for dialysis, use of surgical vascular access at dialysis start, cost savings, and improved quality of life for patients with Stage 5 kidney failure.

Funding: Pharmaceutical Company Support - The International DOPPS Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), and Abbott Laboratories (Since 2009)

TH-PO329

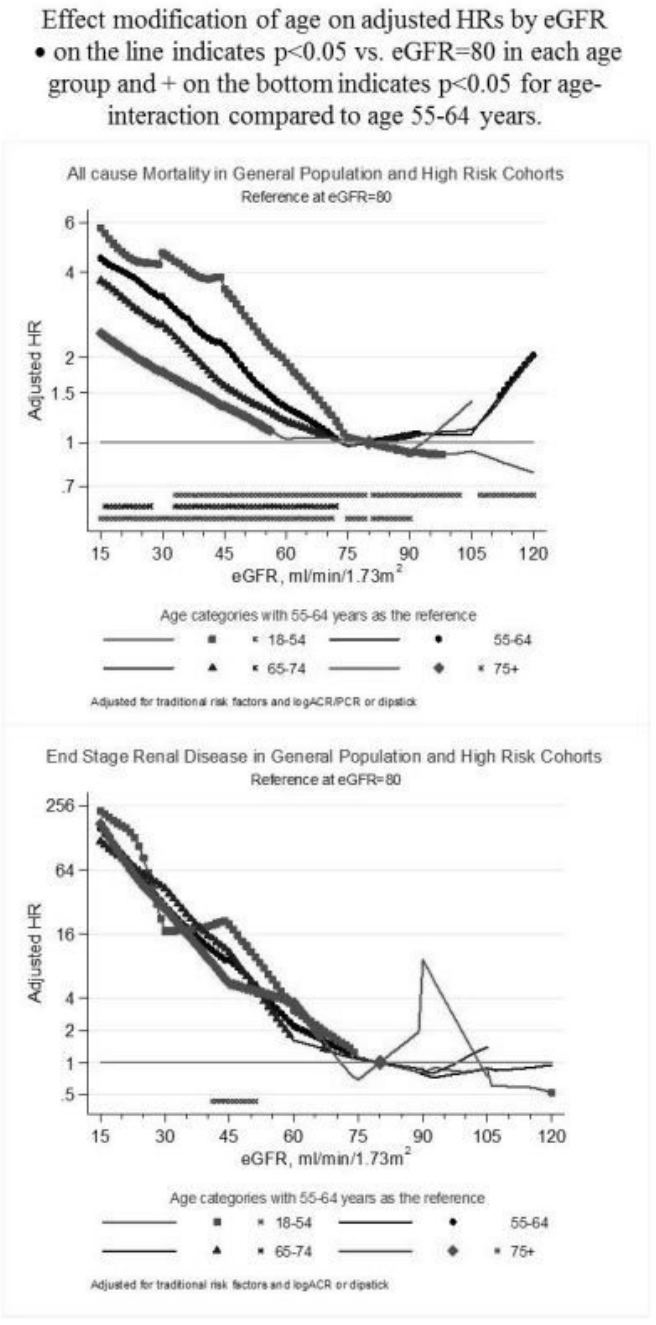
Age and the Association of Kidney Disease Measures with Mortality and End-Stage Renal Disease: A Meta-Analysis of 2 Million Participants from 46 Cohorts (for the CKD-PC Collaborators) Stein I. Hallan, Kunihiro Matsushita, Yingying Sang, B. Khan Mahmoodi, Corri Black, Areef Ishani, Nanne Kleefstra, David M. Naimark, Paul J. Roderick, Marcello Tonelli, Jack F. Wetzels, Brad C. Astor, Ron T. Gansevoort, Adeera Levin, Chi Pang Wen, Josef Coresh. *CKD Prognosis Consortium.*

Background: Chronic kidney disease (CKD) is prevalent in the elderly, but the risk implications of low estimated glomerular filtration rate (eGFR) and high albuminuria across the full age range are controversial.

Methods: We investigated 2,051,244 participants from 46 cohorts. Hazard ratios (HRs) of mortality (112,325 deaths) and ESRD (8,411 events) according to eGFR and albuminuria were meta-analyzed across age categories of 18-54, 55-64, 65-74 and 75+ years. Attributable risks were calculated using HRs and weighted average incidence rates.

Results: Mortality and ESRD risk increased progressively as eGFR decreased and albuminuria increased in every age category. In general/high-risk cohorts, adjusted HRs of mortality for reduced eGFR were lower at older age (e.g., at eGFR 45 [vs. 80] ml/min/1.73m^2, 3.50 [95% CI, 2.55-4.81] in 18-54 years, 2.21 [2.02-2.41] in 55-64 years, 1.59 [1.42-1.77] in 65-74 years, and 1.35 [1.23-1.48] in 75+ years); i.e. a substantial effect-modification by age (*P-values* for overall interaction <0.05 in every age category with 55-64 years as a referent). In contrast, attributable mortality risks were greater at older age (e.g., at eGFR 45, 2.8 extra deaths per 1,000 person-years in 18-54 years, 12.2 in 55-64 years, 26.6 in 65-74 years, and 74.9 in 75+ years). Similar patterns with mortality were observed for albuminuria. Relative and absolute ESRD risk were less influenced by age (*P-values* for interaction >0.1 for eGFR and albuminuria).

Conclusions: Low eGFR and high albuminuria are strongly associated with mortality and ESRD regardless of age.



Funding: Private Foundation Support

TH-PO330

Lifetime Risk of Incident Chronic Kidney Disease Stage 3 Shani Shastri,[1] Lesley Stevens Inker,[2] Hocine Tighiouart,[2] Vilmundur Gudnason,[3] Olafur S. Indridason,[3] Runolfur Palsson,[3] Andrew S. Levey,[2] Mark J. Sarnak.[2] *[1]UT Southwestern; [2]Tufts Medical Center; [3]Landspitali -National University Hospital of Iceland.*

Background: To date there are no studies that have assessed the lifetime risk of incident chronic kidney disease (CKD) in the general population.

Methods: Lifetime risk of CKD stage 3 was calculated in 3741 participants in the prospective population-based Reykjavik Study, who attended at least 3 examinations between 1967 - 2005. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. Incident CKD stage 3 was defined as 2 consecutive eGFR < 60 in those with baseline eGFR ≥ 60 ml/min/1.73/m^2. Sex-specific lifetime risk was calculated using left truncated survival analysis adjusted for competing risk of death.

Results: Mean age was 46 years, 48.6% were males, all were Caucasians, 42% had hypertension, 0.6% had diabetes and 1% had dipstick proteinuria. Mean ± SD baseline eGFR and slope of eGFR decline for men and women were 92 ± 13 and 87 ± 13 ml/min/1.73m^2, and -0.74 ± 0.17 and -0.73 ± 0.19 ml/min/1.73 m^2/year, respectively. 361 subjects (9.6%) developed CKD stage 3 over a median follow-up of 27 years (range 2-38 years) & 1025 (27.4%) died without developing CKD. Short- and long-term cumulative risks of CKD stage 3 stratified by sex, are shown in the table below. Cumulative risk of CKD was higher among those with baseline hypertension for all groups.

Age and Sex-Specific Mortality-Adjusted Estimates of Lifetime Risk of Incident CKD Stage 3

		Years from baseline age			
	Age (years)	5	10	20	30
Women					
	50	0.9 (0.4, 1.4)	2.3 (1.6, 3.0)	8.8 (7.4, 10.1)	16.3 (14.2, 18.3)
	60	3.0 (2.2, 3.8)	6.7 (5.4, 7.9)	14.4 (12.4, 16.4)	
	70	5.6 (4.2, 6.9)	8.8 (6.9, 10.5)		
	80	2.9 (0.7, 4.7)			
Men					
	50	0.6 (0.2, 1.1)	1.5 (0.9, 2.1)	3.8 (2.8, 4.7)	7.2 (5.5, 8.6)
	60	0.9 (0.4, 1.4)	2.4 (1.5, 3.2)	6.0 (4.4, 7.3)	
	70	2.4 (1.4, 3.3)	4.1 (2.7, 5.4)		
	80	1.1 (0.0, 2.3)			

Cumulative risks shown as percentages (95% CI)

Conclusions: The cumulative risk of CKD stage 3 increased with age, though among those ≥ 80 years the short-term risk is lower compared to 70 year olds. Lifetime risk of CKD stage 3 was higher in women at all ages and may reflect lower baseline eGFR and increased survival in women.

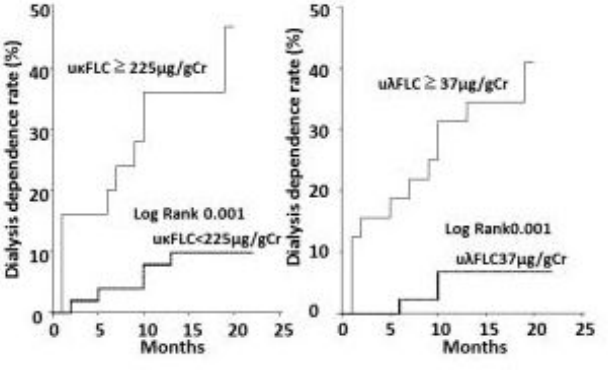
TH-PO331

Clinical Significance of Free Light Chains in Patients with Chronic Kidney Disease Kyoko Kanayama, Midori Hasegawa, Yukio Yuzawa. *Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi-ken, Japan.*

Background: The aim of this study was to investigate the clinical significance of serum and urinary levels of free light chains (sFLCs,uFLCs) in ambulatory patients with chronic kidney disease (CKD).

Methods: A total of 76 ambulatory CKD patients whose estimated glomerular filtration rate was below 60 mL/min/1.73 m^2 and who were not receiving dialysis were enrolled between February 2010 and November 2010. The levels of sFLCs and uFLCs were measured and their relationships with clinical characteristics and renal prognosis were assessed.

Results: Both kappa sFLC and lambda sFLCs levels differed significantly among CKD3, CKD4, and CKD5 (P<0.0001). Kappa and lambda sFLCs were negatively correlated with Hb (r=-0.377, P=0.001, r=-0.393, P<0.0001, respectively) and LDL cholesterol(r=-0.358, P=0.002, r=-0.428, P<0.0001, respectively). Log [kappa uFLCs/gCr] was positively correlated with log [urinary albumin creatinine ratio (UACR)] (r=0.378, P=0.0001) and log [urinary liver-type fatty acid-binding protein (uL-FABP)/gCr] (r=0.610, P<0.0001). Log [lambda uFLCs/gCr] was positively correlated with log [UACR] (r=0.413, P<0.0001) and log [uL-FABP/gCr] (r=0.598, P<0.0001). The patients were clinically followed for a median period of 18months. Fifteen patients became dialysis-dependent. The best cut-off value for dialysis dependence was 225μg/gCr of kappa uFLCs/gCr (AUC0.741; sensitivity66.7%, specificity75.4%) and 37μg/gCr of lambda uFLCs (AUC0.783; sensitivity 80.0% specificity 67.3%) according to the ROC curves. Kaplan-Meier survival curves for dialysis dependence according to kappa and lambda uFLCs are presented in Figure 1.



The rate of dialysis dependence was significantly higher among patients with kappa or lambda uFLCs over cut-off levels.

Conclusions: Levels of uFLCs may be useful for predicting renal outcome in CKD patients.

TH-PO332

Serum Free Light Chains (sFLC) Allow Improved Risk Stratification of Chronic Kidney Disease (CKD) Patients for Progression to End Stage Renal Disease (ESRD) Hannah Louise Church,[1] Anne Bevins,[2] Colin A. Hutchison,[1] Paul Cockwell.[1] *[1]Renal Medicine, University Hospital Birmingham, Birmingham, United Kingdom; [2]Research and Development, The Binding Site Group Ltd, Birmingham, United Kingdom.*

Background: Around 12% of adults have CKD. Only a small proportion of these will progress to ESRD, therefore it is important to risk stratify to allow directed and enhanced care. The aim of this study was to assess the utility of serum immunoglobulin free light chain (sFLC) and urinary (uFLC) as independent risk factors for progression to ESRD.

Methods: Data was collected from 867 patients attending a secondary care CKD clinic with CKD stages 1-5 (pre-dialysis) with a median follow up of 1210 days. At recruitment laboratory markers were measured including serum (s) and urinary (u) FLC. Progression was defined as initiation of dialysis or pre-emptive renal transplantation (renal replacement therapy, RRT).

Results: 169 patients (19.5%) progressed to RRT; this group had higher polyclonal (kappa, κ and lambda, λ) s(κ median 60.20mg/L, range 10.10-278.0, λ 56.80mg/L, 9.09-256.0) and u(κ 115.0, 1.66-640.0, p<0.001, λ 34.2, 0.07-225.0, p<0.001) FLC compared to those who did not progress to RRT (s κ23.25, 0.78-184.0, λ 29.02, 3.31-210.0; u κ 32.3, 0.31-663.0, λ 5.01, 0.7-215.0). Univariate analysis showed that both s(κ OR 10.929, 7.445-16.044, λ 14.723, 9.506-22.803) and u(κ OR 2.045, 1.654-2.528, p<0.001, λ 2.044, 1.689-2.475, p<0.001) FLC were predictive of RRT. Multivariate analysis of categorical results, including all significant variables (p<0.1) without co-linearity showed that ACR (mg/mmol) ≥30 (OR 11.508), eGFR (OR 31.594) and λ sFLC (2.668) were independently predictive. Using the same model with combined λ and κ sFLC (cFLC) showed that eGFR (OR 28.202), ACR ≥30 (OR 12.542, p<0.001), ACR 3.5-30 (OR 3.705, p=0.036), eGFR (OR 28.202, p<0.001) and cFLC (OR 3.521, p=0.006) were independently predictive. Using this model 91.7% of patients progressing to ESRD would have been identified (ROC, AUC = 0.917, p<0.001). uFLC provide no additional utility over ACR and were therefore excluded from the final model.

Conclusions: Combined sFLC allows improved risk stratification of RRT in patients with CKD.

Funding: Pharmaceutical Company Support - The Binding Site Group Ltd

TH-PO333

Combined Serum Free Light Chains Independently Predict Progression in Chronic Kidney Disease James Ritchie,[1] Lakhvir Assi,[2] Richard Hoefield,[1] Paul Cockwell,[3] Stephen Harding,[2] Philip A. Kalra.[1] *[1]Vascular Research Group, Salford Royal NHS Foundation Trust, Manchester, United Kingdom; [2]The Binding Site Group Ltd, Birmingham, United Kingdom; [3]The Renal Research Group, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Background: Chronic kidney disease (CKD) is a heterogeneous condition, in which the majority of patients will not reach end-stage renal disease (ESRD). It is therefore important to accurately risk assess patients for disease progression so that treatment can be targeted accordingly. The aim of this study was to evaluate the prognostic value of polyclonal serum free light chains (FLC) in CKD stage 3-5 patients.

Methods: FLC (Freelite™) were determined in CKD patients enrolled as part of the Chronic Renal Insufficiency Standards Implementation Study (CRISIS). Established normal ranges were used (κ: 3.3-19.4mg/L, λ: 5.71-26.3mg/L). Combined FLC (cFLC; κ+λ) and κ/λ ratios were also calculated. 75/756 (10%) were CKD stage 5 patients. Time to renal replacement therapy (RRT) was assessed using Kaplan Meier and Cox regression analysis, using variables on a linear scale. Mortality was included as a competing endpoint in the analysis. The median follow-up time was 40 months.

Results: At baseline, cFLC were elevated (>50mg/L) in 566/756 (75%) patients and 161/756 (21%) patients had gone on to require RRT. Of these patients, 98% had cFLC>50mg/L (median: 98.6mg/L, IQR: 52.8). Patients who did not require RRT had significantly lower cFLC (median: 63.05mg/L, IQR: 46.33), p<0.001. cFLC were significantly associated with RRT (p<0.001). Multivariate analysis identified elevated cFLC (hazard ratio (HR: 1.007, p<0.001)), gender (HR: 1.9, p=0.004), reduced eGFR (HR: 0.89, p<0.001), high sodium (HR: 1.15, p<0.001) and erythrocyte sedimentation rate (ESR) (HR: 0.9, p=0.05) to be independently associated with reduced time to RRT. Interestingly, proteinuria, a known risk factor for CKD progression, was not significantly associated, suggesting cFLC may be a more sensitive predictor.

Conclusions: cFLC provide independent prognostic information for CKD patients who progress to ESRD requiring RRT. Further work will determine how cFLC can be prognostically used to manage these patients.

TH-PO334

Renal Prognosis in Atherosclerotic Renovascular Disease: Effects of Baseline Proteinuria and eGFR James Ritchie, Darren Green, Philip A. Kalra. *Vascular Research Group, Salford Royal Hospital.*

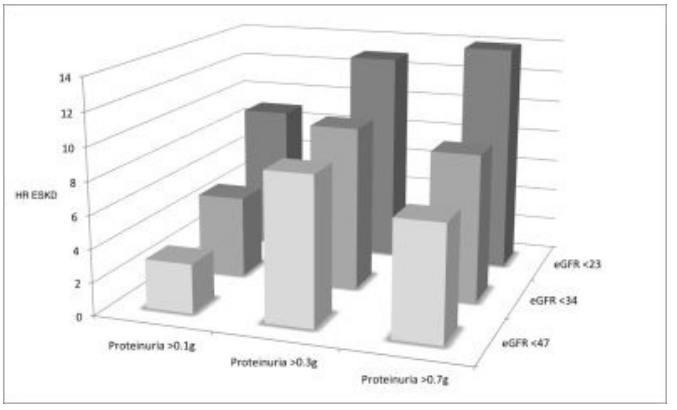
Background: In atherosclerotic renovascular disease (ARVD) associations are described between severity of renal impairment and risk for death, and between proteinuria >0.6g/day and progressive eGFR loss after revascularization. Little data exist describing the effects of these baseline variables on renal prognosis.

Methods: Tertiles of baseline eGFR and 24-hour proteinuria were identified in 333 patients within our ARVD database. Cox regression was used to calculate hazard ratios (HR) for progression to ESKD (eGFR<10ml/min or dialysis) for all combinations of these groups.

Time averaged change in eGFR was calculated for all patients and logistic regression used to calculate odds ratios (OR) for loss of eGFR >1ml/min/year.

Analyses were adjusted for age, gender and blood pressure.

Results: For each eGFR tertile, an increase in proteinuria associated with an increased HR for ESKD. The same was observed for reducing eGFR within each proteinuria tertile. Even mild renal impairment and low-grade proteinuria (eGFR <47ml/min/1.73m^2, proteinuria >0.1g/24 hours) significantly increased HR for ESKD (HR 3.4, p=0.003). The highest risk was observed for patients in the uppermost tertile for renal impairment and proteinuria - eGFR <23ml/min/1.73m^2 and proteinuria >0.7g/24 hours (HR ESKD 13.8, p<0.001).



Baseline renal function was not associated with a change in OR for more rapid eGFR loss, but patients with baseline proteinuria in excess of 0.7g/24 hours were significantly more likely to lose eGFR at a more rapid rate (OR 2.3, p=0.029).

Odds Ratio for eGFR loss >1ml/min/year

eGFR	**<23**	**23-34**	**34-47**	**>47**
OR	Referent	1.3	0.8	0.7
Proteinuria	**<0.1g**	**0.1-0.3g**	**0.3-0.7g**	**>0.7g**
OR	Referent	1.2	1.7	2.3*

* p<0.05

Conclusions: Changes in baseline eGFR and proteinuria have a synergistic effect on risk profile for ESKD in ARVD. Baseline proteinuria, but not eGFR, alters risk for rapid loss of eGFR during follow-up.

TH-PO335

Predicting Progression to ESKD in Atherosclerotic Renovascular Disease: A Decision Tree Analysis James Ritchie, Darren Green, Philip A. Kalra. *Vascular Research Group, Salford Royal Hospital.*

Background: Trial data have shown patients with atherosclerotic renovascular disease (ARVD) have a 2% annual rate of progression to dialysis. Whilst large elevations in serum creatinine (sCr) or proteinuria have been associated with a worse renal prognosis, the values of these variables with the greatest prognostic significance have not been defined. As such it is challenging for clinicians to identify ARVD patients at the highest risk for progression to ESKD.

Methods: Our tertiary renal center has prospectively recorded data on all referred ARVD patients since 1995 (n=819). All records were updated to identify any patients progressing to chronic dialysis or ESKD without dialysis (eGFR<10ml/min^2). Using a combined vector of sensitivity plus specificity, the threshold value of the baseline variable that maximally discriminated for progression to ESKD was identified. This value dichotomized our patient group. The analysis was repeated for both patient groups to generate a decision tree that classified the hierarchal rank of baseline variables and their threshold values. Analyses were repeated until statistical power was exhausted. A scoring system to assess baseline risk of progression to ESKD was generated from the decision tree, with greatest weight placed on most commonly identified variables. The scoring system was applied to the study population, with hazard ratios and c-statistics calculated to internally validate this system.

Results: Identified baseline variables and threshold values were:

1 - sCr (130, 200, 300μmol/L)

2 - urine protein:creatinine ratio (40, 60mg/mmol)

3 - previous stroke or transient ischemic attack

4 - use of statin and angiotensin blockade. Applying an ordinal scoring system to these parameters, patients with a score ≤3 had a significantly reduced, and patients with a score ≥4 a significantly increased HR for ESKD. A score of ≥4 had a sensitivity of 67% and specificity of 72% for identifying patients progressing to ESKD.

SCORE	**HR for ESKD**	**c-statistic**	**Sensitivity/Specificity**
0-3 (n=524)	0.25*	0.72	33/28
4-8 (n=295)	4.06*	0.75	67/72

* = p<0.001

Conclusions: This study suggests identification of ARVD patients with highest and lowest risk for ESKD may be possible at time of diagnosis.

TH-PO336

Chronic Kidney Disease, Lipids and Apolipoproteins and Coronary Heart Disease: The Atherosclerosis Risk in Communities Study Julio A. Lamprea-Montealegre,[1] A. Richey Sharrett,[1] Elizabeth Selvin,[1] Kunihiro Matsushita,[1] Moyses Szklo,[1] Brad C. Astor.[2] *[1]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Medicine, University of Wisconsin, Madison, WI.*

Background: Chronic kidney disease (CKD) is associated with altered apolipoprotein A-1 (apo A-1) and apolipoprotein B (apo B) to A-1 ratios. It is not known whether these ratios are more strongly associated with the risk of coronary heart disease (CHD) in CKD than traditionally measured lipids and lipoprotein cholesterol concentrations.

Methods: We investigated the association of baseline apo B and A-1 ratios with risk of CHD in 10,137 individuals free of CHD at baseline (Visit 4) in the ARIC Study. CKD was defined as an estimated glomerular filtration rate (eGFR)<60ml/min/1.73 m^2 based on a cystatin C measurement. Cox proportional hazards regression models were used to evaluate the association of lipid and apolipoprotein ratios with the risk of CHD in those with and without CKD with adjustment for demographic factors and established cardiovascular risk factors.

Results: The median follow-up time for the entire cohort was 11.13 years. A coronary heart disease event developed in 498 individuals without CKD (incidence rate: 5.2 events per 1,000 person-years) and in 138 individuals with CKD (incidence rate: 12.0 events per 1,000 person-years). CKD was present in 1,217 (12%) individuals at baseline. Individuals with CKD had a lower mean concentration of apo A-1 and a higher apo B to A-1 ratio than those without (p<0.001). Among individuals with CKD, the apo B to A-1 ratio and the non-high density lipoprotein cholesterol (non-HDL-c) to high density lipoprotein cholesterol (HDL-c) ratio were both associated with the risk of CHD: hazard ratios (HR) and 95% confidence intervals (CI) per one standard deviation increase= 1.22 (1.04-1.42) for the apo B to A-1 ratio and 1.30 (1.06-1.59) for the non-HDL-c to HDL-c ratio.

Conclusions: Although prevalent CKD was associated with a lower apo A-1 concentration and with a higher apo B to A-1 ratio at baseline, we found no evidence that these apolipoproteins are more strongly associated with CHD incidence than the non-HDL-c to HDL-c ratio.

TH-PO337

Unrecognised Chronic Kidney Disease: Who Is at Risk? Andrew Smyth,[1,2] Michelle Canavan,[1] Liam G. Glynn,[3] Martin O'Donnell,[1] Donal N. Reddan.[2] *[1]HRB Clinical Research Facility Galway, National University of Ireland, Galway, Galway, Ireland; [2]Nephrology, Galway University Hospitals, Galway, Ireland; [3]General Practice, National University of Ireland, Galway, Galway, Ireland.*

Background: Chronic kidney disease (CKD) is highly prevalent but often unrecognized in primary care. MDRD formula and CKD-EPI definitions of CKD have limitations and may result in misclassification of CKD. In this study, we explore which patient populations are at risk of unrecognised CKD, using either formula.

Methods: The *C*ardiovascu*lar* Mulitmorbid*ity* in Primary Care Study (CLARITY) is a cross-sectional study of community-dwelling adults ≥50 years recruited from a university-affiliated primary care research network. Of 9,816 patients recruited, 8,932 had serum creatinine measured from which estimated glomerular filtration rate (eGFR) was calculated. For this study, we included only participants with CKD defined as an eGFR≤60ml/min/1.73m^2 using either the MDRD or CKD-EPI equation. Risk factors for unrecognised CKD, defined as no documentation of CKD in the patients case file, were identified by step-wise multivariable logistic regression that included the following predictor variables: age, gender, smoking history, outpatient clinic attendance, hypertension, heart disease and diabetes mellitus. Analysis was performed using SPSS version 20.0.

Results: Using the MDRD equation, 1,319 patients were identified with CKD; 81.8% (n=1,079) were unrecognised and independent risk factors included female sex (OR 1.68, 1.22-2.34) and older age (OR 1.02, 1.00-1.04) on multivariable analysis. Using the CKD-EPI equation, 1,587 patients were identified with CKD; 83.9% (n=1,332) were unrecognised and independent risk factors continued to include female sex (OR 1.59, 1.16-2.17) and older age (OR 1.04, 1.02-1.06) on multivariable analysis. Patients with unrecognized CKD had higher systolic and diastolic blood pressure and were less likely to be prescribed ACE/ARB than patients with recognized CKD.

Conclusions: A large proportion of CKD is unrecognised and older females are at particular risk, independent of the equation used for eGFR. Patients with unrecognized CKD have higher blood pressure and are less likely to be prescribed ACE/ARB.

TH-PO338

GFR Variability and Risk of ESRD among Patients with Stage 3 CKD Robert M. Perkins, H. Lester Kirchner, James E. Hartle, Ion D. Bucaloiu. *Geisinger Clinic.*

Background: Dynamic changes in estimated GFR (eGFR) predict death among patients with CKD. Whether variability in serial eGFR measurements is associated with risk of ESRD has not been reported.

Methods: We retrospectively analyzed the risk of ESRD as a function of eGFR variability (defined as the absolute value of the difference between the obtained clinical eGFR value at a given time and the eGFR value estimated by the linear regression line at the same time point) among a cohort of patients with stage 3 CKD. The source population was adult primary care patients enrolled at Geisinger Clinic between January 1, 2004 and December 31, 2006, and who had stage 3 CKD. Patients were excluded for a prior history of solid-organ transplant or metastatic cancer. Cohort members were followed through March 31, 2011 for ESRD (identified through linkage with the USRDS dataset of ESRD, or first outpatient eGFR < 15 ml/min/1.73m^2). A multivariate Cox proportional hazard model (adjusted for demographic factors, co-morbid conditions, medications, hospital-associated acute kidney injury, proteinuria, kidney function, and serum albumin, among other factors) was developed to test the association of eGFR variability with ESRD.

Results: 4219 patients met study criteria. Those with greater eGFR variability were more likely to have diabetes, cardiovascular disease, and better baseline kidney function than those with lesser variability. 193 (4.6%) of the overall cohort developed ESRD during a median follow-up of 3.8 years, while 596 (14.1%) died prior to study end and without ESRD. Results of the multivariate-adjusted Cox proportional hazard model showed that eGFR variability is not associated with ESRD (HR 1.00 for the highest-variability quartile, relative to the lowest; 95% CI 0.66-1.51).

Conclusions: EGFR variability does not predict ESRD among patients with stage 3 CKD, and may have potential as a risk discriminator for the outcomes of death and ESRD among those with stage 3 CKD.

TH-PO339

Predictors of the Rate of Progression to Dialysis Dependence in Stage Five Chronic Kidney Disease David Langsford,[1] Karen Wills,[3] Lisa Shelverton,[2] Matthew D. Jose,[2,3] Geoffrey S. Kirkland,[2] Richard Yu.[2] *[1]Department of Nephrology, St Vincent's Hospital, Melbourne, Australia; [2]Department of Nephrology, Royal Hobart Hospital, Hobart, Australia; [3]Menzies Institute Tasmania, Australia.*

Background: There is little data on markers that predict the progression of the rate of decline of chronic renal failure (CKD) to the commencement of dialysis in incident stage V CKD patients. We investigated whether objective clinical markers may be independent predictors of progression to dialysis dependence in patients with stage V CKD.

Methods: Data from all CKD patients commencing dialysis at the Royal Hobart Hospital between 2009-2011 was captured. The time interval between the first recorded eGFR of 15ml/min/1.73m^2 or less and commencement of dialysis was recorded. Other baseline variables recorded at the time of eGFR 15ml/min/1.73m^2 were age, gender, smoking status, diabetes status, blood pressure, erythropoetin stimulating agent use, body mass index,haemoglobin, albumin, calcium, phosphate, ferritin, bicarbonate and urinary albumin:creatinine. The data was analysed using Cox proportional hazard regression and log-rank test of equality.

Results: Ninety-one patients were recruited, of which sixty-eight have progressed to dialysis dependence. The mean age of the all patients was 60.8 years, 63.7% were male and 38.5% were diabetic. Baseline blood pressure was not predictive of dialysis dependence and older age tended to attenuate rate of progression to dialysis dependence (p=0.06). Of the biochemical markers for every 1gm/litre decrease in serum albumin there was a 6.7% increase in the risk of progression to dialysis dependence (p=0.000). In those patients who progressed to dialysis dependence those with a serum albumin of less than 35gm/litre had a mean rate of decline of eGFR of 1.18ml/min/1.73m^2 per month compared with those with a serum albumin greater than 35gm/litre who declined at a rate of 0.41ml/min/1.72m^2 per month (p=0.01).

Conclusions: Low serum albumin at the time of reaching stage V chronic kidney disease is an independent predictor of time to dialysis dependence. In patients with serum albumin less than 35gm/litre the rate of decline is 2.6 times faster than those with serum albumin greater than 35gm/litre.

TH-PO340

Postoperative Renal Function after Uninephrectomy in Patients with Chronic Kidney Disease Stage 3 to 5: Predictive Factors of Renal Function Loss Dominique Dupuis,[1] Georges Ouellet,[2] Louise Roy.[1] *[1]Nephrology, Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada; [2]Nephrology, Hopital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Background: The rapid compensation of renal function loss by a normal controlateral kidney after uninephrectomy (NPX) is established. The purpose of this study is to determine if this initial compensatory capacity of the controlateral kidney is still present in CKD stage 3 to 5 patients undergoing NPX and which factors influence it.

Methods: We reviewed all cases (142) of NPX in patients with eGFR (following MDRD equation) < 60ml/min/1,73m^2, between 2003 and 2010, in 2 University of Montreal affiliated teaching hospitals. Baseline eGFR, patients comorbidities and surgical characteristics and complications were noted. The expected post-op eGFR, if no compensation by the controlateral kidney following surgery, was estimated according to the pre-op split renal function measured with scintigraphy (DTPA) (or assuming 50% if scintigraphy not available); this result was compared to the actual eGFR at hospital leave.

Results:

Renal Function at Baseline and at Hospital Leave

	Baseline eGFR ≤40ml/min/1,73m2 (n=34)	Baseline eGFR 40 to 59ml/min/1,73m2 (n=108)
Baseline eGFR	33±7	52±5
Expected postoperative eGFR	18±6*	28±6*
Actual eGFR at hospital leave	29±13	47±14
Variation between baseline eGFR and eGFR at hospital leave	-3±10 (-13%)	-6±14 (-10%)
Variation between expected and actual postoperative eGFR	-13±10#	-19±13

Results are Mean ± SEM (eGFR ml/min/1,73m2) / * p<0.001 vs eGFR at hospital leave; # p0.025 vs group baseline eGFR > 40

In the multivariate model, pre-op arterial hypertension and radical NPX significantly decreased the variation between expected and actual post-op eGFR by 7ml/min/1,73m^2 (p<0,05). Our model did not demonstrate a significant impact of other comorbidities and surgical considerations on the outcome.

Conclusions: After NPX, the controlateral kidney in patients with CKD stage 3 to 5 has a clinically significant initial compensatory capacity. The compensation is statistically smaller if initial eGFR is lower, if patient had hypertension or a radical NPX. This compensation is rapid and most probably hemodynamic (hyperfiltration).

TH-PO341

Fibroblast Growth Factor 23 and Peripheral Artery Disease in the Cardiovascular Health Study Pranav S. Garimella,[1] Joachim H. Ix,[2] Ronit Katz,[3] M. Chonchol,[4] Bryan R. Kestenbaum,[3] Ian H. de Boer,[3] David Siscovick,[3] Shani Shastri,[5] Jade S. Hiramoto,[6] Michael Shlipak,[6] Mark J. Sarnak.[7] *[1]Cook County Hospital; [2]UC San Diego; [3]University of Washington; [4]University of Colorado; [5]UT Southwestern Medical Center; [6]UC San Francisco; [7]Tufts Medical Center.*

Background: High serum fibroblast growth factor 23 (FGF23) is associated with increased risk of cardiovascular disease and progression of kidney disease, but its association with the ankle brachial index (ABI) and clinical peripheral artery disease (PAD) is unknown.

Methods: We investigated the cross sectional relationship between serum FGF23 (1996-97) and ABI (1998-99) in 2,432 individuals and the longitudinal association of FGF23 and incident PAD (events committee adjudicated) in 3,143 persons without clinical PAD. Multinomial logistic and Cox regression models were used for the respective analyses.

Results: Mean (SD) age was 77 (4) years, 60% were women and 15% were blacks. Median FGF23 was 68 RU/ml. In cross-sectional analyses, FGF23 was not associated with ABI. For each doubling of FGF (with ABI of 1.0-1.4 as the reference group) the OR's and 95% CI were: ABI<0.90, 0.91 (0.76-1.08); ABI 0.90-1.10, 0.88 (0.77-1.01); and ABI >1.40, 1.06 (95% CI 0.75-1.51).There were 114 incident PAD events with median follow up of 9.8 years. Higher FGF 23 was associated with PAD; however, this relationship was attenuated by measures of kidney function.

Association of FGF23 with incident PAD

Continuous FGF23 (per doubling)	Model 1*	Model 2**	Model 3†
<53.63	1.00 (ref)	1.00 (ref)	1.00 (ref)
53.63 – 70.84	1.11 (0.59, 2.06)	1.03 (0.55, 1.92)	0.89 (0.48, 1.67)
70.85 – 100.38	1.81 (1.02, 3.20)	1.50 (0.84, 2.67)	1.12 (0.66, 2.14)
>100.38	3.25 (1.88, 5.62)	2.40 (1.37, 4.22)	1.51 (0.82, 2.78)

* Age, gender and race ** Smoking, DM, HTN, CHD, CHF, CVA, LDL, HDL, CRP, albumin, triglycerides. † Model 2 plus cystatin C and albuminuria

Conclusions: High FGF23 is associated with incident PAD but this relationship is not significant after adjusting for kidney function. It remains to be determined whether kidney function is a confounding or mediating variable in this relationship.

TH-PO342

FGF23 and Asymmetrical Dimethylarginine (ADMA) Are Interactive Factors in the High Risk for CKD Progression in Stage 2-5 CKD Patients Carmine Zoccali, Daniela Leonardis, Giovanni Tripepi, Giuseppe Enia, Francesca Mallamaci. *On Behalf of MAURO Working Group, Nephrology Unit and CNR-IBIM, Reggio Calabria, Italy.*

Background: FGF-23 is a phosphate-regulating factor which has been associated with endothelial dysfunction and raised plasma ADMA in stage 3-4 CKD patients. Since both FGF23 and ADMA induce renal damage in experimental models, these associations may be implicated in the risk of CKD progression.

Methods: We investigated a) the cross-sectional association between FGF23 and ADMA with the GFR and their interaction in a large cohort (n=759) of stage 2-5 CKD pts and b) tested the interaction between FGF-23 and ADMA for predicting renal disease progression in the same cohort (follow-up: 3 yrs).

Results: FGF23 and ADMA were directly inter-related (r=0.15, P<0.001) and associated with the eGFR (r=-0.48 and r=-0.21, both P<0.001). These relationships held true (P<0.001) in analyses adjusting for potential confounders including proteinuria. The FGF23-eGFR link varied across ADMA quartiles (P=0.016 for effect modification) because a 100 pg/ml difference in FGF23 signalled a 4.0 ml/min decrease in GFR in the 1st ADMA quartile but only a 2.0 ml/min decrease in the 4th ADMA quartile. In the cohort study, 244 pts had renal events (>30% GFR decrease/dialysis/transplantation). Both FGF23 [HR: 1.12, 95% CI: 1.05-1.19, P=0.001] and ADMA [HR:1.08, 95% CI: 1.01-1.15, P=0.027] predicted renal events in analyses adjusting for eGFR, proteinuria and other confounders. Similarly to the cross-sectional analysis, an effect modification by ADMA was found on the relationship between FGF23 and renal events because the risk for these events portended by a 100 pg/ml increase in FGF23 was maximal in patients in the 1st ADMA quartile (HR:1.25, 95% CI: 1.13-1.39, P<0.001), intermediate in those in the 2nd and in the 3rd quartiles and minimal in the 4th ADMA quartile (HR: 1.06, 95% CI: 0.97-1.17, P=NS).

Conclusions: High levels of FGF-23 and ADMA interact for predicting renal disease progression in stages 2-5 CKD pts. The effect of FGF23 on the risk of CKD progression is modified by ADMA suggesting that the nitric oxide system is a relevant pathway whereby FGF23-klotho may affect health outcomes.

Funding: Government Support - Non-U.S.

TH-PO343

A Dynamic Predictive Model for the Progression of Chronic Kidney Disease to Kidney Failure Navdeep Tangri, Lesley Stevens Inker, David M. Kent, John Griffith, Ognjenka Djurdjev, David M. Naimark, Adeera Levin, Andrew S. Levey. *Medicine, University of Manitoba, Winnipeg, MB, Canada.*

Background: Predicting the progression of CKD is important for treatment decisions and for informing patient provider communication. We have previously developed a highly accurate *static* prediction model for the progression of CKD that used one time values. In this analysis, we describe a *dynamic* prediction model for CKD progression that includes changes in laboratory variables between visits as additional predictors of outcome.

Methods: We developed the dynamic prediction model using longitudinally collected data from patients with CKD Stages 3 to 5 referred to nephrologists. Our static model included age, gender, and eGFR, urinary ACR, serum albumin, phosphorous, calcium and bicarbonate. The dynamic model added change in laboratory variables between visits. We used cox proportional hazards models and compared discrimination (IDI), calibration (Hosmer Lemeshow Chi Square), model fit (AIC) and net reclassification (NRI) at 3 years.

Results: We studied 3,004 patients with an average of 5 visits (range 1-22) over a median follow up period of three years. The addition of change in eGFR, as well as an interaction term of the change in eGFR and subsequent eGFR were independently associated with the outcome, and improved model fit and discrimination (Table 1).

Table 1: Performance characteristics of static vs dynamic model

Visit	N	Static Model Chi Square	Dynamic Model Chi Square	Static Model AIC	Dynamic Model AIC	IDI	NRI
1	3,004	140	N/A	3988	N/A	N/A	N/A
2	2,582	327	156	3455	3447	1.0 (-0.7, 3.0)	9.1 (-0.7, 27.3)
3	2,250	179	37	3123	3112	3.2 (1.1, 5.4)	19.6 (5.3, 37.8)
4	1,965	180	21	2770	2743	2.8 (-0.1, 6.1)	10.4 (-6.4, 27.4)
5	1,721	156	11	2449	2435	2.8 (4.5, 6.2)	22.4 (6.7, 42.2)
6	1,512	193	7	2303	2269	4.6 (1.0, 7.4)	42.8 (21.8, 62.1)

Improvements in model fit, calibration and reclassification were also noted after 5 visits. (HL chi square <20 for dynamic model, continuous NRI 22.4 %). Changes in the remaining laboratory parameters did not significantly improve model performance (p IDI > 0.10).

Conclusions: A dynamic predictive model combining changes in eGFR between nephrology clinic visits can improve risk prediction for kidney failure over a static model that uses only a single eGFR. Integration of dynamic models in electronic health records deserves further study.

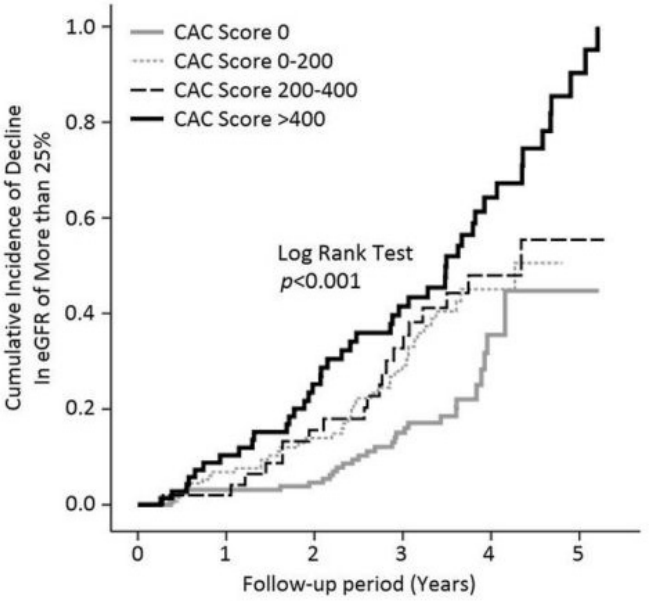
TH-PO344

Coronary Artery Calcification as a Predictor of Chronic Kidney Disease Progression Shuo-ming Ou,[1] Der-Cherng Tarng,[1,2] Yao-ping Lin,[1,2] Jinn-Yang Chen,[1,2] Chih-Ching Lin,[1,2] Wu-Chang Yang.[1,2] *[1]Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan; [2]School of Medicine, National Yang-Ming University, Taipei, Taiwan.*

Background: Chronic kidney disease is known to have a higher risk for increased coronary artery calcification (CAC); however, little is known about whether increased CAC itself has a pathogenic link to microvascular disease, leading to renal function deterioration.

Methods: This retrospective cohort study enrolled 422 adults (M/F 305:117; age= 61.0±11.9 years) who received electron-beam computed tomography examinations of the coronary arteries during physical check-up and had been followed for ≥ 2 years. These patients were categorized into 4 groups according to their CAC score by Agatston system (Q1: 0, n = 163; Q2: 1~199, n= 136; Q3: 200~399, n=51 and Q4: ≥400, n=72). Glomerular filtration rate (eGFR) was estimated by the Modification of Diet in Renal Disease formula. Primary end point was defined as a decline in eGFR ≥ 25%.

Results: The baseline eGFR was comparable among these 4 groups (mean eGFR=93.5~99.2 ml/min/1.73m2, $p = 0.386$). During a follow-up period of 1,120 patient-years, 32% of patients had a decline in eGFR ≥ 25%. The independent risk factors for decline in eGFR ≥ 25% include diabetes (HR= 1.975, $p < 0.001$), age≥60 (HR= 1.824, $p < 0.001$), higher body mass index (HR= 1.055, $p = 0.045$), higher coronary artery calcification score (HR 1.004, $p = 0.003$ per 10 scores). Patients with higher CAC score had significantly higher risks for renal function deterioration.



Conclusions: Our findings showed that the long-term outcome of increased CAC score is associated with a higher risk of renal function deterioration. The possible mechanism is that patients with macrovascular disease might have established pre-existing microvascular diseases leading to further renal function deterioration.

Funding: Government Support - Non-U.S.

TH-PO345

Risk Factors for End Stage Renal Disease and Death in Advanced Chronic Kidney Disease Patients Laura Sola,[1,2] Nancy De Souza,[1,2] Pablo German Rios,[2] Emma Schwedt,[2] Nelson Mazzuchi.[2] *[1]Advanced CKD Clinic, Hospital Maciel, Uruguay; [2]Renal Healthcare Program, Uruguay.*

Background: Patient's referral to nephrologists allows implementing measures to slow progression and reduce the risk (R) of death in patients (Pts) with advanced chronic kidney disease (ACKD). The objective of this study is to evaluate R factors (RF) for death and End Stage Renal Disease (ESRD) in ACKD Pts in the Renal Health Program of Uruguay (RHP-U) Registry.

Methods: Pts registered in the RHP-U (10/1/2004-10/1/2011) were included if estimated glomerular filtration rate (eGFR) was < 30 ml / min in ≥ 2 controls and follow-up≥ 90 days. At each visit were registered: systolic (SBP) and diastolic (DBP) blood pressure, proteinuria, cholesterol and triglycerides levels, renin angiotensin system blockers (RAS-B) use. Late referral was defined as registered in ACKD. Formal multidisciplinary team (FMT) was compared to usual care. ESRD and death rates were calculated and RF were analyzed with Cox regression model. Was considered significant p <0.05.

Results: Of 991 Pts, 483 (48.7%) males, mean age 69.9 ± 13.5 years (ys), 405 (41%) had diabetes (D), and 235 (23.8%) ischemic heart disease (IHD). Most frequent nephropathies were D 187 (18.9%) and vascular 398 (40.2%). During follow-up (median 1.57 (IQ: 0.90-2-57) ys) 123 Pts died (6.63 /100 pts-ys) and 161 Pts reached ESRD (8.68 / 100 pts-ys). Rapid progression (eGFR loss ≥ 3 ml/min/year), in 399 pts (40.3%) increased with higher SBP (OR: 1.015: 1,007-1.024), proteinuria (OR 1.383: 1.166-1.641), and D nephropathy (OR 1,485: 1047-2104). ESRD risk increased with SBP (HR 1.016: 1.006-1.026), proteinuria (HR 1.183: 1.056-1.325), age <45 ys (HR 2.35: 1.25 -4.41), phosphatemia(HR 1.20: 1.09-1.31), and late referral (HR 3.77: 2.39- 5.95); and decreases with RAS-B use (HR 0.59: 041-084) and FMT (HR 0.56:0.36-0.86). Death risk increased in males (HR 1.66: 1.14-2.43), age (HR 1.05: 1.03-1.07) proteinuria (HR 1.24:1.06-1 .44) and IHD (HR1.97:1.44-2.71) and was reduced by FMT (HR 0.58:0.35-0.94).

Conclusions: Proteinuria is a common RF for death and ESRD. Using RAS-B and PAS control reduces ESRD risk. Early referral to nephrologists and a FMT care allow the reduction of CKD progression and death.

TH-PO346

Hyperuricemia Is Associated with Proteinuria in Women, but Not in Men: A Cross-Sectional Study of Japanese General Population Masao Kikuchi,[1] Shouichi Fujimoto,[2] Yuji Sato,[1] Kazuo Kitamura,[1] Tsuneo Konta,[3] Kunitoshi Iseki,[3] Toshiki Moriyama,[3] Hideaki Yoshida,[3] Koichi Asahi,[3] Tsuyoshi Watanabe.[3] *[1]First Department of Internal Medicine, University of Miyazaki Hospital, Miyazaki, Japan; [2]Department of Hemovascular Medicine and Artificial Organs, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan; [3]Steering Committee for the 'Research on the Positioning of Chronic Kidney Disease (CKD) in Specific Health Check and Guidance in Japan', Japan.*

Background: Hyperuricemia is associated with an increased risk of metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease and chronic kidney disease, as well as gout. However, there is pronounced sex difference in the levels of uric acid.

Methods: We assessed the prevalence of proteinuria according to serum uric acid (SUA) among 228,778 patients in a Japanese nationwide database. There were 89,877 men and 138,901 women. We designed the basic model with SUA decile as categorical variable. Proteinuria is defined as 1+ or more by a dipstick method. We examined the association among SUA, age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), fasting glucose, hemoglobin A1c (HbA1c), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and estimated glomerular filtration rate (eGFR) separately in men and women.

Results: In both men and women, TG, LDL-C, BMI, SBP, DBP and PP increased with increased levels of SUA, but HDL-C and eGFR decreased with increased levels of SUA. On the other hand, fasting glucose, HbA1c and age decreased with increased levels of SUA in men, but increased in women. In a multivariable logistic regression analysis, prevalence of proteinuria increased with increased levels of SUA in women, but not in men.

Conclusions: Hyperuricemia is associated with proteinuria in women, but not in men. Further studies are necessary to clarify the cause that the sex difference of uric acid influences proteinuria.

TH-PO347

Predictors of New-Onset of Proteinuria in Japanese Population: MIYAKO (Multicenter Investigation for Yearly Medical Check Assessment in KyotO) Surveillance Shinji Yasuno,[1] Kenji Ueshima,[1] Koji Oba,[2] Sachiko Tanaka,[1] Masato Kasahara,[1] Akira Fujimoto,[1] Yoko M. Nakao,[1] Takashi Miyawaki,[3] Izuru Masuda,[4] Kazuwa Nakao.[1] *[1]Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Hokkaido University, Sapporo, Japan; [3]NTT West Kyoto Hospital, Kyoto, Japan; [4]Takeda Hospital, Kyoto, Japan.*

Background: Hypertension (HT), diabetes mellitus (DM) and dyslipidemia (DL) are known to be modifiable risk factors for proteinuria (PU). Early intervention would be desirable to prevent against new-onset of PU (NOPU), if the risk of NOPU is already increased at the lower levels than those of diagnostic criteria of HT, DM, and DL. We examined the predictors of NOPU in Japanese population, as a retrospective cohort study.

Methods: There were 29,677 and 21,824 persons who underwent an annual medical checkup in Takeda Hospital from 1998 to 2009 and NTT West Kyoto Hospital from 1996 to 2009, respectively. In this study, subjects were 23,322 individuals (mean age, 47.1; mean BMI, 23.0kg/m^2) without PU and treatment of HT, DM, and DL at baseline, who had at least more than two visits and whose first visit was before 2005. PU was defined as ≥1+ of dipsticks. Hazard ratios were calculated with the Cox regression analysis adjusted for age, sex, estimated glomerular filtration rate, body mass index (BMI), systolic blood pressure (SBP), fasting blood sugar (FBS), uric acid, and one of the following lipid parameters: total cholesterol, high density lipoprotein cholesterol (HDL-C), Triglyceride (TG), and non-HDL-C.

Results: For 6.0 years of mean follow-up, 1,806 subjects experienced NOPU. The multiple Cox regression analyses showed that higher level of SBP, FBS, and TG and lower level of HDL-C were significantly associated with the risk of NOPU. Categorical analyses revealed that the risk significantly increased at BP≥130/80mmHg, FBS≥110mg/dL, and HDL-C<40mg/dL.

Conclusions: The risk of NOPU is already increased at the lower levels than those of diagnostic criteria of HT and DM. Early intervention to those people will be needed to prevent against NOPU.

TH-PO348

Association of Visit-to-Visit Variability in Blood Pressure on Renal Function in Chinese Daoxin Yin, Dongliang Zhang, Wenhu Liu. *Department of Nephrology, Beijing Friendship Hospital, Beijing, China.*

Background: This study aimed to clarify the clinical significance of visit-to-visit variability in blood pressure on renal function in Chinese.

Methods: We enrolled 152 stage 3-4 CKD patients with hypertension at our department. All patients had been treated for at least 3 months before participating in this study. We excluded those who had atrial fibrillation, or renal artery stenosis. All patients had BP measured at outpatient visits 15 or more times during the 18 months follow-up. There are no changes in patients' antihyper-tensive regimens within follow-up. Visit-to-visit variability in BP was defined as the SD in BP, which was calculated from BP measurements at all the visits.Subjects underwent biochemical examination of the blood and urine.Clinical parameters included BMI, eGFR, albumin to creatnine ratio(ACR), current smoking, history of diabetes. Correlations between the changes in eGFR and clinical variables were analyzed by the multivariable regression analyses.

Results: Mean age was 61.5±8.8 years, mean eGFR was 41.7±15.1ml·min^{-1}·1.73m^{-2}. 94 patients were men and 58 were women. 134 patients(88.2%) were receiving anti-hypertensive agents, and 38 patients (25%) had diabetes. There is no significant difference on baseline BMI, eGFR, SBP, DBP, ACR, and percentage of diabetes and current smoking between lower SD in SBP group and higher SD in SBP group. Multivariate analysis revealed that SD in SBP and SD in DBP were independent risk factors for renal function. Without adjustment, SD in SBP and SD in DBP were significantly correlated with eGFR decline(P<0.001). In model 1, adjusted for sex, age, BMI, current smoking, baseline eGFR, baseline SBP and DBP, SD in SBP and SD in DBP were also significantly correlated with eGFR decline(P<0.001). In model 2, adjusted for model 1+with or without diabetes, SD in SBP and SD in DBP were still significantly correlated with eGFR decline(P<0.001). In model 3, adjusted for model 2+ACR, SD in SBP and SD in DBP were significantly correlated with eGFR decline too.(SD in SBP: P=0.006; SD in DBP: P<0.001).

Conclusions: Visit-to-visit variability in blood pressure was significantly associated with renal function independent of proteinuria and diabetes.

Funding: Government Support - Non-U.S.

TH-PO349

Visit-to-Visit Varibiability in Estimated Glomerular Filtration Rate Does Not Predict Worsening of Renal Prognosis Keita Uehara, Takashi Yasuda, Naohiko Imai, Yugo Shibagaki, Kenjiro Kimura. *Nephrology and Hypertension, St. Marianna University of Medicine, Kawasaki, Kanagawa, Japan.*

Background: In the AKI setting, subtle increases in serum creatinine (sCr) levels affect prognosis. Although we observed visit-to-visit fluctuations in estimated glomerular filtration rate (eGFR) in ambulatory patients, the significance of these variations on renal outcome is unknown. We examined the significance of visit-to-visit variability in eGFR on subsequent renal events.

Methods: This retrospective cohort study included patients with CKD and/or hypertension in our outpatient clinic who had eGFR measured ≥5 times at ≥4-week intervals over 2 years until August 2009. We used eGFR variability with the coefficient of variation of eGFR (eGFR-CV) and residual eGFR-CV (R-eGFR-CV), derived from a regression line of eGFR. At the time of the latest measurement of sCr among the five serial values, we performed ABPM and various tests as baseline data, and observation period started from this day. The primary endpoint was ESRD during the observation period.

Results: We examined 278 consecutive patients. At baseline, mean age was 65.0±12.7 years and mean eGFR was 47.9±24.0 ml/min/1.73 m^2. Median eGFR-CV was 0.067 (IQR: 0.045-0.079), and median R-eGFR-CV was 0.034 (IQR: 0.017-0.067), we classified subjects into 4 groups (Q1 to Q4) according to eGFR-CV and R-eGFR-CV quartiles, respectively. During a median observation period of 2.9 years (IQR: 2.1-3.5), 39 patients reached the primary endpoint. The cumulative incidence of the primary endpoint differed significantly by eGFR-CV quartiles. Multivariable Cox regression with adjustment for baseline covariates showed that eGFR-CV was a significant factor predicting the primary end point: HR for Q4, 16.9 (95%CI: 2.1-136.5) and HR for Q3, 9.8 (95%CI: 1.2-79.6) compared with Q1. On the other hand, there was no difference in the cumulative incidence of primary endpoint between each R-eGFR-CV quartile in a univariate analysis.

Conclusions: Although eGFR variability is common in patients with CKD and/or hypertension, it does not predict renal outcome when variability was adjusted by the regression line of eGFR.

TH-PO350

Associations of GFR and Albuminuria with Ischemic versus Hemorrhagic Strokes B. Khan Mahmoodi,[1] Hiroshi Yatsuya,[2] Kunihiro Matsushita,[1] Yingying Sang,[1] Rebecca F. Gottesman,[1] Brad C. Astor,[3] Mark Woodward,[1] W. T. Longstreth,[4] Bruce M. Psaty,[4] Michael Shlipak,[5] Aaron R. Folsom,[6] Ron T. Gansevoort,[7] Josef Coresh.[1] *[1]Johns Hopkins University; [2]Nagoya University (Japan); [3]University of Wisconsin; [4]University of Washington; [5]University of California, San Francisco; [6]University of Minnesota; [7]University of Groningen (Netherlands).*

Background: Although low GFR and high albuminuria have been reported associated with incident stroke, few studies compare their contribution to risk of ischemic and hemorrhagic strokes separately.

Methods: Individual participant data from four community-based cohorts from United States (ARIC, CHS, MESA) and The Netherlands (PREVEND) were pooled. To estimate adjusted hazard ratios (HRs) for stroke types, multivariable study-stratified Cox-regression was used. Standard errors for test of differences in HRs of stroke types were obtained by bootstrapping. GFR was estimated using cystatin-C-based equation (eGFR), and albuminuria was quantified by urinary albumin-to-creatinine ratio (ACR). Only adjudicated incident intraparenchymal hemorrhagic and ischemic strokes were considered.

Results: During average follow-up of 9.1 years, 1,151 of 29,429 participants developed stroke (13% hemorrhagic). Both eGFR and ACR risk-associations were roughly linear. Adjusted HRs associated with 1 SD (~22 mL/min/1.73m^2) decrease in eGFR were 1.16 (95%CI, 1.07-1.26) for ischemic versus 1.10 (0.89-1.37) for hemorrhagic stroke. In contrast, ACR showed significant association with both stroke subtypes. In fact, ACR association with hemorrhagic strokes was stronger as compared to ischemic strokes (P difference=0.026).

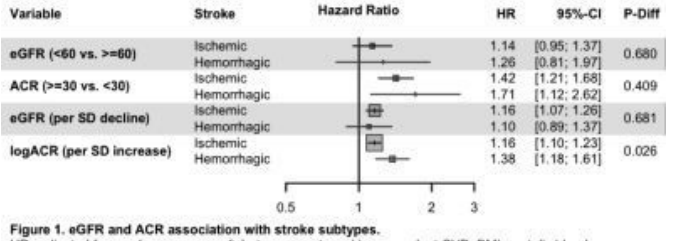


Figure 1. eGFR and ACR association with stroke subtypes.
HRs adjusted for gender, age, race, diabetes, current smoking, prevalent CVD, BMI, systolic blood pressure, cholesterol, antihypertensives and statins use. SD denotes standard deviation; P-Diff is P-value for difference of HRs of ischemic and hemorrhagic strokes.

Conclusions: While both low GFR and high albuminuria were independently associated with ischemic strokes, only ACR showed strong association with hemorrhagic strokes.

Funding: Other NIH Support - NHLBI

TH-PO351

The Association of Parathyroid Hormone (PTH) with ESRD and Pre-ESRD Mortality in the Kidney Early Evaluation Program (KEEP) Georges Saab,[1] Andrew S. Bomback,[2] Samy I. Mcfarlane,[3] Suying Li,[4] Shu-cheng Chen,[4] Peter A. McCullough,[5] Adam Whaley-Connell.[6] *[1]MetroHealth Medical Center, Cleveland, OH; [2]Columbia University, New York, NY; [3]SUNY Downstate, Brooklyn, NY; [4]Chronic Disease Research Group, Minneapolis, MN; [5]St. John Providence Health System, Novi, MI; [6]University of Missouri, Columbia, MO.*

Background: Elevated PTH levels in CKD may lead to an increased risk for progression to ESRD and/or premature death.

Methods: 10,823 participants in the KEEP with CKD (eGFR < 60 ml/min/1.73m^2) were examined from 2005-2010. The association of PTH levels with ESRD and pre-ESRD mortality was ascertained by linking KEEP data to the Social Security Administration Death Master File and the United States Renal Data System (USRDS) and by using competing risk survival models.

Results: Participants were divided into 5 PTH quintiles (1st (<=41), 2nd (>41-59), 3rd (>59-80), 4th (>80-114, and 5th (>114) pg/mL). Higher PTH levels were associated with increasing age, black race, lack of a high school education, CVD, hypertension, and lower GFR. Among the cohort, the incidence of ESRD and pre-ESRD mortality was 6.4 and 20.1 events per 1000 person years with the incidence of both outcomes lowest in the 2nd PTH quintile. After multivariate adjustment, including calcium, phosphorus and GFR, the risk of ESRD was not statistically different from the 2nd quintile in the 1st (subhazard ratio (SHR): 1.42 (95% CI: 0.54-3.77), p = 0.47), 3rd (SHR: 1.62 (95 % CI: 0.65-4.05), p=0.30), 4th (SHR: 0.98 (95% CI: 0.39-2.48), p=0.97), and 5th (SHR: 2.06 (95% CI: 0.91-4.69), p=0.08) quintiles, respectively. Conversely, after similar adjustment, there was a graded risk of pre-ESRD mortality for the 3rd (SHR: 1.52 (95% CI: 1.04-2.24),p=0.03), 4th (SHR: 1.73 (95% CI: 1.19-2.52),p=0.004), and 5th (SHR: 1.86 (95% CI: 1.28-2.52),p=0.001) quintiles, respectively. There was no effect modification for diabetes, gender, race (black vs non-black), and GFR status (<= 45 vs > 45) for either outcome.

Conclusions: Elevated PTH levels are associated with increased pre-ESRD mortality but not with ESRD.

Funding: Pharmaceutical Company Support - Abbott, Amgen, Genentech, Pfizer and Nephroceuticals

TH-PO352

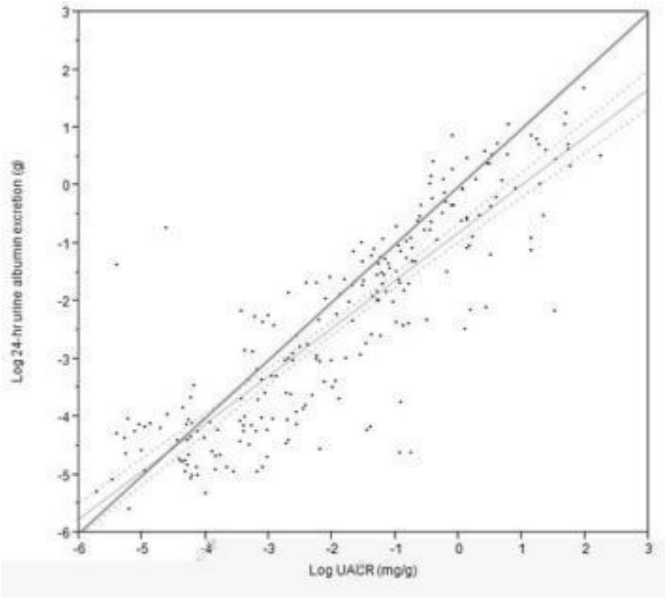
Equations for Converting Estimations of Urine Protein Excretion to 24-hr Urine Collections Ping Tyug Loh, Boon Wee Teo, Titus W. Lau, Evan J.C. Lee. *Medicine, National University Health System, Singapore, Singapore.*

Background: Spot urine protein-creatinine ratio (UPCR, mg/g) and albumin-creatinine ratio (UACR, mg/g) estimations of 24-hour urine protein excretion rate (24UPE, g) and 24-hour urine albumin excretion (24UAE, g), respectively, are used in clinical practice and research. But the established accuracy of these ratios predated the standardization of creatinine assays and proteinuria assessments vary with assay methods. We developed converting equations between ratios and 24-hr measurements from a multi-ethnic Asian population with a variety of CKD.

Methods: The Asian Kidney Disease Study (n=232) prospectively collected 24-hour urine followed by early morning spot urine, which were analyzed for protein (pyrogallol-based assay), albumin (turbidimetry), and creatinine (enzymatic assay). Variables were natural log-transformed before linear regression to correct for non-normal distribution and non-constant variability of observed points around the regression line.

Results: Four patients had 24UPE >3.5g. UACR predict 24UAE better as the slope is closer to 1. UPCR has poorer correlation to 24UPE (r = 0.79743) than UACR is to 24UAE (r = 0.86291). UACR is highly correlated to UPCR (r = 0.99237). 24UAE is also highly correlated to 24UPE (r = 0.99275). UACR is less correlated with 24UPE (r = 0.78334) than UPCR is with 24UAE (r = 0.80716). The prediction equations are (all p <0.001):

Log 24UPE = -0.617019 + 0.7150918 × Log UPCR
Log 24UAE = -0.800153 + 0.8257142 × Log UACR
Log UACR = -0.656352 + 1.3881178 × Log UPCR
Log UPCR = 0.3216439 + 0.6394674 × Log UACR
Log 24UAE = -0.504333 + 1.4810344 × Log 24UPE
Log 24UPE = 0.1624472 + 0.5991885 × Log 24UAE
Log 24UPE = -0.35059 + 0.4767755 × Log UACR
Log UACR = -0.270587 + 1.2870223 × Log 24UPE.



Conclusions: Spot urine albumin to creatinine ratio correlates better with 24-hr urine albumin excretion than proteinuria assessments.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO353

Prevalence of Chronic Kidney Disease and Associated Risk Factors: First Results from a Population Based Screening Program in Morocco (MAREMAR) Mohammed Benghanem Gharbi,[1] Zamd Mohamed,[1] Monique M. Elseviers,[2] Abdelali Belghiti Alaoui,[3] Mohammed El Hassane Trabelssi,[3] Naima Benahadi,[3] Benyounès Ramdani,[1] Bayahia Rabia,[1] Marc E. De Broe.[4] *[1]Moroccan Society of Nephrology; [2]Epidemiological Research, University of Antwerp, Wilrijk, Belgium; [3]Ministry of Health, Morocco; [4]International Society of Nephrology.*

Background: The MaReMar study is a project carried out by the Ministry of Health in Morocco in a partnership with the International Society of Nephrology, the WHO and the Moroccan Society of Nephrology. The aim is to estimate the prevalence of chronic kidney disease (CKD) and its associated risk factors in a representative sample of the population of Morocco aged 26-70.

Methods: The study was performed in two middle sized towns. A stratified random sample of the population, using official voting lists, was taken. Baseline screening was performed using a number of clinical (BMI, blood pressure, hip waist ratio) and laboratory investigations [dipstick, microalbuminuria (Hemocue), serum creatinine, fasting glycemia) and a structured questionnaire.

Results: 10524 subjects were enrolled. The prevalence of CKD [defined as presence of an eGFR < 60 ml/ min/1.73 m^2 or macroalbuminuria or dipstick abnormalities (proteinuria ≥ ++ or haematuria: ≥ ++) or diabetes type 1 associated with microalbuminuria] was 2.9% and increased with age (26-40 y.o: 1.4%; 41-55 y.o.: 2.7%; 56-70 y.o.: 8.5%). The main aetiologies of CKD were diabetes (32.79%), hypertension (28.2%) and nephrolithiasis (9.8%). The prevalence of associated factors was as follow: hypertension: 16.7%; hyperglyceamia (≥ 1.26 g/l): 13.8%; microalbuminuria (30-299 mg/l): 5.26% and obesity (BMI ≥ 30): 23.2%. The most frequent health related habits were traditional medicines (2.9%), analgesics (4.7%), smoking (4.7%) and occupational exposure (3.9%).

Conclusions: Maremar generated, for the first time, quantitative and scientifically correct information on the prevalence of CKD, hypertension, and diabetes in Morocco. This information is highly relevant for future national health strategies in this country and may be in other merging and emerging countries.

Funding: Government Support - Non-U.S.

TH-PO354

JC Polyoma Virus Interacts with Apolipoprotein L1 Genetic Risk in African Americans with Non-Diabetic Nephropathy Barry I. Freedman, Mariana Murea, Michael V. Rocco, Lijun Ma, Donald W. Bowden, David A. Ornelles, Steven Kleiboeker, Carl D. Langefeld, Jolyn Turner, Jasmin Divers. *Wake Forest School of Medicine, Winston-Salem, NC.*

Background: Approximately 50% of pts with untreated HIV infection and 2 *APOL1* nephropathy risk variants develop kidney disease. Other environmental factors, particularly non-HIV viral infections could interact with *APOL1* and contribute to nephropathy. We tested whether urine JC (JCV) and BK polyoma virus (BKV) and plasma Human Herpes Virus 6 (HHV6) and cytomegalovirus (CMV) influenced nephropathy risk via interactions with *APOL1*.

Methods: *APOL1* genotypes and presence of viral DNA via quantitative polymerase chain reaction were tested for impact on urine albumin:creatinine ratio (UACR), serum cystatin C, and estimated glomerular filtration rate (eGFR) in 300 first-degree relatives of African Americans (AAs) with non-diabetic nephropathy. Linear and non-linear mixed models were used to account for familial relationships.

Results: Two, one and zero *APOL1* risk variants were present in 130, 88 and 82 relatives, respectively. Urine JCV and BKV were detected in 30% and 9.7% of subjects; HHV6 and CMV were rare. Demographic data (mean±SD) were age 51.4±12.8 years, UACR 233±840 mg/g, cystatin C 1.0±0.5 mg/L, eGFR 86.5±28.0 ml/min/1.73m^2 and African ancestry 81±10%. Adjusting for family age at ESRD, sex and ancestry, JCV genomic DNA in urine and increasing number of *APOL1* risk alleles were negatively associated with high cystatin C (>0.95 mg/L; p=0.006), albuminuria (UACR>30 mg/g; p=0.0002), and kidney disease (eGFR<60 ml/min/1.73 m^2 and/or UACR>30 mg/g; p=0.000017); whereas BKV genomic DNA in urine was not associated with kidney disease.

Conclusions: AAs at risk for *APOL1*-associated nephropathy with detectible JCV in the urine had a lower prevalence of kidney disease. This suggests that JCV interacts with *APOL1*-associated risk. Potential mechanisms include inhibition of urinary tract replication by other more nephropathic viruses or impact on gene expression profiles that alter susceptibility to *APOL1*-associated nephropathy. These data suggest that non-HIV viral infections can serve as second hits impacting nephropathy risk in those with *APOL1* risk variants.

Funding: NIDDK Support

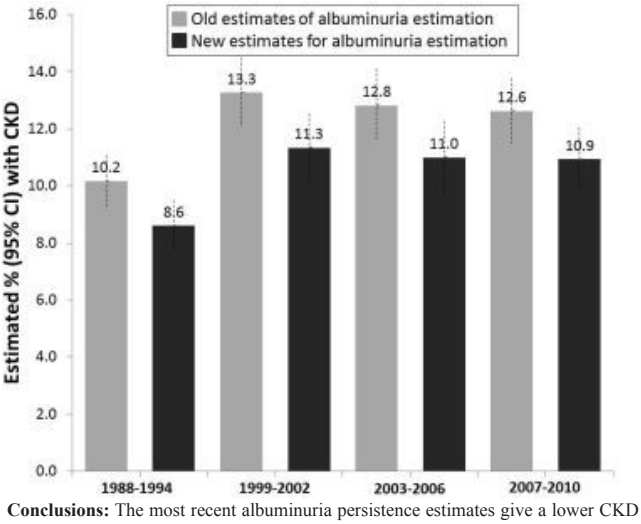
TH-PO355

Time Trends in Chronic Kidney Disease (CKD) Prevalence Appear Similar Regardless of Albuminuria Persistence Estimates Laura Plantinga,[1] Chi-yuan Hsu,[2] Yi Li,[3] Rajiv Saran,[3] Neil R. Powe.[2] *[1]Emory University, Atlanta, GA; [2]University of California, San Francisco, CA; [3]University of Michigan, Ann Arbor, MI.*

Background: CKD prevalence differs based on whether elevated albuminuria is confirmed by two random urine samples or a random and first-morning sample. We explored how the different methods influence estimated time trends in U.S. CKD prevalence.

Methods: "Persistent" albuminuria was defined in 2009-2010 National Health and Nutrition Examination Survey (NHANES) adult (>20 years) non-pregnant participants (N=4,938) by concordance [urinary albumin:creatine ratio (UACR) >30 mg/g in both samples] of data from random spot and first-morning urine samples collected 10 days apart. Persistence of elevated UACR was estimated separately within categories determined by eGFR (ml/min/1.73 m^2) and UACR (mg/g): eGFR ≥90/UACR 30-299, 33.9%; eGFR ≥90/UACR ≥300, 90.5%; eGFR 60-89/UACR 30-299, 41.8%; eGFR 60-89/UACR ≥300, 78.1%). We estimated U.S. population-weighted prevalence of CKD by survey year, using bootstrapping techniques incorporating these estimates vs. older persistence estimates from a subset of NHANES III [1988-1994; eGFR ≥90/UACR 30-299, 50.9%; eGFR 60-89/UACR 30-299, 75.0%; UACR ≥300 (any eGFR), 100%].

Results: Updated albuminuria persistence estimates gave a prevalence of 11.1% (95% CI, 10.3-12.0%) over 1999-2010, vs. 12.9% (12.0-13.8%) with older persistence estimates. There was similar overestimation of prevalence in each time period but similar patterns over time. Changes from 1988-1994 to 2007-2010 were similar for old vs. new persistence estimation (absolute change, 2.5% vs. 2.3%; relative change, 24.2% vs. 27.1%).



Conclusions: The most recent albuminuria persistence estimates give a lower CKD prevalence in the general population, but overall time trends are similar.

Funding: Other U.S. Government Support

TH-PO356

Awareness of Chronic Kidney Disease May Be Underestimated by Random Albuminuria Measurement Laura Plantinga,[1] Delphine S. Tuot,[2] Brenda W. Gillespie,[3] Hal Morgenstern,[3] Sharon Saydah,[4] Nilka Rios Burrows,[4] Neil R. Powe.[1] *[1]Emory University, Atlanta, GA; [2]University of California, San Francisco, CA; [3]University of Michigan, Ann Arbor, MI; [4]Centers for Disease Control and Prevention, Atlanta, GA.*

Background: Estimation of awareness of chronic kidney disease (CKD) may be limited by misclassification of albuminuria, a strong independent predictor of CKD awareness. Single spot urine albumin:creatinine ratio (UACR) may overestimate the prevalence of albuminuria, leading to underestimation of CKD awareness among those classified as having albuminuria.

Methods: In the 2009-2010 National Health and Nutrition Examination Survey, both random spot and first-morning samples were provided by 4,927 non-pregnant adults (≥20 years) and assayed (by the same method in the same laboratory) for UACR. We compared U.S. population-weighted prevalence of awareness ("yes" to questionnaire item "Have you ever been told by a healthcare provider that you have weak or failing kidneys?") among those with albuminuria (UACR ≥30 mg/g) by type of sample and characteristic (age, gender, race/ethnicity, eGFR, diabetes, hypertension), using separate logistic regression models for each UACR measure with estimated margins (adjusted for all characteristics).

Results: When albuminuria was measured by random spot (prevalence, 7.7%) and first-morning (prevalence, 4.6%) measurements, respectively, CKD awareness was estimated at 5.6% (95% CI, 4.1-7.1%) and 8.2% (6.6-9.9%). Those with normal vs. reduced kidney function (eGFR ≥60 vs. <60 ml/min/1.73 m²) had lower prevalence of awareness: 1.5% vs. 19.1% (P<0.001) and 2.5% vs. 23.8% (P<0.001) for the random and first-morning samples, respectively. Regardless of sample type, older, female, and non-Hispanic white individuals with albuminuria were less likely to be aware of CKD and those with diabetes and hypertension were similarly likely to be aware, compared to their counterparts.

Conclusions: CKD awareness differs based on albuminuria measure. Method of albuminuria measurement should be considered alongside population estimates of CKD awareness, particularly those used to gauge the effectiveness of educational interventions.

Funding: Other U.S. Government Support

TH-PO357

Visceral Obesity Is a Risk Factor of Microalbuminuria Hyun Suk Kim,[1] Hyo Eun Park,[2] Su-yeon Choi,[2] Jin Suk Han,[1] Nam Ju Heo.[2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Internal Medicine, Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, Korea.*

Background: Microalbuminuria is known as a marker of cardiovascular and kidney disease. Few studies have examined the effect of visceral obesity on microalbuminuria. The aim of this study is to investigate the association between visceral adipose tissue (VAT) and the prevalence of microalbuminuria.

Methods: We conducted a cross-sectional study of 1,154 subjects who underwent routine checkups, including CT scans of abdominal adipose tissue at the Healthcare System Gangnam Center of Seoul National University Hospital from September 2006 through December 2010. VAT area was defined as intra-peritoneal fat bound by parietal peritoneum or transversalis fascia. Microalbuminuria was defined as albumin to creatinine ratio 30~300 mg/g of morning spot urine. Since the standard for normal abdominal fat has not been established, we used the lowest tertile as a reference group after dividing abdominal fat into three groups.

Results: Overall, 10.4% had microalbuminuria. Individuals with higher VAT showed higher level of albuminuria (*p* for trend of men<0.001, of women=0.049), and higher prevalence of microalbuminuriain in men (*p* for trend<0.001), but not in women (*p* for trend=0.106). Also, higher VAT was associated with higher insulin resistance (HOMA-IR) and higher incidence of metabolic syndrome. (*p* for trend of men<0.001, of women<0.001). After multivariate adjustment, highest tertile group of VAT was associated with higher prevalence of microalbuminuria (odds ratio [OR] 1.882; 95% confidence interval [CI] 1.064-3.330, *p* for trend= 0.030). In subgroup analysis, among men, highest tertiles of VAT was associated with higher prevalence of microalbuminuria (VAT; OR, 2.705; 95% CI, 1.430-5.118). Among women, there was no significant association between VAT and microalbuminuria.

Conclusions: Visceral obesity was significantly associated with microalbuminuria. Higher VAT is also related with higher insulin resistance and higher prevalence of metabolic syndrome.

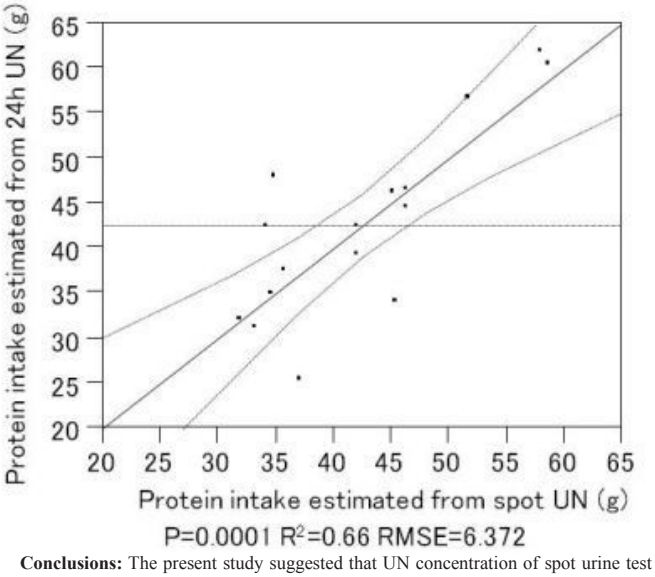
TH-PO358

Estimating Daily Protein Intake by Urea Nitorogen Concentration in Spot Urine Yoshihiko Kanno,[1] Eiichiro Kanda,[2] Kaori Sakamoto,[3] Kei Nakajima,[4] Yoshihiro Matsumoto,[5] Sanae Watanabe,[3] Toshitaka Muneyuki,[6] Tomoya Hirayama.[7] *[1]Apheresis and Dialysis Center, Keio University, Tokyo, Japan; [2]Department of Nephrology, Tokyo Kyosai Hospital, Tokyo, Japan; [3]Kagawa Nutrition University, Sakado, Japan; [4]Department of Medical Dietetics, Josai University, Sakado, Japan; [5]Shizuoka City Hospital, Shizuoka, Japan; [6]Saitama Citizens Medical Center, Saitama, Japan; [7]Department of Nephrology, Kitasaito Hospital, Asahikawa, Japan.*

Background: It is not easy to know the amount of daily protein intake in CKD management. Recording dietary intake is inaccurate, and estimation from total urea excretion is also not easy to ask patients to collect whole urine for 24 hours (h). Spot urine became to be used as indicator of daily sodium intake, and daily urinary protein excretion. In this cross sectional study, we investigated whether urea nitrogen (UN) concentration (C) of spot urine would replace 24h urine collection.

Methods: 17 Japanese young healthy female provided their urine for 24h with diet record. They provided one spot urine on the next day of 24h urine collection. UN and creatinine C were measured in all samples (BML, Inc. Tokyo, Japan).

Results: Bodyweight of objects was 50.0±4.3 kg, UN excretion was 6.08±2.19 g/day. Estimated protein intake was 47.7±13.95 g/day (Maroni formula). Creatinine-adjusted UNC of spot urine on the next morning was significantly correlated with 24h UN excretion than other samples (Spearman's rank correlation coefficient ρ=0.0155, *P=0.*0155). The creatinine-adjusted UNC of spot urine could predict the amount of 24h UN excretion, and protein intake using an univariate linear regression model (Protein intake=24.5 + 3.4 x creatinine-adjusted UNC, adjusted R^2=0.66, P=0.0001).



Conclusions: The present study suggested that UN concentration of spot urine test could estimate daily protein intake, and would help nutritional control in CKD patient.

TH-PO359

Prevalence and Awareness of Chronic Kidney Disease and Cardiovascular Risk Factors in a Large Canadian Survey: The CARTaGENE Study Jacobien Verhave,[1] Stephan Troyanov,[1] Frederic Mongeau,[1] Philip Awadalla,[2] Lorraine Fradette,[2] Josee Bouchard,[1] Francois Madore.[1] *[1]Nephrology, Hôpital du Sacré-Cœur, Montreal, QC, Canada; [2]Medical and Population Genomics Laboratory, University of Montreal, Montreal, QC, Canada.*

Background: Chronic kidney disease (CKD) and cardiovascular (CV) diseases are associated with significant morbidity and mortality. Patient awareness of these conditions is an essential component of CV risk management. We sought to determine the prevalence and awareness of CKD and CV risk factors in the general population and evaluate treatment of CV risk factors.

Methods: CARTaGENE is a cohort study of 20,004 randomly selected Canadian subjects, aged 40-69. Participants had anthropometric and blood pressure (BP) measurements, evaluation of medical conditions using self-administered questionnaires (including medication use) and blood sample collection. Regarding kidney disease, subjects were asked: Has a doctor ever told you that you have kidney disease, renal failure, renal infection or kidney stones?

Results: The prevalence of stage 3 CKD was 6.8% (MDRD) and 3.9% (CKD-EPI). CKD awareness was low: 92.3% of subjects were unaware of their renal condition. The prevalence self-reported hypertension, diabetes and hypercholesterolemia was 25, 7.5 and 28%, respectively. In self-declared normotensive subjects, 12% had a measured BP ≥140/90 mmHg. In those without self-reported diabetes, 2.8% had diabetes defined as HbA1c level ≥6.5% or hyperglycemia. One third of individuals without a history of hypercholesterolemia had LDL levels above ATPIII recommendations. With regard to treatment of known conditions, a measured BP ≥140/90 was present in 31% of the subjects with self-reported hypertension. BP was ≥130/80 in 53% of subjects who reported diabetes or CKD in addition to hypertension. Among self-reported diabetic subjects, 41% had an HbA1c ≥7 and 13% an HbA1c ≥8.5. More than half of the subjects with a moderate or high Framingham risk score had LDL levels above the target.

Conclusions: In this large sample of the general Canadian population, stage 3 CKD and CV risk factors are common. Awareness of these conditions is low and treatment goals are not achieved in the majority of subjects.

Funding: Government Support - Non-U.S.

TH-PO360

Prevalence of Albuminuria in a General Pediatric Population: National Health and Nutrition Examination Survey 2009-2010 Lauren F. Loeffler, Alicia M. Neu, Jeffrey J. Fadrowski. *Pediatric Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Albuminuria is a well-known marker of kidney disease. In children, however, albuminuria is often transient or orthostatic in nature and not pathologic. The prevalence of albuminuria, and microalbuminuria in particular, has been poorly described in the general pediatric population.

Methods: Children aged 6-17 years participating in the National Health and Nutrition Examination Survey (NHANES) 2009-2010, a cross-sectional survey of non-institutionalized US civilians, had albumin and creatinine measured in a random and first morning urine collection. Prevalence estimates were calculated for microalbuminuria (albumin to creatinine ratio ≥ 30 mcg/mg) and for albuminuria (albumin to creatinine ratio ≥ 300 mcg/mg). Glomerular filtration rate was estimated (eGFR) by the bedside Chronic Kidney Disease in Children (CKiD) equation (mL/min/1.73m^2).

Results: Of 1,876 children, 48% were female, 13% Black, 58% White, and 21% Hispanic. The median albumin to creatinine ratio was 8.9 mcg/mg and 6.3 mcg/mg on random and first morning urine samples, respectively.

Prevalence of Albuminuria in Children, NHANES 2009-2010

	Prevalence Microalbuminuria (%)			Prevalence Albuminuria (%)		
Urine Sample	Overall	6-11 y	12-17 y	Overall	6-11 y	12-17 y
Random	13.9	12	15.6	1.4	0.8	2
First Morning	4.5	3.8	5.2	0.4	0.3	0.5
Both	2.2	1.4	2.9	0.2	0.1	0.3

Of those with microalbuminuria and albuminuria on a random sample, 84% and 86%, respectively, did not have albuminuria on the subsequent first-morning urine collection, and thus may be classified as having transient or orthostatic albuminuria. Differences in prevalence by age were not statistically significant. Of those with first morning microalbuminuria, 7% and 15% had an eGFR <75 and <60, respectively. Of those with first morning albuminuria, 72% and 72% had an eGFR <75 and <60, respectively.

Conclusions: Although limited by a single urine collection, the prevalence of albuminuria and microalbuminuria in this population is of interest and supports that a significant proportion of adults with chronic kidney disease may have the earliest signs during childhood or adolescence.

Funding: NIDDK Support

TH-PO361

The Relationship between Vascular Disease and Albuminuria Testing in a Guideline Based and Incentivized Health-Care System Mark David Jesky,[1,2] Paul Cockwell,[1,2] Andrew Felix Burden.[3] *[1]Nephrology Department, University Hospitals Birmingham NHS Foundation Trust, United Kingdom; [2]Department of Infection and Immunity, University of Birmingham, United Kingdom; [3]Birmingham and Solihull NHS Cluster of CCGs, United Kingdom.*

Background: The UK employs national guidelines and financial incentives to optimize the monitoring of individuals at high-risk of chronic kidney disease (CKD), including proteinuria monitoring by albumin:creatinine ratio (ACR). Previous studies have shown the success of this approach. However, utilization of ACR testing in non-diabetic high-risk groups is unclear; a shortfall this study addresses.

Methods: In a UK primary-care catchment area data are collected electronically for individuals identified on vascular disease registers. For patients on these registers monitoring of eGFR and ACR 12-monthly is recommended. Utilizing the 2011 dataset, we analyzed the proportion of this population who had a creatinine checked, an eGFR reported and an ACR analyzed within the previous 15 months. We then focused on utilization of ACR testing in the non-diabetic high risk group.

Results: 47,329 (14.7%) people were on one or more vascular disease register. Creatinine, eGFR and ACR were reported in 83.2%, 66.3% and 46.8% respectively. ACR was checked in 15125 of 21529 (70.3%) diabetics. For those not on the diabetic register, ACR was checked in 7028 of 25800 (27.2%). Amongst the non-diabetic group, multivariate logistic regression demonstrated the key determinant of ACR testing was prior inclusion on the CKD stage 3-5 register (odds ratio 5.84, 95% confidence interval 5.40 – 6.32, p<0.001). Only 20.6% (4,395/21,339) of people not on the CKD 3-5 or diabetes register had their ACR checked. 25.0% of these 4,395 had a high ACR (above 30mg/g); in comparison, 27.9% of registered diabetics and 37.7% of individuals on the CKD register had a high ACR.

Conclusions: As ACR represents a major tool for assessing risk and directing management, continuing primary care initiatives to increase albuminuria testing are critical. This study indicates the need for an enhanced emphasis on non-diabetic, vascular disease risk groups, where there is a high prevalence of albuminuria, but monitoring rates are low.

Funding: Private Foundation Support, Clinical Revenue Support

TH-PO362

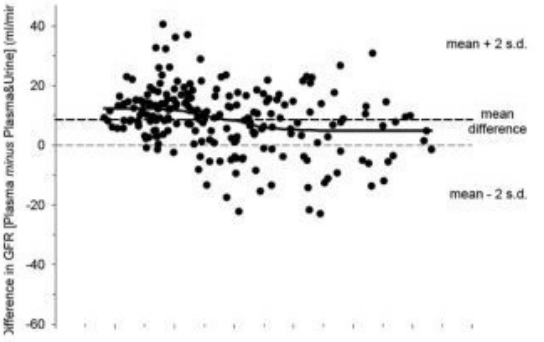
Differences between Plasma and Urinary Isotopic Glomerular Filtration Rate in Diabetic Nephropathy Shih-Han S. Huang,[1] Guido Filler,[2] Andrew A. House.[1] *[1]Medicine, Nephrology, Western University, London, ON, Canada; [2]Paediatric, Nephrology, Western University, London, ON, Canada.*

Background: Kidney function is assessed by measurement of the glomerular filtration rate (GFR). Isotopic GFR (iGFR) measurement is comparable to inulin method. In this study, we compared urinary and plasma iGFR methodologies in patients with diabetic nephropathy.

Methods: Ninety-Three patients were selected from the London Health Sciences Center participants in the Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe) trial. They had both urinary and plasma iGFR measurements during their first clinic visits. The two measurements were compared using intra-class correlation method, paired-t test, non-parametric test, Bland-Altman analysis and multivariable regression analysis. P-value < 0.05 was considered significant.

Results: The median age was 62 years (inter quartile range; IQR= 51.5, 69.0). The median plasma and urinary iGFR values were 65.3 ml/min/1.73m^2 (IQR= 47.2, 89.0) and 53.7 ml/min/1.73m^2 (IQR= 34.0, 82.4), respectively (p<0.001). There was a statistically significant correlation (r = 0.86; p<0.001) and a statistically significant difference between the plasma and urinary values.

Figure 1.The Bland-Altman analysis between plasma and urinary iGFR values (bias: 9.4 ml/min/1.73m^2; standard deviation of bias: 15.1 ml/min/1.73m^2)



Bland-Altman analysis demonstrated an overall bias of 9.4±15.1 ml/min/1.73m^2. The bias was greatest in chronic kidney disease (CKD) stage 4 (16.9±12.9 ml/min/1.73m^2). The multi-variable regression analysis using two variables, height and CKD stage provided a model that explained 26% (p<0.001) of the variation between the urinary and plasma iGFR.

Conclusions: The plasma clearance method of iGFR estimation consistently gave higher results than the urinary method. The bias increased with increasing CKD stage. Plasma iGFR may not be the gold-standard reference method in studies that are aimed to measure GFR in patients with more advanced CKD.

TH-PO363

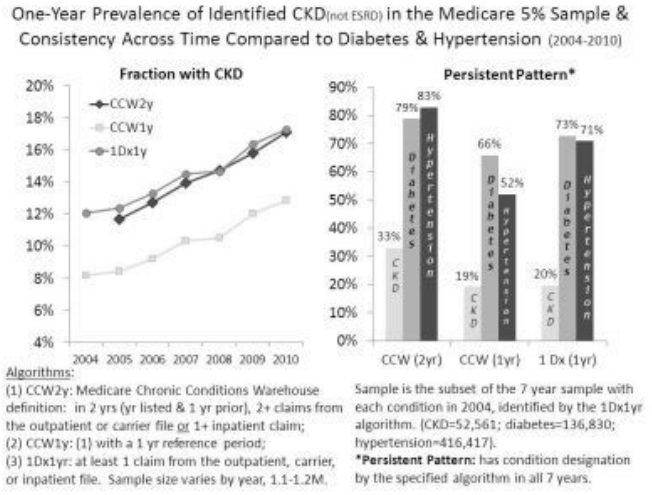
Comparison of Algorithms to Identify Non-ESRD Chronic Kidney Disease (CKD) in Medicare Claims over Time Diane Steffick,[1] Vahakn B. Shahinian,[1] Hal Morgenstern,[1] Tanushree Banerjee,[2] Neil R. Powe,[2] Sharon Saydah.[3] *[1]University of Michigan, Ann Arbor, MI; [2]University of California San Francisco; [3]Centers for Disease Control and Prevention, Atlanta, GA.*

Background: Medicare claims are a valuable data source for CKD surveillance in the US, although they currently lack laboratory values. We used 3 algorithms for identification of non-ESRD CKD from Medicare claims; 2 based on the Medicare Chronic Conditions Warehouse algorithm–2+ hospital outpatient (OP) claims or 2+ non-hospital ambulatory care (AC) claims or 1 inpatient (IP) claim within a 2 yr (CCW2y) or 1 yr period (CCW1y), & one requiring only 1+ OP, AC, or IP claim during 1 yr (1Dx1y). To provide context for the pattern of CKD identification over time, we also show hypertension (HTN) & diabetes (DM).

Methods: We analyzed the 5% Medicare inpatient, outpatient & ambulatory care files. Eligible persons were aged 65+, not in managed care, enrolled in Part A&B all year & had 1+ outpatient visits each year, 2004-2010. A persistent pattern was defined as having the condition (CKD/DM/HTN) detected in every year. For simplicity, we limited the persistent pattern analysis to persons with claims in all 7 yrs & 1+ claim for the condition in 2004.

Results: CCW2y & 1Dx1y gave similar estimates of the prevalence of CKD, while CCW1y was lower. CCW2y had the highest fraction (33%) with a persistent pattern. In contrast, 79% of CCW2y DM cases & 83% of HTN cases had the persistent pattern.

Conclusions: When using only 1 year of claims data, researchers should consider relaxing the requirement for 2 outpatient claims to avoid undercounting CKD. Furthermore, Medicare patients rarely have claims for CKD consistently over time while the majority of patients with DM and HTN claims did. Whether this indicates a lack of attention to CKD, lack of referral to nephrology, or something else requires further investigation.



Funding: Other NIH Support - Centers for Disease Control and Prevention

TH-PO364

GFR Estimating Equations Based on Standardized Serum Cystatin C

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Background: Serum cystatin C (Scys) is a potential alternative GFR marker. We developed GFR estimating equations based on standardized Scys that was traceable to a primary reference material, ERM-DA471/IFCC.

Methods: Development dataset (413 subjects) and validation dataset (350 subjects) were used. GFR was measured using inulin renal clearance. Serum creatinine was measured by IDMS-traceable enzymatic method. Serum cystatin C was measured by nephelometric immunoassay (Siemens, Dade Behring) previously. 727 freezed samples were re-measured by colloidal gold immunoassay (Alfresa Pharma) that was traceable to ERM-DA471/IFCC. Residual 36 of 763 samples were calibrated to the standardized values. GFR equations based on Scys (Eq-Cys1), Scys with constant non-renal elimination of cystatin C (Eq-Cys2) and Scys in combination with serum creatinine (Scr) (Eq-Cr-Cys) were developed. The Eq-Cys1, Eq-Cys2, Eq-Cr-Cys, average value of Eq-Cr and Eq-Cys2 (Eq-average), Japanese GFR equation based on Scr (Eq-Cr), and a equation based on standardized Scys reported by Inker LA (Eq-Inker) were compared.

Results: Ethnic coefficient (95% CI) of Eq-Inker for Japanese was 1.02 (0.99-1.05). In validation dataset, median (IQR) of difference between mGFR and eGFR by Eq-Cr, Eq-Inker, Eq-Cys1, Eq-Cys2, Eq-combined and Eq-average were 7.4 (13.2), 7.1(11.9), 7.3 (11.7), 6.7 (10.3), 6.2(9.9) and 6.1(9.3) ml/min/1.73m^2, respectively. Accuracy within ±30%(P30) of mGFR were 75%, 76%, 77%, 78%, 81% and 82%, respectively. Eq-Cys1, Eq-Cys2 and Eq-Inker were as accurate as or more accurate than Eq-Cr. Performance of the GFR estimation was more improved by Eq-Cr-Cys and Eq-average.

Conclusions: The equation based on Scys was as accurate as or more accurate than the equation based on Scr. Estimated GFR based on both Scr and Scys was more accurate. Ethnic coefficient of Eq-Inker was about 1.0, suggesting the possibility that equations based on serum cystatin C could be used in patients with different race.

Funding: Government Support - Non-U.S.

TH-PO365

Performance of Estimating Glomerular Filtration Rate (eGFR) Formulas in Indigenous Australians with and without Diabetes

Richard J. MacIsaac,[1] Elif I. Ekinci,[2] Jaquelyne T. Hughes,[3] Paul D. Lawton,[3] Graham Ross Dallas Jones,[4] Andrew G. Ellis,[5] Alan Cass,[6] Wendy E. Hoy,[7] Kerin O'Dea,[8] George Jerums,[2] Louise J. Maple-Brown.[3] *[1]St Vincent's Hospital & University of Melbourne, Australia; [2]Austin Health & University of Melbourne, Australia; [3]Menzies School of Health Research & Charles Darwin University, Australia; [4]St Vincent's Hospital Sydney, Australia; [5]Austin Health, Australia; [6]George Institute for Global Health & University of Sydney, Australia; [7]University of Queensland, Australia; [8]University of South Australia, Australia.*

Background: The CKD-EPI formula has been proposed as a more accurate marker of GFR than the MDRD formula. However, the best method for estimating GFF in Indigenous Australians with diabetes is still unclear.

Methods: Indigenous Australians with (n=234) or without (n=345) type 2 diabetes were studied. A reference GFR measurement was obtained using the plasma disappearance of iohexol (mGFR) over 4 hours. Serum creatinine was measured by an enzymatic method. Performance was determined as bias, derived from mGFR-eGFR and accuracy (percentage of eGFR within 30% of mean mGFR).

Results: In the entire study population, the performance of the CKD-EPI formula was superior to the MDRD formula. However, in Indigenous Australians with diabetes, the CKD-EPI formula underestimated mGFR to a greater extend and was less accurate than in those without diabetes (Table, †p < 0.05 compared with no diabetes).

Performance of the MDRD and CKD-EPI formulas in people with and without diabetes

	Diabetes (n=234)	No Diabetes (n=354)
Measured GFR (ml/min/1.73m²)	83 (77, 89)	101 (98, 104)
MDRD		
Bias (ml/min/1.73m², median, 95%CI)	8.8 (6.5,11.1)	8.8 (6.9, 12.3)
Accuracy (%, 95%CI)	79 (74, 84)	89† (89, 92)
CKD-EPI		
Bias (ml/min/1.73m², median, 95%CI)	6.1 (3.5, 7.9)	1.8† (-0.5, 4.3)
Accuracy (%, 95%CI)	81 (76, 86)	92† (88, 94)

Conclusions: Overall, the CKD-EPI formula outperforms the MDRD formula for estimating GFR in Indigenous Australians. However, in Indigenous Australians with diabetes, the CKD-EPI formula has a greater negative bias and is less accurate compared to those without diabetes.

TH-PO366

Validation of Kidney Disease Hospitalizations in a Prospective Cohort: The ARIC Study

M. Grams, Wen Hong Linda Kao, Brad C. Astor, Josef Coresh. *JHU.*

Background: Many cohorts classify kidney disease solely by visit-based laboratory measures. Use of interim hospitalizations improves estimation in time-to-event analyses, event capture among those who miss visits, and identification of AKI, a transient yet potent predictor of outcomes. However, the validity of identifying interim kidney disease hospitalizations by discharge *ICD-9-CM* codes is uncertain, and many previous studies do not adjust for sampling technique.

Methods: Records were randomly selected within 9 *ICD-9-CM* code strata from 9,104 hospitalizations (3,784 participants at a single center, 1996 -2008), oversampling strata containing a kidney code. Inpatient labs, the admission note, nephrology consult, and discharge summary were abstracted. A blinded nephrologist adjudicated events as follows: **CKD**, chart mention or eGFR ≤ 60 (CKD-Epi equation); **Stage 4+ CKD**, eGFR ≤ 30; **AKI**, chart mention or 50% increase in creatinine; **ESRD**, RRT at admission and/or discharge, or death from progression of kidney disease (baseline CKD Stage 3+). Total events were estimated using inverse probability weighting (1/number sampled/total number in strata).

Results: The initial sample contained 191 charts; 91% had sufficient data. Sensitivity ranged from 20.8% (AKI) to 68.5% (Stage 4+ CKD); specificity was >92% for all outcomes.

Validity of ICD-9-CM-coded kidney hospitalizations

	AKI	CKD (Stage 4+ CKD)	ESRD
Sensitivity (%)	20.8	44.3 (68.5)	38.0
Specificity (%)	99.6	98.3 (92.1)	99.1
Positive Predictive Value (%)	91.3	91.2 (49.1)	76.8
Negative Predictive Value (%)	85.4	81.4 (96.3)	95.3

Positive predictive value (PPV) was lower for rarer events. In association studies, these estimates would result in little bias (e.g., the relative risk of AKI would decrease from 2.0 to 1.9, were sensitivity unrelated to exposure). Incorporation of the sampling design is critical for avoiding spectrum bias: ignoring sample weights grossly overestimates sensitivity (61, 86, and 77% for AKI, CKD, and ESRD, respectively) and underestimates specificity (95, 86, and 94%).

Conclusions: *ICD-9-CM* codes for kidney events have high specificity but low sensitivity. Validity varies with the spectrum of disease, and calculations should be adjusted based on sampling technique.

Funding: NIDDK Support, Private Foundation Support

TH-PO367

Renal Function Decline in Older Adults and Association with C-Reactive Protein: Results from the Einstein Aging Study

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Background: Decreased kidney function is an independent risk factor for increased morbidity and mortality in all age-groups, but risk factors for decreased or declining kidney function in older adult population remain unclear. Markers of inflammation including C-reactive protein (CRP) increase with age and have been linked with increased risk for cardiovascular in young and middle-aged adults. Therefore, we examined rates and predictors of estimated glomerular filtration rate (eGFR) decline in a sub-study of the Einstein Aging Study (EAS).

Methods: We identified 369 subjects with 2 to 5 yearly serum creatinine measurements and calculated creatinine-based eGFR using three different formulas (Cockgroft-Gault (CG), Modification-of-Diet-in-Renal-Disease (MDRD), and Chronic-Kidney-Disease-Epidemiology-Collaboration (CKD-EPI) formula. eGFR decline rates were determined by mixed effect modeling. Multivariate linear regression was used to identify factors associated with baseline eGFR and mixed effect eGFR decline rate.

Results: The mean age of 369 subjects was 78 years old (68-95 years; 232 female and 137 male). The mean eGFR decline percentage change was -4.3% in CG, -3.1% in MDRD, and -3.5% in CKD-EPI formula. In addition, the 5th percentile eGFR decline percentage change was -5.8% in CG, -4.6% in MDRD, and -5.0% in CKD-EPI formulas. In multivariate linear regression with baseline eGFR as the outcome, higher CRP levels were associated with lower eGFR (β=-0.006, P-value=0.01 in CG; β=-0.006, P-value=0.005 in MDRD; β=-0.006, P-value=0.007 in CKD-EPI). In multivariate linear regression predictinge GFR

decline percentage change, lower baseline eGFR was associated with more eGFR decline (β=0.02, P-value=0.0001 in CG; β=0.02, P-value=0.0001 in MDRD; not significant in CKD-EPI). However, baseline CRP or CRP change over period of time was not associated with eGFR decline.

Conclusions: Whereas lower eGFR is significantly associated with more rapid eGFR decline, serum CRP levels are associated with cross-sectional eGFR but not with eGFR decline in subjects in the EAS.

Funding: Other NIH Support - NIA

TH-PO368

Two Novel Equations to Estimate Kidney Function in Persons Aged 70 and Above Elke Schaeffner,[1] Natalie Ebert,[1] Pierre Delanaye,[2] Ulrich Frei,[1] Jens Gaedeke,[1] Olga Jakob,[3] Martin K. Kuhlmann,[4] Mirjam Schuchardt,[1] Markus Toelle,[1] Reinhard Ziebig,[6] Markus van der Giet,[1] Peter Martus.[5] *[1]Nephrology, Charité, Berlin, Germany; [2]Nephrology, CHU Sart Tilman, Liège, Belgium; [3]Biostatistics, Charité, Berlin, Germany; [4]Nephrology, Vivantes Klinikum im Friedrichshain, Berlin, Germany; [5]Biostatistics, Eberhard Karls University, Tuebingen, Germany; [6]Laboratory Medicine, Charité, Berlin, Germany.*

Background: In older adults current equations to estimate glomerular filtration rate (GFR) are not validated and might misclassify elderly persons in terms of their chronic kidney disease stage.

Methods: We developed two new equations based on creatinine alone and creatinine and cystatin C in a cross-sectional analysis. We used two separate original data sets for equation development (n=285) and validation (n=285) of an elderly population based sample (mean age 78 yrs) of the Berlin Initiative Study (BIS). GFR was measured using plasma clearance of iohexol. Creatinine and cystatin C assays were traceable to high-level reference materials.

Results: The new creatinine-cystatin C-based BIS2 equation yielded the smallest bias and highest accuracy followed by the creatinine-based BIS1 equation. All other equations overestimated GFR considerably. The BIS equations confirmed a high prevalence of people >70 yrs with a GFR <60 ml/min/1.73m^2 (BIS1 50.4%, BIS2 47.4%, mGFR 47.9%). Total misclassification rate for this criterion was smallest for BIS2 equation, followed by the cystatin C3 equation proposed by the CKD-Epi-Collaboration. Among the creatinine-based equations BIS1 had the smallest misclassification rate followed by the CKD-Epi equation.

Performance of eGFR equations in people >70 yrs

Equation	Bias	Precision (1st/3rd quartile)	Accuracy P_{30}(%)	Total misclassified (%)
BIS1	0.8	-5.03/6.11	95.1	49 (17.2)
MDRD	11.29	3.85/17.68	70.9	66 (23.3)
CKD-Epi	9.69	2.45/15.49	77.9	58 (20.4)
BIS2	0.87	-4.40/4.98	96.1	33 (11.6)
CysC3	9.22	3.46/14.42	81.4	58 (20.4)

CysC3: $177.6 \times \text{Creatinine}^{-0.65} \times \text{Cystatin C}^{-0.57} \times \text{age}^{-0.20} \times 0.82$ (if female)

Conclusions: In people >70 yrs with either no or mild to moderately reduced kidney function the new BIS equations are more precise and accurate tools than current equations to estimate GFR.

Funding: Private Foundation Support

TH-PO369

Impact of Body Composition on the Accurate Assessment of Kidney Function in Indigenous Australians: The eGFR Study Louise J. Maple-Brown,[1,2] Jaquelyne T. Hughes,[1,2] Leigh Ward,[3] Leonard Sunil Piers,[4] Graham Ross Dallas Jones,[5] Paul D. Lawton,[1] Andrew G. Ellis,[4] Richard J. MacIsaac,[4] Alan Cass,[6] Wendy E. Hoy,[3] Kerin O'Dea,[7] George Jerums.[4] *[1]Menzies School of Health Research; [2]Royal Darwin Hospital; [3]University of Queensland; [4]University of Melbourne; [5]St Vincents Hospital; [6]George Institute; [7]University of South Australia.*

Background: Differences in body composition raise the question that creatinine-based estimates of glomerular filtration rate (eGFR) derived for use in European populations may not be appropriate for Indigenous Australians. This study aimed to assess if incorporation of fat-free mass (FFM) measurement improves performance of eGFR in Indigenous Australians.

Methods: We measured GFR (mGFR) by plasma disappearance of iohexol over 4 hours and eGFR by modification of diet in renal disease (MDRD-4) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations, without African-American correction factor. Body composition was assessed by dual-energy X-ray.

Results: Indigenous Australians (n=135) were aged 47±15 years, 41% male, 40% with diabetes, 18% CKD stages 3-5, BMI 31±6 kg/m^2, FFM 58±14kg, mGFR 88ml/min/1.73m^2. On multiple regression of mGFR, addition of FFM (β 0.005, p<0.001) to the model (including age, gender, creatinine) resulted in improved model performance (relative reduction root mean square error 5.3%). An equation was derived for eGFR using FFM(kg), creatinine(μmol/l), age(years), sex: $\text{FFM eGFR} = 4482 \times \text{Creatinine}^{(-0.864)} \times (0.993)^{\text{Age}} \times (1.005)^{\text{FFM}} \times 0.823$ if female. Median bias(95% CI) = mGFR – eGFR:

	MDRD eGFR	CKD-EPI eGFR	FFM eGFR
All (n=135)	9.3 (6.8, 13.6)	2.6 (-0.7, 5.6)	1.2 (-1.8, 4.0)
BMI<30 kg/m2 (n=64)	8.4 (3.9, 16.1)	1.1 (-2.6, 6.3)	4.2 (-2.0, 7.4)
BMI≥30 kg/m2 (n=71)	10.3 (6.3, 14.5)	3.6 (-0.2, 6.5)	-0.1 (-5.5, 2.2)
BMI≥30 & Male (n=27)	19.3 (10.3, 23.7)	9.8 (4.2, 16.0)	-1.4 (-5.9, 3.6)*

*p<0.05 compared to CKD-EPI

Conclusions: Addition of FFM to age, gender and creatinine significantly enhanced the model assessing GFR in Indigenous Australians. However, the FFM eGFR equation derived in this cohort demonstrated significantly improved performance to CKD-EPI eGFR only in obese males.

Funding: Government Support - Non-U.S.

TH-PO370

Predicting Outcomes after Myocardial Infarction by Using the Chronic Kidney Disease Epidemiology Collaboration Equation in Comparison with the Modification of Diet in Renal Disease Study Equation: Results from the Korea Acute Myocardial Infarction Registry Joon Seok Choi, Chang Seong Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: The presence of chronic kidney disease is an independent prognostic factor in patients with myocardial infarction (MI). We compared the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and the Modification of Diet in Renal Disease (MDRD) study equation with regard to prognostic value in patients with MI.

Methods: This study analyzed a retrospective cohort of 11,050 consecutive patients who had myocardial infarction and were enrolled in the Korea Acute Myocardial Infarction Registry (KAMIR) from November 2005 to August 2008. We applied the CKD-EPI equation and the MDRD study equation to determine the estimated glomerular filtration rate (eGFR) in a cohort of patients with MI.

Results: The mean $\text{eGFR}_{\text{CKD-EPI}}$ was slightly higher than $\text{eGFR}_{\text{MDRD}}$ (73.16 versus 72.23 mL/min/1.73m^2, P < 0.001). The prevalence of $\text{eGFR}_{\text{CKD-EPI}}$ <60mL/min/1.73m^2 was 26.9%, whereas that of $\text{eGFR}_{\text{MDRD}}$ was 28.5%. The area under the receiver operator characteristic curve was significantly larger for predicting 1-year major adverse cardiovascular event (MACE) and 1-year all-cause mortality with $\text{eGFR}_{\text{CKD-EPI}}$ (0.648 versus 0.641, 0.768 versus 0.753, respectively; P < 0.001). The net reclassification index for improvement in risk of 1-year MACE and 1-year all-cause mortality were 4.09% (P < 0.001) and 9.25% (P < 0.001), respectively.

Conclusions: The application of the $\text{eGFR}_{\text{CKD-EPI}}$ demonstrated better predictive values for clinical outcomes than $\text{eGFR}_{\text{MDRD}}$ in a cohort of patients with MI.

TH-PO371

Pregnancy Outcomes in Women with Autosonal Dominant Polycystic Kidney Disease Sajeda Youssouf,[1] Matt Hall,[2] Liz Lightstone,[3] Graham Graham Lipkin,[4] Nigel J. Brunskill,[1] Sue Carr.[1] *[1]The John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; [2]Nottingham City Hospital, Nottingham, United Kingdom; [3]Imperial College Kidney and Transplant Institute, London, United Kingdom; [4]Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Background: Pregnancy is associated with adverse fetal and maternal outcomes in women with advanced chronic kidney disease. There is little evidence that the aetiology of maternal renal disease has an effect on outcomes but most information is based upon retrospective, historical data. In this study we analysed fetal and maternal renal outcomes in pregnancy in women with known Autosomal Dominant Polycystic Kidney Disease (ADPKD) at the time of conception.

Methods: Prospective data from three specialist renal obstetric services has been collected since 2003. In this analysis we identified those with a diagnosis of ADPKD at the time of conception and reviewed pregnancy outcomes and long term maternal outcomes in this group.

Results: We identified 17 pregnancies in 12 women aged 26-43. One woman had CKD 4 at conception, there were 5 pregnancies in women with CKD 3 at conception and the remainder (11 pregnancies) had CKD 1-2. Five women had treated hypertension. Five women (42%) had previously had a miscarriage or fetal loss. Fifteen pregnancies (88%) resulted in a live birth, with one stillbirth and one termination due to severe congenital malformation. Two babies (13%) were admitted to NNU and 3 (20%) had a low birth weight. Twelve (75%) were born at term, the remaining 3 were born at 34-37 weeks. Renal function remained stable in pregnancy in all women. Four women developed worsening proteinuria in pregnancy. Two women went on to develop progressive renal impairment post-partum including one woman who developed ESRD at 4 years post-partum. Both women had CKD 3b/4 at the time of conception. No woman with CKD 1-2 had progression of renal dysfunction.

Conclusions: Fetal outcomes in ADPKD are better than reported in historical case series of pregnancy in women with CKD. There is no evidence that ADPKD has an impact on maternal renal dysfunction post-partum, except in those with pre-existing advanced CKD.

TH-PO372

Estimated Glomerular Filtration Rate Equations for Non-Obese Population Can Predict Measured Glomerular Filtration Rate in Obese Population Kearkiat Praditpornsilpa, Krittaya Tiskajornsiri, Somchai Eiam-Ong, Kriang Tungsanga. *Division of Nephrology, Faculty of Medicine, Chualongkorn University, Bangkok, Thailand.*

Background: High body mass index(BMI) has been an independent risk of CKD and ESRD. The currently available methods of estimated GFR(eGFR) were not validated in high BMI or obese population. The difference of body compositions of non-obese and obese patients may affect the precision and accuracy of eGFR equations. This study aimed to validate the eGFR equations used to assess renal function in obese population.

Methods: Thai adults>18years old, BMI≥30kg/m^2 were recruited. Body composition was assessed by bioimpedance analysis. The reference GFR was determined by ^{99m}Tc-DTPA plasma clearance. A single intravenous bolus of ^{99m}Tc-DTPA was injected and blood samples were drawn at5,10,20,30,60,90,120,180,and 240 minutes post ^{99m}Tc-DTPA injection. Serum creatinine was measured by using enzymatic assay and were adjusted by IDMSreference serum creatinine. The eGFR values were calculated by eGFR equations. Thai eGFR equation and re-expressed MDRD equation with Thai racial factor are $375.5xCr^{-0.848}xAge^{-0.364}x0.712$ if female and $175xCr^{-1.154}xAge^{-0.203}x0.742$ if femalex1.129 if Thai.

Results: 104 cases were studied. The average age was43.9±13.2years. The mean BMI, BSA and waist were 36.16±6.15kg/m^2, 1.93±0.22m^2 and40.9±4.7cm, respectively. The mean skeletal muscle mass, body fat mass and percent body fat were 29.6±14.5kg, 39.1±12.6kg and 43.3±6.9% respectively. The mean Cr was 0.86±0.89mg/dL. The mean reference GFR was 109.36±8.75mL/min/1.73 m^2. The mean eGFR by re-expressed MDRD equation, CKD-EPI equation, re-expressed MDRD equation with Thai racial factor, and Thai eGFR equation were 100.0±24.3, 96.9±30.8, 100.8±26.5, and 109.4±34.7mL/min/1.73 m^2,respectively. The mean differences between referenceGFR and re-expressed MDRD,CKD-EPI, re-expressed MDRD formula with Thai racial factor, and Thai eGFR equation were expressed as 12.6±23.6, 9.3±19.2, 0.1±25.5,and 9.0±20.5 mL/minper 1.73m^{2}respectively.

Conclusions: eGFR equations for non obese population can predict measured GFR in obese population. The specific racial eGFR equation or racial correction factor improve the accuracy of eGFR calculation.

Funding: Government Support - Non-U.S.

TH-PO373

Creatinine and/or Cystatin C-Based GFR Estimation in HIV Patients Amandine Gagneux-brunon,[1,2] Pierre Delanaye,[3] Xavier Delavenne,[4] Thierry Basset,[4] Frederic Lucht,[2] Christopher R. Mariat.[1] *[1]Nephrology, Universitary Hospital of Saint-Etienne, Saint-Etienne, France; [2]Infectious Diseases, Universitary Hospital of Saint-Etienne, Saint-Etienne, France; [3]Nephrology, Universitary Hospital of Liege, Liege, Belgium; [4]Pharmacology, Universitary Hospital of Saint-Etienne, Saint-Etienne, France.*

Background: CKD is a major comorbidity in HIV+ individuals. Screening these patients for CKD and its related complications is necessary. Among the numerous GFR-estimating equations available, it is not known whether one provides a better prediction and should be privileged in this population. We aimed to determine and compare the performance of creatinine and/or cystatin C-based equations in a cohort of HIV+ patients whom true GFR was measured by a reference method.

Methods: HIV+ outpatients who have undergone a plasma iohexol clearance (mGFR) along with IDMS-traceable serum creatinine and IFFC-traceable serum cystatin C (cyst) measurement were included. Equations considered were: Cockcroft-Gault, MDRD, CKD-EPI$_{creat}$, CKD-EPI$_{cyst}$ and CKD-EPI$_{creat+cyst}$. We compared bias, precision and accuracy and the performance of each equation to detect mGFR under 60 mL/min/1.73 m².

Results: 191 patients (93% whites, 158 men, 93% under highly active retroviral therapy, mean mGFR of 94 (±26) ml/min/1.73m^2) were included. CKD was found in 19 patients (9.9 %). As compared to others equations, the Cockcroft-Gault formula was significantly most biased, less precise and less accurate. Accuracy-30% for the MDRD and all others CKD-EPI Equations was similar (from 75% to 80%). CKD-EPI$_{creat+cyst}$ was significantly better for detecting stage 3 CKD as compared to the MDRD (ROC AUC of 0.84 vs 0.73, p<0.05) as well as to the CKD-EPI$_{creat}$ (0.84 vs 0.74, p<0.05).

Conclusions: In an apparent "nephrologically" healthy, almost exclusively Caucasian HIV+ population, equations developed by the CKD-EPI group significantly outperform the Cockcroft-Gault formula. In terms of GFR estimation, MDRD and CKD-EPI$_{creat}$ display similar performance. In terms of detection of CKD, CKD-EPI$_{creat}$ is unexpectedly not superior to the MDRD study equation. In contrast, CKD-EPI$_{creat+cyst}$ is significantly better and might be an estimator of choice for this purpose.

TH-PO374

Accuracy of eGFR Equations in Adults with Sickle Cell Disease: Impact on the Estimation of the Prevalence of CKD Antonio Guasch, Mitzi W. Near, Sasikala Selvaraj. *Renal Division, Dept of Medicine, Emory University, Atlanta, GA.*

Background: Renal involvement is common in sickle cell disease (SCD). In prevalence studies in adult patients with HbSS, abnormally high albuminuria occurs in >60% of adults and may reach 80% in individuals over 40 years of age. However, the prevalence of renal insufficiency (CKD) has been reported to be low, 4-7% in previous studies, based on abnormal serum creatinine values. We hypothesized that the apparent low prevalence of CKD in SCD is due to the poor accuracy of serum creatinine and eGFR equations to detect renal insufficiency.

Methods: We determined glomerular filtration rate (GFR) by the urinary clearance of inulin in 112 adults SCD patients (43 males, 63 females, median age 34 years, range 17-65, Hb SS=81, other sickle Hb=41) studied on 203 occasions (median 1, range 1-4). The average GFR and serum creatinine values were 80±4 ml/min/1.73 (range 5-199), and 1.0±0.1 mg/dL (range 0.3-7.7), respectively. The following eGFR equations were calculated: Cockcroft-Gault (C-G), aMDRD, MDRD and CKD-epi.

Results: All estimation equations systematically overestimated GFR: C-G by 51%, aMDRD by 64%, MDRD by 53% and CKD-epi by 50%. The accuracy of the prediction was low; for instance, in CKD stages 2-3, the respective percentage of patients falling within 30% and 50% range of estimates for each equation were: C-G: 23% and 40%, aMDRD 17% and 32%,MDRD 30% and 42%, and CKD-epi 19% and 29%. When we restricted the analysis to individuals with GFR 60-89 ml/min/1.73 (n=68), the percentage of patients correctly identified as CKD-2 were 21% (C-G), 21% (aMDRD), 21% MDRD and 15% (CKD-epi), vs 9% based on abnormal serum creatinine values.

Conclusions: We conclude that current eGFR equations and serum creatinine lack accuracy to estimate true GFR in adults with SCD, and systematically overestimate GFR. In CKD stage 2, less than 25% of patients are correctly identified by eGFR. Thus, the true prevalence of CKD in the SCD population may be grossly underestimated. Specific modifications of eGFR equations may be needed in SCD patients.

Funding: NIDDK Support

TH-PO375

Ethnicity, Proteinuria, and Diabetes Predict Faster Decline in Renal Function among Minority Populations Albert M. Osei,[1] Amit J. Joshi,[1] Kalyani Perumal,[1] Huiyuan Zhang,[2] William Trick,[2] Peter D. Hart.[1] *[1]Division of Nephrology, Stroger Hospital of Cook County, Chicago, IL; [2]Collaborative Research Unit, Stroger Hospital of Cook County, Chicago, IL.*

Background: Ethnic minorities bear a disproportionately higher burden of chronic kidney disease (CKD) in the United States. Improved understanding of CKD risk factors may facilitate the adoption of new prevention and treatment strategies for this vulnerable population. We examined and here in describe the clinical characteristics of such patients at a large inner city hospital.

Methods: Adult patients (≥18 years) with CKD followed in our nephrology clinics from Jan 2008 until March 2012 were identified. CKD was defined as: (i) persistent proteinuria (>2+ on urine dipstick or >100 mg/dL) or (ii) estimated glomerular filtration rate (eGFR) <60ml/min/1.73m^2 BSA; on two separate occasions 3 months apart using MDRD formula. Patients with initial eGFR <15 were excluded.

Results: 2646 (63%) of 4198 patients had adequate data points for clinical analysis. Mean age was 59.4 (±13.5) years, and women accounted for 45% of the study cohort. African Americans constituted 56%, Hispanics 25%, Caucasians 8%, and other races 11%. Patients with CKD stages 1, 2, 3, 4 were 11%, 26%, 51% and 12% respectively; 1700 (64%) of whom had diabetes. Baseline mean eGFR was 55.8 (±23.6), and after an average follow up of 2.9 (±1) years, it declined to 44.1 (±24.6). There was no statistical difference in initial eGFR among the different ethnic groups. Patients with proteinuria had higher initial eGFR (57.3 vs. 51.3, P<0.05). Proteinuria (irrespective of diabetic status) predisposed to faster decline in renal function (-5.6 vs -1.3, P<0.05). Annual eGFR decline was much faster in diabetics compared to non- diabetics (-5.6 vs -2.9, P<0.05), and among Hispanics and African Americans compared to Caucasians (-5.5, -4.7 and -3.7 respectively, P<0.05).

Conclusions: Proteinuria, diabetes, and African American or Hispanic ethnicity is associated with increased risk of CKD progression in minority patients. Further studies are needed to better characterize risk factors and develop interventions to reduce the CKD burden in this high risk patient population.

TH-PO376

Use of Glomerular Filtration Rate Estimating Equations for Drug Dosing in HIV Aghogho A. Okparavero,[1] Hocine Tighiouart,[1] Zipporah Krishnasami,[2] Christina M. Wyatt,[3] Hiba Graham,[4] James Hellinger,[1] Lesley Stevens Inker.[1] *[1]Tufts Medical Center, Boston, MA; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Mt. Sinai Hospital, New York, NY; [4]Gilead Sciences, Inc., Foster City, CA.*

Background: Current HIV treatment guidelines recommend the use of creatinine clearance estimated by the Cockcroft-Gault (CG) equation for drug dosing adjustments in HIV-positive patients. The use of other more accurate estimating equations for drug dosing has not been studied in this population.

Methods: In a cohort of 200 HIV-positive patients on stable antiretroviral therapy (ART) with measured GFR (mGFR), we evaluated the concordance of assigned kidney function categories (>80, 50-80, 30-50 and <30 mL/min), designated by the Food and Drug Administration (FDA) Guidance for Industry for pharmacokinetic studies using mGFR to GFR estimated using the MDRD Study, CKD-EPI and CG equations.

Results: Mean mGFR was 87±26 mL/min per 1.73 m^2. The number of subjects in each of FDA kidney function categories were >80 (135), 50-80 (54), 30-50 (9) and<30 (2). Overall, the CKD-EPI equation showed the highest concordance with mGFR (79%) while the MDRD Study equation had the lowest concordance (71%). All estimating equations led to lower kidney function categories compared to mGFR. This pattern was consistent across most subgroups (Table). There was a high degree of concordance between the CKD-EPI and MDRD Study equations to the CG equation (85% and 79%, respectively).

Concordance between assigned Kidney Function Categories using Measured versus Estimated GFR.

	Concordance to mGFR (%)		
	CKD-EPI	MDRD	CG
Overall	79	71	77
Tenofovir use			
Yes	80	70	78
No	77	72	75
Age group (years)			
≤50	84	75	81
>50	69	63	68
Sex			
Women	80	69	78
Men	79	72	76
Race			
African American	84	79	74
White or other	74	63	79
BMI group (kg/m^2)			
<22	66	66	54
22-30	82	74	79
>30	81	67	89
HIV RNA viral load (copies/mL)			
Undetected	76	66	79
<1000	84	79	79
≥1000	79	79	64

Conclusions: The CKD-EPI estimating equation has the highest concordance with mGFR for FDA assigned kidney function categories. Thus, its use may lead to lower dosing related errors in HIV-infected US adults on stable ART.

Funding: Pharmaceutical Company Support - Gilead Sciences, Inc.

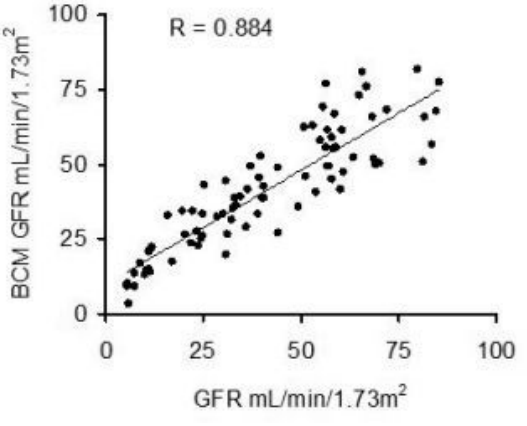
TH-PO377

Prediction of GFR in Aged Patients with a Formula Based on Serum Creatinine Adjusted for Body Composition Carlo Donadio. *Internal Medicine - Nephrology, University of Pisa, Pisa, PI, Italy.*

Background: The measurement of GFR is not feasible to clinical practice. Serum creatinine (SCr) is a simple marker of GFR impairment, unfortunately it lacks sensitivity, particularly in aged patients. Different formulas have been proposed to predict GFR from SCr, avoiding 24-hour urinary collection which is a major problem in aged patients. The aim of this study was to evaluate a new method to predict GFR in aged patients from the body cell mass (BCM) and SCr, avoiding urine collection.

Methods: Eighty adult chronic kidney disease (CKD) patients (43 males), aged 65-81 years, mean 71, with different renal function (SCr 0.7-8.8mg/dL) participated in this study. GFR was measured as the renal clearance of ^{99m}Tc-DTPA. SCr was determined with a standard laboratory method. The values of BCM were obtained by means of body impedance analysis, with a single frequency impedance analyzer. The relationship of GFR with Scr and BCM demonstrated a very high correlation between GFR and the ratio BCM/SCr. Form this relationship we derived a formula to predict GFR from the values of SCr and BCM: $_{BCM}$GFR(mL/min)= Patient's BCM (kg) x 2.69 / SCr (mg/dL). For comparison, GFR was predicted also according to Cockcroft and Gault formula ($_{CG}$GFR), and to the simplified MDRD formula ($_{MDRD}$GFR).

Results: $_{BCM}$GFR gave the most precise estimate of GFR.



In fact, $_{BCM}$GFR had the best agreement with true GFR (^{99m}Tc-DTPA), while $_{CG}$GFR and $_{MDRD}$GFR significantly overestimated true GFR. The coefficients of variation of all prediction formulas were markedly lower than that of 24h CCr (22.4%). Finally, the error of prediction of $_{BCM}$GFR was definitely lower than that of $_{CG}$GFR and $_{MDRD}$GFR.

Conclusions: In aged patients, GFR can be predicted from the values of BCM and serum creatinine. In the mean time, the impedance analysis allows to evaluate the nutritional status and the balance of body fluids compartments.

Funding: Government Support - Non-U.S.

TH-PO378

FGF-23 in Children with a Solitary Functioning Kidney: The KIMONO-Study Rik Westland,[1] Yael Abraham,[1] Michiel F. Schreuder,[2] Joanna Van Wijk.[1] *[1]Pediatric Nephrology, VU University Medical Center, Amsterdam, Netherlands; [2]Pediatric Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Fibroblast Growth Factor 23 (FGF23) has been identified as an important 'early' marker for chronic kidney disease (CKD) in adults. However, data on pediatric patients are scarce. An important patient group with a risk for CKD is children with a solitary functioning kidney. THE KIMONO-STUDY (KIdney of MONofunctional Origin) explores the applicability of FGF23 in children with a solitary functioning kidney.

Methods: 84 children (51 congenital and 33 acquired) were included in this study. Serum FGF23 was measured by C-terminal assay (Immutopics), which measures both active and inactive FGF23. GFR was estimated with the Schwartz-equation (eGFR). Serum PTH and 25OH-vitamin D were simultaneously determined. Renal injury was defined as the presence of hypertension, micro-albuminuria, eGFR < 60 ml/min/1.73m² and/or use of antihypertensive/antiproteinuric medication. CKD stage was classified by the K/DOQI guidelines.

Results: Median serum FGF23 was 106 (interquartile range 55) RU/ml at a mean age of 10.8 (SD 4.8) years, whereas mean eGFR was 100 (SD 31) ml/min/1.73m². Serum FGF23 levels showed an inverse relationship with eGFR (r = -0.403, P < 0.001) and were positively associated with PTH (r = 0.541, P < 0.001) and 25OH-vitamin D (r = 0.139, P = 0.007). Furthermore, children with CKD ≥ stage 3 had increased FGF23 levels compared to children with CKD 1 and 2 (P = 0.031) and children in the highest FGF23 tertile demonstrated a higher incidence of renal injury than children in the lowest FGF23 tertile (64% vs. 36%, respectively; P = 0.043).

Conclusions: THE KIMONO-STUDY shows that FGF23 is a potential marker for CKD in children with a solitary functioning kidney. Furthermore, FGF23 levels are elevated in a subclinical stage of CKD, which suggests an important role for FGF23 to identify early renal damage in these children. We therefore underline the need for further studies to fully substantiate the prognostic impact of FGF23 in children with CKD, and children with a solitary functioning kidney in particular.

Funding: Private Foundation Support

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HARPE Study: Prevalence of Renal Abnormalities (RA) in Chronic Hepatitis B Virus Infection (HBV+) Sabine Amet,[1] Vincent Launay-vacher,[1] Stanislas Pol,[2] Jean-pierre Bronowicki,[3] Dominique Thabut,[4] Fabien Zoulim,[5] Marc Bourliere,[6] Philippe Mathurin,[7] Victor De Ledinghen,[8] Yves Benhamou,[4] Dominique Larrey,[9] Elfie Bruce,[1] Nicolas Janus,[1] Gilbert Deray.[10] *[1]Service ICAR, Nephrology, Pitie Salpetriere, Paris; [2]Hepatology, Cochin, Paris; [3]Hepatology, Brabois, Nancy; [4]Hepatology, Pitie Salpetriere, Paris; [5]Hepatology, Croix Rousse, Lyon; [6]Hepatology, Saint Joseph, Marseille; [7]Hepatology, Claude Huriez, Lille; [8]Hepatology, Haut Leveque, Pessac; [9]Hepatology, Saint Eloi, Montpellier; [10]Nephrology, Pitie Salpetriere, Paris.*

Background: A few data are available on RA prevalence in HBV+ patients (pts). The HARPE study (Hepatitis And Renal Parameters Evaluation), multicentric, prospective, evaluated the prevalence of renal impairment (RI), and RA (electrolytes, urinary sediment) in chronic HBs antigen-positive (HBsAg(+)) pts, with active or inactive infection.

Methods: Included pts were adult, mono-infected and oral anti-HBV treatment (AT)-naïve: 268 pts were prospectively included over 2 years by 8 liver units. Statistical univariate tests and multiple linear regressions comparing the RA between inactive and active pts were performed with the SAS software 8.02 (Inc.,Cary,NC).

Results: Pts characteristics: 58% males, mean age: 42 ± 14 years, 59.6% inactive carriers, 35% had an active HBV infection including 58.2% with abnormal transaminase levels (TL), 3.8% immune tolerant and 1.5% with fluctuating TL. 47 pts (47.2% of the active pts) were supposed to start an AT. Prevalences: proteinuria: 37.4%, hematuria: 20.8%, glycosuria: 3.9%, leukocyturia: 11.7%. 55.8% of pts had a glomerular filtration rate under 90 ml/min/1.73m². 27% of pts had chronic kidney disease (CKD) stage 1 to 3 and none had CKD stage 4 or 5. Diabetes, hypertension and dyslipidemia were observed respectively in 4.6%, 9.2%, 38.8% pts. There were no significant differences in RA between active and inactive pts.

Conclusions: RA are present in 1/3 and CKD in 1/4 of HBsAg (+) pts, whatever the immune stage and before any oral AT, evidencing the need of: 1. a renal evaluation in all; 2. a regular renal monitoring before and during AT to diagnose and manage RI and adjust dose of AT to the renal function.

Funding: Pharmaceutical Company Support - Gilead Sciences

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Association of Hepatitis C Virus Infection with Chronic Kidney Disease: A Meta-Analysis Motaz Ashkar,[1] Amani Yamani,[1] Paweena Susantitaphong,[1,2] Bertrand L. Jaber.[1,2] *[1]Department of Medicine, Division of Nephrology, St. Elizabeth's Medical Center, Boston, MA; [2]Department of Medicine, Tufts University School of Medicine, Boston, MA.*

Background: Recent studies examining the association of hepatitis C virus (HCV) infection with development of chronic kidney disease (CKD) using markers of kidney damage (proteinuria or estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m²)

have yielded conflicting results. We conducted a meta-analysis to examine the association of HCV infection with prevalence and incidence of CKD, including development of end-stage kidney disease (ESKD).

Methods: We searched the literature for cross-sectional and cohort studies using MEDLINE (through December 2011), the Cochrane Central Register of Controlled Trials, the American Society of Nephrology abstracts and the bibliographies cited in the retrieved articles. The search strategy was limited to human studies with no language restrictions. Using random-effects model meta-analyses, we computed pooled adjusted odds ratios (ORs) (for cross-sectional studies) and pooled hazard ratios (HRs) (for cohort studies) of HCV infection status with outcomes of interest including proteinuria, low eGFR (<60 ml/min/1.73 m²), and ESKD.

Results: 9 cross-sectional and 6 cohort studies were identified (n=863,123). By meta-analysis, in 5 cross-sectional studies, there was a significant association between HCV infection and proteinuria (adjusted OR 1.57; 95% CI 1.18, 2.10; P=0.002; I^2 index 74%); however, in 7 cross-sectional studies, there was no association with low eGFR (adjusted OR 1.00; 95% CI 0.81, 1.24; P=0.97; I^2 index 94%). In 4 cohorts, there was no association between HCV infection and incident CKD as defined by low eGFR (pooled adjusted HR 1.12; 95% CI 0.86, 1.48; P=0.40). However, in 2 cohorts, there was a significant association between HCV infection and incidence of ESKD (pooled adjusted HR 1.67; 95% CI 1.54, 1.81; P<0.001; I^2 index 0%).

Conclusions: This meta-analysis demonstrates that HCV infection is associated with proteinuria and incident ESKD. Although there was substantial heterogeneity of effect size estimates across studies, these observations require further study.

TH-PO381

Use of Various Weight Descriptors in the Cockcroft-Gault Equation for Estimating Creatinine Clearance in an Asian Diabetic Population Priscilla P. How,[1,2] Hui Yang Lim,[1] Christine Teng.[1,3] *[1]Pharmacy, National University of Singapore; [2]Medicine (Nephrology), National University Hospital, Singapore; [3]Pharmacy, Tan Tock Seng Hospital, Singapore.*

Background: The Cockcroft-Gault (CG) equation is used to estimate creatinine clearance (CrCl) and determine drug doses for patients with renal impairment. Different weight descriptors such as ideal body weight (IBW), lean body weight (LBW), adjusted body weight (ABW) and no body weight (NBW) have been proposed for more accurate estimated CrCl (eCrCl) for different patients. Rounding of serum creatinine (SCr) to 1mg/dL is also practised in elderly patients though with limited evidence. The objectives of this study were to determine which weight descriptor in the CG equation best approximates measured CrCl (mCrCl) and whether rounding of SCr to 1mg/dL with use of TBW in the CG equation is appropriate in estimating CrCl in our local elderly patients.

Methods: This retrospective cross-sectional study included adult diabetic patients from the Tan Tock Seng Hospital, Singapore. Patients' age, gender, weight, height, SCr and mCrCl from 24-h urine collection were obtained. The eCrCl was calculated using CG equation with different weight descriptors to yield CG_{TBW}, CG_{IBW}, CG_{LBW}, $CG_{ABW0.3}$, $CG_{ABW0.4}$ and $CG_{NBW,}$ respectively. These were compared to mCrCl for bias, precision and accuracy using one-way ANOVA followed by a post-hoc Tukey's test.

Results: A total of 1230 cases (625 male, 605 female) were included for analysis. Mean mCrCl was 61.9±33.3 ml/min. Of the eCrCl calculated using various weight descriptors, $CG_{ABW0.3}$ and $CG_{ABW0.4}$ yielded CrCl of 62.0±29.6 ml/min and 63.5±30.5 ml/min respectively, which were similar to mCrCl (P>0.05). Furthermore, $CG_{ABW0.3}$ and $CG_{ABW0.4}$ had relatively high levels of precision and accuracy to mCrCl than other weight descriptors. For patients aged ≥65 with SCr <1mg/dL, CG_{NBW} (eCrCl 61.5±6.9 ml/min) provided an unbiased estimate of mCrCl (63.1±22.4 ml/min) when SCr was rounded to 1 mg/dL (P>0.05).

Conclusions: The use of ABW in the CG equation to estimate CrCl ($CG_{ABW0.3}$ or $CG_{ABW0.4}$) provided the best estimates of mCrCl in our local diabetic population. CG_{NBW} is most appropriate when using rSCr in elderly patients with SCr <1mg/dL.

TH-PO382

Performance of Untimed Urine Protein-to-Creatinine Ratio for Estimation of 24-Hour Proteinuria among Morbidly Obese Patients: A Prospective Cohort Study Ion D. Bucaloiu, H. Lester Kirchner, Evan Norfolk, James E. Hartle, Robert M. Perkins. *Nephrology Department, Geisinger Medical Center.*

Background: Reduced lean body mass (e.g., in the elderly) results in lower creatinine generation and misleading proteinuria estimates using untimed urine protein-to-creatinine ratios (PCR). In morbidly obese (MO) patients, creatinine generation differs from lean individuals and thus it is unknown whether PCR accurately estimates 24-hr proteinuria. We conducted a prospective cohort study to test the hypothesis that in MO patients, PCR performs poorly for the estimation of proteinuria.

Methods: Adults with BMI > 40 kg/m² evaluated in the Nephrology and/or Weight Management Clinics at Geisinger were eligible; those >65 years, with active malignancy, ESRD, solid organ transplantation, or pregnancy were excluded. Patients returned 24-hr urine collections at the time of blood and untimed urine sample collection. 24-hr urine collection accuracy was defined as measured creatininuria within 35% of expected, with expected creatininuria derived using a previously validated formula. The primary analysis examined the correlation, bias, and precision between PCR and 24-hr proteinuria.

Results: 54 patients submitted 24-hr urine collections. Among those with accurate collections (n = 35), median age was 47 years, 88.6% were Caucasian, and 48.6% male. Median (IQR) BMI was 45.1 (41.7, 52.1) kg/m². Median (range) proteinuria was 0.09 (0.03, 9.8) g. In sensitivity analyses using alternative collection accuracy thresholds (40, 45, 50, and 55 % of expected creatininuria), results were similar.

Performance characteristics of untimed protein-to-creatinine ratio compared with 24-hour proteinuria in morbidly obese. (n=35)

Bias, mean (sd), g proteinuria.	Precision, mean of the square root of the difference between actual and estimated 24-hour proteinuria, g proteinuria	Correlation* (p-value)
-0.05 (0.27)	0.253	0.59 (0.0002)

Conclusions: PCR estimates 24-hr urine poorly among MO patients, and should not be relied upon for clinical decision-making in this population.

Funding: Private Foundation Support

TH-PO383

Contrast Intensity Following CT Scan Correlates with Kidney Function Katherine Yang,[1] Brittany E. Yee,[1] Cynthia Santillan,[2] Ravindra L. Mehta.[1] *[1]Nephrology, School of Medicine, University of California, San Diego, San Diego, CA; [2]Radiology, University of California, San Diego, San Diego, CA.*

Background: Iodinated contrast agents are utilized to measure glomerular filtration rates (GFR) since their elimination correlates with inulin clearance. Since these agents are almost entirely removed by glomerular filtration we hypothesized that the duration and intensity of the kidney image would correlate with underlying kidney function and could be used to approximate GFR.

Methods: Data was reviewed from 191 consecutive patients who had abdominal CT scans at a single center over a 1 month period and had detailed information on the type, amount and timing of contrast agent and had baseline and serial creatinine measurements over the first 7 days following the scan. GFR was estimated (eGFR) from the baseline serum creatinine using the CKD Epi equation. The time to appearance, duration and intensity of the image were recorded using a standardized method for ascertaining the pixel intensity in Hounsfield units over specific regions in each kidney. The rate of change of contrast intensity between the venous and delayed phases was computed as the log slope and was correlated with the eGFR.

Results: 33(17.3%) patients had a baseline sCr >1 mg/dl (mean eGFR of 62 ml/min) and had a slower change in contrast intensity in comparison to those with sCr<1 (mean eGFR 99 ml/min). The change in contrast intensity correlated with the underlying eGFR.

Rate of change of contrast intensity

eGFR ml/min	Number	Mean sCr mg/dl	Mean eGFR	Mean L Kidney Slope*	Mean R Kidney Slope*
<60	14	1.61	45	.29	.28
60-90	89	.87	77	.36	.37
>90	88	.67	110	.36	.36

* p value =.043

Conclusions: Our data shows a good correlation between the rate of change in contrast intensity in kidney CT images and eGFR. This information could be used as a non-invasive method to measure GFR. Further studies are needed to confirm these findings in patients with other co-morbidities.

Funding: NIDDK Support

TH-PO384

Estimated Glomerular Filtration Rate Is a Good Marker of Renal Clearance for Indoxyl Sulfate and p-Cresyl Sulfate Bjorn Meijers, Liesbeth Viaene, Ruben Poesen, Bert Bammens, Kathleen Claes, Pieter Evenepoel. *Department of Nephrology, University Hospitals Leuven, Leuven, Belgium.*

Background: Indoxyl sulfate (IS) and p-cresyl sulfate (PCS) contribute to CKD progression and cardiovascular disease. Clearance of these solutes predominantly is by active tubular secretion. For any given stage of CKD IS and PCS serum concentrations show a wide inter-individual dispersion. From this it is inferred that eGFR is inadequate to estimate renal clearances of IS and PCS. Renal clearance studies have not been performed to date.

Methods: We studied renal clearances of IS and PCS in the Leuven CKD study cohort (NCT00441623). Associations between eGFR (CKD-EPI) and clearances of creatinine, IS and PCS were analyzed using Spearman rank coefficients. Multivariate linear regression models were built to evaluate the relative contribution of eGFR, demographics and generation rates to IS and PCS concentrations.

Results: We analyzed renal clearances in 203 patients with CKD 1-5. IS renal clearances (median 18, min-max 1.6 - 142 ml/min) exceed PCS clearances (median 7, min-max 0.9 - 43 ml/min) about threefold (clearance ratio mean 2.8, SD 0.9). eGFR was closely associated with measured creatinine clearance (r=0.91), but also with renal clearances of IS (r=0.84) and PCS (r=0.82) (all P<0.0001). In multivariate regression, IS concentrations are determined (R^2 0.74) by eGFR, IS generation rate (both P<0.0001), age and sex (both P<0.05). PCS concentrations are determined (R^2 0.74) by eGFR and PCS generation rate (both P<0.0001).

Conclusions: Estimated GFR provides an acceptable estimate of renal solute clearance of indoxyl sulfate and p-cresyl sulfate. Intriguingly, while renal clearances of indoxyl sulfate and p-cresyl sulfate are closely associated, absolute indoxyl sulfate clearances exceed those of p-cresyl sulfate by threefold and show important interindividual variation. These data suggest substantial differences between tubular transporter affinities and/or the involvement of separate transporter systems for IS and PCS.

TH-PO385

Long-Term Renal Function after Tandem High-Dose Chemotherapy and Autologous Stem Cell Transplantation in Pediatric Patients with High-Risk Solid Tumors Heeyeon Cho. *Department of Pediatrics, Samsung Medical Center, Seoul, Korea.*

Background: High-dose chemotherapy and autologous stem cell transplantation (HDCT/autoSCT) has improved the survival of children with high-risk solid tumors. However, long-term organ damage such as renal insufficiency has emerged as a major cause of treatment-related mortality and morbidity in patients who have undergone HDCT/autoSCT. Little is known about the incidence of chronic kidney disease (CKD) in pediatric patients with HDCT/autoSCT.

Methods: We undertook as retrospective study in order to assess the incidence, risk factors and outcome of HDCT/autoSCT-associated CKD in 58 pediatric patients who were transplanted at Samsung Medical Center from 2008 to 2010. Various renal function parameters were evaluated before each HDCT/autoSCT, and 1 year after the second HDCT/autoSCT.

Results: Thirty-nine patients were male, and 19 were female and the median age at first HDCT/autoSCT was 4.2 years. Primary disease included neuroblastoma (42%), retinoblastoma (6%), and brain tumor (52%). Twelve months after second HDCT/autoSCT, CKD [e.g. glomerular filtration rate (GFR) estimated using Schwartz formula < 90 ml/min/1.73m^2] was noted in 27 patients (46.5%) and the mean GFR was 59.1 ± 27.6 ml/min/1.73m^2. CKD with GFR < 60 ml/min/1.73m^2 was noted in 11 patients (18%). Four of these patients had severe CKD stage 4 and 5, with a GFR < 30 ml/min/1.73m^2 and 2 patients needed dialysis. Three patients received renal replacement therapy during early posttransplant period and progressed to CKD. There was no significant difference in the sex, primary disease, or the baseline GFR between the patients with or without CKD. The age at 1st HDCT/autoSCT was younger in the patients with CKD compared to those without CKD (3.9 ± 3.2 vs. 6.1 ± 4.9 years, P=0.053). Subclinical tubular dysfunctions during early posttransplant period were associated with the development of CKD (P=0.037).

Conclusions: CKD is a relatively common late complication of HDCT/autoSCT. Because renal function may decline with time, there is a risk to progress to end-stage renal disease in pediatric patients. The current findings suggest the necessity for long-term follow-up of vulnerable patients with CKD.

Funding: Government Support - Non-U.S.

TH-PO386

Effect of Renal Failure on Metabolic Rate Using the Doubly-Labeled Water Isotope Technique Enric Vilar,[1,2] Beverley Summerhayes,[1,2] Ashwini Machado,[1] Andrew Garrett,[2] David Wellsted,[2] Ken Farrington.[1,2] *[1]Renal Unit, Lister Hospital, Stevenage, Hertfordshire, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: Renal failure is associated with metabolic disturbances including lean body mass depletion. Metabolic rate in CKD is poorly understood and predictive algorithms are required for provision of accurate dietary advice. Defining the effect of renal failure on metabolic rate is required to develop improved algorithms for calculating dialysis dose in end-stage renal failure. We investigated the impact of CKD on Total Energy Expenditure(TEE) using the gold-standard multi-point doubly-labeled water technique.

Methods: Subjects with CKD were prospectively recruited, half with CKD1-3 and half CKD4-5. All undertook a metabolic analysis with indirect calorimetry to measure basal metabolic rate and respiratory quotient. Over 14 days, TEE was measured using doubly-labeled water. After ingestion of ^{18}O and ^{2}H doses, isotope excretion was measured using mass spectroscopy of interval urine samples allowing CO_2 production and TEE to be calculated. Estimates of TEE were compared between those CKD1-3 and CKD4-5. To determine whether eGFR predicted TEE, multiple linear regression was used.

Results: 40 subjects were recruited of mean age 60±SD15 years. There was no significant difference in body size parameters and comorbidities between the CKD1-3 and 4-5 groups. Serum hemoglobin was lower in those with CKD4-5(p=0.02) but albumin and CRP were similar. The doubly-labelled water technique was successfully used with high correlation of isotope excretion curves(r=-0.99 for both ^{18}O and ^{2}H). Basal metabolic rate was non-significantly lower in the CKD4-5 compared to the CKD 1-3 group(mean 1488 v 1638kCal/day, p=0.11). TEE was non-significantly lower in the CKD4-5 group (2429 v 2620, p=0.17). In multiple regression for TEE, eGFR was not a significant predictor of TEE (p=0.94).

Conclusions: The doubly-labelled water technique is effective for measuring TEE in the CKD population. There was a non-significant trend towards lower basal metabolic rate and TEE in those with CKD4-5. We did not find evidence that advanced CKD is associated with substantial changes to metabolic rate.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO387

Prevalence, Outcome, and Predictors of Cardio-Renal Syndrome in Children with Dilated Cardiomyopathy Ahmad Kaddourah,[1] Stuart Goldstein,[1,2] Jeffrey A. Towbin,[2] John L. Jefferies.[1,2] *[1]Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, OH; [2]The Heart Institute, CCHMC, Cincinnati, OH.*

Background: Cardio-renal syndrome (CRS) describes variety of acute or chronic conditions where the primary failing organ can be either the heart or kidney. The prevalence, predictors and outcome of CRS has not been reported in children with dilated cardiomyopathy (DCM).

Methods: Data from patients > 1 year of age with DCM enrolled in the Pediatric Cardiomyopathy Registry (PCMR) was assessed. CRS was defined as glomerular filtration rate <90 ml/min/1.73m^2 estimated by modified Schwartz formula (eGFR). Patients with eGFR>150 ml/min/1.73m^2 were excluded. CRS prevalence was determined by cross-sectional design in patients newly diagnosed with DCM using the baseline PCMR data. Survival analysis compared patients with vs. without CRS using annual PCMR data. Regression models were applied on both baseline and annual data to study the interactions between eGFR and different echocardiographic measures.

Results: The baseline data had 484 children with DCM. Serum Cr data to calculate eGFR were available for 92 patients. Fifty six (62.0%) patients had eGFR < 90 ml/min/1.73m^2, which is greater than the 48% prevalence published in 3256 healthy historical controls (P<0.001). CRS patients had a mean eGFR of 61 (±22.6) vs. 108 (±14) (P<0.001). Death occurred in 8 (14%) of CRS patients compared to no deaths in patients without CRS (P=0.052) after 7 years follow-up. Univariate regression models showed a significant positive correlation between fractional shortening (FS) and eGFR (P= 0.0013, r^2 = 0.09). No correlation was observed between eGFR and ejection fraction (EF), left ventricular end-diastolic dimension (LVEDD) and LVEDD Z-scores.

Conclusions: This is the first study to show that CRS is common in children newly diagnosed with DCM and is associated with higher risk of subsequent death. Worsening FS was associated with decreasing eGFR which suggests that patients with poor FS might have significant CRS. We suggest kidney functions should be evaluated systematically in children with DCM to assess for chronic kidney disease and direct potential management.

TH-PO388

Kidney Injury Molecule-1 (KIM-1) Is a Promising Urinary Biomarker to Detect Cardiorenal Syndrome in Children with Dilated Cardiomyopathy (DCM) Ahmad Kaddourah,[1] Stuart Goldstein,[1,2] John L. Jefferies.[1,2] *[1]Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, OH; [2]The Heart Institute, CCHMC, Cincinnati, OH.*

Background: Type II cardiorenal syndrome (CRS) describes renal dysfunction in the presence of chronic heart dysfunction. Novel urinary acute kidney injury biomarkers (NUB) have not been studied in children with DCM. We aim to 1) Evaluate the correlation between four NUB and severity of LV dysfunction, and 2) Determine the best marker to detect CRS in children with DCM.

Methods: We are conducting a cross-sectional study in children with DCM and LV dysfunction. Enrolled children had an echocardiogram to measure the LV ejection fraction (EF). Urine samples were collected at the same visit to measure the NUB: KIM-1, NGAL, IL-18, and L-FABP. NUB levels were adjusted for each mg of urinary creatinine. Glomerular filtration rate was estimated using modified Schwartz formula (eGFR) when Creatinine level (Cr) is available. Univariate analysis was conducted between patients (pts) with EF< 45% and EF ≥ 45%. ROC analysis was performed for all NUB and GFR. 2x2 tables were constructed from NUB cutoffs to maximize specificity for EF <45%.

Results: 30 pts (14.2 ±6.4 years old) have been enrolled to date. 13 (43%) had EF<45%. Although all NUB levels were higher in pts with EF <45%, only KIM-1 was significantly higher [836.5 (±844.1) vs. 442.8 (±249.5) pg/mg$_{cr}$, $P_{one\text{-}tailed}$=0.04]. Area under the curve for KIM-1 was 0.61 to predict EF <45%, compared to 0.53 for eGFR. A KIM-1 cutoff of 900 pg/mg$_{cr}$ yielded highest correct EF classification (38% sensitivity, 94% specificity). 6/30 (20%) pts had KIM-1 >900; 5/6 (83%) had EF of <45% vs. 8/24 (33%) with KIM-1<900 ($P_{one\text{-}tailed}$=0.03).

Conclusions: Our pilot data suggest DCM pts with EF<45% tend to have higher NUB urinary levels indicating worse CRS than pts with EF≥45%. Urinary KIM-1 may be the most predictive test to detect CRS compared to other NUB and eGFR. We anticipate confirming this finding by increasing the study's power.

TH-PO389

Evaluation of a Non-Invasive Survey to Identify Individuals with Chronic Kidney Disease in a Community-Based Screening Program Donna H. Harward,[1] Heejung Bang,[2] Yichun Hu,[1] Abhijit V. Kshirsagar.[1] *[1]University of North Carolina at Chapel Hill; [2]University of California Davis.*

Background: We previously developed the SCORED questionnaire based on nine readily available characteristics (age, gender, anemia, hypertension, diabetes, cardiovascular disease, heart failure, peripheral vascular disease, and proteinuria)to identify individuals with a high likelihood of having prevalent CKD.Individuals are given a numeric value for the presence of these characteristics, and those having a cumulative integer score of ≥ 4 have a 20% chance of having CKD, defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2. While SCORED is well validated in national datasets such as NHANES and ARIC, its utility has not been demonstrated in community screenings where it could be a simple and inexpensive test. This study sought to evaluate SCORED in an ongoing community screening program in rural North Carolina with a predominantly African American population.

Methods: We conducted screenings as part of the UNC Kidney Education Outreach Program from December 2010 through August 2011. The study employed a cross-sectional design where the SCORED questionnaire was administered at the same time that blood was collected. Serum creatinine was used to calculate MDRD and CKD-EPI eGFR. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SCORED for eGFR < 60 ml/min/1.73 m2 were calculated.

Results: Of the 359 persons who participated in the 16 separate screenings, 169 participants provided blood for measuring creatinine. The average age of the group was 55 years, 80% were African American, and 73% female. About 9% of the participants

providing blood had eGFR < 60 ml/min/m2. The sensitivity and specificity of SCORED was 100% and 34% respectively; PPV was 13%, and NPV was 100% using the MDRD eGFR. Similar test characteristics were seen using the CKD-EPI eGFR to define CKD.

Conclusions: The SCORED questionnaire demonstrated good screening test characteristics in a community based setting with a predominantly African American population. It may provide a simple and cost-effective alternative to invasive screening tests for CKD.

Funding: Other U.S. Government Support

TH-PO390

Association of Body Composition and Estimated Glomerular Filtration Rate in Elderly Males and Females Hrefna Gudmundsdottir,[1,4] Margret B. Andresdottir,[1] Lesley Stevens Inker,[3] Runolfur Palsson,[1,4] Vilmundur Gudnason,[2] Andrew S. Levey,[3] Olafur S. Indridason,[1] Thor Aspelund.[2,4] *[1]Landspitali University Hospital, Iceland; [2]Icelandic Heart Association, Iceland; [3]Tufts Medical Center, Boston, MA; [4]University of Iceland, Iceland.*

Background: Changes in body composition (BC) and kidney function are commonly seen with aging. Limited data is published on the association of kidney function and BC in the elderly population. The goal of the study was to determine if there was an association between BC, strength and estimates of kidney function in elderly males and females.

Methods: The data were obtained from the population based Age, Gene/Environment Susceptibility - Reykjavik Study. A total of 1.610 males and 2.059 females had complete data. BMI (kg/m2) was calculated from height and weight and abdominal circumference (AC) measured using standardized protocols. Fat% was measured by bio-electrical impedance and prediction equations were used to calculate fat free mass (FFM) and total fat mass (TFM). Leg muscle strength (LMS) was measured using an adjustable dynamometer chair. Estimated GFR (eGFR) was calculated by the CKD-EPI equation using IDMS-standardized creatinine. Descriptive statistics and linear regression analysis adjusted for age, hypertension (HTN) and diabetes (DM) were performed.

Results: All participants of the study were white and 56.1% were females. The mean (SD) age of the population was 76.2 (5.5) years, 79.8 % had HTN and 12.1 % DM. The changes in BC measures and LMS associated with 10 ml/min/1.73m^2 increase in eGFRare shown in the Table for males and females, respectively. A significant interaction with sex was found for the association of eGFR with AC and LMS (p<0.05).

Table

	Males	Females
BMI (kg/m^2)	-0.14 ± 0.07 *	-0.31 ± 0.07 ***
FFM (kg)	-0.41 ± 0.13 **	-0.36 ± 0.01 ***
FAT (kg)	-0.12 ± 0.12	-0.37 ± 0.11 ***
Abd (cm)	-0.33 ± 0.19	-0.86 ± 0.19 ***
Str (Nwt)	-5.73 ± 1.81 **	1.70 ± 1.08

*p<0.05, **p<0.005, ***p<0.0001

Conclusions: Measures of BC and strength are negatively associated with GFR estimated from serum creatinine in older adults and this relationship can be different for men and women. These findings need to be confirmed with measured GFR.

TH-PO391

Renal Biopsy in the Elderly Is Safe, Leads to Disease Specific Treatment and Prevents End Stage Renal Failure Candice Clarke, Megan Griffith, H. Terence Cook, David Taube, Khrishanthne Sambasivan. *Imperial College Kidney and Transplant Centre.*

Background: With the growing aging population elderly patients make up a large proportion of new nephrological referrals but pragmatically are less likely to be offered renal biopsies. A caveat to this is that they may be disadvantaged in not receiving disease specific treatment. Evidence on whether older patients benefit from such intervention is limited. The aim of this study was to analyse the indications, histological features and outcomes from biopsies in elderly patients.

Methods: We retrospectively studied patients ≥70yrs of age who underwent a first native renal biopsy at our unit between 2005-2011.

Results: 306/2583 [11.9%] of all native biopsies were performed in patients ≥70yrs. Mean age at time of biopsy was 74.6±3.8yrs. 202[66.0%] were males,198[64.7%] were caucasoid. Mean follow up was 3.2±1.7yrs.

Patient survival at 1,3 and 5yrs was 93.4,84.0 and 70.1%. Dialysis free survival at 1,3 and 5yrs was 89.3,83.4 and 76.2%.24[7.8%] patients were dialysis dependent at the time of biopsy,20 received specific therapy and 8 recovered function. Dialysis dependence was associated with increased risk of death,p=0.0088.

The indications for biopsy were CKD:171[55.9%],AKI:63[20.6%],nephrotic range proteinuria with preserved function:43[14.1%] and nephrotic with renal impairment:29[9.5%]. The main histological findings were:pauci-immune glomerulonephritis [GN] in 34[11.1%],other primary GN:72[23.5%],diabetes:45[14.7%],tubulo-interstitial nephritis:38[12.4%],amyloid/myeloma:16[5.2%],ATN:13[4.4%],non-specific scarring:75[24.8%] and other pathologies 14[4.6%].101[33.0%] had a positive serological screen and 180[58.8%] had an abnormal urinary sediment. Patients with pauci-immune GN were more likely to be dialysis dependent at the time of biopsy,p=0.001.147[48.0%] of patients required disease specific treatment.Complication rates were low with only 5[1.6%] of patients developing perinephric haematomas or macroscopic haematuria.

Conclusions: This study shows that full evaluation of renal disease in elderly patients is important. Specific treatment can be indicated, without which there may be increased risk of ESRF and mortality.

TH-PO392

Comparison of Performance for Estimating Glomerular Filtration Rate Using MDRD and CKD-EPI Equation in the Korean Population Yun Jung Oh,[1] Su Mi Lee,[1] Hajeong Lee,[1] Dong Ki Kim,[1] Yon Su Kim.[1,2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Kidney Research Institute Medical Center, Seoul National University Hospital, Seoul, Republic of Korea.*

Background: The Chronic Kidney Disease Epidemiology Collaboration(CKD-EPI) equation was developed to resolve limitation of the Modification of Diet in Renal Disease(MDRD) equation that was derived from CKD patients. The CKD-EPI equation shows better accuracy in estimating glomerular filtration rate(GFR) than MDRD equation in most studies, but not all. Furthermore, it has not been externally validated in Korean population.

Methods: We compared the estimations of MDRD and CKD-EPI equations to a gold standard GFR measurement using plasma ^{51}Cr-EDTA clearance. All subjects from the study population composed of 30 healthy volunteer and 102 CKD patients underwent a GFR measurement and simultaneous renal function assessment including serum creatinine. Bias, precision, and accuracies between estimated and measured GFR were calculated. These values were analyzed within strata of variables.

Results: Overall, CKD-EPI equation had less bias(-3.4 versus -8.4) and high accuracy than MDRD equation(*P*=0.004). CKD-EPI equation demonstrated better agreement between measured GFR and estimated GFR in the classification into CKD stages (Cohen's κ 0.714 versus 0.575). While both equations underestimated GFR in the subjects with GFR≥60 ml/min/1.73m^2, overestimated in GFR<60 ml/min/1.73m^2. In case of GFR≥60 ml/min/1.73m^2, CKD-EPI equation had higher accuracy (P=0.004) than MDRD equation. In GFR<60 ml/min/1.73m^2, MDRD equation had less bias but, the accuracy was not significantly different from CKD-EPI equation. The accuracy of CKD-EPI equation was higher in younger than 60 years(*P* <0.001), but was not different in subjects older than 60 years.

Conclusions: In general, CKD-EPI equation was superior to MDRD equation for estimating GFR, and these were influenced by GFR and age.

Funding: Government Support - Non-U.S.

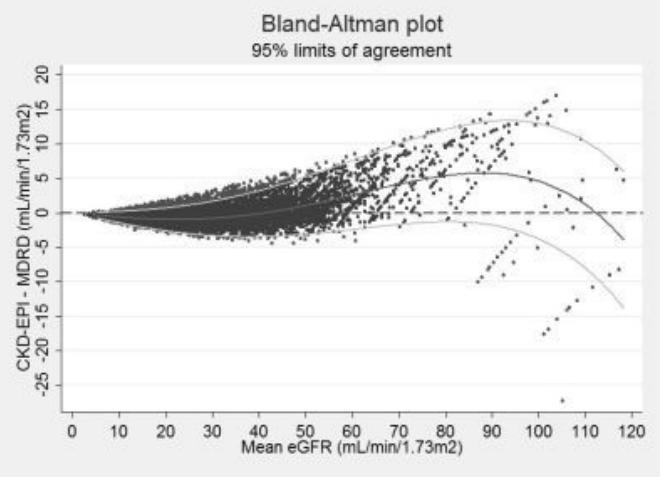
TH-PO393

Comparison of MDRD and CKD-EPI Equations: An Alternative Method Based on Bland-Altman Plot and Fractional Polynomial Regression Antonio Santoro,[1] Jacopo Lenzi,[2] Marcora Mandreoli,[1] Paola Rucci,[2] Mattia Corradini,[3] Dino Gibertoni,[2] Maria Pia Fantini.[2] *[1]Nephrology, Dialysis and Hypertension, Policlinico S. Orsola-Malpighi, Bologna, Italy; [2]Department of Public Health and Statistc, Alma Mater Studiorum University of Bologna, Bologna, Italy; [3]Nephrology and Dialysis, Arcispedale S. Anna IRCSS, Reggio Emilia, Italy.*

Background: Evidence from the literature suggests that the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation more accurately estimates glomerular filtration rate (GFR) than the Modification of Diet in Renal Disease (MDRD) Study equation using the same variables, especially at higher GFR. The Bland-Altman graphical method has been used to compare the two equations, but it is biased because the assumption that the mean and the standard deviation of the difference between the two equations are constant across all GFR values does not hold. This study is aimed to suggest an alternative method, based on polynomial fractional regression, to compute the confidence interval of the difference between the two equations.

Methods: The data used are drawn from the Italian PIRP (Prevention of Progressive Renal Insufficiency) registry, that collects prospectively demographic and clinical information on 10,687 outpatients with CKD in the Emilia-Romagna region since 2004.

Results: Evaluation of the agreement between these formulas showed that there was a good correlation between CKD- EPI and MDRD for values of eGFR between 40 and 10 mL/min/1.73m^2. In this range the two formulas may be interchangeable.



Conclusions: In the patients with CKD in its early stages (CKD2, CKD3a), we suggest to monitor the time course of renal function with the same estimation formula, because the deviation between the values obtained is quite large. Clinical implications of this method in terms of accurate prediction of risk of renal failure will be discussed.

Funding: Government Support - Non-U.S.

TH-PO394

Poor Outcome in Incident Dialysis Patients with High Estimated GFR (eGFR), but Not with High Measured GFR (mGFR), Is due to Confounding by Protein-Energy Wasting (PEW) Olof Heimburger, Peter F. Barany, Peter Stenvinkel, Bengt Lindholm, Abdul Rashid Tony Qureshi. *Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: Several studies have shown that a high eGFR at initiation of dialysis is predictive of a poor outcome. The aim of the present study was to assess to what extent this was due to confounding by PEW.

Methods: We studied 399 patients with CKD 4-5 (248 males, mean age 53 years) close to start of dialysis. Renal function was measured using the average of urinary urea and creatinine clearance from a 24-h urine collection (mGFR) and compared to eGFR from the body surface area corrected Cockroft Gault equation (CG), the 4 and 6 variable MDRD equations, and the CKDepi equation. 3-year survival (censored for transplantation) was calculated using the Cox model. The relationships between eGFR and nutritional variables (e.g., lean body mass DEXA (LBM), hand-grip strength (HGS), subjective global assessment (SGA)) were studied.

Results: There were strong correlations between eGFR CG and the other eGFRs (r^2=0.76-0.81), and particularly between eGFR MDRD 4V, 6V and CKDepi (r^2=0.94-0.89), but the correlations between mGFR and the eGFRs were less strong (r^2=0.38-0.45). Using the Cox model (including age, sex, diabetes, cardiovascular disease and p-CRP) a high eGFR (any method) predicted poor outcome, but there was no difference in survival between the tertile groups of mGFR. When SGA was added in the model, the impact of eGFR on outcome was borderline or non-significant. Though correlations between mGFR and nutritional variables were weak (except for LBM), negative correlations were found between eGFR and nutritional variables, for all eGFR methods:

	mGFR	eGFR CG	eGFR MDRD4V	eGFR MDRD6V	eGFR CKDepi
ml/min/1.73m²	6.7±2.1	10.2±3.1	6.8±2.3	7.0±2.6	6.8±2.7
vs. HGS	rho= -0.02, ns	rho= -0.21***	rho= -0.28***	rho= -0.25***	rho= -0.28***
vs. LBM	rho= -0.17**	rho= -0.11, ns	rho= -0.15**	rho= -0.16**	rho= -0.18***
vs. SGA	rho= 0.03, ns	rho= 0.23***	rho = 0.35***	rho = 0.29***	rho= 0.34***

Values are mean ±SD, **p<0.01, ***p<0.001

Conclusions: Poor prognosis with high eGFR at start of dialysis is to a large extent due to confounding by PEW.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

TH-PO395

A New Risk Scoring System for Predicting ESRD within One Year: A Tool for Judging, When to Prepare for Renal Replacement Therapy (RRT) Fumika Taki,[1] Keita Hirano,[1] Kenichiro Koitabashi,[1] Kumiko Shimasaki,[1] Masahiko Nagahama,[1] Tomio Omata,[2] Yasuhiro Komatsu.[1] *[1]Department of Internal Medicine, Division of Nephrology, St.Luke's International Hospital, Tokyo, Japan; [2]Clinical Epidemiology, St.Luke's International Hospital, Tokyo, Japan.*

Background: On the late stage of CKD patients, nephrologists need to consider RRT. Timing of starting the preparation of RRT for these patients should be, not too early or late. For judging the timing, knowing the last one year risk for ESRD may be useful. We developed the new scoring system predicting ESRD within a year, from our previous clinical data.

Methods: From 2004 to 2011, CKD stage 4 to 5 patients in St.Luke's international hospital, Tokyo, Japan, were participated. We determined these patients' development of ESRD by initiation of dialysis or GFR<5ml/dl/min. Their characteristics of clinical parameters; baseline GFR, laboratory data, co-morbidity and medications were assessed. Logistic regression was used to identify one year ESRD risk factors, and these factors were used to construct a risk score predicting one-year ESRD risk.

Results: 265 patients were included, 52.3% were male, mean age was 64.6 years and their baseline eGFR was 16.5±7.2mg/dl/min. 30 patients (11.2%) turned in ESRD within a year: they tend to have higher rates of diabetes (70% vs. 37%) and higher usage of diuretics (80% vs. 38%), more existing proteinuria (5.2g/day vs. 1.2g/day) and lower baseline GFR (13.8 vs. 16.8 mg/dl/min) compared with non-ESRD patient within a year. We included these factors and made risk scores (Table 1). This scoring system has 0.78 sensitivity and 0.84 specificity, for predicting dialysis within a year. From these scores, "total risk score over 6" patients have more than 80% risk of ESRD within a year. These patients need starting RRT education.

Conclusions: Our new risk scoring system contributes to show appropriate timing for starting RRT education for the late stage of CKD patients.

Risk score predicting ESRD within a year

Risk	Risk Score	Total Risk Score/ ESRD risk within a year (%)
eGFR 20-30mg/dl/min	0	0/12%
eGFR 10-20mg/dl/min	1	1/25%
eGFR <10mg/dl/min	2	2/37%
Proteinuria <1g/day / >1g/day	0/2	3-5/62%
Diabetes -/+	0/2	6-7/82%
Diuretics -/+	0/2	8/94%

TH-PO396

Prevalence of Chronic Kidney Disease in Patients Treated with Lithium Compared to Other Psychiatric Patients Olafur S. Indridason,[1] Engilbert Sigurdsson,[2,3] Matthias Halldorsson,[2] Runolfur Palsson.[1,3] *[1]Division of Nephrology; [2]Division of Psychiatry, Landspitali - the National University Hospital of Iceland; [3]University of Iceland, Reykjavik, Iceland.*

Background: We have previously reported an increased prevalence of chronic kidney disease (CKD) in adult patients on long-term lithium treatment compared to healthy controls. The purpose of this study was to examine if the same holds true when patients with psychiatric disorders are used as controls.

Methods: In this retrospective study, we obtained information on all adult subjects taking lithium during the years 2003-2010 by examining records of serum lithium measurements performed at the University Hospital in Reykjavik and prescriptions for lithium in the National Drug Prescription Database. The control group consisted of all patients above the age of 18 years who attended the Psychiatry Outpatient Clinic at the University Hospital and had at least one serum creatinine (SCr) measurement available in the hospital records, excluding those on lithium therapy. SCr was obtained from medical records and the eGFR was calculated from the lowest SCr value in the last year of measurement for each subject using the MDRD equation. CKD was defined as eGFR <60 mL/min/1.73 m².

Results: A total of 1577 subjects received at least one lithium prescription during the study period, 454 (25.2%) of whom had no recorded measurements of serum lithium or SCr. The remaining 1123 subjects had a median (range) age of 46 (18-98) years, and 659 were female (58.7%). The control group comprised 4549 subjects with a median age of 36 (18-89) years, 2623 (57.7%) of whom were female.

Prevalence of CKD in the lithium and control groups, by age

Age group (years)	Lithium group	Control group	P-value	OR (95% CI)
18-30	0%	0.1%	-	-
30-40	3.2%	0.2%	<0.001	17.0 (3.5-82.4)
40-50	6.9%	0.4%	<0.001	20.2 (6.0-69.1)
50-60	13.9%	1.2%	<0.001	13.4 (6.0-30.0)
60-70	25.5%	2.9%	<0.001	11.6 (5.4-24.8)
>70	41.8%	7.6%	<0.001	8.7 (4.3-17.5)

Conclusions: Patients receiving lithium therapy have a significantly increased risk of CKD, compared to other patients with psychiatric disorders. These findings are comparable to those of our previous study with controls drawn from the general population.

TH-PO397

Creatinine and Cystatin-C Based Equations in Comparison with 51-Chromium EDTA in Prediction of Glomerular Filtration Rate in Malay Population in University Malaya Medical Centre Maisarah Jalalonmuhali, Kok Peng Ng, Soo Kun Lim, Li Ping Tan, Tee Chau Keng, Yip-Boon Chong, Wai Yew Kong, Chew Ming Wong. *Division of Nephrology, University Malaya Medical Centre, Kuala Lumpur, Malaysia.*

Background: Accurate measurement of renal function is very important, however gold standard measurement of GFR can only be used on a very limited scale. Creatinine based eGFR equations are widely used but the performance may vary depending on ethnicity. We propose a study to validate the accuracy of available eGFR formulas in Malay population.

Methods: This was a cross-sectional study recruiting patients from UMMC Renal clinic. All patients underwent 51-Chromium EDTA clearance for measurement of GFR. Blood was obtained for serum creatinine and plasma cystatin-C. Serum creatinine was determined by IDMS reference modified Jaffe kinetic assay. The predictive capabilities of Cockcroft-Gault (CG), CG corrected for body surface area (CGBSA), 4-MDRD, CKD-EPI and cystatin-C based equations were calculated. Data were analysed using SPSS version 20 and bias, precision and accuracy were determined.

Results: A total of 51 subjects with mean age of 58.7 years and BMI of 26.5 kg/m² were recruited. The mean reference GFR was 42.04 (17.70 – 111.10) ml/min/1.73m². Estimated GFR based on CG, CGBSA, 4-MDRD, CKD-EPI and cystatin-C formula were 42.25 (16.90 – 98.20), 40.47 (16.52 – 115.52), 35.90 (14.00 – 98.00), 37.24 (14.00 – 121.00) and 49.76 (21.00 – 90.00) respectively. While all eGFR formulas correlated well with the reference GFR (0.860, 0.877, 0.848, 0.854, 0.811), overall the 4-MDRD equation performed the best. Subgroup analysis showed similar performance for CKD-EPI and 4-MDRD equations in patients with GFR < 60 ml/min/1.73m², while CG was noted to be superior in patients with GFR ≥ 60 ml/min/1.73m² and in patients with BMI < 23 kg/m². For patients with BMI ≥ 23 kg/m², the 4-MDRD equation performed better. Interestingly, in non-diabetics, cystatin-C formula was the most accurate equation.

Conclusions: The 4-MDRD equation was better in estimating GFR in Malay population specifically in the subgroup of GFR < 60 ml/min/1.73m² and BMI ≥ 23 kg/m². Cystatin-C formula was better in non-diabetic group.

TH-PO398

Prevalence and Risk Factors for Chronic Kidney Disease in a Community-Based Population in Japan Hiroyuki Watatani, Yohei Maeshima, Hiroko Yamasaki, Norikazu Hinamoto, Haruyo Ujike, Hitoshi Sugiyama, Hirofumi Makino. *Dept. of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Okayama, Japan.*

Background: Chronic kidney disease (CKD) is associated with increased risk for end-stage renal disease and cardiovascular morbidity and mortality. Recent reports indicate CKD affects 10–15% of the adult population worldwide. We aimed to determine the prevalence and the risk factors for CKD in general population of Okayama, Japan.

Methods: We conducted a community-based cross-sectional cohort study with 8239 adults aged 40 years and over (40-75 yo) who received the national health checkup program in Japan (Specific Health Checkups and Guidance System) in 2011 living in Okayama city. CKD was determined by estimated glomerular filtration rate (eGFR) calculated by modified MDRD equation for Japanese, and proteinuria assessed by urine dipstick. Clinical and anthropometric data were collected. Descriptive statistics, Anova, Chi-square test, multiple-logistic regression test were performed.

Results: Mean age of participants was 67±8 yo, 28% had overweight (BMI>25). The prevalence of CKD was 22.8%. The adjusted risk factors of CKD included age, hyperuricemia (> 7.0 mg/dl) (OR 3.2 [2.72-3.8], p<0.0001), obesity (BMI>25) (OR 1.21 [1.06–1.37], p= 0.0034), and past history of cardiovascular events (OR 1.38 [1.12–1.69], p =0.0021). In subjects with hypertension and prehypertension and a normal blood pressure (over 140/90 mmHg), the risk for CKD was significantly greater (OR 1.19) than those with optimal blood pressure (less than 120/80 mmHg). Subjects with hyperuricemia (7.0-7.9 mg/dL), high normal serum uric acid level (6.0-6.9) exhibited increased risk for CKD (OR 3.89, 2.17, respectively) than those with normal level (less than 6.0). Gender differences and past history of stroke were not identified as significant risk factors.

Conclusions: In the current rather elderly general population, CKD was very common with a prevalence of 22.8%. These results suggest that subjects with elderly age, obesity, mildly elevated serum uric acid, hypertension and previous history of cardiovascular events possess increased risk for CKD, requiring appropriate instruction and follow-ups.

TH-PO399

Prevalence of Chronic Kidney Disease in Primary Care in a Central European Country: A Cross-Sectional Study Yuki Tomonaga,[1] Thomas D. Szucs,[2] Lorenz Risch,[3] Patrice M. Ambuehl.[4] *[1]Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland; [2]European Center of Pharmaceutical Medicine, University of Basel, Basel, Switzerland; [3]Labormedizinisches Zentrum Dr. Risch, Schaan, Liechtenstein; [4]Renal Division, Stadtspital Waid, Zurich, Switzerland.*

Background: As severe chronic kidney disease (CKD) is usually identified based on typical symptoms, little information is available on the prevalence of clinically silent mild to moderate CKD in a primary care setting. Our aim was to estimate the prevalence of CKD in primary care in Switzerland.

Methods: A multicenter, cross-sectional study with randomly selected general practitioners (GP) was performed. Adults visiting the GPs during defined periods were asked to participate. Demographic and social variables, clinical status and co-morbidities were reported on a questionnaire. Urine and blood samples were analyzed in a central laboratory. Renal status was assessed using the KDIGO classification [1]. Extrapolation to national level was adjusted for age and gender.

Results: 1000 individuals were included. 57% were females and the mean age was 57±17 years. Overall, 41% of the patients had normal estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR), whereas 36% of the subjects had slightly reduced excretory renal function with physiological albuminuria based on normal ACR. About one tenth of the patients had a substantially reduced eGFR of <60 ml/min/1.73m^2, and 17% showed relevant proteinuria (ACR >30 mg/g creatinine). Almost one fourth of the subjects had either a substantial reduction in renal function or high levels of proteinuria. Extrapolation to national level suggests that about 18% of primary care patients may suffer from CKD.

Conclusions: CKD prevalence in a primary care population is high. Screening may be advisable, in particular as CDK prevalence is likely to rise over the next decades due to an ageing population and common epidemiological risk factors.

Reference: 1 Levey AS et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney international (2011) 80,17-28.

Funding: Pharmaceutical Company Support - Abbott AG, Switzerland

TH-PO400

Characteristics of Renal Insufficiency in Korean Multiple Myeloma Patients: Reversible Factors from Renal Dysfunction Hyun Chul Whang, Eun Sil Koh, Yu Ah Hong, Sungjin Chung, Cheol Whee Park, Yong-Soo Kim, Yoon-Sik Chang. *Department of Internal Medicine, The Catholic University of Korea College of Medicine, Seoul, Korea.*

Background: Multiple myeloma (MM) is frequently accompanied by renal insufficiency which has been regarded as a poor prognosis factor for MM. It has been known that the incidence and characteristics of MM in Asia are different from those in western countries. The aim of this study is to evaluate risk factors of renal impairment and to investigate reversible factors from renal failure in patients with MM.

Methods: The patients newly diagnosed with MM from 2005 to 2008 at a single center in Korea were included. We investigated factors associated with renal insufficiency and those related to recovery from renal dysfunction after 12 week of treatment of MM.

Results: Renal insufficiency was recognized in 79 (36%) of 221 patients at the time of diagnosis of MM. When comparing characteristics between patients with renal insufficiency (n=79) and patients with normal kidney function (n=142), there were no significant differences in types of immunoglobulin and amounts of serum free light chains. In the binary logistic regression analysis, presence of diabetes (OR=4.12, p=0.02), β2–microglobulin level (OR=1.01, p=0.02) and serum calcium level (OR = 1.936, p<0.01) were independence risk factors of renal failure in MM patients. After 12 weeks treatment, 24 (30%) of 79 patients with renal insufficiency recovered normal renal function. No improvement of renal function was seen mainly in patients with male gender, IgA type MM, high β2–microglobulin level, increased 24 hour urine protein excretion and poor response to chemotherapy. In the binary logistic regression analysis, only good response to chemotherapy (OR=4.9, p<0.01) was associated with renal function recovery.

Conclusions: Diabetes, β2–microglobulin and amount of urine protein were independent risk factor for development of renal failure in Korean MM patients, and the response to chemotherapy significantly influenced recovery of renal function.

TH-PO401

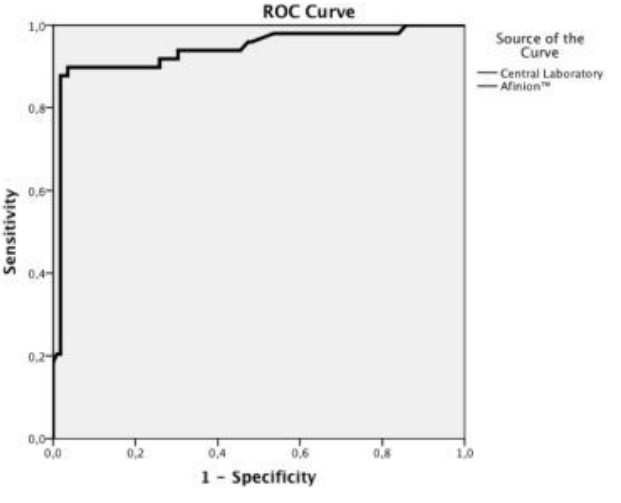
Utility of a Point of Care Device in the Diagnosis of Albuminuria in Type 2 Diabetes Mellitus Patients Laura Cortes-sanabria, Miguel Tapia Alanis, Victor Omar Frías-navarro, Blanca Liliana Maldonado-ruiz, Erika Fabiola Gómez-garcía, Karla Gabriela Meza-torres, Héctor R. Martínez Ramírez, Alfonso M. Cueto-Manzano. *Unidad de Investigacion Medica en Enfermedades Renales, Hospital de Especialidades, CMNO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

Background: Albuminuria (Album) is a key measurement for diagnosis of nephropathy in high risk groups. Afinion™ ACR is a point of care test for quantitative determination of albumin/creatinine ratio (ACR) in urine, which uses a solid phase immunochemical assay for Album and an enzymatic colorimetric test for creatinuria. A portable device may have obvious advantage in certain situations, for example screening programs; however, the accuracy of the method should be adequately demonstrated.

Aim: To establish the accuracy of Afinion™ ACR compared to central laboratory methods (spectophotometry for Album measurement and two-point kinetic measurement for creatinuria) in DM2.

Methods: DM2 patients of any age, sex and duration DM were included. In a first morning urine sample, both Afinion® ACR and central laboratory methods were performed. All measurements in the central laboratory were performed by the same personnal, blinded to details of the samples. Microalbuminuria (MA) was defined as ACR 30-300 mg/g, and macroalbuminuria (MAA)>300 mg/g.

Results: 161 patients with age of 62 ± 11 years were studied; 88 (55%) women, and 79 (49%) with hypertension. MA was found in 31 (19%) patients, and MAA in 18 (11%). Sensitivity of Afinion™ ACR was 90% and specificity 98%; both positive and negative predictive values were 95%. Global accuracy of the test was 0.96% (maximum value 1). The best cut-off point for MA was 28.7 mg/g, and the area under the curve (±SE) was 0.94 ± 0.02 (95% confidence interval of 0.89-0.99).



Conclusions: Afinion™ ACR is a rapid, valid, and reliable method for albuminuria diagnosis in DM2 patients.

TH-PO402

Prevalence and Associations of Proteinuria in Australian Scleroderma Patients: A Retrospective Review Holly L. Hutton,[1] Joanne Maree Sahhar,[2] Gene-siew Ngian,[2] Peter G. Kerr,[3] Kevan Polkinghorne.[3] *[1]Nephrology, The Geelong Hospital, Geelong, Victoria, Australia; [2]Rheumatology, Monash Medical Centre, Clayton, Victoria, Australia; [3]Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.*

Background: Previous studies suggest that proteinuria is a predictor of death in this multisystem, chronic autoimmune disease. However whether the presence of proteinuria is related to traditional cardiovascular risk factors or Scleroderma itself is unclear. We aimed to assess the prevalence and associations of dipstick positive proteinuria in a cohort of 659 patients with scleroderma.

Methods: Subjects with scleroderma without missing data at baseline (N=659 of 1104) were obtained from the Australian Scleroderma Cohort Study (ASCS). Proteinuria was defined as trace or more positive on urine dipstick. Predictors of proteinuria were assessed using logistic regression and mortality assessed using Cox models.

Results: 582 (88%) subjects were female with median age 57.5 years. 67% had limited scleroderma, 26% diffuse disease, 6% mixed connective tissue disease (MCTD). 7.9% had coronary artery disease (CAD), 31.8% hypertension and 2.7% diabetes. Prevalence of proteinuria was 8.5% (3.6% trace, 4.9% one plus or greater). On univariate analysis, scleroderma disease classification (p=0.022) but not hypertension (p=0.065) was associated with proteinuria. On multivariate analysis, subjects with MCTD were three times as likely to have proteinuria (OR 2.99) compared to limited disease. Other independent predictors of proteinuria were eGFR and age, but not diabetes, hypertension or CAD. Pulmonary hypertension (HR 3.49, 95% C.I. 1.69-7.25) but not proteinuria (HR 2.17, 95% C.I. 0.70-6.74) independently predicted mortality.

Conclusions: The prevalence of proteinuria in this cohort was higher than in age matched Australian population estimates. Associations were age, eGFR and scleroderma subtype but not traditional cardiovascular risk factors.

TH-PO403

Estimating Glomerular Filtration Rate in Elderly Chinese Patients with Chronic Kidney Disease Xun Liu,[1] Chenggang Shi,[1] Linsheng Lv,[2] Cailian Cheng,[1] Hua Tang,[1] Tan-qi Lou.[1] *[1]Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China; [2]Operating Room, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China.*

Background: Chronic kidney disease (CKD) has become a world widely problem needed particularly special attention. In the previous study, a group of 103 elderly CKD patients were investigated to evaluate the applicability of formulas based on serum creatinine (SC) levels. In this study, we increased the sample size and sought to evaluate these formulas again.

Methods: 319 elderly Chinese patients with CKD were enrolled. SC was determinedenzymatically. The ^{99m}Tc-DTPA-GFR was used as the standard GFR (sGFR). The mean age was 70.0 ± 6.8 years. The mean sGFR was 39.4 ± 21.8 ml/min/1.73 m^2. The Cockcroft-Gault-equation, 4-variable MDRD equation, 6-variable MDRD equation, Jelliffe-1973-equation and Hull-equation were tested.

Results: On the Bland–Altman plot, the precision ranged from 58.5 ml/min/1.73 m^2 to 76.4 ml/min/1.73 m^2. Median of difference ranged from -0.3 ml/min/1.73 m^2 to -4.3 ml/min/1.73 m^2. Accuracy with a deviation less than 15% ranged from 27.9% to 32.9%. Accuracy with a deviation less than 30% ranged from 53.6% to 57.7%. Accuracy with a deviation less than 50% ranged from 74.9% to 81.5%. CKD stage misclassification ranged from 42.9% to 44.2%. However, none of the equations had accuracies that reached the 70% while differing less than 30% from the sGFR. And the agreement limits of the equations except the Cockcroft-Gault equation exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m^2.

Conclusions: When SC was measured by the enzymatic method, GFR estimation equations mayshow great bias in elderly Chinese patients with CKD. Further improved equations are needed. If conditions are not available, the Cockcroft-Gault equation may be more accurate for elderly Chinese patients with CKD.

Funding: Government Support - Non-U.S.

TH-PO404

Comparison of Calculated CRUSADE Bleeding Risk with MDRD and Cockroft Gault Formulae in Patients Presenting with Non ST Elevation MI Muhammad Ali Abdool,[1] Hsu Pheen Chong,[2] Matthew George Parry, Mahvash Zaman,[1] Andrew Kuk,[1] Bhavna Pandya.[2] *[1]Aintree Cardiac Centre, Aintree University Hospital, Liverpool, United Kingdom; [2]Aintree Renal Unit, Aintree University Hospital, Liverpool, United Kingdom.*

Background: Patients with NSTEMI are at risk of bleeding with antithrombotics and invasive investigations. It is practice to calculate this risk using the evidence-based formula from the CRUSADE trial using the CG formula. However, since 2006, the 4 variable MDRD formula has been used to establish eGFR and define the stage of CKD in the UK and worldwide. This potentially results in the use of two different formulae (MDRD & CG) to calculate renal function and hence CRUSADE bleeding scores.

Methods: A total 165 patients admitted to Aintree University Hospital, Liverpool over a 5 month period between April and August 2011 with the diagnosis of NSTEMI were included in the analysis. MDRD and CG eGFR values and the respective CRUSADE bleeding risks were calculated.

Results:
Calculated CRUSADE risks with different eGFR formulae

	CG risk						
		Very Low	Low	Moderate	High	Very High	Total
	Very Low	11	3	0	0	0	14
Low		10	14	11	2	0	37
MDRD Risk	Moderate	1	6	18	6	0	36
	High	1	0	10	18	7	36
	Very High	0	0	0	3	44	47
	Total	23	23	39	29	51	165

There was adequate correlation between the calculated values for eGFR (R=0.7, p<0.005) although not enough to be considered interchangeable. There was correlation in calculated risk between the 2 formulae for 105 patients (63%). Only 4 (2.4%) patients had more than one grade risk difference and only 1 (0.6%) of these was 2 risk grades different.

There is no impact on the 38 patients who have a low or very low bleeding risk as calculated by both formulae. Out of the remaining 127 patients, there was correlation in 88 (69.3%) patients, with the rest (30.7%) having a discrepancy in bleeding risks. This has an influence on choice of antithrombotic therapy, invasive investigations and most importantly can be an indication of clinical outcome.

Conclusions:

· None of the very high risk patients were missed by either eGFR calculations.

· 2.4% of patients had the risk scores differ by more than 1 grading

· 30.7% of patients with moderate or higher CRUSADE bleeding risk differed in calculated estimations as per the 2 formulae.

TH-PO405

Comparison of Creatinine-Based Estimations of Glomerular Filtration Rate with Iohexol Glomerular Filtration Rate in Obese Subjects Philip D. Evans,[1] Tim J. James,[2] Garry Tan,[1] Chris W. McIntyre.[1,3] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Clinical Biochemistry, Oxford Radcliffe Hospitals, Oxford, United Kingdom; [3]School of Graduate Entry Medicine and Health, University of Nottingham, Derby, United Kingdom.*

Background: Obesity is an established risk factor for chronic kidney disease. Current methods for estimating glomerular filtration rate(GFR) in the obese can be inaccurate and the most appropriate equation is uncertain.

Methods: 23 obese subjects were recruited from a single centre. Each participant underwent iohexol GFR(iGFR) assessment and serum biochemistry tests. GFR was estimated using MDRD and CKD-EPI equations, corrected and uncorrected for body surface area(BSA). The Cockcroft-Gault(CG) formula, using actual and lean body weight(LBW), was used to estimate creatinine clearance(CrCl). Comparison of equations to iGFR were performed using the Bland-Altman method.

Results: The mean age(SD) was 50±11yrs, 61% were male, mean weight(SD) was 150.1±21.6kg, and mean iGFR was 124.7±24.6mL/min. Comparisons between iGFR and estimating equations of GFR are shown in table 1.
Comparison of Estimating Equations with iGFR

	MDRD(mL/min/ 1.73m2)	MDRDBSA (mL/min)	CKD-EPI (mL/min/ 1.73m2)	CKD-EPIBSA (mL/Min)	CGCrCl(mL/min)	CGCrClLBW (mL/min)
Mean±SD	83.1±14.2	124.0±24.3	89.5±12.5	134.2±24.2	191.3±42.5	95.8±24.5
Mean Difference with iGFR (95%CI)	41.6 (33.7 to 49.5)	0.7 (-5.8 to 7.2)	35.2 (27.8 to 42.5)	-9.5 (-15.4 to -3.6)	-66.6 (-76.7 to -56.6)	27.7 (21.7 to 33.6)
Upper Limit of Agreement (95%CI)	87.7 (74.1 to 101.3)	38.7 (27.5 to 49.9)	78.1 (65.5 to 90.7)	25 (14.9 to 35.2)	-7.4 (-24.9 to -10.1)	61.2 (51.3 to 71.1)
Lower Limit of Agreement (95%CI)	-4.46 (-18.1 to 9.1)	-37.3 (-48.5 to -26.1)	-7.7 (-20.3 to 4.9)	-44.0 (-54.2 to -33.9)	-125.8 (-143.3 to -108.3)	-5.8 (-15.7 to 4.1)

MDRDBSA and CKD-EPIBSA had the smallest mean differences with iGFR but both still had large ranges between upper and lower limits of agreement.

Conclusions: Creatinine based equations for estimating GFR show little agreement with iGFR in obese subjects. This may have significant repercussions in the diagnosis and management of kidney disease in the obese. Further study of how these equations perform in obese subjects with reduced GFR would be of benefit.

Funding: Clinical Revenue Support

TH-PO406

Estimation of Glomerular Filtration Rate in South Asians from the General Population in Pakistan Saleem Jessani,[1] Andrew S. Levey,[2] Rasool Bux,[1] Lesley Stevens Inker,[2] Muhammad Islam,[1] Nish Chaturvedi,[3] Christopher R. Mariat,[4] Christopher H. Schmid,[2] Tazeen H. Jafar.[1,5] *[1]Aga Khan University, Karachi, Pakistan; [2]Tufts Medical Center, Boston, MA; [3]Imperial College London, United Kingdom; [4]University de Saint-Etienne, Saint-Etienne, France; [5]Duke-NUS Graduate Medical School, Singapore.*

Background: Unlike the US and UK, laboratories in South Asian countries do not routinely report estimated glomerular filtration rate (eGFR). The objectives of the study were to 1) evaluate the performance of existing eGFR equations in South Asians, and 2) modify the existing eGFR equations and/or develop a new eGFR equation specific to this population.

Methods: We measured GFR (mGFR) using urinary clearance of inulin in 581 adults in Karachi, Pakistan. The performance of the CKD-EPI and MDRD Study equations was assessed. A modified CKD-EPI equation and a new estimating equation with age, gender

and serum creatinine were developed using linear regression. The main outcome measures were bias (median difference between eGFR and mGFR), accuracy (P_{30} percent of eGFR values that were within 30% of mGFR), precision (the inter-quartile range (IQR) of the bias) and root mean squared error (RMSE); reported as bootstrapped estimates and 95% confidence intervals (CIs) based on 1000 replications.

Results: The South Asian correction factor for CKD-EPI was $0.721 \times eGFR^{1.049}$.

Performance of GFR Estimating Equations

GFR Estimating Equations	RMSE (95% CIs)	Median Bias (95% CIs)	IQR (95% CIs) ml/min/1.73 m2	P30 (95% CIs)
MDRD Study Equation	0.273 (0.253, 0.293)	8.4 (6.4, 9.8)	28.7 (25.8, 31.9)	68.6 (64.6, 72.7)
CKD-EPI Equation	0.243 (0.226, 0.259)	7.0 (6.0, 9.3)	22.7 (20.3, 25.3)	76.8 (73.1, 80.2)
South Asian CKD-EPI Equation	0.243 (0.224, 0.260)	NA	22.3 (20.0, 25.0)	82.3 (79.0, 85.4)
South Asian New Equation 1	0.238 (0.223, 0.255)	NA	22.5 (20.3, 24.9)	82.6 (79.7, 85.8)

NA, Not applicable

Conclusions: The original CKD-EPI is more precise and accurate than the MDRD Study equation in estimating GFR in South Asian population in Karachi and would be preferred for eGFR reporting. The application of a South Asian correction factor may further improve the performance of CKD-EPI and should be evaluated in other studies.

Funding: Other NIH Support - NIH-Fograty International Center

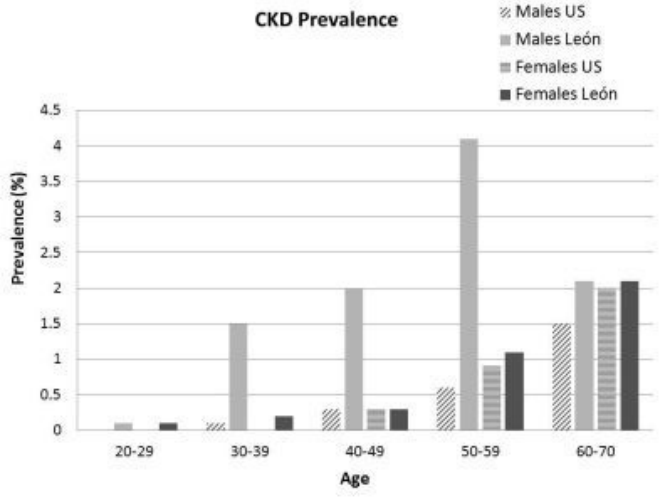
TH-PO407

Prevalence of Chronic Kidney Disease in León, Nicaragua Jill Lebov,[1] Douglas Morgan,[1] Edgar M. Pena,[3] Scott Leonard Sanoff,[2] Yichun Hu,[1] Romulo E. Colindres,[1] Susan L. Hogan.[1] *[1]UNC, Chapel HIll, NC; [2]UVA, Charlottesville, VA; [3]Centro de Investigación e Intervenciones en Salud, León, Nicaragua.*

Background: Several epidemiologic studies have pointed to an excess of chronic kidney disease (CKD) in the Pacific coastal regions of Nicaragua and El Salvador, particularly among males aged 18-50. These studies have been conducted in small, non-random samples, mainly in rural areas. Population prevalence of CKD has not been estimated in this region.

Methods: The Pacific coastal municipality of León, Nicaragua is home to a demographically and geographically diverse population. A random sample was selected from the León Health and Demographic Surveillance system, a unique resource representative of all León residents. Blood specimens collected from 2033 participants were analyzed for serum creatinine level, using the kinetic Jaffe method with calibrated instruments. Glomerular filtration rate was estimated (eGFR) using the CKD-EPI equation, with CKD defined as eGFR<60 ml/min/1.73m^2. León estimates were compared to US estimates based on NHANES 2001-2008 data, stratified by age and sex.

Results: In this large, population-based study, CKD prevalence was 6.1% in Leon versus 2.9% in the US. Leon CKD prevalence was significantly greater among males (9.9%) compared to females (3.7%) and increased with age. León CKD prevalence was highest among males age 50-59 (4.1%), whereas US prevalence was highest among females age 60-70 (2.0%). León estimates were greater than US estimates across all strata of age and sex.



Conclusions: León CKD prevalence estimates are comparable to those found in regional studies, but are more than double the prevalence observed in the US, and validate the growing body of research indicating a CKD epidemic in Pacific coastal Central America.

Funding: Other NIH Support - Fogarty International Center

TH-PO408

Comparison of Elevated Albuminuria: First Morning with Random Urine in National Health and Nutrition Examination Survey Adults, 2009-2010 Sharon Saydah,[1] Meda E. Pavkov,[1] David A. Lacher,[2] Mark Eberhardt,[2] Cindy Zhang,[2] Nilka Rios Burrows,[1] Andrew S. Narva,[3] Paul W. Eggers,[3] Desmond Williams.[1] *[1]DDT, CDC, Atlanta, GA; [2]NCHS, CDC, Hyattsville, MD; [3]NIDDK, NIH, Bethesda, MD.*

Background: In the United States, over 10% of the adult population meets diagnostic criteria for chronic kidney disease (CKD) based on single estimated glomerular filtration rate (eGFR) <60 ml/min/1.72 m² or elevated albumin/creatinine ratio (ACR ≥30 mg/g) in a random urine specimen. Clinical guidelines for diagnosis of CKD, however, recommend elevated ACR to be confirmed in at least two urine specimens. We report measures of agreement between two urine collections in National Health and Nutrition Examination Survey (NHANES) participants and their impact on CKD estimates.

Methods: In 2009-2010, NHANES participants provided two untimed urine specimens for sequential ACR measurement: an initial random urine collected in the NHANES Mobile Examination Center and a subsequent first morning void collected at home. Elevated albuminuria was calculated overall, by demographics, diagnosed diabetes and hypertension status, and eGFR.

Results: The distribution of ACR differed from the random compared to the first morning sample. The median ACR was 6.0 mg/g for the random sample and the median ACR was 4.5 mg/g for the first morning sample. Overall, 43.5% of adults with elevated ACR in a random urine also had elevated ACR in a first morning urine. This percent was higher among those 50 years and older (48.9%), males (53.3%), those with diagnosed diabetes (56.3%), hypertension (51.5%) or eGFR <60 ml/min/1.72 m² (56.9%). The observed agreement for ACR status between random and first morning void urine was 94.3% and the kappa coefficient was 0.51. Using the confirmed elevated ACR to define CKD resulted in a lower prevalence (11.6%, 95% CI 10.2-13.6) than using random spot urine level only (15.2%, 95% CI 13.7-17.4).

Conclusions: The prevalence of elevated ACR was significantly higher in random urine specimens than in first morning urine collections. Measuring persistence of albuminuria and the correct assessment of albumin excretion are both key steps in the early detection of CKD.

TH-PO409

Urinary Excretion of Liver-Type Fatty Acid-Binding Protein as a Marker of Progressive Kidney Function Deterioration in Patients with Chronic Glomerulonephritis Shan Mou, Qin Wang, Beili Shi, Jialin Li, Zhaohui Ni. *Renal Division, Renji Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China.*

Background: Liver type fatty acid binding protein (L-FABP) is expressed in human proximal tubules. The aim of this study was evaluation of urinary L-FABP and the value of basal urinary L-FABP excretion as a prognostic indicator of the progression of kidney function impairment in patients with chronic glomerulonephritis (CGN) was also assessed.

Methods: 123 patients with newly diagnosed, biopsy-proven primary CGN were included in the study. In all patients, and in 28 healthy subjects, L-FABP was measured using an ELISA. In the patients, risk factors of the progression of impairment of kidney function were evaluated. The patients were in follow-up for 5 years.The progression of kidney function impairment was defined as a reduction of GFR > 5 mL/min/ 1.73 m² / year during follow-up.

Results: Urinary excretion of L-FABP in the patients with CGN (76.58±34.61 ug/g. cr) was greater than in the healthy subjects. A significant negative correlation between L-FABP excretion and eGFR (R=-0.565, P < 0.01) and a positive correlation between L-FABP excretion and proteinuria (R=0.501, P < 0.01), serum creatinine (R=0.601, P < 0.01) were found. Kaplan–Meier analysis revealed that L-FABP excretion >76.58 ug/g. cr predicts progression of renal function. The cut off values for L-FABP at 119.8μg/g cr was found to be more sensitive. The sensitivity and specificity of were 87.5% and 90.5%.

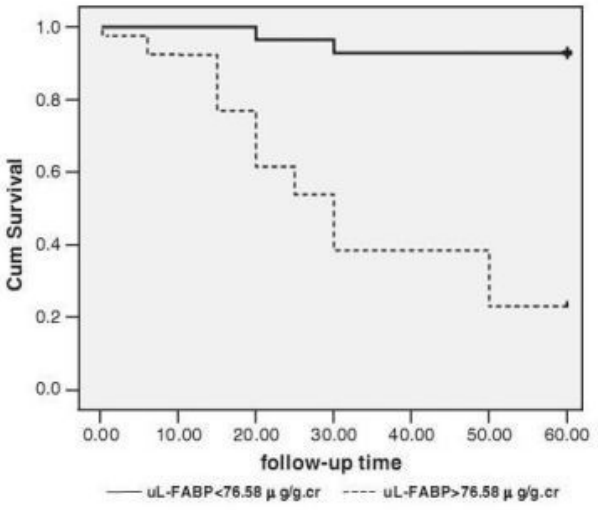


Fig. 1. Survival and uL-FABP in the whole group. uL-FABP > 76.58 µg/g.cr at presentation was associated with a significantly poorer outcome. Log rank = 24.47.

Conclusions: Urinary L-FABP excretion may be considered as a good marker of an activated L-FABP pathway in patients with newly diagnosed CGN and as a potentially risk factor of progressive kidney function impairment.

Funding: Government Support - Non-U.S.

TH-PO410

Double-Blind, Randomized Placebo-Controlled Clinical Trial for the Efficacy of Tacrolimus in the Patients with Albuminuric, Normotensive IgA Nephropathy

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Background: The optimal treatment for IgA nephropathy (IgAN) remained unknown in patients with mild to moderate proteinuria with normal blood pressure who are not tolerable to the renin angiotension aldosterone inhibitor (RASI), well. Tacrolimus reduces proteinuria by suppressing the immune response and by non-immunological mechanisms of stabilization of the podocyte cytoskeletone. We performed this study for 16 weeks to investigate the anti-proteinuric effect of tacrolimus in IgAN with significant albuminuria and normal blood pressure (NCT01224028).

Methods: The patients with biopsy proven IgAN were randomly assigned to tacrolimus treatment (TAC 0.05-0.1 mg/kg/day, 20 patients) and placebo(PLACEBO, 20 patients) with stratification by one RASI. We enrolled normotensive patients (<130/80 mmHg) with urine albumin to creatinine ratio (UACR) 0.3-3.0 g/g creatinine (g/g Cr) who did not use steroid or other immunosuppressive treatment. The primary outcome was the change of albuminuria after 16 weeks of trial [UACR at 16weeks/UACR at baseline] compared to baseline between TAC and PLACEBO.

Results: RASI was used in 11 patients in TAC and 9 in PLACEBO ($p>0.05$). At enrollment, systolic blood pressure and diastolic blood pressure were 118.2±6.5 and 72.7±4.8 mmHg in TAC and 119.3±6.1 and 73.1±5.1 mmHg in PLACEBO, respectively ($P>0.05$). The creatinine clearance was 73.4±18.8 in TAC and 79.8±21.3 ml/min in PLACEBO ($P>0.05$). The UACR was 1.10±0.64 in TAC and 0.96±0.46 g/g Cr in PLACEBO ($P>0.05$). After 16 weeks, The change of UACR was 0.52±0.25 in TAC and 0.18±0.30 in PLACEBO ($P=0.001$). Among users of RASI, the change of UACR was 0.40±0.29 in TAC and 0.22±0.34 in PLACEBO ($P=0.246$) and, among non-users of RASI, it was 0.67±0.12 in TAC and 0.15±0.27 in PLACEBO ($P=0.246$) ($P<0.001$).

Conclusions: Tacrolimus showed an effective anti-proteinuric effect in albuminuric and normotensive patients with IgAN who were not tolerable to RASI during short-term period of clinical trial.

Funding: Private Foundation Support

TH-PO411

Effects of Mycophenolate Mofetil Combined with Corticosteroids for Inducing Remission of Henoch-Schönlein Purpura Nephritis

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Background: Henoch-Schönlein purpura (HSP) can progress to Henoch-Schönlein purpura nephritis (HSPN), and the most effective treatment remains controversial. Our aim was to compare the effects of oral mycophenolate mofetil (MMF) with low dosage prednisone and the full dosage corticosteroids(CS)(prednisone) for the induction therapy of HSPN with large proteinuria.

Methods: This was a retrospective study. The patients were enrolled from January 2007 to September 2011;The diagnosis of HSP was in accordance with the criteria from the American College of Rheumatology in 1990. All the patients received renal biopsy with urine protein ≥2.0g/d. The exclusion criteria included: (1) age less than 14 years or greater than 65 years; (2) prescription of a cytotoxic drug or full dosage of CS within the previous 6 months before the start of MMF or full dosage of CS; (3) severe infection within the previous three months; (4) active phased hepatitis. 53 adults with biopsy-proved HSPN with large proteinuria (greater than 2.0g/24hr) were divided into two groups: MMF group(n=27) that received oral MMF 1.0g/day (1.5g/day for patients with a body weight >70kg) combined with low dosage prednisone (0.4-0.5mg/kg/d), and CS group (n=26) that received the full dosage prednisone (0.8-1.0mg/kg/d).We compared the effects of inducing remission at 6-month follow-up and the overall remission rate at the end of the follow-up between two groups.

Results: At 6 months, the eGFR level remained stable, while the urine protein decreased significantly in both groups, and the remission rate was 76.9% in CS group and 55.6% in MMF group($p=0.101$).With a median follow-up 28.8months in CS group,28.2months in MMF group, the overall remission rate was 80.8% in CS group and 77.8% in MMF group ($p=0.788$). The MMF group had less side effects than the CS group (48.1% VS 76.9% $p=0.031$). the relapse was in 4/21(19.0%) in CS group and 0/21 in MMF group ($p=0.115$).

Conclusions: MMF is useful for inducing remission and maintaining remission in Chinese HSPN with large proteinuria, and may represent an alternative treatment to the full dosage of CS.

Funding: Clinical Revenue Support

TH-PO412

Bortezomib for Treatment of IgA Nephropathy

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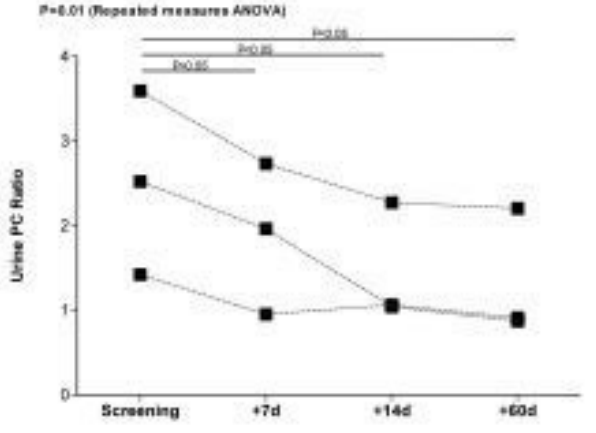
Background: IgA nephropathy is the most common form of glomerulonephritis and its pathogenesis involves an autoimmune antibody response to abnormal galactose-deficient IgA1 antibodies. Bortezomib is a proteasome inhibitor that targets plasma cells and inhibits release of NF-kB-inducible cytokines. We are conducting a pilot study testing the effects of bortezomib in subjects with biopsy proven IgA nephropathy and significant renal disease manifested by greater than 1000mg of daily proteinuria.

Methods: Bortezomib infusion was given at 1.3mg/m^2 for a total of 4 doses over a 2-week period after enrollment. Daily proteinuria, estimated as urine protein to creatinine ratio, was assessed following infusion of bortezomib at predetermined intervals. Any adverse events, renal function, and hematologic parameters were subsequently followed.

Results: Four subjects (1 male, 3 female) met inclusion criteria and were enrolled in the pilot study. The mean (±SD) age, baseline serum creatinine, and proteinuria were 37±17 years, 1.68±0.89 mg/dL, and 2.34±0.95 gram/day respectively. The cumulative mean (±SD) bortezomib exposure was 9.70±1.15 mg. Common side effects of neutropenia, thrombocytopenia, and neuropathy were not seen at 60 days of follow-up. One subject developed varicella zoster at 4 months after bortezomib infusion. The mean (±SD) proteinuria and serum creatinine were 1.33±0.76 gram/day and 1.84±1.0 mg/dL, at 60 days follow-up. Figure 1: Only 3 subjects depicted at 60 days follow-up.



Conclusions: We present our early experience testing bortezomib in 4 subjects with native IgA nephropathy and severe proteinuria. For the first time, we show that a single cycle of bortezomib therapy was tolerated and has the potential to reduce proteinuria in IgA nephropathy.

Funding: Other NIH Support - Weill Cornell Clinical and Translational Science Center (CTSC), Pharmaceutical Company Support - Millennium Pharmaceutical, Inc.

TH-PO413

Validation Study of Oxford Classification of IgA Nephropathy: A Proposal of Prediction of Kidney Outcome According to Numbers of Selected Pathological Parameters Ritsuko Katafuchi,[1] Masaharu Nagata,[2] Toshiharu Ninomiya,[2] Koji Mitsuiki,[3] Hideki N. Hirakata.[3] *[1]Kidney Unit, National Fukuoka-Higashi Medical Center, Koga, Fukuoka, Japan; [2]Department of Medicine and Clinical Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan; [3]Kidney Unit, Fukuoka Red Cross Hospital, Fukuoka, Japan.*

Background: We published a validation study of Oxford classification of IgA nephropathy (IgAN) and found a significant association of extracapillary proliferation (Ex) with risk of endstage renal failure (ESRF). Our next question is how we can use the Oxford classification as a prognosticator in patients with IgAN.

Methods: 702 patients with IgAN were included. Pathologic parameters were estimated according to the definition of the Oxford classification. Mesangial hypercellularity score (M) was defined as M0, 0.5 or less, and M1, over 0.5. Endocapillary hypercellularity (En), segmental glomerulosclerosis (S), and Ex were scored 0 in absence and 1 in presence. Tubular atrophy/interstitial fibrosis (T) was graded according to the percentage of involved area as T0, less than 25; T1, 25 or more. We examined the kidney survival and risk for development of ESRF according to the status of each pathological parameter, separately and also according to the number of pathological parameters (0 or 1, 2, 3 and 4 or 5).

Results: The kidney survivals in M1, Ex1, S1 and T1 significantly lower than in M0, Ex0, S0 and T0, respectively. The presence of M1, Ex1, S1 and T1 showed a significant association with the risk of ESRF compared to M0, Ex0, S0 and T0, respectively. The 5 and 10 years kidney survival was 100 and 98, 97 and 89, 88 and 77, 80 and 60% in patients with 0 or 1, 2, 3, and 4 or 5 pathological parameters, respectively. Three and 4 or 5 pathological parameters were associated with 8.35 and 13.2-fold increased risk of ESRF compared to 0 or 1 parameter.

Conclusions: The prognosis can be predicted according to the number of pathological parameters. The patients with three or more parameters might have poor outcome. This method is simple and could be the first step of clinical use of Oxford classification.

TH-PO414

Histological Predictors of Treatment Efficacy in Severe Childhood IgA Nephropathy (IgAN): Validation of the Oxford Classification of IgAN (Ox C) Yuko Shima,[1] Koichi Nakanishi,[1] Taketsugu Hama,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Shingo Ishimori,[2] Hiroshi Kaito,[2] Ryojiro Tanaka,[3] Kazumoto Iijima,[2] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical University, Wakayama, Japan; [2]Pediatrics, Kobe University, Kobe, Hyogo, Japan; [3]Pediatric Nephrology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan.*

Background: We previously demonstrated that Ox C appears to be valid for predicting renal outcome in children without intensive treatments (Pediatr Nephrol 2012;27:783-92). However, predictive value of Ox C in treatment efficacy has not been fully examined in children.

Methods: We analyzed retrospectively consecutive 92 children newly diagnosed as severe IgAN showing diffuse mesangial proliferation (DMP) from May 1987 to May 2009 and treated with prednisolone (PSL) only or combination (PSL + azathioprine or mizoribine) for 2 years. We assessed the ability of each variable in Ox C as a predictor of the 2-yr treatment efficacy defined as proteinuria remission using logistic regression analysis.

Results: The mean mesangial score (M), and ratios of endocapillary hypercellularity (E), segmental glomerulosclerosis (S), tubular atrophy (T), and crescents (C) were 1.93, 19.9%, 9.6%, 4.7%, and 15.7%. After the 2-yr treatment, 53 of the 92 patients (58%) showed proteinuria remission. There was no significant difference in clinical findings (MAP, urinary protein excretion and eGFR) between remission group and non-remission group at the start of treatment. As to histological variables, there was no significant difference in M, E, S, and C between the groups. Prevalence of T was significantly lower in remission group (37.7% vs 87.2%, *P=0.006* Fisher's exact test). The prognostic factor related to proteinuria remission after the 2-yr treatment was presence of T in univariate and multivariate analyses adjusted by all histological variables (OR 3.8, 95%CI 1.3-13.5, *P=0.018)*.

Conclusions: In severe childhood IgAN showing DMP, the presence of T was a predictor of refractoriness, therefore, other options of treatments may have to be considered in severe IgAN with T. Further studies seem to be needed for evaluation of the total performance of Ox C for prediction of treatment efficacy.

TH-PO415

Bacteria Specific to Tonsil in IgA Nephropathy Patients Predicts the Remission by the Treatment of Tonsillectomy and Corticosteroid Pulse Yasuyuki Nagasawa,[1,2] Kenichiro Iio,[2] Hirotsugu Iwatani,[2] Ryohei Yamamoto,[2] Maki Shinzawa,[2] Enyu Imai,[2] Takeshi Nakanishi,[1] Hiromi Rakugi,[2] Yoshitaka Isaka.[2] *[1]Department of Internal Medicine, Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]Department of Nephrology and Geriatric Medicine, Osaka University, Suita, Osaka, Japan.*

Background: Immunoglobulin(Ig)A nephropathy (IgAN) is the most common form of primary glomerulonephritis in the world. Some bacteria were reported to be the candidate of the antigen or the pathogenesis of IgAN, but systemic analysis of bacterial flora in tonsil with IgAN has not been reported. Moreover, these bacteria specific to IgAN might be candidate for the indicator which can predict the remission of IgAN treated by the combination of tonsillectomy and steroid pulse.

Methods: We made a comprehensive analysis of tonsil flora in 68 IgAN patients and 28 control patients using Denaturing gradient gel electrophoresis methods. We also analyzed the relationship between several bacteria specific to the IgAN and the prognosis of the IgAN.

Results: *Treponema sp.* were identified in 24% IgAN patients, while in 7% control patients (P=0.062). *Haemophilus segnis* were detected in 53% IgAN patients, while in 25% control patients (P=0.012). *Campylobacter rectus* were identified in 49% IgAN patients, while in 14% control patients (P=0.002). Multiple Cox proportional-hazards model for proteinuria revealed that *Treponema sp.* or *Campylobactor rectus* are significant for the remission of proteinuria (Hazard ratio 2.18, p=0.027). There was significant difference in remission rates between IgAN patients with *Treponema sp.* and those without the bacterium (p=0.046) by Kaplan-Meier analysis. There was also significant difference in remission rates between IgAN patients with *Campylobacter rectus* and those without the bacterium (p=0.037). There was no significant difference in remission rates between IgAN patients with *Haemophilus segnis* and those without the bacterium.

Conclusions: This insight into IgAN might be useful for diagnosis of the IgAN patients and the decision of treatment of IgAN.

Funding: Government Support - Non-U.S.

TH-PO416

Mortality of IgA Nephropathy Patients: A Review of 30-Years of Experience Hajeong Lee,[1] Hayne C. Park,[1] Yon Su Kim,[1] Dong Wan Chae,[2] Suhnggwon Kim,[1] Ho Jun Chin.[2] *[1]Internal Medicine, Seoul National University Hospital; [2]Internal Medicine, Seoul National University Bundang Hospital.*

Background: Research on the prognosis in IgA nephropathy (IgAN) has focused on renal survival, with little information being available on the patient survival of IgAN patients. This investigation aimed to explore long-term patient outcome in IgAN patients.

Methods: Clinical and pathologic characteristics at the time of renal biopsy were reviewed from 1979 to 2008 in 1,364 IgAN patients. The outcomes were patient death and end stage renal disease (ESRD) progression. The relative mortality rate of IgAN was estimated by the standardized mortality ratio (SMR) with 95% confidence interval (CI).

Results: 71deaths (5.3%) and 277 ESRD cases (20.6%) were developed during 13,916 person-years. 10-, 20-, and 30-year patient survival rates were 96.3%, 91.8%, and 82.7%, respectively. Among the deceased, more than half died before ESRD progression and their most common cause of death was malignancy. Overall mortality was elevated by 43% from the age/sex matched general population (SMR 1.43, 95% CI 1.04-1.92). Men showed comparable survival (SMR 1.22, 95% CI 0.82-1.75), however, women exhibited an increased mortality rate more than double (SMR 2.17, 95% CI 1.21-3.57). Patients with well-known renal risk factors including renal dysfunction (GFR <60 ml/min/1.73m^2, SMR 1.70 95% CI 1.13-2.46), higher systolic blood pressure (≥130 mmHg, SMR 1.51, 95% CI 1.00-2.18) and proteinuria (≥1 g/day, SMR 1.66, 95% CI 1.16-2.29) also showed elevated mortality rate than general population, whereas patients with preserved renal function, normotension, and proteinuria less than 1 g/day revealed similar mortality rate compared with general population.

Conclusions: This investigation demonstrated that overall patient survival of IgAN was higher than general population, especially in women or patients with renal risk factors. However, patients without renal risk factors survived as similar to general population. Therefore, optimized strategies to alleviate renal risk factors are also warranted to reduce patient mortality.

TH-PO417

Urinary Neutrophils Gelatinase-Associated Lipocalin Level Is Closely Associated with Tubulointerstitial Injury in IgA Nephropathy Su Mi Lee, Seung Min Lee, Dong Ki Kim, Yon Su Kim. *Seoul National University Hospital, Seoul, Republic of Korea.*

Background: IgA nephropathy (IgAN) was thought to be relatively benign but several studies reported that IgAN eventually progresses to end-stage renal failure. Renal tubulointerstitial injury plays an important role in the progression of IgAN. Neutrophils gelatinase-associated lipocalin (NGAL) was thought to be a sensitive biomarker for tubule injury. The aim of this study is to investigate which relationship is present between severity of tubulointerstitial injury and urine NGAL levels in IgAN patients.

Methods: We analyzed data for 123 IgAN patients who were biopsy-proven and 26 healthy controls who were age and sex matched. Urinary NGAL levels were measured by enzyme-linked immunosorbent assay and were corrected by urinary creatinine. We evaluated percentage of global sclerosis, segmental sclerosis, and extent of tubulointerstitial damage. The correlation between urinary NGAL levels with clinical, histopathological features were evaluated.

Results: The patients were 41.2±14.5 years old, presented with 1.7±1.8 g/day proteinuria. Individuals with IgAN had higher urinary NGAL levels than in normal controls (1.8±4.1 versus 0.8±0.8 ng/mg urinary Cr, P = 0.02). Baseline urinary NGAL levels were significantly correlated with urinary protein excretion($r = 0.40$, $p < 0.001$), serum creatinine($r = 0.47$, $p <0.001$), creatinine clearance($r = -0.221$, $p = 0.007$), urinary β_2 microglobulin($r = 0.707$, $p < 0.001$). The urinary NGAL levels and urinaryβ_2 microglobulin in patients with tubular atrophy and interstitial fibrosis Grades 1, 2, and 3 were significantly higher than in normal controls. In addition to, similar results were identified in patients with different grades of interstitial infiltration. The patients with severe mesangial proliferation, global sclerosis had higher urinary NGAL levels than that in patients without.

Conclusions: Our study shows that urinary NGAL levels may be a useful biomarker to evaluate tubulointerstitial injury of IgA nephropathy.

TH-PO418

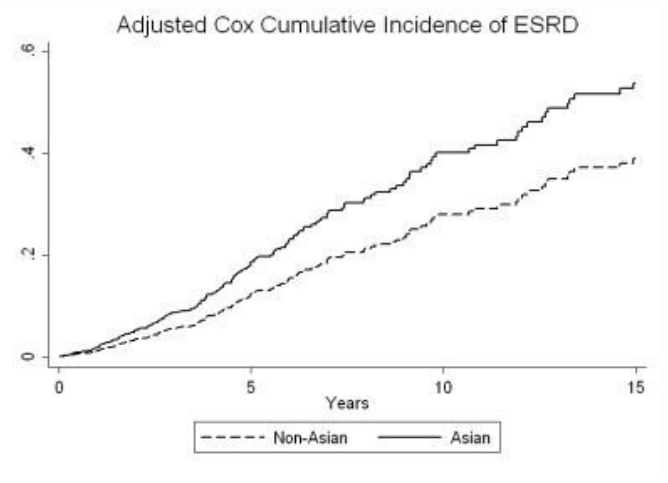
Asians with IgA Nephropathy Have an Increased Risk of Progression to End-Stage Renal Disease Sean Barbour,[1,3] Daniel C. Cattran,[2,3] Joseph Kim,[2] Ron Wald,[2] Adeera Levin,[1] Michelle A. Hladunewich,[2,3] Heather N. Reich.[2,3] *[1]Nephrology, University of British Columbia, Vancouver, BC; [2]Nephrology, University of Toronto, Toronto, ON; [3]Toronto Glomerulonephritis Registry, Toronto, ON.*

Background: IgA nephropropathy (IgAN) is the most common cause of glomuerulonephritis (GN) worldwide, however it accounts for a far higher proportion of end-stage renal disease (ESRD) in Asia compared to North America. It is not known if this is entirely due to higher disease prevalence in Asians or a higher risk of disease progression. The lack of an adequately diverse population followed longitudinally in a single center has previously precluded the ability to address this question.

Methods: To determine if Asians with IgAN have a higher risk of ESRD, we analyzed a cohort of 669 adults from the Toronto GN Registry with biopsy-proven IgAN, in which 30% of subjects were of self-reported Asian race. Patients were followed prospectively for a median of 46 months. The primary outcome was time from kidney biopsy to ESRD (dialysis, transplantation or eGFR<15), which occurred in 213 patients, and was analyzed using Cox survival regression analysis.

Results: The mean age, eGFR and proteinuria were 39.7 years, 59.6ml/min/1.73m^2 and 1.8g/day. After adjusting for age, sex, baseline eGFR, MAP and proteinuria over time, the use of ACEi/ARB and the use of immunosuppression, the risk of ESRD was significantly higher in Asians compared to non-Asians (HR=1.56 95%CI 1.10-2.22 p=0.01, see figure). This was supported by both a 1.62ml/min/1.73m^2/year faster rate of eGFR decline in Asians (95%CI -3.19--0.5 p=0.04), and an increased risk of a 50% reduction in eGFR (HR=1.81 95%CI 1.25-2.62 p=0.002).

Conclusions: We have shown that in a large multi-racial cohort of patients with IgAN, Asians have a higher risk of progression to ESRD compared to non-Asians, after adjusting for known prognostic factors.



TH-PO419

Comparison of Clinical Outcomes between Adult Patients with Henöch-Schönlein Purpura Nephritis and Immunoglobulin A Nephropathy Mi Jung Lee,[1] Dae-Suk Han,[1] Dong Ho Shin,[1] Shin-Wook Kang,[1,2] Hye-young Kang,[2] Seong Hun Kim,[2] Seung Hyeok Han.[1] *[1]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei University, Seoul, Republic of Korea.*

Background: Henöch-Schönlein purpura nephritis (HSPN) is considered a systemic form of immunoglobulin A nephropathy (IgAN). Although these two diseases may be different manifestations of a single disease, little is known whether the long-term outcomes are different between HSPN and IgAN.

Methods: We studied 120 patients with biopsy-proven HSPN and 1,070 patients with IgAN who were recruited between Jan 2000 and Sep 2010 from 4 medical centers in Korea. The primary outcome was the composite of a doubling of the baseline serum creatinine concentrations, the onset of end-stage renal disease (ESRD), or death. Secondary outcomes included the individual renal outcome of a doubling of the baseline serum creatinine concentrations, ESRD, and the rate of decline in estimated glomerular filtration rate (eGFR). We compared the clinical outcomes between the two groups using 1:2 propensity score (PS) matching.

Results: In the unmatched cohort, HSPN patients had more vasculitis symptoms and more favorable histologic features and were more commonly treated with steroids than IgAN patients. The risk of reaching the primary outcome was significantly lower in HSPN than IgAN patients (hazard ratio [HR], 0.67; 95% confidence interval [CI], 0.43-0.96; P=0.044). The 1:2 PS matching yielded matched pairs of 89 HSPN and 178 IgAN patients, resulting in no differences in baseline characteristics. In the matched cohort, 16 patients (18.0%) with HSPN reached the primary outcome compared to 38 patients (21.3%) with IgAN (HR, 0.78; 95% CI, 0.47-1.36; P=0.166). The risk of reaching a doubling of the baseline serum creatinine levels (HR, 0.74; 95% CI, 0.49-1.52; P=0.132) and developing ESRD (HR, 0.77; 95% CI, 0.59-1.53; P=0.181) did not differ between the two groups. The rate of decline in eGFR was also comparable.

Conclusions: There was no significant difference in clinical outcomes between patients with HSPN and IgAN, suggesting that these two diseases have a similar prognosis.

TH-PO420

Effect of Cigarette Smoking on Renal Function and Renal Pathological Injury in IgA Nephropathy Patients Han Zhang, Xiaoyan Zhang, Yi Fang, Xiaoqiang Ding. *Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.*

Background: Immunoglobulin A nephropathy (IgAN) is the most common chronic glomerulonephrititis worldwide. Recently, smoking has been demonstrated as a risk factor in the progression of IgAN. The aim of this study was to illuminate the effect of cigarette smoking on renal function and renal pathological lesions in IgAN patients.

Methods: One hundred biopsy-proved IgA nephropathy patients were involved. Smoking status, gender, age, height, body weight, blood pressure, urine routine, renal function and proteinuria were recorded. The estimated glomerular filtration rate (eGFR) was calculated by MDRD formula. We assessed pathological lesions in glomerular, tubulointersitial and vascular tissue semiquantitatively according to Katafuchi score.

Results: There ware 32 somkers in 100 IgA nephropathy patients. There were more male patients in smokers (31/1) than nonsmokers (47/21) (*P<0.01*). Smokers were older than nonsmokers (44.8±12.9 *vs.* 39.3±12.7, *P<0.05*). Smokers had lower eGFR(64.9±27.9 *vs.* 92.0±29.8, *P<0.01*) and serum HDL(1.04±0.32 *vs.* 1.33±0.41, *P<0.01*) than nonsmokers. Tubulointersitial score(4.23±1.97 *vs.*2.90±1.89, *P<0.01*) and index of intrarenal vessel wall thickening (1.69±1.45 *vs.*0.88±1.31, *P<0.01*) were higher than nonsmokers. The relationship between smoking and intrarenal vessel hyaline change was not proven.Multiple linear regression analyses revealed that hypertension (β=-0.51, *P<0.01*), proteinuria (β=-0.45, *P<0.01*) and smoking (β=-0.22, *P<0.05*) were correlated significantly with lower eGFR in 52 male patients. Multiple binary logistic regression analyses revealed that age (OR=1.05, 95%CI (1.02, 1.09), *P<0.01*) and smoking (OR=2.54, 95%CI (1.02, 6.36), *P<0.05*) were correlated significantly with higher prevalence intrarenal vessel wall thickening.

Conclusions: Smoking was an independent risk factor of eGFR decline in male IgA nephropathy patients, and renal tubulointerstitial lesions and intrarenal vessel wall thickening in IgA nephropathy patients.

TH-PO421

Novel Diagnostic Approach for IgA Nephropathy Hiroyuki Yanagawa,[1] Hitoshi Suzuki,[1] Yusuke Suzuki,[1] Bruce A. Julian,[2,3] Jan Novak,[3] Yasuhiko Tomino.[1] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; [3]Microbiology, University of Alabama at Birmingham, Birmingham, AL.*

Background: There is increasing evidence that galactose-deficient IgA1 (Gd-IgA1) and Gd-IgA1-containing immune complexes are important players in the pathogenesis of IgA nephropathy (IgAN). Moreover, serum levels of Gd-IgA1-specific antibodies, responsible for the formation of immune complexes with Gd-IgA1, are also elevated in IgAN. However, due to the clinical heterogeneity of this disease, there is no biomarker to replace the diagnostic renal biopsy. In the present study, we assessed a novel noninvasive multi-biomarker approach combined with analysis of clinical data by a logistic model as a diagnostic test for IgAN.

Methods: Serum samples were collected from 2006 to 2011 at the time of renal biopsy from 135 IgAN patients and 79 patients with other renal diseases such as lupus nephritis, diabetic nephropathy, and membranous nephropathy. We also collected serum samples from 106 healthy controls. We measured serum levels of IgA, IgG, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA by ELISA. Each data was analyzed by multivariate analysis.

Results: Although serum levels of IgA, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA were elevated in patients with IgAN compared with disease and healthy controls, none of the biomarkers alone fully differentiated IgAN patients from disease controls. Therefore, we re-analyzed these biomarkers and compared them with clinical data, such as age, gender, serum creatinine, urinary protein/creatinine ratio and degree of microscopic hematuriausing a logistic model. This model differentiated IgAN patients from disease controls with 81% specificity and 91% sensitivity.

Conclusions: Our results suggest that serum Gd-IgA1 and Gd-IgA1-specific antibodies (IgG and IgA) are useful biomarkers for diagnosis of IgAN. The novel quantitative scoring system can be applied for diagnosis of IgAN besides renal biopsy.

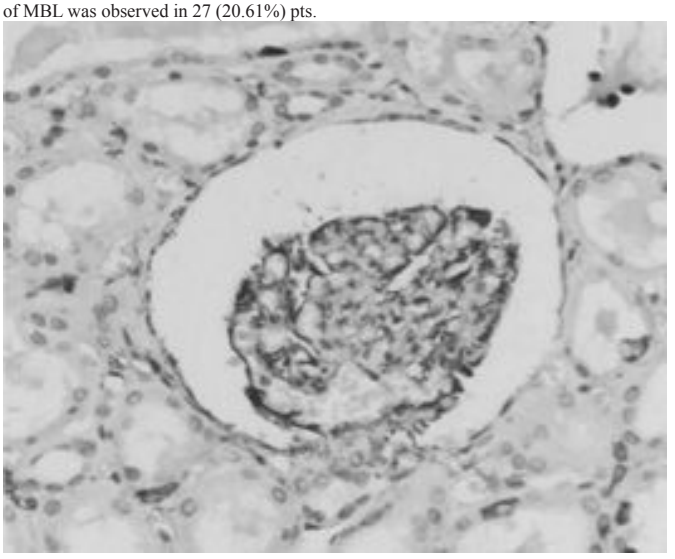
TH-PO422

Mannose-Binding Lectin Gene Polymorphism Is Closely Associated with Clinic-Pathological Manifestation and Renal Prognosis in IgA Nephropathy Patients Beili Shi, Zhaohui Ni, Shan Mou, Liou Cao, Min Zhang, Qin Wang, Yucheng Yan, Mingli Zhu, Wei Fang, Jia Qi Qian. *Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: IgA nephropathy (IgAN) is one of the most worldwide primary GN. Recently, mannose-binding lectin (MBL) was suggested to play an important role in the pathogenesis and progress of IgAN.

Methods: Patients who were diagnoseed as primary IgAN, 18-65 year-old, followed up >=12 months were enrolled. Primary secondary end-point were defined as entering ESRD, and Scr increased >50% of baseline, respectively. 1) Renal biopsy specimens were immuno-histochemical staining by MBL mono-antibody. 2) Integrated capillary electrophoresis was used to detect MBL gene polymorphism in peripheral blood DNA. 3) ELISA was used to detect serum MBL level.

Results: 131 IgAN pts (60 males, 37.59±11.60 years old) were enrolled and followed 52 (20-82) months, among who 12 entered end-point (4 ESRD). 1) Glomerular deposition of MBL was observed in 27 (20.61%) pts.



Compared with MBL+, MBL- cases showed severer glomerular/segmental sclerosis (GGS), higher BP and Scr, accompanied with poorer renal prognosis (P=0.004). 2) In 93 patients' DNA samples, we found SNP in promoter -221 (G→C) and exon 1 +54 (G→A). Compared with wild-type, exon 1 +54 variants had significantly lower serum MBL levels (P<0.001), higher baseline Scr (P=0.048), higher percentage of GGS (P=0.024) and pronouced tubular-interstitial damage (TID) (P=0.049). Among 10 patients entered end-point, exon 1 +54 variants had higher occurrence (P<0.001). Cox analysis found that exon 1 (+54) SNP (β=2.460, P=0.033), baseline Scr (β=0.966, P=0.04) and TID (β=0.202, P=0.048) were independent factors of renal prognosis.

Conclusions: IgAN patients with MBL gene variation had severer clinical and histological manifestation, accompanied with poorer renal prognosis.

Funding: Government Support - Non-U.S.

TH-PO423

Reduction in Urine Protein as a Surrogate Marker for Kidney Disease Outcomes in IgA Nephropathy Lesley Stevens Inker,[1] Hocine Tighiouart,[1] Neal B. Shah,[1] Gerald B. Appel,[2] Bart Dirk Maes,[3] Philip K.T. Li,[4] Manuel Praga,[5] Tom Greene,[6] Christopher H. Schmid,[1] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]Columbia University; [3]Heilig Hartziekenhuis; [4]Prince of Wales Hospital; [5]Hospital 12 de Octubre; [6]University of Utah.*

Background: Clinical trials in IgA nephropathy using GFR decline as an endpoint require long duration of follow-up with high expense and complexity. The use of early reduction in urine protein (delta UP) as a surrogate endpoint may accelerate testing of new therapies.

Methods: In a patient level analysis of 6 randomized controlled trials of drug interventions in IgA nephropathy, we evaluated: 1) Treatment effect for the ratio of delta UP from baseline to early follow-up (defined as first UP between 2.5 and 13 months); 2) Treatment effect for the composite outcome of doubling of serum creatinine or ESRD or death. 3) Patient level association of delta UP with the composite outcome.

Results: The table shows the results by study. There is a strong relationship between delta UP and outcomes. Stronger associations were observed in studies of fish oil and the renin-angiotensin system inhibition than in those of immunosuppression.

Study	Intervention type	N patients/N events	Treatment effect on delta UP in Tx group relative to control*	Treatment effect on composite outcome in Tx group relative to control	Hazard ratios per 2X of UP on composite outcome**
1	Fish oil	90/17	23%	**74%**	2.21 (1.09, 4.51)
2	Fish oil	65/14	-37%	**0**	3.96 (1.82, 8.61)
3	ACEI	44/15	**27%**	**57%**	2.44 (1.09, 5.43)
4	ARB	107/8	**39%**	61%	9.63 (2.63, 35.26)
5	MMF	34/3	-15%	55%	5.25 (0.50, 54.75)
6	MMF	20/4	-41%	55%	0.99 (0.40, 2.50)
Overall		360/61	**15%**	55%	2.37 (1.72, 3.28)

Bolded number indicate statistical significance. *% decline UP from baseline to early follow-up for the ratio of treatment to control groups; **adjusted for baseline urine protein.

Conclusions: Our results may be taken as preliminary evidence for the role of proteinuria as a surrogate marker for kidney disease outcomes in IgA nephropathy.

Funding: Pharmaceutical Company Support - Pharmalink AB

TH-PO424

Urinary Protein of Three Years after Diagnosis Is the Strongest Predictor of Renal Outcome in IgA Nephropathy Takayuki Fujii,[1] Satoshi Suzuki,[1] Tanaka Hiroaki,[1] Kunihiro Yamagata.[2] *[1]Kidney Center, Seirei Sakura Citizen Hospital, Sakura, Chiba, Japan; [2]Department of Nephrology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: Level of proteinuria (UP) has a strong association with poor prognosis in IgA nephropathy. However, recent studies using the Oxford classification suggested that UP at diagnosis of IgA nephropathy was not an independent prognostic factor. We evaluated the usefulness of not only clinical and pathological findings at the time of renal biopsy, but also urinary findings 3 years after diagnosis for the accurate prediction of outcomes.

Methods: Between January 1980 and June 2008, 481 patients were diagnosed as IgA nephropathy in our hospital, and could be followed up for 3 years or more. We performed a retrospective cohort study in 350 out of the 481 patients who showed eGFR≥60 ml/min/1.73m^2. The endpoint of this study was a 50% decrease in eGFR during follow-up. In addition to renal histological findings according to the Oxford classification, eGFR, the mean blood pressure, age, sex, and the treatment method, we applied urinary findings at the time of renal biopsy (UP≥0.5g/day or not, and presence of hematuria or not) in Model A, and we applied urinary findings 3 years after diagnosis in Model B for variables factors. The association between these factors and renal outcomes was evaluated using Cox's proportional hazard model.

Results: Hematuria waa the only risk factor (HR, 2.58; 95% CI, 1.26-5.45) in model A, while UP≥0.5g/day (HR, 7.37; 95% CI, 3.14-20.4), hematuria (HR, 5,57; 95% CI, 2.29-13.1), and the mean blood pressure (HR, 1.56; 95% CI, 1.06-2.32) were significant risk factors for poor renal outcomes in model B. There was no significant association between renal histological findings or treatment methods and renal outcomes in both models.

Conclusions: For the prediction of the outcome of IgA nephropathy with normal renal function, irrespective of the treatment methods, not UP at diagnosis but UP 3-years after diagnosis is useful and strong predicting factor of renal prognosis.

TH-PO425

Urine Podocin:Nephrin mRNA Ratio (U-PNR) as a Useful Biomarker in IgA Nephropathy Akihiro Fukuda,[1] Takashi Iwakiri,[2] Hiroyuki Komatsu,[2] Tatsunori Toida,[2] Mariko Tatsumoto,[1] Hideto Nakagawa,[1] Masao Kikuchi,[2] Yuji Sato,[1] Kazuo Kitamura,[2] Shouichi Fujimoto.[1,3] *[1]Dialysis Division, University of Miyazaki Hospital, University of Miyazaki, Miyazaki, Japan; [2]Division of Circulatory and Body Fluid Regulation, Department of Internal Medicine, University of Miyazaki, Miyazaki, Japan; [3]Department of Hemovascular Medicine and Artificial Organs, University of Miyazaki, Miyazaki, Japan.*

Background: Proteinuria and/or albuminuria are widely used for noninvasive assessment of kidney diseases. Proteinuria is a nonspecific marker of diverse forms of kidney injury, therefore, more specific glomerular disease biomarkers are required. Podocyte depletion is a major mechanism driving glomerulosclerosis. Podocyte cell lineage specific mRNAs can be recovered from urine pellets of model systems and man. In model system, progressive glomerular disease was associated with low level urine nephrin mRNA excretion compared to urine podocin mRNA excretion (Sato Y et al. J Am Soc Nephrol 2009, Fukuda A et al. Kidney Int 2012). Thus, the urine podocin:nephrin mRNA ratio (U-PNR) could serve as a useful glomerular disease progression biomarker (Fukuda A et al. Nephrol Dial Transplant in press). The purpose of this study was to test whether the U-PNR could be a useful biomarker in patients with IgA nephropathy (IgAN).

Methods: Urine sample and kidney biopsy specimen from 34 patients with IgAN (15 males; mean age 38.6yr: range 17 to 60 yr) were analyzed. We examined the relationships between U-PNR and urine protein:creatinine ratio (U-PCR), or renal histological parameters defined by Oxford classification.

Results: Both the U-PNR and U-PCR were significantly elevated in patients with IgAN compared to healthy volunteers (0.85±0.10 vs 0.29±0.06 and 0.49±0.16 vs 0.03±0.01, respectively). U-PNR was significantly correlated with the severity of segmental glomerulosclerosis and of endocapillary hypercellularity lesions, on the other hand, U-PCR did those of mesangial hypercellularity and of endocapillary hypercellularity. These results suggest that U-PNR was highly correlated with glomerular lesions in patients with IgAN.

Conclusions: The U-PNR could be a useful biomarker in patients with IgAN.

TH-PO426

Serum BAFF Is Elevated and Associated with Clinical and Histopathological Features of Patient with IgA Nephropathy Xuli Xia, Zhiming Ye, Shuangxin Liu, Ruizhao Li, Wei Shi. *Department of Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: B cell activating factor belonging to tumor necrosis factor family (BAFF) was found to have the function of activating B cell and participating in the class-switching of B cells, however, its clinical application needs further studies. In the present study, the serum BAFF level of patients with IgAN (IgAN) with different histopathological phenotypes was detected.

Methods: The levels of serum BAFF in 153 patients with IgAN and 55 healthy controls were detected using commercial available ELISA kits. Their correlations with clinical and histopathological features of patients with IgAN were further evaluated.

Results: The levels of serum BAFF in patients with IgAN were significantly higher than normal controls. Serum BAFF levels were significantly higher in patients with mesangial hypercelluarity and segmental glomerulosclerosis than those without. Serum BAFF levels

were associated with the severity of the tubular atrophy/interstitial fibrosis. Serum BAFF levels were significantly correlated positively with eGFR and serum creatinine. The patients with elevated serum BAFF levels showed significantly more severity in clinical and histopathological stages.

Conclusions: The levels of serum BAFF were elevated in patients with IgAN and were associated with clinical and pathological features of the disease. Serum BAFF levels could be a non-invasive biomarker for monitoring disease progression of IgAN.

Funding: Government Support - Non-U.S.

TH-PO427

Role of Innate Immunity in IgA Nephropathy Can Li,[1] Hong Bin Zou,[2] Bi Hu Gao,[3] Shang Guo Piao,[4] Chul Woo Yang.[4] *[1]Nephrology&Dialysis Unit, YanBian University Hospital, YanJi, JiLin, China; [2]Nephrology, The First Affiliated Hospital of JiLin University, ChangChun, JiLin, China; [3]Nephrology, The Affiliated Zhong-Shan Hospital of DaLian University, DaLian, LiaoNing, China; [4]Nephrology, The Catholic University of Korea, Seoul, Korea.*

Background: Recent studies demonstrated that toll-like receptor expression is increased in circulating mononuclear cells or mucosal B cells and dendritic cells in IgA nephropathy. However, its expression in human kidney tissues with IgA nephropathy is undetermined. This study was designed to investigate the expression of toll-like receptors (TLR) and MYD88 in kidney biopsy specimens with IgA nephropathy.

Methods: Total 94 biopsy-proven IgA nephropathy patients were enrolled and 13 patients with nephrectomy for renal cancer served as controls. Intrarenal expression of TLRs (TLR2 and TLR4) and MYD88 was examined by immunohistochemistry. Clinic characteristics such as age, systolic blood pressure (SBP), serum creatinine, glomerular filtration rate (GFR), serum albumin, and 24h urinary protein excretion were recorded.

Results: Compared with the control, IgA nephropathy patients showed a significant increase in the expression of TLR2 and TLR4 in the glomerulus. However, there is no relationship between TLRs expression and the degree of pathologic renal damage. Interestingly, TLR2 expression was significantly increased in the tubulointerstitum of IgA nephropathy but TLR4 expression was unaffected. Concomitantly, this increase in TLRs expression was paralleled with overexpression of MYD88. Furthermore, TLRs expression was positively correlated with 24h urinary protein excretion and negatively correlated with the GFR.

Conclusions: Thus, TLR2, TLR4, and MYD88 expression is upregulated in IgA nephropathy, and that this increase strongly associated with 24h urinary protein excretion and GFR. These findings suggest that innate immunity may be one of the molecular mechanisms in the pathogenesis of IgA nephropathy.

Funding: Other U.S. Government Support, Government Support - Non-U.S.

TH-PO428

Increase in CD208+ Dendritic Cells in Tonsils of Patients with IgA Nephropathy Hanako Takechi,[1] Takashi Oda,[1] Kojiro Yamamoto,[1] Naoki Oshima,[1] Daisuke Kamide,[2] Takeshi Matsunobu,[2] Kensuke Joh,[3] Osamu Hotta,[4] Akihiro Shiotani,[2] Hiroshi Nagura,[5] Hiroo Kumagai.[1] *[1]Department of Nephrology, National Defense Medical College, Saitama, Japan; [2]Department of Otolaryngology, National Defense Medical College; [3]Sendai Shakaihoken Hospital, Sendai, Japan; [4]Hotta Osamu Clinic, Sendai, Japan; [5]Tohokurosai Hospital, Sendai, Japan.*

Background: Therapeutic effect of tonsillectomy for IgA nephropathy (IgAN) has widely been reported. However, the exact mechanism how tonsil immunity leads to glomerulonephritis has been unclear. The participation of dendritic cells (DCs) in pathogenesis of IgAN has recently been suggested. We therefore investigated phenotypes and localization of DCs in tonsils of patients with IgAN. Furthermore, we examined the relationships between the tonsillar DCs with clinical features and renal histological change of IgAN patients.

Methods: We examined tonsils from twenty-two IgAN patients. The tonsils from recurrent tonsillitis patients and tonsil biopsy specimens from healthy controls were also used as controls. Five distinct markers of DCs (CD1a, CD1c, CD303, CD208, and CD209) were analyzed by immunohistochemically as well as by flow cytometry on isolated cells. The quantity of mRNAs of DCs was evaluated using real-time PCR. The clinical and histological data in renal biopsy were statistically compared with immunological data.

Results: Among the five phenotypes of DCs, CD208 (DC-lysosome-associated membrane glycoprotein; DC-LAMP), a marker of interdigitating DCs, positive cells significantly increased in the tonsils of IgAN compared with the tonsil of controls. By double immunofluorescence staining, CD208+DCs were found to localize touching with CD4+ and CD8+ T cells, and they aggregated as nodules in interfollicular area. Interestingly, the numbers per area of CD208+DCs in tonsils significantly correlated with the ratio of crescent positive per total glomeruli in renal biopsy ($p<0.05$). On the other hand, no relations were found between the level of DCs and clinical data.

Conclusions: These observations suggest that increased in CD208+DCs with sensitized T cells in tonsils relates with active lesions, especially crescents, in glomeruli of the patients with IgAN.

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TH-PO429

Expansion of T Cell Nodule and Reduced Lymphoepithelial Reticulation Is Characteristic Features of IgA Nephropathy-Associated Tonsillitis and Correlates with Formation of Crescent and Segmental Sclerosis in the Kidney Kensuke Joh,[1] Atsuhiro Kanno,[2] Hideyuki Kosukegawa,[2] Toshinobu Sato,[2] Osamu Hotta.[3] *[1]Division of Pathology, Sendai Shakaihoken Hospital, Sendai, Miyagi-ken, Japan; [2]Kidney Center, Sendai Shakaihoken Hospital, Sendai, Miyagi-ken, Japan; [3]Kidney Center, Hotta Osamu Klinic, Sendai, Miyagi-ken, Japan.*

Background: Tonsillectomy (TL) with steroid pulse therapy (SPT) can be a choice of therapy against IgA nephropathy (IgAN). The purpose was to characterize histological features of the tonsil of IgAN (Tons A) and to know whether the tonsillar lesions can relate to the renal lesions.

Methods: Tonsils were obtained from the 77 IgAN patients (pts), who underwent TL with SPT including 14 pts with TL after SPT and 63 patients with TL before SPT. As control, tonsils of chronic tonsillitis (Tons C) were obtained from 21 pts. The number of T lymphocyte nodules with assembly of HLA-DR-positive cells in the center, were counted per 2.0mm^2. Cytokeratin-positive tonsillar crypt epithelium showed reticulation and partially non-reticular area indicating an involution of lymphoepithelial symbiosis. Therefore, the reduced area of lymphoepithelial reticulation (RLR) was scored by 5 grades. Histological grading (J Nephrol 2012) and the constituting lesions in the kidney were evaluated according to Oxford classification.

Results: The number of T nodule (3.9+-0.7) and grade of RLR (1.9+-1.0) in Tons A increased significantly than those of Tons C as (3.2 +-1.0) and (1.2+-0.9), respectively ($p<0.01$). Histological grade in the kidney correlated with the grade of RLR (B=0.29 (0.12-0.45), $p<001$), but not to the number of T nodule ($p>0.05$). In multivariate linear regression analysis, only crescent and segmental sclerosis were selected as independent parameters, which correlated with the number of T nodule and the grade of RLR, respectively ($p<0.05$). Between in a group of TL after SPT and of TL before SPT, there was no difference for the number of T nodule and for the grade of RLR.

Conclusions: The number of T nodule correlating with crescent and the grade of RLR correlating with segmental sclerosis, were not diminished by SPT, suggesting the underlying mechanism of the effectiveness of TL combined with SPT.

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TH-PO430

Relationship between Asymmetric Dimethylarginine(ADMA) and Prognosis Factors in IgA Nephropathy Hyung-Jong Kim,[1] Dong Ho Yang,[1] Kyung Mi Park,[1] Hun Jeong,[2] Hyun Ju Oh.[1] *[1]Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Seoul Bukbu Hospital, Seoul, Korea.*

Background: Because of the variety of the clinical course in IgA nephropathy, it is very difficult to predict the prognosis. The risk factors associated with poor prognosis in IgA nephropathy are hypertension, proteinuria, and renal biopsy classification. Asymmetric demethylarginine(ADMA), the intrinsic inhibitor of nitric oxide synthesis, has been known to be associated with cardiovascular disease. But, there is still not enough knowledge about the roles of serum ADMA in IgA nephropathy. Therefore, the goal of this study was to investigate the association between serum ADMA and valuable markers for predicting poor prognosis in IgA nephropathy.

Methods: Twenty patients confirmed IgA nephropathy by renal biopsy were enrolled. We measured insulin, fasting glucose, hsCRP, lipid profile, serum BUN/creatinine, total protein/albumin by patient's blood samples and urien protein/creatinine by urine samples. We divided them into two groups according to Hass histology(Group 1 = I, II vs. Group 2 = III, IV, V). Also, two groups were divided according to urien protein creatinine ratio(PCR) at the time when renal biopsy was performed(Group A<0.5 mg/mg, Group B ≥ 0.5 mg/mg).

Results: Level of serum ADMA(Group 1; 0.5 ± 0.07 vs. Group 2; 0.58 ± 0.1) and urine protein/creatinine ratio(Group 1; 0.76 ± 0.78 vs. Group 2; 2.12 ± 1.76) were increased in group 2 that included more severe pathologic sub-classifications($p<0.05$). There was positive correlation between level of serum ADMA and urine protein/creatinine ratio(PCR)($p<0.05$). Also, serum total cholesterol, triglyceride was positively correlated with urine PCR($p<0.05$). There was negative correlation between serum albumin and urine PCR(<0.05).

Conclusions: Serum ADMA in IgA nephropathy was associated with the amount of proteinuria, which is the well-known conventional risk factor associated with renal failure. These findings strongly suggest that serum ADMA is a valuable biochemistric marker for predicting poor prognosis in IgA nephropathy. Prospective studies may be necessary hereafter.

TH-PO431

Evaluation of Hyaline Change of Afferent Arterioles in Patients with IgA Nephropathy Using Cardio-Ankle Vascular Index (CAVI) Hideo Okonogi, Yasunori Utsunomiya, Keita Hirano, Akihiro Shimizu, Masato Ikeda, Tetsuya Kawamura, Tatsuo Hosoya. *Division of Kidney and Hypertension, The Jikei University School of Medicine, Tokyo, Japan.*

Background: Several studies reported that hyaline change of afferent arterioles (HAA) were related to adverse renal outcome of IgA nephropathy (IgAN). Up to now, there was no tool to evaluate the relationship between HAA and arterial stiffness which reflects athero- or arteriosclerotic disease. Recently, cardio-ankle vascular index (CAVI) was developed as a novel indicator of arterial stiffness. CAVI is non-invasive method and not influenced by the

blood pressure (BP). Therefore, we examined the relationship between clinicopathological findings and CAVI in patients with IgAN.

Methods: Thirty-six IgAN patients whose renal biopsy specimens contain afferent arterioles were included. The presence of HAA, hyaline change and/or wall thickening of interlobular arteries (HTILA) were analyzed. In addition, serum levels of inflammatory parameters were measured.

Results: As a result, 20 patients (55%) had HAA, and 28 patients (78%) had HTILA. In the histological examination, the presence of HAA did not correlate with the degrees of global glomerular sclerosis, segmental glomerular lesions, nor interstitial fibrosis, while HTILA correlated with global glomerular sclerosis and interstitial fibrosis. In clinical parameters, HAA significantly correlated with age, BP, and CAVI ($p<0.05$). By receiver-operating characteristic curves, the areas under the curve for diagnosis of the presence of HAA by CAVI was 0.72. While HAA tended to correlate with eGFR and serum levels of hs-CRP, it did not correlate with proteinuria, HbA1c, HOMA-IR, lipids profiles (LDLC, non-HDLC, RLPC) and thrombomodulin. In addition, HTILA correlated with age, BP and eGFR, but did not correlate with CAVI, proteinuria, HbA1c, HOMA-IR, lipids profiles and thrombomodulin.

Conclusions: These results indicate that HAA correlates with the individual age and BP, and that CAVI may be a novel clinical tool to presume the presence of HAA, not but HTILA, in patients with IgAN.

TH-PO432

Aberrantly Glycosylation Is Not a Characteristic of Minimal Change Disease with IgA Deposition Yu Yan, Wei Chen, Bao Dong, Mei Wang. *Renal Division, Peking University People's Hospital, Beijing, China.*

Background: Some IgAN patients with nephrotic syndrome (NS), who mostly have no hematuria, no hypertension and normal renal function were named minimal change disease (MCD) with IgA deposition (MCD-IgA). Whether it is a special phenotype of IgAN or MCD was in debate. Our previous study revealed that MCD-IgA may be MCD with non-specific deposition rather than a phenotype of IgAN according to clinical and pathological analysis. The current study was to investigate the differences of glycosylation of IgA1 in serum IgA1-containing macromolecules and serum IgA1 among MCD-IgA, with nephrotic proteinuria without hypoalbuminemia (HP-IgAN) and MCD.

Methods: 10 patients from each three groups were enrolled. Polyethylene glycol 6000 was used to precipitate the macromolecules from sera of patients and controls. Biotinylated lectins were used in ELISA to examine different glycans on IgA1 molecules. The a2,6 sialic acid (SA) was detected by elderberry bark lectin (SNA), the exposure of terminal galactose (Gal) and N-acetylgalactosamine (GalNAc) were detected by arachis hypogaea (PNA) and Helix asperas agglutinin (HAA)respectively.

Results: Serum IgA1 levels were comparable among three groups. Exposure of GalNAc was less profound in both MCD-IgA and MCD than in HP-IgAN ($P<0.05$). No significant difference of GalNAc exposure was found between MCD-IgA and MCD. Serum IgA1 from MCD patients had more SA than that from HP-IgAN patients, but similar with that from MCD-IgA patients. In precipitated macromolecules total protein amounts were similar among three groups. Coincided with serum IgA1, exposure of GalNAc in macromolecular IgA1 was less profound in both MCD-IgA and MCD than in HP-IgAN ($P<0.05$). No significant difference of GalNAc exposure was found between macromolecular IgA1 from MCD-IgA and MCD patients. More SA was detected in macromolecular IgA1 from MCD patients than that from HP-IgAN patients. However, comparable sialylation was found between MCD-IgA and MCD patients.

Conclusions: Aberrantly glycosylation is not a Characteristic of MCD-IgA, which implies that MCD-IgA might not be a special phenotype of IgAN.

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TH-PO433

Tonsils of Patients with IgA Nephropathy Contain Cells Producing Aberrantly Glycosylated IgA1 and Anti-Glycan Antibodies: Implications for Tonsillectomy Hitoshi Suzuki,[1] Yusuke Suzuki,[1] Hiroyuki Yanagawa,[1] Jan Novak,[2] Yasuhiko Tomino.[1] *[1]Nephrology, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Microbiology, University of Alabama at Birmingham, Birmingham, AL.*

Background: IgA1 in circulating immune complexes and in the glomerular deposits of patients with IgA nephropathy (IgAN) is aberrantly glycosylated, galactose-deficient in *O*-glycans (Gd-IgA1) and is bound in the complexes by antiglycan IgG/IgA1 autoantibodies. However, the origin of cells producing Gd-IgA1 and the autoantibodies is unknown. Upper respiratory tract infections and/or tonsillitis are frequently associated with clinical presentation and exacerbation of IgAN, suggesting a link with the disease pathogenesis. There is increasing evidence that treatment including tonsillectomy and glucocorticoids prevents IgAN progression.

Methods: In this study, we studied 27 patients with IgAN who underwent tonsillectomy and glucocorticoid therapy (TSP). Tonsillar cells were immortalized with Epstein-Barr-virus, then Gd-IgA1 and anti-glycan IgG secreted by these cells were measured. In addition to urinary protein and hematuria, serum levels of Gd-IgA1, anti-glycan IgG and anti-glycan IgA were measured before and after the TSP therapy.

Results: Sixteen of twenty-seven patients showed less 0.3 g/gCr proteinuria and 5 red blood cells/HPF after TSP (Remission group). A rate of decrease in serum levels of Gd-IgA1, anti-glycan IgG, and anti-glycan IgA were greater in Remission group than in non-Remission group ($P<0.01$). Furthermore, tonsillar B cells from Remission group secreted higher amounts of Gd-IgA1 and anti-glycan IgG than those from non-Remission group ($P<0.01$).

Conclusions: In summary, tonsillar B cells are sources of Gd-IgA1 and anti-glycan antibodies in patients with IgAN. Moreover, these serum biomarkers may be useful disease markers for guiding the therapeutic approaches for IgAN.

TH-PO434

The Deposition of Secretory IgA in Patients with IgA Nephropathy Are Associated with Clinical and Pathological Phenotypes Yan Liang, Junjun Zhang, Liu Zhangsuo. *Zhengzhou University.*

Background: IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis in the world especially in China. Mucosal infection associated with episodic macroscopic haematuria are observed in many patients with IgAN. Secretory IgA (sIgA) is a mainly immunoglobulin involved in mucosal immunity. Our previous study showed it could be deposited in kidney in about 1/3 of IgAN patients and played an important role in the pathogenesis of IgAN. However, the association between renal sIgA deposition and clinical manifestations as well as pathogical phenotypes are still not clear. The aim of this work is to investigate the characteristics of IgAN patients with or without renal sIgA deposition.

Methods: Frozen renal sections from 99 primary IgAN patients without IgM deposition were immunofluorescence stained and examined by confocal microscopy to detect the co-deposition of IgA and secretory component (SC). The association between deposition of sIgA and characteristics of IgAN patients was analysed.

Results: In 99 patients, 32 patients (32.32%) with sIgA deposition, the incidence of infection history and gross hematuria were higher in patients with sIgA deposition compared with patients without. The levels of 24h urinary protein, serum cystatin C and serum creatinine were tendency lower in patients with sIgA deposition than the patients without. The number of crescents was less and the degree of tubulointerstitial lesion was milder in sIgA deposition group than non- sIgA deposition group. However, arterial pressure,hemoglobin and plasma-albumin were not different in these two groups.

Conclusions: It was concluded that sIgA deposited in renal,at least partly, was originated from mucosal immune sites. The deposition of sIgA in kidney might be associated with different clinical manifestation and tubulointerstitial lesion in patients with IgAN.

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TH-PO435

Decrease of Perlecan LG3 Peptide and Free k-Light Chains Urine Excretion in IgA Nephropathy: New Insights for a Non-Invasive Evaluation of Disease Activity and Injury Maria Teresa Rocchetti,[1,3] Massimo Papale,[1,3] Salvatore Di Paolo,[2] Annamaria D'Apollo,[1] Ida Valentina Suriano,[1] Grazia Vocino,[1] Eustacchio Montemurno,[3] Leonarda Varraso,[1] Giuseppe Grandaliano,[1] Loreto Gesualdo.[3] *[1]Nephrology, Dialysis and Transplantation Unit, Dept. of Medical and Surgical Sciences, University of Foggia, Foggia, Italy; [2]Division of Nephrology and Dialysis, Hospital Dimiccoli, Barletta, Italy; [3]Nephrology, Dialysis and Transplantation Unit, DETO, University of Bari, Bari, Italy.*

Background: IgA nephropathy (IgAN) has a highly variable clinical presentation and progression. Its accurate diagnosis and prognosis requires renal biopsy. Therefore, the identification of biomarkers allowing non-invasive diagnosis and monitoring of disease is strongly required.We investigated urine proteome in order to identify a set of proteins potentially helpful in identifying IgAN.

Methods: Urine proteins from 49 biopsy-proven IgAN patients, 42 patients with non-IgA chronic kidney diseases (CKD: 16 Diabetic Nephropathy, 12 Nephroangiosclerosis, 14 Membranous Nephropathy) and 40 healthy subjects (CTRL) were analyzed by SELDI-TOF/MS. Mass peaks in the 3000-30000 m/z mass range were studied by univariate analysis. Mass peaks able to discriminate IgAN were identified by MALDI-TOF-MS/MS. Proteomic findings were confirmed by immunological methods.

Results: Univariate analysis identified 13 mass peaks able to discriminate IgAN from CKD and CTRL. Among them, two mass peaks were identified as LG3 peptide and Ig-k light chains. Western Blot analysis confirmed the decreased urinary excretion of LG3 in IgAN compared to CKD and CTRL. Then, Immunonephelometry analysis confirmed the lower urinary excretion of kappa free LC in IgAN patients compared to CKD and CTRL. Accordingly, tissue data showed an increase of k FLC deposition and a decrease of LG3 in IgAN biopsy specimens. Finally, urinary kappa FLC and LG3 inversely correlated with the severity of clinical and histologic features of IgAN patients.

Conclusions: In conclusion, higher urine level of k FLC and LG3 are associated to IgAN patients with a better prognosis. K FLC and LG3 peptide could serve as candidate non-invasive diagnostic and/or prognostic biomarkers of IgAN.

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TH-PO436

Interleukin 6 Has a Unique and Central Role in the Development of Renal Damage in Two Different Types of Glomerulonephritis Maria Stangou,[1] Aikaterinni Papagianni,[1] Christos Bantis,[1] Efstratios D. Kasimatis,[1] Irene Stavrinou,[1] George Toulkeridis,[1] Dimitrios Moisiadis,[1] Marie Skoularopoulou,[1] Nikoleta-maria Kouri,[2] George Efstratiades,[1] Demitrios Memmos.[1] *[1]Department of Nephrology, Hippokration Hospital, Thessaloniki, Greece; [2]Department of Biochemistry, Hippokration Hospital, Thessaloniki, Greece.*

Background: Focal segmental necrotizing gloemerulonephritis (FSNGN) is characterized by severe inflammation and necrosis leading to rapid decline of renal function. IgA nephropathy (IgAN) leads to kidney damage through gradual development of glomerulosclerosis and tubulointerstitial fibrosis. We evaluated the role of local cytokine production in these two different types of glomerulophritis.

Methods: Epidermal Growth Factor (EGF), Interleukins-1β, -2, -4, -6, -7, -8, -9, -10, -15, -17, Vascular Endothelial Growth Factor (VEGF), Monocyte Chemoattractant Protein-1 (MCP-1) were measured in first morning urine samples from 53 IgAN [M/F 35/18 age 40.5yrs(17-65)]and 38 FSNGN patients[M/F 21/17, age 59.5yrs(25-80)]collected at day of renal biopsy, using a multiplex cytokine assay. Results were correlated with histology and long term outcome of renal function.

Results: In IgAN, the degree of glomerulosclerosis had positive correlation with IL-1β, (p=0.007) and MCP-1 (p=0.007) and negative with EGF (p=0.02) urinary excretion. High IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-17, MCP-1 and low EGF levels were associated with renal function deterioration. Urinary IL-6 was the only independent parameter correlated with the degree of renal failure (r^2=0.5, p=0.02) at the end of follow up (65±34months). In FSNGN, the percentage of crescents had positive correlation with IL-17 (p=0.008) and negative with EGF (p<0.0001). High IL-6, IL-15, VEGF levels were associated with poor outcome of renal function. The only independent predictor factor of long term outcome was IL-6 urinary levels (r^2=0.2, p=0.03). Follow up of the patients was 62±42months.

Conclusions: Different cytokines are involved in the progression of renal damage in two different types of glomerulonephritis, IgAN and FSNGN, but IL-6 seems to be implicated and play a key role in both of them.

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TH-PO437

Circulating microRNAs in Patients with *Escherichia coli* O104:H4-Associated Haemolytic Uraemic Syndrome Johan M. Lorenzen, Bernhard M.W. Schmidt, Mario Schiffer, Jan T. Kielstein, Hermann G. Haller, Thomas Thum. *Nephrology, Hannover Medical School, Germany.*

Background: In early May 2011, an outbreak of hemorrhagic colitis associated with hemolytic–uremic syndrome (HUS) first developed in Northern Germany and spread to 15 other countries in Europe. The outbreak-strain O104:H4, which combined virulence factors of typical enteroaggregative and Shiga-Toxin–producing E. coli was associated with an unusual high rate of hemolytic uremic syndrome. Also an unexpected high rate of coma and seizures leading to mechanical ventilation and ICU treatment was observed. MicroRNAs are small ribonucleotides orchestrating gene expression. We tested whether circulating microRNAs in serum of HUS patients during the 2011 epidemics are altered in this patient cohort and related to clinical manifestations.

Methods: We profiled microRNAs using RNA isolated from serum of patients and healthy age-matched controls. The results were validated in 38 patients at baseline, 29 patients during follow-up and 21 age-matched healthy controls by miRNA-specific quantitative RT-PCR.

Results: Circulating levels of miR-24, miR-126 were increased in HUS patients versus controls. There was no association between these microRNAs and renal function or the need for renal replacement therapy. In contrast, levels of miR-126 were associated with neurological symptoms at baseline and during follow-up. In addition, miR-126 (on admission) and miR-24 (on admission and during follow-up) were associated with platelet count.

Conclusions: Circulating microRNAs are strongly altered in this patient cohort and associated with neurological symptoms as well as platelet count.

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TH-PO438

The Number of Plasma Exchanges and the Extended Need of Dialysis Correlate with Poor Long Term Outcome in Adult Patients with Escherichia Coli Associated Hemolytic Uremic Syndrome during the 2011 German Outbreak Maria von Lewinski, Rolf A. Stahl. *Division of Nephrology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

Background: During the 2011 German outbreak of E. coli associated hemolytic uremic syndrome (HUS) 129 patients with HUS were treated in our institution. 48% of these patients had neurologic symptoms and 51% needed hemodialysis (HD). Patients received supportive care or supportive care and additionally plasma exchange (PE) or Eculizumab or both depending on the severity of the disease.

Methods: In a single center approach we analyzed the ten months outcome of 122 patients. They were divided into two groups depending on the treatment. Group A (n=60) received PE (mean 3.2 courses) or only supportive treatment. Patients in Group B (n=62) were treated with Eculizumab and most individuals additionally with PE (mean 4 courses). Group B was divided into three subgroups according to the number of PE: B1 (n=5) 0-1 PE, B2 (n=33) 2-5 PE, and B3 (n=24) 6-13 PE.

Results: In group B, the course of the disease was much more severe with an average of 11.3 days on HD compared to 1.3 days in group A. In group A, 2.6% of patients had an elevated serum creatinine and 28% of patients had persistent albuminuria after ten months. 23% of patients in group B had an impaired renal function and 31% of patients had a persistent albuminuria.

In group B, patients had a more severe course of the disease and a worse outcome depending on the number of PE. In group B1, patients required renal replacement therapy for an average of two days versus 11 days in group B2 versus 13 days in group B3. The serum creatinine after ten months was above normal in 0% (B1), 21% (B2), and 29% (B3) of patients; the urine albumin-creatinine-ratio was elevated in 20% (B1) and in 33% (B2 and B3) of patients.

Conclusions: In patients who had been severely ill and were treated with Eculizumab, the number of plasma exchanges correlated with the time on dialysis. Furthermore a higher percentage of these patients had an impaired renal function after ten months. These findings suggest that the number of PE and the time on dialysis are predictors for a residual renal damage in severely ill patients with STEC-HUS.

TH-PO439

TCC Generation In-Vitro as a Possible Monitoring Parameter in Patients with Atypical Hemolytic Uremic Syndrome (aHUS) Treated with Eculizumab Magdalena Riedl,[1] Johannes Hofer,[1] Alejandra Rosales,[1] Giacomo D. Simonetti,[2] Tanja Maier,[3] Therese C. Jungraithmayr,[1] Reinhard Würzner.[1] *[1]Medical University, Innsbruck, Austria; [2]University Children's Hospital, Bern, Switzerland; [3]Phillipps-University, Marburg, Germany.*

Background: Eculizumab, an antibody blocking C5, is a novel treatment for aHUS. Here we present 5 patients with aHUS and factor H mutations in whom complement activation was determined during long-term Eculizumab treatment.

Methods: C3a, a marker of proximal complement activation, the terminal complement complex (TCC) concentration and degree of sheep erythrocyte lysis was measured by ELISA. Preincubation of serum with zymosan leads to in-vitro activation of complement and is a measure of the residual capacity of complement. Results are given as median and IQR, normal value (NV) is given as IQR of 92 healthy controls.

Results: Eculizumab, applied after plasma exchange withdrawal, led to decrease of in-vitro TCC formation from 1249 AU/ml (896-1476 AU/ml) prior treatment to 62 AU/ml (49-62 AU/ml) during biweekly Eculizumab application (NV 339-889 AU/ml). TCC concentration in plasma was reduced from 2.9 AU/ml (2.0-3.8 AU/ml) to 2 AU/ml (1.6-2.6 AU/ml; NV 0.7-2.0 AU/ml). C3a concentration was elevated prior to Eculizumab (3830pg/ml; 1412-6000pg/ml) and decreased to normal during biweekly administration (734pg/ml; 499-876pg/ml; NV 188-627pg/ml). In 2 patients lysis was detectable (12.6, 5.8%; NV 0-4.7%) prior Eculizumab, which resolved under treatment. 4/5 patients were switched to a three weeks interval, resulting in a rapid increase of TCC capacity to 366 AU/ml (195-554 AU/ml), an elevation of C3a levels to 1435pg/ml (1018-2278pg/ml) and lysis of 10% in 1 patient. A sustained reduction of renal impairment and an increase of platelet count was reported.

Conclusions: We report the successfull long-term treatment of 5 patients with Eculizumab. The determination of TCC generation in-vitro and C3a concentration seem to be promising monitoring parameters. A normalization of C3a levels during Eculizumab indicates a reduction of proximal complement activation. A three weeks interval, may lead to a less blocked complement activation and therefore an increased risk for recurrence.

Funding: Government Support - Non-U.S.

TH-PO440

The Challenge of Managing Hemophilia A and STEC-Induced Hemolytic Uremic Syndrome Dineke Westra,[1] Eiske Dorresteijn,[3] Auke Beishuizen,[4] Lambertus V. Heuvel,[1] Nicole Van De Kar,[1] Paul Brons.[2] *[1]Pediatric Nephrology, RUMC, Nijmegen, Netherlands; [2]Pediatric Hematology-Oncology, RUMC, Nijmegen, Netherlands; [3]Pediatric Nephrology, Erasmus MC-Sophia, Rotterdam, Netherlands; [4]Pediatric Oncology-Hematology, Erasmus MC-Sophia, Rotterdam, Netherlands.*

Background: Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy leading to acute renal failure in children. In most cases it is triggered by a STEC infection. Endothelial damage plays a central role in the pathogenesis of disease. Haemophilia A is a genetic disorder, leading to Factor VIII (FVIII) deficiency, an important factor in the coagulation system. Here we describe a haemophilia A patient that developed STEC -induced HUS.

Methods: Here we describe a hemophilia A patient in which severe gastro-intestinal and neurological complications occurred during STEC O26-induced HUS. Due to hemorrhagic colitis, severe neurological complications, hypertension, and bleeding at the exit site of the jugular catheter, all in combination with thrombocytopenia and persistently low FVIII levels in serum, FVIII treatment had to be increased enormously. The role of FVIII administration (oral or IV) in the severe outcome of disease is discussed.

Results: It is known that shiga toxin induces the secretion of von Willebrand Factor (vWF) from microvascular endothelial cells, a protein important in adhesion of platelets at the site of injury and a carrier of FVIII in plasma. The adhesion of platelets by vWF will probably lead to increased thrombus formation in the microvasculature of the kidneys of HUS patients The supraphysiological dose of FVIII needed in this case may have contributed further to the thrombotic microangiopathy after shiga toxin-induced endothelial damage in the affected organs.

Conclusions: To our knowledge, this is the first report of a haemophilia patient that developed severe HUS with complications after STEC infection. The patient's treatment schedule of rFVIII during the HUS period was a big challenge and we cannot exclude that it contributed to the severity of the HUS by enhancing the thrombotic microangiopathic process. Continous intravenous rFVIII gave the most stable FVIII levels in the acute phase of STEC HUS.

Funding: Private Foundation Support

TH-PO441

Several Genetic Aberrations in Different Complement Genes in a Patient with Dense Deposit Disease Dineke Westra,[1] Hans Van der Deure,[2] Elena Volokhina,[1] Lambertus V. Heuvel,[1] Nicole Van De Kar.[1] *[1]Pediatric Nephrology, RUMC, Nijmegen, Netherlands; [2]Pediatrics, Deventer Hospital, Netherlands.*

Background: Membranoproliferative glomerulonephritis type II or dense deposit disease (DDD)is characterized by onset of hematuria and/or proteinuria, acute nephritic or nephrotic syndrome. The exact pathogenesis is not known, but there is involvement of the alternative complement system. In some patients, it is associated with C3-nephritic factor (C3NeF), which stabilizes the C3 convertase C3bBb, with mutations in the complement factor H (CFH) and complement C3, or with autoantibodies against CFH (αFH). Here, we identify potentially pathogenic genetic aberrations in genes of the alternative complement pathway and the membrane attack complex (MAC) in a DDD patient with low C3 values.

Methods: In one DDD patient, mutational screening was performed in the alternative pathway genes *CFH*, *CFI*, *MCP*, *CFHR5*, *C3*, *CFB*, and *CFD*, and the MAC genes *C8A*, *C8B*, and *C9*. Potential pathogenicity of aberrations was checked in literature, evolutionary conservation, and *in silico* mutation prediction programs.

Results: The male patient presented at six years of age with hematuria without other symptoms. C3 values were low (<0,5 mg/l; normal values: 0.9-1.8 g/l). DDD was identified in a renal biopsy. The patient is C3NeF positive, but αFH negative; no drusen are present, yet. A genetic aberration was found in *CFD* (A41P), in *C3* (K155Q), and in *C8A* (A221E). Prediction models for structural influence of the mutations will be displayed. Family screening showed that only the patient carried all three genetic variations.

Conclusions: In our DDD patient, three potentially pathogenic variations are found in genes of the alternative complement pathway and the membrane attack complex. In the patient, a combination of three sequence variations was found, while healthy family members carried only one or two of these variations. This indicates that, next to C3NeF, a combination of genetic defects in the complement system may contribute to the low C3 level and might be needed to display DDD.

Funding: Private Foundation Support

TH-PO442

Eculizumab (ECU) Is Effective in Patients (pts) with Atypical Hemolytic Uremic Syndrome (aHUS) Regardless of Underlying Genetic Mutations or Complement Factor H (CFH) Auto-Antibodies Tim Goodship, Richard J. Smith, Christophe M. Legendre, Christoph Licht, Petra Muus, Nancy MacDonald Rodig, Samhar I. Al-Akash, Camille Bedrosian, Veronique Fremeaux-bacchi, Chantal Loirat. *Newcastle University, Newcastle, United Kingdom.*

Background: aHUS is a life-threatening, genetic disease characterized by chronic uncontrolled complement activation and systemic thrombotic microangiopathy (TMA). Despite plasma exchange/infusion, 33-40% of aHUS pts progress to death/ESRD during the first clinical manifestation. Genetic mutations/CFH auto-antibodies are identified in 50-70% of aHUS pts.[1] Overall, risk of death/ESRD is similar in pts with/without an identified mutation.[1,2] ECU, a terminal complement inhibitor, is the only approved treatment for aHUS. In 3 studies, ECU was shown to continuously inhibit TMA and maintain/improve renal outcomes.

Methods: Efficacy parameters were analyzed across 3 studies (2 prospective and 1 retrospective [<18 yrs]), by

presence/absence of genetic abnormalities (Table).

Results: Across the 3 studies, 24-41% of enrolled aHUS pts had no identified complement abnormality. Efficacy of ECU was similar in pts with/without an identified genetic abnormality (Table).

Conclusions: Across 3 studies of ECU in aHUS, improved clinical outcomes were similar in pts with/without identified genetic mutations/CFH auto-antibodies, confirming treatment can be initiated immediately in patients with a clinical diagnosis of aHUS before the analyses of a genetic abnormality are undertaken or the results are available.[3]

Parameter	Pts with Long Duration of Disease (Median ECU Duration 62 Weeks) (N=20)		Pts with Progressing TMA (Median ECU Duration 64 Weeks) (N=17)		Pediatric Pts (Retrospective Study)[1] (N=17)	
	With mutation[2] n=13	No identified mutation[2] n=7	With mutation[2] n=13	No identified mutation n=4	With mutation[2] n=10	No identified mutation n=7
PRIMARY ENDPOINT	TMA event free status[3], n (%)		Mean platelet count change (SE) 10^9/L		None	
	12 (92)	5 (71)	118.8 (19.4)	103.8 (50.0)[4]		
TMA event-free status, n (%)	12 (92)	5 (71)	12 (92)	3 (75)	7 (70)	5 (71)
Platelet count >150×10^9/L, n (%)	12 (92)	6 (86)	12 (92)	3 (75)	10 (100)	6 (86)
Hematological normalization[5] n (%)	12 (92)	6 (86)	12 (92)	3 (75)	4 (40)	3 (43)
Creatinine decrease ≥25%, n (%)	4 (31)	3 (43)	11 (85)	2 (50)	4 (40)	4 (57)
eGFR increase ≥15mL/min/1.73m^2, n (%)	2 (15)	1 (14)	7 (54)	2 (50)	5 (50)	3 (43)
Mean eGFR increase (SE) mL/min/1.73m^2	5.4 (2.3)	7.3 (2.7)	32.9 (9.6)	31.2 (21.0)[6]	NA	NA

[1]Variable follow-up duration
[2]Identified genetic mutations also includes CFH auto-antibodies
[3]A pt with a CFHR1-3 deletion has been included under no identified mutation for this analysis
[4]No decrease in platelet count of >25%, no PE/PI and no new dialysis ≥12 consecutive weeks
[5]Data from a patient who discontinued after 1 dose due to meeting an exclusion criterion are not included
[6]Platelet count >150×10^9/L and LDH ≤ ULN for ≥4 weeks
NA, not available.

1.Noris et al. Clin J Am Soc Nephrol 2010; 2.Caprioli et al. Blood 2006; 3.Ariceta et al. Pediatr Nephrol 2009

Funding: Pharmaceutical Company Support - The Clinical Trials Were Sponsored by Alexion Pharmaceuticals Inc.

TH-PO443

Non Lupus Membranoproliferative Glomerulonephritis Classification According to Immunoglobulin Deposition on Immunofluorescence Raquel Maria Maia, Camila Hitomi Nihei, Loyana Teresa Teofilo Lima Silva, Cristiane Bitencourt Dias, Viktoria Woronik. *Nephrology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.*

Background: A new classification of membranoproliferative glomerulonephritis (MPGN) according to immunoglobulin deposition has been proposed in literature. The aim of this study is to compare the initial clinical presentation and follow up of those patients.

Methods: This retrospective study assessed, among 1641 renal biopsies performed in one center from 1999 to 2011, the clinical and laboratory data at diagnosis and during follow up of patients with non lupus MPGN (n = 95 biopsies). We excluded 25 patients by insufficient clinical on histological data, remaining 70 patients for analysis.

Results: Immunoglobulin deposits on IF were observed in 54 patients (77.1%) while 16 (22.8%) showed no immunoglobulin deposits. Among patients without immunoglobulin deposits 6 showed C3 deposits and 10 were totally negative. Comparison of the initial data between MPGN with immunoglobulin and without immunoglobulin in IF showed no difference in age of 45.9 ±15.1 vs 42.3 ±19.4 years, serum creatinine of 1.8 (1.1-3.2) vs 2.2 ±1.5 mg/dL, proteinuria of 5.1 ±3.2 vs3.7 ±3.0 g/day, hematuria in 75.9 vs 75.0% and low C3 complement in 48.1 vs 50.0% . Among patients with positive immunoglobulin, 23 (42.6%) exhibited an associated disease: 5 schistosomiasis, 4 HCV, 1 HBV, 1 HIV, 2 HCV + HBV, 1 leprosy, 2 autoimmune disease, 2 neoplasia; 4 cirrhosis and 1 spherocytosis. In patients without immunoglobulin deposition we found an association with other disease in 6 (37.5%): HCV in 4 and neoplasia in 2. On follow up, end stage chronic disease was observed in 21.2% if immunoglobulin positive patients and 37.5% of negative patients, not different between both groups.

Conclusions: There was no difference between MPGN groups with and without deposition of immunoglobulin considering initial clinical and laboratory data, as well as, to evolution to chronic disease. Surprisingly in our series and not expected from literature data, HCV and neoplasias were associated with both MPGN groups (immunoglobulin positive and negative).

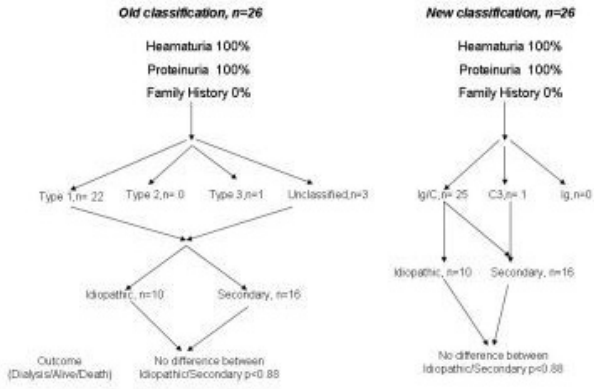
TH-PO444

A Ten Year Diagnostic Review of MPGN Cases in a Single Centre Arvind Ponnusamy, Darren Green, Shiv Bhutani, Edmond O'Riordan. *Renal, Salford Royal NHS Trust Foundation, Salford, United Kingdom.*

Background: MPGN is a rare glomerulonephritis in adults. The existing classification is based on electron microscopy appearances. A new complement-driven pathogenesis-based classification along 3 immuno-fluorescence pattern groups (Ig only, Ig and C3 and C3 only) has been proposed. Little is known of their clinical utility.

Methods: We reviewed the clinical notes of patients who presented to our hospital between 2001 and 2011. Cases were classified by the existing and proposed criteria. Laboratory, clinical parameters and outcome were also analysed.

Results: Mean follow up time was 3 years. The mean creatinine at presentation was 197umol/l ± 132 and proteinuria was 396 ± 251gm/day. On biopsy, 23 cases were Type 1, one was Type 3 and 3 cases were unclassifiable. Both immunoglobulin and complement deposition were present in 25 cases, one patient had only C3 deposition (previously classed type 1), and no patients were negative for both Ig and C3. We found the following secondary causes: SLE [4 patients], CLL [3 patients], IgAN [2 patients], Cryoglobulinemia [4 patients] and rheumathoid arthritis [1 patient], light chain [1 patient], with 10(42%) patients regarded as idiopathic. Comparison of both classification systems revealed neither was useful in predicting patient outcome ((patient/renal survival), p<0.88).



Analysis of baseline data suggests that age >65 years and creatinine > 250 ummo/l were associated with worse outcomes (OR 5.3, OR 4.8, p<0.05) respectively. One patient identified with hereditary C4 deficiency (Type 1) revealed both Ig and complement deposition.

Conclusions: Categorization either by existing or proposed classification results in clustering in 1 subgroup. Despite investigations, many remain idiopathic and patient outcomes are heterogenous. Genetic complement disorders also occur in patients with both Ig and C3 deposition.

TH-PO445

Treatment of C3 Glomerulopathies with Mycophenolate Mofetil or Rituximab: A Comparative Study Sophie Chauvet,[1] Alexandre Karras,[1] Isabelle Tostivint,[2] Eric Thervet,[1] Veronique Fremeaux-bacchi,[3] Aude Servais.[4] *[1]Nephrology, HEGP, Paris; [2]Nephrology, Pitié-Salpétrière, Paris; [3]Immunology, HEGP, Paris; [4]Nephrology, Necker, Paris.*

Background: C3 glomerulopathy is a newly described entity associated with acquired and/or genetic abnormalities in complement alternative pathway. Renal outcome is poor with a ten years renal survival of 50% but optimal treatment is not known. The aim of this study was to describe clinical and biological evolution of patients treated with mycophenolate mofetil (MMF) or rituximab (RTX).

Methods: This retrospective multicentric study included 20 patients. Data were collected at treatment start, at 6, 12 months and last follow up. Renal response was defined as partial (prteinuria Pu ≤ 2.5g/d or a ≥50% decrease of Pu, albuminemia ≥25g/l, stable serum creatinine (sCr)) or complete (Pu ≤ 0.5g/d, albuminemia ≥30gl and normal or stable sCr). Immunological response was defined by normal C3 level and/or negativation of C3 Nephritic factor (C3Nef).

Results: Twenty patients (sex ratio 1, median age 24 years, range 6 to 64) were included. Seventeen patients had low serum C3. C3Nef was found in all cases, associated in 3 with a factor H or I mutation.At the time of treatment start, sCr was 106µmol/l (range 29-600), Pu 6.24g/d (range 1.38-15), albuminemia 27g/l (range 13-38). Patients received MMF (n=9) or RTX (n=11).

Partial and complete renal responses were observed in both groups during follow up. After a median follow up of 49 months (range 6-156), 7 patients reached end stage renal disease (ESRD) (5 in RTX group).

complete and partial renal responses

	MMF	RTX
6 months	5/9	6/11
12 months	5/9	5/11
last follow up	4/9	4/11

Serum creatinine of others patients was 126µmol/l (range 30-575) and 100µmol/l (range 30-175) in MMF and RTX group, respectively. At 6 and 12 months, immunological response was observed in 5 and 3 patients, respectively.

Conclusions: Renal remissions were similar in both groups during the first year of treatment, independently of immunological response. Nevertheless, RTX seems to be associated with a higher risk of ESRD at last follow up.

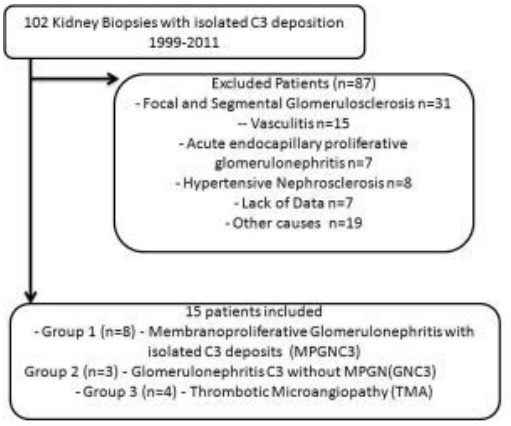
Funding: Clinical Revenue Support

TH-PO446

Clinicopathological Features in Patients with Isolated C3 Deposits in Glomerular Diseases Maria Julia C.L.N. Araujo, Ligia Costa Battaini, Vivian Lumi Onusic, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, University of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Glomerular nephropathies with isolated complement C3 deposits can be observed in different situations: MPGN type II, acute poststreptococcal glomerulonephritis, and glomerulonephritis C3. Glomerulonephritis C3 was associated in rare instances with haemolytic uraemic syndrome. The aim of our study was evaluate clinical and histological features associated with isolated C3 deposits in kidney biopsy.

Methods: A retrospective analysis of 102 kidney biopsies with isolated C3 deposits performed at our hospital was done. Relevant clinical and biological data were collected. A huge heterogeinety of histological diagnosis was found and we chose for analysis 3 pathologies known for their envolvement with imunocomplexes and/or complement cascade activation, as show in fig1.



Results: The results are summarized in Table1.

Clinical and Histological Features

	Group1 (MPGN 3)	Group2 (GN 3)	Group3 (TMA)
Age(y)	43,1±19,3	19,33±4,6	41±14,7
Male	5(63%)	1(33%)	1(25%)
eGFRin(ml/min)	51,4±21,8*†	115,3±52,6	9,3±2,4
Proteinuria(g/day)	3,9±2,2†	7,9±5,3§	1,2±0,4
C3 level<90mg/dl n(%)	3(37%)	0(0%) §	4(100%)
Hemoglobin(g/dl)	11,7±1,64†	14±2,3§	7,6±1,3
Platelets(cells/mm3)	210±61,3	329±12,9§	115±104,4
Hematuria n(%)	6(75%)	3(100%)	2(50%)
Crescents n(%)	3(38%)	0(0%)	2(50%)
Interstitial fibrosis	7(88%)	1(33%)	4(100%)
Vascular injury	2(25%)	0(0%)§	4(100%)
ESRD	4(50%)*	0(0%)§	4(100%)

***p<0.05 1 vs 2; †p<0.05 1 vs 3; §p<0.05 2 vs 3**

Only 15% of the patients are affected by pathologies related to complement cascade activation. Despite the small casuistics, TMA patients showed the worst scenario considering clinical presentation and follow up, while GNC3 was the less agressive disease.

Conclusions: Isolated C3 glomerular deposition should be cautiously overlooked and avaliated on the ground of the underlying clinical disease.

Funding: Government Support - Non-U.S.

TH-PO447

'Persistent' Post-Infectious Glomerulonephritis Is Associated with Abnormalities in the Alternative Pathway of Complement Sanjeev Sethi,[1] Fernando C. Fervenza,[1] Ladan Zand,[1] Yuzhou Zhang,[2] Samih H. Nasr,[1] Richard J. Smith.[2] *[1]Mayo Clinic, Rochester, MN; [2]University of Iowa, Iowa City, IA.*

Background: Post-infectious glomerulonephritis is a common type of glomerulonephritis (GN) that results following an infection. In the majority of cases, there is complete recovery of renal function. However, in a small percentage of patients there is persistence of the GN manifested by hematuria and proteinuria. The cause of 'persistent' post-infectious GN is unknown. We hypothesized that in such patients there is a defect in the regulating mechanisms of the alternative pathway (AP) of complement that prevents its down-regulation following resolution of the infection.

Methods: 11 patients with persistent hematuria and proteinuria were selected. Kidney biopsy showed a proliferative GN, bright C3 staining on IF and subepithelial humps on EM. The AP was evaluated by functional assays including C3 nephritic factors (C3Nefs) and genetic testing.

Results: There were 5 females and 6 males (age range 2 to 71). Serum creatinine ranged from 0.5 to 3.1 mg/dL (mean 1.43) with an eGFR of 53 to 280 ml/minute (mean 72.45). Ten patients had hypertension. C3 levels were low in 6 patients. C4 levels were normal in all patients. All patients had persistent hematuria and/or proteinuria (range 4 to 48 months, mean 15). Autoantibodies or mutations in complement genes were identified in 10 of 11 patients. Seven patients were positive for C3Nefs, and were associated with additional functional abnormalities of the AP in 6 patients. Four patients had mutations of complement genes including 3 patients with mutations in CFH and 1 patient with a mutation in CFHR5. The CFH mutations included a frameshift – c.2171delC, p.Thr724fsSTOP725 – and two missense variants – c.1699A>G,p.Arg567Gly and c.3350A>G,p.Asn1117Ser. The single missense variant in CFHR5 was c.646-647AA>TT,p.Asn216Phe.

Conclusions: Patients with 'persistent' post-infectious GN have a defect in regulation of the AP resulting in an inability to control the AP even after resolution of the infection. The sequela is continual glomerular deposition of complement factors with resultant inflammation and development of 'persistent' post-infectious GN.

Funding: NIDDK Support

TH-PO448

2,8-Dihydroxyadenine Crystal Nephropathy: Report of 11 Cases
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Background: APRT deficiency is a rare autosomal recessive purine enzyme defect that results in recurrent 2,8-dihydroxyadenine (2,8-DHA) urolithiasis and more rarely crystal nephropathy.

Methods: Retrospective multicentric study of patients with 2,8-DHA crystal nephropathy diagnosed by infrared spectroscopy.

Results: 11 patients (5M/6F) were included (age 57±13 years). The mean interval between first consult and diagnosis was 6±5 years but 7 pts had a history of urolithiasis. All patients had CKD attributed to nephrolithiasis and chronic tubulointerstitial nephritis (4 pts), benign nephroangiosclerosis (2 pts) or undetermined nephropathy (5 pts). 8 pts had reached ESRD and 7 pts had a kidney graft before diagnosis presenting with delayed graft function (3 pts), chronic graft dysfunction (1 pt) or acute-on-chronic renal failure (2 pts). In one case, the diagnosis was established after transplantation without graft dysfunction. Mean eGFR at diagnosis was 16±11 ml/min/1,73m² with 4 pts requiring dialysis. In all cases kidney biopsies showed tubulointerstitial lesions with crytals that were birefringent under polarized light. Oxalosis was firstly suspected in 6 pts. Renal 2,8-DHA-crystals were eventually identified by infrared spectroscopy in all cases. Cristalluria showed 2,8-DHA crystals in 3/3 pts. Erythocyte APRT activity was undetectable in 7/7 pts. APRT gene mutations were identified in 6/6 pts. Under therapy including increased water-intake, low-purine diet and allopurinol, renal function improved in 6 pts leading to withdrawal of dialysis in 2 pts. At last follow-up, 4 pts were on dialysis and mean eGFR for 7 pts was 33±16 ml/mn/1.73m².

Conclusions: 2,8-DHA crystal nephropathy is an underdiagnosed cause of kidney injury leading to ESRD and graft loss. A better knowledge of this disease would allow earlier diagnosis and start of an efficient allopurinol therapy.

TH-PO449

Mortality and Renal Outcome of Biopsy-Proven Glomerulonephritis: A Review of 30 Years of Experience
Hajeong Lee,[1] Yun Jung Oh,[1] Yon Su Kim,[1] Dong Wan Chae,[2] Suhnggwon Kim,[1] Ho Jun Chin.[2] *[1]Internal Medicine, Seoul National University Hospital; [2]Internal Medicine, Seoul National University Bundang Hospital.*

Background: Previous epidemiologic studies have focused on the prevalence of glomerulonephritis (GN) and few studies have explored the long-term patient outcomes. This study was conducted to investigate long-term patient and renal outcome of GNs.

Methods: Patients performed a renal biopsy from 1979 to 2008 were included in this retrospective analysis. The outcomes were patient and renal death. The relative mortality rate of GNs was estimated by the standardized mortality ratio (SMR) with 95% confidence interval (CI).

Results: GN comprised 86.7% of 4,998 naïve kidney biopsies, with 3,235 of primary GNs and 1,100 secondary GNs. IgA nephropathy (IgAN) and lupus nephritis was the most common primary (n= 1,404, 43%), and secondary GN (n= 526, 48%), respectively. Membranoproliferative GN of primary GNs and diabetic nephropathy of secondary GNs showed worst prognosis in terms of both renal (20 year survival rate [YSR] 48.4% vs. 10YSR 46.6%) and patient survival (20YSR 64.2% vs. 10YSR 60.9%), respectively. Interestingly, IgAN and Henoch-Schonlein nephritis (HSN) patients revealed relatively fair patient survival (20YSR 89.6% vs. 86.8%), notwithstanding their significant renal progression (20YSR 60.3% vs. 62.9%). Mortality of GN patients is significantly higher than age/sex matched general population in both primary GN (SMR 1.67, 95% CI 1.42-1.95) and secondary GN (SMR 4.77, 95% CI 3.95-5.70). In primary GN, men showed comparable mortality except with focal segmental glomerulosclerosis (SMR 1.63, 95% CI 1.05-2.43), whereas most of women showed higher mortality except with minimal change disease (SMR 2.21, 95% CI 0.72-5.16), compared to general population. In secondary GN, most patients exhibited elevated mortality except men with HSN (SMR 1.72, 95% CI 0.47-4.41).

Conclusions: We demonstrated that mortality of GN is significantly higher than general population, especially in secondary than primary GN, and in women than men. IgAN and HSN revealed relatively fair survival rate, allowing for their significant renal progression.

TH-PO450

Recurrent and De Novo Glomerular Disease in Renal Allografts
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Background: The usual question about glomerular disease in renal transplants is how often does each type recur. Here we answer 3 different questions: 1) What is the frequency of different types of glomerular disease in renal transplant biopsies? 2) When a specific type of glomerular disease is observed in a transplant, how often is it recurrent vs de novo? 3) When a transplant patient develops glomerular disease, how does the likelihood of recurrent vs de novo disease vary with the initial cause for ESRD?

Methods: Frequencies of specific categories of glomerular diseases were determined in 385 patients with glomerular disease in allograft biopsies evaluated at UNC. The frequency of recurrent versus de novo disease was determined in 84 patients who had paired native kidney and transplant biopsies.

Results: Selected glomerular disease frequencies were FSGS 139, membranous GN 45, IgA GN 31, TMA 30, immune complex GN NOS 29, diabetic GS 27, lupus GN 18, type I MPGN 11, C1q GN 5, ANCA GN 4, MIDD 3, DDD 3, amyloidosis 2, anti-GBM GN 1. 139 FSGS cases include 30 collapsing FSGS but only 2 tip lesion FSGS. 30 TMA cases include 7 in the setting of calcineurin inhibitor toxicity. Table 1 compares renal biopsy diagnosis in native kidney (x-axis) with transplant kidney (y-axis).

Native kidney diagnosis (x-axis), transplant kidney diagnosis (y-axis)

	FSGS	Mem GN	Lupus GN	IgA GN	GN NOS	Diabetes	Other
FSGS	20	1	2	1	2	0	4
Mem GN	2	6	0	0	1	0	3
Lupus GN	0	0	8	0	0	0	0
IgA GN	1	0	0	4	0	0	1
GN NOS	3	0	1	0	3	0	7
Diabetes	0	1	0	0	0	1	0
Other	1	0	0	1	0	0	4

When observed in a transplant biopsy, 100% lupus GN, 67% FSGS, 67% IgA GN, and 50% membranous GN were recurrent. Glomerular disease developing in a transplant was recurrent in approximately 75% of patients with native kidney lupus GN, membranous GN and FSGS; and less often recurrent in patients with other native glomerular diseases.

Conclusions: In our cohort, glomerular diseases in renal transplants is most often FSGS, membranous GN, and IgA GN; and all glomerular diseases in transplants are more often recurrent than de novo.

TH-PO451

Profiling Human Urinary mRNA
Larysa T. Wickman,[1] Yan Yang,[1] Akihiro Fukuda,[2] Ryuzoh Nishizono,[1] Debbie S. Gipson,[1] David B. Kershaw,[1] Marie Tanzer,[1] Roger C. Wiggins.[1] *[1]University of Michigan, Ann Arbor, MI; [2]University of Miyazaki, Japan.*

Background: Depletion of podocytes drives the progression process in animal model systems and human glomerular diseases. Increased urine podocyte specific product measurements, such as podocin and nephrin, are associated with both acute glomerular injury and progression. We previously analyzed urine podocin and nephrin mRNAs in 4 different model systems of progression. Two model systems relied on initial podocyte injury to trigger progression (PAN and podocin hDTR transgenic rat), while two relied on growth to trigger progression (5/6 nephrectomy and podocin-AA4EBP1 transgenic rat). We defined patterns of podocyte injury as: "Podocyte Loss" (high podocin:creatinine ratio), and "Podocyte Stress" (high podocin:nephrin ratio). For comparison we used urine aquaporin2 mRNA as a marker of tubular injury and TGFb1 mRNA as a marker of pro-fibrotic milieu.

Methods: Using insights developed from the model systems we compared 682 urine samples from adult and pediatric kidney clinics to 110 control samples. Spot urine samples were collected, processed for RNA purification, reverse transcribed and assayed using the TaqMan system for podocin, nephrin, aquaporin2 and TGFb1 mRNA, protein and creatinine concentrations. A two sample comparison was used to analyze urine podocin:creatinine ratio and urine protein:creatinine ratio of clinic samples vs controls.

Results: Compared to controls, clinical samples had elevated urine protein:creatinine ratio ($P=3x10^{-21}$) and elevated urine podocin:creatinine ratio ($P=3.6x10^{-33}$). Podocin:nephrin mRNA ratio was increased in clinic samples compared to control samples ($P<0.001$). Urine podocin:creatinine ratio did not correlate highly with the urine protein:creatinine ratio ($r^2=0.12$), thereby demonstrating that urine podocin mRNA provides additional information.

Conclusions: Urine mRNA biomarkers can provide clinical information complimentary to proteinuria. Longitudinal analysis of phenotypically well-characterized patients will be required to establish the potential utility of urine mRNA biomarkers for clinical decision-making within defined settings.

TH-PO452

Comparison of Glomerular Density and Size Distributions in Renal Biopsies of Primary Glomerular Diseases
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Background: We have recently reported that glomerular number per renal cortical area in biopsies (glomerular density: GD) differs significantly between individuals, and that a low GD predicts a worse renal outcome in patients with IgA nephropathy (IgAN) and idiopathic membranous nephropathy (IMN) (CJASN2010, NDT2011). Differences in GD values between etiologically distinct glomerular diseases remain undetermined.

Methods: The values of the GD and the glomerular volume (GV) were measured in a total of 302 biopsies using a computed imaging analyzer. The measured values were compared between the patients including minimal change disease (MCD), IgAN, IMN, membranous proliferative glomerulonephritis (MPGN) and focal segmental glomerulosclerosis (FSGS). All biopsies were performed at the time of preserved renal function (eGFR≥60ml/min/1.73m²) and the biopsies of kidney transplant donors (KTD) were used as comparison.

Results:
Comparison of GD/GV

	KTD (n=34)	MCD (n=50)	IgAN (n=98)	IMN (n=65)	MPGN (n=32)	FSGS (n=23)
GD (/m^2)	3.1±1.1	3.6±1.1	3.5±1.5	3.4±1.1	3.4±1.1	2.1±1.0*
GV (x10^{6}µm^3)	2.6±0.8	2.5±0.8	2.9±1.1	4.1±1.1*	3.9±1.0*	5.0±0.3*
r (GD vs. GV)	-0.438	-0.420	-0.544	n.s.	n.s.	-0.420

*p<0.01 vs. KTD

Of note, there are three distinct patterns of the GD/GV distribution in primary glomerular diseases.The values of these measures in MCD and IgAN were similar to those of KTD. In this first patients group, the GD showed inverse correlations to the GV (normal pattern). In the second patients group of IMN and MPGN, the GV values were large regardless of the GD levels and there were no significant correlations between these measures (MN pattern). In the third group of FSGS patients, the GD was extremely low, and the GV showed marked enlargement (FSGS pattern). Analyses of the serial biopsies in IgAN (n=18) and IMN (n=10) patients showed the same trends toward FSGS pattern during the progressive loss of renal function.

Conclusions: The differences in the GD/GV distribution in primary glomerular diseases are independent of those observed between the individuals, and may represent the disease characteristic status.

TH-PO453

Glomerular Volume in Patients Who Died due to Intracranial Hemorrhage Marcin Adamczak, Katarzyna Kwiecien, Katarzyna Konat, Henryk Karkoszka, Magdalena Szotowska, Andrzej Wiecek. *Department od Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland.*

Background: Results of some quantitative histopathological studies suggest that patients with essential hypertension are characterized by lower number and higher mean volume of kidney glomeruli (MGV). Intracranial hemorrhage is one of the common causes of death in the patients with hypertension. The aim of the study was to evaluate MGV in kidney donors died due to intracranial hemorrhage.

Methods: Into the retrospective study medical records from cadaveric kidney donors harvested between 2005 and 2010 were included. In all cases preimplantation kidney biopsies ("zero biopsies") were performed. MGV was evaluated in specimens of "zero biopsies". The results were shown as a median and 95 CI.

Results: Analyzed group consisted of 34 cadaveric kidney donors who died due to intracranial hemorrhage [18 females and 16 males; age 49 years (42-51), kidney weight 191.0g (174.1-208.7) and serum creatinine concentration 104 µmol/l (89-174)]. The control group consist of 20 patients who died due to brain trauma [3 females and 17 males, age 41 years (30-44), kidney weight 187.0g (164.2-212.7) and serum creatinine concentration 97 µmol/l (81-236)]. Entire medical history of cadavers (including history of hypertension) were not available for all kidney donors. Kidney donors died due to intracranial hemorrhage characterized by significant higher MGV than donors died due to brain trauma [4.95x10^{6}µm^3 (4.02-6.14) vs 3.78 x10^{6}µm^3 (2.93-4.22); p<0.042]. There were also significant (p=0.025) differences of MGV in tercils of cadaveric donors age [tercile 1 (<42 years) – 3.62x10^{6}µm^3 (2.90-3.98), tercile 2 (42-49 years) – 4.68x10^{6}µm^3 (4.00-5.38) and tercile 3 (>50 years) – 5.11x10^{6}µm^3 (3.56-7.97)]. The significant positive correlation between median MGV and donors age (p=0.024, R=0.42) was found.

Conclusions: 1. Kidney from patients who died due to intracranial hemorrhage are characterized by higher MGV. 2. It is necessary to perform further studies analyzing long term results of transplantation of kidney with higher MGV.

Funding: Government Support - Non-U.S.

TH-PO454

Changes in the Incidence of Glomerular Diseases in Uruguay during the Last Ten Years Oscar A. Noboa,[1,4] Liliana Gadola,[1,4] Hena Maria Caorsi,[2,4] Mariela Garau,[1,4] Nelson Acosta,[2,4] Panuncio Ana,[3] Maria Auchayna,[4] Silvia Melesi,[3] Ana Marino,[3] Francisco Gonzalez-martinez.[1,4] *[1]Centro de Nefrologia, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay; [2]Sociedad Uruguaya de Nefrologia, Uruguay; [3]Depto Anatomia Patologica, Universidad de la Republica, Montevideo, Uruguay; [4]Program of Prevention and Treatment of Glomerulopathies.*

Background: The National Program of Prevention and Treatment of glomerular Diseases collects clinical and histological data from all the patients in whom a renal biopsy is performed in the country since 1980. In 2004 glomerulopathies were included among mandatory notifiable diseases.

The aim of this study is to analyze the changes in the incidence of glomerular diseases during the last ten years.

Methods: All Renal biopsies (RB) performed in three periods were analyzed, 1998-99 (I), 2003-4 (II) y 2008-09 (III), (728 biopsies). RB were processed for optical microscopy and immunofluorescence.

The Uruguayan population > 14 years old was 2.511.296, 2.555.068 and 2.625.826 in each period. The rate of each glomerular disease per millon population, per year was calculated (pmp). Statistic analysis was performed comparing the incidence rate ratio (IRR) per millon adult population during the period of time for each diagnosis along with their 95% confidence interval (CI).

Results: The frequency of renal biopsies performed pmp per year was similar for the three periods: 46.7 (I), 46.2 (II) and 51.9 (III). The incidence rate (IR) was calculated for each glomerulopathy. Focal and segmental glomerulosclerosis significantly decreased, IR, 10.2, 8.0 and 4.2 with an (IRR 2.49, CI 1,47-3.99) (p = 0.005). IgA Nephropathy significantly increased from, 5.6, 7.2 and 12.6 (IRR 0.44 CI 0.28- 0.69 (p= 3.10^{-4}) as well as Vaculitis IR increased from 3.0, to 7.0 and 6.3 (IRR CI 0.26-0.87 p= 0.02).

Conclusions: The incidence rate of glomerular diseases significantly changed in the last 10 years although the rate of biopsy performed pmp did not change. While Focal and segmental glomerulosclerosis significantly decreased, IgA nephropathy and vasculitis increased. This national epidemiologic surveillance would have an impact on public health policies and clinical practice.

Funding: Government Support - Non-U.S.

TH-PO455

Development of a Novel Technique to Visualize In Vivo Renin Activity and Analysis of the Renoprotective Actions of a Renin Inhibitor Kengo Kidokoro, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Despite the systemic renin activity is suppressed in diabetes, a renin-angiotensin system (RAS) inhibitor exert renoprotective effects in diabetes. Activation of local/tissue RAS, therefore, appears to be involved in pathogenesis of diabetic nephropathy. In an attempt to examine the possible involvement of local RAS in diabetic nephropathy, we tried to develop a novel technique to visualize tissue renin activity in vivo by using the fluorescence resonance energy transfer (FRET) system and multiphoton laser microscopy(MP-LM). We also examinedrenprotective mechanisms of aliskiren in diabetic nephropathy.

Methods: Streptozotosin (STZ)-induced diabetes model was made in C57/BL6 mice. Following 4 groups were prepared; Control (Cont), STZ, STZ + aliskiren (25 mg/kg/day, 4 weeks) (Alis), and the STZ + hydralazine (100 mg/kg/day, 4 weeks) groups (Hyd). Renin FRET peptide was given intravenously and generated fluorescence was detected by MP-LM in the kidney tissue in vivo. Glomerular hyperfiltration of macromolecules was also examined in vivo imaging system using fluorescence-labeled 70-kDa dextran. The production of reactive oxygen species (ROS) was also evaluated by a fluorescent indicator, dichlorodihydrofluorescin diacetate.

Results: Local renin activity was successfully visualized in the kidney tissue of living mice and was markedly enhanced in the glomeruli of the STZ group. Both plasma and tissue renin activities were significantly decreased in the Alis group. Hyperfiltration of the macromolecules precedent to the development of albuminuria and increased oxidative stress were observed in the STZ kidney. These changes were ameliorated in the Alis group.

Conclusions: We have successfully developed a novel in vivo imaging technique to directly visualize renin activity in living animal. Our data indicated that aliskiren inhibited both plasma and tissue renin activities, ameliorated tissue oxidative stress and attenuated urinary albumin excretion in a mouse diabetes model. The combination of in vivo imaging technique and FRET peptide will provide a novel approach to explore pathophysiology of kidney diseases.

TH-PO456

eNOS Deficiency Predisposes to Renin Angiotensin Aldosterone System Sensitive Podocyte Injury in Diabetes Darren A. Yuen,[1] Kathryn E. White,[2] Tomoko Takano,[3] Lei Zhu,[3] Takamune Takahashi,[4] Raymond C. Harris,[4] Andrew Advani.[1] *[1]Dept. of Medicine, St. Michael's Hospital, Toronto, ON, Canada; [2]EM Research Services, Newcastle University, Newcastle, United Kingdom; [3]Dept. of Medicine, McGill University, Montreal, QC, Canada; [4]Dept. of Medicine, Vanderbilt University, Nashville, TN.*

Background: Endothelial nitric oxide synthase (eNOS) deficiency has been implicated in the pathogenesis of diabetic nephropathy in both experimental and clinical studies. In the present study we explored the mechanisms by which eNOS deficiency may lead to renal injury, focusing on two common sequelae of endothelial dysfunction in diabetes: glomerular capillary growth and effects on neighbouring podocytes.

Methods: Experiments were performed in control and diabetic wildtype and eNOS$^{-/-}$ mice treated with a VEGF receptor antagonist, ACE inhibitor (ACEi) or angiotensin II receptor blocker (ARB), as well as in cultured podocytes and in wildtype and eNOS$^{-/-}$ glomerular endothelial cells (GECs).

Results: After 12 weeks of diabetes, glomerular capillary volume was increased in both steptozotocin (STZ)-diabetic C57BL/6 and eNOS$^{-/-}$ mice. Although VEGF receptor inhibition attenuated glomerular capillary enlargement in both groups, and albumin excretion in STZ-C57BL/6 mice, the albuminuria that occurred in STZ-eNOS$^{-/-}$ mice was unaffected. Heavy albuminuria occurred in STZ-eNOS$^{-/-}$ mice as early as two weeks after diabetes induction, associated with an acute podocytopathy, also observed in hyperglycemic db/dbeNOS$^{-/-}$ mice. Treatment of STZ-eNOS$^{-/-}$ mice with the ACEi captopril prevented podocyte injury and albuminuria development, as did the ARB losartan when administered at non-hypotensive dosing. Exposure of cultured podocytes to conditioned medium derived from eNOS$^{-/-}$ GECs exposed to high glucose and angiotensin II activated podocyte RhoA, a pivotal actin cytoskeleton regulator.

Conclusions: Podocytopathy occurring as a consequence of eNOS deficiency and hyperglycemia highlights the importance of endothelial-podocyte crosstalk in diabetes. Elucidation of RAS-sensitive mediators of this communication may lead to the development of novel therapies targeting endothelial dysfunction in albuminuric individuals with diabetes.

Funding: Private Foundation Support

TH-PO457

Early Activation of RAS in Diabetic Mice Urine Is Angiotensinogen-Dependent and ACE-Independent Jan A. Wysocki, Laura Garcia-Halpin, Minghao Ye, Daniel Batlle. *Div. of Nephrology&Hypertension, Feinberg School of Medicine, Northwestern University, Chicago, IL.*

Background: Urinary renin-angiotensin system (RAS) components could be used as potential biomarkers of RAS overactivity to predict development of diabetic kidney disease. Urinary angiotensinogen (AOG) has been found to be increased in urine of diabetic patients. We studied urinary angiotensin in conjunction with other components of RAS system in the urines from the db/db model of type 2 diabetes at a time when urine albumin excretion is only slightly increased and kidney histology is normal.

Methods: Urinary AOG and angiotensin II were measured by ELISA. Urinary activities of Ang II-forming (ACE) and AngII–degrading enzyme (ACE2) were measured using synthetic fluorogenic substrates in urines from 8 weeks old female db/db and db/m mice.

Results: At 8 weeks of age, there was a slight increase in albumin/creatinine ratio (303±32vs. 87±4 ug/mg, respectively) but a marked increase in urinary AOG levels in db/db as compared to db/m mice (41.2±9.7 vs. 5.9±1.7 ng/mg creatinine, $p<0.05$, respectively). The increase in AOG levels was associated with an increase in urinary angiotensin II (203±54 vs. 45±13 pg/mg creatinine, $p<0.05$, respectively). At this early age, ACE2 activity in db/db mice was also increased as compared to db/m (38±5 vs. 19±3 RFU/ug creat/hr, $p<0.05$, respectively). In contrast to the increase in ACE2 activity and AOG levels, ACE activity in the urine from db/db mice was reduced as compared to that of db/m mice (1.42±0.36 vs. 2.78±0.34 RFU/ug creat/hr, $p<0.05$, respectively).

Conclusions: Early activation of the kidney RAS system in the db/db mice is evident from increased levels of urinary Ang II and high levels of this peptide result from increased angiotensinogen and not from increased ACE activity. ACE2 activity in the urine is increased likely reflecting the increased metabolism of angiotensin II.

Funding: NIDDK Support

TH-PO458

Role of Angiotensin Type 2 Receptor in Diabetic Nephropathy Rita de Cassia Cavaglieri, Robert T. Day, Denis Feliers. *Medicine/Renal Disease, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Background: Angiotensin II plays a major role in the development of diabetic nephropathy through activation of the AT1 receptor. The other receptor, AT2 is believed to oppose the effect of AT1. Its role in the pathogenesis of diabetic nephropathy is the focus of this study.

Methods: We induced type 1 diabetes in AT2 knockout mice (KO) and their wild-type littermates (wt) by injection of streptozotocin and studied renal function after 3 months of diabetes. Markers of inflammation (monocyte/macrophage infiltration, MCP1 and VCAM1) and fibrosis were measured by immunohistochemistry, immunoblot and RT-qPCR.

Results: Absence of AT2 did not affect blood pressure in control and diabetic mice, but increased glycemia slightly in control mice and very significantly in diabetic mice. Interestingly, diabetic AT2KO mice did not lose body weight in spite of higher glycemia. Normoglycemic AT2KO mice showed signs of kidney injury: renal and glomerular hypertrophy and increased fibrosis. Induction of diabetes in AT2KO mice increased kidney weight, and worsened glomerular hypertrophy and fibrosis. Absence of AT2 did not affect development of albuminuria in diabetic mice, but it prevented development of inflammation (monocyte/macrophage infiltration, MCP1 and VCAM1 expression) in kidneys from diabetic mice. AT1 and AT2 receptor were upregulated in diabetic AT2wt mice. Absence of AT2 led to increased AT1 expression in kidneys from control mice to the same level as in diabetic wt-mice, that was not further increased with diabetes.

Conclusions: Our data show that absence of AT2 worsens some aspects of renal injury during diabetes but does not affect albuminuria and paradoxically prevents development of renal inflammation. Activation of AT2 in kidneys from diabetic mice could limit the extent of kidney injury.

Funding: Private Foundation Support

TH-PO459

Increased Renal Uptake of Exogenous Human Prorenin and Angiotensinogen Enhances the Renal Renin-Angiotensin System in Diabetic Rats Satoshi Kinugasa,[1] Akihiro Tojo,[1] Yasunobu Hirata,[1] Toshiro Fujita,[1] Christopher S. Wilcox.[2] *[1]Department of Internal Medicine, University of Tokyo, Tokyo, Japan; [2]Department of Medicine, Georgetown University Medical Center, Washington DC.*

Background: The renal renin-angiotensin system (RAS) is considered to be regulated independently of systemic RAS. Recently, renal tissue angiotensinogen (AGT) was shown to originate from the liver, uptaken by tubules via megalin. To clarify the mechanism of renal RAS, we examined the renal endocytosis of exogenous human prorenin and AGT, and the effect on blood pressure in diabetic rats.

Methods: Streptozotocin-diabetic rats with microalbuminuria (DM) and controls were injected iv with 20 μg human prorenin, and subsequently human AGT every 5 minutes with stepwise increased doses (0.02–20 μg). Arterial pressure was measured continuously. Kidneys were harvested, renal angiotensin II (Ang II) levels were measured, and both immunohistochemistry (IHC)and western blotting were performed for prorenin, AGT, and prorenin receptor (PRR).

Results: Light and electron microscopic IHC showed that human prorenin and AGT uptake was increased in endocytotic vesicles of podocytes, proximal tubules and cortical collecting ducts (CCD) in DM compared to controls, which was confirmed by western blotting (densitometry of bands for prorenin 116±11 vs. 60±11, $p<0.01$). PRR was expressed in podocytes, macula densa, and CCD, and weakly in proximal tubules. Immunoprecipitation of kidney homogenate with antibody against PRR or megalin showed increased binding of human prorenin with PRR or megalin in DM, implying enhanced prorenin endocytosis via these receptors. Renal Ang II levels did not differ between DM and controls. However, when human prorenin and AGT were selectively injected into the left kidney, renal Ang II increased by 37% compared to the contralateral kidney. Systolic blood pressure (sBP) increased dose-dependently in DM, which did not occur in controls (sBP variation after 20 μg AGT injection 33±5 mmHg vs. 8±2 mmHg, $p<0.01$).

Conclusions: The endocytosis of exogenous human prorenin and AGT was enhanced in podocytes and renal tubules in diabetic rats with microalbuminuria, causing increased blood pressure.

TH-PO460

Detailed Localization of Augmented Angiotensinogen mRNA and Protein in Proximal Tubule Segments of Diabetic Kidneys in Rats and Humans Masumi Kamiyama,[1] Michelle K. Garner,[1] Tadashi Sofue,[2] Takashi Morikawa,[4] Yoshio Konishi,[4] Masahito Imanishi,[4] Akira Nishiyama,[3] Hiroyuki Kobori.[1,3] *[1]Department of Physiology, and Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center; [2]Department of Cardiorenal and Cerebrovascular Medicine, Kagawa University School of Medicine, Japan; [3]Department of Pharmacology, Kagawa University School of Medicine, Japan; [4]Department of Nephrology and Hypertension, Osaka City General Hospital, Japan.*

Background: Increased levels of intrarenal angiotensinogen (AGT) have been shown in diabetic humans, mice, and rats. However, the precise localization of the augmented AGT mRNA and protein in proximal tubule segments of kidneys in diabetes is still unknown.

Methods: To investigate the detailed localization of AGT in 3 proximal tubule segments of the kidneys in diabetes, we used Otsuka Long-Evans Tokushima fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats as genetic controls. Kidney tissues were also prepared from OLETF rats that were administered with angiotensin II type 1 receptor blocker, olmesartan or a combination of vasodilator agents (hydralazine, reserpine, and hydrochlorothiazide; HRH). In addition, biopsied samples of human kidney cortex were studied. Using kidney tissues, we examined the co-localization of AGT mRNA or protein with segment-specific protein markers by double staining technique.

Results: Results showed that AGT mRNA and protein were higher at 15 weeks in OLETF rats than in LETO rats. In the 15 week-old rat kidneys, AGT mRNA expression was not recognized much in Segment (S)1 segments of either OLETF or LETO rat kidneys. AGT mRNA expression area in S3 was augmented in the OLETF rats (2.31 ± 0.02-fold; $P = 0.0029$). AGT protein expression areas in S1 and S3 were also increased (8.50 ± 2.78-fold; $P = 0.0476$, and 3.71 ± 0.83-fold; $P = 0.0317$, respectively). Chronic treatment with olmesartan, but not with HRH, ameliorated the augmented AGT expression areas. Human kidney cortical biopsied samples confirmed that AGT protein in S1 was augmented in diabetes.

Conclusions: These data suggest that the augmented AGT mRNA in S3 and AGT protein in S1 and S3 play an important role in the development and the progression of diabetic nephropathy.

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TH-PO461

Podocyte JAK2 Augments Glomerular Injury Induced by Diabetes and Angiotensin II Hongyu Zhang, Jharna Saha, Kevin B. Atkins, Frank C. Brosius. *Internal Medicine/Nephrology, University of Michigan, Ann Arbor, MI.*

Background: In the best mouse models of diabetic nephropathy generally only the earlier manifestations of the human disease reliably occur. The lack of progression in diabetic mouse models could be due to absent or muted pathogenic responses and/or the presence of distinctive protective mechanisms. JAK2 has been implicated in the pathogenesis of human diabetic nephropathy (DN). In transcriptomic analyses, humans but not mice with type 2 diabetes were found to express enhanced JAK2 levels in podocytes early in DN (Berthier, et. al. Diabetes, 2009;58:469).

Methods: In order to test the role of increased JAK2 expression in podocytes in early DN we knocked in a STOP/FLOX JAK2 construct to the ROSA26 locus in 129S6 mice and generated homozygous podocyte-specific enhanced JAK2 expression with or without Akita type 1 diabetes in mice. To test the role of angiotensin II (Ang II) in this process, 10 wk-old mice received Ang II via osmotic minipump at 1.4 mg/kg/d.

Results: The podocyte JAK2 (pJAK2) mice had a 2-fold increase in total glomerular JAK2 mRNA and protein similar to the increase seen in humans with progressive DN. After 4 wks of Ang II infusion and 8 wks of diabetes, pJAK2 diabetic mice developed a ~35-fold increase in albuminuria compared to non-diabetic Ang II infused mice ($p<0.001$) and 2.5-fold increase compared to diabetic wild-type Ang II mice ($p<0.05$). In addition, mesangial expansion ($p<0.001$) and mesangial sclerosis were greater in the pJAK2 diabetic Ang II mice compared to diabetic wild-type Ang II mice. Podocyte number, though less in non-diabetic pJAK2 vs. non-diabetic wild-type mice ($p=0.01$) was similarly reduced in diabetic pJAK2 and wild-type mice.

Conclusions: These data demonstrate that increased expression of podocyte JAK2 enhances Ang II induced glomerulopathy in diabetic mice.

Funding: NIDDK Support, Private Foundation Support

TH-PO462

Efficacy of Aliskiren, Compared with Angiotensin II Blockade, in Slowing the Progression of Diabetic Nephropathy in db/db Mice: Should the Combination Therapy Be a Focus? Guangyu Zhou, Alfred K. Cheung, Yufeng Huang. *Internal Medicine, University of Utah, Salt Lake City, UT.*

Background: Although Ang II blockade has become standard therapy for diabetic nephropathy, decline in kidney function is common. A feedback increase in renin often accompanies Ang II blockade. We therefore examined whether aliskiren, a direct renin inhibitor, confers better renoprotection than Ang II blockade and whether dual RAS blockers with aliskiren and ACEI or ARB would further slow nephropathy progression vs monotherapy.

Methods: We examined the uninephrectomized db/db mouse, a model of type 2 diabetes where overt nephropathy is evident by 18 wks of age.

Results: Untreated uninephrectomized db/db mice developed progressive albuminuria and mesangial matrix expansion between wks 18 and 22, associated with increased renal expression of TGFß1, PAI-1, fibronectin (FN) and type IV collagen (Col IV). Although body weight and blood glucose were comparable in all groups (n=9 for each group), aliskiren at all doses (3 to 50 mg/kg BW/d) from wk 18 to 22 significantly retarded the increases in albuminuria and glomerular matrix accumulation seen in db/db mice. However, increasing doses of aliskiren resulted in increasing reduction in renal FN and Col IV production, with the 30 mg/kg dose showing maximal reduction by 80% and 72% for FN and Col IV, respectively. Notably, the therapeutic effect of aliskiren given at this dose was similar to that of either enalapril or valsartan given alone at maximally effective doses ($p>0.05$). Combined therapy with aliskiren and enalapril/valsartan further reduced albuminuria but yielded no further reduction in markers of renal fibrosis and podocyte injury. Further, the groups received combined therapy lost 10% to 20% of total mice while all mice without treatment or with monotherapy survived.

Conclusions: These results suggest that aliskiren, enalapril and valsartan are almost identical in slowing the progression of diabetic nephropathy in db/db mice. Dual RAS blockade treatment yields no additional effect in diabetic nephropathy but may increase the mortality. Results from these animal studies do not support the use of combination therapy with aliskiren and ACEI/ARB.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

TH-PO463

Protective Effects of Enalapril in Uninephrectomized ZSF1 Rats: An Accelerated Model of Diabetic Renal Disease Steven M. Weldon,[1] Xiaomei Zhang,[1] Susan Goldrick,[1] Damian C. Matera,[1] Michael Thibodeau.[2] *[1]CardioMetabolic Disease Research, Boehringer-Ingelheim Pharm. Inc.; [2]Non-Clinical Drug Safety, Boehringer-Ingelheim Pharm. Inc., Ridgefield, CT.*

Background: The ZSF1 rat exhibits many features of human type 2 diabetes including obesity, hyperglycemia, dyslipidemia and hypertension, all of which contribute to progressive nephropathy and symptoms of renal failure within 12-18 months of age.

Methods: We performed uninephrectomy (UNx) in 9 wk old ZSF1 rats to accelerate the development of nephropathy and evaluate the effect of enalapril (ENA,30 mg/kg/d) initiated prophylacticaly (ENA-P) upon UNx or therapeutically (ENA-T) 6 weeks post-UNx. We measured blood pressure, glucose, creatinine, BUN, urine albumin excretion (UAE), tissue biomarkers (mRNA qPCR) and renal pathology at term, 12 weeks post UNx.

Results: Compared to naïve ZSF1 rats, UNx accelerated and exacerbated development of renal damage. Renal pathology in UNx ZSF1 included segmental glomerulosclerosis (GS), mesangial thickening and tubular nephropathy (TN) that included hyaline casts and fibrosis. BP, UAE, proinflammatory and profibrotic biomarker expression were also increased in vehicle treated UNx ZSF1. Treatment with ENA-P or ENA-T attenuated increases in BP and reduced UAE to near normal levels. GS was attenuated by both dosing regimens of enalapril however; the incidence and severity were less following treatment with ENA-T. Tubular nephropathy was attenuated by ENA-P only. Proinflammatory and profibrotic biomarker expression were increased in kidneys of vehicle treated UNx ZSF1 rats. ENA-P attenuated increases in MMP2, PAI-1, FN, TGFb, Osteopontin and MCP-1 expression while ENA-T attenuated only MCP-1.

Conclusions: Although we and others have successfully demonstrated that intact ZSF1 rats provide a robust preclinical model of diabetic complications, the time required for spontaneous disease development can be challenging. These new data show that UNx accelerates and exacerbates renal pathology in these animals over a shorter, 12 week study period. In addition, the accelerated disease process can be effectively attenuated by enalapril even following a therapeutic dosing regimen initiated up to 6 weeks after UNx.

Funding: Pharmaceutical Company Support - Boehringer-Ingelheim Pharm. Inc.

TH-PO464

Insulin Inhibits Angiotensinogen Gene Expression and Prevents Hypertension in Diabetic Akita Mouse Kidney via Heterogeneous Nuclear Ribonucleoprotein K Expression Shaaban Abdo,[1] Chao-Sheng Lo,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hospital, Montreal, QC, Canada; [2]Res. Ctr., Maisonneuve-Rosemont Hosp., Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp., Boston, MA.*

Background: Heterogeneous nuclear ribonucleoprotein K (hnRNP K) binds to the insulin-responsive element in the rat angiotensinogen (Agt) gene promoter and inhibits Agt gene transcription in vitro. We investigated whether insulin can stimulate hnRNP K expression in renal proximal tubular cells (RPTCs), inhibit Agt gene expression and prevent systemic hypertension (sHTN) in type 1 diabetic Akita mice.

Methods: Adult male Akita mice (12 weeks of age) were treated ± insulin implants for 4 weeks. Non-Akita mice served as controls. Plasma glucose, systolic blood pressure and albuminuria were monitored weekly. Kidneys were processed for histology. Renal proximal tubular (RPT) Agt and hnRNP K mRNA and protein expression were evaluated by real time- qPCR and Western blotting, respectively. Urinary Agt and angiotensin II (Ang II) levels were quantified by ELISA. We also studied immortalized rat RPTCs (IRPTCs) ± stable transfection with a plasmid, pGL4 containing the rat Agt gene promoter fused with a luciferase reporter *in vitro.*

Results: Akita mice developed sHTN (~136 ± 3.8 mm Hg vs. ~108 ± 0.7 mm Hg in non-Akita mice) and exhibited renal hypertrophy. Insulin treatment normalized plasma glucose levels and sHTN, attenuated renal hypertrophy, decreased urinary albumin/creatinine ratio and urinary Agt and Ang II levels in Akita mice. Furthermore, RPT Agt expression was significantly increased whereas hnRNP K expression was markedly decreased in Akita mice and these changes were normalized by insulin treatment. *In vitro*, high glucose (25 mM D-glucose) stimulated Agt gene promoter activity and inhibited hnRNP K expression in IRPTCs. Insulin reversed these effects, and its action was prevented by transfecting RPTCs with small interfering RNA of hnRNP K.

Conclusions: Our data suggest that insulin prevents sHTN and RPTC injury in diabetes through, at least in part, hnRNP K-mediated suppression of intrarenal Agt gene expression.

Funding: Government Support - Non-U.S.

TH-PO465

Insulin Increases Angiotensin Converting Enzyme 2 Short and Long Term Expression in Podocytes: A Renoprotective Effect on Diabetic Nephropathy? Eva Marquez, Marta Riera, Julio Pascual, Maria Jose Soler. *Kidney Disease Research Group, Nephrology Department, Hospital del Mar- IMIM, Barcelona, Spain.*

Background: Angiotensin-converting enzyme 2(ACE2) cleaves angiotensin II to angiotensin 1-7, a vasodilator and anti-proliferative peptide. ACE2 is present in glomerulus, mainly in the glomerular epithelial cells(podocytes). ACE2 inhibition in experimental diabetic nephropathy increases albuminuria and worsens glomerular injury. The podocyte is a key cell involved in the development of albuminuria since early stages of diabetic nephropathy. Our aim is to study the effect of short and long term insulin incubation on ACE2 expression in podocytes.

Methods: A conditionally immortalized mouse podocyte cell line proliferated in permissive conditions (32ºC γ-interferon). After that, cells were induced to differentiate in non-permissive conditions (37ºC without γ-interferon) for 14 days. Cells were then incubated without (control group; PODc) or with insulin(200nM) for 1 hour and 48 hours (POD1 and POD48; each group n=8). Podocyte were collected for protein studies (Western Blot; expressed as index ACE2/β-actin) and enzymatic activity (expressed as relative fluorescence units (RFU/µg protein/hour). Cells were stained for immunofluorescence using the antibody against ACE2.

Results: ACE2 protein expression, determined by Western Blot, was higher in podocytes after 1 hour of insulin incubation as compared to controls (POD1 1.29±0.4 vs PODc 0.74±0.2, $p<0,05$). After 48 hours of insulin incubation the increase in ACE2 expression was observed as compared to control group (POD48 1.07±0.07 vs PODc 0.8±0.1, $p<0,05$). In the immunofluorescence study, ACE2 expression was increased in insulin treated podocytes. The enzymatic activity was concordant with protein ACE2 expression but without statistical significance (POD1 1.91±0.5 vs PODc 1.65±0.8; POD48 1.75±0.9 vs PODc 1.53±0.4, p=ns).

Conclusions: In the podocyte, a cell that expresses a functional intrinsic RAS, insulin incubation increases ACE2 expression, and this elevation is maintained over the time. This finding may suggest a specific role of insulin in maintaining intraglomerular RAS balance and protect against the progression of diabetic kidney disease.

Funding: Private Foundation Support

TH-PO466

Apelin Ameliorates Renal Function in Diabetes Robert T. Day, Rita de Cassia Cavaglieri, Denis Feliers. *Medicine/Renal Disease, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Background: Apelin and its receptor APJ have pleiotropic effects in mice and humans, and play a protective role in cardiovascular diseases. We studied the effect of apelin administration on the progression of kidney disease in mice with established type 1 diabetes.

Methods: Ove26 mice with 10 weeks of type 1 diabetes received daily subcutaneous injections of apelin, for 2 weeks or for 14 weeks. Glycemia, blood pressure, kidney histology and albuminuria were measured. APJ and markers of inflammation were measured by immunohistochemistry and RT-qPCR.

Results: In the kidneys, APJ was localized in the glomeruli and blood vessels. Its expression was reduced in mice with 3 months of diabetes and was upregulated by apelin. Treatment with apelin did not affect glycemia, body weight or blood pressure in diabetic mice at any time point. Apelin significantly reduced albuminuria at 6 months. Kidney and glomerular hypertrophy, as well as renal inflammation (monocyte infiltration, MCP1 and VCAM1 expression) were inhibited by apelin at 3 and 6 months. Finally, expression of the anti-oxidant enzyme catalase was reduced in kidneys from diabetic mice and was upregulated by apelin.

Conclusions: These findings show for the first time that apelin exerts a protective effect on the diabetic kidney. A short treatment was sufficient to reduced kidney and glomerular hypertrophy as well as renal inflammation, but a prolonged treatment was required to improve albuminuria. Apelin may represent a novel tool in the therapeutic arsenal for the treatment of diabetic nephropathy.

Funding: Private Foundation Support

TH-PO467

Effect of Losartan on Epigenetic Changes in the Renal Glomeruli of Diabetic db/db Mice Marpadga A. Reddy,[1] Sumanth Putta,[1] Linda L. Lanting,[1] Hang Yuan,[1] Mei Wang,[1] Charles E. Alpers,[2] Karol Bomsztyk,[2] Rama Natarajan.[1] *[1]Beckman Research Institute of COH, Duarte, CA; [2]University of Washington, Seattle, WA.*

Background: Diabetic nephropathy (DN) is characterized by glomerular hypertrophy and ECM accumulation leading to proteinuria and renal failure. The renoprotective effects of Angiotensin II type 1 receptor blockers suggest a key role for AT1R signaling in DN. Epigenetic mechanisms such as histone H3 lysine methylation (H3Kme) and acetylation (H3KAc) are involved in gene regulation and metabolic memory implicated in vascular complications. However, the role of HKme/Ac and AT1R signaling in these events is not clear. Here we tested the hypothesis that AT1R blockade with Losartan (Los) can ameliorate DN and reverse epigenetic modifications associated with glomerular gene expression.

Methods: Male type 2 diabetic db/db mice were treated with Los (10 mg/kg/day) or without Los for 10 wks. Age-matched non-diabetic db/+ mice without Los were controls. We then determined DN parameters, glomerular gene expression (RT-PCR/PCR arrays) and histone modifications (Matrix chromatin immunoprecipitation (ChIP) assays) at gene promoters.

Results: There was significantly increased blood pressure, mesangial expansion, proteinuria and glomerular expression of RAGE, MCP-1, PAI-1 and Col1a2 genes in db/db mice relative to db/+. These were reversed by Los. Matrix ChIP assays showed decreases in repressive marks (H3K27me3) and increases in activation marks (H3K9/14Ac, H3K4me1, H3K4me2, H3K36me3) at the RAGE and PAI-1 promoters in db/db mice. PCR arrays revealed alterations in the expression of several histone modification enzymes in db/db mice. Los reversed some but not all changes in chromatin marks and expression of key histone modification enzymes.

Conclusions: These results demonstrate the involvement of epigenetic mechanisms in the expression of key glomerular genes associated with DN that may be mediated in part by AT1R. Thus, epigenetic therapies in conjunction with ARBs and other drugs may be more effective in DN treatment.

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Effect of Losartan on the Glomerular Protein and miRNA Expression Profile of Type 2 Diabetic KKAy Mice Qiuling Fan. *Department of Nephrology, First Hospital, China Medical University.*

Background: To investigate the pathogenesis of diabetic nephropathy and to search novel therapeutic targets, the glomerular protein and miRNA expression profile of KKAy mice treated by losartan was analyzed.

Methods: The 8-week-old KKAy mice were divided into the losartan treatment group and the non-treatment group, and C57BL/6 mice were used as the control group. 12 weeks after the treatment, glomeruli were isolated by abdominal perfusion with magnetic beads.The glomerular protein expression profiles were investigated using 2D-DIGE and MALDI-TOF mass spectrometry. Western blot analysis was used to confirm the results of proteomics. miRNA expression profiles were analyzed using miRNA arrays. Real-time PCR was used to confirm the results.

Results: Losartan treatment improved albuminuria and renal pathological lesion of KKAy mice. 6 proteins were found to be differentially expressed between the KKAy non-treatment mice and C57BL/6 mice glomeruli, and their differential expression were suppressed by losartan treatment, including mitochondrial ATP synthase subunit d, GRP75 and selenium-binding protein 1 et al. The expression of 10 miRNAs was higher and the expression of 12 miRNAs was lower in the glomeruli of the KKAy non-treated mice than that of the CL57BL/6 mice. The expression of 4 miRNAs was down-regulated in the glomeruli of the KKAy losartan-treated mice compared with that of the non-treated mice. Among them, the expression of miRNA-503 and miRNA-181d was higher in the glomeruli of the KKAy non-treated mice and was inhibited by losartan treatment.

Conclusions: The over-expression of miR-503 and miR-181d in KKAy mice glomeruli may be responsible for the pathogenesis of DN by regulating the expression of the target genes, such as heat shock protein 75, GRP75 and GRP78 et al.

Funding: Government Support - Non-U.S.

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Renoprotective Effects of Dipeptidyl Peptidase IV Inhibition in Diabetic Nephropathy Yuliya Sharkovska,[1] Thomas Klein,[3] Sebastian C. Bachmann,[1] Berthold Hocher.[2] *[1]Charite Universitätsmedizin Berlin, Institute of Vegetative Anatomy, Berlin, Germany; [2]University of Potsdam, Institute for Nutritional Science, Potsdam, Germany; [3]Boehringer Ingelheim, Dept. CardioMetabolic Diseases Res. Ger., Biberach, Germany.*

Background: Inhibition of dipeptidyl peptidase IV (DPPIV) stabilizes glucagon-like-peptide-1 (GLP-1) levels and is considered as a new approach in the treatment of type 2 diabetes. We examined the renoprotective effects of linagliptin in diabetic nephropathy (DN) using *db/db* mice. Results were compared with the known, beneficial effects of RAS blockade by enalapril.

Methods: Biochemical and pathological analyses were performed in 10 week-old male diabetic *db/db* mice treated for 3 months with either vehicle (n=10), 3 mg linagliptin/kg/day (n=8), or 20 mg enalapril/kg/day (n=10). Heterozygous *db/+* mice treated with vehicle served as controls (n=8).

Results: Diabetic *db/db* mice showed elevatedfasting levels of glucose and insulin. Like enalapril, linagliptin did not affect plasma glucose levels. The urinary *albumin-to-creatinine ratio* was *4-fold elevated* in vehicle-treated *db/db* compared to controls ($p < 0.001$). Linagliptin decreased urinary *albumin-to-creatinine ratio in db/db mice at 8 weeks* (-35%; $p < .05$). Glomerular and tubulointerstitial injury was decreased by linagliptin, and to a lesser extent also by enalapril treatment in sirius red, PAS, collagen I, and a-SMA staining.The abundance of podocalyxin, a marker for glomerular damage, was decreased in db/db mice compared to controls (-40%, $P < .01$), whereas linagliptin and enalapril normalized its expression. DPPIV and GLP-1 receptor were colocalized in podozytes. At steady state, only GLP-1 receptor was lowered in db/db mice (-30%, $p < .05$). Linagliptin normalized its expression.

Conclusions: This study suggests that the DPPIV inhibitor linagliptin has beneficial effects on DN-associated kidney damage without affecting blood glucose levels. The renoprotective action of linagliptin may be due to the attenuation of podocyte injury and inhibition of myofibroblast transformation.

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Blockade of KCa3.1 Attenuated Diabetic Nephropathy Chunling Huang, Sylvie Shen, Xinming Chen, Carol A. Pollock. *Renal Medicine, Kolling Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia.*

Background: Transforming growth factor-β1 (TGF-β1) plays a central role in the development of renal fibrosis. KCa3.1, a potassium channel protein, is associated with vascular inflammation, atherogenesis, and proliferation of endothelial cells, macrophages, and fibroblasts. Blockade of KCa3.1 has been shown to ameliorate renal fibrosis in a mice model of unilateral ureteric obstruction. However, the role of KCa3.1 in the development of diabetic nephropathy associated with an elevation in TGF-β1 is unknown.

Methods: Primary interstitial fibroblasts isolated from human kidneys were incubated with TGF-β1 (2ng/ml) +/- the selective inhibitor of KCa3.1, TRAM-34 (2uM/L), for 48 hours. Markers of fibroblast activation (α-smooth muscle actin (α-SMA), fibroblast specific protein 1(FSP1), vimentin), inflammation (monocyte chemoattractant protein-1(MCP-1)), and fibrotic responses (CTGF (connective tissue growth factor), plasmin activator inhibitor-1(PAI-1), collagen 1 and 4, and fibronectin) were measured by qRT-PCR, western blot or ELISA. Downstream TGF-β1 signalling molecules Smad2/3, ERK1/2, P38, and JNK were measured by western blot. The activities of MMP2/9 were examined using gelatine zymography. ENos-/- mice with streptozotocin-induced diabetes were treated with TRAM-34 for 24 weeks and the expression of KCa3.1, α-SMA and collagen 1 in kidneys were examined using real-time qRT-PCR and immunohistochemistry.

Results: TRAM-34 reversed TGF-β1-induced upregulation of α-SMA, FSP1, vimentin, MCP-1, CTGF, collagen 1, collagen 4, fibronectin, PAI-1, and MMP2/9 activity ($P<0.01$) in renal interstitial fibroblasts. TRAM34 also reduced TGF-β1-induced phosphorylation of Smad2/3 and ERK1/2 but did not affect p38 and JNK MAPK pathway. In animal studies, TRAM-34 suppressed KCa3.1, α-SMA and collagen 1 expression in diabetic kidneys compared to vehicle control at both mRNA and protein levels ($P<0.05$).

Conclusions: KCa3.1 mediated TGF-β1-induced activation of renal interstitial fibroblasts and renal fibrogenesis through Smad2/3 and ERK1/2 but independent of p38 and JNK MAPK. Blockade of KCa3.1 could be an effective strategy for the treatment of diabetic nephropathy.

Funding: Government Support - Non-U.S.

TH-PO471

Acute Hemodynamic and Renal Effects of a Glucagon-Like Peptide 1 Analog in Rats Xiaoyan Zhou, Chin-hu Huang, Julie Lao, Alessandro Pocai, Olga Price, Gail M. Forrest, Michael J. Forrest, Kathleen A. Sullivan. *Merck Research Laboratories, Merck & Company, Rahway, NJ.*

Background: Liraglutide is a glucagon-like peptide 1 (GLP-1) receptor agonist approved for the treatment of type II diabetes. GLP-1 receptor agonism lowers blood glucose mostly by enhancing the secretion of insulin in a glucose dependent manner and by lowering plasma glucagon. GLP-1 analogs have demonstrated beneficial cardiovascular effects both pre-clinically and clinically. However, there is limited information on the renal effects of GLP-1 analogs. Therefore, we investigated the acute hemodynamic and renal effects of liraglutide.

Methods: Experiments were performed on anesthetized male Sprague-Dawley rats. Three ascending doses of liraglutide (30, 100, and 300 μg/kg/h) were administered by intravenous infusion. Blood pressure (BP) was recorded from an indwelling catheter. Glomerular filtration rate (GFR) and renal blood flow (RBF) were assessed by Inutest and para aminohippurate clearance, respectively. Renal excretory function was examined by recording urine volume and urinary electrolytes excretion.

Results: Heart rate was significantly increased by liraglutide (100 and 300 μg/kg/h), whereas BP was not affected at any tested dose. Liraglutide (100 and 300 μg/kg/h) also evoked significant diuresis (6.7 and 7.0 fold), natriuresis (38.3 and 56.4 fold), chloruresis (20.3 and 22.9 fold), kaliuresis (4.7 and 3.4 fold), and decreased urinary H^+ excretion. As a consequence of effects on renal excretory function, liraglutide caused hypokalemia, hypochloremia, and volume contraction. Moreover, Liraglutide (300 μg/kg/h) increased GFR (1.5 fold), but had no significant effect on RBF.

Conclusions: Liraglutide is a potent natriuretic/diuretic agent with a favorable effect on GFR. Our findings are consistent with previous reports of a natriuretic/diuretic effect of GLP-1 in rats and humans. The data support the hypothesis that GLP-1 receptor agonism may have a direct and/or indirect effect on the renal handling of sodium and water which may ultimately contribute to favorable cardiovascular effects.

Funding: Pharmaceutical Company Support - Merck & Company

TH-PO472

Renal Epigenetic Changes in Mouse and Rat Models of Type 1 Diabetes Karol Bomsztyk,[1] Oleg N. Denisenko,[1] Daniel S. Mar,[1] Terry T. Oyama,[2] Bei Xu,[2] Radko Komers.[2] *[1]UW Medicine SLU, University of Washington, Seattle, WA; [2]Division of Nephrology, Oregon Health and Science University, Portland, OR.*

Background: Epigenetic processes are likely to play a role in kidney disease including diabetic nephropathy (DN) via altered expression of genes involved in the pathophysiology of these disorders.We hypothesized that changes in expression of selected genes in DN are associated with epigenetic modifications mediated by alterations in histone modifying enzymes.

Methods: We used RT-PCR, immunoblots of histone modifying enzymes, and Matrix chromatin immunoprecipitations to examine renal gene expression, RNA polymerase II (Pol II) levels and epigenetic marks at genes in the mouse (OVE26) and streptozotocin-induced rat models of type 1 diabetes (T1D) that share DN features with human disease including common sets of aberrantly expressed genes. We focused on genes implicated in DN.

Results: Diabetes-induced expression of several renal transcripts in both the mouse and the rat models of T1D nephropathy were seen including Cox2, Fsp-1, vimentin (Vim) and laminin gamma 1 (Lamc1) genes. While the differences in mRNA levels between control and diabetic mice for some genes persisted (Cox2,Fsp-1), for others the differences seen at an earlier time points where not observed later (Vim, Lamc1). In the rat, where the DN is less severe, the mRNA differences between diabetic and normal animals were smaller and less sustained. In both the diabetic mice and rats elevated transcript levels were associated with increased Pol II suggesting that the mRNA alterations are transcriptionally mediated. These changes were associated with decreased levels of the histone H3K27m3 repressive epigenetic mark suggesting one cause for the increased transcription. Immunoblots showed higher levels of the H3K27m2/3 demethylase, Kdm6a, in kidneys of the diabetic OVE26 mice compared to controls. In contrast, Ezh2, a H3K27-specific methyltransferase, remained unchanged.

Conclusions: Increased expression of Kdm6a could be one of the primarily changes induced by diabetes contributing to the aberrant mRNA expression in DN. Kdm6a and other chromatin enzymes might be targets to explore biomarkers and DN epigenetic therapies.

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TH-PO473

Increased Kinase Recruitment to Epigenetically Altered Renal Profibrotic Gene in a Mouse Model of Type 2 Diabetes Karol Bomsztyk, Steve Flanagin, Daniel S. Mar, Oleg N. Denisenko, Kelly L. Hudkins, Tomasz A. Wietecha, Charles E. Alpers. *UW Medicine, University of Washington, Seattle, WA.*

Background: The BTBR mouse strain with the ob/ob leptin-deficiency mutation develops severe type 2 diabetes (T2D) and diabetic nephropathy (DN). Connective tissue growth factor (CTGF) is key mediator of fibrogenesis in DN. We used this T2D model to test a hypothesis that increased expression of *CTGF* in DN is associated with aberrant epigenetic changes at this gene including direct recruitment of kinases to this locus.

Methods: RT-PCR and multiplex Matrix chromatin immunoprecipitations were used to compare renal gene expression, epigenetic marks, RNA polymerase II (Pol II) and levels of several kinases at the *CTGF* and other loci in the tubulointerstitium of BTBR wild-type (wt) and BTBR ob/ob.

Results: Diabetes-enhanced expression of *CTGF* mRNA in BTBR ob/ob kidneys (5.5±1.7-fold, $p<0.05$) was associated with increased Pol II levels at the gene (1.5±0.1-fold, $p<0.01$) suggesting that the *CTGF* mRNA alteration is transcriptionally mediated. Upregulation of *CTGF* in BTBR ob/ob mice was associated with increased levels of histone H3K9/14Ac activating epigenetic mark (1.5±0.2-fold, $p<0.05$) while H3 levels were not different (1.1±0.2-fold, n.s.) at this locus between the ob/ob and wt mouse strains. These results suggest that histone modifications contribute to increased transcription of *CTGF* in the tubulointerstitium of BTBR ob/ob mice. There was also higher density of the kinase Fyn (1.5±0.2-fold, $p<0.05$) along with increased levels of active B-Raf (1.4±0.1-fold, $p<0.01$) and Erk1/2 (1.6±0.2-fold, $p<0.05$) kinases at this locus suggesting that kinase recruitment to chromatin is directly involved in abnormal expression of *CTGF* in BTBR ob/ob mice.

Conclusions: Detailed knowledge of epigenetic and transcription changes in relation to gene-bound kinases at key fibrogenic loci provides comprehensive way to explore genomic processes that contribute to fibrosis in DN. The translational application of these findings is that kinases and other epigenetic enzymes tethered to fibrogenic genes provide identifiable targets for potential drug interventions to control expression of these genes and renal fibrosis.

Funding: NIDDK Support, Private Foundation Support

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Genome-Wide Cytosine Methylation Differences in the Tubule of Human Diabetic Kidney Sang Youb Han,[1,2] Ae Seo Deok Park,[1] James M. Pullman,[3] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Division, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Division of Nephrology, College of Medicine Inje University, GoYang, Republic of Korea; [3]Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Diabetic kidney disease (DKD) remains one of the most devastating complications of diabetes. Clinical observational studies indicate the role of fetal and adult programming/long-term memory effect on disease development. In vitro studies suggest that epigenetic differences might mediate such long term changes. Here we analyzed differences of DNA cytosine methylation in tubule of patients with DKD (21), hypertension(23), diabetes(19) and healthy control (22).

Methods: Genomic DNA from 85 manually microdissected tubule tissue was used for hybridization on Infinium Human methylation 450K BeadChip. Corresponding gene expression was analyzed using Affymetrix U133 expression arrays.

Results: DKD group showed significantly lower eGFR (31.18±18.46 ml/min) compared to healthy controls (81.54±16.06) and samples with hypertension (76.62±14.94) or diabetes (73.55±12.10) and increased proteinuria. Histological analysis confirmed changes consistent with DKD.

The statistical analysis identified 4162 differential methylated regions (DMR), 2896 (70%) of them showed loss of methylation in DKD and 1266 (30%) loci were hypermethylated. We rarely observed differential methylation of CpG islands. Most differences localized to >2000 bp away from CpG islands. Most DMR localized to Refseq gene annotated gene body regions and we rarely observed differential methylation of promoters. Gene ontology analysis indicated the concordant differences both in gene expression and DNA methylation levels in genes related immune pathways, cell adhesion and development, genes and pathways that we and others have shown to play role in DKD development. Interestingly while diabetic samples in absence of renal disease showed significant gene expression changes, these samples showed little if any consistent differences in their cytosine methylation patterns.

Conclusions: In summary, human DKD is associated with changes in cellular epigenome and these changes may contribute to phenotype development.

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miRNA Microarray Expression Profiling in Human Diabetic Nephropathy Francesca Conserva,[1] Paola Pontrelli,[1] Matteo Accetturo,[1] Giorgia Cordisco,[1] Anna Maria Di Palma,[1] Giuseppe Grandaliano,[2] Loreto Gesualdo.[1] *[1]DETO-Nephrology Unit, Univ. of Bari, Italy; [2]Dept. of Medical and Surgical Sciences, Univ. of Foggia, Italy.*

Background: Diabetic Nephropathy (DN) is the primary cause of End Stage Renal Disease. Up to date there is no cure for DN and it is still unclear what determines its evolution in some individuals but not in others. Aim of our study was to identify a dataset of differentially expressed miRNAs in human biopsies of patients with type 2 DN.

Methods: Agilent miRNA arrays (8x15K slides) were used to characterize miRNA expression in Formalin-Fixed Paraffin-Embedded human kidney biopsies. Three sample groups were included: 8 DN patients with increased proteinuria and decreased glomerular filtration rate; 4 stage 1 membranous nephropathy samples, a condition also associated to glomerular basement membrane thickening and proteinuria not induced by hyperglycemia; 4 control kidney tissues from patients with no signs of nephropathy. Differentially expressed miRNAs in DN tissues *vs* controls, as well as membranous nephropathy tissues *vs* controls, were identified by unpaired t test and Benjamini-Hochberg multiple testing correction ($p\leq.05$; $FC\geq1.5$). Ingenuity Pathway Analysis was then employed to define the potentially deregulated pathways.

Results: We identified 76 differentially expressed miRNAs, 42 upregulated and 34 downregulated in DN tissues when compared to normal kidney, of which, 23 specific for DN (10 upregulated and 13 downregulated) and 54 in common with the membranous nephropathy group. The investigation of all miRNA potential targets in DN predicted a statistically significant upregulation of EphrinB ($p=3.16*10^{-5}$), Axon guidance ($p=3.12*10^{-4}$) and Notch ($p=1.48*10^{-3}$) pathways. *In silico* analysis on miRNA experimentally validated targets only, revealed vascular endothelial growth factor (VEGFA) and phosphatase and tensin homolog (PTEN) as the molecules targeted by a greater number of miRNAs in our DN tissues.

Conclusions: Our study profiles miRNA expression in DN human tissues and represents the first miRNA microarray benchmark study aimed to identify novel mechanisms of post-transcriptional modifications involved in type 2 DN pathogenesis and novel potential biomarkers.

Funding: Government Support - Non-U.S.

TH-PO476

Regulation of Ubiquitination in Diabetic Nephropathy: The Role of microRNAs Paola Pontrelli,[1] Francesca Conserva,[1] Matteo Accetturo,[1] Massimo Papale,[1] Giorgia Cordisco,[1] Anna Maria Di Palma,[1] Giuseppe Grandaliano,[2] Loreto Gesualdo.[1] *[1]DETO-Nephrology Unit, Univ. of Bari, Italy; [2]Dept. of Medical and Surgical Sciences, Univ. of Foggia, Italy.*

Background: Diabetic Nephropathy (DN) results from the unbalance of several pathways and ubiquitin has emerged as a novel key-player in its onset. Along with classical lys48, lys63 ubiquitination affects protein localization and signaling. Aim of our study was to explore DN, joining information from different high-throughput technologies, in order to identify new specific genes and microRNAs and clarify their role in DN onset and progression.

Methods: Agilent miRNA arrays (8x15K) were performed on formalin-fixed paraffin-embedded biopsies of 8 patients with DN and 4 healthy subjects. Gene expression arrays (Affymetrix HG-U133A) were obtained from human proximal tubular cells (HK2) grown in high glucose (HG) [30 mM], compared to normal glucose (NG) [5.5 mM] and mannitol [30 mM] as controls. Data were analyzed with GeneSpring GX12.0 (p-value<.05; FC≥1.5). qPCR, immunoblotting and immunohistochemistry were used for validation.

Results: miRNA arrays on DN patients showed that 42 out of 76 differentially expressed miRNAs belonged to the ubiquitination cascade, of which, 19 out of 34 downregulated miRNAs target the E2 class of the ubiquitinating enzymes. The second most down-regulated specifically targets UBE2V1 which mediates lys63-linked ubiquitin chain formation (26.1 fold). Microarray gene expression data confirmed an increase of UBE2V1 in HK2 under HG, validated by qPCR after 1h (1.51 fold HG vs NG). UBE2V1 protein expression after 24h (2.6 fold) and lys63 ubiquitinated proteins after 48h (2.05 fold) were detected by western blotting. Higher UBE2V1 tubular expression was observed in vivo (p=.004) along with lys63 ubiquitinated proteins (p=.008) in patients with DN vs normal controls. UBE2V1 expression and lys63 polyubiquitination are increased in DN and miRNAs could play a prominent role in this process.

Conclusions: The validation of miRNA data and the identification of ubiquitinated target proteins will provide a molecular signature of DN and lead to the identification of possible therapeutic targets and/or predictive biomarkers of disease.

Funding: Government Support - Non-U.S.

TH-PO477

Involvement of Inflammation-Related microRNAs: miR-155 and miR-146-a in Diabetic Nephropathy: Implication in Inflammation-Mediated Glomerular Endothelial Injury Ping Fu, Fang Liu. *Division of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.*

Background: The role of microRNAs (miRs) in diabetic nephropathy is unknown. Here, we sought to identify the repertoire of miRs in diabetic patients with diabetic nephropathy and their potential regulatory role on inflammation-mediated glomerular endothelial injury.

Methods: Microarray analysis was performed to examine the expression profile of miRs in kidney tissues from renal biopsies (7 from diabetic nephropathy patients and 3 from normal control). In situ hybridization was used to localize miR-155 and miR-146a. Meanwhile, the expressions of miR-155 and miR-146a were detected in STZ-induced type 1 diabetic nephropathy by real time PCR. Human renal glomerular endothelial cells (HRGEC) were cultured under high glucose, transfected with miR-155 and miR-146a mimics and inhibitors and PDTC (NF-κB inhibitor). Inflammatory cytokines (TNF-α, IL-1β, ICAM-1, MCP-1) were examined by western blot and real-time PCR. NF-κB activity was examined by EMSA.

Results: Ex vivo study showed there were 71 miRs with different expressions, 32 of which were up-regulated and 39 of which were down-regulated. Expressions of miR-155 and miR-146a of diabetic nephropathy were up-regulated by more than five-fold in kudney tissue of diabetic nephropathy patients compared with normal control. Furthermore, the expressions of the two miRs were not correlated with eGFR and urinary protein excretion. In situ hybradyzation showed up-regulated miR-155 and miR-146a mainly expressed in glomerular mesangial area and endothelial cells. In STZ-induced type 1 diabetic nephropathy, miR-155 and miR-146a were up-regulated in early stage. In vitro, high glucose induced the expressions of miR-155 and miR-146a in HRGECs and overexpressions of miR-155 and miR-146a increased the expressions of inflammatory cytokines via NF-κB pathway.

Conclusions: Increased expressions of miR-155 and miR-146a associated with increased expressions of inflammatory factors in diabetic nephropathy, which contribute to inflammation-mediated glomerular endothelial injury via NF-κB-dependent pathway.

Funding: Government Support - Non-U.S.

TH-PO478

FOG2, a Target of microRNA-200b/c, Controls Glomerular Mesangial Hypertrophy by Regulating TGF-β-Induced Akt Kinase Activation in Diabetic Nephropathy Jung Tak Park, Mitsuo Kato, Linda L. Lanting, Sumanth Putta, Rama Natarajan. *Department of Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.*

Background: Diabetic nephropathy (DN) is characterized by glomerular hypertrophy. TGF-β-activated Akt kinase mediates cellular hypertrophy under diabetic conditions. Recently, miR-200 and its target FOG2 were reported to regulate the activity of PI3K (Akt activator) to modulate insulin signaling and body size in flies. However, the role of FOG2 in Akt activation by TGF-β in mammalian kidneys is not clear. We hypothesized that TGF-β activates Akt in glomerular mesangial cells (MC) by inducing miR-200b/c which target FOG2, a PI3K inhibitor, and this contributes to MC hypertrophy.

Methods: mRNA and protein levels were examined by RT-qPCR or Western blotting in TGF-β-treated mouse MC (MMC) with or without miR-200b/c mimic, inhibitor, or FOG2 siRNA transfection. Glomerular FOG2 was detected by immunostaining.

Results: FOG2 protein and mRNA expression were reduced while miR-200b/c levels were increased in diabetic mouse glomeruli compared to non-diabetic controls. In addition, FOG2 protein and mRNA levels were down-regulated while miR-200b/c were reciprocally up-regulated in TGF-β-treated MMC. Down-regulation of FOG2 in TGF-β treated MMC was accompanied by increased Akt activation, while FOG2 knockdown by siRNAs in MMC also activated Akt, suggesting a causal relationship between FOG2 down-regulation and Akt activation. Transfection of MMC with miR-200b/c mimics decreased FOG2 expression and increased Akt phosphorylation. Conversely, when miR-200b/c inhibitor oligos were transfected into MMC, TGF-β induced down-regulation in FOG2 expression and increases in Akt activation were both reversed. Furthermore, transfection of MMC with miR-200b/c mimics increased the protein content/cell ratio, while conversely, miR-200b/c inhibitors abrogated the TGF-β-induced increases in protein content/cell. Moreover, FOG2 knockdown with siRNAs increased protein content/cell ratio, suggesting cellular hypertrophy.

Conclusions: These results suggest a new mechanism of Akt activation by TGF-β through FOG2 down regulation by miR-200b/c which can lead to MC hypertrophy related to DN.

Funding: NIDDK Support

TH-PO479

microRNA-26a Is Upregulated in Glomeruli of Diabetic Mice and Attenuates TGFβ1-Induced Extracellular Matrix Expression in Podocytes by Inhibiting Both CTGF and SMAD2 Kenichi Koga, Masashi Mukoyama, Hideki Yokoi, Kiyoshi Mori, Masato Kasahara, Takashige Kuwabara, Hirotaka Imamaki, Tomoko Kawanishi, Akira Ishii, Keita P. Mori, Yukiko Kato, Shoko Ohno, Naohiro Toda, Moin Saleem, Akira Sugawara, Kazuwa Nakao. *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: MicroRNAs (miRNAs) are small non-coding RNAs which negatively regulate target genes. Recently, we showed miR-26a targeting connective tissue growth factor (CTGF) is upregulated in glomeruli of *db/db* mice and attenuates the expression of col1a1 and col4a3 in TGFβ1-stimulated podocytes.

Methods: We examined miR-26a expression in glomeruli of streptozotocin (STZ)-induced diabetic mice by TaqMan PCR and *in situ* hybridization (ISH). We transfected its mimic into podocytes by nucleofection and examined target gene expressions by Western blot and CTGF 3'UTR luciferase reporter assay. In addition, we analyzed smad activity in TGFβ1-treated podocytes using luciferase reporter plasmid containing smad binding element (SBE).

Results: miR-26a was upregulated in glomeruli of STZ mice by 2.7-fold compared with control mice by TaqMan PCR ($p < 0.05$) and was mainly localized in glomeruli of STZ mice by ISH. Transfection of miR-26a mimic into podocytes significantly decreased CTGF protein level by 49% and reduced CTGF 3'UTR luciferase activity by 30%. We also showed that miR-26a mimic inhibited SBE luciferase activity by 91% ($p < 0.001$), indicating that miR-26a inhibits TGF-β/SMAD signaling. We analyzed other target genes of miR-26a involved in TGF-β/SMAD signaling and identified SMAD2 as a target gene of miR-26a by showing that miR-26a mimic significantly reduced SMAD2 protein level by 39%.

Conclusions: These results indicate that miR-26a attenuates TGF-β/SMAD signaling and TGFβ1-induced fibrosis in podocytes by downregulating CTGF and SMAD2. We hypothesize that increased expression of miR-26a prevents the progression of DN via inhibiting TGF-β/SMAD signaling. miR-26a could be a therapeutic agent for DN.

TH-PO480

Inhibitory Effect of the Benfotiamine on Hyperglycemia-Induced Inflammatory and Fibrogenic Signals in DM Nephropathy Eun Jin Cho,[1] Kyung Don Ju,[2] Eun Kyoung Shin,[2] Dong Ki Kim,[1] Kwon Wook Joo,[1] Yon Su Kim,[1] Curie Ahn,[1] Jin Suk Han,[1] Kook-Hwan Oh.[1] *[1]Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Clinical Research Institute, Seoul National University Hospital, Seoul, Korea.*

Background: Benfotiamine is an anti-oxidant and anti-inflammatory substance. We aimed at verifying the protective effect of benfotiamine against the development and progression of diabetic nephropathy in an in-vivo and in-vitro model.

Methods: Diabetic rats were prepared by injecting streptozotocin (STZ, 65mg/kg). Those that developed diabetes after 72 h were treated with benfotiamine (70mg/kg). Diabetic rats without benfotiamine treatment and nondiabetic rats were used for control. Rat mesangial cells were treated in high glucose (HG, 50mM) and normal glucose (NG, 5mM) conditions.

Results: Benfotiamine-treated diabetic rats exhibited decreased albuminuria, and NADPH-dependent ROS generation. Immunohistochemistry showed reduced type IV collagen and fibronectin deposition in the glomeruli compared with nontreated diabetic rat. Parallel changes were shown in the tissue mRNA and protein expression level of MCP-1, TGF-β1, fibronectin and type IV collagen in the kidney. These findings suggest that benfotiamine protects against kidney injury via transketolase upregulation.

Conclusions: Benfotiamine exhibited a renoprotective effect in the progression of experimental diabetic nephropathy. Future research is warranted to investigate the protective mechanism of benfotiamine more specifically and to help set the basis for clinical use of this substance in the prevention of diabetic nephropathy.

TH-PO481

Collagen XVIII and Endostatin Have Differential Roles in Early Stages of Diabetic Nephropathy Yuki Hamano,[1] Yoshihiko Ueda,[2] Makoto Ogawa.[1] *[1]Nephrology, Chiba University Hospital, Chiba, Japan; [2]Pathology, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Japan.*

Background: Collagen XVIII is distributed throughout glomerular basement membrane, mesangial matrix and Bowman's capsule. Proteolytic cleavage within its C-terminal domain releases the fragment endostatin, which has antiangiogenic properties. Diabetic nephropathy is characterized by thickening of the glomerular basement membrane and excessive extracellular matrix accumulation in the mesangium. Streptozotocin (STZ) has been commonly used to induce hyperglycemic diabetes. Because its renal toxicity accompanies extracellular matrix accumulation and vascular injury, we investigated the role of collagen XVIII and endostatin in this disorder.

Methods: Diabetic nephropathy was induced in collagen XVIII/endostatin-null (KO) and wild-type (WT) mice by injection of STZ. Animals injected with citrate buffer only served as controls. At 14 weeks, blood and urine samples were collected and mice were sacrificed to obtain tissues. The resulting matrix accumulation and neovascularization were compared.

Results: No differences in the degree of hyperglycemia and blood pressure were seen between diabetic KO and WT mice. In diabetic KO mice compared with diabetic WT mice, the albuminuria, blood urea nitrogen and serum creatinine levels, mean glomerular volume and mesangial matrix accumulation were increased significantly, whereas the thickening of glomerular basement membrane was completely blocked. Lack of collagen XVIII/endostatin led to an elevation of the renal cortical expression of MCP-1, CTGF, SDF-1 and MMP-2 but not of VEGF-A, TGF-β1 and TSP-1. Levels of nephrin, CD2AP and podocin were not reduced in diabetic KO mice compared with diabetic WT mice. The microvascular density and influx of macrophages were increased in the glomeruli of diabetic KO mice compared with diabetic WT mice. The endothelial progenitor cells were detected in the peripheral blood and bone marrow of diabetic KO mice more than those of diabetic WT mice.

Conclusions: Collagen XVIII and endostatin play important roles in preserving the integrity of extracellular matrices and capillary vessels in the kidney and have a protective effect against diabetic kidney disease.

Funding: Government Support - Non-U.S.

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Oral Treatment with a Novel First-in-Class Anti-Fibrotic Compound PBI-4050 Reduces Kidney Hyperfiltration, Proteinuria and Hepatic Steatosis in the Diabetic db/db Mouse Model Lyne Gagnon, Lilianne Geerts, André Doucet, François Sarra-Bournet, Martin Leduc, Shaun Abbott, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin, Brigitte Grouix. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: Diabetic nephropathy is increasing in incidence and is now the number one cause of end-stage renal disease**.** Obese and diabetic db/db mice exhibit a consistent and robust increase in albuminuria and mesangial matrix expansion, and develop hepatic steatosis. PBI-4050, a potential treatment for fibrotic diseases, possesses a pleiotropic mechanism of action with anti-inflammatory, anti-oxidant and anti-fibrotic properties.

Methods: The aim of this study was to investigate the effect of PBI-4050 on uni-nephrectomized diabetic (db/db) mice. Total nephrectomy of the right kidney was performed on day 0 and animals were treated with vehicle or PBI-4050 (100 mg/kg, oral once a day) from day 1 through 130. Kidney function (urinary creatinine, GFR, albuminuria and proteinuria), kidney mesangium lesions and hepatic steatosis were examined.

Results: Urinary creatinine was significantly reduced in uni-nephrectomized db/db mice compared to background C57BL/6 mice, but returned to the normal level in PBI-4050-treated db/db mice. Albuminuria was also increased by 1.5 to 2 fold in uni-nephrectomized mice (C57BL/6 and db/db) compared to sham C57BL/6 mice. Treatment of db/db mice with PBI-4050 reduced both albuminuria and proteinuria to the sham C57BL/6 mice level. Kidney GFR was significantly reduced in uni-nephrectomized C57BL/6 mice. Uni-nephrectomized db/db mice demonstrated a significant increase in GFR (hyperfiltration), which was significantly reduced by treatment with PBI-4050. Db/db non-treated mice had larger glomeruli with increased mesangial matrix, as shown by periodic acid-Schiff (PAS) staining. Mesangium lesions score were also reduced in db/db mice treated with PBI-4050. Hepatic steatosis, observed in db/db mice, was significantly (50%) reduced with PBI-4050-treatment.

Conclusions: These results suggest that PBI-4050 offers the potential as a novel therapy for the reduction of diabetic kidney disease.

Funding: Pharmaceutical Company Support - ProMetic BioSciences Inc.

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Endogenous Anti-Fibrotic Peptide N-Acetyl-Seryl-Aspartyl-Lysyl-Proline Inhibits Endothelial-Mesenchymal Transition and Diabetes-Associated Kidney Fibrosis Takako Nagai, Keizo Kanasaki, Daisuke Koya. *Diabetology and Endocrinology, Kanazawa Medical University, Kahoku, Ishikawa, Japan.*

Background: Fibrosis is critical determinant of the progression in kidney diseases. Endothelial to mesenchymal transition (EndMT) has emerged as one of the important source of kidney fibroblasts. Angiotensin-converting enzyme (ACE) inhibition increases plasma concentration of endogenous anti-fibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP).

Methods: Human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMVEC) were stimulated with transforming growth factor (TGF)-β2 or combination of TGF-β2, interleukin (IL)-1β and tumor necrosis factor (TNF)-α, with or without AcSDKP incubation. In vivo experiments were performed in streptozotocin (STZ)-induced diabetic CD1 mouse, the model displaying diabetes-associated kidney fibrosis.

Results: Here we found that AcSDKP exhibited anti-EndMT, anti-apoptosis and anti-kidney fibrosis effects in vivo and in vitro. TGF-β2 induced HUVEC/HMVEC cells into spindle shapes and reduced endothelial markers CD31/VE-cadherin expression together with the induction of mesenchymal markers such as αSMA or SM22α expression, suggesting TGF-β2 induced EndMT. Such TGF-β2-induced EndMT was restored by the co-incubation with AcSDKP in either HUVEC or HMVEC. Furthermore, combination of TGF-β2, IL-1β and TNF-α stimulated HMVEC cells into EndMT and apoptosis; AcSDKP inhibited such cell phenotypic changes induced by these cytokines combination. When analyzing the kidney fibrosis in STZ-induced diabetic CD1 mice, the conventional ACE-inhibitor plus AcSDKP add-on therapy significantly ameliorated kidney fibrosis in association with EndMT inhibition. Interestingly microRNA array analysis revealed that anti-EndMT effects of AcSDKP were correlated with the restoration in TGF-β2-induced profibrotic microRNA profiles.

Conclusions: These results suggest that AcSDKP is potential valuable endogenous anti-fibrotic molecule via partially inhibition of EndMT.

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TGFβ Inhibition in the KK.CG-AY/J Mouse Differentially Affects Canonical and Non-Canonical TGFβ Signaling Stephen O'Brien, John N. Vassiliadis, Mandy M. Smith, Hong Ling, Steven R. Ledbetter, Cynthia M. Arbeeny, Stefan Wawersik. *Tissue Protection & Repair Unit, Sanofi-Genzyme R&D Center, Framingham, MA.*

Background: We previously reported that uninephrectomized KK^{y} mice fed a moderately high fat diet (24% of calories) develop renal injury characteristic of human diabetic nephropathy (DN). This model exhibits upregulation of genes associated with fibrosis (fibronectin 1, MMP9, Col3a1) and inflammation (IL-1b, IL-6, MCP-1), and decreased podocyte markers nephrin and podocin. We have also demonstrated that a 5m/kg dose of the TGFβ neutralizing antibody 1D11, administered 3x/week for 12 weeks, protects KK^{y} mice from renal injury.

Methods: To establish the PK/PD profile for TGFβ antagonism we examined the short-term effects of blocking TGFβ. Female KK^{y} mice were uninephrectomized at 8 weeks old and started on a 24% fat diet. At 12 weeks old, the 1D11 antibody was administered IP 3x/week at either 0 (vehicle control), 0.5, 1, or 5 mg/kg. After 3 weeks of treatment, animals were sacrificed and the effect of 1D11 treatment on markers of fibrosis and inflammation was analyzed by quantitative RT-PCR on total kidney RNA.

Results: Compared to untreated KK^{y} mice, Col3a1 expression was moderately reduced, but expression of the fibrotic markers fibronectin and MMP9 were unchanged. The inflammation markers Il-6 and IL-1b were also markedly downregulated. Since we observed only minor effects on fibrotic gene expression in this short-term study, we assessed the early effects of 1D11 treatment on canonical (Smad 2/3) and non-canonical (TAK1) TGFβ signaling pathways. At all 3 doses of 1D11, we see no measurable inhibition Smad2 phosphorylation in total kidney lysates, and a weak but not statistically significant trend towards inhibition of Smad3 phosphorylation. However, we find that 1D11 treatment at both 1 and 5 mg/kg significantly reduces phosphorylation of TAK1.

Conclusions: These data suggest that 1D11 has distinct effects on canonical and non-canonical TGFβ signaling, and that inhibiting signaling through TAK1 may play an important role in countering the inflammatory response that contributes to initiation and progression of renal pathology in this DN model.

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TH-PO485

Differential Regulation of Tubule Endoplasmic Reticulum Chaperones in OVE26 Type I Diabetic Mice Michelle T. Barati, Susan M. Isaacs, Jon B. Klein. *Nephrology, University of Louisville, Louisville, KY.*

Background: Endoplasmic reticulum (ER) stress response occurs in diabetic nephropathy (DN), yet progression of this response in different tubule subtypes is not known. The goal of this study was to define progression of ER stress response markers in proximal and distal tubules and determine if severity of proteinuria during diabetes alters activation of the PERK branch of stress response signaling.

Methods: This study used OVE26 and OVE26-Nmt3 diabetic and FVB control mice. OVE26-Nmt are cross of OVE26 and Nmt mice (overexpress metallothionein in podocytes) and have decreased proteinuria. Expression of ER stress response-induced chaperones GRP78, GRP94, and PDI was determined in isolated cortical tubules of OVE26 and FVB mice. Tubule localization determined by immunofluorescent staining of kidneys for ER chaperones and FITC-lotus tetragonolobus agglutinin (LTA), to mark proximal tubules (PT). Phospho-PERK and CHOP analyzed by immunostaining in age-matched FVB and severe or mildly proteinuric OVE26 and OVE26-Nmt mice, respectively. For *in vitro* studies, cultured PT cell fibronectin (FN) secretion analyzed following knockdown of PERK by siRNA.

Results: GRP78, GRP94, and PDI were increased in isolated tubules of 7 month old diabetic mice. Immunostaining showed upregulation of GRP78 in all cortical tubule types and PDI in distal tubules. PDI and GRP78 were decreased in tubules of 2 and 3 month old diabetic mice, respectively, in all tubule subtypes. Phospho-PERK and CHOP were expressed similarly in abundance and tubule subtype between OVE26 and OVE26-Nmt diabetic mice. Knockdown of PERK by siRNA *in vitro,* increased FN secretion by PT cells, defining a role for PERK in PT function relevant to DN.

Conclusions: ER chaperones are differentially regulated in tubule subtypes in diabetes and functional outcome of decreased chaperone expression in young diabetic mice remains to be defined. ER chaperones may be subject to degradative or autophagy pathways. Severe proteinuria does not alter signaling via the PERK branch of ER stress response in tubules. Increased PT cell FN secretion with PERK knockdown suggests that the ER stress response may inhibit pro-fibrotic mechanisms in DN.

Funding: NIDDK Support

TH-PO486

Localization of the Apelinergic System within the Kidney Anastasia Z. Kalea,[1,2] Minghao Ye,[1] Jan A. Wysocki,[1] Ivy Hsieh,[1] Daniel Batlle.[1] *[1]Div. of Nephrology &Hypertension, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2]Centre for Cardiovascular Genetics, University College London, United Kingdom.*

Background: The Apelin receptor (APJ)–Apelin system has been implicated in cardiovascular function. Apelin's effects appear related, in part, to an interaction with the RAS. Since Apelin, like angiotensin II, is a substrate for ACE2, which we previously showed to localize in glomerular epithelial cells (podocytes), we wanted to investigate the localization of this peptide within the glomerulus and the overall expression of apelin and its receptor APJ within the kidney.

Methods: We employed RT-real time PCR, western blot, immunostaining, tissue and cell confocal microscopy to study apelin and APJ in kidneys from female C57BLKS mice (8 wks of age) and in cultured immortalized podocytes. Mouse podocytes were stimulated with Pyr[1]Apelin-13 (100nM) for 0, 1, 5, 15 and 60min and tested for phospho-status of AKT, p70S6K and ERK.

Results: Immunohistochemical analysis showed that both Apelin and APJ were abundantly present in the kidney being expressed in most glomerular tufts, proximal tubules, and arterioles. In glomeruli, apelin colocalized with nephrin, PECAM-1, and a-SMA indicating its presence in podocytes, endothelial cells, and mesangial cells. APJ, by contrast, did not colocalize with PECAM-1 but also colocalized with several podocyte markers. In the proximal tubule, the APJ receptor colocalized with ACE2. In renal arterioles, APJ showed colocalization only with smooth muscle markers, while apelin colocalized both with endothelial and smooth muscle cell markers. Apelin and APJ mRNA were also expressed in cultured mouse podocytes where apelin-13 induced AKT, p70S6K and ERK signaling at 15min.

Conclusions: Apelin and its receptor are widely expressed in the kidney, including podocytes where apelin-13 is involved in AKT, p70S6K and ERK signaling. Since receptor expression usually correlates with its physiological function, the presence of the newly identified apelinergic system in the kidney and its possible interaction with the podocyte RAS could be relevant to disease conditions, such as diabetic kidney disease, where there is overactivity of the RAS.

TH-PO487

Acute Disruption of Megalin Expression in Renal Cortex Reveals Compensatory Reabsorption Capacity of Low Molecular Weight Proteins in S3 Segment of Proximal Tubules and Collecting Ducts Keita P. Mori,[1] Kiyoshi Mori,[1] Masashi Mukoyama,[1] Hideki Yokoi,[1] Masato Kasahara,[1] Takashige Kuwabara,[1] Hirotaka Imamaki,[1] Akira Ishii,[1] Kenichi Koga,[1] Yukiko Kato,[1] Akira Sugawara,[1] Tomomi Endo,[2] Motoko Yanagita,[2] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Department of Nephrology, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: Reabsorption activity of proximal tubules gives profound effects upon urinary excretion levels of chronic and acute kidney injury biomarkers such as albumin and low molecular weight (LMW) proteins, including neutrophil gelatinase-associated lipocalin (Ngal) and retinol-binding protein 4 (RBP). Megalin (fl/fl) x ApoE-Cre mice have been used to analyze the effects of megalin gene deletion, but the efficiency of disruption in the kidney was only 60%.

Methods: In the present study, to understand the impact of more efficient elimination of renal cortical megalin expression upon renal reabsorption activity after normal development, we generated megalin (fl/fl) [kindly provided from Prof. Thomas Willnow, Max-Delbrueck-Center] x N-myc downstream-regulated gene 1-CreERT2 (NDRG1) mice.

Results: By real-time RT-PCR, renal megalin gene expression was reduced by 80% after intermediate dose of Tam administration, and this dose was chosen for further analysis. (With higher dose, deletion efficiency became > 90%.) By ELISA, urinary albumin excretion was elevated by 8-fold and Ngal excretion was elevated by 20-fold after Tam injection in megalin (fl/fl) x NDRG1 mice. In normal mice, by immunofluorescence, megalin protein was expressed along brush borders in S1-S3 segments of proximal tubules, and reabsorption of iv injected RBP was observed selectively in S1 and S2. After Tam treatment, distribution of exogenously given RBP was shifted towards S3 and it was also observed in cortical and medullary collecting ducts.

Conclusions: These findings suggest that renal cortical tubules are the predominant site of LMW protein reabsorption in physiologic conditions, and S3 and collecting ducts have a reservoir capacity for reabsorption.

TH-PO488

Microalbuminuria: Is Proximal Tubule the Culprit? Elif Erkan,[1] Bob Monks,[2] Morris J. Birnbaum.[2] *[1]Pediatrics, University of Pittsburgh, Pittsburgh, PA; [2]University of Pennsylvania.*

Background: Diabetes mellitus (DM) is the leading cause of end-stage renal disease. Microalbuminuria (MA) is utilized as a marker for target organ damage. We previously reported that protein kinase B (Akt), a major protein involved in insulin signaling, mediates albumin endocytosis in the proximal tubule. We hypothesize that in DM where there is downregulation of insulin-Akt pathway, alterations in the function of endocytic machinery may lead to microalbuminuria.

Methods: We examined the effect of insulin (100nm) on albumin endocytosis in human kidney (HKC-8), opposum kidney and primary mouse proximal tubule cell lines by fluorometry and western blotting (WB). Inhibition of Akt was accomplished with a double dominant mutant (DDN). The role of upstream Akt mediator adaptor protein containing a PH domain, PTB domain, and leucine zipper motif (APPL1) was evaluated by transfecting the cells with siRNA. Protein-protein interactions between cytoplasmic tail (CT) of megalin and Akt substrate AS160 were assessed by coimmunoprecipitation (Co-IP) and GST pull down (PD) assays. Disabled-2 (Dab2), megalin and cubilin expression of Akt2 knock-out (KO) mice was examined by WB and immunofluorescence.

Results: We demonstrated that insulin induces albumin endocytosis in multiple proximal tubule cell culture models. Insulin induced albumin endocytosis was perturbed by downregulation of Akt and APPL1 showing that this novel pathway is driven by Akt activation. Over expression of AS160 potentiated the insulin induced albumin endocytosis in association with an increase in megalin expression. An interaction between AS160 and megalin CT was demonstrated by co-ip and GST-PD assays. Furthermore Akt2 KO mice displayed decreased renal expression of megalin, cubilin and Dab2 confirming the role of insulin-Akt cascade in albumin endocytosis.

Conclusions: We showed a novel pathway that connects insulin-Akt signaling to albumin endocytosis in the kidney. Further delineation of protein-protein interactions surrounding insulin-Akt signaling in the proximal tubule may allow us to implement early screening tools and develop strategies to prevent proximal tubule damage and MA in diabetic nephropathy.

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Thrombomodulin in the Renal Microcirculation in Diabetes Is Regulated by Protein Kinase C-alpha Joon-Keun Park, Torsten Kirsch, Jan Menne, Hermann G. Haller. *Clinic of Hypertension and Nephrology, Hannover Medical School, Hannover, Germany.*

Background: Thrombomodulin (TM) is a cell surface glycoprotein which is a cofactor for thrombin binding that mediates protein C activation and inhibits thrombin activity. In addition, TM, has potent anti-inflammatory function through a variety of molecular mechanisms. Its role in diabetic vascular complications has not been investigated. We hypothesized that TM contributes to the pathogenesis of diabetic nephropathy and tested whether glucose-induced activation of PKC is involved.

Methods: Expression of TM was analyzed in mouse models (SV) and in PKC-alpha and PKC-beta -/- mice using immunohistochemistry, immunoblotting and real-time PCR. Mice were made hyperglycemic by streptozotocin treatment. Microvascular endothelial cells were obtained from Lonza Inc.

Results: Exposure of cultured microvascular endothelial cells to high glucose concentration (20 mM) rapidly down-regulated TM. This down-regulation was accompanied by the loss of perlecan and other glycocalyx molecules. The effect of glucose was prevented inhibition of PKC-alpha (antisense) but not by other PKC isoforms. Hyperglycemia in a mouse model led to a rapid decrease in TM immunoreactivity in glomerular endothelial cells. TM was also less in interstitial capillaries, although to a lesser level. TM expression was not altered in larger renal vessels. The diabetes-induced down-regulation of TM was almost completely prevented in PKC-alpha -/- mice, while the deletion of PKC-beta did not influence TM expression. Inhibition of PKC-alpha with a specific inhibitor (Goe 8862) prevented the effect of glucose on TM.

Conclusions: TM is rapidly influenced by high glucose concentrations and disappears under diabetic conditions in microvascular endothelial cells. This effect is mediated specifically by PKC isoform-alpha. The loss of TM may contribute to the altered coagulation and the increased inflammatory state in the diabetic microvasculature. Specific inhibition of PKC-alpha ameliorates glomerular inflammatory mechanisms in diabetic nephropathy.

TH-PO490

CD2AP Phosphorylation Is Enhanced in the Diseased Kidney Irini Schaefer, Beina Teng, Kirstin Worthmann, Hermann G. Haller, Mario Schiffer. *Nephrology, Medical School Hannover, Hannover, Germany.*

Background: CD2AP is an adaptor protein that can transmit intracellular signals involved in survival and cytoskeletal regulation of the cell. We have shown that CD2AP phosphorylation determine binding to nephrin. Until now it is unknown if phosphorylation of CD2AP is regulated in renal diseases as it was shown for nephrin. The aim of these studies was to analyze the involvement of CD2AP phoshorylation in kidney diseases.

Methods: Highly conserved tyrosine residues are in every SH3 domain of CD2AP on positions Y4/8/10, Y119 and Y273/280. We analyzed localization of phosphorylated CD2AP in cultured podocytes, in mice and human biopsies by immunofluorescence, immunohistochemistry and subcellular fractionization.

Results: Stimulation of murine and human podocytes with VEGF showed a typical phosphorylation profile of CD2AP with the generated phospho-specific antibodies.

Immunofluorescence stainings on murine and human parafine sections reveal that the phospho-specific antibodies against Y119 and Y273/280 showed a specific slit-diaphragm staining whereas the phospho-specific antibody against Y4/8/10 showed a more cytosol-specific staining. To further investigate the localization of CD2AP phosphorylation we performed subcellular fractionization. We can show that fulllength CD2AP is phosphorylated on tyrosine position Y4/8/10 in the cytosol, but a band of approximately 40 kDa can be found at the membrane and in the nucleus which could be a spliced variant or a cleaved form of CD2AP. Furthermore in patients with diabetic nephropathy, where VEGF expression is enhanced and nephrin expression is downregulated in the kidney, CD2AP phosphorylation on position Y4/8/10 is increased in the cytoplasm and especially in the nucleus of podocytes. Lysates from glomeruli of diabetic mice show an increased phosphorylation on position Y4/8/10 of the short 40 KDa form of CD2AP in the nucleus.

Conclusions: These results propose that CD2AP phosphorylation in regulated in the diseased glomerulus and could be of fundamental importance to understand initiation and development of glomerulopathies. Furthermore a short phosphorylated form could be determined in the nucleus of podocytes especially in diseased conditions.

Funding: Government Support - Non-U.S.

TH-PO491

Circulating Klotho Levels Are Reduced in Type 2 Diabetes Mellitus Independent of Hyperglycemia Joris van Ark,[1] Hans-Peter Hammes,[3] Marcory van Dijk,[1] Harry Van Goor,[1] Bruce H. Wolffenbuttel,[2] Jan-luuk Hillebrands.[1] *[1]Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands; [2]Endocrinology, University Medical Center Groningen, Groningen, Netherlands; [3]5th Medical Department, University Hospital Mannheim, University of Heidelberg, Mannheim, Baden-Württemberg, Germany.*

Background: Circulating α-Klotho is produced in the kidney and is a putative anti-aging and vasculoprotective hormone. Reduced circulating Klotho levels may increase cardiovascular risk. Type 2 diabetes (T2DM) patients are at increased cardiovascular risk. We therefore investigated if T2DM is associated with reduced circulating Klotho levels and whether hyperglycemia alters renal Klotho production.

Methods: Peripheral blood was obtained from T2DM patients without documented diabetic nephropathy (n=8) and healthy control subjects (n=10). Serum Klotho levels were determined with ELISA. Primary proximal tubular epithelial cells (PTEC) were isolated and cultured *in vitro* in the presence of various concentrations of glucose (7.8, 15, 30 and 50 mM). Klotho expression in PTECs was determined after 4 days using real-time PCR analysis and quantitative Klotho immunofluorescence. To study the effect of hyperglycemia on renal Klotho production *in vivo*, serum Klotho levels and renal Klotho expression were determined using ELISA and real-time PCR, respectively in hyperglycemic $Ins2^{Akita}$ mice (n=7) and normoglycemic C57BL/6 mice (n=7).

Results: Serum Klotho levels were significantly reduced in T2DM patients compared with healthy control subjects (21.6± 3.1 vs 78.7 ± 11.5 ng/mL, resp., p<0.001). Cultured primary PTECs exposed to high glucose did not display altered Klotho mRNA or protein expression. Long-term hyperglycemic $Ins2^{Akita}$ mice were characterized by similar circulating Klotho and renal Klotho mRNA expression levels when compared with normoglycemic control mice.

Conclusions: Circulating Klotho levels are reduced in patients with T2DM without overt diabetic nephropathy. Our *in vitro* and *in vivo* data indicate that hyperglycemia *per se* does not reduce renal Klotho production. Future studies will have to elucidate the mechanism underlying the reduced circulating Klotho levels in T2DM.

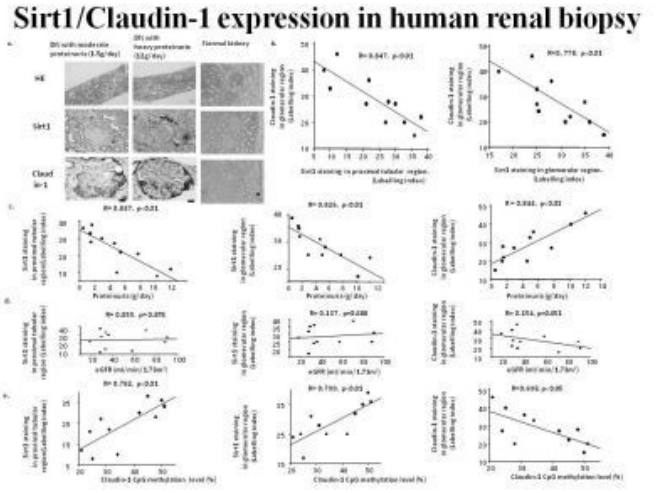
TH-PO492

Sirt1 and Claudin-1 Expression in Human Renal Biopsy Specimens of DN Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio University, Shinjuku-ku Shinanomachi 35, Tokyo, Japan.*

Background: We reported **epigenetic regulation of claudin-1** (oral presentation in 2010, 2011 ASN). In diabetic nephropathy (DN), reduced Sirt1 decreased claudin-1 CpG methylation and increased Cldn-1 in podocytes. Sirt1 overexpression reversed these changes. However, **the expression or role of Sirt1 and Cldn-1 in humans has not been reported**.

Methods: Renal biopsy specimens were obtained from 11 patients with DN. As controls, pre-transplant biopsies from 5 living donors were obtained. Written informed consent was obtained and the study was performed in accordance with the declaration of Helsinki.

Results: All patients were with an age of 61.8±13.2 years, and had a serum creatinine level of 1.57±0.66 mg/dl, daily urinary protein excretion of 4.79±3.80 g/day, eGFR of 48.8±24.9 ml/min/1.73m^2, and HbA_{1c} of 6.24±0.39%. Sirt1 in both proximal tubules (PTs) and podocytes (Pods) was lower with DN (a). In contrast, Cldn-1 in Pods increased with DN. As a reference, Sirt1 were detected in both PT and Pods, and no Cldn-1 in Pods was detected in controls. Cldn-1 was correlated with lower Sirt1 in both PTs and Pods (b). Of clinical parameters, only proteinuria was correlated with a lack of Sirt1 staining. In addition, proteinuria was correlated with Cldn-1 (c). There were no correlations between eGFR and the staining of these proteins (d). **Cldn-1 CpG methylation was correlated with a lack of Sirt1 staining and inversely correlated with Cldn-1 expression (e).**



Conclusions: We first found that in human kidney **1) Sirt1 decreased in DN, 2) Cldn-1, which is correlated with Sirt1 down-regulation, was up-regulated in DN, and 3) both increase in Cldn-1 and decrease in Sirt1 were correlated with the extent of proteinuria in DN.** Since the clinical data are consistent with the results in mice, these findings can provide useful insight into the pathogenesis and treatment of DN in clinical practice.

TH-PO493

Podocyte-Specific Overexpression of SIRT-1 Increases Nephrin in Obese Mice Fed a High-Fat Diet Jae Won Yang,[1] Seung-Ok Choi,[1] Byoung Geun Han,[1] Minseob Eom.[2] *[1]Nephrology, Yonsei University Wonju College of Medicine, Wonju, Gangwon, Korea; [2]Pathology, Yonsei University Wonju College of Medicine, Wonju, Gangwon, Korea.*

Background: Obesity can lead to inflammation, hyperlipidemia, diabetes, hypertension, and renal dysfunction, and is also associated with proteinuria. Histopathological changes in the glomeruli in obesity were characterized by glomerulomegaly and focal segmental glomerulosclerosis. This study was designed to investigate the effect of sirtuin 1 (SIRT-1), podocyte-specific overexpressed, on nephrin levels in obese mice induced by high fat diet. SIRT-1 has been proposed as a chemotherapeutic target for type II diabetes mellitus.

Methods: After establishing the SIRT-1 transgenic mice, experimental groups were divided into following three groups: Normal diet-normal group (ND-NL), high fat diet-normal group (HFD-NL), and high fat diet-SIRT-1 group (HFD-SIRT1). The background of transgenic mice was C57BL/6. High fat diet group were fed with a high calorie diet (60%) for up to 21 weeks to examine a progressive development of obesity. Body weight, 6 hours fasting blood glucose, and HbA1c were regularly measured. Albumin-Creatinine Ratio (ACR) in 24 hours urine was measured 21 weeks after the experiment. The expression levels of SIRT-1 and nephrin in the kidney by using western-blot and RT-PCR were compared.

Results: With repeated measures ANOVA test, both high fat diet groups were showing that the body weight was significantly higher than normal diet group (P<0.0001) and showing that 6 hours fasting blood glucose was also significantly different (P<0.05). Although statistically not different, urinary ACR of the HFD-SIRT1 group was lower than the HFD-NL group (P=0.09). The nephrin protein expression in the HFD-SIRT1 group was significantly increased than the HFD-NL (P<0.05). The nephrin mRNA level in the HFD-SIRT1 group showed a tendency to increase compared with the HFD-NL group.

Conclusions: Taken together the results, deterioration of the kidney disease caused by obesity and hyperglycemia could be prevented by increasing the level of the nephrin expression through SIRT-1 activation. SIRT-1 may have the ability to protect the podocyte from injuries caused by obesity and hyperglycemia.

TH-PO494

Inhibition of TNFα Decreases Nephrin Endocytosis and Protects Mice against Hyperglycemia-Induced Proteinuria Magdalena Woznowski, Sebastian Alexander Potthoff, Eva Koenigshausen, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin, Ivo Quack. *Nephrology, Heinrich Heine University, Duesseldorf, Germany.*

Background: In diabetes TNFα is upregulated and has been recognized as an important mediator of diabetic nephropathy and proteinuria. However, the underlying pathomechanism remained elusive so far. Thus we wanted to analyze if TNFα is critical for the regulation of slit diaphragm integrity in hyperglycemic states.

Methods: HEK293T cells and podocytes were treated with high levels of glucose (30mM). C57BL/6 mice were treated intraperitoneally with streptozotocin to induce hyperglycemia. For inhibition of TNF-alpha activity, Etanercept, a soluble dimeric fusion protein that binds soluble TNFα, was administered in cells and animals. For quantification of endocytosis in vitro and in vivo all extracellular proteins were labeled with a reactive sulfo-biotin-esther. Precipitation of nephrin with subsequent western blot analysis for the biotinylation status of was performed. Total mRNA was isolated from in vitro differentiated podocytes and subjected to a Real-Time-PCR analysis for the expression level of TNFα mRNA. TNFα serum levels were measured by ELISA. Albuminuria was quantified as albumin/creatinin ratio.

Results: Cell culture: treatment of HEK 293T cells or podocytes with high glucose leads to a rapid upregulation of TNFα and induces the endocytosis of nephrin. Simultaneous treatment with etanercept prevented nephrin endocytosis. Animal model: mice with streptozotocine induced diabetes develop elevated serum levels of TNFα and proteinuria within hours. Inhibition of TNFα via intraperitoneal administration of etanercept strongly reduces nephrin endocytosis and proteinuria.

Conclusions: Hyperglycemia leads to elevated TNFα serum levels. TNFα in turn induces the endocytosis of nephrin in podocytes and thereby weakens the integrity of the glomerular slit diaphragm. Inhibition of TNFα prevents proteinuria without influencing the hyperglycemia. Our results suggest TNFα as a pivotal mediator of hyperglycemia-induced damage of slit diaphragm integrity. Inhibition of TNFα might become a promising treatment option to prevent diabetic proteinuria.

Funding: Government Support - Non-U.S.

TH-PO495

A PAI-1 Mutant Slows Diabetic Nephropathy Progression in db/db Mice via Podocyte Protection Chunyan Gu, Jiandong Zhang, Alfred K. Cheung, Yufeng Huang. *Internal Medicine, University of Utah, Salt Lake City, UT.*

Background: PAI-1R, a mutant plasminogen activator inhibitor (PAI-1), aimed at reducing endogenous PAI-1 activity, has been shown to inhibit albuminuria and prevent glomerular mesangial matrix expansion in experimental diabetes. The mechanism of albuminuria reduction is unclear but may be related to amelioration of podocyte injury.

Methods: This study sought to determine whether administration of PAI-1R could protect podocyte from injury directly thereby slowing the progression of glomerulosclerosis in the db/db mouse, a model of type 2 diabetes.

Results: Untreated uninephrectomized db/db mice developed progressive albuminuria and mesangial matrix expansion between wks 20 and 22, associated with significant segmental podocyte foot process effacement, reduction of renal nephrin, podocin and ZO-1 production and induction of renal desmin and B7-1 production. Treatment with PAI-1R at 0.5mg/Kg BW, ip, twice daily from wk 20 to 22 repeatedly prevented the increased albuminuria and even lowed the levels of urinary albumin excretion than before treatment by 31.4% ($p<0.05$). PAI-R also repeatedly reduced glomerular matrix accumulation, FN and Col production by 36%, 62% and 65%, respective ($p<0.05$) without affecting body weight or blood glucose levels. Of note, the dramatic changes in podocyte morphology and podocyte markers seen in diabetes were all significantly ameliorated by PAI-1R administration. In vitro, recombinant PAI-1 at the concentration of 150 nM down-regulated nephrin and ZO-1 by more than 50% but increased desmin and B7-1 mRNA expression and protein production by podocytes by more than 2-fold compared with the normal cells ($p<0.05$), similar to the effects of TGFß1 at concentration of 2ng/ml.

Conclusions: These observations provide the evidence that PAI-1, similar to TGFß, may signal directly to regulate podocyte disorder particularly in diabetes, which is characterized by increased plasma and tissue PAI-1. Reducing the increased PAI-1 activity by PAI-1R in vivo may, in fact, restore podocyte injury; therefore PAI-1R may provide additional therapeutic effect in slowing progression of diabetic nephropathy through maintaining podocyte integrity.

Funding: NIDDK Support

TH-PO496

Fusion of Bone Marrow-Derived Cells with Podocytes Increases in Diabetic Nephropathy Tomohisa Yamashita,[1] Mineko Fujimiya,[2] Masayuki Koyama,[1] Yusuke Okazaki,[1] Marenao Tanaka,[1] Masato Furuhashi,[1] Hideaki Yoshida,[1] Tetsuji Miura.[1] *[1]2nd Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan; [2]2nd Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan.*

Background: We previously reported that diabetes induces appearance of proinsulin-producing bone marrow-derived cells (BMDCs) in various organs and their fusion with somatic cells including renal tubular epithelial cells, leading to diabetic nephropathy (FASBJ. 26, 1559-1568; 2012). However, it remains unclear whether BMDCs fuse with glomerular cells such as podocytes and have any adverse effects on kidney function in diabetes. We examined this issue by use of a mouse model of diabetes.

Methods: We performed bone marrow transplantation (BMT) from male green fluorescent protein (GFP) transgenic mice to female C57BL/6J mice before induced diabetes by streptozotocin. Non-diabetic controls received sham treatment (saline injection). Four months after streptozotocin or sham treatment, kidneys were removed, and fixed by Zamboni's fixation. Sections were immunoflurostained for analysis by confocal laser scanning microscopy (Nicon A1) and NIS element AR 4.0.

Results: First, in slides without immunostaining, we confirmed presence of GFP positive cells in the glomeruli. In the glomeruli of the diabetic mice with BMT, GFP positive area and the number of GFP positive cells were increased compared with those in non-diabetic mice (5.2% vs 2.5%, $p<0.01$ and 4.4 vs 9.1 cells /glomerulus, $p<0.01$). Three dimensional analysis showed that BMDCs were larger in diabetic mice than in controls and fused with the other cells. Detailed spectrum analysis indicated that approximately 10% of BMDCs contained triple spectrums of GFP, nephrin and desmin in diabetes.

Conclusions: Diabetes induces fusion between BMDCs and podocytes. Fusion with BMDCs might be responsible for podocytes injury in diabetic nephropathy.

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Podocyte Deletion of LKB1 Results in Increased Severity of Diabetic Nephropathy in Mice Kimberly J. Reidy,[1] Zhongfang Du,[1] Katalin Susztak.[2] *[1]Pediatrics/ Nephrology, Albert Einstein College of Medicine, Bronx, NY; [2]Medicine/ Nephrology, University of Pennsylvania, Philadelphia, PA.*

Background: LKB1 (also known as Par4 or stk11) is a serine-threonine kinase expressed in the developing kidney and in podocytes. LKB1 is a key regulator of cell polarity and metabolism. Recently LKB1 has been shown to play a role in regulation of AMPK kinase and cellular metabolism, suggesting it may play a role in cellular response to the diabetic milieu. In vitro studes showed decreased activation of LKB1 in podocytes exposed to high glucose. As LKB1 is a critical integrator of polarity and metabolism hypothesize that LKB1 contributes to maintenance of podocyte structure, and may be protective in the setting of metabolic stress.

Methods: Conditional deletion of LKB1 in podocytes was achieved by generating NPHS2-Cre:LKB1flox/flox mice. Diabetes was induced by intraperitoneal streptozotocin (STZ) injection (50mg/kg) for 5 days in five week old male mice. Controls were non-diabetic (vehicle injected) NPHS2-Cre:LKB1flox/flox and LKB1flox/flox male mice as well as diabetic (STZ injected) LKB1flox/flox littermates. Severity of diabetic nephropathy was assessed by urine albumin:creatinine ratios, and histologic examination of 14-20 week old male using light and electron microscopy.

Results: Nondiabetic mice with podocyte deletion of LKB1 were indistinguishable from controls on light microscopy, and appear to have normal polarity, with majority of foot process intact and normal distribution of Pard3 expression. Control diabetic mice developed only mild mesangial expansion and mild albuminuria, diabetic mice with podocyte deletion of LKB1 developed nephrotic range albuminuria, and Kimmelstein-Wilson like glomerular lesions.

Conclusions: Podocyte LKB1 deletion results in severe diabetic nephropathy, suggesting that podocyte LKB1 contributes to maintenance of the glomerular filtration barrier in the diabetic mileiu.

Funding: NIDDK Support

TH-PO498

Ezrin Is Downregulated in Glomeruli of Streptozotocin-Induced Diabetic Rats and Regulates Glucose Transport in Cultured Podocytes Anita A. Wasik,[1] Susanna Koskelainen,[1] Mervi E. Hyvonen,[1] Luca Musante,[1] Eero Lehtonen,[1] Kerttu Koskenniemi,[2] Csaba Imre Szalay,[3] Pekka Varmanen,[4] Tuula A. Nyman,[5] Peter Hamar,[3] Harry B. Holthofer,[1] Sanna H. Lehtonen.[1] *[1]Haartman Institute, University of Helsinki, Finland; [2]Department of Veterinary Biosciences, University of Helsinki, Finland; [3]Semmelweis University, Budapest, Hungary; [4]Department of Food and Environmental Sciences, University of Helsinki, Finland; [5]Institute of Biotechnology, University of Helsinki, Finland.*

Background: Diabetic nephropathy is a major cause of end-stage renal disease but the pathophysiological mechanisms associated with its development are poorly characterized.

Methods: We performed quantitative proteomic profiling of glomeruli isolated from streptozotocin-treated diabetic or control rats by fluorescence-based two-dimensional difference gel electrophoresis followed by mass spectrometry.

Results: We identified 29 differentially expressed proteins in glomeruli four weeks after induction of diabetes. Among the proteins that were altered significantly, ezrin was found to be downregulated by 50% in the streptozotocin group compared with controls. The cytoskeletal protein ezrin functions as a cross-linker between the actin cytoskeleton and the plasma membrane and participates in insulin granule trafficking and docking to the plasma membrane in the pancreatic beta cells. Immunofluorescence and quantitative Western blot analyses confirmed downregulation of ezrin in glomeruli of streptozotocin-treated rats. We found that ezrin is expressed in cultured mouse podocytes and that its localization does not depend on an intact actin cytoskeleton. However, knockdown of ezrin led to loss of actin stress fibers. We also found that ezrin forms a complex with VAMP2, the regulator of vesicle trafficking on GLUT4 storage vesicles, and that depletion of ezrin by siRNA affects the insulin stimulated glucose uptake activity of podocytes. Furthermore, ezrin was activated via phosphorylation in a glucose- and insulin-dependent manner in podocytes.

Conclusions: Our findings suggest that ezrin may play an important role in the regulation of glucose transport in podocytes.

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Podocyte *VEGF-A*: Gain-of-Function Induces Nodular Glomeruloesclerosis in eNOS Knockout Mice Delma Veron, Pardeep Kumar Aggarwal, Heino Velazquez, Gilbert W. Moeckel, Michael Kashgarian, Alda Tufro. *Yale University, New Haven, CT.*

Background: Increased vascular endothelial growth factor-a (VEGF-A) and decreased nitric oxide (NO) availability play a pathogenic role in the development of diabetic nodular glomerulosclerosis in mice. The objective of this study was to define the role of local VEGF-A and NO availability in the development of glomerulopathy in the absence of diabetic milieu.

Methods: We examined the effects of increased podocyte VEGF-A in glomeruli from endothelial nitric oxide synthase (*eNOS*) knockout mice. *eNOS* knockout mice (*C57BL/6j-Nos3tm1Unc*) were crossbred with *podocin-rtTA:tet-O-VEGF*$_{164}$ mice that overexpress VEGF$_{164}$ in podocytes upon doxycycline exposure. *Podocin-rtTA:tet-O-VEGF*$_{164}$*: eNOS*$^{-/-}$ (PTE)mice were fed standard diet (control=PTE) or doxycycline containing diet for 1-3 months (VEGF$_{164}$ overexpressing mice=PTE+dox).

Results: PTE mice were viable, fertile and had normal litter size. Podocyte $VEGF_{164}$ overexpression for 1 month resulted in advanced glomerulosclerosis, increased systolic blood pressure and decreased glomerular filtration rate compared to control mice. The glomerular lesions in PTE mice progressed to nodular glomerulosclerosis after 3 months of podocyte $VEGF_{164}$ overexpression, associated to massive proteinuria and renal failure, in the context of normal blood glucose. Microaneurisms, mesangiolysis, glomerular basement membrane thickening and foot process effacement were also observed in PTE + dox mice by light microscopy and TEM. Immunoreactive collagen IV and laminin increased significantly in the nodules; nephrin expression decreased, whereas podocin and WT1 were not altered in PTE + dox mice.

Conclusions: The present findings suggest that simultaneous upregulation of podocyte VEGF-A signaling and decreased NO bioavailability is sufficient to induce the development of nodular glomerulosclerosis, massive proteinuria and renal failure, i.e. advanced diabetic nephropathy-like phenotype, in the absence of diabetic milieu.

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TH-PO500

Simultaneous or Separate Inhibition of Vascular Endothelial Growth Factor Receptors 1 and 2 Aggravates Diabetic Nephropathy in *db/db* Mice

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Background: It is a still controversy over manipulating vascular endothelial growth factor (VEGF) receptors (VEGFRs) may be promising therapeutic tools in diabetic nephropathy. Therefore, we examined the renal effects of anti-flt 1 hexamer (VEGFR1 inhibitor) or anti-flk 1 heptamer (VEGFR2 inhibitor) or both of them in *db/db* mice treating for 12 wks in male *db/db* mice.

Methods: Male db/db amd db/m mice were treated with VEGFR1 inhibitor and VEGFR2 inhibitor simultaneously or separately for 12 weeks from 8 weeks of age.

Results: Induction of diabetes suppressed the VEGFR1 and increased VEGFR2 expressions in kidneys. In the >*db/db* mice with VEGFR1 or VEGFR2 inhibition, albuminuria, glomerular mesangial matrix expansion, and inflammatory cell infiltration were more prominent than those of control *db/db* mice although there were no differences in blood glucose levels in all *db/db* groups. They exhibited an increase in the number of apoptotic glomerular cells without cell proliferation and increased 24-h urinary 8-OH-deoxyguanosine concentrations. Interestingly, more severe albuminuria and renal lesions were noted in the *db/db* mice with both VEGFR1 and VEGFR2 inhibition compared with either VEGFR1 or VEGFR2 inhibition. All of these changes were associated with the inactivation of renal PI3K-Akt-eNOS-NO pathway. In contrast, VEGFR1 or VEGFR2 blockade-induced renal phenotypes were not observed in any *db/m* groups. In glomerular endothelial cells, high-glucose media containing VEGFRs inhibitors, especially media with both VEGFR1 and VEGFR2 inhibition, induced more apoptotic cell death and oxidative stress than did high-glucose media associated with an inactivation of both the PI3K-Akt-eNOS pathway and SOD1 and SOD2.

Conclusions: Our results reveal that the blockade of VEGFR1 or VEGFR2 or both VEGFR1 and VEGFR2 caused severe renal injury related to the inactivation of the PI3K-Akt-eNOS pathway resulting in the oxidative stress-induced apoptosis in type 2 diabetic nephropathy.

TH-PO501

A Small Bioactive Peptide of Pigment Epithelium-Derived Factor Reduces Diabetic Renal Injury

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Background: Pigment epithelium derived factor (PEDF) is a multifunctional, pleiotropic protein with antiangiogenic, anti-oxidative and anti-inflammatory properties. However, the direct role of PEDF in the kidney remains unclear. We hypothesize that a minimal fragment of PEDF (P78-PEDF) confers kidney protection in diabetic nephropathy (DN).

Methods: The expression, localization, and effects of P78-PEDF were determined in DBA mice following multiple low doses of streptozotocin (STZ; 50 mg/kg ip for 5 days; n=5 each group) treated with continuous subcutaneous infusion of vehicle or P78-PEDF for 6 weeks.

Results: Using immunohistochemistry, we confirmed the localization of PEDF in the kidney; mainly in the vasculature, interstitial space, tubules, glomeruli, and in the macula densa under normal condition. Kidney PEDF protein and mRNA expression were reduced significantly after 15 wks in a vehicle-treated STZ-induced mouse model. Continuous infusion with P78-PEDF for 6 wks resulted in kidney protection as evident by reduced albuminuria ($p<0.05$), reduced blood urea nitrogen ($p<0.0001$), decreased kidney macrophage recruitment ($p<0.05$), and reduced kidney inflammatory cytokines (TNF-α: $p<0.05$, IFN-γ: $p<0.05$) and urinary TNF-α ($p<0.05$) compared with vehicle-treated diabetic mice. The increase in albuminuria in the vehicle-treated diabetes group was associated with a reduction in nephrin expression, which was reversed by P78-PEDF treatment. *In vitro*, P78-PEDF blocked the increase in podocyte permeability to albumin and disruption of the actin cytoskeleton, induced by puromycin aminonucleoside (PAN)-induced injury.

Conclusions: These findings highlight the importance of P78-PEDF as a potential therapeutic modality in diabetic renal injury, at least in part, by reducing macrophage recruitment and levels of inflammatory cytokines. In addition, P78-PEDF has a direct effect on preserving the normal structure of podocyte foot processes, slit diaphragms, and actin cytoskeleton.

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TH-PO502

Autophagy in Diabetic Nephropathy and Non-Diabetic CKD In Vitro and In Vivo

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Background: Macroautophagy(Atg), a process activated by cell stress, culminates in the degradation of cytoplasmic constituents by lysosomes. Atg is regulated by major multifunctional signaling pathway nodes at mammalian target of mTORC1, AMP-kinase, PI3-kinase, and IP3. Atg fosters cell survival, but in insurmountable injury, induces cell death. In diabetes and DN, insufficient Atg may contribute to ineffective tissue repair and organ failure. Hematopoietic growth factor inducible neurokinin-1(HGFIN) acts downstream of the nodal signaling pathways to enhance Atg. We showed that HGFIN is a tissue and urine biomarker of CKD (KI 79:1138-48, 2011). Chloroquine (Cq) inhibits Atg.

Methods: 1. We present a case of chronic medicinal Cq intoxication leading to CKD. **2.** We stained human control and DN tissue to evaluate the expression of HGFIN. **3.** We cultured WT and HGFIN-overexpressing mouse distal convoluted tubule (MDCT) cells in 5.5 mM (NG) vs 10-25 mM (HG) to measure the relative capacity of HG and HGFIN overexpression to induce Atg, and used Cq to assess Atg flux by measuring the ratio of the Atg proteins LC3BII/BI over time.

Results: 1. We reported proteinuria and CKD due to Cq toxicity in the treatment of rheumatoid arthritis (Mod Pathol.18:733-8, 2005). Renal biopsy showed podocyte inclusions and dense mesangial granules, both in "lysosomes." New ultrastructural analysis (x320,000) shows the material as lamellae, sometimes with a trilamellar appearance, in autophagosomes defined by double-membrane structures. These data are consistent with the development of CKD from Cq-induced Atg inhibition. 2. In DN, HGFIN was increased in all renal cortical epithelia, including podocytes, parietal epithelial cells. 3. HGFIN overexpression was substantially more effective in increasing Atg and Atg flux than HG.

Conclusions: These data demonstrate that in vivo, inhibition of Atg may induce CKD. In vitro, the data show that in mDCT cells, HG and HGFIN overexpression both activate Atg. Methods to increase Atg may enhance renal repair in DN.

Funding: Private Foundation Support

TH-PO503

Autophagy-Deficiency in Obese Mice Exacerbates Proteinuria-Induced Tubulointerstitial Lesions

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Background: Autophagy is an intracellular catabolic process to maintain intracellular homeostasis. It had been proposed that autophagy-deficiency is involved in the pathogenesis of obesity-related diseases. However, relationship between autophagy and obese-mediated renal damage has remained unclear. Thus, we examined the role of autophagy on the obesity-mediated exacerbation in a protein-overload mouse nephropathy model.

Methods: Recent studies have shown that excess free fatty acid (FFA) bound to albumin are filtered through glomeruli and reabsorbed into the renal proximal tubules, which causes tubulointerstitial inflammation in the kidney.

Intraperitoneal injection of FFA-bound albumin (0.3g/kg•BW) was also used to induce proteinuria-mediated tubular damage in this study. Autophagy was analyzed by measuring LC3II formation by immunoblotting and LC3-dot formation in GFP-LC3 transgenic mouse.

Results: We found that protein-overload-induced proximal tubular cell damages, (determined by histological analysis, cleavage of caspase 3 (an apoptosis marker), and Ngal expression), were significantly worse in obese mice (HFD: 60% of fat for 4 weeks) compared to lean mice. In addition, protein-overload induced autophagy in the proximal tubules of lean mice, whereas it was suppressed in obese mice. To clarify the pathological significance of autophagy-deficiency on proteinuria-induced tubular damage in obese mice, we generated proximal tubule-specific Atg5-deficient mice. Atg5-deficient mice developed exacerbated protein-overload-induced histological damage, proximal tubular cell apoptosis and Ngal expression, similar to obese mice.

Conclusions: Collectively, these results showed that a protective action of autophagy against proteinuria in proximal tubular cells was significantly suppressed in diet-induced obese mice. Restoration of autophagy activity may be a new therapeutic option to improve renal outcome in obese patients with proteinuric kidney disease.

TH-PO504

High Protein Diets Alter Renal Cortical Autophagy and Worsen Protein Oxidation in Early Streptozotocin-Induced Diabetes Harold A. Franch,[1,2] Sara Zoromsky,[1,2] Changlin Ding.[1,2] *[1]Renal Division, Atlanta VAMC; [2]Department of Medicine, Emory University.*

Background: Inhibiting autophagy leads to tissue damage from decreased destruction of protein oxidation by chaperone-mediated autophagy (CMA) and accumulation of protein aggregates by reducing macroautophagy (MA). In the renal cortex, diabetes increases MA, but reduces CMA. High protein diets increase diabetic kidney damage but their effect on renal cortical autophagy and oxidized proteins (OX) has not been examined.

Methods: Sprague-Dawley rats (200 g) were made diabetic with 60 mg/kg streptozotocin (D) or injected with citrate buffer (C) and fed isocaloric 12% (L) or 40% (H) protein diets for 21 days (n= 7-11/group). L diets had carbohydrates replacing protein. MA, CMA, were estimated by western blotting for light chain 3b II/I ratio, lysosome associated membrane protein 2a, p62/sequestisome 1 labeled aggregates (p62), targets for macroautophagy, were measured by western blotting, while OX was measured by Oxyblot.

Results: Blood glucose was lower in DH vs.DL (408±25 vs. 482±23 mg/dl, $p<0.05$), but kidney wt/body wt was greater in DH than DL (104±13 vs. 66±11% of CL, $p<0.02$). Compared to CL, DL reduced renal cortical CMA by 66±9%% ($p<0.05$), CH by 34±13%% (NS) and CD by 84±6% ($p<0.05$, but NS with DH). Compared to CL, DL increased renal cortical OX by 35±24% (NS), CH by 5±25% (NS) and DH by 95±31% ($p<0.05$, vs CL & DL). Compared to CL, MA increased ~ 3 fold with in CH and DL (both NS), but was ~30 fold higher in DH ($p<0.01$ vs CL, $p<0.02$ vs CH and DL). p62 did not change significantly with diet or diabetes.

Conclusions: Despite lower glucose in early diabetes, high protein diets diets worsen diabetic renal hypertrophy and cortical protein oxidation, while reducing CMA and accelerating MA. One mechanism of protein oxidation could occur via reduced destruction by suppressing CMA, while increases in MA serve to prevent p62 protein aggregates from accumulating. Reduced CMA could provide a mechanism for diabetic tissue damage from high protein diets.

Funding: NIDDK Support, Veterans Administration Support

TH-PO505

Selective Insulin Receptor Substrate 1 and Its Functions in Glomeruli of Diabetic and Insulin Resistant Rats Akira Mima,[1,2] George L. King,[2] Toshio Doi.[1] *[1]Nephrology, Tokushima University, Tokushima, Japan; [2]Vascular Cell Biology, Joslin Diabetes Center, Boston, MA.*

Background: Insulin resistance has been associated with the progression of chronic kidney disease in diabetes and obesity. However, detailed characterization of insulin signaling of the renal tissue has not been reported in these pathophysiological states.

Methods: Diabetes of eight weeks duration was induced by streptozotocin (STZ) in six weeks old Sprague-Dawley (SD) rats. Zucker lean (ZL) and fatty (ZF) rats of 14 weeks of age were used as models of obesity. Insulin (10mU/g) was injected via inferior vena cava and insulin signaling in the renal glomeruli and tubules were analyzed. Insulin signaling and actions on endothelial nitric oxide synthase (eNOS) in glomerular endothelial cells isolated from SD rats were studied on their responses to elevated levels of glucose.

Results: Insulin-induced phosphorylation of insulin receptor substrate-1 (IRS1), Akt, and eNOS were selectively inhibited in the glomeruli but not in the renal tubules of both STZ-diabetic and ZF rats compared to non-diabetic and ZL rats. Protein levels, but not the mRNA expression, of IRS1 were decreased only in the glomeruli of STZ-diabetic rats and increased its association with ubiquitination. RBX treatment enhanced insulin actions and elevated IRS1 expression. In rat glomerular endthelial cells (RGECs), high glucose levels (25mM) inhibited insulin-induced phosphorylation of Akt and eNOS, decreased IRS1 protein expression and increased association with ubiquitination. Also, in podocytes, insulin signaling was inhibited in high glucose condition.

Conclusions: There is selective inhibition of insulin signaling via the IRS1/PI3K/Akt pathway and loss of eNOS activation only in the glomeruli in diabetes and insulin resistance, partly due to increased IRS1 degradation and protein kinase C (PKC)β activation. The selective loss of insulin's effect on eNOS activation may contribute to the glomerular pathologies observed in diabetes and obesity.

Funding: NIDDK Support

TH-PO506

Effects of Lipid-Lowering Treatment on Inflammatory Markers in Diabetic Patients with Chronic Kidney Disease Tora Almquist,[1,2] Stefan H. Jacobson,[2] Per-Eric Lins,[3] Paul Hjemdahl.[1] *[1]Karolinska Institutet, Dept. Medicine Solna, Clinical Pharmacology Unit, Karolinska University Hospital, Stockholm, Sweden; [2]Dept. Clinical Sciences, Div. Nephrology, Danderyd Hospital; [3]Dept. Clinical Sciences, Div. Diabetology, Danderyd Hospital, Stockholm, Sweden.*

Background: Diabetes mellitus (DM) is the most common cause of chronic kidney disease (CKD). Inflammation plays an important role in atherosclerosis and may contribute to the high incidence of cardiovascular disease in DM and CKD. Monocyte chemoattractant protein-1 (MCP-1) plays a central role in recruiting monocytes into early atherosclerotic lesions and is involved in the pathogenesis of diabetic nephropathy. Interferon gamma (IFNγ) is considered to be a key player in the progression of atherosclerosis and increases synthesis of endothelial cell chemokines including MCP-1. Lipid-lowering treatment (LLT) with statins may have favorable effects on inflammation, and ezetimibe co-treatment provides additional cholesterol-lowering.

Methods: After a placebo run-in period, the effects of simvastatin alone (S) or simvastatin+ezetimibe (S+E) were compared in a randomized, double-blind, cross-over study on inflammatory markers including proinflammatory cytokines, chemokines and adhesion molecules.18 DM patients with estimated glomerular filtration rate (eGFR) 15-59 ml/min x 1.73 m^2 (CKD stages 3-4) (DM-CKD) and 21 DM patients with eGFR >75 ml/min (DM only) were included.

Results: At baseline, levels of MCP-1 ($p=0.03$), IFNγ ($p<0.01$), tumor necrosis factor-α (TNF-α) ($p=0.001$), and soluble vascular adhesion molecule (sVCAM) ($p=0.001$) were elevated in DM-CKD compared to DM only patients. LLT with S and the combination of S+E reduced MCP-1 levels ($p<0.01$ by ANOVA; post hoc test $p=0.04$ and $p<0.001$ resp.) and IFNγ levels ($p<0.01$ by ANOVA; post hoc test $p=0.02$ and $p=0.001$ resp.) in DM-CKD patients but not in DM only patients. The reduction was most prominent with the combination treatment.

Conclusions: DM patients with CKD stages 3-4 had increased inflammatory activity compared to DM patients with normal GFR. Lipid-lowering treatment with simvastatin alone and in combination with ezetimbe decreased levels of MCP-1 and IFNγ in DM patients with concomitant CKD.

Funding: Pharmaceutical Company Support - MSD Medical School Grant

TH-PO507

Urinary Proteomics Predict Onset of Microalbminuria in Normoalbuminuric Type 2 Diabetic Patients in the DIRECT 2 Study Morten Lindhardt,[1] Frederik I. Persson,[1] Petra Zürbig,[4] Angelique Stalmach,[3] Harald Mischak,[3,4] Dick de Zeeuw,[5] Hiddo Jan Lambers Heerspink,[5] Anne Katrin Sjoelie,[6] Ron Klein,[6] Trevor Orchard,[6] Massimo Porta,[6] John Fuller,[6] Rudolf W. Bilous,[6] Nish Charturvedi,[6] Hans-Henrik Parving,[6] Peter Rossing.[1,2] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]University of Aahus, Denmark; [3]University of Glasgow, United Kingdom; [4]Mosaiques Diagnostics GmbH, Hannover, Germany; [5]University Medical Centre, Groningen, Netherlands; [6]The DIRECT Steering Group.*

Background: Early prevention of diabetic nephropathy is not successful as early interventions have shown diverging results. Urinary proteomics has shown promise as an early indicator of future development of diabetic nephropathy and could guide need for treatment.

Methods: In a post-hoc study of the DIRECT-Protect 2 study, a randomized, controlled clinical trial of candesartan for slowing the progression of retinopathy, we studied patients with type 2 diabetes and normoalbuminuria (n=792), followed for a mean of 4.7 years. We determined a previously defined CKD risk score based on proteomic measurement of 273 urinary peptides (CE-MS), the risk-score was selected from previous cross sectional case-control studies. A Cox regression model for progression of albuminuria was built. The primary endpoint was development of persistent microalbuminuria (MA) (3 out of 4 samples).

Results: Persistent MA developed in 92 patients (11,6%). At baseline the CKD risk score was able to predict development of MA during follow-up, independent of treatment (candesartan/placebo), age, gender, baseline systolic BP, baseline UAER, baseline eGFR, baseline HbA_{1c} and diabetes duration (HR 2.0 (95% CI 1.17-3.45), p=0.012). In the placebo treated group the HR was 2.1 (1.1 to 4.2) compared to 1.6 (0.8 to 3.1) in the candesartan group.

Conclusions: In this cohort of patients with type 2 diabetes and normoalbuminuria from a large intervention study, the CKD classifier was an independent predictor of MA. This may provide a better opportunity to select normoalbuminuric patients for early prevention of diabetic nephropathy as treatment with candesartan seems to mitigate this risk.

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TH-PO508

Urine Haptoglobin Predicts Development of Diabetic Nephropathy in Patients with Type 2 Diabetes Nishant M. Bhensdadia,[1] Kelly J. Hunt,[1] Maria Lopes-virella,[1,2] Michael Tucker,[1] Benjamin Neely,[1] Michael G. Janech,[1,2] John M. Arthur.[1,2] *[1]Medical University of SC; [2]Ralph H Johnson VA Medical Center.*

Background: Diabetic nephropathy(DN) is the leading cause of End Stage Renal Disease. Urine albumin to creatinine ratio(ACR) is commonly used as a predictor for the development of nephropathy but it is neither sensitive nor specific.

Methods: Liquid chromatography/mass spectrometry was performed on urine of 8 normoalbuminuric patients with type 2 diabetes(T2DM) from the VA Diabetes Trial(VADT) to identify candidate markers for loss of renal function. Verification of the ability to predict the future development of DN was done for 7 candidate markers by selective reaction monitoring(SRM) in urine from 30 patients. Finally we measured the concentration of the leading candidate, haptoglobin, by ELISA in 204 patients to determine the ability of haptoglobin to creatinine ratio(HCR) to predict early renal functional decline(ERFD) (≥3.3% eGFR decline per year).

Results: Proteomic analysis identified 24 candidate biomarkers to predict loss of renal function. Verification of 7 candidates (haptoglobin, angiotensinogen, agrin, mannan-binding lectin serine protease 2, LAMP-2, NGAL and uromodulin) by SRM in 30 patient's urine showed haptoglobin, angiotensinogen and NGAL predict ERFD. HCR was predictive of ERFD in 204 patients with eGFR≥60 ml/min and ACR<300 mg/g. In separate models comparing the highest to lowest tertile, the odds ratio for having ERFD was 2.70 (95% CI: 1.15, 6.32) for HCR and 2.50 (1.14, 5.48) for ACR after adjusting for treatment group and

use of ACE inhibitors. Although both HCR and ACR predicted the outcome, the Spearman rank correlation between them was only 0.25 suggesting that the two biomarkers reflect different pathophysiologic processes.

Conclusions: We identified urine HCR as a new biomarker which can predict ERFD in T2DM prior to the development of macroalbuminuria or reduced GFR. HCR appears equivalent to, but independent of ACR as a predictor; therefore, the discriminatory power of models that combine the two biomarkers may be superior to that of models using one biomarker. The clinical use of HCR may enable better prediction of patients at risk for adverse renal outcomes.

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TH-PO509

Copeptin as Biomarker for Cardiovascular and All-Cause Mortality in Type 2 Diabetes (ZODIAC-31) Ineke J. Riphagen,[1] Wendy E. Boertien,[1] Alaa Alkhalaf,[2] Nanne Kleefstra,[1,2] Ron T. Gansevoort,[1] Klaas Groenier,[2,3] Kornelis J. Van Hateren,[2] Joachim Struck,[4] Gerjan Navis,[1] Henk Bilo,[1,2] Stephan J.L. Bakker.[1] *[1]Internal Medicine, UMC, Groningen, Netherlands; [2]Diabetes Centre, Isala Clinics, Zwolle, Netherlands; [3]Gerenal Practice, UMC, Groningen, Netherlands; [4]BRAHMS GmbH, Thermo Fischer Scientific, Hennigsdorf, Germany.*

Background: Copeptin, a surrogate marker for vasopressin, is associated with renal function and albuminuria and has been reported to be of prognostic value in type 2 diabetes (DM2) patients with acute cardiovascular (CV) events. It is not known whether copeptin is associated with outcome in stable DM2 patients. We investigated this issue in a cohort of community dwelling out-patients.

Methods: Patients with DM2 participating in the observational Zwolle Outpatient Project Integrating Available Care (ZODIAC) study were included. Cox regression analyses were used to assess the relation of baseline copeptin levels with CV and all-cause mortality.

Results: We included 1,195 patients (age 67±12 yrs,44% male). Baseline copeptin levels (median [IQR]; 5.4 [3.1-9.6] pmol/L) were associated with eGFR (r =-0.14, p<0.01) and albumin-to-creatinine ratio (ACR) (r=0.13, p<0.01). After median follow-up for 5.9 [IQR 3.2-10.1] yrs, 345 patients died (29%), with 148 CV deaths (12%). In univariate cox regression analyses, log(2) copeptin was significantly associated with CV (HR(95%CI)=1.74(1.42-1.74), p<0.001) and all-cause mortality (1.57(1.42-1.74), p<0.001). These associations remained significant after adjustment for age, gender, BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, ACE inhibitors/ARB, history of CV diseases, eGFR, and log ACR both for CV (HR=1.24(1.05-1.46), p=0.01) and all-cause mortality (1.25(1.11-1.39), p<0.001).

Conclusions: In stable DM2 patients, we found copeptin to be associated with CV and all-cause mortality independent of CV risk factors, eGFR, and ACR. Intervention studies should show whether the high CV risk in patients with DM2 can be reduced by suppression of vasopressin, e.g. by increasing water intake.

Funding: Other NIH Support - CTMM (PREDICCt)

TH-PO510

Bilirubin and Protection against Progression of Diabetic Nephropathy Ineke J. Riphagen,[1] Petronella E. Deetman,[1] Stephan J.L. Bakker,[1] Gerjan Navis,[1] Mark E. Cooper,[2] Julia Lewis,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Clinical Pharmacology and Internal Medicine, UMC, Groningen, Netherlands; [2]Baker Heart Institute, Melbourne, Victoria, Australia; [3]Nephrology, Vanderbilt University, Nashville, TN.*

Background: Serum bilirubin is a potent endogenous antioxidant found to protect against progression of diabetic nephropathy (DN) in rodents. In humans, cross-sectional studies show an inverse relation between bilirubin and DN. We aimed to prospectively investigate this relation in a post-hoc analysis of the Reduction of Endpoints in Non insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials.

Methods: We included patients participating in RENAAL and IDNT trials (evaluating the effect of losartan and irbesartan vs placebo, respectively, on renal outcome in DN). Patients with liver enzymes (ALT, AST, bilirubin) 1.5 times above upper limit of normal were excluded. Cox regression analyses were used to assess the association of baseline serum bilirubin with the combined renal endpoint consisting of doubling of serum creatinine (DSCR) or end-stage renal disease (ESRD) during a median follow-up of ~3 years.

Results: Serum bilirubin was available for 1,498 patients in RENAAL and 1,707 in IDNT. Mean (SD) baseline bilirubin levels were 0.57 (0.19) and 0.54 (0.21) mg/dL. Age, gender, smoking, total cholesterol, and log albumin-to-creatinine ratio (ACR) were significantly associated with bilirubin levels in both trials. Log(2) transformed bilirubin was significantly inversely associated with the renal endpoint after adjustment for age and gender, with hazard ratios of 0.44 (95% CI 0.35-0.55; p<0.001) in RENAAL and 0.53 (0.45-0.64; p<0.001) in IDNT. The association persisted after further adjustment for race, BMI, smoking, total cholesterol, systolic blood pressure, HbA_{1c}, treatment, eGFR, and log ACR with HR of 0.68 (0.54-0.86; p=0.001) in RENAAL and 0.79 (0.65-0.96; p=0.02) in IDNT.

Conclusions: We found an independent inverse association of serum bilirubin with DSCR/ESRD in both the RENAAL and IDNT trials. These data suggest treatments raising serum bilirubin may confer renoprotective effects.

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TH-PO511

Relationship between Plasma Copeptin and Degree of Renal Function Impairment in Patients with Type 2 Diabetes María de la Luz Villela,[1] Anel Gomez Garcia,[2] Bengt Lindholm,[4] Elvia Garcia-lopez,[4] Cleto Alvarez Aguilar.[3] *[1]División de Posgrado, Ciencias Médicas y Biológicas, Universidad Michoacana, Morelia, Mexico; [2]Biomedical Research Center of Michoacan, Morelia, Mexico; [3]HGR No. 1, Instituto Mexicano del Seguro Social, Morelia, Mexico; [4]Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: Copeptin, the carboxy-terminal fragment of the vasopressin precursor (pre-pro-vasopressin) is a surrogate marker of plasma levels of vasopressin. Copeptin is a prognostic marker of mortality in chronic heart failure and has been associated with microalbuminuria. Here we investigated the association between plasma copeptin and degree of renal function impairment in patients with type 2 diabetes (T2-DM).

Methods: Plasma copeptin and microalbuminuria were determined in a cross-sectional study of 643 T2-DM patients (mean age 58 ± 10 years) who were classified into six groups: Normal renal function (n=86), and chronic kidney disease (CKD) stages 1-5: CKD 1 (n=33), CKD 2 (n=246), CKD 3 (n=102), CKD 4 (n=34), and CKD 5 (n=142). Medical history, anthropometry, and blood pressure were recorded, and blood glucose, creatinine, lipids, and cystatin-C were determined. The estimated glomerular filtration rate (eGFR) was calculated by Cockroft-Gault equation.

Results: Copeptin correlated negatively with eGFR (r = - 0.445, p <0.0001) and positively with cystatin-C (r = 0.408, p = 0.0001). The highest concentrations of copeptin were found in CKD stage 5 (2.51 ± 1.33 ng / mL). Copeptin differed significantly between patients with normal renal function and CKD stage 1-3 versus CKD stage 5 (p<0.001). In a sub-group of 63 patients undergoing hemodialysis (HD), there was no difference between pre-and post-HD levels of copeptin.

Conclusions: Copeptin is associated with the degree of decline of renal function in T2-DM patients. Prospective studies are required to establish whether this marker vasopressin may be a predictor of progression rate of GFR and cardiovascular complications as well as mortality in T2-DM patients with CKD.

TH-PO512

Circulating Levels of FGF23 and TNFR1 and Their Effects on Progression to ESRD and Mortality Unrelated to ESRD in Type 2 Diabetes Jung Eun Lee, William Walker, Adam Smiles, Jan Skupien, Rita R. Holak, Kevin Patrick Mcdonnell, Jackson Jeong, Andrzej S. Krolewski, Monika A. Niewczas. *Research Division, Joslin Diabetes Center, Boston, MA.*

Background: We have recently reported that circulating Tumor Necrosis Factor Receptor 1 (TNFR1) is a very robust and independent predictor of progression to end-stage renal disease (ESRD) in a prospective study of subjects with type 2 diabetes (T2D) (JASN 2012). In the literature, circulating level of fibroblast growth factor-23 (FGF-23) has been reported to be associated with chronic diabetic complications and progression of chronic kidney disease to ESRD. We aimed to investigate the independent contributions of these two markers on nephropathy progression to ESRD and on mortality unrelated to ESRD in patients with T2D.

Methods: This study included 410 patients with T2D who were followed for 8-12 years and were used previously to examine the effect of TNFR1. Plasma FGF-23 levels detecting C-terminal form were measured at baseline with ELISA. The mean estimated glomerular filtration rate of the study group was 92 ± 31 ml/min per 1.73m^2. Eighty subjects (20%) had overt proteinuria at baseline. During follow-up, 59 patients (14%) developed ESRD and 84 patients (21%) died without ESRD.

Results: Baseline plasma concentrations of both markers, TNFR1 and FGF-23 were higher in subjects who developed ESRD in comparison with subjects that remained alive without ESRD. Cox proportional hazard model revealed that the strong predictive effect of TNFR1 on ESRD was not confounded by FGF-23 levels in the model adjusted by clinical covariates. Increased concentrations of FGF-23 were associated with increased risk of ESRD in the univariate analysis, however the effect of FGF-23 was no longer significant after controlling for TNFR1 (HR 1.25, p=0.15). FGF-23 remained an independent predictor of mortality unrelated to ESRD (HR 1.54, p<0.001).

Conclusions: In our study, TNFR1 was a superior predictor of progression to ESRD over FGF-23. However, circulating levels of FGF-23 had an independent effect on mortality unrelated to ESRD in subjects with T2D.

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TH-PO513

FGF-23 and Cardiovascular Outcomes in Type 2 Diabetic with Mild to Moderate Kidney Disease Ana Paula Silva, André Fragoso, Ana Pinho, Cláudia Silva, Nélio Santos, Fatima Rato, Nelson Almeida Tavares, Marilia Faisca, Pedro Neves. *Nefrologia, Hospital de Faro, Portugal.*

Background: Increased levels of fibroblast growth factor 23 (FGF-23) are associated with a greater risk of cardiovascular and renal events. The mechanisms that lie beneath these association are unknown. It is possible that FGF-23 has a direct and harmful action on the heart muscle and the vascular wall. The aim of our study was to evaluate the relationship of FGF-23 and cardiovascular mortality in type 2 diabetics with mild to moderate kidney disease.

Methods: In this prospective study, we included 92 type 2 diabetic patients (f=38 m=54), with a mean age of 62.4 years and a mean eTGF of 42.1 ml/min. At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed: insulin resistance (HOMA-IR), inflammation (interleukin 6), mineral metabolism (phosphorus (P), PTH, vitamin D), and oxidative stress (oxLDL), adiponectin and eTFG. We evaluated the mean arterial pressure (MAP) and also the pulse pressure (PP), and the left ventricular mass index (LVMI) was calculated using the Penn convention criteria. We divided the population in 3 groups, according to the FGF-23 tertiles. GI (n=29) FGF-23 <65.4rU/mL; GII (n= 32) - FGF-23 >65.5 and < 168.6 rU/mL; GIII (n=31) FGF-23 ≥ 168.7 rU/mL.

Results: We found that G=III patients were older (p=0.0001) and showed higher levels of PTH (p=0.0001), IL6 (p=0.0001), oxLDL (p=0.0001), HOMA-IR (p=0.0001), LVMI (p= 0.042), MAP (p= 0.042), PP (p=0.0001) and lower levels of 25 (OH)D3 (p= 0.0001), adiponectin (p=0.0001) and eTFG (p=0.0001) when compared with the other groups. Using the Kaplan Meier analysis we found that the survival of the GI, GII, and GIII at 54 months was respectively: 100%, 92.8 %, 14.5% (Log-rank = 22.3 p=0.0001).

Conclusions: In our study, elevated levels of FGF-23 are associated with cardiovascular mortality in type 2 diabetics with mild to moderate kidney disease. Further studies are needed to elucidate these potential mechanisms and if an early intervention regarding the FGF-23 will be associated with improved outcomes in our patients.

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TH-PO514

FGF-23 and Resistin: Are the New Predictors of Mortality Cardiovascular in Type 2 Diabetic? Ana Paula Silva, André Fragoso, Ana Pinho, Cláudia Silva, Nélio Santos, Nelson Almeida Tavares, Pedro Neves. *Nefrologia, Hospital de Faro, Portugal.*

Background: Cardiovascular disease is the primary cause of morbidity and premature mortality in chronic kidney disease. More recent data suggest that the mineral metabolism and adipokines, may play a role in the development of cardiovascular disease in chronic kidney disease The purpose of this study was to evaluate the role of new biomarkers on the cardiovascular mortality in type 2 diabetic with mild to moderate kidney disease.

Methods: In this cross-sectional study, we included 78 type 2 diabetic patients (f=30 m=48), mean age 61,9 years, mean eGFR 43,5ml/min. At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed: mineral metabolism (phosphorus (P), PTH, fibroblast growth factor 23 (FGF-23), vitamin D),oxidative stress (oxLDL), adipokines (visfatin, resistin). Our population was divided in two groups. G I (n=65) survivors and G II (n= 13)non survivors (CVD).

Results: We found that G=II II was older (73 *vs* 59.6 years p=0.0001) showed higher levels of P (5.2 *vs* 4.1 mg/dL p=0.0001), PTH (243.9 *vs* 114.7 mg/dL p=0.0001), FGF -23 (502.7 *vs* 118.4 Ru/mL p=0.0001), resistin (9.0 *vs* 5.3 pg/mL p=0.0001), visfatin (143.5 *vs* 43.8 pg/mL p=0.0001), LVMI (135.2 vs 97.8 g/m^2 p=0.0001), oxLDL (78.9 vs 38.3 U/L p=0.0001), and G II also showed lower levels of eGRF (23.6 vs 45.5 ml/min p=0.001), 25 (OH)D3 (9.3 vs 21.4 ng/mL p= 0.0001). In multivariate Cox proportional hazard stepwise LR to identify independent risk factors of cardiovascular mortality. FGF-23 and resistin were found to predict patient survival (HR= 0.2,95% CI, 0.4 to 0.6, p=0.015)and (HR= 40.7, 95% CI, 20.1 to 65, p=0.0001),respectively. ROC curve analysis showed that FGF-23 (AUC=0.962 p=0.001) and resistin (AUC=0.774 p=0.002) are predictors of mortality cardiovascular in type 2 diabetic with mild to moderate kidney disease.

Conclusions: In our study we found that FGF-23 and resistin are new biomarkers predictors of mortality cardiovascular in type 2 diabetic with mild to moderate kidney disease.

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TH-PO515

Lower Magnesium and FGF-23 Level: New Predictors of Pulse Pressure in Chronic Kidney Disease Diabetic Patients André Fragoso, Ana Pinho, Anabela Malho, Hercilia Lopes Quintas Martins, Cláudia Silva, Nélio Santos, Ana Paula Silva, Pedro Neves. *Nephrology Department, Hospital de Faro, Faro, Portugal.*

Background: It is well documented that large-artery stiffness is the main determinant of Pulse Pressure (PP) and several epidemiological studies have shown that PP is an independent risk factor for cardiovascular morbidity and mortality. Magnesium deficiency has been associated with increased risk for arterial stiffness. The purpose of this study was to evaluate the relationship between magnesium deficiency with increased arterial stiffness.

Methods: In a cross-sectional study, we included 80 type 2 diabetic patients (63.75% males) with CKD stages 3 and 4. The mean age was 65.7 years and the mean eGFR was 49.8ml/min. We analyzed mineral metabolism (iPTH, 25(OH)D3, FGF23, osteocalcin, phosphorus, calcium, magnesium), inflammation (interleukin6), oxidative stress (malonaldehyde), insulin resistance (HOMA-IR) and lipid profile. Our population was divided in two groups:G-1(PP≥50mmHg, n=34) and G-2 (PP<50mmHg, n=46).

Results: We found that G-1 patients showed lower calcium (9.12 vs 9.56mg/dl p=0.004), GFR (41.64 vs 55.81ml/min p=0.001), magnesium (1.79 vs 2.50mg/dl p=0.000), osteocalcin (4.45 vs 15.62ng/ml p=0.000), 25(OH)D3 (12.51 vs 24.73ng/ml p=0.001), and higher PTH (162.51 vs 102.76 µg/ml p=0.001), FGF23 (214.32 vs 80.25ng/ml p=0.0001), malonaldehyde (4.46 vs 3.30mg/dl p=0.0001), Interleukin6 (7.45 vs 5.29pg/ml p=0.001) and HOMA-IR (4.22 vs 3.01 p=0.033). No differences were found between the two groups regarding age, duration of disease, Hg, HgA1c, cholesterol and phosphorus. In a multivariate analysis, we found that FGF23 and magnesium independently influenced the PP (OR=3.5, 95% CI, 0.1 to 7, p=0.045) and (OR=- 99.1, 95% CI, -100 to – 39.7, p= 0.028), respectively.

Conclusions: In our diabetic population with early stages of CKD the FGF23 and lower magnesium levels were associated with higher PP levels, a known marker of cardiovascular mortality. Further studies are needed not only to better understand the relationship between FGF-23 levels and PP but also to ascertain if magnesium correction in CKD diabetic patients would reduce PP and consequently cardiovascular events.

TH-PO516

Nephronectin Is a Novel Protein Associated with Diabetic Nephropathy Shinya Nakatani,[1,2] Eiji Ishimura,[1] Akihiro Tsuda,[2] Yu Tateishi,[2] Shinya Fukumoto,[2] Masaaki Inaba.[2] *[1]Nephrology, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Metabolism, Endocrinology, and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan.*

Background: Comparative proteome analysis is a new technology which can be used to identify disease-specific proteins, and is considered to be a powerful diagnostic tool for the definition of the onset, progression, and prognosis of human diseases. In the present study, we performed proteome analysis of diabetic glomeruli in human autopsy cases to identify new proteins which are related to diabetic nephropathy.

Methods: We performed proteome analysis for lasermicrodissected glomeruli from paraffin-embedded tissues of patients with diabetic nephropathy (n=10) and those of non-diabetic patients (n=10). Immunohistochemistry of identified proteins of renal biopsy was performed in a total of 190 patients.

Results: There were 55 up-regulated and 45 down-regulated proteins that were differently expressed in glomeruli of diabetic patients, compared to those of non-diabetic patients. Nephronectin, which is an integrin α8β1 ligand and functions as assembly of extracellular matrix, was found to be up-regulated. Nephronectin was strongly expressed in the mesangial matrix expansion of diabetic nephropathy, but was not significantly expressed in glomeruli from other kidney diseases. The percentage of nephronectin-positive areas in the glomeruli from diabetic nephropathy (15±4.7%, n=18) was significantly higher than that for any other kidney diseases[IgA nephropathy; 4.8±2.9% (n=46), membranous nephropathy; 6.0±3.9% (n=30), minimal change nephrotic syndrome; 4.1±2.0% (n=28), membranoproliferative glomerulonephritis; 7.0±5.7% (n=14), lupus nephritis; 4.0±3.0% (n=11), crescentic glomerulonephritis; 7.6±3.4% (n=9), focal segmental glomerulosclerosis; 4.5±3.3% (n=9), hypertensive nephrosclerosis; 8.1±4.7% (n=8) and others; 4.7±2.0% (n=17), p<0.05 respectively].

Conclusions: The present study demonstrated that nephronectin is a novel protein of increased extracellular matrix in diabetic glomerulosclerosis, and also suggests that nephronectin staining could be a novel, useful tool for the diagnosis of diabetic nephropathy.

TH-PO517

Expression of Podocyte-Associated Molecules in Various Stages of Diabetic Nephropathy and in Pre Diabetes Francisco José Veríssimo Veronese,[1] Jonathan Fraportti do Nascimento,[1] Patrícia Garcia Rodrigues,[1] Gabriel Joelsons,[1] Luis Henrique Canani.[2] *[1]Nephrology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [2]Endocrinology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Background: This study investigated the mRNA profile of podocyte-associated molecules (PAM) in the different stages of diabetic nephropathy (DN) and in pre diabetes (PD).

Methods: Fifty-seven diabetic patients (DM) were included: normoalbuminuric, NA (n=35), microalbuminuric, MI (n=14) and macroalbuminuric, MA (n=8); and also 19 patients with PD and 10 healthy individuals (controls, C). Nephrin (nephr), podocin (pod), podocalyxin (pdx), synaptopodin (syn), TRPC6, and alpha-actinin 4 (actin4) mRNA were quantified by real time PCR in the urinary sediment; TGFbeta (TGFb) was also measured. Gene transcripts were correlated with albuminuria, glomerular filtration rate (GFR) and glycemic control (HbA1C); mRNA was log transformed (median/IIQ).

Results: Comparing the five groups, PAM mRNA did not shown a good correlation with level of albuminuria. Only nephr was higher in NA (vs. PD, p=0.046), pdx was higher in NA (vs. C, p=0.028), syn was higher in MA (vs. PD, p=0.033) and in NA (vs. PD, p=0.04), and Actin4 was higher in NA (vs. C, p=0.023). However, comparing the patients by diabetes status (yes/no), almost all genes were significantly higher expressed in DM (table 1). mRNA of nephr, pod, pdx, syn and TRPC6 were associated with poor glycemic control (p<0.05), but did not correlate with GFR.

PAM mRNA in diabetics and nondiabetics

Urinary mRNA	Non diabetic (n=34)	Diabetic (n=57)	P
Nephr	2.00(1.24-2.65)	3.08(1.86-3.89)	0.001
Pod	2.14(1.46-2.96)	2.66(1.81-3.98)	0.045
Pdx	2.17(1.28-3.04)	3.07(2.09-3.80)	0.002
Syn	1.81(1.29-2.32)	2.96(1.77-3.39)	0.001
Actin4	2.48(1.77-3.18)	2.90(2.00-3.99)	0.048
TRPC6	1.56(1.02-2.61)	2.76(1.66-3.94)	0.008
TGFb	2.49(1.84-3.29)	2.76(2.09-3.85)	0.121

Conclusions: A higher expression of PAM was found in DM, even in the stage of normoabuminuria, suggesting podocyte damage and podocyturia. Poor glycemic control correlated with higher amounts of PAM mRNA. TGFb mRNA in DM did not differ from non-DM, probably due to less advanced disease.

TH-PO518

Clinical Validation of the Novel Panel of Urinary Biomarkers to Monitor the Progression of Type 2 Diabetic Nephropathy Tzu-ling Tseng,[1] Wei-ya Lin,[1] Yen-Peng Li,[1] Kuo-hsiung Shu,[2] Chi-Hung Cheng,[2] Han-hsiang Chen,[3] Chih-Jen Wu,[3] Chwei-Shiun Yang,[4] Yuh-feng Lin,[5] Jin-Shuen Chen.[5] *[1]Industrial Technology Research Institute; [2]Taichung Veterans General Hospital; [3]Mackay Memorial Hospital; [4]Cathay General Hospital; [5]Tri-Service General Hospital, Taiwan.*

Background: The development of new markers is critical to improving the diagnosis, staging and treatment of diabetic nephropathy (DN). In this study, we conduct a clinical validation study of a urinary marker panel, including Alpha2-HS-glycoprotein precursor (AHSG), alpha-1-antitrypsin (A1AT) and alpha-1 acid glycoprotein 1 (AGP1).

Methods: Urine samples were collected from subjects included healthy control (HC), type 2 diabetic mellitus (DM) and DN. Group DN were further subgrouped by microalbuminuria and macroalbuminuria.at participating medical centers of the Taiwan Renal Biomarker Consortium. In the discovery phase, a series of biomarkers were identified with by differential proteomics platforms established at ITRI. Western blot of urine and immunohistochemical staining of renal tissues were evaluated. In the next validation phase, the assay of candidate biomarkers was performed by ELISA in a large scale, +300 samples.

Results: With respect to immunohistochemistry, increased A1AT, AHSG and AGP1 staining were evident in DN renal tissues. Evaluating the performance of these three markers in combination revealed an apparent staging effect with high sensitivity and specificity for discriminating between early and late stages of DN. Moreover, the three proteins in this DN biomarker panel were assigned to the same functional network with the insulin receptor as one of the major hubs. Therefore, these three markers may be not only statistically associated with each other but also functionally related to each other.

Conclusions: We have successfully developed a novel biomarker panel in combination of diagnostic algorithms with three urinary markers, AHSG, A1AT and AGP. Combination of three biomarkers is superior in function to a single marker for the staging of DN. An on-going longitudinal study will further elucidate applications of the biomarker panel in disease diagnosis and monitoring.

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TH-PO519

Urinary Exosomal WT1 Is Increased in T1DM Patients with Microalbuminuria Marcela Ururahy,[1,2] Karla Souza,[1] Yonara Monique Oliveira,[1] Melina Bezerra Loureiro,[1] Heglayne P. Silva,[1] Magali Araujo,[2] Francisco Paulo Freire Neto,[1] Joao F. Bezerra,[1] Ricardo Fernando Arrais,[3] Rosario D.C. Hirata,[4] Mario Hiroyuki Hirata,[4] Maria Das Graças Almeida,[1] Adriana Augusto de Rezende,[1] Sonia Q. Doi.[2] *[1]Clinical and Toxicological Analysis, UFRN, Natal, RN, Brazil; [2]Medicine, Uniformed Services University, Bethesda, MD; [3]Pediatrics, UFRN, Natal, RN, Brazil; [4]Clinical and Toxicological Analysis, USP, Sao Paulo, SP, Brazil.*

Background: Urinary exosomes are released by cells from every segment of the nephron, including podocytes, and may be a source of biomarkers. The Wilm's Tumor-1 (WT1), a marker of podocytes, has been found in urinary exosomes in various glomerular diseases. Using the bovine growth hormone transgenic (bGH) mouse, a model of glomerulosclerosis, we have shown that WT1 protein was decreased in the kidney and increased in exosomes of bGH compared to control mice, suggesting that increased exosomal WT1 is associated with glomerular podocyte damage.

Methods: Urine samples were collected from 13 patients with type 1 diabetes (T1DM) and 8 normoglycemic (NG) subjects, age 5-17 y.o. to measure urinary albumin/creatinine ratio (ACR) and WT1 in exosomes isolated by ultracentrifugation. Relative quantification of WT1 was obtained by immunobloting and densitometry of WT1 bands normalized to urinary creatinine.

Results: Five of 13 T1DM patients were microalbuminuric (MALB, ACR≥30mg/g) and 8 had ACR<30mg/g (N-MALB). The mean value of WT1/creatinine was significantly increased in MALB compared to both N-MALB and NG groups (Table 1). WT1 band was strongly positive in 4 out of 5 MALB patients. Conversely, only 1 out of 8 N-MALB patients showed a strong WT1 signal.

TABLE 1 – Relative quantification of exosomal WT1 according to ACR

	NG	N-MALB	MALB
ACR (mg/g of creatinine)	7.8 (6.7-12.9)	10.8 (8.2-22.1)	45.6 (38.4-128.7)*
WT1/creatinine	0.05±0.02	0.21±0.15	0.87±0.12*

Results are mean±SEM or (median interquartile range). * significant vs. NG and N-MALB ($p<0.05$).

Conclusions: This is the first demonstration that urinary exosomal WT1 can distinguish T1DM patients with from those without microalbuminuria. We are currently conducting a longitudinal study to determine whether exosomal WT1 precedes the increase in ACR.

Funding: Other U.S. Government Support, Government Support - Non-U.S.

TH-PO520

Regulation of Urine Metabolomics in Diabetic Kidney Disease Bethany E. Karl, Joachim H. Ix, Robert K. Naviaux, Kumar Sharma. *Department of Medicine, University of California San Diego, San Diego, CA.*

Background: Analysis of urine metabolomic data in patients with diabetic kidney disease (DKD) may lead to better understanding of its pathophysiology to direct development of therapeutics, isolate biomarkers and determine risk of chronic disease or progression.

Methods: 106 diabetic patients with CKD (eGFR<75ml/min/m²), 52 diabetic patients without CKD and 24 controls completed 24hour urine collections after which targeted urine metabolites were measured by GC/MS. The targeted metabolites were selected for their role in inborn and acquired disorders of intermediary metabolism. This same metabolite panel was measured in the plasma of a subset of the DKD (n=12) and control (n=13) groups. Fractional excretions of each metabolite were then calculated.

Results: Mean serum creatinine in the DKD group was 2.18 mg/dl (+/- 1.05) and 0.85 (+/-0.13) in controls. Statistical analysis revealed 9 metabolites to correlate significantly with eGFR ($P<0.007$). These were in lower concentration in DKD compared to the other two groups. Of the 9 metabolites, only 3 had calculable fractional excretions including citric acid, glycolic acid and 3-hydroxyisobutyric acid. The other 6: aconitic acid, 3-hydroxyisovaleric acid, 3-methyl-adipic acid, tiglylglycine, uracil, and 2-ethyl-3-hydroxypropionic acid, had undetectable concentrations in either plasma or urine. In the DKD group, the median fractional excretion of citric acid was decreased over 40-fold ($p=0.0005$) while the fractional excretions of both glycolic acid and 3-hydroxyisobutyric acid were increased with difference between means of 36% ($p=0.01$) and 14% ($p=0.003$) respectively.

Conclusions: To our knowledge this is the first evaluation of fractional excretion of metabolites in the urine metabolome in DKD vs. controls. Though all 9 of the measured urinary metabolites are lower in DKD compared to those without, the fractional excretions were surprisingly varied. The results support renal excretion differences for individual metabolites which do not simply reflect differences in serum levels.

Funding: NIDDK Support

TH-PO521

Proximal and Distal Tubular Markers in Patients with Type 1 Diabetes Bernt Johan von Scholten, Simone Theilade, Maria Lajer, Christel Joergensen, Stine Nielsen, Peter Rossing. *Steno Diabetes Center, Gentofte, Denmark.*

Background: The isoenzymes alpha and pi glutathione S-transferase (α- and π-GST) are secreted from proximal and distal renal tubules, respectively. Enzyme levels are elevated in type 2 diabetes and acute tubular damage. We evaluate if levels of α- and π-GST were elevated and associated with albuminuria in patients with type 1 diabetes.

Methods: Cross sectional study including 187 patients and 16 controls (C). Patients were (mean±SD) 53±14 years, with 29±18 years diabetes duration, 123(66%) male. Normoalbuminuria (<30mg/24-h) (Na) was present in 54(29%), microalbuminuria (30-300mg/24-h) (Mi) in 63(34%) and macroalbuminuaria (>300mg/24-h) (Ma) in 70(37%). Controls were 40±12 years and 11(69%) were male. Morning spot urines were analysed with ELISA (Argutus Medical).

Results: Median(range) π-GST was in C, Na, Mi and Ma 9.4(1.5-77.5), 9.3(0.3-172.0), 9.0(0.3-340) and 6.1(0.3-138) ug/l, respectively ($p=0.191$). Alpha-GST was 9.3(0.3-50.5), 4.3(0.3-50.8), 2.5(0.3-170.8) and 3.0(0.3-18.3) ug/l, respectively ($p=0.025$). Pi-GST was lower in men *vs.* women 7.5(0.3-90.3) *vs.* 15.0(0.8-340.0) ug/l; ($p<0.001$). Alpha- and π-GST-levels were similar in patients with and without cardiovascular disease ($p>0.05$). In all patients *vs.* C, levels of α-GST were 3.0(0.3-170.8) *vs.* 9.3(0.3-50.5) ug/l, ($p<0.05$), while π-GST levels were 8.8(0.3-340.0) *vs.* 9.4(1.5-77.5) ug/l in C, ($p>0.05$). Alpha- and π-GST correlated with each other ($r=0.357$; $p<0.001$), and α-GST correlated with HbA_{1c} ($r=0.179$; $p=0.001$) and eGFR ($r=0.169$; $p=0.016$); whereas π-GST correlated with none of these covariates ($p>0.05$). Neither GST isoenzymes correlated with age, diabetes duration, total cholesterol, 24-h systolic blood pressure or urinary albumin excretion rate ($p>0.05$).

Conclusions: Urinary levels of π-GST were similar in controls and patients regardless of albuminuria degree, whereas urinary levels of α-GST were lower in patients and decreased further with increasing albuminuria degree. These findings of altered tubular marker levels in patients with type 1 diabetes with or without chronic renal damage are in contrast to findings in patients with acute tubular damage where increased levels of the markers are present.

Funding: Private Foundation Support

TH-PO522

Neutrophil Gelatinase-Associated Lipokalin and Cathepsin as an Early Predictors of Kidney Dysfunction in Diabetic Children Jacek Zachwieja,[1] Jolanta Soltysiak,[1] Danuta Ostalska-Nowicka,[1] Piotr Fichna.[2] *[1]Department of Pediatric Nephrology, Poznan University of Medical Sciences, Poznan, Poland; [2]Department of Pediatric Diabetes and Obesity, Poznan University of Medical Sciences, Poznan, Poland.*

Background: One of the most dangerous complications of diabetes mellitus (DM) is diabetic kidney disease (DKD). At present, the measurement of albuminuria and glomerular filtration is used as a standardized non-invasive test for the diagnosis of DKD. However, early renal dysfunction may be overlooked despite using that method. On the other hand, the gold standard in DKD detection—that is, renal biopsy—is highly invasive.The aim of this study was to evaluate the level of neutrophil-gelatinase associated lipocalin in serum (sNGAL) and in urine (uNGAL) as well as urinary excretion of cathepsin L (uCathL) and angiotensinogen (uAGT) in children with type 1 DM (DM1) considered as not presenting DKD.

Methods: The study group consisted of 63 children with DM1 (28 males and 35 females) with mean age of 13.46±2.95 yr. The average time of DM1 treatment was 5.16±3.39 yr. All patients presented a normal albumin/creatinine ratio(ACR< 30 mg/g) and normal eGFR, based on cystatin C (>90 ml/min/1.73m²).

Results: Children with DM1 compared to controls showed significantly higher levels of uNGAL (43.02±45.68 vs. 6.85±6.05 ng/ml; p<0.001), as well as lower sNGAL (109.96±50.06vs. 151.34±53.95 ng/ml; p<0.001) and uCathL (4.32±1.61 vs 5.18±0.95 ng/ml).These changes were observed even in children with optimal glycemic control (HbA1c<7.5%), treated for less than 5 years. uAGT levels were similar in both groups

(0.148±0.474vs. 0.00 ng/ml; p=0.628). A possitive correlations between uNGAL and ACR (r=0.313; p=0.013) and between uCathL and eGFR (r=0.501; p=0.034) were found. HbA1c levels showed a positive correlation with eGFR (r=0.399; p=0.014) and with ACR (r=0.268; p=0.034).

Conclusions: Normal-range albuminuria and normal eGFR do not exclude DKD, if defined as changes of sNGAL, uNGAL and uCathL concentration. These changes may occur even in children with relatively short lasting diabetes and with optimal glycemic control. Our study highlights the value of sNGAL, uNGAL and uCathL as potential markers for early DKD detection.

Funding: Government Support - Non-U.S.

TH-PO523

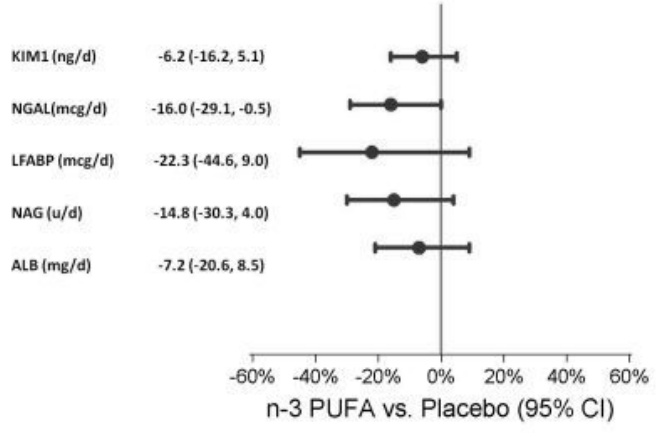
The Effects of N–3 Polyunsaturated Fatty Acid Supplementation on Biomarkers of Kidney Injury in Adults with Diabetes: Results of the GO-FISH Trial Edgar R. Miller, Stephen P. Juraschek, Cheryl A. Anderson, Sharon Turban, Eliseo Guallar, Lawrence J. Appel. *Medicine, Johns Hopkins University, Baltimore, MD.*

Background: The effect of n–3 long-chain polyunsaturated fatty acid supplementation (n-3 PUFA) on kidney disease is uncertain.

Methods: We conducted a randomized controlled trial to test the effects of n-3 PUFA supplementation on markers of kidney injury in patients with adult-onset diabetes and ≥ trace dipstick proteinuria. Participants were randomized in a 2-period cross-over trial to 4 g/d of n-3 PUFA or placebo. Each period lasted 6 weeks separated by a 2-week wash-out. Outcomes were urine markers of kidney injury [albumin, kidney injury molecule-1 (KIM-1), N-acetyl β-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL) and liver fatty acid-binding protein (LFABP)] and serum markers of eGFR [cystatin C, β2-microglobulin, creatinine].

Results: Of the 31 participants, 29 finished both periods (55% were female, 61% were African-American, mean age was 67 years, 68% on medications that block the renin-angiotensin-aldosterone system (RAAS). At baseline, median eGFR was 75 ml/min/1.73m^2 and median 24-hour urine albumin excretion was 161 mg/d. By repeated measures analyses, n-3 PUFA lowered 24-hour urine excretion of NGAL by 16% (95% CI-29.1%, -0.5%) compared with placebo. There was a consistent trend of reduction in each of the other urine biomarkers.

Figure. Difference in urine markers of kidney injury in n-3 PUFA compared with placebo periods



There was no effect on serum markers of eGFR. In a secondary analysis, there was a significant decrease in 24-hour excretion of albumin, NGAL, LFABP, and NAG, in the subgroup of participants on RAAS blockers.

Conclusions: These results highlight the potential role of n-3 PUFA supplementation as a means to prevent further kidney injury in patients with diabetes and evidence of kidney disease. Long-term trials with clinical outcomes are warranted.

Funding: NIDDK Support, Pharmaceutical Company Support - Galaxo-Smith-Kline

TH-PO524

Serum Albumin Globulin Ratio as a Predictor of Worsening Renal Function in the Diabetic Population Basem Azab, Suchita J. Mehta, Neeraj N. Shah, Deepak Asti, Morton J. Kleiner, Suzanne E. El Sayegh. *Staten Island University Hospital, Staten Island, NY.*

Background: Hypoalbuminemia has been associated with worse renal function in prior studies. The use of serum albumin globulin ratio (AGR) as a predictor for worsening renal function among diabetics has not been elucidated.

Methods: Our study included 334 diabetics followed at our clinic from 2007 to 2009. We divided them into quartiles based on the 2007 AGR. The primary outcome was a decrease of GFR > 12 with the last (2009) GFR < 60ml/minute. Linear & logistic regression models using respectively GFR at year 3 & primary outcome as the dependent variables were constructed. Additional analysis was performed after exclusion of patients with albumin<3gm/dl.

Results: Mean rate of decline of GFR per year was -0.11 ml/min/year (95% CI +0.69 to -0.91). 1st AGR quartile had a rate of decline of -1.97 ml/min/year (95% CI -0.78 to -3.17), while the 2nd, 3rd & 4th quartiles had GFR change of +1.06, +0.46 & +0.16 ml/min/year respectively. Linear regression with GFR at year 3 as the dependent and GFR at year 1 & AGR quartiles as the independent variables yielded coefficients of 9.4 (p=0.007), 7.8 (p=0.026) and 6.9 (p=0.048) of 2nd, 3rd & 4th AGR quartiles relative to 1st quartile, thus illustrating a linear relation between GFR at year 3 and AGR. In multivariate analysis, the coefficients of 2nd & 4th quartile relative to 1st quartile remained significant (p<0.05) at 8.0 & 7.6 respectively. 1st AGR quartile had the highest number of patients with primary outcome (14%) compared to 2nd, 3rd and 4th quartiles (7%, 5% and 4% respectively), with chi-square p of 0.04.

Dropping of GFR according to AGR.

	AGR < 1.10	AGR 1.10-1.25	AGR 1.26-1.42	AGR >1.42
Dropping of GFR > 12 ml/minute/3 years	12/84	6/84	4/83	3/83

Univariate logistic regression analysis showed odds ratio (OR) of 0.23 (p=0.025) of 4th vs. 1st AGR quartile, which persisted in multivariate analysis (OR 0.2, p=0.034) and after exclusion of patients with low albumin (OR 0.26, p=0.046).

Conclusions: Low serum AGR was an independent predictor of lower GFR and a worse renal function over a 3 year follow up period in diabetics. This effect was independent of hypoalbuminemia.

Funding: Private Foundation Support

TH-PO525

Abnormalities in 24-Hour Plasma Glucose Variation on CKD: Review of Using CGMs Kozi Hosoya, Shu Wakino, Hitoshi Minakuchi, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio University School of Medicine, Sinjyuku-ku Sinanomati 35, Tokyo, Japan.*

Background: Although CKD patients were complicated with insulin resistance even without diabetes (DM), the more detailed information on temporal change in plasma glucose (PG) has not been reported. Recently, continuous daily plasma glucose (PG) profiles can be monitored by using CGMS (continuous glucose monitoring system). We studied one of CKD patients without diabetes mellitus (DM) using this system.

Methods: We compared various parameters in CGMS between 15 patients of Stage 5 CKD without DM (CKD) and 10 patients with normal renal function (control). The correlations were analyzed with multi-regression analysis (M-RA) and simple-regression analysis (S-RA). CGMS parameters analyzed were as follows; 24-hour average PG (ave-PG), standard deviation (SD) of 24-hour PG, area under the curve (AUC) of 24-hour PG, average PG of sleeping time, SD of PG of sleeping time, the difference between fasting PG and maximum PG for 2-hour postprandial (MAX-PG) (breakfast, lunch, dinner, sum of three meals(S-mesls)), the difference between 2-hour postprandial PG and fasting PG(2-hr PG), and postprandial 2-hour AUC(2-hr AUC) (breakfast, lunch, dinner, S-meals).

Results: Fasting glucose, HbA1c, and GA were not different between CKD and control. However, 24-hour ave-PG, SD of 24-hour PG and AUC of 24-hour PG were significantly higher in CKD than those in control. In 2-hour PG, significant differences in breakfast and sum of all meal were also observed between CKD and control. In 2-hr AUC, we also recognized a significant difference between CKD and control in breakfast, lunch, dinner, and S-meals.By S-RA, SD values were correlated with FFA (free fatty acid) and ave-PG was correlated with GA (Glycated albumin), FFA, and e-GFR. 2-hr PG was correlated with serum total protein, serum calcium, FFA, Hb (hemoglobin) and eGFR, and 2-hr AUC was correlated with GA, FFA, and eGFR. By M-RA, SD, ave-PG, and 2-hr PG were correlated with FFA.

Conclusions: CKD patient without DM have significant postprandial glucose spike. And this variation had strong relationship with FFA metabolism. It can be concluded that this glucose spike contributes to cardiovascular disease in CKD.

Funding: NIDDK Support

TH-PO526

Validation of a Novel Method to Determine the Renal Threshold for Glucose (RT_G) in Untreated and Canagliflozin (CANA)-Treated Subjects with Type 2 Diabetes David Polidori,[1] Sue Sha,[1] Paul Rothenberg,[1] Leona Plum-Mörschel,[2] Tim Heise.[2] *[1]Janssen Research & Development, LLC, Raritan, NJ; [2]Profil Institute for Metabolic Research, Neuss, Germany.*

Background: Nearly all glucose filtered by the kidney is reabsorbed until blood glucose (BG) exceeds RT_G. By inhibiting the sodium glucose co-transporter 2, CANA decreases RT_G, leading to increased urinary glucose excretion (UGE). The established method for determining RT_G requires stepwise hyperglycemic clamps. This study was performed to validate a new method for estimating RT_G using data collected from a mixed-meal tolerance test (MMTT).

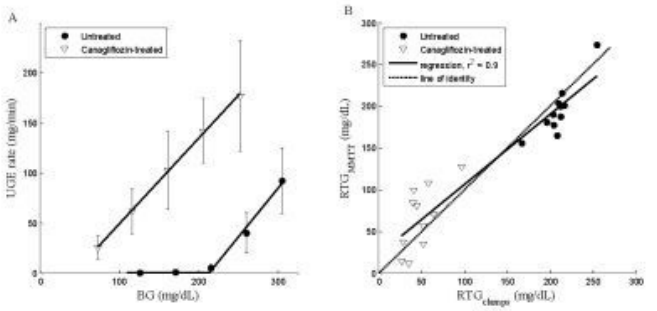
Methods: Untreated (N=14) and CANA-treated (100 mg once daily for 8 days; N=14) subjects with type 2 diabetes (mean A1C=8%) underwent an MMTT and a stepwise hyperglycemic procedure on consecutive days. During the MMTT, UGE and BG were measured and the glomerular filtration rate was estimated. $RT_{G:MMTT}$ was estimated based on these data using an extension of the established method for determining $RT_{phosphate}$. During the clamp procedure, UGE rates during the 5 BG clamp steps were measured and $RT_{G:clamp}$ was determined by nonlinear regression. The 2 methods were compared using the concordance correlation coefficient and geometric mean ratio (GMR).

Results: In untreated subjects, the UGE versus BG relationship was well-described by a threshold relationship and CANA left-shifted the relationship (Figure A). There was strong agreement between $RT_{G:MMTT}$ and $RT_{G:clamp}$, with a concordance correlation coefficient

of 0.94 and GMRs (90% confidence interval) of 0.93 (0.89, 0.96) in untreated subjects and 1.03 (0.78, 1.37) in CANA-treated subjects (Figure B). The safety and tolerability of CANA were consistent with previously reported studies.

Conclusions: The effects of CANA on UGE are well-described by lowering RT_G and the new MMTT-based method for estimating RT_G provides good agreement with the clamp method.

Figure. (A) UGE versus BG during the clamps and (B) comparison of $RT_{G:MMTT}$ versus $RT_{G:clamp}$



Funding: Pharmaceutical Company Support - Janssen Research & Development, LLC

TH-PO527

Vitamin D Analogue Therapy, Cardiovascular Risk and Kidney Function in Type 1 Diabetic Patients with Diabetic Nephropathy: A Randomized Trial Christel Joergensen,[1] Lise Tarnow,[1,2] Jens Peter Goetze,[2,3] Peter Rossing.[1,2] *[1]Steno Diabetes Center, Denmark; [2]Aarhus University, Aaarhus, Denmark; [3]Rigshospitalet, Denmark.*

Background: Vitamin D is associated with decreased cardiovascular morbidity and mortality and is suggested to have renoprotective effects. ProBNP is a plasma marker for cardiovascular risk and mortality in diabetic patients. Estimates of renal function decline with vitamin D analogue (VDA) therapy. We evaluated the impact of therapy with the VDA paricalcitol on cardiovascular risk and kidney function in patients with type 1 diabetes and diabetic nephropathy.

Methods: Double-blind, placebo controlled, randomized cross-over trial in 45 patients with type 1 diabetes and diabetic nephropathy, all in stable RAAS blockade and diuretic treatment. Paricalcitol (1-2µg/day) and placebo therapy was given in random order for 12 weeks interrupted by one month of wash-out. Before and after each treatment period the primary and secondary endpoint, a change in plasma proBNP concentration and urinary albumin excretion rate (UAER) from three consecutive 24h urines, were measured. End of therapy endpoints were estimated glomerular filtration rate (eGFR) and measured GFR (^{51}Cr-EDTA plasma clearance).

Results: At baseline mean (SD) age was 56(9) years, 32(71%) subjects were men, mean HbA_{1c} was 70(9)mmol/mol, mean eGFR was 47(15)ml/min/1.73m^2 and mean 24h blood pressure was 135(17)/76(11)mmHg. None of the patients had heart failure. Compared to placebo Paricalcitol had no significant effect on plasma proBNP concentrations, p=0.6. During paricalcitol therapy mean UAER was significantly reduced by 19% (range 1-35%) from 127mg/24h, $p = 0.019$ for comparison with change during placebo. eGFR was significantly lower at the end of paricalcitol therapy compared to placebo, 41 vs. 46ml/min/1.73m^2 respectively, p<0.001, whereas only a non significant reduction of 1.5ml/min/1.73m^2, p=0.2,was observed in GFR.

Conclusions: Therapy with the VDA paricalcitol has no effect on plasma proBNP concentrations but significantly lowers albuminuria in type 1 diabetic patients with diabetic nephropathy. Although estimates of GFR decline, "true" GFR is unchanged, suggesting paricalcitol therapy may affect creatinine handling.

Funding: Pharmaceutical Company Support - The Study Is Financed by an Unrestricted Grant from Abbott Laboratories

TH-PO528

Efficacy and Safety of the Glucagon-Like Peptide-1 Analogue Liraglutide in Diabetic Patients on Hemodialysis Masao Toyoda, Moritsugu Kimura, Tomoya Umezono, Daisuke Suzuki, Masafumi Fukagawa. *Division of Nephrology and Metabolism, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: Despite the development of several oral hypoglycemic agents and insulin preparations, glycemic control in hemodialysis (HD) patients still remains a task of great difficulty with high risk of hypoglycemia. Liraglutide (LG), a recently developed glucagon-like peptide-1 analogue, lowers the blood sugar levels through a glucose-dependent insulin secretagogue effect, thus with lower risk of hypoglycemia. Nevertheless, efficacy and safety of LG in HD patients have not been fully evaluated yet. In the present study, Japanese diabetes patients who were on HD and switched to LG from basal-bolus therapy or pre-mixed insulin therapy were evaluated to determine the efficacy of LG.

Methods: Blood C-peptide levels in these patients were measured, and patients without severe defects in endogenous insulin secretion were switched to LG. In these patients, we measured HbA_1c levels, dry weight, body weight gain between dialysis treatments (Δ BW), and number of hypoglycemic episodes at the time of switching (pre-LG) and 3 months later (post-LG). Further, the impact of the switch on the quality of life was investigated using a questionnaire.

Results: HbA_1c levels significantly improved from 6.0±0.6 at pre-LG to 5.8±0.6 at 3 months post-LG (p<0.05). Further, the dry weight and ΔBW decreased significantly after the switch. The number of hypoglycemic episodes also decreased after switching.

Conclusions: It is possible for LG to improve HbA_1c without increasing body weight and frequency of hypoglycemia in diabetic Japanese HD patients if it is used appropriately. Further studies are necessary in patients of different ethnic background and dietary habits.

TH-PO529

Vildagliptin Improves Glycaemic Control and Fluctuation in Hemodialysis Patients with Type2 Diabetes Kunihiro Ishioka, Machiko Oka, Hidekazu Moriya, Takayasu Ohtake, Sumi Hidaka, Shuzo Kobayashi. *Nephrology, Immunology, and Vascular Medicine, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan.*

Background: Since the dipeptidyl peptidase-4 (DPP-4) inhibitor, therapeutic agent of diabetes mellitus through incretin, emerged, it has brought a paradigm shift in the treatment of diabetes mellitus. Among DPP-4 inhibitor, Vildagliptin is available not only for patients with renal insufficiency, but also for hemodialysis patients. The purpose of this study is to assess the efficacy and safety of vildagliptin (50mg once daily), and to see the effect on glucose fluctuations using continuous glucose monitoring (CGM) in hemodialysis patients with type 2 diabetes mellitus (T2DM).

Methods: The study included 42 hemodialysis patients (26 men: mean age ±SD, 68.0±11.2 years) with T2DM who had inadequate glycaemic control. We devided them into two groups. Fifty mg/day of vildagliptin was administered to one group (V group) in addition to the original treatment. The other group (C group) continued the original treatment. We assessed HbA1c, glycated albumin (GA), and blood glucose value in two groups before and 1 month after the treatment. Additionally, we evaluated averaged glucose value (AGV), blood glucose fluctuation (SD) for 24 hours using continuous glucose monitoring (CGM).

Results: In V group, HbA1c and blood glucose value decreased significantly 1 month after administration of vildagliptin (6.5±1.1% to 5.6±1.1%, 184±79mg/dl to 135±50mg/dl, p<0.05), respectively. GA also showed improvement in V group (25.1±5.3% to 22.9±4.4%, P=0.16). CGM showed that AGV and SD improved both one day and one month after vildagliptin administration.

Conclusions: Our data showed that vildagliptin improved blood glucose control very soon as well as glycaemic fluctuations without any adverse effects.

TH-PO530

Renal Safety and Outcomes with Linagliptin: Meta-Analysis of Individual Data for 5466 Patients with Type 2 Diabetes Maximilian von Eynatten,[1] Angela Emser,[1] Mark E. Cooper,[2] Vlado Perkovic,[3] Julio Rosenstock,[4] Christoph Wanner,[5] Hans-Juergen Woerle.[1] *[1]Boehringer Ingelheim, Ingelheim, Germany; [2]The Baker IDI Heart and Diabetes Institute, Melbourne, Australia; [3]The George Institute for Global Health, Sydney, Australia; [4]Dallas Diabetes and Endocrine Center at Medical City, Dallas, TX; [5]University of Würzberg, Würzberg, Germany.*

Background: Long-term glycemic control in diabetes is associated with reduced risk of renal microvascular complications. Linagliptin has shown nephroprotective effects in animal models and significantly reduced albuminuria in type 2 diabetes (T2D)-associated nephropathy. As these effects do not appear to be directly related to short-term glycemic improvements, it was speculated that linagliptin may have potential nephroprotective effects. The aim of these analyses was to evaluate renal outcomes with linagliptin in completed Phase 3, randomized, double-blind, placebo-controlled trials (≥12 wks).

Methods: Predefined events from 13 trials were analyzed using a composite primary endpoint: new onset of a) micro- or b) macroalbuminuria, c) CKD (serum creatinine increase ≥2.83 mg/dL), d) worsening of CKD (loss in eGFR >50% vs baseline), e) acute renal failure (ARF; standardized MedDRA query), and f) death (any cause).

Results: Of 5466 participants (mean baseline HbA1c: 8.2% and eGFR: 91 mL/min/1.73m^2), 3505 received linagliptin 5mg qd and 1961 placebo; cumulative exposure (person yrs) was 1756 and 1057, respectively. Events occurred in 448 (12.8%) patients receiving linagliptin vs 306 (15.6%) for placebo. The HR of 0.84 (95% CI: 0.72-0.97, P<.05), was not significantly altered by race, but tended to be stronger in patients <65 vs >65 yrs (HR: 0.77 vs 1.04). The descriptive RRs for individual renal endpoints were: micro- (0.85) and macroalbuminuria (0.88), new onset (0.44) or worsening of CKD (0.76), ARF (0.93), and death (0.77).

Conclusions: In this large meta-analysis, renal safety and outcomes were significantly improved in patients with T2D treated with linagliptin. These data support a potential direct nephroprotective effect of linagliptin that warrants future long-term controlled trials designed to confirm these findings.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim

TH-PO531

Safety and Efficacy of Linagliptin in Combination with Insulin in Patients with Type 2 Diabetes (T2D) and Declining Renal Function Janet McGill,[1] Hannele Yki-Järvinen,[2] Angela Emser,[3] Maximilian von Eynatten,[3] Hans-Juergen Woerle.[3] *[1]Washington University in St. Louis, St. Louis, MO; [2]University of Helsinki, Helsinki, Finland; [3]Boehringer Ingelheim, Ingelheim, Germany.*

Background: Renal impairment (RI) and fluctuating kidney function limits glucose-lowering treatment options in T2D. Hence, insulin is commonly used in this population, despite the increased risk of hypoglycemia. Linagliptin, a primarily non-renally excreted oral DPP-4 inhibitor, can be given to T2D patients with any degree of RI, without dose adjustment. Two Phase 3 trials (n=1394) assessing linagliptin 5 mg qd vs placebo in combination with insulin were included in this pooled analysis of safety and efficacy in patients with T2D and RI.

Methods: In these trials, the primary efficacy endpoint was change from baseline to wk 12 (NCT00800683) or wk 24 (NCT00954447) in HbA1c. Adverse events (AEs) were also assessed.

Results: At baseline 565, 124, and 104 patients had mild, moderate, or severe RI, respectively, and were inadequately controlled on insulin; mean HbA1c was 8.3, 8.2, and 8.3% and mean daily insulin dose was 40.7, 42.7, and 64.9 IU, respectively. Over 85% of all patients had a diabetes duration >5 yrs. The placebo-adjusted mean change ±SE in HbA1c with linagliptin was -0.59± 0.07%, -0.71±0.15%, and –0.43±0.16%, respectively (all $P<0.01$). Drug-related AEs in mild, moderate, and severe RI occurred in 17.6, 14.3, and 46.3% of linagliptin patients vs 23.1, 25.9, and 43.6% of placebo patients; corresponding incidences of drug-related hypoglycemia were 6.5, 8.4, and 42.6% on linagliptin vs 6.6, 11.6, and 40.0% with placebo. Overall, most hypoglycemic events on linagliptin were of mild intensity and the incidence of serious hypoglycemic events was low with linagliptin (n=4) and placebo (n=1).

Conclusions: In T2D patients with mild, moderate, or severe RI, inadequately controlled on insulin, linagliptin was well tolerated and achieved clinically meaningful improvements in glycemic control without excess risk of hypoglycemia. These data support linagliptin as a treatment alternative in patients with declining renal function.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim

TH-PO532

The Effect to Sodium Excretion and Blood Pressure with DPP-4 Inhibitors Koichi Kanozawa, Hajime Hasegawa, Juko Asakura, Takatsugu Iwashita, Taisuke Shimizu, Tokushi Nakajima, Tetsuya Mitarai. *Division of Nephrology and Hypertension, School of Medicine, Saitama Medical University, Kawagoe, Saitama, Japan.*

Background: The diabetes patients are known to be easy to present with salt sensitive hypertension. On the other hand, the GLP-1 receptor is expressed in renal proximal tubule, and acts on sodium diuresis through Na(+)/H(+) exchanger NHE3. However, the effect to sodium diuresis and the blood pressure with the DPP-4 inhibitors were unknown. We examine sodium diuresis and the antihypertensive effect with the DPP-4 inhibitors by the short-term effect in the inpatient and the long-term effect in the outpatient.

Methods: We gave DPP-4 inhibitor (either of sitagliptin 50-100 mg, vildagliptin 50-100 mg, alogliptin 12.5-25 mg) for Japanese patients with Type 2 diabetes less than 2.0 mg/dl of serum creatinine level. In the inpatients, we calculated urinary salt excretion using urine collection for 24 hours within one week around administration. In the outpatients, we calculated sodium excretion quantity estimation level using first or second urinary in the morning, and measured the mean of home and office blood pressure, before and after administration. Futhermore, we examinated with sulfonyl ureas which strongly promoted insulin secretion, and between the drug of DPP4 inhibitors.

Results: In 23 inpatients, urinary salt excretion significantly increased 1.8±0.6g ($p<0.01$), for 24 hours with the DPP-4 inhibitors. On the other hand, systolic and diastolic blood pressure significantly had decreased 6±2mmHg ($p<0.01$) and 5±2mmHg ($p<0.05$), each. In 45 outpatients, quantity of salt excretion estimation level significantly increased 0.8±0.2g/day ($p<0.01$) with the DPP-4 inhibitors. The systolic home and office blood pressure significantly had decreased 2±1mmHg ($p<0.05$) and 4±2mmHg ($p<0.01$), each. In contrast, these changes were not seen with the sulfonyl urea. Furthermore, the meaningful these effects were seen in alogliptin and sitagliptin, but was not seen in vildagliptin.

Conclusions: By the administration of the urine excretion type DPP-4 inhibitors for the diabetes, urinary sodium excretion increase, and blood pressure decrease.

TH-PO533

Efficacy and Tolerability of Vildagliptin in Chronic Kidney Disease (CKD) Stage 3-5 Patients with Type 2 Diabetes Mellitus Yoshihiko Inoue, Tomoaki Miyazaki, Shinya Oomiya, Daisuke Komukai, Kiyoko Inui, Ashio Yoshimura. *Division of Nephrology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

Background: It is important to establish the efficacy and tolerability of a new antidiabetic agent in patients with renal impairment. Dipeptidyl peptidase-4 (DPP-4) inhibitor Vildagliptin (VG) is one of the newest class of oral antidiabetic agents. We evaluate the usefulness of it on CKD stage 3-5 patients with diabetes mellitus (DM).

Methods: 51 patients (25 cases with CKD stage 3 [eGFR 49.5±1.5 ml/min/1.73m^2], 16 with stage 4 [24.6±1.2], 10 with stage 5 [10.9±1.0]) were followed for 36 weeks after the start of VG treatment. 50mg/day (36 cases, VG50) or 100mg/day (15 cases, VG100) of VG was newly started or was added to current antidiabetic therapy. HbA_{1C}, eGFR, BMI and the amount of proteinuria were studied at before treatment, after 4-week, 12-week, 24-week and at the end of the study.

Results: Result 1: There was no difference in HbA_{1C}, serum albumin levels, BMI, or protein excretion between stages ofCKD before VG treatment. BMI was normal ranges (24.1±0.5 kg/m^2). After 4 weeks, VG elicited statistically significant decrease in HbA_{1C} to the end of the study (36-week of HbA_{1C}: 6.53±0.14 vs. baseline: 7.41±0.20 %, $p<0.001$). The statistical decrease in HbA_{1C} was also shown in each CKD-stage group respectively from after 4 weeks until the end of the study. There was no difference in serum albumin levels, BMI, urine protein excretion, or eGFR between baseline and the end of the study (36-week of eGFR: 36.09±2.81 vs. baseline: 34.32±2.78 ml/min/1.73m^2, p=0.072).

Result 2: There was no difference in eGFR between groups of VG100 and VG50 before treatment (36.5±3.8 vs. 33.2±3.0 ml/min/1.73m^2, p=0.53). The dosage of VG (mg/kg) was higher in VG100 group rather than VG50 group (1.75±0.43 vs. 0.84±0.24 mg/kg, $p<0.01$). Patients in VG100 group were higher than those in VG50 group in HbA_{1C} before treatment (8.1±0.6 vs. 7.1±0.2 %, $p<0.05$). HbA_{1C} was significantly suppressed in both groups of VG50 and VG100.

Result 3: There were no severe adverse events including hypoglycemia in all patients.

Conclusions: VG is effective and tolerated in CKD stage 3-5 patients with type 2 DM.

TH-PO534

Association between Milk Consumption and Cardiovascular Risk Factors in Children: NHANES 1999-2000 Kathleen Perez,[3] Kamyar Kalantar-Zadeh,[2] Keith C. Norris,[3] Gangadarshni Chandramohan.[1] *[1]Pediatrics, LA Bio-Medical Research Inst. at Harbor-UCLA Medical Center, Torrance, CA; [2]Department of Internal Medicine, University of California, Irvine, CA; [3]Charles Drew University School of Medicine, Lynwood, CA.*

Background: Recent studies have shown that low milk consumption is associated with increased risk of hypertension in adults. To date, there are no data on the association between milk and various cardiovascular (CV) risk factors in the pediatric population. In this study we looked at the relationship between milk consumption and age, gender, ethnicity and CV risk factors in children between the ages of 6 – 17 years using data from National Health and Nutrition Survey (NHANES) 1999-2000.

Methods: We analyzed data of 5387 children, 58% were 6-12 yrs and 51% males. Two or more days per week of milk consumption is considered as some.

Results: There was a significant difference in the rate of milk consumption by age and gender. A significant positive association with abnormal fasting blood sugar (FBS) and a negative association with hyperlipidemia were observed in those who drank some milk compared to those who never drank milk. No association noted between milk consumption and ethnicity, high BP, obesity and waist circumference.

Relationship Between Milk Consumption and Cardiovascular Risk Factors in Children Between Ages 6 - 17 years Using Chi Square Analysis

Variables	Never Drank Milk (%)	Drank Some Milk (%)	p Value
Normal WC	60	63	
High WC	47	37	0.47
Non-Obese	80	81	
Obese	20	19	0.88
Normal BP	88	91	
High BP	12	9	0.26
Normal Lipids	41	53	
Hyperlipidemia	59	47	0.01
Normal FBS	98	93	
Abnormal FBS	2	7	0.001

WC: waist circumference, High WC: >75th percentile for age and gender, BP: blood pressure, FBS: fasting blood sugar

Conclusions: The negative association between milk consumption and hyperlipidemia suggests that most children may be consuming low fat milk. However, a positive association between milk and abnormal FBS may suggest a high lactose intake from milk. This should be considered as a possible culprit for the development of Type 2 Diabetes. However, further prospective studies is warranted to confirm these findings.

Funding: NIDDK Support

TH-PO535

Combined Use of Amlodipine and Irbesartan in Patients with Diabetic Kidney Disease and Hypertension: A Multi-Center Randomized Prospective Controlled Trial Ping Fu, Hui Zhong. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: We designed a multi-center randomized controlled trial (RCT) to investigate the efficacy and safety of combined use of Amlodipine and Irbesartan in reducing urinary protein excretion and blood pressure control in patients with diabetic kidney disease (DKD) and hypertension.

Methods: This is a multi-center, prospective, single-blind RCT study. A total of 200 patients (18<age≤80y) with type 2 DKD (phage 3 and 4) and hypertension were enrolled and randomly assigned to double dose Irbesartan group (II group, Irbesartan 300mg/d) or combined treatment group (AI group, Amlodipine 5mg/d and Irbesartan 150mg/d) at 1:1 ratio, followed up for 8 weeks. The primary endpoints included the change of urinary albumin: creatinine ratio (ACR) and the effective rate of the two groups (It was defined as effective if the change rate of ACR was over 50% or the ACR of 8th week was normal). The secondary endpoints included urinary protein: creatinine ratio (PCR), serum creatine, eGFR, and blood pressure.

Results: Data of 181 patients was analyzed as 19 patients were lost to follow-up. The effective rate was 16.48% in AI group and 8.89% in II group (P=0.1251), and there was no difference in change rate of ACR between the two groups either (AI group 0.28±0.24,

II group 0.22±0.21, P=0.0824). However, the change rate of PCR was significantly higher in AI group (0.34 ±0.81, 0.24±0.46, P=0.0032). Both groups showed favorable control of blood pressure when compared to baseline (all P value <0.0001), but the AI group deceases both SBP (P=0.0044) and DBP (P=0.0007) more than II group. There was no difference in changes of serum creatinine, serum glucose and eGFR (P>0.05).

Conclusions: There was no significant difference in decreasing ACR between Irbesartan 300mg/d and Amlodipine 5mg/d combined with Irbesartan 150mg/d, but the combination therapy had better effect on blood control and decreasing PCR. The combined therapy may bring benefits to both cardiovascular and renal protection, which needs to be confirmed by further studies.

Funding: Pharmaceutical Company Support - Pfizer

TH-PO536

Efficacy and Safety of Canagliflozin (CANA) in Subjects with Type 2 Diabetes Mellitus (T2DM) and Moderate Renal Impairment George L. Bakris,[1] Jean-Francois Yale,[2] Ewa Wajs,[3] Liwen Xi,[3] Keith Usiskin,[3] Gary Meininger.[3] *[1]Univ of Chicago Pritzker School of Medicine, Chicago, IL; [2]Royal Victoria Hospital and McGill Univ, Montreal, Canada; [3]Janssen Research & Development, LLC, Raritan, NJ.*

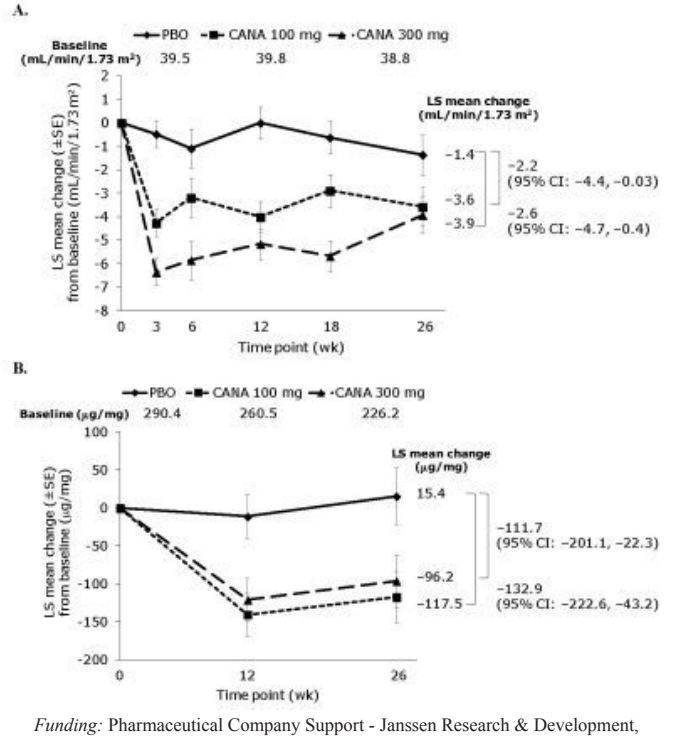
Background: This Phase 3 study evaluated the efficacy and safety of CANA, a sodium glucose co-transporter 2 inhibitor, in subjects with T2DM and moderate renal impairment.

Methods: In this randomized, double-blind, placebo (PBO)-controlled study, subjects (N=269) with T2DM and moderate renal impairment (estimated glomerular filtration rate [eGFR] ≥30 and <50 mL/min/1.73m^2) received CANA 100 or 300 mg or PBO (mean baseline characteristics: age 68.5 y; A1C 8.0%; fasting plasma glucose [FPG] 164.0 mg/dL; BMI 33.0 kg/m^2; eGFR 39.4 mL/min/1.73m^2). Mean and median albumin/creatinine ratios (ACR) were 256.8 and 30.0 µg/mg. 31% of subjects were on sulfonylureas and 74% on insulin.

Results: At Week 26, CANA 100 and 300 mg significantly lowered A1C relative to PBO (least squares mean change vs PBO of –0.30% [*P*<0.05] and –0.40% [*P*<0.001]). Both CANA doses reduced FPG, body weight, and systolic blood pressure compared to PBO. Incidence of adverse events (AEs), serious AEs, and AE-related discontinuations was similar across groups. Slight increases in urinary tract infections and osmotic diuresis-related AEs were seen with CANA 300 mg, but led to few discontinuations. Modest decreases in eGFR were seen with CANA 100 and 300 mg relative to PBO; mean changes from baseline were maximal at Week 3 (–4.3, –6.4, and –0.5 mL/min/1.73m^2) and lessened over time (–3.6, –3.9, and –1.4 mL/min/1.73m^2 at Week 26). Decreases in ACR were observed with CANA 100 and 300 mg relative to PBO at Week 26 (median change from baseline: –7.2, –3.8, and –0.8 µg/mg).

Conclusions: CANA 100 and 300 mg improved glycemic control, reduced body weight, and was well tolerated in subjects with T2DM and moderate renal impairment.

Figure. Change in (A) eGFR and (B) ACR over time.



Funding: Pharmaceutical Company Support - Janssen Research & Development, LLC

TH-PO537

Copeptin, a Surrogate Marker for Vasopressin, Is Associated with Progression of Diabetic Nephropathy (ZODIAC-33) Wendy E. Boertien,[1] Ineke J. Riphagen,[1] Iefke Drion,[2] Alaa Alkhalaf,[2] Klaas Groenier,[2] Stephan J.L. Bakker,[1] Joachim Struck,[3] Henk Bilo,[2] Nanne Kleefstra,[2] Ron T. Gansevoort.[1] *[1]Nephrology, UMC, Groningen; [2]Diabetes Centre, Isala Clinics, Zwolle, Netherlands; [3]BRAHMS, ThermoFisher Scientific, Hennigsdorf, Germany.*

Background: Vasopressin (VP) has been hypothesized to have deleterious renal effects. In rodent models of diabetes, it has been shown to cause hyperfiltration, albuminuria and glomerulosclerosis. A potential role of VP in diabetic nephropathy has not yet been investigated in human epidemiological studies. We aimed to investigate whether copeptin, a surrogate for VP, is associated with progression of diabetic nephropathy.

Methods: Type 2 diabetes patients treated in primary care were included in the ZODIAC cohort study. We analyzed the association of baseline plasma copeptin with (change in) albumin/creatinine ratio (ACR) and eGFR (MDRD). Linear regression models were applied, crude and adjusted for possible confounders: gender, age, baseline ACR/eGFR, and CKD risk factors (e.g. HbA1c, body mass index, smoking).

Results: Samples of 976 patients were available (46% male, median age 68 (IQR 58-75) y, ACR 16 (8-50) mg/g, eGFR 66.8 ±14.3 mL/min/1.73m^2). Copeptin (5.4 (3.1–9.5) pmol/L) was significantly associated with ACR (r=0.13, p<0.001) and eGFR (r=-0.14, p<0.001). These associations remained significant after adjustment for confounders (std B=0.09, p=0.005 and std B=-0.21, p<0.001 resp.). Patients were followed for 9.0 (6.0-9.9) y, during which change in ACR was 0.2 (-1.3 to 3.5) mg/g/y (n=439) and change in eGFR was -1.0 (-2.1 to 0.1) mL/min/1.73m^2/y (n=753). Baseline copeptin level was significantly associated with rate of change in ACR and eGFR during follow-up independent of potential confounders and baseline ACR and eGFR (std B=0.10, p=0.04; std B=-0.08, p=0.04, resp.). The association of copeptin with both increase in ACR and decrease in eGFR was stronger than that of several established risk factors for diabetic nephropathy.

Conclusions: In diabetic patients, higher baseline copeptin level is significantly associated with an increase in ACR and a decline in eGFR during follow-up, independent of and stronger than several traditional CKD risk factors.

TH-PO538

Structural–Molecular Relationship in Early Diabetic Nephropathy Claudiu V. Komorowsky,[1] Viji Nair,[1] E. Jennifer Weil,[2] Berne Yee,[3] Kevin V. Lemley,[4] Robert G. Nelson,[2] Matthias Kretzler.[1] *[1]Division of Nephrology, University of Michigan, Ann Arbor, MI; [2]Diabetes Epidemiology and Clinical Research Section, NIDDK, NIH, Phoenix, AZ; [3]Southwest Kidney Institute, Phoenix, AZ; [4]Children's Hospital LA, University of Southern California, Los Angeles, CA.*

Background: Morphological changes in kidney ultrastructure precede functional impairment in diabetic nephropathy (DN). Integrating structural changes with genome wide molecular profiles may identify molecular pathways associated with ongoing intra–renal damage in patients with minimal clinically detectable disease.

Methods: Protocol kidney biopsies were obtained from 40 participants with type 2 diabetes (T2DM) in the "Renoprotection in Early Diabetic Nephropathy in Pima Indians Trial" (NCT00340678). Urine albumin/creatinine ratio (ACR) near time of biopsy was 44 (15–106) mg/g and GFR was 139 (112–167) ml/min (geometric mean and interquartile range). Gene expression profiling and quantitative morphometric analysis of fractional interstitial area (FIA) were performed on the kidney tissue. Transcripts correlating significantly with FIA (False discovery rate q≤0.05, |r|=0.36–0.68) were analyzed for underlying regulatory concepts and related to clinical disease manifestation (ACR).

Results: FIA was higher in T2DM than in living donors (29.5% *vs*. 11.3%; p<0.05). Transcripts correlating positively with FIA suggested activation of migratory (*e.g*. CDC42 and integrin signaling) and inflammatory (*e.g*. interleukin signaling) pathways; negative correlations were seen for transcripts enriched for differentiated tubular metabolic functions. Morphogenomic signatures correlated with ACR at time of biopsy in only 3/651 FIA–linked genes. However, 345 FIA–linked genes correlated strongly (q≤0.05, |r|=0.50–0.75) with disease progression as determined by ACR 4.2 years (range 2–6) post biopsy.

Conclusions: Integration of intra–renal transcripts and quantitative structural changes facilitates identification of molecular mechanisms in DN. FIA–linked genes may enable identification of urinary markers of detrimental intra–renal disease processes already active in patients with no or minimal clinical disease activity.

Funding: NIDDK Support, Other NIH Support - P30 DK081943

TH-PO539

The Association of Gene Variants within the Renin Angiotensin System with Diabetic Nephropathy in a Malay Population in Malaysia Joyce Siew Mei Tan,[1] Munavvar Zubaid Abdul Sattar,[2] Wan Ahmad Hafiz Wan Md Adnan,[1] Nor Azizan Abdullah.[1] *[1]University Malaya, Kuala Lumpur, Malaysia; [2]Universiti Sains Malaysia, Penang, Malaysia.*

Background: Diabetes mellitus (DM) could lead to microvascular complications such as diabetic nephropathy (DN) and this is the predominant cause of end stage renal disease in Malaysia. The renin-angiotensin system (RAS) has commonly been implicated in the development of DN. Association studies linking the development of DN and genetic variants within the RAS produce conflicting results because of the heterogeneity in a population. To rule out this discrepancy, we carried out a hospital-based study on a cohort of Malay population, the main ethnic group in Malaysia, to determine whether variants in the RAS genes were associated with the development of DN.

Methods: A total of ten single nucleotide polymorphisms (SNPs) within the RAS genes were analysed by real-time polymerase chain reaction in 497 malay individuals consisting of 290 cases with Type 2 DM and 207 patients with DN. The DN cases included in the study were T2DM cases with serum creatinine >132 µmol/L in men or >115 µmol/L in women and microalbuminuria (urine albumin-to-creatinine [uACR] >2.5 mg/mmol creatinine in men and >3.5 mg/mmol creatinine in women). Haplotypes were estimated using the Haploview software.

Results: A significant association was observed in ACE gene variant rs1800764 (T>C) SNP with DN (χ^2=8.309, p=0.016) and this association was shown to be influenced by the male gender (χ^2=6.295, p=0.043). The ACE Ins/Del polymorphism showed an almost significant association with DN and significant association was observed in the female gender (χ^2=7.285, p=0.026). Logistic regression analysis of a co-dominant model revealed that the Ins/Del genotype is significantly associated with less risk of DN (OR=0.426, CI 0.193-0.941) and stratification to female gender maintained that only the Ins/Del heterozygote confers protection against the progression to DN. Haplotype analysis exposed a tight linkage between the two variants.

Conclusions: Our data enables us to provide evidence of association between genetic variants in the RAS genes with type 2 diabetic nephropathy in the malay population in Malaysia.

Funding: Government Support - Non-U.S.

TH-PO540

Acidosis and Mortality in Intensive Care Unit (ICU) Patient's on Continuous Renal Replacement Therapies (CRRT): Classical versus Stewart's Approach Paolo Lentini,[1] Luca Zanoli,[2] Massimo de Cal,[3] Andrea Contestabile,[1] Anna Basso,[1] Claudio Ronco,[3] Graziella Berlingò,[1] Antonio Granata,[1] Valentina Pellanda,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, St Bassiano Hospital, Bassano del Grappa (Vi), Italy; [2]University of Catania, Italy; [3]Nephrology, St Bortolo Hosp, Vicenza, Italy; [4]Neph, St G di Dio Hosp, Agrigento, Italy.*

Background: Acid-base disorders are common indications for CRRT in ICU.If unrecognized they may result in poor outcomes.Stewart approach may be superior for acid-base analysis in the critically ill. AIM:Assessment of Classical and Stewart approaches for the analysis of acid-base disturbances during CRRT and mortality risk at 30 days.

Methods: We enrolled 40 consecutive adult patients on CVVH and mechanical ventilation. All patients received a 35 ml/Kg/h infusion of a standard buffer [5 Liters(mmol/L):[HCO^{-3}]35,[Na^+]140,[K^+]2,[Ca^{2+}]1,75,[Mg^+]0.5,pH 7,4]. We calculated[pH] and SBE with the Henderson-Hasselbach and Siggaard-Andersen equations. Physicochemical analysis was performed using the Stewart equations modified by Figge et al.Apparent strong ion difference (SIDa) was calculated as:SIDa,mEq/L=[Na^+]+[K^+]+[Mg^+]+[Ca^{2+}]-[Cl^-]-[lactate]. Effective SID(SIDe) was calculated as:SIDe, mEq/L=1000x2.46x10-11x$PaCO_2$/(10-pH)+[albuminemia]x(0.123 x pH-0.631)+[PO4-]x(0.309xpH-0.469).Acidosis was defined by SBE<-5mEq/l, pH<7.35, SIDa<40mEq/l or SIDe<38mEq/l.

Results: The prevalence of acidosis,assessed by pH,SBE,SIDa or SIDe,was significantly different (P<0.001)at each step. At 0,6,12 and 24hr from start of CVVH,pH<7.35 was present in 60,33,20 and 10% of patients;SBE<-5mEq/l in 35,10,10 and 5%; SIDe<38mEq/l in 100,88,65 and88%; SIDa<40mEq/l in 73,60,48 and 43% respectively. 58% of patients (n=23)died within 1 month from ICU admission.The risk of death was significantly higher for reduction of SIDa(P<0.01), SIDe(P<0.05) and SBE(P<0.05) but not pH (P=NS),independently to APACHE II score and gender.16 of 17 patients (94%) with SIDa<40mEq/l at 24h died within 1 month from ICU admission(the relative risk of death was 3.1).

Conclusions: Stewart approach seems to be more sensitive for detection of acidosis in ICU patients on CRRT and has a greater impact to mortality than Classical approach.

TH-PO541

Continuous Renal Replacement Therapy Outcomes in Acute Kidney Injury and End Stage Renal Disease Andrew Allegretti, David J.R. Steele, Jo Ann David-kasdan, John Niles, Ishir Bhan. *Department of Medicine, Division of Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Continuous renal replacement therapy (CRRT) is a resource-intensive therapy used in critically ill patients. Assessment of prognosis is needed to optimize use of limited resources and provide appropriate guidance to patients. CRRT is used in critically ill patients with either acute kidney injury (AKI) or end-stage renal disease (ESRD), but most prior studies have restricted inclusion to AKI.

Methods: All adult patients who were initiated on CRRT at an academic tertiary care center between January 1, 2008 and September 30, 2011 were followed prospectively. Baseline demographic and laboratory data were collected, and comorbid illness was assessed with the Charlson score. Subjects were followed to assess survival (in all patients) and renal recovery (in patients with AKI). Predictors of inpatient outcomes were assessed with univariate models and multivariate logistic regression. For patients who survived until discharge, predictors of survival post-discharge were assessed with Cox proportional hazards models.

Results: 725 patients with AKI and 138 patients with ESRD were included in the analysis, with similar in-hospital mortality in both groups (60.7% vs. 54.4%). In patients with AKI, factors independently associated with in-hospital mortality included age > 60 years (1.6, 95% CI 1.2-2.2) and Charlson score > 3 (HR 1.5, 95% CI 1.0-2.1. Outpatient predictors of mortality were unchanged from their inpatient counterparts for these subjects. In patients with ESRD, Charlson score > 3 (OR 2.8, 95% CI 1.1-6.8) and admission to a medical (vs. surgical) intensive care unit (ICU) (OR 2.6, 95% CI 1.2-5.5) predicted in-hospital mortality, but age demonstrated no such association. In contrast, age was the single independent predictor for survival in ERSD patients following discharge (HR 3.9, CI 1.4-11). 11% of subjects with AKI recovered renal function prior to discharge.

Conclusions: Mortality rates are similar in both AKI and ESRD associated CRRT. While age, comorbid illness, and ICU type predict outcomes, the relative weight of these predictors differs depending on baseline renal function.

Funding: Clinical Revenue Support

TH-PO542

RIFLE and Acute Kidney Injury: Old Recipe for New Problems? Pedro Francisco Azevedo,[1] Raquel Gil,[2] Hugo Mário Silva,[1] Pedro V.A. Aguiar,[1] Anibal Marinho.[3] *[1]Nephrology, CHP-HSA, Oporto, Portugal; [2]ICBAS, Oporto, Portugal; [3]Intensive Care Unit, CHP-HSA, Oporto, Portugal.*

Background: The wide variation in definitions for acute kidney injury (AKI) has made it difficult to compare results across studies and populations, with controversial results in incidence and prognosis. The RIFLE and AKIN classifications were proposed in an attempt to solve the dilemma and several studies support its clinical relevance in assessing AKI severity and predictive ability for mortality in the intensive care unit (ICU). However, the extent to which one classification is superior to another remains unanswered. The purpose of the study was to evaluate the incidence of AKI, defined and stratified according to the RIFLE and AKIN classification and its impact on mortality.

Methods: Prospective study conducted over a period of ten months, in a polyvalent ICU. Demographic data, mortality rate, serum creatinine prior to admission, variability in the amount of serum creatinine in the current admission and its stratification for RIFLE and AKIN. We excluded all patients with less than 5 days in this Unit.

Results: We studied 104 patients with a mean age of 66,5±14,8; 57,7% male (n=60), mostly admitted after emergent surgery, an average length of stay in ICU of 19,2±16,0 days and a mortality rate of 11,5%. In 2 patients (1,9%) it was not possible to obtain baseline serum creatinine. The remaining 102 patients were categorized, according to the RIFLE criteria: 19 patients in class R(mortality 0%), 25 patients in class I(mortality 8%) and 12 patients in class F(mortality 25%). Applying the AKIN criteria: 23 patients were in the stage 1(mortality 17,4%); 19 patients in stage 2(mortality 0%) and 7 patients in stage 3(mortality 42%).

Conclusions: The incidence of AKI in critically ill patients is high and with a significant impact on mortality. Applying both criteria, RIFLE and AKIN, we found that RIFLE classification was more sensitive evaluating the mortality rate in patients with AKI than AKIN classification. Contrary to some published studies, the AKIN classification did not further improve the sensitivity, robustness and ability of RIFLE to predict short-term outcome, such as in-hospital mortality in critically ill patients.

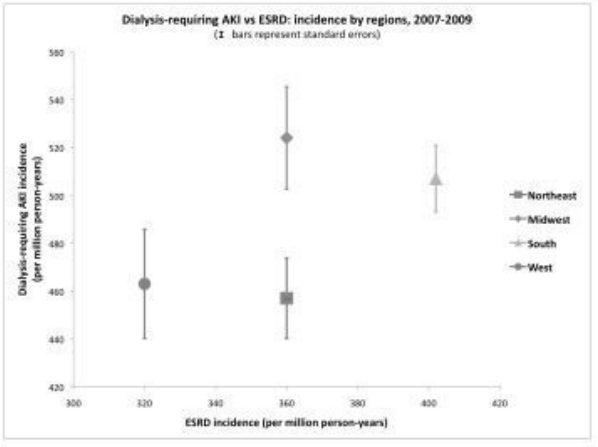
TH-PO543

Regional Variation in the Incidence of Dialysis-Requiring Acute Kidney Injury in the United States Raymond K. Hsu, Charles E. McCulloch, R. Adams Dudley, Chi-yuan Hsu. *UCSF.*

Background: While geographic variation in the incidence of end-stage renal disease (ESRD) in the U.S. is well characterized, little is known about geographic differences in the incidence of dialysis-requiring acute kidney injury (AKI).

Methods: We performed a cross-sectional analysis using data from the Nationwide Inpatient Sample (NIS), a nationally representative sample of hospitalizations, to determine the incidence rates of dialysis-requiring AKI between 2007-2009 amongst the four U.S. Census Bureau designated regions. Cases were identified using validated ICD-9-CM codes (Waikar JASN 2006). Poisson regression models were used to estimate overall regional rates and standard errors, accounting for the NIS survey sampling scheme. Results were compared with regional incidence rates of renal replacement therapy-requiring ESRD, obtained from the U.S. Renal Data System.

Results: In 2007-2009, the population incidence rates of dialysis-requiring AKI differed across the four Census designated regions. Incidence was highest in the Midwest (523 cases/million person-years, 95% CI 483-568) and lowest in the Northeast (457 cases/million person-years, 95% CI 426-492). The pattern of regional variation does not follow that for ESRD, as ESRD incidence was highest in the South and lowest in the West.



Conclusions: Based on the latest nationally representative hospital discharge data available, there appears to be notable regional variation in the incidence of dialysis-requiring AKI. The pattern of regional variation in incidence of dialysis-requiring AKI appears to differ from the pattern of regional variation in incidence of ESRD. More studies are needed to understand whether these observed geographic variations are due to regional differences in AKI risk factors, practice patterns, or some other cause.

Funding: NIDDK Support

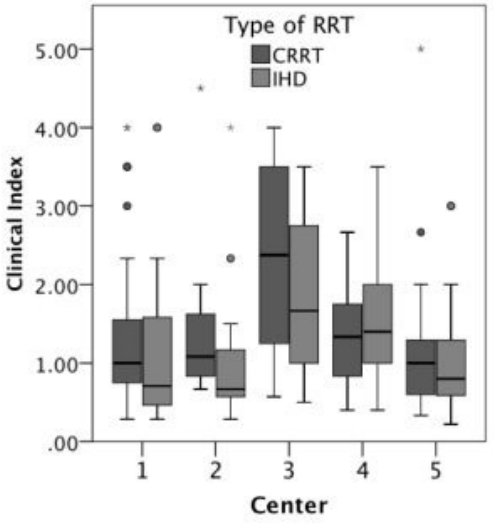
TH-PO544

Practice Variations in Timing of Renal Replacement Therapy Initiation and Modality Choice in Acute Kidney Injury Etienne Macedo,[1] Glenn M. Chertow,[2] Jonathan Himmelfarb,[3] Emil P. Paganini,[4] T. Alp Ikizler,[5] Ravindra L. Mehta.[6] *[1]University of Sao Paulo; [2]Stanford University School of Medicine; [3]University of Washington; [4]Cleveland Clinic Foundation; [5]Vanderbilt University Medical Center; [6]University of California San Diego.*

Background: Currently, there is considerable variation in the timing of initiation for renal replacement therapy (RRT) in patients with AKI. Comparative data has been limited by the absence of standardized measurements for assessing the timing for RRT initiation. We have developed a clinical index (CI) based on clinical and lab data incorporating organ failure scores, solute and fluid balance and renal functional parameters to track changes in a patient's underlying condition. Within our CI, higher values represent a greater severity of renal and non-renal organ dysfunction. We evaluated the applicability of the CI to determine the extent of practice variations for RRT in AKI.

Methods: We analyzed data from 327 critically ill patients with AKI at 5 centers included in the PICARD study from Feb99 until Aug01. We computed a daily value for the CI starting from 48h prior to peak sCr (non dialyzed 140) and RRT start (dialyzed 187).

Results: The reasons for starting RRT were different across centers. The CI was progressively higher in dialyzed patients. There was a significant difference across centers at the level of CI at RRT initiation. Within each center, there was a wide range of CI to start RRT. Intermittent modalities tended to be started in less severely ill patients. The patterns for initiating RRT and modality choice remained consistent.



Conclusions: We demonstrated a considerable variation in the start of RRT and the choice of modality for management of AKI. The utilization of a standardized CI could help quantify changes in practice patterns for RRT within and across centers and providers.

Funding: NIDDK Support

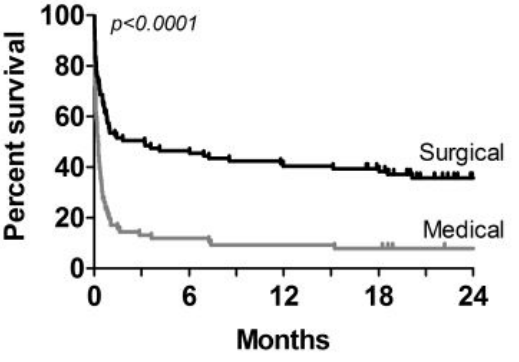
TH-PO545

The Use of Continuous Renal Replacement Therapy in Surgical versus Medical Patients Vijay G. Menon, Alagappan A. Annamalai, Rex Chung, Catherine Bresee, Hector J. Rodriguez, Steven D. Colquhoun. *Cedars-Sinai Medical Center.*

Background: The use of continuous renal replacement therapy (CRRT) is resource intensive. The optimal patient populations to benefit are yet to be defined. Two groups with different clinical end-points include those patients in surgical versus medical intensive care units. This study was carried out to discern difference in outcomes between those surgical vs. medical patients.

Methods: A retrospective study assessing survival of all patients undergoing CRRT in 2010. Surgical vs. Medical patients were compared. Surgical patients were defined as pre and post-operative at the time of CRRT initiation. Medical patients were defined as all others. All patients were first assessed by a single nephrology consult service using standard criteria: 1) oligura and volume overload with failure of non-renal replacement therapy, or severe metabolic acidosis requiring administration of high dose sodium bicarbonate, or severe alterations of extracellular fluid composition and 2) hemodynamic instability precluding intermittent dialysis.

Results: Data was from from 175 ICU patients undergoing CRRT. The median survival time for surgical was 97 days vs. 7 days for medical (p<0.0001). Further analysis showed significant differences in short and long term outcomes between surgical and medical cases, respectively, with one week, one month and one year survival rates of 73.7% vs. 50%, 53.5% vs. 19.7% and 41.4% vs. 7.9% (p<0.0001).



Conclusions: High costs and intensive resources required for the use of CRRT justify a closer assessment of candidacy. Different end-points in clinical care can be used to define and identify patients who may derive maximal benefit. Among patients undergoing CRRT, there is a significant difference in survival between patients defined as Surgical versus Medical. Further analysis of such sub-groups appears relevant to the decision to initiate CRRT.

TH-PO546

Sunday Dialysis Initiation: The Impact in Severe Acute Kidney Injury Francis P. Wilson, Wei (Peter) Yang, Harold I. Feldman. *Department of Medicine, Perelman School of Medicine at University of Pennsylvania, Philadlphia, PA.*

Background: Initiation of dialysis for acute kidney injury (AKI) occurs at a lower rate on Sundays, although the magnitude of the relationship between day of the week implementation of dialysis therapy has not been evaluated. It is unclear whether lower rates of dialysis on Sundays leads to adverse outcomes.

Methods: We identified adults who suffered severe in-hospital AKI from 1/1/2004 – 8/31/2010 at three hospitals in the University of Pennsylvania Health System. Patients were included if baseline creatinine was ≤1.4mg/dl for men or ≤1.2 mg/dl for women, and serum creatinine doubled during the hospital admission. A logistic regression model was fit to evaluate the likelihood of initiation of dialysis on a given day. Patients who initiated dialysis on a Sunday were propensity-score matched to patients not dialyzed on a Sunday. Cox proportional hazards models examining overall mortality were used to evaluate the potential benefit associated with dialysis therapy in this matched cohort.

Results: In the overall cohort (n=6,119), Sunday was negatively associated with dialysis with adjusted OR 0.55 (0.39 – 0.75) versus other days of the week. However, patients initiated on dialysis on a Sunday (n=56) were similar to those initiated on other days of the week (n=546) in terms of severity of illness and comorbidities, and suffered a similar rate of mortality. After propensity-score matching, the overall risk for Sunday initiation (vs. non initiation) was 0.93 (0.55 – 1.57, p=0.791). Initiation of dialysis on a non-Sunday (vs. non-initiation) carried an overall HR for mortality of 0.93 (0.78 – 1.10, p=0.390). These HRs were not statistically different (p=0.987). Stratifying across categories of BUN or serum creatinine did not modify this relationship.

Conclusions: Patients with AKI are less likely to initiate dialysis on a Sunday. Patients initiated on Sunday do not seem to be more ill than those initiating on any other day of the week. No excess mortality was detected that could be attributable to non-dialysis on a Sunday, even among those with markers of worse renal dysfunction.

Funding: NIDDK Support

TH-PO547

Early Initiation of Continuous Renal Replacement Therapy Improves Survival in Severe Progressive Sepsis Patients with Acute Kidney Injury

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Background: The definition of 'early' in terms of continuous renal replacement therapy (CRRT) initiation has not been uniformly used. Therefore, we tried to elucidate whether the timing of CRRT application, based on the interval between the start time of vasopressors infusion and CRRT initiation, was an independent predictor of mortality in progressive sepsis patients with acute kidney injury (AKI).

Methods: Progressive sepsis patients with AKI, in whom the infusion doses of vasopressors were increased compared to the initial dose during the first 6-hour of vasopressor treatment and CRRT was performed between 2009 and 2011, were collected and divided into two groups based on the median interval between the two points. Cox proportional hazard analysis was performed to determine the independent prognostic power for all-cause mortality of early initiation of CRRT treatment. Kaplan-Meier curves were also constructed to compare the difference in all-cause mortality between the two groups.

Results: A total of 210 patients were included. The mean age was 62.4 years, and 126 patients (60.0%) were male. The most common comorbid disease was malignancy (53.8%), followed by hypertension (35.7%) and diabetes mellitus (29.0%). The median interval between the start time of vasopressor infusion and CRRT commencement was 2.0 days. During the study period, 156 patients (74.3%) died within 28 days of CRRT application. The interval between the two points was significantly shorter in the survivor compared to the death group (P<0.001). In addition, 28-day overall mortality rates in the early CRRT group were significantly lower than those in the late CRRT group (P=0.034). Moreover, early CRRT treatment was independently associated with lower mortality rates even after adjustment for age, gender, causative organisms, and infection sites (P=0.032).

Conclusions: Early initiation of CRRT may be beneficial in severe progressive sepsis patients with AKI, in whom the requirement dose of vasopressor is increasing.

TH-PO548

How Common Is Cardiorenal Syndrome in Patients Admitted to the Intensive Care Unit?

Kelly V. Liang,[1] Mark L. Unruh,[1] Florentina E. Sileanu,[2] John A. Kellum.[2] *[1]Renal, University of Pittsburgh; [2]Critical Care, University of Pittsburgh.*

Background: Critically ill patients with cardiorenal syndrome (CRS) have poor outcomes and commonly require renal replacement therapy (RRT). We aimed to examine characteristics associated with risk, timing, and modality of RRT as well as influence of RRT on outcomes in critically ill CRS patients.

Methods: We examined critically ill subjects admitted to UPMC ICU over 4 years (2000-2004) using the High Density Intensive Care (HiDenIC) database. CRS was defined based on heart failure (HF) ICD-9 code on admission and estimated GFR 15-60 mL/min. Three patient groups were identified: 1) CRRT (either initially or after IHD), 2) IHD only, and 3) No RRT. Prevalence of CRS and incidence of RRT were determined. Patient characteristics and clinical outcomes were compared.

Results: Of the 21,152 patients in the cohort, 3,680 had HF codes on admission, and 2,809 had both HF and renal dysfunction; thus, CRS was present in 76% of the HF population admitted to the ICU and 13.3% of the total ICU population. Of the 2,809 patients with CRS, 1,272 patients had acute kidney injury (AKI) with maximum RIFLE score of F. Of these patients, 362 (28.5%) received RRT (175 IHD, 187 CRRT). Patients receiving RRT were younger (62 vs. 70 years) and had higher APACHE 3 scores, especially the CRRT group (87.7 vs. 76.2 vs. 64.4 in CRRT vs. IHD vs. none). The CRRT group had higher hypotensive index than the IHD group (27.8 vs. 8.0). Hospital mortality was greater in patients receiving RRT, especially the CRRT group (mortality rates 64% vs. 25% vs. 16% in CRRT vs. IHD vs. none). Hospital and ICU LOS were greater in the RRT vs. no RRT group, especially the CRRT group (mean hospital LOS 35 vs. 26 vs. 16 days; mean ICU LOS 22 vs. 12 vs. 7 days in CRRT vs. IHD vs. none).

Conclusions: The majority (76%) of patients admitted to ICU with HF have CRS. Severe (RIFLE-F) AKI accounts for nearly a third of these patients and RRT is used in 28.5% of those with severe AKI. Hospital mortality, hospital LOS, and ICU LOS are greater in those receiving RRT. These findings underscore the need for further studies to determine the appropriate use and timing of RRT in the CRS population in order to improve outcomes.

Funding: NIDDK Support

TH-PO549

Extracorporeal Oxygen Delivery and Carbon Dioxide Removal Using Hemodialysis Equipment

Scott D. Bieber,[2] Suhail Ahmad.[2] *[1]Medicine, Nephrology, University of Washington, Seattle, WA; [2]Scribner Kidney Center Research, Northwest Kidney Centers, Seattle, WA.*

Background: Abnormal pulmonary function is a common problem in acutely ill patients requiring dialysis in intensive care units. Lung protective ventilation and fluid removal by dialysis are often the only options for these patients with grave prognosis. Improving oxygenation and reducing pCO2 may help improve outcome. Extracorporeal membrane oxygenation systems (ECMO) have been utilized for such a purpose but they are expensive and require highly trained staff to operate them. As an alternative, a simple, cost effective modification of existing hemodialysis technology could provide enhanced gas exchange in critically ill patients already on continuous renal replacement therapy. The modified dialysis set up and preliminary in vitro data in support of our hypothesis is presented here.

Methods: A typical hemodialysis circuit was modified by placing an additional gas exchanging dialyzer in series with the traditional hollow fiber dialyzer used for dialysis. Oxygen was delivered at varying rates to the empty dialysate compartment of the gas exchanging dialyzer. Blood was then circulated in the blood compartment of both dialyzers and blood gas measurements were taken from sample ports in the circuit before and after each dialyzer.

Results: There was a significant increase in blood pO2 of 116 mm Hg and decrease in pCO2 of 18 mmHg across the dialyzer.

Results

	Before O2 Delivery	After O2 Delivery	Delta	p value
Mean pO2 (mmHg)	158 (SD 27.2)	273.5 (SD 46.7)	+116	0.01
Mean pCO2 (mmHg)	60.4 (SD 5.7)	42.4 (SD 5.8)	-18	0.009

Conclusions: Our data shows that significant gas exchange can be provided by dialysis machines with a simple modification of existing technology. Future studies will utilize additional modifications to further enhance gas exchange.

TH-PO550

Nationwide Japanese Survey of Continuous Renal Replacement Therapy for Acute Kidney Injury in Children

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Background: Continuous renal replacement therapy (CRRT) and apheresis therapy are used for children with a life-threatening illness, including acute kidney disease (AKI). However, the current state of CRRT for childhood AKI is unknown in Japan.

Methods: A retrospective nationwide survey of CRRT in April 2007–March 2009 was performed. A total of 669 patients were reported from 121 institutions and 283 patients received CRRT due to AKI.

Results: The median age was 2.3 years (<1year; 40%), median weight was 12.8 kg (<10 kg; 40%). The median duration of CRRT was 15.3 d. Major treatment modalities were CVVHDF (n=139), CVVHD (n=113), CVVF (n=28), plasmapheresis (n=38), and direct hemoperfusion with polymyxin B (n=38). Major anticoagulant agents were nafamostat mesylate(80%) and heparin sodium (17%). Main primary diseases of AKI were renal (34%), cardiovascular (19%), hematological and oncological (14%), sepsis (8%), neuromuscular (7%) and gastrointestinal (5%). The total survival rate was 58%. Multivariate analysis for the risk of death showed that respiratory failure (odds ratio (OR)5.4; 95%CI, 1.6-18) and conscious disturbance (OR5.2; 95%CI, 1.6-18) had a high risk. However, sepsis (OR2.1; 95%CI, 0.9-4.6) and body weight less than 10 kg (OR1.6; 95% CI, 0.76-3.1) did not affect survival. The survival was poor for cardiovascular (74%), hematological and oncological (71%), sepsis (79%) and gastrointestinal (62%), but excellent for renal (6%) diseases. Long-term prognosis approximately 4 years after CRRT among 131 of 283 patients was evaluated (117 living, 5 dead, 9 unknown). Twenty-seven patients had progressed to end stage renal diseases (peritoneal dialysis n=15, hemodialysis n=2, renal transplantation n=2). Interestingly, 22 of 27 patients suffered renal disease as the primary disease.

Conclusions: Although many young children received CRRT for AKI, the survival was relatively satisfactory. Non-renal disease has a poor life prognosis in the acute phase, but renal diseases have poor long-term renal prognosis.

Funding: Government Support - Non-U.S.

TH-PO551

Phosphate Mobilization Clearance during Continuous Renal Replacement Therapies

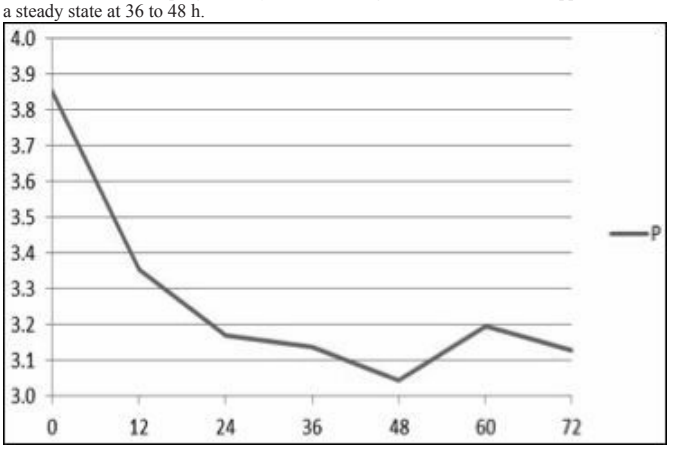
Rolando Claure-Del Granado,[1] Soo Young Yoon,[2] Ravindra L. Mehta.[3] *[1]Universidad del Valle, Bolivia; [2]Kwandong University, Korea; [3]University of California San Diego.*

Background: The kinetics of plasma phosphorus (P) during CRRT is incompletely understood. Various models for P kinetics have been described, however neither conventional one nor two-compartment models can be used. Leypolt et al have developed a pseudo one-compartment model to describe P kinetics. We evaluated the applicability of this model for P mobilization clearance (K_mP) in patients with AKI treated with CRRT.

Methods: We analyzed data from 76 critically ill patients with AKI treated with pre-dilution CVVHDF. P concentrations were measured at the begining and at the end of each treatment. Filter efficacy was assessed by measuring effluent to blood urea nitrogen ratio (FUN/BUN). We compute dialyzer P clearance (K_DP). K_mP was estimated using the

following equation: $K_mP = Pf((K_DP–Quf)/(Pi – Pf))$; where *Pi and Pf* are P at the begining and at the end of treatment, *Quf* is ultrafiltration rate, and K_DP is dialyzer P clearance.

Results: P levels progressively decline during CRRT treatment and appear to reach a steady state at 36 to 48 h.



K_DP (mL/min) was not constant during treatment (12 h 26.9±7.9; 24 h 24.5±7.8; 36 h 24.3±7.5; 48 h 26.8±8.0; 56 h 25.7±13.3; 72 h 25.3±10.0; p <0.001). K_mP(mL/min) increased in consecutive days of treatment (118.9±137.0, 144.7±101.5, and 161.3±198.6; p < 0.001). Mean FUN/BUN ratio was 0.98±010 (day 1), 0.93±0.17 (day 2), and 0.94±0.16 (day 3), filter efficacy appear not to influence K_mP. No correlation was found between K_mP and post CRRT body weight ($r^2 = 0.071$; p = 0.170), effective treatment time ($r^2 = 0.005$; p = 0.552), or net UF ($r^2 = 0.015$; p = 0.901).

Conclusions: The estimation of K_mP provides a tool to better assess P kinetics during CRRT. This parameter could help to optimize and individualize the CRRT prescription and determne the amount of phosphate supplementetion required.

Funding: NIDDK Support

TH-PO552

Inflammatory Mediator Clearance and Mortality among Critically Ill Patients on CRRT Ilson Jorge Iizuka,[2] Beata Marie Redublo Quinto,[1] Maria Dalboni,[1] Bento Santos,[2] Marcelo Costa Batista.[1,2] *[1]Medicine/ Nephrology Division, UNIFESP; [2]Hospital Israelita Albert Einstein.*

Background: Critically ill patients with acute kidney injury (AKI) present high mortality rates. The magnitude of inflammatory response ultimately determines the prognosis of such patients. Continuous renal replacement therapy (CVVHDF) may play role in removing inflammatory mediators in patients with AKI. The aim of this study was to investigate whether the magnitude of inflammatory mediators (TNF-a) removal is associated with mortality among critically ill patients on CVVHDF.

Methods: This study consisted of 64 critically ill patients requiring CVVHDF. Plasma levels of C3a, TNF-a, IL-10, IL-6, IL-1b, sTNFRI and sTNFRII were determined by enzyme-linked immunosorbent assay (ELISA) at the beginning of CVVHDF and after 24h (outlet). Clearance of cytokines during the first 24 hours of CVVHDF was calculated. Clinical and laboratory data were acquired from patient's records data. Statistical analyses were carried out using Q-Square test, T test and ANOVA. Binary logistic regression was processed to investigate mortality predictors in this population.

Results: Mean age of patients requiring CVVHDF was 63 years, 67.2% were men and 87.3% were caucasian. Thirty-five (35) patients (54.7%) died. Comparing non-survivors with the group of survivors we observed higher incidence of sepsis (68.6 versus 37.9%, p<0.05), higher APACHE II score (34.8±7.6 versus 29.2±7.1, p<0.05) and higher lactate levels (23.2±17.6 versus 16.4±6.6, p<0.05). According to the inter-tercile range of TNF-a clearance (ITR1 (<0.008); ITR2 (0.008–1.17); ITR3 (1.18–3.48)) we found that those patients with higher TNF-a removal by RRT (ITR3) had a better survival. Multivariable analysis showed that lower clearance of TNF-a remained independently associated with high mortality after adjustment for sex, age, use of vasoactive drugs, APACHE II score sepsis, creatinine and lactate before CVVHDF (HR: 0.179, 95% IC: 0.049 – 0.661, p<0.01).

Conclusions: The attenuation of inflammatory response may be related to the lower mortality observed on those patients with higher TNF-α removal by CRRT. This study was supported by FAPESP.

Funding: Government Support - Non-U.S.

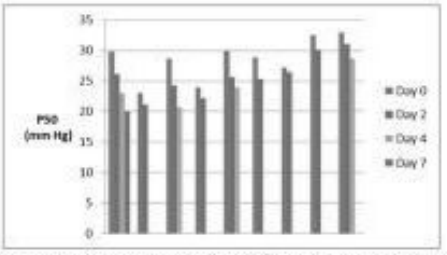
TH-PO553

Effects of Continuous Veno-Venous Hemofiltration on RBC Oxygen-Hgb Curve & 2, 3 Diphosphoglycerate (2,3-DPG) Levels Shilpa Sharma,[1] Carlo Brugnara,[2] Sushrut S. Waikar.[1] *[1]Brigham & Women's Hospital; [2]Children's Hospital, Boston, Boston, MA.*

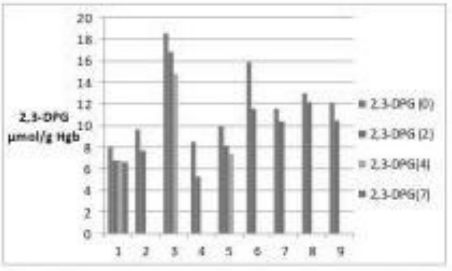
Background: Hypophosphatemia is a frequent complication during continuous renal replacement therapy(CRRT), and may contribute to poor patient outcomes due to phosphate's critical role in energy metabolism in every organ system. Because intracellular phosphate is a constituent of 2,3-DPG and therefore a determinant of the affinity of oxygen to hemoglobin, we sought to determine the physiological effect of phosphate depletion by measuring RBC 2,3-DPG concentrations and Hgb:O2 dissociation curves in critically ill patients with AKI during CRRT.

Methods: Venous blood was collected on day 0, 2, 4, 7 of Continuous Veno-Venous Hemofiltration (CVVH). P50 was calculated using blood gas analyzer with co-oximeter. 2,3-DPG measurement was done by enzymatic cleavage method. We calculated the difference in 2,3-DPG concentrations and P50 from baseline to the last sample collected during CVVH.

Results: We collected multiple blood samples on 9 critically ill, oligo-anuric patients with AKI undergoing CVVH. CVVH was used with citrate or bicarbonate solutions at 1600-3000ml/hr. Blood flow ranged between 200-250 ml/min. Both P50 and 2,3-DPG declined after initiation of CVVH as shown in Figure 1 (p< 0.001). Serum phosphate concentrations correlated with p50 (r=0.72; p<0.001) and 2,3-DPG (r= 0.5; p<0.05).



Mean P50 at CVVH initiation: 28.5 mmHg; Mean decrease at Day 2: 2.8 mmHg(10%)



Mean 2,3-DPG at CVVH initiation: 11.9µmol/g Hgb; Mean decrease at Day 2 by 1.9 µmol/g Hgb (17%)

Conclusions: CVVH can lead to significant phosphate depletion and may cause physiologically important effects on oxygen-hemoglobin affinity causing inadequate tissue oxygen extraction by reducing intracellular RBC 2,3-DPG levels and p50 levels. Our results suggest that CVVH may have unintended adverse effects from excessive small solute clearance. Hypophosphatemia should be anticipated and avoided during the course of therapy with CRRT.

Funding: Other NIH Support - NIH Academic Nephrology Training Grant T32 DK007527

TH-PO554

Fibroblast Growth Factor-23 Clearance by Continuous Veno-Venous Hemofiltration in Acute Kidney Injury Shilpa Sharma, Sushrut S. Waikar. *Brigham & Women's Hospital, Boston, Boston, MA.*

Background: Fibroblast Growth Factor (FGF)-23 is a bone derived regulator of phosphate homeostasis. Emerging evidence suggests FGF-23 levels are elevated in acute kidney injury (AKI) and may be associated with adverse clinical outcomes. We evaluated FGF-23 clearance during continuous veno-venous hemofiltration(CVVH).

Methods: We collected plasma/CVVH effluent samples using a partial effluent collection device, continuously sampling 1% of total effluent volume from 8 oligo-anuric patients with AKI. C-terminal FGF-23 levels were measured in effluent and corresponding plasma samples over multiple time points during the course of CVVH. CVVH was performed using biocompatible polyether sulphone membranes (hemofilter size:1.6 m^2), pre-dilution mode using bicarbonate or citrate replacement solution at 1600-3000ml/hr. Sieving coefficients (SC) were determined by dividing the effluent by plasma FGF-23 concentration. Clearance was calculated by multiplying SC's with 24 hr effluent volume.

Results: Baseline characteristics are shown in the table. Mean daily effluent volumes were 60L(range 50–76). We collected 23 timed CVVH effluent/plasma samples. Median plasma FGF-23 concentration at CVVH initiation was 6612.5 RU/ml(IQR: 2368.1–32566.9). Mean SC of FGF-23 was 0.27 (±0.08) and mean estimated FGF-23 clearance was 11.3 ml/min (± 3.25).

Table

Age/Sex	ICU Admission Cause	AKI cause	Peak Phos, mg/dl	Peak Cre, mg/dl	CVVH days	Outcome
54/M	S S	ATN	6	3.6	5	Death
50/M	S S	M	8	2.9	5	Death
66/M	O	ATN	5.9	5.3	15	Dialysis
54/M	S S	ATN	4	3.7	4	Death
56/F	S S	ATN	7.4	2.9	6	Death
79/M	U RF	M	7.1	5	1	Recovery
62/M	O	M	4.4	4.1	3	Death
47/F	U RF	ATN	7.6	3.6	1	Death

Legend: ATN, Acute tubular necrosis; M, Multifactorial; O, Causes of ICU admission other than major surgery, septic shock, respiratory failure; S S, Septic Shock; U RF, Respiratory failure secondary to ARDS

Conclusions: FGF-23 is a 26-kD protein with key role in maintenance of normophosphatemia. We found FGF-23 levels are markedly elevated in patients with AKI requiring CVVH, and that FGF-23 is cleared during CVVH. Future studies will need to clarify FGF-23's role in AKI as a biomarker or mediator of disease outcomes and whether therapies aimed at reducing FGF-23 levels may be beneficial.

Funding: Other NIH Support - NIH Academic Nephrology Training Grant T32 DK007527

TH-PO555

Enhanced Vascular Endothelial Growth Factor and Inflammatory Cytokine Removal with Online Hemodiafiltration overHigh-Flux Hemodialysis in Sepsis-Related Acute Kidney Injury Patients Khajohn Tiranathanagul,[1] Wiwat Chancharoenthana,[1] Nattachai Srisawat,[1] Paweena Susantitaphong,[1] Asada Leelahavanichkul,[1,2] Kearkiat Praditpornsilpa,[1] Kriang Tungsanga,[1] Somchai Eiam-Ong.[1] *[1]Division of Nephrology, Department of Medicine, King Chulalongkorn Memorial Hospital and Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*

Background: Hypercytokinemia plays a central role in the pathogenesis and is related to the high mortality in sepsis-related acute kidney injury (AKI). Besides the established cytokines, vascular endothelial growth factor (VEGF) is demonstrated as an important factor in enhancing vascular leakage in sepsis.

Methods: This prospective randomized trial was conducted to compare the efficacy of cytokine removal between online hemodiafiltration (HDF), which combines convective and diffusive solute removal, and high-flux hemodialysis (HD). Twenty eight sepsis-related AKI patients were included and randomized into online HDF and high-flux HD. The percentages of the reduction ratio in plasma cytokines were measured as primary outcomes. Other clinical parameters were determined as secondary outcomes.

Results: When compared with high-flux HD Online HDF provided significantly greater percentages of the reduction ratio in plasma cytokine levels, including VEGF (52.6±47.6 vs. 11.7±18.9%, p<0.001), IL-6 (44.8±38.8 vs. 6.04±10.4%, p=0.001), IL-8 (58.4±53.8 vs. 13.1±13.9%, p=0.021), IL-10 (47.2±45.9 vs. 11.4±18.4%, p=0.011), and tumor necrosis factor-α (52.9±49.7 vs. 32.9±26.1 %, p=0.029). There were no significant differences in intradialytic blood pressure parameters. Online HDF revealed better 30-day dialysis free (60.7 vs. 35.7%, p=0.01) and shorter median length of hospitalization (16 vs. 24 days, p=0.04) than high-flux HD.

Conclusions: On-line HDF in sepsis-related AKI could provide significantly better removal of VEGF and other cytokines and these were associated with better renal outcome than high-flux HD. Thus, online HDF would offer a potential role in hypercytokinemic state in sepsis-related AKI.

TH-PO556

Hemodiafiltration with High-Flux Dialyzer as an Economic Alternative to High Cut-Off Dialysis among Patients with Kappa Cast Nephropathy Mathieu Rousseau-Gagnon, Mohsen Agharazii, Simon Desmeules. *Service of Nephrology, CHUQ-Hôtel-Dieu de Québec, Quebec, QC, Canada.*

Background: In acute kidney injury due to cast nephropathy, free light chain (FLC) removal with High Cut-Off (HCO) dialysers combined with chemotherapy may be associated with better renal recovery. However, HCO dialysers are expensive and their use is complicated by necessity to replace albumin losses. We examined whether FLC could be efficiently removed by hemodiafiltration (HDF) using less expensive high-flux dialysers.

Methods: In 16 patients with cast nephropathy, 3- to 6-hour HDF sessions were conducted on a daily or alternate-daily basis. Pre-dilution, low volume post-dilution and high volume post-dilution HDF were performed with high-flux dialysers (Bellco's Phylther HF22SD®, Gambro's Polyflux 210H®, Nephral 500® and Fresenius' F250®), and two HCO dialysers (HCO 1100® and Theralite 2100®). Serum free light chains were measured before and after each session to determine FLC reduction ratio for each dialyzer and dilution technique.

Results: Among the high-flux dialysers evaluated, only the Phylter HF22SD® showed clinically adequate kappa (Molecular Weight(MW): 22,500 kDa) FLC clearance. None of the high-flux dialysers cleared lambda (MW: 45,000kDa) FLC effectively. For lambda FLC, HDF with HCO dialysers was optimal with a median reduction ratio(RR) in Postdilution >15L of 82% using TheraLite®. Clearance of kappa FLC by Phylter HF22SD® was influenced by infusion volume and infusion mode. Median kappa RR were as followed; Postdilution >15L: 83%, Predilution >15L: 67%, Postdilution <15L: 59%. Considering that patients with kappa cast nephropathy received a median of 20 HDF treatments and a cost differential between HCO and HF dialysers of 850$, savings of 17,000$ per patient treated can be expected.

Conclusions: In conclusion, HDF with Phylther HF22SD® is a cost-effective alternative to HCO dialysis for kappa FLC removal, whereas HCO-HDF (Theralite®) provides the adequate reduction ratios for lambda FLC removal.

TH-PO557

Ultrafiltration Dosing in Continuous Renal Replacement Therapy: Are We on Target? William Weber, Sagar Patel, Nand K. Wadhwa. *Division of Nephrology, Stony Brook University, Stony Brook, NY.*

Background: The optimal target of ultrafiltration (UF) in continuous renal replacement therapy (CRRT) in fluid overloaded critically ill patients with acute kidney injury (AKI) remains a clinical challenge. The optimal delivery of UF depends on the accurate volume status assessment involving multidisciplinary health care professionals. We investigated physician's CRRT orders compared to actual delivered CRRT treatments to evaluate if goals were achieved.

Methods: We retrospectively reviewed the records on physician prescription orders compared to actual delivered treatments in critically ill patients with AKI. 18 consecutive patients (mean±(SD) age 53±17 years; 12 males, 6 females), admitted to the Intensive Care Unit (ICU) from Feb to Nov, 2011 with AKI requiring CRRT were monitored up to 5 consecutive days. All patients received CRRT using Prisma M100 set with AN69 hemofilter on citrate anticoagulation with an effluent rate of 25-30 ml/kg/hr. Data, including admission weight (WT), daily WT and daily fluid balance calculations, were obtained from electronic medical records and ICU flow sheets which were compared to physician prescription orders.

Results: The mean hours of filter survival was 53hrs. The mean hrs of treatment was 19.9±7.3.The mean pre-CRRT WT (99.0±25.6 kg) was markedly increased when compared to the mean admitting WT (80.2±24.9 kg). After 2 consecutive days on CRRT, the mean weight decreased to 98±23.1 kg which was not significant. However the mean weight on day 5 was 92.9±25.3 which was significantly (p=.01) lower from day 2. Mean total fluid loss over 5 days was 3200mL. The mean prescribed ultrafiltration (UF) rate was 35.3±28.4 mL/hr while delivered UF rate 52.7±48.6 mL/hr on day 2 (p=.02). On day 2, the mean fluid removal set on the Prisma machine was 6495 mL/day while mean net fluid removal was lower at 6014 mL/day which was significantly lower (p=.003).

Conclusions: The net fluid removal was significantly lower than what was set at the Prisma machine. The significant weight loss and fluid removal was achieved by day 5. The actual delivered UF during CRRT treatments exceeded physician prescription orders. However, UF goals still needed to be optimized.

TH-PO558

Lactated Ringer Can Be Safely Used as Part Replacement Fluid in Therapeutic Plasma Exchange (TPE) Chaitanya P. Prabhu, Rajanna Sreedhara. *Nephrology, Fortis Hospitals, Bangalore, Karnataka, India.*

Background: TPE is a major treatment option in GB syndrome (GBS), Myasthenia gravis (MG), etc. Routine TPE involves replacement of plasma with colloids such as albumin, fresh frozen plasma (FFP) and cryosupernatant. Use of colloids is attendant with excessive cost and complications. There have been very few studies looking at partial replacement of plasma by crystalloids such as lactated ringer (RL) and saline. In India, where cost is a major deterrent for TPE, part replacement of plasma by crystalloids can significantly reduce overall cost of treatment. Our study evaluates the safety and efficacy of partial replacement of plasma by RL in TPE.

Methods: This retrospective study included all patients who underwent TPE from July 2007 till June 2011. Up to 1/3 to ½ of replacement fluid was RL, the rest being 4% albumin and FFP. Clinical outcomes and treatment related adverse events were recorded. Other parameters analysed were number of plasma filters used, amount of heparin, serum albumin, serum calcium, haemoglobin, and PT-INR. Results were expressed as mean ± SD.

Results: A total of 35 patients underwent 194 sessions of TPE. Male/Female: 28/7; Age: 54.3 ± 18.2 years; Indication for TPE: MG in 15, GBS in 14, and other causes in 6 patients. Mean volume of plasma removed 3106 ± 417 ml per TPE session. Mean volume of replacement fluid: RL, 1239 ± 348 ml, 4% Albumin, 993 ± 379 ml; FFP, 930 ± 285 ml. Mean BP: Pre-procedure, 128/76 mmHg; lowest during TPE, 116/70 mmHg; and end-procedure 129/77 mm/Hg. Transient hypotension was recorded in 24 sessions and allergic reaction in 2 patients but controlled without interruption of TPE. Clinical Outcomes: Complete improvement in 18; partial improvement in 10, no improvement in 2 and worsening in 5 patients (4 of whom died and 1 left the Hospital against medical advice). All laboratory variables were stable despite 40% of plasma volume replacement done with crystalloid fluid. Cost of replacement fluid was 40% less when compared to use of colloids alone.

Conclusions: In TPE, partial replacement of plasma with RL is safe, clinically-effective and cost-effective. This strategy decreases the cost of replacement fluid by nearly 40% in India.

TH-PO559

Low Dose Enoxaparin versus Regional Citratefor Anticoagulation in Critically Ill Patients Undergoing Continuous Renal Replacement Therapy Ming Shi, Changjian Qiu, Hui Cheng, Wei Liang, Ruhan Jia, Guohua Ding. *Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China.*

Background: Continuous renal replacement therapy (CRRT) is widely used in the treatment of critically ill patients, but the extracorporeal circuit clotting is still one of the greatest problems. In the present, it recommend citrate as an anticoagulation agent in patients who require CRRT but are at high risk of bleeding. Complexity of the regional citrate anticoagulation (RCA) is the major hurdle preventing widespread application. Therefore, alternative anticoagulation methods should be more widely adopted. The purpose of this study is to evaluate the safety and efficacy of low dose enoxaparin for anticoagulation during CRRT.

Methods: From March 2010 to March 2012 a total of 76 patients were included in the study with a total of 305 filters. The patients undergoing predilute continuous venovenous hemofiltration (CVVH) were randomly divided into enoxaparin group and citrate group. The following recommendation for enoxaparin dosing during CVVH: a loading dose of 40IU/kg followed by a maintenance infusion rate (per 4-hours 20IU/kg). Citrate group adopt the 4% sodium citrate and conventional RCA anticoagulant regiment.The following indicators were collected for analysis, including the filter patency, ionized calcium level,blood pH, activated the partial thromboplastin time (APTT), prothrombin time (PT), platelet count and bleeding event.

Results: The circuit life span in the enoxaprin group was no significantly difference compared with that in the citrate group (23.5 ± 12.7 h vs. 25.1 ± 13.8 h, p = 0.04). Two patients developed minor bleeding and no patient developed severe bleeding episodes in enoxaparin group and only one patient developed minor bleeding in citrate group. Two patients with hypocalcemia and two patients with metabolic alkalosis in citrate group but are transient.

Conclusions: The efficacy of enoxaparin andcitrate anticoagulation for predilution CVVH was similar. We recommend low dose enoxaparin as an alternative anticoagulation agent in critically ill patients undergoing continuous renal replacement therapy.

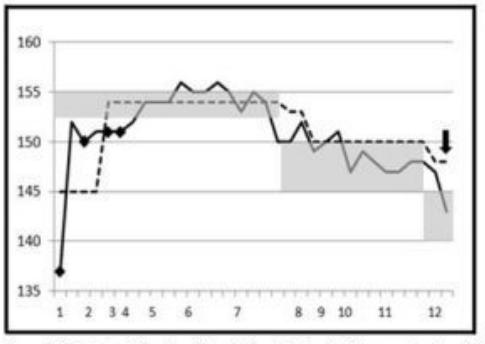
TH-PO560

High Sodium Continuous Renal Replacement Therapy for Liver Failure Patients with Renal Failure and Cerebral Edema Tamim Hamdi, Mark D. Faber. *Nephrology, Henry Ford Hospital, Detroit, MI.*

Background: Acute liver failure is a rare but devastating condition associated with a high mortality rate. Cerebral edema is the leading cause of death. Osmotherapy consisting of IV mannitol or hypertonic saline remains the cornerstone of managing of cerebral edema. Significant disadvantages of this approach include poor or unpredictable control of serum sodium concentration and extracellular fluid volume.

Methods: We used 24-hour sustained low efficiency dialysis with regional citrate anti-coagulation (SLED-RCA) to accurately control the serum sodium (S_{Na}) (150-155 mEq/L) in five patients with acute liver failure, renal failure, and cerebral edema. We used a Fresenius 2008 K machine in hemodialysis mode to deliver a blood flow of 60 ml/min and dialysate flow of 400 ml/min. Our previously published protocol results in complete removal of infused citrate by the dialyzer. On-line clearance calculations were used to model the time required to reach to target S_{Na}.

Results: All five patients achieved a S_{Na} within 2 mEq/L of target without fluctuations or rebound. Four patients had complete recovery of their renal and hepatic function with reversal of cerebral edema. One patient died of cerebral edema despite reaching the prescribed S_{Na} target. The course of a typical patient who recovered completely is shown.



Legend: X axis indicates time (days), Y axis indicates Na concentration (mEq/L). Solid line indicates achieved S_{Na}. Broken line indicates dialysate Na concentration. Diamonds indicate the administration of 23.4 % saline bullets to increase S_{Na} faster than SLED-RCA alone. Grey zone indicates desired S_{Na}. Arrow indicates end of SLED-RCA therapy.

Conclusions: We describe a novel approach for delivering continuous osmotherapy to patients with acute liver failure, renal failure, and cerebral edema. In comparison to standard therapy, SLED-RCA enables precise titration of SNa without undesirable fluctuations in extracellular fluid volume. A particular advantage of our high efficiency protocol, that results in zero delivery of citrate to patients, is the safety of use in patients with severe liver failure.

TH-PO561

High-Volume Hemofiltration Plus Hemoperfusion as an Adjuvant Therapy for Hyperlipidemic Severe Acute Pancreatitis Shiren Sun,[1] Lijie He,[2] Hanmin Wang.[3] *[1]Xijing Hospital, FMMU; [2]Xijing Hospital, FMMU; [3]Xijing Hospital, FMMU.*

Background: To evaluate the efficacy of high-volume hemofiltration (HVHF) in combination with hemoperfusion (HP) on hyperlipidemic severe acute pancreatitis.

Methods: Twenty patients with hypertriglyceridemic severe pancreatitis admitted to XiJing hospital between May 2009 and May 2011 were included in the present study. Patients were randomly divided into the control group (conventional treatment) and the HVHF+HP group. Patients in the HVHF+HP group received 48 h of HVHF and 2 sessions of hemoperfusion (HP) treatment in addition to conventional treatment after admission. The blood and effluent samples were taken to measure the changes of serum triglyceride and serum cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, IL-10).

Results: With 48 h of HVHF and 2 sessions of hemoperfusion (HP) treatment, there was an improvement in clinical features in HVHF plus HP-treated patients.The patients who accepted HVHF plus HP had significantly better Acute Physiology and Chronic Health Evaluation II scores and Sequential Organ Failure Assessment scores. The serum triglyceride levels were markedly decreased in the HVHF+HP group than in the control group. All serum cytokines after HVHF plus HP treatment were significantly lower than those at the start of HVHF plus HP treatment ($p<0.05$). In contrast, there was no significant change in control patients.

Conclusions: This study indicates that HVHF plus HP, as compared with routine treatment, is a safe and effective treatment for hyperlipidemic severe acute pancreatitis.

TH-PO562

Continuous Blood Purification in the Treatment of Patients with Multiple Organ Failure after Wasp Stings: A Report of 9 Cases Peng Zhang, Shiren Sun, Huang Chen. *Nephrology, Xijing Hospital, Xi'an, China.*

Background: The aim of this study was to evaluate of the effects of continuous blood purification (CBP) in the treatment of patients with multiple organ failure (MOF) after wasp stings.

Methods: Nine patients with multiple organ failure after wasp stings admitted to XiJing hospital between January 2007 and January 2012 were included in the present study. These patients all received CBP treatment in addition to conventional treatment after admission. Among them, six patientsreceivedtreatment of HP (2-3 h/day) using activated charcoal for two days and consecutive continuous veno-venous hemofiltration (CVVH) for 72 hours, three patientsreceivedtreatment of plasma exchange (3000ml) followed by CVVHfor 72 hours. Patients' clinical features, hemodynamics variables and serum chemical tests were monitored every day after admission.

Results: Eventually, 8 patients survived with complete clinical recovery, 1 case died. In the survived patients, there was a significant improvement in clinical features and hemodynamics variables during CBP treatment. Serum glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), blood urea nitrogen (BUN) and creatinine (Cr) returned to normal level. The Glasgow scores increased significantly from 6.4±2.7 to 14.3±3.6 and APACHE II scores decreased significantly from 18.2±6.1 to 6.6±2.3 ($P<0.05$).

Conclusions: Our findings suggest that CBP treatment is significantly effective in improving the outcome of patients with MOF after wasp stings which may be due to its rapid removing the plasma wasp venom and inflammatory mediators from the circulation of patients.

TH-PO563

Citrate Dialysis: An Alternative to Heparin Free Dialysis in Intensive Care Unit (ICU) Patients Alan F. Almeida, Rajaram Rambhau Jagdale, Rasika A. Sirsat, Jatin Piyush Kothari. *Nephrology, P. D. Hinduja National Hospital, Mumbai, Maharashtra, India.*

Background: Renal Replacement Therapy (RRT) for Acute Kidney Injury [AKI] is often complicated by a bleeding tendency precluding the use of heparin. Citrate Dialysis [CD], an alternative to heparin-free saline flush dialysis (HFSFD) has not been prospectively studied in India till date. The present study seeks to compare the effectiveness of CD with and without flushing on dialyzer clotting, reuse of dialyzer and to compare it with HFSFD.

Methods: In this prospective study, 198 heparin free dialysis sessions were performed in 25 patients randomized to 3 groups (Group A-HFSFD, Group B- CD without saline flushing, and Group C- CD with saline flushing). Clinical, biochemical, dialyzer reuse data, etc were collected and analyzed.

Results: The study population was elderly [Mean ±SD 61.4±14.5 years] with high SAPS II scores [Mean ±SD 60.24±17.71]. Out of 198 dialysis sessions, 72, 66 and 60 sessions were done in group A, B and C respectively. When Groups A & B were compared, hemodynamic instability and hence the requirement of inotrope support was higher in group B [p=0.04] than group A. Inspite of this, average reuse of the dialyzer was higher in group B [p=0.018] than A. Though there was no significant difference in dialyzer clotting among the 3 groups, there was a trend towards significantly less dialyzer clotting in the citrate groups (B and C). The delivered dose of dialysis (urea reduction ratio in this study) was not different among the 3 groups. Pre to post Dialysis ionized calcium difference in patients with and without liver dysfunction was not different, indicating the safety of citrate use in patients with liver dysfunction. The dialysis sessions in Group A required more frequent flushing, was more time consuming and labor intensive.

Conclusions: Citrate dialysis appears to be a safe and effective alternative to heparin free saline flushing dialysis in ICU patients with AKI in terms of flushing frequency, dialyzer clotting and reuse of the dialyzer.

Funding: Private Foundation Support

TH-PO564

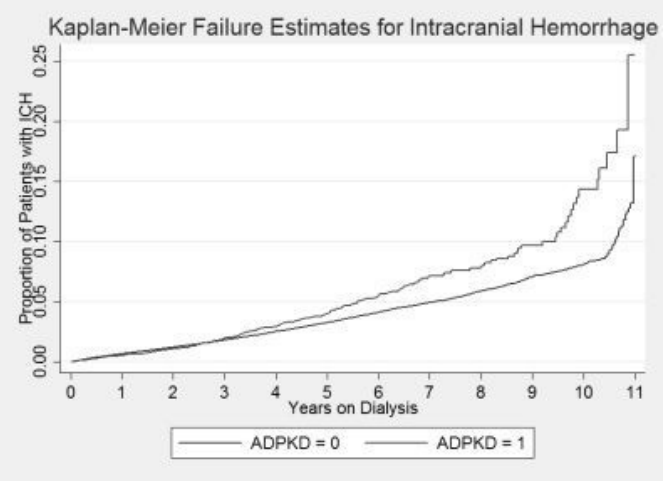
Intracranial Hemorrhage in Patients with Autosomal Dominant Polycystic Kidney Disease on Maintenance Dialysis Robert Nee,[1] David J. Yoo,[1] Lawrence Agodoa,[2] Kevin C. Abbott.[1] *[1]Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [2]NIDDK, National Institutes of Health, Bethesda, MD.*

Background: A ruptured intracranial aneurysm is a potentially life-threatening complication of autosomal dominant polycystic kidney disease (ADPKD). An analysis of intracranial hemorrhage (ICH) in a national sample of ADPKD patients receiving long-term dialysis has not been reported.

Methods: We identified 512,772 Medicare patients in the United States Renal Data System who were initiated on chronic dialysis between 1 January 1999 and July 2009, followed until 31 December 2009. We then assessed Medicare claims for subarachnoid and intracranial hemorrhage.

Results: A total of 8,793 patients (1.71%) developed ICH during the study period, and 6,749 (1.32%) had ADPKD as the primary cause of end stage renal disease. 3.67% of ADPKD patients developed ICH vs 1.96% of non-ADPKD patients (p<.001). ADPKD and non-ADPKD patients had 9.78 and 7.18 episodes of ICH per 1000 person-years, respectively, with an incidence rate ratio of 1.3 (95% CI 1.19-1.55). 0.71% of ADPKD patients had ruptured berry aneurysm vs 0.33% of non-ADPKD patients (p<.001). In Cox regression analysis, ADPKD was a significant predictor for ICH (AHR 1.45, 95% CI 1.26-1.66). Other significant risk factors in the regression included age/year (AHR/year 1.03, 95% CI 1.02-1.03), year of first dialysis treatment (AHR 1.07/year, 95% CI 1.06-1.08) and black race (AHR 1.11, 95% CI 1.06-1.17). Gender, diabetes mellitus, hypertension, alcohol dependence, tobacco use and malnutrition were not significantly associated with ICH.

Conclusions: ADPKD is a significant risk factor for ICH among patients on maintenance dialysis. "The views expressed in this abstract are those of the authors and do not reflect the official policy of the National Institutes of Health, the Department of Defense, or the US government".



TH-PO565

Characteristics and Outcomes of Dialysis Patients with Subarachnoid Hemorrhage Ankit Sakhuja, Jesse D. Schold, Sankar D. Navaneethan. *Cleveland Clinic, Cleveland, OH.*

Background: Chronic dialysis patients are at higher risk for subarachnoid hemorrhage (SAH). SAH has been shown to be associated with over 25% mortality in general population. We studied the characterisics and outcomes of chronic dialysis patients admitted with SAH.

Methods: Using Nationwide Inpatient Sample Database from 2005-2009, patients with primary discharge diagnosis of SAH were identified. Primary outcomes included all-cause inpatient mortality and discharge disposition. Logistic regression analysis was used to examine associations of inpatient mortality in those on chronic dialysis after adjusting for demographics, Charlson's comorbidity index (CCI), primary payer, hospital characteristics and year of admission. Predictors of mortality among those on chronic dialysis with SAH were also studied.

Results: Of 127,274 patients with primary SAH 1400(1%) were on chronic dialysis. Prevalence of SAH admissions by US Census & USRDS data was $77/10^5$ for dialysis vs $11/10^5$ for rest population. Dialysis patients with SAH were younger, more likely African Americans/Hispanics with higher CCI(≥3 in 88.2% vs 17.5%, p<0.0001). Patients on chronic dialysis were admitted less often to teaching hospitals(69% vs 75.3%, p=0.02) and to medium/high volume hospitals(60.8% vs 71.6%, p<0.001). In multivariate adjusted model chronic dialysis was associated with 2.62 times higher odds (95% CI 1.91, 3.62) of mortality. In patients with SAH who required mechanical ventilation, need for chronic dialysis was associated with 3.83 times higher odds(95% CI 2.42, 6.08) of death. Among those with SAH and on chronic dialysis increasing age and need for mechanical ventilation were independent predictors for inpatient mortality. Chronic dialysis patients with SAH were discharged more often to other health care facilities or with additional home care(35.9% & 12.3% vs 33.9% & 7.2% p=0.002).

Conclusions: Chronic dialysis patients appear to have a higher prevalence of SAH and higher mortality and need for specialized care post discharge than those not on chronic dialysis with SAH. Further studies are needed to devise specific treatment protocols to improve outcomes for this high risk population.

TH-PO566

Outcomes of Thrombolytic Treatment for Acute Ischemic Stroke among Dialysis Patients Fahad Saeed,[1] Nauman Tariq,[2] Saqib A. Chaudhary,[2] Malik M. Adil,[2] Adnan I. Qureshi.[2] *[1]Nephrology, Dartmouth Hitchcock Medical Center, Lebanon, NH; [2]Zeenat Qureshi Stroke Research Center, University of Minnesota, Minneapolis, MN.*

Background: The aim of this study was to determine the outcomes of IV thrombolytic therapy (tPA) for acute ischemic stroke among dialysis patients with in the United States.

Methods: We analyzed the data from Nationwide Inpatient Sample (NIS 2005-2008) for all thrombolytic treated patients presenting with acute ischemic stroke with or without dialysis dependence. Patients were identified using the ICD 9 CM codes. Baseline characteristics, in-hospital complications including secondary intracerebral hemorrhage (ICH), deep venous thrmobosis, urinary tract infections, sepsis, pneumonia, sepsis, pulmonary embolism and discharge outcomes (mortality, minimal disability, and moderate to severe disability) were compared between the groups.

Results: Of the 82142 patients with ischemic stroke who received thrombolytic treatment, 1072 (1.3%) were dialysis dependent. Out of 4215004 patients with ischemic stroke who did not receive thrombolytic treatment, 61400 (1.4%) were dialysis dependent. Baseline characteristics were mainly age, sex, race, medical comorbidities including hypertension, diabetes mellitus, presence of congestive heart failure and chronic lung diseases. The ICH rates did not differ significantly between patients with ischemic stroke with or without dialysis who received thrombolytics (5.2% vs 6.1%). The in-hospital mortality rate was higher in dialysis dependent patients treated with thrombolytics. (22% vs 11%) (*P*= <.0001). After adjusting for age and sex, and co morbidities, dialysis dependence was associated with higher rates of in-hospital mortality in patients treated with thrombolytics (odds ratio [OR], 2.47; 95% CI, 1.80-3.38 vs. odds ratio [OR], 3.06; 95% CI, 2.86-3.26) as compared to those who did not.

Conclusions: The three folds higher odds of in-hospital ischemic stroke mortality associated with administration of IV thrombolytics among dialysis patients warrants a careful assessment of risk benefit ratio before giving this therapy.

TH-PO567

Stroke Incidence and Risk Factors in Peritoneal Dialysis Albert J. Power,[1] Andrew Davenport,[2] Edwina A. Brown,[1] Neill D. Duncan,[1] Stanley Fan.[3] *[1]Imperial Renal & Transplant Centre, Imperial College Healthcare NHS Trust, London, United Kingdom; [2]Royal Free Hampstead NHS Trust, London, United Kingdom; [3]Barts & the London NHS Trust, London, United Kingdom.*

Background: Stroke is the leading cause of disability in the US with markedly higher rates in dialysis cohorts. There are very few studies examining stroke in peritoneal dialysis [PD] despite increasing numbers of patients on this modality. Of concern these suggested greater stroke mortality in PD compared to hemodialysis [HD] in analyses of cohorts over 10yrs old. We aimed to describe stroke epidemiology in contemporary PD cohorts.

Methods: This multicentre retrospective study [Jan 2002-Mar 2012] examined patients incident to PD. Stroke was defined as an acute neurological event >24hrs duration with compatible findings on neuroimaging. Subdural hematoma was excluded. Factors associated with stroke risk were examined with multivariate Cox models and a competing-risks approach. The effect of stroke on survival assessed using Weibull models.

Results: 1511 patients were studied [mean age 54.7±16.5yrs, 35% diabetic, 7% with pre-existing cerebrovascular disease] over 3672 total patient years follow-up. 36 strokes occurred [overall incidence 9.8/1000 pt years] of which 31/36 [86%] were ischemic, 14% hemorrhagic. Stroke occurred in older patients [mean age 60.5±12.5 vs 54.2±16.6yrs, p=0.01] after a median of 11 months on PD. After age-adjustment, established cerebrovascular disease was the only factor independently associated with a greater risk of stroke [HR 4.2, p<0.001] whereas gender, ethnicity, diabetes, atrial fibrillation were not. One year mortality after stroke was 19% with no significant difference by subtype. On multivariate analysis acute stroke was independently associated with worse patient survival [HR 4.6, p<0.001].

Conclusions: Stroke incidence is ten-fold greater in PD compared to the general population. In the largest study of its kind to date we found incidence rates lower in PD cohorts compared to HD [15-30/1000 patient years] and in contrast to previous studies. The relatively fewer hemorrhagic events seen may relate to casemix, anticoagulation exposure, residual renal function and volaemic control.

TH-PO568

Hemodialysis Patients with Atrial Fibrillation Have a 1.7-Fold Higher Risk for All-Cause Death Despite of Lacking the Elevated Risks for Cardiovascular Mortality Masaki Ohsawa. *Department of Hygiene and Preventive Medicine, Iwate Medical University, Iwate Prefecture, Japan.*

Background: Atrial fibrillation (AF) contributes to increased risks for cardiovascular morbidity and mortality in general populations. However, whether AF contributes to increased risks for these endpoints has not been fully examined in hemodialysis patinets (HD pts).

Methods: Two prospective cohort studies of 1,109 HD pts and 26,469 community-dwelling people (controls) were carried out. Sex- and age-adjusted mortality rates (all-cause, cardiovascular and infectious-related) and incidence rates (acute myocardial infarction (AMI) and stroke) and adjusted rate ratios were calculated using Poisson regression model (see table).

Results:
Sex- and age-adjusted mortality and incidence rates (/1000 pys) and incidence rate ratios in the groups stratified by AF

HD vs ctls	HD pts (n=1,109)		controls (n=26,469)	
AF groups	non-AF (n=1,074)	AF (n=35)	non-AF (n=26,067)	AF (n=402)
all-cause death	68.7 (60.0 to 77.4)	117 (67.8 to 166)	3.37 (3.01 to 3.74)	5.93 (4.22 to 7.65)
RR (95% CI)	REF	1.70 (1.13 to 2.56)	REF	1.76 (1.34 to 2.31)
CVD death	37.2 (30.5 to 43.9)	53.0 (30.0 to 76.0)	0.61 (0.46 to 0.76)	2.38 (1.27 to 3.49)
RR	REF	1.27 (0.64 to 2.50)	REF	3.91 (2.58 to 5.91)
Infectious death	16.5 (12.2 to 20.7)	36.5 (9.52 to 63.5)	0.15 (0.09 to 0.22)	0.29 (0.07 to 0.51)
RR	REF	2.22 (1.09 to 4.50)	REF	1.89 (0.99 to 3.61)
AMI	8.91 (5.74 to 12.1)	9.65 (0.00 to 23.8)	0.35 (0.23 to 0.46)	0.62 (0.03 to 1.20)
RR	REF	1.08 (0.26 to 4.56)	REF	1.78 (0.72 to 4.41)
stroke	45.4 (38.5 to 52.3)	38.9 (3.92 to 73.8)	3.13 (2.78 to 3.48)	10.7 (7.61 to 13.8)
RR	REF	0.86 (0.35 to 2.11)	REF	3.41 (2.59 to 4.51)

Adjustment for for persons aged 60 yrs old and male/female ratio beeing 1.0.

There were 1.7-fold higher risks of all-cause death in persons with AF as opposed to those without AF both in HD pts and controls. AF contributed to increases in risks for all-cause death and cardiovascular morbidity and mortality in a general population, whereas it only increased a risk for all-cause death without increasing risks for cardiovascular morbidity and mortality in HD pts.

Conclusions: AF increaseed a risk for death without increasing risks for cardiovascular morbidity and mortality in HD pts.

Funding: Government Support - Non-U.S.

TH-PO569

Attributable Risk of Atrial Fibrillation for Stroke in Dialysis Patients

James B. Wetmore,[1] Jonathan D. Mahnken,[3] Milind A. Phadnis,[3] Sally K. Rigler,[2] Xinhua Zhou,[3] John Spertus,[5] Purna Mukhopadhyay,[3] Theresa I. Shireman.[4] *[1]Division of Nephrology, University of Kansas Medical Center, Kansas City, KS; [2]Medicine, University of Kansas Medical Center, Kansas City, KS; [3]Biostatistics, University of Kansas Medical Center, Kansas City, KS; [4]Preventive Medicine & Public Health, University of Kansas Medical Center, Kansas City, KS; [5]St. Luke's Mid America Heart Institute, University of Missouri - Kansas City, Kansas City, MO.*

Background: Both stroke and chronic atrial fibrillation (CAF) are common in dialysis patients. We sought to determine the association between CAF and stroke and to establish the incidence of new strokes.

Methods: A cohort of incident Medicare & Medicaid patients was constructed. A Medicare claims were used to determine the onset of CAF, which was treated as a time-dependent covariate. Strokes were determined from Medicare claims. Cox proportional hazards models were constructed to determine the association between predictor variables and times to stroke.

Results: CAF was independently associated with time to ischemic stroke (HR 1.26, P = 0.0005) and to all (combined) strokes (HR 1.21, P = 0.0019) but not with hemorrhagic stroke (HR 0.93, P = 0.70). Race played a striking role in hemorrhagic strokes: African-Americans (HR 1.46), Hispanics (HR 1.64), and other minorities (HR 1.76) all had significantly shorter times-to-event than did Caucasians (P < 0.001 for all). Overall, the ratio of ischemic to hemorrhagic strokes was approximately 4.5:1. There were 22.8 ischemic, 5.0 hemorrhagic, and 27.3 combined strokes per 100 patient-years. Stratifying CAF patients into $CHADS_2$ scores of 0-1, 2, or 3-6, Kaplan-Meier curves varied from each other at the level of P = 0.01.

Conclusions: CAF has a highly significant, but modest, association with ischemic stroke. The most striking association with hemorrhagic stroke is race/ethnicity. The ratio of hemorrhagic to ischemic strokes is much higher than in the general population. The $CHADS_2$ risk score appears to work well in broadly stratifying CAF dialysis patients for ischemic stroke.

Funding: NIDDK Support, Private Foundation Support

TH-PO570

Safety of Warfarin Use in Chronic Hemodialysis Patients: A Prospective Case-Control Study

Matsuhiko Hayashi, Ichirou Takamatsu, Yoshihiko Kanno, Tadashi Yoshida. *Apheresis and Dialysis Center, University of Keio, School of Medicine, Shinjuku-ku, Tokyo, Japan.*

Background: Since there are limited data concerning the risks associated with warfarin use in hemodialysis (HD) patients, we conducted a prospective case-control study in the patients on chronic HD in Japan.

Methods: The patients on chronic HD with warfarin and control HD patients were recruited from 103 HD centers in Japan. For each patient on warfarin, two dialysis-vintage matched controls were selected at each HD center. Clinical data were collected at the beginning and 12 months after the registration.

Results: At the end of the study, 344 cases with warfarin and 645 controls were qualified for further analysis. The occurrence of composite incidences (18.9%), which include death, new stroke, and new cardiovascular events, and the occurrence of each of these incidences were not significantly different as compared to the controls (composite incidences 17.7%). The incidence of major bleeding (gastrointestinal, intramuscular, and hemorrhagic stroke) in warfarin users (8.1%), however, was significantly higher than in the controls (2.6%, p<0.0001). By multivariate logistic regression model analysis, age (OR 1.039 for each 1-year increase, 95% CI 1.021-1.057, p<0.0001) and the history of cardio- and cerebrovascular events (OR 1.84, CI 1.27-2.67, p<0.05), but not warfarin use or atrial fibrillation (Af), were identified as the risk factors for composite incidences, while warfarin use was the sole risk factor for major bleeding (OR 3.22, CI 1.50 – 6.94), p<0.005). Patients with warfarin for preexisting Af (184 cases) and their corresponding controls (350 cases) were analyzed separately, and warfarin use did not significantly increase composite incidences, mortality, new stroke, and new cardiovascular events in the patients with Af, either.

Conclusions: The present study showed that warfarin use increased the risk of major bleeding, while the occurrences of composite and individual incidences except major bleeding were not significantly different from the controls in either total cases or in the cases with Af. Considering the high cardiovascular mortality in patients on chronic HD and Af, warfarin use may be beneficial for these patients.

Funding: Government Support - Non-U.S.

TH-PO571

Appropriate Intensity Anticoagulation with Warfarin Reduces Risk of Mortality in Hemodialysis Patients with Atrial Fibrillation

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Background: Atrial fibrillation (AF) is a common comorbidity in hemodialysis (HD) patients. However, there is a paucity of data concerning risk-benefit tradeoff in anticoagulation therapy with warfarin (WF). The aim of this study was to investigate the effects of WF use in HD patients with AF on clinical outcomes.

Methods: We registered all patients on regular HD (n=1110) in 24 affiliated hospitals on Jan 2009 and prospectively followed up for 3 years. After exclusion of 173 patients who had missing data or were dropped out during follow-up, 937 subjects (age 65.7 ± 12.2 years; mean duration of HD 82.0 months) were divided into Sinus Rhythm group (SR, n=819) and AF group (AF, n=118), and AF was subdivided into AF with WF (AFWF, n=31) and AF without WF (AFNWF, n=87). Pre-specified end-points were the new onset of stroke, bleeding events and death.

Results: The mortality in the AF was higher than in the SR (24.6% vs. 14.6%, P<0.01). Compared with SR, the AF was significantly older (70.9 ± 9.4 vs. 65.0 ± 12.4 years, P<0.01) and had previous history of stroke (23.7% vs. 14.5%, P<0.01), ischemic heart disease (21.2% vs. 12.1%, P<0.01), and peripheral artery disease (22.0% vs. 12.8%, P<0.01). Logistic regression analysis indicated that age, duration of HD, history of ischemic heart disease and peripheral artery disease, and $CHADS_2$ score were independent factors associated with the mortality. In Kaplan-Meier survival curves analysis, AFWF had significantly lower mortality (P = 0.03) and lower incidence of new-onset stroke (P = 0.02) compared with the AFNWF, though the incidence of bleeding events was similar (12.9% vs. 10.8%). In the AFWF, the prothrombin time-international normalized ratio was 2.2 ± 1.0.

Conclusions: The present results suggest that proper anticoagulation with WF reduces the risk of mortality and that of stroke without increasing bleeding events in HD patients.

TH-PO572

Is the High Prevalence of Atrial Fibrillation in End Stage Renal Disease Related to the Hemodialysis Procedure Itself?

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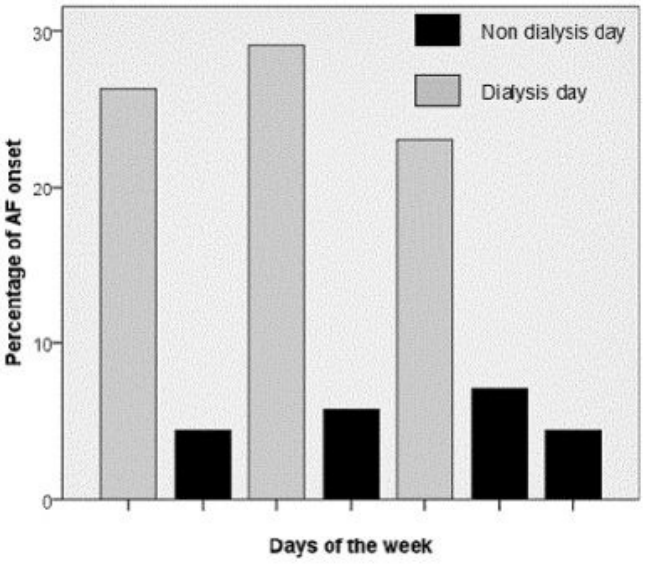
Background: Atrial fibrillation(AF) is common in patients with end stage renal disease(ESRD) and is associated with increased morbidity. Pathophysiology might be related to common risk factors of both AF and ESRD or to hemodialysis(HD) specific factors. The purpose of this study was to determine the onset of AF in relation to the HD procedure itself.

Methods: All HD patients currently enrolled in the ICD2 trial, implanted with an ICD were included. All days recorded by the ICD were analyzed for AF onset and the relationship between AF and the HD procedure was assessed. All procedures during which AF developed were further analyzed and compared to procedures in which AF did not occur.

Results: A total of 41 pts were included (follow-up 27±16 mths, 81% male, 70±8 yrs). In this group, 14 pts developed a total of 428 AF episodes. The onset of AF was seen more frequently on the days of HD (p<0,001) with the highest percentage of episodes starting around the last hour(s) of the procedure. HD procedures in which AF developed were associated with a higher HD volume (p<0,008) and a lower potassium concentration in the HD fluid (p<0,001), compared to procedures in which AF did not occur.



Conclusions: This is the first study to show a clear relationship between the onset of AF and the HD procedure itself. Volume shifts are larger in HD procedures in which AF start and the time of onset clusters around the last hours of the HD procedure. These findings could point to an important role for intravascular volume depletion and potassium flux in the pathophysiology of AF.

TH-PO573

Evolution of Death Risk Following Initiation of Hemodialysis in the United States, 2005-2009 Robert N. Foley,[1,2] Allan J. Collins,[1,2] David T. Gilbertson,[1] Shu-cheng Chen.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Although initiation of dialysis is a period of great clinical flux, the evolution of mortality risk early in the course of hemodialysis is not well understood. This lack of information is surprising, as it could help with clinical decision-making.

Methods: We studied the evolution of mortality risk in two-week increments for one year in US hemodialysis patients (2005-2009, N = 506,506, mean age 63.2 years).

Results: Mortality risk for the overall population (expressed as the probability of death at 1 year if risk remained constant) was at its lowest in the initial two-week interval (0.12, 95% confidence interval [0.11, 0.12], rose gradually to a peak at week 8 (0.41 [0.40, 0.42]) and declined gradually to an intermediate level at week 52 (0.18 [0.17, 0.19]). This biphasic mortality was present in all subgroups studied, whether defined by age, sex, race, cause of ESRD, presence of diabetes and cardiovascular disease, GFR, mode of vascular access and pre-dialysis nephrology care. Among the 21 subgroups examined, the median change in mortality risk between initiation of dialysis and week 8 was +0.30; the largest and smallest absolute changes were in patients ≥ 65 years (0.18 to 0.66) and patients < 40 years (0.03 to 0.09), respectively. Analysis of hemodialysis patients in other eras (1995-2000 and 2001-2004) also showed a biphasic mortality pattern.

Conclusions: Morality risk accelerates rapidly in the first two months following initiation of dialysis, especially in older patients.

Funding: NIDDK Support

TH-PO574

Comparative of Transplanted Listed Hemodialysis and Peritoneal Dialysis in the United States, 2005-2009 Robert N. Foley,[1,2] Allan J. Collins,[1,2] David T. Gilbertson,[1] Shu-cheng Chen.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Although markedly different in terms of dialysis technique, many studies have shown similar survival expectations with hemodialysis (HD) and peritoneal dialysis (PD). Concern remains, however, that unmeasured comorbidity threatens the validity of existing survival comparisons. As exclusion of serious ongoing medical issues is a prerequisite for transplant listing, survival comparisons by mode of dialysis in listed patients has the potential to mitigate these concerns.

Methods: We compared annual dialysis mortality estimates by mode of dialysis at the time of first listing for transplantation in the United States between 2005 and 2009 (N = N 93459, 14.5% PD).

Results: Compared to HD, PD patients were younger (mean age 46.1 Vs. 50.8 years), and less likely to be male (55.6% Vs. 62.3%), on dialysis for ³ 1 year (47.1% Vs. 56.1%), Black (26.2% Vs. 35.0%), Hispanic (16.5% Vs. 20.0%) and to have diabetes as cause of ESRD (33.0% Vs. 41.6%). Death rates were similar, at 4.7 (4.6,4.9) Vs. 4.5 (4.1,4.9) per 100 person-years, respectively in the HD and PD populations. Unadjusted and adjusted PD-to-HD mortality hazards ratios were 0.96 and 1.02 (P = 0.6), respectively.

Conclusions: Among transplant-listed patients, survival on dialysis is similar for HD and PD.

Funding: NIDDK Support

TH-PO575

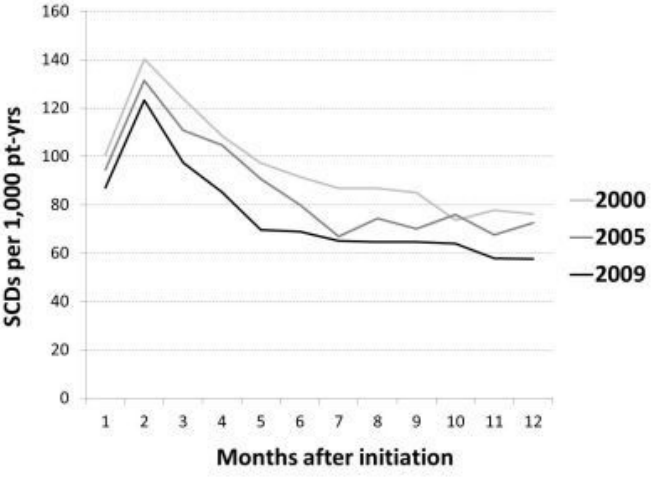
Temporal Trends in Sudden Cardiac Death in Incident US Hemodialysis Patients Shuling Li,[1] Charles A. Herzog.[1,2] *[1]CVSSC, United States Renal Data System, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

Background: The initiation of hemodialysis (HD) is a vulnerable period with a markedly heightened risk of death. Few published data exist regarding sudden cardiac death (SCD) in the first 90 days after dialysis initiation. We hypothesized that the rate of SCD is markedly higher in newly incident HD pts and we analyzed temporal trends in SCD over a decade.

Methods: Incident HD pts in 2000 (n= 84,526), 2005 (n= 97,090) and 2009 (n= 105,644) were identified in the USRDS database. Pts were followed from HD initiation to the earliest of death, transplant, recovery of kidney function, loss-to-follow-up, or one year after initiation. SCD was determined from CMS Form 2728. Cumulative probability of SCD and monthly SCD rates per 1000 pt-yrs, estimated by Kaplan-Meier method, were adjusted for age, sex, race, and ESRD cause with 2009 cohort as reference.

Results: Demographic characteristics of incident HD pts were similar from 2000-2009: 26-27% age 75+, 54-57% male, 64-65% white, 45-46% DM as primary cause of ESRD. One year unadjusted probability of SCD for 2000, 2005, 2009 were respectively 8.7%, 8.0%, and 7.0%, and after adjustment, 9.0%, 8.2%, and 7.2%. Peak SCD rates occurred 2 months after HD initiation: unadjusted monthly SCD rate/1000 pt-yrs for 2000, 2005, and 2009 were respectively 138, 131, and 122 and after adjustment 140, 132, and 123 SCD's/1000 pt-yrs (See Figure for adjusted rates).

Conclusions: The hazard of SCD is markedly increased in incident HD pts in the first 90 days after dialysis initiation, with the highest SCD rate at two months after initiation of HD. The rates of SCD have declined over the past decade, but even in 2009 7% of HD pts succumbed to SCD in the first year of dialysis.



Funding: NIDDK Support

TH-PO576

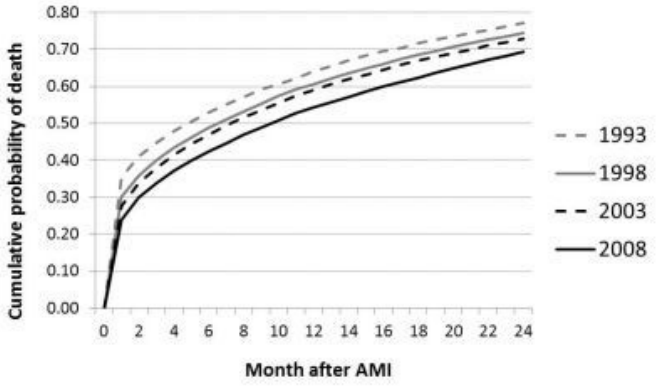
Long-Term Survival after Acute Myocardial Infarction among Patients on Long-Term Dialysis: Is It Still Dismal? Shuling Li,[1] Gautam R. Shroff,[2] Charles A. Herzog.[1,2] *[1]CVSSC, USRDS, MMRF, Minneapolis, MN; [2]Hennepin County Medical Ctr., Minneapolis, MN.*

Background: Acute myocardial infarction (AMI) is a catastrophic event associated with dismal long-term survival in dialysis pts (Herzog et al, NEJM, 1998): the one and two yr mortality for AMI was respectively 55% and 71% in 1977-84, 62% and 74% in1990-95. We sought to determine if there has been any change in outcome of dialysis pts sustaining AMI in the most recent treatment era.

Methods: We searched the US Renal Data System database to identify prevalent HD pts hospitalized for an index AMI in 1993, 1998, 2003, and 2008. Follow-up time was 2 yrs from the index AMI admission and censored at transplant, recovery of kidney function or loss-to-follow-up. Cumulative 2-yr mortality was estimated by Kaplan-Meier method with subsequent adjustment for age, gender, race, dialysis vintage (time on dialysis), and etiology of ESRD with 2008 as reference.

Results: There were 43,168 dialysis pts with AMI (4,494 in 1993, 8,081 in 1998, 14,232 in 2003, and 16,361 in 2008). Over time, there was a progressive proportional increase in older pts, black pts, diabetic ESRD, and older vintage, but no change in gender. For 1993 vs 2008 there were 23% vs 31% age 75+, 25% vs 31% black, 42% vs 55% diabetic ESRD, 15% vs 26% vintage 5+ yrs, (and 53% vs 54% male). In-hospital death decreased from 32% in 1993 to 19% in 2008 (P< .0001). The **Figure** shows adjusted cumulative probability of death by AMI yr. 30 day mortality for 1993, 1998, 2003, and 2008 were respectively 35%, 30%, 28%, and 24%. Mortality for 1993, 1998, 2003, and 2008 were respectively 64%, 61%, 59%, and 54% at 1 year and 77%, 75%, 73%, and 69% at 2 yrs.

Conclusions: There has been an improvement in survival in dialysis pts sustaining AMI in the most recent treatment era, predominantly due to a marked reduction in 30 day mortality. Aggressive interventions targeting long-term mortality are warranted.



Funding: NIDDK Support

TH-PO577

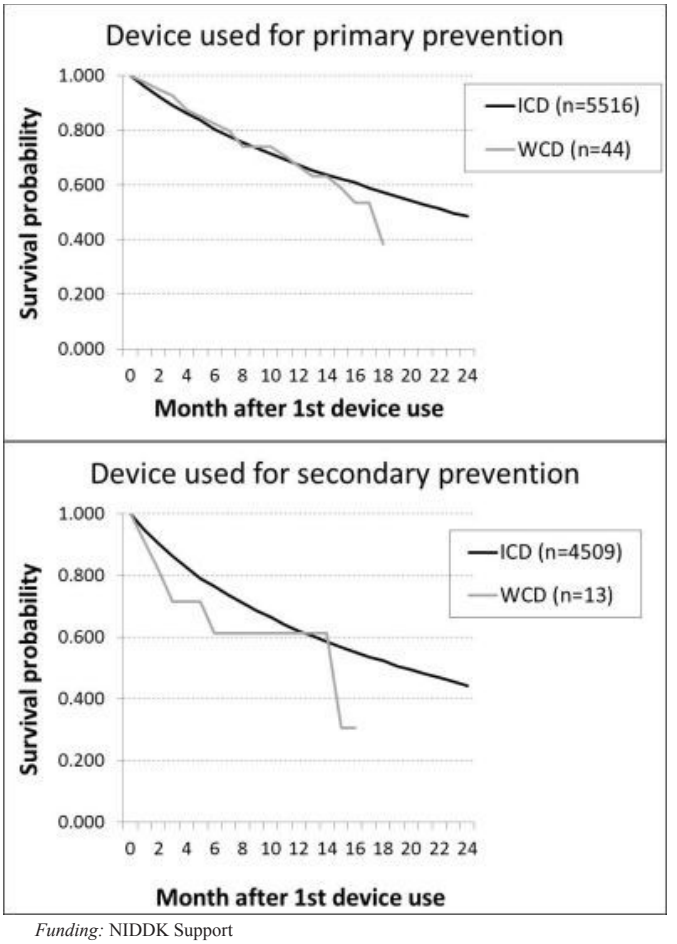
Comparative Survival of Hemodialysis Patients with Implantable and Wearable Cardioverter Defibrillators Shuling Li,[1] Charles A. Herzog.[1,2] *[1]CVSSC, United States Renal Data System, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

Background: Sudden cardiac death (SCD) accounts for 26% of all-cause mortality in dialysis pts, and implantable cardioverter defibrillators(ICD/CRT-D) are widely used for primary prevention (PP) to prevent SCD and secondary prevention (SP) of SCD (in cardiac arrest survivors). Few data exist on outcome of dialysis pts with wearable cardioverter defibrillators (WCD).

Methods: Prevalent HD pts receiving first ICD/CRT-D or WCD in 2005-2010 were identified in the US Renal Data System (USRDS) database (ICD-9-CM codes 37.94 and 00.51 for ICD/CRT-D and CPT codes 93292 and 93745 for WCD). SP was defined by ICD-9-CM codes 427.1, 427.4 and 427.5 on the same device claims. F/u time from first device use was two yrs (to 6/30/11) with censoring at transplant, device change, or switch to PD. Survival probability was estimated by Kaplan-Meier method with differences in survival between devices tested by Log-rank test.

Results: There were 10,082 HD pts with ICD/CRT-D (55% PP) and 57 pts with WCD (77% PP). Across prevention types, ICD/CRT-D cohort had more pts aged 65+ yrs, female and white, and fewer pts with ESRD vintage 5+ yrs than WCD cohort. More diabetic ESRD pts were seen in PP ICD/CRT-D (50%) than PP WCD (39%), but fewer in SP ICD/CRT-D (49%) than SP WCD (54%). 10 WCD pts subsequently received ICD's. Median time to switch was 40 days. The Figure shows 2-year cumulative survival probability comparing ICD/CRT-Ds with WCD by prevention type. For PP, WCD had similar survival with ICD/CRT-D. For SP, WCD had inferior survival than ICD/CRT-D. Both comparisons were not statistically significant.

Conclusions: Wearable defibrillators may provide comparable one yr survival to ICD's, particularly for primary prevention of SCD. Randomized clinical trials of WCD's for prevention of SCD in HD pts are warranted.



Funding: NIDDK Support

TH-PO578

Mortality Rates among Prevalent Hemodialysis Patients in Beijing: A Comparison with USRDS Data Li Zuo, Mei Wang, Wen Huang, Wenhu Liu, Xuemei Li, Kai Wang, Li Jijun, Chunhua Zhou, Xu-yang Cheng. *Department of Medicine, Peking University First Hospital, Peking University Institute of Nephrology, Beijing, China.*

Background: The reported rude mortality rate in Chinese patients on maintenance hemodialysis (MHD) was around 10%, it was around 20% in the US reported by the United States Renal Data System (USRDS). Our hypothesis was that the large survival difference was cause by difference in race, practice pattern between nations if baseline patients' characteristics were adjusted.

Methods: Annual mortality of Beijing prevalent MHD patients in each year of 2007 through 2010 was reported, and relative risks of death compared with corresponding mortality of USRDS prevalent MHD patients after age, gender and primary cause of end stage renal disease were adjusted were calculated. 11,675 MHD patients from 104 dialysis facilities in Beijing from December 31, 2006 to December 31, 2010 were included. 1,937,819 MHD patients (only White, African-American and Asian were eligible for inclusion) were subtracted from USRDS No-60-Day prevalent dataset from the year 2004 to 2009 using RenDER system. Rude annual mortality for each race was reported as number per 1000 MHD patients at risk for each year. Adjusted annual mortality and relative risk of death after age, gender and primary cause of ESRD was adjusted comparing Beijing and each of the USRDS race.

Results: The annual mortality for Beijing cohort increasing gradually from 47.8 per 1000 patient-years in 2007 to 76.8 in 2010. The annual mortality for White cohort2007 was 250.7 per 1000 patient-years, and gradually decreased to 236.3 in 2009. Annual mortality for African-American was much lower than that for White. Annual mortality for Asian was slightly lower than that for African-American. After adjustment, Beijing MHD still had survival benefit comparing each of the examined USRDS race.

Conclusions: Annual mortality for Beijing MHD patients was lower than their USRDS counterparts, and this difference existed after baseline demographics were adjusted. This survival difference between Beijing and USRDS MHD cohorts could be attributable to race or practice pattern difference. More study needed to validate our hypothesis.

TH-PO579

Pre-Dialysis Factors and the Comparative Effectiveness of Early versus Conventional Dialysis Initiation among Older Adults Deidra C. Crews,[1,4] Julia J. Scialla,[2,4] Haifeng Guo,[3,4] Jiannong Liu,[3,4] Karen J. Bandeen-roche,[1,4] L. Ebony Boulware.[1,4] *[1]Johns Hopkins Univ.; [2]Univ. of Miami; [3]Chronic Disease Research Group; [4]For the DeCIDE Network Patient Outcomes in ESRD Study.*

Background: Recent studies of the timing of dialysis initiation have not accounted for several pre-dialysis clinical factors that could contribute to poor outcomes observed among early initiators. These factors may be especially important among older adults.

Methods: We identified patients initiating dialysis at age ≥67 years from 2006-2008 with 2 prior years of Medicare coverage in the US Renal Data System. We reviewed 6 months of pre-ESRD claims for acute kidney injury (AKI), uremia, hyperkalemia, congestive heart failure (CHF) admissions, and total hospital days. We used Medicare claims and the Medical Evidence form to ascertain demographics, lab values, comorbidities at dialysis initiation and outcomes (mortality and hospitalization). Using propensity scores for likelihood to initiate dialysis early (eGFR ≥10 ml/min/1.73m^2) versus later/conventional (eGFR <10), we constructed a cohort matched 1:1. We estimated risks of post-ESRD mortality (all-cause and cause specific) and hospitalizations (Cox model) among early versus later initiators.

Results: Among 84,654 older US adults with pre-ESRD claims, 58% initiated dialysis early. Early initiators were more likely to have had AKI, CHF admissions and all-cause hospitalizations when compared to later initiators. Propensity matching was successful for 61,930, with 50% in each initiation group. Early initiation was associated with greater risk of all-cause, cardiovascular (CV) and infectious mortality; and greater risk of all-cause and infectious hospitalizations.

Hazard Ratios Comparing Early versus Later Dialysis Initiation

Outcome	Early Incidence per 1000 person yrs	Later Incidence per 1000 person yrs	Hazard Ratio (95% Confidence Interval) Comparing Early (referent) to Later
All-Cause Mortality	402.1	360.5	1.11 (1.08-1.14)
CV Mortality	146.7	131.8	1.11 (1.06-1.15)
Infectious Mortality	41.7	36.0	1.15 (1.06-1.24)
All-Cause Hospitalizations	2079.8	2017.8	1.03 (1.01-1.05)
CV Hospitalizations	623.7	629.9	0.99 (0.96-1.01)
Infectious Hospitalizations	538.5	487.5	1.10 (1.07-1.13)

Conclusions: After accounting for pre-dialysis clinical factors, early dialysis initiation is associated with greater mortality and infectious hospitalizations among older US adults.

Funding: Other U.S. Government Support

TH-PO580

Trends in Mortality in Pediatric Chronic Dialysis Patients Blanche M. Chavers,[1,2] Julia T. Molony,[1] Craig Solid.[1] *[1]USRDS Coordinating Center, MMRF, Mpls, MN; [2]Dept. of Pediatrics, University of MN, Mpls, MN.*

Background: There are few published data describing survival trends over time of pediatric dialysis patients. Our goal is to describe mortality rates over a 15 year period.

Methods: From the USRDS database, we identified chronic dialysis (HD or PD) patients aged 18 and under for each year between 1995 and 2010. Patients were followed for up to 1 year and censored by a change in the type of renal replacement therapy. We calculated the number of deaths, percent of deaths, and the mortality rate per time at risk within each cohort year. Results were considered separately by type of dialysis and patient characteristics.

Results: The average yearly cohort of patients studied included about 1,200 at risk patients of whom 60% were HD and 40% were PD, and the majority were over 10 years of age. There were slightly more males (55%) than females (45%), and the most common causes of dialysis were congenital/reflux/obstructive (45%) and glomerulonephritis (30%). From 1995 to 2010, the yearly number of deaths has generally declined (Table 1). The mortality rates were higher for patients under 10 years old (average of 6.5 per 100 pt yrs) as compared to patients over 10 years old (average of 3.3 per 100 pt yrs). Mortality rates were also lower for PD patients than for HD patients. Overall, there appears to be some decline in the unadjusted mortality rates over the study period, although the change is slight.

Period Prevalent HD and PD Pediatric Patients from 1995-2010

Year	Patients at Risk	Deaths	Mortality per 100 pt years
1995	1221	47	5.21
1996	1255	47	5.13
1997	1263	45	4.74
1998	1214	39	4.33
1999	1173	50	5.83
2000	1103	33	4.05
2001	1136	34	4.06
2002	1215	31	3.49
2003	1282	55	6.00
2004	1271	29	3.10
2005	1290	46	3.80
2006	1227	40	4.56
2007	1176	34	4.05
2008	1146	29	3.52
2009	1179	28	3.37
2010	1161	26	3.10

Conclusions: In general, mortality rates among pediatric dialysis patients have not changed dramatically over the past 15 years. However, younger patients have almost double the mortality rate when compared to older pediatric patients, and PD patients have a lower mortality rate than HD patients. Of note are older pediatric PD patients who show some improvement in mortality rates over time.

Funding: NIDDK Support

TH-PO582

Valve Replacement in Patients with Dialysis-Dependent Renal Failure: Choice of Prosthesis and Impact of Hyperparathyroidism on Mortality Qi Qian,[1] Sameh M. Said,[2] Hartzell Schaff,[3] Joseph A. Dearani,[3] Soon J. Park,[3] Kevin L. Greason,[3] Rakesh M. Suri,[3] Richard C. Daly.[3] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Thoracic and General Surgery, Mayo Clinic, Rochester, MN; [3]Cardiovascular Surgery, Mayo Clinic, Rochester, MN.*

Background: Controversy exists regarding the prosthesis of choice in patients with dialysis-dependent renal failure.

Methods: Between Jan 1980 & Dec 2010, 95 pt (63 males, 66%) with dialysis-dependent ESRD underwent left-sided valve replacement with biological (n=44) or mechanical valves. The mean dialysis duration prior to valve replacement was 4.6±4.8 & mean age 63±14 yr. 27 pts (28%) were re-do valve replacement. Comorbidities included diabetes mellitus in 49%, chronic lung disease 17%, & hypertension 97%; 20 pt (21%) had bacterial endocarditis preoperatively. 68 pt (72%) underwent isolated aortic valve replacement, while 15 pt had isolated mitral valve replacement, and both aortic & mitral valves were replaced in the remaining 17 pt (13%). Preoperatively, the serum PTH, phosphate, calcium and calcium-phosphate product were 421±993 pg/ml, 5.9±1.4 mg/dl, 9.7±0.9 mg/dl, and 57.6±16 mg/dl, respectively.

Results: Early mortality was related to preop risk factors. Among pts without endocarditis operative risk was 9%. For pt having isolated AVR, early mortality was 4%. For pt with endocarditis, risk was 20%. For all pt, survival at 1-, and 5-yr was 62%, and 27%. Type of the prosthesis did not have a significant impact on survival. The 5-year survival was lower in pt with elevated PTH, phosphate, & calcium-phosphate product (25vs33%, 17vs38% and 21vs33%) but this difference was not significant. Late stroke occurred in 7 pt with biologic valves and only 1 pt with a mechanical valve (p=0.01). Reoperation due to structural valve degeneration was documented in two pt with biological valves.

Conclusions: Mortality, both early & late is relatively high for patients with ESRD undergoing valve replacement. There is no demonstrable survival advantage to bioprostheses. Hyperparathyroidism may have an impact on survival in dialysis-dependent pt undergoing valve surgery.

TH-PO583

Left Atrial Appendage Closure (LAAC) as New Option for ESRD-Patients with Atrial Fibrillation and Discontinued Oral Anticoagulation after Severe Bleedings: Pilot Data on Three Patients Karl August Brensing,[1] Peter M. Raab,[1] Thomas M. Gerhardt,[1] Uwe Poege,[1] Peter Heidkamp,[1] Heyder Omran.[2] *[1]Dialysis Unit, Nephrology Center Bonn, Bonn, NRW, Germany; [2]Internal Medicine, Marien Hospital, Bonn, NRW, Germany.*

Background: Patients with atrial fibrillation (AF) need oral anticoagulation (OAC), but for ESRD-patients this approach is unproven, since no prospective data exist, current scores for embolism (CHADS2-VASC) and bleeding (HASBLED) are not validated for ESRD and two large register studies reported higher morbidity and mortality in HD-patients on OAC (1, 2). Thus, HD/PD-patients with OAC related severe bleedings might benefit from left atrial appendage closure (LAAC) by avoiding further OAC but safety and outcome data are missing.

Methods: We report on three older ESRD patients (Pt 1: 81 yrs, HD; Pt 2: 84 yrs, HD; Pt 3: 77 yrs, IPD) with AF and discontinued OAC after severe bleedings (1x hematothorax, 2x gastrointestinal) with follow-up for 3, 10 and 21 months (Mo) after LAAC using an Amplatzer Cardiac Plug occluder set.

Results: LAAC was well tolerated without any interventional complications. After initial heparin (hirudoid Pt 2 due to HIT-II) for 2 days all patients received only low-dose aspirin (50-100 mg/day) and HD (3x/week=wk high-flux HD for 4 h, with heparin or hirudoid) or 3x/wk IPD (for cardio-renal syndrome, Pt.3). In Pt 1 a device fixed small thrombus (2 mm) was detected 1 Mo after LAAC which disappeared on increased hirudoid dose (at HD) after 2 Mo. Up to 10 (Pt. 1), 21 (Pt. 2) and 3 Mo (Pt. 3) we saw no embolic or bleeding event under low-dose aspirin therapy. However, 9.5 Mo after LAAC Pt. 1 needed shunt-thrombosis-OP and presented 2 wks later (mechanical) ileus but refused all (diagnostic/therapeutic) procedures and died.

Conclusions: 1. In our pilot data LAAC was safe and without apparent embolic (cerebral) or bleeding events up to 21 Mo. 2. In ESRD patients with AF and contraindicated OAC the new LAAC can prevent embolic events from the left atrium. 3. Now more long-term data is needed and even RCTs are warranted (LAAC vs. coumarin/new OAC) in ESRD patients with AF and known high bleeding risk.

Ref: 1.) Chan KEJ et al. JASN 2009; 20:872 2.) Wizemann V et al. KI 2010; 77: 1098.

TH-PO584

Trends of Infective Endocarditis in End Stage Renal Disease in the Last Decade Karthik Murugiah,[1] Gagan Kumar,[1] Priyanka Khatri,[2] Abhishek Deshmukh,[3] Puneet Sood.[1] *[1]Medical College of Wisconsin, WI; [2]Hospital of St. Raphael, CT; [3]University of Arkansas for Medical Sciences, AR.*

Background: Infective endocarditis (IE) is a common complication of vascular access in End Stage Renal Disease (ESRD). There is paucity of national level epidemiologic data.

Methods: Using the Nationwide inpatient sample (NIS) 2000-08 we identified patients ≥ 18 yrs with a discharge diagnosis of bacterial endocarditis using the ICD-9 code 421.x. Data was divided into 3 year periods to study trends. Population data was obtained from the US Census Bureau and for ESRD from the United States Renal Data system. Outcomes

of interest were in-hospital mortality, length of stay (LOS) and receipt of valve surgery. We used logistic regression to examine risk factors for in-hospital mortality.

Results: Incidence of IE in ESRD patients was 63.4 times that of general population. Proportion of patients >65 yrs was 40.2% in ESRD with IE group compared to 47.1% in all patients with IE. Among ESRD, 50.4% were male compared to 58% in all patients. LOS was 16.4±17 days vs 14.8±16 days in all patients with IE. Mortality was higher in ESRD patients at 20% vs 13% in all patients. (all p<0.001) In logistic regression analysis adjusting for age, sex, race, payer status, hospital characteristics and other comorbidities patients with ESRD and IE were 1.7 times more likely to die during the hospitalization and 2 times less likely to undergo valve surgery. During the study period there was a dramatic increase in IE among ESRD patients compared with the rest of the population. There has been an improvement in mortality albeit modest.

Trends of IE in ESRD (2000-08)

	2000-02	2003-05	2006-08	All Years
Estimated nationwide discharges with IE	91765	106970	117980	316715
Incidence (per 100000 person-years) in general population*	14.4	16.2 (12.7% increase)	17.3 (6.5% increase)	16
ESRD (%)*	11.3	13	15.8	13.5
Incidence (per 10000 person-years) in ESRD*	84.1	98.8 (17.4% increase)	117.3 (18.8% increase)	101.5
Valve replacement or repair (%)	5.2	4.6	6.1	5.4
In hospital mortality (%)*	23.2	20.2	17.9	19.9

*P value sig at <0.001

Conclusions: There is an alarming increase in IE in ESRD patients over the last decade with significant diagnostic and therapeutic implications.

TH-PO585

Remote Ischaemic Pre-Conditioning in Haemodialysis: A Pilot Study
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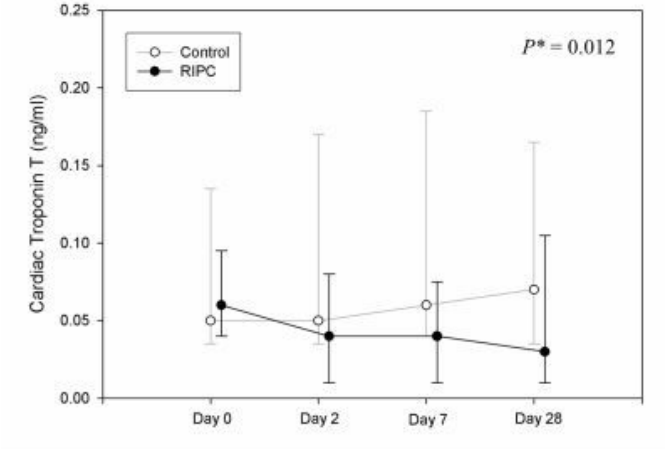
Background: Myocardial stunning induced by transient myocardial ischaemia has been reported during haemodialysis (HD) and it would be important issue to reduce it. Remote ischaemic pre-conditioning (RIPC) has a protective effect against myocardial ischaemia-reperfusion injury.

Methods: Chronic HD patients were randomized to the control, or the RIPC group. RIPC was induced by transient occlusion of blood flow to the arm with a blood-pressure cuff. The procedure was undertaken before every HD session for 1 month (total 12 times). The primary outcome was the change in cardiac troponin T (cTnT) level at day 28 from baseline.

Results: Demographic and baseline laboratory values were not different between the control (n=17) and the RIPC group (n=17). cTnT levels tended to decrease from day 2 in the RIPC group through to 28 days, in contrast to no change in the control group. There were significant differences in the change of cTnT level at day 28 from baseline [Control, median; -0.002 ng/ml (IQR; -0.008 ~ 0.018) versus RIPC, median; -0.015 ng/ml (IQR; -0.055 ~ 0.004), *p = 0.012*].



*Mann-Whitney U test was used to compare the change of cTnT level at day 28 from baseline.

Conclusions: RIPC reduced cTnT release in chronic conventional HD patients, suggesting that this simple, cheap, safe, and well-tolerated procedure has a protective effect against HD-induced ischaemia.

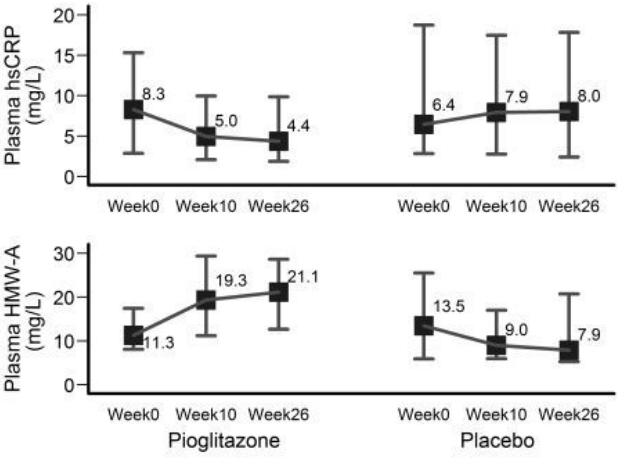
TH-PO586

RCT: Pioglitzaone ↓ Plasma hsCRP and ↑ HMW Adiponectin in Hemodialysis Patients
S. Beddhu,[1,2] Tom Greene,[1] G. Wei,[1] Jill L. Neilson,[1] R. Filipowicz,[1] Y. Zhang,[1] Alfred K. Cheung,[1,2] Abinash C. Roy,[1] Yufeng Huang,[1] Beverly Farmer,[3] M. Chonchol.[3] *[1]Univ of Utah; [2]VHASLC, SLC, UT; [3]Univ of Colorado, Denver, CO.*

Background: Inflammation is thought to play a major role in cardiovascular disease and is a strong predictor of mortality in hemodialysis (HD) patients (pts); however, there is no established Rx for ↓ inflammation in HD pts. Pioglitazone (pio) by inhibiting NFκB pathway ↑ anti-inflammatory adiponectin and ↓ pro-inflammatory cytokines in adipose tissue with consequent ↓ in systemic inflammation. We examined the effects of pio in a double-blind RCT on the primary endpoints of Δ in plasma high sensitivity C-reactive protein (hsCRP) and high molecular weight adiponectin (HMW-A) levels in HD pts.

Methods: 95 overweight or obese HD pts with diabetes mellitus or insulin resistance (HOMA > 2.1) were randomly assigned to pio 30 mg/d (n=48) or matching placebo (pla) (n= 47) for 26 wks. Immunoassays were used to measure hsCRP and HMW-A. Mixed effects models were used to compare Δ from baseline to weeks 10 and 26 in log hsCRP and HMW-A between treatment groups after controlling for their baseline level.

Results: Baseline characteristics were balanced between groups. 81% in pio and 79% in pla groups (p =0.8) completed the study. Baseline and follow-up hsCRP and HMW-A levels are summarized in figure.



Compared to pla, pio treatment resulted in 28% ↓ in hscCRP (p=0.053) and 53% ↑ in HMW-A (p=0.01) in mixed effects models.

Adverse advents were not different in either group: death (0% pio, 4% pla), fracture (2% pio, 6% pla), and fluid overload (6% pio, 0% pla), (all p>0.2). There were no incidences of serious hypoglycemia, liver failure or ↑ in liver enzymes in either group.

Conclusions: Pioglitazone treatment ↓ inflammation in HD pts. Larger RCTs with hard endpoints of cardiovascular events and mortality are warranted.

Funding: NIDDK Support

TH-PO587

Heparin-Based Haemodialysis Is a Potent Inductor for sFlt1 Release
Frederic Lavainne,[1,2] Emmanuelle Meffray,[1] Ruth J. Pepper,[3] Melanie Neel,[1] Catherine Delcroix,[2] Alan D. Salama,[3] Fadi Fakhouri.[1,2] *[1]INSERM UMR 1064, ITUN, Université de Nantes, Nantes, France; [2]Nephrology Department, CHU de Nantes, Nantes, France; [3]Center for Nephrology, University College London, Royal Free Hospital, London, United Kingdom.*

Background: Soluble Flt1 (sFlt1) is a potent inhibitor of vascular endothelial growth factor secreted mainly by placenta, endothelial cells and monocytes. sFlt1 serum levels correlate with endothelial dysfunction and cardiovascular complications in chronic kidney diseases patients.

Methods: We first assessed sFlt1 kinetics during routine haemodialysis (HD) and post dilution haemodiafiltration (HDF) sessions for 48 patients. Then we compared kinetics with two different membranes in HD. Finally, we assessed the impact of heparin on the sFlt1 secretion with initially heparin free HD (with heparin delayed administration after 30 minutes) and predilution HDF without heparin.

Results: In 48 patients, sFlt1 serum levels increased as early as 1 minute after the start of dialysis and peaked at 15 minutes before returning to baseline at 4 hours (mean peak level 2551 pg/ml, vs 102 pg/ml before HD and 69 pg/ml in controls (p<0.0001)). There were no differences in the kinetics between the two membranes in HD. If either unfractionated heparin (UH) or low molecular weight heparin (LMWH) was omitted during the dialysis session (HD or predilution HDF), no significant increase in sFlt1 serum levels occurred. Conversely, delayed administration of LMWH (after 30 minutes of a heparin-free dialysis) induced a sharp increase in sFlt1. In vitro, UH and LMWH failed to induce sFlt1 release by monocytes from controls or HD patients. These findings suggest that either priming of monocytes on the extracorporeal circuit is required for heparin-induced sFlt1 release or that endothelial cells contribute to the increase in sFlt1 levels.

Conclusions: Our results indicate that heparin-based haemodialysis induces a major sFlt1 release and therefore may exacerbate the sFlt1associated anti-angiogenic state in end-stage renal disease (ESRD) patients and could aggravate their endothelial dysfunction and cardiovascular burden. This should be taken into account for the assessment and establishment of optimal dialysis procedures.

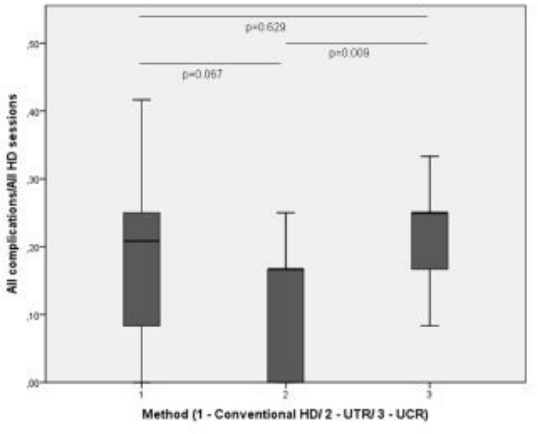
TH-PO588

Blood Volume-Monitored Regulation of Ultrafiltration in Fluid Overloaded Hemodialysis Patients Manfred Hecking, Marlies Antlanger, Walter Hoerl, Gere Sunder-plassmann, Marcus Saemann. *Medical University of Vienna, Nephrology, Vienna, Austria.*

Background: Chronic fluid overload in hemodialysis patients is associated with poor survival. We tested whether blood-volume monitored regulation of ultrafiltration and dialysate conductivity (UCR) and/or regulation of ultrafiltration and temperature (UTR) would facilitate dry weight reduction, in comparison to conventional hemodialysis.

Methods: Patients with fluid overload ≥15% extracellular water (ECW; by BCM [Fresenius/Germany]) were randomized 1:1:1 into conventional, UTR and UCR hemodialysis groups at 3 centers. Target dry weight was set as minus7% ECW postdialysis, in systematic steps following the DRIP-trial protocol (Agarwal et al., Hypertension 2009). Primary endpoint: complications (symptomatic and asymptomatic intradialytic hypotension, nausea, vomiting, cramps, malaise; NCT01416753).

Results: Of the 245 patients who were assessed for eligibility, 99 had fluid overload ≥15% ECW, 50 patients received the allocated intervention and all completed the study. The rate of intra- and postdialytic complications was 17% per 12 HD sessions for all groups (details in the figure).



Measurements of quality control indicated 26 of 79 randomly checked UCR treatments were inadequately executed, despite extensive training efforts. Dry weight reduction was 3.0±2.4% in the conventional group, 3.8±2.1% in the UTR, and 2.4±1.7% in the UCR group (mean±SD in % of body weight; all p ≥0.05).

Conclusions: Even in patients with bioimpedance-proven fluid overload, dry weight reduction was challenging, despite BVM-based dialysis techniques. A randomized controlled clinical trial is needed to demonstrate reduction of chronic fluid overload can prospectively reduce mortality and is therefore worth the strains imposed on patients and their caretakers.

Funding: Pharmaceutical Company Support - Nikkiso Germany

TH-PO589

Clinical Evaluation of Hemocontrol in Korean Hypotension-Prone Hemodialysis Patients: A Multicenter Prospective Crossover Study Hyo-Wook Gil,[1] Kitae Bang,[2] So-young Lee,[2] Byoung Geun Han,[3] Jin Kuk Kim,[1] Young Ok Kim,[5] Ho Cheol Song,[5] Young-Joo Kwon,[4] Yong-Soo Kim.[5] *[1]Medicine, Soonchunhyang University College of Medicine, Korea; [2]Medicine, Eulji University School of Medicine, Korea; [3]Medicine, Yonsei University Wonju College of Medicine, Korea; [4]Medicine, Korea University Medical College, Korea; [5]Medicine, The Catholic University of Korea College of Medicine, Korea.*

Background: The Hemocontrol biofeedback system automatically monitors and regulates blood volume contraction during HD through the adjustments of ultrafiltration rate and dialysate conductivity. The effect of hemocontrol on the incidence of intradialytic hypotension (IDH) in Korean hypotension-prone HD patients was evaluated.

Methods: In this prospective crossover study, 60 (males 32%; age 57±11 years; diabetics 67%; HD duration 58±46 months) hypotension-prone patients from 9 HD centers completed the study. Artis® machines were used. The study included period A (current best practice HD for 8 weeks), B0 (HD with Hemoscan blood volume monitoring for 2 weeks), and period B1 (Hemocontrol HD treatments for 8 weeks).

Results: The number of HD sessions symptomatic IDH occurred was significantly decreased (39% ↓) in period B1 compared with period A (37.6% vs. 61.4%, $p < 0.001$). The number of nursing interventions per HD session was also significantly decreased in period B1 compared with period A (0.56 vs. 0.96, $p < 0.001$). Although the pre-dialysis BPs were not different, the post-dialysis systolic, diastolic, and mean arterial pressures were significantly higher in period B1 compared with period A, leading to the small variation of BPs between pre- and post-dialysis (ΔBP) in period B1. Furthermore, ΔBP in period A was positively correlated with the incidence of IDH in period B1. The recovery time after dialysis was significantly faster in period B1 compared with period A. There was no difference in the interdialytic weight gain, URR, serum electrolytes, hemoglobin and serum albumin levels between period B1 and period A.

Conclusions: These data suggest that Hemocontrol biofeedback system may improve the patient tolerability to HD through the reduction of IDH occurrence in Korean hypotension-prone HD patients.

Funding: Pharmaceutical Company Support - Gambro AB

TH-PO590

Clinical Usefulness of a Bioimpedance Analysis Aimed to Control Ambulatory Blood Pressure in Hemodialysis Patients Jung-ho Shin, Youn-su Park, Min-jee Han, Su Hyun Kim, Suk-hee Yu. *Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea.*

Background: Hypertension is very common in hemodialysis patients and is strongly associated with an increased cardiovascular mortality. We investigated the relationship between ambulatory blood pressure monitoring (ABPM) and an edema index and investigated if the edema index can be used to control blood pressure in hemodialysis patients.

Methods: Patients who had hemodialysis for more than three months in Chung-Ang University Hospital were recruited. ABPM was measured for twenty four hours after hemodialysis. Bioimpedance analysis (BIA) was conducted before hemodialysis in the supine position. We checked clinical results including brain-natriuretic peptide (BNP) levels. Patients were divided into controlled and uncontrolled groups by ambulatory systolic blood pressure. We anticipated and adjusted accordingly dry weight based on BIA results.

Results: Thirty-three patients were recruited, there were nineteen men and fourteen women. In regards to ABPM, the mean systolic and diastolic blood pressures were 140.2±19.7 mmHg and 79.0±10.6 mmHg, respectively. There was significant association between mean systolic blood pressure and the edema index ($p=0.007$, $R^2=0.628$). Mean systolic blood pressure was also associated with the BNP level ($p=0.016$, $R^2=0.607$). At night, the edema index in controlled group was 0.384±0.008 and that in the uncontrolled group was 0.398±0.015 ($p=0.018$). However, the edema index during daytime hours was not different between the two groups. There was no difference in the BNP level both during daytime hours and at night. After adjustment of the dry weight, we compared the changes. The edema index had decreased from 0.395±0.014 to 0.386±0.032 ($p=0.150$). Office systolic blood pressure had significantly decreased from 135.2±26.0 mmHg to 126.1±14.2 mmHg ($p=0.039$). BNP level also decreased, but there was no statistical difference ($p=0.067$).

Conclusions: The edema index obtained by the bioimpedance analysis is more significantly correlated with ambulatory systolic blood pressure than the BNP level. An adjustment of dry weight regarding the edema index can be used to control blood pressure in hemodialysis patients.

TH-PO591

Long-Term Effects of a Negative Sodium Gradient on Blood Pressure, Arterial Stiffness and Left Ventricular Hypertrophy in Hypertensive Hemodialysis Patients Yi-Lun Zhou, Jing Liu, Li-jie Ma, Fang Sun, Yang Shen, T.G. Cui. *Department of Nephrology, Chao-Yang Hospital, Capital Medical University, Beijing, China.*

Background: Sodium overload plays an important role in the pathogenesis of hypertension, and has harmful effects on the cardiovascular system.The aim of the present study is to examine the long-term effects of a mild negative sodium gradient on cardiovascular risk factors in hemodialysis (HD) patients.

Methods: Sixteen hypertensive HD patients were enrolled in this pilot trial. These patients had achieved their "dry weight" assessed by bioimpedance methods, and their pre-HD plasma sodium levels were slightly higher than the facility dialysate sodium concentration (138mmol/L). After 1-month period of dialysis with standard dialysate sodium of 138mmol/L, the patients were switched to low sodium dialysis with dialysate sodium of 136mmol/L and followed up for a 12-month period. No changes in instructions to patients about dietary sodium control were made. During the period of study, the dry weight was adjusted monthly under the guidance of bioimpedance spectroscopy. 44h ambulatory blood pressure (BP),aortic pulse wave velocity, left ventricular mass index, interdialytic weight gain were measured, and adverse events were recorded.

Results: After 12-month period of dialysis with negative sodium gradient, there were significant reductions in 44-hour ambulatory systolic and diastolic BP (-12 and -7 mmHg), and a small reduction in interdialytic weight gain (-0.30kg). There were also significant decreases in pulse wave velocity (from 12.68±2.37 to 11.75±2.71 m/s, $p=0.001$), and left ventricular mass index (from 149.29±13.75 to 139.61±14.41 g/m^2, $P < 0.001$). The post-HD volume parameters were kept constant throughout the study period. There were no significant changes in the frequency of adverse events.

Conclusions: In selected hypertensive HD patients, a small negative sodium gradient for 12 months resulted in a significant reduction in BP, accompanied by improvements in aortic stiffness and left ventricular hypertrophy with good tolerance.

TH-PO592

Dialysis Dose and Intra-Dialytic Hypotension Finnian R. McCausland, Steven M. Brunelli, Sushrut S. Waikar. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Intra-dialytic hypotension associates with increased morbidity and mortality in chronic hemodialysis patients. Intra-dialytic decline in osmolality from rapid urea removal may predispose to hypotension resulting from transcellular fluid shifts into the intracellular compartment. We wished to determine the association of clinical measures of urea removal with the occurrence of intra-dialytic hypotensive events in chronic hemodialysis patients.

Methods: We performed a post-hoc analysis of the HEMO Study (n=1826), a multi-center trial that randomized participants on the basis of dialysis dose (high vs standard Kt/V) and membrane flux (higher vs lower). Detailed hemodynamic and urea kinetic modeling data (URR) was abstracted from 62,557 dialysis sessions.

Results: In unadjusted analyses, higher target Kt/V was associated with a trend towards greater odds of intra-dialytic hypotension (OR 1.11; 95% CI 1.00-1.25). Upon adjustment for HEMO Study pre-specified covariates, this achieved significance (OR 1.13; 95% CI 1.01-1.26). In sensitivity analyses, higher URR (per 5%) was also associated with greater odds of intra-dialytic hypotension (OR 1.08; 95% CI 1.01-1.06).

Conclusions: Metrics of urea removal are independently associated with greater odds of developing intra-dialytic hypotension. These findings support a role for intra-dialytic decline in osmolality in the genesis of hemodynamic instability. Future studies should address the safety and efficacy of minimizing osmolality decline during hemodialysis.

TH-PO593

Isolated Ultrafiltration Induces Reduced Microcirculatory Flow without Changes in Systemic Blood Pressure in Chronic Hemodialysis Patients Marc H. Hemmelder,[1] Gerke Veenstra,[2] E.C. Boerma.[2] *[1]Nephrology, Medical Center Leeuwarden, Leeuwarden, Netherlands; [2]Intensive Care, Medical Center Leeuwarden, Leeuwarden, Netherlands.*

Background: It has been reported that microcirculatory blood flow decreases during hemodialysis with ultrafiltration treatment, while macrovascular hemodynamic parameters were unaffected. We tested whether this decrease of microcirculatory blood flow is dependent on the method of ultrafiltration in 9 chronic hemodialysis patients who had no previous hypotensive complications.

Methods: Three methods of ultrafiltration were performed at random order at a 4 hour midweek session during 3 consecutive weeks: 1. Isolated ultrafiltration after the hemodialysis session (IUF); 2. Linear ultrafiltration during hemodialysis (LUF); 3. Ultrafiltration by blood volume monitoring during hemodialysis (UF-BVM). Microcirculatory flow index (MFI) was assessed with sidestream darkfield imaging in the sublingual capillaries at each hour during the sessions. The amount of ultrafiltration was estimated by body composition measurement. Primary outcome parameter was the change in MFI from baseline to the end of a session. Differences were non-parametrically tested with Wilcoxon signed rank test ($p<0.05$).

Results: All patients were of Caucasian origin, with a mean age of 66±12 years and 37±29 months of dialysis vintage. Only IUF resulted in a significant decrease of MFI from 2.8 [2.8-2.9] to 2.5 [2.2-2.8], whereas MFI remained unchanged during LUF and UF-BVM. The amount of ultrafiltration did not differ between the sessions and amounted 1.7 [1.2-1.9] l, 1.7 [1.2-2.1] l and 1.9 [1.7-2.1] l respectively. During the sessions no differences in blood pressure, pulse or laboratory parameters were detected. No hypotensive period was present during the study.

Conclusions: We conclude that MFI only decreases during IUF, without a reduction of baseline blood pressure and pulse rate. In contrast LUF and UF-BVM had no adverse effects on MFI. Further research is needed to determine whether these observations are associated with adverse effects on the cardiovascular risk profile or residual renal function of chronic hemodialysis patients.

TH-PO594

Systolic Blood Pressure Was Reduced in Hypertensive Hemodialysis (HD) Patients during a Year-Long Quality Improvement Project on Fluid Management at One Facility Linda H. Ficociello,[1] Len A. Usvyat,[2] Michael Black,[1] Patrice B. Taylor,[2,3] Antoinette M. Ordish,[2,3] Paul Balter,[2] Paul M. Zabetakis,[2] Claudy Mullon,[1] Jose A. Diaz-Buxo.[1] *[1]Fresenius Medical Care - NA, Waltham, MA; [2]Renal Research Institute (RRI), New York, NY; [3]University of North Carolina Hospitals, Chapel Hill, NC.*

Background: Inadequate volume control is associated with hypertension in HD patients. An on-going quality improvement (QI) project on fluid management using Crit-Line® Blood Volume Monitors (CLM) has been conducted at an RRI clinic. CLM measure changes in hematocrit, oxygen saturation and relative blood volume during HD.

Methods: Systolic blood pressure pre (preSBP) and post dialysis (postSBP) were tracked from Month 0 (Baseline), month before QI project began, to Month 11 of QI project. Eligible patients were all 45 patients dialyzed at the clinic at start of Month 0. For this analysis, we classified patients with average preSBP≥140mmHg in Month 0 as "Baseline Hypertensive" (BL-HYP) and patients with preSBP<140 as "Baseline Normotensive" (BL-NOR). For patients with clinical data in Months 0 and 11 (n=36), paired t-tests compared monthly mean preSBP and postSBP before and after QI initiation. Longitudinal data analysis (linear mixed effects modeling) was conducted to estimate average change per month using all patients.

Results: The majority of patients were black (53%), hypertensive (62%), and without a catheter (80%). Reductions in preSBP and postSBP were observed in BL-HYP and not in BL-NOR.

	BL-HYP (n=26)			BL-NOR (n=10)		
	Month 0	Month 11	Pvalue	Month 0	Month 11	Pvalue
PreSBP (mmHg)	164.4±13.5	155.6±16.3	0.02	127.8±7.1	133.8±15.5	0.22
PostSBP (mmHg)	150.9±17.1	135.6±15.2	0.0003	121.5±12.3	123.1±13.3	0.78

The average change per month was modeled for BL-HYP to confirm changes observed Month 0 to 11. In BL-HYP, average change in PreSBP was -0.75 mmHg per month ($p=0.04$) and PostSBP was -1.86 mmHg per month ($p<0.0001$) in BL-HYP. There was no PreSBP or PostSBP decrease in BL-NOR.

Conclusions: Over a year-long QI project on fluid management, an average reduction of 8.9 and 15.3 mmHg was observed in preSBP ($p=0.02$) and postSBP ($p=0.0003$), respectively, among patients with hypertension.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

TH-PO595

Enhanced Sympathetic Activity Is Associated with Improved Long-Term Survival in Hemodialysis Patients Dvora Rubinger, Rebecca Backenroth, Yosef S. Haviv, Dan Sapoznikov. *Nephrology and Hypertension, Hadassah University Medical Center, Jerusalem, Israel.*

Background: Sympathetic activity is increased in patients with end stage renal disease, but its predictive value in chronic hemodialysis (HD) patients is not well established.

Methods: Multivariate analysis was performed to assess the prognostic role of blood pressure variability in the low frequency range (LF SBP) and of the standard deviation (sd) of interbeat intervals (sd IBI), two measures of sympathetic activity, as predictors of mortality and cardiovascular morbidity in HD patients. Continuous beat-to-beat IBI, systolic (SBP) and diastolic (DBP) blood pressure were monitored in 111 HD patients. LF SBP and sd IBI were calculated from recordings of SBP and IBI spontaneous variations. The study population was subdivided into tertiles for both variables. Primary (death) and secondary (fatal and non-fatal cardiovascular events) outcomes were recorded during a mean follow-up period of 60 months (range: 2-113).

Results: Kaplan-Meier analysis showed significantly decreased 5 year survival for patients with lower (1st tertile) LF SBP ($p=0.006$) and sd IBI ($p=0.040$) as compared with those with highest indices (3rd tertile). Patients in the lowest LF SBP and sd IBI tertiles had also an increased probability of cardiovascular events ($p=0.020$ and 0.025, respectively). On univariate analysis, age, diabetes mellitus, diffuse arterial disease, intradialytic hemodynamic stability, lower plasma albumin, increased CRP and decreased LF SBP, sd DBP and sd IBI were associated with both decreased survival and increased occurance of cardiovascular events. Multivariate Cox regression analysis with clinical data, biochemical and nutritional markers identified diffuse arterial disease, CRP and sd DBP, a marker of arterial mechanics, as the most significant predictors of mortality and cardiovascular morbidity.

Conclusions: Our data show that in HD patients, sympathetic activity is inversely correlated with mortality and cardiovascular morbidity. Lower sympathetic activity is significantly associated with diffuse arterial disease and increased inflammatory markers. In HD patients both outcomes are improved in the presence of a more robust autonomic nervous system.

TH-PO596

Interdialytic 44-Hours Ambulatory Blood Pressures Predict Left Ventricular Hypertrophy in Pediatric Hemodialysis Patients Chryso P. Katsoufis,[1] Wacharee Seeherunvong,[1] Nao Sasaki,[2] Carolyn L. Abitbol,[1] Jayanthi Chandar,[1] Michael Freundlich,[1] Gaston E. Zilleruelo.[1] *[1]Pediatric Nephrology, University of Miami, Miami, FL; [2]Pediatric Cardiology, University of Miami, Miami, FL.*

Background: Cardiovascular (CV) disease results in high mortality of patients with end-stage renal disease. Children undergoing chronic hemodialysis (HD) often exhibit left ventricular hypertrophy (LVH). 24-Hour ambulatory blood pressure monitoring (ABPM) has been shown to predict CV morbidity better than casual blood pressure (CBP). Given the extreme blood pressure (BP) variability attributed to interdialytic fluid overload, we hypothesized that 44-hour ABPM should better predict the CV morbidity in pediatric HD patients.

Methods: We conducted a cross-sectional study in 17 patients, 16.7±2.9 years, maintained on chronic HD. 44-Hour interdialytic ABPM was performed. Data of averaged pre and post-dialytic CBP measurements and routine echocardiogram within 6-month of ABPM were collected. Left ventricular mass index (LVMI) was calculated by the standard equation; LVH was defined as LVMI>95%ile for height-age and gender. Hypertension (HTN) was defined by the recommendations of the Fourth Report of the National High BP Education Program for CBP, and by those of the American Heart Association for ABP.

Results: 24% of patients had HTN by post-dialytic systolic CBP, whereas 47% were HTN by ABPM. 53% of patients had LVH, while 88% had abnormal cardiac geometry. 6/17 patients (35%) had masked HTN, including 2 with concentric LVH, 1 with eccentric LVH, 1 with concentric remodeling and 2 with normal cardiac geometry. LVMI correlated well with ABP, but not with CBP measurements. The strongest association with increased LVMI ($p=0.04$, $r^2=0.24$) and LVH ($p=0.04$) was seen in 2nd day ABP parameters, specifically nighttime measurements.

Conclusions: CBP underestimates HTN in pediatric HD patients and does not correlate with LVMI or LVH. In contrast, 44-hour interdialytic ABPM better characterizes HTN, with the nightime-BP of the 2nd day most strongly predicting increased LVMI and LVH. Prospective studies to investigate the benefit of targeting nocturnal HTN in this population are very much warranted.

TH-PO597

Mortality and the Variability of Blood Pressure and Heart Rate in Chronic Hemodialysis Patients Ying Wang, Jinghua Xia, Yan Qin, Xuemei Li, Xuewang Li, Limeng Chen. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: To identify the association between the variability in blood pressure and heart rate and mortality in hemodialysis patients.

Methods: The variation in blood pressure and heart rate was examined in 14,704 times' hemodialysis of 99 patients in a single university hospital between Jan to Dec 2006. The mortality was recorded during the 5 years follow-up from Jan, 2007 to Jan 2012. The Coefficient of variation and standard deviation were used as indicators of variability in blood pressure and heart rate respectively. Baseline demographic data and predialysis and postdialysis weights were also reviewed. Albumin, serum creatinine, calcium, phosphate, Hb, iPTH, KT/V and nPCR, were averaged over a year period. Cox regression analysis was used to identify the independent predictors for mortality.

Results: During the 5 year follow up period 35 patients died. Cardiovascular event was the most common cause of death (62.9%). Predialysis systolic blood pressure(SBP)<120 mmHg was an independent predictor for mortality(HR19.28, 95%CI3.37-110.13). Predialysis SBP variability (HR1.18[95%CI:1.03-1.35]per 1% increase in SBP variability) and predialysis heart rate variability(HR1.23[95%CI:1.07-1.42]per 1-bpm increase in HR variability) were independent predictors of total mortality. Mutiple linear regression analysis identified the variability of weight lose during dialysis was positively correlated with the variability of systolic blood pressure(r=3.291, P=0.008), while serum albumin was negatively(-0.218, P=0.010).The variability of heart rate was only negatively correlated with the use of ACEI(-0.20,P=0.048).

Conclusions: Lower predialysis SBP, larger variability of SBP and HR appears associated incrementally with higher mortality in HD patients. The volume variation may be correlated with the blood pressure variability.

TH-PO598

Autonomic Dysfunction and Inflammation in Hemodialysis (HD) Eric Seibert,[1] Kristina Zohles,[1] Christof Ulrich,[1] Alexander Kluttig,[2] Sebastian Nuding,[3] Jan Kors,[4] Cees A. Swenne,[5] Karl Werdan,[3] Roman Fiedler,[1] Matthias Girndt.[1] *[1]Nephrology, Halle University, Halle, Germany; [2]Medical Epidemiology, Biostatistics, Informatics, Halle University, Halle, Germany; [3]Cardiology, Halle University, Halle, Germany; [4]Medical Informatics, Erasmus University, Rotterdam, Netherlands; [5]Cardiology, Leiden University, Leiden, Netherlands.*

Background: Autonomic nervous dysfunction (AD) is common in HD patients (pt). Sympathetic and parasympathetic activation may influence inflammatory responses. We investigated a role of AD for inflammation in HD pt.

Methods: 30 HD pt and 15 healthy controls (ct) were studied for heart rate variability (HRV) using Fourier analysis of 5 min ECG recordings according to the CARLA study protocol. HRV was estimated by standard deviation of the R-R distance (SDNN) and the percentage of pairs of adjacent RR intervals differing by >50 ms (pNN50). Sympathetic/Parasympathetic activity was estimated using high- and low-frequency variation of RR distances (HF, LF). Inflammation was detected by CRP, IL-6 and monocyte subpopulation numbers. Immune cells were characterized by ACh receptor expression. 323 additional ct for HRV were matched for gender and diabetes from CARLA.

Results: Pt differed from ct in terms of age (66.8±9.4yrs vs. 54.5±7.4yrs, p<0.05) and HRV (SDNN ctr. 40.7±20.2ms, pt. 23.4±24.7ms; p<0.01). This finding was not restricted to diabetics although diabetes is an important cause of AD (SDNN,diab. 20.9±19.6ms, non-diab.25.7±29.2ms). LF and HF were reduced to 1/3 of those in ct, being best attributed to lowered baroreflex sensitivity. CARLA ct population confirmed SDNN, HF and LF findings in an extenuated but statistical significant manner. Pt had chronic inflammation (CRP 11.2±11.5 mg/l, ctr 1.8±1.3 mg/l, p<0.01) and expanded proinflammatory monocyte subpopulations (CD14/CD16 pos. cells 47±25/µl, ctr 28±19/µl, p<0.01). ECG parameters did not correlate with inflammation. Monocyte ACh receptor expression was enhanced in pt.

Conclusions: HD pt show strongly impaired HRV and signs of AD. Chronic inflammation is not directly related to AD, although monocytes express the ACh receptor at enhanced density making them potentially more sensitive to parasympathetic effects.

Funding: Pharmaceutical Company Support - Amgen

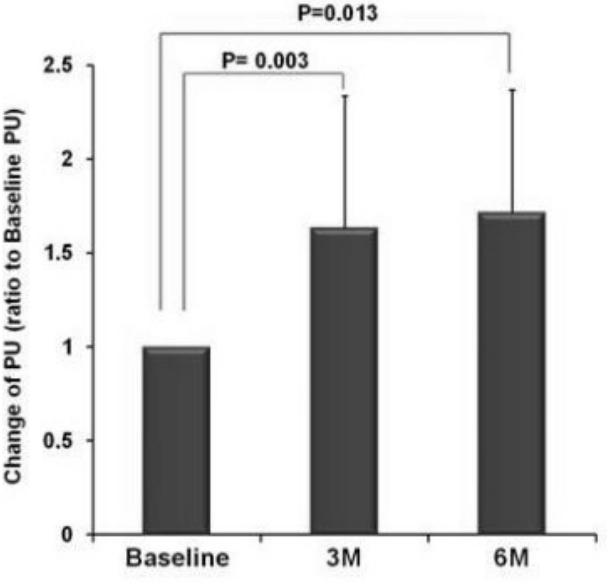
TH-PO599

AST-120 Improves Microvascular Endothelial Dysfunction in Hemodialysis (HD) Patients: A Preliminary Report Jung-hwa Ryu,[1] Mina Yu,[1] Shina Lee,[1] Sung Chul Hong,[1] Sang Hee Lee,[2] Dong-Ryeol Ryu,[1] Seung-Jung Kim,[1] Duk-Hee Kang,[1] Kyu Bok Choi.[1] *[1]Division of Nephrology, Department of Internal Medicine, Ewha Womans University School of Medicine, Seoul, Republic of Korea; [2]Division of Nephrology, Ewha Womans University Mokdong Hospital, Seoul, Republic of Korea.*

Background: Endothelial dysfunction(ED) is a pivotal phenomenon in the cardiovascular diseases(CVD), which are critical causes for mortality in HD patients. Indoxyl sulfate(IS) has been known to induce ED in chronic kidney disease. The aim of this study is to investigate whether AST-120, an absorbent for IS improves micorvascular or macrovascular ED in HD patients.

Methods: Twelve and 4 stable HD patients were included in AST-120group and the control, respectively. The subjects in AST-120 group were treated with AST-120(6g/day) for 6M. Microvascular function was assessed by laser Doppler flowmetry using iontophoresis of Acetylcholine(Ach) and sodium nitroprusside(SNP) at baseline,3M,and 6M,respectively. Change of Perfusion unit(PU) was measured.Carotid IMT and flow-mediated vasodilation(FMD) were assessed at baseline and 6M.

Results: Ach-induced iontophoresis(endothelium-dependent response) in AST-120 group was significantly improved in 3M and 6M than baseline, respectively.



Whereas, that of control didn't. SNP-induced response was not changed in either groups. The serum level of IS was decreased at 3M, but didn't show more decrease at 6M. IMT was significantly reduced at 6M than baseline in AST-120 group(meanIMT:0.78 at baseline *vs.*0.75mm at 6M,P=0.012). The FMD at 6M did not show significant changes compared to baseline in either groups. No specific side effect from AST-120 was not reported.

Conclusions: AST-120 ameliorated a microvascular ED in HD patients, furthermore, decreased IMT. Further studies with extended population will be needed to prove a crucial role of AST-120 for the prevention of CVD in HD patients.

Funding: Pharmaceutical Company Support - Kureha Chemical Industry

TH-PO600

Lower Oxygen Saturation Levels and Lack of Its Stability Are Associated with Higher Hospitalization Rates in Non-Catheter Hemodialysis (HD) Patients Patrice B. Taylor,[1,3] Lisa A. Pacelli,[1] Antoinette M. Ordish,[1,3] Nancy Ginsberg,[1] Len A. Usvyat,[1] Linda H. Ficociello,[2] Michael Black,[2] Claudy Mullon,[2] Jose A. Diaz-Buxo,[2] Mary T. Sullivan,[1] Peter Kotanko,[1] Paul Balter,[1] Paul M. Zabetakis.[1] *[1]RRI, NY, NY; [2]FMC, Waltham, MA; [3]University of North Carolina, Chapel Hill, NC.*

Background: An on-going quality improvement (QI) project using Crit-Line Blood Volume Monitors (CLM) has been conducted in 5 RRI clinics. CLM measures changes in Hct, oxygen (O2_SAT) and relative blood volume during HD. We aim to understand if minimum O2_SAT and changes in O2_SAT are associated with differing outcomes.

Methods: Data from 178 active HD patients (pts) that utilize Crit-line on a routine basis bn May 1, 2011 and Apr 30, 2012 were analyzed. We included only pts with arteriovenous fistulas and grafts. For every pt, we calculated lowest O2_SAT (O2_MIN) during the tx and the % change in O2_SAT computed as [max O2_SAT – min O2_SAT]/[min O2_SAT] (O2_CHANGE). Pts were divided into tertiles of O2_MIN and O2_CHANGE.

Results: Mean O2_MIN=90.7±8.0%; mean O2_CHANGE=5.4±6.2%. In bivariate correlation, O2_MIN was significantly & positively associated with albumin (r=0.20, p<0.05) and blood flow (r=0.32, p<0.05) but not with age, gender, race, diabetic status,

body weight, systolic blood pressure or on-line clearance (OLC). O2_CHANGE was significantly & negatively associated with albumin (r=-0.18, p<0.05), blood flow (r=-0.26, p<0.05), and OLC (r=-0.17, p<0.05).

Tertiles of O2_MIN	O2_MIN Range	Hospital admissions per patient year	Hospital days per patient year
1	45-91	1.60	10.52
2	91-93	1.50	9.23
3	93-97	0.73	4.54

Tertiles of O2_CHANGE	O2_CHANGE Range	Hospital admissions per patient year	Hospital days per patient year
1	0.9-3.1%	0.92	6.87
2	3.1%-5.2%	1.23	8.36
3	5.3%-20%	1.70	9.11

Pts with highest O2_SAT have fewest hospitalizations; similarly, pts with the most stable O2 levels during the tx (tertile 1 of O2_CHANGE) have fewest hospitalizations.

Conclusions: In non-catheter pts, higher O2_SAT are associated with higher albumin. Higher O2_SAT during tx are associated with fewer hospitalizations; stability of oxygen saturation during the tx is also associated with improved outcomes.

TH-PO601

Blood Oxygen Saturation (SO2) and Intradialytic Hypotension (IDH): The Final Results of the SOGLIA Study Elena Mancini,[1] Stefano Severi,[2] Claudia Perazzini,[2] Antonio Santoro.[1] *[1]Nephrology Dialysis Hypertension, S.Orsola Malpighi, Bologna, Italy; [2]HST-CIRI, University of Bologna, Bologna, Italy.*

Background: IDH is a frequent problem complicating HD. SO_2 affected by cardiac output, pH, Hb concentration, can be considered a marker of hemodynamic instability. Online, non-invasive monitoring of SO_2 is today possible, by means of an optical sensor measuring SO_2 in blood entering the dialyzer. The aim of the present multicenter observational trial was to assess, in a large number of HD sessions, whether the short-term variability of SO_2 may predict the occurrence of symptomatic IDH.

Methods: Fifty-one hypotension-prone patients were enrolled and monitored along 3 months, during their regular HD treatment, without any change in the medical prescription. Mean dry weight 72±16 kg, weight loss 3.0±0.8 kg; blood flow and session duration, 292±16 mL/min and 230±10 min respectively. SO_2 signal was recorded from the *Hemox* (Formula Therapy, Bellco, Italy). Blood pressure, at 30-min interval, symptoms, and onset time of IDH were collected. Based on IDH definition (EBPG), sessions with symptomatic IDH were counted offline as *positive* (IDH yes) or *negative* (IDH no). SO_2 standard deviation (SD) was computed on a 5 min length mobile windows. In the *positive* sessions, the SO_2 variability analysis was truncated at IDH onset.

Results: Out of 1636 HD sessions collected, 274 were discarded due to incorrect data collection: a total of 1362 sessions were then analysed. On the basis of the IDH presence, 314 (23%) were classified as *positive* and 1048 (77%) as *negative*.

The ROC analyses on the whole data set was performed and the critical threshold of SO_2 SD was chosen equal to 2.2%.

Based on the SO_2 variability, sensitivity was 67% (211/314 *positive* sessions identified) and the specificity was 64% (667/1048 *negative* sessions identified).

Conclusions: The SO_2 short term variability seems to show, on a large number of sessions, a fair predictive power of IDH. This study may pave the way to the implementation in a future generation of dialysis monitors of an automatic alarm system including SO_2 variability oscillations as a warning variable, offering the opportunity of preventive manoeuvres to avoid hypotension.

Funding: Clinical Revenue Support

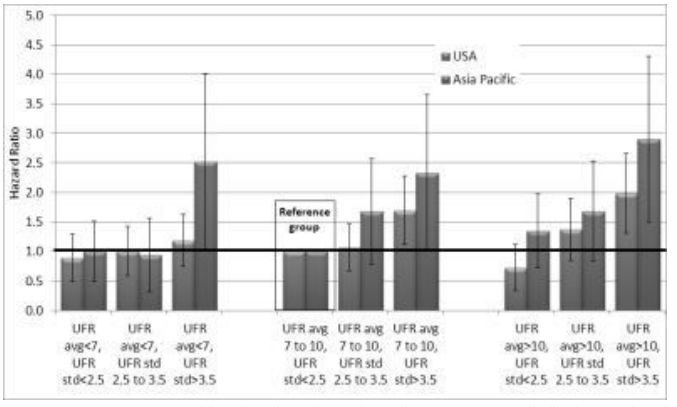
TH-PO602

Variability of Ultrafiltration Rate Is Associated with Poor Outcomes in US and Asian Pacific Dialysis Patients Len A. Usvyat,[1] Paul Balter,[1] Penny Faith Sheppard,[1] Adrian Marcos Guinsburg,[4] Aileen Grassmann,[3] Adam Tashman,[1] Cristina Marelli,[4] Eric Liu,[2] Daniele Marcelli,[3] Frank van der Sande,[6] Inga Bayh,[3] Jeroen Kooman,[6] Laura Scatizzi,[3] Michael Etter,[2] Stephan Thijssen,[1] Edwin B. Toffelmire,[5] Yuedong Wang,[7] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute, NY, NY; [2]Fresenius Asia Pacific Ltd, Hong Kong, Hong Kong; [3]Fresenius Medicare Care, Bad Homburg, Germany; [4]FMC, Buenos Aires, Argentina; [5]FMC Canada, Toronto, Canada; [6]Maastricht University Hospital, Maastricht, Netherlands; [7]University of California, Santa Barbara, CA.*

Background: We aim to determine whether level and variability in ultrafiltration rate (UFR) is associated with outcomes in incident HD patients (pts).

Methods: The MONitoring Dialysis Outcomes (MONDO) consortium consists of HD databases from RRI clinics (USA), FMC clinics in Europe, Asia, Latin America and Canada, Maastricht University (Netherlands) and KfH clinics (Germany). Only databases from RRI and FMC Asia were queried for incident HD pts who survived >12 months. "Baseline" UFR was computed as avg of <=6 months. "Follow up" UFR variability was computed as standard deviation (std) bn mns 7 and 12. Pts were stratified into 9 grps of baseline UFR and UFR variability. Pt survival was assessed in months 13 to 24 from HD initiation and Cox proportional hazards models were constructed.

Results: We studied 3979 pts from Asia and 6342 from US. Higher UFR variability was associated with poorer outcome in all pts. Notably, differences between pts with least UFR and highest variability were much more pronounced in Asia.



Conclusions: Our multi-national study confirms that UFR>10 mL/hr/kg is associated with poor survival. While the negative effects of higher UFR variability are noted in both US and Asian pts, it is particularly pronounced in the latter.

TH-PO603

Cardiovascular Risk in Hemodialysis Patients: Fluid Overload and Heart Rate Variability Anna Clementi,[1] Dinna N. Cruz,[1] Francesco Garzotto,[1] Manuela Ferrario,[2] Alessandra Brendolan,[1] Claudio Ronco.[1] *[1]Nephrology, Dialysis, Transplantation, St Bortolo Hosp, Vicenza, Italy; [2]Bioengineering, Politecnico, Milano, Italy.*

Background: Cardiovascular (CV) disease is the leading cause of mortality in hemodialysis (HD) patients. Fluid overload (FO) and abnormalities in the autonomic control of heart rate are important in its pathogenesis. We hypothesized that HD patients with high FO would have altered heart rate variability (HRV), and would experience worse CV outcomes.

Methods: We enrolled 76 stable chronic HD patients. FO was assessed with the Body Composition Monitor (Fresenius) before a mid-week HD session, and 24h ECG recordings were performed. The traditional HRV indices *SDNN*, *SDANN*, *RMSDD*, *pNN50%,VLF*, *LF*, *HF, LF/HF* were computed. Patients were classified into 5 groups depending on FO and pre-HD systolic blood pressure (SBP). Group I FO>2.5L-SBP>140mmHg; Group II normal hydration-SBP>140mmHg; Group III underhydration-SBP<140mmHg; Group IV FO>2.5L-SBP<140mmHg; Group N+Dx (reference group)-1L<FO<2.5L and 100<SBP<150 mmHg.The groups were compared using ANOVA for HRV indices, and survival curve analysis for death and hospital admissions.

Results: The patients were grouped as follws: Group I (n=34),II (n=4),III (n=0),IV (n=15),N+Dx (n=23). Group II was excluded from analysis due to small sample size. Group IV had significantly higher RMSSD, pNN50% and HF(p<0.05), which are related to parasympathetic nervous system (PNS). Group IV did worse in terms of all-cause death (p=0.02) and CV-related death (Fig.1,p=0.001). There was no difference among the groups with regards to all-cause or CV-related hospitalization.

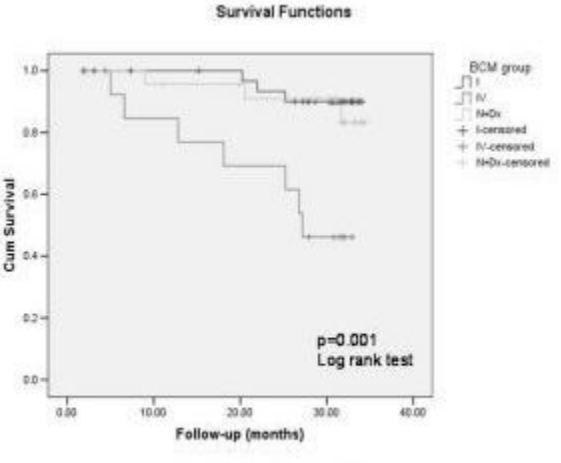


Fig. 1 CV-related death

Conclusions: In this study, HD patients with high FO(>2.5L) but low-normal SBP(<140mmHg) showed an overdrive of PNS, and this alteration in HRV may explain their higher mortality. This is the first study to analyze CV outcomes in HD patients considering both FO and HRV analysis.

TH-PO604

Time-Dependent Modeling of the Effectiveness of Cardioprotective Antihypertensives in Dialysis Patients Theresa I. Shireman,[1] Milind A. Phadnis,[1] James B. Wetmore,[1] Sally K. Rigler,[1] Qingjiang Hou,[1] John Spertus,[2] Jonathan D. Mahnken.[1] *[1]University of Kansas School of Medicine, Kansas City, KS; [2]Mid-America Heart Institute/UMKC, Kansas City, MO.*

Background: Cardiovascular disease is a leading cause of death in patients with end-stage renal disease (ESRD).There is limited clinical trial or observational data support for the benefits of renin-angiotensin system antagonists, beta-blockers, and calcium channel blockers in ESRD.

Methods: We developed a time-dependent model of medication exposure to determine the survival benefits of these medications in Medicare-Medicaid eligible ESRD patients linking Medicaid pharmacy claims with USRDS core files and Medicare institutional and physician/supplier service claims. We selected a cohort of hypertensive ESRD patients initiating dialysis between 1/1/2000 to 9/30/2005. We followed the cohort until death, loss of eligibility, or 12/31/2005. Medication exposure was defined with three time-dependent covariates: weekly drug availability (per days supply from claims), cumulative percentage of weeks with medication available (compliance), and cumulative number of weeks where drug availability switched (available vs not available). Models were adjusted for demographic, functional status, and comorbidity covariates. We used a Cox proportion hazards model with time-dependent covariates including a three-way interaction between the medication exposure measures.

Results: The final sample included 62,776 patients. Adjusted hazard ratios (AHR) for current use vs non-current use of any of the agents were significantly less than 1.0 for all combinations of switches and compliance: AHRs ranged from a low of 0.566 (1-2 switches with 90% compliance) up to 0.837 (10 switches with 10% compliance). For current users as compared to never users, AHRs ranged from 0.468 (0 switches & 100% compliance) up to 0.643 (1 switch & 10% compliance). We identified a significant 3-way interaction between time-dependent medication exposure that demonstrates a range of effectiveness for cardioprotective medications in ESRD.

Conclusions: The degree of mortality reduction associated with cardioprotective medications depends on the patterns of use over time.

Funding: NIDDK Support, Private Foundation Support

TH-PO605

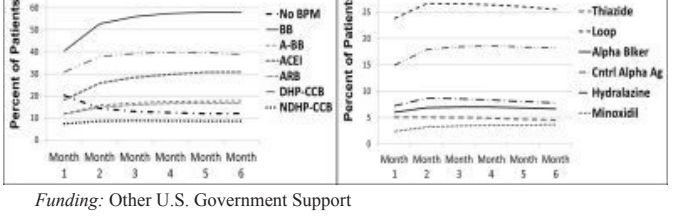
Blood Pressure Medications during the First 6 Months of Dialysis Wendy L. St. Peter,[1,4] Stephen M. Sozio,[2,4] Tariq Shafi,[2,4] Jason Luly,[2,4] P. Zager,[3,4] L. Ebony Boulware.[2,4] *[1]University of Minnesota; [2]Johns Hopkins University; [3]Dialysis Clinic, Inc; [4]The DEcIDE Network Patient Outcomes in End Stage Renal Disease Study Investigators.*

Background: Most incident dialysis patients have hypertension, but, there is a paucity of data on blood pressure medication (BPMs) in these patients. Our study describes trends in BPM prescription in first 6 months of dialysis.

Methods: We studied 15,056 Dialysis Clinic, Inc. patients (2003-2008). Data were linked to United States Renal Data System data for added information on patient characteristics and comorbidities.

Results: Mean age was 61 ± 15 yrs, 55% were male, 60% were White and 36% were African American (AA), 91.5% were on hemodialysis; 43%, 54% and 62% had congestive heart failure, cardiovascular disease and diabetes, respectively. 88% were prescribed ≥1 BPM in month 6. Over 6 months, use of beta-blockers (BB), alpha-beta blockers (A-BB), renin angiotensin system (RAS) agents increased, while other BPMs remained relatively stable. (Figures) AAs and Whites had a mean (± SD) of 2.7 (1.4) and 2.3 (1.3) BPMs in month 6, respectively. Older patients were prescribed less BPMs than younger. BPMs varied considerably by race and by comorbidity, but not by sex. Among those on BPMs, the most common prescriptions were BB alone (9.7%); BB+ RAS(7.3%); BB+dihydropyridine calcium channel blocker (DHP)(5.3%); BB+RAS+DHP(6.1%); BB+RAS+other (not DHP)(9.4%); BB+DHP+other (not RAS) (8.0%); BB+other (not DHP or RAS) (8.9%) and BB+DHP+RAS+other (10%). 72% and 54% of patients on BPMs were prescribed combination regimens containing BB and RAS agents, respectively bu month 6.

Conclusions: BPM patterns are established early after dialysis initiation. Prescription of specific BPM classes varied by race, age and comorbidity. Except for BB, single BPM use was uncommon. Interventions to change prescription patterns should focus on this early period.



Funding: Other U.S. Government Support

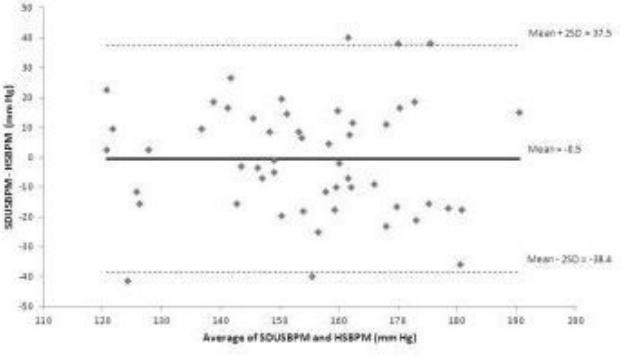
TH-PO606

Adherence and Comparison of Standardized Dialysis Unit and Home Blood Pressure Measurements in the BID Study A. Gul,[1] D. Miskulin,[2] Jennifer J. Gassman,[3] B. Horowitz,[1] P. Zager.[1,4] *[1]UNM, ABQ, NM; [2]Tufts, Boston, MA; [3]Cleveland Clinic Foundation, Cleveland, OH; [4]DCI, ABQ, NM.*

Background: The best blood pressure measurement to guide treatment in hemodialysis (HD) patients is uncertain. Routine dialysis unit measurements of systolic blood pressure exhibit significant variability and are poor predictors of outcomes. Standardized systolic blood pressure measurements, made in accord with American Heart Association guidelines in the dialysis unit (SDUSBPM) and at home (HSBPM) may be less variable but long term adherence is uncertain. The BID (Blood Pressure in Dialysis) Study, a pilot randomized clinical trial (RCT) assessing usual vs. intensive control of systolic blood pressure (SBP), is comparing SDUSBPM and HSBPM.

Methods: We studied the first 52 BID participants. We assessed adherence by computing the percentages of the prescribed pre-dialysis SDUSBPM and HSBPM obtained. We compared midweek pre-dialysis SDUSBPM with HSBPM obtained the following day.

Results: During months 1, 2, and 3 the percentages of prescribed SDUSBPM and HSBPM obtained were 96.4, 92.7 and 97.9% and 84.5, 80.3 and 80.4%, respectively. The mean difference between SDUSBPM and HSBPM was -0.48 mm Hg (SD 18.9; range -41.5 to 40 mmHg). A Bland-Altman plot revealed no overall bias between SDUSBPM and HSBPM. Differences between paired readings were < 2 SD from the mean difference in 86% of participants.



However, some participants exhibited significant differences between the two measurements (mm Hg) 5-10 (21.2%), 11-20 (42.3%) and >20 (23.07%). Analyses of diastolic blood pressure had similar results.

Conclusions: It is feasible to obtain both SDUSBPM and HSBPM in a RCT of HD patients. The relative differences were modest and HSBPM were not systematically lower than SDUSBPM. However, some participants had large differences between the paired measurements.

Funding: NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic Inc.

TH-PO607

Associations of Heart Failure and Cardiovascular Medications with Wall Motion Abnormalities during Hemodialysis Ruth F. Dubin,[1] Alexis L. Beatty,[2] John R. Teerlink,[2] Nelson B. Schiller,[2] Dean Alokozai,[3] Carmen A. Peralta,[1] Kirsten L. Johansen.[1] *[1]Nephrology, San Francisco VAMC/UCSF, San Francisco, CA; [2]Cardiology, San Francisco VAMC/UCSF, San Francisco, CA; [3]Cardiocore, South San Francisco, CA.*

Background: Worsening of cardiac wall motion abnormalities (WMA) during hemodialysis has been observed by prior investigators and may contribute to the high rate of cardiovascular death in persons with end-stage renal disease. We sought to identify risk factors and changes in biomarkers of ischemia (highly sensitive cardiac troponin T (hs-TnT)) associated with intradialytic WMA.

Methods: A trained sonographer performed echocardiograms before and during the last hour of dialysis in 40 patients (80 dialysis sessions). A single, blinded reader at a core laboratory analyzed WMA in 16 myocardial segments. In a subset of 26 patients (40 dialysis sessions) we measured pre- and post-dialytic hs-TnT, adjusted for hemoconcentration. We evaluated relative risk of worsening WMA using a generalized linear model and performed multivariable adjustment for covariates that were associated with WMA at $p<0.2$: dialysis vintage, post-dialysis fatigue, congestive heart failure (CHF), hemoglobin, angiotensin converting enzyme (ACE) or angiotensin receptor blocker (ARB) use, beta blockers, and aspirin.

Results: Among 40 patients, WMA worsened in 11(28%) and improved in 4(10%) during dialysis. Intradialytic hemodynamic measures, ultrafiltration, and baseline hs-TnT or changes in hs-TnT were not associated with WMA. After multivariable adjustment, participants with CHF had a 6.1-fold higher risk of worsened WMA (95%CI (2.8, 13), $p<0.001$). A reduced risk of worsened WMA was observed for participants using ACE/ARB (RR 0.22, 95%CI (0.1, 0.4), $p<0.001$) or beta blockers (RR 0.44, 95%CI (0.2, 0.8), $p=0.01$). Further adjustment for ultrafiltration, change in mean arterial pressure, diabetes or atherosclerosis did not significantly change these associations.

Conclusions: Subjects with CHF may be at increased risk of worsened WMA during hemodialysis. Further studies are needed to understand whether medications commonly used for CHF may ameliorate this risk.

TH-PO608

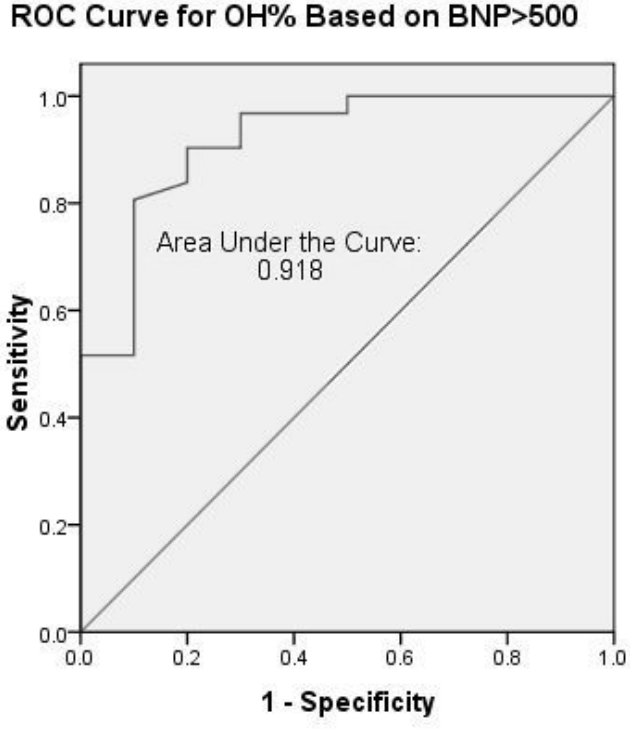
Volume Estimation in Dialysis Patients: The Concordance of Brain-Type Natriurtic Peptide Measurements and Bioimpedence Values

Mihaly Tapolyai,[1] Maria Faludi,[1] Virag Reti,[1] Zsolt Lengvarszky,[2] Tibor Szarvas,[2] Tibor Fulop,[4] Gabriella Beko,[3] Klara Berta.[1] *[1]Dialysis, Semmelweis University Budapest, Budapest, Hungary; [2]Mathematics, Louisiana State University Shreveport, Shreveport, LA; [3]Laboratory Medicine, Semmelweis University Budapest, Budapest, Hungary; [4]Nephrology, University of Mississippi, Jackson, MS.*

Background: The estimation of hydrational status in dialysis patients remains and important but difficult quest. Bioimpedance measurements have been validated by various physiological tests and the use of Brain-type natriuretic peptide (BNP) has been validated by inferior vena cava diameter measurements.

Methods: This is an observational study to evaluate the correspondence of bioimpedance measured overhydration percentage (OH%) over the extracellular water with concurrent BNP measurements. We measured OH% by a bioimpedance apparatus (BCM) and BNP pre-dialysis in 41 prevalent HD patients: 19/41 women, age 58.9 ±14.5 years.

Results: The average BNP was 2694 ±3278 pg/mL and 10 (24.4%) of the 41 patients had a BNP less than 500; average 260.7 ±108.5 pg/mL. The OH% was 8.5 ±7.0% among those with a BNP of less than 500 while the rest of the population had an OH% of 21.4 ±8.0%, corresponding to an excess volume of 1.6 ±1.3 and 4.4 ±3.8 L, respectively. The OH% vs. BNP relationship is best described by an exponential regression analysis: of $y = 216.4e^{0.097x}$ (r 0.6996). The regression line predicted BNP 216.4 pg/mL at 0% overhydration status. Receiver Operator Curves (ROC) revealed an AUC was 0.885 for the BNP when the OH% was set ≥15% of overhydration and an AUC for OH% is 0.918 when the BNP is set to be ≥500 pg/mL for the abnormal values.



Conclusions: We conclude that in our cohort there is a high degree of correspondence between these two tests with an exponential relationship between the measurements.

TH-PO609

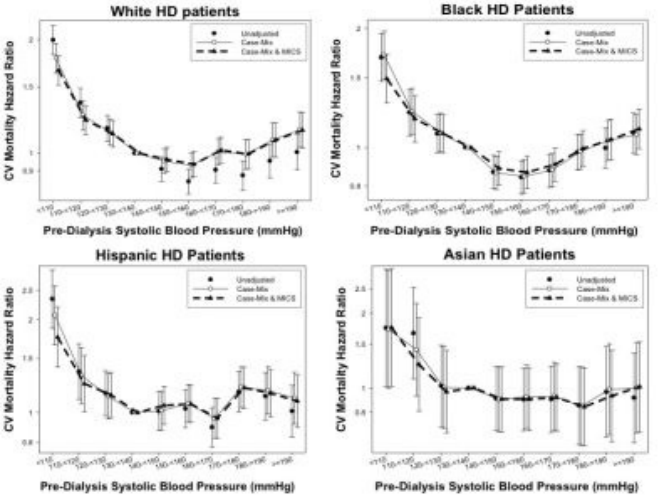
Racial-Ethnic Differences in the Association of Pre-Hemodialysis Systolic Blood Pressure and Cardiovascular Mortality

Joni L. Ricks,[1] John J. Sim,[2] Miklos Zsolt Molnar,[1] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology at Los Angeles Biomedical Research Institute at Harbor-UCLA; [2]Kaiser Permanente; [3]University of Tennessee; [4]Harbor-UCLA.*

Background: While stroke and mortality have been associated with rising systolic blood pressure(SBP), the relationship between cardiovascular(CV) disease and SBP has not been evaluated among maintenance hemodialysis patients(MHD). We sought to examine whether low vs. high SBP at the start of hemodialysis(HD) treatment session was a predictor of CV mortality across different racial-ethnic groups in a large cohort of MHD patients.

Methods: Pre-Dialysis SBP and CV mortality was examined in a cohort of 111,030 MHD outpatients including 47,813 White, 35,874 Black, 16,180 Hispanic and 3,304 Asian outpatients. Three levels of statistical adjustment are shown for each stratified Cox Proportional models. The pre-dialysis SBP group of 130 to <140 was used as the reference in each model.

Results: Among White, Black, Hispanic and Asian MHD patients, the mean age was 65±15, 57±15, 58±15 and 63±16 yrs and included 42%, 48%, 45% and 48% women; and 54%, 56%, 69%, 56% diabetics, respectively. Across the 10 a priori SBP groups from <110 to ≥190 mmHg, very low pre-dialysis SBP (<110 mmHg) was associated with higher CV mortality in all racial-ethnic groups examined. Additionally, the 3 lowest pre-dialysis SBP groups were associated with increased CV mortality in White MHD patients, while the 2 lowest pre-dialysis SBP groups were associated with of higher mortality among Black and Hispanic MHD patients.



Conclusions: Similarly worse CV mortality is observed in the lower pre-HD SBP groups across all racial and ethnic groups of MHD patients; although this association appears most pronounced among White MHD patients. Our findings beg the question of where optimal pre-HD SBP should be.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

TH-PO610

Association of Achieved Levels of Blood Pressure with Cardiovascular Disease and Death among Hypertensive Hemodialysis Patients: A Sub-Analysis of OCTOPUS

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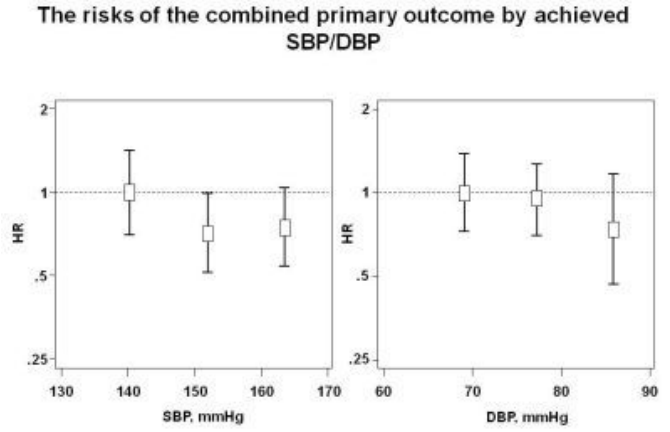
Background: Hypertension is a major risk factor for death and cardiovascular disease in patients with chronic haemodialysis (HD), but there is uncertainty surrounding the target levels of blood pressure (BP) in this high-risk patient group.

Methods: In a multicentre, prospective, randomised, open-label, blinded-endpoint trial, we assigned 469 patients with chronic HD and elevate BP (140-199/90-99 mmHg) to receive an ARB olmesartan (at a dose of 10-40 mg daily; n=235) or other treatment except for ARB and ACEi (n=234). A total of 449 patients with information on achieved BP were included in the present analysis. Three patient groups were defined by tertiles of achieved systolic (SBP) and diastolic BP (DBP). Primary outcomes were (1) composite of death, nonfatal stroke, nonfatal myocardial infarction, and coronary revascularization, and (2) all-cause death.

Results: During a mean follow-up of 3.9 years, 125 combined primary outcome events and 67 deaths were observed. Incidence rates of combined primary outcomes were 9.7% in achieved SBP<145 mmHg, 7.2% in achieved SBP 145-156 mmHg, and 8.6% in achieved SBP≥157 mmHg and those of achieved DBPs were 11.2% (<73 mmHg), 9.3% (73-81 mmHg) and 5.2% (≥82 mmHg). Mortality rates were 5.5% in achieved SBP<145 mmHg, 3.0% in achieved SBP 145-156 mmHg, and 4.0% in achieved SBP≥157 mmHg and that of achieved DBPs were 6.4% (<73 mmHg), 4.4% (73-81 mmHg) and 1.8% (≥82 mmHg). Although trends for both primary composite outcomes and mortality rate were not significant in both achieved SBP and DBP, events rates were lowest in patients who achieved SBP of 145-156 mmHg and DBP of ≥82 mmHg.



Conclusions: Target levels of blood pressure seemed to be higher than those of non dialysis CKD population.

Funding: Private Foundation Support

TH-PO611

Progression of Aortic Arch Calcification over 1 Year Is an Independent Predictor of Mortality in Incident Peritoneal Dialysis Patients Mi Jung Lee,[1] Dae-Suk Han,[1] Seung Hyeok Han,[1] Dong Ho Shin,[1] Hye-young Kang,[2] Seong Hun Kim,[2] Shin-Wook Kang.[1,2] *[1]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei University, Seoul, Republic of Korea.*

Background: The presence and progression of vascular calcification have been demonstrated as important risk factors for mortality in dialysis patients. However, since most previous studies were conducted in hemodialysis, limited information was available in peritoneal dialysis (PD) patients. Therefore, this study was aimed to investigate the prevalence of aortic arch calcification (AoAC) and prognostic value of AoAC progression in PD patients.

Methods: We prospectively determined AoAC on chest X-ray at the time of PD commencement and after 12 months in 415 incident PD patients. Progression of AoAC was defined as an increase in AoAC score on the follow-up chest X-ray. Patients were divided into 4 groups according to the presence and progression of baseline AoAC. Cox proportional hazard analysis was performed to determine the independent prognostic value of the presence and progression of baseline AoAC for all-cause and cardiovascular mortality.

Results: Of 415 patients, 169 patients (40.7%) had AoAC at baseline with a mean of 18.1±11.2%. The presence of baseline AoAC was an independent predictor of all-cause (Hazard ratio [HR], 2.181; 95% confidence interval [CI], 1.336-3.561; P=0.002) and cardiovascular mortalities (HR, 3.582; 95% CI, 1.577-8.132; P=0.002). In addition, among 363 patients with available follow-up chest X-rays at 12 months after PD start, all-cause and cardiovascular death rates were significantly higher in the progression group compared with the non-progression group (P<0.001). Moreover, multivariate Cox regression analysis revealed that AoAC progression was an independent predictor for all-cause (HR, 2.491; 95% CI, 1.115-5.560; P=0.026) and cardiovascular mortalities (HR, 3.450; 95% CI, 1.044-11.401; P=0.042) in patients with AoAC at baseline.

Conclusions: The presence and progression of AoAC assessed by chest X-ray were independently associated with unfavorable outcomes in incident PD patients.

TH-PO612

Genetic Approach to Evaluate the Role of the PDE3 Subfamilies in Polycystic Kidney Disease (PKD) Hong Ye,[1] Xiaofang Wang,[1] Caroline R. Sussman,[1] Peter C. Harris,[1] Vincent C. Manganiello,[2] Christopher James Ward,[1] Vicente E. Torres.[1] *[1]Mayo Clinic, Rochester, MN; [2]NHLBI, NIH, Bethesda, MD.*

Background: A large body of evidence indicates that cAMP and protein Kinase A (PKA) play a central role in the pathogenesis of PKD. Accumulation of cAMP in cystic tissues may in part be due to enhanced adenylyl cyclase activity, but inhibition of cAMP degradation by phosphodiesterases (PDE) likely plays an important role. Of the large PDE superfamily, the PDE1 and PDE3 families may be particularly important. PDE1 accounts for most PDE activity in renal tubules, is the only PDE activated by calcium (which is reduced in PKD cells), and its activity is reduced in cystic kidneys (Wang etal KI 77:129 2010). PDE3 is inhibited by cGMP (which is degraded by PDE1) and affects tubular epithelial cell proliferation.

Methods: To assess the role of the two PDE3 subfamilies in PKD, we generated double *Pde3a* or *Pde3b* and *Pkd2* mutants that were sacrificed at 16 weeks. Blood was collected by cardiac puncture and kidneys (K) and liver (L) harvested to evaluate the extent of the cystic disease.

Results:

Pkd2$^{-/WS25}$ mice	BW, g	K/B Wt, %	Cyst area, %	Fibrosis area, %	L/B Wt, %
Pde3a$^{-/-}$ (M, n=11)	30.9±3.8	2.14±0.49	37.8±18.4	7.2±4.1	5.80±0.52
Pde3a$^{-/-}$(F, n=7)	21.9±4.6	2.21±0.76	39.1±15.6	8.4±4.1	5.58±0.63
Pde3a$^{+/+}$(M, n=11)	31.1±2.2	1.61±0.49	13.6±17.5	4.5±2.1	4.74±0.52
Pde3a$^{+/+}$(F, n=10)	21.2±2.5	1.60±0.34	16.8±9.0	4.7±1.9	4.88±0.32
Pde3a$^{+/+}$(M, n=10)	28.5±2.7	1.73±0.42	20.8±13.8	3.3±2.0	4.55±0.21
Pde3a$^{+/+}$(F, n=10)	20.7±2.8	1.74±0.72	19.7±8.9	3.7±1.9	4.55±0.58
P values*	NS	0.0013	<0.0001	<0.0001	<0.0001

* *Pde3a* genotype effect (two-way ANOVA)

The *Pde3a* knockout was associated with worse PKD and polycystic liver disease (PLD) in *Pkd2*$^{-/WS25}$ mice. The *Pde3a* knockout was also associated with worse PLD (but not PKD) in *Pkd2*$^{+/-}$ and *Pkd2*$^{+/WS25}$ mice (not shown). The *Pde3b* knockout, contrary to the *Pde3a* knockout, was not associated with detectable worsening of PKD or PLD in *Pkd2*$^{-/WS25}$ mice (not shown).

Conclusions: These results suggest that a *Pde3a* variant may regulate a cAMP pool important for cystogenesis. Triple *Pde3a/Pde3b/Pkd2* mutants are being generated to rule out functional redundancy of the two *Pde3* subfamilies.

Funding: NIDDK Support

TH-PO613

Effects of PDE1 or PDE3 Inhibition on Cyst Development in *Pkd2-/WS25* Mice Hong Ye, Xiaofang Wang, Caroline R. Sussman, Peter C. Harris, Christopher James Ward, Vicente E. Torres. *Mayo Clinic, Rochester, MN.*

Background: A large body of evidence indicates that cAMP plays a central role in the pathogenesis of Polycystic Kidney Disease (PKD). DDAVP administration markedly aggravates the renal cystic disease in PCK rats. However, it has no or only a negligible effect in *Pkd2*$^{-/WS25}$ mice. This may be due to higher PDE1 activity in murine compared to rat kidney (Wang et al Kid Int 77:129, 2010). PDE1 accounts for most PDE activity in renal tubules, is the only PDE activated by calcium (which is reduced in PKD cells), and its activity is reduced in cystic kidneys. PDE3 is inhibited by cGMP (which is degraded by PDE1) and affects tubular epithelial cell proliferation.

Methods: To determine whether a cystogenic effect of DDAVP in male (M) or female (F) *Pkd2*$^{-/WS25}$ mice can be unmasked by simultaneous treatment with a PDE1 or PDE3 inhibitor, we administered DDAVP (D) via osmotic pump (30 ng/100 g bw/hour, sc) together with vinpocetin (VP, a PDE1 inhibitor, 40 mg/kg bw/day by gavage), cilostazol (CZ, a PDE3 inhibitor, 60 mg/kg bw/day by gavage) or vehicle alone (CN) between 4 and 13 (VP) or 16 (CZ) weeks of age.

Results: In both cases administration of DDAVP aggravated PKD development.

Group (n)	BW, g	Kid cAMP [a]	Kid PDE [b]	Kid/B Wt, %	Cyst area, %	P Urea [c]
CN M (10)	26.1±2.4	2.7±0.9	10.1±2.0	1.52±0.19	15.1±9.2	62±12
CN F (10)	21.2±1.1	4.0±1.3	9.6±1.8	1.63±0.33	13.7±6.5	68±11
VP+D M (9)	23.0±1.5	7.2±4.2	8.3±1.3	2.22±0.75	31.5±19.3	80±21
VP+D F (9)	19.7±1.8	6.8±3.3	7.9±0.8	2.19±0.69	29.7±17.9	79±11
P value [d]	<0.001	<0.001	0.001	<0.001	0.001	0.005
CN M (14)	27.1±1.9	3.0±1.2	1.27±0.23	1.56±0.19	11.4±7.9	82±15
CN F (12)	21.3±1.9	3.8±1.3	1.10±0.19	1.64±0.38	16.4±11.8	75±12
CZ+D M (14)	25.6±1.8	7.3±3.4	0.80±0.23	2.03±0.34	22.2±15.7	94±13
CZ+D F (14)	21.5±1.8	5.6±1.8	0.33±0.26	2.12±0.36	27.7±16.5	98±22
P value [d]	NS	<0.001	<0.001	<0.001	0.003	<0.001

[a] pmol/mg renal lysate protein [b] PDE activity (pmol/min/mg renal lysate protein PDE1 in rows 2-5 and PDE3 in rows 7-10) [c] mg/dl [d] Treatment effect (two-way ANOVA)

Conclusions: Coadministration of DDAVP and either a PDE1 or a PDE3 inhibitor aggravates PKD development in *Pkd2*$^{-/WS25}$ mice. The results may underestimate the role of PDE1 and PDE3 since their activity was only moderately suppressed by VP or CZ administration.

Funding: NIDDK Support

TH-PO614

Hsp90 Inhibition Slows Cyst Growth in a Mouse Model of Autosomal Dominant Polycystic Kidney Disease (ADPKD) Tamina Seeger-Nukpezah,[1] David A. Proia,[2] Erica A. Golemis.[1] *[1]Fox Chase Cancer Center, Philadelphia, PA; [2]Synta Pharmaceuticals, Lexington, MA.*

Background: ADPKD affects about 1:700 individuals and typically manifests in middle age, with 50% of affected individuals progressing towards end stage renal disease. Development of ADPKD is associated with elevated activity of numerous signaling proteins that control proliferation and cell-environment interactions. These include SRC, STAT3, ERK, Aurora-A, and others. We recognized that many of these signaling proteins are clients and effectors of the molecular chaperone HSP90, suggesting that treatment with HSP90 inhibitors might provide a useful therapy for PKD by simultaneously depressing activity of multiple PKD-promoting signaling factors.

Methods: Tamoxifen-inducible PKD1 conditional knockout mice, in which cysts normally appear at 4 months of age, were used as a model for ADPKD. Mice were dosed with vehicle or the second generation HSP90 inhibitor STA-2842 once weekly for 10 weeks, from 4 - 6.5 months of age. Using monthly MRI-imaging, histopathology and blood urea nitrogen measurements we quantitated cyst growth and renal function. Reverse phase protein arrays (RPPA) were implemented to measure >150 protein analytes in vehicle and STA-2842 treated PKD1-/- cell lines.

Results: STA-2842 was well tolerated during treatment. Cyst growth was greatly reduced and renal function significantly improved in mice treated with STA-2842, in comparison to the control group. A follow-up experiment indicated that prevention therapy, with treatment starting directly after induced *PKD1* deletion, delayed cystogenesis. RPPA analysis showed a reduction in receptor tyrosine kinase expression (EGFR, HER2, IGF-1R) by STA-2842 in PKD-/- cell lines, as well as deactivation of numerous signaling proteins (AKT, SRC, MAPK, STAT3) and upregulation of pro-apoptotic factors (BIM, ANNEXIN).

Conclusions: Inhibition of HSP90, in a mouse model of ADPKD, demonstrates encouraging signs of activity, both in the reduction of cyst number and volume as well as improved renal function. With weekly dosing schedule being well tolerated and effective over a long period of time, HSP90 inhibition may be suitable for long-term treatment of ADPKD patients.

Funding: Other NIH Support - RO1 CA63366

TH-PO615

Network Analysis of a *Pkd1*-Mouse Model of Autosomal Dominant Polycystic Kidney Disease Identifies HNF4α as a Disease Modifier

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD; MIM ID's 173900, 601313, 613095) is caused by mutations in *PKD1* or *PKD2* and leads to end stage kidney disease. Inactivation of *Pkd1* before or after P13 in mice results in distinct early- or late-onset disease.

Methods: Using a mouse model carrying floxed *Pkd1* alleles and inducible Cre recombinase, we applied network analysis to transcriptomics and metabolomics data, following disease progression in a large number of mice induced before P10. We analyzed 1114 published gene expression arrays, and tracked phenotypic and metabolomics changes in *Pkd1/Hnf4*α double conditional mice.

Results: Weighted gene co-expression network analysis suggests that *Pkd1*-cystogenesis does not cause developmental arrest and occurs in the context of gene networks similar to those that regulate normal kidney morphology/function. Pathway analysis identifies HNF4α as a network node. The results are supported by a meta-analysis of 1114 published gene expression arrays, and predict metabolic pathways are key elements in postnatal kidney maturation and cystogenesis. Urinary metabolomics show that *Pkd1* cystic mutants have a distinct profile of excreted metabolites, suggestive of altered metabolism. We perturbed metabolic networks by inactivating *Hnf4*α. *Pkd1/Hnf4*α double mutants have significantly more cystic kidneys, indicating that metabolic pathways could play a role in *Pkd1*-cystogenesis.

Conclusions: The data suggest that *Pkd1*-cystogenesis does not require/cause developmental arrest, and occurs in the context of gene networks essentially similar to those that regulate normal kidney morphology/function. We propose that postnatal kidney maturation is accompanied by changes in metabolic pathways that are likely modifiers of *Pkd1*-cystogenesis and could underlie the differences in the kinetics of cyst formation in early- or late-onset disease models. We show that HNFα is a modifier of *Pkd1*-cystogenesis.

Funding: NIDDK Support

TH-PO616

A Novel Kidney-Selective AMPK Activator NT1021 Inhibits Proliferation and *In Vitro* Cyst Growth of ADPKD Cells

Gail Reif,[1] Emily Nivens,[1] Archana Raman,[1] Cibele S. Pinto,[1] Ken W. Batchelor,[2] Darren P. Wallace.[1] *[1]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]NovaTarg Inc, Research Triangle Park, NC.*

Background: In polycystic kidney disease (PKD), inappropriate activation of mTOR signaling increases proliferation of cyst-lining epithelial cells and contributes to PKD progression. AMP-activated protein kinase (AMPK) is an energy sensor that orchestrates energy utilization and regulates cell growth and protein synthesis through mTOR inhibition. AMPK also inhibits CFTR Cl^- channels. Metformin, an approved drug that activates AMPK, was shown to inhibit phosphorylation of S6 kinase, a downstream mediator of mTOR and reduce cyst growth in PKD mice, suggesting that AMPK is a therapeutic target for PKD. Metformin is transported by organic cation transporters, including OCT2 on the basolateral membrane of proximal tubules, and OCT1 expressed by hepatocytes. We generated a panel of 60 biguanide analogues for selective transport by OCT2 to increase renal drug exposure. NT1021 was identified as the most highly OCT2-selective biquanide.

Methods: Effects of NT1021on S6 kinase and S6 phosphorylation in human ADPKD cells were determined by Western blot analysis. To test the drug's effect on CFTR, ADPKD monolayers were treated with forskolin, a cAMP agonist, in the absence and presence of NT1021. Changes in anion current were measured by short circuit current. For cyst growth assays, ADPKD cells were seeded within a collagen matrix and treated with EGF and forskolin ± NT1021. Total surface area of the cysts per well was calculated from individual cyst diameters.

Results: EGF increased levels of phosphorylated S6 kinase and S6 and the rate of ADPKD cell proliferation, consistent with activation of mTOR signaling. NT1021 decreased EGF-induced S6 kinase and S6 phosphorylation and cell proliferation at lower concentrations compared to metformin. NT1021 also caused an inhibition of cAMP-induced anion secretion across ADPKD cell monolayers and completely blocked *in vitro* cyst growth.

Conclusions: A highly selective OCT2 transported biguanide NT1021 inhibits mTOR activation and cell proliferation and blocks *in vitro* cyst growth of human ADPKD cells.

Funding: NIDDK Support, Pharmaceutical Company Support - NovaTarg

TH-PO617

Two Ranges of Rapamycin for Treatment of Autosomal Dominant Polycystic Kidney Disease: 12-Month Open-Label Pilot Study

William E. Braun, Jesse D. Schold, Rita A. Spirko, Brian R. Stephany, Brian R. Herts. *Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH.*

Background: Rapamycin studies in human ADPKD that used change in total kidney volume (TKV) as the primary endpoint and change in estimated glomerular filtration rate (eGFR) have not substantiated the protective effect of rapamycin seen in animal models. Change in iothalamate GFR (iGFR) at 12 months was the primary endpoint here; rapamycin dosing was directed at different trough ranges.

Methods: Thirty patients with ADPKD were randomized in an open-label 12-month pilot study to low-dose rapamycin (trough level 2-5 ng/mL) (Group A, n=10), standard dose rapamycin (trough level >5-8 ng/mL) (Group B, n=10), or standard care (Group C, n=10). There were no significant differences in demographics or indicators of rapid progression between the groups. Evaluations were also done at 6 months and post-treatment at 18 months. Rapamycin levels were measured by liquid chromatography-tandem mass spectometry, and renal volume by non-contrast computed tomography (CT).

Results: 87% of patients (26/30) had 12-month data for the primary end-point of change in iGFR (9 in Group A, 8 in Group B, and 9 in Group C). Change in iGFR (mL/min/1.73m2) at 12 months was significantly improved in Group A (+7.7 ± 12.5) but not in Group B (+1.6 ± 12.1) when compared to Group C (-11.2 ± 9.1) (A vs C p=0.005, B vs C p=0.07, A vs B p=0.52, A + B vs C p=0.002). Group A iGFR change was also significant at 6- but not at 18-month follow-up. Change in TKV at 12 months was not significantly different: +196.7 ± 201.2 cc in Group A, +82.9 ± 111.3 cc in Group B, and +152.7 ± 129.4 cc in Group C (A vs C, B vs C, A vs B, A + B vs C, all p=NS). Trough rapamycin levels in Group A were significantly lower than in Group B: 6 months (n=9) (2.49 ± 0.75 vs 4.48 ± 1.33 ng/mL, respectively, p=0.001); 12 months (n=6) (2.75 ± 0.29 vs 5.28 ± 1.42 ng/mL, respectively, p=0.002). Adverse events were most frequent in Group B.

Conclusions: Low-dose rapamycin (Group A) was associated with significantly higher iGFR after 6 and 12 months of ADPKD treatment without improvement in TKV. Adverse events were most frequent in those receiving standard dose rapamycin (Group B).

Funding: Pharmaceutical Company Support - Wyeth/Pfizer

TH-PO618

mTORC1 and 2 Inhibition in Polycystic Kidney Disease

Kameswaran Ravichandran, Iram Zafar, Zhibin He, Charles L. Edelstein. *Univ of Col Denver.*

Background: mTOR exists in association with two different complexes, mTORC1 and mTORC2. Both mTORC1 and 2 are pro-proliferative. mTORC1 inhibition with rapamycin has been disappointing in human studies. mTORC1 and 2 inhibition with mTOR kinase inhibitors offers a new therapeutic approach. mTORC2 signals downstream via Akt1, PKCα, Rho and Rac1.

Methods: A mTOR kinase antisense oligonucleotide (ASO) that selectively binds to the ATP-binding site in mTOR catalytic domain, inhibiting mTORC1 and mTORC2 was injected at 50mg/kg/wk dose i.p. in PKD2WS25mice (an orthologous model of human ADPKD) from 4 to 16 weeks of age. The role of mTORC2 was validated in cyst formation by silencing Rictor the functional component of mTORC2 in an in vitro model of Type 1 MDCK cells that forms cysts in collagen culture.

Results: On immunoblot there was a 2 fold increase in PKC-α(P<0.05), a marker of mTORC2 activation, in kidneys of PkdWs25 vs wild type mice. In PKD2Ws25 mice, the 2K/TBW, CVD and BUN were significantly decreased in mTOR ASO treatment groups.

mTOR ASO in pkd2WS25 mice

	+/+ Veh	WS25 Veh	WS25 mTOR ASO
BW (g)	23 ± 0.3	30 ± 1.8	27± 2.2
2K/TBW (%)	1.27 ± 0.03	2.2 ± 0.3*	1.4 ± 0.1 **
CVD (%)	1.0 ± 0.01	29 ± 6*	10 ± 5 **
BUN (mg/dL)	18 ± 1	34 ± 5 *	25 ± 2 **

*P<0.001 vs +/+ Veh, **P<0.05 vs WS25 Veh, n=4

In MDCK cells in culture, on day7 the cyst diameter was decreased by 28.8% in Rictor silenced MDCK cells as compared to MDCK control cells (P<0.05) and cyst diameter was increased by 23.6% with addition of constitutively active Akt1. On day10 the cyst diameter was decreased by 48.2% in Rictor silenced MDCK cells as compared to MDCK control cells (P<0.05) and was increased by 42% with addition of constitutively active Akt1.

Conclusions: 1) mTOR ASO-treated mice had lower kidney weight, CVD and better kidney function than vehicle-treated Pkd2WS25 mice. The effect of mTOR inhibition on PKD in Pkd2WS25mice was more effective than we have previously published with rapamycin in the same model, 2) mTORC2 silencing in an in vitro model reduces the cyst size that is reconstituted by addition of constitutively active Akt1. In conclusion combined mTORC1 and mTORC2 inhibition may have better therapeutic potential in ADPKD and merits further study in ADPKD.

Funding: Other NIH Support - 5R01DK074835-04

TH-PO619

Folate-Conjugated Rapamycin Slows Progression of Polycystic Kidney Disease with Increased Renal Specificity Thomas Weimbs,[1] Jonathan M. Shillingford,[1] Christopher P. Leamon,[2] Iontcho Vlahov.[2] *[1]Department of Molecular, Cellular & Developmental Biology, University of California Santa Barbara, Santa Barbara, CA; [2]Endocyte Inc., West Lafayette, IN.*

Background: Autosomal-dominant polycystic kidney disease (ADPKD) frequently leads to renal failure necessitating kidney transplantation or life-long dialysis. Currently, no treatment is available to prevent or slow disease progression. The mTOR signaling pathway has been shown to be aberrantly activated in ADPKD. In PKD rodent models, treatment with mTOR inhibitors such as rapamycin is highly effective in ameliorating the disease. However, recent clinical trials did not show significant benefits of treatment with mTOR inhibitors in ADPKD patients. It is likely that the relatively low, tolerable doses of mTOR inhibitors are insufficient to achieve effective mTOR inhibition in the target tissue in humans.

Methods: To overcome this limitation we have synthesized a folate-conjugated form of rapamycin (EC0371, FC-rapa) designed to be taken up by folate-receptor (FR)-mediated endocytosis, and cleaved intracellularly to reconstitute the active drug.

Results: We show that the FR is highly expressed in renal cyst-lining cells in ADPKD and mouse models. *In vitro* experiments demonstrate that FC-rapa inhibits mTOR activity in a dose- and folate receptor-dependent manner. Treatment of a PKD mouse model with folate-rapa results in inhibition of mTOR in the target tissue, strong inhibition of proliferation and renal cyst growth, and preservation of renal function. Dosing experiments show that FC-rapa inhibits mTOR activity in the kidney but not in other organs.

Conclusions: These results suggest that renal drug targeting using FC-rapa may be useful to overcome the significant side effects and lack of renal efficacy observed in clinical trials with mTOR inhibitors in ADPKD patients.

Funding: Other U.S. Government Support

TH-PO620

Everolimus, an Oral mTOR Inhibitor, Is Not Nephrotoxic in Patients with Tuberous Sclerosis Complex or Sporadic Lymphangioleiomyomatosis John J. Bissler,[1] Chris Kingswood,[2] Bernard Zonnenberg,[3] Michael Frost,[4] Elena Belousova,[5] Elzbieta Radzikowska,[6] Petrus J. de Vries,[7] Karen Stein,[8] Gaurav Shah,[8] Sara Miao,[8] Jeremie Lincy,[8] Klemens Budde.[9] *[1]Cincinnati Children's Hosp Med Center; [2]Royal Sussex County Hosp; [3]University Hospital Utrecht; [4]Minnesota Epilepsy Group; [5]Moscow Research Institute of Pediatrics & Pediatric Surgery; [6]National Tuberculosis & Lung Diseases Research Institute; [7]University of Cape Town; [8]Novartis Pharmaceuticals Corp.; [9]Charite-Universitätsmedizin Berlin.*

Background: Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutations in the *TSC1* or *TSC2* gene, resulting in constitutive mTOR pathway upregulation. TSC is characterized by growth of nonmalignant tumors in multiple organs including the kidneys (angiomyolipoma, AML). AML is also associated with the rare pulmonary disorder sporadic lymphangioleiomyomatosis (sLAM).

Methods: EXIST-2 (NCT00790400) is an international, randomized, double-blind, placebo-controlled, phase 3 trial in patients with TSC or sLAM who have AML. Eligible patients (≥1 AML with longest diameter ≥3 cm) were randomized 2:1 to oral 10 mg/d everolimus (n=79) or placebo (n=39), stratified by TSC and enzyme-inducing anti-epileptic drug use, and presence of sLAM. As of 14 Oct 2011, 90 days of additional safety data from the original cutoff date (30 Jun 2011) became available and are presented herein.

Results: At the 90 day updated analysis, 53% and 41% of everolimus and placebo patients, respectively, had ≥48 weeks of exposure to study drug. Between the original cutoff and updated analysis, discontinuations occurred in only placebo patients, primarily due to disease progression. Glomerular filtration rate (GFR) remained stable for both treatment groups; 98% and 92% of everolimus and placebo patients maintained a GFR ≥30 mL/min/1.73 m^2. In the everolimus and placebo groups, respectively, blood creatinine increased in 3% and 8% and proteinuria was detected in 4% and 13%.

Conclusions: This updated safety analysis is consistent with the original analysis. Renal function data from everolimus patients suggests stable renal function in TSC patients with AML.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

TH-PO621

Identification of Novel Pathways Affected by Intracellular Ca^{2+} in Human ADPKD Cells by Comprehensive Gene Analysis Darren P. Wallace,[1] Gail Reif,[1] Corey White,[1] Yafeng Dong.[2] *[1]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS.*

Background: In ADPKD, loss of polycystin function disrupts intracellular Ca^{2+} regulation and transforms renal epithelial cells into poorly differentiated hyperplastic cells that give rise to cysts. cAMP stimulates the proliferation of ADPKD cells, but inhibits the proliferation of normal renal cells. Restoration of intracellular Ca^{2+} by BayK8644, a Ca^{2+} channel activator, rescues a normal response, such that cAMP inhibits ADPKD cell proliferation; however, the underlying mechanism remains unclear.

Methods: ADPKD cells were treated with cAMP ± Bay K8644 for 24h. RNA was isolated and evaluated for integrity. Affymetrix Human Genome U133A 2.0 arrays were used to identify genes with a 1.3-fold expression change ($P<0.05$) and gene signaling pathways were analyzed by GeneGo MetaCore Bioinformatics software.

Results: Of 14,500 well-characterized genes, only 312 genes were altered by increased Ca^{2+}. The five top canonical pathways were "Cell cycle", "Inhibition of Telomerase Activity and Cellular Senescence", "Role of Parkin in the Ubiquitin-Proteasomal Pathway", "Regulation of G1/S transition", "Start of DNA Replication in Early S Phase", "Sister Chromatid Cohesion", and "Regulation of Actin Cytoskeleton by Rho". Specific genes included cyclin-dependent kinase 6 (CDK6), a recognized component of cystic disease, and novel genes including mini-chromosome maintenance protein MCM 4, retinoblastoma-associated protein p107, elongation factor E2F3, DBF4 homolog A (ASK), IGF-binding protein 2, Inhibitory G protein-specific GPCRs, Interleukon-1α and Proliferation-inducing gene-2. Ubiquitin conjugating enzyme UBC7 and alpha-synuclein, not normally expressed in adult kidney, had altered expression, suggesting a role of proteosomal degradation. Integrated pathway maps generated by Enrichment Pathway Network Analysis provided a comprehensive analysis of gene-gene interactions.

Conclusions: We identified unique genes involved in cell proliferation and ubiquitin-proteasomal pathways that may underlie the phenotypic switch induced by Ca^{2+}restoration in ADPKD cells.

Funding: NIDDK Support

TH-PO622

HIF-1α Causes Renal Cyst Expansion through Calcium-Activated Chloride Secretion Bjoern Buchholz,[1] Gunnar Schley,[1] Diana Faria,[2] Sven Kroening,[1] Johannes Schoedel,[1] Bernd Klanke,[1] Karl Kunzelmann,[2] Kai-Uwe Eckardt.[1] *[1]Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Department of Physiology, University of Regensburg, Regensburg, Germany.*

Background: Polycystic Kidney Diseases (PKD) are characterized by a multitude of bilateral renal cysts that enlarge gradually and lead to a decline of kidney function. Cyst growth involves chloride-dependent transepithelial fluid secretion. Cyst enlargement causes regional hypoxia which results in the stabilization of the hypoxia-inducible transcription factor HIF-1α in the cyst epithelium.

Methods: To assess the functional relevance of HIF-1α activation for cyst growth, we interfered with the HIF pathway in two *in vitro* cyst models – MDCK cells in a collagen matrix and cultured embryonic mouse kidneys stimulated with forskolin. In addition, we performed Ussing Chamber analyses with MDCK cells deficient for HIF-1α. Furthermore, we used a forward genetic screen to identify novel HIF-1α target genes and analysed their expression and function in both cyst models. Beyond that, we analysed human ADPKD kidneys in regard to the expression of HIF-1α target genes that played a role in cyst growth.

Results: Chronic stabilization of HIF-1α promoted MDCK cyst growth and cyst enlargement in cultured embryonic kidneys stimulated with forskolin whereas genetic and pharmacological inhibition of HIF-1α inhibited cyst growth. In addition, MDCK cells deficient for HIF-1α lacked calcium-activated chloride secretion in Ussing Chamber analyses. In a forward genetic screen, we identified both, the purinergic receptor $P2Y_2R$, and the calcium-activated chloride channel anoctamin 6 (ANO6) as HIF-1α-regulated genes. Consistently, pharmacological inhibition of anoctamins, significantly impaired cyst growth in both cyst models. In addition, ANO6 was co-expressed with HIF-1α in the cyst epithelium of human ADPKD kidneys.

Conclusions: We conclude that in PKD regional hypoxia aggravates cyst expansion via HIF-1α-dependent calcium-activated chloride secretion. Thus the HIF pathway including the HIF-inducible genes ANO6 and $P2Y_2R$ may offer novel targets for inhibition of cyst growth.

Funding: Government Support - Non-U.S.

TH-PO623

Shh Inhibition Improves Renal Functions in *Pkd2 Mutant Mice* Yuan Li,[1,2] Wenyi Wang,[1,2] Ao Li,[1,2] Haichao Liu,[1,2] Elizabeth Green,[2] Dan Liang,[2] Guanqing Wu.[2] *[1]State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China; [2]Genetic Medicine, Vanderbilt University, Nashville, TN.*

Background: ADPKD is one of the most common monogenic diseases in the kidneys and is characterized by numerous fluid-filled renal cysts. ADPKD is caused by mutations in either *PKD1* or *PKD2*, each of which respectively encodes the proteins, polycystin-1 (PC1) and polycystin-2 (PC2). Recent studies indicate that PC1 and PC2 form a protein complex through interaction between their COOH-terminals, and loss of either polycystins dysregulates multiple cell signaling pathways. Such disruption may lead to aberrant ionic transportation, polarity, and proliferation/apoptosis in renal epithelial cells and eventually cause renal cyst formation.

Since Shh protein has been reported to closely associate with the cilia of diverse epithelial cells including those of the kidneys, we hypothesized that polycystins and Shh may have epistatic effects and contribute to cyst formation in ADPKD. To this end, we employed *Pkd2* and *Shh* mutant mice to study their functional relationship *in vivo*. In this study, we have crossed heterozygous *Shh* mice (*Shh*$^{+/-}$) and Vil-Cre-derived *Pkd2* conditional knockout mice (Vil-Cre:*Pkd2*$^{f3/f3}$) to generate compound Vil-Cre:*Pkd2*$^{f3/f3}$:*Shh*$^{+/-}$ mutant mice. We performed a cohort study for Vil-Cre:*Pkd2*$^{f3/f3}$ mice with and without *Shh*$^{+/-}$ mutant alleles (n>20 for each group). By examining their survival rate, kidney or liver/body ratio, renal cystic index, and renal function of these mice, we found that there are no significant differences in lethal phenotypes or renal cystic severity between mice with Vil-Cre:*Pkd2*$^{f3/f3}$:*Shh*$^{+/-}$ and Vil-Cre:*Pkd2*$^{f3/f3}$ alleles. However, significantly improved renal function (BUN and Cr) can be seen in Vil-Cre:*kd2*$^{f3/f3}$:*Shh*$^{+/-}$ mice compared to Vil-Cre:*Pkd2*$^{f3/f3}$ mice. We have also investigated PC2-deficient effects in *Shh*$^{+/-}$ mice. Interestingly, we found that the lack of PC2 induces solitary kidney in *Shh*$^{+/-}$ mice (20.6%). These results indicate that downregulation of Shh may have a protective role in ADPKD models/patients and PC2 may engage in Shh-associated kidney development.

Funding: Government Support - Non-U.S.

TH-PO624

Modulation of Wnt/β-Catenin Signaling by GSK3 Inhibition Accelerates Polycystic Disease Progression a Model of Nephronophthisis
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Background: Polycystic kidneys diseases (PKDs) are a family of genetic disorders characterized by renal cystic growth and progression to end stage renal disease. Cystogenesis is associated with dysregulation of multiple signaling pathways, including intracellular calcium homeostasis, cAMP signaling, mitogenic cascade, mTOR and Wnt signaling, some of which are regulated by the primary cilium. Because ciliary dysfunction appears to be a common abnormality leading to multiple forms of PKD, including nephronophthisis, we set out to address the role of the ciliary-regulated Wnt/beta-catenin pathway in promoting PKD in the jck mouse model of nephronophthisis. To understand whether canonical Wnt signaling is active in jck cystic kidneys, we performed RT PCR gene expression array analysis and found upregulation of several Wnt ligands, Frizzled receptors and downstream target genes. Also, changes in beta-catenin expression and subcellular distribution in cystic tissue early in disease suggested aberrant activation of Wnt/beta-catenin pathway. To clearly define whether this pathway is a key signaling component in promoting cytogenesis, we directly tested the effects of glycogen synthase kinase-3 (GSK-3) inhibition on PKD progression in jck mice. Treatment with GSK3 inhibitors lithium or SB216763 significantly accelerated disease progression resulting in increased cystic volumes and decreased renal function, accompanied by increased phosphorylation of GSK-3β at Ser9 and elevated expression of beta-catenin. Additionally, GSK-3 inhibition further elongated cilia of renal cystic epithelial cells. These data provide in vivo evidence that pharmacological activation of the Wnt/beta-catenin pathway promotes PKD.

Funding: Pharmaceutical Company Support - Genzyme Corp., a Sanofi Co.

TH-PO625

Postnatal Inhibition of Glycogen Synthase Kinase-3 Reduces Renal Cystogenesis in cpk/cpk Mice
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Background: Polycystic kidney diseases are a large family of inherited disorders characterized by the formation of multiple fluid filled cysts in the renal tubules, often leading to end stage renal disease. Progressive cyst enlargement in PKD is known to be associated with elevated cAMP levels, and the inhibition of vasopressin mediated adenylate cyclase activity has been shown to dramatically diminish the formation and growth of renal cysts in rodents. We previously found that inhibition or gene deletion of glycogen synthase kinase 3ß (GSK3ß) reduces arginine vasopressin (AVP) -mediated adenylate cyclase activity and reduced cAMP levels in the kidney. GSK3 is a serine/ threonine protein kinase which plays a crucial role in Wnt signaling and cell proliferation. The aim of the current study was to examine if inhibition of GSK3 could ameliorate the development and progression of cysts in PKD.

Methods: For the studies we used cpk/cpk mice, a model for autosomal recessive PKD, characterized by rapid postnatal renal cyst progression resulting in death around weaning. Mouse litters were injected daily starting on P3 with vehicle or TDZD-8 (5mg/Kg BWt, IP), a highly specific ihhibitor of GSK3. Pups were sacrificed on P14.

Results: When compared to wild type mice, in the cpk/cpk mice we detected high GSK3ß expression in whole kidney lysate and specifically in the cyst-lining epithelia by immunoblot and immunostaining. Treatment with TDZD-8 retarded renal cyst development and progression in cpk/cpk mice. The kidney weight/body weight ratio was significantly lower in the TDZD-8 treated mice compared to vehicle treated mice. Examination of the gross morphology in H&E stained sections revealed more intact parenchyma in the TDZD-8 treated group than in the vehicle treated group. Renal cyst volume and BUN were also significantly reduced in the TDZD-8 treated mice compared to vehicle treated mice.

Conclusions: These studies suggest that inhibition of GSK3 reduces renal cystogenesis in cpk/cpk mice.

Funding: NIDDK Support

TH-PO626

Very Low Dose Rosiglitazone Inhibits Cyst Growth in the PCK Rat Model of Polycystic Kidney Disease
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Background: Autosomal dominant polycystic kidney disease (ADPKD), the most common genetic renal disease, is characterized by the slow growth of fluid-filled cysts in kidney tubules and liver bile ducts. Cyst enlargement is due, in part, to Cl^- secretion via the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel. Our previous studies demonstrated that PPARγ agonists, insulin sensitizing drugs used to treat diabetes, inhibit Cl^- secretion by collecting duct principal cells via decreased CFTR synthesis. For 4 PPARγ agonists tested, the IC_{50}s calculated from the dose response inhibition curves for Cl^- transport paralleled the EC_{50}'s for receptor transactivation with a leftward shift, suggesting an approximately 10 fold increase in sensitivity for drug induced inhibition of Cl^- secretion. These data suggest that the FDA-approved PPARγ agonists should be effective in inhibiting cyst expansion when used at very low doses. Our previous preclinical studies showed that high (20 mg/kg BW) dose pioglitazone, an FDA approved PPARγ agonist, inhibited cyst growth in the PCK rat model of PKD. The current study was designed to show a class action of PPARγ agonists by determining if rosiglitazone, another PPARγ agonist, inhibits cyst growth and if so, does this agonist work at concentrations lower than those typically used to treat diabetes.

Methods: A 24 week feeding study in the PCK rat model used 3 doses of rosiglitazone (4, 0.4, 0.04 mg/kg BW). 4 mg/kg BW rosiglitazone is analogous to the previous dose of 20 mg/kg BW pioglitazone.

Results: The lowest dose, 0.04 mg/kg BW, which is two orders of magnitude less than previously used concentrations, significantly slowed renal cyst growth.

Conclusions: Based on these preclinical findings it is hypothesized that PPARγ agonists could be used to inhibit cyst expansion at doses that are low enough to avoid the characteristic side effects of these drugs.

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TH-PO627

Genetic Modulation of Glycosphingolipid Network Identifies GM3 Synthase as a New Target for Polycystic Kidney Disease
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Background: Sphingolipids and glycosphingolipids are important regulators of cellular proliferation, differentiation, and survival, all processes which are disrupted in polycystic kidney diseases (PKD), including ADPKD and nephronophthisis. Recent studies have demonstrated that inhibition of glucosylceramide (GlcCer) accumulation attenuates PKD in several preclinical models. Because GlcCer is a precursor to multiple complex glycosphingolipids, including LacCer, the globosides and gangliosides, it is important to determine which specific glycosphingolipid modulates cytogenesis in search of optimal therapeutic targets. We sought to define the role of the simplest ganglioside, GM3 in nephronophthisis-associated PKD using jck model. We show, for the first time, that GM3 plays a pivotal role in cystogenesis by crossing jck mice with mice carrying a targeted mutation in the GM3 synthase gene (*St3gal5*). Homozygous loss of GM3 synthase prevented GM3 synthesis and inhibited cystogenesis in jck mice. Several pathways were modulated in response to GM3 loss including cell cycle, apoptosis, mitogenic, Akt-mTOR and Wnt signaling. To assess the impact of GM3 synthase deletion on flux through the glycosphingolipid pathway, we measured changes in the levels of GlcCer, LacCer (the immediate precursor to both the gangliosides and globosides), and the globosides Gb3 and Gb4 in mutant mice. Only kidney GM3 and GlcCer were correlated with disease severity, identifying these two lipids as key mediators of cystogenesis. These data suggest that genes involved in glycosphingolipid metabolism may function as modifier loci to determine the severity of polycystic kidney disease, and further, suggest GM3 synthase as a new target for the treatment of PKD.

Funding: Pharmaceutical Company Support - Genzyme Corp., a Sanofi Co.

TH-PO628

Curcumin and Analog 2a Inhibit ADPKD Cyst-Cell Proliferation and Cyst Growth *In Vitro* but Not in Cysts Growing in Nude-Mouse Implants
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Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst development, destruction of the native normal kidney and finally kidney failure requiring dialysis or transplantation. Treatments using targeted inhibition of specific signaling pathways are presently actively pursued. However, since cyst growth is associated with concomitant activation of many proliferative pathways, multiple-pathway inhibition could be advantageous for ADPKD treatment. It is known that curcumin inhibits several cell-proliferation pathways activated both in cancer and in ADPKD cyst cells. However, no therapeutic levels are achieved by oral (PO) administration in rodents and humans due to poor intestinal absorption and liver inactivation.

Methods: We studied the effects of Curcumin and Analog 2a (a compound lacking glucuronidation sites, thus avoiding decreased absorption and liver metabolism) on the proliferation and cyst formation by ADPKD cyst cells in primary cultures and on the active β-catenin levels using normal kidney cells as control; the effects of intraperitoneal (IP) curcumin on cyst development in nude-mouse implants; and the blood and kidney levels of both drugs, after PO administration to mice.

Results: We found that: 1. Curcumin and Analog 2a were more cytotoxic to cyst cells than to normal kidney cells: curcumin IC50 = 19.6 ± 1.4 vs 46.5 ± 4.3 μM; $P < 0.003$ and Analog 2a IC50 = 1.0 ± 0.2 vs 4.4 ± 0.5 μM; $P < 0.001$, respectively. 2. Curcumin and Analog 2a inhibited active β-catenin in cyst cells. 3. Curcumin was undetectable in mouse blood after PO administration. 4. Its IP administration decreased cyst volume in the implants, but not cyst number. 5. Analog 2a was detectable in mouse blood and concentrated in the kidney after PO administration.

Conclusions: We conclude that Analog 2a increased effectiveness, decreased toxicity to normal cells and improved bioavailability supports the use of new curcumin analogs for ADPKD treatment.

Funding: Private Foundation Support

TH-PO629

Branched-Chain Amino Acid Supplementation Accelerates Cyst Growth in a Mouse Model of Autosomal Dominant Polycystic Kidney Disease
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Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most commonly inherited renal disease. ADPKD is characterized by progressive development and enlargement of renal and liver cysts, leading to chronic kidney disease (CKD). Dietary protein restriction prevents progression of CKD, because most CKD symptoms, for example uremia, metabolic acidosis and bone disease, are caused by protein intolerance. However, the role of dietary protein restriction in patients with ADPKD is unknown. We investigated the effects of branched-chain amino acids (BCAA), which developed for the purpose of improving hypo-albuminemia in patients with uncompensated liver cirrhosis, We investigated the effects of BCAA in *Pkd1*$^{flox/flox}$: *Mx1-Cre* mice.

Methods: *Pkd1*$^{flox/flox}$*:Mx1-Cre* mice were intraperitoneally injected with 10 μg/g body weight of polyinosinic-polycytidylic acid for 6 consecutive days at 2 weeks of age. The ratio of Aminofeel BCAA is 0.250 g isoleucine/g, leucine 0.500 g/g and 0.250g/g valine. BCAA was dissolved in the drinking water of the mice at a concentration of 20mg BCAA/mL (BCAA treated group) for 18 consecutive weeks (4-22 weeks of age), whereas a control group received vehicle alone. Mice were sacrificed at 22 weeks of age, and measured body, kidneys and liver weight. Kidney and liver sections were stained with hematoxylin and eosin, and determined the cystic index (CI), defined as the percent of total cross-sectional area occupied by cysts.

Results: The two kidney/body weight ratio was signigficantly greater in BCAA treated group than in control group (2.86 ± 0.98 vs. 1.31 ± 0.16%, $p<0.01$). The liver/body weight ratio was no significantly different. CI was greater in BCAA treated group than in control group (kidney; 37.4 ± 15.3 vs. 7.0 ± 4.6%, $p<0.01$, liver; 29.6 ± 8.1 vs. 13.3 ± 10.2%, $p<0.05$).

Conclusions: Branched-chain amino acid supplementation accelerates polycystic kidney disease in *Pkd1*$^{flox/flox}$*:Mx1-Cre* mice.

TH-PO630

Induction of Glycosuria via Phlorizin-Mediated SGLT Inhibition Prevents Renal Disease Progression in the Han:SPRD Rat Model of Polycystic Kidney Disease
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Background: The pathogenic role of the apical renal sodium-glucose cotransporters SGLT1 and SGLT2 in polycystic kidney disease (PKD) is not known. We hypothesized that induction of glycosuria and osmotic diuresis with the SGLT1/SGLT2 inhibitor phlorizin inhibits cyst growth and retards loss of renal function in the Han:SPRD rat model of PKD.

Methods: We induced glycosuria in 5-week old heterozygous Cy/+ male Han:SPRD rats (n=8) by s.c. injection for 5 weeks with phlorizin (400 mg/kg/d). Phlorizin induced immediate and sustained glycosuria which was accompanied by a 2.7-fold increase in the diuresis (64 vs. 24 ml/d), and by a 2.7-fold increase in osmolaluria (43 vs. 16 mosm/d).

Results: After 5 weeks the BUN and creatinine were 22% and 38% lower in the phlorizin- compared with the vehicle-treated group, whereas the creatinin clearance was 59% higher. Furthermore the protein/creatinine ratio was reduced from 113 ± 19 to 64 ± 9 mg/day ($P<0.05$). The 2 kidneys/total body weight (2K/TBW) ratio was 18% lower with phlorizin ($P<0.001$). A reduction in the size of megalin-positive cysts was apparent in the cortex upon phlorizin treatment, and the cyst index was 28% lower ($P<0.05$). Western blot analysis of Han:SPRD kidney extracts demonstrated that the activated MAPK pathway was inhibited, whereas the mTOR pathway remained activated. Ki67 staining revealed that phlorizin-treated rats displayed a 35% lower proliferation of epithelial cells in cysts.

Conclusions: The significant beneficial effect of phlorizin-mediated glycosuria and osmotic diuresis on cystic disease progression in the Han:SPRD model of PKD supports the hypothesis that renal SGLT inhibition and induction of glycosuria could have a therapeutic effect in polycystic kidney disease.

TH-PO631

Hyperglycemia Accelerates Cystogenesis and Alters Wnt and mTOR Signaling in a Mouse Model of Polycystic Kidney Disease
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Background: The rate of cyst formation and disease progression in polycystic kidney disease (PKD) is highly variable. The lack of predictability in disease progression may be due to accompanying environmental or pathophysiological processes. Diabetes, a growing epidemic worldwide, results in hypertrophic signaling in the kidney, which has previously been shown to enhance cystogenesis. The goal of this study was to determine if diabetes (hyperglycemia) enhances the rate of cyst formation and to identify the signaling pathways involved in this process.

Methods: Adult *ift88* conditional floxed allele mice were administered tamoxifen to induce cilia loss in the presence of cre. Subsequent administration of streptozotocin at 50 mg/kg for 5 days was used to induce hyperglycemia (blood glucose ≥ 200 mg/dL). Streptozotocin administration resulted in equivalent hyperglycemia in both cilia (+) and cilia (-) mice, although cilia (-) mice trended to have elevated blood glucose compared to cilia (+) mice. Histology, Western blot, and gene array analysis were used to assess cystic development and differences in signaling pathways.

Results: Hyperglycemia with loss of cilia increased the rate of cyst formation and cell proliferation, and resulted in interstitial inflammation, formation of primitive renal tubules, focal foot process effacement, polyuria, and increased proteinuria. Hyperglycemic cilia (-) mice had increased mTOR and Wnt signaling, including increased β-catenin staining specifically in cysts and primitive renal tubules. Additionally, hyperglycemic cilia (-) mice had increased markers of epithelial-mesenchymal transformation.

Conclusions: These data suggest that hyperglycemia in the absence of cilia accelerates cyst formation and expansion, through increased cell proliferation and loss of polarity, suggesting that diabetes is a risk factor in the progression of polycystic kidney disease.

Funding: NIDDK Support, Veterans Administration Support

TH-PO632

Short-Term Renal Hemodynamic Responses and Safety of Tolvaptan in Subjects with ADPKD at Various Levels of Kidney Function
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Background: Vasopressin V2-receptor antagonists may delay disease progression in autosomal dominant polycystic kidney disease (ADPKD). Human trials with these agents have been performed predominantly in patients with normal kidney function (eGFR >60 mL/min/1.73m^2). In this trial, we investigated renal hemodynamic responses and safety of initiating tolvaptan in patients at various levels of kidney function.

Methods: ADPKD patients were included in 3 groups based on eGFR (A: >60, B: 30-60 and C: <30 mL/min/1.73m^2) for a 3 week treatment period with tolvaptan (titrated up to 120 mg/day). Glomerular filtration rate (GFR, by ^{125}I-iothalamate clearance), effective renal plasma flow (ERPF, by ^{131}I-hippuran clearance), and safety variables were measured before, after 3 weeks of treatment, and 3 weeks after the last dose of tolvaptan.

Results: 29 subjects entered the study, with 27 completing, 9 patients in each study group. With tolvaptan, a reversible decrease in GFR was observed, which reached statistical significance in Groups A and B: median -8.2 (interquartile range -14.1 to -1.8) and -4.0 (-12.2 to -1.0) mL/min, resp. The percentage change in GFR, ERPF and filtration fraction with tolvaptan did not differ between the 3 study groups. No differences between groups were observed, besides increases in urine volume, free water clearance, ALP, ALT and GGT during tolvaptan treatment, which were significantly smaller in Group C. Tolvaptan was well tolerated, with only 2 subjects withdrawing study treatment (1 in Group A (polyuria) and B (dry mouth), resp.). The most frequently observed adverse events (AEs) were thirst, dry mouth, polyuria and nocturia. No differences in AE incidence rate between the 3 study groups were noted. All observed changes were reversed upon withdrawal of treatment.

Conclusions: Doses of tolvaptan typically used in ADPKD patients (60-120 mg/day) are not associated with a different safety profile (adverse event incidence or renal hemodynamic responses) in ADPKD patients with impaired kidney function.

Funding: Pharmaceutical Company Support - Otsuka Pharmaceutical Development and Commercialization, Inc

TH-PO633

Telmisartan Ameliorates Fibrocystic Liver Disease in an Orthologous Rat Model of Human ARPKD
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Background: We previously reported that a peroxisome proliferator-activated receptor (PPAR)-γ agonist ameliorated kidney and liver disease in PCK rats with autosomal recessive polycystic kidney disease (ARPKD). Telmisartan is an angiotensin receptor blocker (ARB) which is a partial PPAR-γ agonist. In the current study, we determined whether Telmisartan ameliorates polycystic kidney and fibrocystic liver disease progression in the PCK rat, an orthologous model of human ARPKD.

Methods: Male and female PCK and normal control (+/+) rats (n=10 per gender) were randomly assigned to one of two groups: treatment with 3 mg/kg Telmisartan or vehicle (0.3% DMSO) by gavage every day from 4 to 20 weeks of age. Blood pressure (BP), blood levels of urea nitrogen (BUN) and aspartate amino transferase (AST) were measured. Cystic area and fibrotic index were obtained from hematoxylin-eosin and sirius red stained kidney and liver sections. Ki67- and TGF-β-positive cells were detected by immunohistochemistry.

Results: Treatment of Telmisartan decreased BP in both PCK and +/+ rats. Liver weight (% of body weight, LB%), liver cystic area, hepatic fibrosis index, and the number of Ki67- and TGF-β-positive cells in interstitial tissue of the liver were significantly decreased in Telmisartan treated PCK rats. AST and BUN levels were in the normal range in both genders and were unaffected by Telmisartan treatment. No effect on kidney disease progression was observed.

Conclusions: Telmisartan ameliorates fibrocystic liver disease possibly through the inhibition of cell proliferation and TGF-β signaling in PCK rats, an orthologous model of human ARPKD. The current results support the potential use of ARB with partial PPAR-γ agonistic action as therapeutic agents for the treatment of fibrocystic liver disease in ARPKD patients.

Funding: Government Support - Non-U.S.

TH-PO634

Aliskiren Ameliorates Cyst Progression in an Orthologous Mouse Model of Autosomal Dominant Polycystic Kidney Tasuku Nakagaki,[1] Saori Nishio,[1] Sekiya Shibazaki,[1] Yasunobu Ishikawa,[1] Stefan Somlo,[2] Tatsuya Atsumi.[1] *[1]Internal MedicineII, Hokkaido University Graduate School of Medicine; [2]Department of Internal Medicine, Yale University School of Medicine, New Haven.*

Background: Hypertension is a well-recognized complication of autosomal dominant polycystic kidney disease (ADPKD). Hypertension relates to progressive kidney enlargement and is significant independent risk factor for progression to ESRD. It has been reported that renin-angiotensin system (RAS) inhibitors had a more favorable renoprotective effect than calcium channel blockers. However, the mechanisms leading to hypertension in ADPKD are not well understood. Involvement of the RAS has been postulated, but no consistent relationship has been found between blood pressure and plasma renin activity or plasma aldosterone concentrations.The purpose of this study is to examine the effects of antihypertensive drugs for *pkd1* conditional knockout mice.

Methods: We generated *Pkd1* conditional knockout mice carrying the Cre transgene under the control of the *Mx1* promoter (*Pkd1 $^{flox/flox}$;Mx1-Cre* mice). All mice were injected with polyinosinic-polycytidylic acid (pI:pC) to induce the expression of Cre recombinase and to inactivate *Pkd1* at 2 weeks of age. To evaluate the effects of antihypertensive drugs, *Pkd1$^{flox/flox}$;Mx1-Cre* mice were treated by osmotic mini-pumps with amlodipine or olmesartanor aliskiren, or vehicle for 12 weeks. Blood pressure, kidney weight to body weight ratio,cystic index, BUN, albuminuria, and proliferation rate as determined by PCNA were usedto assess efficacy of treatment. Fibrosis progression was examined by immunostaining for α-smooth muscle actin (α-SMA) and Elastica-Masson.

Results: There was no difference in kidney weight to bodyweight ratio, renal cystic index, urinary albumin excretion, and BUN between amlodipine, olmesartan and vehicle. Aliskiren treatment significantly reduced the kidney weight to bodyweight ratio, renal cystic index, urinary albumin excretion, and BUN.

Conclusions: These studies suggest RAS inhibition with aliskiren may be effective in slowing cyst growth in ADPKD.

TH-PO635

Cardiorenal Effects of Paricalcitol and ACE Inhibition in Rats with Polycystic Kidney Disease Kristina Gjerdrum Schwensen, David C. Harris, Gopala K. Rangan. *Centre for Transplant and Renal Research, Westmead Millennium Institute, University of Sydney at Westmead Hospital, Sydney, NSW, Australia.*

Background: Vitamin D receptor agonists (VDRAs) reduce tubular epithelial cell proliferation and interstitial inflammation/fibrosis, and attenuate proteinuria and hypertension in experimental and human chronic kidney disease. In this study, we tested hypothesis that VDRAs prevent cyst growth in the early stage of polycystic kidney disease (PKD), and have synergistic cardiorenal protective effects with angiotensin converting enzyme (ACE) inhibitors in established disease.

Methods: In **Study 1**, the preventative effects of VDRAs on cyst growth were assessed, and Lewis polycystic kidney disease (**LPK**) rats (a hypertensive rat model of cystic renal disease) received either paricalcitol (**P**, 0.2μg/kg/d by daily i.p.i) or vehicle (**V**) from postnatal weeks 3 to 10 (n=6). In **Study 2**, the therapeutic effects of VDRAs were assessed and LPK rats received either P (800 ng/kg by i.p.i. 3x/week), V (i.p.i 3x/week), enalapril (**E**, 50 mg/L in drinking water) or a combination of E+PC from postnatal weeks 10 to 20 (n=4-5).

Results: In **Study 1**, P did not affect the progression of serum calcium, proteinuria or renal dysfunction in LPK rats (data not shown). The increase in kidney enlargement in LPK rats was also unaffected by P (kidney to body weight ratio: Lewis±V: 0.8+0.0; Lewis±P: 0.8+0.0; LPK±V: 5.9±0.2;LPK+P: 5.7+0.5%; mean±SE). In **Study 2**, E, P and E+PC reduced proteinuria and improved endogenous creatinine clearance at week 20 (Table 1). E+PC attenuated hypertension and both E and E+PC reduced cardiac enlargement. However, neither E, PC or E+PC altered the progression of kidney enlargement (Table 1).

Table 1

Parameter	LPK+V	LPK+P	LPK+E	LPK+P/E
Proteinuria (mg/mmol)	1276±124	687±315*	345±68*	374±55*
CrCl (μl/min/g BW)	0.91±0.11	1.45±0.13*	1.58±0.07*	1.28±0.11*
Systolic BP (mm Hg)	129±8	130±8	119±10	93±4*#
Kidney weight:BW%	7.2±0.4	6.9±0.3	7.2±0.4	7.1±0.4
Heart weight:BW%	0.44±0.02	0.42±0.03	0.39±0.01*	0.39±0.03*

*P<0.05 vs LPK+V; #P<0.05 vs LPK+P

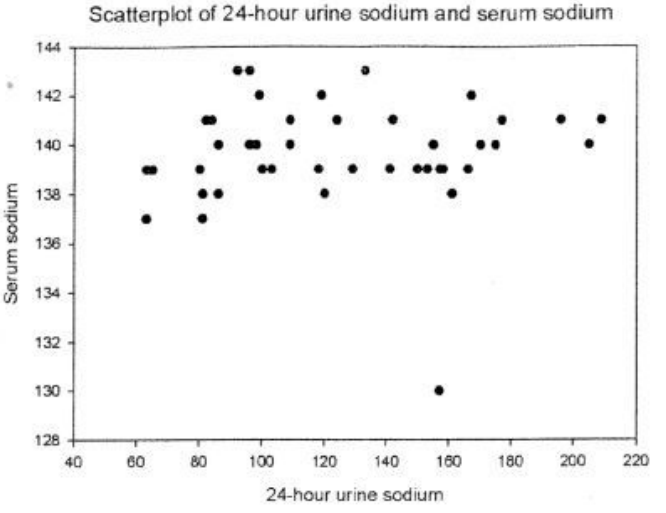
Conclusions: Although paricalcitol does not attenuate cyst growth, it reduces proteinuria, improves renal function and potentiates the anti-hypertensive effects of ACE inhibitors in established PKD.

Funding: Government Support - Non-U.S.

TH-PO636

Apparent Safety of Low Salt Diet to Suppress Arginine Vasopressin (AVP) in Patients with Polycystic Kidney Disease (PKD) Nabil J. Haddad, Rose Marie Shim, Lee A. Hebert. *Division of Nephrology, The Ohio State University, Columbus, OH.*

Background: Mechanisms that contribute to cyst growth in PKD include generation of adenosine 3.5 cyclic monophosphate induced by increased AVP secretion. Increased water intake is recommended to suppress (AVP) release (ref). Another strategy to suppress AVP is to limit solute intake, especially NaCl (Kidney Int 81:407-411, 2012). The goal is to reduce average 24-h Uosm to 250 Uosm/kg. This can be achieved at 24-h urine volume of ~ 2 L/day by limiting solute intake, especially NaCl. However, it has been suggested that low salt intake along with a fluid intake adequate to achieve a urine output of 2 L/day could result in hyponatremia in PKD patients. We tested this hypothesis.



Methods: Seven PKD patients who were instructed in low salt diet were studied. Each provided at least one 24-h urine collection with sodium content ≤ 100 mmol. Completeness of the collection was assessed by the creatinine content.

Results: Patients (pts) mean age was 50.3 ± 18, weight 66.3 ± 14kg, sex M:F1:6, sCr 1.19 ± 10mg/dl, systolic blood pressure119 ± 10, 2 pts had HTN, 4 pts were receiving ACEI and/or ARB, 1 received a diuretic. There were 51 samples of paired 24-h urine collections and serum Na levels. There was no relationship between 24-h urine Na and serum Na.[figure]The range of urine Na was 60-210 mmol/day, median 135 mmol/day. The range of 24-h urine volume was 550-3500 cc, median 1490 cc/day. Only 1 instance of hyponatremia was identified. In that case, the 24-h urine Na was 159 mmol/day and urine volume was > 2 L, and pt was receiving a diuretic.

Conclusions: We found no relationship between salt intake and serum Na in PKD pts, who usually followed a low salt diet. This suggests that reducing salt (and other solute) intake to suppress AVP release should not lead to hyponatremia in PKD patients.

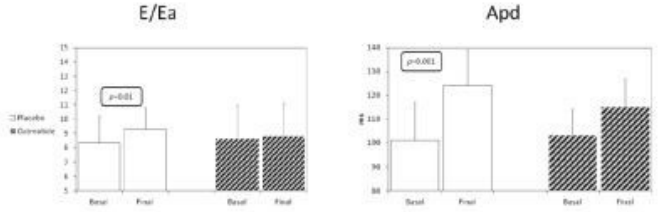
TH-PO637

Favorable Effects of Somatostatin Analogue on Left Ventricular LV Diastolic Function in Autosomal Dominant Polycystic Kidney Disease (ADPKD): A Prospective Randomized Echocardiographic Study Bianca Visciano,[1] Antonio Pisani,[1] Piero Ruggenenti,[2] Norberto Perico,[2] Giuseppe Remuzzi,[2] Giusi Rosaria Mozzillo,[1] Massimo Sabbatini,[1] Emiliano Assante di Panzillo,[1] Letizia Spinelli.[1] *[1]University of Neaples Federico II, Neaples, Italy; [2]Mario Negri Institute, Bergamo, Italy.*

Background: ADPKD is associated with early onset LV hypertrophy.Since LV hypertrophy is associated with LV diastolic function impairment,we aimed to assess the changes of LV diastolic function in ADPKD patients and whether they were affected by treatment with the somatostatin analogue,octreotide.

Methods: 35 ADPKD patients(14males)aged 34±8 years(GFR 82±26 mL/min/1.73m^2) were randomly assigned to 36 months treatment with placebo(18)or octreotide(17).An echocardiography was performed at baseline and study end.LV mass(M)and ejection fraction(EF)were calculated according to Devereux formula and biplane Simpson's algorithm,respectively.LV filling was assessed by mitral and pulmonary vein flow velocity curves and mitral annulus early diastolic velocity peak(Ea)by tissue Doppler imaging.

Results: At baseline and follow up blood pressure,heart rate,LVM,EF and the proportion of patients on ACEi or ARBS treatment were similar between groups.The ratio between mitral early diastolic flow velocity peak(E)and Ea and the duration of pulmonary vein reverse flow at atrial contraction(Apd)were also similar at baseline.They significantly increased on placebo (E/Ea,8.3±1.9and 9.3±1.5,p<0.01;Apd,101±16and 124±15,p<0.001,basal and final,respectively)and did not change appreciably(E/Ea,8.6±2.4and 8.8±2.3,ns;Apd,103±11 and 115±12,ns,basal and final,respectively)on octreotide.



Conclusions: In ADPKD patients there is a progressive worsening of LV diastolic function that appears to be prevented or limited by somatostatin therapy.Whether this functional effect may be cardioprotective in the long term is worth investigating.

TH-PO638

Young Women with Polycystic Liver Disease Benefit the Most from Treatment with Somatostatin Analogues: A Meta-Analysis on Individual Patient Data Tom J.G. Gevers,[1] Joanna Inthout,[2] Anna Caroli,[3] Piero Ruggenenti,[4] Marie C. Hogan,[5] Vicente E. Torres,[5] Frederik Nevens,[6] Joost P.H. Drenth.[1] *[1]Gastroenterology and Hepatology, Radboud University Nijmegen Medical Center; [2]Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Center; [3]Biomedical Engineering, Mario Negri Institute for Pharmacological Research; [4]Kidney Disease, Mario Negri Institute for Pharmacological Research; [5]Division of Nephrology and Hypertension, Internal Medicine, Mayo Clinic; [6]Hepatology, UZ Leuven.*

Background: Somatostatin analogues (SA) reduce polycystic liver volumes by 4.5-5.9% in patients with autosomal dominant polycystic liver or kidney disease (PCLD/ADPKD). However, it is unknown if certain patient subgroups have better treatment responses. The aim of this study is to estimate the impact of age, gender, baseline liver volume and diagnosis on treatment response to SA in patients with polycystic liver disease (PLD).

Methods: We performed a meta-regression analysis to estimate the impact of age, gender, baseline liver volume, and diagnosis on the effect of SA on liver volumes in 108 PLD patients (3 randomized controlled trials), including 12 patients of a cross-over trial that were measured twice (SA=67, placebo=53).

Results: SA therapy decreased polycystic liver volume with 5.3% (95% CI: 3.4–7.2%) when compared to placebo ($p<0.001$) using linear modeling, and this effect was not significantly affected by diagnosis, age, baseline liver volume or gender. Untreated young women (≤47y) showed the largest growth in polycystic liver volume (5.3%, 95% CI: 2.7–8.1%), whereas livers did not grow in older women and men. We performed an additional analysis including subgroups for gender combined with age, using the median of 47 years as a cut-off value. Women ≤47 and >47 years treated with SA experienced reductions in liver volume of 8.5% ($p<0.001$) and 4.0% ($p=0.017$) respectively when compared to placebo using linear modeling, with significantly better responses in the ≤47 years group ($p=0.044$).

Conclusions: Young female PLD patients appear to have the most substantial benefit from SA therapy, possibly by averting the progressive course of the disease observed in this specific group.

TH-PO639

Excessive Activation of the Complement System in the Progression of ADPKD Changlin Mei,[1] Zhen Su,[1] Xueqi Wang,[1,2] Jing Zhou,[3] Andreas L. Serra,[2] Rudolf P. Wuthrich.[2] *[1]Kidney Institute of PLA, Department of Nephrology, Shanghai Changzheng Hospital, SMMU, Shanghai, China; [2]Division of Nephrology, University Hospital, Zurich, Switzerland; [3]Harvard Institutes of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Background: Mutations in the *PKD1* and *PKD2* genes are the primary cause of ADPKD, however, the additional molecular pathogenic mechanisms remain poorly understood. Here we analyzed the importance of the complement system in ADPKD disease progression.

Methods: We analyzed the glycoproteome of urine samples obtained from ADPKD by MS for components of the complement system. We also tested the effect of the complement inhibitor romaric acid in animal models of PKD. RMA (150 mg/kg/d and 300mg/kg/d) was administered by gavage from P29-P112 in Han:SPRD rats, and from P14-P49 in *Pkd1*$^{-/-}$ mice.

Results: Analysis of the glycoproteome of urine samples from ADPKD patients revealed that disease progression is associated with excessive activation of the complement system, which was characterized by significantly increased urine concentrations of complement factor B (CFB), SERPING1 and C9. In contrast, the levels of C1RL, CD55 and CD59 were markedly decreased. Enhanced expression of CFB and C9 was confirmed by IHC staining and Western blot in diseased kidneys from ADPKD patients, Han:SPRD Cy/+ rats and *Pkd1*$^{-/-}$ mice. Treatment with RMA which inhibits the complement system by covalently binding with activated C3b significantly delayed the deterioration of renal function in Han:SPRD rats and *Pkd1*$^{-/-}$ mice.

Kidney functional parameters in Han:SPRD rats after RMA treatment for 12 weeks

	Cy/+ (n=10)	Cy/+R150 (n=10)	Cy/+R300 (n=10)	+/+ (n=8)	+/+ R300 (n=8)
BUN(mmol/L)	24.4±5.32	19.6±4.63**	19.53±3.44**	6.06±0.87	6.8±0.84
SCr(umol/L)	92.4±5.58	74.5±9.89**	76.5±3.66**	26.7±4.61	23.0±3.15
Urine osmolarity (mosm/H2O)	694.5±127	1022.4±177*	1102.1±152*	1670.1±142	2213.3±174
Urine AH50# (KU/L)	12.52±2.16	7.30±3.66**	1.36±1.44**	1.84±1.80	1.98±1.08

#: alternative complement pathway hemolytic activity

Conclusions: Our results suggest that excessive activation of the complement system is associated with ADPKD progression. Targeting the complement system might be a new therapeutic strategy.

Funding: Government Support - Non-U.S.

TH-PO640

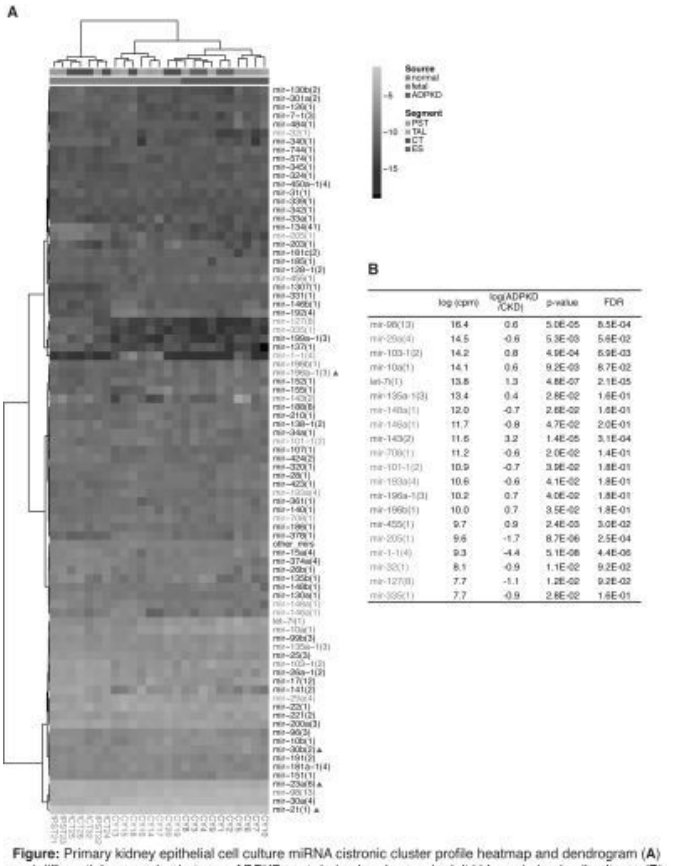
MicroRNA as Biomarkers in Autosomal Dominant Polycystic Kidney Disease Iddo Z. Ben-Dov,[1] Ying-cai Tan,[2] Pavel Morozov,[1] Hanna Rennert,[2] Jon D. Blumenfeld.[2] *[1]Rockefeller University, New York, NY; [2]Rogosin Institute, New York, NY.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) has unpredictable progression and complications. Biomarkers are needed to inform prognosis and guide treatment. MicroRNAs (miRNA) are present in biofluids and can be profiled efficiently. We profiled miRNAs in ADPKD to gain insight on molecular abnormalities and evaluate potential use as biomarkers.

Methods: MiRNA profiles were obtained by RNA sequencing of primary cultures of renal epithelium from cysts (N=10), normal (N=8) or fetal (N=7) kidneys, and from urine and plasma of ADPKD patients (N=20) and matched controls (N=20) with other chronic kidney diseases (CKD). The study was approved by Weill Cornell Medical College and Rockefeller University Institutional Review Boards.

Results: MiRNA profiles distinguished between cells cultured from healthy adult and ADPKD kidneys (panel A), with members of mir-98 (let-7s), mir-103, let-7i and mir-143 upregulated and mir-1 and mir-29a downregulated in ADPKD (panel B). With exception of the let-7s, expression of the noted miRNA in fetal cells resembled ADPKD rather than healthy cells. Compared to non-ADPKD patients, mir-143(2) was upregulated in ADPKD urine, while mir-1(4) and related mir-133b(2) were downregulated. Additional findings included increases in mir-223(1), mir-199a(3) and mir-199b(1) cluster members. miR-1(2), miR-133a(2) and miR-499 were decreased in ADPKD urine ultrafiltrate samples compared to other CKD. Several plasma miRNAs were linked with *stage* of CKD, but only negligible differences were noted between ADPKD and other CKD at similar stage.

Conclusions: MiRNA in ADPKD patients' urine correspond to patterns occurring in cyst-derived cells. Alterations in miRNA composition in ADPKD also imply mononuclear cell infiltration and fibrosis-related changes. We suggest a role for miRNA in pathogenesis and denote biomarker potential.



	log (cpm)	log(ADPKD /CKD)	p-value	FDR
mir-99(13)	16.4	0.6	5.0E-05	8.5E-04
mir-29a(4)	14.5	-0.6	5.3E-03	5.6E-02
mir-103-1(2)	14.2	0.8	4.9E-04	6.9E-03
mir-10a(1)	14.1	0.6	9.2E-03	8.7E-02
let-7i(1)	13.8	1.3	4.8E-07	2.1E-05
mir-135a-1(3)	13.4	0.4	2.8E-02	1.6E-01
mir-148a(1)	12.0	-0.7	2.6E-02	1.6E-01
mir-146a(1)	11.7	-0.8	4.7E-02	2.0E-01
mir-143(2)	11.6	3.2	1.4E-05	3.1E-04
mir-708(1)	11.2	-0.6	2.0E-02	1.4E-01
mir-101-1(2)	10.9	-0.7	3.0E-02	1.8E-01
mir-193b(4)	10.6	-0.6	4.1E-02	1.8E-01
mir-196a-1(3)	10.2	0.7	4.0E-02	1.8E-01
mir-199b(1)	10.0	0.7	3.5E-02	1.8E-01
mir-455(1)	9.7	0.9	2.4E-03	3.0E-02
mir-205(1)	9.6	-1.7	8.7E-06	2.5E-04
mir-1-1(4)	9.3	-4.4	5.1E-08	4.4E-06
mir-32(1)	8.1	-0.9	1.1E-02	9.2E-02
mir-127(8)	7.7	-1.1	1.2E-02	9.2E-02
mir-335(1)	7.7	-0.9	2.8E-02	1.6E-01

Figure: Primary kidney epithelial cell culture miRNA cistronic cluster profile heatmap and dendrogram (**A**) and differential expression between ADPKD cyst-derived and normal adult kidney-derived cell cultures (**B**). ▲, signifies that a difference was detected between ADPKD and normal sampels in miRNA precursor seuquece variation in a member of the cluster (p<0.05).

Funding: Other NIH Support - Supported in Part by Grant # UL1RR024143 from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health

TH-PO641

Systematic Analysis of microRNAs in Urine Exosome-Like Vesicles and Correlation with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Christopher Y. Chen, Christopher James Ward, Katharina Hopp, Peter C. Harris, Marie C. Hogan. *Division of Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

Background: MicroRNAs (miRs) are non-coding RNAs that canonically bind to the 3' UTR of target mRNAs and inhibit their translation. Urine exosome-like vesicles (ELVs) shed from epithelial cells of the nephron may be involved in ADPKD pathogenesis and are enriched for miRs. The miR content of urine ELVs is currently unknown. To characterize ELV miR content, explore the role of miRs in cystogenesis, and identify new biomarkers for ADPKD progression, miR expression was analyzed in human and mouse urine ELVs.

Methods: We isolated urine ELV miRs from PKD1 patients, *Pkd1* hypomorphic mice, and age/gender matched controls. ELV miR expression was analyzed by Illumina MiSeq and RT-PCR then compared to miR data from normal human kidneys (NHK). Novel miRs were predicted by *miRDeep2*. Functions of mis-regulated miRs were determined by *mirPath*.

Results: Next-Gen sequencing of normal urine ELVs defined a specific subset of 478 miRs (134 miRs with >10 reads/million). Furthermore, 13 putative novel miRs were identified, including two related miRs on chrs 15 and 10 with 586 and 30 reads/million, respectively. Relative to NHK, urine ELVs were most enriched for miRs -200a*, -423-3p, 22*, -200b*, -28-3p, -101*, 23b*, 7a*, and 29* that are involved in regulating TGF-β, Wnt, focal adhesions, MAPK, mTOR, and ErbB pathways. Star (*) sequences, which comprised 3% of miRNA pairs in NHKs, were enriched to 24% in urine ELVs, suggesting a role for ELVs in transporting these generally rare miRs. Preliminary results comparing PKD and normal urine ELVs show the greatest depletion of miRs -451, -23a, -24, -29b, -199a-3p, -23b, -497, -365, and -29c, which are predicted to regulate the TGF-β, Wnt, and focal adhesion pathways, previously implicated in ADPKD pathogenesis.

Conclusions: We have established the first miR-nome for normal human urine ELVs and made a comparison with ADPKD ELVs. Our results show urine ELVs are enriched for miRs regulating several renal developmental pathways. Reduction of Wnt, TGF-β, and focal adhesion regulating miRs in *Pkd1* mutant ELVs suggests a novel mechanism for modulating cystogenesis.

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TH-PO642

Urine Sodium Excretion and Plasma proANP as Markers of Disease Progression in ADPKD

Maria V. Irazabal,[1] Wendy E. Boertien,[2] Doug Landsittel,[3] Jie Li,[3] Joachim Struck,[4] Michael F. Flessner,[5] Ron T. Gansevoort,[2] Vicente E. Torres.[1,6] *[1]Mayo Clinic, Rochester, MN; [2]U of Groningen; [3]U of Pittsburgh; [4]ThermoFisher Scientific; [5]NIDDK; [6]Consortium for Radiologic Imaging Studies of PKD (CRISP).*

Background: CRISP has shown that higher urinary sodium excretion (UNaE), lower serum HDL-cholesterol, and younger age at baseline associate with more rapid renal enlargement (CJASN 6:640 2011). High UNaE and plasma proANP have been associated with non-diabetic CKD progression (KI 75:408 2009; JASN 23:165 2012).

Methods: Associations of baseline UNaE plasma proANP (sandwich immunoluminometric assay) and their interaction with rates of renal enlargement (MRI) and GFR decline (iothalamate clearance) using mixed (with and without interactions with time) and regression models for specific time points adjusting for gender, age, height, MAP, plasma glucose, HDL cholesterol, smoking, and baseline GFR or TKV.

Results: Baseline lnTKV and UNaE, but not plasma proANP, predict lnTKV across all time points and show a significant interaction with time. Plasma proANP, but not UNaE, predict GFR in all longitudinal models; the significance level increases over time and shows a significant interaction with time.

lnTKV	**Baseline**	**Year 3**	**Year 6**	**Repeated**	**Interactions**
Variable	β | p-value	β | p-value	β | p-value	β | p-value	p-value
Time	--- | ---	--- | ---	--- | ---	0.045 | <0.001	---
lnTKV0	--- | ---	1.067 | <0.001	1.087 | <0.001	1.042 | <0.001	<0.001
lnANP	.185 | 0.04	-0.006 | 0.76	0.060 | 0.10	0.011 | 0.39	0.01
UNaE	0.060 | 0.21	0.024 | 0.03	0.043 | 0.03	0.019 | 0.01	<0.001
Age0	0.015 | <0.01	-0.001 | 0.20	-0.007 | 0.001	-0.001 | 0.05	<0.001
HDL	-0.005 | 0.18	-0.002 | 0.07	-0.005 | 0.001	-0.001 | <0.01	<0.001
GFR	**Baseline**	**Visit 30**	**Visit 60**	**Repeated**	**Interactions**
Variable	B | p-value	B | p-value	B | p-value	B | p-value	p-value
Time	--- | ---	--- | ---	--- | ---	-2.42 | <0.001	---
GFR0	--- | ---	0.63 | <0.001	0.60 | <0.001	.071 | <0.001	0.001
lnANP	-6.82 | 0.09	-10.34 | 0.01	-19.24 | 0.001	-9.41 | <0.001	<0.001
UNaE	-1.44| 0.50	3.48 | 0.08	-0.94 | 0.75	0.97 | 0.43	0.74
Age0	-0.97 | <0.001	-0.28 | 0.19	-0.74 | 0.03	-0.36 | 0.01	0.01

Conclusions: UNaE is an independent predictor of lnTKV increase whereas ANP is an independent predictor of GFR decline. Longer follow-up may be needed to detect an association of UNaE with GFR decline.

TH-PO643

Urinary Extracellular Vesicles from Healthy and ADPKD Urine Prepared by Three Different Methods Display Consistent Lectin Microarray Profiles

Jared Q. Gerlach,[1] Anja Krüger,[1,2] Marie C. Hogan,[3] Christopher James Ward,[3] Lokesh Joshi,[1] Matthew D. Griffin.[2] *[1]Glycosciences Group, NCBES, National University of Ireland, Galway, Ireland; [2]Regenerative Medicine Institute, National University of Ireland, Galway, Ireland; [3]Dept Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Glycosylation of urinary extracellular vesicles (uEVs, "exosomes") may reflect aetiology/severity of kidney diseases but uEV isolation remains problematic. In this study, intact uEVs, isolated from healthy urine by 3 methods, were profiled for glycosylation using lectin microarrays and were compared with uEVs from ADPKD subjects.

Methods: uEVs were isolated from 3 healthy subjects by ultracentrifugation (UC) and centrifugal filter concentration then from an age-matched set of 7 ADPKD and 7 healthy controls by gradient UC and were fluorescently labeled (CM-DiI or PKH26). 43 lectins with affinity to a broad range of carbohydrate epitopes were printed on Nexterion hydrogel slides. After incubation with uEVs, arrays were washed and laser-scanned at 5 µm resolution. Numerical intensity values were extracted, normalized, and intensity profiles established. Arrays of purified AF647-labeled Tamm-Horsfall protein (THP) were similarly performed.

Results: Unsupervised clustering of normalized lectin intensity data showed that, while minor differences were detectable between the 3 uEV isolation methods, the degree of profile similarity was very high. uEVs demonstrated binding to lectins specific for a variety of carbohydrate epitopes indicative of both N- and O-linked glycans and these contrasted to the glycoprofiles of THP from multiple donors which displayed fewer and less diverse lectin interactions. Responses for healthy and ADPKD uEVs differed significantly at lectins AIA (Galβ-/T-antigen), RCA-I (Galβ-/LacNAc), and AAL (Fucα1-3/α1-6) although both sets of uEVs displayed similar overall profiles.

Conclusions: Filtration and simplified UC produced uEV lectin array glycoprofiles comparable to the gold standard of gradient UC. All uEV profiles were readily distinguishable from THP. uEV from ADPKD subjects have subtle changes in glycosylation compared to healthy subjects.

Funding: Government Support - Non-U.S.

TH-PO644

Identification of Novel Biomarkers for Vascular Complications Associated with Autosomal Dominant Polycystic Kidney Disease Using Patient-Specific iPSCs Kenji Osafune,[1] Daisuke Taura,[2] Fumihiko Shiota,[1] Masakatsu Sone,[2] Tomonaga Ameku,[1] Toshikazu Araoka,[1] Satoshi Matsui,[1] Isao Asaka,[1] Eri Muso,[4] Akio Koizumi,[3] Kazuwa Nakao,[2] Shinya Yamanaka.[1] *[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan; [2]Department of Medicine and Clinical Science, Kyoto University, Kyoto, Japan; [3]Department of Environmental and Health Sciences, Kyoto University, Kyoto, Japan; [4]Division of Nephrology and Dialysis, Kitano Hospital, Osaka, Japan.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenic disorder, characterized by the development of multiple renal cysts and various extrarenal manifestations. Cardiovascular complications are the main cause of death in ADPKD patients and intracranial aneurysms, causing subarachnoid hemorrhage, are among the most serious. The pathogenesis of vascular lesions as well as cyst formation remains largely unknown.

Methods: We derived induced pluripotent stem cells (iPSCs) from skin fibroblasts from seven ADPKD patients, among whom four had intracranial aneurysms, and differentiated them into vascular endothelia and smooth muscle cells to model the vasculopathy in ADPKD.

Results: Vascular cells differentiated from ADPKD-iPSCs recapitulated the defective intracellular Ca^{2+} regulation, similar findings to those reported in vascular cells of mouse ADPKD models. By microarray analyses, we have identified several molecules whose expression levels are specifically altered in the iPSC-derived vascular cells from ADPKD patients and in those from ADPKD patients with aneurysms. Among the molecules, both mRNA and protein expression of an enzyme and its secretion into culture media show statistically significant elevations in the iPSC-derived endothelia from ADPKD patients with aneurysms as compared to the patients without.

Conclusions: Vascular cells differentiated from patient-specific iPSCs can be used for studying the mechanisms of vascular complications in ADPKD and for identifying novel molecular diagnostic and therapeutic targets in ADPKD.

Funding: Government Support - Non-U.S.

TH-PO645

Urine and Plasma Biomarkers Evaluation in a Cross-Sectional Cohort of Autosomal Dominant Polycystic Kidney Disease (ADPKD) Patients Carolina Haefliger,[1] Ivan Formentini,[1] Bengt C. Fellstrom,[2] Paolo Piraino,[5] Jan Melin,[2] Peter F. Barany,[3] Anders Fernstrom,[4] Markus Ramsauer,[1] Lucy Rowell,[1] Guillemette Duchateau-Nguyen,[1] Maria Bobadilla,[1] Jacques Mizrahi.[1] *[1]Roche, Switzerland; [2]Nephrology, Uppsala, Sweden; [3]CLINTEC, Karolinska, Sweden; [4]Nephrology, Linkoping, Sweden; [5]StatsConsulting.*

Background: Although the diagnosis of ADPKD is well established, no markers for predicting disease progression are available. Biomarkers for disease monitoring and for the identification of patients with higher risk of progression to end stage renal disease (ESRD) are key to support the development of new therapeutic interventions.

Methods: Fourteen candidate biomarkers were assessed in urine and plasma samples collected from a cross-sectional cohort of ADPKD patients (n=56) and controls (n=20). Renal function was characterized by estimated glomerular filtration rate (eGFR), creatinine clearance in 24 h urine, urine albumin, urine albumin to creatinine ratio (UACR), plasma BUN and cystatin C. All subjects were classified in chronic kidney disease (CKD) stages. Biomarkers were measured by multiplexed protein immunoassays.

Results: Controls and ADPKD patients had similar distribution of gender and ethnicity, whereas mean age was higher in the ADPKD group. Controls eGFR ranged between 114 and 70.1 mL/min/l whereas ADPKD patients ranged from 114.5 to 12.4 mL/min/l being CKD stages 2 and 3 the most prevalent ones. Candidate biomarkers in plasma such as alpha 1 microglobulin, NGAL, TIMP1, TFF3, VEGF and osteopontin increased with CKD stages. Most of the biomarkers had a similar, though less pronounced, increase in urine according to the degree of renal impairment whereas osteopontin urine levels decreased with CKD stages. Multivariate analysis will be performed to assess covariates.

Conclusions: Urine and plasma candidate biomarkers showed correlation with CKD stages in this ADPKD population. They have the potential to be used for monitoring of disease progression and therapeutic intervention in ADPKD patients. Further, longitudinal studies are required to confirm the applicability of the findings.

Funding: Pharmaceutical Company Support - F Hoffmann LaRoche Ltd

TH-PO646

Expression of Biomarkers during Different Phases of Polycystic Kidney Disease in the *Pkd1*^{nl,nl} Mouse Model Danielle Leuning,[1] Hester Happe,[1] Wouter N. Leonhard,[1] Martijn H. Breuning,[1] Emile De Heer,[2] Dorien J.M. Peters.[1] *[1]Human Genetics, Leiden University Medical Centre, Leiden, Netherlands; [2]Pathology, Leiden University Medical Centre, Leiden, Netherlands.*

Background: We previously described the histopathology of the hypomorphic *B6Ola-Pkd1*^{nl,nl} mouse model for Polycystic Kidney Disease (PKD) with reduced expression of the normal *Pkd1* transcript (Happé *et al*, 2009: F-PO1658). These mice develop large cystic kidneys within 4 weeks. Interestingly, cyst expansion is followed by renal volume regression due to collapse of cysts accompanied by focal formation of fibrotic areas.

In this study, we analyzed renal function and the expression of genes encoding potential biomarkers at different stages of renal cystic disease in *B6Ola-Pkd1*^{nl,nl} mice. i.e. Neutrophil gelatinase-associated lipocalin (NGAL), Sulfated glycoprotein-2 (Clusterin), Prostaglandin D2 synthase, all tubular injury markers, and complement component 3 (*C3*), important in innate immunity.

Methods: Blood urea concentrations were determined using Reflotron Plus (Roche Diagnostics). RT-MLPA was used to analyze the expression of biomarker encoding genes in *Pkd1*^{nl,nl} and *Pkd1*^{wt,wt} mice at different ages (5,8,15,24,50 weeks old) and in inducible ksp-*Pkd1*^{del} mice (+1,2,3,4 months).

Results: After a dramatic drop within the first 4 weeks, renal function did not decline further and remained stable over time.

The genes encoding NGAL (*Lcn2)*, Clusterin (*Sgp2)* and Prostaglandin D2 synthase (*Ptgds)*, all showed increased mRNA expression levels in *B6Ola Pkd1*^{nl,nl}mice compared to wild-type animals, at all ages. The dynamics of expression of *Lcn2* and *Sgp2* was remarkably similar to that of renal volume, showing an initial peak at 4 weeks and a strong reduction afterwards. This correlation was less pronounced for *Ptgds* and in contrast with the pattern seen for C3, which continued to increase during disease progression.

Conclusions: In this hypomorphic mouse model, markers related to tubular injury are more indicative for the cyst expansion phase rather than for later fibrotic stages of renal cystic disease.

Funding: Private Foundation Support

TH-PO647

Wolffian Duct Involvement in Initial Cyst Formation of ADPKD: The Pkd1;Hoxb7-Cre Mouse James B. Tee. *Pediatrics, University of Calgary, Calgary, AB, Canada.*

Background: Autosomal dominant polycystic kidney disease type 1 (ADPKD) is a leading cause of kidney failure and is associated with a germ-line mutation of one of the two Pkd1 alleles. Evidence from the analysis of cystic epithelia in ADPKD indicate that for the disease to occur, a second event that disrupts the expression of the other inherited Pkd1 allele must occur. Cysts in ADPKD can be detected in embryos, but their earliest point of initiation remains unknown.

Methods: To evaluate this, Pkd1(+/flox);HoxB7-Cre and Pkd1(flox/flox) mice were mated to generate a novel mutant mouse strain that would model the inheritance of the mutant Pkd1 allele in humans, with the distinction that knockdown of the gene occurs only in the earliest kidney structure from which cysts can form: the Wolffian duct.

Results: In contrast to previously published models with targeted homozygous mutations of Pkd1 that have been uniformly characterized by embryonic lethality, the Pkd1(flox/flox);HoxB7-Cre mutant mice generated from these pairings survived delivery and died at an average of 18 days of age from complications of renal failure. H&E sections of these kidneys revealed enlarged kidneys and massive cyst formation. Pkd1(+/flox);HoxB7-Cre mutant mice with heterozygous knockdown of the Pkd1 gene did not demonstrate cysts at the same age. However, despite the phenotypic similarity between the kidneys of these heterozygous mice compared to wildtype (non-HoxB7-Cre expressing) mice, a striking difference in genotypic expression was revealed by whole-transcript mRNA microarray analysis. 40 genes in particular featured >= 2-fold change in ANOVA-1way differential expression (p-value+FDR < 0.05) and a unique hierarchical clustering. Pearson's correlation with miRNA expression in these kidneys also revealed significant differences between the heterozygote and wildtype kidneys.

Conclusions: The presented results suggest that the Pkd1;Hoxb7-Cre mouse is a novel model for ADPKD that will be useful in the study of the genotypic changes underlying cyst formation, in the study of perinatal factors given the model's survival postpartum, and for the first time reveals a role for the origin of cystogenesis in the earliest structure of kidney formation: the Wolffian duct.

TH-PO648

Reduced Ciliary Polycystin-2 in Induced Pluripotent Stem Cell Lineages from Patients with Polycystic Kidney Disease Benjamin S. Freedman,[1] Albert Q. Lam,[1] Jamie L. Sundsbak,[2] Rossella Iatrino,[1] Sarah J. Koon,[2] Maoqing Wu,[1] Jing Zhou,[1] Peter C. Harris,[2] Joseph V. Bonventre.[1] *[1]Renal Division/Center for PKD Research, Brigham and Women's Hospital, Harvard, Boston, MA; [2]Division of Nephrology and Hypertension and Translational PKD Center, Mayo Clinic, Rochester, MN.*

Background: Induced pluripotent stem (iPS) cells are a powerful new technology for investigating human disease and potential therapeutics in diverse cell types. We tested the potential of iPS cells to model the ciliopathy, polycystic kidney disease (PKD).

Methods: Fibroblasts with characterized mutations from three autosomal dominant (ADPKD) and two autosomal recessive (ARPKD) patients were reprogrammed into iPS cells by retroviral expression of OCT4, SOX2, KLF4, and c-myc. iPS cells were differentiated into ZO-1+ somatic epithelial cells or AFP+CK19+HNF4+ hepatic progenitor cells and quantitatively evaluated for ciliation, polycystin (PC)-1 and -2 protein expression patterns, and cyst formation, and compared to iPS and ES cells from five controls.

Results: Ciliation occurred efficiently in PKD iPS cells, but 50-75% fewer PC2-positive primary cilia were observed in undifferentiated iPS cells, derived epithelial cells, and hepatic progenitor cells from the three ADPKD patients ($p = 10^{-5}$). In contrast, PC2 staining was normal in iPS cell lineages derived from ARPKD patients or controls. The finding of a single PKD1 mutation in the ADPKD parental fibroblasts and iPS cells suggested that reduced ciliary polycystin-2 localization occurred without complete PC1 loss. During differentiation in 3D culture, ARPKD and ADPKD derived embryoid bodies showed a 2-3 fold increased tendency to form fluid-filled cysts, compared to controls ($p = 0.01$).

Conclusions: We show that features of polycystic kidney disease pathophysiology can be recapitulated in patient-derived iPS cells. Reduced PC2 at the primary cilium is specific to ADPKD, not ARPKD, consistent with differences in the pathophysiology of cystogenesis between these diseases. These PKD iPS cell lines provide a novel resource for understanding the cellular features of the disease and can serve as cellular substrates for drug screens and personalized or regenerative medicine approaches.

Funding: NIDDK Support

TH-PO649

Exome Sequencing Identifies a Novel *EEA1* Variant in Japanese Familial IgA Nephropathy Shin Goto,[1] Kazuyoshi Hosomichi,[2] Hiroyasu Tsukaguchi,[3] Ichiei Narita.[1] *[1]Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Division of Human Genetics, National Institute of Genetics, Shizuoka, Japan; [3]Second Department of Internal Medicine, Kansai Medical University, Osaka, Japan.*

Background: A genetic predisposition of IgA nephropathy (IgAN) has been suggested by the familial clustering of the disease. Previously, genome-wide linkage analyses of IgAN have revealed several susceptibility loci, however, causative gene have not been identified until now. From the point of view of genetic heterogeneity of familial IgAN, oligo/polygenic and multiple susceptibility gene model for the disease is proposed. Recently, exome sequencing has been demonstrated to be a powerful and cost-effective strategy for dissecting genetic basis of diseases.

Methods: To identify the genetic causality of familial IgAN, we applied exome sequencing to a family in which four biopsy-proven IgAN patients clustered in a dominant transmission mode. Whole exome sequencing of four affecteds, two asymptomatic carriers, and two non-affected individuals was performed on a HiSeq 2000 Platform. Several-step filtering including annotation and functional expectation was carried out.

Results: After exome sequencing, eight variants turned out to be the promising candidates. Among these, we found a novel missense variant F161Y in *EEA1,* encoding Early Endosome Antigen 1, a Rab5 effector protein that facilitate the docking and tethering incoming endocytic vesicles, located within a linkage locus with a maximum LOD score of 1.68 obtained by linkage analysis of the family. The F161Y variant furthermore co-segregated completely within the family and the amino acid position were highly conserved across zebrafish to human. A probabilistic prediction program, VAAST, placed *EEA1* F161Y in top 20 ranking among 23000 gene variants.

Conclusions: Identification of *EEA1* F161Y variant in our family indicate that defective endosomal trafficking may be implicated in the pathogenesis in familial IgAN. The possible role of *EEA1* F161Y variant in trans-cellular transport of IgAs is now being under investigation.

TH-PO650

The Effect of Kidney Function on Loci for Hematocrit (Hct) Identified from Genome-Wide Association Studies (GWAS) Adrienne Tin,[1] Wen Hong Linda Kao,[1] Santhi Ganesh,[2] Josef Coresh,[1] Eric Boerwinkle,[3] Meredith A. Atkinson.[4] *[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Department of Internal Medicine, University of Michigan, Ann Arbor, MI; [3]Human Genetics Center, University of Texas School of Public Health, Houston, TX; [4]Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Anemia affects over half of the 10 million Americans with chronic kidney disease (CKD). Previous GWAS have identified 8 genetic loci for Hct, but it is not known whether the effects of these loci are modified by kidney function.

Methods: We conducted a GWAS of Hct in 8645 European Americans from the Atherosclerosis Risk in Communities (ARIC) study, adjusted for age, sex, and estimated glomerular filtration rate (eGFR, using the CKD-EPI equation) and stratified the analysis by CKD status, with CKD defined as eGFR<60mL/min/1.73m^2.

Results: After controlling for eGFR and its square term, the GWAS of Hct did not identify additional loci. The associations of the 8 known Hct loci were similar with and without controlling for eGFR. Two of the 8 Hct loci (*PRKAG2* and *SH2B3*) were also independently associated with eGFR. The A allele of *PRKAG2* rs10224002 was associated with higher levels of Hct ($p=8.7x10^{-5}$) and eGFR ($p=1.0x10^{-2}$). At *SH2B3*, the A allele of rs11065987 was associated with lower level of Hct ($p=4.5x10^{-4}$) but higher level of eGFR ($p=5.6x10^{-2}$). The association of the 8 loci with Hct were similar across CKD except for the *HFE* locus, where the A allele of rs1800562 (C282Y) showed stronger association with Hct among individuals with CKD (beta=1.7%, $p=1.0x10^{-2}$) than those without (beta=0.4%, $p=5.2x10^{-6}$, heterogeneity p=0.04).

Conclusions: In 8645 European Americans, adjustment for eGFR did not change the GWAS results for Hct. The missense variant of *HFE* (rs1800562), a known risk variant of hemochromatosis, showed a stronger association with Hct among individuals with non-dialysis CKD. This novel observation suggests common genetic factors may confer susceptibility or resistance to the anemia associated with CKD, and may have pathophysiologic and therapeutic implications.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO651

***APOL1* Is Associated with Proteinuria in HIV** Michelle M. Estrella,[1] Man Li,[1] Wen Hong Linda Kao,[1] Stephen J. Gange,[1] Rulan S. Parekh.[2] *[1]Johns Hopkins; [2]University of Toronto.*

Background: HIV-infected persons have increased risk for proteinuria likely due to viral and host factors.

Methods: To determine if APOL1 genotype is associated with proteinuria in HIV infection, 1498 Caucasian, African American or Hispanic other women in the Women's Interagency HIV Study were genotyped for APOL1 G1 (rs73885319) and G2 (rs71785313) and 168 ancestry markers. Ancestry was estimated by principal components using EIGENSOFT. Proteinuria (urine protine-to-creatinine ratio >200mg/g) was measured on 2 visits (median 355d) from 10/94-03/03. We used recessive logistic models to estimate the odds of proteinuria associated with 2 APOL1 alleles vs 1/0.

Results: Of 1285 women successfully genotyped, 124 and 78 had proteinuria at 1 and 2 visits, respectively; 80 had 2 APOL1 alleles. Characteristics were: mean age 36y; 22% Caucasian, 61% African American and 17% Hispanic other; 3% diabetic; and 25% hypertensive. Median CD4 count and HIV1 RNA were 389 cells/mm^3 and 5600 cps/mL respectively; 1/3 injected drugs, were hepatitis C coinfected, had prior AIDS or received HAART. In adjusted analyses, 2 APOL1 alleles were associated with 4-fold higher odds of proteinuria vs. 1/0 APOL1 variants in women without prior AIDS (Table). APOL1 was not associated with proteinuria in women with prior AIDS (P-int=0.02). Similar odds ratios were observed with proteinuria based on 2 visits. Hepatitis C or HAART did not modify the association between proteinuria and APOL1.

Odds of Proteinuria

	Unadjusted	Adjusted†	
		AIDS-	AIDS+
2 vs. 1/0 APOL1	3.26 (1.88, 5.57)	4.17 (2.08, 8.33)	0.56 (0.11, 2.79)
Age, per 1y older	1.05 (1.02, 1.07)	1.01 (0.98, 1.05)	1.03 (0.67, 1.61)
eGFR, per 10 mL/min lower	1.18 (1.10, 1.28)	1.12 (1.01, 1.25)	1.20 (1.02, 1.41)
Hypertension	2.22 (1.51, 3.27)	1.71 (0.96, 3.04)	1.83 (0.89, 3.75)
Hep C	1.64 (1.12, 2.42)	1.31 (0.75, 2.26)	0.57 (0.27, 1.19)
HIV RNA, per ln 1000 copies/mL higher	1.18 (1.10, 1.27)	1.13 (1.02, 1.26)	1.26 (1.09, 1.45)
HAART	0.57 (0.35, 0.92)	0.92 (0.46, 1.85)	1.26 (0.51, 3.08)
AIDS history	1.77 (1.20, 2.60)		

† Adjusted for all variables listed

Conclusions: APOL1 variants are associated with higher risk of proteinuria in HIV-infected women without prior AIDS independent of eGFR, traditional risk factors and HIV-related factors.

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TH-PO652

Apolipoprotein L1 Risk Variants Associate with Lupus Nephritis-Induced End-Stage Renal Disease in African Americans Barry I. Freedman,[1] Carl D. Langefeld,[1] Mary E. Comeau,[1] Lee A. Hebert,[2] Mark S. Segal,[3] Jeff Edberg,[4] Bruce A. Julian,[4] Robert P. Kimberly.[4] *[1]Wake Forest School of Medicine, Winston-Salem, NC; [2]Ohio State University Medical Center, Columbus, OH; [3]University of Florida, Gainesville, FL; [4]University of Alabama at Birmingham, Birmingham, AL.*

Background: The G1 and G2 coding variants in the apolipoprotein L1 gene (*APOL1*) are strongly and reproducibly associated with focal segmental glomerulosclerosis (FSGS), HIV-associated collapsing glomerulopathy, and hypertension-attributed end-stage renal disease (ESRD) in AAs. The role of *APOL1* in lupus nephritis (LN) remains less clear. We tested for association between *APOL1* risk variants and severe LN in a national sample of unrelated AAs with systemic lupus erythematosus (SLE).

Methods: The study sample included 668 AA cases with LN-ESRD (456 with kidney biopsy documentation; 212 physician-reported) and 697 AA patients with longstanding SLE lacking nephritis. Allele frequency differences between LN-ESRD cases and SLE non-nephropathy cases were analyzed using logistic regression multivariable models, adjusting for non-muscle myosin heavy chain 9 gene single nucleotide polymorphism rs4821480 under a recessive genetic model.

Results: In cases with LN-ESRD, 87.1% were female, 89% received cytotoxic therapy, mean±SD age at SLE onset was 26.6±0.4 years, and duration of SLE to ESRD was 7.2±0.3 years. In non-nephritis patients, 93.5% were female with age at SLE onset 35.2±0.8 years. Contrasting all cases with and without ESRD, *APOL1* risk variants were significantly associated with LN-ESRD (odds ratio 2.35 (1.77-3.3 95% CI); p=4.25E-^{9}); significant differences in association were not observed when comparing cases with or without kidney biopsy documentation to those lacking lupus nephritis.

Conclusions: This study demonstrates strong association between *APOL1* and LN-associated ESRD in AAs. This finding extends the spectrum of *APOL1*-associated nephropathy to include that associated with SLE ESRD. It appears likely that *APOL1* contributes to nephropathy progression, as it strongly associates with LN-ESRD, FSGS, and other non-diabetic etiologies of ESRD in individuals with African ancestry.

Funding: Other NIH Support - NIAMS

TH-PO653

APOL1 Rare Variants Do Not Contribute to Sporadic Focal Segmental Glomerulosclerosis in African or European Americans Sophie Limou,[1] Ping An,[1] George W. Nelson,[1] Jeffrey B. Kopp,[2] Cheryl Ann Winkler.[1] *[1]Frederick National Laboratory for Cancer Research, NIH, SAIC-Frederick, Frederick, MD; [2]NIDDK, NIH, Bethesda, MD.*

Background: Carriage of two common *APOL1* renal risk alleles (termed the G1 and G2 coding variants) explains much of the excess of HIV-associated nephropathy (HIVAN), primary focal segmental glomerulosclerosis (FSGS), and hypertensive nephrosclerosis (OR ~ 29, 17, and 7, respectively) in African Americans (AA). However, approximately 28% of FSGS and HIVAN AA cases carry only one or no *APOL1* risk alleles, and these risk alleles are rare to absent in European Americans (EA). We thus sought to determine if additional *APOL1* coding or non-coding variants contribute to glomerular disease in the subgroup carrying 0 or 1 *APOL1* risk alleles.

Methods: We resequenced the promoter, 5' and 3' untranslated region, 7 exons and all intron-exon junctions of *APOL1* in 468 FSGS cases (54% AA, remainder EA) and 737 (55% AA) healthy controls, as well as 57 HIVAN AA cases and 261 HIV-infected AA controls without kidney disease.

Results: Resequencing revealed only 2 novel (not present in dbSNP or the 1000 Genomes Project) non-synonymous SNPs in *APOL1*, both seen in AA. In addition, we observed 5 rare (frequency 0.1% to 0.3% in AA controls) and 6 private (identified in only one individual in the heterozygote state) novel non-coding variants among AA; also 2 additional rare and 2 private novel variants were observed in EA. No *APOL1* non-synonymous variants, either previously or newly observed, had a significant association with FSGS/HIVAN when G1/G2 were taken into account. Further, rare and private variations were not collectively associated with disease risk.

Conclusions: *APOL1* resequencing did not identify additional variants that are associated with glomerular disease beyond the known G1 and G2 variants.

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TH-PO654

Apolipoprotein-L1 Kidney Risk Variants Demonstrate Differential Intracellular Retention and Altered Cell Biological Pathways Patrick Daniel Dummer,[1] Alison Hickman,[1] Dominic Esposito,[2] Cheryl Ann Winkler,[2] Trairak Pisitkun,[3] Jeffrey B. Kopp.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]FNLCR, NIH, Frederick, MD; [3]NHLBI, NIH, Bethesda, MD.*

Background: Coding variants in *APOL1* (termed G1, G2, compared to ancestral G0) are strongly associated with kidney disease in the African American population. Over-expression of ApoL1 induces autophagic cell death in colon cancer cells, but effects of ApoL1 variants on cell function have not been studied.

Methods: Recombinant GST-tagged ApoL1 was expressed in insect cells and purified using a glutathione column. HeLa cells were used for transient transfection.

Results: Using immunostaining and western blotting for LC3B, we confirmed that ApoL1 G0 increased autophagy and found that G1 and G2 had a greater effect. In transiently transfected HeLa cells, ApoL1 subcellular distribution was similar among G0, G1, and G2 variants, but signal intensity was altered. Cycloheximide-chase experiments revealed that G1 had a 20.3% decreased steady-state protein level and similar half-life compared to G0, and G2 showed a 83.2% increased steady-state and 6.6-fold increased half-life. Using protein degradation inhibitors (MG132, epoxomicin, chloroquine, E64/pepstatin A) we found G2 was resistant to proteosomal degradation. No evidence was seen for lysosomal degradation among the variants. Size-exclusion chromatography of recombinant G2 protein and human cell lysates of G1 and G2 indicated altered multimerization, which may contribute to differences in degradation rates. ApoL1 decreased total proteasome activity, G0 by 20.2%, G1 by 46.2%, and G2 by 60.1%. Western blotting for canonical ER stress markers revealed that ApoL1 induced the unfolded protein response, and that the risk variants lead to significantly more ER stress (G0, G1, G2 compared to control: BiP protein 1.3, 1.8 and 3.9 fold; pPERK, 3.3, 7.9, 4.5 fold; and cleaved ATF6, 3.7, 6.0, 11.6 fold).

Conclusions: ApoL1 G1 and G2 variants exhibit distinct cellular profiles compared to G0, with enhanced autophagy, altered protein half-life, decreased proteosomal activity, increased ER stress and altered multimerization. It remains to be determined whether these phenomena, observed in cells with forced over-expression, are relevant to human glomerulopathy.

Funding: NIDDK Support

TH-PO655

***APOL1* Null Alleles from a Village in India Do Not Correlate with Glomerulosclerosis** Duncan B. Johnstone,[1] V. Shegokar,[2] Deepak Nihalani,[1] Yogendra Singh Rathore,[3] Leena Mallik,[3] Fnu Ashish,[3] Halil O. Ikizler,[1] V. Zare,[4] Rajaram Powar,[2] Lawrence B. Holzman.[1] *[1]Renal, U Pennsylvania; [2]Microbiology, GMH, Nagpur, India; [3]CSIR-IMTech, Chandrigarh, India; [4]PHI, Nagpur, India.*

Background: African-American glomerulosclerosis correlates by genome wide association (GWAS) with the G1 and G2 alleles of *APOL1*. In the current hypothesis, individuals heterozygous for G1 and G2 benefit from increased resistance against Trypanosoma, resulting in positive allele selection, but individuals homozygous for G1 and G2 are predisposed to glomerulosclerosis. Although there is substantial circumstantial evidence, the causative effect of G1/G2 on glomerulosclerosis has not been proven, and it remains possible that the cause at this locus is linked to *APOL1* rather than *APOL1* itself. Linkage is an important caveat of GWAS and may explain why only 5% of individuals homozygous for G1 and G2 develop glomerulosclerosis.

Methods: We approached the possibility of linkage using classical genetic inference. G1 and G2 correlate with the incidence of glomerulosclerosis as recessive alleles, suggesting they are loss of function mutations. If so, causality for glomerulosclerosis can be tested by generating *APOL1* homozygous null alleles, but this cannot be done in rodents as the *APOL* gene cluster evolved in primates. However, there is one human being known to be *APOL1* homozygous null living in a rural village in India. We obtained clinical data, blood and urine from this patient and 50 related villagers.

Results: Based on blood pressure, BUN, creatinine, albuminuria, DNA sequencing and immunoblotting, this *APOL1* null individual does not have glomerulosclerosis, nor do relatives who carry *APOL1* null alleles.

Conclusions: This small study cannot be definitive but the absence of glomerulosclerosis in this Indian cohort is consistent with the possibility that African-American glomerulosclerosis is caused, not by *APOL1* loss of function, but either by subtle semi-dominance or by "genetic hitchhiking," a recently appreciated mechanism in which positive selection for one variant inadvertently caries along linked, deleterious mutations. Other tests for genetic hitchhiking are underway.

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TH-PO656

Diabetic Nephropathy Fails to Associate with the *APOL1/MYH9* Locus or Type 2 Diabetes Mellitus Susceptibility Genes: The Family Investigation of Nephropathy and Diabetes (FIND) Consortium Wen Hong Linda Kao.[1,2] *[1]Case Western Reserve University; [2]Johns Hopkins.*

Background: Diabetic nephropathy (DN), a devastating complication of types 1 and 2 diabetes mellitus (DM), is the leading cause of end-stage renal disease in the United States. Its molecular pathogenesis has not been clearly defined nor are there specific susceptibility alleles with strong effects leading to the development of DN. Variants in the *APOL1/MYH9* locus have not been rigorously tested in patients with DN across multiple ethnic groups.

Methods: We conducted candidate SNP case-control analyses using samples from FIND. DN cases and DM controls with no kidney disease of African American (AA, n=1932), American Indian (AI, n=1017), European American (EA, n=1020), and Mexican American (MA, n=2498) ancestry were genotyped for the *APOL1* G1 and G2 variants, the *MYH9* E1 risk haplotype, and sentinel SNPs previously associated with T2DM or estimated glomerular filtration rate (eGFR) and chronic kidney disease (CKD). Generalized estimating equations were used to determine the population-specific associations between SNPs and DN adjusting for age, gender, DM duration, center, and population substructure. Significance level was established at 0.001 (0.05/43 SNPs).

Results: Among AA, *APOL1* and *MYH9* variants were nominally associated with DN in a recessive model ($p = 0.0002$ to 0.02, model adjusted for age, sex, center, and duration), but not after correction for multiple testing. Neither *UMOD* nor *SHROOM3,* nor *SOD1 were* associated with DN in any FIND ancestry group. While several SNPs reached nominal significance, none were consistent across all populations, nor remained significant after multiple testing corrections were applied. An additive DM genetic risk score was also not associated with DN ($p = 0.04 – 0.67$ after adjusting for age, sex, center and duration).

Conclusions: Neither *APOL1/MYH9* risk variants nor previously reported DM or eGFR variants associate with DN. Additional approaches need to be implemented to define the underlying genetic architecture of DN. Determination of the molecular pathways altered by causal variants will lead to better approaches for prevention and treatment of DN.

Funding: NIDDK Support

TH-PO657

Partial ADAMTS13 Deficiency in Atypical Hemolytic Uremic Syndrome Stephen J. Eyler,[1] Shuju Feng,[2] Yuzhou Zhang,[1] Tara Maga,[1] Carla M. Nester,[3] Michael Kroll,[2] Vahid Afshar-kharghan,[2] Richard J. Smith.[1,3] *[1]Department of Otolaryngology, University of Iowa, Iowa City, IA; [2]Division of Internal Medicine, Benign Hematology, University of Texas M. D. Anderson Cancer Center, Houston, TX; [3]Department of Internal Medicine, Division of Nephrology, University of Iowa, Iowa City, IA.*

Background: Thrombotic microangiopathies (TMAs) are a group of microvascular disorders characterized by microangiopathic hemolytic anemia and thrombocytopenia. Atypical hemolytic uremic syndrome (aHUS) and thrombotic thrombocytopenic purpura (TTP) are two such TMAs that share phenotypic similarities. Despite their clinical overlap, these two diseases have distinct etiologies: aHUS is caused by dysregulation of the alternative complement system, while TTP is caused by severe deficiencies in ADAMTS13. Given the phenotypic similarities, we hypothesized that ADAMTS13 deficiency may coexist with abnormalities in the alternative complement pathway in aHUS.

Methods: We screened 26 patients with the clinical diagnosis of aHUS for functional and genetic abnormalities of the alternative complement system and ADAMTS13. ADAMTS13 activity was assessed by measuring the cleavage of a recombinant von Willebrand factor-A2 domain (vWF-A2) and a fluoregenic peptide comprising 73 amino acids of vWF-A2 (FRETS vWF73). Genetic variants in *ADAMTS13* and complement genes were assessed by bidirectional Sanger sequencing.

Results: Mutations and/or variants in complement genes and *ADAMTS13* were identified in 14 (54%) and 19 (73%) patients, respectively. The *ADAMTS13* variants were associated with partially reduced activity (<60%) in 15 patients (58%) as measured by the FRETS vWF73 and recombinant vWF-A2 cleavage assays. The coexistence of both ADAMTS13 deficiency AND excessive complement activation was found in 13 (50%) patients.

Conclusions: Partial ADAMTS13 deficiency is a common finding among aHUS patients. Decreased activity of this von Willebrand factor-cleaving protease contributes to the pathogenesis of aHUS. Clinical evaluation of ADAMTS13 activity in aHUS patients may provide finer phenotypic characterization of aHUS, which may be of help in defining the role of various treatments for this disease.

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TH-PO658

Family-Based Association Analysis in the Joslin Study of Genetics of Nephropathy in Type 2 Diabetes Family Collection Further Confirms the Role of Chromosomes 9q21.32 and 13q33.3 in the Susceptibility of Diabetic Nephropathy in Diabetes

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Background: A genome-wide association scan of the GoKinD collections identified four novel diabetic nephropathy (DN) susceptibility loci, located on chromosomes 7p14.3, 9q21.32, 11p15.4, and 13q33.3, that were associated with DN in T1D and subsequently shown to be associated with DN in unrelated patients with T2D. To follow up these findings, we examined whether single nucleotide polymorphisms (SNPs) at these susceptibility loci were associated with DN in patients from the Joslin Study of Genetics of Nephropathy in Type 2 Diabetes Family Collection.

Methods: Six SNPs across the four loci identified in the GoKinD collections were genotyped in 1,221 individuals from 66 extended families of European ancestry. Pedigrees from this collection contained an average of 18.5 members (range: 6 to 39 members), with 4 to 26 individuals from each family, including 2 to 14 members with T2D, having DNA available. Genotype data were tested for association using FBAT.

Results: Among 798 family members, a significant association with DN was identified at the 9q21.32 locus (rs1888747, P=5.7x10^{-3}). This same locus was significantly associated with variation in urinary albumin excretion in all relatives (P=0.02) and in analyses restricted to those with T2D (P=0.03). Quantitative analysis of estimated glomerular filtration rate (eGFR) was strongly associated with variants at the 13q33.3 locus (rs1411766, P=2.2x10^{-3}) in diabetic relatives.

Conclusions: These data further increase support that associations identified in the GoKinD collections on chromosomes 9q21.32 and 13q33.3 are true DN susceptibility loci. Quantitative analysis of variation in urinary albumin excretion and renal function suggests that these loci may have distinct roles in the pathogenesis of DN.

Funding: NIDDK Support

TH-PO659

The Discovery Phase of the CRIC Study GWAS Identifies Novel Candidate Markers Associated with Progression of CKD

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Background: The rate of decline of renal function and development of end stage renal disease (ESRD) varies significantly among individuals with established chronic kidney disease (CKD). Traditional risk factors for CKD only partially explain this variability. To better understand the contribution of inherited genetics to CKD progression, we performed a genome-wide association study (GWAS) within the Chronic Renal Insufficiency Cohort (CRIC) Study cohort.

Methods: We genotyped over 900,000 SNP markers in a total of 1502 African-Americans (AA) and 1586 Caucasian study participants with established CKD. Outcome measures were either change in eGFR over time or the composite of occurrence of ESRD or 50% decline in eGFR from baseline (ESRD+). Linear regression models and Cox proportional hazards models were used to detect associations of SNP markers with eGFR slope and time to ESRD+, respectively. All primary analyses were stratified by genetically inferred race. Within race strata, analyses also were stratified by diabetes mellitus (DM) status.

Results: In our Caucasian cohort, we identified 3 markers associated with progression to ESRD+, one of which reached genome-wide significance. In our AA cohort, we discovered 3 SNP markers that were suggestively associated ($p<1.0 \times 10^{-7}$) with eGFR decline, one of which attained genome-wide significance ($p<5.0 \times 10^{-8}$) among participants without DM. Stratified analyses further revealed 2 markers that were associated with eGFR decline at genome-wide significance and 6 markers that were suggestively associated with progression to ESRD+.

Conclusions: We identified 4 genome-wide significant markers mapping to 4 unique genes associated with eGFR decline and/or renal events. We also have identified an additional 10 markers (10 unique genes) that have suggestive associations with CKD progression. Replication efforts to confirm these signals are underway. These findings may lead to a better understanding of genetic influences on progressive CKD.

Funding: NIDDK Support

TH-PO660

Homozygozity Mapping for Steroid-Sensitive Nephrotic Syndrome

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Background: Familial and sporadic steroid-sensitive (SS-) nephrotic syndrome (NS) due to minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) may be underpinned by mutations of rare recessive genes. Runs of homozygosity (ROH) analysis is a powerful approach for mapping recessive gene loci in inbred families and outbred populations.

Methods: We performed a genome-wide ROH scan in 58 patients with SS-MCD/FSGS including 7 families with 2 or 3 affected using Illumina Human CNV370 and 660W arrays. Using PLINK and genotype data from 862 unrelated ethnic-specific samples from HapMap III reference populations as controls, we assigned 58 patients to 5 ethnic groups by multidimensional scaling. We calculated the inbreeding coefficient F for each study subject and catalogued all ROHs ≥ 1Mb.

Results: We found a higher F-value only in our Caucasian cases compared to controls ($p<0.05$; Wilcoxon rank sum test) and cryptic relatedness in the parents of 5 patients ($F>0.016$). We catalogued 50 ROH tracts in 38 Caucasian pts, 50 ROH tracts in 10 Native Indian/Mexican pts, 5 ROH tracts in 4 South Asian pts, and 2 ROH tracts in 2 Black pts.

ROH analysis of 58 patients with SS-NS and 862 HapMap III control samples from 5 ethnic groups after LD-pruning

Groups	Sample Size	Ethnicity	No. SNP (with LD pruning)	# ROH (> 1MB)	# ROH shared by ≥ 2 persons	# ROH enriched in cases vs. controls ($p < 0.05$)*	# unique ROH shared by ≥ 2 pts
Pts	38 (including FSSN52, 6, 7, 8)	Caucasian	70,064	50 (1.3)**	68	2	2 ROH tracts each shared by 2 unrelated cases
CTRL	215	CEU_TSI		186 (0.76)			
Pts	10 (including FSSN54)	Native Indian	61,083	50 (5)	14	10	2 ROH tracts each shared by all 3 affected relatives (2 sibs and 1 cousin) from a Native Indian family; 1 ROH shared by 2 unrelated cases
CTRL	54	MEX		37 (0.68)			
Pts	4 (including FSSN53)	Chinese	59,556	0 (0)	36	0	0
CTRL	359	CHB_CHD_JPT		124 (0.35)			
Pts	4 (including FSSN55)	South Asian	67,690	5 (1.25)	153	0	0
CTRL	97	GIH		412 (4.25)			
Pts	2	Black	105,450	2 (1)	190	0	0
CTRL	140	ASW_LWK		660 (4)			
Total # Pts	58						
Total # CTRL	862						

*p-value by Fisher exact test; **mean number of ROH tracts/individual in bracket

Among these tracts, 2 and 10 were uniquely present in our Caucasian and Native Indian/Mexican pts, respectively, and several of them were shared by ≥2 pts. In one Native Indian family, only 2 ROH tracts were shared by all 3 affected relatives.

Conclusions: All ROH tracts enriched in cases compared to controls or shared by affected members within the same family are candidate regions for recessive genes in SS-NS. ROH mapping coupled with exome sequencing (currently in-progress) represent a promising approach to identify these genes.

Funding: Private Foundation Support

TH-PO661

A Novel Mutation in KCNJ1 (ROMK1) in a Kindred with Bartter's Syndrome

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Background: We report clinical and genetic data of a Pakistani kindred with 3 affected female members with nephrocalcinosis and features of atypical Bartter's Syndrome.

Methods: The eldest sibling presented with developmental delay at 3 years of age and was diagnosed with rickets. She was treated with Vitamin D supplements. Subsequently, she developed hypercalciuria and nephrocalcinosis and was initially treated with thiazide diuretics and potassium supplements. She had learning difficulties but remained normotensive with no evidence of metabolic acidosis or alkalosis. The second sibling had an identical presentation and treatment and was left with nephrocalcinosis, learning difficulties and hypokalemia. She went on to develop gestational diabetes and advanced renal impairment. The third child was born premature with a history of maternal polyhydramnios.She was initially diagnosed with hypocalcaemia, followed upon correction by hypercalciuria. When hypercalciuria was observed, she was treated with indomethacin, thiazides and a low-salt diet. With such treatment she frequently showed a hypokalemic alkalosis.Once it was possible to determine the levels of renin and aldosterone in the youngest child a suspected diagnosis of Bartter's syndrome was made.There was no history of deafness. Because of possible parental consanguinity, we initially performed homozygosity mapping of the 3 affected individuals. However, there were very few regions of shared homozygosity by descent, and no overlap of these regions with SLC12A1, KCNJ1, CLCKNB, BSND or CASR. We proceeded to examine, using exon PCR and Sanger sequencing, known genes for Bartter's syndrome.

Results: We detected a compound heterozygous change in KCNJ1 gene, encoding the potassium channel ROMK1. In silico modelling of the missense amino acids suggested deleterious changes.

Conclusions: In this family, molecular investigation provided a precise diagnosis and allows further medical management and genetic counselling to be given appropriately.

TH-PO662

Mutation Identification in the Adenine Phosphoribosyltransferase Gene in a Rare Form of Chronic Kidney Disease due to Crystalline Nephropathy

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Background: A 44 year old Caucasian woman was referred for chronic kidney disease (creatinine[Cr] 3.6mg/dl). She has mental impairment since childhood. She has an identical twin with similar cognitive limitation who developed renal failure requiring dialysis 1 year ago due to nephrolithiasis. Parents had a consanguineous marriage and two female siblings are healthy.

Methods: Kidney biopsy revealed numerous brown rod shaped crystals in tubular lumens, epithelium and histiocytes in the interstitium. Chronic tubulointerstitial nephritis was noted with interstitial inflammation due to lymphocytes and plasma cells. Crystals were strongly birefringent and appeared yellow when parallel to the polarizer suggesting uric acid composition, though uric acid levels in serum and urine were normal.

Results: Reference laboratory identified the crystals as 2,8-dihydroxyadenine (DHA) based on morphology, while X-ray microanalysis ruled out oxalate. Adenine Phosphoribosyltransferase (APRT) activity in red blood cells was absent (<0.01 nmol/min/mg protein, normal:0.26-0.46). Allopurinol 300mg/day improved renal function (follow-up Cr 1.7mg/dl at 4 months). For mutation analysis, DNA was isolated from blood and a 2.4 kb fragment spanning *APRT* was amplified and then sequenced. A single T insertion was identified at the intron 4 splice donor site (TGgtaa to TGgttaa:IVS4+2insT) in both alleles from the proband and her twin. The same mutation was identified in a younger sister, while an older sister and both parents were heterozygotes for the mutation. *Tru91* digestion of the PCR product spanning exon 4 confirmed the mutation.

Conclusions: APRT deficiency is an autosomal recessive disorder of purine metabolism that can cause DHA crystalline nephropathy. The IVS4+2insT mutation, known to lead to complete loss of APRT activity, has been identified in several families from Europe, suggesting a founder effect. Differentiating DHA from uric acid crystals and confirming APRT deficiency with enzyme assay and mutation analysis have important implications for preventing further renal injury to native or transplanted kidney.

TH-PO663

Genetic Variants in the Vitamin D Pathway Are Associated with End-Stage Renal Disease

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Background: Vitamin D deficiency has been associated with progressive renal disease, and vitamin D analogues reduce RAAS activation, inflammation and proteinuria. Recent studies linked common variants in vitamin D-related genes to low vitamin D levels. We hypothesized that genetic variants in the vitamin D pathway are associated with the risk of end-stage renal disease (ESRD) or graft loss after kidney transplantation (KTx).

Methods: In 1271 KTx donor/recipient pairs we genotyped 44 single nucleotide polymorphisms (SNPs) in 4 genes in the vitamin D pathway: *CYP27B1* (1α-hydroxylase), *CYP24A1* (24-hydroxylase), *VDR* (vitamin D receptor) and *GC* (vitamin D binding protein). Minor allele frequencies of these SNPs were compared between donors and recipients. In survival analysis, the effect of SNPs in donors and recipients on the risk of death-censored graft loss and all-cause mortality was investigated by multivariate Cox regression adjusting for known risk factors of graft loss and mortality after KTx.

Results: Seven SNPs were identified with higher minor allele frequencies in ESRD patients than in donors: in *CYP24A1,* rs8124792 (odds ratio 2.2 [95%CI 1.8-2.9]), in *VDR,* rs757343 (2.7 [2.1-3.5]) and rs11574143 (0.5 [0.3-0.6]), and in *GC,* rs3755967 (1.5 [1.3-1.7]), rs4364228 (1.5 [1.2-1.9]), rs222017 (1.7 [1.4-2.0]) and rs3733359 (1.6 [1.3-2.0]) (all $p<0.0001$). The cumulative risk of these SNPs on ESRD expressed as a genetic risk score was highly significant (1.57 [1.44-1.70] per risk allele copy, $p=9\times10^{-28}$). At 5.4 [3.0-8.8] years after KTx, the minor allele of rs4809957 (*CYP24A1*) in recipients was the only locus in our study that was significantly associated with an increased risk of mortality (hazard ratio 1.45 [95%CI 1.15-1.84], $p<0.00001$). No associations with death-censored graft failure were found.

Conclusions: We identified 7 SNPs in the vitamin D pathway independently contributing to the risk of ESRD, and one SNP in the recipient *CYP24A1* gene associated with mortality after KTx. Genetic variations in the vitamin D pathway may contribute to progressive renal disease, and provide a target for intervention.

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TH-PO664

A QTL for the Coefficient of Variation in 24-h Urinary Calcium in a Rodent Model of Hypercalciuria

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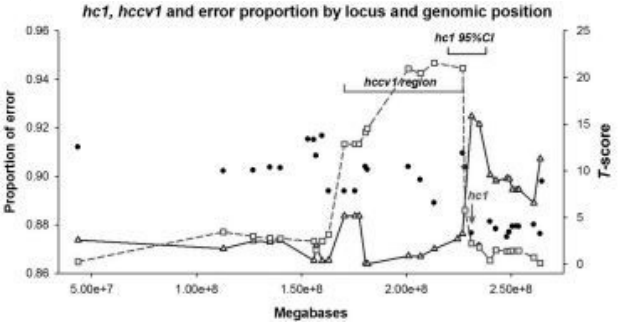
Background: Genes for residual variation might represent an important source of error in linkage or association mapping of genes for renal solutes. Previous work of ours indicates the heredity of residual variation in urinary calcium, a major risk factor for stone disease. We investigated the effects of genes for residual variation on the estimation of classical genetic parameters.

Methods: We estimated heritability for the coefficient of variation in urinary calcium (CV_{Ca}) in a chromosome 1 congenic Genetic Hypercalciuric Stone-forming × normocalciuric Wistar-Kyoto rat pedigree being used to isolate a classically-acting locus for urinary calcium (*hc1*). Marker-CV_{Ca} association mapping was performed using PLINK.

Results: CV_{Ca} was heritable ($h^2_a=0.067\pm0.047$). We detected a quantitative trait locus (QTL) ($P_{FDR}<0.001$) for CV_{Ca}, termed here *hypercalciuria coefficient of variation 1* (*hccv1*; 170-227 MB; *D1Rat204–D1Rat73*) orthologous to human chromosomes 9 (70-80 MB) and 11 (0-70 MB) and enriched for insulin receptor genes ($-\log_{B\text{-}H}=1.58$). Within the *hccv1* region, estimates of error in untransformed mean calcium excretion were 2.4% higher than over the region for the classically-acting *hc1*.

Conclusions: Our findings indicate that *hccv1* influences the mapping of classically-acting loci for renal solute output. Such systems could affect the genetic mapping of other traits, including renal solute indicators of renal pathology.

Figure 1. Association of microsatellites with mean (blue solid line, triangles for markers) and *CV* (green dashed line; squares) urinary calcium on rat chromosome 1 (right axis). Proportions of error by marker from linear analysis (σ^2_e/σ^2_p) indicated as dots (left axis).



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TH-PO665

Genetic Loss of Function Mutations in Zebrafish and Human CRB2, a Regulator of Epithelial Polarity, Are Associated with Podocyte Foot Process Effacement and Focal Segmental Glomerulosclerosis

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Background: Podocytes are polarized epithelial cells, central to the filtration barrier, and are a major pathological target in kidney glomerular disease. Podocytes cell polarity is manifested in different apical and basal membrane domains, slit diaphragms, and well defined actin rich foot processes. In published work, we identified a requirement for zebrafish *crb2b*, a conserved regulator of epithelial polarity, in protein trafficking events leading to podocyte morphogenesis. Crb2b is required for trafficking Nephrin to slit diaphragms. Crb proteins are EGF-like domain transmembrane proteins which regulate apical basal epithelial polarity and membrane biogenesis.

Results: We use zebrafish *crb2b* gene as an entrance point into exploring cell polarity in podocyte stucture and function. A recessive, null allele in zebrafish, *crb2b^{m}*, phenocopies the morpholino results and genetically confirms a need for *crb2b* in podocyte differentiation. Co-immunoprecipitation shows that Crb2b protein associates with Nephrin. Crb2b and Nephrin co-localize in vesicles when transiently expressed in cell culture.

We extended our zebrafish Crb2 studies into human nephrotic syndrome. SNP homozygosity mapping and exome re-sequencing were used to screen for mutations in human CRB2. Analysis of 30 consanguinous sibling pairs, displaying early onset FSGS, identified a homozygous missense mutation in CRB2.

Conclusions: Zebrafish Crb2 is required for podocyte differentiation. Mutations in human CRB2 are associated with FSGS, indicating an evolutionarily conserved function for CRB2 in podocyte structure and function. Cell polarity is central to podocyte function, and these studies suggest that a fraction of podocyte based nephrotic syndromes may be due to fundamental misregulation of podocyte apical basal polarity. Lastly, our studies underscore the importance of zebrafish in studying the genetics of human kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO666

Role of Kynurenine 3-Monooxygenase for the Integrity of the Glomerular Filtration Barrier

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Background: Kynurenine 3-monooxygenase (KMO), a flavin monooxygenase in the tryptophan pathway, hydroxylates L-Kynurenine to L-3-Hydroxykynurenine. While its inhibition seems to have a neuroprotective effect, a SNP in KMO has been associated with an elevated albumin/creatinin-ratio in a genome wide association study in mice. In order to investigate the functional role in an animal model, we used zebrafish (*Danio rerio*) as a model organism.

Methods: KMO was knocked down in zebrafish embryos using an ATG-blocking-morpholino. The fish were then screened for the development of edema. To assess renal protein leakage, we injected an FITC labeled dextran (70 kDa) into the cardinal vein of the zebrafish and compared the amount of systemic fluorescence in the retinal vessel plexus 24 and 48 hours post injection. In addition we performed KMO knockdowns in a transgenic fishline that expresses a GFP labeled liver-FABP (78 kDA) in the circulation and measured systemic fluorescence development over time as well. A significant loss of fluorescence in knockdown-fish would indicate glomerular leakage. To confirm our findings we examined zebrafish glomeruli on transmission electron microscopy (TEM).

Results: Morpholino injected fish displayed a severe edematous phenotype. In the functional assays, the knockdowns showed a significant loss of systemic fluorescence (28.79%, p<0.0001), compared to control injected animals (0.54%, p=0.86). The transgenic knockdowns developed only 1/30 of the fluorescence of the control-group. The TEM analysis revealed an intact basement membrane and endothelial cells, but wide stretches of podocyte effacement. Our current investigations focus on the KMO activity in NZM-mice, where the SNP was initially found. The mice develop spontaneous proteinuria and die within 12 months.

Conclusions: To our knowledge this is the first evidence provided that a dysregulated tryptophane metabolism can directly influence glomerular filtration barrier function.

Funding: Government Support - Non-U.S.

TH-PO667

A Clinical, Pathological and Genomic Targeted Customized Multi-Indexed Exome Sequencing Approach Identifies Novel and Rare Variants in Patients with Proteinuria, Atypical Alport Syndrome or CAKUT

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Background: There has been limited application of genomics in kidney diseases. This is much needed particularly in patients where clinical, radiological and pathological findings are equivocal and clinical presentation is atypical.

Methods: We performed custom targeted capture and exome sequencing of 292 genes using next generation sequencing (NGS) in an index case of a Caucasian woman with VUR who presented with nephrotic range proteinuria at 44 years of age. We then selected a panel of genes implicated in proteinuria and CAKUT and applied our approach on a small cohort of patients with either confirmed or presumed Alports or FSGS.

Results: Initial biopsy findings in the index case showed FSGS. Sequencing analysis of 4,041 exons revealed two deleterious nonallelic *COL4A3* mutations, one novel and the other previously reported in an Alport syndrome patient, and a novel deleterious *SALL1* mutation; all confirmed by Sanger sequencing. Bioinformatic analysis demonstrated that these variations altered highly conserved amino acids. Pedigree analysis suggested a compound recessive mode of inheritance. Immunohistochemistry in the renal biopsy revealed markedly diminished *COL4A3*. Several years after initial presentation, the patient presented with hematuria and sensorineural hearing loss. Later biopsies revealed histological findings of FSGS and ultrastructural changes diagnostic of Alport syndrome. We then extended this approach to a small cohort of proteinuria patients using multi-indexed targeted NGS. We found that five of nine patients with clinical diagnosis suggestive of Alports had mutations in *COL4A3, A4* or *A5*. *APOLI_G2* deletion was noted in 2 out of 3 African American patients (one homozygous) with FSGS. Further, novel deleterious *LAMA5* mutations were observed in 3 out of 5 FSGS patients.

Conclusions: These studies demonstrate that customized gene panels and targeted capture-based exome sequencing is a powerful approach in revealing genetic mutations as a potential factor in complex or atypical renal phenotypes.

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TH-PO668

Refinement of the HIVAN1 Susceptibility Locus on Chr.3A1-A3 via Generation of Subcongenic Strains

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Background: HIV-1 transgenic mice on the FVB/NJ background (TgFVB) represent a validated model of HIV- associated nephropathy (HIVAN). A mapping study between TgFVB and CAST/EiJ (CAST) strains previously identified a major susceptibility locus on chromosome 3A1-A3 (*HIVAN1*), with CAST alleles associated with increased risk of disease. Previously reported TgFVB-HIVAN1^{CAST}congenic strain, carrying a 50 Mb CAST interval (encompassing the *HIVAN1* locus), introgressed into the TgFVB genome, showed accelerated development of collapsing glomerulopathy compared to TgFVB strain.

Methods: We generated two sub-congenic strains (SubII and SubIII) which encompass proximal (5.8-41.8 Mb) or distal (37.7-55Mb) regions of the previously reported FVB-HIVAN1^{CAST} locus (build 37.1). These sub-congenic strains were crossed with TgFVB strain and HIVAN pathology traits were compared in F1 and backcross (BC) generations to better localize the *HIVAN1 gene.*

Results: At 6-9 weeks of age, HIV transgenic homozygous SubIII mice display significant glomerulosclerosis (54.8 %± 10.4% vs. 15.6 ±5, p<0.01) and tubulointerstitial injury (58% ± 11 vs. 14.1%± 4, p <0.01) compared to SubII-FVB-HIVAN1^{CAST} mice. In contrast, SubII-FVB-HIVAN1^{CAST} mice were not different from Tg-FVB (glomerulosclerosis score 15.6 %± 5.2% vs. 16.5 ±5, p=0.9) and tubulointerstitial injury (14.1% ± 4 vs. 8.3%± 2, p =0.24). The severity of disease correlated with the number of SubIII CAST alleles: glomerulosclerosis score (54.8 %± 10.4% vs. 30.1 ±8.1) and tubulointerstitial injury score (58% ± 11.1 vs. 31.9%± 6.6) were significantly higher in BC mice compared to F1 mice. The identified *SubIII-HIVAN1* locus spans 17.3 Mb and encodes 59 genes, of which 4 have been reported to play a role in kidney development and 8 have non-synonymous coding SNPs that differentiate CAST from FVB.

Conclusions: These data further confirm that a gene on chr3A1-A3 increases susceptibility to HIVAN and reduce the *HIVAN1* locus to a maximal 17Mb interval with 11 high priority candidate genes.

TH-PO669

Impact of Initial Serum Uric Acid Levels on Mortality in Diabetic Hemodialysis Patients

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Background: Little is known about whether serum uric acid (UA) levels are independently associated with mortality in diabetic (DM) hemodialysis (HD) patients. The purpose of this study was to assess the predictive role of serum UA levels at initiation of dialysis therapy in DM HD patients.

Methods: A total of 319 incident HD patients (182 males; age of 60±14 years; estimated glomerular filtration rate of 7.5±3.8 ml/min; 193 DM) were included. Based on initial serum UA levels, and limit for normal UA in our laboratory (8.3 mg/dL for men, 6.6 mg/dL for women), patients were divided into hyperuricemia (HUA) group (n=165) and non-hyperuricemia (non-HUA) group (n=154).

Results: On Cox proportional hazards multivariate analysis including all patients, old age, presence of cardiovascular disease (CVD) and other comorbid disease (CMD), and low serum albumin were independent predictors of mortality but *not* high UA levels. In contrast, in DM patients, old age, presence of CVD and other CMD, and high UA were independent predictors of mortality. In all patients, patient survival was not different between HUA group and non-HUA group but in DM patients, 2-year patient survival was significantly lower in HUA group than in non-HUA group (74.5% and 88.9% respectively; log rank test, p=0.01). In the subgroups of DM patients, 2-year patient survival was worse among those with HUA and CVD (52.3%; n=30) than in those with non-HUA with CVD (81.1%; n=36), HUA without CVD (88.6%; n=62), and non-HUA without CVD (93.9%; n=65). In DM patients with HUA and CVD, the risk ratio for mortality was 5.98 times that of DM patients with non-HUA without CVD.

Conclusions: An initial high uric acid level is associated with increased mortality in those diabetic HD patients (but not in non-diabetic HD patients) and this risk is mainly attributed to higher risk of mortality in diabetic HD patients who had high UA and CVD at the start of HD. These data suggest that the predictive value of serum UA as a risk marker in end-stage renal disease patients is influenced by presence of diabetes and CVD.

Funding: Private Foundation Support

TH-PO670

Decreased Plasma Formic Acid in Patients with End Stage Renal Disease (ESRD) and Its Contributing Factors

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Background: Formic acid (FA) is an essential substance for de novo synthesis of purine nucleotides. FA is combined with folic acid and incorporated into purine synthesis pathway as formyl-tetrahydrofolate. FA is used as a constituent of C1 and C8 of purine bodies. Exogenous FA administration enhances nucleic acid production. In ESRD, it is known that nucleic acid metabolism is altered, e.g. enhanced elimination of nucleic acid in erythrocyte, and thus, it is supposed that there may be an altered FA consumption under this condition. Low concentration of endogenous FA exists in the healthy subjects, but only a limited number of reports examined about it, and FA levels in ESRD remain unknown.

Methods: Plasma FA levels and purine metabolite, uric acid (UA) were measured by High Performance Liquid Chromatography methods in (a) non-CKD (n=300), (b) CKD 5 (n=80), and (c) CKD 5 under dialysis (D) patients (n=30), and examined influencing factors for FA levels.

Results: Plasma levels (mean±SD) of FA and UA were as follows; FA (mg/L): (a) 3.71±1.17, (b) 1.43±1.07, (c) 1.96±1.10, UA (mg/dL): (a) 5.6±1.4, (b) 9.8±2.1, (c) 8.3±1.1. Significant differences were seen in respective levels by one-way analysis of variance (P<0.05), and furthermore, there was a significant difference in plasma FA levels between CKD5 and 5D (P=0.02). No relationships were found between FA and UA, and other parameters in respective groups. Regarding the influencing factors for FA levels, subjects with smoking habit exhibited lower FA levels in respective groups, and smoking was revealed to be an independent contributing factor in non-CKD by multivariate linear regression analysis.

Conclusions: In ESRD, there was a significant decrease in plasma FA level, and it was indicated that the dialysis therapy could partly restore it. Since plasma FA levels were low in smokers, and no relation was found between FA and purine metabolite in ESRD, the mechanism of the decreased FA needs to be addressed in relevance to cellular injury and FA consumption in uremic condition.

TH-PO671

Endothelial Glycocalyx Alterations in Hemodialysis Patients Tom Cornelis,[1] Jeroen Kooman,[1] Natascha Broers,[1] Karel M.L. Leunissen,[1] Frank van der Sande,[1] Hans Vink.[2] *[1]Internal Medicine, Division of Nephrology, Maastricht University Medical Center, Maastricht, Netherlands; [2]Cardiovascular Research Institute, Physiology, Maastricht University, Maastricht, Netherlands.*

Background: Chronic kidney disease patients are at increased risk for endothelial disease. Whether hemodialysis treatment itself exacerbates endothelial damage, is unknown. The endothelial glycocalyx mediates vascular protection. We speculated that hemodialysis might cause endothelial glycocalyx changes.

Methods: The glycocalyx forms the interface between flowing blood and the vascular wall. Red blood cells (RBCs) penetrate deeper into a damaged glycocalyx, suggesting that measurements of RBC column width might serve as a marker for glycocalyx damage. Glycocalyx damage can be tested by measuring the dimension of the RBC perfused boundary region (PBR), which fills up the space between median RBC column width and the outer edge of RBC permeable vascular lumen. PBR will increase when glycocalyx is damaged. We studied PBR in 21 prevalent hemodialysis patients with sublingual Sidestream Dark Field Imaging before the start of hemodialysis (baseline) and subsequently at 30, 60, 120, 180, 240 (end of treatment) and 270 minutes.

Results: A statistically significant increase of PBR in vessels with diameters between 5 – 30 microns was observed from 2.18 ± 0.06 microns to 2.35 ± 0.06 microns and 2.37 ± 0.07 microns at 30 and 120 minutes respectively. A similar trend persisted during the entire treatment. A secondary finding was a statistically significant greater baseline PBR and hence more damaged glycocalyx in the ultrafiltration patients (PBR = 2.27 ± 0.06 microns) as compared to the non-ultrafiltration group (PBR = 2.02 ± 0.09 microns).

Conclusions: Hemodialysis causes a significant increase in the width of the RBC accessible component of the sublingual microvascular glycocalyx, indicating loss of vascular protection and increased susceptibility to vascular complications. We also found a greater baseline PBR in the ultrafiltration group, suggesting that residual renal function may protect the endothelial glycocalyx. The pathophysiology and implications of these novel observations require further investigation.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

TH-PO672

New Target of ACT in Heparin Anticoagulation during Hemodialysis Ki-won Kwon,[1] Young Hye Song,[1] Young-Il Jo.[1,2] *[1]Dialysis Center, Konkuk University Hospital, Korea; [2]Nephrology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul, Republic of Korea.*

Background: Unfractionated heparin (UFH) is currently the most widely used anticoagulant during hemodialysis (HD). In order to prevent over-anticoagulation, UFH can be monitored using the activated coagulation time (ACT) aiming for ACT of 80% above baseline during HD and 40% above baseline at end of HD. However, even in the patients without significant bleeding risks, stressful bleeding complications may be caused by UFH anticoagulation despite ACT is maintained within the recommended ACT goal. This study was designed to evaluate the suitability of the recommended ACT goal and the proper level of ACT goal in HD patients.

Methods: A total 74 chronic HD patients without significant bleeding risks were enrolled. In phase I study, the doses of UFH were titrated to maintain the recommended ACT goal. In phase II study, the doses of UFH were titrated according to the time for needle sites to stop bleeding and visual assessment of clotting within the dialyzer for the patients who were dropped out from phase I study. ACT was monitored at 0, 120 min and 240 min after initiation of HD. All patients were divided into clotting (CG) and non-clotting group (NCG).

Results: All patients were dropped out from phase I study due to the increased time to stop bleeding from needling site after termination of HD. In phase II study, there were no significant differences in age, gender, cause of ESRD, Kt/V and use of anticoagulants between CG (n=13, 17.5%) and NCG (n=61, 82.4%). ACT at 120 min did not differ between two groups (CG vs. NCG; 123.9±6.2 vs. 136.3±3.5 sec, p>0.05) although ACT in NCG was slightly higher than in CG group. The doses of heparin in NCG group were significantly higher than in CG group (CG vs. NCG; 8.3±1.1 vs. 11.6±0.7 IU/kg of BW, p=0.038). ACT was significantly correlated with the dose of heparin (r=0.326, p<0.001).

Conclusions: These results suggest that the currently recommended ACT goal is too high even for the patients without significant bleeding risks and the new target of ACT for UFH anticoagulation during HD is needed. We are conducting the phase III study, a prospective randomized trial, to establish a new ACT goal.

TH-PO673

Use and Safety of Heparin-Free Maintenance Hemodialysis Jenny Shen, Aya Alice Mitani, Tara I. Chang, Wolfgang C. Winkelmayer. *Stanford University, Palo Alto, CA.*

Background: Although heparin is commonly used to anticoagulate the dialysis circuit for chronic hemodialysis (HD), some patients undergo heparin-free HD for reasons not well characterized. We describe the determinants of heparin-free HD and its association with clinically important outcomes using data from a national dialysis provider merged with Medicare claims.

Methods: We identified patients aged ≥67 years who initiated maintenance HD from 2007-08. Using logistic regression, we examined whether patient or provider factors were associated with the use of heparin-free HD on day 90 after dialysis initiation. We applied Cox regression to a propensity-score matched cohort to estimate the hazards of bleeding events (gastrointestinal (GI) hemorrhage, hemorrhagic stroke, other hemorrhage), thrombotic events (myocardial infarction, ischemic stroke, pulmonary embolism, deep vein thrombosis), and all-cause mortality.

Results: Among 12,917 patients, 885 (6.9%) were dialyzed heparin-free. In multivariable-adjusted analyses, a history of GI bleeding, hemorrhagic stroke, lower hemoglobin and platelet counts, and warfarin use were associated with higher odds of heparin-free HD; black race and central venous catheter use were associated with lower odds of heparin-free HD. The use of heparin-free HD also varied significantly by facility region (e.g., a 4-fold variation between the New England and West North Central census divisions). We found a modest association of heparin-free HD with all-cause mortality (hazard ratio (HR) 1.27; 95% confidence interval (CI): 1.07-1.52) compared to HD with heparin, but no significant association with bleeding events (HR 1.18; 95%CI: 0.79-1.78) or thrombotic events (HR 1.18, 95%CI: 0.98-1.44).

Conclusions: Patient markers of increased risk of bleeding along with facility region associated with use of heparin-free HD. Despite the potential benefits of avoiding heparin use, heparin-free HD was not associated with decreased hazards of bleeding or thrombotic events. The association between heparin-free HD and increased mortality may be residually confounded by indication, but suggests it is at least no safer than HD with heparin as currently practiced in the U.S.

Funding: NIDDK Support, Private Foundation Support

TH-PO674

Variations in the Circulating Heparin Levels during Maintenance Hemodialysis in End Stage Renal Disease Patients Kristiyana Kaneva,[1] Vinod K. Bansal,[2] Debra Hoppensteadt,[1] Joesphine Cunanan,[1] Jawed Fareed.[1] *[1]Pathology, Loyola University Medical Center; [2]Nephrology, Loyola University Medical Center.*

Background: Unfractionated heparin has remained the anticoagulant of choice for hemodialysis patients but wide variations in the heparinization responses have been observed. The purpose of this investigation was to measure circulating heparin levels in patients prior to and after hemodialysis.

Methods: This study included 119 End Stage Renal Disease (ESRD) patients undergoing maintenance hemodialysis. For the 3-4 hour hemodialysis duration, a heparin loading dose of 1000 Units followed by two additional dosages of 500 Units/hour were administered. Citrated blood samples were collected prior to and immediately after the dialysis session and analyzed utilizing such clot based methods as Activated Partial Thromboplastin Time (APTT), Heptest and Prothrombinase Induced Clotting Time (PiCT). Circulating Anti-Xa levels, Antithrombin (AT) levels and Thromboplastin induced thrombin generation were also measured. The circulating levels of heparin were determined using a calibration curve constructed from the heparin used in the dialysis unit.

Results: Heparin levels ranged from 0 to 1.08 U/ml with a mean of 0.07 ± 0.11 for the APTT and a range of 0 to 1.98 for the Heptest with a mean of 0.09 ± 0.26 U/ml. There was no significant difference in circulating levels of heparin between pre and post hemodialysis samples using APTT, Heptest and PiCT, whereas the Thrombin generation and Anti-Xa resulted in a statistically significant p value < 0.05 when comparing the two groups.

Conclusions: The presence of detectable levels of heparin in the pre dialysis plasma samples for almost two thirds of the patients (87 out of 119) suggests that residual heparin circulates in these patients for a longer period of time. A significant number of post dialysis samples, 6 out of 119 (5%), contained greater than 0.25 U/ml of heparin. These results also suggest that the use of heparin in maintenance hemodialysis patients in repeated regimen results in a steady state hypocoagulation as evidenced by the inhibition of thrombin generation, circulating Anti-Xa level and the prolongation of various clotting times.

TH-PO675

Effects of Citrate-Enriched Dialysate and Reduced Heparin Dose on Reuse of Dialyzers Jorge P. Strogoff-de-Matos,[1] Amanda Rocha,[1] Esther Oliveira,[2] Marcia Guimaraes,[2] Vanessa Padua,[1] Jocemir R. Lugon.[1] *[1]Universidade Federal Fluminense, Niteroi, Brazil; [2]CDR- Clínica de Doenças Renais, Ingá, Niteroi, Brazil.*

Background: We evaluated if the use of citrate-enriched dialysate would allow the reduction of heparinization without affecting the efficiency of reprocessed dialyzers.

Methods: A cross-over study in which 30 patients on maintenance HD were randomized to 2 different regimens of heparinization and dialysate acid buffer along 12 HD sessions, split by a 2-week washout period: Phase A- regular 4.0 mmol/l acetic acid containing dialysate and standard heparin dose (~100 UI/Kg), and Phase B- 2.4 mmol/l citric acid containing dialysate and reduced heparin dose (~70 UI/Kg). Dialysate calcium concentration (1.25 mmol/l) was similar in both phases, as well as the remaining solutes. All patients used high flux polysulfone membrane dialyzers which were reprocessed with peracetic acid/hydrogen peroxide mixture. Dialyzers were discharged before the 12th use if total cell volume (TCV) <80% of baseline.

Results: Twenty eight patients accomplished both phases. Nine dialyzers were discharged before 12th use in Phase A and 11 in Phase B. The total of successful HD sessions accomplished in phases A and B were 290 and 286, respectively. The utilization rate of dialyzers was similar in both phases (86.3% and 85.1%, respectively; P=0.74). Urea Kt/V did not change along both phases. As expected, systemic anticoagulation during and post-HD was more intensive in Phase A (P<0.001). Activated partial tromboplastin time at pre, 120min and post-HD in Phase A was 38±8", 95±56" and 68±36", respectively, whereas it was 36±4", 73±48" and 47±18" in Phase B. Ionized calcium in post-dialyzer blood line segment significantly increased during HD in Phase A (P=0.03), but did not change in Phase B. The incidence of cramps (8.4% and 1.0% of sessions) and hypotensive episodes (9.1% and 3.1% of sessions) were significantly more frequent during Phase B.

Conclusions: Use of citrate-enriched dislysate allowed a 30% reduction in heparin dose and caused less systemic anticoagulation, without compromising reuse performance. However, a significant increase in cramps and acute hypotensive episodes were seen.

TH-PO676

Intradialytic Kinetic Shifts of Protein-Bound Solutes Sunny Eloot, Raymond C. Vanholder. *Nephrology, Ghent University Hospital, Gent, Belgium.*

Background: Patients with renal failure retain a large variety of solutes, of which urea is commonly applied as marker for dialysis adequacy. It was previously found that urea kinetics are not representative for the removal of other solutes. But to the best of our knowledge, no in depth kinetic analysis is available for protein-bound solutes, in spite of the known association between their concentration and outcome parameters. We therefore studied the kinetics of protein-bound solutes with different degree of protein binding.

Methods: This study included 10 stable patients on high flux hemodialysis with 300/700mL/min blood and dialysate flow. Blood samples were collected from inlet blood line at the start, and after 15, 30, 60, 120, and 240min, and from outlet blood line at 30 and 120min. Total and free concentrations of hippuric acid (HA), indoxyl sulfate (IS), indole acetic acid (IAA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), p-cresylsulfate (PCS), and p-cresylglucuronide (PCG) were determined and used to calculate dialyzer clearance (K), reduction ratio (RR), and protein binding (PB). A two-pool kinetic model was fitted to the concentrations to calculate the plasmatic volume (V_1), total distribution volume (V_{tot}), and intercompartment clearance (K_{12}).

Results: K as well as RR correlate well with PB (R=0.94;P=0.006 and R=-0.91;P=0.013). V_1 is significantly different for PCG vs IAA, IS, and PCS (all P<0.01), and V_{tot} and K_{12} are different for PCG vs IAA and PCS (all P<0.05). CMPF (100%PB) was not found removed from the patient. V_1, V_{tot}, and K_{12} of IS and PCS, known to share the same binding site on albumin were correlated (R=0.89, R=0.84 and R=0.84; all P≤0.002).

Solute	V_1 (L)	V_{tot}(L)	K_{12} (mL/min)	K (mL/min)	PB (%)
HA	8.8 ± 4.0	23.4 ± 5.3	176 ± 87	132 ± 12	40 ± 14
IS	4.3 ± 1.9	17.5 ± 7.1	96 ± 78	27 ± 5	93 ± 3
IAA	5.2 ± 1.6	29.2 ± 14.8	127 ± 52	52 ± 8	70 ± 9
PCS	3.8 ± 0.9	11.5 ± 2.6	87 ± 44	21 ± 4	94 ± 3
PCG	8.2 ± 2.4	22.7 ± 12.4	103 ± 47	152 ± 28	13 ± 6

Conclusions: Protein-bound solute removal is complex and different per compound. Even related compounds show fairly divergent patterns (PCG vs PCS). Dialyzer clearance differs depending on %PB, but differences in intercompartment shifts further decrease removal possibilities as well.

TH-PO677

Kt/V Is a Poor Predictor of Uremic Toxin Concentration Sunny Eloot, Griet L.R.L. Glorieux, Raymond C. Vanholder. *Nephrology, Ghent University Hospital, Gent, Belgium.*

Background: Kt/V is used as marker of dialysis adequacy. The purpose of this study was to investigate whether Kt/V is representative for the concentration of a broad array of uremic toxins in patients on hemodialysis (HD).

Methods: Predialysis blood samples were taken during a midweek session in 75 stable chronic maintenance HD patients. Samples were analyzed by colorimetry, HPLC, or ELISA for urea, creatinine (Crea), uric acid (UA), symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA), beta-2-microglobulin (β_2M), and free and total hippuric acid (HA), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indoxyl sulfate (IS), indole acetic acid (IAA), p-cresylsulfate (PCS), and p-cresylglucuronide (PCG). Associations were evaluated between concentrations and different parameters: i.e. age, body weight (BW), Kt/V, Residual Renal Function (RRF), normalized Protein Catabolic Rate (nPCR), and vintage. Multifactor analyses were performed per solute.

Results: Kt/V only showed non consistent correlations with concentrations of Crea and UA (both R=-0.2), HA and free HA(both R=0.2), and ADMA (R=0.3), while all other correlations were non-significant. nPCR, on the contrary, showed significant associations for the majority of solutes with R~1 for urea, 0.4-0.6 for Crea, UA, SDMA, PCS, and free PCS, and 0.2-0.4 for ADMA, HA, IS, PCG, free HA, and free PCG. RRF showed inversed associations with β_2M (R=-0.5) and with Crea, ADMA, SDMA, HA, IS, PCG, free HA, free IS, free PCS, and free PCG (R=-0.2 to -0.4).In multifactorial analysis, nPCR and RRF but not Kt/V were significantly determining the concentrations of ADMA, SDMA, HA, IS, PCG, free HA, free PCS, and free PCG. Age, BW, and dialysis vintage did not contribute significantly to any correlation, except for Crea.

Conclusions: In HD patients, uremic toxin concentration seems more dependent on nPCR and RRF than on dialysis dose as assessed by Kt/V. Hence, Kt/V cannot be considered as a major representative for the evolution of solute concentration in HD patients, and by extension for the toxicity which conceivably is concentration-dependent. Nutritional intake and RRF seem to have a more substantial impact, and might thus be more interesting to modify.

TH-PO678

Direct Calculation of Kt/V as Total Blood Clearance of Urea over Total Body Water Volume Carlo Donadio. *Internal Medicine - Nephrology, University of Pisa, Pisa, Italy.*

Background: Most commonly Kt/V is estimated from the values of BUN (postdialysis/predialysis), ultrafiltration volume and postdialysis body weight. The aim of this study was to evaluate the possibility to calculate Kt/V as the total dialyzer urea clearance during the dialysis session, indexed for the volume of total body water of the patient, measured by electrical body impedance analysis (BIA).

Methods: Seventy eigth patients, 34-86 years, body weight 43-130 kg (mean 72.8), in maintenance hemodialysis treatment since 1-31 years (mean 6.5). All patients were on treatment with 3 dialysis, with bicarbonate dialysis (n=55), hemodiafiltration (n=16), or acetate free biofiltration (n=7). Dialysis monitors employed: Fresenius (n=37), Hospal (n=24), Gambro or others (n=17). BIA was measured using a single frequency tetrapolar impedance analyzer at the end of the dialysis session. TBW was calculated from the values of resistance and reactance, combined with body height and weight. Kt/VBIA was calculated as total blood clearance of urea during dialysis session over TBW. First, urea extraction ratio (UER) was calculated as (predialysis-postdialysis)/predialysis values of urea). The total volume of blood flow (QB, L) during dialysis was recorded. Then, Kt/V was calculated as: UER x QB / TBW; where UER x QB is the total dialyzer blood clearance of urea during the dialysis session. For comparison, Kt/V was calculated with Daugirdas formula (spKt/V).

Results: A very high correlation coefficient (r = 0.818) was found between the results of Kt/V measured with the two formulas. The slope was 1.13 x and the intercept non significantly different from 0. The mean difference sp Kt/V (Daugirdas) Kt/V (present study) was -0.03 (NS). The range of agreement between the two measurements was between -0.31 and + 0.37. The correlation between the two formulas was even better when clustering the patients in groups according to the dialysis monitor. Correlation coefficient r ranged 0.853-0.912, indicating that the accuracy of the measurement of blood flow is different according to the different monitors.

Conclusions: The adequacy of dialysis can be assessed from the direct calculation of Kt/V as total blood clearance of urea during dialysis session over TBW.

TH-PO679

Lung Ultrasound for the Evaluation of Extravascular Lung Water in Maintenance Hemodialysis Patients: Comparison with Total Body and Lung Electrical Impedance Carlo Donadio,[1] Laura Bozzoli,[1] Elisa Colombini,[1] Giovanna Pisanu,[1] Guido Ricchiuti,[1] Luna Gargani.[2] *[1]Internal Medicine, Nephrology, University of Pisa, Pisa, PI, Italy; [2]Insitute of Clinical Physiology, CNR, Pisa, PI, Italy.*

Background: In maintenance hemodialysis patients (MHD) dyspnea frequently occurs due to the increase in extra-vascular lung water (EVLW), due to heart failure or to the increase in extra-cellular water (ECW). In the presence of EVLW, the reflection of the ultrasound beam creates some reverberation artifacts, called B-lines. The direct evaluation of EVLW, by means of lung ultrasounds (LUS) together with the measurement of ECW, should help in assessing the "ideal" body weight and to evaluate the mechanism of dyspnea in individual MHD patients. Aim of this study is to compare LUS with total body and segmental lung electrical impedance analysis (BIA).

Methods: Eighteen adult MHD patients, aged 44-85 years, dialysis vintage 0.2-6.5 years, treated by standard or high-flux HD, have been examined immediately before and after a dialytic session. LUS was performed by means of a by-dimensional grey scale ultrasound apparatus equipped with a convex probe. Total body and segmental lung electrical impedance were analyzed by means of a single frequency (sf) and of a multiple frequency (mf) impedance analyzer.

Results: The mean reduction in body weight during the dialysis session was 2.8±1.3 kg. B-lines at LUS decreased significantly (from 36 to 16). In the mean time total body BIA demonstrated a significant reduction in ECW (from 20.2 to 17.6 kg sf-BIA, and from 18.8 to 16.8 mf-BIA), accompanied by a significant increase in resistance and reactance (sf) or impedance (mf). Lung BIA also demonstrated a highly significant increase in resistance and reactance (sf) or impedance (mf).

Conclusions: These preliminary data indicate that lung ultrasound can evaluate extra-vascular lung water in MHD patients and its results are correlated with total body and segmental lung electrical impedance analysis.

Funding: Government Support - Non-U.S.

TH-PO680

A Potential Route to the Identification of Uremic Toxins: Use of Mass Spectrometry to Identify Solutes Efficiently Removed by the Native Kidneys Tammy L. Sirich,[1] Pavel A. Aronov,[1] Natalie Plummer,[1] Thomas H. Hostetter,[2] Timothy W. Meyer.[1] *[1]Medicine, VA Palo Alto HCS and Stanford University, Palo Alto, CA; [2]Medicine, Case Western Reserve University, Cleveland, OH.*

Background: Identifying uremic toxins has been notoriously difficult. We hypothesize that uremic toxins might be included in the group of solutes normally maintained at low plasma concentrations by very high native kidney clearances. The current study employed untargeted high accuracy mass spectrometry (MS) to identify such solutes.

Methods: LC-MS was performed with a C18 column and orbitrap mass spectrometer on samples of plasma, plasma ultrafiltrate, and urine from 5 normal subjects. The clearance k_{free} was calculated as the rate of solute excretion divided by the concentration in the plasma ultrafiltrate, representing the free solute concentration.

Results: Out of a total of 1808 features identified in both urine and ultrafiltrate by LC-MS, 379 had values for k_{free} which exceeded the renal plasma flow (eRPF) estimated as five times the creatinine clearance. For 82 of these 379 features, solutes with corresponding mass were found in the Human Metabolome Database, including solutes known to accumulate in renal failure of which examples are listed below (mean±sd).

Solute	% free	k_{free}(ml/min)	k_{free}/eRPF
Indolelactate	4 ± 1	8350 ± 905	11 ± 1
Androsterone glucuronide	2 ± 1	5270 ± 1819	6.9 ± 2.6
Indoxyl sulfate	2 ± 1	3289 ± 490	4.3 ± 0.8
1-Methylinosine	14 ± 2	1810 ± 836	2.9 ± 0.6
Hippurate	27 ± 5	2254 ± 512	2.3 ± 0.9
p-Cresol sulfate	2 ± 1	1149 ± 129	1.5 ± 0.2

Efficiently cleared solutes with k_{free} greater than eRPF were bound to plasma proteins as reflected by the free percentages of 2 to 27%. For such solutes, the combination of protein-binding and tubular secretion serves to keep the free plasma concentration to which tissues are exposed very low. Our results show that the number of such solutes is large and that many of them remain to be chemically identified.

Conclusions: Presumably, the kidneys have evolved to provide efficient removal of toxic solutes. Further analysis of the list of solutes most efficiently removed by the native kidneys may therefore provide a route to the identification of uremic toxins.

Funding: NIDDK Support

TH-PO681

An Increased Permeability of the Dialyser Membrane Has a Positive Effect on the Removal Rate of the Protein-Bound Uremic Toxins Phenylacetic Acid and p-Cresylsulfate Falko Brettschneider,[1] Sonja Steppan,[2] Markus van der Giet,[1] Mirjam Peter,[2] Jutta Passlick-Deetjen,[2] Joachim Jankowski.[1] *[1]Med. Klinik IV, Charite, Berlin, Germany; [2]Fresenius Medical Care GmbH, Bad Homburg, Germany.*

Background: Innovative modifications are essential to improve the adequacy and permselectivity of dialyser membranes. This study focuses on the effect of opening up the support region of the dialyser membrane to increase permeability for middle molecules on the removal rate of protein-bound uremic toxin, since the hydrophobic, protein-bound uremic toxins contribute to the development of atherosclerotic vascular lesions, affecting endothelial cell, leukocyte, platelet and/or vascular smooth muscle cell function in CKD patients.

Methods: In a small prospective cross-over study, five CKD stage 5D outpatients were treated with a conventional high-flux hemodialyser (FX-800; Fresenius Medical Care) in comparison with a modified high-flux FX-800 hemodialyser (Helixone® plus membrane) with an increased permeability for middle molecules but the same sieving properties for albumin. The removal rate of phenylacetic acid and p-cresylsulfate were quantified at different time points by using gradient ion-pair reversed phase chromatography.

Results: The mean age of the patients was 63 ± 7, systolic RR 122 ± 15 mmHg and diastolic RR 68 ± 6. Removal rates of phenylacetic acid and p-cresylsulfate tend to increase using the modified high-flux FX-800 hemodialyser compared to the conventional high-flux FX-800hemodialyser (phenylacetic acid: 51.90 ± 13.5 vs. 72.4 ± 10.0; p-cresylsulfate: 29.2 ± 3.0 vs. 45.9 ± 8.9; in %).

Conclusions: An increased porosity of the support region of high-flux membranes is highly efficient for better removal of middle molecules and additionally, has a positive effect on the removal of protein-bound uremic toxins. Due to the high impact of protein-bound, hydrophobic uremic toxins on progression of CKD and CVD, the use of modified high-flux hemodialyser may be a therapeutic option to increase the removal rate of these uremic toxins. However, larger, long-term prospective clinical trials are needed to demonstrate the relevance for an improved outcome.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

TH-PO682

Effects of Protein-Leaking Dialyzer on Plasma Pentosidine Concentration, a Marker of Carbonyl Stress, in Hemodialysis Patients Hidenori Yamazaki, Fumihiro Tomoda, Tsutomu Koike, Takeshi Hayashi, Satoshi Kagitani, Taizo Nakagawa, Hiroshi Inoue. *The Second Department of Internal Medicine, University of Toyama, Toyama, Japan.*

Background: Over 90% of plasma pentosidine (PEN), a marker of carbonyl stress is bound to albumin. Because only the free fraction of PEN is available for diffusion through dialysis membrane, the removal of PEN is low in conventional hemodialysis (HD). However, the effects of protein-leaking dialyzer on albumin-bound solutes such as PENT remained to be elucidated in hemodialysis patients.

Methods: In the present study, the removability of PEN was compared between two types of triacetate dialyzer with different membrane pore sizes, FB-UH and FB-G (having pore radius of 76 and 60Å, respectively). Eight HD patients were treated with FB-UH and FB-G by crossover manner in the same condition for three months each. Plasma PEN and the leakage of albumin into dialysate effluent were measured at the end of each treatment. Removal rate of solutes was calculated from their pre-HD and post-HD blood levels.

Results: Although the removal rate of small molecular solutes such as creatinine and urea did not differ between the two dialyzers, β_2-microglobulin was removed more efficiently by FB-UH compared with FB-G. At pre-HD levels, plasma PEN was lower in FB-UH compared with FB-G. Plasma PEN decreased after HD in FB-UH, but did not change in FB-G (0.35±0.10 to 0.26±0.07 vs 0.70±0.09 to 0.70±0.14 μg/mL; HD × dialyzer interaction, $p< 0.05$). Consequently, the removal rates of PEN were greater in FB-UH than in FB-G (24.7±2.5 vs 4.9±7.0 %, $p<0.05$). The leakage of albumin into dialysate effluent was also greater in FB-UH compared with FB-G (2.41±0.41 vs 0.51±0.02 g/HD, $p<0.05$). Additionally, the removal rate of PEN was correlated positively with the leakage of albumin into dialysate effluent ($r=0.56$, $p<0.05$) in the combined measurements during the treatment with both dialyzers.

Conclusions: The protein-leaking dialyzer, FB-UH reduced carbonyl stress efficiently in HD patients. The reduction of carbonyl stress in FB-UH might be attributed to the removal of albumin-binding PEN via leakage into dialysate effluent.

TH-PO683

High Flux Hemodialysis with New Membranes Is as Efficient as Hemodiafiltration for Middle Weight Molecules Extraction Patrick Fievet. *Néphrologie Hémodialyse, Groupement Hospitalier du Sud de l'Oise, Creil, France.*

Background: Previous studies suggest that middle weight molecules (MWM) removal is a determinant factor of dialysis patient's prognosis. New technologies provide better control of pore size of dialyser membranes allowing reaching greater permeability. We have studied MWM removal of 5 high flux (HF) membranes in order to verify their ability to get the same performance for removal of MWM in HF-HD as hemodiafiltration (HDF).

Methods: Following HF membranes (ultrafiltration rate > 25 ml/h/mmHg) have been studied in the same conditions (membrane surface = 2.0 to 2.2m², duration = 4h, blood flow =340 to 360 ml/min and dialysate flow = 500 ml/min): PMMA, AN69, Polyethersulfone, PEPA and Polyphenylene.

Results: 1st : On 144 dialysis sessions with Kt/V > 1.2 in 77 patients, no difference has been found for low molecular weight reduction rate (RR). Beta 2 microglobulin (β_2M) RR were significantly higher with Polyphenylene, PEPA and Polyethersulfone than with AN69 and PMMA (respectively: 73.2±4.2, 70.4±6.2, 70.0±4.7, 51.0±5.5, 46.8±12.0%) and myoglobin (MY) RR were significantly higher with Polyphenylene than with PEPA, Polyethersulfone, AN69 and PMMA (65.8±5.3, 50.8±12.4, 47.4±7.6, 47.8±4.4, 37.8±7.5%).

2nd: A non linear relationship (mathematical model: $y = k.(1-e^{-x})$) have been found between β_2M and KT/V with Polyphenylene, Polyethersulfone and PEPA membranes ($p<0.01$) and between MY RR and KT/V with Polyphenylene ($p<0.02$), Polyethersulfone ($p<0.05$) and PMMA ($p<0.05$).

3rd: 12 volunteers patients with predialytic β_2M > 27,5 mg/l treated with the less efficient membranes (AN69 and PMMA) have been switched on the most efficient membrane (Polyphenylene). β_2M significantly decreases (35.0±5.1 vs 30.3±4.5%, $p<0.01$) after 2 months.

Conclusions: 5 currently used HF membranes are significantly different as regard MWM RR whereas low molecular weight RR are identical. With polysulfone derived membranes β_2M RR is dependant of delivered dose of dialysis and can reach 80% at maximal level. These results are in the range of those published with HF-HDF. Polyphenylene membrane is the most effective and can reduce predialytic β_2M plasma level in patients previously treated with less effective HF membranes.

Funding: Other NIH Support - Groupement Hospitalier du Sud de l'Oise

TH-PO684

Evaluation of Oxidative Stress and Inflammatory Markers on Hemodialysis Patients with and without Dialyzers Reuse Carla Barbosa Muraro Furlan,[1] Rosilene M. Elias,[1] Denise Frediani Barbeiro,[1] Manuel C. Castro,[1] Hugo Abensur,[1] Francisco Garcia Soriano.[1] *[1]Nephrology, University of São Paulo, São Paulo, SP, Brazil.*

Background: Dialyzers reuse (DR) is common practice in the United States and South America, but has been decreasing in several countries in Europe. The dialyzer reuse-associated mortality is still controversial, and the main reason for this continuing practice is economical. The effect of DR on oxidative stress and inflammation was not well investigated. Here, we evaluated whether there is any effect of DR on oxidative stress and inflammation markers in patients on hemodialysis (HD). Also, N-acetylcysteine (NAC) was tested for possible protective effect on patients submitted to DR.

Methods: We studied 29 patients on high-flux polysulfone membrane HD. Membraneswere reprocessed with hydrogen peroxide. We collected blood samples at baseline (patients on reuse) and at the end of each period (single use; reuse again and reuse plus NAC) for determination of serum thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), glutathione (GSH), albumin, interleukin 6 (IL6), ultrasensitive CRP (uCRP).

Results: TBARS was higher on single use period. All other variables showed no significant difference.

Variables (Mean ± SD)	Reuse	Single Use	Reuse	Reuse+ NAC	P value
TBARS, nmol / ml	7,27±5,47	11,24±4,95	9,90±6,45	7,12±4,99	0,002
uCRP, mg/L/L	3,69±3,43	6,60±8,15	5,26±6,95	5,01±6,38	0,136
GSH, μM/L	0,43±0,78	0,64±1,69	0,83±2,11	0,92±2,35	0,243
SOD ,U/mL	1,71±0,07	1,72±0,09	1,73±0,09	1,69±0,10	0,227
IL 6, pg/dL	6,50± 4,95	9,37± 10,94	6,87±5,99	8,93±8,57	0,053
Albumin, g/dL	4,24±0,29	4,21±0,31	4,25±0,33	4,14±0,20	0,069
KT/V standard	1,47±0,31	1,39± 0,21	1,37±0,21	1,38±0,26	0,108
Serum iron, mg / dL	65,03±22,61	63,48±23,79	62,72±29,72	60,21±19,01	0,775
Ferritin, ng / mL	645,5±358,6	571,5±483,7	559,3±440,0	598,8±468,9	0,271

Conclusions: These findings indicate that DR may provide an improvement on oxidative stress. The single use was even associated with higher oxidative stress. We found no additional benefit of NAC.

Funding: Government Support - Non-U.S.

TH-PO685

In Vitro Results for a Novel Device Designed for Selective Removal of Blood Phosphates in Hemodialysis Melanie S. Joy,[1] Quan Shi,[2] Michael Jolly,[2] Marian Mccord.[2,3] *[1]School of Medicine and Pharmacy, University of North Carolina, Chapel Hill, Chapel Hill, NC; [2]TECS, North Carolina State University, Raleigh, NC; [3]Joint Department of BME, UNC and NCSU, Raleigh, NC.*

Background: Hyperphosphatemia is prevalent in ~90% of hemodialysis patients and is linked to significant morbidity and mortality. Despite dietary modifications and oral phosphate binder therapy, 50% of patients fail to normalize blood phosphate levels to 3.5 to 5.5 mg/dL. The current abstract describes *in vitro* study results from a new technology being formulated as a medical device (the PHOSFILTER) to reduce blood phosphate levels during the hemodialysis procedure.

Methods: The efficiency of selective phosphate removal by the adsorptive material was tested in phosphate buffer solutions (concs 1- 10 mg/dL, pH=7.8), porcine plasma and bovine blood, respectively. Phosphate adsorption of the adsorbent material was tested up to 4 hours at 37°C. Phosphate concentrations were measured by a colorimetric assay kit for buffer solution and plasma or by the hospital lab for bovine blood with assessment of complete blood counts and chemistries to evaluate safety. The adsorptive material was then placed into the PHOSFILTER and evaluated in a simulated hemodialysis circuit to study phosphate adsorption over time.

Results: The PHOSFILTER adsorption material showed an adsorption capacity of 28 to 32 mg/g in buffer solution. Significant phosphate reduction (50-60%) was also observed in plasma and blood. Serum calcium was decreased by ~30%. No changes were noted in serum albumin, total protein, chloride, potassium, sodium or magnesium. No changes in red blood cell, white blood cell, or platelet counts were documented. In the studies performed in a simulated hemodialysis circuit, the phosphate level of bovine blood was reduced by 42% after 240 minutes.

Conclusions: The *in vitro* studies with the PHOSFILTER adsorbent material demonstrated high capacity, selectivity, and safety for removing phosphates from blood and plasma. Studies are ongoing to assess blood phosphate kinetics during combined use of a dialyzer and PHOSFILTER. Future studies are planned to assess the efficacy, utility, and safety in vivo.

Funding: Private Foundation Support

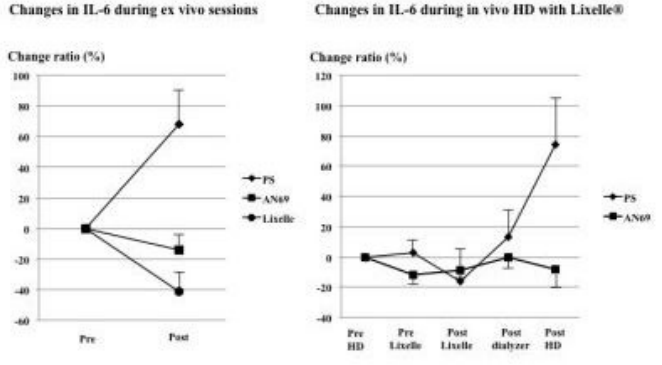
TH-PO686

The Synergy Effect on Inflammatory Cytokines of Adsorbent Column for β2-Microglobulin and AN69 Membrane Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Division of Nephrology and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

Background: Lixelle, an adsorption column, has been developed for the elimination of β2-microglobulin(MG) has been reported to remove other substance such as IL-6. AN69 also removed various cytokines by its negative charge at the surface of membrane. We estimated the synergy effect on inflammatory cytokines during hemodialysis(HD) with Lixelle using polysulfone(PS) or AN69.

Methods: EX vivo study: Mini-modules were made using PS, AN69, and Lixelle. 50 mL of whole blood was circulated for 2h in microcircuit through mini modules without dialysate compartments. Blood samples were drawn at 0 and 120min, and measured IL-6, and TNF-α. All experiments were repeated thrice. In vivo study: 5 HD patients were treated using PS with Lixelle, while 5 HD patients were treated using AN69 with Lixelle. IL-6 and TNF-α levels were measured at start and 15 minute, and the end of HD session. Blood samples were also collected in the circuit between Lixelle and PS or AN69(I) and after PS or AN69(A).

Results: There were no significant differences in TNF-α levels during EX vivo sessions with PS and Lixelle. IL-6 levels significantly decreased after EX vivo session with AN69 and lixelle, while, IL-6 increased with PS. TNF-α levels significantly decreased after HD with Lixelle using either PS or AN69 in vivo study. IL-6 levels significantly decreased at collection (I), however, using PS, it significantly increased at collection (A) and at the end of HD session. On the other hand, there was no significant increase in IL-6 levels during HD with Lixelle using AN69.



Conclusions: IL-6 was adsorbed to both Lixelle and AN69. PS significantly increased IL-6 levels while, the removal rate of β2MG is significantly lower in AN69 than PS. Therefore combination therapy of Lixelle and AN69 could effectively remove the β2MG and inflammatory cytokines.

TH-PO687

Results of Initial U.S. Mid-Dilution On-Line Hemodiafiltration Clinical Trial Leonard Stern,[1] Gregory Collins,[2] James Summerton.[2] *[1]Medicine/ Nephrology, Columbia University Medical Center, New York, NY; [2]Research and Development, Nephros, Inc, River Edge, NJ.*

Background: Hemodialysis (HD) is primarily a diffusive process with limited convective capability. Mid-dilution HDF is a unique configuration that optimizes convective and diffusive clearance in a single mid-dilution HDF filter (Nephros OLpur MD 220). The Nephros H_2H Module, when coupled with a conventional UF-controlled HD machine with ultrapure dialysate, and the Nephros MD 220 HDF filter are all the necessary components needed to perform on-line mid-dilution HDF therapy in the USA. Substitution fluid is produced on-line when the H_2H Module draws off a portion of fresh dialysate and passes it through its unique on board dual-stage ultrafilter producing a dialysate for infusion meeting AAMI RD52:2004 requirements.

Methods: A multicenter, prospective, non-randomized clinical trial was conducted to assess the safety and efficacy of on-line mid-dilution HDF using the Nephros OLpur MD 220 HDF Filter and H_2H Module compared to high flux HD. Adverse events, adverse symptoms and blood chemistries, including β_2-microglobulin (β_2m) and dialysis adequacy markers, were monitored. Dialysate and infusion fluid were evaluated for compliance with AAMI quality standards for bacteria and endotoxin. For 4 weeks, each patient received their normal 3X weekly high flux HD (control period). For the next 12 weeks, each patient underwent mid-dilution HDF with the H_2H Module and MD 220 HDF Filter (test period). The dialysis prescriptions (treatment times, blood and dialysate flow rates) were unchanged between the two periods. 28 and 23 patients completed the control and test period, respectively.

Results: There was no difference in adverse symptom or adverse event rates between HDF vs. HD (p=NS). For HDF vs HD, the mean Alk P decreased from 129.7 units/l to 121.5 units/l (p=0.019), the mean β_2m decreased from 37.3 mg/l to 30.3 mg/l (p=0.0016), and the β_2m reduction ratio increased from 57.1% to 79.7%. (p<0.001).

Conclusions: There are significant biochemical benefits associated with mid-dilution HDF compared to high flux HD. Longer term studies to fully assess the potential clinical benefits of mid-dilution HDF are needed.

Funding: Pharmaceutical Company Support - Nephros, Inc

TH-PO688

Role of Residual Renal Function in the Clinical Management in Hemodialysis Patients Sonoo Mizuiri,[1] Yoshiko Nishizawa,[1] Kazuomi Yamashita,[1] Touru Nakazono,[1] Kohji Usui,[2] Kenichiro Shigemoto.[1] *[1]Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan; [2]Nephrology, Ichiyokai Ichiyokai Clinic, Hiroshima, Japan.*

Background: The relationship between residual renal function (RRF) and improved phosphate and anemia control in HD patients has been reported.

Methods: We evaluated clinical data associated with RRF in 354 incident HD patients. Inclusion criteria were thrice weekly, 4 h HD using a high-flux dialyzer. GFR was calculated as (Ccr and Curea)/2 with 24 h urinary samples in patients with urinary output ≥100 ml/day (n=75), and urinary output <100 ml/day (anuria) for 279 patients. Univariate comparisons and multivariate comparisons with SPSS II were performed.

Results: There were significant differences in median values for duration of dialysis (23.0 vs. 95.1 months, P<0.001), prevalence of DM (45.3 vs. 32.6 %, P<0.05), predialysis diastolic blood pressure (77 vs. 81 mmHg, P<0.05), cystatin C (6.09 vs. 7.49 mg/L, P<0.001), ß2-microglobulin (ß2MG) (22.0 vs. 28.8 mg/L, P<0.001), calcium (Ca) (8.8 vs. 9.4 mg/dl, P<0.001), and nPCR (0.78 vs. 0.86 g/kg/day, P<0.01) in patients with RRF vs. anuric patients. However, there were no significant differences in age, water removal, CTR, the use of ACEI or ARB, PTH, Hb, phosphate, serum albumin, erythropoiesis stimulating index, high-sensitivity CRP, and Kt/Vurea in the groups. On univariate comparisons, DM, duration of dialysis, predialysis systolic blood pressure, water removal, cystacin C, β2MG and Ca were associated with urinary output. A multivariate comparison found a significant effect for duration of dialysis, ß2MG and cystacin C on urinary output. In univariate analysis, duration of dialysis, water removal, cystacin C, ß2MG, Ca, phosphate, serum albumin, K/ Vurea and nPCR were associated with (Ccr+Curea)/2. A multivariate comparison found a significant effect only with serum albumin on (Ccr+Curea)/2 and a correlation between (Ccr+Curea)/2 and serum albumin was observed (r=-0.49, P<0.001).

Conclusions: A relationship between urinary output and ß2MG and cystacin C was observed in this study. However, no relationship was observed between RRF and phosphate control or anemia, and maintenance of RRF may worsen hypoalbuminemia in proteinuric HD patients.

Funding: Private Foundation Support

TH-PO689

Cystatin C and β2-Microglobulin as Plasma Markers of Residual Renal Function in Hemodialysis Enric Vilar,[1,2] Capella Boltiador,[1] Ashwini Machado,[1] Adie Viljoen,[1] Ken Farrington.[1,2] *[1]Renal Unit, Lister Hospital, Stevenage, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: Residual Renal Function (RRF) is an important predictor of outcomes in peritoneal and hemodialysis(HD). Measurement of RRF in HD is useful for ensuring that minimum clearance targets are achieved but urine collection is inconvenient and expensive. We studied the potential use β2-microglobulin and cystatin C as plasma markers of RRF.

Methods: Patients on high-flux HD or hemodiafiltration were recruited. Over two consecutive HD sessions (HD1, HD2), blood was drawn pre and post-HD1 and pre-HD2 and analysed for urea, creatinine, cystatin C and β2-microglobulin. Inter-dialytic urine collection allowed GFR estimation (mean of urea and creatinine clearance). Relationship between β2-microglobulin and cystatin C plasma levels and RRF were examined. Receiver Operating Characteristic(ROC) curves were used to determine whether pre-dialysis middle-molecule levels may identify those with significant RRF.

Results: 341 subjects were recruited(111 high-flux HD and 230 on-line HDF). Mean Kt/V was 1.33±SD0.22 and dialysis time 190mins±SD32. Urea and creatinine clearance correlated closely(r^2=0.78). The parameter best correlating with GFR, urea and creatinine clearance was 1/pre-HD1 β2-microglobulin(r^2 0.67, 0.57 and 0.68 respectively). 1/pre-HD1 cystatin C level correlated less well(r^2=0.50, 0.44, 0.48 for GFR, urea and creatinine clearance respectively). The inter-dialytic cystatin C and β2-microglobulin rise correlated less well with GFR. Regression equations for GFR based on pre-HD1 cystatin C and β2-microglobulin levels were derived.Using ROC analysis we determined that pre-HD β2-microglobulin <19.2mg/L identified those with >2ml/min/1.73m^2BSA urea clearance with a 10% false positive rate but poor sensitivity(65%).

Conclusions: Pre-HD β2-microglobulin and cystatin C correlate closely with RRF but urea and creatinine are poor indicators. Regression equations for RRF based on pre-HD β2-microglobulin and cystatin C have been derived. Cutoff β2-microglobulin levels identify those with significant RRF. Use of plasma RRF markers may be useful as clinical or research tools and could be performed routinely in HD.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO690

Effect of Lower Dialysate Bicarbonate Concentration on Pre-Dialysis Alkalosis in Patients on Maintenance Hemodialysis Anip Bansal, Pablo A. Casares, Clinton Colaco, Marcelo R. Barrios Gini, Ira S. Meisels. *Medicine, St Luke's Roosevelt Hospital Center, New York, NY.*

Background: The possible deleterious effects of acidemia in patients with End Stage Renal Disease(ESRD) have led to the emphasis on the avoidance of metabolic acidosis by the use of a high alkali concentration in dialysates. Recent studies have highlighted the association of high Pre-Dialysis Bicarbonate(PDB) i.e. >27 mmol/L with higher mortality and risk for cardiac arrest. The objective of this study was to assess the effect of the use of lower dialysate bicarbonate(DB) concentration on PDB levels.

Methods: This was a retrospective study from a single, urban dialysis center where the DB was decreased from 35 mmol/L to 33 mmol/L in January 2012. The medical charts of all patients on HD at any time between September 2011 & May 2012 were screened and all the PDB levels extracted. After exclusion of patients who did not have monthly test results, the means of the PDB levels before and after the intervention were compared using the paired t-test.

Results: A total of 170 HD patients' records were reviewed. The distribution of various PDB levels is summarized in the table below.

Frequency distribution of patients based on incremental PDB levels

PDB (mmol/L)	Mean % Patients while on Higher DB	Mean % Patients while on Lower DB	p value
0-18	4.20	6.21	0.23
18-22	25.42	37.62	0.19
0-22 (i.e. Acidosis)	29.62	43.83	0.19
22-26	55.92	47.50	0.19
26-30	13.13	8.26	0.25
>30	1.33	0.41	0.05
>26	14.46	8.67	0.23

The mean percentage of patients with acidosis (PDB<22 mmol/L) before and after was 29.03% (SD= 9.43) and 43.83% (SD =10.26) ; and of those with alkalosis(PDB>26 mmol/L) was 12.4% (SD= 5.6) and 8.67% (SD= 3.68) respectively. These differences were statistically not significant. There was a significant difference in the mean PDB before (Mean= 23.52 mmol/L, SD=2.01) and after the intervention (Mean=22.9 mmol/L, SD=2.09).

Conclusions: A reduction in the DB concentration lead to an expected decline in the PDB levels. There was a statistically insignificant trend towards increased incidence of pre-dialysis acidosis and decreased incidence of pre-dialysis alkalosis.The optimal DB concentration and PDB levels are unknown.

TH-PO691

In Acidotic Hemodialysis (HD) Patients, Mid-HD Alkalosis Occurs Even on 33.5-34 mEq/L and More on 40 mEq/L Dialysate Bicarbonate (BIC) Concentration, due to Large Initial 2 Hours Increase in Serum BIC Level (SBIC) Inversely Correlated with Pre-HD SBIC David Tovbin,[1] Seungjin Kim,[3] Lone Solling Avnon,[2] Moshe Zlotnik.[1] *[1]Nephrology, Soroka Medical Center; [2]Pulmonary, Soroka Medical Center; [3]Medical School for International Health, Ben-Gurion University; [4]Biomedical Engineering, Ben-Gurion University, Beer-Sheva, Israel.*

Background: Acidosis correction in hemodialysis (HD) is beneficial for outcome. However, NKF-K/DOQI guideline of pre-HD serum bicarbonate (BIC) level (SBIC) ≥22 mEq/L is frequently not achieved, since BIC-transfer increases end-HD SBIC but SBIC falls afterwards frequently to acidotic range. We hypothesized that in patients with pre-HD SBIC <22 mEq/L ("acidotic") on currently used dialysate BIC concentration (DBIC) of 33.5-34 mEq/L (CDBIC), high DBIC (HDBIC) of 40 mEq/L corrects inter-dialytic acidosis without inducing intra-dialytic alkalosis. Thus, intra-dialytic serum BIC changes and their relation with pre-HD SBIC on HDBIC vs CDBIC were assessed.

Methods: In a prospective bi-center study, 15 patients were assessed for 3-week periods on CDBIC and afterwards HDBIC. Blood gases & electrolytes were assessed weekly at start, after 2 hours (mid-HD) and end of HD. Data is presented as mean±SD. Statistical evaluation used non-parametric tests.

Results: On CDBIC, pre-HD & mid-HD SBIC were 21.5±2.7 and 27±1.9 mEq/L, respectively. Initial 2 hours SBIC increase (Δ2h) was 5.5±1.9 mEq/L and inversely correlated with pre-HD SBIC (r=-0.71,p<0.005). Mid-end SBIC increase (lateΔ) was 1.3±2 mEq/L. On HDBIC, pre-HD & mid-HD SBIC were 24.7±2.3 and 33.2±2 mEq/L, respectively. Δ2h was 8.5±1.7 mEq/L and inversely correlated with pre-HD SBIC (r=-0.85,p<0.05). LateΔ was 1.75±2 mEq/L. In the 8 acidotic patients, on CDBIC pre-HD, mid-HD & end-HD SBIC were 19.3±1.5, 26±2 and 28±2.9 mEq/L and on HDBIC 24±2.6, 33.2±2.4 and 34.9±3.2 mEq/L, respectively.

Conclusions: HDBIC corrects inter-dialytic acidosis. However, acidotic patients develop mid-HD alkalosis even on CDBIC and augmented intra-dialytic alkalosis on HDBIC, in line with inverse correlation of Δ2h with pre-HD SBIC. DBIC individualization, gradual increase between sessions & intra-dialytic changes may be suggested.

TH-PO692

Comparison of Techniques of Fluid Assessment in Hemodialysis Patients Flavio Basso, Sabrina Milan Manani, Dinna N. Cruz, Alessandra Brendolan, Monica Zanella, Catarina Teixeira, Claudio Ronco. *Department of Nephrology, St Bortolo Hosp, Vicenza, Italy.*

Background: Because volume status assessment is critical in hemodialysis (HD) patients, several methods have been developed to ascertain this parameter. Bioimpedence by Body Composition Monitor (BCM) is a validated method, B-type natriuretic peptide (BNP) levels increase with fluid overload (FO), ultrasound (US) Lung Comets Score (LCS) have a linear correlation with the extravascular lung water and inferior vena cava diameter (IVCD) US reflects volume status. We aim at comparing these four methods in the optimisation of UF prescription and dry weight estimation in HD.

Methods: We evaluated weight, BCM (L), LCS, ICVD (mm) during inspiration (IVCDmin) and expiration (IVCDmax), Collapsibility Index (IVCCI) and BNP (pg/mL) before and after HD in 31 patients. US measurements were performed by two trained operators with the same device.

Residual overload (RO) was calculated by the difference between BCM before HD and weight loss by HD. Overhydration (OH) was considered if RO after HD was >0.5.

Results: There was a significant reduction in mean BCM (1.7±1.8 to 0.0±1.8 p<0.001), in mean LCS (20±11.5 to 12.8±7.1 p<0.001), in mean IVCDmin (9.6±3.8 to 7.0±3.3 p<0.001), in mean IVCDmax (11.8±4.9 to 9.3±4.1) and in mean BNP(621.0±847.0 to 436.4±644.2) after HD. Less significant reduction was observed in IVCCI (p=0.13).

There was a significant correlation between BCM and LCS, BNP, IVCDmin and IVCDmax (p<0.05) before and after HD.

Between OH (n=5) and non-OH (n=26) groups, there was a significant difference in LCS (p<0.001), IVCDmin (p=0.019) and IVCCI (p=0.001). No differences were found for BNP and IVCDmax.

Conclusions: All parameters showed a significant decreasing trend along HD session. Assuming BCM as a standard, all others methods were statistically correlated before and after HD, allowing to assess possible residual FO. Both LCS and IVCD are reliable and useful techniques that should be considered for monitoring volume status and prescribing UF in HD patients.

TH-PO693

Radioistope Blood Volume (BV) Measurement in Conjuction with Crit-Line (CLM-III) in Hemodialysis (HD) Patients Jun-Ki Park, Aditya Mattoo, Frank Modersitzki, David S. Goldfarb. *Nephrology, NYVAMC & NYUSoM, New York, NY.*

Background: Accurate assessment of the BV and distribution of body fluids is essential for prescribing HD and for reducing complications related to hypovolemia and volume overload. Monitoring relative changes in BV using hematocrit (Hct), e.g. CLM-III, an indirect method, cannot be used to determine the absolute levels of BV. Here we report the first study of isotope BV measurement (IBVM) for assessing volume status in HD patients using indicator dilutional method.

Methods: 10 adult HD patients were enrolled in this prospective observational study. Multi-point IBVM before and after HD was performed using BVA-100 (Daxor, New York, NY). BVA-100 calculates BV with an accuracy of ±2.5%, by using <25μCi of iodinated I-131 albumin. It assumes normal BV for a given individual on the basis of patients' deviation from ideal body weight. Fluid loss from the extravascular component of the extracellular space (EV) was calculated by subtracting absolute BV change from total weight loss. Intradialytic relative BV changes were measured by CLM-III during the same HD session. Bland-Altman plot was used to compare relative BV change pre- and post-HD by IBVM and CLM-III.

Results: 8 of 10 cases had significant hypervolemia, 2 cases were normovolemic. The range of BV variation from predicted normal was 156ml to 1990ml. Higher pre-HD BV was associated with lower pre-HD albumin and higher pre-HD BNP. Significant interindividual differences in EV fluid loss ranged from 54% to 99% of total weight loss. In 8 of 10 patients Bland-Altman plot showed excellent agreement in the measurement of relative BV by IBVM and CLM-III with a mean difference of -0.8% ± 3.2% (mean ± SD) and the 95% CI -7.21% to 5.51% (p=NS).

Conclusions: IBVM is useful to determine absolute BV and changes in BV and correlates with CLM-III measurements. Fluid removal from EV space can be calculated based on absolute BV changes to describe individual refilling ability. This may prove useful in prescribing and monitoring ultrafiltration (UF) rates and establishment of optimal BV in HD patients. More accurate prescription of UF has the potential to reduce the morbidity and mortality associated with chronic HD.

Funding: Veterans Administration Support, Pharmaceutical Company Support - Daxor

TH-PO694

Accuracy of Whole and Segmental Bioimpedance Measurements to Assess Extracellular Volume in Hemodialysis Patients Samer Rateb Abbas,[1,2] Fansan Zhu,[1] Peter Kotanko,[1] Nathan W. Levin.[1] *[1]Nephrology, Renal Research Institute, New York, NY; [2]Nephrology, Beth Israel Medical Center, New York, NY.*

Background: If estimation of extracellular fluid volume (ECV) is accurate, the divergence between ultrafiltration volume (UFV) and change in the ECV (ΔECV) during HD should approximate zero. The aim of this study was to compare the whole body (wBIS) and segmental bioimpedance spectroscopy (sBIS) techniques in HD patients in terms of this divergence.

Methods: wBIS and sBIS (arm, trunk and leg) were performed simultaneously pre and post-HD on chronic HD patients using a a Hydra 4200 (Xitron technologies). ECV_{wBIS} (De Lorenzo, JAP 1997) and sum of ECV_{sBIS} (Zhu F, JAP 2006) were calculated pre- and post HD. ΔECV_{wBIS} and ΔECV_{sBIS} were obtained. Bland-Altman plots were used to compare the divergences obtained by wBIS and sBIS.

Results: 45 patients (53% males, age 62.6±14.5 year) were studied twice. Intradialytic weight loss was significantly decreased (ΔWt, 2.62±0.96 kg, p<0.01) and UFV was 2.7±0.92 L. Significant difference in the same patients at the same time between ΔECV_{wBIS} and ΔECV_{sBIS} (2.29±0.75 vs 2.83±0.89 L, p<0.001) was observed. Correlations (R^2) of UFV to ΔECV_{wBIS} and to ΔECV_{sBIS} were 0.72 and 0.73 respectively. Bland-Altman plots show the bias (divergences) in sBIS significantly (-0.14±0.48 vs 0.41±0.49 L, p<0.001) lower than in wBIS.

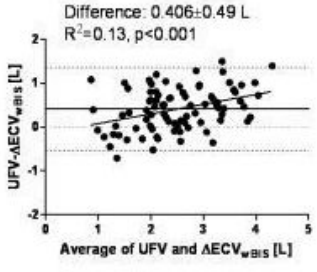


Fig. 1

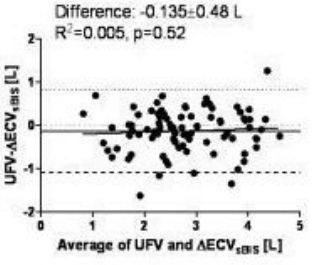


Fig. 2

In addition, the slope of the regression line was significant (p<0.001) with wBIS.

Conclusions: Study shows change in ECV by wBIS was significantly underestimated with proportional bias. This did not occur with use of sBIS. This error could be due to the incorrect assumption that the whole body can be modeled as a cylinder with uniform conductivity because in the trunk, resistance does not reflect its fluid volume. sBIS calculates ECV separately in each segment so that total ΔECV by sum of segments is close to UFV.

TH-PO695

The Role of Acetate-Free Biofiltration (AFB) on Hypotension and Nurse Workload Piergianni Calzavara,[1] Denis Steckiph,[2] Biagio Polverino,[1] Niloufar Tadayyon,[1] Giulia Adriana Nicolai,[1] Alessandro Ciavatti.[1] *[1]Conegliano's Hospital, Italy; [2]Gambro Hospal, Italy.*

Background: Intradialytic hypotension (IDH) is still the most common acute complication in hemodialysis (HD) patients and occurs in approximately 20% of patients on dialysis. The AFB is a dialysis treatment that can reduce the occurrence of IDH, eliminating the undesirable effects of acetate, customizing the control of acid-base and reducing the inflammatory status in ESRD patients. The purpose of this study was to evaluate if AFB therapy is able to reduce the load and the complexity of the work of the nursing staff associated to IDH.

Methods: Seven hypotension-prone patients (IDH monthly frequency> 40%), were selected for the study. In each patient 42 sessions in HD standard (control) and 42 AFB (intervention) were performed. The monitored variables were: number of sessions complicated by hypotension, number and type of nursing interventions, weight loss, blood pressure and heart rate pre and post dialysis. All treatments were performed with Integra and ARTIS dialysis machines (Gambro Dasco, Italy).

Results: The two treatments were found similar on the main dialytic parameters (UF: HD 2.3±0.8, AFB 2.1±0.6 L, p=0,386; Td: HD 218±221, AFB 21±22 min, p=0,189; Qb: HD 282±25, AFB 279±26 ml/min, p=0,583), except for the convective volume (HD 2.3±0.8, AFB 9.6±1.2 L, p<0.0001). The number of dialysis sessions with IDH was reduced by about 70% in treatment AFB (from 191 to 62, p<0.0001). In AFB the nursing workload was coherently reduced for all types of intervention: saline infusion (from 129 to 46, p<0.0001), infusion of NaCl 20mg (from 77 to 23, p<0.0001) and Stop UF (from 103 to 23, p<0.0001).

Conclusions: Our results confirm the usefulness of therapy AFB in the reduction of IDH frequencies in haemodynamically unstable patients, leading to a reduction of the load and the complexity of nursing work.

TH-PO696

Prediction of Intradialytic Morbid Events in Chronic Hemodialysis Patients Fansan Zhu, Peter Kotanko, Stephan Thijssen, Nathan W. Levin. *Research, Renal Research Institute, New York, NY.*

Background: Intradialytic morbid events (IME) complicate over 30% of all hemodialysis (HD) sessions because no reliable method is available to predict these. The aim of this study was to explore hemodynamic indicators to predict IME.

Methods: We reduced post-HD target weights systematically in chronic HD patients to achieve dry weight defined by calf bioimpedance spectroscopy (DW_{cBIS}). Systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) were measured every 10 minutes during HD. In addition, we calculated the SDH [min / beat] = (SBP/DBP)/HR at the same time points. IME were defined as low blood pressure (SBP<90 mmHg), or muscle cramps or dizziness during HD. SDH_{IME} denotes the nadir SDH prior to IME. Ultrafiltration rate (UFR) was recorded.

Results: 45 HD patients were studied during a total of 245 HD sessions. IME occurred in 124 sessions (51%). Pre-HD SBP, DBP, HR and UFR did not separately differ between sessions with and without IME (Table 1). SDH_{IME} was lower in the same sessions with IME (0.017±0.001 vs. 0.023±0.004, min/beat, p<0.001). SDH_{IME} was lower than average SDH in non IME sessions (0.017±0.001 vs. 0.024±0.005, min/beat, p<0.001). An SDH criterion defined as mean+2SD of SDH_{IME} (0.017+0.001*2=0.019) of <0.019 min/beat resulted in a sensitivity of 69 % and specificity of 57 % for prediction of IME. On average SDH_{IME} preceded an IME by 31 minutes (Interquartile range 7 to 55 min).

Conclusions: Physiologically, a blood pressure drop results in a HR rise. Absolute levels of SBP, DBP and HR vary greatly among HD patients while the SBP / DBP ratio shows less variability. The SBP / DBP ratio over HR may provide an improved signal-to-noise ratio to indicate intravascular hypovolemia and thus herald IME more accurately compared to the three components individually. Prospective studies in diverse populations of HD patients are required to further explore the clinical utility of SDH.

Table 1

	Pre-HD Weight, kg	Post-HD Weight, kg	Pre-HD SBP, mmHg	Pre-HD DBP, mmHg	Pre-HD HR, beat/min	SDH, min/beat	Ultrafiltration rate, mL/h
With IME	78.9±17	75.9±17	135.4±22.6	73.2±12.7	79.8±12	0.017±0.001**	906.1±294
Without IME	79.8±15.8	76.6±15.3	137.7±22.7	72.8±13	78.6±13	0.024±0.005	890.8±239

Funding: Private Foundation Support

TH-PO697

Is There a Gold Standard for Measuring Dry Weight in Hemodialysis Patients? Fansan Zhu, Peter Kotanko, Nathan W. Levin. *Research, Renal Research Institute, New York, NY.*

Background: Dry weight (DW) has classically been assessed by clinical signs including blood pressure (SBP) changes and hypotensive symptoms. Many techniques, such as calf bioimpedance spectroscopy (cBIS) and relative blood volume (RBV), have been applied to assess dry weight. Our aim was to evaluate relationships of SBP, cBIS and RBV with progressive reduction of fluid status towards DW.

Methods: In 64 patients post HD weight was gradually reduced until DW (DW_{cBIS}) was obtained by two criteria: 1) flattening of the curve of change in calf resistance during HD and 2) reaching a normal range of calf normalized resistivity (CNR) post HD. cBIS (Hydra 4200) was continuously performed during HD. Patients who did not reach DW_{cBIS} by three months or quit the study were categorized as no DW (NDW).

Results: 32 patients (440 sessions) reached DW_{cBIS} and 32 patients (286 sessions) were NDW (Table 1). Pre and post Wt decreased between baselines (BL) and DW or NDW. CNR at DW reached the normal range (m: 18.5, f: 20, $Ohm*m^3/kg\ 10^{-2}$) but not at NDW. SBP was higher at NDW than in DW but decreased in both groups. RBV changes did not differ significantly between BL and at DW or NDW. The number of measurements at DW was higher than at NDW (13.8±7 vs 6.9±3.3 sessions, p<0.001).

Conclusions: 32 patients using two separate criteria (Zhu et al, Physiol Meas 2008) reached DW_{cBIS} after approximately 14 sessions. NDW patents with higher SBP and presumably more fluid overload did not reach DW_{cBIS} in about 7 sessions. RBV change in two groups did not reflect fluid status. We propose that the combination of the two criteria above may be considered currently as a candidate for a gold standard for assessment of DW.

Table 1

	Pre HD Wt, kg	Post HD Wt, kg	Pre HD SBP, mmHg	Post HD SBP, mmHg	Pre HD CND, Ohm*m^3/ kg*10^-2	Post HD CND, Ohm*m^3/ kg 10^-2	RBV. %
BL	75.1±15	72.3±15	127.1±18	122.6±22	14.4±2.3	18.1±3	84.5±6
DW	73.7±15	71.1±15	121.4±18	114.2±21	15.6±3.4	20.4±1.9	84.7±5.7
p value	<0.0001	<0.0001	<0.05	<0.01	0.07	<0.0001	0.8
BL	87.3±19	83.6±19	155.1±29**	127.6±33	11.7±1.7**	14.1±1.9**	87.1±5*
NDW	86.8±19	83.4±19	150.3±27**	130±31**	12.2±1.8**	14.7±2**	87.5±6**
p value	<0.001	<0.001	0.18	0.8	<0.001	<0.001	0.57

*, ** indicate difference between DW and NDW groups

Funding: Private Foundation Support

TH-PO698

Comparison and Reproducibility of Three Methods for Volume Assessment in Hemodialysis Patients Sabrina Milan Manani, Flavio Basso, Dinna N. Cruz, Alessandra Brendolan, Federico Nalesso, Claudio Ronco. *Nephrology, San Bortolo Hospital, Vicenza, Italy.*

Background: The assessment of fluid overload is critical in hemodialysis (HD) patients. It can be done using bioimpedance or ultrasonography (US) of inferior vena cava (IVC). Recently, lung US has also been shown to be useful to assess extravascular lung water. However, all ultrasound methods are somewhat operator dependent, precluding their generalized use. Our aim was to establish the reproducibility and ease of performance of these three techniques.

Methods: Two certified sonographers measured the following parameters in 28 stable HD patients in a blinded fashion: IVC diameter during inspiration (IVCDmin) and expiration (IVCDmax), Collapsibility Index(IVCCI) by US; lung comet score (LCS) by US; and overhydration by Body Composition Monitor (BCM). Subjects were also classified into 3 levels of hydration based on established cut-offs of IVCDmax, IVCCI and LCS. Correlation and agreement between the 2 observers were evaluated by Spearman correlation and kappa test, respectively.

Results: Correlation between the 2 observers were good to excellent for all US parameters: LCS (r=0.99) (Fig. 1), IVCDmax (r=0.85) (Fig.2), IVCDmin (r=0.95), IVCCI (r=0.93) (p<0.001 for all). The agreement for IVCDmax and IVCCI categories was good (k=0.79 and 0.78, respectively, p<0.001 for both), while that for LCS categories was perfect (k=1.0). Similarly, correlation between 2 observers of BCM overhydration was excellent (r=0.99 p<0.001). IVC US and lung US were considered both easy and quick to perform. Median time was 2.5 (IQR 2,3) min for IVC US and 6 (IQR 5,7) min for lung US.

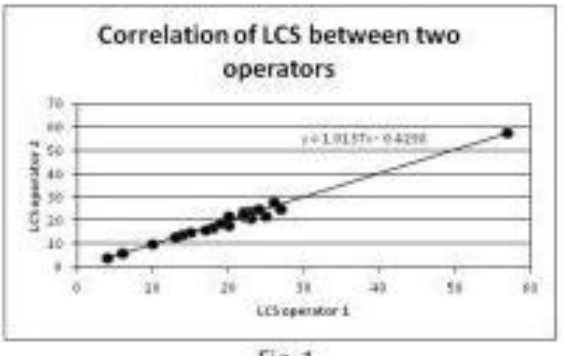


Fig. 1

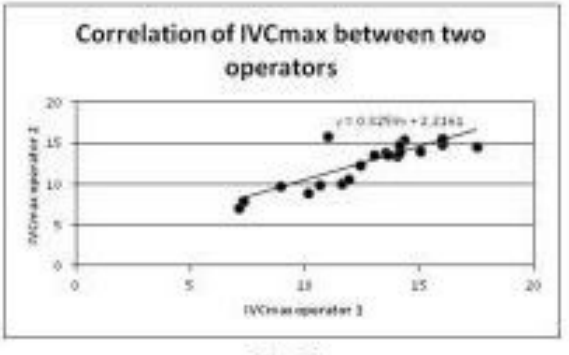


Fig. 2

Conclusions: The reproducibility of measurements from IVC US, lung US and BCM between two trained operators were very good. All methods were quick and easy to perform.

TH-PO699

Monitoring Intracellular, Intersititial and Intravascular Volume Changes during Dialysis Susie Q. Lew,[1] Manuel T. Velasquez,[1] Leslie Montgomery,[2] Wayne A. Gerth,[2] Richard W. Montgomery.[2] *[1]Medicine, George Washington University, Washington, DC; [2]LDM Associates, San Jose, CA.*

Background: Clinicians need to be able to measure the volume in the intravascular, interstitial, and intracellular compartments with a non-invasive real-time device. The aim is to test the feasibility and validity of a novel electrical impedance spectroscopic (EIS) technique that permits on-line measurements of compartmental volume changes in human subjects.

Methods: EIS technique validation was performed during 3 consecutive HD treatments in 20 ESRD patients by analyzing the timed profiles of fluid redistribution to mathematically derive *post hoc* hematocrit profiles. These profiles were then compared to the simultaneously measured hematocrit values recorded independently using the CritLine® monitor. Regression and Bland Altman analyses were used to compare the EIS data and Critline® hematocrits at sequential times during 60 routine HD sessions.

Results: The statistical and graphical evidence confirms that the EIS system reliably monitors the continuous and real-time *rates of change* of the fluid volumes of the three compartments. An example of one profile is shown in Figures 1 and 2.

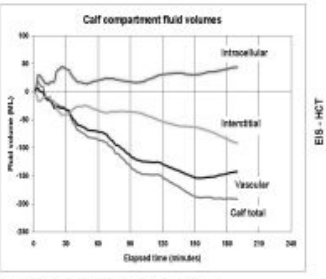


Figure 1. Kalman-smoothed fluid compartment volumes relative to initial values.

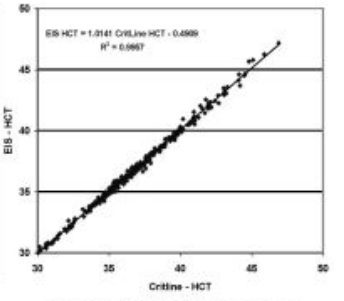


Figure 2. Correlation between optical sensor hematocrits (CritLine-HCT) and values predicted from EIS (EIS-HCT) measurements at 30, 60,90, 120 min elapsed time and from the last minute of the dialysis period.

Conclusions: The present study demonstrates for the first time that the EIS method can reliably provide on-line continuous measurements of compartmental intravascular, interstitial, and intracellular fluid volume changes that occur during HD treatment. Such information may prove valuable in the diagnosis and management of changes in body fluid balance in clinical situations such as hemodialysis, volume resuscitation or diuretic therapy.

Funding: Other NIH Support - SBIR

TH-PO700

Blood Volume Monitoring Prevents Intradialytic Hypotension: A Pilot Study Anita Saxena, Raj K. Sharma, Amit Gupta. *Nephrology, SGPGIMS, Lucknow, Uttar Pradesh, India.*

Background: During dialysis, maintenance of blood pressure is related to two mechanisms, blood volume preservation and cardiovascular compensation. Arterial hypotension occurs when central hypovolemia causes an underfilling of the cardiac chambers, thereby compromising the circulatory load. Objectiive: Estimation of blood volume during hemodialysis.

Methods: Blood Volume (BVM) and Blood temperature(BTM) was monitored on 12 non diabetic ESRD patients on MHD who were prone to intradialytic hypotension. Plasma and water compartments were evaluated using bioelectrical impedance analysis.

Results: Patients, moderately malnourished, had not achieved dry weight. Critical relative blood volume was fixed at 90%. Changes in red blood volume and hematocrit were noted during dialysis. Mean Hemoglobin was 7.5 mg%, albumin 3.2 mg, CRP 1.5, KT/V 1.2. Predialysis to post dialysis changes were: Hematocrit changed from 20.2 to 26.5, plasma volume 3.8 to 3.6, TBW 30.4 to 25.5 ECW 18.9 to 14.5, ICW 14.7 to 13.1, plasma 3.8 to 3.4, interstitial fluid, 12.3 to 12.0, blood pressure 138/84HGmm to 131.5/81HGmm. Net ultrafiltratio was 3.2L. Discussion: Results show significant changes in blood volume and water compartments during dialysis. With use of BVM, none of the patients went into hypotension, or had headache and drop in blood pressure despite a net ultrafiltration ranging between 2.0 L to 4.5 L as the ultrafiltration is cut off automatically when the critical levels of blood volume (which was fixed at 90% in this study) and HCT are reached.

Conclusions: Study demonstrates that BVM can prevent intradialytic hypotension and save patient from life threatening condition.

TH-PO701

Rethinking Intradialytic Blood-Pressure: Asymptomatic Short-Term Blood Pressure Variability Is Common Scott Wilson, Eugenia Pedagogos, Gavin J. Becker. *Dept Nephrology, Royal Melbourne Hospital.*

Background: The prognostic significance of asymptomatic blood-pressure (BP) variability to independently predict cardiovascular disease is recognised in non-dialytic populations. BP swings during haemodialysis (HD) are commonly observed, however the magnitude and directions of such changes are usually unprofiled. By convention, intradialytic hypotension (IDH) is defined by a symptomatic absolute drop in systolic BP by 20mmHg, ignoring asymptomatic change and failing to consider the relativity of BP change to an individual patient's baseline.

Methods: We hypothesised that patients on HD would demonstrate significant short-term intradialytic BP fluctuations below symptomatic thresholds.

Systolic BP was continuously recorded by Finometer during standard 4-hour, short-break HD in 26 stable outpatients without clinical history of IDH. All patients underwent concomitant blood-volume monitoring.

Using the continuous BP time series, short-term BP variability was measured against a moving baseline of 500 heart-beat intervals. Dynamic changes in BP >15% (equating a change of 20mmHg at 135mmHg systolic) between sequential intervals defined significant BP variability.

Results: Haemodynamic instability was common, with 299 significant BP changes observed (mean 11/HD treatment). Sudden rises (n=173) in BP were more frequent than acute declines (n=126). Surprisingly no event was associated with a coincident variation in pulse rate. Whole-of-treatment linear trends identified a paradoxical intradialytic rise in BP in 17 (65%) HD sessions. BP variability was not predicted by blood-volume reduction slope, pre-HD electrolytes, renin, aldosterone or NT-ProBNP, dialysate prescription, or by the use of antihypertensives.

Conclusions: This study reveals that significant, clinically silent BP fluctuations are both common and unrecognised in standard HD when defined relative to an individual patient's baseline pressure and intradialytic BP trajectory. Such instability may be associated with the excess of cardiovascular mortality observed in this patient group. Current IDH definitions warrant reconsideration as they may miss these potentially important events by relying on an arbitrary absolute symptomatic BP change.

TH-PO702

The Effect of Oral Ferric Citrate on Intravenous Iron Dose and Serum Iron Markers T. Christopher Bond,[1] Tracy Jack Mayne,[1] Jamie P. Dwyer,[2] Mohammed Sika,[2] Robert M. Niecestro.[3] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Vanderbilt University, Nashville, TN; [3]Keryx Biopharmaceuticals, New York, NY.*

Background: Ferric citrate is a phosphate binder in phase 3 clinical development to treat hyperphoshatemia in patients with end-stage renal disease. In addition to managing patients' serum phosphorus, earlier clinical studies have shown that some iron (Fe) in the drug is absorbed, increasing saturated transferrin (TSAT) and serum ferritin levels.

Methods: A 28-day, phase 2, multicenter open-label study was conducted to assess drug safety and tolerability in dialysis patients. Subjects received starting doses of 4.5 or 6.0 g/day ferric citrate, titrated until serum phosphate was in-range (3.5 to 5.5 mg/dL). The study design allowed concomitant intravenous (IV) Fe for subjects with serum ferritin <500 ng/mL, TSAT <25%, and/or at the discretion of the physician. We conducted a post-hoc analysis to compare the iron storage measures between patients receiving IV Fe prior to the study (visit 0), and those patients who did not receive IV iron prior to the study.

Results: 55 subjects were enrolled. 30 subjects were receiving IV Fe prior to beginning the study, and 25 of these 30 received IV Fe during the study. 5 subjects (17%) who received IV Fe prior to the study were able to discontinue its use while receiving ferric citrate. No subjects without IV Fe at baseline added IV Fe while taking ferric citrate.

	Visit 0 (Day 28)			
	Serum Fe, mcg/dL	Ferritin, ng/mL	*TIBC, mcg/dL	%TSAT
IV Fe at baseline [n=30]				
IV Fe during study [n=25]	67.5 (76.8)	513.9 (586.7)	226.6 (220.5)	30.1 (35.8)
No IV Fe during study [n=5]	61.2 (58.4)	639.8 (674.4)	202.8 (212.4)	29.4 (27.8)
No IV iron at baseline [n=25]				
No IV Fe during study [n=25]	71.2 (77.2)	603.2 (617.8)	233.8 (214.3)	30.8 (36.5)
IV Fe during study [n=0]	n/a	n/a	n/a	n/a

*TIBC=total iron-binding capacity

Conclusions: In this short-term study, some subjects were able to discontinue IV Fe while maintaining adequate Fe level with oral ferric citrate, with the added benefit of serum phosphorus maintenance. Results from a long-term, phase 3 clinical study of ferric citrate will be available in late 2012.

Funding: Pharmaceutical Company Support - Keryx Biopharmaceuticals

TH-PO703

Vancomycin Is Safe to Administer in Haemodialysis without Levels but Optimal Dosing Is Dependent on Body Weight Rhys David Russell Evans, Manish Verma, Mark Blunden, Ravindra Rajakariar. *Nephrology Department, Royal London Hospital.*

Background: Vancomycin is frequently used in haemodialysis. Most published literature uses an accepted lower limit of 5mg/l to achieve therapeutic benefit. This equates to a pre-haemodialysis concentration of 7.5mg/l based on the effect of haemodialysis on levels.

Methods: In this retrospective observational study, we compared two vancomycin dosing regimes in haemodialysis patients: Either 1g or 500mg loading followed by 500mg given during the last hour of each haemodialysis session. All patients were dialysed three times per week, for 3-4 hours, using a high flux polysulphone dialyser. The primary end-point was the proportion of patients achieving a mean pre-haemodialysis vancomycin concentration of greater than 7.5mg/l. We also investigated correlation with body weight.

Results: Out of 850 patients undergoing haemodialysis over a 6-month period, 24 patients met the selection criteria for this study. There were 13 in the 500mg loading group and 11 in the 1g loading group. The median length of treatment was 2 weeks in both groups (range 1-7 weeks). 89% of patients (9/11) in the 1g loading group had mean pre-haemodialysis vancomycin concentrations above the target of 7.5mg/l compared with only 46% (6/13) in the 500mg loading group (p=0.07). There was no drug accumulation in either group with pre-haemodialysis concentrations less than 25mg/l in all patients. Subsequent analysis of the 1g loading group demonstrated that 100% of patients less than 80kg achieved a mean level greater than 7.5mg/l compared with 0% of those greater than 80kg (p<0.01).

Conclusions: Vancomycin with a loading dose of 1g followed by 500mg given during the last hour of each haemodialysis provided better therapeutic concentrations than a 500mg loading dose. Both regimes were safe without evidence of drug accumulation in either group suggesting that levels do not need to be done to assess safety. Patient weight requires consideration as those who weigh greater than 80kg require a larger cumulative dose than was used in this study.

TH-PO704

Cardiac Dysautonomia in Patients on Chronic Hemodialysis: A One-Year Follow-Up Study Muhammad Zaman Khan Assir,[1] Syed Rizwan Bokhari,[1] Hafiz I. Ahmad,[1] Arif Asif.[2] *[1]Department of Nephrology, Allama Iqbal Medical College, Lahore, Punjab, Pakistan; [2]Division of Nephrology, University of Miami School of Medicine, Miami, FL.*

Background: Cardiac dysautonomia is associated with increased frequency of cardiac arrhythmias. However, the frequency of new onset cardiac dysautonomia in dialysis patients is not known.

Methods: We conducted a prospective study on cohort of fifty chronic hemodialysis patients. Of these thirty eight (76%) patients were alive at end of one year. These patients underwent three tests of cardiac autonomic dysfunction at base line (start of the study) and at end of 1-year follow-up. This tests included abnormal heart rate response to deep breathing (E:I ratio less than 1.17), abnormal Valsalva ratio (longest to shortest R-R ratio less than 1.2) and abnormal 30:15 ratio (30th to 15th R-R ratio on standing less than 1.03). Patients with 2 or more abnormal tests were considered to be having cardiac dysautonomia.

Results: Out of total fifty patients, 88% had dysautonomia at baseline. Of the remaining six (12%) patients who did not have cardiac dysautonomia at baseline, five (83%) developed cardiac dysautonomia at the end of one year. One patient was excluded from analysis due to inability to perform tests. Patients with baseline cardiac dysautonomia showed no change in dysautonomia scores at the end of one year.

Conclusions: Our study showed that majority of chronic hemodialysis patients who did not exhibit cardiac dysautonomia initially had developed this abnormality at the end of one year follow up.

TH-PO705

Cardiac Dysautonomia and Mortality in Chronic Hemodialysis Patients Hafiz I. Ahmad,[1] Syed Rizwan Bokhari,[1] Muhammad Zaman Khan Assir,[1] Ali Jawa,[1] Arif Asif.[2] *[1]Department of Nephrology, Allama Iqbal Medical College, Lahore, Punjab, Pakistan; [2]Division of Nephrology, University of Miami School of Medicine, Miami, FL.*

Background: Sudden cardiac death is common in chronic hemodialysis patients. Recent data have emphasized that cardiac dysautonomia is present in 88% of chronic hemodialysis patients (Ahmad et al: ASN 2011). Cardiac dysautonomia has been shown to be associated with increased frequency of cardiac arrhythmias and higher incidence of sudden cardiac death.

Methods: In this prospective analysis we followed a cohort of fifty patients who were diagnosed with cardiac dysautonomia. Patients were followed for a one-year period and mortality data were collected. Cardiac dysautonomia tests included abnormal heart rate response to deep breathing (E:I ratio less than 1.17), abnormal Valsalva ratio (longest to shortest R-R ratio less than 1.2) and abnormal 30:15 ratio (30th to 15th R-R ratio on standing less than 1.03).

Results: Of the fifty patients included in this prospective study, 12 (24%) had died at one-year follow-up. Sudden cardiac death was reported in 7(58%) of the 12 patients. Pneumonia, sepsis and hypertensive encephalopathy were reported to be the cause of death in rest of the 5 (42%) patients. All 7 patients who died of sudden cardiac death had three abnormal cardiac dysautonomia tests. On multivariate regression analysis age, sex, diabetic status and duration on dialysis had no effect on mortality.

Conclusions: In this study, a significant number of patients with cardiac dysautonomia had died at a one-year follow-up period. Cardiac dysautonomia appears to be strong predictor of death in end stage renal disease patients on long-term hemodialysis.

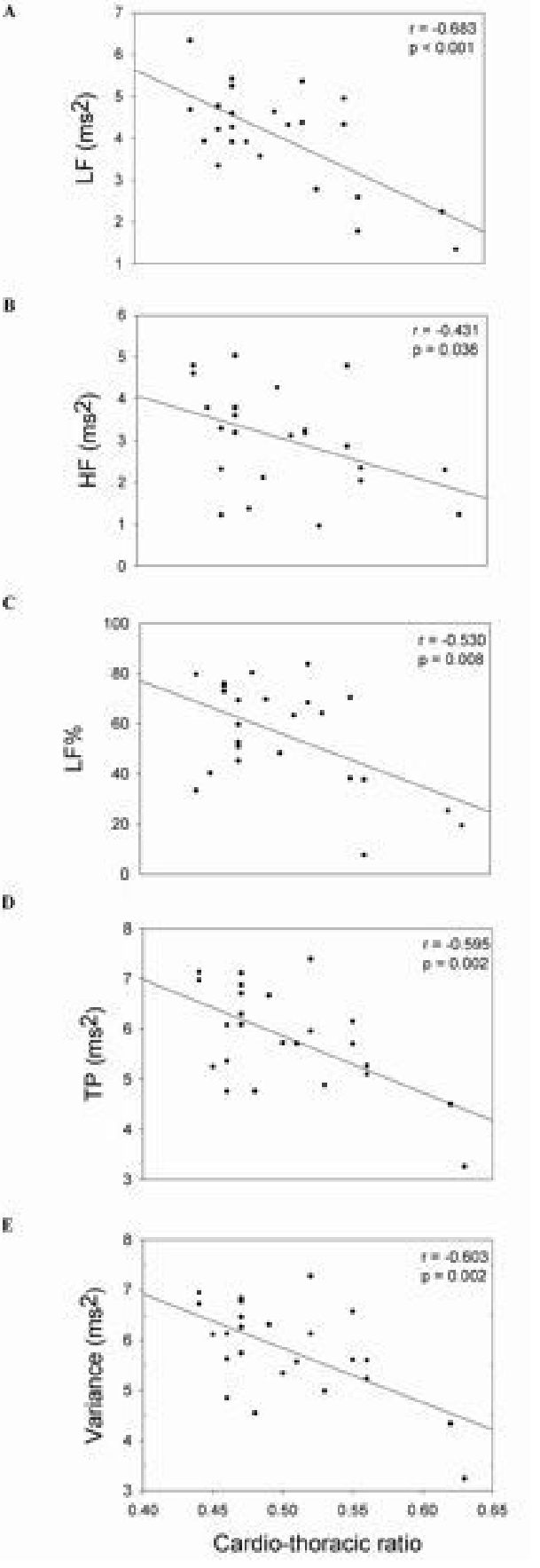
TH-PO706

Cardiothoracic Ratio in Hemodialysis Patients Predict Autonomic Function Yi Shin Chen,[1] Ming-Ju Wu.[2] *[1]Division of Nephrology, Department of Medicine, Taichung Tzu Chi General Hospital, Taichung, Taiwan; [2]Division of Nephrology, Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan.*

Background: Autonomic dysfunction is common in dialysis patients and associated with mortality. Heart rate variability (HRV) assessment is a noninvasive measure of autonomic function. The cardiothoracic ratio is an indicator of fluid status in dialysis patients. This study investigated the correlation between cardiothoracic ratio and HRV in hemodialysis patients.

Methods: This was a cross-sectional study. 24 male dialysis patients were recruited in a hemodialysis center. Cardiothoracic ratio was calculated from chest X-ray and taken at the time of interdialytic day. Power spectral analysis of successive RRs from 5 minutes ECGs performed at the day of chest X-ray taken was performed to determine the variance (variance of RR-interval values), high-frequency power (HF), low-frequency power (LF), the ratio of LF power to HF (LF/HF) and total power (TP).

Results: There were significantly negative correlations between LF, HF, TP, and variance with cardiothoracic ratio.



In particular, TP decreased by 13 fold for each fold increase in cardiothoracic ratio.

Conclusions: Cardiothoracic ratio was found to be significantly related to TP and HF. Higher cardiothoracic ratio was associated with lower autonomic function and parasympathetic activity. Our findings suggest that the higher cardiothoracic ratio predicts autonomic dysfunction in hemodialysis patients.

TH-PO707

Evaluation of E.Coli Contamination in Dialysate by Bacteria Plate Counts, Flow Cytometry and LAL Test Huan Chen,[1] Mei Wang.[2] *[1]Renal Department, Beijing Hospital, Beijing, China; [2]Renal Department, Beijing University People's Hospital, Beijing, China.*

Background: Morbidity in hemodialysis patients is associated with chronic inflammation. Microbiological contaminants derived from dialysate are thought to be one inflammatory stimulus. According to AAMI standard from USA, dialysate should contain less than 200CFU/mL colonies and less than 2EU/ml of endotoxin. Whereas European Best Practice Guidelines recommend 0.1CFU/ml and 0.03EU/ml respectively.What is the most accurate method for bacterial contaminated dialysate and how about the relationship between bacterial counts and endotoxin level?

Methods: E.Coli ATCC25922 as contaminant source was diluted by serial 10-fold. 6 different contaminant concentations were obtained. We tested each sample of 6 different concentrations of E.Coli by TGEA culture plate counts, flow cytometry and LAL kinetic turbidimetric assay. Comparison of sensitivety of different methods and the correlation among 3 assays by Pearson correlation and Linear regression analysis were finished. To evaluate the sensitivity of TGEA culture plate counts, flow cytometry and LAL test for E.Coli contaminated dialysate in vitro and to exlpore the relationship between E.Coli counts and endotoxin level.

Results: E.Coli contaminated dialysate of 6 levels in ascending sequence, results from flow cytometry were 2.63×10^2, 2.63×10^3, 2.63×10^4, 2.064×10^5, 2.671×10^6, 2.02899×10^7 cells/ml. Corresponding values were 0.01687, 0.4665, 4.7663, 45.8333, 588.8333 and 4987.3333EU/ml by LAL test. TGEA plate counts were 0, 8±4.47, 36±27.02, 648±150.23CFU/ml and other two higher contaminated concentration samples couldn't be measured beacause of occurence of lamellar bacteria plaque. Linear correlation was observed between flow cytometry counts and LAL levels. One-dimensional linear regression equation was established as follow: Quantity of E.Coli by FCM(cells/ml) = 56240.545+4062.207×LAL(EU/ml). According to the equation, 60303 cells/ml E.Coli corresponded to 1EU/ml of endotoxin.

Conclusions: Flow cytometry is more accurate than TGEA plate counts in evaluation of E.Coli contaminated dialysate. It doesn't show the concordance in E.Coli plate counts and endotoxin level from guidelines defined limits.

Funding: Government Support - Non-U.S.

TH-PO708

Cold Disinfection with Low-Dose Ozone Achieved High-Level Microbiological Fluid Quality in a Non-Heat Resistant PVC Water Loop and Favorable Outcome Data in Prevalent HD Patients Karl August Brensing,[1] Peter Heidkamp,[1] Eva Neugebauer,[2] Peter M. Raab,[1] Uwe Poege,[1] Thomas M. Gerhardt,[1] Thomas Kistemann,[2] Martin Exner,[2] Juergen Gebel.[2] *[1]Dialysis Unit, Nephrology Center Bonn, Bonn, NRW, Germany; [2]Institute for Hygiene & Public Health, University of Bonn, Bonn, NRW, Germany.*

Background: Good HD-outcome requires optimal fluid quality to avoid infections. However, frequent (≤weekly) water loop disinfection is costly and unfeasable (heat >80°C) for many PVC loops. Thus, cold disinfection by ozone may help but long-term efficacy is unknown.

Methods: During 3-months (=Mo) run-in and 60 Mo on ozone we tested permeate for colony forming units (=CFU/ml; water bacteria/R2A, mycobacteria/Middlebrook, pseudomonas/Cetrimid agars) and endotoxin (=ENDO; sensitive limulus assay: ≥0.005 EU/ml). Ozone was used weekly (9 Mo) then daily (51 Mo) by low levels (20-50 ppb: 0-3 pm) in an 8-year old PVC loop. In addition, we tested ozone (daily) impact on biofilm bacteria in dialysis inflow-stubs (used 3-5 Mo), dialysate fluid (CFU/100ml) and outcome data (quarterly albumin=ALB + CRP; survival) in 53 prevalent HD patients.

Results: Baseline permeate ENDO was 0.03 EU/mL (median; Range: 0.01-0.31) and we had in 12 probes 8x water bacteria (16/mL, range: 0-330), 4x mycobacteria or pseudomonas. Finally, ENDO (0.005 EU/ml, <0.005 – 0.069; $p<0.001$) and water bacteria CFU (4/mL, 0-12; $p<0.01$) declined significantly. Daily ozone reduced CFU in dialysis inflow-stubs biofilm (initial: $1.2x10^4$ per cm^2 final: $2.4x10^2$ per cm^2 ; $p<0.01$) and in dialysate fluid (CFU/100ml: 13; 2-32 vs. 0.0/ml; 0.0 - 0.9; $p<0.001$). We saw favorable biomarkers (ALB: initial: 41.7 g/L, 33-49; final: 40.1 g/L; 28-48; $p<0.01$ and CRP: initial: 0.6 mg/dl, 0.1-3.6; final: 0.6 mg/dl, 0.1-4.2; NS) and actual 5-year survival of 51% (27/53; = 9.8% mortality/year).

Conclusions: 1. Ozone achieved sustained high-level fluid quality and decreased biofilm contamination even in an 8-year old PVC loop. 2. Favorable inflammation biomarkers and a rather low mortality suggest clinical benefits. 3. Cold disinfection with low-dose ozone appears reliable and cost-effective to provide optimal microbiological fluid quality even in non-heat resistant PVC water loops.

TH-PO709

Implementation of Medication Therapy Management (MTM) Pharmacist Services in Dialysis Care and Impact on Patient Outcomes Amy W. Williams,[1] Lance J. Oyen,[2] Robert C. Albright,[1] Jan Anderson,[2] Robert Hoel,[2] Laura Odell.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Pharmacy Services, Mayo Clinic, Rochester, MN.*

Background: The role of the pharmacist in providing care to dialysis patients has often been limited to hospital settings, or ambulatory settings or specific drug classes. Our hypothesis is that pharmacists providing comprehensive MTM services will reduce the risk of medication adverse events, identify nonadherence, and optimize drug therapy for outpatient hemodialysis patients.

Methods: As a quality improvement project we initiated pharmacist services for chronic dialysis patients focusing on patients new to dialysis or hospitalized within 60 days. The service was provided over a 6 month period in one dialysis unit which cares for the most medically complex dialysis population in our system. The goals were to reduce patient medication risks by optimizing drug therapies leading to a reduction in healthcare and patient costs and an improvement in patient outcomes.

Results: From November 2011 to April 2012, 3 pharmacists provided MTM services to 68 patients, average age of 61 years, averaging 1.7 visits per patient. Patients averaged 17.5 medications (range 6-35) and 13 unique conditions targeted by the medications. (range 6-25). The average patient medication burden was 25 unique doses/d at an average wholesale price drug cost of $5983/month. An average of 7 drug therapy problems (DTPs) per patient were identified and resolved by the pharmacist, with 85% of patients having 3 or more DTPs. DTPs were related to indication (32%), safety (15%), effectiveness (27%) and adherence (26%). Using a standardized tool for costs of care estimation, MTM resulted in estimated drug cost savings of $311/pt/yr and estimated healthcare savings of $3387/pt/year through avoidance of unnecessary lab testing and clinic visits, selection of cost effective therapy and avoidance of serious adverse drug reactions.

Conclusions: Comprehensive pharmacist provided MTM care to outpatient dialysis patients optimizes complex medication regimens leading to improved adherence, reduced patient risks, as well as both drug and total cost of care savings.

TH-PO710

Improving Comprehensive CKD & ESRD Care Using Quality & Design Methods Amy W. Williams, Kathryn Zavaleta, Krisa Ryan, John J. Dillon, Mary Tibor, Susan Dornack, Mary Ann Ryan, Michelle C. Hedin, Paul Klugherz, James T. McCarthy, Stephen F. Gudgell, Robert C. Albright. *Mayo Clinic, Rochester, MN.*

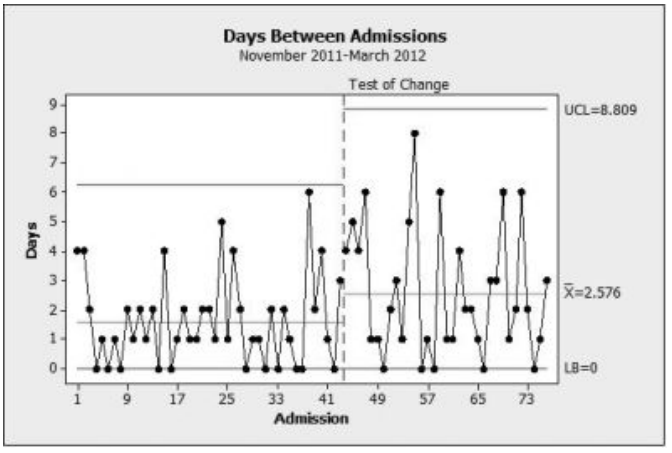
Background: CMS-mandated changes have the potential to fragment & increase total cost of care for ESRD patients. This quality project focused on achieving highest quality, lowest cost care in a multidisciplinary setting.

Methods: The multi-disciplinary team used design-engineering & quality improvement methods to create patient-centered care processes. Process mapping of current & future ideal states led to interventions tested with PDSA cycles. Research on advanced care planning & care management was incorporated. Improvement strategies were integrated into electronic information flows, clinic/hospital workflows & educational materials, establishing consistency among care teams & seamless patient transitions.

Results: Improvements paralleled disease trajectories from CKD to ESRD.

Process Gaps	Improvements
Early CKD referral	EMR alert for referral
Consistent CKD care	Standard template
Team communication	multi-authored EMR tool
Pt-centered ed	Design theory process for new ed
Discuss goals/preferences prior to dialysis referral	Decision aids,care team palliative care training,palliative care staff on CKD/ESRD team
High cost care overuse	Alert when pt in ER/hosp,Huddles:nursing/provider
Med therapy risks	Pharm D. med management
inpt unit assignment	Central process
inpt safety	Standard admit orders
Hosp dismissal	2/d huddles:inpt/outpt teams,Discharge checklist,Critical info packet

An initial 20-40% reduction in inpatient care(50% of total costs)& positive feedback from patients/staff were realized.



Conclusions: Design-engineering & quality improvement methods are valuable tools to determine needed improvements in CKD/ESRD care. The interventions discovered in this project can be translated to improve care in other chronic disease populations.

TH-PO711

In Dialysis: The Association between Social Support and Psychosocial Factors upon Mortality and Quality of Life Ali Mohammed Allehbi,[2] Archie Dumdum Bunani.[1] *[1]Clinical Services, DaVita Lehbi Care - Saudi Arabia, Riyadh, Central Province, Saudi Arabia; [2]Medicine - Nephrology, DaVita Lehbi Care - Saudi Arabia, Riyadh, Central Province, Saudi Arabia.*

Background: Treating End Stage Renal Disease (ESRD) by hemodialysis (HD) creates changes to relationship patterns, social life, activities of daily living and the ability to attain a satisfactory Quality of Life (QoL). There is an absence of information in Saudi Arabia on the impact of these changes in the renal population. This study investigated the influence of social support and other psychosocial factors upon mortality, adherence to medical care recommendations, and physical QoL amongst patients.

Methods: 272 HD patients were examined using the QoL questionnaire to determine self-reported inclinations. Logistics regression through Weighted K was used to analyze data.

Results: 53.5% of patients reported *health had interfered with their social activities* demonstrating a strong associated with risk towards All-Cause (sp=1.33) and Cause-Specific Mortality including cardiac diseases (sp=1.28). These patients had a greater risk of withdrawing (sp=1.67) from treatment, non- adherence to Phosphorus (sp=1.06) greater than 7.5 mg/dL and increased risk towards an albumin of less than 3.5 g/dL (sp=1.23). Patients reporting *dissatisfied with family support* (12.0%) were at highest risk to non-adherence to intra-dialytic weight gain (sp=1.27), shortening the dialysis session (sp=1.21) and increased risk of Potassium level greater than 6 mEq/L (sp=1.14). However, patients reporting *dissatisfied with staff support* (14.1%) revealed a higher risk of decreased physical QoL (sp=0.76).

Conclusions: This study demonstrated that physical QoL was not only affected by medications and other laboratory work-ups but also with additional psychosocial support. The study led to the development of programs empowering patients and families to participate in their treatment plans. The program includes various counselling approaches directed to patient, families, and health team.

Funding: Private Foundation Support

TH-PO712

Changes in Dialysis Social Workers' Caseloads and Job Tasks Since the Implementation of the 2008 Conditions for Coverage Joseph R. Merighi,[1] Teri Browne.[2] *[1]School of Social Work, Boston University, Boston, MA; [2]College of Social Work, University of South Carolina, Columbia, SC.*

Background: This study examines changes in U.S. dialysis social workers' caseloads and job tasks since the implementation of the 2008 Conditions for Coverage (CfC). Studies indicate that high caseloads are associated with a decreased ability to provide social work interventions (Browne, 2012), and high task demands with little time to provide psychosocial and counseling services are linked to suboptimal patient care (Callahan et al., 1998; Merighi & Collins, 2011).

Methods: 1,322 part-time (PT) and full-time (FT) social workers were obtained from all 18 End Stage Renal Disease Networks. Respondents were recruited between 3/31/10 and 6/21/10 using the Council of Nephrology Social Worker listserv. A 130-item online survey was administered by the National Kidney Foundation to gather data on renal social workers' caseloads, job responsibilities, and workplace resources.

Results: Since the implementation of the 2008 CfC, 41.2% of PT and 50.1% of FT dialysis social workers reported increases in their caseloads, with FT social workers reporting a greater proportionate increase ($p<.05$). The caseloads for PT social workers who reported that it increased (M=87.1) was significantly higher than those who reported that it stayed the same (M=73.2; $p<.001$). The caseloads for FT social workers who indicated that it increased (M=130.9) was significantly higher than those who reported that it stayed the same (M=111.9) or decreased (M=95.7; $p<.001$). With regard to job tasks, increases were reported by 80.2% of PT and 85.9% of FT social workers, with FT social workers reporting a greater proportionate increase in tasks being performed ($p<.01$). In addition, 70.4% of PT and 76.6% of FT social workers reported that they had insufficient time to provide psychosocial services to patients.

Conclusions: Findings demonstrate notable increases in social workers' caseloads and job tasks between 2008 and 2010. Together, these factors make it challenging for them to satisfy all CfC mandates. Additional research is needed to examine how these workload demands affect dialysis patients' quality of life and health outcomes.

TH-PO713

Incidence of Missed and Shortened Hemodialysis Treatments in a Large Urban Hemodialysis Unit: Effect on Mortality. Impact of QAPI Derived Multidisciplinary Protocol on Outcomes John F. D'Avella, Stephanie Antonelli, Maryjane Porado, Donna Kelly. *Dialysis, Hartford Hospital, Hartford, CT.*

Background: There is no recent data on the incidence of missed or shortened hemodialysis treatments particularly in a urban setting. There is little data on why it happens and whether it impacts on mortality. We track missed and shortened traetments as a quality indicator When the incidence was noted to increase a QAPI project was undertaken to study why and design a protocol to correct it.

Methods: Missed and shortened treatments are tracked by social workers. In 2010 a increase of 28% in missed treatments was noted. This was brought to QAPI as a problem .Patients were interviewed and a root cause analysis was done Results were discussed with the Nephrology section. A protocol was developed: This involved notifying the nephrologist for missed treatments. Barriers to treatment were discussed and the patient was educated about the risk of missing treatments. If a patient missed more then 3 treatments per quarter; the family is involved with patient consent. In addition there was a unit wide education initiative addressing the risks of missed /shortened treatments.

Results: The unit delivers approx 26208 treatments (RX)per yr In 2010 640 RX were missed(2.4%) Patients that missed 4 or more RX had a mortality of 31% vs unit mortality of 19.6%. With the protocol in 2011 missed RX fell to 516 (decrease 19%)and 2012 1st quarter data annualized to 392 (decrease 39%) This will result in added revenue of $55,056. Shortened RX did not initially change 198/199 2010/2011(they were included in the protocol)Since inclusion; the annualized rate for 2012 is 172(decrease of14%) The most common reason for missed treatments (RX) was not feeling well /similar for shortened RX along with cramping These improved with individual attention and patient education as to the risks of missing/shortening treatments.

Conclusions: Missed treatments have a negative impact on patient mortality and unit revenue. Through a protocol which discovers individual reasons for missed/shortened treatments ; relies on patient and family education and involves all members of the health care team; it is possible to reverse this trend.

TH-PO714

Incidence of False Positive PF4 Antibody Screen for Heparin Induced Thrombocytopenia in Hemodialysis Patients Using Rapid Assay by Akers Bioscience and a Unconvential Cause of Thrombocytopenia in Hemodialysis Patients John F. D'Avella, Debbie Cofrancesco, Donna Kelly. *Dialysis, Hartford Hospital, Hartford, CT.*

Background: Heparin is a necessary part of hemodialysis. The incidence of heparin-induced thrombocytopenia in patients is said to be 3.2%. This syndrome is the result of heparin-induced antibodies against platelet factor IV heparin complex. This not only can result in arterial and venous thrombosis but prohibits the use of heparin. This alone in hemopatients increases the risk and cost of dialysis. The testing for PF4 antibodies used for the diagnosis HIT is said to have a sensitivity of 95% and specificity 50 to 90%. False positive tests are known to occur in patient's with anti-phospholipid syndrome and lupus. There are reports of false positive tests in hemodialysis patients but the incidence is unclear and ther is no mention of specific test specificity or variation.

Methods: On screening 11 patient's had platelet count < 100,000. They were screened for HIT with the PF4 antibody rapid assay Akers Biosciences. None of the patient's had lupus or APS. Positive antibody screens were confirmed with a serotonin release assay. Two patient's after a negative workup for thrombocytopenia were tested for electron beam sterilized hemodialysis membrane induced thrombocytopenia.

Results: 11 patients with platelet cts <100,000 were screened with the rapid PF4 assay by Akers Biosciences. All 11 patient's were positive. All had the serotonin release assay to confirm HIT .Only 1 of 11 was positive. 91% were false positives. Two patient's because of persistent thrombocytopenia post dialysis had platelet counts drawn pre-and post dialysis with a F 160 NR dialyzer (electron beam sterilized) and post ethylene oxide sterilized dialyzer. Patient #1 Platelet counts fell 44% with E beam vs 12% with ethylene oxide. . Patient #2 platelet count fell 33% with E beam vs 12% with ethylene oxide.

Conclusions: Rapid PF4 assay by Akers Biosciencesis has too many false positivesand should not be used in hemodialysis patients In patients with persistent thrombocytopenia consideration should be given to electron beam sterilized hemodialysis membrane induced thrombocytopenia.

TH-PO715

Regional and Ethnic Variations in Adherence to Prescribed Dialysis Treatment and the Effect of Clinic Size on Missed and Shortened Hemodialysis Treatments Chamberlain I. Obialo,[1] O. Myers,[2] William C. Hunt,[2] P. Zager.[2] *[1]Medicine, Morehouse School of Medicine, Atlanta, GA; [2]Medicine, University of New Mexico, Albuquerque, NM; [3]Dialysis Clinic, Inc, Albuquerque, NM.*

Background: Differences between the United States [US] and other countries in the adherence to dialysis prescriptions have been well documented. However, ethnic and regional variations within the US have not been clarified. This study explores the relationship of race/ethnicity, geographic region and clinic size with the frequency of missed and shortened hemodialysis [HD] treatments.

Methods: We studied a prevalent cohort of 15,340 HD patients treated in facilities operated by Dialysis Clinic, Inc. [DCI] between January 2007 and June 2008. The cohort consisted of 48% non-Hispanic whites [NHW], 41% African Americans [AA], 6% Hispanics [H], 2% Native Americans [NA], 2% Asians [AS], and 1% unknown. We divided the US into 4 regions, Northeast [NE], Midwest [MW], South [S], and West [W].

Results: The demographics and select variables of the patients by geographic region are shown in the table below. Comparisons between regions revealed that patients were older in the NE vs. the S ($p<0.001$) and W ($p=0.0052$). Rates of missed and shortened treatments were lower in the NE vs. other regions ($p<0.0001$). Hospitalizations were lower in the W vs. NE ($p<0.01$). Mortality rates were similar across regions. Missed (odds ratio 1.31; 955 CI 1.14-1.52) and shortened (odds ratio 1.86; 95%CI 1.73-2.0) treatments were more common in clinics with >100 patients vs. those with <50 patients. Compared to NHW, the rates of missed and shortened treatments were higher in AA, H, and NA ($p<0.001$) and lower among AS ($p<0.001$).

	NE	MW	S	W
N (%)	3972 (25.9)	2237 (14.5)	7003 (45.7)	2128 (13.9)
Median Age (yr)	66	62	61	61
Missed HD (%)	1.6	2.6	2.7	2.8
Shortened HD (%)	13	15.4	16.8	15.5
Hospitalization/ pt-yr	1.88	1.87	1.74	1.39
Mortality/100 pt-yr	14.3	15.9	16.03	14.14

Conclusions: The frequency of missed and shortened treatments varies significantly by race/ethnicity, geographic region and clinic size. The association of missed and shortened treatments with larger clinic size should be considered when planning new HD facilities.

Funding: Pharmaceutical Company Support - Dialysis Clinic, Inc.

TH-PO716

Prospective Study of the Impact, Burden and Outcomes of Hospitalisation in Maintenance Hemodialysis Shiv Bhutani, Milind Nikam, Arvind Ponnusamy, Sandip Mitra. *Biomedical Research Centre, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: Hospitalization in dialysis patients leads to significant morbidity, huge impact on healthcare costs and provides an index of quality of care. Although hemodialysis (HD) patients are at a higher risk of hospitalization, its true rates and outcomes in the UK has not been studied prospectively. The objective was to obtain admission rates for HD patients and identify risk factors, outcomes and impact on resources.

Methods: Prospective admission episodes data were collected using electronic records on all HD patients (n=450) from tertiary centre and associated satellite unit between June to November 2011.

Results: 159 hospitalization episodes in 82 patients were recorded over 6-month period with total duration of 2137 inpatient bed days and hospitalization rate of 700 per 1000 patient-years. The leading causes of hospitalization were cardiovascular (24.5%), infection (16.4%) and access related (18.7%). 54% (n= 20) of cardiovascular related admission episodes were due to fluid overload or hypotension on dialysis. 81 % (n=67) patients were discharged and 12% (n=10) died. Total deaths of 20 were reported for same cohort during the follow up period.

In a multivariate analysis, higher risk of death was associated with cardiovascular (OR 39.5, p=0.03) and infective (OR 39.3, p=0.03) causes for hospitalization as well as underlying diabetes (OR 29.9, p=0.027).

42% (n=67) of episodes were re-hospitalization event and 28% (n=19) had more than two 2 hospital admissions. Multivariate analysis revealed that patients who were re-hospitalized were younger with a mean age of 56.7yr as compared to those with single hospitalization whose mean age was 61.1y (p=0.09).

An estimate of resource utilization suggested a cost of over $ 1.9 million annually ($462 per bed day).

Conclusions: The 2 leading causes of hospitalization in HD are also those predicting mortality. It appears that risk of re-hospitalization is related to younger age, this needs further exploration. Fluid imbalance on dialysis is the leading cause of admissions, and potentially amenable to intervention that may lead to significant reduction in healthcare costs for HD.

Funding: Government Support - Non-U.S.

TH-PO717

Association of Health Literacy with Intermediate Outcomes in Hemodialysis Patients Divya Singh, James J. Paparello, Michael J. Fischer, Shubhada N. Ahya. *Division of Nephrology and Hypertension, Northwestern Memorial Hospital, Chicago, IL.*

Background: Health literacy is defined as the ability to understand basic health information to make appropriate health decisions. Low health literacy has been associated with a higher mortality in chronic hemodialysis patients. Little is known about the association of health literacy and dialysis related intermediate outcomes.

Methods: This is a single center, cross sectional study of 101 hemodialysis patients in whom the association of heath literacy and secondary outcomes was assessed. Health literacy was measured using the STOFHLA (Short form Test of Functional Health Literacy in Adults), where score <23 defined low (inadequate/marginal) and ≥23 defined adequate. Secondary outcomes such as infections, hospitalizations, dialysis access and laboratory parameters were examined. Descriptive statistics were used to characterize the findings.

Results: Among 101 subjects, the range of STOFHLA scores was 10-36, with a mean of 31 (SD 5.4). Eight had low (8%) health literacy. Of these, the mean age was 71 years (51-85), 2 were listed for transplant (25%), 3 had catheters (37.5%), 6 were women (75%), 6 were African-American (75%), 5 had high school education or less (62.5%). With the exception of age (P 0.01), no significant associations between health literacy and intermediate measures were found.

Health Literacy (STOFHLA) & Secondary Outcomes in Hemodialysis Patients

	Low	Adequate	P Value
Sex- Male/Female	2/6	47/46	0.672/0.539
#Hospitalization: 0	5	47	0.329
#Hospitalizations ≥1	3	46	0.577
# Infections 0	5	41	0.337
# Infections ≥1	2	52	0.432
Phos <5.5	3	39	0.346
Phos ≥.5	5	54	0.678
PTH <500	6	67	0.509
PTH ≥500	2	26	0.673
Albumin <2.5	0	6	0.804
Albumin ≥2.5	8	87	0.773
spKt/v<1.2	1	10	0.524
spKt/v≥1.2	6	83	0.487
HD Access- Catheter/ AVA	3/5	41/52	0.562/0.511
Dialysis Vintage <6mo	1	14	0.611
Dialysis Vintage ≥6	7	79	0.804
Received preHD Care	7	63	0.535

Conclusions: This study of prevalent urban hemodialysis patients showed no statistically significant associations between health literacy and intermediate outcomes. Studies are ongoing in a larger population with more intermediate outcomes.

TH-PO718

Reliability of Self-Report Co-Morbidity Questionnaire in Haemodialysis Patients Sivakumar Sridharan, Jocelyn Berdeprado, Ken Farrington. *Renal Department, Lister Hospital, Stevenage, United Kingdom.*

Background: Extra-renal co-morbidities have major influences on outcomes in haemodialysis (HD) patients. Obtaining co-morbidity data from medical records can be cumbersome and relies on the availability and quality of these records. A self-report co-morbidity questionnaire could be a useful adjunct. Our objective was to validate a self-report comorbidity questionnaire in HD patients.

Methods: Each study subject was administered a self-report co-morbidity questionnaire when they attended for HD sessions. The questionnaire was based on that developed by Sangha et al, previously validated in general medical and surgical patients. Patients were asked to indicate whether they suffered from any of eight co-morbid conditions, and for positive responses whether they received any treatment for the condition and whether it limited their activities. We examined the concordance between each of the reported conditions and data obtained from medical records using Cohen's kappa score. We also evaluated the influence of age, sex and ethnicity on the accuracy of co-morbidity reporting.

Results: 282 HD patients (105 female, 177 male) completed the self-report questionnaire. Mean age was 64.1 (±15.2). There were 201 White, 51 Asian and 30 Black patients. There was substantial agreement between reported and recorded co-morbidity for cardiovascular disease (Kappa=0.639), cancer (0.669) and liver disease (0.658), almost perfect agreement for diabetes mellitus (0.957) and only fair agreement for lung disease (0.272), arthritis (0.348) and depression (0.288). All levels of agreement were highly significant ($p<0.001$). There was no significant difference in concordance rates among different gender or ethnic groups. Whilst the presence of lung disease was over-reported in medical records compared to patient self-report, arthritis and depression were under-reported in medical records.

Conclusions: Self-report co-morbidity is a simple, reliable and useful tool to obtain co-morbidity data in HD patients. The poor concordance for arthritis and depression may be largely due to sub-optimal documentation of these conditions in medical records. The approach may be a useful tool in improving medical record-keeping.

TH-PO719

Serum Albumin Variability and Prognosis of Maintenance Hemodialysis Patients Pu Lei, Hong Daqing, Li Wang. *Renal Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: Serum albumin indicates patient nutritional status, its mean concentrations were reported to be initmate relationship with MHD patients' prognosis, but none was report to indetify the fluctuation of albumin and clinical situation. So the objective of this study was to analyze the serum albumin levels and fluctuation of maintenance hemodialysis patients, and its impaction on prognosis, retrospectively.

Methods: We retrospectively analyzed 129 MHD patients from Augsust 2010 to December 2012, serum albumin was measured more than 3 times. The occurrence of hospitalization and deaths were recorded. Hospitalization and/or deaths were defined as an adverse event. The average level of serum albumin of every patient was calculation .the mean serum albumin of all patients as a standard to measure the level fluctuation of serum albumin. SDALBmean=$\sqrt{\sum}(x_i\text{-}40)^2/N\text{-}1$ was the equation to calculation the fluctuation of serum albumin. Chi-square test analyze the association between the levels and fluctuation of serum albumin with the adverse events.

Results: 1) The occurrence of adverse events was 30%. 2) Adverse events rate 38.2% of patient in the ≤40g/L group significantly higher than those in the >40g/L group, the incidence of 21.3% (p=0.037). 3) Adverse events rate 38.2% of patient in the >2.5 group significantly higher than those in the ≤2.5 group, the incidence of 21.3%. 4)According to the mean serum albumin and SDALBmean, patients were divided into four groups: ALB≤40g/L+ SDALBmean>2.5group, ALB≤40g/L+ SDALBmean≤2.5group, ALB>40g/L+ SDALBmean>2.5group and ALB>40g/L+ SDALBmean≤2.5 group. The incidence of adverse events of every group was 45.7%, 27.3%, 27%, 20%, respectively. The incidence in the first group was significantly higher than that in the fourth group (p=0.022).

Conclusions: Serum albumin and its fluctuation is association with the adverse events of MHD patients. Serum albumin>40g/L, and SDALBmean≤2.5 may be help to reduce the incidence of adverse events, improve the prognosis of maintenance hemodialysis patients.

Funding: Clinical Revenue Support

TH-PO720

ESRD in the Very Elderly Population Jeena Sandeep,[1] Mark E. Williams.[2] *[1]Internal Medicine, Mount Auburn Hospital, Cambridge, MA; [2]Nephrology, Joslin Diabetic Center, Boston, MA.*

Background: Diabetes is a major risk factor for progression to ESRD in the geriatric population. However, data on incidence, prevalence and survival trends of diabetic (DM) and nondiabetic patients with ESRD on dialysis in the very elderly population (>90 years) are limited. The objective of this study was to compare the incidence, prevalence and mortality effect of diabetes on ESRD on dialysis in the very elderly population. We hypothesized that the mortality rates are higher in the group with DM and ESRD.

Methods: We have conducted a retrospective study using the USRDS database from 1999-2009 to evaluate the trends in incidence and outcomes in the very elderly group of patients with ESRD on dialysis, in the DM and non diabetic groups. Our patient population included all races, sex and ethnicity and all modalities of dialysis.

Results: The incidence of both DM and nondiabetic population above 90 years of age with ESRD on dialysis has increased over the years from 1999 through 2009 (P=0.0001). The percentage prevalence of DM-ESRD in the very elderly population on dialysis has gone up from 15.3 % to 20.3%. The unadjusted mortality rate per 1000 patient years was the same with both groups (P=0.086, 95 % CI-4.2 to 52.6). We studied the baseline characterestics, BMI and mean GFR between diabetic and nondiabetic population and found that mean eGFR in DM patients at intiation of dialysis was higher (P= 0.0058) than nondiabetic population and BMI was significantly higher in DM-ESRD (P=0.0001). For both groups combined, vascular access in the very elderly group was observed to be catheter only (76%) compared to catheter with AV graft and AV fistula. Among comorbidities during the same period, diabetes was reported in 27%, while hypertension was reported in 83 % and CHF in 48 %.

Conclusions: Mortality rates are comparable between diabetic and non diabetic groups with ESRD on dialysis in the very elderly population. Incidence and prevalence of diabetic population with ESRD on dialysis has increased over the period from 1999 to 2009.

TH-PO721

Characteristics of Peripheral Neuropathy and Quality of Life Evaluation in Hemodialysis Patients Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: To investigate the characteristics of peripheral neuropathy and evaluation of their quality life in homedialysis patients.

Methods: 278 cases ofpatients undergoing hemodialysis treatment were enrolled in a hemodialysis center belong to hospital from January 2011 to June 2011. Patients were divided into the lesion group (110 cases) and non-lesion group (168 cases) according to with or without peripheral neuropathy. EMG was used to detect sensory nerve conduction velocity (SCV), sensory nerve action potential amplitude (SNAP), and motor nerve conduction velocity (MCV) and distal latency (ML). SF-36 scale was used to evaluate the quality of life.

Results: In lesions group, patients appeared paresthesia including numbness and burning sensation in upper limbs (3.6%) and lower limbs (48.2%) respectively. Weakness was accounted for 1.8% in upper limbs, and 11.8% in lower limbs. 9.09% tendon reflexes, 55.45% knee-tendon reflex and 35.5% Achilles'-tendon reflex were disappeared. Average subjects of nerve SCV, MCV and ML extending were slowing down in lesion group compared with non-lesion group (P <0.01). SF-36 score in the lesions group was lower than the control group, but the total score was not statistically different than the non-lesion group (t = 1.896, P = 0.060), only a statistical difference in body pain (t=5.301, P <0.001).

Conclusions: The common symptoms of peripheral neuropathy were numbness of the limbs, weakness in and signs for the change of the tendon reflexes. Sensory nerve abnormal was more popular than that in the motor nerve. Clinicians need to pay sufficient attention to peripheral neuropathy in patients undergoing hemodialysis.

Funding: Government Support - Non-U.S.

TH-PO722

Is the Dialysis Amyloidosis (Carpal Tunnel Syndrome) Preventable? It Is the Cohort Study for 23 Years β2-Microgrobrin, Dialyzer Membrane Junji Uchino, Toyohiko Yoshida. *Mihama Hospital, Chiba, Japan.*

Background: Complications in long-term dialysis patients include dialysis amyloidosis (CTS;carpal tunnel syndrome). Gejyo et. al. reported that dialysis amyloidosis causes by accumulation of β2-Microgrobrin (β2-MG). **Purpose:** We examine relationship with dialyzer membrane, change of the serum β2-MG level and onset of the CTS in the dialysis patients. **Object:** Total of 3,639 patients received maintenance dialysis in our hospital for 1988-2011 year was included.

Methods: During this period, we measured β2-MG level before and after dialysis every year. We examined change the dialyzer membrane according to a year. Furthermore, we examined relationship with history of the carpal tunnel opening resection (including the universal subcutaneous endoscope), serum β2-MG level and the dialyzer membrane. We used ultrapure dialysis fluid (Endotoxin activity level, less than detection limit 0.001EU/mL) from 1994.

Results: The mean β2-MG level before dialysis decreased with maximum of 40.2+-9.3 (1993), 30.0+-5.6 (2001), at least 26.8+-6.0 mg/L (2007) over time. The mean β2-MG level after dialysis decreased with maximum of 18.4+-11.8 (1998), 10.2+-7.6 (2001), at least 7.5+-2.5 (2007) over time. The dialyzer membrane was all cellulose for 1988 years (86% of regenerated cellulose, CTA14%). We started use of Polyslufon(PS) in 1995 and used 7% of PS, cellulose 76%, EVAL 17% in this year. We used 83% of PS, cellulose 5%, EVAL 12% in 2001 and PS 95%, cellulose 2%, EVAL 3% in 2007. After 1996, there is no need to receive carpus trunk opening, and it was almost matched the spread of PS membrane. With the spread of PS membrane, there is no need to receive the carpal tunnel opening resection in patients with β2-MG before dialysis 35-30 mg/L or less and serum β2-MG afer dialysis 10 mg/L or less. These β2-MG level was supposed with the clinical management targeted value. This finding came from the effect of use of high β2-MG removal performance of the PS membrane and use of the ultrapure dialysis fluid.

Conclusions: It is considered that management of β2-MG before dialysis less than 30 mg/L and β2-MG after dialysis less than 10 mg/L can prevent dialysis amyloidosis.

Funding: Private Foundation Support

TH-PO723

Medication Adherence and Its Relationship with Outcome in Pediatric Dialysis Patients Douglas M. Silverstein. *Office of Device Evaluation-Gastroenterology and Renal Devices Branch, Food and Drug Administration, Silver Spring, MD.*

Background: Medication adherence is a major factor determining outcome in children with chronic disease. Children with end-stage renal disease are challenged with requirements for renal replacement therapy in addition to complicated medication regimens.

Methods: We assessed medication adherence in 22 pediatric patients receiving chronic dialysis using a published adherence survey called the Adolescent Medication Barriers Scales (AMBS), designed to assess barriers to medication adherence in adolescent transplant recipients.

Results: Twenty-two patients were receiving chronic dialysis (63.6% receiving hemodialysis (HD) and 36.4% receiving peritoneal dialysis (PD); age 15.9±0.7 years with dialysis vintage 31.6±6.5 months. Adherence was assessed by a 16 question survey with a maximum score (difficulty) of 64. Among all patients, the mean adherence score was 30.9±2.4 (range 16-49; median=27.5). Adherence scores trended lower in patients receiving HD (27.5±2.9 [HD] compared to PD (36.8±3.7) [PD], p=0.06). Compared to HD patients, the mean overall score for all questions was significantly higher in PD patients (1.7±0.2 [HD] compared to PD (2.4±0.2) [PD], p=0.006). 5/16 questions resulted in a mean response ≤1.2 in HD patients compared to 0/16 in PD patients. Neither gender, age nor dialysis vintage was related to adherence scores. Finally, adherence scores trended higher in females compared to males (35.6±3.7 [Females] versus (27.5±2.9) [Males, p=0.1), but this did not reach statistical significance. Markers of mineral bone disease were similar in HD and PD patients. Among all targets in HD and PD patients combined, there was no relationship between adherence scores and the number of targets reached (r= -0.09, p=0.7).

Conclusions: We conclude that there are many barriers to medication adherence in pediatric patients receiving dialysis. The difficulties were more evident in patients receiving PD compared to HD.

TH-PO724

Neointima Formation in AV Fistulas: Role of the Transcription Factor ETS-1 Wenguang Feng,[1] Michael Allon,[1] James George,[1] Silvio H. Litovsky,[2] Ping Hua,[1] Phillip H. Chumley,[1] Gabriel Rezonzew,[1] Edgar A. Jaimes.[1,3] *[1]Nephrology, University of Alabama at Birmingham; [2]Pathology, University of Alabama at Birmingham; [3]Nephrology, VA Medical Center, Birmingham, AL.*

Background: Arteriovenous fistula (AVF) is the preferred vascular access for dialysis but still exhibits high rates of dysfunction, in large part due to excessive neointimal hyperplasia. Better understanding of the mechanisms of neointimal hyperplasia in AVF is essential to prevent access dysfunction. We have previously demonstrated the role of the transcription factor ETS-1 as mediator of neointima formation after balloon injury (HTN'10). Herein, we determined whether ETS-1 contributes to AVF neointimal hyperplasia in an animal model and in hemodialysis patients.

Methods: AVFs were surgically created in mice (C57BL/6) between the right carotid artery and right jugular vein. After AVF the mice were infused with either vehicle or an ETS-1 dominant negative peptide (ETS-1 DN, 1 mg/kg/day SQ, N=6 per group). ETS-1 was measured by immunofluorescence (IF) and RT-PCR. Quantification of wall thickness and lumen to cross-sectional ratio was performed in AVF sections. In human studies, ETS-1 was measured by IF in native vein samples from CKD patients at the time of AVF creation (N=6) and in fistula samples from the same subjects (N=6) at the time of surgical revision 2-7 months after AVF.

Results: In mice we observed ETS-1 expression in areas of neointima 7 days after AVF creation but not in control veins. ETS-1 mRNA expression increased 2.53 ± 0.23 and 4.22 ± 2.08 fold 1 and 3 weeks after AVF respectively ($p<0.05$, N=6). ETS-1 DN decreased neointima by 40% ($p<0.05$, N=6) and increased the lumen to cross-sectional ratio 7 days after AVF: 0.34 ± 0.05 µm to 0.52 ± 0.06 µm ($p<0.05$, N=6). In the human studies normal veins exhibited ETS-1 staining only in the adventitia. In contrast in human AVFs, ETS-1 staining was intense in both neointima and adventitia.

Conclusions: We have demonstrated increased ETS-1 in the neointima of mouse and human AVF. We also demonstrate that ETS-1 blockade reduces neointimal hyperplasia in mouse AVF suggesting that ETS-1 could be a potential target to prevent excessive neointima formation after AVF.

Funding: NIDDK Support, Veterans Administration Support

TH-PO725

Elastin Modulates Outward Remodeling in a Murine Model of Arteriovenous Fistula Failure ChunYu Wong,[1] Margreet De Vries,[2] Carolien Rothuizen,[1] Anton Jan Van Zonneveld,[1] Ton J. Rabelink,[1] Paul Quax,[2] Joris I. Rotmans.[1] *[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Surgery, Leiden University Medical Center, Leiden, Netherlands.*

Background: AVFs have a 1 year primary patency of only 60%, mainly as a result of maturation failure that is caused by insufficient outward remodeling (OR) and neointimal hyperplasia (NH). Elastin is a key component of the extracellular matrix in the vasculature that contributes to the compliance of the vessel. Furthermore, elastin inhibits vascular smooth muscle cell proliferation and migration. We evaluated the effect on vascular remodeling in AVFs in haplodeficient elastin KO mice ($eln^{+/-}$) in comparison with wildtype (WT) C57bl6/j mice.

Methods: Using our novel murine model, we created an end-to-side AVF between the jugular vein and the carotid artery with interrupted 10.0 sutures in $eln^{+/-}$ and WT mice. Animals were sacrificed at 21 days and histological sections were stained with Hematoxillin, Phloxin and Saffron(HPS), Weigert's Elastin and against αSMA, ki67 and fibulin. Histomorphometric analysis was performed by using computer software and included the parameters: internal elastic lamina (IEL) area, lumen area and neointimal area.

Results: At day 21 after surgery, the mean IEL-area and luminal area was significantly higher in elastin KO mice compared to WT mice (both p=0.02, figure 1). No significant change was observed in neointimal area between the two groups. The underlying mechanisms responsible for this beneficial effect on vascular remodeling in AVFs are currently being evaluated.

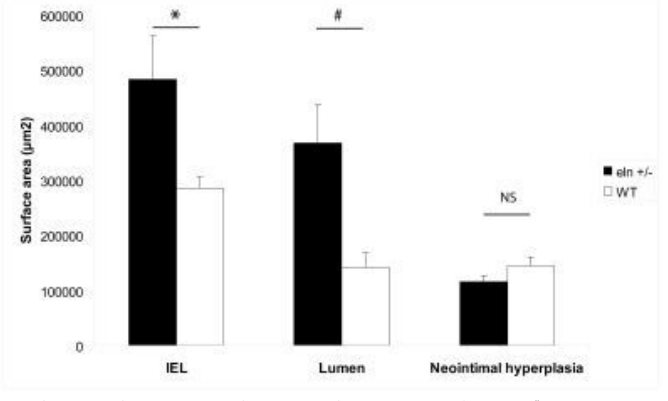


Fig.1 Vesselparameters at day 21.Data in mean±SEM. *p=0.02, #p=0.02, NS=not significant.

Conclusions: Elastin haplodeficiency results in improved outward remodeling in murine AVF. This suggests that elastin might be a suitable target for new interventions aimed to enhance maturation and to improve patency of AVFs.

Funding: Government Support - Non-U.S.

TH-PO726

In Vivo Tissue Engineered Blood Vessels for Hemodialysis Vascular Access Carolien Rothuizen,[1] Febriyani Damanik,[2] Martijn Cox,[3] Ton J. Rabelink,[1] Lorenzo Moroni,[2] Joris I. Rotmans.[1] *[1]Dept of Nephrology, LUMC, Netherlands; [2]Dept of Tissue Regeneration, University Twente, Netherlands; [3]QTIS/e, Netherlands.*

Background: Vascular access is the Achilles' heel of hemodialysis. A tissue engineered blood vessel (TEBV) may provide a durable vascular access. Here we present a novel in vivo tissue engineering approach where the TEBV is grown in the body.

Methods: Cylindrical polymer rods were developed that upon implantation evoke an inflammatory response culminating in the formation of a fibrocellular capsule around the rod. By modulating the implant material characteristics the tissue response can be tailored. Both unmodified, gas plasma treated, chemical etched and growth factor coated PEOT/PBT rods of 4.2mm diameter and 8cm length were implanted subcutaneously in pigs to assess its effect on the tissue response and form a solid basis for a TEBV. Tissue capsules formed around the rods were harvested 4 and 8 weeks after implantation and after extrusion of the rod characterized by histology and mechanical tests.

Results: Macroscopically the tissue capsules appeared as homogenous tubular capsules resembling a human vein (Fig.1). After 4 and 8 weeks the tissue capsules were mainly composed of collagen and α-SMA+ myofibroblasts, both circumferentially aligned (Fig.1). Tissue capsules exhibited burst pressures >2000mmHg and suture strength of >4N, both exceeding the mechanical properties of a human vein. The proof of concept was demonstrated by successful grafting of tissue capsules as arteriovenous (AV)-graft between the jugular vein and carotid artery in pigs.

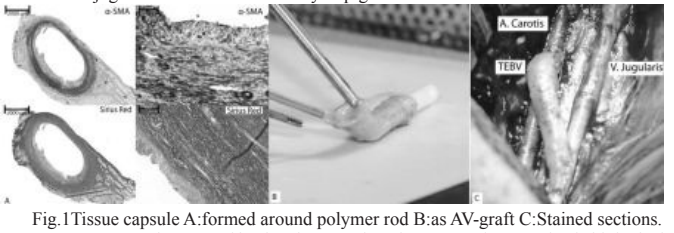


Fig.1Tissue capsule A:formed around polymer rod B:as AV-graft C:Stained sections.

Conclusions: Using a novel in vivo tissue engineering approach a completely biological and autologous TEBV was created with sufficient mechanical strength enabling autologous vascular grafting. Current studies will reveal functionality and durability as AV-graft.

Funding: Government Support - Non-U.S.

TH-PO727

Chronic Kidney Disease Causes Endothelial Barrier Dysfunction in a Mouse Model of Arteriovenous Fistula Anlin Liang,[1,2] Yun Wang,[1] William E. Mitch,[1] Jizhong Cheng.[1] *[1]Division of Nephrology, Baylor College of Medicine, Houston, TX; [2]Department of Orthopedics, First Affiliated Hospital of Chongqing Medical University, Chongqing, China.*

Background: Hemodialysis patients depend on an arteriovenous (AV) fistula for permanent vascular access. The 2-yr primary patency rate is 60%, generally because AV fistula clogged due to intimal hyperplasia at the venous anastomosis. Chronic kidney disease (CKD) can increase neointima formation, but the mechanism is unknown.

Methods: We created AVFs in mice by anastomosing the common carotid artery and internal jugular vein. This new AVF model had the similar pathological changes as human's. CKD was created and the serum BUN from control and CKD mice were assessed (33.25 ± 3.92 mg/dl VS 114.23 ± 12.08 mg/dl, $P < 0.05$).

Results: There was endothelial loss and platelet aggregation ($CD41^+$ cells) especially in the venous anastomosis in 1 day after surgery. Macrophages and neutrophils were present and peaked at 1 week after AVF surgery. Neointima formation (smooth muscle cell accumulation plus extracellular matrix deposition) increased progressively over 1 to 4 weeks. Mice with CKD had ~ 45% more neointima formation VS results in control mice ($P < 0.05$). CKD decreased VE-cadherin expression in endothelial cells and delayed regeneration of the endothelium. There was more inflammatory cell ($Mac\text{-}2^+$ or $CD45^+$) in AVFs of CKD mice. Finally, the AVF was more "leaky" (increased accumulation of Evan's blue) in AVF of CKD mice at 7 days and 14 days VS results in control mice.

Conclusions: In conclusion, we created a new mouse model of AVF and documented that CKD increases neointima formation and endothelial barrier dysfunction. This model allows testing of new agents directed at improving AVF function in CKD patients.

Funding: NIDDK Support, Other NIH Support - American Heart Association

TH-PO728

Women Have Better Endothelial Function, but Need More Time for Arteriovenous Fistula Maturation Tamara K. Jemcov. *Clinic of Nephrology, Clinical Center of Serbia, Belgrade, Serbia.*

Background: Using arteriovenous fistulas (AVF) as vascular access for hemodialysis provides significant benefits, such as greater long term patency, fewer complications and lower infection rates than other vascular access options. Despite these advantages women are still underrepresented among the AVF population. Possible explanations could be the smaller vessels' diameters and the higher rates of primary fistula failure in female HD patients. The aim of the study was to explore morphological and functional parameters in men and women, which can influence the early AVF outcome in the first four weeks after creation.

Methods: Prospective, observational study was performed on 122 patients (66 male) who underwent primary non-dominant forearm AVF creation for HD. Internal diameter of cephalic vein (IDV) and radial artery (IDA), venous distensibility (VD), resistance index (RI) and endothelial function by flow mediated dilatation (FMD) were determined by Color Doppler ultrasound (CDU) examination before the AVF placement. AVF maturation was observed by measuring the blood flow (Qa) and IDV 0, 14 and 28 days after the creation. Depending on the time when the criteria of AVF maturation were reached (Qa≥500ml/min, IDV≥ 5mm, punction with two needles and blood flow ≥ 250ml/min during HD) pts were divided in three goups: group 1- successful maturation (within four weeks), group 2-prolonged maturation after eight weeks and group 3- failure to mature.

Results: Successful maturation was reached in 53% of patients, and prolonged maturation in 36%. Only 11% patients failed to mature the AVF. Sex differences were significant in IDA (p = 0.012) and FMD (p=0.02) and not in IDV, DV and RI. There were more successful AVFs in men (65% vs 39%) and more prolonged maturations AVF in women (46% vs 27%). Significant correlation was found between the successful AVF maturation and male sex (r=0,25; p=0.05). Univariate analysis found male gender to be associated with successful AVF maturation outcome (OR 2.85; 95% CI 0.17-0.72; p=0.005). Nevertheless, after maturation period of eight weeks,there were no differences in maturated AVFs of the two sexes.

Conclusions: Women have smaller IDA, better FMD, and their AVFs need longer period of maturation.

TH-PO729

Arterial Microalcification Is Associateid with Coronary Artery Calcium Score in Hemodialysis Patients Yu-seon Yun, Young Ok Kim, Young Soo Kim. *Department of Internal Medicine, Uijeongbu St. Mary's Hospital, Kyonggi-do, Korea.*

Background: Coronary artery calcium score (CACS) is known as independent predictor of future cardiovascular events, cardiovascular death, and all cause death in hemodialysis (HD) patients as well as general population. We have reported that radial artery micro-calcification (AMC) is closely related to early access failure and aortic stiffness, which is risk factor of cardiovascular mortality in HD patients. This study was designed to evaluate relation of AMC and CACS in HD patients.

Methods: Thirty-four HD patients who received vascular access operation were included in this study. The AMC was diagnosed by pathologic examination of arterial specimen by von Kossa stain, which was acquired during the operation. All patients underwent a multi-detector computed tomography (MDCT) imaging procedure and CACS was calculated. Patients were classified into two groups, according to the CACS, as low (<100), in 17 patients, and high (≥100), in 17 patients. We compared CACS between the patients with and without AMC.

Results: Mean age was 63.8 ± 12.7 years and the male gender was 16 (47.1%). The incidence of AMC was 52.9% (n=18). The mean CACS was 339.8 ± 968.8 (0-5674.1), and the median value was 89.25. Patients with the positive AMC group showed a significantly older age (63.8 ± 11.1 vs 58.8 ± 12.8, p=0.037) and a higher prevalence of diabetes (94.4% vs 37.5%, p=0.004). Positive AMC group showed high incidence of high CACS compared to negative AMC group (88.5% vs 11.8, p=0.000). By binary logistic regression, high CACS was independently associated with positive AMC (OR 25.967, 95% CI 1.361-495.315, p=0.030).

Conclusions: The present study suggests that AMC is closely associated with CACS in HD patients.

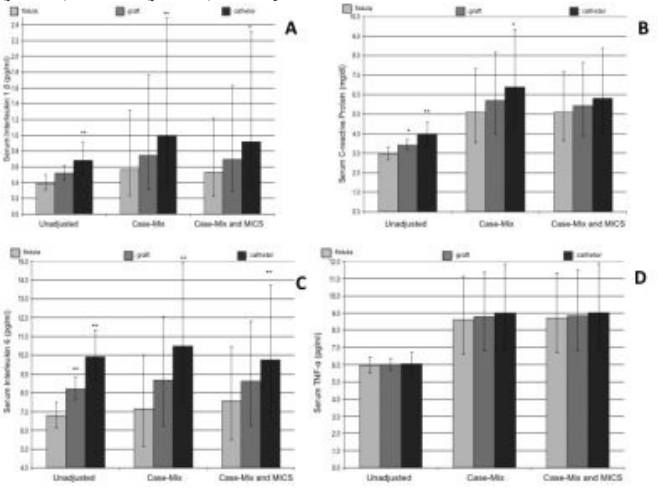
TH-PO730

Association of Vascular Access Type with Inflammatory Marker Levels in Maintenance Hemodialysis Patients Ramanath B. Dukkipati,[1,2] Miklos Zsolt Molnar,[1] Manoch Rattanasompattikul,[1] Jongha Park,[1] Jennie Jing,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LABioMed at Harbor-UCLA; [2]Harbor-UCLA; [3]University of Tennessee.*

Background: High levels of inflammatory markers, which may also be associated with protein energy wasting, are common in hemodialysis (HD) patients and associated with increased mortality risk in these patients. The associations between inflammatory markers level and type of vascular access in HD patients are not clear.

Methods: In a cross-sectional cohort study of 790 HD patients, we examined the associations between inflammatory markers including serum interleukin (IL) 1β, IL-6, C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) and type of vascular access. Unadjusted and multivariate adjusted linear regression models were used.

Results: Mean age of patients was 53±15 years; 54% of patients were men, 52% were Hispanic, 30% were African-American and 53% were diabetic. At baseline 41%, 39% and 20% of patients had fistulas, grafts and catheters, respectively. Compared to patients with fistulas (0.34 [0.16-0.84] pg/ml), patients with grafts (0.41 [0.19-0.79] pg/ml) and catheters (0.61 [0.23-1.61] pg/ml) had significantly higher IL-1β level (p=0.002). Using multivariate linear regression analysis, patients with catheters had significantly higher levels of IL-1β (panel A) and IL-6 (panel C) after adjustment for co-variables.



Similar non-significant trend was found for CRP (panel B), but not for TNF-α (panel D) levels.

Conclusions: Compared to patients with fistulas, patients with catheters have higher levels of IL-1β and IL-6 including after adjustment for important co-variables. The association between types of dialysis access and inflammatory markers may be the underlying link between vascular access and mortality differential and warrant additional studies.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK078012

TH-PO731

A Computational Fluid Dynamic Model of Hemodialysis Fistula Access Mary S. Hammes,[1] Kevin Cassel,[2] Michael Boghosian,[2] Brian Funaki,[1] Jane E. Hines.[1] *[1]University of Chicago; [2]Illinois Institute of Technology.*

Background: The outcomes of ESRD patients on hemodialysis are directly dependent on their vascular access. Regional and national indicators promote placement of arteriovenous fistula (AVF). However, complications occur with cephalic arch stenosis (CAS) being a leading cause of access failure in patients with brachiocephalic fistula (BCF). The etiology of CAS is unknown. This study describes methods used to create computational fluid dynamic modeling (CFD) enabling an understanding of hemodynamic changes when a fistula is created.

Methods: ESRD patients receiving hemodialysis were referred for permanent vascular access with a decision to place an upper arm BCF. The subjects enrolled by written consent with a mapping venogram, Doppler and whole blood viscosity (WBV) preformed pre-fistula and three months. Geometric measurements (GM) and hemodynamic parameters (HDP) were preformed using CFD at stated time intervals.

Results: Two subjects had a venogram, Doppler and WBV measured at baseline and three months. Both had a past history of hypertension and one was diabetic. The Doppler blood flow measured 10 cm from the proximal cephalic arch was 3-6 cm/sec. The vein diameter increased from 0.36 and 0.21 mm pre-fistula to 0.45 and 0.4 mm at 3 months. The Reynolds number from baseline to 3 months, increased from 20.749/27.958 to 260.898/383.238. GM and CFD preformed in 6 subjects at 3 months are shown below.

GM and CFD at 3 months for BCF

Patient	Vein Diameter	Angle of Arch	Min WSS	Max WSS	Reynolds
1	0.52 mm	115°	0.0066	0.0304	101.048
2	0.39 mm	95°	-0.0101	0.0100	850.722
3	0.50 mm	131°	-0.0197	0.0186	245.503
4	0.45 mm	120°	-0.0021	0.0438	260.898
5	1.10 mm	141°	-0.0483	0.0164	422.187
6	0.40 mm	119°	-0.0216	0.0328	383.238

Conclusions: CFD was done using simple clinical tools. A lower Reynolds number was associated with a lower WSS. All subjects at 3 months had minimum WSS which was < 0.076 *Pa,* which is below the threshold where endothelial injury, which could lead to intimal hyperplasia, would be expected to occur. As the incidence of CAS is high in patients with BCF access, the tool of CFD will be used to follow these subjects to observe hemodynamic and geometric changes over time and association with development of stenosis.

Funding: Other NIH Support - NIH 1 RO1 DK90769-01A1

TH-PO732

Effect of Outflow Vein Diameter on Wall Shear Stress Levels in a Benchtop Model of Arteriovenous Fistula Nicholas Franano,[1] Elyse G. Bailey,[1] Howard M. Loree,[1] Geoff D. Tansley.[2] *[1]Flow Forward Medical, LLC, Lowell, MA; [2]School of Engineering, Griffith University, Gold Coast, QLD, Australia.*

Background: Maturation of an arteriovenous fistula (AVF) is highly dependent on flow-mediated outflow vein and inflow artery dilation in response to persistently elevated wall shear stress (WSS) levels, in the setting of an intact and healthy endothelium. Prior studies suggest that moderately increased outflow vein WSS levels of around 2.5–5 Pa result in rapid AVF outflow vein dilation. Prior studies also indicate that very high WSS levels can damage endothelium, which can inhibit flow-mediated dilation. This study examined the effect of outflow vein diameter on WSS levels in a benchtop model of AVF.

Methods: A mock circulatory loop was constructed to model a radiocephalic fistula using a blood analog solution of 35% w/w glycerine at 22 °C. A HeartMate 2000 IP LVAS was used to generate a mean pressure of 120 mmHg in a 55 cm radial artery of 3 mm ID. Flow in the radial artery was adjusted to 41 mL/min (WSS = 0.9 Pa) prior to AVF creation. Cephalic outflow veins of 50 cm length and variously 2, 3, 4, 5, or 6 mm ID were connected to the radial artery in a side (artery) to end (vein) configuration. After AVF creation, arterial and venous flows were measured and WSS levels were calculated assuming Hagen-Poiseuille flow.

Results: As vein diameter increased from 2 to 6 mm, vein WSS decreased from 12.7 to 1.6 Pa, while artery WSS increased from 3.0 to 7.8 Pa (**Table 1**).

Table 1: Flow and WSS Levels in Radial Artery and Cephalic Vein after Mock AVF Creation

	Flow (mL/min)		WSS (Pa)	
Cephalic Vein Diameter (mm)	Radial Artery	Cephalic Vein	Radial Artery	Cephalic Vein
2	135	171	3.0	12.7
3	277	439	6.1	9.7
4	390	647	8.6	6.0
5	335	554	7.4	2.6
6	355	570	7.8	1.6

Conclusions: These data suggest that AVFs with smaller or larger outflow vein diameters have WSS levels that are not optimal for flow-mediated vasodilation, a finding that may shed new light on the causes of AVF maturation failure. Flow Forward Medical is currently developing a medical device to dilate peripheral veins prior to AVF creation that can maintain optimal vein WSS levels across a wide range of vessel diameters.

Funding: Pharmaceutical Company Support - Novita Therapeutics, LLC

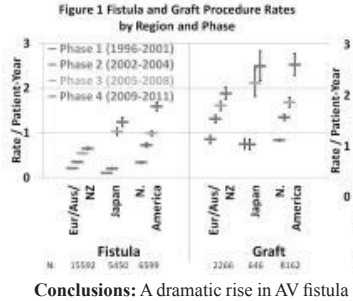
TH-PO733

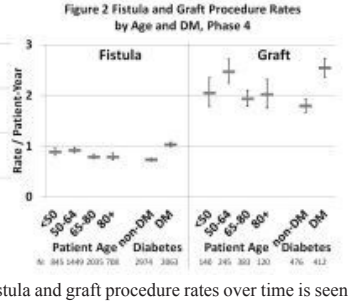
Rising Vascular Access (VA) Procedure Rates over Time Internationally: Findings from the DOPPS Charmaine E. Lok,[1] Keith McCullough,[2] Richard J. Fluck,[3] Lawrence M. Spergel,[4] Vittorio E. Andreucci,[5] Martin P. Gallagher,[6] Mahesh Krishnan,[9] Rachel B. Fissell,[8] Hideki Kawanishi,[11] Rajiv Saran,[10] Friedrich K. Port,[2] Bruce M. Robinson,[2,10] Ronald L. Pisoni.[2] *[1]U. Health Network; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]Derby Hospitals Foundation Trust; [4]Dialysis Management Medical Group; [5]Italian Kidney Foundation; [6]Concord Repatriation & General Hospital; [7]Cleveland Clinic Foundation; [8]DaVita; [9]U. of Michigan; [10]Tauchiya General Hospital.*

Background: Interventional procedures are used to manage hemodialysis (HD) VA. We characterize procedure rates by VA type, region, patient characteristics, and year for AV fistulas and grafts.

Methods: DOPPS data were used from four study phases (1: 1996-2001, 2: 2002-04, 3:2005-08, and 4: 2009-11) to determine VA procedure rates. Rates are calculated as total numbers of VA procedures reported during follow-up divided by total VA follow-up time during the study. Procedures included angioplasty, stent, angiogram, surgical revision, banding, thrombectomy, thrombolysis, and other.

Results: VA procedure rates increased over time in each DOPPS region. Unadjusted procedure rates were significantly lower for fistulas vs grafts (Fig 1). In phase 4, procedure rates were ~40% higher in diabetic than in non-diabetic patients (p<0.001); rates declined slightly with age for fistulas (p=0.01) and had no significant trend for grafts (Fig 2).





Conclusions: A dramatic rise in AV fistula and graft procedure rates over time is seen in North America, Europe/Australia/New Zealand, and Japan. VA procedure rates were higher for diabetic patients, as expected, but they were not higher for older patients. Future work is needed to differentiate and assess whether VA procedures can facilitate initial VA use, and whether more procedures for VA maintenance translate into greater VA longevity.

TH-PO734

Exceeding DOQI Fistula Target-Successful Multidisciplinary Interventions for Arterio-Venous Fistula Creation: The Experience by NW Renal Network 16 Vascular Access Quality Improvement Program Vo D. Nguyen,[1] Chad Lennox,[2] Jim Buss,[2] Lynda K. Ball.[3] *[1]Nephrology, Memorial Nephrology Associates, Olympia, WA; [2]Northwest Renal Network, Seattle, WA; [3]Vascular Access, Fresenius Medical Corporation, Everett, WA.*

Background: In 2001, Network 16 fistula (AVF) prevalent rate was 44%. Substantial variation among individual units (20% to 90% AVF) suggested wide variation in physician practice patterns within the Network.

Methods: GOAL: providing education, tools to providers within INTERVENTION facilities (<40% AVF) to promote AVF creation. METHODS: In 2002, Network 16 sponsored 7 workshops for nephrologists, surgeons, interventional radiologists, vascular access managers. STRATEGIES: 1 Collaborative multidisciplinary teamwork-nephrologist as team leader. 2 Educating local medical community: early referral of CKD patients to nephrologists.3 Training nephrologists, surgeons, and radiologists in Hemodynamics of AVF creation, preop Mapping by physical exam, Doppler of all patients, long term vascular access planning to promote secondary AVF creation when grafts fail, all spectrum of AVF surgery, deep vein Transposition, prevention of Steal. 4 Establishment of Vascular Access Manager, training of dialysis staff in AVF cannulation, including Buttonhole.

Results: AVF in use rate in intervention units (43 facilities, 2913 patients): Pre-intervention: 31.4%. Post: 67.6%%, an increase of 116% (±7.0%) exceeding the 28% (±4.6%) increase in other facilities (49 facilities, 2561 patients). Network 16 AVF prevalent rate was 68.2% in 2011, exceeding DOQI target for AVF rate (65%).

December AVF in use Rate

	2001	2002	2003	2004	2011	2001-2011% Improvement	95% Confidence Int.
Network	44.2%	48.5%	52.9%	57.6%	68.2%	54.1%	±3.4%
Intervention	31.3%	39.8%	48.2%	54.0%	67.6%	116%	±7.0%
Others	53.9%	54.6%	54.7%	59.4%	69%	28%	±4.6%

Conclusions: The deficit of training in AVF creation during and after fellowship programs in the US prevents implementation of DOQI goal of 65% of AVF prevalent rate. The success of interventions at the NW Renal Network 16 in promoting increased use of AVF in units with <40% AVF demonstrates the need for Renal Network sponsored multidisciplinary educational meetings in the US.

Funding: Clinical Revenue Support

TH-PO735

Comparison of Vein Size on Maturation of Radiocephalic AVF Ajith Kumar Puram,[1] Amit Kumar Misra,[1] Andi Qipo,[1] Stuart M. Greenstein,[2] Amit Shah,[1] Erian Shehu,[1] Anjali Acharya.[1] *[1]Jacobi Medical Center, Bronx, NY; [2]Montefiore Medical Center.*

Background: Native AVF is the preferred modality for HD access due to lower rates of complications and a longer survival. KDOQI vascular access guidelines recommendations suggest that maturation rate would be high if the AVF is created with vein size greater than 2 mm. We looked into outcomes of Radiocephalic AVF created with vein size less than 2 mm and clinical factors that affect maturation.

Methods: Retrospective chart review was of patients with CKD/ ESRD with Radiocephalic AVF placement during 2008-2011 with vein sizes less than 2 mm. Various clinical variables were collected as given in the table. Size of the vein was determined by USG vein mapping, or by the impression of the surgeon during procedure.

Primary maturation is considered when fistula is mature at 8-12 weeks after AVF creation. Mature AVF is defined here as successful use of the AVF with blood flow of at least 300 ml/min during HD or removal of catheter. Secondary maturation is considered when the fistula is matured after 12 weeks.

Results: There were 30 patients who had a Radiocephalic AVF created. Results are tabulated in Table 1.

Results

	Vien size < 2 mm	Vien size > 2 mm	Mann Whitney
Total patients 30	14 (46.6%)	9 (30%)	
Age	50.5 (41.0;59.0)	52.0 (41.5;64.0)	P = 0.82
BMI (median, IQR)	24.6 (22.4;26.3)	29.0 (24.0;38.5)	P = 0.05
Diabetes	5 (35%)	6 (66.6 %)	P = 0.19
HTN	13 (92.8%)	7 (77.7 %)	P = 0.89
PVD	0	1 (11.1 %)	P = 0.79
CAD	3 (21%)	4(44.4 %)	P = 0.20
Smoking in past	4 (28.5%)	5(55.5 %)	
Smoking current	2 (14.2%)	3 (33.3 %)	
ASA	10 (71.4 %)	5(55 %)	P = 0.54
Plavix	0	1 (11.1 %)	P = 0.79
No of BAM procedures	10	3	
Average Catheter days	108 days	226 days	P = 0.004
Primary patency	4 (28%)	0 (0%)	P = 0.03
Secondary patency	7 (50 %)	8 (88.8 %)	P = 0.17
Total patency	11 (84.6%)	8 (88.8%)	P = 0.89

Conclusions: Our preliminary data shows that patients with vein size less than 2 mm can have functional AVF created with better outcomes. Vein size alone should not be exclusion criteria for placement of an AVF. This is important as it will help reach a higher AVF rate especially with the proposed new CMS QIP program. This data needs to be confirmed in a larger cohort of patients.

Funding: Clinical Revenue Support

TH-PO736

Vascular Function Prior to AV Fistula Creation in the Hemodialysis Fistula Maturation (HFM) Cohort Study Laura M. Dember,[1] Peter B. Imrey,[2] Maiann Duess,[3] Joseph Vita,[3] The HFM Study Group.[4] *[1]U Penn; [2]Cleveland Clinic; [3]Boston U; [4]NIDDK.*

Background: Little is known about the plausible relationship between hemodialysis fistula maturation and underlying vascular function, in part because previous studies have included small numbers of patients.

Methods: Prior to fistula creation surgery, participants in the ongoing NIDDK HFM Study undergo flow-mediated (FMD) and nitroglycerin-mediated (NMD) brachial artery dilation, carotid-femoral (CF) and carotid-radial (CR) pulse wave velocity (PWV), and venous occlusion plethysmography (VP) to assess endothelium-dependent and -independent arterial function, central and peripheral arterial stiffness, and venous distensibility, respectively. We examined the individual associations of age, sex, diabetes, and dialysis treatment with each vascular function measure in joint, additive multiple linear regression models.

Results: Measurements available as of 6/5/12 are summarized in the **Table**. For all tests, the range of values is broad. As in non-CKD populations, arterial function was better in female (FMD +1.10%, p=0.047; NMD +3.09%, p<0.0001; CR-PWV -0.46 m/sec, p=0.021), younger (FMD -0.50%/decade, p=0.012; NMD -0.99%/decade, p<0.0001; CF-PWV +0.74 m/sec/decade, p<0.0001), and non-diabetic (FMD +2.02%, P=0.0002; NMD +3.01%, p<0.0001; CR-PWV -0.44 m/sec, p=0.024; CF-PWV -1.67 m/sec, p<0.0001) patients. Venous distensibility did not vary with age or sex, but was lower in diabetics (-0.6%/mmHg, p=0.038). Patients treated with dialysis had better FMD than those not yet receiving dialysis (absolute difference 1.1%, p=0.033).

Vascular Function in HFM Participants

	FMD (%)	NMD (%)	PWV-CR (m/sec)	PWV-CF (m/sec)	VP Capacitance %/mmHg	VP MVO %/min/mmHg
	N=393	N=333	N=313	N=312	N=421	N=421
Median	3.85	5.90	8.8	10.5	0.048	0.35
25th-75th percentile	1.13-7.66	2.56-10.91	7.6-9.9	8.6-12.8	0.034-0.060	0.24-0.48

Abbreviations: MVO, maximum venous outflow

Conclusions: Among individuals with advanced CKD, older age, male sex and diabetes are associated with greater impairment in arterial function. The broad range of values for all types of vascular function tests will facilitate assessment of the association of vascular function with fistula maturation in the HFM study.

Funding: NIDDK Support

TH-PO737

Maturation of Arteriovenous Fistulas Placed Prior to Dialysis Initiation Craig Solid,[1] Robert N. Foley.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Few data exist describing the percent of placed arteriovenous fistulas (AVFs) that reach maturation or how long they take to mature. Starting on July 1, 2010, Medicare claims billed by outpatient (OP) dialysis clinics include information on the type of vascular access (VA) used at each dialysis session.

Methods: Using the USRDS database, we identified patients who initiated hemodialysis (HD) between July and December of 2010, were aged 67 years or older at initiation, and had a Medicare claim for an AVF insertion within 1 year prior to initiating HD. OP dialysis claims were used to determine the type of VA the patient used at their first OP dialysis session. The percent of patients whose AVF was used at their first OP dialysis session was calculated, stratified by the number of days prior to initiation when it was placed.

Results: We identified a total of 2,476 patients aged 67+ who initiated in the last half of 2010 and had a AVF placed within a year prior. As Table 1 displays, only 3% of those who had their AVF placed less than 30 days prior used it at their first OP dialysis session. Almost one-third of those who had it placed at least 60 days prior had a functioning AVF upon initiation. However, only 37% of patients whose AVF was placed 6 to 12 months prior to initiation used it on their first OP dialysis session.

Patients Whose AVF Matures in Time for Initiation

Days Prior to ESRD that AVF was Placed	% Who use AVF at first OP dialysis session
< 30 Days	3%
31 - 60 Days	20%
61 - 90 Days	30%
91 - 180 Days	36%
181 - 365 Days	37%

Incident Patients July-Dec 2010, aged 67+ with an AVF insertion claim prior to ESRD

Conclusions: Within this population, less than 40% of patients who had an AVF placed prior to initiation used that access at their first OP dialysis session, although that percent was influenced by the length of time between placement and initiation.

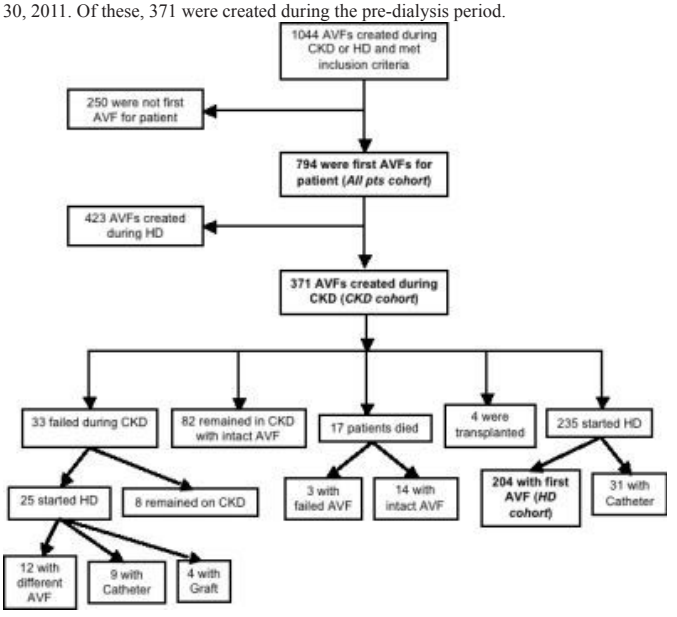
Funding: NIDDK Support

TH-PO738

Arteriovenous Fistulas Created before Dialysis Start: Ongoing Evaluation of Preemptive AVF Outcomes Monica C. Beaulieu,[1,2] Alexandra Romann,[2] Mercedeh Kiaii.[1] *[1]Division of Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]BC Renal Agency, BC, Canada.*

Background: The Provincial Vascular Access Services Team in British Columbia (BC), focuses on improving vascular access (VA) outcomes. Since 2008 we have been able to capture 100% of key VA data elements due to provincial funding for data entry. We describe the outcomes of preemptive fistulas created in CKD patients between 2008-2010.

Methods: A retrospective analysis of prospectively collected data identified 794 first AVFs created between April 1, 2008 and March 31, 2010 with follow-up data until Sept 30, 2011. Of these, 371 were created during the pre-dialysis period.



Results: Patients with a preemptive fistula created were followed by nephrology for a median of 16 (5-39) months prior to creation. Median eGFR at AVF creation was 13 (11-16) mmol/L. In the CKD cohort (n=371), primary failure occurred in 81 (22%) of AVFs.

260/371 (72%) patients started HD by the end of follow-up. 216/260 (83%) started HD with a fistula (first fistula in 204, subsequent fistula in 12). 17 patients died prior to dialysis start (14 with a mature fistula, 3 with a failed fistula). Of first AVFs that were created during CKD and then used for HD, 1, 2 and 3 year patency rates were 78, 64, and 64% for primary patency; 99, 96, and 94% for secondary potency and 97, 94, and 91% for functional patency.

Conclusions: We report the outcomes of pre-emptive fistula creation in British Columbia. In our cohort, 72% of patients started dialysis within the follow-up period and very few died before dialysis start, indicating appropriate selection of patients. As vascular access guidelines continue to promote fistula creation in the pre-dialysis period, ongoing outcome evaluation is required to ensure appropriate patient selection.

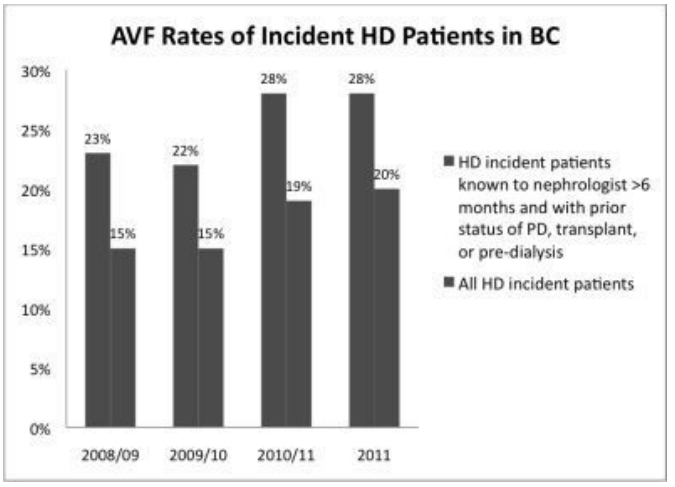
TH-PO739

The Power of Data: British Columbia's Provincial Initiative of Regular Vascular Access Outcome Reporting Improves Arteriovenous Fistula Rates Monica C. Beaulieu,[1,2] Alexandra Romann,[2] Mercedeh Kiaii.[1] *[1]Division of Nephrology, University of British Columbia, Vancouver, BC, Canada; [2]British Columbia Renal Agency, Vancouver, BC, Canada.*

Background: The interdisciplinary Provincial Vascular Access Services Team (PVAST) in British Columbia, supported by the BC Renal Agency, focuses on improving vascular access (VA) outcomes. Phase 1 of the initiative focused on ensuring standardized guidelines, clinical pathways, education and targets (2005-2008). Phase 2 of the initiative (2009-present) involves regular reporting of VA outcomes at the provincial, health authority, hospital, and provider level. We hypothesized that regular reporting of VA outcomes in a real world environment would improve AVF incidence over time.

Methods: Resources were required to ensure provincial engagement, development of metrics, and the accurate capture of data and data analysis. These included a part time project manager, physician champion, and statistical support. Estimated total provincial costs were $27,000/yr. Data capture was possible through an electronic provincial renal database with a customized vascular access module. Reports included patient demographics, vascular access incidence and prevalence, vascular access performance and wait times. A detailed "why catheter" report looked at provider, patient or system barriers.

Results: Through this initiative, the provincial AVF incidence rate improved from 15% in 2008/09 to 20% in 2011. For patients known to nephrologists > 6 months, an increased % of patients started HD with an AVF (23% in 2008/09 to 28% in 2011).



Conclusions: Regular reporting of vascular access outcomes has lead to an improvement in the AVF incidence rate in British Columbia despite no change in the resources available for creation and maintenance of AV fistulas. The regular reporting of VA outcome data may be an important and cost effective strategy to improve VA outcomes.

TH-PO740

Progression to End-Stage Renal Disease among Patients with Preemptive Vascular Access Placement Chenyin He, Kirsten L. Johansen, Chi-yuan Hsu, Nisha Bansal. *UCSF.*

Background: National guidelines recommend that patients approaching ESRD be referred for preemptive vascular access placement within 1 year prior to the start of hemodialysis (HD). Whether these recommendations translate into timely and appropriate vascular access placement is unknown. We studied the rate of progression to ESRD among patients with preemptive vascular access placement.

Methods: In this retrospective chart review, we examined the clinical course of consecutive adults with advanced chronic kidney disease (CKD) cared for in the nephrology ambulatory faculty practice at the University of California, San Francisco (UCSF) between July 1, 1999 and December 31, 2010 and the San Francisco Veterans Affairs Medical Center (SFVAMC) between July 1, 2009 and June 30, 2010. We reviewed all patients who had preemptive vascular access placement, defined as either an arteriovenous fistula (AVF) or graft (AVG).

Results: Among 116 patients from UCSF with preemptive vascular access (109 AVF and 7 AVG), mean age was 64 years, 48% were men, 24% were black, 40% had diabetes and mean estimated GFR 16.1 ml/min/1.73m^2 based on MDRD equation. Median follow-up time was 10.8 [IQR 3.1, 24.3] months. Within 1 year from vascular access placement, 57 (49%) began HD [Figure 1]. Extending following through December 31, 2011, a cumulative 81% patients started HD. Overall median time from vascular access placement to HD initiation was 9.1 (IQR 2.4, 18.9) months. Of the 20 patients from SFVAMC who had preemptive vascular access placement, 7 patients (35%) proceeded to hemodialysis within one year.

Conclusions: In a diverse population of advanced CKD patients followed at two academic medical centers, among those with preemptive vascular access placement, fewer than half started HD within one year. There is opportunity to improve the timing of preemptive vascular access by advancing tools to predict progression to ESRD.

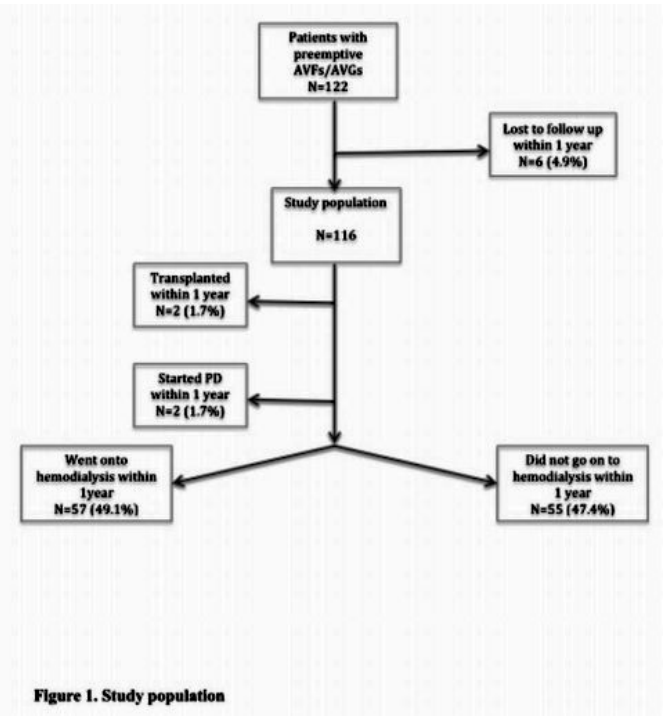


Figure 1. Study population

Funding: NIDDK Support

TH-PO741

Vascular Access Choice at Hemodialysis Initiation: A Decision and Cost Analysis David A. Drew,[1] Joshua Cohen,[1] Navdeep Tangri,[2] Charmaine E. Lok,[3] Daniel E. Weiner.[1] *[1]Tufts Medical Center; [2]University of Manitoba; [3]University of Toronto.*

Background: Current vascular access recommendations promote fistulas first; however there has been no demonstration that this strategy is optimal for all hemodialysis patients. Accordingly, we performed a decision analysis evaluating fistulas (AVF), grafts (AVG), and central venous catheters (CVC) for a patient initiating dialysis with a CVC, incorporating age, sex, and diabetes, to address the mortality and costs associated with each vascular access option attempted.

Methods: A decision tree model was constructed to reflect typical vascular access initiation and courses of incident hemodialysis patients. Patients were assigned one of three vascular access choices: maintain CVC, attempt fistula, or attempt graft. Probabilities of primary and secondary patency for each access type were explicitly modeled, with success modified by age, sex and diabetes. Access specific mortality was incorporated using pre-existing cohort data including terms for age, sex and diabetes. Costs were ascertained from the 2010 USRDS report for annual access-related costs and CMS for procedure costs.

Results: An AVF attempt strategy was superior in regards to mortality and cost compared to AVG and CVC for the majority of possible patient characteristic combinations, especially for younger, non-diabetic males. In contrast, female diabetic patients had more similar outcomes, regardless of access type.

Table: Mean Life Expectancy (Years) and Total Cost over this Life Expectancy

Model patient characteristics	AVF Attempt Strategy		AVG Attempt Strategy		Maintain CVC Strategy	
	Life Expectancy	Total Cost	Life Expectancy	Total Cost	Life Expectancy	Total Cost
40 year old, non-diabetic, man	8.6	$31,435	6.2	$43,962	5.0	$28,507
60 year old, non-diabetic, man	4.7	$19,084	3.6	$26,347	3.0	$18,128
80 year old, non-diabetic, man	2.6	$12,129	2.2	$16,690	2.0	$12,267
40 year old, diabetic, woman	5.7	$23,637	5.4	$39,380	4.6	$26,528
60 year old, diabetic, woman	3.2	$14,619	3.2	$25,880	2.8	$16,901
80 year old, diabetic, woman	2.0	$10,344	2.0	$17,025	1.9	$11,872

Conclusions: An AVF attempt strategy was superior in regards to mortality and cost in most patients, but this difference lessened considerably when patients were older, female and diabetic. Further decision analyses can evaluate the impact of other patient combinations and access complications, such as infections to assist in individualization of the optimal access.

Funding: Other NIH Support - T32 Training Grant

TH-PO742

Predictors of Arteriovenous Fistula Use at the Time of Hemodialysis Initiation Varun Chawla, Ranil N. Desilva, Bhanu K. Patibandla, Akshita Narra, Yael Vin, Jan Flesche, Alexander S. Goldfarb-Rumyantzev. *Beth Israel Deaconess Med Ctr, Boston, MA.*

Background: One of the challenges we face to start patients on HD with AV fistulas is- primary fistula failure. In this study, we investigate the factors associated with primary fistula failure.

Methods: Study cohort (n =21,436) is derived from the USRDS data linked to Medicare Claims from 03-08 to identify the first access placed/tried on the patient and included incident HD patients from 05-08, >67 years of age, with AVF as the first access placed. Patients were excluded if they had been transplanted before the initiation of HD and if there was another AVF placed prior to HD initiation. After these exclusions, the study cohort consisted of 20,360 patients. We defined fistula as success when the AVF initially placed was used at the time of HD initiation (n=9,794) and as failure when the arteriovenous graft or catheter was used at the time of HD initiation (n=10,566). Multivariable logistic regression analysis was used to estimate the independent contribution of specific patient characteristics in predicting the success or failure of the AVF.

Results: Primary AVF success is associated with early fistula placement (OR 1.07 per month prior to HD initiation, p<0.001). Pre-ESRD nephrology care also has a significant impact on AVF success (i.e. OR for <6 months, 6-12 months and >12 months compared to no care were 3.22, 3.33, and 4.12 respectively). Glomerulonephritis as the primary cause of ESRD is associated with increased fistula success compared to those whose ESRD is due to diabetes (OR 1.22 p=0.01). Age (OR 0.99 per year, P=0.009), female gender (OR 0.64, P<0.001), black race (OR 0.75, P< 0.001), body mass index (^{3}30kg/m2 compared to <18.5 kg/m2 OR 0.86, P=0.001), history of diabetes (OR 0.84, P<0.001), cerebrovascular disease (OR 0.88, P=0.008), and congestive heart failure (OR 0.77, P<0.001) are associated with primary AVF failure.

Conclusions: Early timing of fistula placement and pre-ESRD nephrology care are the strongest predictors of primary AVF success. Furthermore, the results can be used to design a model to predict the success or failure of an AVF placed based on the patient characteristics.

TH-PO743

Disparities in Arteriovenous Fistula Placement Prior to Hemodialysis Initiation Varun Chawla, Ranil N. Desilva, Bhanu K. Patibandla, Akshita Narra, Yael Vin, Jan Flesche, Alexander S. Goldfarb-Rumyantzev. *Beth Israel Deaconess Med Ctr, Boston, MA.*

Background: In this study we assessed and quantified the effect of the clinical and nonclinical factors determining the access type initially placed on a hemodialysis patient by using a large national cohort.

Methods: Study cohort (N=111,953) is derived from the USRDS data with linked Medicare Claims (from 03-08). We identified the first access placed before HD initiation using medicare claims data. The data from form 2728 was used to identify the patient characteristics. Multivariable logistic regression analysis was used to estimate the independent contribution of specific patient characteristics on predicting the placement of AVF prior to start of HD.

Results: Pre-ESRD nephrology care significantly increases the likelihood of AVF being the first access placed(OR for < 6 months, 6-12 months, > 12 months compared to no care were 9.35, 9.78, and 17.21 respectively). Age (OR 0.98 per year; P < 0.001), females (OR 0.80; P < 0.001), blacks (OR 0.93; P = 0.005), history of diabetes (OR 0.79; P < 0.001), history of malignancy (OR 0.91; P=0.003), history of peripheral vascular disease (0R 0.96 P = 0.04), history of cardiac failure (OR 0.62; P < 0.001), and history of cerebrovascular disease (OR 0.88; P < 0.001) are the factors associated with decreased likelihood of AVF placement as the initial access compared to a catheter. In addition, hypertension (OR 0.85; P = 0.007) or glomerulonephritis (OR 0.95; P < 0.001) as the primary cause of ESRD (compared to diabetes as the primary cause of ESRD) are associated with decreased likelihood of AVF being the first access placed. Living in an urban location (compared to rural) was also associated with reduced chances of AVF as the initial access placed (OR 0.81; P < 0.001).

Conclusions: In spite of the increasing efforts to start hemodialysis patients on AVF, there still exist some differences based on age, race, sex, and comorbidities. Our results suggest that pre-ESRD nephrology care significantly increases the placement of AVF. Identification of these factors is important so that the potential interventions can be suggested to increase the prevalence of AVF.

TH-PO744

Pain Resulting from Arteriovenous Fistulae: Prevalence and Impact Emma L. Aitken, David Kingsmore, Marc J. Clancy. *Department of Renal Surgery, Western Infirmary, Glasgow, United Kingdom.*

Background: The burden of pain from cannulation of AVF and the impact on quality of life is poorly described in the literature.

Methods: We undertook a questionnaire-based study evaluating pain scores in all patients in the West of Scotland dialyzing via an AVF (n=480). Pain on cannulation and chronic pain were assessed using Visual Analogue scores (VAS) and McGill pain score. Chi-squared test was used to compare patients with "severe pain" (VAS >5) to those with minimal pain. Results are presented as median (IQR) (p<0.05 is significant).

Results: 97.5% of patients completed the questionnaire. Median VAS pain score on cannulation was 3 (IQR 2, 5). 24.4% had "severe" pain on cannulation and 3.2% had "severe" chronic pain. Compliance with dialysis was good, however 53 patients (11.3%) reported having cut a dialysis session short due to pain. Most pain was described as sharp (41.7%) or aching (25%). However, a number of patients used words such as "shooting" (4.5%), "burning" (5.3%) and "tingling" (12.4%) indicating a neuropathic element to their pain. 9.6% of patients with severe pain on cannulation and 46.7% of patients with severe chronic pain were found to have a physical complication affecting the AVF (e.g. venous stenosis/pseudoaneurysm). Following treatment of the underlying problem chronic pain improved in 71.4% and resolved completely 14.3% of patients. Brachiobasilic AVF were associated with a higher incidence of severe pain than either brachiocephalic or radiocephalic AVF (50%, 23.3% and 24.4% respectively; p=0.03). There was a trend towards more "severe" pain with rope-ladder cannulation (27.7%) compared to button-hole cannulation (18.2%), however this difference did not reach statistical significance (p=0.09).

Conclusions: Pain from AVF is a poorly recognised and under-reported problem. Whilst severe pain resulting in the avoidance of dialysis is rare, it can lead to significant difficulties and ultimate abandonment of an AVF. Pain is often suggestive of an underlying anatomical problem. This should always be investigated in the first instance. Our findings suggest that pain is multi-modal and neuropathic pain may play a significant role, particularly in chronic pain.

Funding: Government Support - Non-U.S.

TH-PO745

Venous Conductance: A Reliable Predictor of Fistula Thrombosis David H. King,[1] Graeme Taylor,[2] Mo Al-qaisi,[1] Anthony Chan,[1] Sumith C. Abeygunasekara,[1] Yiannis Panayiotopoulos,[1] William D. Paulson,[3] Eric Chemla,[4] Abdelgalil Abdelrahman Ali.[1] *[1]Renal Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Department of Physics, Guy's and St Thomas Hospital, London, United Kingdom; [3]Medical Center, Georgia Health Sciences University, Augusta, GA; [4]Renal Department, St Georges Hospital, London, United Kingdom.*

Background: Venous stenosis is a major cause of thrombosis in native arterio-venous fistulae (AVF). The effectiveness of access surveillance in predicting thrombosis is controversial, and there is no consensus that any test is effective. In this study, we tested the hypothesis that Venous Conductance (VC) can fill this role.

Methods: VC is defined as AVF blood flow (Q) divided by mean blood pressure (MBP) in the feeder artery upstream to the arteriovenous anastomosis; thus VC = Q/MBP. Brachial artery Q was measured by colour duplex scanner. MBP in the distal brachial artery was measured by a novel non-invasive method (BlueDop* www.bluedop.co.uk). We determined the relation between AVF thrombosis and VC <10ml.min^{-1}.mmHg^{-1}. We have previously shown this threshold is associated with significant venous stenosis.

Results: We studied 111 AVF patients with 75 brachio-cephalic and 36 radio-cephalic AVFs, created between 0.5 and 30 months previously. Ninety-three AVFs had VC greater than threshold (range 10 to 52). 92 of these remained patent at 90 days and 1 thrombosed. Eighteen AVFs had VC below threshold; 14 thrombosed within 90 days (range = 0 to 85 days, mean = 24 days). Three had already thrombosed by the time of measurement, with VC values close to zero. One underwent intervention but then thrombosed; 4 remained patent at 90 days. Table shows VC sensitivity = 93%, specificity = 96% and accuracy = 95%.

Table 1 Prediction of thrombosis by VC

	Fail (VC <10)	Pass (VC>=10)
Thrombosed within 90 days	14	1
Patent at 90 days	4	92

Conclusions: Venous Conductance appears to be a practical first line test for AVF intervention. Further studies are needed to confirm these promising results.

TH-PO746

A Study of the Variability of Access Flow Measurements by Thermodilution Method Osasuyi A. Iyasere, Rachel Westacott, Mary Anastasia Quashie-howard. *John Walls Renal Unit, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom.*

Background: Vascular access surveillance by blood flow measurements (Qa) is widely recommended but there is no conclusive evidence for use of any one technique.The blood thermodilution method (BTM) correlates well with the more established transonic method and offers advantages in terms of ease of use.Following the recent adoption of BTM at our dialysis unit, we noticed some unusually variable results. We sought to determine the extent of intraobserver and interobserver variability in access flow measurements by thermodilution (BTM) method and determinant factors.

Methods: Monthly BTM measurements were prospectively obtained from haemodialysis patients at a single centre ,between june and august 2011.Patients were selected into an intervention group,where all measurements were carried out by one person according to protocol, and a control group, with multiple unselected observers.Information relating to patient comorbidities, access and dialysis parameters were recorded for each patient.The coefficients of variability for measured access flow(cvBTM) were determined using SPSS.Measurements with a coefficient value greater than 20% were considered highly variable.

Results: Of the 60 dialysis patients selected, 48 had more than one BTM measurement, 27 of which were from the intervention group. 28.6% of BTM measurements from the intervention group had a coefficient value (cvBTM) greater than 20% (range – 0.3 to 54%;mean -17.86%), compared to 52.3% of measurements from the controls (range – 0.7 to 109.5%; mean – 26.06%). This difference was statistically significant (p -0.038). Whilst the coefficients of variability for mean arterial pressure (cvmap) and ultrafilration rate (cvuf) correlated positively with cvBTM in the control group (Rcvmap=0.426, Rcvuf=

0.242) and intervention group (Rcvmap=0.222, Rcvuf= 0.089), the relationship did not reach statistically significance. There were no significant associations between cvBTM and other dialysis parameters.

Conclusions: Access flow readings were more variable in the control group, suggesting that having a single user improves the reliability of BTM measurements.

TH-PO747

Elevated Pulse Pressure Predicts Arteriovenous Fistula Non-Maturation at 3 Months Austine Y. Mengnjo, Robert M. Perkins, Anil Kotru, Taher M. Yahya. *Geisinger Clinic.*

Background: Up to 50% of all newly created arteriovenous fistulas (AVF) fail to mature (FTM). Among factors previously associated with FTM, hemodynamic characteristics have not been reported.

Methods: Using prospectively collected hemodynamic data from all patients with stage 4 or 5 CKD not yet receiving renal replacement therapy evaluated in a single nephrology clinic in central Pennsylvania between January 1, 2010 and June 30, 2011 and referred for first AVF creation, we retrospectively tested the hypothesis that elevated pulse pressure independently predicts FTM at 3 months from date of access creation. Pulse pressure was defined as the mean of the difference between the 4 most recent systolic and diastolic clinic blood pressure readings prior to the date of surgery. Baseline demographic and clinical information was extracted from the electronic health record. AVF maturation was defined as attainment of all 3 of the following within 12 weeks of access placement: venous segment depth ≤ 6 mm, length ≥ 6 cm, and diameter ≥ 6 mm. Measurements were obtained by duplex scans performed by clinic protocol at 4, 8, and 12 weeks after surgery. A multivariate logistic regression model was created using all variables associated ($P \leq 0.10$) in univariate analysis with FTM at 12 weeks to quantify the association between pulse pressure and FTM.

Results: 79 patients met inclusion criteria. Median age was 62 years, 51% were female, 62% had diabetes, 38% had coronary artery disease, 28% had CHF, and the median estimated GFR was 18 ml/min/1.73 m^2. The baseline median (IQR) pulse pressure was 63 (55.5, 69.5) mm Hg. 49% of fistulas failed to mature at 3 months. In univariate analysis, only pulse pressure and diastolic blood pressure predicted FTM at 3 months. Adjusted for diastolic blood pressure, pulse pressure independently predicted FTM at 12 weeks: each 1 mm Hg increase in pulse pressure was associated with a 7.2% (95% CI 2.4-11.9%) increased risk of AVF non-maturation (P=0.003).

Conclusions: Wide pulse pressure independently predicts FTM at 3 months and may represent a clinically expedient discriminator for PTFE graft placement over AVF creation for individuals in need of timely permanent hemodialysis access.

TH-PO748

The Usefulness of Vasc-Alert® to Identify Significant Dialysis Access Stenosis Gerald A. Beathard, Aris Q. Urbanes, Terry Litchfield. *Lifeline Vascular Access, Vernon Hills, IL.*

Background: An optimally functional vascular access (VA) is the lifeline for a hemodialysis (HD) patient. As a result of FFBI and other QI programs, the use of AV accesses for HD is on the rise. VA is not without complications. The primary complication that causes AV accesses to fail is stenosis with subsequent thrombosis. Surveying for stenosis can be performed in a variety of ways. Clinical monitoring, measuring flow, determining pressure, and measuring recirculation are all methods of monitoring the VA. In addition, stenosis can be directly visualized, through noninvasive techniques such as CFD, or through minimally invasive venography.

Methods: In this study, several dialysis centers of a large dialysis organization (LDO) utilized a software system that has developed an algorithm that derived access pressure ratios (intra access pressure/MAP) Vasc-Alert®. 254 patients with functional vascular access were included in the study group. A referral for venogram and angioplasty (if the stenosis was greater than 50%) to a dedicated vascular access center was completed if the patient met the criteria for stenosis alert.

Results: One hundred twenty nine (129) patients were sent for diagnostic angiography. All but five (5) patients (3.8%) had a significant stenosis and were successfully treated with angioplasty. No patients had a thrombosis episode in the study period. None missed any dialysis treatments and the stenosis pre-procedure was significantly less than those sent from other methods of surveillance (71% stenosis for study patients vs. 89% for patients referred from other surveillance methods). In addition, procedure times were reduced as well by 5.3 minutes per procedure.

Conclusions: Vasc-Alert® is an effective tool to detect VA stenosis. To maintain the functionality of the access for the HD patient, a team approach is imperative. The collaboration and cooperation of the patient, nephrologist, dialysis nurse and technician, vascular access coordinator, interventionalist, and vascular surgeon is necessary to preserve this lifeline. The dialysis organization and interventional center can optimize access care with the use of tools to identify access stenosis.

TH-PO749

Should the Current Access Blood Flow Threshold for Arteriovenous Fistula Stenosis Repair Reassessed? Interim Analysis of a Randomized Controlled Trial Nicola Tessitore,[1] Valeria Bedogna,[1] Albino Poli,[2] Alessia Pendino,[1] Antonio Lupo.[1] *[1]Div. Nephrology - Hemodialysis Unit Policlinico Borgo Roma, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; [2]Dept. Public Health, Azienda Ospedaliera Universitaria Integrata, Verona, Italy.*

Background: Current guidelines recommend to refer for imaging and treatment for stenosis arteriovenous fistulae (AVF) with an access blood flow (Qa) <400-500 ml/min. Significant (>50%) stenosis, however, can be detected in AVF with Qa>500 ml/min, but whether correcting such stenosis with "high" blood flow is of value, is currently unknown.

Methods: From July 2006, we performed a Randomized Controlled Trial enrolling adult pts with mature AVF with >50% stenosis and Qa>500 ml/min (measured by ultrasound dilution). AVFs were randomized either to elective stenosis repair by angioplasty or surgery (TX) or control (C) with intervention triggered by signs of impending thrombosis (Qa<350 ml/min or clinical abnormalities such as persistent difficult cannulation, low blood pump flow (Qb) or a decline in dialysis dose). Outcomes of the trial were access failure (a composite of thrombosis and impending thrombosis) and access loss.

Results: Until May 2012, 58 pts were enrolled, 26 in C and 30 in TX. The two arms had similar pts age, gender, diabetes, cardiovascular disease, AVF age, follow-up (28±18 vs 33+22 mo), and Qa (798[95%CI 710-886] vs 693[95%CI 613-773]). During the follow-up, 16 AVFs failed in C (10 thrombosed) and 9 in TX (7 thrombosed), while 12 were lost in C and 5 in TX. Failure rate was 0.27[95%CI 0.15-0.43] event/AVF-y in C and 0.12[95%CI 0.06-0.24] in TX, equal to an incidence rate ratio(IRR) for TX of 0.47[95%CI 0.18-1.13],p=0.066. Access loss rate was 0.19[95%CI 0.09-0.32] event/AVF-y in C and 0.06[95%CI 0.02-0.14] in TX, equal to a IRR for TX of 0.32[95%CI 0.09-0.92],p=0.024.

Conclusions: Our interim analysis shows that elective stenosis repair in AVF with Qa>500 ml/min portends an unsignificant halving in the risk of access failure and a significant three-fold reduction in the risk of access loss, raising the question of whether the Qa threshold for evaluation and treatment for stenosis recommended by the current guidelines should be reassessed.

TH-PO750

Time-Course Analysis of Access Blood Flow Revealed AVF Was Needed Much Longer Time to Maturate in Diabetic Patient Kyung Nam Lee, Dong Won Lee, Soo Bong Lee, Il Young Kim, Ihm Soo Kwak, Eun Young Seong, Harin Rhee, Byeong Yun Yang. *Pusan National University School of Medicine, Busan, Republic of Korea.*

Background: Access blood flow (Qa) is helpful for early identification of native arteriovenous fistula (AVF) stenosis in hemodialysis (HD) patients. Diabetes mellitus (DM) is one of the most important risk factors of AVF stenosis. Therefore, we analyzed the changes of Qa with time in DM and non-DM HD patients.

Methods: Forty five patients were enrolled, who started AVF cannulation for the first time in our dialysis center after a minimum maturation period of 12 weeks. Qa was measured monthly using ultrasound dilution (Transonic® Flow QC HD02) for 4 months after the first cannulation. Three patients were excluded, who have taken any intervention for AVF during follow up periods. Time courses of Qa in DM and non-DM groups were analyzed using general linear model for repeated measurement (SPSS18).

Results: We included 29 DM and 13 non-DM patients. There were no statistically significant differences in demographic, chemical, hemodynamic parameters and use of anti-platelets agents. Mean values of Qa at the first month were 576.6 ± 293.2 and 1003.9 ± 320.1mL/min respectively in DM and non-DM patients ($p < 0.001$). Eighteen patients showed Qa under 600mL/min in DM patients.Considering not only the first measurement per patient, but also another 3 times consecutive measurements, Qa was significantly lower in DM patients compared to non-DM patients (672.9 ± 392.0 vs. 933.9 ± 439.6mL/min, 679.7 ± 446.0 vs. 918.5 ± 372.9mL/min, 750.3 ± 552.6 vs. 933.9 ± 495.1mL/min respectively, p=0.030). Interestingly, Qa showed the tendency to increase continuously in DM patients. In contrast, Qa of non-DM patients showed no significant changes with time and were within normal range, over 600mL/min during follow up period.

Conclusions: In conclusion, Qa in DM patients was lower than in non-DM patients throughout the follow up period. However, Qa in DM showed improved slowly with time. It suggests that newly created AVF in DM patients should be needed longer time to be fully maturated, and have higher risk for stenosis in earlier period after first cannulation.

TH-PO751

Optimizing Vascular Access Outcomes: Surveillance Using Ionic Dialysance Based Vascular Access Flow Improves Fistula Survival Rates Thyago Proença de Moraes,[3] Zoe C.L. Pittman,[2] Chris W. McIntyre,[1] Maarten W. Taal,[2] Richard J. Fluck.[2] *[1]School of Graduate Entry Medicine and Health, University of Nottingham Medical School at Derby, Derby, United Kingdom; [2]Department of Renal Medicine, Derby Hospitals NHS Foundation Trust, Derby, United Kingdom; [3]Health and Biological Sciences, Pontificia Universidade Catolica do Parana, Curitiba, Parana, Brazil.*

Background: Vascular access in hemodialysis (HD) is crucial for patient outcomes but arteriovenous fistulas (AVF) survival rates remains low and the optimal surveillance strategy is still not defined. Intra-access flow measurement (Qa) has been used to monitor AVFs and grafts but data looking at its impact on clinical outcomes is lacking. The aim of

this study was to evaluate the impact of a regular access management program based on an iterative cycle of ionic dialysance based measurement of Qa and radiological intervention.

Methods: All 187 patients undergoing HD from a single center in January 2009 were followed during 3 years and their Qa assessed monthly by ionic dialysance technique. Those with either an initial Qa of less than 600ml/min, a decremental change of 25% or clinical suspicion of stenosis were referred for fistulogram studies. For each procedure, the lesion types, site, severity, the angioplasty balloon and the outcome were recorded. The Qa measurement was repeated after the procedure.

Results: A total of 158 fistulograms were performed in 92 patients with a mean of 0.45 interventions per patient per year. In only 9 studies no lesion was found. A single venous stenosis was confirmed in 77, 2 in 37 and >3 in 12 studies. An anastomotic stenosis was demonstrated in 31 exams, and arterial stenosis in 10. 20 studies had mixed lesions. 148 angioplasties were performed. Median Qa prior to vascular intervention was 373 ml/min increasing to 566 ml/min after angioplasty ($p < 0.001$). Overall patency rates of AVF in these prevalent patients at 1, 2 and 3 years were 99%, 94% and 90.7% respectively.

Conclusions: Routine use of this surveillance technique predicts the existence of functionally significant vascular lesions. A procedural program based on this is associated with high AVF survival rates and low per patient intervention burden.

TH-PO752

Vascular Access Management in Patients with Failing Kidney Transplants

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Background: Despite recommendations by NKF/KDOQI and Fistula First to increase pre-dialysis vascular access (VA) placement in CKD patients, only ~20% of U.S. patients initiate HD with a permanent access. The proportion is higher among those patients with at least 1 year of pre-HD nephrology follow-up. There has been extensive publication on pre-HD VA management in patients with advanced CKD, but very little on VA planning in those with failing kidney transplants (KTx). The goal of our study was to compare pre-HD VA management in the 2 groups.

Methods: We compared VA management in 2 groups of patients who initiated HD during a 5-year period: (1) those with a failed KTx, and (2) those with advanced CKD. The analysis was restricted to patients with ≥1 year of follow-up and ≥3 nephrology visits prior to initiating HD. Patient demographics, comorbidities, and pre-HD VA surgery were extracted. Differences between groups were analyzed by the t-test and Chi-square test.

Results: As compared to patients initiating HD after advanced CKD, those initiating HD after a failed KTx were younger, less likely to be black, and less likely to have diabetes or CHF (Table). The proportion of patients with pre-HD VA surgery was lower in the group with the failing KTx (41 vs 60%; hazard ratio 0.69; 95% CI 0.54-0.87, p=0.0008). The proportion of fistulas placed was similar in both groups (70 vs 75%, p=0.54).

Variable	*Failed KTx*	*Advanced CKD*	*p-value*
N pts	123	219	
Pt age, yrs	47±14	60±14	<0.0001
Age >65 yrs	14 (11%)	84 (38%)	<0.0001
Male sex	67 (54%)	109 (50%)	0.40
Black race	71 (58%)	170 (78%)	<0.0001
Diabetes	57 (38%)	130 (59%)	0.02
CHF	11 (9%)	41 (19%)	0.02
Pre-HD VA surg	51 (41%)	132 (60%)	0.0008

Conclusions: Patients with a failing KTx are substantially less likely to receive VA surgery prior to initiation of HD, as compared to patients with CKD. This difference occurred despite the younger age and lower comorbidity in the failing KTx group, factors that would favor fistula success. The reason for this discrepancy is unknown, but may be due to a greater focus on salvaging the failing KTx, at the expense of advanced VA planning. Systematic efforts are needed to increase VA surgery in patients with failing KTx.

Funding: Clinical Revenue Support

TH-PO753

Buttonhole Cannulation versus Rope Ladder Technique and Hemodialysis Access Patency

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Background: Compared with the rope-ladder (RL) technique, buttonhole cannulation (BHC) provides a higher level of patient comfort, easier needle placement and reduction in hematoma and aneurysms. Recent studies have documented a higher risk of infection with the BHC technique. To the best of our knowledge, AVF patency has not been studied in this population. We analyzed the association of BHC with primary patency and clinical outcomes in our center.

Methods: 45 prevalent dialysis patients using BHC were compared with 38 patients using the RL technique over a median of 12 months (inter-quartile range: 4-27 months). We compared outcomes of primary unassisted patency, episodes of bacteremia, access blood flow (Qa), and quality of life (QoL) scores.

Results: The two groups did not differ significantly in demographics except that diabetes was more common in those with BHC as compared to RL (69% vs. 34%; p=0.002). The 3-month primary patency (PP) was 89.5% and 85.8% in the BHC and RL groups, respectively. The 12-month PP was 59.8% and 56.6% respectively. Risk factors associated with diminished PP were age (HR=1.19 per decade; 95% CI: 1.01-1.39; p=0.03) and female gender (HR=2.01; 95% CI: 1.13-3.58; p=0.02). Having BHC was not significantly associated with PP (HR=1.22, 95% CI: 0.65-2.28). Episodes of bacteremia (n=8; p=0.62), mean Qa before intervention (n=39; p=0.42) and after intervention (n=42; p=0.19) did not differ between BHC or RL technique. Having diabetes did not modify the association between BHC and patency (p=0.59). The physical and mental component, burden and symptoms scores from QoL were not different between groups (all p>0.05).

Conclusions: This study shows for the first time that there is no clear association between BHC use and access patency. Despite previous evidence on higher rates of bacteremia and better overall patient satisfaction, we found no significant differences in these clinical outcomes. Given the impending increase in home dialysis, quotidian therapies and emphasis on patient satisfaction, the role of BHC on access patency requires further study.

TH-PO754

Risk of Infectious and Non-Infectious Complications of Permanent Hemodialysis Access

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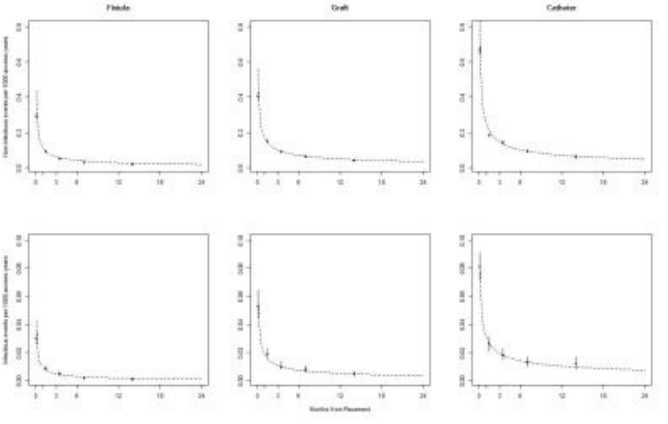
Background: Vascular access complications are a major cause of morbidity in hemodialysis patients. Understanding how the risks of different complications vary over time may help design prevention strategies.

Methods: We used data from the Dialysis Outcomes and Practice Patterns Study I-III to study temporal profiles of risk for infectious (access infection or sepsis) and non-infectious complications (dysfunction requiring interventions) of each permanent access type (fistula, graft and catheter) in incident hemodialysis patients. We used longitudinal data on access-related interventions and infections to define the risk set. We considered multiple accesses per subject, and baseline and time-varying covariates. We studied how rates changed over time to select the best survival model.

Results: Of 8,052 patients identified, 7,140 received at least one permanent access. During a median followup of 14 months there were 10,574 non-infectious events and 1169 access-related infections (568 hospitalizations for sepsis). The hazard for both non-infectious and infectious complications declined over time in all access types, without changing direction. The hazard for both complications declined more quickly in fistulas than in grafts and catheters (Figure 1); difference in Weibull parameter P<0.001). However, only 10% of the cohort had a followup >2.5 years.



Conclusions: The risks for non-infectious complications and access-related infections decline over time in all types of access. Prevention strategies should target the first 6 months after access placement.

Funding: Government Support - Non-U.S.

TH-PO755

Fistula Failure Leads to AV Grafts: Need for Re-Evaluation of CMS Standards

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Background: The 2012 CMS payment schedule incorporates two sub-measures; fistula prevalence and catheter prevalence but dialysis graft prevalence not included. In anticipation of longer dialysis duration, access site availability needs to be preserved by acceptance of graft as viable alternative.

Since 2003 CMS effort has been successful in increasing fistula prevalence from 32.2% to 55.8% and decreasing catheter rate prevalence from 26.9% to 23.8 %. Catheter incidence rate of 82% remains unchanged. The drawback to this is a high primary fistula failure rate compared to grafts with need for catheter dependence. The performance standard is set at 58% with benchmark set at 74% for fistula prevalence. Catheter performance standard is set at 14% with benchmark set at 5%. Financial penalty is to be incurred for failing the goal. The current standards may actually cause patients to lose access sites. For example if the fistula creation is not possible, then instead of using a graft in the forearm, the upper arm fistula would be considered.

Methods: We reviewed amongst our 124 patients, 59(47.6%) have fistulas only, 11(8.9%) have a newly created fistula with catheters being used for dialysis, making the total fistula prevalence 56.4%. This would subject our unit to financial penalty. Forty four of 124 (35.5%) have grafts and only 9/124 catheters (7.3%). Of patients with grafts, 50%(

22/44) had functioning fistula in the past with grafts inserted only after fistula failure. Of 44 patients 13/44 (29.5%) had primary fistula failure, 9/44 (20.4%) had inadequate vessels for fistula and 2/44 (4.5%) patients had unclear reason. Counting all fistula at one time in patients, our unit fistula placement would be 80/124 or 64.5%, surpassing the current set performance standards for primary fistula placement.

Results: Our evaluation suggests that grafts are primarily placed after fistula failure. A graft in the same site after a failed fistula should be considered within the fistula category to avoid loss of access sites, unnecessary procedures and long term catheter use.

Conclusions: CMS recommendations need to be revised so that the dialysis units are not penalized for improving patient care.

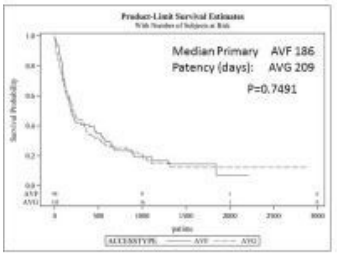
TH-PO756

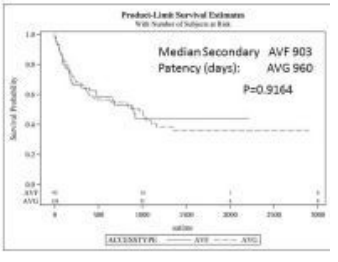
Arteriovenous Fistulae and Grafts Have Similar Outcomes in an Urban Population Senthil Nathan Jayarajan,[1] Abhigna Chowdary Madineni,[1] Sridhar K. Reddy,[2] Vishnu Koganti,[1] Jean Lee,[2] Iris J. Lee,[2] Eric T. Choi.[1] *[1]Department of Surgery, Temple University, Philadelphia, PA; [2]Department of Nephrology, Temple University, Philadelphia, PA.*

Background: Dialysis access failure is a major cause of morbidity in End-Stage Renal Disease patients. The Fistula First Initiative dictates arteriovenous fistulae (AVF) should be preferred over grafts (AVG) as first-line for surgically placed accesses. The purpose of this study was to compare patency rates of surgical dialysis accesses in the North Philadelphia.

Methods: Current dialysis patients with accesses placed between 2006 and 2011 were included. Patient characteristics, access outcomes, and interventions were analyzed. Primary patency was defined as time from access placement to first intervention, while secondary patency was the time from access placement to abandonment.

Results: 156 patients and 239 accesses were included in the study. Of the accesses, 101 were AVF and 138 were AVG. AVF was placed in younger patients than AVG (56 vs. 61 years, p=0.02), while AVG were more often black than AVF (96.4 vs. 83.3%, p = 0.001). The groups were evenly matched in terms of prevalence of hypertension, diabetes, CAD, and PVD. Average number of complications requiring intervention per access was greater with AVG than AVF (1.01 vs. 0.46, p=0.02). AVF and AVG had similar primary patency (median: 186 vs. 209 days, p=0.74) and secondary patency (median: 903 vs. 960 days, p=0.92).





Conclusions: More patients in North Philadelphia are likely to be dialyzed via AVGs. While complications requiring intervention are greater with AVG, primary and secondary patency rates are similar between AVF and AVG, despite implementation of Fistula First.

TH-PO757

Strategies to Maintain Primary Patency of Hemodialysis Arteriovenous Grafts: A Cost Utility Analysis Austin Parker,[1] Dustin J. Little,[1] Kevin C. Abbott,[1] Jonathan Himmelfarb,[2] Robert Nee.[1] *[1]Department of Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [2]Kidney Research Institute, Seattle, WA.*

Background: US Healthcare expenditures for ESRD reached $29 billion in 2009, including the placement of 33,600 new AV grafts (AVG) in hemodialysis patients. The probability of AVG loss is 75% at one year, primarily due to thrombosis. Attempted salvage of AVG is costly, and largely unsuccessful. Therefore, a cost-effective strategy to maintain AVG primary patency is needed.

Methods: We performed a cost utility analysis of pharmacologic interventions aimed at preventing loss of AVG patency; strategies included aspirin, dipyridamole alone, aspirin+dipyridamole, aspirin+dipyridamole with and without concurrent aspirin, and placebo. Probabilities were based upon published studies performed by the Dialysis Access Consortium Study Group while costs of medications and procedures were drawn from public sources. Utilities of health states were derived from published reports and the SF-6D tool. Calculations were performed using TreeAge Pro® decision analysis software.

Results: One-way sensitivity analysis showed that for any probability of AVG loss <0.75, aspirin therapy dominated alternate strategies. In a Monte Carlo probabilistic sensitivity analysis, aspirin was the optimal strategy in 72% of scenarios, while 21% favored dipyridamole monotherapy, at a willingness-to-pay of $50,000 per quality-adjusted life year (QALY).

Conclusions: In our analysis, aspirin monotherapy dominated other strategies based on cost per QALY. Aspirin therapy has the potential to significantly reduce healthcare costs through decreased vascular access interventions. If all patients with incident AVGs in the US were given aspirin, more than $33 million could be saved annually without decrement in QALY, as compared to no prophylaxis.

Funding: Other U.S. Government Support

TH-PO758

Descriptive Analysis of Vascular Access Outcomes in a Single-Center 40-Year Home Hemodialysis (HHD) Experience Eric Goffin,[1] Tony Goovaerts,[1] Krystel Carlier,[2] Veronique Chapalain,[3] Michel Y. Jadoul.[1] *[1]Nephrology, Université Catholique de Louvain, Brussels, Belgium; [2]Keyrus Biopharma, Lasnes, Belgium; [3]Keyrus Biopharma, Paris, France.*

Background: In a context of expanding HHD with longer and more frequent dialysis sessions (nocturnal, short daily), there is a concern that frequent use of vascular access might lead to more complications. We analyzed vascular access outcomes and causes of failure in all our patients with HHD as first RRT over a period of 40 years.

Methods: A total of 246 ([169 males; mean age: 43 (15-79) years] patients started HHD during the study period. Median follow-up time on HHD was 2.4 (IC 95%: 2.1-3.0) years. The study duration was divided in four periods (before 1980; 1980-1990; 1999-2000; 2000-2011). All vascular access complications were recorded.

Results: During the study period, a mean (range) of 1.3 (1-5) vascular access/patient was created. Native AV Fistula (AVF) represent 92.2% overall, but only 81.9% during the last period due to an increase (transient or permanent) use of tunnelled venous catheter (p<0.001). The mean duration use was 3.5 years; 2.3 during the last period of the study (p<0.001). AVF cannulation was mainly performed by a family member until 1990 (86.8% for 1990-1999) but self cannulation concerned 51.1 % of the patients (p<0.001) after 2000. HHD practices changed over time: nocturnal, short daily and more than 3 sessions/week represented 58.5% of the HHD schedule during the last period. 82% of native AVF did not require any intervention. Incidence of AVF complication was 0.34, 0.01, 0.03 and 0.03 case/100 patients/months for thrombosis, infection, pseudo-aneurysms and stenosis, respectively, and 0.32 for catheter infection incidence. After 1997, the use of the buttonhole technique for AVF cannulation became systematic. Despite the use of this technique and an increase in self cannulation, infection, stenosis and pseudo-aneurysms rates did not increase; thrombosis incidence slightly increased (p: 0.09).

Conclusions: In this analysis, we did not observe a significant increase in vascular access complications despite the more frequent use of the AVF and of the buttonhole technique but a slight increase in thrombotic events.

Funding: Pharmaceutical Company Support - Baxter Healthcare

TH-PO759

Optiflow™ Anastomotic Connector: Safety and Efficacy: Results from the OPEN Study Milind Nikam,[1] Afshin Tavakoli,[1] Jackie Evans,[1] Angela M. Summers,[1] Paul E. Brenchley,[4] Eric S. Chemla,[2] Prabir Roy-Chaudhury,[3] Sandip Mitra.[1] *[1]Manchester Royal Infirmary, Manchester, United Kingdom; [2]St George's Healthcare NHS Trust, London, United Kingdom; [3]University of Cincinnati; [4]Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: Early arteriovenous fistulae (AVFs) failure remains a significant clinical problem with high early failure rates. Optiflow™ is a novel device which acts as an anastomotic connector and could address some of the factors associated with early failure. The Optiflow™ Patency and Maturation (OPEN) study was set up to investigate the safety and efficacy of the Optiflow™ device.

Methods: Forty device and 40 hand-sewn control patients undergoing AVF creation were recruited prospectively. AVFs were created using the Optiflow™ device in an end-to-side configuration using a 3 or 4mm device. Device size was determined by arterial and venous diameter obtained pre-operatively by ultrasound vessel mapping and also by clinical evaluation intra-operatively. AVF patency was determined in this interim analysis by clinical evaluation and / or ultrasound scan and defined as an AVF with diameter ≥ 5mm and blood flow 500ml/min.

Results: Ultrasound measurements and/or clinical evaluation were performed at 2, 6 and 13 weeks post-operatively according to a defined protocol. Early results suggest that the average blood flow at 90 days was 1298 ml/min in the device group and 988 ml/min in the control group (p=0.037). The average diameter at 90 days, 3 cm from the anastamosis, was 8.2mm and 7.8mm in the device and control groups respectively (p=0.062). Primary unassissted maturation at 3 months was 71% in the device arm. It is expected that at the time of presentation, complete dataset from the trial would be available.

Conclusions: Optiflow™ device appears to be a safe and effective intervention in creation of primary AVFs. The potential advantages of the device are: standardisation of the procedure and thus reducing skill dependency, optimising anastomotic configuration and shielding the peri-anastomotic region from neo-intimal hyperplasia. Optiflow™ device is novel, safe and effective technology for creation and maturation of AVFs. Its potential for improving AVF outcomes is attractive and merits further consideration.

Funding: Pharmaceutical Company Support - Bioconnect Systems, Inc.

TH-PO760

Endovascular Salvage of Failed Arteriovenous Dialysis Access: Long Term Results and Factors Predicting Access Survival Milind Nikam, Leonard Ebah, Anuradha Jayanti, Angela M. Summers, Paul E. Brenchley, Nicholas Chalmers, Sandip Mitra. *Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: A patent arteriovenous (AV) access is crucial to improving outcomes in patients on haemodialysis (HD). AV access failure, often as a result of thrombosis, is common and often leads to significant morbidity to the patient and increased costs. Here we report results of an interim analysis of endovascular salvage of AV access from our centre.

Methods: A regional AV fistula salvage service was set up in 2008 to cover 2 large renal centers. Failed AV access was defined as any AV access which could not be used for HD. Co-morbidity and follow up information was obtained from electronic case records.

Results: A total of 445 episodes of failed AV access were referred for treatment in the period between 1/1/2008 to 12/31/2011. Thrombosis accounted for 71% of episodes. Native fistulae accounted for 68% episodes whereas 32% were grafts (centre graft prevalence ~5%). Mean patient and access age were 58 years and 24 months respectively, with 18% diabetes prevalence. The initial anatomical success rate was 90%. Primary patency rates for fistulae and grafts were 43% and 14% at 6 months, 30% and 7% at 1 year; and 18% and 3.5% at 2 years respectively. Secondary patency rates were 66% and 38% at 6 months, 61% and 25% at 1 year; and 48% and 12% at 2 years respectively. Multivariate analysis of anatomical success outcome revealed no impact of having a graft vs. fistula, age, diabetic status and time from onset to procedure. Cox regression analysis revealed that poor access survival (primary patency) was associated with having a graft vs. fistula (OR 1.7, p=0.03), presence of ischemic heart disease (OR 5.9, p<0.001), peripheral vascular disease (OR 9.5, p=0.04) whereas improved survival was associated with being on statin therapy (OR 0.285, p<0.001) and antiplatelet therapy (OR 0.72, p=0.02).

Conclusions: Despite good anatomical patency, longer-term access patency after an episode of failure remains significantly poor especially for grafts. Statin and antiplatelet therapy seem to be associated with improved outcomes - these are simple interventions and merit further investigation.

TH-PO761

Treatment of Vitamin D Deficiency with Ergocalciferol Is Associated with Reduced Vascular Access Dysfunction in Chronic Hemodialysis Patients

Christine L. Gear, Neena M. Penagaluru, Jonathan A. Gelfond, Wajeh Y. Qunibi. *Nephrology, University of Texas Health Science Center, San Antonio, TX.*

Background: Vitamin D deficiency (VDD) is prevalent among CKD and Hemodialysis patients (HDP). Previous studies reported an association between VDD and various clinical disorders including cardiovascular disease, vascular calcifications, arterial stiffness, peripheral vascular disease and vascular endothelial dysfunction. Complications of vascular access in HDP contribute to morbidity and increased healthcare cost. In our previous observational study, we showed that VDD is an independent risk factor for the development of vascular access dysfunction (VAD) in HDP. The purpose of the current study was to examine the effect of treatment of VDD with ergocalciferol on future development of VAD.

Methods: Data from medical records of 256 HDP were analyzed to evaluate the relationship between treatment of VDD with ergocalciferol and VAD (defined as vascular access intervention for dysfunction).

Results: Of the 256 patients in the initial cohort, 145 patients were treated with ergocalciferol, and 73 were not. Mean age was 56±13 years; 51% females. Age and gender were not associated with treatment status (p>0.05). Mean 25OHD level was 19.1±11 ng/ml. Subjects treated with ergocalciferol had lower baseline 25OHD levels (17±11 vs. 23±14, p<0.001). The observed, unadjusted rate of any vascular intervention was 57% in the untreated group compared to 43% in the treated group (p=0.06). Logistic regression that adjusts for multiple factors including baseline 25OHD, and treatment with ergocalciferol was associated with a significant reduction in the risk of vascular access interventions for dysfunction (OR=0.37; 95%CI [0.19, 0.07], p=0.002).

Table 1.

Variable	Log Odds	Std Error	OR	CI 95	Pvalue
Ergocalciferol	-1.001	0.33	0.37	[0.19, 0.7]	0.0023
PreVit.D.level	-0.036	0.02	0.96	[0.94, 0.99]	0.0164
Ca	-0.474	0.19	0.62	[0.43, 0.9]	0.0122
Alb	-0.818	0.44	0.44	[0.19, 1.05]	0.0641

Conclusions: Our study suggests that treatment of VDD with ergocalciferol in HDP is associated with fewer vascular access interventions. Further randomized controlled trials are warranted to determine whether treatment of VDD contributes to reduction in VAD in HDP.

Funding: Clinical Revenue Support

TH-PO762

The Effect of Lowering LDL-Cholesterol with Simvastatin Plus Ezetimibe on Vascular Access Patency: Results from the Study of Heart and Renal Protection (SHARP)

William G. Herrington. *On Behalf of the SHARP Collaborative Group, Clinical Trial Service Unit, University of Oxford, United Kingdom.*

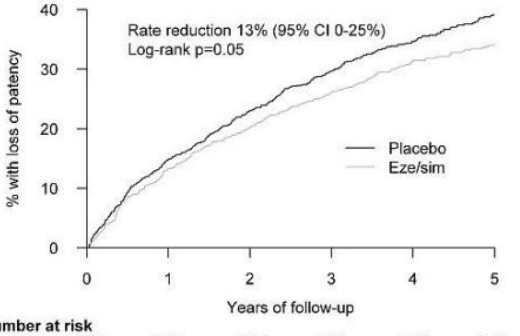
Background: The SHARP trial, which randomized 9270 patients with chronic kidney disease to simvastatin 20mg plus ezetimibe 10mg daily versus matching placebo, showed that lowering LDL-cholesterol (LDL-C) for an average of five years safely reduces the risk of major atherosclerotic events. The potential effect on vascular access patency, however, is unknown.

Methods: Vascular access status was recorded at baseline and events related to vascular access were recorded routinely at each follow-up visit. Post-hoc analyses were performed of the potential effect of allocation to simvastatin plus ezetimibe on loss of vascular access patency (defined as any access revision procedure, access thrombosis, or the removal of old/formation of new permanent dialysis access). Analyses of the time to first event were done according to the intention-to-treat principle.

Results: 2353 patients had functioning vascular access at randomization (2204 had an arteriovenous fistula and 149 an arteriovenous graft). In these patients, allocation to simvastatin plus ezetimibe resulted in a 13% proportional reduction in loss of vascular access patency (355 [29.7%] simvastatin/ezetimibe *vs.* 388 [33.5%] placebo; rate ratio 0.87, 95% CI 0.75-1.00; p=0.05). Consistent results were seen for each measure corresponding to loss of patency. There was no significant heterogeneity of treatment effect among those treated with antiplatelet therapy and those not.

Conclusions: This analysis raises the hypothesis that lowering LDL-C may have beneficial effects on maintaining vascular access patency.

Life table plot of effects of allocation to simvastatin plus ezetimibe versus placebo on loss of patency among 2353 patients with a fistula or AV graft at randomisation



Number at risk						
Placebo	1157	919	777	651	450	249
Eze/sim	1196	963	823	682	478	262

Funding: Pharmaceutical Company Support - The SHARP Study Was Funded Mainly by Merck/Schering-Plough Pharmaceuticals (North Wales, PA, USA), Government Support - Non-U.S.

TH-PO763

AVF.SIM: A Web-Application for Planning Vascular Access Surgery in Hemodialysis Patients

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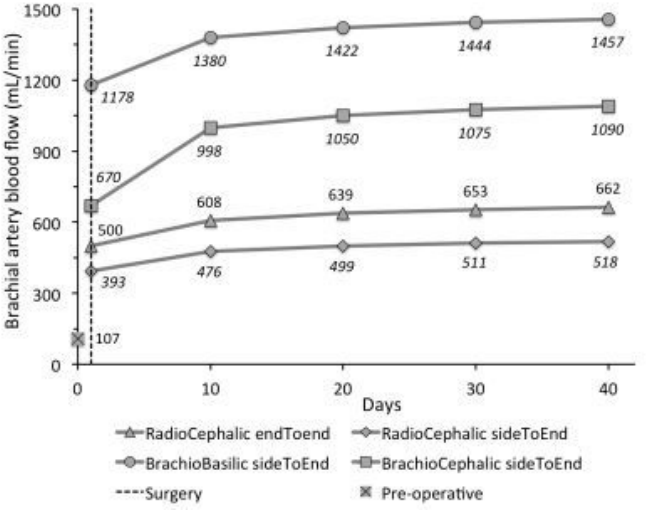
Background: The planning of surgical procedure for vascular access (VA) is based only on physical examination and eventually on ultrasounds (US) investigation. These evaluations do not allow to predict the outcome of the VA surgery. We developed a computer-based simulation tool to quantitatively predict the post-operative VA blood flow and its changes during VA maturation.

Methods: AVF.SIM is a clinical web-application for predicting hemodynamic changes induced by different configurations of arteriovenous fistula (AVF) surgery at patient-specific level. AVF.SIM is based on a numerical solver (pyNS) which implements 1D wave propagation computational models and a vascular adaptation algorithm based on the assumption that vessel diameter changes upon increase in blood flow volume to maintain a physiological value of the peak wall shear stress. This tool allows to predict the increase in blood flow in arterial and venous segments of the arm vasculature, immediately after surgery and during VA maturation (about 6 weeks).

Results: On the basis of structural and functional information obtained by US on patient-specific vasculature AVF.SIM allows to simulate the potential results of different surgery procedures (distal and proximal, end-to-end and side-to-end) in terms of changes in time of vessel diameters and expected blood flow.



Conclusions: Simulation of the potential effects of different type of anastomosis, as well as different anastomosis location, may help the surgeon to select the most appropriate AVF surgical planning. The use of this simulation tool may reduce the incidence of AVF primary non-function and may allow to avoid very high blood flow in the VA, exposing the patient to the risk of cardiac failure.

TH-PO764

Analysis of Vascular Access Interventional Therapy: 6-Year Follow Up Survey in 2,000 Cases with Vascular Access Failure Teruhiko Maeba, Shigeru Owada. *Asao Kidney Clinic, Kawasaki, Kanagawa, Japan.*

Background: Keeping a functional vascular access (VA) is one of the most important factors in the maintenance of well controlled HD modality. The application of vascular access interventional therapy (VAIVT) for VA trouble is growing recently but the effectiveness of VAIVT has not been fully satisfactory because of the relatively higher rate of re-stenosis.

Methods: We have experienced 2,000 cases of VA trouble treated with VAIVT over the last 6 years in which primary assisted and secondary assisted rates were analyzed.

Results: 1. We have used a noncompliant type of balloon catheter for 96.4%of cases and primary assisted rates were 99.0%. 2. Assisted patency rates were 85.0% at one year, 75.3% at 3 years and 67.2% at 6 years in all patients. 3. In diabetic patients, assisted patency rate was 61.5% at 6 years and it was 70.8% in non-diabetic patients (p=0.03, Log rank). There were no significant differences found between the arteriovenous fistulas and the arteriovenous grafts (67.3% and 63.3% at 6 years, respectively). 4. Failure of the secondary assisted patency was observed in 135 patients. In these, a new arteriovenous fistula was made on the same side in 60.0% of the patients, a contralateral side shunt was made in 5.9% and an AV graft in 28.0%, superficialized reposition of brachial artery in 2.3% and death occurred in 3.7%.

Conclusions: 1. The results in primary assisted patency rates were good. 2. It was possible to reduce the economical burden for patients using a noncompliant type of balloon catheter. 3. Good patency rates for vascular access were maintained using management charts of VA.

TH-PO765

Early Changes in Upper Arm Blood Vessels Following Forearm Arteriovenous Loop Graft Placement Troy J. Plumb,[1] Michael Morris,[2] Valerie K. Shostrom,[3] Gernon Matthew Longo.[2] *[1]Internal Medicine, University of Nebraska Medical Center, Omaha, NE; [2]Surgery, University of Nebraska Medical Center, Omaha, NE; [3]Biostatistics, University of Nebraska Medical Center, Omaha, NE.*

Background: The timing and extent of changes in the vasculature of the upper arm following forearm arteriovenous loop graft (fAVG) placement are not known. When, and to what degree changes occur are important when considering the placement of a fAVG and the potential for future secondary AV fistula creation. In this study we describe the changes that occur in the artery and veins of the upper arm following fAVG placement.

Methods: We prospectively evaluated the basilic vein, cephalic vein and brachial artery of the upper arm in 10 subjects undergoing fAVG placement. Subjects underwent ultrasound imaging to assess vessel diameter at baseline and at 1, 4, 12 and 27 weeks following fAVG placement. The brachial artery was measured near the antecubital fossa. Cephalic and basilic vein diameters were measured at the antecubital fossa (AC), the mid upper arm (MID), and proximal upper arm (UP). A repeated measures model with adjustment for multiple comparisons was used to evaluate differences in artery and vein diameters across time points.

Results: Basilic vein diameters (millimeters) increased from baseline to 1 week at AC: 2.9±1.3 (baseline) to 5.8±1.9 (1 week), MID: 3.6±1 to 6.2±1.5, UP: 3.7±1.1 to 7.2 ±1.6 ($p<0.001$ for all locations). Brachial artery diameter in AC increased from 4.1±0.8 at baseline to 5±1.1 at 1 week (p=0.02). These early increases in artery and vein diameters persisted for the duration of the study. There were fewer patients with patent cephalic veins at baseline. Cephalic vein diameters increased to a lesser degree, and only became significant in the MID location.

Conclusions: Marked increases in upper arm artery and vein diameters occurred following fAVG placement. These changes occurred within one week and persisted for the duration of the study. Given the timing of these changes, even patients with early failure or failing fAVGs should be considered for prompt conversion to an upper arm AVF.

Funding: Private Foundation Support, Clinical Revenue Support

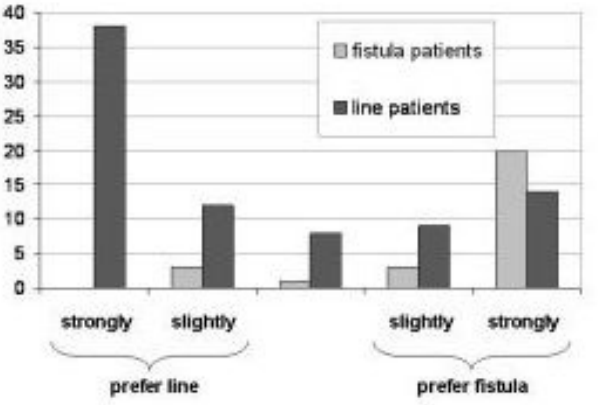
TH-PO766

Haemodialysis Access: Determinants of Patient Preferences Damien Ashby,[1] James John Rowland Budge,[2] Courtney Burtenshaw,[2] Neill D. Duncan,[1] Jeremy Crane.[1] *[1]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; [2]Imperial College School of Medicine, Imperial College, London, United Kingdom.*

Background: Compared to other forms of access, arteriovenous fistulae are associated with improved outcomes in haemodialysis patients. Various guidelines and incentives have been developed to increase fistula prevalence within institutions but some patients are reluctant to undergo fistula formation.

Methods: Patient opinions and the influences informing them were determined using structured interviewer led questionnaires in a group of prevalent haemodialysis patients.

Results: In 108 patients (aged 18 – 87, 69% male) opinions were highly polarised, with two thirds expressing a strong access preference and only 8% expressing no opinion.



Of 81 patients dialysing on a tunnelled line, 50 (62%) were unwilling to have a fistula. The principle reasons were body image and pain during dialysis, cited by 70 and 74% of patients respectively. Most of these patients claimed to understand the risks of non-fistula access, but failure to appreciate this was a contributory factor in 28%.

Opinions in patients who had never had a fistula were overwhelmingly informed by their interactions with other patients, with only 27% reporting any influence due to professional advice. Those who had previously had a fistula were informed by their own experience as much as other patients, and not at all by professionals. The negative effect of unsightly fistulae in other patients was clearly substantial.

Conclusions: Access opinions are polarised according to personal experiences and interactions with other patients, with large fistulae in particular having a strong negative impact. A better understanding of patient reasoning could lead to improved access choices.

Funding: Clinical Revenue Support

TH-PO767

Thigh Graft Outcomes in Hemodialysis Patients Song Ching Ong, Michael Allon. *Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL.*

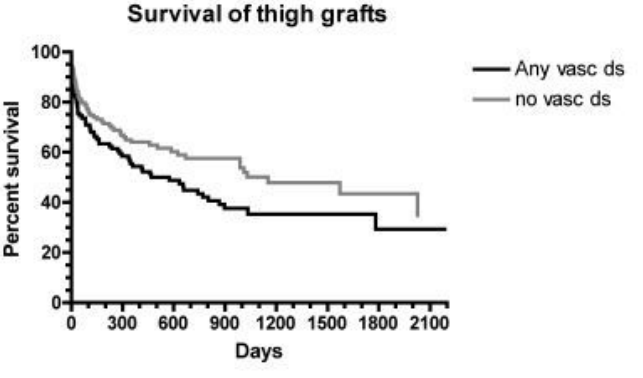
Background: Prosthetic arteriovenous thigh grafts are an important option for hemodialysis patients who have exhausted their upper limb vascular access sites. We evaluated our experience with the outcomes of thigh grafts at a large dialysis center.

Methods: We retrospectively analyzed a prospective vascular access database to identify all thigh grafts placed during an 8.5-year period. The rates of graft survival, thrombosis and infection were calculated. In addition, we evaluated the association of graft survival with patient demographics and comorbidities.

Results: A total of 255 grafts were placed during the study period. The surgical technical failure rate was 7.8%. The median graft survival was 802 days (2.2 years) and median time to thrombosis was 189 days (0.5 year). The 1, 3 and 5 year cumulative graft survival was 60%, 43% and 36% and the respective thrombosis free survival was 38%, 22% and 13%. Graft infection occurred in 55 cases (17%). Cumulative graft survival was lower among patients with vascular disease, as compared to those without vascular disease (HR 1.41, 95% CI 1.00-2.04, p= 0.05), but was not associated with patient age, sex, diabetes or heart failure.

Clinical factor	HR	95% CI	p-value
Any vascular disease (Y vs N)	1.41	1.00-2.04	0.05
Age (>65 vs <65yr)	0.78	0.50-1.24	0.30
Sex (male vs female)	0.87	0.61-1.25	0.46
Diabetes (Y vs N)	0.95	0.66-1.36	0.79
Congestive heart failure (Y vs N)	1.20	0.78-1.90	0.38

Conclusions: Thigh grafts have a fairly good cumulative survival rate and should be considered as the access of choice in patients who have exhausted options in both upper extremities. Thigh graft survival is decreased in patients with vascular disease.



TH-PO768

Surgical Management of Cephalic Arch Occlusive Lesions: Advantages and Limitations Shouwen Wang,[1] Ammar Almehmi.[2] *[1]AKDHC-ASC, Arizona Kidney Disease and Hypertension Center, Phoenix, AZ; [2]University Vascular Access Center, University of Tennessee College of Medicine, Memphis, TN.*

Background: Cephalic arch occlusive lesions (CAO) are common cause for dysfunction of upper extremity arteriovenous fistulas (AVFs). These lesions are usually resistant or not amenable to endovascular interventions. Therefore, various surgical interventions have been employed to manage CAO. We here report the outcomes of the largest case series of surgical interventions for CAO.

Methods: This series included 40 hemodialysis patients who had dysfunctional AVFs due to CAO and underwent surgical revisions from 2009 to 2012.

Results: Of the 40 patients: 65% were males, mean age was 59.4 ± 14.0 years, and 70% were diabetics. Mean age of fistulas was 2.1 ± 1.6 years and mean post-operative follow-up was 16.6 ± 7.9 months. The surgical indications were: frequently recurrent stenosis (17/40), high-grade elastic stenosis (5/40), occlusions (17/40), and localized tortuous stenosis (1/40). The procedures performed included: cephalic vein transposition (37/40), basilic vein transposition (1/40), stenotic segment resection (1/40), and cephalic-jugular vein bypass graft (1/40). Of the new cephalo-basilic anastomoses after cephalic transposition, 25% required no interventions (follow-up 8-24 months) and 75% required angioplasties (follow-up 1-32 months). The development of neo-anastomosis stenosis correlated with angioplasty of the cephalic veins prior to transposition (correlation coefficient 0.556, $p<0.001$). In a subset of 14 patients who presented with recurrent stenosis and had at least 12 months of follow-up after surgery, the number of angioplasties/year for cephalic lesions was decreased to 1.3 ± 1.4 after transposition from 4.2 ± 1.4 before transposition ($p<0.001$). The primary assisted patency of the AVFs at 12 months after cephalic transposition was 77.9%.

Conclusions: Surgical interventions are safe and effective in salvaging AVFs complicated with CAO. They can prolong access life and reduce the need for endovascular interventions in selected patients. Further investigations are needed to identify factors that may affect the outcomes of surgical interventions.

TH-PO769

A Comparative Analysis of Vascular Access and Immunization between For-Profit and Non-Profit Dialysis Facilities Using Medicare Claims Data Mahesh Krishnan,[1] Allen R. Nissenson,[1] Rachel Feldman,[2] Mark Desmarais,[2] Lianna Weissblum.[2] *[1]DaVita Inc., Denver, CO; [2]The Moran Company, Arlington, VA.*

Background: Previous limited analyses have suggested differences in outcomes between for- (FP) and non-profit (NP) dialysis providers. We hypothesized that FP facilities would have superior outcomes when compared with non-profit facilities for process-driven outcomes that are resource intensive. We used Medicare data to test this hypothesis.

Methods: 2009-10 Medicare Standard Analytic File Claims data were analyzed per dialysis facility to compare identification data for FP and NP providers from the files downloaded from that site. USRDS Annual Data Report definitions were used to determine immunization rates and v5, v6, and v7 modifiers in place on ESRD claims since July 2011 were used to determine vascular access status.

Results: We analyzed data from 4,242 dialysis clinics' Medicare claims for 2010. FP dialysis facilities showed better outcomes for vascular access than NP dialysis facilities as measured by the difference in percent of catheter use at (17.5% FP vs 20.1% NP).

Similarly, FP dialysis facilities showed better outcomes in percent of vaccination rates for influenza, (54.8% FP vs 52.3% NP). These trends continued for Pneumococcal Pneumonia immunization (26.1% FP, vs 21.3% NP) and Hepatitis B immunization (25.2% FP and 21.1% NP). All data reflect Medicare claims data rates only and differ from internal rates of the large dialysis organization population.

Conclusions: FP providers had superior vascular access and vaccination outcomes compared to NP providers. Intense focus on care processes like vaccination and vascular access can significantly augment population-based and patient-based goals, improving population health and constraining healthcare costs. Sufficient infrastructure support and scaling capability, however, are needed as well as high prioritization, as is seen in for-profit dialysis facilities.

Funding: Pharmaceutical Company Support - DaVita Inc.

TH-PO770

Association between Vascular Access Handling and the Outcome of Arteriovenous Fistula:International Comparison between Facilities Using Data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Manabu Asano,[1] Jyothi R. Thumma,[5] Kenichi Oguchi,[1] Ronald L. Pisoni,[5] Tadao Akizawa,[2] Takashi Akiba,[3] Akira Saito.[4] *[1]Renal Unit, Ikegami General Hospital, Tokyo; [2]Showa University School of Medicine, Tokyo; [3]Tokyo Women's Medical University, Tokyo; [4]Tokai University School of Medicine, Kanagawa, Japan; [5]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Background: Vascular access (VA) failure is a momentous cause of morbidity in hemodialysis (HD) patients. Our objective was to corroborate whether VA handling factors are associated with arteriovenous fistula (AVF) survival by means of results obtained from the DOPPS 2 and 3.

Methods: Analyses included 1,183 incident HD pts (on HD≤7 days and using an AVF at study entry) and 949 prevalent pts (on HD>7 days). Medical directors and nurse managers from each participating facility were asked to complete a self-administered questionnaire that included questions on VA practices including: Physician-related factors (existence of preferred surgeon, prompt surgical/radiological VA procedure, technical skill of surgical/radiological VA procedure) and staff-related factors (response to a failed VA and cannulating skill). The hazard ratio of AVF failure with VA handling factors were compared by the three regions(Europe/Australia/New Zealand:EUR/ANZ, North America:North Am, Japan)using Cox regression models.

Results: The perception by unit staff that radiological VA procedures were performed "promptly" in their unit vs not promptly was associated with a tendency towards a lower hazard of final AVF failure in Japan and EUR/ANZ. (EUR/ANZ:HR 0.80, CI 0.54-1.19; Japan:HR 0.48, CI 0.09-2.46; North Am:HR 2.61, CI 0.63-10.75,). A tendency towards lower AVF failure also was seen in dialysis units in Japan and EUR/ANZ which reported that radiological VA procedures were performed with the highest level of skill. (EUR/ANZ:HR 0.73, CI 0.48-1.11; Japan:HR 0.23, CI 0.06-0.93; North Am:HR 2.77, CI 0.62-12.33).

Conclusions: Regional variability was observed in VA management. Both in Japan and EUR/ANZ, radiological VA procedures when performed promptly or with highest technical skill were inclined to contribute to greater AVF patency.

TH-PO771

Surgical Site Infection Surveillance Post Vascular Access Interventions Aris Q. Urbanes, Terry Litchfield, Kevin Graham. *Lifeline Vascular Access, Vernon Hills, IL.*

Background: By definition, a surgical site infection (SSI) is an infection that develops within 30 days after an operation or within one year if an implant was placed and the infection appears to be related to the surgery. Post-operative SSIs are the most common healthcare-associated infection in surgical patients, occurring in up to 5 percent of surgical patients. In the United States, between 500,000 and 750,000 SSIs occur annually. Patients who develop an SSI require significantly more medical care. Endovascular surgical procedures are quite common in the dialysis population and can be a cause of SSIs. A large outpatient vascular access system developed and implemented a surveillance program to measure and monitor SSI in their population.

Methods: This surveillance was achieved through a survey given at discharge to all patients having interventional procedure performed with the survey returned at 30 days. The annual implant data collection was done via a telephonic voice response system. The data was collected and analyzed using SPSS software.

Results: During the time period, 41,233 patients underwent interventional procedures. 6,218 of those patients returned the survey for a 15% response rate. The mean response was received 32.52 days post intervention. 99.6% of the respondents reports their surgical site healed well, without evidence of infection. Using a standard definition of SSI as being either antibiotic prescribed; 2 or more clinical signs; dehiscence, the rate of Surgical Site infection was 1.4%.

Conclusions: SSIs are a serious medical problem associated with increased morbidity and mortality and increased medical care costs. This study showed that the rate of post procedure SSIs are quite low in outpatient vascular access procedures. All providers should consider an active surveillance program after these types of procedures given the co-morbidities associated with the dialysis population.

TH-PO772

Intra-Access Pressure Change after Angioplasty and Post Interventional Patency Bushra Joarder,[1] Alexander S. Yevzlin,[2] Charmaine E. Lok.[1] *[1]Nephrology, Toronto General Hospital and University of Toronto, Toronto, ON, Canada; [2]Nephrology, University of Wisconsin, Madison, WI.*

Background: Fistula and grafts are often complicated by stenosis and thrombosis and require percutaneous transluminal angioplasty (PTA) to maintain graft patency. Generally, less than a 30% residual stenosis indicates intervention success. However, this subjective measure may not correlate with the hemodynamic effect and patency anticipated. Rather, direct intra-access pressure measurement before and after PTA may be an objective reflection of the effect of reducing the stenotic lesion. We aimed to determine an association between intra-access pressure change with PTA and the time to the next PTA.

Methods: We retrospectively evaluated a random 59 vascular accesses (36 fistulas) that underwent underwent PTA between 2004-2010. Pre and post PTA pressure measurements at different intra-access locations within an access were reviewed; the greatest difference in pressure (delta) at any location in the access largest (delta circuit), at the arterial (delta AP) and venous (delta VP) access segments were obtained. Associations between intra-access pressure changes and the time to the next angioplasty (TTAP) was determined by correlational statistics (SAS, 9.2).

Results: No correlation was found between delta circuit and TTAP; however the maximal delta VP was positively correlated to the TTAP ($r=0.38$; $p=0.02$). Further, delta circuit and delta AP was highly correlated ($r=0.94$; $p<0.0001$) as was delta circuit and delta VP ($r=0.85$; $p<0.0001$).

Conclusions: The maximal intra-access pressure change by AP anywhere in the circuit is not associated with primary patency and is more highly associated with changes in AP than VP; however, the maximal change in VP is positively correlated with a longer time before next PTA is required. Larger studies are required with specific evaluations of fistulas and grafts to further elucidate the role of intra-access pressure measurements to indicate PTA success and time to next procedure.

Funding: Other NIH Support - , Other U.S. Government Support, Government Support - Non-U.S.

TH-PO773

Percutaneous Transluminal Angioplasty (PTA) in Cephalic Arch and Juxta-Anastomotic Stenosis of Hemodialysis Arteriovenous Fistula Gun Hee An, Yul Hee Cho, Myung Hyun Lee, Jeong Gwan Kim, Hyun Chul Whang, Yong-Soo Kim. *Section of Interventional Nephrology, Division of Nephrology, The Catholic University of Korea College of Medicine, Seoul, Republic of Korea.*

Background: Venous stenosis is a major cause of vascular access dysfunction. We evaluated the outcome of the PTA in cephalic arch stenosis (CAS) and juxta-anastomotic stenosis(JAS) of hemodialysis arteriovenous fistula (AVF).

Methods: A cohort of 171 PTA performed in 108 patients with dysfunctional AVF diagnosed by clinical assessment or intra-access flow surveillance, from January 2010 to February 2012 in Seoul St. Mary's Hospital were retrospectively reviewed. Out of 171 PTA, 20 PTA in 11 patients with CAS and 56 PTA in 26 patients with JAS were analyzed.

Results: The prevalence of CAS and JAS in total AVF dysfunction was 10% (11 of 108) and 24% (26 of 108), respectively. In 11 patients with CAS, mean age was 62 years old, male 63%, mean access age was 41 months. Ten out of 11 patients had brachiocephalic fistula. In 26 patients with JAS, mean age was 61 years old, male 63%, mean access age was 37 months. Seventy three percent patients of JAS had radiocephalic fistula, and the rest had brachiocephalic fistula.

In both CAS and JAS, the most reason for angiography were serial decrease in access flow (50% vs 38%) and a half had multiple stenotic lesion. Forty two percent of radiocephalic AVF group had JAS and 46% of brachiocephalic AVF group had CAS.

The primary patency of CAS and JAS after 3, 6 and 12 months was 80 / 57 / 57% (mean duration 7.6 months) and 85 / 68 / 37% (mean duration 8.6 months) respectively. No patient had any PTA-related complication.

Conclusions: Our results suggest that the location of stenosis can be specific to the fistula type and the outcome of PTA in cephalic arch and juxta-anastomotic stenosis is clinically acceptable.

TH-PO774

Medicare Reimbursement for Provider Visits and Mortality in Patients on Hemodialysis Kevin F. Erickson,[1,2] Wolfgang C. Winkelmayer,[1] Glenn M. Chertow,[1] Jay Bhattacharya.[2] *[1]Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Health Research and Policy, Stanford University, Stanford, CA.*

Background: In January 2004, the Centers for Medicare and Medicaid Services changed reimbursement for physicians caring for patients on hemodialysis from a capitated system to a tiered fee-for-service system referred to as "G-code" reimbursement, creating an incentive for providers to see patients more frequently. The effects of this policy on outcomes and costs are unknown.

Methods: Using data from the United States Renal Data System (USRDS), we compared adjusted mortality hazards among patients commencing hemodialysis in the 3 years before and after enactment of G-code reimbursement. We used a difference-in-difference approach to compare adjusted mortality hazards in patients covered by: (1) Medicare (Parts A&B) versus Medicare Advantage HMOs (who remained on a capitated payment system); and (2) patients living in urban areas and large towns versus in remote areas (whose providers were less able to respond to the G-code incentive).

Results: While mortality rates for all Medicare beneficiaries on hemodialysis on fee-for-service plans declined 3.4% (95% CI 2.2 to 4.6%) after enactment of G-code reimbursement, mortality rates declined more among beneficiaries who remained under capitated payment plans. Medicare beneficiaries from rural areas and small towns experienced a more pronounced decline in mortality compared with those in more densely-populated areas. Inflation-adjusted physician payments for dialysis visits increased 6.0% per patient-month in the year following G-code enactment.

Conclusions: The tiered payment system designed to increase provider visits to hemodialysis patients led to a temporary increase in Medicare costs with no evidence of a benefit to survival.

Funding: Other NIH Support - Agency for Healthcare Research and Quality

TH-PO775

Variation in Medicare Prospective Payment System Drug Costs by Dialysis Organization and Projection to 2014 Wendy L. St. Peter,[1,2] Eric D. Weinhandl.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

Background: Beginning in 2011, injectable dialysis-related medications and their oral equivalents were included in the ESRD Prospective Payment System (PPS). In 2014, cinacalcet and phosphate binders will also be included in the PPS. We examined variation in PPS drug costs by dialysis organization, according to the 2011 and 2014 designs, as well as variation in total Medicare drug costs and gross Part D costs.

Methods: Linked Medicare Parts A, B and D data were used. Prevalent adult patients who received dialysis during all of 2009, had Medicare Parts A and B as primary payer, and were enrolled in a standalone Part D plan were included (n=140,978). Adjusted per person per year (PPPY) total Medicare drug costs, ESRD PPS drug costs, and gross Part D costs in 2009 were calculated, with stratification by dialysis organization, low-income subsidy (LIS) status, and race. Costs were adjusted by age, sex, and either race or LIS status.

Results: Total Medicare drug costs in 2009 ranged from $10,312 to $12,021 PPPY in non-LIS (maximum:minimum, 1.17) and from $15,597 to $18,074 in LIS patients (1.16). Costs among DaVita patients were higher than among patients in other dialysis organizations, regardless of LIS status. In all organizations, black patients had the highest total Medicare drug costs. PPS drug costs in the 2011 and 2014 designs were highest for DaVita and generally lowest for small organizations and independent providers. Gross Part D costs were highest among DaVita patients and lowest among Fresenius patients; costs per medication per day were also highest among DaVita patients. When cinacalcet and phosphate binders are included in the PPS in 2014, the largest shift in gross Part D drug costs is projected to occur among DaVita patients: $2,409 and $5,020 PPPY will shift from Part D to the PPS in non-LIS and LIS patients, respectively.

Conclusions: There is significant variation in drug costs among dialysis organizations. Shifting coverage of cinacalcet and phosphate binders from Part D to PPS will increase costs for dialysis organizations. Some patients may face increased out-of-pocket drug costs attributable to higher copayments with the PPS versus Part D.

Funding: NIDDK Support

TH-PO776

Variation in Medicare Part D Prescription Drug Fill Rates and Coverage Phase Progression by Dialysis Organization Wendy L. St. Peter,[1,2] Eric D. Weinhandl.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

Background: 73% and 61% of Medicare hemodialysis (HD) and peritoneal dialysis (PD) patients are enrolled in Part D, respectively. Number of medications, refill rates, and prescription costs influence whether patients progress from the initial coverage phase to the coverage gap (all costs paid out-of-pocket) and subsequently to catastrophic coverage (5% of costs paid out-of-pocket). We investigated whether patterns of coverage phase progression and medication utilization were similar across dialysis organizations.

Methods: Linked Medicare Parts A, B and D data were used. Prevalent adult patients who received dialysis during all of 2009, had Medicare Parts A and B as primary payer, and were enrolled in a standalone Part D plan without the low-income subsidy (LIS) were included (n=29,414). Percentage of patients reaching the coverage gap and catastrophic coverage and mean number of medications per day in each phase were calculated. Results were stratified by modality and dialysis organization and adjusted for age, race, and sex.

Results: Across organizations, 52-58% of HD and 55-67% of PD patients reached the coverage gap (CG), and 12-17% of HD and 14-24% of PD patients reached catastrophic coverage (CC) in 2009. DaVita had higher percentages of HD and PD patients reach each phase than other dialysis organizations. However, among patients who did not reach the CG (46%); who reached the CG, but did not reach CC (40%); and who reached CC (14%), DaVita patients uniformly had the lowest mean number of medications per day in each phase. There was more inter-organization variability in the mean number of medications per day in PD versus HD patients.

Conclusions: Independent of modality, a higher percentage of DaVita patients without the LIS reached the coverage gap and catastrophic coverage, despite the fact that they had the lowest mean number of Part D-covered medications per day. This combination is possible only if medications taken by DaVita patients are more costly, on average, than medications taken by patients in other organizations. Research is needed to assess whether higher Part D expenditures reduce costs of inpatient and outpatient care.

Funding: NIDDK Support

TH-PO777

Future Eligibility for Medicare Part D Medication Therapy Management among Dialysis Patients Eric D. Weinhandl,[1] Wendy L. St. Peter.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN.*

Background: Medicare Part D plan sponsors are required to provide medication therapy management (MTM) to targeted beneficiaries. One eligibility criterion in 2012 is that the beneficiary is likely to incur >$3,100 in annual drug costs. In 2014, coverage of cinacalcet and phosphate binders will shift from Part D to the ESRD Prospective Payment System (PPS) ("the dialysis bundle"), thereby reducing Part D drug costs among dialysis patients. We assessed the projected impact of this shift on MTM eligibility.

Methods: Linked Medicare Parts A, B and D data were used. Prevalent adult patients who received dialysis during all of 2009, had Medicare Parts A and B as primary payer, and were enrolled in a standalone Part D plan were included (n=140,978). Per person per year (PPPY) gross Part D costs in 2009, with and without cinacalcet and binders, were calculated, with stratification by low-income subsidy (LIS) status. Excluding cinacalcet and binders, predictors of annual drug costs <$3,100 were identified from logistic regression.

Results: With cinacalcet and binders, gross Part D costs were $7,897 and $4,410 PPPY in LIS (79%) and non-LIS (21%) patients, respectively. Percentages of LIS and non-LIS patients with costs <$3,100 were 26% and 52%, respectively. Without cinacalcet and binders, gross Part D costs were $3,904 and $2,599 PPPY in LIS and non-LIS patients, respectively. Percentages of patients with costs <$3,100 were 57% and 76%, respectively. In this setting, predictors of costs <$3,100 were age 18-44 (odds ratio, 1.51) vs. 45-64 yr; black (1.90) and Asian (1.47) vs. white race; Hispanic ethnicity (1.78); male sex (1.27); hypertension (2.02), glomerulonephritis (2.17), and cystic kidney (2.52) vs. diabetes as the primary cause of ESRD; and non-LIS status (3.37).

Conclusions: Cincalcet and phosphate binders account for almost half of gross Part D costs. Shifting coverage of these medications from Part D to the ESRD PPS will reduce Part D expenditures to such an extent that the majority of dialysis patients will be ineligible for MTM offered by Part D plan sponsors. The Centers for Medicare and Medicaid Services should consider how to encourage dialysis providers to offer MTM.

Funding: NIDDK Support

TH-PO778

Association of Insurance Status and Outcome of Hemodialysis Patients Utilizing the Emergency Department for Acute Care Subodh J. Saggi, Moro O. Salifu, Eli A. Friedman. *Nephrology, SUNY Downstate, Brooklyn, NY.*

Background: End Stage Renal Disease patients on hemodialysis frequently use the emergency department (ED) for acute care. The aim of this study was to define the characteristics and the impact of insurance status specifically of being uninsured and undocumented on outcomes.

Methods: We evaluated 3065 patients over a three year period (2008-2010) who visited the ED at our public health safety net hospital necessitating the performance of emergent dialysis. The primary outcome was in-hospital mortality based on insurance classification. Secondary outcomes were presenting symptoms, distribution of ED visits, time to activation for dialysis and time to disposition. Data were abstracted from the existing electronic medical record system and analyzed using descriptive statistics, correlation and logistic regression.

Results: Mean age was 65.4±16.7 years, 54.3% were female, 88% were Black and 58.7% presented with volume overload. Average wait time in the ED for dialysis was 1.07±1.3 hours. During hospitalization 2.3% of these visitors died. Combined uninsured and undocumented patients represented 47.1%. When adjusted for age, race, time to dialysis and length of stay, the adjusted OR of death for undocumented patients was 2.56 (95% CI 1.56-4.82, p=0.003). Undocumented patients were older (p=0.01), had significantly higher rate of unknown race (p=0.001), time to dialysis (p=0.009) and length of stay (p=0.038) compared with documented patients.

Conclusions: Undocumented status predicted in-hospital mortality. Volume overload was highest in undocumented patient's visits but failed to predict in-hospital mortality. Further studies are needed to determine the mechanisms underlying the protective effect of sustainable medical insurance on mortality.

TH-PO779

Differences in Dialysis Modality and Mortality by Proximity to a Pediatric Dialysis Centre Anke M. Banks,[1] Andrea Soo,[1] R. Todd Alexander,[2] Bethany J. Foster,[3] Susan M. Samuel.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada; [3]McGill University, Montreal, QC, Canada.*

Background: Canadian children with end-stage renal disease (ESRD) may live a great distance from renal care. It is unknown if this geographical barrier influences dialysis modality or mortality.

Methods: Using data from the Canadian Pediatric ESRD Database, pediatric patients (<18 years) who began dialysis in Canada between 1992-2010 were followed until death, first kidney transplant, date of last contact or study end (December 31, 2010). We calculated the distance from a patient's residence to the nearest pediatric dialysis centre (PDC) using geographic information software and categorized them as <50 km, 50-150 km, 150-300 km or ≥300 km. We analyzed mortality by distance to PDC and modality using Cox proportional hazard models adjusted for etiology of ESRD, age at start of dialysis, gender, ethnicity and income quintile.

Results: 893 patients were followed for a median of 1.3 (IQR 0.7-2.4) years (total follow-up 1799 patient-years). The median age at dialysis onset was 13.1 (IQR 7.5-15.8) years. Most patients (49.9%) lived <50 km from a PDC. A larger proportion of Aboriginals than Caucasians lived ≥300 km from a PDC (50.0% vs. 21.0%, p<0.001). Of patients living <50 km away, more started on hemodialysis (HD) than peritoneal dialysis (PD) (59.2% vs. 40.8%, p<0.001), and 85.6% remained on HD after initiation. Almost equal numbers of patients living ≥300 km away started on HD and PD (45.6% vs. 54.4%, p=0.71), but 31.3% who started on PD changed to HD. There were 54 deaths prior to transplantation with a median age at death of 15.1 (IQR 3.7-20.2) years. In HD patients, living ≥300 km away was associated with an increased risk of mortality compared to those living <50 km away (hazard ratio 5.17; 95% CI 2.14-12.46).

Conclusions: The majority of Canadian pediatric ESRD patients living close to a PDC started on HD and stayed on HD. A large proportion of patients living ≥300 km from a PDC also started on HD or switched to HD from PD. Pediatric HD patients living ≥300 km from a PDC have an increased risk of mortality.

Funding: Private Foundation Support

TH-PO780

Centre/Provider Specific Performance Indicators for Renal Services Using Routine Linked Data: Survival, Frequency of Admission and Hospitalisation to Start Renal Replacement Therapy James Fotheringham,[2,3] Richard M. Jacques,[3] Damian G. Fogarty,[1] Meguid El-Nahas,[2] Michael J. Campbell.[3] *[1]The UK Renal Registry, Bristol, United Kingdom; [2]Sheffield Kidney Institute, University of Sheffield, Sheffield, South Yorkshire, United Kingdom; [3]School of Health and Related Research, University of Sheffield, Sheffield, South Yorkshire, United Kingdom.*

Background: Renal Replacement Therapy (RRT) registries have the capacity to report centre specific outcomes. Case-mix needs to be adjusted for and missing data minimised to allow robust comparisons, with measures meaningful to the patient and clinician.

Methods: 21,633 patients starting RRT between 2002 and 2006 in England were linked to 11,546 mortality records and 290,433 hospitalizations. Comorbid conditions at the start of RRT were identified from discharge diagnoses. In patients surviving beyond 90 days, survival, all cause frequency of admission, hospitalization to start dialysis and hospital standardized mortality were modelled using Cox proportional hazards, the negative binomial distribution and logistic regression. Centre specific survival proportions and observed/expected ratios for admissions and mortality rates were compared using funnel plots.

Results: Ninety-eight percent of incident patients were suitable for analysis across 46 centres. Age, ethnicity and socio-economic status explained a large proportion of performance variation. Following adjustment for these and comorbidity, the number of outliers for incident 3-year survival uncensored for transplantation reduced from 6 to 1. Outliers for admission rate in the first 12 months of dialysis changed from 4 to 3 in hemodialysis and 4 to 5 in peritoneal dialysis. Six centres remained outliers for hospital associated mortality and for hospitalization to start dialysis. All measures had centres moving both in and out of control following adjustment.

Conclusions: Routine linked data combined with robustly calculated and adjusted performance measures presents a powerful way of comparing centre performance. In England adjusted survival on RRT is similar across centres. However, patients in some centres are admitted four times as often as in others, with associated cost and reduction in quality of life.

TH-PO781

Early Referral to Nephrologist Improved Mortality of Newly Diagnosed End Stage Renal Disease Patients Do Hyoung Kim,[1,4] Jung Pyo Lee,[2,4] Yun Jung Oh,[1,4] Hyun-Seop Cho,[1] Jiwon Ryu,[1] Yong-Lim Kim,[3,4] Yon Su Kim.[1,4] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, National University Boramae Medical Center, Seoul, Korea; [3]Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea; [4]Clinical Research Center for End Stage Renal Disease, Daegu, Korea.*

Background: Over the past several years, interest has evolved in evaluating the timing of nephrology referral as an important variable related to prognosis. The purpose of this study was to explore the impact of early referral to nephrologist on patient survival in a subset of patients from Comprehensive Prospective Study of Clinical Research Center for End Stage Renal Disease (CRC ESRD) in Korea.

Methods: A total of 1028 incidental ESRD patients newly diagnosed from July 2008 to October 2011 were enrolled. Data were served from CRC ESRD nationwide web-based multi-center joint network prospective cohort in the Korea. Patients were classified arbitrarily as early referrals if their first encounter with a nephrologist occurred longer than 1 year, number of visit to nephrologist >2 times, and education for dialysis (from nurse or nephrologist) prior to initiate dialysis.

Results: Time from referral to dialysis was significantly longer in 522 early referral patients (ER) than in 506 late referral patients (LR) (63.6±59.6 vs. 10.7±28.1 mo, P<0.001). Emergent hemodialysis was required in 217 (41.6%) of ER compared with 268 (53.0%) of the LR (P< 0.001). The survival rate in ER was better than that in LR (P =0.046). The 1-year and 2-year survival rates in ER were better than that of LR (p=0.049). The 1-year and 2-year survival rates of ER were 95.9% and 90.9%, respectively, compared to 92.1%, and 80.3% of LR. In diabetic ESRD patients, the survival rate of ER was significantly higher than that in LR (P=0.032). The difference was significant after adjusting for covariates (OR 0.16 95% CI 0.03-0.93, P=0.042).

Conclusions: Early referral to nephrologist is associated with reduced mortality especially in diabetic chronic kidney disease patients.

TH-PO782

Bouncing Back: Patterns of Early Hospital Readmissions in ESRD Patients on Dialysis Priyanka Khatri,[1] Lisa A. Pacelli,[2] Peter Juergensen,[2] Shirin Shirani,[2] Fredric O. Finkelstein.[2] *[1]Hospital of Saint Raphael, New Haven, CT; [2]Hospital of Saint Raphael, Renal Research Institute, New Haven, CT.*

Background: Hospital readmissions are an important measure for assessing performance of the health care system as well as dialysis facilities. According to the 2011 report by United States Renal Data System, up to 36% of ESRD patients are rehospitalized within 30 days of discharge. There is a need for investigations to examine the profile of readmissions in this population to identify potentially modifiable factors. Only a few studies have focused on readmissions specifically in ESRD patients.

Methods: We conducted a retrospective review of discharge summaries of all dialysis patients readmitted within 30 days of their last discharge during the year 2009-2011 at 3 dialysis centers in New Haven, CT. Data was collected regarding the basic patient demographic profile, cause of index hospitalization and cause of readmission. Two authors independently reviewed the causes of hospitalizations and characterized the readmissions as being for the same diagnosis, a problem directly related to, or a problem unrelated to the index hospitalization.

Results: Admissions averaged about 2 admissions per patient year. The 30 days readmission rate was 30.8 %. 237 readmissions amongst 121 patients were examined in detail .The mean+/- SD age of study population was 67+/- 14 years. 50% were male. 34% of the readmissions occurred within 1 week of discharge. 17 % of patients were readmitted with the same diagnosis and an additional 22 % with a cause related to the initial diagnosis.

Conclusions: This study suggests that nearly 40% of ESRD patients who are readmitted within 30 days of discharge are readmitted with the same or a diagnosis related to the index hospitalization. 1/3 of readmissions occur within the first week. These findings underscore the importance of improving immediate discharge planning and post hospitalization care as a means of trying to reduce the high rehospitalization rate observed in these patients. Further studies focusing on possible areas of intervention including better communication between hospital and dialysis care providers need to be done.

TH-PO783

Mortality Trends in Wait-Listed Dialysis Patients versus the General Population in the United States, 2000-2009 Robert N. Foley,[1,2] Allan J. Collins,[1,2] David T. Gilbertson,[1] Shu-cheng Chen.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of MN, Minneapolis, MN.*

Background: Apparently encouraging survival gains continue to accrue in the US dialysis population. It is unknown, however, whether these salutary trends reflect improving survival in the general population or differences in comorbidity in the dialysis population.

Methods: In order to minimize these potential effects, we evaluated annual mortality trends in US dialysis patients from the time of first transplant waitlisting between 2000 and 2009 and compared these estimates with expected mortality rates in the US general population over the same period.

Results: Among patients first wait-listed for transplant in 2000, the observed mortality on dialysis was 5.8 per 100 person years; based on the distribution of age, sex and race ethnicity, this value was 6.2 times that expected from general population estimates. By 2009, the ratio of actual-to-expected mortality ($M_{A/E}$) had declined significantly to 4.9, a change of 21%. When trends within subgroups were examined, there were notable disparities in findings by gender ($M_{A/E}$ declining from 4.8 to 4.1 in males and from 9.3 to 6.9 in females), race-ethnicity (White: 8.1 to 6.4; Black 5.0 to 3.4; Hispanic 7.0 to 7.9) and mode of dialysis (hemodialysis: 5.9 to 4.8; peritoneal dialysis 7.7 to 6.1).

Conclusions: Survival gains trends in patients wait-listed for transplant mirror those seen in the overall dialysis population and are greater than expected from general population estimates.

Funding: NIDDK Support

TH-PO784

Comparing the First Two Years of the CMS ESRD Quality Incentive Program (QIP) Alissa Kapke,[1] Jeffrey Pearson,[1] Marc Turenne,[1] Erik Roys,[2] Emily E. Messersmith,[1] Matthew Paul,[2] Claudia Dahlerus,[1] Joseph M. Messana.[2] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]University of Michigan KECC, Ann Arbor, MI.*

Background: The impact of the ESRD QIP on dialysis facility payments may vary over time due to changes in program design or facility performance. Changes in the QIP for payment year 2013 (PY13) include retiring the hemoglobin (hgb)<10 measure, equal weighting of hgb>12 and hemodialysis adequacy measures, payment reductions for any facility with less than the maximum total performance score (TPS<30), and elimination of the 0.5% reduction. Changes in clinical practice between performance years PY12 (2010) and PY13 (2011) may have occurred in anticipation of the PY13 QIP or due to the expanded ESRD PPS.

Methods: We compared actual PY 2012 results with 9 months of 2011 CMS data to simulate PY13 results.

Results: In PY12, 31% (1528) of scored facilities received a payment reduction while 12% (619) are projected to receive a reduction in PY13. Of those with a reduction in PY12, 20% (305) are also projected to receive one in PY13. Among facilities with no PY12 reduction, 9% (312) are projected to receive a reduction in PY13.

Comparison of facilities receiving PY 2012 payment reductions and simulated PY 2013 reductions

PY 2013 Simulation				
PY 2012	**No Reduction**	**Some Reduction**	**Not Scored**	*Total*
No Reduction	2991	312	108	*3411*
Some Reduction	1166	305	57	*1528*
Not Scored	226	2	639	*867*
Total	*4383*	*619*	*804*	*5806*

Among facilities with a reduction in PY12, 66% (1010) received a reduction due to their hgb<10 score. In PY13 only 109 of these facilities are projected to receive a reduction. Among facilities with hgb>12 scores in both years, maximum hgb scores are expected to increase from 98% in PY12 to 99.6% in PY13. Maximum URR scores are expected to increase from 85% to 87%. Finally, less than 4% of facilities with scores from 26-29 are estimated to incur a reduction in PY13 but would not have received a reduction using the PY12 scoring system.

Conclusions: In year two of the QIP substantially fewer payment reductions are expected due to removal of the hgb<10 measure. Changes in other aspects of the QIP and in clinical practices are expected to have a smaller impact on facility payments.

Funding: Other U.S. Government Support

TH-PO785

Current Status of Dialysis in Developing Asian Countries and Future Support Toru Hyodo.[1,2] *[1]NGO Ubiquitous Blood Purification International, Yokohama, Japan; [2]The Program Committee of the 17th Annual Meeting of Japanese Society for Hemodiafiltration (JSHDF), Sagamihara, Japan.*

Background: In Asian countries, details of dialysis practices have not been clarified due to a lack of statistical data. The actual status was identified as a result of a special investigation conducted by the program committee of the 17th annual Meeting of Japanese Society for Hemodiafiltration.

Methods: With the cooperation of 9 dialysis-related companies that have sales distribution in Asia, the actual status of dialysis practices as of 1st July, 2011 was investigated. Based on the data collected by each company, we investigated the number of dialysis facilities and patients in each country, ranking of primary diseases at dialysis initiation, number of patients undergoing hemodialysis (HD), online hemodiafiltration (HDF), and continuous ambulatory peritoneal dialysis (CAPD), number of hemodialysis monitoring devices, number of online HDF monitoring devices, cost per dialysis session (US dollar) and the self-pay rate (%), and 1-year survival rate of dialysis patients (%).

Results: Dialysis facility numbers in Asia are increasing every year. Diabetes is now the most common cause for initiating dialysis in all Asian countries. The 1-year survival rate of dialysis patients was approximately 90% in developed countries. Although, there are many countries with an unknown survival rate, that in Myanmar was 30%, whereas, that in the Philippines and Malaysia was approximately 90%. The precise data is shown below.

Conclusions: Since explosive growth of the dialysis population is expected in developing countries, developed countries should provide support to facilitate proper dialysis practices by nurturing clinical engineers.

The Status of Asian Dialysis

Country	Dialysis patients number	Hospital number	On-line HDF patients number	HD patients number	CAPD patients number	The cost of HD(US dollar)	Private charge(%)
Cambodia	200	10	0	200	0	55	100
Myanmar	600	28	0	600	0	55	100
Philippine	10000	270	unknown	9300	700	50	80
Vietnam	12000	130	5-10	10000	2000	30	0
Malaysia	23500	600	unknown	21700	1800	50	0
Thailand	29500	500	1400	26500	1500	25	0-25
Corea	45009	614	4000	37391	7618	130	10
Taiwan	63655	552	2200	55825	6110	170	0
China	272000	3500	unknown	260000	12000	90	5-30
Japan	297126	4152	unknown	297126	9728	350	0

Funding: Pharmaceutical Company Support - Nikkiso, Toray, Kawasumi, KRD, Nipro, JMS, Asahi Medical

TH-PO786

Cleanliness of Hemodialysate and Future Support in South East Asian Developing Countries Toru Hyodo.[1,2] *[1]NGO Ubiquitous Blood Purification International, Yokohama, Japan; [2]The Program Committee of the 17th Japanese Society for Hemodiafiltration (JSHDF), Sagamihara, Japan.*

Background: There have been no studies reporting the cleanliness of dialysate used at dialysis facilities in Asian developing countries. Its actual status was identified as a result of a special investigation conducted by the program committee of the 17th Japanese Society for Hemodiafiltration (JSHDF) and NGO Ubiquitous Blood Purification International.

Methods: The research team including a specialist of dialysate purification was dispatched from the JSHDF, and investigated the quality of dialysis water used at dialysis facilities in Vietnam and Cambodia with the cooperation of three facilities in Vietnam (including one dialysis facility which was established in the Department of Nephrology with the support of NPO Ubiquitous Blood Purification International) and one facility in Cambodia (dialysis facility established with the support of NPO Ubiquitous Blood Purification International). The endotoxin (ET) level (EU/L) and bacterial count (CFU/mL) in tap water, in water purified by a reverse osmosis (RO) system, and in dialysates were measured.

Results: There were no facilities that fulfilled criteria for bacterial counts specified in the ISO 23500 guideline (Standard dialysate:<500 EU/L, <100 CFU/mL, Ultra pure dialysate: <30EU/L, <0.1CFU) in both standard and ultrapure dialysates. Two facilities fulfilled criteria for the ET level only in standard dialysate.

ET level and Bacterial Counts of Dialysate in Asian Developing Countries

Hospital	ET(EU/L)	Bacteria(CFU/mL)
A	741	500
	903	800
B	38	150
	49	100
C	201	1000
	395	1000
D	309	800
	245	800

Conclusions: Physicians in these countries have read many studies, and desire to adopt online HDF in their own countries. Not only the provision of medical devices, but also organic support for implementing dialysate purification are needed. A support program for nurturing clinical engineers in developing countries is now about to begin in Japan and South East Asia.

Funding: Private Foundation Support

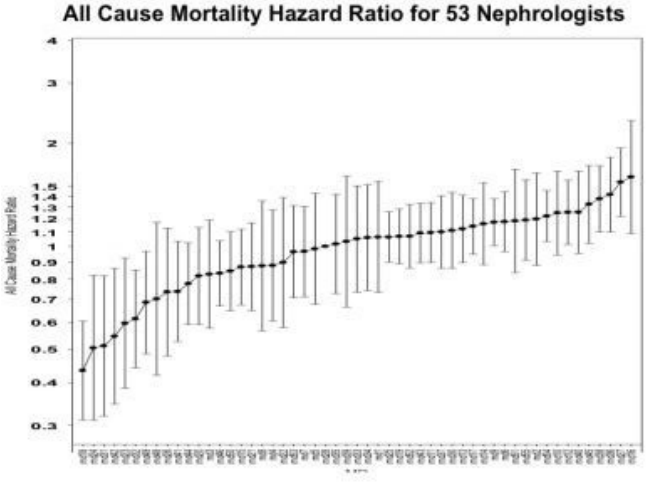
TH-PO787

Nephrologist Practice Pattern and Survival of Hemodialysis Patients in a Densely Populated Urban Area in California Kevin T. Harley,[1] Elani Streja,[2] Alpesh Amin,[2,3] Kamyar Kalantar-Zadeh.[2,3] *[1]Nephrology, University of California, Irvine; [2]Harold Simmons Center, LABioMed Harbor-UCLA; [3]David Geffen School of Medicine at UCLA.*

Background: California nephrologists care for a diverse group of maintenance hemodialysis (HD) patients. There is wide variability in patient characteristics and practice patterns. We examined the association between nephrologist-related factors and all-cause mortality in a densely populated urban area in California.

Methods: We anonymously ranked all nephrologists in the selected region according to their HD patient mortality rate (from lowest to highest) over a six year period (7/2001-6/2006), based on data derived from the dialysis clinics of a large dialysis organization and the United States Renal Data System. We calculated hazard ratios for all-cause mortality for each nephrologist and compared patient characteristics of the ten highest and ten lowest scoring nephrologists to detect meaningful differences.

Results: A total 53 nephrologists, each with at least 50 HD patients cumulatively over the study period, provided care to 8,947 HD patients. After ranking all 53 nephrologists according to their HD patient hazard ratios (see Figure), the ten nephrologists with the lowest mortality averaged 72 HD patients while the ten nephrologists with the highest mortality averaged 139 HD patients [72 (64- 80) vs. 139 (92- 185), p< 0.0001]. Additionally, the ten nephrologists with the lowest mortality ratios had patients whose average dialysis time was 10 minutes longer than their ten colleagues with the highest mortality ratios (212±26 vs 202±23, p < 0.0001).



Conclusions: Number of patients carried per nephrologist and longer dialysis treatment time appear associated with better quality of care including patient survival. These findings need to be examined in additional studies.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

TH-PO788

Predictors of Provider-Patient Visit Frequency on Hemodialysis

Yelena Slinin,[1] Haifeng Guo,[2] Suying Li,[2] Jiannong Liu,[2] Benjamin R. Morgan,[1] Kristine E. Ensrud,[1] Areef Ishani.[1,2] *[1]Veterans Administration Health Care System, Minneapolis, MN; [2]Chronic Disease Research Group, Minneapolis, MN.*

Background: In 2004, CMS tied provider reimbursement for outpatient hemodialysis services to a number of times per month providers see their patients on dialysis. Determinants of provider visit frequency to patients on dialysis are uncertain. We aim to identify patient, provider, and facility characteristics associated with provider visit frequency to hemodialysis patients.

Methods: Using USRDS data for point-prevalent patients on in-center hemodialysis on 1/1/2006 (n = 144860), we defined patient characteristics during 1/1/2006-6/30/2006 and calculated provider-patient visit frequency during 7/1/2006-12/31/2006. Provider data were obtained from the AMA. We determined associations between patient, provider, and facility characteristics and provider visit frequency on dialysis using logistic regression.

Results: Patient characteristics independently associated with provider visit frequency (≥4 visits compared to <4 visits/month) included age: Adjusted Odds Ratio (95% Confidence Interval) 1.23(1.18-1.28) for 65+, compared to 20-44 years; African-American race:1.20(1.17-1.24), dialysis duration: 1.08(1.05-1.10) for > 5 vs 1-5 years; comorbidity score:1.13(1.0-1.17) for 4-8, 1.20(1.16-1.24) for ≥8, compared to <4; eligibility for Medicaid:1.06(1.03-1.08); urban status:1.21(1.17-1.25); and greater number of dialysis sessions:1.31(1.25-1.35). Provider characteristics associated with visit frequency included number of years in practice:1.13(1.09-1.16) for >22 compared to <8 years; being a foreign graduate:1.15(1.13-1.18); distance from the practice location to the dialysis unit: 0.70(0.68-0.72) for 2.5-11.5, 0.52(0.51-0.54) for >11.5, compared to<2.5 miles; and number of dialysis patients per provider:1.18(1.15-1.22) for 50-100, 1.49(1.44-1.54) for>100 compared to<50 patients. Facility characteristics associated with provider visit frequency included chain 0.85(0.83-0.88) and hospital 0.79(0.74-0.83) affiliations.

Conclusions: Several patient, provider, and facility characteristics were independently associated with frequency of provider-patient visits on dialysis.

Funding: NIDDK Support

TH-PO789

A Comparitive Study of Outcomes in Fellow Managed versus Non-Fellow Managed Dialysis Units in New York City

Adedoyin G. Akintide,[1] George N. Coritsidis,[1] Marie France R. DeLeon,[1] Kasun I. Navarathna,[1] Carol Lyden.[2] *[1]Division of Nephrology, Elmhurst Hospital Center, Elmhurst, NY; [2]IPRO, Lake Success, NY.*

Background: Many private dialysis units are staffed primarily by technicians and supervised by a few nursing staffs with overseeing nephrologists. The literature has suggested that for-profit units may have worse outcomes in terms of mortality than not-for-profit units. Our literature search did not find any studies looking at outcomes in fellow managed vs non-fellow managed dialysis units. We were interested to see what impact the involvement of an academic fellowship program has on outcomes in dialysis units.

Methods: We reviewed de-identified records from Network 2 of 19 units in the borough of Queens, New York for 2009 and 2010, and compared outcomes. Three fellow managed units (FM) were compared to the remaining sixteen non-fellow managed units (NF).

Results: In 2010, there were no significant differences in age or the prevalence of African Americans, Hispanics or diabetics. However, there were more Asians in the NF units (30 vs 14%, p=0.0065). The urea reduction ratio metric of ≥96% of patients per unit (URR metric) was met by more of the FM units than the NF units. However, the difference was not statistically significant. A higher percentage of the FM patients compared to NF patients met the URR metric (URR % pt, p=.0015). There were fewer hospitalized days per patient (Hosp) in the FM units (P=.0176). Fistula, graft, catheters in use for >90days as well as the standardized mortality ratio (SMR) did not differ. Similar findings were observed for 2009 data except only 47% of the NF units met the URR metric vs 100% of the FM units.

Table of Results

2010	age	DM	African Amer	URR(metric)	*URR(pt average)	*Hospitalizations	SMR
FM units N=3 (549 pts)	60±3.0	48%	40%	100%	99.8%	3.8±0.6	1.0± 0.9
NF units N=16 (3515 pts)	62±0.6	46%	48%	77%	97.6%	6.9±0.5	1.1± 0.3

Conclusions: The presence of academic fellows at dialysis centers may improve URRs and minimize hospitalization days. Our findings were consistent over a 2-year period in similar dialysis populations and may reflect greater physician presence. Mortality was not affected possibly due to small patient samples.

TH-PO790

Estimating US Dialysis Transportation Costs: An Exploratory Analysis

Mark Stephens,[1] Samuel Brotherton,[1] Stephan C. Dunning,[2] Larry C. Emerson,[3] David T. Gilbertson,[2] Ann C. McClellan,[4] William M. McClellan,[4] Sanatan Shreay,[5] Matthew Gitlin.[5] *[1]PHA; [2]CDRG; [3]Dialysis Ctr of Lincoln; [4]Emory; [5]Amgen.*

Background: The costs of transportation to dialysis for US patients have not been previously reported on a national level, but have been estimated in the billions of dollars annually, with a heavy share of costs borne by patients and their families. Previous research suggests that substantial cost savings could be achieved if medically-appropriate transport was made available and covered by insurance.

Methods: We estimated the total costs of transportation for 360,000 US dialysis patients for travel to routine dialysis. Per trip travel distance estimates were calculated from patient ZIP codes and dialysis facility addresses. The costs of ambulance trips were calculated from Medicare's 2012 Ambulance Fee Schedule. Other cost and utilization estimates were derived from government reports, transportation websites and peer-reviewed literature. We estimated potential savings of reduced ambulance use.

Results: The estimated annual cost of transportation to dialysis in the US is nearly $3 Billion, about 6-7% of total dialysis patient costs. Costs per patient per year are estimated at over $8000. One-third of these costs are not covered by patients' primary or secondary insurance. Ambulance services account for 46% of all costs, but only 5% of transports. Based on prior research on ambulance use, the potential savings of substituting more appropriate means of transport for ambulances could be $200-$700 million per year.

Estimated 2012 Transportation Costs For Routine Dialysis By Transport Type and Payer ($ Millions)

Transport Type	Medicare	Medicaid	Private Insurance	Patient/Other	Total
Ambulance	$996.5	$148.2	$35.7	$169.2	$1,349.6
Stretcher Van		$164.5	$25.8	$163.6	$353.9
Chair Car		$425.4	$85.7	$335.1	$846.2
Taxi, Public Transit		$74.9		$155.6	$230.5
Private Auto, Other		$27.2		$125.0	$152.2
Total	$996.5	$840.2	$147.2	$948.5	$2,932.4

Conclusions: Policy-makers should consider the potential to reduce unnecessary dialysis transportation costs, while providing appropriate levels of care.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO791

State Level Variations in Nephrology Workforce and Dialysis Stations and Timing of Dialysis Initiation in the U.S.

Elaine Ku, Kirsten L. Johansen, Anthony A. Portale, Barbara A. Grimes, Chi-yuan Hsu. *UCSF, San Francisco, CA.*

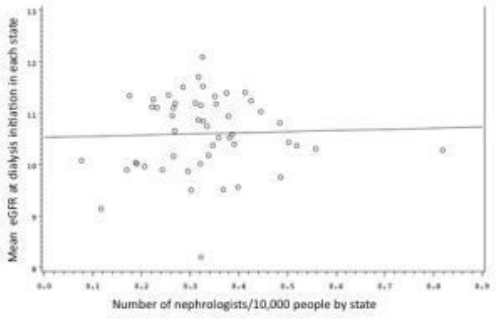
Background: Multiple factors influence timing of dialysis initiation. Impact of systems-based factors such as supply of nephrology workforce is not well known. We hypothesized that dialysis is initiated at higher eGFR in states with higher numbers of adult and pediatric nephrologists per capita and higher number of dialysis stations per capita.

Methods: We determined the number of board-certified pediatric and adult nephrologists in each state using American Boards of Pediatrics and Internal Medicine data as of 12/31/2010 and 2/17/2011, respectively. State population was derived from 2010 US Census. USRDS data were used to determine number of dialysis stations and mean estimated GFR at start of dialysis for children (< 18 years) and adults (≥18 years) using the revised Schwarz and MDRD equations respectively, among the subset with serum creatinine and height available.

Results: We included for analysis 1937 children initiated on peritoneal and hemodialysis between 2007-2009 and 100,983 adults initiated on hemodialysis in 2008. The median number of pediatric and adult nephrologists per state was 0.056 (IQR 0.038 to 0.078) and 0.32 (IQR 0.26 to 0.38) per 10,000 persons, respectively. Mean eGFR at

start of dialysis was 9.7 mL/min/1.73 m^2 (SD 6.0) in children and 10.8 mL/min/1.73 m^2 (6.0) in adults. No significant correlation between eGFR at start of dialysis and number of nephrologists/population/state was apparent with Pearson r of -0.15 (95% CI -0.41 to 0.13 p=0.29) for children and 0.04 (95% CI -0.24 to 0.31 p=0.80) for adults (Figure 1). eGFR at hemodialysis initiation also did not correlate with number of dialysis stations/ adult population/state, with r of -0.02 (95% CI -0.29 to 0.26 p=0.89).

Figure 1: Mean eGFR at dialysis initiation among adult patients vs. number of nephrologists per 10,000 adult population by state



Conclusions: Timing of dialysis initiation was not associated with state-level variations in nephrologists or availability of dialysis stations.

Funding: NIDDK Support

TH-PO792

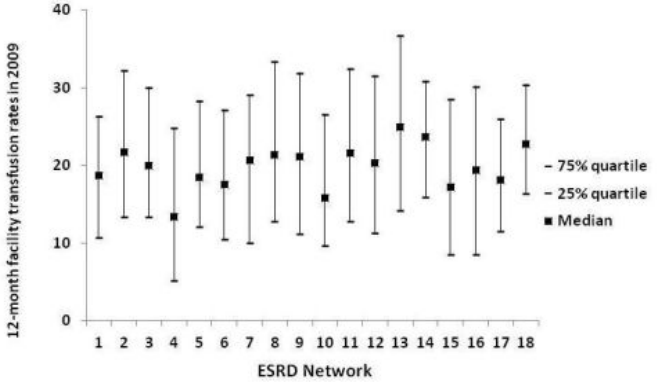
Regional Variation in RBC Transfusions in Hemodialysis Patients

Suying Li,[1] Jiannong Liu,[1] Keri Monda,[2] Tom Arneson,[1] Brian D. Bradbury,[2] David T. Gilbertson,[1] Allan J. Collins.[1] *[1]Chronic Disease Research Group, MMRF, Minneapolis, MN; [2]Amgen, Inc, Thousand Oaks, CA.*

Background: Transfusion avoidance is the primary indication for anemia treatment in dialysis patients. However, there are little data available describing recent trends in the use of transfusions. We investigated transfusion rates in 2008 and 2009 by ESRD network (proxy for geographic region) and facility size.

Methods: The study population was composed of prevalent hemodialysis patients on 1/1/2008 and on 1/1/2009 with ≥ 90 days on dialysis with Medicare as primary payer. To assess transfusions related more to anemia management, patients with sickle cell disease and inpatient (IP) transfusions concurrent with hospitalizations due to major bleeding, surgery, and trauma were excluded. Patients were followed from January 1 to the earliest date of death, transplant, lost to follow-up, or the end of follow up (Jun 30 or Dec 31, depending on 6- or 12-month analysis). Transfusion rates were obtained by dividing total events by the total follow-up time and expressed as per 100 patient-years (pt-yrs). Data were derived from Medicare claims.

Results: The overall 6-month transfusion rates were 23.5 and 22.9 and 12-month transfusion rates were 24.2 and 23.2 per 100 pt-yrs for 2008 and 2009, respectively. The majority of transfusions occurred in the IP setting (~70%). Transfusion rates were stable across provider size (12-month rate ranged from 22.6 to 25.5 in 2008 and 22.1 to 24.3 in 2009). As shown in figure, more variation was observed by ESRD network (12-month rate ranged from 17.9 to 28.5 in 2009).



Conclusions: RBC transfusions were relatively consistent between 2008 and 2009, although there was sizable variation in the rates across ESRD network within the US. Transfusion event evaluation may enable comparisons regarding direct assessment of anemia management practices.

Funding: Pharmaceutical Company Support - Amgen, Inc., Private Foundation Support

TH-PO793

The Initial Impact of Medicare's Bundled, Prospective Payment System for Renal Dialysis

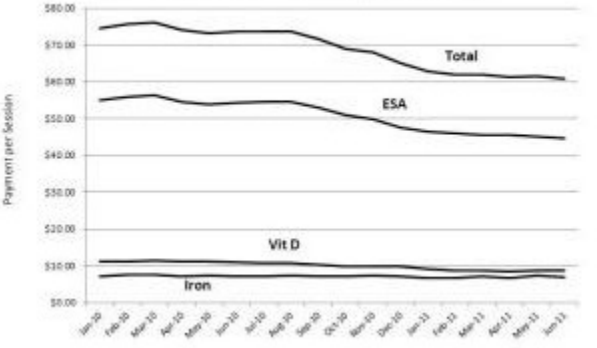
Richard Hirth,[1] Marc Turenne,[2] John R.C. Wheeler,[1] Tammie A. Nahra,[1] Kathryn Sleeman,[1] Wei Zhang,[1] Joseph M. Messana.[1] *[1]Univ. of Mich, Ann Arbor, MI; [2]Arbor Research, Ann Arbor, MI.*

Background: Medicare implemented an expanded prospective payment system (PPS) on January 1, 2011, including services previously paid on a fee-for-service basis. One intent of the PPS was to incentivize providers to be more efficient in providing dialysis services and modality choice.

Methods: We used Medicare claims to assess monthly trends during January 2010 to June 2011 for key injectable drugs that were previously billed separately. We evaluated dialysis modality trends during the first six months of each year from 2007 to 2011. Of note, incentives for reporting use of erythropoiesis stimulating agents (ESAs), iron and vitamin D analogs changed with implementation of the PPS. For 2010, we assessed actual spending. For 2011, we projected spending based on reported utilization.

Results: ESA use declined substantially in the months immediately before and after the PPS, while use of iron products, often therapeutic substitutes for ESAs, increased. For ESAs and vitamin D analogs, the largest changes occurred during the last quarter of 2010. Less expensive vitamin D products were substituted for more expensive ones. Drug spending fell by $14 per session, nearly three times the mandated reduction in the base payment rate of $5. The use of peritoneal dialysis increased from 6.45% in 2010 to 6.80% in 2011, representing a break from the flat 2007-2010 trend. The use of home hemodialysis increased from 1.20% in 2010 to 1.41% in 2011, representing a continuation of the pre-existing trend.

Payments per Session for Select Injectable Drugs



Conclusions: The expanded bundle dialysis PPS provided incentives for the use of lower cost therapies. These incentives seem to have motivated movement toward lower cost methods of care.

Funding: Other U.S. Government Support

TH-PO794

Did the Dialysis Prospective Payment System (PPS) Result in More Patients Receiving Transfusions?

Richard Hirth,[1] Marc Turenne,[2] John R.C. Wheeler,[1] Tammie A. Nahra,[1] Kathryn Sleeman,[1] Wei Zhang,[1] Joseph M. Messana.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]Arbor Research, Ann Arbor, MI.*

Background: On January 1, 2011, Medicare implemented an expanded bundle PPS that included injectable medications previously paid via fee-for-service. This created incentives to use less of these services, particularly ESAs.

Methods: To assess the impact of this policy change on use of blood transfusion, an alternative to ESA for anemia treatment, we used Medicare claims to compare the % of patients receiving ≥1 transfusion during the first 6 months under the PPS (January–June 2011) (4.69%) to the % receiving ≥1 transfusion during the first 6 months 2008 (4.79%), 2009 (4.67%) and 2010 (4.42%). We estimated generalized logit models to control for patient race, sex, age, comorbidity count and year.

Results: Transfusions were more likely among patients who were female (adj. odds ratio (AOR) 1.105, p<.01), non-black (AOR 1.220, p<.01), older (AOR 1.004 per year, p<.01) or had more comorbidities (AOR 1.039, p<.01). The adjusted time trend was similar to the unadjusted transfusion rates, with the lowest rate in 2010 and the highest in 2008. A 2nd model specification added race*year and sex*year interactions to determine if relative use of transfusion by race and sex changed after implementation of the PPS. These interactions were insignificant.

Conclusions: A modestly higher % of patients received transfusions during the first 6 months of 2011 under the PPS than during the first 6 months of 2010. However, the 2011 rates were similar to the rates prevailing in 2008 and 2009. Therefore, it is not clear that the PPS caused a change in the % of patients receiving≥1 transfusion. Further research will explore if there was a change in the frequency of transfusions and ESA use among those patients who received at least 1. The clinical implications of a substitution of transfusions for ESAs among those being transfused may be different than the implications of expanding transfusions into a greater % of the patient population. It should be noted that the data used in this study primarily reflected outpatient transfusions because inpatient claims generally do not contain this level of detail.

Funding: Other U.S. Government Support

TH-PO795

Recent Trends in Anemia Management in Small Dialysis Organizations: The STEPPS Study John M. Burkart,[1] Keri Monda,[2] Margarita Symonian Silver,[3] Susan V. Yue,[2] Robert J. Rubin.[4] *[1]Wake Forest University, Winston Salem, NC; [2]Amgen, Inc., Thousand Oaks, CA; [3]Private Practice, Los Angeles, CA; [4]Georgetown University School of Medicine, Washington DC.*

Background: In Jan 2011 the Prospective Payment System (PPS) was implemented for bundled dialysis care payment. Small dialysis organizations (SDOs, defined as ≤50 facilities) may be particularly susceptible to the financial implications of the PPS. The prospective observational Study to Evaluate the PPS Impact on SDOs (STEPPS) was designed to describe dialysis treatment and outcomes over this period. Herein we examine trends in anemia management between Oct 2010 and Jun 2011.

Methods: Data are collected monthly or quarterly. Trends were assessed via repeated cross-sectional measurements and limited to hemodialysis patients (94.5% in-center and 0.6% home) from 51 SDOs. Lab values are reported as the last quarterly measurement. Intravenous (IV) epoetin alfa (EPO) and darbepoetin alfa (DPO) dose is reported as cumulative monthly and per-administration during the last month of each quarter. Transfusion is expressed as incidence proportion and rate per 100 person-years.

Results: Between Q4 2010 and Q2 2011 median per-administration IV EPO and DPO doses decreased, disproportionately in the 75th percentile; cumulative monthly doses fell to an even greater degree; mean hemoglobin (Hb) declined 0.2 g/dL (table). The percent of patients with Hb <10 g/dL rose from 13% to 17% (32% change); those with Hb >12 fell from 29% to 19% (36% change). Percent increase in transfusion incidence and rate was 61% and 41%, respectively.

Mean±SD; median(p25, p75)	Q4 2010 (N=1730)	Q1 2011 (N=1699)	Q2 2011 (N=1597)
Labs			
Hb (g/dL)	11.3 ± 1.3	11.2 ± 1.3	11.1 ± 1.2
Ferritin (ng/mL)	645 (401, 966)	720 (487, 1034)	768 (485, 1053)
TSAT (%)	30.6 ± 12.1	32.2 ± 12.6	32.5 ± 12.6
Medications			
EPO (units)			
Cumulative monthly	36350 (17000, 71500)	32500 (15000, 62500)	31100 (14000, 60000)
Per-administration	3472 (2100, 6714)	3025 (2000, 5692)	3240 (2000, 6000)
DPO (mcg)			
Cumulative monthly	242.5 (125, 375)	100 (65, 180)	120 (75, 180)
Per-administration	56 (32.5, 95)	31.9 (25, 45)	32.5 (25, 45)
IV iron use (% of patients)	54.2%	56.6%	52.5%
Transfusions			
Proportion (95% CI) (%)	1.8 (1.2, 2.4)	3.0 (2.2, 3.8)	2.9 (2.1, 3.7)
Rate (95% CI)*	13.7 (10.3, 17.7)	21.2 (16.9, 26.3)	19.3 (15.2, 24.3)

*per 100 person-years; SD, standard deviation; p25, 25th percentile; p75, 75th percentile; TSAT, transferrin saturation; CI, confidence interval

Conclusions: During the initial phase and preparation of PPS implementation there was a marked reduction in ESA use, with the greatest reduction among the highest doses, and declines in Hb. Also observed were increases in transfusion outcomes.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO796

Improving Kidney Transplant Parity: Suggestions for Dialysis Providers Teri Browne,[1] Avrum Gillespie.[2] *[1]College of Social Work, University of South Carolina, Columbia, SC; [2]Department of Medicine, Temple University, Philadelphia, PA.*

Background: Research suggests that black dialysis patients are significantly less likely than their white peers to be evaluated and listed for a kidney transplant. We present the findings of two research studies that survey black dialysis patients in two different locations (Chicago and Philadelphia) about their attitudes and knowledge related to kidney transplantation, and use these findings to make recommendations to the interdisciplinary dialysis team members that may help reduce kidney transplant disparities.

Methods: In the Chicago study, 228 black hemodialysis patients in 5 dialysis centers in the Chicago area were surveyed about their kidney transplant status, interest, information, and attitudes about kidney transplant, and attributes of their social networks. In the Philadelphia study, 116 urban, predominantly black hemodialysis patients were surveyed using the *Dialysis Patient Transplant Questionnaire*, and we matched their survey data with their UNOS listing and computerized medical record at time of interview.

Results: In the Philadelphia study, the majority (80%) of patients were interested in a kidney transplant, (71.6%) had been evaluated, yet only 39% were on the transplant waiting list. Moreover, of the patients being evaluated 52.9% incorrectly believed they were on the kidney transplant waiting list. In the Chicago study the barrier was access to transplant, in the Philadelphia study patients had difficulty navigating the transplant system. In both studies, black patients had poor knowledge and understanding about the process related to getting a kidney transplant.

Conclusions: These findings suggest that barriers to kidney transplantation are complex and multidimensional. Furthermore, dialysis professionals can augment their standard course of patient care to identify and attend to this lack of knowledge and understanding.

Funding: NIDDK Support

TH-PO797

Kidney Waitlist and Transplant Rates by Dialysis Facility Characteristics: Race and Rural Classification Valarie B. Ashby, K.A. Wisniewski, Joseph M. Messana, Alan B. Leichtman. *University of Michigan, Ann Arbor, MI.*

Background: Kidney transplant waitlist and transplantation rates are inconsistent across the US. This study examines the contribution of dialysis facility characteristics to this disparity by analyzing the differences in waitlist and transplantation rates among dialysis patients < age 70 at rural vs. urban facilities and at facilities with predominantly Black patients.

Methods: Data were obtained from the 2011 Dialysis Facility Reports, which were derived from CMS and OPTN databases. All comparisons were made using Poisson (Standardized Transplantation Ratio for 2007-10) and linear (% of patients on waitlist in 2010) regression with adjustments for chain affiliation, profit status, hospital association, facility size, state, and facility case-mix for 2,959 dialysis facilities with at least 10 patients age < 70.

Results: Compared to urban, rural facilities had fewer patients on the waitlist (Δ=-2.9%, p<0.001). This difference was larger (Δ=-4.3%, p<0.001) when not adjusting for state. Facilities with predominantly black patients had fewer patients on the waitlist overall (Δ=-3.7%, p<0.001; Δ=-5.0%, p<0.001 when not adjusting for state). The White and Black waitlist rates were lower (Δ=-3.7%, p<0.001 and Δ=-4.0%, p<0.001, respectively) at predominantly Black facilities compared to the White and Black waitlist rates at facilities that are not predominantly Black. Transplantation rates were 23% lower at rural than at urban facilities (p<0.001) and 27% lower at predominantly Black facilities (p<0.001).

Conclusions: Kidney waitlist and transplantation rates for dialysis patients < age 70 are lower in rural dialysis facilities and in predominantly Black facilities. Of note, White patients dialyzing in predominantly Black facilities also have lower kidney waitlist rates than Whites not in predominantly Black facilities. These differences are not explained by chain affiliation, profit status, hospital association, facility size, case-mix, or state. Given the health and cost benefits of kidney transplant vs. dialysis, resources should be (re) allocated to improve the waitlist and transplant rates in rural dialysis facilities and in those with high percentages of Blacks.

Funding: Other U.S. Government Support

TH-PO798

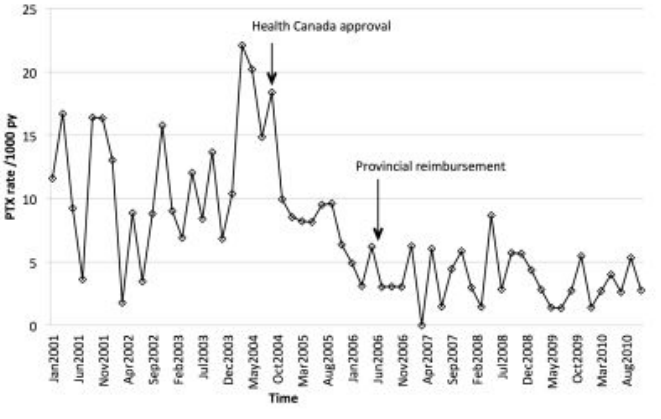
Effect of Cinacalcet Availability and Formulary Listing on Parathyroidectomy Rate Trends Jean-Philippe Lafrance, Heloise Cardinal, Martine Leblanc, Francois Madore, Vincent Pichette, Louise Roy, Jacques Lelorier. *University of Montreal, Montreal, QC, Canada.*

Background: Recent trends in parathyroidectomy (PTX) rates are not known. The aim of this study was to investigate PTX rates trend, and to evaluate if recent availability and reimbursement of cinacalcet modified that trend.

Methods: Using a provincial administrative database, we conducted a time-series analysis including all adult patients receiving chronic dialysis treatments between 2001 and 2010 (incident and prevalent) in Quebec, Canada. We excluded patients with less than 90 days of follow-up, a prior PTX or a kidney transplant. PTX (primary outcome) was identified through hospital discharge summary procedure codes and physician claim codes. We modeled the effect of cinacalcet availability on PTX bimonthly rates using an ARIMA intervention model. Since cinacalcet became available progressively, we used different cut-off dates: Sept. 2004 (Health Canada approval), Jan. 2005, June 2005, Jan. 2006, June 2006 (provincial reimbursement), and Jan. 2007.

Results: We followed 12 795 chronic dialysis patients (mean age 64 years, 39% female, 82% hemodialysis) for a mean follow-up of 3.3 years. We identified 267 PTX during follow-up, translating to an average rate of 7.0 per 1000 person-years (py). The average parathyroidectomy rate before cinacalcet availability was 11.4 /1000 py, and 3.6 /1000 py after cinacalcet formulary listing. Only January 2006 as an intervention date in the ARIMA model was associated with a change in PTX rates (estimate: -5.58, p=0.03). Other intervention dates were not associated with lower PTX rates.

Conclusions: We found decreased rates of PTX after January 2006, corresponding to cinacalcet availability. However, decreased rates may be due to other factors occurring simultaneously with cinacalcet introduction and further studies are needed to confirm these findings.



Funding: Pharmaceutical Company Support - Amgen Canada

TH-PO799

Daily Patient Reported Outcome Reporting Is Practical and Demonstrates Differential Patient Experience in CKD Zoe C.L. Pittman,[1] Stephen G. John,[1] Suzanne K. Roberts,[1] Chris W. McIntyre.[1,2] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]School of Graduate Entry Medicine and Health, University of Nottingham, Derby, United Kingdom.*

Background: Patient reported outcomes (PRO) are increasingly recognised as a critical metric in chronic disease. Whilst a variety of generic and disease specific tools have been reported in CKD, these are mainly cross-sectional with limited longitudinal data. Specifically, there are no data on frequent PRO assessment and no tool designed for frequent use. We therefore designed and implemented a CKD specific online PRO for daily use.

Methods: PRO selection was informed by integration of a patient survey with published data. 6 separate domains of interest (general well being (GWB), pain, sleep, breathing, energy, appetite) were selected. A custom website was built to capture these PRO daily utilising visual analogue scales (allows free selection of status from 0 (worst) to 100(best)). 44 haemodialysis (HD) and 30 pre dialysis patients (CKD) agreed to receive individual website registration details and to complete data daily from home.

Results: 19 HD and 21 CKD patients subsequently registered, 57% male, mean age 61±13 yrs. 3 HD patients withdrew (1 died, 2 transplanted). 75% (68% HD, 81% CKD) submitted data for >30 days, with 70% submitting at least three times per week. Patients reported lower scores (median ± IQR), for energy 78±44, GWB 83±35 and sleep 85± 55 than pain 90±41, appetite 94±44 and breathing 95±21 (p<0.001 overall). HD patients had significantly lower median scores for sleep (48± 53, 95± 22, p=0.012) and appetite (54±45, 97±13, p=0.020) and a trend to lower score for energy (60±53, 84±25, p=0.079). HD patients had greater individual variability for sleep (p=0.022) and appetite (P=0.015). 63% of registered patients still submit data at 90 days.

Conclusions: We have successfully introduced a web based system for recording frequent PRO. This is acceptable to patients and has shown ability to discriminate symptom severity cross-sectionally and longitudinally. Ongoing work will expand scope (other patient groups and vascular access), increase access (data input at HD sessions) and deepen analysis (linkage to other patient data).

TH-PO800

Peritoneal Dialysis and In-Center Hemodialysis: A Cost Effectiveness Analysis Frank Xiaoqing Liu,[1] Catrin Treharne,[2] Murat Arici,[3] Lydia Lees,[2] Ira D. Davis,[1] James A. Sloand.[1] *[1]Baxter Healthcare Corp., Deerfield, IL; [2]Abacus International, Oxfordshire, United Kingdom; [3]Baxter Healthcare Corp., Compton, United Kingdom.*

Background: The National Institute of Clinical Excellence has estimated that £37 million annual savings could be generated in England by increasing the number of prevalent peritoneal dialysis (PD) patients from current 15% to 39%. Achieving this goal will require that the number of new PD patients increases significantly from current 20%. The aim of this study is to estimate the benefits and costs associated with hypothetical scenarios of PD use vs. the current situation.

Methods: We constructed an Excel-based Markov model to estimate the costs and quality adjusted life years (QALYs) associated with different modality distributions from the UK payer's perspective. We modeled an incident patient population over a time horizon of 5 and 10 years. The current UK dialysis modality distribution (scenario 0) of 20% PD, 79% in-center HD (ICHD), and 1% home HD, was compared to 3 hypothetical scenarios: Scenario 1: 50% PD; Scenario 2: 39% PD; Scenario 3: 0% PD. In all of these scenarios, the level of ICHD changes accordingly while home HD stays constant. Model parameters and data inputs were from published articles, annual reports of the UK Renal Registry, NHS Payment by Results, and ERA-EDTA registry. All future costs and benefits were discounted to their present value at an annual rate of 3.5%.

Results: The number of incident dialysis patients was estimated to be 6,366 in UK in 2012. Scenarios 1 (50% PD) & 2 (39% PD) demonstrate lower costs and higher QALYs compared to scenario 0 (20% PD), whereas opposite findings were seen for scenario 3 (0% PD) compared to scenario 0. Increasing PD to 39%-50% saves between £2,519-4,579 per patient and results in an increase of 0.018-0.036 QALYs per patient. Reducing the PD use to 0% results in an increase of costs between £2,652-3,052 per patient and a decrease in QALY between 0.020-0.024 per patient.

Conclusions: Our analysis indicates that increasing the use of PD among incident ESRD patients can generate healthcare savings while improving health benefits. Our results are consistent with NICE PD clinical guideline.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

TH-PO801

Measuring the Effect of ESRD Medicare Facility Surveys on Dialysis Facility Outcomes Joseph M. Messana, Deanna Chyn, Valarie B. Ashby, N. A. Lueth, Tempie H. Shearon, Richard Hirth. *Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI.*

Background: Each year, CMS evaluates approximately 1500 chronic outpatient dialysis facilities for adherence to Medicare's ESRD Conditions for Coverage (CfCs). A facility's failure to fully meet a CfC results in a citation(s), classified as either standard or, for more severe violations, condition-level (CfC citation). This study explores the association between FY 2009 CfC citation status and changes in facility outcomes by comparing outcomes in the year before (CY 2008) and after (CY 2010) the survey.

Methods: Using survey data from the CMS Computing System, we determined the presence of any CfC on FY09 facility surveys. We combined these data with Dialysis Facility Report data, which are based on national CMS data, from CY 2008–10. Three years of outcomes data were available for 5177 facilities, of which 1083 were surveyed in FY09. Using each facility's citation status, we fitted linear models to predict changes in primary (mortality (SMR), hospitalization (SHR)) and clinically important intermediate (% patients with URR >65%, % patients with catheter as dialysis access) outcomes over the 2-year period. We weighted the models by facility size and adjusted for patient demographics and diabetes as cause of ESRD.

Results: Non-surveyed dialysis facilities experienced a magnitude and direction of two-year change in outcomes similar to that of surveyed facilities that received no CfC citations. In contrast, surveyed facilities that did receive a CfC citation had small but statistically significant improvements in % patients with catheter dialysis access and % patients with URR >65%. Primary outcomes did not differ by facility citation status.

Change in Facility Outcomes (2010 v 2008) by FY 2009 Facility Citation Status

Status	SMR	SHR	% Catheter	% URR >65%
Not surveyed	+0.004	-0.02	-3.67	+0.45
Surveyed, no CfC	+0.03	-0.001	-3.50	+0.30
Surveyed, at least 1 CfC	-0.02	-0.02	-4.83	+1.51
p-value (CfC vs. no CfC)	0.09	0.35	0.01	<0.001

Conclusions: Facilities cited for CfC deficiencies may be more strongly motivated than non-cited facilities to identify and improve modifiable intermediate outcomes.

Funding: Other U.S. Government Support

TH-PO802

Dialysis Treatment Time, Dialysis Frequency, and Dialysis Adequacy among Hemodialysis Patients in the Saudi Arabia Dialysis Outcomes and Practice Patterns Study (DOPPS) Ayman Karkar,[2] Brian Bieber,[1] Saad Alghamdi,[2] Faissal A. Shaheen,[2] Jamal S. Alwakeel,[2] Fayez F. Alhejaili,[2] Mohammed A. Al-Ghonaim,[2] Haroun Zakaria Ahmed,[2] Sylvia Paz B. Ramirez,[1] Ronald L. Pisoni.[1] *[1]Arbor Research; [2]Saudi Arabia DOPPS Country Investigators.*

Background: In order to understand practice patterns of hemodialysis (HD) care in Saudi Arabia (SA), a pilot study of SA DOPPS was conducted from November 2011-May 2012. We present practice patterns for HD prescription in SA compared to other DOPPS regions.

Methods: 20 HD facilities treating at least 23 chronic HD patients were randomly selected from a comprehensive roster of dialysis units to be representative of >95% of SA HD patients. Descriptive results shown for SA, based on a random sample of 20-25 HD patients in each study facility, are compared to other DOPPS regions. Preliminary results in SA are based on 315 HD patients from 16 dialysis units. Results are presented as weighted estimates, accounting for the sampling fraction in each unit.

Results: Mean # prescribed HD sessions/week was similar in SA (2.96) versus the 3 other DOPPS regions, with 5% of patients dialyzing <3x/week (Table). Mean HD session length in SA (205 min) was lower than in other DOPPS regions (range 220-245). The mean blood flow rate of 290 mL/min was lower than in EUR/ANZ (317) and North America [NA] (406), but higher than in Japan (203). Mean BMI was lower in SA than in EUR/ANZ and NA, but higher than in Japan. Mean URR in SA (66%) was slightly lower than in Japan (69%), but significantly lower than in EUR/ANZ (74%) and NA (74%).

Dialysis Adequacy Measures, by Region

Measure	Saudi Arabia (N=315)	EUR/ANZ (N= 4340)	North America (N= 4930)	Japan (N= 1575)
Prescribed dialysis sessions/week	2.96±0.53	3.02±0.48	2.99±0.43	2.96±0.37
< 3 dialysis sessions/week, %	5%	2%	3%	3%
Dialysis session length, min	205±60	245±63	220±53	238±50
Blood flow rate, mL/min	290±103	317±91	406±107	203±56
Urea reduction ratio (URR), %	66±22	74±13	74±12	69±13
URR ≥ 65, %	60%	89%	92%	72%
Body mass index (BMI), kg/m^2	25.1±15.9	26.0±8.8	28.3±10.2	21.3±6.2

mean values are shown with (± Standard Deviation); URR was restricted to patients having ESRD > 1 year, and receiving 3 HD sessions per week; EUR/ANZ includes Belgium, France, Germany, Italy, Spain, Sweden, United Kingdom, Australia, and New Zealand

Conclusions: The DOPPS represents a unique opportunity to evaluate practice patterns in SA, with comparisons to other DOPPS countries. A longitudinal component of SA DOPPS is planned, to determine the relationship of practice differences in SA on clinical outcomes.

Funding: Pharmaceutical Company Support - The Initial Cross-Sectional DOPPS Study in Saudi Arabia Was Supported by Amgen without Restrictions on Publications. The International DOPPS Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), and Abbott Laboratories (Since 2009)

TH-PO803

Twice Weekly Hemodialysis in China: Frequency and Determinants in a Cross-Sectional Analysis from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Shuchi Anand,[1] Mei Wang,[2] Brian Bieber,[3] Mia Wang,[3] Li Zuo,[4] Ronald L. Pisoni,[3] Xueqing Yu,[4] Xiao Yang,[4] Jia Qi Qian,[4] Nan Chen,[4] Yucheng Yan,[4] Friedrich K. Port,[3] Bruce M. Robinson,[3] Sylvia Paz B. Ramirez.[3] *[1]Stanford University; [2]Co-First Author, China DOPPS Country Investigators; [3]Arbor Research; [4]China DOPPS Country Investigators.*

Background: While published studies on HD practices in China are limited, several single-center reports and city registries suggest widespread 2x/wk HD. To understand practice patterns of HD care in China, an initial cross-sectional study was conducted in Beijing, Guangzhou and Shanghai. This abstract reports data on prevalence and correlates of <3x/wk HD in these Chinese cities.

Methods: 1379 pts were randomly sampled from 45 HD units in 2011. Logistic regression was used to identify predictors of 2x/wk HD. Linear mixed models were used to explore the cross-sectional association with intermediate outcomes.

Results: 26% of pts were on <3x/wk, compared with < 5% in a majority of core DOPPS countries. The facility % of pts prescribed <3x/wk ranged from 4-55% (26% for avg facility). Females, and pts with residual kidney function (RKF) were more likely treated with 2x (vs 3x)/wk while those with DM, HTN, and more yrs on HD, were less likely on 2x/wk. Adjusting for pt characteristics in the table, 2x/wk was associated with longer treatment time (by 12 mins), lower s. Ca^{2+} and Hb, and higher s. PO_4, PTH, and albumin. Pt reported QoL was similar.

Table: Patient characteristics associated with twice weekly HD

Measure	2 sessions per week (vs. 3)					
	Unadjusted			Adjusted* OR		
	OR	95% CI	p	OR	95% CI	p
Age (per 10 years)	0.95	(0.86, 1.05)	0.31	0.97	(0.87, 1.09)	0.63
Female	1.33	(1.07, 1.64)	<0.01	1.32	(1.06, 1.63)	0.01
Vintage (per 1 year)	0.92	(0.88, 0.96)	<0.01	0.94	(0.90, 0.98)	<0.01
Urine output > 200 mL/day	3.05	(2.09, 4.46)	<0.01	2.81	(1.83, 4.32)	<0.01
Co-morbidity: Diabetes (DM)	0.56	(0.41, 0.76)	<0.01	0.49	(0.36, 0.67)	<0.01
Co-morbidity: Hypertension (HTN)	0.53	(0.33, 0.83)	<0.01	0.51	(0.32, 0.81)	<0.01

* Adjusted for all variables in table, plus body mass index and other cardiovascular co-morbidities. N=1339 patients; N with HD frequency 2x/week =323. Associations for, body mass index and other cardiovascular comorbidities were not significant. Once weekly HD accounted for < 2% of patients in the China sample and was excluded from analyses.

Conclusions: A substantial % of HD pts in 3 major Chinese cities receive <3x/wk HD. These pts had a lower co-morbidity burden, shorter yrs on HD, and greater RKF. Whether this practice is due mainly to provider preference or resource limitations remains to be determined. The long term impact of less frequent HD in this selected population is unclear and will be studied in the longitudinal China DOPPS.

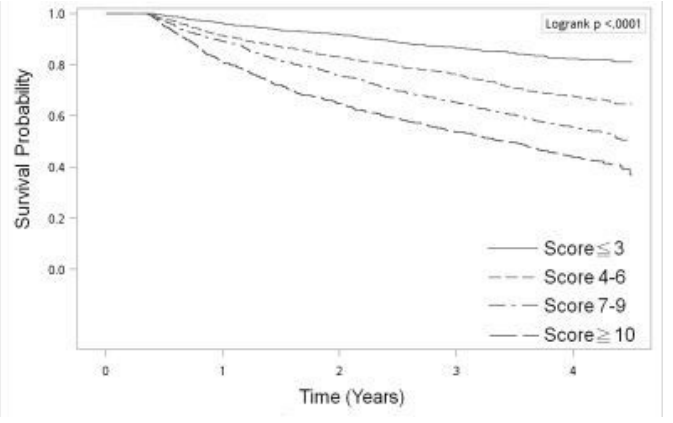
TH-PO804

A Novel Comorbidity Index for Outcome Analyses of Chinese Dialysis Patients Jinn-Yang Chen. *Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: Comorbid conditions are highly prevalent among patients with end-stage renal disease. In order to depict the comorbidity burden, to predict outcomes and to adjust confounders, we developed a new index for mortality analyses of Taiwan dialysis patients.

Methods: Cox proportional hazards model for mortality was used to estimate coefficient estimates of each comorbid condition. Numerical weights were assigned to 9 comorbid conditions, including atherosclerotic heart disease, congestive heart failure, cerebrovascular accident/transient ischemic attack, dysrhythmia, chronic obstructive pulmonary disease, gastrointestinal bleeding, liver disease, cancer, and diabetes. A patient's comorbidity score was the sum of the weights corresponding to the individual conditions present.

Results: This new index was developed among dialysis patients based on the 2006 (n=7999) incident cohort of Taiwan, and was validated using the 2005 (n=4071) and 2007 (n=8473) incident patients. Our new index performed almost identical to the individual comorbid conditions regarding model fit, predictive ability, and effect on inference. In Chinese ESRD patients, this index outperformed the Charlson's Comorbidity Index. The index predicted dialysis survival very well.



The new index also works well for hospitalization frequency and hospitalization days.

Conclusions: The index developed from Taiwan dialysis incident cohort was very suitable for outcome analysis of mortality and hospitalization. This index would be an important tool for observational research in Chinese ESRD patients.

Funding: Government Support - Non-U.S.

TH-PO805

The Carbon Footprint of Satellite Hemodialysis in Australia Allan Lim, Anthony D. Perkins, John W.M. Agar. *Renal Unit, Geelong Hospital, Barwon Health, Geelong, Victoria, Australia.*

Background: HD services create a significant environmental footprint. Knowing the key service contributors to this footprint, and understanding how these are affected by geographical variations in resource emissions factors, will allow HD services to play a more effective role in primary climate-change mitigation.

This study aimed to determine the carbon footprint (CFP) of a 6-chair suburban satellite hemodialysis (HD) unit in Victoria, Australia: to calculate the per-patient CFP of satellite HD: and to understand the relative contributions of energy, transportation, pharmaceuticals and medical equipment to the total CFP.

Methods: Activity data were collected (01/01/11 – 31/12/11) for energy and water use, patient and staff travel, and procurement of the consumables, pharmaceuticals and medical equipment associated with the provision of satellite HD, and were converted to a common measurement unit of tonnes of CO_2 equivalents (t CO_2-eq) via established emissions factors.

Results: In 2011, the total unit CFP was 98.3 t CO_2-eq, corresponding to a per-patient CFP of 8.2 t CO_2-eq / year. In comparison, an average 4 person Australian household emits 18 t CO_2-eq / year. Emission data are shown in Table 1.

Table 1

SECTOR	SUB-SECTOR	EMISSIONS (tCO2-eq)	% of EMISSIONS
Energy	HD power	2.4	2.4
	Non-HD power	4.4	4.5
	SUB-TOTAL	6.8	6.9
Water	Mains supply & treatment	0.2	0.2
	Waste water disposal	1.3	1.3
	SUB-TOTAL	1.5	1.5
Travel	Staff	3.7	3.8
	Patient	7.0	7.1
	SUB-TOTAL	4.2	4.3
Waste	Landfill	1.4	1.4
	Incineration	3.8	3.9
	Recycled	-1.0	-1.0
	SUB-TOTAL	4.2	4.3
Procurement	Pharma	43.5	44.3
	Equipment	28.5	29.0
	Food	1.9	1.9
	Laundry/Sanitation	1.0	1.0
	Other	0.2	0.2
	SUB-TOTAL	75.1	76.4
TOTAL		98.3	100

Conclusions: Modest emissions reductions appear achievable through targeted energy-saving initiatives in our region. While energy use in other regions where less carbon-intensive electricity generation methods are used may contribute less to the overall CFP, financial and environmental benefits may still accrue if energy-saving initiatives are broadly introduced. More importantly, a more detailed analysis of pharmaceutical and medical equipment emission data should identify effective nation-wide interventions that will reduce HD-related carbon emissions.

TH-PO806

Problem-Solving Therapy to Improve Depression Scores among Older Hemodialysis Patients: A Pilot Randomized Trial Shiloh D. Erdley,[1] Zvi D. Gellis,[2] Hillary Bogner,[2] Robert M. Perkins.[1] *[1]Geisinger Clinic; [2]University of Pennsylvania.*

Background: Depression is the most common mental health problem reported among dialysis patients. Effective depression interventions in this population are limited.

Methods: In this randomized, controlled, un-blinded pilot study we examined the feasibility and impact of 6 protocolized, once-weekly, 30-minute problem solving therapy (PST) sessions conducted during routine maintenance hemodialysis sessions by a trained social worker on BDI and PHQ-9 depression scores in non-hospitalized adult patients with ESRD at a single dialysis unit in central Pennsylvania. Control subjects received standard interventions by the unit social worker according to unit policy. Outcomes were 6-week BDI and PHQ-9 scores, and change-from-baseline BDI and PHQ-9 scores.

Results: 35 subjects were randomized between January 1 and January 31, 2012. 33 subjects completed the study; one subject died and one subject withdrew due to illness (both randomized to intervention group). At baseline, subjects in each arm were similar with the exception that mean PHQ-9 and BDI scores were higher in the intervention group (P=0.009 and 0.05, respectively). At 6 weeks, there were no significant differences in mean PHQ and BDI scores between the control and intervention groups (PHQ-9 5.8 vs. 3.3, P=0.1; BDI 11.3 vs. 9.3, P=0.6); however, mean change-from-baseline scores were significantly improved in the intervention group relative to the control group (change in PHQ-9 7.2 vs. 0.3, P<0.001; change in BDI 6.3 vs. -0.6, P=0.008). When adjusted for baseline depression scores, mean 6-week BDI and PHQ-9 scores were significantly lower in the intervention group.

Conclusions: The results demonstrate that PST is feasible and well-tolerated, and may positively impact depression among maintenance hemodialysis patients.

TH-PO807

A Propose for Diagnostic Criteria Concerning Hemodialysis Related Amyloidosis Shinichi Nishi, Hideki Fujii, Keiji Kono, Kentaro Nakai. *Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Hygo, Japan.*

Background: Hemodialysis related amyloidosis (HDRA) is a major complication of chronic hemodialysis patients and shows various kinds of bone-articular symptoms and sings. Among them carpal tunnel syndrome (CTS) and trigger finger are famous sings, however, we cannot easily determine their incidence is simply derived from HDRA, because pathological proof of B2MG protein is sometimes impossible in the operative specimens. We proposed a new diagnostic criteria concerning HDRA and evaluated its clinical availability in Japanese chronic HD patients.

Methods: We configured five major clinical findings; polyarticular pain, CTS, trigger finger, HD spondyloarthorpathy and bone cysts, and three associate findings; bone fracture, ischemic colitis and other minor findings. We determined a patient who has two or more major findings is a definitive case and one major finding and one or more associate findings is a probable one. We surveyed the prevalence of each finding and the rates of definitive and probable cases in 60 patients with long-time HD history.

Results: In the prevalence rate of each finding, polyariticular pain (48.3%), CTS (23.3%) and trigger finger (21.7%) were most frequent ones. These rates increased according to the duration of HD. The definitive cases were 26.7% (16/60) in total cases. The rates dramatically increased after 10 years HD duration; 6.7% (1/15 cases) in 5 to 10 years HD duration, 26.7% (4/15 cases) in 10 to 15 years HD duration and 73.3% (11/15 cases) in more than 15 years HD duration. However, there was a week relationship between ages and definitive rates.

Conclusions: The prevalence rate of HDRA increases according to duration of HD. Ten year duration may be necessary for incidence of HDRA. A combination criteria concerning HDRA is useful for the correct analysis of HDRA.

Funding: Government Support - Non-U.S.

TH-PO808

Routine Use of an Abbreviated 4-Item Scale to Assess Dependence in Essential Activities of Daily Living amongst Elderly Hemodialysis Patients: A Validation Study Farhat Farrokhi,[1] Sarbjit Vanita Jassal.[1,2] *[1]Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada; [2]Division of Nephrology, University Health Network, Toronto, ON, Canada.*

Background: Poor functional status is associated with reduced survival and poor outcomes in older dialysis patients. The Geriatric Nephrology Advisory Group recommends routine evaluation of functional status on all older patients; however, assessments can be time consuming and burdensome to clinical care providers. The objective of this study was to validate an abbreviated 4-item self report screening tool for use in elderly hemodialysis patients.

Methods: The functional dependence of community-dwelling hemodialysis patients, aged≥65 years, was measured by trained evaluators. The accuracy of a 4-item self report activities of daily living (ADL) score was compared against formal evaluation by the Barthel Index and the outcomes using agreement statistics and Cox regression analysis.

Results: The cohort included 167 patients with a mean age of 74.8 ± 5.9 years (57% males). The 4-item scale correctly identified 83% of the patients dependent in ≥1 ADL. Those incorrectly identified as independent on the abbreviated scale were uniformly unable to climb stairs without assistance. The sensitivity and specificity, and coefficient for agreement between the 4-item scale and the Barthel Index were 83.2%, 100% and 0.78, respectively. The positive and negative predictive values of the 4-item scale were 100% and 76.9%, respectively. Using the 4-item scale, the presence of severe disability was predictive of increased mortality (HR, 12.5; 95%CI, 2.5–65.0; P=0.03).

Conclusions: The 4-item scale is a simple, valid screening test for disability which can be used in the elderly population on dialysis as a screening tool. Difficulties with stair-climbing may be overlooked using this score.

TH-PO809

Validation of the Perceived Kidney Disease Self-Management Scale in Hemodialysis Julia M. Steed, Kenneth A. Wallston, T. Alp Ikizler, Kerri L. Cavanaugh. *Vanderbilt University.*

Background: Self-management skills, such as adherence with diet, medication and dialysis prescriptions, are required to ensure effective hemodialysis therapy for treatment of end-stage renal disease (ESRD). High self-efficacy, or the perceived ability to perform self-management, may be an important factor related to optimizing self-care behaviors. Validated condition-specific self-efficacy assessments are available for other chronic conditions (HIV, arthritis, and diabetes), but an instrument for ESRD is lacking.

Methods: In a cross-sectional study, we adapted the 8-item Perceived Medical-Condition Self-Management Scale to kidney disease. We evaluated its internal consistency and pearson's correlations with a priori determined variables including: patient characteristics, affect (PANAS), perceived expectencies (PEI), despressive symptoms (CESD), dialysis knowledge (CHeKS), and self-care behaviors.

Results: In 146 prevalent maintenance hemodialysis patients, mean age 52(14) years, 50% male, 75% non-White, vintage 4.7 (5.4) years, the mean (SD) total PKDSMS score was 30.7 (6.0). Cronbach's alpha was 0.75. Correlations with PKDSMS included: depressive symptoms (r=-0.55, p<0.001), affect (negative: r=-0.44, p<0.001; positive: r=0.41, p<0.001), perceived expectencies (r=0.59, p<0.001), dialysis knowledge (r=0.20, p=0.01) and self-care (r=0.27, p=0.001). No differences by patient characteristics were observed.

Conclusions: Hemodialysis patent's perceived ability to perform goal-oriented tasks is significantly related to self-report of actual behaviors, dialysis knowledge, and an improved psychologic state. The PKDSMS is reliable and demonstrates evidence for construct validity. Evaluation of self-efficacy may be a target for nephrology providers and researchers to consider as a target for understanding and improving self-care in ESRD.

Funding: NIDDK Support

TH-PO810

Immune Response after Monovalent Influenza A (H1N1) Vaccine in Hemodialysis Patients Ellen T. McCarthy,[1] Karen Tamano,[1] Sumanth Mulamalla,[1] Lisa A. Clough.[2] *[1]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Infectious Diseases, University of Kansas Medical Center.*

Background: Chronic hemodialysis (HD) patients have significant impairment of immunity, including decreased antibody response to vaccinations. Chronically ill patients, such as those on HD, are at increased risk for morbidity and mortality due to influenza. We hypothesize that patients with ESRD on chronic HD will have a blunted antibody response to the H1N1 vaccination.

Methods: We studied 45 patients on chronic HD. Of these, 57.8% were male; age range 32-85 (mean 56.4); 42% were ≥ 60 yrs of age. Patients received a single injection of monovalent H1N1 influenza vaccine administered in Dec 2009. Serum antibody titers were measured using hemagglutination-inhibition (HAI) assay at baseline and 21-28 days following immunization. A positive response to vaccination was defined as 1) pre-vaccination titer 1:10 with post-vaccination titer ≥ 1:40, or 2) ≥4-fold increase in antibody titer following vaccination.

Results: Twenty-seven of the 45 patients (60%) responded to vaccination with H1N1 vaccine. Patients ≥60 yrs of age were more likely to have post-vaccination titers ≥1:40 or four-fold rise in titers though the difference did not reach statistical significance. Responders did not differ from nonresponders in duration of dialysis, underlying disease, previous administration of seasonal influenza vaccine or baseline Hg, ferritin or vitamin D levels. Seven of 9 patients who had received prior renal allograft and were maintained on low-dose immunosuppression responded to vaccination.

Conclusions: Patients on chronic HD had an immunologic response to the H1N1 vaccine that was blunted compared to the general population (60% vs >80-90% reported in literature). Surprisingly, patients on chronic immunosuppression responded well. The introduction of the H1N1 vaccine was a unique opportunity to study immune response to influenza vaccines in this population. Despite lower response rates, an adequate response to the vaccine was seen in the majority of patients. Influenza vaccination is likely an important step in controlling influenza infections and outbreaks among dialysis patients.

TH-PO811

Analysis of USRDS Dataset Indicates Viral Infections Are Associated with Increased Risk of Fistula Failure in Incident Hemodialysis Patients Hari Garapati,[1] William D. Paulson,[1] John White,[1] Pamela J. Fall,[1] Mufaddal F. Kheda,[1] Tushar J. Vachharajani,[3] Earnest J. Baulkmon,[1] Kristina W. Kintziger,[1] Puja Chebrolu,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] N. Stanley Nahman.[1,2] *[1]Georgia Health Sciences University, Augusta, GA; [2]Charlie Norwood VAMC, Augusta, GA; [3]WG Hefner VAMC, Salisbury, NC.*

Background: ESRD is associated with arteriosclerosis and increased arterial stiffness. This stiffness impairs vessel dilatation after native arteriovenous fistula (AVF) creation, thereby promoting AVF thrombosis and failure (Kheda NDT 2010). Viral infections, such as HIV and hepatitis C (HCV), are also associated with increased stiffness. We tested the hypothesis that HIV, HCV, and hepatitis B (HBV) infection are independently associated with AVF failure in incident hemodialysis (HD) patients.

Methods: All incident HD cases in the USRDS dataset for calendar years 2005-07 were queried. Demographics were obtained from validated USRDS data, and access type was derived from form CMS-2728, submitted to Medicare on every new outpatient HD

patient. The criteria for AVF failure were derived from Medicare A datasets containing ICD-9 and CPT billing codes for AVF related complications. HIV, HCV or HBV infection was inferred from serologies and determined from the appropriate ICD-9 billing codes.

Results: For the 3 year study period, 259,224 patients with 80,825,083 claim observations were analyzed. AVF was the access used in the first dialysis in 33,925 patients, and 49.8% met criteria for AVF failure. Mean age was 64 years; 65% were male, 68% were Caucasian, 26% were African American. Positive viral serologies: HIV (N=204, 0.6%), HCV (N=712, 2.1%) and HBV (N=274, 0.8%). Relative risk (RR) of AVF failure was highest among HIV positive patients (RR 1.54, 95% confidence interval [CI] 1.42-1.68). RR of AVF failure in HCV was 1.25 (CI 1.20-1.31) and in HBV was 1.21 (CI 1.13-1.30).

Conclusions: AVF failure occurred in nearly 50% of incident HD patients. Positive serologies for HIV, HCV, and HBV were associated with increased risk of AVF failure, with HIV having the highest risk. We speculate that increased arterial stiffness in these patients may help explain these adverse outcomes.

Funding: Clinical Revenue Support

TH-PO812

Detection of Antibodies to HCV Core among Anti-HCV-Screening-Negative Hemodialysis Patients at Risk of Occult HCV Infection Guillermina Barril,[1] Juan Antonio Quiroga,[2] Dolores Arenas,[3] Secundino Cigarran,[4] Nuria Garcia-Fernandez,[5] Jose A. Herrero,[6] Emilio E. Gonzalez-parra,[7] Adoración Martin,[8] Javier Bartolome,[9] Inmaculada Castillo,[9] Vicente Carreño.[9] *[1]Nephrology, H. Princesa, Madrid, Spain; [2]FEHV, Madrid; [3]H. Perpetuo-Socorro, Alicante, Spain; [4]H. DaCosta, Burela; [5]CUN; [6]Clinico, Madrid; [7]FJD; [8]Hospital Poniente; [9]FEHV.*

Background: Testing for HCV RNA in PBMC allows identification of occult HCV infection in a proportion of anti-HCV and serum HCV RNA-negative hemodialysis patients with abnormal liver enzymes (JASN2008). Antibodies HCVcore are detectable among anti-HCV-negative patients with occult HCV infection without renal disease. Up to now anticore has not been tested among hemodialysis patients to detect occult HCV.

Methods: To evaluate detection of antibodies HCV core and correlate to HCV RNA testing in PBMC for diagnosing occult-HCV infection in anti-HCV and serum HCV RNA negative hemodialysis patients with abnormal liver enzymes 113 HD patients were studied. HCV-RNA was tested in PBMC by real-time PCR. Antibodies to HCV anti-core were assessed by recently developed ELISA.

Results: HCV RNA was detected in PBMC from 40/113 (35%)patients indicating occult HCV infection.Anti-core antibodies were detectable in 26(23%) HDpatients supporting HCV exposure. Overall, 56/113 (49%had either marker detectable: 10 were positive for anti-core and RNA in PBMC; 30 only positive for viral RNA in PBMC whereas 16 had anti-core alone. Anti-core detection iidentified 16/73 anti-HCV-screening-negative HD-patients with undetectable HCV-RNA in PBMC may have occultHCV infection. 24patients were re-tested after 12 months who remained anti-HCV-screening-negative, including 10 with and 14 without anti-core detectable baseline 4/14 anti-core-negative at baseline seroconverted 30/113 reacted the anti-core assay at any time-point.

Conclusions: 1 Anti-core antibodies are often detectable among anti-HCV screening-negative HDpatients who may have an occult HCV infection, including 25% with confirmed occult infection and 22% of those exposed to HCV might have developed occult HCV infection

2 Repeated anti-core testing identifies 28% more cases.

TH-PO813

Variability in Response to Hepatitis B Vaccine in Hemodialysis Patients Majed Samarneh, Yorg Azzi, Pranab Sharma Acharya, Chadi Saifan, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Hemodialysis patients are exposed to blood and blood products more than the general population and are also at higher risk for Hepatitis B contamination. For these reasons, it is highly recommended that this patient population gets the Hepatitis B vaccine. The efficacy of the vaccine is measured by measuring titers of Antibody in the serum of the patient. A minimum titer of 10 mIU/mL is considered to be a response. The conversion rate in hemodialysis patients ranges from 50-80%, as compared to the general population where the conversion rate is over 95%. As opposed to the general population, end stage renal patients on hemodialysis do not always respond to the vaccine. The main objective in this study is to try to identify factors that may hinder the response. Correction of these factors in the future may help non-responders.

Methods: This was a retrospective chart review at a single hemodialysis center to compare the laboratory and clinical differences between responders and nonresponders. Inclusion criteria are hemodialysis patients who received the hepatitis B vaccine and patients with concomitant hepatitis C. Exclusion criteria are patients who refused the vaccine and patients who did not complete the vaccine course.

Results: There are a total of 108 subjects included in the study, out of which 44 (42.3%) are responders to the HepB vaccine. A multivariate logistic regression was performed using the statistically significant risk factors as identified by the univariate logistic regression, including age range, albumin, hemodialysis vintage, vascular access and diabetes status. The results from the multivariate logistic regression show that advanced age (P-value=0.005) and diabetes status (P-value=0.003) are found to be strong independent risk factors of responder status. The type of vascular access (AVF or other types) is also marginally statistically significant (P-value=0.05).

Conclusions: In this retrospective chart review comparing hepatitis B vaccine responders versus non-responders, we found that advanced age and a history of diabetes are independent risk factors in predicting responder status.

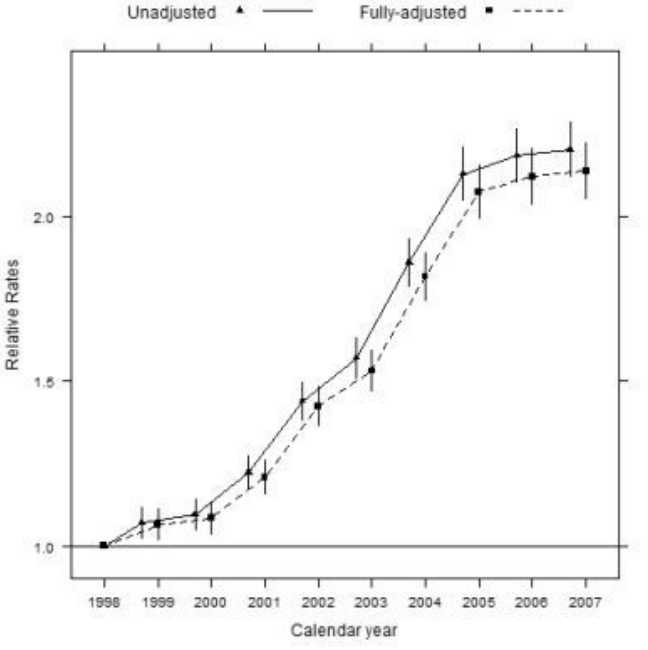
TH-PO814

Trends in the Incidence of *Clostridium difficile* Infection in U.S. Dialysis Patients (1998-2007) Juyeh Yang,[1] Tsung-chun Lee,[2] Maria E. Montez-Rath,[3] Glenn M. Chertow,[3] Wolfgang C. Winkelmayer.[3] *[1]Division of Nephrology and Department of Internal Medicine, Far Eastern Memorial Hospital, New Taipei, Taiwan; [2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; [3]Divisions of Nephrology, Stanford University School of Medicine, Palo Alto, CA.*

Background: In the U.S., the number of *Clostridium difficile* (*C. diff.*) infections tripled between 2000 and 2009. Patients with ESRD are at particular risk for contracting and dying from >*C. diff.* infection, and have high recurrence rates. However, the current literature contains mostly cross-sectional data or small case series. Little is known about the long-term trends of the incidence and outcomes of *C. diff.* infections among dialysis patients.

Methods: We studied all dialysis patients (1998-2007) who had Medicare A+B as their primary payor. Episodes of *C. diff.* infection were ascertained from ICD-9 diagnosis code 8.45. We used Poisson regression and general estimation equation methods for correlated data to model the annual number of *C. diff.* infections.

Results: Overall, 948,345 patients contributed 2.3 Million patient-years. We observed 89,669 distinct *C. diff.* infections among 68,644 patients, corresponding to an occurrence rate of 39 infections per 1000 person-years (p-y). The occurrence rates increased from 25 episodes per 1000 p-y in 1998 to 52 episodes per 1000 p-y in 2007. After adjustment for socio-demographics and comorbidities, the occurrence rate increased by 10.1% per year (95% CI: 9.8% to 10.5%).



The 30-day mortality associated with *C. diff.* infection episodes was 15.5% and it did not change over the decade of study.

Conclusions: Our longitudinal study indicated that episodes of *C. diff.* infections doubled in U.S. dialysis patients between 1998 and 2007, and these episodes remained associated with high short-term mortality.

TH-PO815

Prevalence of Oral Lesions in Hemodialysis Patients: The Oral-D Prospective Multinational Cohort Study Suetonia Palmer,[1] Marinella Ruospo,[2] Patrizia Natale,[2] Letizia Gargano,[2] Mariacristina Vecchio,[3] Valeria Maria Saglimbene,[3] Fabio Pellegrini,[3] Eduardo Jorge Celia,[2] Paul Stroumza,[2] Jorgen B.A. Hegbrant,[2] Jonathan C. Craig,[4] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Diaverum Medical Scientific Office; [3]Mario Negri Sud Consortium; [4]University of Sydney.*

Background: Oral diseases are common in the general population and associated with socioeconomic status. It is plausible that the prevalence of oral diseases is increased in people on hemodialysis due to impaired role functioning and health status, but not formally established. We conducted a systematic prospective survey of oral lesions in adults on hemodialysis.

Methods: ORAL-D is a multinational prospective cohort study. We consecutively enrolled adults receiving hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Europe and South America. A dental surgeon conducted a standardized examination of dental, periodontal, mucosal and salivary lesions based upon standard dental practice methodology. We analyzed prevalence of oral diseases using descriptive statistics.

Results: 4720 (mean age 62.85 years (SD 15.76) adults on hemodialysis in the participating clinics received a complete oral examination. Of these, 922 (20%) were edentulous, 1693 (39%) had tooth attrition and dental erosion, and 109 (2%) had enamel hypoplasia. The mean decay/missing/filled teeth (DMFT) score was 21.9 (9.18), salivary pH was 7.48 (1.32). There was a high prevalence of patients with high buffer capacity (n=1834 [61%]), and only 96 patients (8%) with low buffer capacity. Salivary flow rate before dialysis was 0.84 ml/min (0.78), versus 0.77 ml/min (0.72) post dialysis. 1491 (32%) patients had mucosal lesions, 2074 (45%) patients reported mouth dryness, 229 (5%) had oral burning and 351 (8%) reported mouth pain. Periodontitis was present in 1516 (42%) of 3672 dentate patients.

Conclusions: Oral lesions are prevalent in people receiving hemodialysis and may indicate impaired healthcare practices, although further research on the predictors of oral disease in this population is needed.

TH-PO816

The Effect of Online Hemodiafiltration on Infections: Results from the CONvective TRAnsport Study (CONTRAST) Claire H. Den Hoedt,[1,2] Muriel Grooteman,[3] Michiel Bots,[2] Peter J. Blankestijn,[2] Neelke C. Van Der Weerd,[3] Renee Levesque,[4] Pieter M. Ter Wee,[3] Menso Jan Nube,[3] Marinus A. Van Den Dorpel.[1] *[1]Maasstad Hospital, Rotterdam; [2]UMCU, Utrecht; [3]VU MC, Amsterdam, Netherlands; [4]St-Luc Hospital, Montréal, Canada.*

Background: Hemodialysis (HD) patients have a high risk of infections. Immune function is worsened by uremia and frequent damaging of the skin facilitates the entry of micro-organisms into the body. Online hemodiafiltration (HDF) might reduce the infectious risk by ameliorating the uremic milieu by enhanced clearance of middle molecules. On the other hand the infusion of substitution fluid might impose a larger risk if endotoxin fragments are transferred into the bloodstream. Since there are no data on infectious outcomes in HDF, we compared the effects of HDF and low-flux HD on the incidence and type of infectious complications.

Methods: We used data of the 714 prevalent chronic HD patients (age 64 ±14, 62% men, 25% DM, 7% catheters) included in the CONvective TRAnsport Study (CONTRAST), a randomized controlled trial evaluating the effect of HDF as compared to low-flux HD. The risk of infectious events, adjudicated by an independent committee, was compared with Cox regression for repeated events and Cox proportional hazard models. The distributions of infection types were compared between the groups.

Results: The risk for infections during the entire follow-up did not differ between the two treatment arms (HDF 205 and HD 171 infections in 1073 and 1083 patient yrs respectively, hazard ratio HDF vs. HD 1.06 (0.86-1.30), P=0.58, adjusted for clinical center). No difference was found in the occurrence of the first infectious event (either fatal, non-fatal or type specific) except for an increased combined risk for catheter-related infection and sepsis in HDF patients, HR 1.94 (1.06-3.55), P=0.03. Miscellaneous infections (such as wound-/skin infections, osteomyelitis etc) were the most common (36% in HDF, 30% in HD), followed by respiratory infections (25% in HDF, 28% in HD).

Conclusions: HDF as compared to HD did not result in a reduced risk of hospitalization for infection. In the CONTRAST cohort, miscellaneous infections and respiratory infections were the most common infections.

Funding: Pharmaceutical Company Support - Fresenius Medical Care (The Netherlands) and Gambro Lundia AB (Sweden), Roche Netherlands; the International Society of Nephrology/Baxter Extramural Grant Program

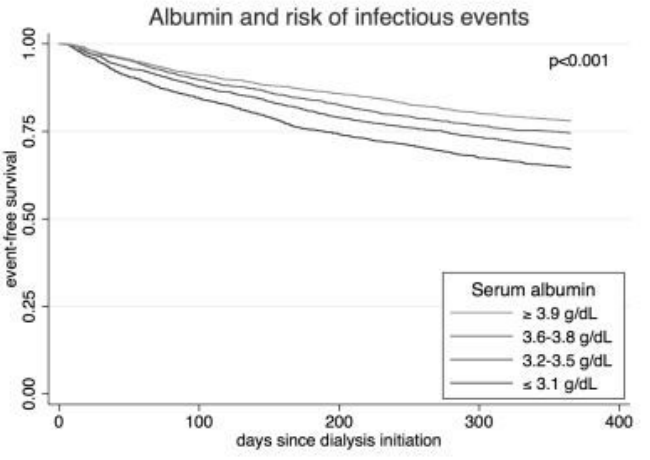
TH-PO817

Infection-Associated Events in Hemodialysis: Characteristics and Predictors Ishir Bhan, Elizabeth D. Ankers, Ravi I. Thadhani. *Division of Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Infection is commonly seen in patients on hemodialysis, but little is reported about the frequency and nature of infectious events or underlying risk factors.

Methods: We analyzed infection-associated deaths and hospitalizations in Accelerated Mortality in Renal Replacement, a cohort of 10,044 incident hemodialysis patients followed for one year. Cause of death was determined from discharge diagnosis forms. Diagnosis codes from hospital admissions were evaluated. Death diagnoses and admissions were classified as infection-associated based on physician review. Admissions were considered infection-associated if the primary or secondary diagnosis was consistent with an infectious cause. Laboratory, comorbidity, and demographic data were determined at the time of dialysis initiation. Predictors of infectious events were determined by multivariable Cox models, with continuous variables analyzed by quartile.

Results: A total of 16,661 hospital admissions occurred during the study period, representing 6620 individuals, or 66% of the cohort. 3,233 (19.4%) of admissions were consistent with infection, representing 23% of the overall population. Only 224 admissions (1.3%) were defined as being of viral etiology. 1,528 (15.2%) subjects died within one year of dialysis initiation, with infectious causes representing 10.5% of these deaths. In multivariate analysis, diabetes (HR 1.2), catheter use (HR 1.5), and higher calcium (HR 1.1) were associated with increased risk of infectious events, while black race (HR 0.84), high systolic blood pressure (HR 0.94), and higher albumin (HR 0.84) were protective.



Conclusions: Infection represents an important cause of death in hemodialysis, but is a more important contributor to hospitalization. The most potent risk factors for infectious events include non-black race, diabetes, catheter use, and low albumin.

Funding: NIDDK Support

TH-PO818

Association between Dialysis Facility Report (DFR) Infection Measures and Dialysis Survey Deficiencies Joseph M. Messana, Natalie Scholz, Deanna Chyn, Valarie B. Ashby, Tempie H. Shearon. *Univ of Michigan, Ann Arbor, MI.*

Background: There is increasing awareness of the burden of vascular access infections, particularly access-related bacteremia, in patients receiving dialysis in the US. This study evaluates the relationship between assignment of a condition level infection control deficiency citation (CFC V110) & facility hospitalization for septicemia in the years before, during & after survey and whether these citations are associated with subsequent improvement in facility outcomes.

Methods: Infection citation data (V110-148) from 2009 facility surveys were combined with 2008-10 DFR data, based on national CMS data. Analyses include 5092 facilities with septicemia data available for all 3 yrs, of which 1265 were surveyed in 2009. GLM models were fit to predict % of patients hospitalized due to septicemia in each year based on 2009 infection survey citations. The differences in hospitalization for septicemia between each pair of yrs were also fit to assess the relationship between survey & change in infection outcomes over time. The models were weighted by facility size & adjusted for patient demographics, diabetes as cause of ESRD, & catheter use >90 days.

Results: Facilities with a CFC V110 in 2009 had significantly more hospitalizations for septicemia each year compared to facilities without a CFC V110. From 2008-10, facilities not surveyed or surveyed without a CFC V110 had smaller decreases in % of patients hospitalized for septicemia (0.46% & 0.48%, respectively) than facilities with a CFC V110 (1.55%), although it was not statistically different (p=.057).

Percent of Patients Hospitalized due to Septicemia in 2008-10 by Infection Citation Status in 2009*

2009 Survey Status	2008	2009	2010	Change from 2008 to 2010
Received a CFC V110 citation (n=86)	14.6 (p<0.001)	12.3 (p<0.001)	13.1 (p=0.001)	-1.55 (p=0.057)
Surveyed - No CFC V110 citation (n=1,265)	11.9 (ref)	10.2 (ref)	11.5 (ref)	-0.48 (ref)
Not surveyed (n=3,741)	11.7 (p=0.23)	9.8 (p=0.03)	11.3 (p=0.23)	-0.46 (p=0.89)

*Values shown for facility with average patient characteristics

Conclusions: These findings suggest an association between ESRD surveyor citations & facility infection control practices that contribute to better patient outcomes.

Funding: Other U.S. Government Support

TH-PO819

Infectious Events in Chronic Hemodialysis Patients: Risk Factors, Practice Patterns and Yield of Obtained Blood Cultures Golaun Odabaei, Don N. Chang, Burl R. Don, Andrew I. Chin. *Div. of Nephrology, UC Davis Medical Center, Sacramento, CA.*

Background: Infection is a major problem for chronic hemodialysis patients (pts) and nephrologists are generally contacted by the dialysis unit when an infectious event occurs. Blood cultures may be obtained during dialysis when pts manifest signs and/or symptoms of an infection. The practice patterns of community nephrologists in contending with an infectious event at an outpatient dialysis unit (where the nephrologist is generally not present) and the yield of an initiated evaluation for these events are not known.

Methods: Over a two year period of time (2010-2011), there were 100,050 dialysis treatments at the four DCI Sacramento dialysis units for an average prevalence of 347 pts.

Results: During this period, 512 new infectious events on dialysis were reported (fever, chills, signs of catheter infection etc.) in 242 unique pts with a frequency of 5 new infectious events per 1000 dialysis treatments. For the general dialysis population, the prevalence of catheters, fistulas and grafts were 23%, 58% and 19%, respectively, whereas in the

infectious events group, 51% had catheters, 42% had fistulas and 8% had grafts. For each new infectious event, the nephrologist was contacted and blood cultures were obtained in 355 of the 512 events (69%) in 189 unique pts. Empiric antibiotic therapy was initiated in 55% of the new infectious events. Only 59/355 blood cultures were positive (17%). The organisms isolated from blood cultures were gram + cocci (46/59), gram - rods (11/59) and yeast (1/59). In comparing the general dialysis population with the infectious event group, there was no significant relationship between gender, age, race and presence of diabetes mellitus and the occurence of infectious events.

Conclusions: Although infection is a major problem for chronic dialysis pts, the frequency of infectious events in an outpatient hemodialysis unit is low. When contacted, nephrologists order blood cultures and empiric antibiotics for a majority of the hemodialysis pts. Only a minority of blood cultures obtained (17%) were positive. The type of vascular access (catheter vs. fistula/graft) is the most important factor predicting an infectious event.

TH-PO820

Infection Rates for U.S. Dialysis Facilities: Comparing Sources

Erik Roys,[1] Natalie Scholz,[1] Casey Parrotte,[1] John Kalbfleisch,[1] Rajiv Saran,[1] Carol Chenoweth,[1] Stephen C. Hines,[2] Joseph M. Messana.[1] *[1]University of Michigan, Ann Arbor, MI; [2]HRET, Chicago, IL.*

Background: Reducing infections in hemodialysis facilities is a national priority to improve patient outcomes and reduce cost. Tracking infection rates at the facility level is essential for quality improvement. Several sources of infection rate information are currently available to facilities and other ESRD community members. This study examines the differences and similarities in infection rates based on Medicare claims and the CDC's National Health Safety Network (NHSN) data.

Methods: Four infection rates were examined for 34 facilities enrolled in the NOTICE project from 4 ESRD Networks for August-November 2011: 1) hemodialysis access-related infections based on ICD-9 code 996.62 reported on Medicare inpatient, physician/supplier, and outpatient claims; 2) vascular access-related bacteremia based on the V-modifier (V8) reporting in Medicare outpatient claims; 3) NHSN reported vascular access infections, defined as either a local access site infection or an access-related bloodstream infection; and 4) NHSN reported bloodstream infections, defined as any positive blood culture. Rates were expressed as events per 100 hemodialysis patient months. NHSN data were unavailable for 5 facilities.

Results: Rates obtained from NHSN were highly correlated with each other (r=.73, p<0.0001). However, there was a weak correlation between the vascular access infection rate from NHSN and the similar hemodialysis access-related infection rate based on ICD-9 reporting (r=.37, p=0.049). Infection rates based on V-modifier reporting were not correlated with any other source and had the lowest reported numbers of infections.

Correlation	n	r	p-value
ICD-9 vs V-Modifier	34	0.25	0.15
ICD-9 vs NHSN VA	29	0.37	0.05
ICD-9 vs NHSN Bact	29	0.03	0.87
V-Modifier vs NHSN VA	29	0.01	0.96
V-Modifier vs NHSN Bact	29	0.08	0.69
NHSN VA vs NHSN Bact	29	0.73	<0.0001

Conclusions: Despite these measures having somewhat different definitions, significant overlap would be expected. The lack of strong correlation between these measures suggests the need for additional investigation. Possible explanations will be explored including differences in definition and reporting factors.

Funding: Other U.S. Government Support

TH-PO821

Associations of Hemoglobin Concentration with Quality of Life and Mortality in Hemodialysis Patients on Erythropoietin in a Brazilian Population of Predominantly African Descent: A 6-Year Cohort Study

Gildete Barreto Lopes,[1] Cacia Mendes Matos,[2] Antonio Alberto Lopes.[1] *[1]Universidade Federal da Bahia; [2]INED, Salvador, Brazil.*

Background: Erythropoiesis stimulating agents improve outcomes in maintenance hemodialysis (MHD) patients but the target serum hemoglobin (sHb) level remains controversial. The objective was to investigate associations of baseline sHb with health-related quality of life (HRQOL) and survival in MHD patients on erythropoietin (EPO) in a population of predominantly African descent.

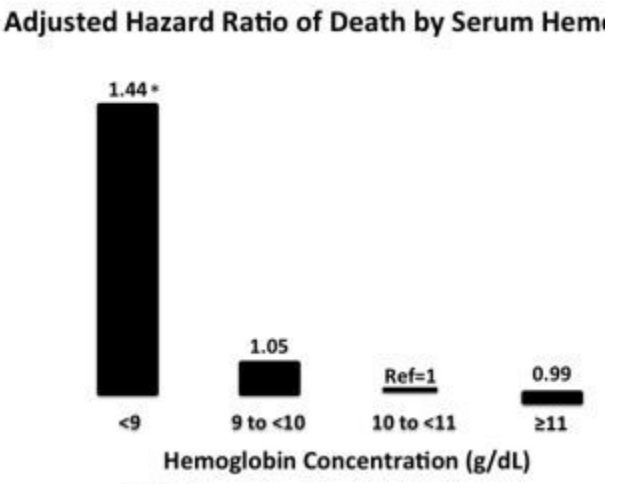
Methods: Prospective cohort study (PROHEMO) of 1244 MHD patients in Salvador, Brazil (2005-2011). Analysis restricted to 912 with more than 3 months on dialysis and on EPO (age 48.3±14.4 yr, 90.2% black or black-white admixture). SF-36 was used for physical (PCS) and mental (MCS) HRQOL. Cox regression was used for hazard ratios of death and linear regression for differences in HRQOL scores with adjustments for demographics, vintage and comorbidities.

Results: HRQOL scores were lower (P<0.05) in patients with sHb <9 and ≥11 compared with 10 to <11 g/dL (Table). Compared with sHb 10 to <11, the hazard of death was higher (P<0.05) for sHb <9 g/dL (Figure).

Mean Scores and Adjusted Differences in Health-Related Quality of Life by Serum Hemoglobin Concentration

	SERUM HEMOGLOBIN (g/dL)			
	<9 (N=230)	9 to < 10 (N=193)	10 to <11 (N=209)	≥11 (N=280)
PCS, mean(SD)	38.9(10.4)	41.1(10.2)	42.2(9.9)	39.9(9.8)
PCS, Adj Dif	-2.76*	-1.05	Ref=0	-2.00*
MCS, mean(SD)	47.0(12.3)	47.2(12.7)	49.8(12.3)	47.1(12.4)
MCS, Adj Dif	-2.79*	-2.47	Ref=0	-2.60*

* *P<0.05, MCS=Mental Component Summary, PCS = Physical Component Summary*, Adj Dif = Differences adjusted for several covariates



Conclusions: This study in a MHD population of predominantly African descent suggests a sHb between 10 and 11 g/dL to maximize the EPO benefits in terms of HRQOL and survival. The study could not assess effects of sHb variation on outcomes.

TH-PO822

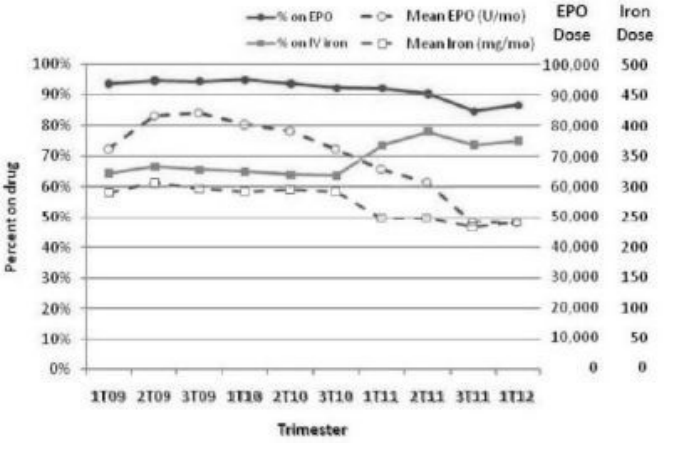
Changing Hemoglobin Targets: Effects on Epoetin Alfa, Intravenous Iron, and Iron Storage Measures from 2009-2012

T. Christopher Bond, Carey Colson, Tracy Jack Mayne. *DaVita Clinical Research, Minneapolis, MN.*

Background: The paradigm for anemia treatment in patients with end-stage renal disease (ESRD) is evolving in response to label revisions for erythropoiesis-stimulating agents and changes in Medicare reimbursement. A USRDS study reported that in 2011, epoetin alfa (EPO) dosing and hemoglobin (Hb) levels decreased significantly in dialysis patients. To better understand how anemia is being treated in patients with ESRD, we examined monthly EPO and intravenous (IV) iron dosing, Hb, as well as serum ferritin levels and percent saturated transferrin (TSAT).

Methods: Data were assessed from records of a large dialysis organization for incident and prevalent hemodialysis (HD) patients who received in-center HD between January 1, 2009 and April 30, 2012. Values were reported by 4-month periods (trimesters) over the study period.

Results: Between 2009 and 2012, the proportion of patients on EPO each month dropped from 95% to 85%, suggesting an increase in dose holds; mean EPO dose/month decreased >30,000 Units/month over the study period. While the average dose of IV iron was reduced over the study from 291 to 241 mg/month, the proportion of patients receiving any IV iron increased by 10-14%, as did the frequency of iron dosing in the population: from 2.54 doses/patient-month in April 2009 to 2.95 doses/patient-month in April 2012. These changes coincided with increases in mean serum ferritin (606 and 769 ng/mL in April 2009 and April 2012, respectively) and TSAT (29.9% and 30.9% in April 2009 and April 2012, respectively). Over the 3-year study period, patients' mean Hb fell from 11.6 to 10.8 g/dL.



Conclusions: Compared to 2009, in 2012 more HD patients received IV iron each month, leading to increased serum ferritin and TSAT levels. EPO administration fell considerably over the same time period, as did Hb levels. The effects of these dosing trends are unknown.

Funding: Pharmaceutical Company Support - Keryx Biopharmaceuticals

TH-PO823

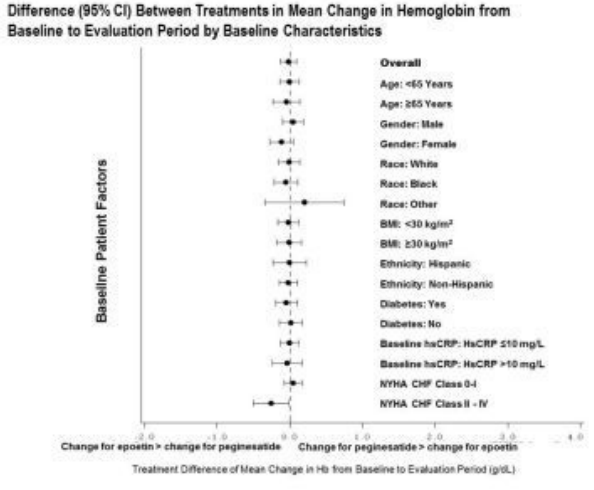
Efficacy of Peginesatide versus Epoetin by Baseline Characteristics in Hemodialysis (HD) Patients (Pts) with Anemia due to Chronic Kidney Disease (CKD)

Nathan W. Levin,[1] Bruce S. Spinowitz,[1] Carol Francisco,[2] Anne-Marie Duliege,[2] Krishna R. Polu,[2] Andrzej Wiecek.[1] *[1]AFX01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: In two large (N~1600) randomized open-label, active-controlled trials comparing peginesatide (OMONTYS®) vs epoetin alfa/beta for anemia treatment due to CKD in HD pts (EMERALD 1, 2), demographic and clinical characteristics were generally representative of the dialysis population. In these mutually supportive studies, peginesatide was noninferior to epoetin in maintaining hemoglobin (Hb) levels, with similar cardiovascular safety. Here we evaluate efficacy in subgroups defined by baseline characteristics.

Methods: Data were pooled from EMERALD 1 and 2, assessing (2:1 ratio) peginesatide Q4W (n=1066) vs epoetin 1-3X weekly (n=542) in HD pts on stable epoetin doses ≥4 weeks. Doses were titrated to maintain target Hb levels of 10-12 g/dL. The primary efficacy endpoint was mean change in Hb from baseline to evaluation period; noninferiority was defined as the lower limit of the two-sided 95% confidence interval (CI) ≥-1.0 g/dL (peginesatide minus epoetin).

Results: Overall, the peginesatide and epoetin groups, respectively, were well matched at baseline: mean age, 58.1 yr and 58.1 yr; male, 58.5% and 54.8%; Black, 37.4% and 38.9%; BMI ≥30 kg/m^2, 36.6% and 34.1%; diabetes as a primary cause of CKD, 37.1% and 39.5%; and high-sensitivity C-reactive protein >10 mg/L, 30.9% and 31.5%. Overall, peginesatide maintained Hb levels similarly to epoetin, least squares mean difference (95% CI) between groups: -0.02 (-0.13, -0.08) g/dL, with generally consistent results in subgroups (Figure).



Overall safety in subgroups was similar between agents.

Conclusions: In this pooled analysis, consistent with overall findings, peginesatide Q4W maintained Hb similarly to epoetin 1-3X weekly across a broad range of patient subgroups.

Funding: Pharmaceutical Company Support - Affymax, Inc., and Takeda Pharmaceutical Company Ltd.

TH-PO824

Linagliptin, a Dipeptidyl Peptidase-4 (DPP-4) Inhibitor, May Potentially Improve Glycemic Variability in Diabetic Hemodialysis Patients: Assessment Using Continuous Glucose Monitoring (CGM)

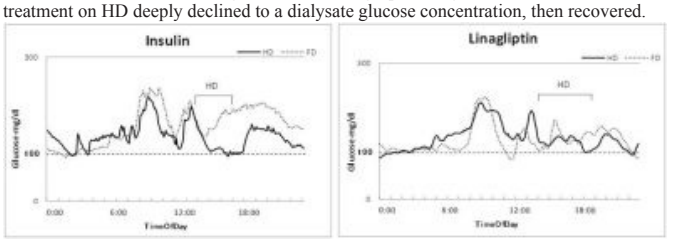
Satoshi Funakoshi,[1] Yoshiaki Lee,[1] Tomoya Nishino,[2] Yoko Obata,[2] Jyunichiro Hashiguchi,[1] Kenichi Miyazaki,[1] Takashi Harada,[1] Kazunori Utsunomiya,[3] Shigeru Kohno.[2] *[1]Nagasaki Renal Center, Japan; [2]Nagasaki University Graduate School of Medicine, Japan; [3]Jikei University, Japan.*

Background: Previous reports indicate that plasma glucose decreases during hemodialysis session, and then increases to relatively hyperglycemic state in diabetic hemodialysis patients. This glycemic variability may play a major role in the development and progression of cardiovascular diseases in hemodialysis patients with diabetes.

In this study we evaluated the efficacy of linagliptin, a biliary-excretion type of DPP-4 inhibitors, in controlling glycemic variability in diabetic hemodialysis patients using CGM.

Methods: Six relatively well-controlled (HbA1c<7.0) diabetic hemodialysis outpatients treated with insulin were enrolled in this study after appropriate IC. Subjects were then monitored overall 48-hours glycemic control, on both hemodialysis day (HD) and non-hemodialysis days (free day: FD), by CGM. Various types of insulin (<15 units per day) were converted to 5 mg of replaced with linagliptin, and glycemic controls were compared in each patient.

Results: As shown in Figure 1, the average plasma glucose curve under insulin treatment on HD deeply declined to a dialysate glucose concentration, then recovered.



On the other hand, the average plasma glucose in linagliptin-treated patients had the similar pattern on HD. More importantly, the mean amplitude of glycemic excursions (MAGE) under insulin treatment on HD was significantly higher than in linagliptin-treated group (38.0±10.5 mg/dL vs 25.1±14.7 mg/dL, p=0.021).

Conclusions: In our study, linagliptin, with its biliary excretion and low hepatic metabolism, could be promising candidate in the treatment in diabetic hemodialysis patients.

Funding: Private Foundation Support

TH-PO825

Oral Protein Supplementation during Dialysis in Patients with Elevated C-Reactive Protein

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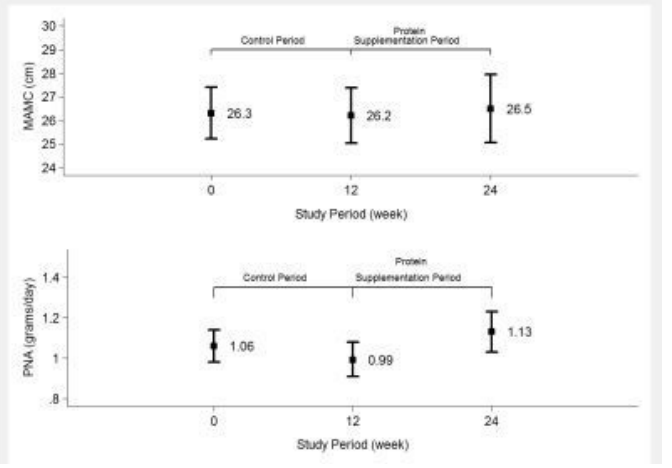
Background: Inflammation is thought to increase catabolism and it is unknown whether oral protein supplementation during hemodialysis (HD) treatment will increase muscle mass in chronic HD patients.

Methods: In this 24 week open labeled, prospective study, 49 chronic HD patients (pts) with a serum high sensitivity C-reactive Protein (hsCRP) of >3 mg/ were included. During the 12 week observation phase, participants received dietary advice to ↑protein intake to 1.2 g/kg/day. During the subsequent 12 week Rx phase, each participant was given 45 grams of oral liquid protein supplement during each HD Rx. Compliance and tolerance of the supplement were monitored. Primary outcome was mid-arm muscle circumference (MAMC). Secondary outcomes included serum albumin and Physical Composite Score (PCS) calculated from SF-12 questionnaire. Protein Nitrogen Appearance (PNA) was calculated using the pre/post BUN, body weight, ultrafiltration, and residual renal function. Statistical analysis was done using the mixed effect models.

Results: A total of 38 patients completed the study. The mean age was 64±17 yrs, 92% white, 14% had malignancy, 65% had DM, mand 14% had a catheter for dialysis access. As shown in the figure, PNA increased significantly in the study participants during the Rx phase. However, there was no significant change in MAMC. No changes in mean serum albumin (3.7 +/- 0.32 at 12 wks vs 3.7+/-0.34 at 24 wks) or the mean PCS scores (30.5+/- 9.9 at 12 wks vs 29.1 +/- 8.9 at 24 wks) were observed during the Rx phase of the study. Three patients experienced diarrhea and stopped taking the supplements.



Conclusions: In chronic HD with high hsCRP, protein supplementation during HD increases protein intake, but does not appear to increase muscle mass, physical quality of life, or serum albumin.

Funding: Private Foundation Support

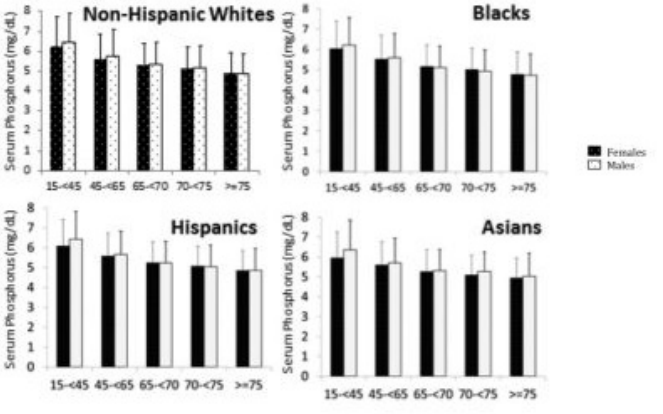
TH-PO826

Serum Phosphorus Concentrations across Different Age Groups of Hemodialysis Patients with Different Races/Ethnicities
Paungpaga Lertdumrongluk,[1,2] Miklos Zsolt Molnar,[1] Wei Ling Lau,[3] Joshua Zaritsky,[6] Csaba P. Kovesdy,[4] Keith C. Norris,[5] Kamyar Kalantar-Zadeh.[1,6]
[1]Los Angeles Biomedical Research Institute, Harbor-UCLA; [2]Srinakharinwirot University, Thailand; [3]University of Washington; [4]University of Tennessee; [5]Charles R. Drew University; [6]David Geffen School of Medicine at UCLA.

Background: Previous studies have shown that elderly maintenance hemodialysis patients (MHD) tend to have worse nutritional parameters compared to their younger counterparts. The differences in serum phosphorus levels between young and elderly MHD across race/ethnicity have not been closely examined in large epidemiological studies.

Methods: We divided 111,302 outpatient MHD patients treated in a large dialysis organization including 50,741 Whites, 38,825 Blacks, 18,078 Hispanics and 3,658 Asians into 5 age groups (15-<45, 45-<65, 65-<70, 70-<75 and ≥75 years old). We compared serum phosphorous levels between different age groups across race/ethnicity and gender using trend analysis and adjusted for demographics, dialysis dose, residual renal function, serum calcium and intact parathyroid hormone concentrations.

Results: The average serum phosphorus levels (mean±SD) were progressively lower within each older age group, 6.24±1.4, 5.64±1.21, 5.25±1.08, 5.10±1.06 and 4.87±1.03 mg/dl, respectively (P-trend <0.001). In the subgroup analysis of each race and gender, this trend persisted whereby older MHD patients had significantly lower serum levels of phosphorus compared to younger MHD patients (P-trend<0.001).



Serum phosphorus did not differ across gender or race/ethnicity. The results remained similar after case-mix adjustment.

Conclusions: The serum phosphorus concentrations were significantly lower in elderly hemodialysis patients compared to younger patients. Our findings were consistent among both genders and each racial/ethnic group.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK078012

TH-PO827

Comparison of Outcomes for Veterans Receiving Dialysis from VA and VA-Outsourced Providers
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Background: Demand for dialysis exceeds its supply within the Department of Veteran Affairs (VA), requiring VA to purchase dialysis for Veterans in the private sector on a fee-for-service basis. It is unclear whether outcomes are similar for Veterans receiving dialysis in these settings. This study assessed the extent of dialysis utilization and differences in all-cause hospitalizations and mortality among Veterans receiving outpatient dialysis from VA and VA-outsourced providers.

Methods: We constructed a cohort of Veterans in 2 regions who received chronic dialysis financed by VA in 2007-2008. From VA administrative data, we identified Veterans who received outpatient dialysis in 1) VA, 2) VA-outsourced, and 3) both ("dual") settings. We used two-part and logistic regression to examine associations between dialysis setting and 1-year all-cause hospitalization and mortality.

Results: Of 1,388 patients who received outpatient dialysis treatment financed by VA 27% received dialysis in VA only, 47% used outsourced dialysis only, and 25% were dual users. VA users were more likely to be younger, non-White, unmarried, have higher comorbidity burden, and live closer to the nearest VA dialysis unit than patients in VA-outsourced or dual settings. Half of the Veterans in our cohort were hospitalized and 13% died. Veterans receiving VA-outsourced dialysis were less likely to be hospitalized (OR= 0.35, p<0.001) and incurred fewer hospitalizations (β= -0.16, p<0.05) and shorter hospital stays (β= -0.37, p<0.05) than VA dialysis users. Patients in dual-settings had lower 1-year mortality than Veterans receiving VA dialysis (OR=0.52, p<0.05).

Conclusions: A significant proportion of VA-funded dialysis is delivered through VA-outsourced providers. Veterans receiving outsourced dialysis are systematically less complex, less likely to be hospitalized, and had shorter lengths-of-stay but no difference in mortality than Veterans receiving VA dialysis. For dual dialysis patients, the likelihood of hospitalization is similar but mortality is reduced.

Funding: Other U.S. Government Support, Veterans Administration Support

TH-PO828

Endothelial Progenitor Cells Induce Macrophage Phenotypic Switch and Improve Renal Recovery Capacity in Swine Renal Artery Stenosis
Alfonso Eirin,[1] Xiang-Yang Zhu,[1] Zilun Li,[1] Behzad Ebrahimi,[1] Xin Zhang,[1] Hui Tang,[1] Michael James Korsmo,[1] Alejandro Chade,[2] Amir Lerman,[1] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Mayo Clinic, MN; [2]Univ. of Mississippi, MS.*

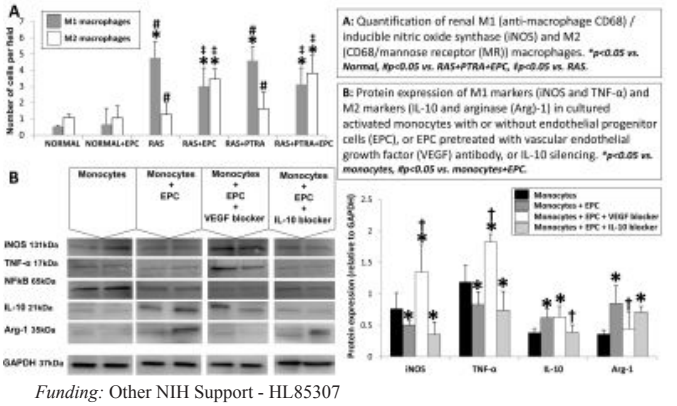
Background: Endothelial progenitor cells (EPC) decrease inflammation and improve endothelial repair, but their potential for modifying the pro-inflammatory macrophage (M1) phenotype remains unknown. Inflammation is a key component of kidney injury in renal artery stenosis (RAS), and may account for its persistence despite restoring renal flow. We hypothesized that EPC would decrease M1 macrophages and improve renal recovery potential in RAS.

Methods: Pigs with 10 wks of RAS were studied 4 wks after percutaneous transluminal renal angioplasty (PTRA) or sham, with or without adjunct intra-renal delivery of autologous EPC expanded from peripheral blood, and compared to normal controls with or without EPC infusion (n=7 each). Single-kidney function, microvascular and tissue remodeling, inflammation, and fibrosis were evaluated.

Results: Mean arterial pressure (MAP), but not renal blood flow (RBF), was normalized 4 wks after PTRA. Injected EPC engrafted in RAS kidneys (~12%). Stenotic-kidney GFR was restored in RAS+EPC, RAS+PTRA, and RAS+PTRA+EPC pigs, while RBF and microvascular structure were improved and fibrosis attenuated only in EPC-treated pigs (Table). EPC decreased the ratio of M1/M2 (inflammatory/reparative) macrophages (Figure A) and induced a phenotypic switch (M1-to-M2) in cultured monocytes, which was blocked by vascular endothelial growth factor (VEGF) blockade (Figure B).

Conclusions: Intra-renal infusion of EPC after PTRA induced VEGF-mediated M1-to-M2 switch, preserved microvascular architecture and function, and decreased inflammation and fibrosis in the stenotic kidney, suggesting a novel mechanism and therapeutic potential for adjunct EPC delivery in improving renal repair in RAS.

	NORMAL	NORMAL+EPC	RAS	RAS+EPC	RAS+PTRA	RAS+PTRA+EPC
MAP (mmHg)	103.4±10.4	102.3±11.2	142.4±15.5*#	148.9±14.2*#	106.3±21.8‡	105.6±18.2‡
RBF (ml/min)	580.7±30.5	596.9±46.8	315.8±62.5*#	484.4±44.2‡	386.0±17.4*#	521.8±50.1‡
GFR (ml/min)	82.9±13.3	95.5±10.4	45.2±9.7*#	68.5±7.9‡	72.5±4.3‡	79.6±5.5‡
Spatial density (microvessels/mm²)	2.96±0.44	2.58±0.09	1.35±0.12*#	2.33±0.11‡	1.77±0.32*#	2.90±0.39‡
Fibrosis (%)	1.95±0.21	2.10±0.22	6.10±0.49*#	4.24±0.11*‡	5.21±0.20*#	4.01±0.11*‡



Funding: Other NIH Support - HL85307

TH-PO829

Mitochondrial Targeted Peptides Attenuate Myocardial Damage after Renal Revascularization in Experimental Atherosclerotic Renovascular Hypertension
Alfonso Eirin, John A. Crane, Xin Zhang, Zilun Li, Xiang-Yang Zhu, Sandra Herrmann, Amir Lerman, Stephen C. Textor, Lilach O. Lerman. *Mayo Clinic, MN.*

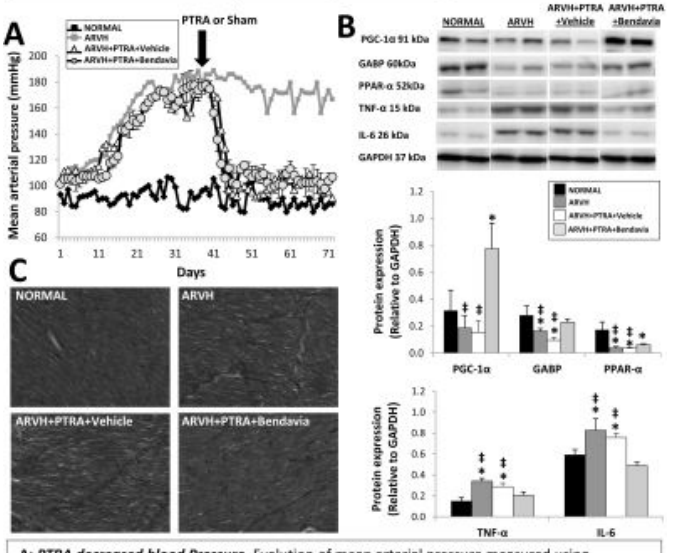
Background: Atherosclerotic renovascular hypertension (ARVH) increases cardiovascular morbidity and mortality. Renal revascularization with percutaneous transluminal renal angioplasty (PTRA) may not fully regress cardiac remodeling and damage, likely due to persistent myocardial ischemic insults. Mitochondrial targeted peptides like Bendavia reduce ischemic cardiomyopathy by preventing mitochondrial dysfunction. However, their potential for attenuating myocardial damage in ARVH has not been explored. We hypothesized that treatment with Bendavia as an adjunct to PTRA would improve cardiac function and decrease myocardial inflammation and fibrosis in swine ARVH.

Methods: After 6 weeks of ARVH (unilateral renal artery stenosis) or control, pigs underwent PTRA+stenting (or sham), with adjunct continuous infusion of Bendavia (0.05 mg/kg IV, 30 min before to 3 hrs after PTRA) or vehicle (n=7 each). Four weeks later, systolic and diastolic function was assessed by multidetector CT and myocardial morphology, inflammation, mitochondrial biogenesis and fibrosis evaluated ex-vivo.

Results: PTRA restored blood pressure to normal levels, yet myocyte cross sectional area remained increased and E/A ratio decreased, while both were normalized in Bendavia-treated pigs (Table). Furthermore, mitochondrial biogenesis was upregulated, and myocardial inflammation and fibrosis normalized in ARAS+PTRA+Bendavia animals (Figure).

Conclusions: Adjunct Bendavia during PTRA prevented myocardial hypertrophy, improved diastolic function, and reversed myocardial inflammation and fibrosis, underscoring the benefits of this strategy for preservation of cardiac function and structure in swine ARVH.

	NORMAL	ARVH	ARVH+PTRA +Vehicle	ARVH+PTRA +Bendavia
Heart rate (bpm)	76.8±17.5	81.6±13.7	83.7.±28.0	81.3±8.9
Cardiac output (L/min)	3.6±1.1	2.9±0.7	3.5±0.9	3.7±0.7
E/A ratio	1.3±0.2	0.7±0.2*‡	0.9±0.3*‡	1.0±0.1
Myocyte cross sectional area (μm)	3800±1756	15680±3623*‡	17320±8940*‡	8400±961
Myocardial fibrosis (%)	2.1±0.6	6.0±0.4*‡	5.4±1.5*‡	3.2±1.4



A: *PTRA decreased blood Pressure.* Evolution of mean arterial pressure measured using telemetry. **B: *Mitochondrial biogenesis and inflammation was upregulated, and inflammation normalized in Bendavia-treated pigs.*** Myocardial protein expression of peroxisome proliferator-activated receptors gamma co-activator (PGC)-1α, GA-binding protein (GABP), peroxisome proliferator-activated receptor (PPAR)α, tumor necrosis factor (TNF)-α, and interleukin (IL)-6. *p<0.05 vs. Normal; ‡p<0.05 vs. ARAS+PTRA+Bendavia. **C: Bendavia abolished myocardial interstitial fibrosis.** Representative myocardial trichrome staining (blue, x40) in all groups.

Funding: Pharmaceutical Company Support - This Study Was Supported by a Grant from Stealth Peptides

TH-PO830

Renal Vein Cytokine Release as an Index of Renal Parenchymal Inflammation in Chronic Experimental Renal Artery Stenosis

Alfonso Eirin, Xin Zhang, Hui Tang, Kyra L. Jordan, Xiang-Yang Zhu, Sandra Herrmann, Amir Lerman, Stephen C. Textor, Lilach O. Lerman. *Mayo Clinic, MN.*

Background: Renal parenchymal inflammation is a critical determinant of kidney injury in renal artery stenosis (RAS), but is difficult to assess in the single kidney without tissue samples. Whether renal vein (RV) levels of inflammatory markers reflect active parenchymal inflammation remains unknown. This study was designed to evaluate the relationship between net RV cytokine release and tissue inflammation in the post-stenotic kidney.

Methods: Pigs were studied after 10 weeks of RAS treated 4 weeks earlier with intra-renal vehicle or anti-inflammatory mesenchymal stem cells (MSC), or normal control. Single-kidney renal blood flow (RBF) was measured by fast CT. RV and inferior vena cava (IVC) levels of tumor necrosis factor (TNF)-α, interferon (IF)-γ, and monocyte chemoattractant protein (MCP-1) were measured by ELISA, and their net release calculated ((RV-IVC)*RBF). Immunostaining of the same inflammatory cytokines was correlated with their net release.

Results: Net release of TNF-α, IF-γ, and MCP-1 was higher in the RAS kidney compared to normal (all p<0.05), and decreased in MSC-treated pigs (Table). Similarly, TNF-α, IF-γ, and MCP-1 immunoreactivity was upregulated in RAS, but ameliorated in pigs treated with MSC (Figure A). Furthermore, net release of TNF-α, IF-γ, and MCP-1 directly correlated with their tissue expression (Figure B-D).

Conclusions: Our findings demonstrate that the net release of inflammatory markers from the affected kidney is a useful index of renal tissue inflammation in experimental RAS. Further studies are needed to establish the clinical value of these measurements.

	NORMAL	RAS	RAS+MSC
Inflammatory markers (Net release)			
TNF-α (pg/min)	-4973.6±2638.7	1460.6±611.1*	-2400.9±2035.4‡
IF-γ (ng/min)	-3.2±11.7	31.5±10.6*	14.1±9.9*‡
MCP-1 (pg/ml)	-9146.9±6505.8	1962.5±2699.8*	-7074.8±4469.8*‡
Renal hemodynamics			
RBF (ml/min)	652.1±92.3	299.3±78.3*	579.8±174.8*‡
GFR (ml/min)	104.5±9.6	49.7±7.1*	103.9±21.9‡

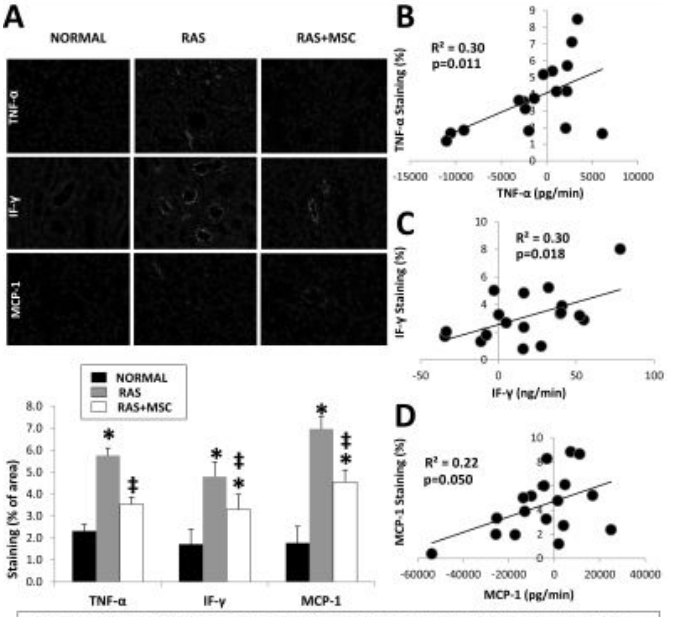


Figure. A: Representative immunostaining (40x) of TNF-α, IF-γ, and MCP-1 (top) and their quantification (B). A direct correlation was found between net renal release of inflammatory markers and their tissue expression (C-E). * p≤0.05 vs. Normal, ‡p<0.05 vs. RAS.

Funding: Other NIH Support - HL85307

TH-PO831

Bendavia, a Mitochondrial Permeability Transition Pore Inhibitor, Preserves Oxygenation and Renal Blood Flow in Swine Atherosclerotic Renovascular Disease

Alfonso Eirin, Behzad Ebrahimi, Xin Zhang, James Krier, Michael James Korsmo, John A. Crane, Xiang-Yang Zhu, Sandra Herrmann, Amir Lerman, Stephen C. Textor, Lilach O. Lerman. *Mayo Clinic, MN.*

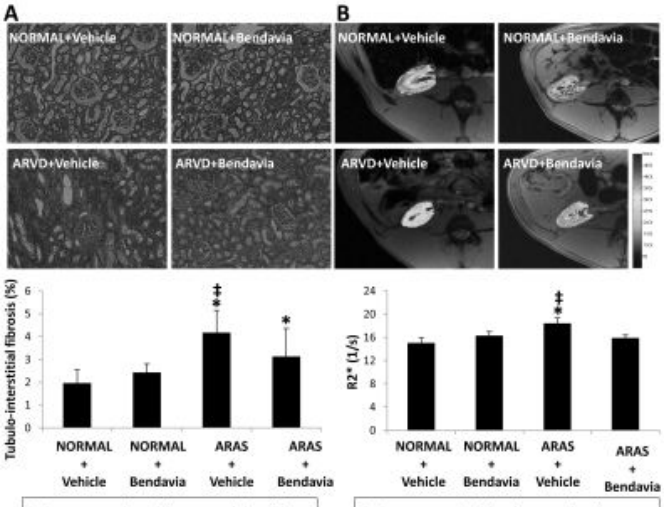
Background: The preservation of renal function in patients with Atherosclerotic Renovascular Disease (ARVD) remains challenging. Bendavia is a peptide drug that inhibits mitochondrial permeability transition pore (MTP) formation and is known to attenuate ischemia reperfusion injury. We hypothesized that chronic treatment with Bendavia would reduce renal fibrosis and dysfunction in ARVD pigs even without revascularization.

Methods: Domestic pigs were studied after 10 weeks of ARVD or sham, treated for the last 4 weeks with daily SC injections (5 d/wk) of Bendavia 0.1mg/kg (or vehicle). Single-kidney volume, perfusion, renal blood flow (RBF), and glomerular filtration rate (GFR) were studied using multi-detector CT, and cortical oxygenation using blood oxygen level-dependent (BOLD) MRI. Renal fibrosis was assessed by trichrome staining.

Results: All ARVD pigs achieved high-grade stenosis and hypertension (Table). Stenotic kidney volume, perfusion, RBF, and GFR decreased in ARAS+Vehicle, associated with cortical hypoxia (manifest as elevated cortical R2*). Daily treatment with Bendavia alone in ARAS pigs was associated with normal cortical R2*, RBF, and GFR, although blood pressure was unaffected.

Conclusions: Daily treatment with Bendavia improved function and cortical oxygenation, and decreased scarring in the stenotic kidney despite sustained renovascular hypertension.These findings support mitochondrial injury in kidney damage in ARVD and underscore a potential role for an MTP inhibitor such as Bendavia to protect the stenotic kidney.

	NORMAL + Vehicle	NORMAL + Bendavia	ARVD + Vehicle	ARVD + Bendavia
Degree of stenosis (%)	0	0	89.8±5.2*	81.0±5.3*
Mean blood pressure (mmHg)	82.2±41.6	84.4±23.7	173.4±19.4*	171.5±15.8*
Renal Volume (cc)	102.9±4.0	96.1±6.3	66.4±6.3*‡	104.9±3.3
Cortical Perfusion (ml/min/cc)	4.5±0.5	4.0±0.3	3.1±0.2*‡	4.4±0.2
RBF (ml/min)	553.8±82.8	589.7±71.8	318.8±61.0*‡	535.1±24.9
GFR (ml/min)	84.0±3.8	75.8±6.8	48.0±4.0*‡	86.6±11.2
Tubulointerstitial fibrosis (%)	1.9±0.6	2.4±0.4	4.2±0.9*‡	2.8±1.0



A: Representative trichrome staining (blue, x40) of renal tissue sections (top) and its quantification (bottom) in normal and ARVD animals either untreated or treated with chronic Bendavia. *p<0.05 vs. Normal; ‡p<0.05 vs. ARVD+Bendavia.

B: Representative blood oxygen level-dependent (BOLD) MRI images (top) and quantification of cortical blood oxygenation index, R2*,in all groups. *p<0.05 vs. Normal; ‡p<0.05 vs. ARVD+Bendavia.

Funding: Pharmaceutical Company Support - This Study Was Supported by a Grant from Stealth Peptides

TH-PO832

A Novel Regulatory Mechanism of Blood Pressure by Midkine through Epoxyeicosatrienoic Acids (EETs) Yuka Sato, Waichi Sato, Tomoki Kosugi, Hiroshi Kojima, Kayaho Maeda, Shoichi Maruyama, Enyu Imai, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.*

Background: The endothelium regulates vascular tone through the release of a number of vasoactive factors, including endothelin, NO and endothelium-derived hyperpolarizing factor (EDHF). Midkine (MK) is a multi-functional growth factor and affects the progress of ischemic renal injury, diabetic nephropathy and hypertension (HTN). We have previously reported that endothelial MK activated RAS through lung ACE after 5/6 nephrectomy. However, its regulatory mechanism remains unclear. In the present study, we focused on NO and EETs, and investigated the role of MK in HTN and endothelial dysfunction.

Methods: Wild-type mice (WT) and MK-deficient mice (MK-KO) were treated with unilateral nephrectomy and L-NAME, NO synthase inhibitor (UNx+L). They were measured BP and sacrificed on 4 months. We also administered charybdotoxin, K_{Ca} channel blocker, and 14, 15-EEZE, EETs blocker, respectively and recorded BP using radio-telemetry systems. Primary renal endothelial cells were isolated from WT and MK-KO, and evaluated EETs.

Results: In the UNx+L group, BP of WT developed marked HTN, while MK-KO exhibited normotension. Proteinuria and glomerular sclerosis were also attenuated in MK-KO. Urine nitrate/nitrite was decreased to the same extent in both WT and MK-KO with UNx+L. eNOS and cGMP showed no significant differences between WT and MK-KO. Consequently, HTN induced by NOS inhibitor was attenuated in MK-KO, however MK did not regulate NO-cGMP directly. Vasoconstractive factors; RAS and endothelin were also unchanged in MK-KO. We measured another vasodilatory factor; EETs. DHETs, EETs metabolites, were increased in urine and medium of primary endothelial cells of MK-KO compared to WT. The EETs pathway blockage by charybdotoxin or 14, 15-EEZE elevated BP higher in MK-KO than in WT. WT treated with MK antibody also exhibited marked BP elevation after 14, 15-EEZE injection.

Conclusions: MK regulated BP through EETs pathway. EETs have cardiovascular protective properties, and are of interest as therapeutics. MK might have the potential as a novel therapeutic target of HTN and endothelial dysfunction.

TH-PO833

Thromboxane Receptors in Smooth Muscle Promote Hypertension, Vascular Remodeling, and Sudden Death Matthew A. Sparks, Natalia A. Makhanova, Thomas M. Coffman. *Division of Nephrology, Duke University, Durham, NC.*

Background: The prostanoid thromboxane (TxA_2) is a potent vasoconstrictor and platelet aggregant that has been implicated in the pathogenesis of cardiovascular diseases including hypertension (HTN). Actions of thromboxane receptors (TP-R) in platelets and the vasculature have both been implicated in cardiovascular pathogenesis. To distinguish the contributions of vascular TP-R in isolation, we generated mice with cell-specific deletion of TP-R in smooth muscle cells (TP-SMKOs) using Cre/Loxp technology.

Methods: To examine the contributions of TP-R in the vasculature, we generated mice with cell-specific deletion from vascular smooth muscle cells (VSMCs). We used the KISM22α-Cre mouse, with Cre recombinase "knocked-in" to the Sm22α gene locus, to excise the conditional Tp receptor allele specifically in smooth muscle.

Results: We crossed the KI*SM22*α-Cre with the mTmG reporter mouse to map Cre expression, documenting Cre in small arterioles in each organ including the kidney. Furthermore, we generated mice homozygous for the conditional *Tp* allele also bearing the KI*SM22*α-Cre allele (TP-SMKOs). mRNA for the TP-R was easily detected in aortae from control mice, but not from TP-SMKOs (P<0.005). Similarly, TP-R mRNA expression in mesenteric arteries, with intact endothelium and adventitia, was decreased by ~80% in TP-SMKOs (P=0.05). In TP-SMKOs, acute responses to the TP agonist U46619 were attenuated by ~60% in both the peripheral and renal circulations (P<0.05), whereas acute responses to AngII were unaffected. Infusion of high-dose U46619 caused circulatory collapse and death in a majority of control mice, but TP-SMKOs were completely protected from U46619-induced sudden death (P<0.05). Baseline BP measured by radiotelemetry were similar in TP-SMKOs (111±1 mmHg) and Controls (114±1 mmHg; P=NS). However, the absence of TP-R in VSMCs caused significant attenuation of AngII-induced HTN (controls: 159±2 mm Hg; TP-SMKO: 145±8 mm Hg, P<0.05) and diminished aortic medial hypertrophy in TP-SMKOs (59±4 μm) vs. Controls (79±7 μm; P<0.05).

Conclusions: Vascular TP-R play a major role in shock, AngII-induced hypertension, and vascular remodeling.

Funding: Veterans Administration Support

TH-PO834

SIRT1 Activation Protects the Endothelial Dysfunction by Inhibiting MCP-1 and ICAM-1 Generation Hideyuki Negoro. *Medicine, Harvard Medical School, Boston, MA.*

Background: SIRT1 is a conserved NAD(+)-dependent deacetylase and possesses beneficial effects against aging-related diseases, but little information is available on a putative role of SIRT1 in endothelial and vascular homeostasis. Endothelial senescence causes endothelial dysfunction to promote atherosclerotic change and contribute to age-related vascular diseases. Cellular adhesion molecules, such as monocyte chemotactic protein-1 (MCP-1) and intracellular adhesion molecule-1 (ICAM-1) play an important part in the progression of age-related vascular diseases.

Methods: We established an in vitro senescence model by prolonged culture of primary endothelial cells isolated from bovine aorta. The freshly isolated young endothelial cells gradually underwent senescence during 1 month of repetitive passages. We knocked down SIRT1 to evaluate the protein levels of LKB1, phosphorylated AMPK, MCP-1 and ICAM-1 in the knocked down cells.

Results: It was observed that protein expressions of SIRT1 were decreased time dependently in the senescent endothelial cells. In contrast, the protein levels of LKB1, a serine/threonine kinase, the phosphorylation of its downstream target AMP-activated protein kinase (AMPK), MCP-1 and ICAM-1 generation were dramatically increased in the senescent cells. On the other hand, resveratrol activated SIRT1 in the endothelial cells. SIRT1 activation with resveratrol inhibited the increase of LKB1, AMPK. At the same time, SIRT1 activation with resveratrol reduced MCP-1 and ICAM-1 generation in the endothelial cells significantly. We knocked down SIRT1 and the protein levels of LKB1, phosphorylated AMPK, MCP-1 and ICAM-1 elevated in the knocked down cells. The protein levels of LKB1, phosphorylated AMPK, MCP-1 and ICAM-1 generation did not changed in the SIRT1 knocked down cells even if they were stimulated with resveratrol.

Conclusions: These findings indicate that activation of SIRT1 provides beneficial effects against the endothelial dysfunction to promote atherogenesis by inhibiting MCP-1 and ICAM-1 generation.

Funding: Government Support - Non-U.S.

TH-PO835

The Increase of Reactive Oxygen Species in Vascular Smooth Muscle Cells Using Juglone Results in Mineralization *In Vitro* Mirjam Schuchardt, Jasmin Pruefer, Markus Tolle, Markus van der Giet. *Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany.*

Background: Vascular alterations like mineralization of vascular smooth muscle cells (VSMCs) in the media of the vascular wall contributes to the high mortality in patients with chronic kidney disease (CKD). Different factors are known that induce calcification, but the underlying mechanisms are not fully understood so far. CKD patients suffer from an increased level of reactive oxygen species (ROS). The aim of this study was to investigate the influence of an increased ROS level on the mineralization of VSMCs *in vitro*.

Methods: VSMCs from rat aorta were used for this study. Differences in gene expression were measured via real-time PCR. The alkaline phosphatase enzyme activity

were determined photometrically. The extracellular calcium content was quantified using the o-cresolphtalaine method. The superoxide production was measured in dihydroethidium-labeled cells. The quantification of hydrogen peroxide was determined in CM-H_2DCFDA-labeled cells via flow cytometry.

Results: The chinone derivate juglone induces a dose-dependent increase in superoxide and hydrogen peroxide production in VSMCs. Stimulation of VSMCs for 21 days using juglone induces the *in vitro* mineralization detected via an increase in extracellular calcium content and an increase in alkaline phosphatase enzyme activity compared to the control. Furthermore, juglone stimulation increases the mRNA expression of osteogenic proteins like alkaline phosphatase and osteocalcin, as well as the transcription factors core-binding factor alpha-1 and osterix.

Conclusions: A chronic increase in the ROS level results in the mineralization of VSMCs *in vitro*. Therefore, the ROS level may play a central role in the molecular signal transduction pathway of vascular calcification.

Funding: Government Support - Non-U.S.

TH-PO836

p38 MAP Kinase Mediates Aldosterone-Induced Podocyte Injury in Natriuretic Peptide Receptor (GC-A)-Deficient Mice Yukiko Kato,[1] Masashi Mukoyama,[1] Hideki Yokoi,[1] Yoshihisa Ogawa,[1] Kiyoshi Mori,[1] Masato Kasahara,[1] Takashige Kuwabara,[1] Hirotaka Imamaki,[1] Akira Ishii,[1] Kenichi Koga,[1] Keita P. Mori,[1] Ichiro Kishimoto,[2] Akira Sugawara,[1] Kazuwa Nakao.[1] *[1]Department of Medicine and Clinical Sicinece, Kyoto University Graduate School of Medicine; [2]Department of Atherosclerosis and Diabetes, National Cerebral and Cardiovascular Center.*

Background: Aldosterone plays an important role in the pathogenesis of renal injury. Natriuretic peptide receptor/guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects by eliciting natriuresis, reducing blood pressure, and inhibiting fibrosis. We demonstrated that uninephrectomized GC-A knockout (KO) mice with aldosterone and sodium overload exhibited accelerated hypertension with massive proteinuria (Ogawa et al. J Am Soc Nephrol 2012). These mice exhibited enhanced phosphorylation of p38 MAP kinase (MAPK) mainly in podocytes. To explore the interaction between p38 MAPK and GC-A signaling, we examined the effect of p38 MAPK inhibition on renal findings of GC-A KO mice with aldosterone.

Methods: GC-A KO mice or wild-type mice were uninephrectomized and then treated for 4 weeks with high salt diet and aldosterone infused subcutaneously using an osmotic minipump. They were also administered with hydralazine or p38 MAPK inhibitor (FR 167653) by drinking water. We examined BP, urinary albumin excretion, renal histology, and immunohistochemistry for nephrin, podocin and downstream targets of p38 MAPK including CHOP and p53.

Results: Administration of FR remarkably suppressed urinary albumin excretion by 90% in GC-A KO mice ($p < 0.01$). Glomerular hypertrophy, mesangial expansion and interstitial fibrosis were decreased in FR-treated aldosterone-given GC-A KO mice. Immunofluorescent study showed that FR treatment kept high intensity of nephrin and podocin in glomeruli. FR treatment abrogated the phosphorylation of p38 MAPK in podocytes, and greatly reduced glomerular expression of p53 protein.

Conclusions: These results suggest that renoprotective properties of the endogenous natriuretic peptide/GC-A system may result from the local inhibition of aldosterone/p38 MAPK pathway in podocytes, involving p53 singnaling.

Funding: Government Support - Non-U.S.

TH-PO837

Loss of Renal or Systemic Angiotensin Converting Enzyme 2 Contributes to the Hypertensive Response to Chronic Angiotensin II David I. Ortiz Melo, Natalie Mattocks, Thomas M. Coffman, Susan B. Gurley. *Medicine, Durham VA and Duke University Medical Centers, Durham, NC.*

Background: Angiotensin Converting Enzyme 2 (ACE2) is a carboxypeptidase that converts Angiotensin II (AngII) to Ang(1-7). Deletion of the *Ace2* gene in mice induces an exaggerated response to AngII-induced hypertension (HTN) associated with elevated renal AngII levels. We hypothesize that renal ACE2 regulates AngII levels and is therefore responsible for its anti-hypertensive effects. To test this hypothesis, we used renal cross-transplantation strategy to separate the effect of selective ACE2 deficiency within the kidney from ACE2 deficiency in all other tissues.

Methods: We studied male ACE2 knockout (KO) mice and wild-type (WT) littermates on (129/SvEv x C57BL/6)F_1 background. Kidney transplants were performed between genetically matched mice to generate four experimental groups: 1) WT, 2) renal ACE2 KO, 3) systemic ACE2 KO, and 4) global ACE2 KO. All kidney function was provided entirely by the single transplanted kidney. After baseline measurements, mice were infused with AngII (1µg/kg/min) for two weeks while mean arterial blood pressures (MAP) were measured with radiotelemetry.

Results: There were no significant differences in MAP at baseline among groups. Global ACE2 knockout mice had an enhanced hypertensive response to AngII as compared to WT mice (152±6 vs.144±7 mmHg; p=0.03), consistent with our previous report. However, the presence of ACE2 in either the kidney or systemic tissues restored MAPs to levels similar to that of the WT group (renal ACE2 KO 140±7mmHg, $p<0.01$ vs. global ACE2 KO; systemic ACE2 KO 140±5 mmHg, $p<0.01$ vs. global KO). This effect was similar between the renal KO and systemic KO groups.

Conclusions: ACE2 has the potential to regulate cardiovascular responses by metabolizing Ang II, thereby acting as a brake on the RAS. Global ACE2 deficiency leads to an exaggerated hypertensive response to chronic AngII administration. Our kidney transplant studies now show that restoration of ACE2 in either the kidney or in extra-renal tissues is sufficient to abrogate this enhanced hypertensive response. Our data suggest equivalent effects of both renal and extra-renal ACE2 in hypertension.

Funding: Private Foundation Support

TH-PO838

Liver-, but Not Kidney-Specific Angiotensinogen Gene Knockout Abrogates the Enhancement of Renal Angiotensin II Synthesis that Follows Podocyte Injury in Progressive Glomerulosclerosis Taiji Matsusaka,[1] Fumio Niimura,[1] Masafumi Fukagawa,[1] Akira Nishiyama,[2] Iekuni Ichikawa.[1,3] *[1]Tokai University, Japan; [2]Kagawa University, Japan; [3]Vanderbilt University.*

Background: Intrarenal angiotensin (A)II is increased in a variety of kidney diseases independently of plasma AII, and thought to act detrimentally on the renal architecture. The NEP25 mouse model allows induction of podocyte-specific injury leading to progressive glomerulosclerosis. Using this model, we have recently demonstrated that podocyte injury leads to a marked increase in renal angiotensinogen (Agt) and AII content (JASN 2012), suggesting that the integrity of the barrier function of podocytes is critically important to prevent augmentation of renal AII synthesis. Subsequently, we also found that podocyte injury concurrently enhances transcription of renal Agt.

Methods: To verify that renal AII is indeed originated to the liver/circulating Agt in glomerular diseases, we analyzed NEP25 mice in which liver or kidney *Agt* is selectively knocked out (KO).

Results: Western analysis and immunohistochemistry revealed that renal Agt protein was markedly increased in NEP25 and similarly in Kidney Agt KO/NEP25 mice 8 days after podocyte injury. By contrast, in Liver Agt KO/NEP25 mice, the level of renal Agt was undetectably low both before and after podocyte injury. In parallel, renal AII, measured by RIA, was markedly increased in NEP mice (750±87 (n=7) vs. 134±21 (n=10) fmol/g tissue). Liver KO, but not kidney KO, reduced the level of renal AII measured after podocyte injury (103±8.2 (n=10)($p<0.001$) and 746±128 (n=10), respectively). Renal AII had no relationship with renal Agt mRNA, which was low in Kidney Agt KO, or with renal renin activity, which was elevated in Liver Agt KO. Moreover, edema was significantly attenuated in Liver Agt KO/NEP25, but not Kidney Agt KO/NEP25. Kidney and Liver dual Agt KO (n=12) showed phenotypes similar to those of Liver Agt KO mice.

Conclusions: The results establish that the detrimental increase in renal AII synthesis in glomerular diseases that follows podocyte injury results from increased filtered Agt of liver origin, not from increased transcription of renal Agt.

Funding: Government Support - Non-U.S.

TH-PO839

Angiotesin II Regulation of Blood Pressure via Modulation of DPPIV Activity and Sodium Hydrogen Exchange (NHE3) in the Renal Proximal Tubules Vinayak Ramanath, James R. Sowers, Adam Whaley-Connell, Ravi Nistala. *Nephrology, University of Missouri-Columbia, Columbia, MO.*

Background: Sodium homeostasis and blood pressure regulation is modulated by the effector peptide (angiotensin II, Ang II) for renin angiotensin system (RAS) via activation of Na^+/H^+ exchanger-3 (NHE3) in the kidney proximal tubule (PT). Excessive sodium retention and activation of RAS in obesity and diabetes is thought to contribute to hypertension. Recently, the role of nutrient sensor mTOR/S6K1 (mTORC1) has been implicated in hyperfiltration seen in early diabetes. Similarly, obesity is characterized by increased mTORC1 signaling and NHE3 activity. In addition, the incretin Glucagon-Like Peptide-1 (GLP-1) or exendin-4 has been shown to decrease NHE3 activity, increase sodium excretion and ameliorate hypertension. However, it is unknown how Akt-mTORC1 interacts with DPPIV enzyme and if nephron hypertrophy is related to NHE3 activity. Therefore, we hypothesized that DPPIV inhibition may suppress Ang II-mediated activation of mTORC1 and NHE3 activity.

Methods: Opossum PT cells stably expressing rat Ang-II Type-1 receptor (AT1R) were stimulated with Ang II (100nM) and pre-treated for 60min, with either MK-0626 (5uM) or olmesartan (1uM). Western blots, IHC and enzyme inhibitor assays were used. Mice fed high sucrose-high fat diet (DIO) for 16 wks were analyzed for BP, proteinuria, NHE3/DPPIV activity and mTORC1 activation.

Results: Ang II stimulation of PTCs for 10min, activated mTORC1 and ERK1/2 as demonstrated by increased P-S473-Akt, P-S2448-mTOR, P-T389-S6K1 and P-T202/204-ERK respectively. Ang II increased NHE3 activity and decreased megalin protein and endocytosis. Olmesartan completely blocks Ang II activation of mTORC1 and ERK while MK-0626 inhibition was partial. MK-0626 also improved megalin-mediated albumin endocytosis. In addition, MK-0626 abrogated Ang II-mediated activation of DPPIV in the PTCs. DPPIV binds to GLP1R, NHE3, megalin and PI3-K/Akt pathway components possibly via PH domains and adaptor proteins.

Conclusions: These results suggest that NHE3 regulation, renal hypertrophy and proteinuria by Ang II may be via activation of DPPIV in the brush border of PTCs.

Funding: Other NIH Support - NIH R01 to James Sowers and NIH R03 to Adam Whaley-Connell, Pharmaceutical Company Support - Merck Pharmaceuticals

TH-PO840

Angiotensin-Stimulated Nitric Oxide and Superoxide Are in Different Compartments in the Thick Ascending Limb Jagannath H. Saikumar,[1] Katherine J. Massey,[2] Mark D. Faber,[1] Jerry Yee,[1] Jeffrey L. Garvin.[2] *[1]Nephrology, Henry Ford Hospital, Detroit, MI; [2]Hypertension and Vascular Research, Henry Ford Hospital, Detroit, MI.*

Background: Angiotensin (Ang II) stimulates both nitric oxide (NO) and superoxide (O_2^-) in the thick ascending limb (TAL). In this segment, Ang II stimulates NO production by activating NOS3 via the AT_2 receptor while Ang II induced O_2^- production is via the AT_1 receptor. Luminal flow-induced NO inhibits flow-mediated O_2^- in the TAL via a cGMP/PKG dependent pathway. Therefore, we hypothesized that Ang II-stimulated NO inhibits Ang II induced O_2^- in the TAL.

Methods: To address this hypothesis, we measured the ability of Ang II to stimulate NO production in the presence and absence of a O_2^- scavenger and O_2^- in the presence and absence of L-arginine, the substrate for NO, and L-NAME, a NOS inhibitor. NO production was measured by fluorescence microscopy in isolated TALs and O_2^- production was measured using the lucigenin assay.

Results: Ang II dose-dependently increased NO production in TALs across the physiological range of 1pM to 1nM by 59±8% ($p<0.03$), 79±49% ($p=0.15$), 129±32% ($p<0.05$) and 91±20% ($p<0.001$). O_2^- production was stimulated by 49% over baseline when the TALs were exposed to Ang II at 1nM (589±88 vs. 875±121 AU/mg/sec, $p<0.05$). NO production was not different when Ang II was used to stimulate the TAL in the presence of the O_2^- scavenger, tempol. When TALs were stimulated with 1nM Ang II in the presence of L-arginine, no difference was seen in O_2^- production (764±97 vs. 779±74 AU/mg/sec). L-NAME reduced O_2^- production from 633±114 to 360±49 AU/mg/sec. L-arginine in the presence of L-NAME did not affect O_2^- production (651±139 vs. 594±71 AU/mg/sec).

Conclusions: These data suggest that Ang II-stimulated NO and Ang II-induced O_2^- do not interact with each other and are likely in different compartments of the TAL epithelial cell. Also Ang II likely causes NOS uncoupling.

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TH-PO841

Decreased Blood Pressure in Tamm-Horsfall Protein Deficient Mice during Chronic Salt Loading James M. Bates, Hajamohideen S. Raffi, Satish Kumar. *Medicine/Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: The Tamm-Horsfall Protein (THP) deficient mouse ($THP^{-/-}$) displays a reduced ability to concentrate urine. The activity of the Na^+, K^+ $2\,Cl^-$ cotransporter (NKCC2) is reduced in the $THP^{-/-}$ mouse. Recent genome wide association studies have shown that a single nucleotide polymorphism in the promoter region of the THP gene is correlated with blood pressure. In this study, we compared the effect of chronic dietary salt loading between $THP^{-/-}$ mice and wild-type ($THP^{+/+}$) mice, on blood pressure, urine volume, food consumption, water consumption, urine sodium and urinary THP.

Methods: Six each of $THP^{-/-}$ and $THP^{+/+}$ mice were fed a high salt diet (8 % NaCl) for 5 months. On day 5, 40 and 140, systolic and diastolic blood pressure (BP), pulse, body weight, urine volume, and water and food intake were measured. Twenty four hour urine samples were collected and analyzed for sodium, THP and creatinine. The data were analyzed with Student's t test and linear regression.

Results: Systolic and diastolic BP and pulse were lower in the $THP^{-/-}$ mice on day 140 (mean systolic BP ± SE, $THP^{+/+}$ 122 ± 2 mm Hg vs $THP^{-/-}$ 114 mm Hg ± 3, $p = 0.03$; mean diastolic BP $THP^{+/+}$ 86 ± 3 mm Hg vs $THP^{-/-}$ 75 mm Hg ± 4, $p = 0.005$; mean pulse $THP^{+/+}$ 595 ± 7 mm Hg vs $THP^{-/-}$ 627 mm Hg ± 10, $p = 0.007$). Urine volume and water consumption were lower in the $THP^{-/-}$ mice on day 5 (mean urine volume ± SE, $THP^{+/+}$ 10.7 ± 1.5 ml vs $THP^{-/-}$ 6.7 ± 1.2 ml, $p = 0.032$; mean water consumption ± SE, $THP^{+/+}$ 19.1 ± 1.9 ml vs $THP^{-/-}$ 14.0 ± 1.7 ml, $p = 0.037$) and day 140 (mean urine volume ± SE, $THP^{+/+}$ 14.8 ± 1.8 ml vs $THP^{-/-}$ 9.9 ± 1.7 ml, $p = 0.04$; mean water consumption ± SE, $THP^{+/+}$ 21.9 ± 2.5 ml vs $THP^{-/-}$ 15.7 ± 2.3 ml, $p = 0.047$). Twenty four hour urinary excretion of sodium was not different between the two groups on day 5 and day 40. Urinary THP excretion (measured on day 40) was positively correlated with excretion of urinary sodium ($R^2 = 0.92$).

Conclusions: We conclude that THP plays a role in renal sodium handling and impacts systemic blood pressure.

TH-PO842

Differential Regulation of Ste20/SPS1-Related Proline/Alanine-Rich Kinase (SPAK), Na-Cl Cotransporter (NCC), and Na-K-2Cl Cotransporter (NKCC) in the Cortex versus Medulla in Angiotensin II (AngII)-Infused Rats Mien T.X. Nguyen, Donna Lee, Alicia A. McDonough. *Cell and Neurobiology, USC Keck School of Medicine, Los Angeles, CA.*

Background: SPAK colocalizes with and phosphorylates NKCC and NCC in the TAL and DCT, respectively. We aimed to determine whether SPAK differentially regulates NKCC and NCC in the cortex vs. medulla in AngII-dependent hypertension.

Methods: Male Sprague-Dawley rats were infused with AngII (400 ng/kg/min) via osmotic minipumps. Kidney cortex and medulla were separated, homogenized and subjected to immunoblotting.

Results: AngII-infused rats developed hypertension after 2 wks: increased mean arterial pressure (161.97 ± 7.09 vs. control 115.23 ± 4.75 mmHg, $p<0.05$), increased heart weight (0.35 ± 0.01 vs. control 0.26 ± 0.004 g/100 g BW, $p<0.05$), and increased overnight urinary Na excretion (2.52 ± 0.18 vs. control 1.50 ± 0.14 mmol, $p<0.05$). Immunoblot density values in AngII-infused rats were normalized to those in control, defined as 1.00 ± SEM (*$p<0.05$).

	Control (n=8)	AngII-infused (n=8)
Cortex		
NKCC	1.00 ± 0.06	1.58 ± 0.23 *
NKCC-P	1.00 ± 0.05	2.14 ± 0.31 *
NCC	1.00 ± 0.05	2.17 ± 0.24 *
NCCpS71	1.00 ± 0.14	3.28 ± 0.53 *
SPAK	1.00 ± 0.08	2.27 ± 0.42 *
Medulla		
NKCC	1.00 ± 0.07	0.55 ± 0.06 *
NKCC-P	1.00 ± 0.15	1.47 ± 0.37
SPAK	1.00 ± 0.04	0.79 ± 0.04 *

Conclusions: AngII infusion significantly increased SPAK abundance, NCC and NKCC total abundance and phosphorylation in the cortex while decreasing SPAK and NKCC total abundance in the medulla. This suggests more Na reabsorption in the distal nephron and less in the loop of Henle, contributing to hypertension in this model.

TH-PO843

Regulation of NKCC1 by AMP-Activated Protein Kinase (AMPK) Matthew R.P. Davies,[1,2] Scott Andrew Fraser,[3] Marina Katerelos,[3] Kurt Gleich,[3] Peter F. Mount,[1,2] David A. Power.[1,2,3] *[1]Department of Nephrology, Austin Health, Melbourne, VIC, Australia; [2]Department of Medicine, Austin Health, Melbourne, VIC, Australia; [3]Institute for Breathing and Sleep, Austin Health, Melbourne, VIC, Australia.*

Background: The Sodium-Potassium-Chloride cotransporter NKCC1 plays a significant role in regulating blood pressure via modulation of vascular tone. Phosphorylation at regulatory sites (including Thr203, Thr207,Thr212 & Thr 217) increases NKCC1 activity and is a major mechanism through which the cotransporter is regulated. The energy-sensitive kinase AMPK plays a key role in cellular metabolism. Activators of AMPK produce vasorelaxation and a fall in blood pressure, the mechanisms of which are largely undetermined. AMPK has previously been shown to regulate the function of NKCC2 in the kidney. We sought to determine if AMPK also regulates the function of NKCC1.

Methods: We studied the effects of stimulating AMPK on the function of NKCC1 in MDCK cells using bumetanide-sensitive $Rb86^+$-flux. Phosphorylation of NKCC1 was analysed with Western Blotting using R5 antibody (recognises phosphorylated Thr212 & Thr217). The effect of AMPK on expression of NKCC1 was compared using transfection of Cos cells. Membrane localisation of NKCC1 was determined by immunofluorescence with confocal microscopy and surface biotinylation.

Results: Stimulation of AMPK in MDCK cells with A769662 resulted in a dramatic reduction of bumetanide-sensitive $Rb86^+$-flux ($p<0.001$) and reduced phosphorylation of NKCC1 detected with R5 antibody ($p<0.005$). Surprisingly, co-transfection of AMPK with NKCC1 resulted in enhanced expression of NKCC1. However, active but not kinase-dead AMPK led to a reduction in membrane localisation of NKCC1 despite increased expression.

Conclusions: This data suggests that activation of AMPK leads to dephosphorylation of NKCC1 at key regulatory sites, possibly by recruitment of a phosphatase, so reducing its membrane localisation and co-transporter activity. Binding of AMPK appears to provide structural stability to NKCC1, indicating the importance of AMPK in its function. The data helps explain the blood pressure lowering effects of AMPK activators and provides a potential link between metabolic disease and hypertension.

TH-PO844

Acute Adrenergic Stimulation Activates the Renal Sodium Chloride Cotransporter Andrew Terker, Chao-Ling Yang, James A. McCormick, David H. Ellison. *Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR.*

Background: The sodium chloride cotransporter (NCC) in the distal nephron is a key regulator of sodium reabsorption and blood pressure homeostasis. The kinase SPAK activates NCC. It has been shown that adrenergic stimulation causes salt-sensitive hypertension and upregulates NCC, but a detailed molecular mechanism and the timing of this pathway remain unclear.

Methods: Blood pressure (BP) was measured by tail-cuff. **Chronic adrenergic stimulation**:Baseline BP was measured for 1 week. Vehicle (V) or norepinephrine (NE, 2.5 mg/kg/d) infusion was administered by osmotic minipump for weeks 2 and 3. Diet was 0.49% NaCl during weeks 1 and 2 and 8% NaCl during week 3. **Acute adrenergic stimulation**: Mice were injected IP with V, NE (750 ug/kg), phenylephrine (750 ug/kg), or isoproterenol (750 ug/kg) as indicated. Kidneys were harvested 30 min later for Western blot. **Western blots** were performed on whole kidney lysate. **Immunofluorescence** was performed on kidneys that were fixed 30 min post-treatment.

Results: Chronic norepinephrine infusion has been shown to stimulate NCC and cause salt-sensitive (SS) hypertension in mice. As this finding was controversial, we first reproduced this observation. During chronic norepinephrine (NE) infusion, blood pressure increases were shown to be SS and both total (tNCC) and phosphorylated NCC (pNCC, a marker of activation) were increased 50-100%. Given this finding, we hypothesized that adrenergic stimulation activates NCC acutely. Kidneys from mice acutely treated with NE showed 300% increases in pNCC with no changes in tNCC. This experiment was then repeated using α- and β-adrenergic specific agonists and both stimulated pNCC. Lastly, SPAK KO mice were given acute injections of NE. These mice also experienced increases in pNCC with no changes in tNCC. Results were demonstrated by Western blot and immunofluorescence.

Conclusions: Chronic adrenergic stimulation causes salt-sensitive hypertension in the mouse. While changes in blood pressure are seen after several days of adrenergic stimulation, NCC is upregulated within minutes This mechanism is mediated by both α- and β-adrenergic receptors and is SPAK-independent.

Funding: NIDDK Support, Veterans Administration Support

TH-PO845

The Kidney Cyp2c44 Epoxygenase Regulates Distal Sodium Excretion and the Blood Pressure Response to Increased Dietary Salt Jorge H. Capdevila,[1] John D. Imig,[2] WenHui Wang.[3] *[1]Medicine, Vanderbilt University Medical School, Nashville, TN; [2]Pharmacology, Medical College of Wisconsin, Milwaukee, WI; [3]Pharmacology, New York Medical College, Valhalla, NY.*

Background: Roles for the mouse Cyp2c44 and rat CYP2C23 arachidonic acid (AA) epoxygenases in blood pressure regulation were suggested by reports that: a) their kidney expression was dietary salt sensitive, b) CYP2C23 inhibition caused salt sensitive hypertension, and c) their 11,12- and 14,15-epoxyeicosatrienoic acid (EET) metabolites inhibited the kidney epithelial sodium channel (ENaC).

Methods: To define roles for the Cyp2c44 epoxygenase in kidney physiology and blood pressure control, we develop a line of mice carrying a disrupted *Cyp2c44* gene, and used polarized collecting duct (CD) cells for mechanistic studies of EET effects on transcellular sodium transport.

Results: Mice lacking a functional Cyp2c44 epoxygenase develop dietary salt sensitive hypertension, a common form of the human disease. Thus, *Cyp2c44(-/-)*(KO) mice are normotensive and become hypertensive when fed high salt diets (systolic pressures: 124± 8 and 168 ±18 mm Hg, respectively). Compared to salt loaded WTs, hypertensive KO mice show a hyperactive ENaC and Amiloride, an ENaC inhibitor, normalizes the pressures of hypertensive KO mice. Moreover, KO mice on high salt fail to induce AA epoxidation, and their urinary output, body weights and free fluid volumes are higher than those of salt loaded WT controls (by approximately 27; 3.7; and 30%; respectively). Studies in polarized M1 cells show that 14,15-EET inhibits amiloride-sensitive transcellular sodium transport by increasing an ERK1/2-catalyzed phosphorylation of the ENaC beta and gamma subunits.

Conclusions: These studies: a) identify an anti-hypertensive role for the Cyp2c44 epoxygenase as an endogenous regulator of distal sodium re-absorption, and b) point to a mechanism that involves a Cyp2c44/EET-controlled, ERK1/2 catalyzed, phosphorylation of ENaC subunits. It is expected that these studies could serve as a basis for future efforts to develop novel strategies for the early diagnosis and treatment of hypertension.

Funding: NIDDK Support, Other NIH Support - NIHLBL

TH-PO846

Regulation of Water and Sodium Balance in Erythropoietin-Induced Hypertension Jeonghwan Lee,[1] Nam Ju Heo,[2] Sejoong Kim,[3] Kwon Wook Joo,[1] Jin Suk Han.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [2]Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, Korea; [3]Internal Medicine, Seoul National University Bundang Hospital, Seoul, Korea.*

Background: The present study was designed to investigate the regulation of sodium and water homeostasis in erythropoietin (EPO)-induced hypertension.

Methods: Renal failure was induced by a two-stage 5/6 nephrectomy. Uremic rats were divided into two groups and received vehicle and EPO (150 U/kg, intraperitoneal injection, two times per week) for 4 weeks.

Results: Serum creatinine, hematocrit, body weight, and systolic blood pressure (BP) were similar in both groups of uremic rats before treatment. After 1 week of treatment, hematocrit increased significantly in the EPO group (50.9±1.2% vs. 43.8±1.6%, *P*=0.002). Systolic BP was significantly higher in the EPO group than the control (155.5±8.8 mmHg vs. 140.0±6.7 mmHg, *P*=0.007). Although intake of diet and water were almost same, urine output (13.7±2.0 ml/d vs. 18.2±4.7 ml/d, *P*=0.034) and urinary excretion of sodium (0.77±0.10 mmol/d vs. 0.90±0.06 mmol/d, *P*=0.018) decreased significantly in the EPO group. After 4 week of treatment, increased systolic BP was sustained (159.5±8.7 mmHg vs. 146.6±6.2 mmHg, *P*=0.007). Urine output (17.1±4.2 ml/d vs. 21.2±5.4 ml/d, *P*=0.141) and urinary excretion of sodium (0.70±0.10 mmol/d vs. 0.79±0.09 mmol/d, *P*=0.142) decreased in the EPO group, but the statistical significance were insufficient. The renal abundances of ENaC α, γ, and NHE3 significantly decreased (56.1±16.0%, 49.4±25.6%, and 38.6±24.8% of the control, *P*=0.011, 0.026, and 0.007, respectively) in the EPO group. Other apical sodium transporters did not change in abundance. Aquaporin-2 significantly increased in the EPO group (116.7±9.0% of the control, *P*=0.002). Renal endothelin levels seemed to be higher in the EPO group (56.1±26.2 pg/ml vs. 50.1±27.9 pg/ml), but the difference was not significant (*P*=0.8048). Plasma renin and serum aldosterone levels were not different between groups.

Conclusions: EPO induces sodium and water retention, which can contribute the EPO-induced hypertension in uremic remnant kidney model.

TH-PO847

Role of Bradykinins in Renal Development of Diabetic Complications Syed J. Khundmiri,[1] Michael Merchant,[1] Jon B. Klein.[1,2] *[1]Medicine-Nephrology, University of Louisville, Louisville, KY; [2]Robley Rex VAMC, Department of Veterans Affairs, Louisville, KY.*

Background: A role for kinins in the development and progression of diabetic nephropathy (DN) remains controversial. Largely research has focused on bradykinin (BK) and des-Arg(9)-BK (DABK) and their signaling through constitutive (BK2R) or inducible (BK1R) receptors. Recent plasma peptidomic studies of type-1 DN patients with microalbuminuria indicated a correlation between BK, DABK and related prolyl-hydroxylated kinins and the onset of early progressive renal function decline. We hypothesized these kinins play a role in the development of the early diabetic nephropathy phenotype. To address this hypothesis we measured the glucose modified kinin-mediated regulation Na-K ATPase (Na-K) activity in renal tubular cells lines.

Methods: Immortalized canine distal tubular (MDCK), human or mouse proximal tubule (HKC11 and MPTEC) cell lines were cultured in normal glucose (NG), high glucose (HG), or NG plus osmotic control culture conditions and the effects of kinin peptides on ouabain sensitive Na-K activity was measured. Receptor specificity for kinin regulation of Na-K activity was characterized using BK2R and BK1R specific antagonists.

Results: As expected in NG conditions BK increased ($p<0.05$) Na-K activity by 25% through BK2R. Unexpectedly, hydroxyprolyl-3-BK (BK3) decreased ($p<0.05$) Na-K activity by 40% in NG conditions through BK1R in MDCK, HKC11 and MPTEC cell lines. In HG conditions, both BK (49%) and BK3 (76%) increased ($p<0.05$) Na-K activity via BK1R. The novel BK variant, bis-3,7-hydroxyprolyl-BK (BK3,7) decreased ($p<0.05$) Na-K activity by 20% in all culture conditions through BK2R signaling.

Conclusions: These results suggest BK signaling in renal tubular cells is affected by both kinin post-translational modification and by hyperglycemia. HG shifts BK signaling from BK2R to BK1R, BK continues to inhibit natriuresis. While HG does not shift BK3 signaling away from BK1R, HG shifts BK3 from natriuresis toward sodium retention. These data suggest a pathophysiologic role of modified kinins in the dysregulation of Na+ transport in periods of uncontrolled hyperglycemia such as occur during poorly controlled diabetes.

Funding: NIDDK Support, Veterans Administration Support

TH-PO848

Characteristic Expressions of GABA Receptors and GABA Producing/ Transporting Molecules in Rat Kidney Kozue Takano,[1] Junichi Yatabe,[1,3] Midori Sasaki Yatabe,[1] Hironobu Sanada,[2] Tsuyoshi Watanabe,[3] Junko Kimura.[1] *[1]Pharmacol., Fukushima Med. Univ., Fukushima, Japan; [2]Health Science Res., Fukushima Welfare Federation of Agricultural Cooperatives, Fukushima, Japan; [3]Nephrol., Hypertens., Diabetol., Endo. and Metab., Fukushima Med. Univ., Fukushima, Japan.*

Background: γ-Aminobutyric acid (GABA) production and signaling have been found in peripheral non-neural tissues. Administration of GABA induces natriuresis and lowers blood pressure, which suggests renal GABA targets. However, precise compositions of GABA receptors and other GABA-related molecules in the kidney have not been elucidated.

Methods: Messenger RNAs of 16 GABA(A) receptor subunits, two GABA(B) receptor subtypes, three GABA(C) receptor subunits, two GABA producing enzyme (glutamate decarboxylase; GAD) subtypes, three GABA transporter (GAT) subtypes, and GABA degrading enzyme (4-aminobutyrate aminotransferase; ABAT) were examined by RT-PCR in the kidney cortex of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Quantitative PCR, western blot and immunohistochemistry for GABA(A) α1, β3 and π subunits were also performed.

Results: WKY kidney cortex expressed GABA(A) receptor subunits α1, β3, δ, ε and π, whereas α2, α3, α4, α5, α6, β1, β2, γ1, γ2, γ3 and θ were not detectable. Both types of GABA(B) receptor, R1 and R2, and GABA(C) receptor ρ1 and ρ2 subunit mRNAs were also found, but not ρ3. Kidney cortex showed expressions of GAD65, GAD67, ABAT and peripheral-type GABA transporter, GAT2, but not GAT1 or GAT3. As this is the first report of π subunit in the kidney, western blot and immunohistochemistry were also performed to confirm the protein expression of GABA(A) receptor π subunit in addition to α1 and β3 subunits. Renal cortical levels of α1 and β3 subunits were not different between WKY and SHR. However, GABA(A) receptor π subunit expression was significantly less in SHR, which may be involved in the pathophysiology of hypertension.

Conclusions: A unique set of GABA receptor subunits and subtypes were found in rat kidney cortex. As GABA producing enzymes, transporters and degrading enzyme are also expressed, we suggest a possible existence of local renal GABAergic system with an autocrine/paracrine mechanism.

TH-PO849

Renalase Metabolizes Catecholamines Using a Mechanism Distinct from that of Monoamine Oxidase A and B Gary V. Desir, Heino Velazquez. *Med, Yale Sch Med, VACHS.*

Background: Renalase, a flavoprotein secreted from kidney and heart, uses NADH to metabolize catecholamines via a superoxide (O_2^-) dependent mechanism. Its crystal structure was recently solved, and it confirmed its flavoprotein nature, and its ability to bind NADH. However, the mechanism mediating its enzymatic function, and the identity of the metabolites generated are not well understood. Recombinant renalase decreases plasma epinephrine (EPI), L-DOPA and dopamine (DA), measured 24 hours post injection, by 82, 63 and 31% respectively. However, the urinary excretion of the deaminated (DOPAC), methylated (3-MT) and deaminated plus methylated (HVA) metabolites does

not change, suggesting that renalase's action on catecholamines differs significantly from that of catechol-O-methyl-transferase (COMT) and monoamine oxidase (MAO) A and B.

Methods: Given that O_2^- formation is critical for the action of renalase on catecholamines, we hypothesized that the enzyme generates adrenochrome, dopachrome and noradrenochrome from the oxidation of EPI, DA and norepinephrine (NE) respectively. These metabolites have an absorption maximum at 480 nm, and their rate of formation were studied by spectrophotometry.

Results: *In vitro* studies using steady state kinetics analysis indicate that, in the presence of NADH, renalase catalyzes the formation of adrenochrome from EPI (kcat=33.33 min^{-1}, Km=822.52 μM). The oxidation rates for DA, NE and L-DOPA are ~10 fold slower than that for EPI. Renalase does not require the presence of NADH to metabolize EPI, L-DOPA, DA, and NE. The reaction can proceed as shown in equations 1 and 2, but the overall reaction rate is ~ 6 fold slower than in the presence of NADH (EPI: kcat= 5.68 vs 33.33 min^{-1}).

FAD_{ox} + EPI → EPI_{ox} + FAD_{red} Eq 1

FAD_{red} + O_2 → FAD_{ox} + O_2^- Eq 2

FAD_{ox}, oxidized FAD; FAD_{red}, reduced FAD; O_2, oxygen; O_2^-, superoxide anion; EPI, epinephrine; EPI_{ox}, adrenochrome.

Conclusions: In summary, renalase metabolizes catecholamines (EPI>>L-DOPA>DA=NE) by a mechanism distinct from than of MAO-A and MAO-B, and does not have an absolute requirement of NADH as a cofactor. These findings should help achieve a full understanding of renalase's physiological and pathophysiological roles.

Funding: NIDDK Support, Veterans Administration Support

TH-PO850

Kidney Restricted, siRNA-Mediated Gene Silencing: A Novel Subcapsular Approach Laureano D. Asico, Van Anthony M. Villar, Crisanto Escano, Yu Yang, Santiago Cuevas, Jun B. Feranil, Ines Armando, Pedro A. Jose. *Medicine, University of Maryland-School of Medicine, Baltimore, MD.*

Background: Essential hypertension represents a set of genetic dysfunctions that involve several organ systems, including the kidney. Elucidating the mechanistic underpinnings of hypertension was facilitated by the availability of knockout mouse models that allow the functional analysis of specific genes on the regulation of blood pressure (BP) and ion transport. Recently, innovations on knockout mouse models, e.g., conditional knockouts, allow the reversible silencing of genes in specific tissues; however, this technology is tedious, costly and not always successful.

Methods: As an alternative, we tested the feasibility and efficiency of using gene-specific siRNA infused into the subcapsular space of the kidney of previously uninephrectomized and acclimated mice to evaluate renal mechanisms involved in hypertension.

Results: A 7-day siRNA infusion—which ensured optimum gene silencing—did not result in gross or histological changes in the renal parenchyma or spill over into other organ systems. We tested several siRNAs (vs. *DJ-1*, and *PON2*, whose absence should increase BP) which resulted in about 55% decrease in gene expression and an increase in systolic BP (DSBP=34±4 and 41±5 mm Hg, n=4), but not when non-silencing siRNA was infused. Conversely, infusion of siRNA against a gene (e.g., *USP48*) whose absence should decrease BP effectively decreased the SBP (DSBP=-21±2 mm Hg, n=4). We also compared the effect of unilateral infusion of *DRD2*-specific siRNA in mice with both kidneys intact; *DRD2* encodes for the dopamine D2 receptor (D_2R) whose absence results in hypertension. The BP was unchanged after a 7-day infusion (n=7), but increased after 28 days (DSBP=26±2 mm Hg, n=5), suggesting that the contralateral, non-infused kidney could initially compensate for the D_2R loss but not for long-term. The D_2R expression was unchanged in the non-infused kidney, regardless of the duration.

Conclusions: Our novel subcapsular siRNA infusion offers the advantage of restricted gene silencing in one kidney only and precludes unforeseen compensatory mechanisms that may develop in classical knockout models.

Funding: NIDDK Support

TH-PO851

Morphological Evidence for Afferent Reinnervation after Renal Denervation in Rats Tilmann Ditting,[1] Christian Fiedler,[1] Wolfgang Freisinger,[1] Kirsten Siegel,[1] Sonja Heinlein,[1] Winfried Neuhuber,[2] Stephanie T. Schmidt,[1] Christian Ott,[1] Roland E. Schmieder,[1] Kerstin U. Amann,[3] Roland Veelken.[1] *[1]Med 4, Nephrology & Hypertension, Friedrich-Alexander Universtiy Erlangen, Erlangen, Germany; [2]Dept. of Anatomy, Friedrich-Alexander Universtiy Erlangen, Erlangen, Germany; [3]Friedrich-Alexander Universtiy Erlangen, Erlangen, Germany.*

Background: Renal denervation is a new treatment for refractory arterial hypertension. Howerever, the underlying mechanisms are not completely clear: Efferent sympathetic renal nerves play a key role in salt retention and renin release, but the role of the afferent nerves is not clearly defined, although evidence exists for sympathoinhibitory action. It is accepted that there is some re-innervation of efferent sympathetic nerves after the denervation, but re-innervation of afferent nerves is doubted. We wanted to test the hypothesis that besides renal sympathetic reinnervation a considerable afferent renal reinnervation occurs after renal denervation in rats.

Methods: 50μm kidney slices from 12 male SD rats were stained for thyrosin hydroxylase (TH), calctonine gene related peptide (CGRP) and smooth muscle actin (SMA). Kidneys were examined at 1, 4 and 12 weeks after left sided surgical renal denervation. The right kidney served as control. Image stacks were generated with a confocal laser scanning microscope. Analysis of nerve density was done visually by 4 blinded investigators in 183 image stacks. Staining for TH (i.e. efferent) and CGRP (i.e. afferent) was scored (0-3). Fiji Image J software was used.

Results: At week 1 both efferent (TH+) and afferent (CGRP+) fiber density was reduced (right 2.43±0.10 vs. left 1.47±0.11 [TH+]; right 1.96±0.17 vs. left 0.86±0.12 [CGRP+]; *P<0.001, each*). After 4 weeks an increase in nerve densities were detected, which increased until week 12 (right 2.67±0.07 vs. left 3.35±0.12 [TH+], *P<0.03*; right 2.06±0.16 vs. left 1.82±0.17 [CGRP+], *P=ns*).

Conclusions: Our study shows that there is not only a relevant sympathetic re-innervation but also afferent re-innervation. The afferent re-innervation process even seems to be more complete. Further studies have to be performed to prove the functional relevance of our findings.

TH-PO852

Defective Dopamine D1 Receptor Function and Na^+, K^+-ATPase Phosphorylation in Ovariectomized Adult Wistar Rats during Salt Loading Luis Di Ciano,[1] Pablo J. Azurmendi,[1] Elisabet Monica Oddo,[1] Gloria Levin,[2] Jorge Toledo,[1] Veronica De Luca Sarobe,[1] Elvira Arrizurieta,[1] Fernando Raul Ibarra.[1] *[1]Lab de Riñón Experimental, Instituto de Investigaciones Médicas A Lanari, UBA, Buenos Aires, Argentina; [2]Hospital de Niños R Gutierrez, CEDIE-CONICET.*

Background: We showed that ovariectomized (oVx) Wistar rats consuming high sodium (HS) had both a less phosphorylated Na+, K+-ATPase (NKA) and a reduced natriuresis resulting in Na^+ sensitive hypertension (ASN 2011). Being NKA phospho-state regulated by dopamine (DA) via D1 receptor mediated mechanism, we test whether NKA and renal DA may be involved in the hypertensive response to salt load.

Methods: Rats oVx at 60 days old and intact female (IF) were studied at 150 days of life consuming the last 5 days normal (NS) or HS diet (1% NaCl in drinking water). Afterwords, rats received D1R antagonist (SCH 23390, 1 mg/kg bwt/d sc), DA infusion (1 μg/kg bwt/min) or vehicle. Mean blood pressure (MBP), urinary volume (V), Na^+ excretion (UNa^+*V) and DA (uDA) were measured. Expression of D1R, NKA α1 subunit (t-NKA) and its phospho-state at Ser23 (p-NKA) were determined in renal homogenates.

Results: Under HS, MBP increased in oVx to 135±4 ($p<0.05$) while UNa^+*V was lower in oVx 2.09±0.12 vs IF 2.88±0.21 mmol/d/100g bwt ($p<0.05$). uDA increased from NS to HS in all groups (465±25 to 660±35 ng/100 bwt/day, $p<0.05$).

After D1R antagonist treatment in IF HS UNa^+*V and V diminished by 50% and MBP increased to 140±2 mmHg ($p<0.05$), while its effect was negligible in oVx HS.

D1R blockade blunted the increase in p-NKA induced by HS in IF ($p< 0.01$). Instead, D1R antagonist had no effect in oVx. t-NKA expression was similar in all groups.

DA infusion enhanced UNa^+*V (μEq/30 min/100g bwt) in IF HS from 14.63±5.2 to 42.2±7.6 ($p<0.05$). This response was absent in oVx HS.

Abundance of D1R was not different in IF vs oVx.

Conclusions: These findings suggest that natriuresis following salt loading in IF is accompanied by an *in vivo* increment of NKA phosphorylation mediated by renal DA through D1R. oVx HS, instead, had a decreased p-NKA and a diminished response to D1R blockade or stimulation pointing to a defect in D1R regulation of NKA as a possible mechanism of hypertension.

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TH-PO853

A Protective Role for Interleukin 17A in Hypertension Induced Renal Injury in Mice Sascha Lange,[1] Alexander Lehners,[1] Ulf Panzer,[1] Joachim Velden,[2] Rolf A. Stahl,[1] Christian Krebs,[1] Ulrich O. Wenzel.[1] *[1]Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University Medical Center Erlangen, Erlangen, Germany.*

Background: T cells are required for the full development of Ang II-induced hypertension. However, the specific subsets of T-cells that are important in the endorgan damage are unknown. Th17 cells are a new subset that produces interleukin 17A (IL-17A).

Methods: Hypertension was induced with DOCA + Ang II in wildtype (WT, n=30) and IL-17A knockout mice (KO, n=25). Hypertensive renal and cardiac endorgan damage was evaluated at day 4 and day 14.

Results: An increased infiltration of IL-17-producing T cells was found in the kidney from WT hypertensive mice. 3 days after induction of hypertension with DOCA + Ang II a significantly higher albuminuria was found in IL-17 KO than in WT mice (197±65 vs. 45±15 mg/mg albumin/creatinine, controls 0.09±0.02). The table shows the data of day 14.

	Systolic blood pressure (mmHg)	Albuminuria (mg/mg)	Glomerular injury (score)	Protein casts (number/high power field)	Urea-N (mg/dl)	Plasma cholesterol (mg/dl)
Controls	93±4	0.11±0.0	0.05±0.1	0.15±0.1	27±2	90±7
DOCA+Ang II wildtype	140±3	111±27	0.67±0.01	6.0±1.5	39±2	216±19
DOCA+Ang II IL-17 KO	139±4	116±35	1.04±0.06**	17.2±3.9**	54±8*	311±44*

*=p<0.05, **=p<0.01 vs. wildtype DOCA + Ang II

Although blood pressure and albuminuria did not differ between hypertensive IL-17A KO and WT mice at day 14, the histological analysis revealed significantly more glomerular injury and more proteineous casts in IL-17A KO than in WT mice. In addition, serum urea N and cholesterol levels were significantly higher in IL-17A KO than in WT mice. DOCA + Ang II also induced a massive cardiac damage as assessed by heart weight, cardiac fibrosis as well as expression of fetal and matrix genes but no significant difference was found between IL-17A KO and WT mice at both time points.

Conclusions: IL-17A deficiency has no effect on the DOCA + Ang II induced cardiac endorgan damage. In contrast, IL-17A deficiency aggravates early albuminuria in response to DOCA+Ang II and induces more renal injury at the late time point.

TH-PO854

Renoprotective Effect of Sildenafil in DOCA-Salt Hypertensive Rats

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Background: Sildenafil was the first selective inhibitor of phosphodiesterase-5 (PDE5) to be widely used for treating erectile dysfunction. Many recent studies have investigated the cardioprotective role of sildenafil in animal models. We evaluated the protective effects of sildenafil represent potential mechanisms for the treatment of end-organ damage in hypertension. This study has investigated the effects of sildenafil (50 mg/kg/day) on renal function impairment, glomerulosclerosis and tubulointerstitial fibrosis in deoxycorticosterone acetate (DOCA)-salt hypertensive (DSH) rat.

Methods: Rats were implanted with DOCA strips (200 mg/kg) on 1 week after unilateral nephrectomy. Rats received control diet with or without sildenafil for 2 weeks. The systolic blood pressure (SBP) was measured by tail cuff method and urinary albumin excretion ratio (UAE) was calculated by urine microalbumin and creatinine. The glomerulosclerosis and tubulointerstitial fibrosis was determined by masson's trichrome stain. The expression of ED-1, transforming growth factor-β (TGF-β), Bax, and Bcl-2 was determined in the kidney by semiquantitative immunoblotting and immunohistochemistry. TUNEL stain was performed for detecting apoptic cell.

Results: In DSH rats, SBP was increased, which was not attenuated by sildenafil treatment. Creatinine clearance was decreased while UAE was increased in DSH rats compared with controls, which were attenuated by sildenafil treatment. Glomerulosclerosis and tubulointerstitial fibrosis in DSH rats were attenuated by sildenafil treatment. The expression of ED-1, TGF-β, and Bax was increased and its of Bcl-2 was decreased in the kidney of DSH rats, which was counteracted by sildenafil treatment. The number of apoptic cells was increased in DSH rats, which was attenuated by sildenafil treatment.

Conclusions: Sildenafil is effective in preventing progression of renal injury in DSH rat, the mechanism of which is associated with anti-inflammatory anti-fibrotic and anti-apoptotic effects.

Funding: Private Foundation Support

TH-PO855

RAS Fingerprinting: A Novel Paradigm to Concurrently Evaluate Multiple Angiotensin Peptides and Their Enzymatic Regulation

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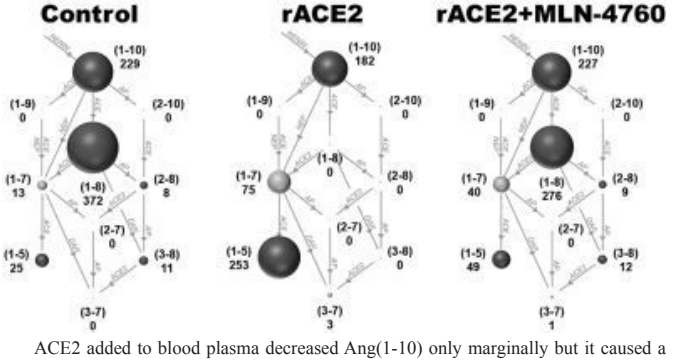
Background: RAS overactivity is implicated in diabetic nephropathy, hypertension and cardiovascular disease but the evaluation of the various components of an increasingly complex system has been limited by available techniques. Here we used a newly developed LC-MS/MS based method to concurrently measure 10 angiotensin peptides to examine their steady state and their regulation by murine recombinant ACE2 in the presence and absence of a specific inhibitor, MLN-4760.

Methods: We produced mrACE2 and its effect on ten endogenous RAS peptides was studied ex vivo in plasma using LC-MS/MS analysis and data processing by Marko Poglitsch, APEIRON-Biologics AG.

Results: In control plasma (no rACE2 added), there was a high abundance of Ang I(1-10) and Ang II(1-8) while other Ang metabolites were present in relatively low concentrations. The diameter of the spheres and the numerical value given next to it reflect concentration of the respective Ang metabolite.



ACE2 added to blood plasma decreased Ang(1-10) only marginally but it caused a complete disappearance of (1-8) and lead to the formation of Ang(1-7). As a result of the increase in Ang(1-7) formation, ACE2 lead also to the formation of Ang(1-5), a process driven by ACE which, like other peptidases, is present in the plasma ex vivo assay. The effects on the above mentioned Ang peptides were ACE2-specific as they all were inhibited by the selective ACE2 inhibitor, MLN-4760 (10-5M).

Conclusions: mrACE2 added to blood plasma degrades Ang(1-8) forming Ang(1-7) and, in the presence of endogenous ACE, leads to increased formation of large amounts of Ang(1-5). RAS fingerprinting is a powerful novel approach to simultaneously evaluate several Ang peptides and their regulation by key enzymes present in plasma.

Funding: NIDDK Support

TH-PO856

Vitamin D Increases Plasma Renin Activity Independently of Plasma Calcium, through Polyuria-Induced Dehydration

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Background: 1,25 OH_2 dihydroxycholecalciferol (Calcitriol) is the active form of Vitamin D and has been reported to inhibit the renin-angiotensin system. However, Calcitriol also increases plasma Ca^{2+}, which can cause polyuria, dehydration, and volume contraction. We hypothesized Calcitriol increases plasma renin activity (PRA) due to hypercalcemia-mediated polyuria and dehydration.

Methods: All experiments were run in male Sprague-Dawley rats over 6 days.

Results: 500 ng/day Calcitriol, i.p., increased PRA from 4.4±0.5 to 17.4±4.6 ngAngI/ml/hr ($p<0.05$). Calcitriol increased plasma Ca^{2+} from 1.26±0.02 to 1.64±0.06 mM ($p<0.001$) and increased urine flow from 9.0±1.1 to 26.3±1.1 ml/day ($p<0.01$). To test the role of plasma Ca^{2+}, we treated rats with 100 ng/day of the vehicle, Calcitriol, or Paricalcitol, a non-calcemic analog of Vitamin D. 100 ng/day Calcitriol increased plasma Ca^{2+} from 1.24±0.01 to 1.52±0.03 mM ($p<0.001$), while Paricalcitol plasma Ca^{2+} (1.23±0.02 mM) did not differ from control. However, Calcitriol and Paricalcitol both increased PRA from 3.1±0.3 to 10.3±1.0 and 8.8±2.0 ngAngI/ml/hr, respectively ($p<0.01$). Also, Calcitriol and Paricalcitol both increased urine flow from 11.3±1.0 to 29.3±3.4 and 24.6±3.2 ml/day, respectively ($p<0.01$). Thus, Vitamin D increased PRA *independently* of the increase in plasma Ca^{2+}. To test if Calcitriol increased PRA *via* polyuria-induced dehydration, we rehydrated Calcitriol-treated rats subcutaneous 0.9% NaCl. Rehydrating Calcitriol-treated rats completely normalized PRA from 9.2±1.8 to 3.1±0.8 ngAngI/ml/hr ($p<0.05$).

Conclusions: Thus, our data demonstrate that Vitamin D increases PRA *independently* of increased plasma Ca^{2+}, *via* polyuria-induced dehydration. Contrary to the recent literature, our data demonstrate that treatment with Vitamin D does not inhibit PRA, but actually *increases* it. Our data suggest that treatment with Vitamin D is not a candidate to directly decrease renin-angiotensin system activity.

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TH-PO857

Evidence for an Interaction between Systemic and Renal Angiotensinogen in the Control of Arterial Pressure

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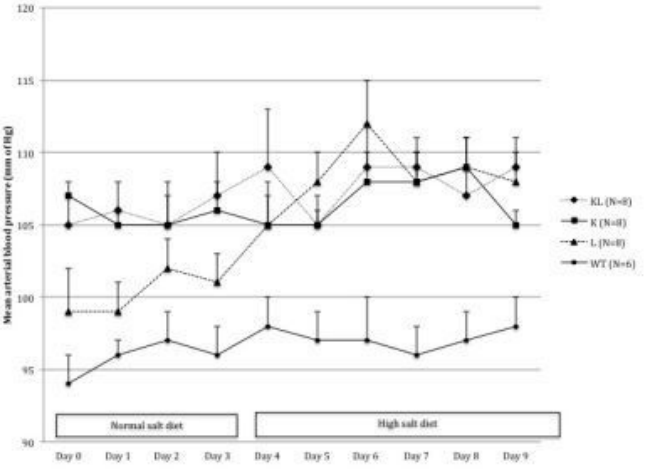
Background: Both the liver and proximal tubule make angiotensinogen (AGT), and overexpression of AGT in either region increases blood pressure (BP). To determine the relative contributions of renal and systemic AGT in modulating BP, mice were used with AGT overexpression in kidney (K), liver (L) or both sites (KL).

Methods: Male transgenic and wildtype (WT) controls aged 24 weeks underwent BP recording via telemetry and metabolic cage studies on normal (0.25%) and high (3.2%) sodium diets.

Results: Mean BP (MAP) was higher in K and KL vs L and WT mice fed normal salt, while MAP in L mice was higher than controls. High salt intake did not alter MAP in WT, K or KL mice but increased it in L mice. Plasma AGT levels, plasma renin concentration, urine Na excretion and urine volume were similar between groups regardless of sodium intake. Urinary AGT (ng/day) on normal sodium intake was: WT: 5±1, L: 4±1, K: 20±5 and KL: 34±9. High salt increased urine AGT in all groups (WT: 19±4, L: 42±8, K: 290±75, KL: 407± 72). High salt did not alter plasma AGT levels but reduced plasma renin concentration in all groups.



Conclusions: Taken together, these data show a correlation between urinary AGT and BP. Mice with renal AGT overexpression may be maximally hypertensive on a normal sodium diet since renal AGT is already high. Mice with liver AGT overexpression have mild hypertension on a normal sodium diet that is exacerbated by high sodium intake; this is associated with a significant increase in urinary AGT as compared to WT animals. Thus, systemic AGT may have the potential to regulate renal AGT and this effect may be a key determinant of BP.

TH-PO858

Renal and Vascular Response to Angiotensin II after Experimental Preeclampsia Anne Marijn van der Graaf,[1,2] Mienke van der Wiel,[1] Tsjitske Toering,[3] Anne-Roos Sophie Frenay,[2] Harry Van Goor,[2] Pieter A. Klok,[2] Robert H. Henning,[4] Hendrik Buikema,[4] Gerjan Navis,[3] Titia Lely,[1] Marijke M. Faas.[2] *[1]Obstetrics & Gynaecology; [2]Pathology and Medical Biology; [3]Nephrology; [4]Clinical Pharmacology UMCG, Groningen, Netherlands.*

Background: Formerly preeclamptic women (PE) have an increased risk for cardiovascular (CVD) and renal disease, possibly due to persistent alterations in the renin-angiotensin system. Previously, we have shown vascular hypersensitivity to angII in rats during experimental PE as compared to healthy pregnant (HP) rats. We investigated whether vascular hypersensitivity to angII persists postpartum in these rats.

Methods: Never-pregnant (NP), formerly HP (fHP), and formerly experimental PE Wistar rats (low dose endotoxin on day 14 of pregnancy; fPE) were treated with angII (200ng/kg/min; i.p.) or sham treated (n=10-14 in all groups), starting 6 weeks after delivery. Systolic blood pressure (SBP) and proteinuria was measured at baseline and weekly for 3 weeks. Aortic rings of sham treated rats were mounted for isotonic measurement of vasotonus. AngII sensitivity was assessed by obtaining response curves in the presence of vehicle, AT2-R blocker PD123319 or AT1-R blocker losartan.

Results: SBP was higher in NP vs fPE at baseline (NP:129(±11); fPE:123(±9); p=0.012; fHP:128(±10)), with no difference in proteinuria (mg/24h) (NP:4.3(±1.2); fHP:4.2(±2.3); fPE:3.3(±1.8); p=0.1). After 3 weeks of angII, a trend was found towards a higher increase in SBP in fPE vs NP (50.2(±21)% vs 41.3(±20)%; p=0.07), and a significantly higher increase in proteinuria in fPE (24.8(±12) vs both NP (21.9 (±20);p=0.04) and fHP (18.7(±14);p=0.05). No differences in in-vitro angII sensitivity were seen between the groups.

Conclusions: These data show that fPE rats have an increased response to angII, as reflected by increased proteinuria and a trend towards increased SBP, compared to fHP and NP rats. Our data show that experimental PE per se induces permanent increased angII sensitivity which may be involved in the higher risk for CVD and renal disease in formerly preeclamptic women. The exact mechanism by which PE induces such permanent changes is subject of further research.

TH-PO859

Genetic Variants in Pre-Eclampsia: A Meta-Analysis Aletta Buurma,[1] Rosanne Jane Turner,[1] Annemariek Driessen,[2] Antien Mooyaart,[1] Jan W. Schoones,[3] Jan A. Bruijn,[1] Kitty Bloemenkamp,[4] Olaf Dekkers,[5] Hans J. Baelde.[1] *[1]Pathology, Leiden University Medical Center; [2]Medical Statistics and Bio Informatics, Leiden University Medical Center; [3]Walaeus Library, Leiden University Medical Center; [4]Obstetrics, Leiden University Medical Center; [5]Epidemiology, Leiden University Medical Center.*

Background: Preeclampsia (PE) frequently affects the kidney, resulting in proteinuria and hypertension. PE has a clear familial component, suggesting that the syndrome may be partly attributable to genetic susceptibility. The search for susceptibility genes has lead to a massive increase in the number of published studies involving genetic associations in PE. However, attempts to replicate these findings have yielded inconsistent results. This meta-analysis aims to assess the pooled effect of each genetic variant that is reproducibly associated with PE.

Methods: Studies assessing the association between genes and PE were searched up to February 2012 in PubMed, EMBASE and Web of Science. We selected all genetic variants that were significantly associated with PE in an initial study and then independently reproduced in at least one additional study. Subsequently, all studies (regardless of p-values) assessing these reproduced variants were included. The association between genetic variants and PE was calculated at the allele level and the main measure of effect was a pooled odds ratio in a random effects model.

Results: The literature search resulted in 2965 citations, of which 542 were genetic association studies investigating PE. We identified 23 replicated genetic variants, of which 8 remained significantly associated with PE in a random-effects meta-analysis. These variants were in or near the following genes: *ACE, AGT, CTLA4, F2, FV, LPL* and *SERPINE1.*

Conclusions: This meta-analysis found eight genetic variants associated with PE. Importantly, many of the variants that were associated with PE are risk factors for the development of hypertension and cardiovascular disease, indicating that PE and cardiovascular disease have shared genetic risk factors. The relative contribution and relevance of the identified genes in the pathogenesis of PE should be the focus of future studies.

TH-PO860

Plasmin Plays Deleterious Roles in Aldosterone-Induced Kidney Injury: A Beneficial Effect of Serine Protease Inhibitor Tomoaki Onoue,[1] Yutaka Kakizoe,[1] Manabu Hayata,[1] Kohei Uchimura,[1] Jun Morinaga,[1] Rika Yamazoe,[1] Teruhiko Mizumoto,[1] Miki Ueda,[1] Sakai Yoshiki,[2] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [2]Research Headquarters, Ono Pharmaceutical Co., Ltd, Osaka, Japan.*

Background: Recently deleterious effects of aldosterone (ALD) have been reported on many organs independent of its hemodynamic effect. However, the mechanisms of these unfavorable effects have been poorly understood. Previously we reported that administration of a synthetic serine protease (SP) inhibitor camostat mesilate (CM) attenuated hypertension and kidney injuries in Dahl salt-sensitive rats fed high salt diet (HSD). Therefore, we hypothesized that SP (s) is/are involved in the pathogenesis of salt-sensitive hypertension and organ damages.

Methods: To prove this hypothesis, we extracted kidney proteins from uninephrectomized rats treated with ALD and HSD, and performed proteomics analysis combined with a fluorescent zymography. We also examined the effect of a SP inhibitor on ALD-induced kidney injury in rats and on SP-induced fibrotic and inflammatory changes in renal fibroblast cells.

Results: LC-MS/MS analysis revealed that plasmin is a candidate SP in the development of kidney injuries mediated by ALD and HSD. Induction of plasmin by ALD was significantly reduced by eplerenone, suggesting that it is mediated by the mineralocorticoid receptor activation. Since CM suppressed plasmin activity in vitro, we administrated CM to rats treated with ALD and HSD. CM significantly ameliorated glomerulosclerosis and tubulointerstitial fibrosis, suggesting the possibility that plasmin could be involved in the pathogenesis of kidney injuries induced by ALD and HSD. In addition, treatment of renal fibroblast cells with plasmin substantially increased mRNA expressions of fibrotic and inflammatory markers and they were significantly reduced by CM.

Conclusions: Our results suggest that plasmin plays deleterious roles in the development of kidney injuries induced by ALD and HSD, and a SP inhibitor CM might be a new class of therapeutic strategy for the treatment of ALD-mediated kidney injuries.

TH-PO861

CXCL116 Regulates Angiotensin II-Induced Inflammation, Proteinuria, and Renal Fibrosis Yunfeng Xia, Jiyuan Chen, William E. Mitch, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Recent studies have shown that inflammation plays a critical role in the pathogenesis and progression of hypertensive kidney disease. However, the signaling mechanisms underlying the induction of inflammation are poorly understood. We have found that CXCL16, a recently discovered chemokine, is induced in the kidney in a murine model of angiotensin II (Ang II)-induced hypertension. Therefore, we examined whether CXCL16 regulates Ang II-induced inflammation and renal injury.

Methods: Wild-type (WT) and CXCL16-KO mice were treated with Ang II via subcutaneous osmotic minipumps at 1500 ng/kg/min for up to 4 weeks.

Results: WT and CXCL16-KO mice had virtually identical blood pressure at baseline. Ang II treatment led to an increase in blood pressure that is similar between WT and CXCL16-KO mice. Immunohistochemical analysis showed that targeted disruption of CXCL16 inhibits infiltration of $F4/80^+$ macrophages and $CD3^+$ T cells in the kidney of Ang II treated mice compared with WT mice. Real time RT-PCR demonstrated that targeted disruption of CXCL16 reduced gene expression of inflammatory cytokines in the kidney of Ang II treated mice. Furthermore, targeted deletion of CXCL16 reduced Ang II-induced proteinuria and podocyte loss. Histological analysis revealed that CXCL16 deficiency inhibited Ang II-induced renal injury and fibrosis. Moreover, CXCL16 deficiency suppresses bone marrow-derived fibroblast accumulation in the kidney of Ang II treated mice.

Conclusions: Our results indicate that CXCL16 plays a pivotal role in the development of Ang II induced renal injury through regulation of macrophage and T cell infiltration and bone marrow-derived fibroblast accumulation.

Funding: Other NIH Support - NHLBI, Private Foundation Support

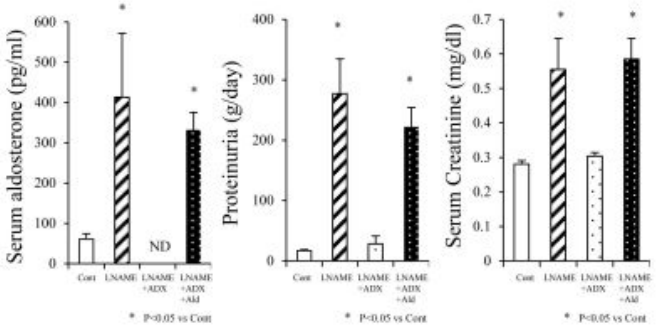
TH-PO862

Aldosterone Plays a Central Role in the Pathogenesis of Renal Injury in Nitric Oxide-Deficient Rat Takaichi Suehiro,[1] Kazuhiko Tsuruya,[1] Hirofumi Ikeda,[2] Toshiaki Nakano,[1] Takanari Kitazono.[1] *[1]Kyushu University; [2]National Kyushu Medical Center Hospital.*

Background: Nitric oxide synthase (NOS) inhibition is a known cause of a renin-angiotensin-aldosterone system (RAAS) activation, hypertension and renal injury. We previously reported both Angiotensin II (AII) receptor blocker and aldosterone (Ald) antagonist almost completely suppressed the renal injury induced by N^{ω}-nitro-L-arginine methyl ester (L-NAME). We hypothesize Ald is a main player of this injury. In this study, we investigated the direct effect of Ald on renal injury under chronic NOS inhibition.

Methods: Male Wistar rats were divided into the following four groups; control rats given vehicle (Cont, n=10), L-NAME rats given 50 mg/kg/day L-NAME (L-NAME, n=11), adrenalectomized rats given L-NAME (L-NAME+ADx, n=10) and adrenalectomized rats with Ald infusion given L-NAME (L-NAME+ADx+Ald, n=12). All rats fed 4% salt diet.

Results: Following treatment for 8 weeks, plasma Ald and AII levels were elevated in L-NAME rats. Ald were not detectable in L-NAME+ADx rats, but elevated in L-NAME+ADx+Ald rats. AII were elevated in L-NAME+ADx rats, but were not different from Cont rats in L-NAME+ADx+Ald rats. Blood pressure were elevated in three L-NAME treated groups, but comparable among those groups. L-NAME rats developed proteinuria, elevated serum creatinine, glomerulosclerosis, interstitial fibrosis and vascular injury. Adrenalectomy almost completely prevented the changes, whereas Ald infusion reversed the effects. The cortical mRNA expression of Sgk1, OPN, MCP-1 and infiltrating macrophages were significantly elevated in L-NAME rats. Adrenalectomy markedly suppressed the changes, but Ald infusion counteracted the effects.



Conclusions: The present study demonstrates that Ald plays a central role in the pathogenesis of renal injury by chronic NOS inhibition, independent of its systemic hemodynamic effects.

TH-PO863

AT_{1a} and/or Mas Receptor-Mediated Long-Term Blood Pressure and Renal Responses to Low Doses of Angiotensin II and Angiotensin (1-7) in Mice

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Background: The blood pressure and renal responses to angiotensin II (Ang II) or Ang (1-7) are most commonly studied in rodents with acute or short-term infusion of high doses of Ang II or Ang (1-7) .

Methods: In the present study, we infused equi-low doses of Ang II or Ang (1-7) (400 ng/kg/day, i.p.) alone or together for 3 months in C56BL/6J (WT) or AT_{1a}-KO mice. Systolic blood pressure (SBP) and renal responses were determined before and weekly after Ang II or Ang (1-7) was infused.

Results: In WT mice, the low dose of Ang II caused moderate and time-dependent increases in SBP (basal: 110 ± 4; week 4: 120 ± 3; week 8: 139 ± 5; and week 12: 134 ± 3 mmHg, $p<0.01$ vs. basal). Infusion of a higher dose of Ang II (800 ng/kg/day, i.p.) further increased SBP to 150 ± 7 mmHg 3 months later ($p<0.01$). These responses were blocked by losartan and were absent in AT_{1a}-KO mice. 24 h urinary sodium excretion were lower in WT mice infused with Ang II than in AT_{1a}-KO or losartan-treated, Ang II-infused WT mice ($p<0.05$). The heart wt to body wt ratio was higher in Ang II-infused WT mice than in Ang II-infused AT_{1a}-KO mice ($p<0.01$). Infusion of an equi-dose of Ang (1-7) alone (800 ng/kg/day, i.p.) had no effect on SBP (week 12: 119 ± 3 mmHg, vs. basal, n.s.) or on Ang II-induce SBP responses in WT mice (week 12: 146 ± 8 mmHg, n.s.). In the kidney, Ang II increased the levels of phosphorylated MAP kinase ERK1/2 ($p<0.01$) and the sodium and hydrogen exchanger-3 (NHE-3, $p<0.01$) in the renal cortex of WT mice. However, Ang (1-7) had no effect on 24 h urinary sodium responses, nor on MAP kinases ERK1/2 and NHE-3 levels in WT mice. Nevertheless, Ang (1-7) decreased blood pressure (basal: 97 ± 3 vs. week 12: 90 ± 2 mmHg, $p<0.01$) and increased 24 h urine excretion in AT_{1a}-KO mice ($p<0.05$).

Conclusions: These results suggest that infusion of Ang II, even at an initially non-pressor dose, for 3 months can increase blood pressure and cause cardiovascular and renal injury. These effects are mediated by AT_{1a} receptors and may not significantly be altered by the Ang (1-7)/Mas receptor axis.

Funding: NIDDK Support

TH-PO864

Renoprotective Effects of DSP-9599, a Novel Human Renin Inhibitor, in Double-Transgenic Rats

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Background: Inhibition of the renin-angiotensin-aldosterone system (RAAS) pathway is important in hypertensive patients with CKD. The rate-limiting enzyme in the RAAS is renin. We have assessed renoprotective effects of DSP-9599 in double-transgenic rats (dTGR), over-expressing both human renin and angiotensinogen genes.

Methods: The inhibitory effects of compounds on enzymes were examined using plasma and recombinant enzymes. The antihypertensive effect after single oral administration was determined by telemetry in enalapril-pretreated dTGR. The renoprotective effects were evaluated in dTGR [urinary albumin creatinine ratio (UACR), 2-6 mg/mg] by 3-week treatment with DSP-9599 (3 and 10 mg/kg, p.o., DSP3 and 10) or aliskiren fumarate (30 mg/kg, p.o., ALI30). Kidney damage was assessed every 1 week by measuring UACR and at the end of experiment by histochemical analysis.

Results: DSR-35894 free base, an active metabolite of DSP-9599, selectively inhibited the human renin activity, and its efficacy was almost equal to ALI (IC_{50} 0.75±0.17 nM vs 0.68±0.20 nM). A single oral administration of DSP-9599 (1-10 mg/kg) and ALI (10-100 mg/kg) decreased mean blood pressure in a dose-dependent manner, and the antihypertensive effect of DSP-9599 at a dose of 1.8 mg/kg was estimated equivalent to that of ALI at a dose of 30 mg/kg. Three-week treatment with DSP-9599 or ALI improved the survival rate (DSP3 83%, DSP10 100%, ALI30 67%, vehicle 0%). DSP10 effectively lowered systolic blood pressure (225±20 mmHg) compared with ALI30 (257±3 mmHg). In addition, DSP10 showed significantly lower albuminuria (UACR 0.5±0.5mg/mg), compared with ALI30 (6.1±5.2 mg/mg). Histological severity score (including incidence and mean severity of hyaline cast, tubular basophilia and tubular dilatation) showed that DSP10 lowered kidney damage compared with ALI30 (0-4 scale, 0.4±0.5, 1.2±0.4 and 0.2±0.4 for DSP10, 1.8±1.0, 2.3±0.5 and 2.3±0.5 for ALI30, respectively).

Conclusions: DSP-9599 showed stronger antihypertensive and renoprotective effects than ALI in dTGR that develop severe hypertension with end-organ damage.

TH-PO865

Renal Human L-Type Fatty Acid Binding Protein Attenuates Angiotensin II-Induced Renal Injury without Affecting Blood Pressure

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Background: Angiotensin II (Ang II) causes renal disorder due to hypertension and oxidative stress. L-type fatty acid-binding protein (L-FABP) is expressed in human proximal tubules and has an endogenous anti-oxidative function.

Methods: In order to investigate the dynamics of human L-FABP (hL-FABP) and its role in an Ang II-induced renal injury model, systemic Ang II infusion into mice (5μg/kg/min; subcutaneous osmotic minipumps) was performed for 14 or 28 days using hL-FABP chromosomal transgenic (Tg) mice and wild-type (WT) mice (Tg-Ang II, WT-Ang II). Control mice in each group of Tg mice and WT mice were injected with saline only (Tg-control, WT-control).

Results: Although mouse L-FABP was not expressed in WT mice, hL-FABP was expressed in the proximal tubules of Tg mice. After a high-dose injection of Ang II, renal gene and protein expression of hL-FABP in Tg-Ang II increased significantly compared to Tg-control. Urinary excretion of L-FABP was significantly greater in Tg-Ang II than in Tg-control mice. Blood pressure levels and urinary albumin levels in both groups increased to a similar extent for 28 days. The degree of glomerular sclerosis in Tg-Ang II was similar to that in WT-Ang II. Up-regulation of gene or and protein expression of monocyte chemoattractant protein-1, macrophage infiltration in the interstitium, tubulointerstitial damage and depositions of type I and III collagens were observed in both Tg-Ang II and WT-Ang II mice. However, these effects were less pronounced in Tg-Ang II compared to WT-Ang II mice. The level of renal N-(hexanoyl)lysine, which is known to be a oxidative stress marker, was significantly higher in WT-Ang II than in Tg-Ang II mice.

Conclusions: In conclusion, renal hL-FABP reduced oxidative stress in Ang II-induced renal injury and attenuated tubulointerstitial damage. The renal protective function of hL-FABP was not associated with blood pressure-dependent effects.

TH-PO866

Lack of Vasohibin-1 Exacerbates Renal Alterations Induced by Infusion of Angiotensin-II

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Background: Hypertensive nephrosclerosis is one of the major pathogenic disorders predisposing ESRD. Angiotensin-II (A-II) infusion induces hypertension and glomerular as well as focal renal tubulointerstitial injuries in experimental animal models. We recently reported the protective role of Vasohibin-1(VASH-1), a negative feedback regulator of angiogenesis, in diabetic nephropathy, but its role on hypertensive nephrosclerosis remains to be elucidated. In the present study, we aimed to evaluate the role of endogenous VASH-1 in regulating renal alterations in an A-II-infusion model.

Methods: Male VASH1+/- or wild-type (VASH1+/+) littermates (C57/BL6J background) received continuous infusion of saline or A-II (1000 ng/kg/min) via osmotic minipumps. Mice were sacrificed on Day 28 and the kidneys were obtained. Morphometric analysis, immunohistochemistry and real-time PCR were performed.

Results: Hypertension was observed in the A-II-infused animals, and blood pressure was not significantly different between A-II-infused wild-type and VASH1+/- mice. A-II-induced increase of proteinuria, glomerular volume and mesangial matrix index (assessed by the computer-image analysis) were significantly exacerbated in the VASH1+/- mice compared with the VASH1+/+ mice. Increase in the glomerular accumulation of type IV collagen, interstitial accumulation of $F4/80^+$ monocytes/macrophages, renal mRNA levels for TGF-beta1, CTGF, PAI-1, type IV collagen and CCL2 in the A-II-infused wild-type mice were significantly aggravated in the A-II-infused VASH1+/- mice.

Conclusions: Taken together, these results suggest the protective role of endogenous VASH1 on A-II-induced glomerular as well as tubulointerstitial alterations via regulating inflammation and fibrosis, and thus suggesting its beneficial effects on hypertensive nephrosclerosis.

TH-PO867

Cardiovascular and Renal Implications of the Ang II/AT1Rs-Jak2-Rho Kinase Pathway Activation Salt Sensitive but Not in Salt Independent Hypertension Ramiro Juncos,[1,2] Kiranmai Chadipiralla,[1] Ming-sheng Zhou,[2] Leopoldo Raij.[1,2] *[1]Renal-Hypertension, VAMC, Miami, FL; [2]Renal-Hypertension, University of Miami, Miami, FL.*

Background: Aortic (Ao) stiffness is a determinant of increased systolic (SBP) and pulse-pressure (PP). Clinically, increases in central SBP and PP (hemodynamic changes linked with Ao stiffness), are associated with CKD progression. In hypertension increased pressure-workload has been considered cause and effect of Ao stiffening but the mechanisms are poorly understood. Rho Kinase decreases eNOS and NO production in the endothelium. In VSMC Ang II via AT1Rs specifically activates Rho Kinase via phosphorylation of Jak2/Arhgef1 which phosphorylates (inhibits) myosin light chain phosphatase (MYPT1) thereby promoting vasoconstriction/remodeling.

Methods: Groups (n= 6) of DS rats fed either 0.5% or 4% NaCl diets for 10 weeks were matched with SHR of similar age (6 and 16weeks old) and SBP (147±8 & 211±7).

Results: Hypertensive DS rats, but not SHR showed increased Ao weight/length (17%, P<0.05) and LV/ body weight (16%, P<0.05). Compared to normotensive DS, in hypertensive DS Ao AT1Rs rose 230% accompanied by increased pJak2/Jak2, 3.5 fold and pMYPT1/MYPT1 3.95 fold, (W.blot) p<0.05. Switch of hypertensive DS to 0.5 % NaCl diet for 4 weeks reduced neither SBP nor the activated Ang II/Rho Kinase pathways. ARB prevented Angll/AT1-Jak2-Rho Kinase activation in hypertensive DS fed 4% NaCl diet and normalized SBP, LVH and Ao weight/length in DS switched to a 0,5% NaCl diet. Hypertensive SHR neither showed Ao upregulation of AT1Rs nor activation of the Angll/Jak2-Rho Kinase pathway.We previously reported that hypertensive DS but not SHR develop progressive kidney disease.

Conclusions: We show for the first time that in salt sensitive but not in salt independent hypertension there is specific Ao activation of the Ang II/Jak2-Rho Kinase pathway. Clinically the variable CVD and renal disease observed in individuals with similar severity of hypertension may be linked, at least in part, to genetically conditioned differences in Angll/AT1Rs, Jak-2 activation in response to high dietary salt.

Funding: Private Foundation Support

TH-PO868

An Excess of Circulating Prorenin Results in Hypertension, Renal and Cardiac Fibrosis in cyp1a1-Prorenin Transgenic Rats Guangyu Zhou, Alfred K. Cheung, Yufeng Huang. *Internal Medicine, University of Utah, Salt Lake City, UT.*

Background: To examine if prorenin is an effector of organ injury, independent of its ability to generate renin, a transgenic, inducible, hepatic prorenin-overexpressing rat model was generated.

Methods: In this model, the rat prorenin was incorporated under the cytochrome p450 (cyp1a1) promoter. Four groups of 5 rats (transgenic and wild-type male and female rats) were assigned to receive a diet containing 0.3% of the gene activator, indole-3-carbinonl (I3C) for 4 wks. Four corresponding groups of 5 rats receiving normal diets served as controls.

Results: Plasma prorenin concentration rose from 23±6 µg/ml to 208±44µg/ml and mean arterial pressure increased from 77±5 to 138±17 mm Hg, whereas renal prorenin/renin protein expression was suppressed, in transgenic rats treated with I3C diet. These alterations in plasma prorenin and arterial pressure were not observed in transgenic rats receiving the normal diet or the wild-type rats. Western blot analysis further suggested that this circulating prorenin was in the precursor former with its prosegment attached. Plasma aldosterone concentrations increased by 10-fold for a period of 4wks in transgenic rats. Importantly, after 4 wks of treatment with I3C, transgenic rats developed significant albuminuria, glomerular mesangial matrix expansion and tubulointerstitial fibrosis in the kidney associated with significantly increased expression of TGFß1, PAI-1, type 1 and type IV collagen and fibronectin mRNA and protein production when they were chronically exposed to the high levels of prorenin. After 4 wks of I3C, the rats also exhibited cardiac hypertrophy, with the ratio of heart weight to body weight being 6.3±1.2 vs 3.3±0.5 mg/g (p<0.05). Cardiac interstitial fibrosis and perivascular fibrosis were prominent, accompanied by a significant increase in mRNA contents of ANP, BNP, TGFß1, PAI-1, collagen I and collagen III in the heart tissue. These genes are known to be the markers of cardiac hypertrophy and fibrosis.

Conclusions: These results indicate that high circulating prorenin levels are pathogenic and cause arterial hypertension, renal and cardiac fibrosis in both male and female rats.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO869

Relationship between Marrow Adipocyte and Aortic Calcification in Chronic Dialysis Patients Aiji Yajima,[1,4] Yasuo Imanishi,[2] Masaaki Inaba,[2] Kosaku Nitta.[3] *[1]Medicine, Indiana University, Indianapolis, IN; [2]Medicine, Osaka City University, School of Medicine, Osaka, Japan; [3]Medicine, Tokyo Women's Medical University, Shinjuku-ku, Japan; [4]Renal Replacement Therapeutic Science, Akita University, Akita, Japan.*

Background: Mesenchymal stem cells give rise to adipogenic or osteogenic differentiation and vascular smooth muscle cellls are transformed to osteoblast-like cell in uremic patients. Relationship of bone marrow adipocyte with aortic calcification was investigated.

Methods: 1, Total marrow adipocyte volume (Ad.V), adipocyte number (N.Ad) and marrow volume (Ma.V) were measured in cancellous bone in 28 hemodialysis (HD) patients (Age; 59.7 ± 6.0 (46-70) yrs, HD duration; 13.6 ± 8.0 (2-28) yrs, intact PTH; 755.1 ± 525.5 (5-1750) pg/mL). And adipocyte volume (Ad.V/Ma.V;%) and adipocyte number (N.Ad/Ma.V; N/mm^2) were calculated. Ten slices of the abdominal aorta above the bifurcation were obtained on CT scan at 1 cm intervals and a total of 120 regions of the abdominal wall were obtained to calculate aortic calcification index (ACI). The relationship between adipocyte parameters and aortic calcification was investigated by linear regression analysis.

2, ACI was compared between Group I (the patients with severe suppression of marrow adipogenesis (Ad.V/Ma.V<15.0 % (n=6), N.Ad/Ma.V<110.0 N/mm^2 (n=7))) and Group II (those without severe suppression of marrow adipogenesis).

Results: 1, As to Ad.V/Ma.V (33.6 ± 21.0 (6.2-81.1) %) and N.Ad/Ma.V (183.2 ± 76.8 (63.1-384.8) N/mm^2), they were nearly associated with ACI (42.2 ± 21.8 %) in 28 patients ((r=-0.311, p=0.107) and (r=-0.315, p=0.102), respectively).

2, As to Ad.V/Ma.V and N.Ad/Ma.V, ACI was greater in Group I than Group II (66.8 ± 12.3 vs 35.6 ± 18.9, p=0.001 and 68.0 ± 10.8 vs 33.7 ± 17.3, p<0.001, respectively).

Conclusions: Mesenchymal stem cells are present in many adult tissues. Decreased marrow fat mass is associated with high bone mass phenotype and osteoblast-like cells increase bone-like lesion on the vascular walls. As a conclusion, it is possible that there is a reciprocal relationship between adipogenesis in bone marrow and osteogenesis presented by aortic calcification in HD patients.

Funding: Pharmaceutical Company Support - Bayer, Chugai, Kirin

TH-PO870

Better Correlation of Pulse Wave Velocity (PWV) than Coronary Artery Calcium Score (CACS) with Parameters of Mineral Bone Disturbance in Chronic Kidney Disease (MBD-CKD) Shin-young Ahn,[1] Hayne C. Park,[2] Kook-Hwan Oh,[2] Ho Jun Chin,[1] Curie Ahn,[2] Dong Wan Chae.[1] *[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Republic of Korea; [2]Department of Internal Medicine, Seoul National University Hospital, Republic of Korea.*

Background: CACS, a hallmark of vascular calcification (VC) in many studies about MBD-CKD, mainly reflects intimal calcification, while VC in MBD-CKD occurs largely in media. Hence we hypothesized that PWV, reflecting vascular stiffness from medial VC, might correlate with the parameters of MBD-CKD better than CACS.

Methods: KNOW-CKD (KoreaN cohort study for Outcome in patients With Chronic Kidney Disease) is an on-going prospective, hospital-based, observational cohort study being conducted in 10 major university hospitals under the sponsorship of Korean Center for Disease Control and Prevention. We performed cross-sectional analysis of a relationship between PWV and CACS and parameters of MBD-CKD in this cohort. Upper most tertile value of PWV was regarded as a significant arterial stiffness. Severe CAC was defined as calcium score ≥ 100 Agatston units.

Results: 753 adult patients were enrolled from Aug 2011 to Nov 2011, 58.4% were male and median age was 53 years (20 – 75 years). The prevalence of hypertension, diabetes, and coronary heart disease (CHD) was 89.9%, 26.3%, and 5.6%. In univariate analysis, both the CACS and PWV were associated with traditional risk factors of atheroscrelosis and also the parameters of MBD-CKD. But, after binary logistic regression analysis, only traditional risk factors such as age, male, diabetes, and CHD were significant risk factors for severe CAC (p<0.001, p<0.001, p=0.014, and p<0.001 respectively). In case of arterial stiffness, parameters of MBD-CKD such as P, CaxP product, eGFR, random urine protein to creatinine ratio, and T-score of DEXA were independent risk factors for significant arterial stiffness (p=0.014, p=0.021, p=0.041, p=0.024, and p=0.005 respectively).

Conclusions: We compared the adequacy of CAC and PWV in analyzing VC in MBD-CKD in this cohort. Because PWV correlated with parameters of MBD-CKD better than CACS, PWV rather than CACS is suggested as a marker of VC in MBD-CKD.

Funding: Government Support - Non-U.S.

TH-PO871

Smooth Muscle Phenotype in Medial Arterial Calcification W. Charles O'Neill,[1] Amy Adams.[2] *[1]Renal Division, Emory University; [2]Department of Pathology, Emory University.*

Background: Development of medial arterial calcification in patients with chronic kidney disease (CKD) has been ascribed to phenotypic changes in vascular smooth muscle but histologic analysis in humans has been limited to advanced lesions in vessels also prone to atherosclerosis and intimal calcification. We have previously shown that breast arterial calcification is exclusively medial and is increased in CKD. Thus, breast tissue provides an opportunity to examine early stages of medial calcification in arteries devoid of atherosclerosis.

Methods: Tissue was obtained from prior breast excisions in 9 women with CKD (serum creatinine 1.2 to 3.9 mg/dl) and 10 with end-stage kidney disease (duration 0 to 7 yr). Sequential sections were prepared and stained for calcification (von Kossa stain) and apoptosis (TUNEL stain), as well as immunostaining for the bone-specific protein osteocalcin, the osteoblastic transcription factor Runx2, and the smooth muscle marker SM22α.

Results: Arterial calcification was present in 18 specimens and was exclusively medial, unrelated to intimal thickening, and occurred in vessels as small as arterioles. No atherosclerosis was observed. The mildest calcification appeared as small punctate lesions scattered throughout the media (all 18 specimens), with linear calcification of the internal elastic lamina in 9 specimens. Large confluences of calcification were seen in 4 specimens. Smooth muscle cells appeared normal, even in heavy calcifications, with no apoptosis. Staining for osteocalcin was absent in regions of early calcification. Osteocalcin was detected

in more advanced lesions (9 specimens) but was not associated with cells and appeared bound to calcifications. There was no staining for Runx2, even in heavily calcified regions. All medial cells stained for SM22α, indicative of a normal smooth muscle phenotype.

Conclusions: Medial arterial calcification in humans can occur in the absence of atherosclerosis in vessels of all sizes, without osteogenic trans-differentiation or apoptosis of smooth muscle cells. This indicates that medial calcification is distinct from intimal disease and that neither osteogenic trans-differentiation nor apoptosis of smooth muscle is a necessary initiating event.

TH-PO872

Determinants of Arterial Stiffness, Valvular and Vascular Calcifications in Prevalent Hemodialysis Patients Anabela Malho, Ana Pinho, André Fragoso, Elsa Morgado, Ana Paula Silva, Pedro Neves. *Centro de Hemodiálise de Faro, NephroCare, Portugal.*

Background: Large-artery stiffness is the main determinant of pulse pressure (PP) and it has independent predictive value for total and cardiovascular mortality. Vascular and valvular calcifications are highly prevalent complications associated with exceedingly high cardiovascular mortality in ESRD patients. The aim of this study was to evaluate the determinants of arterial stiffness, vascular and valvular calcifications, using a 11 point score, in a population of hemodialysis patients.

Methods: We included all stable patients attending our clinic and evaluated the relationship of arterial stiffness, valvular and vascular calcifications with inflammation, mineral metabolism, magnesium and bicarbonate levels in a cohort of hemodialysis patients. Arterial stiffness was accessed by calculating the PP, valvular calcifications were determined by echocardiography and vascular calcifications were diagnosed using plain X-ray of hands and hips. An 11 point score was applied (1 point if PP >75 mmHg, 8 point Simple Vascular Calcification Score, and 1 or 2 points for the affected valves). Statistical analysis was based on descriptive statistics and multiple linear regression.

Results: A total of 162 patients (70 females, 92 males, 19.5 % diabetic), with mean age of 64.1 years and mean time on dialysis of 61.5 months were included. In this population 30.8%, 86.8% and 30.6%of the patients presented PP above 75 mmHg, vascular and valvular calcifications, respectively. The mean values of the evaluated parameters were: CaxP=37.4 mg^2/dL^2, PTH=442.8 pg/mL, magnesium=2.2 mg/dL, bicarbonate=23.8 mmol/L, ferritin=559.3 ng/mL, mean score=3.5. Using multiple linear regression we found a correlation between higher score and age (r=0.355, p=0.001), time on hemodialysis (r=0.424, p=0.001), male gender (r=-0.152, p=0.033), ferritin (r=0.141, p=0.046) and diabetes (r=0.336, p=0.001).

Conclusions: In our population, the arterial stiffness, vascular and valvular calcifications score was directly associated with age, dialysis vintage, male gender, inflammation and diabetes. More studies are needed to validate such score as a predictor of cardiovascular mortality.

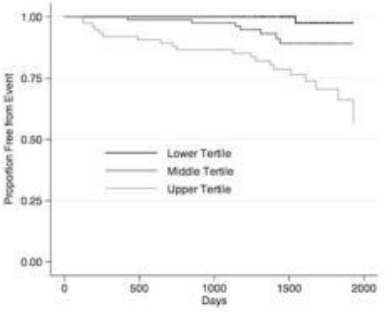
TH-PO873

Coronary Artery Calcification Predicts Death and Cardiovascular Events Independent of Traditional Risk Factors in Patients with Stages 3-5 CKD David C. Wheeler,[1] Ben Caplin,[1] Michael B. Rubens,[2] Joanne Marks,[1] John Cunningham.[1] *[1]UCL Medical School; [2]Brompton and Harefield NHS Foundation Trust, United Kingdom.*

Background: The clinical significance of arterial calcification in CKD patients is uncertain.The aims of the London Arterial Calcification, Kidney and Bone Outcomes (LACKABO) study were to investigate the risk factors for coronary calcification (CoCa) in CKD patients and to evaluate if screening for calcification is useful in predicting clinical outcomes.

Methods: A cohort of 289 patients with CKD stages 2-5 (median MDRD eGFR 39.7 ml/min/1.73m^2, IQR 26.8-51.1) consented to baseline electron beam tomography (EBT) scans (Agatston protocol). Subjects attended follow-up scans (n=154) after a mean of 49 months and were followed for cardiovascular (CV) events and death over a similar period. Determinants of baseline calcification scores, progression of calcification and outcomes were examined using logistic regression and cox proportional hazards models.

Results: Baseline CoCa was detectable in 68% patients, with scores predicted by history of CV events and recognised CV risk factors i.e. age, male gender, smoking and a surrogate for blood pressure (number of antihypertensive medications) but not by eGFR. Progression of CoCa was associated with age, the surrogate for blood pressure and baseline levels of fibroblast growth factor 23. Baseline CoCa scores predicted the combined outcome of death and CV events in both univariable (figure) and multivariable models (Hazard Ratio: 19.8, 95%CI 1.9-210.7 for highest versus lowest tertile of CoCa).



Kaplan-Meier Plot of Proportion of Subjects Remaining Free from the Combined Endpoint of Death or Cardiovascular Event Stratified by Tertile of Baseline Coronary Calcification

Conclusions: CoCa reflects underlying vascular disease in stages 3-5 CKD. Progression of calcification may be driven in part by metabolic disturbances associated with the mineral bone disorder. Assessment of calcification allows more accurate prediction of clinical outcomes compared to knowledge of traditional CV risk factors alone.

Funding: Private Foundation Support

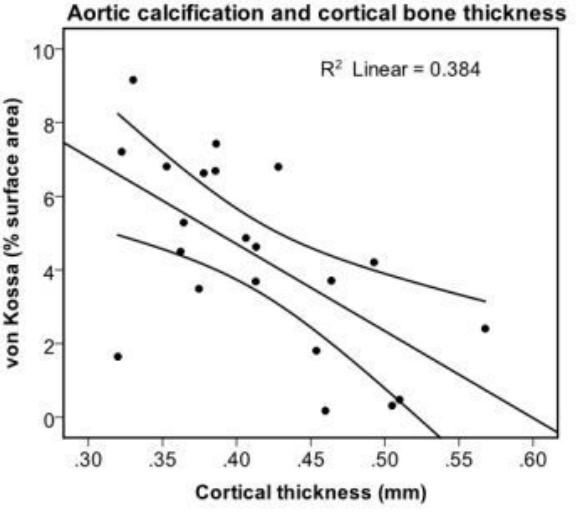
TH-PO874

Vascular Calcification Development and Bone Anomalies in Uremic Rats Fabrice Mac-Way, Alexandra Gauthier-bastien, Caroline Basoni, Roth-visal Ung, Richard Lariviere, Mohsen Agharazii. *CRCHUQ, L'Hotel-Dieu de Quebec, Laval University, Quebec, QC, Canada.*

Background: Anomalies of bone metabolism have been linked to vascular disorders in chronic kidney disease (CKD). We aimed to evaluate the anomalies of bone microarchitecture and mineral density in the development of vascular calcification in uremic rats under Doxycyclin (anti-Matrix Metalloproteinase) and Tempol (antioxidant).

Methods: Forty eight male Wistar rats were included in this sudy. CKD was induced by 5/6 nephrectomy. Vascular calcification was induced by high Ca/P and 1.25 (OH)vitamin D diet (Ca/P/Vit D diet). Four groups were studied: (1) CKD + standard diet (control); 2) CKD + Ca/P/Vit D diet; 3) CKD + Ca/P/Vit D diet + Doxycycline; 4) CKD + Ca/P/Vit D diet + Tempol. After 4-6 weeks of diet, the animals were sacrificed for blood biochemistry, bone specimens and thoracic aorta removal. Von Kossa quantification was used for aorta calcification and left tibiae of each rats were imaged with high resolution micro-CT for architectural analysis.

Results: Von Kossa analysis showed a significantly higher thoracic aorta calcification in the high Ca/P/Vit D diet compared to standard diet (0,30 vs 5,79 %, p<0.05). Neither Doxyxyclin or Tempol diet have affected the degree of calcification. Analysis of cortical BMD, trabecular BMD, inner and outer cortical perimeter of the left tibia with micro-CT did not show any differences between groups. However, the cortical thickness was significantly lower in the CKD + Ca/P/Vit D vs control. Globally, the thoracic calcification was inversely correlated to the cortical bone thickness.



Conclusions: High aortic calcification is related to decreased cortical bone thickness of tibiae in uremic rats, which is probably explained by an increased bone resorption at the expense of Ca/P deposition in vessels.

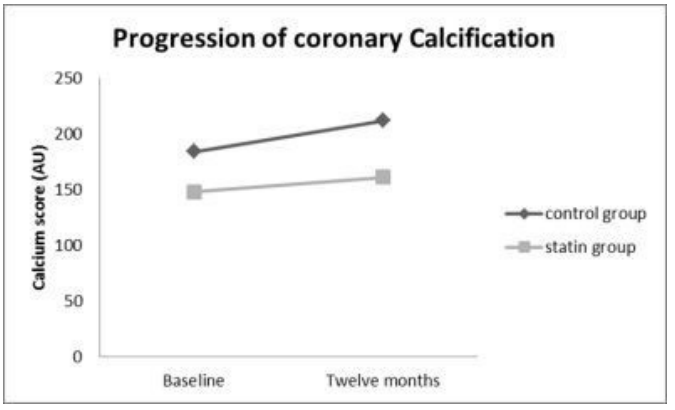
TH-PO875

Is There an Impact of Statin on Coronary Calcification Progression in Kidney Transplantation Recipients? Daniel Constantino Yazbek, Aluizio B. Carvalho, Cinara Barros de Sá, J. Medina-Pestana, Maria Eugenia F. Canziani. *Nephrology, Federal University of São Paulo, São Paulo, SP, Brazil.*

Background: Coronary artery calcification (CAC) is highly prevalent among chronic kidney disease (CKD) patients even after kidney transplantation. CAC strong association with mortality has been recognized in all stages of CKD. The aim of the present study was to test the effect of statin on the progression of CAC in kidney transplant recipients (KTRs).

Methods: A randomized, controlled and open-labeled pilot study was conducted including 100 KTRs (56% men, 41.1±10.0 years). Fifty one patients were randomly assigned to statin (10 mg/day), and 49 to the control group. CAC (multislice computed tomography) and biochemical analyses were performed at baseline and after 12 months.

Results: Statin treatment was associated with increase of eGFR (drug effect p=0.60; time-effect p<0.001; interaction p=0.008) and decrease in LDL-cholesterol (drug effect p=0.83; time-effect p<0.001; interaction p<0.001). At baseline, CAC was observed in 20% and 13% of patients in the statin and control groups, respectively (p=0.18). Calcium score at baseline as well as its absolute and relative changes during 12 months were similar among the groups. The analysis showed that the time but not the statin treatment was associated with the progression of CAC (drug effect p=0.56; time-effect p=0.002; interaction p=0.43).



Conclusions: Despite improvement of kidney function and LDL-cholesterol, the treatment with statin was not able to delay the progression of CAC in KTRs.

TH-PO876

High Glucose May Not Directly Affect High Phosphate-Induced Calcification of Vascular Smooth Muscle Cells Tadashi Yoshida, Maho Yamashita, Matsuhiko Hayashi. *Apheresis and Dialysis Center, School of Medicine, Keio University, Tokyo, Japan.*

Background: Vascular calcification is often seen in patients with chronic kidney disease and diabetes and is associated with increased mortality, myocardial infarction, stroke, and limb amputation. Results of the previous studies by our laboratory and others showed that high phosphate-induced phenotypic switching of vascular smooth muscle cells (SMCs) into osteogenic cells plays an important role in the calcification process. In the present studies, we examined if glucose concentration affected high phosphate-induced calcification of vascular SMCs.

Methods: (1) Male Sprague-Dawley rats were divided into 4 groups: adenine-fed uremic group; streptozotocin-injected diabetic group; adenine-fed and streptozotocin-injected uremic/diabetic group; and controls. Vascular calcification in the aorta of these rats was analyzed. (2) Cultured rat aortic SMCs were incubated in the medium with various concentrations of phosphate (0.9, 1.8, 2.7, and 4.5 mmol/L) and glucose (5, 25, and 50 mmo/L) for 2, 4, 6, and 8 days, and calcium deposition was measured.

Results: (1) By using von Kossa staining, aortic calcification was obvious in adenine-induced uremic rats and adenine-fed/streptozotocin-injected uremic/diabetic rats, whereas it was not observed in diabetic rats and controls. In addition, calcium deposition in uremic/diabetic rats was more abundant than in uremic rats, as determined by the o-cresolphthalein complexone method. (2) High phosphate concentration significantly increased calcium content in cultured SMCs in a dose-dependent manner. However, glucose concentration did not affect the amount of calcium deposition in SMCs incubated with various concentrations of phosphate.

Conclusions: Results suggest that the diabetic status enhances vascular calcification in uremia, but this effect may not be directly mediated by high glucose concentration.

TH-PO877

Study on the Relationship among Circulating Soluble Receptor of Advanced Glycation End Product (sRAGE), S100A12 (EN-RAGE) Levels and Vascular Calcification in Patients with Hemodialysis Ji Yong Jung,[1] Jiyoon Sung,[1] Han Ro,[1] Jae Hyun Chang,[1] Hyun Hee Lee,[1] Chungsik Lee,[2] Wookyung Chung.[1] *[1]Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea; [2]Department of Internal Medicine, Cheju Halla General Hospital, Jeju, Republic of Korea.*

Background: The soluble receptor for advanced glycation end products (sRAGE) exerts a protective effect on the development of atherosclerotic vascular complications by inhibiting RAGE-mediated inflammatory response. In contrast, extracellular newly identified S100A12 (EN-RAGE) contributes to increased vascular damage as a pro-inflammatory ligand for RAGE. We determined the levels of sRAGE and EN-RAGE in hemodialysis (HD) patients and evaluated their relationship with vascular calcification.

Methods: We performed a cross-sectional study with 199 hemodialysis patients. Plain X-ray images of lateral lumbar spine from all subjects were studied for calculation of semiquantitative vascular calcification scores (VCS) as described by Kauppila. For quantification of serum concentration of sRAGE and EN-RAGE, commercially available ELISA kit were used according to the instructions of the manufacturer.

Results: Patients were 57.1 ± 13.7 years of age, 54.3% males, 49.2% diabetics and 36.2% with a history of CVD. We also found a high prevalence of vascular calcification (180 patients, 54.3%) in this population. Kauppila scores revealed 40 patients (20.1%) with higher VCS (>7). In univariate analysis, serum sRAGE was negatively associated with higher VCS (P = 0.003), whereas EN-RAGE showed a positive tendency (P = 0.235). Even after adjustments for confounding risk factors, sRAGE was independently associated with higher VCS (OR = 0.505, 95% CI: 0.269 – 0.948, P = 0.033).

Conclusions: This study shows circulating sRAGE levels are associated in an inverse manner to VCS in HD patients. Longitudinal observations and intervention studies are warranted to establish whether this link is causal in nature.

TH-PO878

The German Calciphylaxis Registry Vincent Brandenburg,[1] Jürgen Floege,[3] Markus Ketteler.[2] *[1]Cardiology, RWTH University Hospital Aachen, Aachen, Germany; [2]Nephrology, Klinikum Coburg, Coburg, Germany; [3]Nephrology, RWTH University Hospital Aachen, Aachen, Germany.*

Background: Calcific uremic arteriolopathy (CUA, calciphylaxis) is a rare disease associated with high morbidity and mortality. CUA is characterised by painful, ischemic, partly necrotic skin ulcerations due to malperfusion based on media calcification of cutaneous arterioles. The aims of the German CUA registry are 1) to collect data about incidence and potential risk factors; 2) to gain overview about current treatment strategies; 3) to link these data to the clinical course.

Methods: The internet-based registry (www.calciphylaxis.eu) allows online notification for all cases of established or suspected CUA. The diagnosis is based on clinical judgement and / or histology by evaluation of both treating physician as well as registry leaders. A comprehensive data base including 66 items concerning patient characteristics, laboratory data, clinical background and presentation as well as therapeutic strategies was established. Follow-up of the patients has been performed by queries of long-term outcome.

Results: Altogether 151 CUA patients have been documented during 5.5 yrs: 60% female; median age 68 yrs (range 21 – 89); 78% dialysis patients. PTH levels were overall low with 54% below 150 pg/mL. Oral anticoagulation with coumadins was common among all patients (45%). Cutaneous lesions were typically localized at the lower extremities or gluteal region (> 80%). Median survival time after registry notification was 516 days. Both serum fetuin-A and MGP levels were significantly lower in CUA dialysis compared to non-CUA dialysis pts. Treating physicians commonly applied i.v. sodium-thiosulfat three times per week, which influenced survival insignificantly (uncontrolled intervention; Kaplan-Meier analysis).

Conclusions: CUA is a rare disease among ESRD pts which increases mortality. PTH levels vary substantially but do not exceed current KDIGO target levels in most cases. Decisions on therapeutic strategies also vary significantly among centers. The present internet based ICCN registry is a valuable tool to collect data upon CUA cases and may become a basis for prospective systematic evaluations of treatment modalities in the near future.

Funding: Pharmaceutical Company Support - Amgen

TH-PO879

Serum Sclerostin Levels and Clinical Outcomes in Incident Dialysis Patients: Results from the NECOSAD Study Vincent Brandenburg,[1] Marc G. Vervloet,[4] Friedo W. Dekker,[4] Markus Ketteler,[3] Johanna Van den Broek,[4] Christiane Drechsler.[2] *[1]Cardiology, RWTH University Hospital Aachen, Aachen, Germany; [2]Nephrologie, Universität Würzburg, Würzburg, Germany; [3]Nephrology, Klinikum Coburg, Coburg, Germany; [4]Nephrology, VU Medical Center, Amsterdam, Netherlands.*

Background: Sclerostin is Wnt pathway antagonist which regulates osteoblast activity and bone turnover. Sclerostin offers interesting perspectives as novel biomarker for CKD-MBD. The present study tests the hypothesis that serum sclerostin levels may be associated with outcome in incident dialysis patients.

Methods: We used data from a prospective cohort study of incident dialysis patients (NECOSAD). All patients for whom serum analysis for sclerostin was available at three months after initiation of dialysis (baseline) were included (n= 362, age = 63±14 yrs; 59% males, 93% hemodialysis). Serum sclerostin was assessed by TECO® Sclerostin EIA Kit. Based on the median of serum sclerostin (1.10 ng/mL) the pts were stratified into two groups for statistical analysis. Cumulative mortality curves were calculated using Kaplan-Meier analysis for all-cause mortality and we calculated Cox proportional hazard ratios (HR) with 95% confidence intervals to assess the impact of sclerostin upon mortality.

Results: Median serum sclerostin levels were similar between PD pts (1.12 ng/mL) and HD pts (1.10 ng/mL). There was a negative correlation to PTH levels (Spearman corr. coeff. r= -0.22 p<0.001) and bone alk phos (r= -0.19, p<0.001) at baseline in the entire cohort. After 3 yrs of FU, 31% of the pts had died. After adjustments for potential confounders including age, sex, dialysis modality and primary kidney disease, there was no significant difference in terms of survival between the two groups with a trend for better survival for those pts with higher serum sclerostin (HR 0.79, 95% CI 0.54 to 1.16).

Conclusions: Serum sclerostin levels in incident dialysis patients were negatively correlated to biomarkers for bone metabolism (PTH, BAP) supporting its role as bone-suppressive factor also in ESRD. In the NECOSAD cohort, there was a trend for improved survival over 3 yrs with higher baseline serum sclerostin levels at three months after start of dialysis.

TH-PO880

Acetylsalicylic Acid, an Antiplatelet Agent Prevents Arterial Calcification in Chronic Kidney Disease: An Old Drug for a New Application Tzong-Shi Lu,[1] Kenneth Lim,[1,2] Daniel Zehnder,[2] Li-Li Hsiao.[1] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom.*

Background: Vascular calcification (VC) is a significant contributor to cardiovascular mortality in patients with chronic kidney disease and coronary artery disease. Aspirin, also known as acetylsalicyclic acid (ASA) is a widely used antiplatelet medication. However, the role of ASA in the development of VC is unknown. Our preliminary data showed that inducible heat shock protein 72 (HSP72) prevents VC. Previous reports suggested that ASA

can induce HSP72 expression. We postulate that ASA may exert cardio-protective effects by stabilizing smooth muscle cell phenotype and inhibiting mitochondrial dysfunction through the induction of HSP72.

Methods: *In vitro* long-term calcification model: human aortic smooth muscle cells (HA-SMCs) treated with calcification medium (CM) containing 5mM $CaCl_2$ and 5mM β-glycerophosphate for 21 days. ASA treatment: 4mM, daily.

Results: We have shown that ASA significantly inhibited the development of VC through the induction of HSP72 in our long-term vascular calcification model, in vitro. Furthermore, anti-calcific effects of ASA were abolished by HSP72 SiRNA. Similar observations were found using heat shock treatment, an established HSP72 inducer, as a control. We next showed that by inducing HSP72, ASA suppressed Runx2, Osteocalcin and Alkaline Phosphatase, major osteogenic regulators involved in the initiation and progression of VC. ASA also restored myocardin and serum response factor (SRF), regulators of HA-SMC contractile genes. In addition, ASA treated HA-SMCs under CM stress had preserved JC-1 complex, indicating stabilization of mitochondrial membrane potential.

Conclusions: ASA inhibits VC through a HSP72-dependent pathway by inhibiting osteogenic transformation and stabilizing smooth muscle cell contractile phenotype. Furthermore, its anti-calcific effects may be associated with stabilization of mitochondrial membrane potential. We suggest treatment strategies involving ASA or other inducers of HSP72 as a new approach to inhibit VC.

Funding: Private Foundation Support

TH-PO881

Study on the Function of Nrf2 in Vascular Calcification in ESRD Li Wang. *Renal Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: Advanced glycation end-products were reported to be a cause of oxidative stress. Nrf2 was a defensive factor against ROS and inflammation in vascular smooth cells. The role of AGEs and its induction of oxidative stress in vascular calcification remains unclear. So the objectives were to explore the relationship between oxidative stress caused by Nrf2 of AGEs-RAGE signaling pathway and vascular calcification.

Methods: 29 ESRD patients were enrolled and samples of radial arteries were taken during the arteriovenous fistula surgery. Six patients with upper extremity injuries and normal renal function were enrolled as control with samples of internal radial artery taken in the operation. The vessels were examined for calcification by alizarin red staining and for the presence of bone matrix protein (osteopontin,OPN), marker of antoxidative stress (nuclear factor erythroid-2-related factor2,Nrf2), marker of oxidative stress(malondialdehyde-LDL, MDA-LDL), Runx2, SM22α, and receptor for advanced glycation end products(RAGE) by immunohistochemistry. Plasma dvanced glycation end products (AGEs) were measured. Relationship between vascular calcification and other markers and clinical parameters including gender, age, BMI, Cr, HB, PTH, Ca, P, ALP, ALB, CHOL, hs-CRP and so on were analyzed.

Results: Vascular calcification was found in the media of the vessels of 15 uremic patients, while none in the controls. OPN, MDA-LDL, RAGE and Runx2 expressed in the vessels and plasma levels of AGEs of uremic patients were significantly higher than those in the control, while Nrf2 and SM22α expressed in patients with ESRD was significantly lower than those in the control($P<0.01$). Pearson correlation analysis showed that the degree of calcification positively correlated with plasma AGEs($r=0.838$, $P<0.001$), MDA-LDL($r=0.922$, $P<0.001$), RAGE($r=0.911$, $P<0.001$), Nrf2($r=-0.711$, $P<0.001$), plasma phosphate($r=0.623$, $P=0.001$), serum iPTH($r=0.398$, $P=0.032$), age($r=0.591$, $P=0.001$), and duration of dialysis ($r=0.437$, $P=0.018$).

Conclusions: Decreased Nrf2 which expressed in the vessels were found in patients with ESRD, the Nrf2 levels were closely related to vascular calcification which maybe induced by AGEs-RAGE signal transmission.

TH-PO882

L-Lysine Ameliorates Vascular Calcification in Adenine-Induced Uremic Rats Akihiro Shimomura,[1] Isao Matsui,[1] Takayuki Hamano,[2] Kazunori Inoue,[1] Yoshitsugu Obi,[1] Chikako Nakano,[1] Yasuo Kusunoki,[1] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Vascular calcification (VC) is common in patients with chronic kidney disease (CKD) and is associated with increased cardiovascular morbidity and mortality. Although numerous studies have revealed the pathogenic mechanism of VC, it still remains unresolved, and satisfactory therapies have not been established. Price et al. reported that low protein diet exacerbates VC in adenine-induced uremic rats Kidney Int. 2006;70(9):1577-83]. We hypothesized that insufficiency of some amino acids worsened VC in this model. Because L-lysine is the first-limiting amino acid in most of cereal grains, we examined whether L-lysine supplementation ameliorates VC.

Methods: Male Sprague-Dawley rats at age 13 weeks were divided randomly into four groups; low protein (LP) diet (group LP), LP diet + 0.75% adenine (group Ade), LP diet + 0.75% adenine + 2.5% glycine (group Gly), or LP diet + 0.75% adenine + 2.5% L-lysine•HCl (group Lys). Glycine, the simplest amino acid was served as control.

Results: At age 18 weeks, rats in group LP showed no VC, whereas those in groups Ade and Gly exhibited comparable levels of severe VC. L-lysine supplementation almost completely ameliorated VC. Body weight, food intake, water intake, serum levels of creatinine, urea nitrogen, and phosphate were not different among groups Ade, Gly, and Lys. Intriguingly, serum calcium level in group Lys was slightly but significantly higher than those of groups Ade and Gly. To investigate the underlying mechanism for VC-prevention, we performed in vitro analysis and found that L-lysine, but not glycine, dose-dependently attenuates spontaneous precipitation of minerals in a solution of supersaturated calcium/phosphate.

Conclusions: L-lysine ameliorated vascular calcification in adenine-induced uremic rats. Our findings provide a novel approach for the treatment of VC in CKD.

TH-PO883

Hemodialysis Reduces the Calcification Propensity of Serum Andreas Pasch,[1,2] Stefan Farese,[1,2] Georg Schlieper,[3] Jürgen Floege,[3] Dominik E. Uehlinger,[1] Willi Jahnen-dechent.[2] *[1]Department of Nephrology and Hypertension, University Hospital Bern, Inselspital, Bern, Switzerland; [2]Biomedical Engineering, Biointerface Laboratory, RWTH Aachen University, Aachen, Germany; [3]Department of Nephrology, RWTH University of Aachen, Aachen, Germany.*

Background: Vascular and soft tissue calcification is a major cause of death in hemodialysis (HD) patients.We have developed an in vitro test, which measures serum calcification propensity by detecting the spontaneous transformation of colloidal primary calciprotein particles (CPPs) to crystalline secondary CPPs. The effect of hemodialysis on serum calcification propensity has not been determined yet.

Methods: The intrinsic calcification propensity of pre- and post-HD sera obtained from 98 prevalent HD patients were analyzed with our novel nephelometry test. Calcium, phosphate, magnesium, fetuin-A, albumin, and total protein concentrations were related to the test results and integrated into a multivariate model with stepwise selection.

Results: HD treatment reduced serum calcification propensity by delaying transformation time (T_{50} pre-HD 244 ± 112 min., post-HD 340 ± 114 min., $p < 0.0001$) and reducing precipitation intensity (relative nephelometric units, RNU_{50} pre-HD 6892 ± 2404, post-HD 5234 ± 1789, $p < 0.0001$). A multivariate model showed, that the T_{50} of pre-HD sera depended mainly on magnesium (transformation delay, $p < 0.0001$) and fetuin-A (delay, $p < 0.0001$), and the HD-induced delay of T_{50} on phosphate (acceleration, $p < 0.0001$), magnesium (delay, $p = 0.0094$) and fetuin-A (delay, $p < 0.0001$) serum concentrations. In contrast, the reduction of precipitation intensity RNU_{50} induced by HD depended on the change of the total serum protein concentration ($p = 0.0407$), which was closely correlated to the albumin and fetuin-A concentration changes induced by HD ($p < 0.0001$). Calcium concentrations did not contribute significantly to the test results.

Conclusions: HD vastly improves the intrinsic calcification propensity of patient sera, with phosphate, magnesium and fetuin-A as major influencing factors. Monitoring serum-inherent calcification propensity may help improve morbidity and mortality of HD patients in the future.

TH-PO884

Chronic Kidney Disease Leads to Osteogenesis and Calcification of Intraperitoneally Transplanted Mesenchymal Stem Cells in Rats Rafael Kramann,[1,2] Rebekka K. Schneider,[2] Vincent Brandenburg,[3] Barbara Mara Klinkhammer,[1] Uta Kunter,[1] Jürgen Floege.[1] *[1]Nephrology, RWTH University, Aachen, Germany; [2]Pathology, RWTH University, Aachen, Germany; [3]Cardiology, RWTH University, Aachen, Germany.*

Background: Once considered as a passive process, vascular calcification has emerged as a tightly regulated, coordinated and osteoblastic process resembling bone morphogenesis. Executive cell types familar to bone biology are seen in calcified vasculature. As osteoblasts, smooth muscle cells, adipocytes, fibroblasts and chondrocytes all share a common mesenchymal progenitor stem cell (MSC), the present study is based on the hypothesis that MSC contribute to vascular calcification and ectopic osteogenesis in chronic kidney disease (CKD).

Methods: Rat MSC were intraperitoneally transplanted in an established 3D collagen-based model of the vascular wall in healthy control animals (n=12) and two rat models of CKD and vascular calcification: a) 5/6 nephrectomy + high phosphorus diet b) adenine nephropathy (n=18 each). As internal controls, collagen gels without MSC were transplanted in the same animals.

Results: After 4 and 8 weeks, MSC were still detectable and proliferating in the collagen gels (FACS analysis and confocal microscopy after PKH26-labelling prior to implantation). Aortae and MSC-containing collagen gels in the CKD animals showed distinct similarities in calcification (von Kossa-staining, micro-computed tomography, EDX analysis, calcium content measurement) and up-regulation of osteogenic markers as BMP-2, Runx2, ALP and the osteocytic marker sclerostin. An equivalent extracellular matrix (ECM) remodelling process occurred in both the aortae and the heterotopically implanted MSC in CKD with up-regulation of osteopontin, collagen I/III/IV, fibronectin and laminin. Neither calcification nor osteogenesis or matrix remodelling were observed in healthy control animals and non-MSC containing collagen gels of all groups.

Conclusions: CKD induced similar calcification, osteogenesis and ECM remodelling in the vascular wall and heterotopically implanted MSC supporting our hypothesis of MSC as critical cells in heterotopic calcification processes of CKD patients.

Funding: Private Foundation Support

TH-PO885

The Responsiveness of Serum Calcium Level on Soluble Klotho in CKD Patients Ichiro Ohkido, Keitaro Yokoyama, Yukio Maruyama, Tatsuo Hosoya. *Division of Nephrogy and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

Background: The extracellular domain of Klotho is cleaved and released into various extracellular fluids, such as blood, urine, and cerebrospinal fluid, as circulating soluble Klotho (sKlotho). Circulating sKlotho might be a biologically active molecule. However, there are few reports concerning sKlotho level in human.

Methods: we measured sKlotho using enzyme-linked immunosorbent assay kits in 157 CKD patients (CKD1-CKD5=109, CKD5D=48) to examine its role in CKD-MBD.

Results: The sKlotho level of the CKD patients without dialysis patients was 654 pg/mL (280-1777 pg/mL), which was higher than that of hemodialysis patients 430 pg/mL (386-540 pg/mL). In single regression analysis, the serum sKlotho level showed a positive correlation with serum corrected Calcium (cCa) (rho=0.22, P<0.05) and eGFR (rho=0.27, P<0.01), while serum phosphorus (Pi) tended to be negatively correlated with sKlotho (rho=-0.34, P<0.01) and intact-PTH (rho=-0.21, P<0.05). However, there was no significant association between serum sKlotho and age or FGF23. Multiple regression analysis showed that cCa was independently associated with the serum sαKl levels (P=0.05) in CKD patients without dialysis, on the other hand, in CKD 5Dpatients, Pi was independently associated with the serum sKlotho levels (P<0.05). In CKD patients without dialysis, the positive correlation between serum sKlotho level and cCa level in single regression analysis should be recognized as renal function. However those also were found in multiple regression analysis.

Multiple regression model for circulating sKlotho (log-normalized)

Variable	Regression Coefficient	Standard error	t value	95% Confidence Interval	p value
Male gender	0.118	0.0663	1.78	-0.0139 to 0.249	0.08
Age	-0.000351	0.00217	-0.16	-0.00466 to 0.00396	0.87
eGFR	0.000914	0.00122	0.75	-0.00151 to 0.00334	0.46
Collected Ca	0.105	0.044	1.92	-0.00332 to 0.212	0.05
Pi	-0.0432	0.0266	-1.62	-0.0960 to 0.00970	0.11
intact PTH	0.000463	0.000323	1.43	-0.000178 to 0.00110	0.16

total adjusted R2=0.30, P=0.01

Conclusions: These results might indicate that the responsiveness of calcium on sαKl impair in CKD status. Soluble Klotho may thus plays a role in CKD/MBD.

Funding: Government Support - Non-U.S.

TH-PO886

Circulating Sclerostin Levels and Vascular Calcification in Chronic Kidney Disease: A Complex Relationship Kathleen Claes,[1] Liesbeth Viaene,[1] Sam Heye,[1] Bjorn Meijers,[1] Patrick C. D'Haese,[2] Pieter Evenepoel.[1] *[1]Nephrology, University Hospitals Leuven, Leuven, Belgium; [2]Laboratory of Nephrology, University Antwerp, Antwerp, Belgium.*

Background: Sclerostin, a Wnt antagonist, regulates osteoblast activity and is a key player in bone turnover. The Wnt pathway may also play an important role in the process of vascular calcification. We tested the hypothesis that serum sclerostin levels are associated with vascular calcification in patients with chronic kidney disease (CKD) not yet on dialysis.

Methods: 154 CKD patients were recruited from an observational study evaluating the natural history of mineral metabolism. Aortic calcification (AC) was assessed by lumbar X-ray and scored according to Kaupilla. In addition to traditional and non-traditional risk factors, serum sclerostin levels were measured (ELISA, Tecomedical). Regression analysis was performed to identify determinants of serum sclerostin levels and AC.

Results: AC was present in 59% of patients. Patients with AC had higher sclerostin levels. Higher age (p <0.0001), male gender (p= 0.004), and lower eGFR (0.004) were independent determinants of higher serum sclerostin levels. In univariate logistic regression, age, diabetes, cardiovascular history, BMI, serum CRP and sclerostin were all directly associated with the presence of AC, whereas eGFR was inversely associated. However, in multivariate analysis lower, not higher, sclerostin levels (p=0.04; OR per ng/ml decrease 0.237), higher age (p<0.0001, OR per year increase 1.172) and cardiovascular history (p=0.02,OR for a positive cardiovascular history 3.83) were identified as independent determinants of AC.

Conclusions: In CKD patients not yet on dialysis we found that patients with aortic calcifications had higher sclerostin levels. However, in multivariate analysis we observed an inverse association between sclerostin and aortic calcification. We speculate that extraskeletal sclerostin may contribute significantly to circulating levels and may be part of a local feedback suppressing further progression of vascular calcification. Additional clinical and experimental studies are required to elucidate whether or not sclerostin protects against progression of vascular calcification.

TH-PO887

Changes in Skeletal Osteopontin Expression Occur in Response to Skeletal Mineralization R.C. Pereira,[1] Harald Jüppner,[2] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Endocrine Unit, Harvard Medical School and Massachusetts General Hospital, Boston, MA.*

Background: Defects in skeletal mineralization persist in pediatric ESKD despite suppression of bone turnover with active vitamin D sterols. OPN, a mineralization inhibitor, is regulated by $1,25(OH)_2D$. However, the effect of active vitamin D on skeletal OPN and its relationship to skeletal mineralization are unknown.

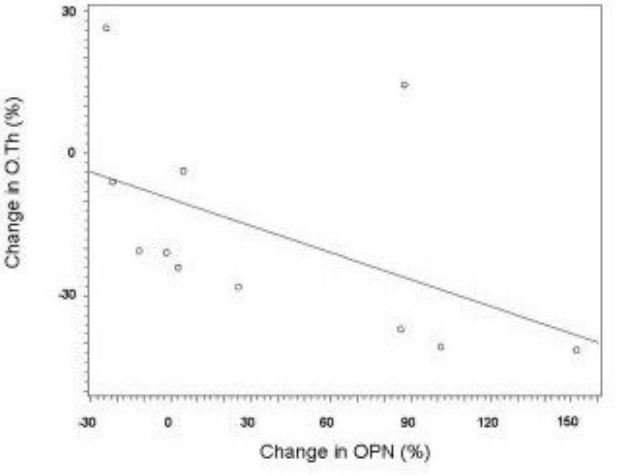
Methods: 11 ESKD pts (7M, 4F), age 16 ± 1 years, underwent BBx before and after 8 months of active vitamin D therapy. OPN protein expression was assessed in undecalcified bone by immunohistochemistry and quantified by Ariol scanning. Biochemical values were obtained at the time of BBx.

Results: Biochemical values and OPN expression remained unchanged during therapy, while osteoid accumulation decreased.

	Pre	Post
Biochemicals		
Calcium (mg/dl)	8.6 ± 0.2	8.8 ± 0.2
Phosphorus (mg/dl)	6.3 ± 0.4	6.0 ± 0.4
Alk p'tase (IU/l)	298 (146, 488)	175 (124, 414)
PTH (pg/ml)	517 (378, 1048)	559 (375, 922)
FGF23 (RU/ml)	705 (318, 1196)	1696 (565, 3973)
Bone Histomorphometry and Osteopontin Expression		
BFR/BS (um3/mm2/d)	50.0 ± 10.4	30.4 ± 7.5
OV/BV (%)	7.0 ±1.1	4.4 ± 0.8*
O.Th (um)	12.7 ± 0.9	10.2 ± 0.6*
OS/BS (%)	41.1 ± 3.9	31.7 ± 3.8*
OMT (d)	15.3 ± 1.4	14.6 ± 2.1
MLT (d)	28 (24, 47)	36 (30, 46)
BV/TV (%)	37.2 ± 3.7	36.1 + 2.7
Tb.Th (um)	173 ± 19	159 ± 6
OPN (pixels/mm2)	22,823 ± 3,969	26,300 ± 2,549

*p<0.05 from baseline

OPN correlated with osteoid accumulation (OV/BV: r= - 0.44, o<0.05; OS/BS: r= - 0.46, p<0.05; O.Th: r= - 0.39, p=0.07) and changes in O.Th were inversely related to changes in OPN expression (r= -0.65, p<0.05).



Conclusions: Skeletal OPN expression increases in response to mineralization. Whether OPN can be used as a target for therapies directed at healing mineralization defects remains unknown.

Funding: NIDDK Support, Other NIH Support - UL1 RR-033176

TH-PO888

Circulating and Bone Sclerostin Expression in Peritoneal Dialysis Patients Rodrigo Azevedo de Oliveira,[1] Fellype Carvalho Barreto,[1,2] Monique Mendes,[1] Juliana C. Ferreira,[1] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Rosa M.A. Moyses,[1] Vanda Jorgetti.[1] *[1]Nephrology, Universidade de São Paulo, São Paulo, SP, Brazil; [2]Medicine, Health Science, Universidade Nove de Julho, São Paulo, SP, Brazil.*

Background: Sclerostin (SOST) is a soluble inhibitor of wnt signaling, produced by osteocytes, that inhibits osteoblast function. Previous studies have shown that SOST is increased in hemodialysis, as well as in predialysis patients. Trying to elucidate the role of SOST in peritoneal dialysis (PD) patients, this study sought to investigate the association between circulating and bone SOST expression with biochemical and bone histomorphometric parameters in this population.

Methods: In this cross-sectional study, thirty PD patients (50% male; 49 ± 10.4 years; mean length on PD: 19 months) were submitted to an iliac crest bone biopsy followed by histomorphometric analyses. Levels of circulating SOST, iPTH, 25(OH)vitamin D and other biochemical markers of mineral metabolism were analyzed. Bone expression of SOST was evaluated by imunohistochemistry.

Results: Serum SOST was increased in PD patients, and correlated with age, iPTH and alkaline phosphastase (r = 0.63, -0.44 and -0.48, respectively; p< 0.05 for all). Higher serum SOST was found in patients with lower trabecular bone volume [BV/TV (2.56±0.92 vs 1.74 ± 0.75 ng/ml in those with normal BV/TV; p=0.02). We also found significant differences between those patients with low bone turnover in comparison with high bone turnover (2.46±0.76 vs 1.58 ± 0.78 ng/ml; p=0.009). Furthermore, serum levels of SOST were negatively associated to osteoblast surface, osteoid thickness, mineralizing surface and bone formation rate (r = -0.68 -0.66, -0.59, -0.58, respectively; p<0.05). Regarding the bone expression of SOST, it was higher in PD patients compared to normal subjects (65.6 ± 44.3 vs 24.6 ± 18.4 ng/ml; p= 0.02) and negatively associated to bone formation rate (r =-0.41; p <0.05).

Conclusions: Serum levels of SOST are increased in PD patients, as well as its bone expression. Our data confirms SOST as a biochemical marker of bone volume and formation. The role of SOST in the pathogenesis of CKD-MBD, by affecting bone structure and turnover, remains to be determined.

Funding: Government Support - Non-U.S.

TH-PO889

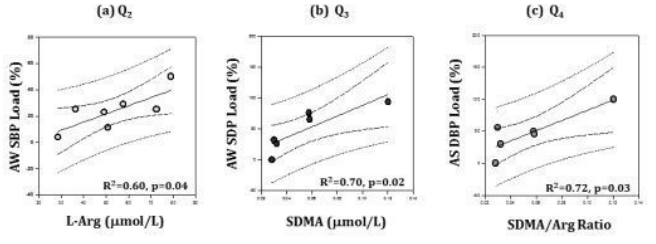
CKD-MBD in Children: Mineral and Vascular Inter-Relationships Are Mediated by FGF23 Ellen Brooks, Craig B. Langman, Heather E. Price, Nisa Desai, Neziha Celebi. *Pediatric Kidney Diseases, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Background: ↑FGF23 is the 1st hormonal change of CKD-MBD producing ↓ of both Pi reabsorption (%TRP) & $1,25(OH)_2D_3$ [1,25D] synthesis. Ca dynamics change too, with ↓bone balance & ↑vascular calcification & dysfunction. We studied these relationships in pediatric CKD using biomarkers.

Methods: We collected cross-sectional data on 28 pts (CKD stage 1-4; Mean±SD eGFR=40.9±13.7 ml/min/1.73m^2; Age=12.3±3.9y) & 10 healthy sibling controls (C) & measured Pi, intact PTH (*i*PTH), c-terminal FGF23, & bone markers: OPG, RANKL, TRAP, BALP. Vascular factors included were plasma methylated arginine derivatives (MADs) [L-Arg, ADMA & SDMA]. 24-hr ambulatory BP (ABP) for awake (AW) & asleep (AS) BP loads was measured. Data were log transformed & analyses completed using Student's T test, Spearman Rank Correlation & Linear Regression.

Results: CKD had ↑FGF23 v C (148±196 v 29±25 RU/mL; p=0.004). Inverse correlations in the highest CKD FGF23 quartile ($FGF23Q_4$) were with %TRP (r= -0.86,p=0.006) & OPG/RANKL ratio (r= -0.88, p=0.03). Other associations included [CaxPi] & eGFR (r =-0.75,p=0.040), *i*PTH & [L-Arg] (r= -0.90, p=0.02), Pi & Arg/ADMA ratio (r= -0.94, p=0.02), BALP & TRAP (r=0.89, p=0.03) & %TRP with OPG/RANKL ratio (r=0.94, p=0.02). In Q_4 regression models, [L-Arg] explained 83 & 99% of TRAP & BALP variability (p=0.01; p<0.001), respectively. In Q_4, FGF23 & 1,25D explained 42% of CKD [CaxPi] variability in stepwise regression (p=0.003; 0.03). For ABP, $FGF23Q_3$-Q_4 had ↑AW SBP loads v C & $FGF23Q_4$ had ↑AS SBP & DBP loads v C (p<0.05-0.008). $FGF23Q_2$-$Q_4$24 hr ABP loads correlated with MADs.

Figure 1a-c. ABP Loads Explained By MADs in Quartiles of CKD-FGF23 Q_2-Q_4



Conclusions: Taken together our findings suggest a coordinated activation of CKD-MBD mechanisms of bone loss & vascular dysfunction in children with moderate CKD through upregulation of FGF23.

Funding: Private Foundation Support

TH-PO890

A Cross-Sectional Chronic Kidney Disease-Mineral and Bone Disorder in MHD Patients Hong Daqing, Li Wang, Pu Lei. *Renal Department, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: As risk factors for cardiovascular disease in maintenance hemodialysis (MHD) patients, CKD-MBD has become a challenge for nephrologists. It's important to find out prevalence, risk factors and treatment status of mineral disorders, and it's the objective for this study.

Methods: 217 MHD patients were enrolled from July, 2010 to March, 2011 with a questionare, serum biochemistry, X-ray, pulse wave velocity (PWV), ankle-brachial index (ABI), and coronary artery CT taken. Prevalence of CKD-MBD and its components were analyzed, single and multiple logistic regression was applied to study the risk factors. Relationship between different techniques for vascular calcification (VC) were analyzed.

Results: The prevalence of hyperphosphatemia, hypophosphatemia, hypercalcemia, hypocalcemia, decreased PTH and elevated PTH was 45.16%, 10.14%, 31.80%, 21.66%, 20.74% and 48.39% respectively (Figure 1~3). Patients in biochemistry treatment targets were only 9.22%. 71% patients had VC by X-ray (Figure 4). 96.31% patients was diagnosed CKD-MBD by biochemistry abnormalities, VC or combination of both. Risk factors for VC were old age, serum ALP, hyperphosphatemia and high level of CRP.

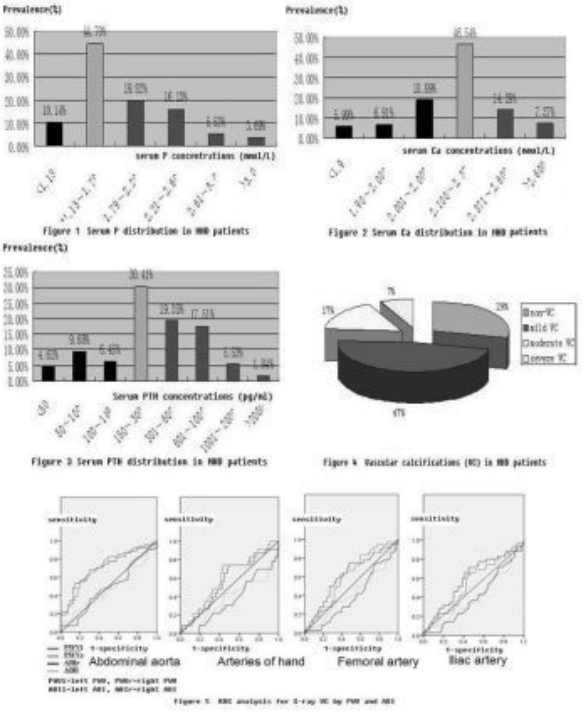
Table 1 Risk factors for vascular calcifcation in MHD patients

Parameters	ß	OR	95% CI	p
Age (year)	0.117	1.124	1.003-1.260	0.045
ALP(mmol/L)	0.026	1.026	1.001-1.052	0.040
P(mmol/L)	1.283	3.608	1.033-12.598	0.044
CRP(mg/L)	0.557	1.745	1.060-2.874	0.029

PWV correlated better than ABI in diagnosis of VC by X-ray (Figure 5). Iliac and femoral showed best correlation with coronary artery calcification in 36 MHD patients (table 2).

Table 2 Various and combined parts of the X-plain film of two categorical variables mode (ie, calcification or without) and CACs ≥ 100

	OR	*P*	sensitivity	specificity
Illiac artery	12.250	0.007	91.30%	53.85%
Femoral artery	8.556	0.015	60.87%	84.62%
Abdominal aorta	5.714	0.036	86.96%	46.15%
Radial artery	1.778	0.467	34.78%	76.92%
Finger artery	1.158	0.877	17.39%	84.62%



Conclusions: Prevalence of CKD-MBD in MHD patients is high. Risk factors for VC are old age, serum ALP, hyperphosphatemia and CRP. PWV is better than ABI in diagnosis of VC. Femoral and iliac X-ray may be better than lateral abdominal X-ray as a subsitute for coronary artery calcification evaluated by CT.

TH-PO891

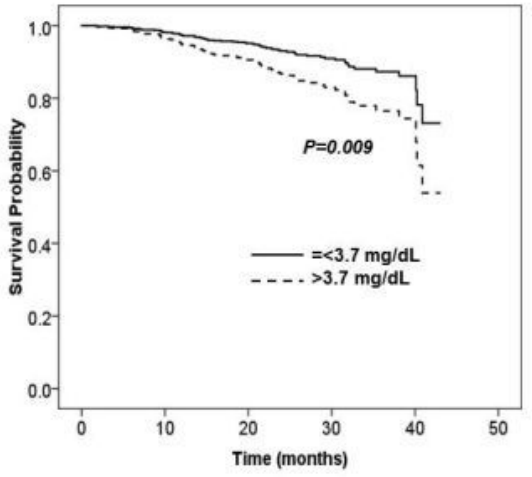
Mineral Metabolism and Outcomes in Predialysis Chronic Kidney Disease Sinee Distha-Banchong, Kotcharat Vipattawat, Arkom Nongnuch. *Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Phayathai, Bangkok, Thailand.*

Background: Marked hyperphosphatemia and hyperparathyroidism and 25-hydroxyvitamin D deficiency are known to predict mortality in dialysis patients. Less data is available in predialysis chronic kidney disease (CKD) population. It was suggested that high-normal serum phosphate might be associated with worse renal and patient outcomes. The data regarding PTH in the predialysis population is limited.

Methods: The present study examined the abnormalities of mineral metabolism and their associations with the development of end-stage renal disease (ESRD) and mortality in 446 predialysis CKD patients. Mineral parameters were obtained from all patients and the patients were followed prospectively for 25 (1-44) months or until they reached the endpoints of ESRD or mortality.

Results: Hyperparathyroidism and 25-hydroxyvitamin D deficiency occurred since CKD stage 2, whereas significant hyperphosphatemia only developed in CKD stage 4 or later. Increasing serum phosphate (>3.7 mg/dL) and PTH (>42 pg/mL) despite being within the normal ranges were associated with the development of ESRD and the composite outcomes of ESRD and mortality after adjustments with cardiovascular risk factors and other mineral parameters (Figure 1). 25-hydroxyvitamin D deficiency (<15 ng/mL) was also associated with worse outcomes.

Figure 1. Adjusted survival curves for serum phosphate



Conclusions: In the course of CKD, hyperparathyroidism developed prior to significant hyperphosphatemia confirming the presence of a state of phosphate retention since the early CKD period. The associations between the increasing serum phosphate and PTH, despite the values being within the normal limits, and worse renal and patient outcomes emphasized the need for early intervention in the care of CKD patients.

Funding: Government Support - Non-U.S.

TH-PO892

Osteoprotegerin Replaces Coronary Artery Calcification Score as a Predictor of Incident Cardiovascular Events in Diabetic Chronic Kidney Disease Chikako Nakano,[1] Takayuki Hamano,[2] Naohiko Fujii,[3] Yoshitsugu Obi,[1] Isao Matsui,[1] Kazunori Inoue,[1] Akihiro Shimomura,[1] Yasuo Kusunoki,[1] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan.*

Background: Coronary artery calcification score (CACS) was reported to predict cardiovascular disease (CVD). However, CACS has several disadvantages, such as radiation exposure and inconvenience. Our aim was to identify a more readily-measurable predictor of CVD among vascular calcification-related factors: osteoprotegerin (OPG), fibroblast growth factor 23, fetuin-A, and fetuin mineral complex (FMC).

Methods: This is a prospective cohort study of 97 diabetic outpatients with chronic kidney disease (CKD). CACS was measured by multi-detector computed tomography. The endpoint was a fatal/non-fatal CVD requiring hospitalization. Multiple imputation method was performed for missing values. After confirming that CACS predicted CVD with adjustment for conventional risk factors, further adjustment for which biomarker changed the coefficient of CACS by ≥15% was scrutinized in Cox models. We compared model performance between models with that biomarker or CACS by computing C-statistics, Bayesian information criterion, net reclassification improvement, and integrated discrimination improvement.

Results: Median estimated GFR was 25 mL/min/1.73m^2. During a median follow-up period of 5.0 years, 32 patients reached the endpoint. At baseline, only OPG and FMC were related to CACS. CACS predicted CVD after adjusting for conventional risk factors. Among 4 factors, only further adjustment for OPG changed the coefficient of CACS by ≥15%. The effect size was higher for OPG than for CACS (hazard ratios of CACS and OPG, 4.34 [1.07-17.6] and 18.1 [2.60-125] in the highest vs. lowest quartile, respectively). Model performance was better for the model with OPG than that with CACS.

Conclusions: In diabetic patients with CKD, OPG is more useful than CACS in predicting incident CVD.

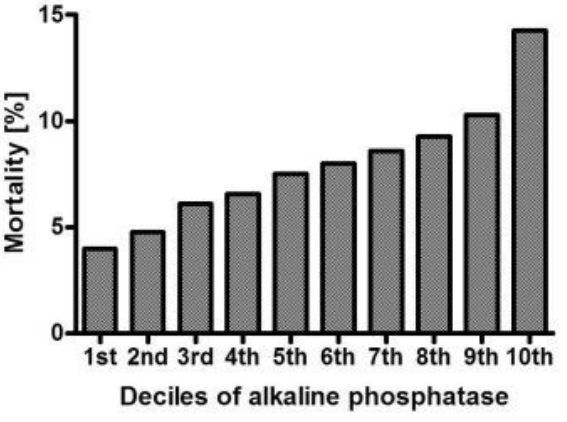
TH-PO893

A Higher Serum Alkaline Phosphatase Is Associated with Increased Mortality among Patients Receiving Hemodialysis in Japan Yukio Maruyama,[1] Keitaro Yokoyama,[1] Takashi Shigematsu,[2] Masatomo Taniguchi,[3] Junichiro J. Kazama,[4] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, The Jikei University School of Medicine, Tokyo, Japan; [2]Division of Nephrology and Blood Purification Medicine, Wakayama Medical University, Wakayama, Japan; [3]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [4]Division of Blood Purification Therapy, Niigata University Medical and Dental Hospital, Niigata, Japan.*

Background: The monitoring of serum alkaline phosphatase (ALP), a biochemical marker of bone turnover, is widely recommended in the management of chronic kidney disease-mineral and bone disorder (CKD-MBD). However, unlike calcium, phosphate, or parathormone, the relationship between serum ALP and mortality of the patients receiving hemodialysis (HD) in Japan is unknown.

Methods: We collected the baseline data of 187,792 patients receiving HD thrice weekly (66 ± 12 years, 116,314 males, and median HD vintage of 7.9 years) extracted from a nationwide dialysis registry at the end of 2009 in Japan. Then we evaluated the patient survival using the registry at the end of 2010.

Results: A rise in baseline serum ALP was incrementally associated with increasing death risk (Figure 1), and a receiver operating characteristic (ROC) curve revealed that the sensitivity was 57% and the specificity was 58% when a serum ALP of 253 IU/L was taken as the cut-off value.



In a multiple logistic regression analysis including serum ALP, Ca, P, PTH, medication and comorbidity, higher serum ALP was independently associated with increased mortality.

Conclusions: In this large observational cohort study, higher levels of serum ALP were independently associated with increased mortality among HD patients. Close monitoring of serum ALP may be useful for consideration of the therapy for CKD-MBD.

TH-PO894

Relationship of Bone-Specific Alkaline Phosphatase to Parathyroid Hormone in Hemodialysis Patients Ghassan Bandak, Beth Adams, Meredith Mahan, Anatole Besarab. *Henry Ford Hospital, Detroit, MI.*

Background: Metabolic bone disease (MBD) is common in patients with chronic kidney disease (CKD) and is a major source of morbidity and mortality in this patient population. Control of parathyroid hormone (PTH) levels is a major aspect in managing MBD. Reliability of PTH assays has been questioned. Other markers of bone disease have been suggested to help with MBD management, such as alkaline phosphatase (ALP) and bone specific ALP (bSAP). We therefore assessed the relationship of PTH to bSAP and ALP levels over a period of one year in which MBD management depended chiefly on active vitamin D therapy with only a small subset receiving a calcimimetic.

Methods: 251 prevalent HD patients as of March 2011 had measurements made of serum ALP, bSAP, total calcium (Ca), and ionized calcium (Ca2+), phosphorus (P) and PTH levels every 3 months using methods available at Spectra Laboratories over a period of 9 months. Most patients had 4 measurements. All patients in our unit were included to avoid selection bias.

Results: The population was predominantly Afro-American. At the first time point The PTH averaged 611±450 (±SD), the bSAP 38.6±35.1, P 4.8±1.4, Ca 8.9±0.8, Ca2+ 4.8±0.5. These remained unchanged over the next 9 months except for bSAP which decreased to 25±17 and as a percent of ALP from 32 to 23%. bSAP was strongly significantly correlated to ALP at all time points (r=0.75 to 0.83, p<0.001). Moderate correlations of bSAP to PTH, (r=0.37 to 0.52, p<0.001), weak correlations of bSAP with URR (r=0.14 to 0.34, p<0.05) and no significant correlations with Ca, Ca2+ or P were noted at any time point. Using a repeated-measures mixed model we found that female gender and changes in PTH, Ca2+,

and P, had significant effects on bSAP. Although within-subject variance was smaller than between-subject for PTH and bSAP, the intra class correlations were 0.64 and 0.57 indicating a high degree of variability between and within subjects.

Conclusions: PTH has moderate positive correlations with ALP and bSAP and changes in PTH over time significantly affects the levels of both. PTH in turn is strongly affected by both Ca2+ and P. Future studies should evaluate the possible need to use both parameters for MBD management.

Funding: Clinical Revenue Support

TH-PO895

Accumulated Indoxyl Sulfate Aggravate Bone Mechanical Property with Chronic Kidney Disease Yoshiko Iwasaki,[1] Junichiro J. Kazama,[2] Hideyuki Yamato,[3] Masafumi Fukagawa.[4] *[1]Oita Univ. of NHS, Oita; [2]Niigata Univ. Medical and Dental Hospital, Niigata; [3]Kureha Industry, Tokyo; [4]Tokai Univ. School of Medicine, Isehara, Japan.*

Background: Progression of renal dysfunction is associated with increasing fracture risk in chronic kidney disease (CKD) patients. Recent clinical studies have failed to find out the significant relationship between the severity of hyperparathyroidism and fracture risk. Thus, accumulated uremic toxins other than PTH may play an important role in the promotion of bone fragility in CKD condition. We investigated the role of indoxyl sulfate (IS), which is one of uremic toxins, in the bone mechanical property in experimental CKD rats.

Methods: We made CKD rats without hyperparathyroidism using method by partial nephrectomy, thyroparathyroidectoy (TPTx) and continuous exogenous PTH supplementation. CKD rats were divided into two groups; those administered oral charcoal absorbent which decreases the circulating levels of IS (CKD-A), and those received vehicle (CKD-V). The Control (C) group underwent TPTx and PTH supplementation only. Femoral bone elasticity was non-invasively assessed by a DMA method. Bone chemical compositions were analyzed by a raman spectroscopy.

Results: Compared with the C group, bone elasticity was significantly deteriorated in the CKD-V group, indicating decreased mechanical strength in the uremic cortical bone. However, the elasticity was maintained in the CKD-A group. Raman spectroscopic analyses revealed that mineral and collagen compositions, including carbonate phosphate ratio and non-physiological collagen crosslinks pentosidine were increased while crystallinity was decreased in the CKD-V group. These parameters were comparable between the CKD-A and the C groups. Serum parameters were comparable between the CKD-A and the CKD-V groups except the IS level was significantly higher in the CKD-V group. Multiple regression analysis revealed that IS concentration and collagen crosslinks were independently associated with bone elasticity.

Conclusions: Accumulated IS and possibly other uremic toxins other than PTH are likely candidate that promote bone fragility by deteriorating bone elasticity through changing the chemical compositions in CKD.

Funding: Government Support - Non-U.S.

TH-PO896

The Relationship between Sclerostin, FGF23 and PTH in Dialysis Patients: A Multicenter Study Joanna Matuszkiewicz-Rowinska,[1] Mariusz Mieczkowski,[1] Pawel Zebrowski,[1] Ewa Wojtaszek,[1] Tomasz Stompor,[2] Antoni Sokalski,[3] Jerzy Przedlacki,[1] Wieslaw Klatko,[4] Edyta Gieglis,[5] Janusz Grochowski,[6] Ignacy Jarzyllo,[7] Robert Malecki,[8] Stanislaw Niemczyk,[9] Zofia Wankowicz.[9] *[1]Medical University of Warsaw; [2]Warmia-Mazury University; [3]Radom Hospital; [4]Ciechanow Hospital; [5]Otwock Nephrocare; [6]Makow Hospital; [7]Wolomin Hospital; [8]MSSW Miedzylesie; [9]Military Institute of Medicine, Poland.*

Background: Sclerostin, an inhibitor of Wnt/beta-catenin signaling pathway, has emerged as a potent regulator of bone formation. Recently, its increased serum levels have been described in pts on hemodialysis (HD). The aim of our study was to assess the possible relationships between sclerostin and the other mineral metabolism regulators in HD and peritoneal dialysis (PD) pts.

Methods: 252 pts (age 59±15 years, on dialysis 45±49 months, 210 HD & 42 PD) and 36 healthy controls (age 56±20 years) were enrolled. Pts taking vitamin D or calcimimetics were excluded. Serum sclerostin (ELISA), iPTH, cFGF23 & routine tests were analysed.

Results: Compared to the controls, in pts sclerostin levels were 4x higher (117±64.5 vs 26.9±11.1 pmol/l; p<0.001), with lower values in women (106±62.1 vs 126±65.3 pmol/l in men; p=.016), diabetics (98±53 vs 123±66 pmol/l; p=.014) and non-significantly in PD pts (103±65.0 vs 120±64.2 pmol/l; p=.057). Serum sclerostin highly correlated with dialysis vintage (r=0.35, p<.001 in all, r=0.29, p<.001 in HD, r=0.54, p<.001 in PD), serum creatinine (0.39, p<.01 in all, r=0.30, p<.001 in HD, r=0.61, p<.001 in PD), FGF23 (r=0.31, p<.001 in all, r=0.22, p<.001 in HD, r=0.60, p<.001), and negatively with PTH (r=-0.24, p<.001) in all, r=-0.28, p<.001 in HD). Serum PTH correlated weakly with FGF23 in all (r=0.18, p=.007) and HD pts (r=0.17; p=.02). There were no differences in serum PTH between HD and PD groups.

Conclusions: Serum sclerostin levels are much higher in dialysis pts compared to healthy controls, with the lower values in women, diabetics and PD pts. Its negative correlation with PTH may reflect its role in the PTH signal transduction in osteocytes.The high positive correlation with FGF23 deserves further study.

TH-PO897

KDOQi- or KDIGO-Recommended PTH Levels Are Associated with Low Bone Turnover in Pre-Dialysis Chronic Kidney Disease Patients Romulo Nina Azevedo,[1] Fellype Carvalho Barreto,[2] Maria Eugenia F. Canziani,[1] Aluizio B. Carvalho.[1] *[1]Nephrology, Federal University of São Paulo, São Paulo, Brazil; [2]Nephrology, University of São Paulo, São Paulo, Brazil.*

Background: KDOQi and KDIGO-recommended PTH levels have been based on opinions and not in the bone turnover of pre-dialysis chronic kidney disease (CKD) patients. The aim of this study was to evaluate if the guidelines target PTH levels proposed are in accordance with bone turnover in that population.

Methods: Fifty consecutive asymptomatic CKD outpatients (51.7±10.4 yrs, 68% male, 36% african-american, body mass index: 26.2±4.3kg⁄m², eGFR:36.3±18 mL⁄min⁄1.73m² by CKD-EPI) were submitted to a tetracycline pre-labeled bone biopsy, followed by histomorphometric analysis. Bone turnover was classified as low, normal or high according to bone formation rate (BFR⁄BS; reference values: 0.13±0.07 and 0.07±0.03 μ^3/μ^2⁄day, for male and female, respectively). Low bone turnover was defined as BFR⁄BS < -1 standard deviation (SD) below mean (< 0.06 or < 0.04 μ^3/μ^2⁄day, for male or female, respectively). Normal to high bone turnover was defined as BFR⁄BS ≥ 1SD above mean (≥ 0.06 or ≥ 0.04 μ^3/μ^2⁄day, for male or female, respectively). Laboratorial evaluation included intact-PTH (iPTH) whose levels were classified as normal or high based on KDOQi or KDIGO recommendations.

Results: BFR/BS was 0.02±0.02 μ^3/μ^2⁄day, for male and female. Low turnover bone disease (LTBD) was found in 86% of the patients. Median of iPTH levels were 83 pg/mL (26 - 548). A positive correlation between iPTH and BFR⁄BS was observed (r=0.40;p=0.04). According to KDOQi, iPTH levels were normal or high in 42 and 58%, respectively. According to KDIGO, iPTH levels were normal or high in 28 and 72% of the patients, respectively. Overall, 56% or 70% of the LTBD patients had high iPTH levels, according to KDOQi or KDIGO, respectively.

Conclusions: In CKD patients: 1. PTH levels are frequently high, according to KDOQi or KDIGO targets recommendation; 2. PTH is correlated with bone formation rate; 3. However, high PTH levels are associated with LTBD.

Funding: Government Support - Non-U.S.

TH-PO898

Correlations of Tubular Reabsorption of Phosphate (TRP) According to Renal Function in CKD Patients Gun Hee An, Yu Ah Hong, Yul Hee Cho, Myung Hyun Lee, Jeong Gwan Kim, Hyun Chul Whang, Chul Woo Yang, Cheol Whee Park, Yong-Soo Kim. *Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital,College of Medicine, the Catholic University of Korea, Seoul, Republic of Korea.*

Background: Hyperphopshatemia are key contributors to chronic kidney disease-mineral and bone disorder. Recently, FGF23 and soluble α-Klotho is an emerging potential biomarker of phosphorus and vitamin D metabolism bur its use is limited. A tubular reabsorption of phosphate may be a simple and effective biomarker in CKD patients. The aim of this study investigates the correlation between TRP and renal function in CKD patients.

Methods: We performed an observational study in stable patients with CKD stage 1-5. TRP was calculated with the formula; 1- [(Up/Pp) x (Pcr/Ucr)] x100. Normal TRP was defined as over the 80%. The renal function was evaluated by calculating the estimated GFR with MDRD equation. Serum FGF23 and urine soluble α-Klotho were determined by a sandwich enzyme-linked immunosorbent assay system.

Results: From April 2011 to February 2012, 76 stable patients with CKD stage 1-5 enrolled. In normal TRP (>80%) group, while renal function was significantly higher than that of low TRP group (<80%), serum phosphate, alkaline phosphatase, intact PTH were significantly lower than those of low TRP group. Serum FGF23 level in lower TRP group was higher than that of normal TRP group. Urine Klotho level was the other way round. As glomerular filtration rate declined, TRP significantly decreased (R^2=0.561, P<0.001) and intact PTH increased (R^2=0.330, P<0.001) for the augmentation of phosphaturia. In predialysis CKD stage 1-4, TRP was significantly correlated with serum FGF23 (r=-0.348, P= 0.013), intact PTH (r=-0.411, P=0.003), whereas was not correlated with urine Klotho and serum phosphate. TRP also showed a significant association of GFR and intact PTH, but did not associate with FGF23 and soluble α-Klotho in multivariate linear regression analysis.

Conclusions: TRP is a useful early surrogate marker compared with serum FGF23 and urine soluble Klotho for the assessment of the phosphate metabolism of CKD patients.

TH-PO899

Pulse Wave Analysis Predicts Cardiovascular Mortality in Pre-Dialysis Chronic Kidney Disease Darren Green, Richa Sinha, James Ritchie, Smeeta Sinha, Helen Eddington, Philip A. Kalra. *Dept Renal Medicine, Salford Royal Hospital, United Kingdom.*

Background: Vascular stiffness is an independent predictor of outcome in end-stage renal disease. The aim was to assess whether it also predicts outcome in pre-dialysis CKD.

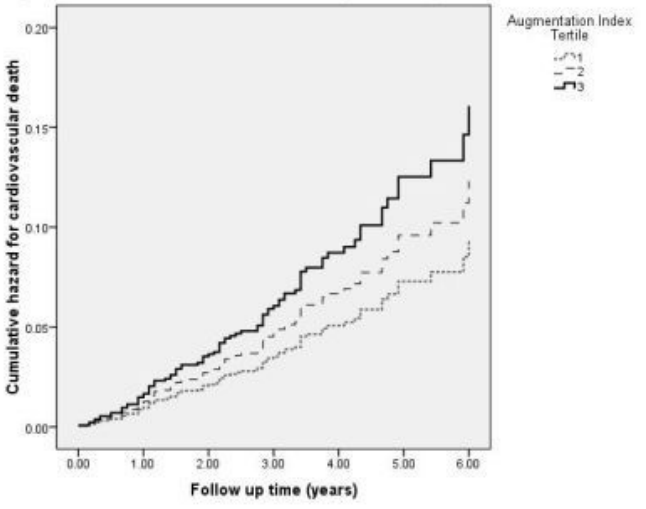
Methods: We performed pulse wave analysis using applanation tonometry in a cohort of 956 patients with CKD stages 3-5. We measured ejection duration (ED), augmentation index corrected for ED and heart rate (AIx), and systolic peak times (T_1 & T_2). Patients also had serum biochemistry. Follow up was to dialysis, death, or last appointment. Predictors of outcome were from forward stepwise Cox regression (inclusion in model if $\alpha \leq 0.05$ on univariate analysis). End-points were all-cause mortality (ACM) & cardiovascular death (CVD).

Results: Cohort characteristics were age 66±14 years, eGFR 45±12 mL/min/1.73m^2, 61% male, 29% diabetes, follow up 3.4±1.7 years, 201 deaths (21%), 98 CVD (49% of ACM).

The predictors of ACM were AIx, T_2, old age, low serum albumin, high urea and parathyroid hormone (PTH), smoking, coronary artery disease (CAD), and peripheral vascular disease (PVD). For CVD, only AIx, age, smoking, CAD, and PVD remained significant. eGFR was a better predictor of CVD than urea. Phosphorus & calcium were not significant in any model that included AIx.

The hazard ratios (HR) of AIx were: ACM HR=1.045 per % increase; CVD HR=1.040 (figure 1). The HR of AIx for CVD became less significant as eGFR fell: CKD 3 HR=1.090, p<0.001; CKD 4 HR=1.048, p=0.008; CKD 5 HR=1.007, p=0.877.

Figure 1. Rates of cardiovascular mortality according to tertiles of AIx



Conclusions: AIx is predictive of ACM and CVD in CKD. Serum biochemistry associated with vascular stiffness & previously shown to predict CVD are not significant in this model. Thus, these factors may increase CV risk by way of their role in vascular stiffness. The impact of AIx on outcome falls with eGFR. This is likely because of the influence of other risk factors.

TH-PO900

Changes in Insulin-Like Growth Hormone-1 (IGF-1) during First Year on Dialysis: Implications for Bone Status, Nutritional Status and Patient Survival Ting Jia, Thiane Gama Axelsson, Hong Xu, Sun-Hee Park, Tobias E. Larsson, Olof Heimburger, Peter F. Barany, Bengt Lindholm, Peter Stenvinkel, Abdul Rashid Tony Qureshi. *Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: Abnormalities in the IGF-1/growth hormone system contribute to catabolism in chronic kidney disease (CKD) patients (pts). In incident dialysis pts, a low IGF-1 level has been reported to be associated with low bone mineral density (BMD). We investigated relations between changes in IGF-1, nutritional status and BMD during the first year of dialysis, and the predictive power of changes in IGF-1 on mortality.

Methods: In 207 CKD stage 5 pts (median glomerular filtration rate 7 mL/min, median age 56 years, male 61%) starting on dialysis, fasting samples were obtained at baseline and after one year on dialysis for measurements of IGF-1 and biochemical parameters. Nutritional status was assessed by subjective global assessment (SGA) and bone mineral density (BMD) was measured by dual energy X-ray absorptiometry. Determinants of IGF-1 changes were analyzed by Mixed Model. Associations between levels of IGF-1, IGF-1 variation and mortality were evaluated by Kaplan Meier curve, and conventional and competing risks Cox proportional hazards models.

Results: The median concentration of IGF-1 increased from 181 (87-320) to 240 (113-413) ng/ml (p<0.05) after one year on dialysis. Both at baseline and after one year, IGF-1 was negatively associated with signs of poor nutritional status (SGA score >1) and correlated with BMD (baseline: rho=0.17; p<0.001; one year: rho=0.16; p=0.03). According to the Mixed model, phosphate, calcium and fat mass were significant determinants of IGF-1 change during first year dialysis. After adjusting for confounding factors, patients with stable high or increased IGF-1 had improved survival during 5-years follow-up.

Conclusions: During the first year on dialysis, a low IGF-1 level was associated with low BMD and poor nutritional status. Changes in IGF-1 were associated to phosphate, calcium and fat mass, and predicted survival. These results suggest that IGF- 1 has a key role in the interplay between nutritional status, fat mass and bone status in dialysis pts.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

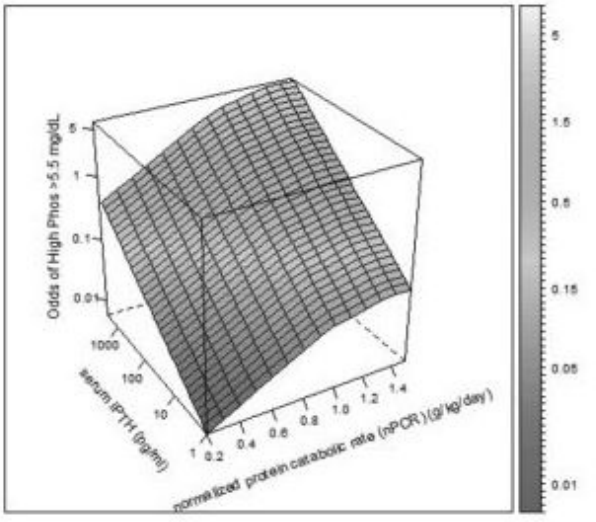
TH-PO901

Modeling the Likelihood of Hyperphosphatemia as a Combined Function of Serum Parathyroid Hormone and Dietary Protein Intake in Chronic Kidney Disease Leanne Streja,[1,2] Miklos Zsolt Molnar,[1] Elani Streja,[1] Joshua Zaritsky,[3] John J. Sim,[4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LABioMed at Harbor-UCLA; [2]City of Hope; [3]David Geffen School of Medicine at UCLA; [4]Kaiser Permanente; [5]University of Tennessee.*

Background: High phosphorus levels are associated with higher risk of death in hemodialysis patients. Previous studies have suggested that both higher serum intact parathyroid hormone (iPTH) levels and higher dietary protein intake may contribute to higher serum phosphorus. However, it is not well known how these two factors contribute to the risk of hyperphosphatemia simultaneously if both are taken into account. We hypothesized that the likelihood of hyperphosphatemia increases across higher serum iPTH and higher normalized protein catabolic rate (nPCR), a surrogate of protein intake.

Methods: Over an eight-year period, we identified 69,355 MHD patients with iPTH, nPCR and phosphorus data in a large dialysis provider. Logistic regression models were examined to assess the association between likelihood of hyperphosphatemia (P>5.5 mg/dL) across serum iPTH and nPCR increments.

Results: Patients were 61±15 years old and included 46% women, 33% blacks, and 57% diabetics. Both higher serum iPTH level and higher protein intake were associated with higher risk of hyperphosphatemia.



Compared to patients with iPTH level 150-<300 pg/ml and nPCR level 1.0-<1.2 g/kg/day, patients with iPTH>600 pg/ml and nPCR>1.2 g/kg/day had a 3 fold higher risk of hyperphosphatemia (OR:3.17, 95%CI:2.69-3.75).

Conclusions: Our findings demonstrate an association between higher serum phosphorus levels with both dietary protein intake and serum PTH level in MHD patients. Dietary interventions and correction of hyperparathyroidism should be considered in hyperphosphatemia management.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

TH-PO902

Parathormone and Bone-Specific Alkaline Phosphatase for the Follow-Up of Bone Turnover in Hemodialysis Patients: Is It so Simple? Pierre Delanaye, Francois Jouret, Jean-marie H. Krzesinski, Olivier Moranne, Etienne Cavalier. *Nephrology-Dialysis-Transplantation, University of Liège, CHU Sart Tilman, Liège, Belgium.*

Background: Chronic kidney disease (CKD) is associated with mineral and bone disorders (MBD). International guidelines suggest that levels of serum parathormone (PTH) or bone-specific alkaline phosphatase (b-ALP) can be used to evaluate MBD in dialysis patients. The evidence for such recommendation remains moderate and mostly based on transversal studies.

Methods: We retrospectively investigated the variations of PTH (ΔPTH) and b-ALP (Δb-ALP) serum concentrations over a short (6-weeks) and a long (one-year) period in a monocentric hemodialysis population. The proportion of patients reaching the critical difference (CD) (50% for PTH and 25% for b-ALP) was calculated.

Results: Seventy-seven patients were included. A significant correlation between PTH and b-ALP levels was found at baseline (r=0.75). By contrast, no correlation was observed between ΔPTH and Δb-ALP over a 6-week interval (r=0.14). Furthermore, contradictory variations were observed in 39 patients (51%). The CD for PTH and b-ALP was reached by 19 and 11 patients, respectively, with 2 patients showing consistent variations of both biomarkers. One year later, measurements of PTH and b-ALP were repeated in 48 survivors. No correlation was found between ΔPTH and Δb-ALP (r=0.27). Contradictory evolution

of PTH and b-ALP was observed in 39 patients (81%). The CD for PTH or b-ALP was reached by 24 patients and 28 patients, respectively, with 6 patients (12.5%) showing opposite results for both biomarkers.

Conclusions: This study shows the lack of correlation between ΔPTH and Δb-ALP over time in patients under chronic hemodialysis. Such discrepancy urges prospective and longitudinal trials to identify which biomarkers clinicians should rely on.

TH-PO903

CKD-MBD Therapy in Peritoneal Dialysis: Multicenter Retrospective Analysis (OPTIMHYST Study) Roberto Dell'Aquila,[1] Gianpaolo Amici,[2] Graziella Berlingò,[1] Laura Cappuccino,[3] Alex Cosaro,[4] Mario Cozzolino,[5] Emilio Giulio Galli,[6] Paolo Ghiringhelli,[7] Gian Maria Iadarola,[8] Alessandro Laudon,[9] Carlo Rugiu,[10] Stefano Saffioti,[3] Roberto Scarpioni,[11] Alessandra Trubian.[10] *[1]Neph & Dial, Hosp, Bassano; [2]Hosp, Treviso; [3]University, Genova; [4]Hosp, S Bonifacio; [5]Hosp, Milano; [6]Hosp, Treviglio; [7]Hosp, Cremona; [8]Hosp, Torino; [9]Hosp, Trento; [10]University, Verona; [11]Hosp, Piacenza, Italy.*

Background: CKD-MBD therapy in peritoneal dialysis (PD) is prescribed specifically orally. Our study evaluates clinical evolution and therapy of CKD-MBD with a data collection of 11 PD centers.

Methods: A retrospective, polycentric cohort study of PD patients (2010-2011) was carried out with a 1 year follow-up of 371 pts at baseline, 362 at 6 months and 326 at 12 months, 287 (77%) were prevalent and 84 (23%) were incident. At baseline, 6 and 12 months have been recorded: Ca, P, PTH, ALP, Alb, BMI, CLCR, KT/V, comorbidity and mortality. Therapy records were collected: VDRAs, natural vitamin D, phosphate binders and cinacalcet. A MANOVA RM model and Kaplan-Meier curves with the Log-Rank test were applied.

Results: Patients were 64±15 yrs-old, median time on dialysis was 15 months (IQ range 6-35). We recorded 30 deaths, 15 for CV causes. Prescription was: CAPD 38%, APD 54% and incremental 8% at baseline, baseline PTH was 314±264, at 6 months 317±272, at 12 months 329±282 ng/ml (p=ns); baseline P was 4.98±1.30, at 6 months 4.97±1.29, at 12 months 5.12±1.34 mg/dl (p=ns); baseline Ca was 9.06±0.79, at 6 months 9.15±0.78, at 12 months 9.10±0.75 mg/dl. At start 227 patients (61%) were treated with VDRAs, paricalcitol therapy was 10%, natural vit D was 7.8%, binders therapy was 75.5%, cinacalcet was 12.9%. We observed a significantly lower PTH level in paricalcitol vs calcitriol treated patients at 6 months (p=0.013). Survival analysis showed an advantage in patients treated from baseline with VDRAs (Log-Rank p=0.044).

Conclusions: At the first analysis of this PD database we found a good control of CKD-MBD with oral therapy. Similar to hemodialysis, in PD we observed a survival advantage in patients treated with VDRAs.

TH-PO904

The Costs of Treating Mineral Bone Disease in Dialysis Patients: Results of All Wales MBD Audit Shafi Malik,[1] Rajiva Ibakkanavar,[1] James A. Chess,[2] Aled O. Phillips,[1] Steve Riley.[1] *[1]Institute of Nephrology, University Hospital of Wales, Cardiff, United Kingdom; [2]Renal Medicine, Swansea Marriston Hospital, Swansea, United Kingdom.*

Background: Mineral bone disease significantly adds to the morbidity and mortality associated with CKD. CKD-MBD treatment targets are still disputed and we await outcome data to confirm benefit for patients. Newer agents that help achieve targets have led to increased treatment cost. We set out to audit the use and associated expenditure of CKD-MBD medications in Wales.

Methods: Renal units in Wales were contacted to provide information on all dialysis patients over a 3-month period in 2010. Demographics,dialysis adequacy and MBD results were collected. MBD drugs and dosages were confirmed with patients in the middle of the data collection period. A 3 month average for Calcium,Phosphate and a single PTH result was used for each patient. Drug expenditure was calculated by using publicly available prices.

Results: 1193 patients were included (76% HD, 19% PD, 5% HHD).

Mean phosphate level for the population was 1.59 ± 0.46 mmol/l. 62%, 72% and 55% of patients reached the phosphate, calcium and PTH targets respectively. The achievement of all 3 targets was seen in 17% of patients on UHD, 19% on PD and 20% on HHD.

25% were not on a phosphate binder. 42% were on calcium-based binders and 75% were on non-calcium binders(44% Sevelamer,15% Lanthanum,5% Aluminium). 46 patients with phosphate >1.8 mmol/l were not on a binder. Of those patients who did not achieve the phosphate target majority were on a non-calcium binder(75% v 50%).

The total annual cost was £1.2 million for all MBD medications. £970k came from non-calcium based phosphate binders(£695k Sevelamer, £275k Lanthanum) and £180k from Cinacalcet. The mean total cost per patient per year is around £1000. Cost decreased as number of MBD targets achieved increased(zero £1070; 1 £1050; 2 £1000; 3 £795).

Conclusions: Achievement of targets were similar across the different renal units and modalities. There was no significant difference in MBD control. The cost of achieving these targets is substantial and with increasing financial constraint further work is needed to optimize cost benefit ratio in this area of prescribing.

TH-PO905

Impact of Mineral Metabolism on Mortality in Hemodialysis Patients: Serum Phosphate Level Should Be Primarily and Consistently Controlled Masatomo Taniguchi,[1] Masafumi Fukagawa,[2] Naohiko Fujii,[3] Takayuki Hamano,[4] Kunitoshi Iseki,[5] Yoshiharu Tsubakihara.[4] *[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan; [3]Medical and Research Center for Nephrology and Transplantation, Hyogo Prefectural Hospital, Nishinomiya, Japan; [4]Department of Comprehensive Kidney Disease Research, Graduate School of Medicine, Osaka University, Osaka, Japan; [5]Dialysis Unit, University Hospital of the Ryukyu, Naha, Japan.*

Background: Mineral metabolism is affects mortality in hemodialysis patients and is identified by imbalances in serum phosphate (P), calcium (Ca), and parathyroid hormone (PTH).Most of previous studies have examined associations between baseline values at the start and subsequent survival.

Methods: We examined associations between annual mineral values (P, Ca, PTH) and mortality in a 3-year cohort (Dec 2006-2009) of 128,125 hemodialysis patients using three models, i.e. baseline, time-dependent and time-average Cox models. We also examined associations between achieved Japanese guideline targets (P: 3.5-6.0 mg/dL, corrected Ca 8.4-10.0 mg/dL, intact PTH 60-180mg/dL) and all-cause survival to elucidate which parameter should be controlled as a priority.

Results: High and low serum P (> 6.0 or ≤ 3.5 mg/dL), high Ca (> 9.5 mg/dL), higher PTH (> 300 pg/mL) and lower PTH (≤ 60 pg/mL) were significantly associated with high mortality in all three models (p< 0.01). A hazard ratio for mortality in hyperphosphatemia was higher when compared to other mineral levels. Patients (20% of subjects) who achieved all parameters simultaneously showed lowest mortality. Those who only met P targets had a lower risk of death (hazard ratio=1.17) compared to those that achieved Ca and PTH targets (1.41, 1.47, respectively). As time increased post achieving P and Ca targets, all-cause death risks diminished incrementally.

Conclusions: Among mineral values, Pwould be the strongest predictor for high mortality and should be the priority for control. Consistent achievement of P and Ca targets would lead to good survival in hemodialysis patients.

TH-PO906

Cinacalcet Increases FGF Receptor 1 Expression in Hyperplastic Parathyroid Glands from Uremic Patients Keiichi Sumida, Yoshifumi Ubara, Koki Mise, Masayuki Yamanouchi, Tatsuya Suwabe, Junichi Hoshino, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: In uremic patients, the depressed expression of both fibroblast growth factor receptor 1 (FGFR1) and its co-receptor Klotho in hyperplastic parathyroid glands has been demonstrated to underlie the pathogenesis of secondary hyperparathyroidism (SHPT) and its resistance to extremely high FGF23 levels, however, there have been no reports regarding the expression of both Klotho and FGFR1 in patients with SHPT after administration of cinacalcet hydrochloride (cinacalcet). This study was aimed to elucidate the effect of cinacalcet on the expression of FGFR1 and Klotho in hyperplastic parathyroid glands.

Methods: A total of 77 parathyroid glands, including 73 hyperplastic parathyroid glands from 18 dialysis patients with severe SHPT who required parathyroidectomy and 4 normal parathyroid glands resected together with thyroid tumor, were obtained at our institute. The 18 dialysis patients were divided into those treated with (cinacalcet group, n = 10) and without (non-cinacalcet group, n = 8) cinacalcet. Immunohistochemical staining for Klotho and FGFR1 was performed and the expression of Klotho and FGFR1 were semiquantitatively analyzed.

Results: Compared with normal glands, the hyperplastic parathyroid glands in each group showed significantly decreased Klotho and FGFR1 expression. Compared with the non-cinacalcet group, FGFR1 expression was significantly increased in the cinacalcet group (39 glands) ($P < 0.05$), while Klotho expression was not different between the groups ($P > 0.05$).

Conclusions: Our results suggest that cinacalcet increases the expression of FGFR1 in hyperplastic parathyroid glands with severe SHPT, and may contribute to an inhibitory effect on progression of SHPT in uremic patients. Furthermore, the insufficient Klotho expression may imply one of the reasons that parathyroidectomy is necessary in patients showing cinacalcet resistance.

TH-PO908

Treatment of Secondary Hyperparathyroidism in Maintanence Hemodialysis Patients: A Randomised Clinical Trial Comparing Paricalcitol, Calcitriol and Cinacalcet Siren Sezer,[1] Emre Tutal Tutal,[1] Zeynep Bal,[1] Fatma Nurhan Ozdemir Acar.[2] *[1]Nephrology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Istanbul, Turkey.*

Background: Secondary hyperparathyroidism is a common complication of chronic kidney disease. Treatment of SHPT includes VDR analogs and calcimimetics. The aim of this study is to evaluate and compare the effectiveness of these treatments as monotherapies and in combination in maintenance hemodialysis (MHD) patients with SHPT.

Methods: 114 subjects were included. After a washout period, patients who were already receiving calcitrol and having PTH levels >300 pg/mL were randomized into 4 groups: a) total serum calcium < 10.5mg/dL, serum Ca × P <75 and PTH level between

300-1000 pg/ml were randomized to recieve either paricalcitol (n: 31, group 1) or calcitriol (n: 32, group 2), b) normalized total serum calcium > 10.5mg/dL, serum Ca × P<75 and PTH level >1000 pg/ml were randomized to recieve either cinacalcet plus paricalcitol (n: 29, group 3) or cinacalcet plus calcitriol (n: 22, group 4).

Results: Study groups were similar in means of demographic characteristics. Patients in group 3 and 4 had significantly higher Ca, P and CaxP product levels at the first 3 months of study (p<0.01) however in following 9 months these levels were statistically similar in all groups. When PTH levels were assessed and compared to basal values we observed a significant reduction (-9.8%) in group 1 while there was an increament in group 2 (%24.3, p< 0.008). When we compared groups 3 and 4 in means of iPTH reduction ratio at the end of 1 year group 3 had higher reduction ratios (-33.7%) compared to group 4 (-6.1%, p:0.011). The mean cinacalcet dose initiated were similar in group 3 and 4 while the mean cinacalcet dose significantly increased in patients receiving calcitriol versus paricalcitol (42.0±15.2 mg and 25.2±16.1 mg, p<001).

Conclusions: This study showed that paricalcitol treatment reduced PTH concentrations more effectively without causing hypercalcemia and hyperphosphatemia than calcitiriol therapy either alone or in combination with calcimimetics. We suggest that paricalcitol should be prefered to calcitriol for the treatment of MHD patients with moderate to severe SHPT.

TH-PO909

Beneficial Effects of Paricalcitol on Left Ventricular Hypertrophy and Myocardial Fibrosis in Rats with Chronic Renal Failure Sara Panizo, Natalia Carrillo-Lopez, Sara Barrio-Vazquez, Amalia Fernandez-Vazquez, Jose L. Fernandez-martin, Manuel Naves, Jorge B. Cannata-Andia. *Bone and Mineral Research Unit, HUCA, Instituto Reina Sofía de Investigación, REDinREN del ISCIII.*

Background: Cardiovascular disease is the main cause of death in CKD patients. Abnormalities of left ventricular structure and function are frequent among these patients. Vitamin D is associated with decrease in cardiovascular mortality. To further explore this topic we investigated in rats with chronic renal failure (CRF) the heart morphological and molecular effects of an active vitamin D compound, paricalcitol (PC).

Methods: CRF Wistar rats, carried out by 7/8 nephrectomy, were treated intraperitoneally with 30ng/kg/day of PC 5 days per week over 4 weeks. CRF+vehicle and Sham groups served as control. Morphological and molecular parameters were measured to evaluate cardiomyocytic and fibrotic changes.

Results: PC reduced the adaptive left ventricular hypetrophy (LVH) that has 2 main components 1)the hypertrophy of the muscular tissue evidenced by the increase of cardiomyocytes size, 2)the increase of fibrosis triggered by the hypertrophic myocytes and the increase of collagen by fibroblasts. On one hand, PC decreased the morphological parameters of LVH, wall thickness (2.29±0.2 vs 1.88±0.18) and cardiomyocytes size (10.67±1.16 vs 9.78±0.48), as well as the molecular parameters: BNP heart expression (5.64±2.45 vs 0.94±0.70) (marker of ventricular myocyte stress that precedes the cardiac hypertrophy) and erk1/2 phosphorilation (1.95±0.37 vs 0.85±0.19) (hypertrophic growth signal). On the other hand, PC reduced the fibrosis assessed by Masson and Sirius staining by increasing heart levels of collagenase MMP1 (0.55±0.26 vs 2.07±0.24) avoiding collagen accumulation.

Conclusions: In non-treated CRF rats all these changes lead to an increase of heart fibrosis at the expense of losing muscle tissue. In contrast, in the PC treated rats a significant reduction in the fibrosis was observed. The advantageous proportion of muscle/fibrotic tissue observed with the use of PC that can not be detected using conventional methods (nuclear magnetic resonance/echocardiography) can have a beneficial effect on cardiac function.

Funding: Pharmaceutical Company Support - Abbott Pharmaceuticals (ACA-SPAI-08-22), Government Support - Non-U.S.

TH-PO910

Fibroblast Growth Factor 23, Left Ventricular Mass Index, and Left Ventricular Hypertrophy in Community-Dwelling Older Adults Anna Jeanette Jovanovich,[1] Joachim H. Ix,[2] Kim Mcfann,[1] Ronit Katz,[3] Bryan R. Kestenbaum,[3] Ian H. de Boer,[3] Mark J. Sarnak,[4] Michael Shlipak,[5] Kenneth J. Mukamal,[6] David Siscovick,[3] M. Chonchol.[1] *[1]University of Colorado; [2]University of California San Diego; [3]University of Washington; [4]Tufts Medical Center; [5]University of California San Francisco; [6]Beth Israel Deaconess Medical Center.*

Background: Fibroblast growth factor 23 (FGF23) regulates phosphorus and calcitriol homeostasis. In chronic kidney disease (CKD), high FGF23 levels are associated with left ventricular hypertrophy (LVH) and death. The relationship between FGF23 with left ventricular mass index (LVMI) and LVH in the general population and the influence of CKD remains uncertain.

Methods: Plasma C-terminal FGF23 concentrations were measured in 2255 women and men >65 years in the Cardiovascular Health Study with echocardiogram data for LVMI and LVH. Cross-sectional associations of plasma FGF23 levels with LVMI and LVH were examined with linear and logistic regression models adjusted for age, sex, race, body mass index, clinic site, smoking, diabetes, hypertension, systolic blood pressure, C-reactive protein, Cystatin C-estimated glomerular filtration rate ($eGFR_{cys}$) and urine albumin-creatinine ratio (ACR).

Results: Participant characteristics were as follows: mean age, 78±5 years; 64% women; 29% black; 32% CKD ($eGFR_{cys}$ < 60ml/min/1.73m^2or ACR ≥ 30mg/g); and median FGF23 level 70 (IQR 53-99) RU/mL. Higher plasma FGF23 levels were associated with greater LVMI (ß=3.90, [95% CI 2.58, 5.22]) and greater odds of LVH (OR=1.35 [95% CI 1.17, 1.57]) per doubling FGF23 after multivariate adjustment. These associations were stronger among those participants with CKD (p interaction=0.005): LVMI β=5.66 [95% CI 3.51, 7.81] and LVH OR=1.46 [(95% CI 1.20, 1.77] per doubling FGF23. The corresponding associations in those without CKD were (β=2.01 [95% CI 0.58, 3.57]) for LVMI and (OR=1.19 [95% CI 0.96, 1.47]) for LVH per doubling FGF23.

Conclusions: In a large cohort of community-dwelling adults >65 years, higher FGF23 levels were associated with greater LVMI and LVH, with a stronger relationship in participants with CKD.

Funding: NIDDK Support

TH-PO911

2MD, a Highly Potent Analog of Calcitriol, Suppresses Parathyroid Hormone Levels in Rat Models of Hyperparathyroidism Julia B. Zella,[1,2] Lori A. Plum,[1,2] Margaret Clagett-Dame,[1,2] Hector F. Deluca.[1,2] *[1]Biochemistry, University of Wisconsin-Madison, Madison, WI; [2]Deltanoid Pharmaceuticals, Inc., Madison, WI.*

Background: 1α,25-Dihydroxyvitamin D and its analogs have been used successfully to suppress parathyroid hormone (PTH) levels in chronic kidney disease (CKD) patients with secondary hyperparathyroidism (SHPT). In order to identify new vitamin D analogs with therapeutic potential, we screened over two dozen novel vitamin D compounds with modified side chains and/or A-rings using the 5/6 nephrectomy (NX) rat model of CKD. Select compounds were also evaluated in combination with the angiotensin-converting enzyme inhibitor, enalapril. A novel, less-invasive model of hyperparathyroidism secondary to vitamin D deficiency in normo-calcemic rats is also described.

Methods: Young male Sprague-Dawley rats were used for both models of SHPT. A high phosphorus-containing purified diet was used to induce elevated PTH levels in NX rats. Rats were administered vitamin D analogs either orally or intraperitoneally, depending on the experiment. In a second model of hyperparathyroidism, normal calcium levels were restored in vitamin D deficient rats through the use of a purified diet containing high levels of lactose, calcium and phosphorus.

Results: Of the compounds we tested, one particular analog, 2-methylene-19-nor-20S-1α,25$(OH)_2D_3$ (2MD or DP001), shows particular promise. 2MD is a highly-potent analog of calcitriol that has recently been shown to suppress PTH in post-menopausal women. In NX rats, 2MD significantly suppresses PTH levels without raising serum calcium and when combined with enalapril, significantly decreases proteinuria compared to untreated NX rats. 2MD also normalizes PTH levels in vitamin D deficient rats, independent of calcium.

Conclusions: We have identified several new analogs of vitamin D that have potential therapeutic use for the treatment of SHPT. 2MD is the lead candidate for development due to its potency and safety profile. An oral formulation of 2MD is currently in Phase 2 clinical trials in hemodialysis patients with SHPT.

Funding: Pharmaceutical Company Support - Deltanoid Pharmaceuticals, Inc., Private Foundation Support

TH-PO912

The Switch from Calcitriol to Paricalcitol Decreases Calciuria in Predialysis CKD Patients Isabel Martínez,[1] Ramon M. Saracho,[2] Adriana S. Dusso.[3] *[1]Nephrology, Hospital de Galdakao, Usansolo, Vizcaya, Spain; [2]Nephrology, Hospital Universitario de Alava, Vitoria, Alava, Spain; [3]Experimental Nephrology Lab, IRBLleida, Lleida, Spain.*

Background: Serum levels of calcitriol decrease early in the course of CKD. Because reductions in serum calcitriol (CTRL) contribute to the development of secondary hyperparathyroidism (SHPT), CTRL replacement has been the therapy of choice to prevent/slow the progression of SHPT. However, the doses of CTRL required to suppress SHPT in CKD can cause hypercalcemia and/or hyperphosphatemia. Therefore, a CRTL analog, paricalcitol, was developed to suppress SHPT with less hyperphosphatemic and hypercalcemic actions. Several reports dispute the actual achievement of this goal. Thus, the aim of this study is to compare the effects of paricalcitol and CTRL treatment on plasma and urinary calcium and phosphate levels and on serum PTH.

Methods: We studied 73 patients (40 women) CKD stages 3 to 5, with average (SD) values for Age: 73 (11) years; CrCl corrected by 1.73 m^2= 34.9 (17) ml/min. Patients were treated with CTRL first and then switched to paricalcitol.

Results: The table shows similar levels of serum calcium, phosphate and PTH, urinary phosphate excretion, and proteinuria during CTRL or paricalcitol treatments. Importantly, calciuria was lower during paricalcitol treatment (p= 0.04). As expected, paricalcitol reduced serum CTRL through inducing its degradation.

	Mean(SEM) Calcitriol	Mean(SEM) Paricalcitol	**p (significance)**
Serum Creatinine. mg/dl	2.7(0.2)	2.8(0.2)	NS
eGFR (MDRD) ml/min	26.4(2.1)	25.8(2.1)	NS
Creat. Clearance ml/min	31.1(2.4)	28.8(2.3)	NS
Calcitriol pg/ml	32.7(2.6)	28.5(2.0)	NS
PTH pg/ml	135.0(12.0)	134.0(8.8)	NS
Total Calcium mg/dl	10.11(0.10)	10.08(0.10)	NS
Phosphorus mg/dl	3.7(0.1)	3.8(0.1)	NS
Ionized Calcium mg/dl	5.08(0.05)	5.06(0.05)	NS
Calciuria mg/day	58.1(7.4)	48.9(6.1)	0.04
Phosphaturia mg/day	591.1(27.8)	581.4(34.4)	NS
TPR %	58.7(2.0)	58.4(1.8)	NS
Urinary Protein/Creat. g/g	1.10(0.21)	1.17(0.26)	NS

Conclusions: A lower effect of paricalcitol on intestinal calcium absorption decreases urinary calcium excretion compared to CTRL, suggesting that paricalcitol treatment of SHPT results in a lower systemic calcium overload.

Funding: Government Support - Non-U.S.

TH-PO913

Loss of Function Mutations of *CYP24A1*, the Vitamin D 24-Hydroxylase Gene, Cause a Novel Adult Dent-Like Kidney Stone Disease

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Background: Kidney stone disease is a common problem with diverse etiology, including genetic and environmental factors. Dent's disease is an X-linked hereditary renal tubular disorder, characterized by nephrolithiasis, nephrocalcinosis, hypercalciuria, low-molecular-weight proteinuria and renal failure**.** Recently, loss-of-function mutations of *CYP24A1*, the gene encoding for 1,25-dihydroxyvitamin D_3, 24-hydroxylase, were identified in idiopathic infantile hypercalcemia. Here we describe the clinical and molecular basis of a long standing kidney stone disease, with a hypercalcemic adult Dent-like phenotype caused by *CYP24A1* mutations.

Methods: Three subjects from two Jewish Israeli families with a Dent-like phenotype were clinically characterized. DNA was extracted and the genes responsible for Dent's disease, *CLCN5* and *OCRL1*, as well as *CYP24A1*, were sequenced.

Results: All subjects presented with nephrolithiasis at a young age and had hypercalcemia, hypercalciuria and nephrocalcinosis. Serum PTH levels were very low, serum $25(OH)D_3$ and $1,25(OH)_2D_3$ were high and serum $24,25(OH)_2D_3$ was low. All subjects suffered from severe kidney stone disease, the cause of which was not deciphered for decades despite extensive workup. The oldest subject had low-molecular-weight proteinuria and developed chronic renal failure (serum creatinine is 2.3 mg%). Sequencing of *CLCN5* and *OCRL1* was normal. Instead, we detected three *CYP24A1* loss-of-function mutations: a homozygous deletion (del E143) in family 1 and compound heterozygous mutations, L409S and the novel W268-stop, in family 2.

Conclusions: Mutations in *CYP24A1* gene may cause severe kidney stone disease with a Dent-like phenotype. It may present in adults with no vitamin D supplement and may lead to chronic renal failure. Our results support a recessive mode of inheritance. Further studies are needed to ascertain the prevalence and clinical significance of altered *CYP24A1* function.

Funding: Government Support - Non-U.S.

TH-PO914

Role of Parathyroid Hormone in Renal Failure Induced Downregulation of Liver Cytochrome P450 in a Knockout Mouse Model

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Background: Chronic renal failure (CRF) is associated with a decrease in drug metabolism secondary to a reduction of cytochromes P450 (CYP450) and phase II metabolism enzymes such as N-acetyltransferase. This downregulation was observed in rat and mouse models of chronic renal failure. However, the mechanisms involved remain poorly understood. One potential mechanism involves parathyroid hormone (PTH). Indeed, it was shown, in rats, that parathyroidectomy prior to nephrectomy prevented the decrease in Cyp3a expression and activity. The purpose of this study is to confirm these results using PTH knockout (PTH-/-) mice.

Methods: Four groups of mice were studied: CRF induced by 3/4 nephrectomy in PTH-/- and WT (PTH+/+) mice and two control groups of mice which underwent sham surgeries (CTL-/-, CTL+/+). Liver protein expression and mRNA level of the major CYP450 isoform Cyp3a11 was measured by western blot and real-time PCR, respectively. Cyp3a activity was also assessed by the N-demethylation of erythromycin.

Results: Our results demonstrate that liver Cyp3a11 protein and mRNA expression is similar in CTL+/+ and CTL-/- mice. While CRF+/+ mice exhibit a 30% decrease in Cyp3a11 liver protein expression (as observed in WT C57BL/6 mice), CRF-/- mice do not show any significant reduction in Cyp3A11. Similar results were obtained for Cyp3a11 specific mRNA. Finally, Cyp3a11 activity was similar in CTL+/+, CTL-/- and CRF-/- mice, while we found a reduction of Cyp3a11 activity in CRF+/+ mice.

Conclusions: These results strongly support a significant role for PTH in the downregulation of liver CYP450 enzymes in CRF. We have yet to identify the mechanisms by which PTH can downregulate CYP450 expression and activity.

Funding: Private Foundation Support

TH-PO915

Impact of Lanthanum Carbonate on FGF23 in Chronic Kidney Disease Stages 3-5

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Background: FGF23 plays an important role in the regulation of phosphate (PO4), vitamin D and parathyroid hormone (PTH) metabolism. As CKD progress, PO4 retention with hyperparathyroidism develops. Serum PO4 levels are not a good early marker of PO4 retention, whereas increases in FGF23 which help maintain normal PO4 balance may be a better indicator of PO4 retention and subsequent development of hyperparathyroidism. The purpose of this study was to determine if reducing FGF23 levels with lanthanum could prevent or reverse the development of hyperparathyroidism.

Methods: A double blind randomized placebo controlled study was performed in subjects with CKD stages 3-5 to determine if treatment with lanthanum carbonate 500 mg pc or placebo could reduce FGF23 and PTH. Subjects were treated for 60 days and doses increased to 1000 mg pc for phosphorus > 5.5 mg/dl. We report data on 19 randomized patients. One way ANOVA was used to assess significance.

Results: Twenty two patients were randomized to placebo versus treatment. Two patients in treatment group and 1 in placebo group dropped out secondary to GI side effects. Data from 10 patients on Lanthanum and 9 patients in placebo are presented. There was no change in FGF23(%) calcium, PO4, 25 vitamin D and change in PTH (%) levels in patients treated for 60 days with placebo. There were significant declines in FGF23 (%) levels treated with lanthanum for 60 days.

Impact of Lanthanum Carbonate versus Placebo on Markers of Mineral Metabolism

	PLACEBO GROUP			LANTHANUM GROUP		
Serum values	Time 0 (Mean) SD	60 Days (Mean) SD	P value	Time 0 (Mean) SD	60 Days (Mean) SD	P value
% Change FGF23 (RU/ml)	100	4.92±25.3	0.69	100	-30.5±23.5	**0.03**
Calcium (mg/dl)	9.7±0.5	9.6±0.4	0.98	9.7±0.3	9.6±0.3	0.598
Phosphorus(mg/dl)	3.9±0.7	4±0.67	0.51	4±0.6	4.2±0.6	0.644
% Change PTH	100	37±123.1	0.54	100	-19.6±27	0.10
25(OH) Vitamin D(ng/ml)	42.1±12.7	41.7±16	0.96	32.9±14.7	28.5±14.9	0.78
1,25 Vitamin D(ng/ml)	37.7±19.3	28.2±12.4	0.24	23.5±4.6	28.1±9.4	0.30

Conclusions: Lanthanum carbonate given with meals significantly reduces FGF23 with no changes in calcium, PO4 and vitamin D levels.

Funding: Pharmaceutical Company Support - Shire

TH-PO916

Upregulation of FGF23 by Glucocorticoid Decreases Bone Longitudinal Growth and Mineralization

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Background: Glucocorticoids (GC) are used as immunosuppressant therapy in renal transplant. However, in pediatric kidney transplant (PKT) patients GC reduce bone mineralization and longitudinal growth. FGF23 is a phosphaturic protein secreted by osteocytes that reduces mineralization of bone cells in culture. In a previous clinical study we demonstrated a increased $[FGF23]_p$ in PKT patients treated with GC, as compared to patients that didn't receive GC. We hypothesize that GC directly stimulate FGF23 synthesis in bone, increasing $[FGF23]_p$.

Methods: Male SD rats (60-80g) were treated with prednisone (PD, 2mg/Kg) or vehicle (control) for 20 days. We evaluated longitudinal bone growth and mineralization, $[FGF23]_p$, bone FGF23 mRNA and protein abundance (10 and 20 days).

Results: PD treated rats showed lower weight gain (79±6 %, P<0,05). As compared to control group, the length and weight of tibia in PD group were lower (92±4% and 86±5%, 10 and 20 days respectively; P<0,01), with thinner epiphyseal cartilage (39±5%, P<0,01). PD group showed a significant increase of $[FGF23]_p$ at 10 and 20 days (324±23% and 160±39% of control levels respectively, P<0,03). Moreover, bones from PD rats showed uprgulation of FGF23 mRNA and protein (5 fold vs control; P<0,05). To evaluate the direct effect of GC on FGF23 expression, rat metatarsal bones were cultured with dexamethasone (Dex; 1nM) or vehicle for 8 days. Dex decreased the longitudinal growth, but increased the osseous abundance of FGF23 (3±1 fold vs control; P<0,05). Pharmacological blockade of GC receptor (RU486) prevented the effects of Dex. Finally, we evaluated the effect of recombinant FGF23 on metatarsal growth in vitro (6 days). 450 pM FGF23 decreased growth and mineralization, an effect prevented by PD173074 (competitive inhibitor of the FGF23 receptor, FGFr1c.)

Conclusions: Our results suggest that GC directly upregulate FGF23 in bone via the glucocorticoid receptor, reducing mineralization and growth.

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Funding: Government Support - Non-U.S.

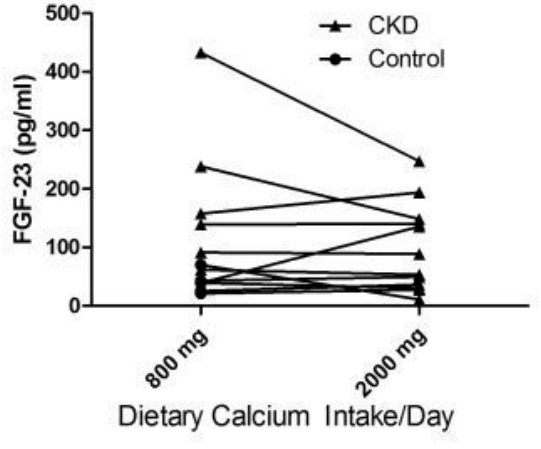
TH-PO917

Dietary Calcium Intake Does Not Modulate FGF-23 Levels David M. Spiegel,[1] Leigh Darryl Quarles,[2] Valentin David.[2] *[1]Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO; [2]Division of Nephrology, University of Tennessee Health Science Center, Memphis, TN.*

Background: The understanding of mineral and bone disorders seen in progressive chronic kidney disease (CKD) now incorporates FGF-23 as an early hormonal marker of the disease process. The factors responsible for the regulation of FGF-23 include serum $1,25(OH)_2D$, phosphorus levels and PTH and bone mineralization. There is some evidence in VDR null mice that a diet containing high calcium also stimulates FGF-23, either through changes in serum concentrations or modulation of bone mineralization.

Methods: We questioned whether dietary calcium modulates FGF-23 in 6 control and 6 subjects with advanced CKD (eGFR 20-33 ml/min/1.73m^2) who received 2 experimental diets for 9 days each in a cross-over study with at least 1 week between diets. Diets contained either 800 or 2000 mg of elemental calcium and 1600 mg of phosphorus. Following 7 days of outpatient diet subjects were admitted to the clinical trials unit and continued on the control diet for 48 hrs while formal calcium balance studies were performed (KI 81:1116-22,2012). For this analysis, FGF-23 levels were measured fasting on the AM of their second inpatient day. CKD subjects were compared to controls by the unpaired t-test while differences within individuals on the 2 diets were evaluated by the paired t-test.

Results: FGF-23 levels were significantly higher in CKD than control subjects (187±55 vs. 40±7; p=0.02). There was no significant change in FGF-23 levels in the entire group (p=0.43) or in either the control (p=0.69) or CKD (p=0.27) subgroups when ingesting the 800 or 2000 mg calcium diets (fig). The serum calcium levels were not different on these 2 diets.



Conclusions: While calcium may modulate FGF-23 in some experimental setting, we found no evidence for an effect of dietary calcium on serum FGF23 levels.

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TH-PO918

Broadening of the Target Range for Parathyroid Hormone Levels: The Impact on Facility-Level Practice Patterns and Patient Control David M. Spiegel,[1] Jamie Heise,[2] J. Brian Copley,[2] Moshe Fridman.[3] *[1]University of Colorado Denver; [2]Shire Pharmaceuticals; [3]AMF Consulting.*

Background: In chronic kidney disease, management of mineral and bone disorders is challenging, and a lack of randomized outcome trials means optimal therapeutic targets are controversial. Over-suppression of parathyroid hormone (PTH) can lead to low turnover bone disease and high levels promote high bone-turnover. While both are associated with increased fracture risk, the optimal PTH level remains uncertain. A large dialysis organization modified their target PTH range in Sep 2010 from 150–300 pg/mL to 150–600 pg/mL to be more consistent with KDIGO guidance (~130–600 pg/mL). This analysis aimed to identify changes in practice patterns and achievement of target PTH levels following this change.

Methods: Attainment of the PTH target range by ≥50%, and ≥75% of facility patients during Sep 2010 and Aug 2011 was compared. The proportion of patients receiving active vitamin D analogues, cinacalcet, and different phosphate binders during these two months was also compared. Data were provided by 1930 facilities (112,724 patients), and 1993 facilities (105,882 patients), in Sep 10 and Aug 11, respectively.

Results: Comparing Sep 10 with Aug 11, the percentage of facilities with ≥50% of patients in the target PTH range decreased from 93.2% to 90.6% (p=0.003), and those with ≥75% of patients in range decreased from 37.4% to 31.2% (p<0.0001). For the same months, the proportion of patients using active vitamin D compounds increased from 81% to 84% (p<0.0001), the use of cinacalcet decreased from 30% to 28% (p<0.0001), calcium-based binder use increased from 44% to 46% (p<0.0001), and the use of lanthanum and sevelamer decreased from 15% to 13% (p<0.0001) and 66% to 64% (p<0.0001) respectively.

Conclusions: Between Sep 10 and Aug 11, the percentage of facilities achieving the target PTH range of 150–600 pg/mL decreased. This may have occurred if physicians placed less emphasis on PTH control following the range revision. This finding also highlights the differences between targeted and achieved outcomes and suggests that if the PTH goal is <600 pg/mL, intervention needs to be implemented before this value is reached.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

TH-PO919

Targeted Deletion of Klotho in Kidney Distal Tubules Causes a Disturbed Mineral Metabolism Hannes Olauson,[1] Karolina Lindberg,[1] Risul Amin,[1] Ting Jia,[1] Annika Wernerson,[2] Göran Andersson,[2] Tobias E. Larsson.[1] *[1]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden; [2]Department of Laboratory Medicine, Karolinska Institutet and Karolinska University Hospital Huddinge, Stockholm, Sweden.*

Background: Renal Klotho controls mineral metabolism through direct regulation of tubular phosphate (Pi) and calcium (Ca) reabsorption, and functions as an obligate co-receptor for the phosphaturic and vitamin D regulating hormone fibroblast growth factor-23 (FGF23).

Methods: We generated a novel mouse model with a partial deletion of Klotho in distal tubular segments by crossing floxed Klotho to Ksp-cadherin-Cre mice (Ksp-KL$^{-/-}$).

Results: In contrast to systemic Klotho *null* mice, Ksp-KL$^{-/-}$ mice were fertile, had a normal gross phenotype and an essentially normal renal histology without signs of vascular or tubular calcification. However, Ksp-KL$^{-/-}$ mice were hyperphosphatemic with an abundant expression of the sodium-Pi co-transporter Npt2a at the brush border membrane and increased FGF23 levels corresponding to the degree of Klotho deletion. Serum Ca and 1,25D were unaltered, whereas parathyroid hormone was decreased. TRPV5 protein, which mediates tubular Ca reabsorption, was reduced paralleled by a mild increase in Ca excretion. Renal expression of vitamin D regulatory enzymes Cyp27B1, Cyp24A1 and the vitamin D receptor (VDR) were higher compared to wild-type controls, suggesting increased turnover of vitamin D metabolites and possibly a functional increase in VDR signaling. There was a threshold effect of residual Klotho expression, where approximately 70% deletion of Klotho resulted in an exponential increase in FGF23. Importantly, FGF23 was elevated in a subgroup of Ksp-KL$^{-/-}$ mice with normal serum Pi and 1,25D levels, suggesting a Klotho-derived renal-bone feedback loop.

Conclusions: Renal FGF23-Klotho signaling, which is disrupted in chronic kidney disease, is essential for homeostatic control of mineral metabolism.

Funding: Government Support - Non-U.S.

TH-PO920

Fibroblast Growth Factor 23 Is a Risk Factor for Left Ventricular Hypertrophy in the Chronic Kidney Disease in Children (CKiD) Cohort Anthony A. Portale,[1] Lisa Aronson Friedman,[2] Mark Mitsnefes,[2] Myles S. Wolf,[3] Susan L. Furth,[2] Bradley A. Warady,[2] Harald Jüppner,[4] Isidro B. Salusky.[5] *[1]Pediatrics, UCSF, San Francisco, CA; [2]CKiD Investigator; [3]Medicine, Univ. of Miami, Miami, FL; [4]Massachusetts General Hospital, Boston, MA; [5]Pediatrics, UCLA, Los Angeles, CA.*

Background: Cardiovascular disease is the leading cause of death in children with chronic kidney disease (CKD). Plasma fibroblast growth factor 23 (FGF23) concentration increases early in the course of progressive CKD, and in adults is a risk factor for left ventricular hypertrophy (LVH) and death. Whether FGF23 is associated with cardiovascular outcomes in children across the spectrum of CKD is not known.

Methods: We performed echocardiograms and measured plasma C-terminal FGF23 in 385 children, ages 1-19 years, mean 11 ± 4 (SD), with CKD stages 2-4 enrolled in the observational CKiD study. A second assessment was made in 175 patients, and a third in 23, at 2 year intervals. GFR was determined by plasma clearance of iohexol (n=313) or the CKiD estimating equation. We used multivariate, longitudinal regression analysis to model the prevalence of LVH using all available data, accounting for repeated measures.

Results: Median GFR was 45 ml/min/1.73 m^2 (IQR: 34-57). The overall prevalence of LVH, defined as LVMI/height^2.7 >95%tile for age and gender, was 16%. The overall prevalence of either systolic (11%) or diastolic (10%) hypertension was 17%. Median FGF23 was 133 RU/ml (IQR: 88-199), 2.3-fold higher than that in healthy children, and values increased progressively with declining GFR. Median FGF23 was 23 RU/ml higher in the children with LVH than in those without LVH. We found that log FGF23 was a significant predictor of LVH (OR 1.6, 95% CI 1.1-2.4, p=0.025), after adjusting for diastolic hypertension (OR 2.8, CI 1.3-5.9, p<0.01), female gender (OR 2.5, CI 1.3-4.6, p<0.005), body mass index (OR 1.1, CI 1.1-1.2, p<0.001), age (OR 0.9, CI 0.8-0.97, p<0.01), and age-adjusted serum phosphorus (OR 1.2, CI 0.98-1.4, p=0.08); GFR was not a predictor.

Conclusions: Increased plasma FGF23 concentration is an independent risk factor for the presence of LVH in children with pre-dialysis CKD.

Funding: NIDDK Support, Pharmaceutical Company Support - Genzyme, Abbott

TH-PO921

Intravenous Phosphate Loading Increases Fibroblast Growth Factor 23 in Uremic Rats Ai Nakazawa,[1] Masahide Mizobuchi,[1] Hiroaki Ogata,[2] Chiaki Kumata,[2] Fumihiko Koiwa,[3] Eriko Kinugasa,[2] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Japan; [2]Department of Internal Medicine, Showa University Northern Yokohama Hospital, Japan; [3]Division of Nephrology, Department of Medicine, Showa University Fujigaoka Hospital, Japan.*

Background: Fibroblast growth factor 23 (FGF23) plays a crucial role in phosphate metabolism in chronic kidney disease (CKD). Oral phosphate loading and calcitriol stimulate FGF23 secretion although precise mechanisms underlying the stimulation of FGF23 remain to be studied. We compared the effect of oral phosphate loading with that of intravenous loading on FGF23 levels in normal and 5/6th nephrectomized uremic rats.

Methods: Uremic rats (Nx) and sham-operated rats (Sham) were fed a 0.5% phosphate diet for 2 weeks and then divided by 3 groups, 1) with the same 0.5% phosphate diet (NP), 2) with a high phosphate (0.8%) diet (HP), and 3) the NP rats with intravenous phosphate infusion (2M of solution, 20μl/hr) using a micro infusion pump (IV). Blood and urine were obtained 1 day (acute phase) and 7 days (chronic phase) after the interventions.

Results: Both in acute and chronic phases, serum phosphate levels and fractional excretion of phosphate (FEP) were comparable between HP and IV in both Sham and Nx rats, respectively. Serum phosphate levels in both HP and IV were significantly higher than those in NP only in chronic phase of Sham and Nx rats, respectively. FEP in both HP and IV were significantly higher than that in NP in acute and chronic phase of Sham and Nx rats, respectively. In acute phase of Sham and Nx rats, FGF23 levels were comparable among 3 groups (NP, HP, and IV) while in case of chronic phase FGF23 levels in HP were significantly higher than those in NP, and this increase was further pronounced in IV only in Nx rats.

Conclusions: These results show that chronic intravenous phosphate loading increases FGF23 indicating alternative sensing mechanisms for FGF23 other than dietary route (gut) may exist in CKD.

TH-PO922

Fibroblast Growth Factor 23, Interleukin-6 and Mortality in CKD Jair Munoz Mendoza,[1] Tamara Isakova,[1] Julia J. Scialla,[1] Amanda Hyre Anderson,[2] Jing Chen,[3] Jiang He,[3] Matthias Kretzler,[4] Sankar D. Navaneethan,[5] Lavinia A. Negrea,[6] Lisa C. Nessel,[2] Dawei Xie,[2] Sylvia E. Rosas,[2] Huiliang Xie,[1] James P. Lash,[7] Dominic S. Raj,[8] Myles S. Wolf.[1] *[1]U Miami; [2]U Pennsylvania; [3]Tulane U; [4]U Michigan; [5]Cleveland Clinic; [6]Case Western U; [7]U Illinois; [8]G. Washington U.*

Background: Elevated levels of FGF23 are associated with inflammation and each is associated with increased mortality. We tested whether interleukin-6 (IL6) contributes to the relationship between FGF23 and death in CKD.

Methods: We used Cox proportional hazard regression in 3879 individuals enrolled in the Chronic Renal Insufficiency Cohort Study. We adjusted for demographics, kidney function, and traditional risk factors.

Results: During a median follow-up of 3.5 years, 268 participants died (20.4/1000 person-years). Individually, higher levels of natural log (ln) transformed FGF23 (HR per 1SD of lnFGF23, 1.6; 95%CI 1.4-1.8) and IL6 (HR per 1SD of lnIL6, 1.4; 95%CI 1.3-1.6) were independently associated with greater risk of death in adjusted analyses. When lnFGF23 and lnIL6 were both included in a single model, the risk of death associated with lnFGF23 (HR per 1SD of lnFGF23 1.4; 95% CI 1.3-1.6) and lnIL6 (HR per 1SD of lnIL6, 1.4; 95%CI 1.2-1.5) were minimally attenuated. In crude analyses, there was significant interaction between IL6 and FGF23 on the risk of death (p for lnFGF23 x lnIL6=0.03). In stratified analyses above and below the respective medians, participants with high levels of both FGF23 and IL6 had the highest event rates. However, there was no interaction in adjusted analyses (p for interaction=0.6).

	Rate/1000-person-years	HR (95% CI)	
		Crude	Adjusted
Low IL6 low FGF23	6.2 (3.9-8.5)	Ref	Ref
Low IL6 high FGF23	16.5(11.4-21.5)	2.7(1.6-4.3)	2.2(1.3-3.8)
High IL6 low FGF23	17.0(11.8-22.1)	2.7(1.7-4.6)	2.2(1.3-3.7)
High IL6 high FGF23	40.8(34.5-47.1)	6.6(4.4-9.9)	4.2(2.6-6.8)

Conclusions: Elevated levels of FGF23 and IL6 are each independently associated with greater risk of death in CKD. These independent and additive risks suggest that high FGF23 and IL6 levels may influence risk of mortality through distinct pathways.

Funding: NIDDK Support

TH-PO923

What Do We Get when We Measure Serum 25(OH)Vitamin D Levels? Ramon M. Saracho,[2] Isabel Martínez,[1] Iratxe Ajuria Morentin,[3] Edurne Bereciartua,[3] Carmen Mar,[3] Adriana S. Dusso.[4] *[1]Nephrology, Hospital de Galdakao, Usansolo, Vizcaya, Spain; [2]Nephrology, Hospital Universitario de Alava, Vitoria, Alava, Spain; [3]Clinical Lab, Hospital de Galdakao, Usansolo, Vizcaya, Spain; [4]Experimental Nephrology Lab, IRBLleida, Lleida, Spain.*

Background: CKD patients have a high prevalence of nutritional vitamin D (VitD) deficiency. Guidelines recommend to correct it through supplementation with this pro-hormone, based on its potential beneficial effects. However, a previously unrecognized determinant of the "VitD deficiency epidemic" could be an underestimation of serum VitD levels. In fact, several cases of VitD intoxication with hypercalcemia, and even ARF presented at our institution, which were caused by excessive VitD supplementation. These findings raised concerns about the accuracy of current methods to measure VitD status. The aim is to compare the accuracy of the 5 most commonly used immunochemical methods to measure serum 25(OH)D3+25(OH)D2 compared to the gold standard: liquid chromatography-mass spectrometry LC-MS/M.

Methods: We measured 25(OH)D2+D3 in aliquots of 107 serum samples from CKD patients obtained during the same month using all 5 available methods: Architect, Advia Centaur®, Cobas® e411, Liaison®, IDS-iSYS and LC-MS/MS. Results were compared using Passing Bablok regression.

Results: The table depicts the estimates of the regression lines comparing all 5 methods vs. LC-MS/MS. There is a clinically significant variability between different analytical methods of 25(OH)D3+25(OH)D2. The Liaison method used in our institution underestimated serum 25(OH)D2+25(OH)D3 levels by 27.3%. Only one method, Cobas, has a regression line superimposed to that of LC-MS/MS. Equations are provided to correct serum values from any of the available methods by the most accurate reference LC-MS/MC.

	Slope	Intercept	R coefficient
Architect	.750 (.681;.850)	4.7 (3.0;5.9)	.877
Centaur	.631 (.562;.690)	5.3 (4.3;7.0)	.921
Cobas	.993 (.900;1.116)	-.7 (-2.5;1.3)	.907
Liaison	.727 (.667;.818)	.5 (-1.2;1.7)	.824
IDS-iSYS	1.030 (.961;1.123)	5.8 (4.4;7.5)	.924

Conclusions: When considering initiating VitD supplementation, we should be cautious about the analytical method used in our medical center to measure VitD to avoid overdosage.

Funding: Government Support - Non-U.S.

TH-PO924

Serum FGF-23 Levels Predicts Low Bone Turnover in Adult CKD-5D Patients Marie-Claude M. Faugere,[1] Valentin David,[2] Hanna W. Mawad,[1] Leigh Darryl Quarles,[2] Hartmut H. Malluche.[1] *[1]Division of Nephrology, University of Kentucky, Lexington, KY; [2]Division of Nephrology, University of Tennessee, Memphis, TN.*

Background: Low bone turnover (LBT) is a common histologic finding of renal osteodystrophy. It has deleterious consequences, including increased risk of fractures and vascular calcifications.

Methods: To test the hypothesis that serum levels of FGF-23 may reflect the level of bone turnover in CKD-5D patients better than customarily used tests, 36 CKD-5D patients (15 men and 21 women, mean age: 50±2 yrs, dialysis vintage: 65±5.83 mos.) underwent iliac crest bone biopsies and blood drawings for determination of concentrations of FGF-23, intact PTH (iPTH), bone specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRAP), calcium and phosphorus.

Results: There were 19 patients with LBT and 17 patients with normal to high bone turnover. Serum FGF-23 levels ranged from 18 to 37,349 pg/mL. Patients with LBT had significantly lower serum FGF-23 than patients with normal to high bone turnover (3,128±1,294 vs. 15,094±3,207 pg/mL, resp., $p<0.001$). FGF-23 correlated with parameters of bone mineralization, in particular with mineralization lag time (rho=-0.654, $p<0.001$), but not with osteoid thickness (rho=0.113, ns). In addition, it correlated weakly with serum phosphorus (rho=0.53, $p=0.001$) and iPTH (rho=0.39, $p<0.05$). IPTH correlated with histomorphometric parameters of static and dynamic parameters of bone remodeling (rho=0.42-0.78), but not with mineralization parameters. Logistic regression to predict LBT showed that among FGF-23, iPTH, BAP and TRAP, the only significant predictor was FGF-23 ($p<0.01$). With a cut-off level of FGF-23<2,000 pg/mL, the sensitivity of FGF-23 to diagnose LBT was 79% with a specificity of 80%. The positive predictive value was 94% and the negative predictive value was 80%. No comparable cut-off level could be established for iPTH and the other measured parameters.

Conclusions: Determination of FGF-23 in serum is useful for noninvasive diagnosis of LBT (adynamic bone disease) in patients with CKD-5D. Serum measurements of iPTH, BAP and TRAP do not provide the same diagnostic information.

Funding: NIDDK Support

TH-PO925

Cinacalcet Lowers Serum Fibroblast Growth Factor-23 Concentration Independently from Serum Ca, P, PTH and Active Vitamin D Treatment in Peritoneal Dialysis Patients Hyo-jin Kim, Yon Su Kim, Curie Ahn, Ki Young Na, Kook-Hwan Oh. *Division of Nephrology, Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea.*

Background: Elevated serum levels of fibroblast growth factor-23 (FGF23) is associated with adverse outcomes in dialyzed patients. The CUPID study compared the efficacy of a cinacalcet-based regimen with conventional care (vitamin D and P binders) for achieving the stringent NKF-K/DOQI targets for PD patients. Additionally, we analyzed change in FGF23 levels between two treatments to explore the cinacalcet effect in lowering FGF23.

Methods: This is a multicenter and open-label study. Sixty six patients were randomly assigned to treatment with either cinacalcet + oral vitamin D (cinacalcet group, n=33) or oral vitamin D alone (control group, n=33) to achieve K/DOQI targets. CUPID included a 4-week screening for vitamin D washout, a 12-week dose-titration, and a 4-week assessment phases. We calculated mean values of iPTH, Ca, P, Ca x P, during assessment phase and final FGF23 to assess the outcome.

Results: 72.7% (n=24) of the cinacalcet group and 94% (n=31) of the control group completed the study. Cinacalcet group received 30.2±18.0 mg/day of cinacalcet and 0.13±0.32 μg/d oral vitamin D ($P < 0.001$ vs control with 0.27±0.18 μg/d). Achievement of iPTH goal of 150 to 300pg/ml (24.3% vs 18.2%, $P = 0.764$) was not different for both groups. Cinacalcet group achieved targets of Ca x P and Ca earlier than control group($P < 0.01$) and significantly decreased corrected Ca level ($P < 0.01$). Cinacaclet group had a significant decrease in median FGF23 levels (from 3,960 to 2,325 RU/ml, $P = 0.002$), compared with control group (2,085 to 2,415 RU/ml) and a significant difference in percent change of FGF23 (-42.54% vs 15.83%, $P = 0.008$). In multivariate regression analyses, cinacalcet treatment was an independent determinant of the serum FGF23 reduction, which was not associated with age, sex, diabetes, serum Ca, serum P, serum iPTH, and active vitamin D dose.

Conclusions: Cinacalcet lowers the FGF23 independently from its effects on the serum Ca, P, and iPTH.

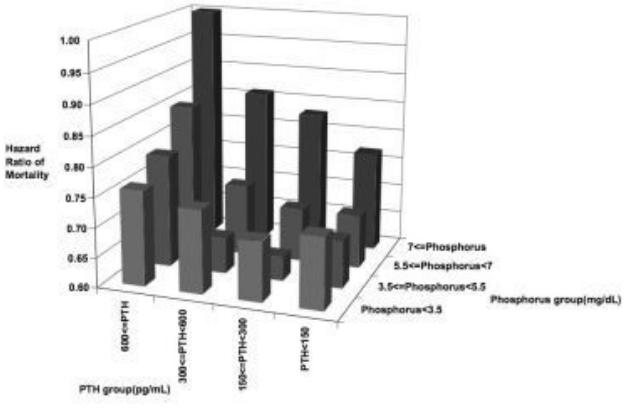
TH-PO926

Association of the Combined Serum Parathyroid Hormone and Phosphorus Levels with Mortality in 107,000 Maintenance Hemodialysis Patients Hsin-yi Wang,[1] Miklos Zsolt Molnar,[1] Joshua Zaritsky,[2] John J. Sim,[3] Elani Streja,[1] Csaba P. Kovesdy,[4] Isidro B. Salusky,[2] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LABioMed at Harbor-UCLA, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Kaiser Permanente; [4]Nephrology, University of Tennessee, Memphis, TN.*

Background: Both high and low phosphorous and parathyroid hormone (PTH) levels are associated with higher mortality risk in patients on maintenance dialysis (MHD). However, the associations of PTH and phosphorus combinations are based on cross-sectional studies; therefore this study is aimed to explore the influences of survival rate in MHD patients based on different combinations of PTH and phosphorus levels.

Methods: We examined the survival impact of serum PTH and phosphorous level in a 5-year (7/2001-6/2006) cohort of 107,299 outpatients in dialysis clinics of a large dialysis organization using Cox models adjusted for case-mix and surrogates of Malnutrition-Inflammation Complex.

Results: In this cohort the mean age (mean±SD) was 64±15 years old and included 45% women, 33% African-Americans, and 59% diabetics. In the fully adjusted models, patients with highest level of PTH (≥600 pg/mL) combined with highest phosphorus (≥7 mg/dL) had the highest mortality risk, whereas the best survival (HR: 0.64, 95%CI: 0.61-0.67) was observed with PTH of 150-300 pg/mL was combined with phosphorus of 3.5 to 5.5 mg/dL(Figure).



Conclusions: Patients with PTH between 150 and 300 (pg/mL) combined with phosphorus between 3.5 and 5.5 (mg/dL) have the best survival; thus, new target serum P levels in MHD will need to be defined.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

TH-PO927

Fibroblast Growth Factor 23 Is a New Predictor of Aortic Artery Calcification in Maintenance Haemodialysis Patients Zijin Chen,[1] Xiaonong Chen,[1] Jingyuan Xie,[1] Xiaobo Ma,[1] Fang Zhong,[1] Liang Hou,[2] Huawei Ling,[2] Xiao Li,[1] Hong Ren,[1] Nan Chen.[1] *[1]Nephrology Department, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China; [2]Radiology Department, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China.*

Background: To investigate the risk factors associated with high plasma FGF23 levels in MHD patients and to determine whether plasma FGF23 level is related to aortic artery calcification(AAC).

Methods: The study included 120 MHD patients and 20 healthy controls. We collected clinic data and pre-dialysis blood tests. FGF23 was measured by ELISA. AAC was detected by a lateral lumbar X-ray plain and read by two radiologists who were blinded to the participants using a semi-quantitative scoring method independently.

Results: Plasma FGF23 levels were significantly higher among MHD patients compared to controls(median FGF23 level 5601.27RU/m in MHD patients).Significant correlations were observed between FGF23 and LgVintage($r=0.413, P<0.001$),LgiPTH ($r=0.283, P=0.002$),P($r=0.587, P<0.001$),Ca($r=0.419, P<0.001$),25(OH)D($r=0.195, P=0.033$),BUN($r=0.263, P=0.004$) and SCR($r=0.415, P<0.001$).Stepwise multiple regression analysis showed that multiple variables were independently associated with FGF23. They were P($\beta=0.206, P<0.001$),Ca($\beta=0.392, P<0.001$),LgPTH($\beta=0.318, P<0.001$), SCR($\beta=0.001, P=0.001$) and prealbumin($\beta=-0.002, P=0.015$)(adjusted for sex,age,LgVintage,ALP,25(OH)D,BUN,UA,TG,TC,HDL and LDL).There were 60.83% patients had visible calcification in the abdominal aorta.Median AAC score was 2.0(0-21) and median AAC segments affected was 1.0(0-4).According to the FGF23 quartiles, AACscore(AACS) in the first quartile was 0.97±1.59,increased to 6.50±6.92 in the forth. Bivariate analysis showed that AACS correlated with LgFGF23($r=0.371, P<0.001$),P($r=0.201, P=0.028$),Ca($r=0.216, P=0.018$),Lgvintage($r=0.404, P<0.001$),age($r=0.395, P<0.001$) and DBP($r=0.395, P=0.026$).Stepwise logistic analysis showed that the independent parameters associated with AAC were age (OR=1.088,P<0.001),TP(OR=2.366,P=0.005) and LgFGF23(OR=0.921,P=0.06).

Conclusions: Plasma FGF23 level is significant increased in MHD patients and is independently associated with aortic artery calcification detected by simple X-ray plain.

TH-PO928

Long-Term Cholecalciferol Supplementation in Hemodialysis Patients: A One-Center Randomized Pilot Study Mariusz Mieczkowski,[1] Pawel Zebrowski,[1] Jerzy Przedlacki,[1] Stanislaw Niemczyk,[2] Janusz Sierdzinski,[3] Joanna Matuszkiewicz-Rowinska.[1] *[1]Nephrology, Medical University of Warsaw; [2]Nephrology, Military Institute of Medicine; [3]Medical University of Warsaw, Poland.*

Background: Vitamin D plays an essential role not only in mineral metabolism, but has also potent pleiotropic extraskeletal effects. Despite a widespread 25(OH)D (25D) deficiency, data on its supplementation in hemodialysis (HD) pts are scarce with only one small unpublished randomized study.We assessed the effect of cholecalciferol on vitamin D status, parathyroid activity and bone mineral density (BMD) in this population.

Methods: 19 pts (age 53±10years, on dialysis 55±63 months) with 25D<20 ng/ml were randomized into: Group A receiving cholecalciferol (2000 IU p.os 3x/week) during HD) and Group B (control). Patients with hypercalcemia, hyperphosphatemia and those receiving vitamin D or calcimimetics were excluded. In addition to routine testing serum 25D, 1,25D, iPTH and alkaline phosphatase (ALP) were examined every 2 months. Dual-energy X-ray absorptiometry was performed at the proximal femur, lumbar spine and forearm, before and after the study.

Results: There was a significant increase in serum 25D in Group A, from 13.5±4.67 to the average of 43.5±8.44 ng/ml (vs 14.0±3.09 to 19.3±7.42 ng/ml in Group B, $p<0.001$). As a result, in Group A serum 1,25D increased from 18.2±14.6 to the average of 41.6±8.86 pmol/l, while in Group B, the corresponding values were 12.1±5.50 and 25.0±9.85 pmol/l ($p=0.009$). Opposite to 25D, serum 1,25D didn't demonstrate any seasonal variations in both groups.The treatment was associated with a moderate increase in serum calcium (from 2.22±0.20 to the average of 2.31±0.15 mmol/l, $p=0.043$) and phosphates (from 1.66±0.32 to 1.98±0.25, mmol/l NS), while serum iPTH and ALP, as well as BMD Z-scores remained unchanged in both groups.

Conclusions: Oral cholecalciferol at a weekly dose of 6000 IU is a cheap and safe way to treat vitamin 25D deficiency in HD pts leading to a significant increase in serum1,25D, however it may be insufficient for parathyroid glands suppression.

TH-PO929

FGF-23 in Patients with Chronic Kidney Disease Stage 5D: Intact or C-Terminal? Mariusz Mieczkowski,[1] Pawel Zebrowski,[1] Ewa Wojtaszek,[1] Antoni Sokalski,[2] Edyta Gieglis,[3] Tomasz Stompor,[4] Wieslaw Klatko,[5] Janusz Grochowski,[6] Ignacy Jarzyllo,[7] Jerzy Przedlacki,[1] Stanislaw Niemczyk,[8] Janusz Sierdzinski,[1] Zofia Wankowicz,[8] Joanna Matuszkiewicz-Rowinska.[1] *[1]Nephrology, Medical University of Warsaw; [2]Radom Hospital; [3]Otwock Nephrocare; [4]Warmia@Mazury University; [5]Ciechanow Hospital; [6]Makow Hospital; [7]Wolomin Hospital; [8]Military Institute of Medicine.*

Background: High serum FGF-23 concentration is an important negative predictor of morbidity and mortality in patients with chronic kidney disease (CKD). The aim of the study was to assess the usufulness of two assays evaluating different FGF23 fragments: intact FGF and C-terminal in this population.

Methods: 252 patients with CKD stage 5D from 9 units were enrolled into the study. 210 were on hemodialysis (HD) and 42 on peritoneal dialysis (PD). Mean age was 59±15 years, and dialysis vintage 45±49 months. All blood samples were centrifuged and serum immediately transferred on ice to our core lab, where stored in -70°C until further use. Both iFGF-23 and cFGF23 concentrations were measured using the immunoenzymatic sandwich type assays (ALPKO, Salem, USA).

Results: Both studied tests correlated positively (r=0.24, p<0.001) with each other. The study showed very high serum iFGF23 (438±362 pg/ml) and cFGF23 (4447±3783 RU/ml) in all patients. The values were more than 1.5 x lower in PD group and about 3 x lower in diabetics. There were high correlations of both iFGF23 and cFGF23 with serum phosphate: in all patients (r=0.51, P<0.001 and r=0.45, p<0.001), in HD (r=0.50, p<0.001 and r=0.43, p<0.001) and PD (r=0.48, p=0.002 and r=0.53, p<0.001) and the weaker correlations with serum calcium: in all patients (r=0.20, p=0.002 and r=0.23, p<0.001) and in HD r=0.22, p=0.00 and r=0.28, p<0.001), as well as with serum iPTH in all patients (r=0.24, p<0.001 and r=0.18, p=0.007) and in HD (r=0.25, p<0.001 and r=0.17, p=0.02). A positive correlation between FGF-23 and the dialysis vintage may reflect the impact of residual renal function, diminishing with time.

Conclusions: The usefulness of both FGF-23 tests in patients with CKD stage 5D is similar.

TH-PO930

New Insights into Vitamin D-Related Mineral Metabolism in Nephrotic Syndrome Michelle Denburg,[1] Ian H. de Boer,[2] Dean Carlow,[1] Amy York,[1] Rene Chun,[3] Martin Hewison,[3] Mary B. Leonard.[1] *[1]Children's Hosp of Philadelphia, Philadelphia, PA; [2]Univ of Washington; [3]UCLA Orthopaedic Hosp.*

Background: Patients with nephrotic syndrome (NS) have low 25-hydroxyvitamin D (25D) levels, attributed to urinary loss of vitamin D-binding protein (DBP) and albumin (Alb), but vitamin D (vitD) metabolism in NS remains poorly understood.

Methods: Cross-sectional study of 20 NS and 30 control participants (ages 3-40 yr). NS participants were younger (p <0.001); sex, race and season did not differ between groups. Serum measures: 25D, 24,25D, DBP, Alb, PTH, fibroblast growth factor 23 (FGF23), ionized calcium (ICal), and phosphorus (Pi). Free 25D was calculated using total 25D, DBP and Alb. Urine measures: total protein, creatinine (cr), DBP, and 25D, the latter by a novel HPLC-MSMS assay. Multivariate linear regression was used to identify correlates of 25D and PTH.

Results: Total and free 25D, 24,25D, and ICal were lower, and FGF23 higher in NS. Serum DBP and PTH did not differ between groups.

Comparison of NS vs. Control

Measure*	NS	Control	p**
SERUM			
25D ng/ml	8.8 ± 8.8	22.4 ± 8.5	<0.0001
Alb g/dl	2.6 (2.1, 3)	4.3 (4.2, 4.5)	<0.0001
DBP mg/dl	16.9 (9.6, 27.6)	17.1 (10.1, 24.6)	0.81
Free 25D pg/ml	2.9 (2.2, 3.8)	8.8 (6.9, 16.6)	<0.0001
24,25D ng/ml	1.5 (1.0, 1.7)	2.5 (1.8, 4.0)	0.0034
ICal	1.17 ± 0.06	1.22 ± 0.05	0.0015
Pi mg/dl	4.7 ± 0.9	4.1 ± 0.8	0.021
PTH pg/ml	32.8 (20.8, 47.0)	42.3 (32.4, 51.7)	0.14
FGF23 pg/ml	52.1 (37.7, 65.1)	31.2 (28.1, 39.5)	0.0012
URINE			
Protein:cr	6.2 (3.5, 9.2)	0.04 (0.03, 0.05)	<0.0001
DBP:cr	0.004 (0.0005, 0.02)	0.00003 (0.00002, 0.00005)	<0.0001
25D pg/ml	197 (64.1, 685)	0 (0, 0)	0.019

*Median (IQR) or mean ± SD. **Rank-sum or t-test.

In multivariate analysis, the association between NS and both forms of 25D was explained by Alb but not serum DBP. The expected inverse relationship between PTH and 25D was found in the control (p <0.02) but not the NS group (p=0.6; interaction p=0.07). Higher FGF23 levels in NS were independent of Pi and eGFR.

Conclusions: This is the first study to demonstrate reduced free 25D in NS. Despite heavy urinary loss of DBP in NS, serum DBP was preserved. PTH was not increased in NS despite lower ICal and vitD. These findings suggest that aberrant vitD-related mineral metabolism in NS extends beyond DBP loss.

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TH-PO931

α-Klotho in CKD Sandro Mazzaferro, Silverio Rotondi, Lida Tartaglione, Cristiana Leonangeli, Giuliana Pirrò, Marzia Pasquali. *Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, Sapienza University, Rome, Italy.*

Background: Membrane α-Klotho is a co-factor for FGF23 and a decrease in membrane a-Klotho in CKD may prevent the actions of FGF23. Secreted α-Klotho results from the shedding of membrane α-Klotho, which is expressed in renal tubules. A decrease in soluble α-Klotho could indicate a decrease of membrane α-Klotho. The possible clinical significance of circulating α-Klotho in CKD is still a matter of debate.

Methods: We assayed serum α-Klotho, FGF23, vitamin D and parameters of mineral metabolism in 70 pts(59±16y) with CRF stage 2 to 4(eGFR 45±22ml/min) and in 10 controls(34±12y;eGFR 95±19ml/min).

CKD parameters

Klotho,pg/ml	Ca s,mg/dl	P s,mg/dl	BALP,U/l	PTH,pg/ml	25D,ng/ml	1,25D,pg/ml	FGF23,pg/ml
519,8±183,4	9,4 ±0,6	3,5 ±0,7	24,2 ±13,0	60,0 ±31,7	23,7 ±11,1	24,8 ±13,2	73,8 ±51,8

Results: Levels of α-Klotho were lower than normal(519±184vs983±523 pg/ml,p<.0001), a difference that was evident since stage 2 CRF(611±191vs983±523,p<.007). In front of mild vitamin D insufficiency(25D:23±11) patients had 1,25D values at the lower limit of normality, mild increment of PTH and normal mean values of Ca, P and bone AP. Serum levels of FGF23 were increased as compared to controls(73±51vs30±12,p<.03).

α-Klotho correlated with renal function(r=.427,p<.001) but not with age. Moreover, the relationship was negative with FGF23(r=-.331,p<.01), P(r=-.251,p<.05) and PTH(r=-.279,p<.05) and positive with Ca(r=.305,p<.01). Importantly no correlation existed with fractional excretion of P.

Conclusions: Our data indicate a significant negative effect of renal function on circulating levels of α-Klotho which seems to start very early in the disease. Low circulating α-Klotho could be responsible for reduced phosphaturia(lower direct effect) thus becoming a stimuli for FGF23 secretion. In addition, if soluble Klotho mirrored transmembrane Klotho, low circulating levels could indicate the occurrence of FGF23 resistance in renal tubuli. As for Ca homeostasis, soluble α-Klotho, even though reduced, seems to preserve its direct action on serum Ca. In summary, soluble α-Klotho drops early in the course of renal disease and seems to contribute, in this setting, to some derangements of mineral metabolism.

TH-PO932

Low PTH and High Circulating Sclerostin Levels Are Independently Associated with Low Bone Specific Alkaline Phosphatase Levels in Hemodialysis Patients Liesbeth Viaene,[1] Geert J. Behets,[2] Pieter Evenepoel,[1] Patrick C. D'Haese.[2] *[1]Laboratory of Nephrology, Catholic University Leuven, Leuven, Belgium; [2]Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium.*

Background: Sclerostin, a Wnt antagonist, produced by osteocytes inhibits osteoblastic activity and is hypothesised to counteract PTH-induced high bone turnover. Preliminary bone histomorphometry data indicate that sclerostin may be superior to PTH as a non-invasive indicator of bone turnover. The present study aimed to identify determinants of circulating sclerostin levels in hemodialysis patients and tests the hypothesis that sclerostin and PTH have opposite effects on bone-specific alkaline phosphatase (bsAP) and carboxyterminal cross-linking telopeptide(CTX).

Methods: Biochemical parameters of bone and mineral metabolism, including whole PTH (in-house bio-intact immunoradiometric assay and Liaison, Diasorin), calcidiol (Total 25 OHD, Liaison, DiaSorin), calcitriol (RIA, DiaSorin), FGF23 (ELISA, Kainos), DKK-1 (ELISA, Biomedica), sclerostin (ELISA, Biomedica), bsAP(BAP Ostase, Liaison, DiaSorin) and CTX (Serum Crosslaps ELISA, IDS) were assessed in 100 hemodialysis patients (40 male, age 68±13yrs, dialysis vintage 40 (16-69) months, residual renal function (RRF) 0.6±1.3 ml/min/1.72m^2).

Results: In multivariate linear regression analysis, the presence of diabetes (p=0.04), male gender (p=0.01), lower bsAP (p<0.0001) and lower RRF (p=0.002) were identified as independent determinants of higher levels of circulating sclerostin levels(R^2=0.33, p<0.0001). The correlation between both PTH assays was good (r^2=0.99; p<0.0001). Both higher PTH and lower serum sclerostin levels are independently associated with higher bsAP levels (R^2=0.34, p<0.0001). Serum PTH, but not sclerostin was independently associated with serum CTX levels in hemodialysis patients (R^2=0.11, p=0.005).

Conclusions: Diabetes mellitus, male gender, low bsAP, and low RRF are independent risk factors for high circulating sclerostin levels. High circulating sclerostin levels and low PTH levels are independently associated with low bsAP levels, reflecting low bone formation.

TH-PO933

Association between Vitamin D Receptor Polymorphisms and PTH Levels in CKD5 Patients on Dialysis Olynka Vega,[1] Maria de Los Angeles Mendoza-De la Garza,[1] Mara Medeiros,[2] Ricardo Correa-Rotter.[1] *[1]Nephrology, National Institute of Medical Sciences, Mexico, Distrito Federal, Mexico; [2]Nephrology, Hospital Infantil de Mexico, Mexico, Distrito Federal, Mexico.*

Background: The possible influence of vitamin D receptor (VDR) gene polymorphisms on the regulation of the calcium-PTH-vitamin D axis is highly relevant in chronic kidney disease. The aim of the study was to explore the frequency of VDR polymorphism rs7975232 (Apa 1), rs731236 (Taq 1), and rs1544410 (Bsm 1) and their association with PTH, calcium, and phosphate serum levels in Mexican patients on dialysis.

Methods: 143 prevalent dialysis patients were included. Biochemical determinations: calcium, phosphate, and PTH. VDR genotypes were analyzed as RFLPs using Apa 1, Bsm 1, and Taq 1 enzymes.

Results: Mean age 45.6±16.9, 60% male, 55.5% HD. All allelic and genotypic frequencies were in Hardy-Weinberg equilibrium. Genotypic frequencies for Apa 1: 73% Aa, 20% aa and 7% AA; for Bsm 1: 77% Bb, 15% BB and 8% bb; for Taq 1: 52% Tt, 46% TT and 2% tt. Patients who were homozygous for allele A and B had lower serum PTH concentrations (p=<0.001), independent to calcium and phosphate levels. Five patients were homozygous for A, B and T and presented lower PTH levels [median PTH 301 (range: 76 - 475) Vs 548 (range: 114 - 5422), P= 0.001]. Patients homozygous for T had higher serum calcium concentrations (9.8 vs 9.5, p=0.028). There were no significant differences in use of phosphate binders and oral calcitriol between compared groups (Table 1). Likewise, time on dialysis was also no different between groups.

Table 1. Biochemical parameters between different groups of polymorphisms.

	AA n=9	Aa + aa n=134	p	BB n= 22	Bb+ bb n= 121	p
PTH, pg/mL**	337 (77-979)	727 (23-5423)	0.003	429 (77-1240)	760.9 (23-5423)	0.008
Ca+, mg/dL	9.9±1.2	9.6± 0.9	0.49	10.2±1.1	9.5±0.9	0.06
P+, mg/dL	5.1±2.1	5.8±2.0	0.29	6.0±2.1	5.7±2.0	0.50

**Median (min-max)

Conclusions: The most common genotype for Apa 1, Taq 1 and Bsm 1 in Mexican patients on dialysis is the heterozygous. Homozygous patients for the allele A or B had lower PTH levels. This diversity of the VDR gene expression may confer variable sensitivity to the vitamin D in specific individuals.

TH-PO934

Vitamin D Insufficiency or Not? It Depends on the Used Assay! Hamid R. Dezfoolian, Ola G. Samuelsson, Maria Svensson. *Department of Nephrology, Sahlgrenska Academy, Sahlgrenska University Hospital, Gothenburg, Sweden.*

Background: Vitamin D deficiency and insufficiency have been linked to a variety of chronic diseases. Vitamin D deficiency is defined as a serum concentration of 25-OH-vitamin D <20 ng/mL (50 nmol/L) and insufficiency as 21–29 ng/mL (50-75 nmol/L). Different assays may show different levels of serum 25-OH-D, and thereby affect treatment strategies. The aim of this sub-study was to evaluate the performance of two different assays of serum 25-OH-D and how vitamin D_3 supplementation may affect the performance of these methods.

Methods: Twenty-one non-diabetic patients with chronic kidney disease (CKD stage 3-4) and vitamin D insufficiency measured by the radioimmunoassay (RIA) method were included. Patients were randomized in a placebo-controlled, two-way cross-over study to receive daily either 3200 IU (80 µg) vitamin D_3 (cholecalciferol) or placebo in 10 weeks. At the end of each treatment period we measured the serum 25-OH-D levels using both a radioimmunoassay (RIA) (DiaSorin®) and a High-Performance Liquid Chromatography (HPLC) (Shimadzu®) to compare these methods.

Results: After placebo treatment vitamin D insufficiency was found in all 21 patients (100%) using the RIA method, while only in 15 patients (71%) using the HPLC method. Mean (SD) serum 25-OH-D level was 52 (13) nmol/L using RIA, compared with 66 (15) nmol/L using HPLC, P<0.0001. After treatment with vitamin D3 there were still five patients (23%) with vitamin D insufficiency using RIA, but only one patient (4%) using HPLC method. The mean (SD) serum 25-OH-D levels rose 67% to 87 (17) nmol/L using RIA, (P<0.0001) compared with 58% to 104 (17) nmol/L using HPLC, (P<0.0001). The difference between the means using these different methods was significant (P=0.001). The Pearson correlation's coefficient between the two methods was 0.8 (P<0.0001).

Conclusions: Results from these two different 25-OH-D assays differed markedly, when measuring serum 25-OH-D among non-diabetic patients in chronic kidney disease (stage 3-4). Diagnosis of vitamin D deficiency or insufficiency and consequential recommended supplementation may thus, depends on analysis method. Methods for analysis of 25-OH-D should therefor be standardized.

Funding: Private Foundation Support

TH-PO935

Vitamin D Seasonal Variations in Dialysis Patients: A Prospective Multicenter Study Joanna Matuszkiewicz-Rowinska,[1] Mariusz Mieczkowski,[1] Ewa Wojtaszek,[1] Pawel Zebrowski,[1] Antoni Sokalski,[2] Tomasz Stompor,[3] Janusz Grochowski,[4] Edyta Gieglis,[5] Wieslaw Klatko,[6] Jerzy Przedlacki,[1] Robert Malecki,[7] Ignacy Jarzyllo,[8] Stanislaw Niemczyk,[9] Zofia Wankowicz.[9] *[1]Nephrology, The Medical University in Warsaw; [2]Radom Hospital; [3]Warmia@ Mazury University; [4]Makow Hospital; [5]Otwock Nephrocare; [6]Ciechnow Hospital; [7]MSSW Warsaw; [8]Wolomin Hospital; [9]Military Institute of Medicine, Warsaw, Poland.*

Background: The prevalence of 25(OH)D (25D) deficiency is widespread in pts on dialysis, and has recently been shown to be associated with increased mortality. As most of 25D is of skin origin and sunshine dependent, its levels differ according to a season. This cross-sectional study is the most extensive prospective assessment of these fluctuations to date in this population.

Methods: It involved 252 prevalent pts (210 on hemodialysis (HD), 42 on peritoneal dialysis (PD), age 59±15 years) from 9 units. Pts taking any vitamin D derivatives were excluded. In all of them serum 25D (RIA), PTH and routine parameters were measured (centrally), 3x a year: in summer, autumn and winter.

Results: The study showed a significant 25D deficiency in the studied population. Its serum levels decreased from 23.0±10.4 (summer) to 14.3±9.4 ng/ml (winter). Normal serum 25D was observed in summer in 22%, in autumn in 11%, and only in 3.5% in winter! The proportion of pts with the deficiency (<20 ng/ml) rose from 43 to 82%, and severe deficiency (<10 ng/ml) from 7.2 to 32%. PD pts were particularly prone to the deficit: in autumn and winter, none of them had normal serum 25D, while its deficiency was observed in 89 and 96%(vs 51 and 80% of HD patients), and severe deficiency in 32 and 61% (vs 3.6 and 28% of HD, p <0.001). Multivariate analysis showed that independent predictors of 25D deficiency were: female gender (OR 3,45, CI 95%, 1,97–6,12, p<0,0001) and age >65 years (OR 1,87, 95% CI, 1,05–3,34, p=0,034).

Conclusions: Prevalence of 25D deficiency is widespread in our dialysis population, in winter being present in 78 to 96% pts. It can be very deep in PD pts, in whom in autumn and winter serum 25D can reach dramatically low levels posing a risk of osteomalacia.

TH-PO936

Effects of a High Protein Diet on Regulation of Phosphorus Homeostasis Robin Amy Kremsdorf,[1] Andrew N. Hoofnagle,[2] Mario Kratz,[2] Holly Callahan,[2] Angela Horgan,[3] Jonathan Q. Purnell,[3] David S. Weigle,[2] Ian H. de Boer,[2] Bryan R. Kestenbaum.[2] *[1]Seattle Children's Hospital, Seattle, WA; [2]University of Washington, Seattle, WA; [3]Oregon Health and Science University, Portland, OR.*

Background: High protein diets that are typically used for weight loss contain large quantities of phosphorus. Phosphorus excess and associated regulatory hormones are implicated in cardiovascular disease, in populations with and without kidney disease. It is not known whether moderate changes in dietary phosphorus from natural sources can impact phosphorus responsive hormones in the general population.

Methods: We studied 19 healthy subjects from a sequential dietary modification trial for weight loss. Subjects received the following prepared diets in order: (1) a two-week isocaloric, low-protein (15%) diet, (2) a two-week isocaloric, high-protein (30%) diet, and (3) a 12-week *ad libitum* high-protein (30%) diet. We measured fibroblast growth factor-23 (FGF-23), parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, and 24,25-dihydroxyvitamin D from integrated plasma samples that that were collected at 8 timepoints at the end of each diet phase.

Results: Mean phosphorus intake during each diet phase was 1,556, 2,071, and 1,622 mg/day, respectively. There were minimal changes in plasma concentrations of FGF-23, PTH, and vitamin D metabolites upon changing to an isocaloric or *ad libitum* high protein diet.

	N	Isocaloric 15% protein diet	Isocaloric 30% protein diet	Ad Libitum 30% protein diet
Dietary phosphorus (mg/day)		1,556	2,071	1,622
FGF-23 (pg/mL)	19	33.6±7	29.1±5.7	32.6±10.7
PTH (pg/mL)	19	35.4±11.1	35±11	35.4±11.7
1,25(OH)2D (pg/mL)	16	49.1±10.7	50±10.2	46.9±12.2
24,25(OH)2D (ng/mL)	19	3.8±2.3	3.8±2	4.1±2.3

Values shown as mean ± SD

Conclusions: Among healthy people, an approximately 33% increase in dietary phosphorus from a high-protein diet does not meaningfully alter plasma levels of phosphorus regulatory hormones.

Funding: NIDDK Support, Private Foundation Support

TH-PO937

Serum Fibroblast Growth Factor 23 Is Independently Correlated with Inflammation and Chemokine Expression in Healthy Predialysis CKD Patients Nihil Chitalia, Geeta Hampson, David Goldsmith. *King's Health Partners, London, United Kingdom.*

Background: Serum vitamin D, along with FGF23, is an independent risk factor for CV disease and overall survival in CKD. Evidence now supports an immunoregulatory effect of vitamin D, but the effect of FGF23 on the immune system is unknown. To investigate effects of these on immune system, we measured serum FGF-23, along with substrate and active serum vitamin D levels, and circulating markers of inflammation and chemokine expression, in a CKD cohort.

Methods: 114 adult stage 3-5 pre-dialysis ambulant vitamin D naïve outpatients were examined. A 25-plex panel Invitrogen™ immunoassay was used for quantitative analysis of inflammatory cytokines. Serum FGF-23 was measured by two-site ELISA detecting both C-terminal and intact fragments. Serum 25 hydroxy vitamin D [25(OH)D] and 1,25 dihydroxy vitamin D [1,25$(OH)_2$D] were measured by radioimmunoassay. Panels for Inflammation, Cytokine II, Chemokine and Th1/Th2 protein expression were statistically analysed using canonical correlation analysis (CCA) with known 'clinical variables', namely 25(OH)D, 1,25$(OH)_2$D and eGFR.

Results: The patients were aged 55±15 years, males 60% and MDRD eGFR 45±23ml/min/1.73m^2. Median±IQR FGF-23, PTH & hsCRP concentrations were 65±75 RU/ml, 58±69ng/L and 2.03±3.07mg/L respectively. Mean±1SD serum 25 hydroxy vitamin D and1,25$(OH)_2$D concentration were 51±24 nmol/L and 64±39 pmol/L respectively.

On CCA, there was a positive correlation between inflammatory panel variables and clinical variables at 5% significance (Wilk's=0.659,df=24,p=0.031). FGF23 positively correlated with IL8 accounting for 58.02% of the overall correlation variance of the model. On testing clinical variables with the Chemokine panel, there was a positive correlation (Wilk's=0.616,df=28,p=0.022). Monokine induced by INFγ (MIG) was positively correlated with FGF23, accounting for 54.14% of the correlation variance. Serum vitamin D did not show an independent correlation with cytokines when adjusted for eGFR.

Conclusions: Serum FGF-23 may have pro-inflammatory, and chemoattractant, roles in CKD via expression of inflammatory cytokines and chemokines. This hypothesis now needs testing in an in-vitro model.

TH-PO938

Differential Associations of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D with Impaired Renal Function in Patients with Diabetes Keitaro Yokoyama,[1] Akio Nakashima,[1] Ichiro Ohkido,[1] Tatsuo Hosoya,[1] Mitsuyoshi Urashima.[2] *[1]Division of and Hypertension, Department of Internal Medicine, the Jikei University School of Medicine, Tokyo, Japan; [2]Division of Molecular Epidemiology, the Jikei University School of Medicine, Tokyo, Japan.*

Background: Among patients with diabetes, both 25-hydroxyvitamin D (25OHD) and 1,25-dihydroxyvitamin D (1,25OHD) were shown to associate with urinary albumin-to-creatinine ratio (UACR) in patients with diabetes, separately, through either cross sectional studies or double blinded placebo controlled randomized trials. By measuring both 25OHD and 1,25OHD simultaneously, we aimed to clarify differential associations of 25OHD and 1,25OHD with eGFR stage and UACR as well as other metabolic parameters.

Methods: Cross sectional study of 542 patients with type 2 diabetes from eGFR stage 1 to 5. eGFR stages and UACR were set as primary outcomes and their associations with 25OHD and 1,25OHD were evaluated with multiple linear regression models with adjustments using age, gender, body mass index, usage of ACEI or ARB, usage of statin, and duration of diabetes.

Results: Mean (SD) of 25OHD and 1,25OHD were 24.2 (9.5) ng/mL and 43.6 (22.0) pg/mL, respectively. Under multi-variate adjustment, eGFR stages were negatively associated with 1,25OHD (P<0.001), but not with 25OHD. Similarly, 1,25OHD, but not 25OHD, was positively associated with levels of Ca (P=0.001), while negatively with levels of Pi (P<0.001). On the other hand, UACR were negatively associated with 25OHD (P=0.002), but not with 1,25OHD, in patients within eGFR stage 2 and stage 3.5. Similarly, fasting blood glucose levels were significantly associated with 25OHD (P=0.015), but not with 1,25OHD.

Conclusions: Our data suggest that 25OHD may associate with UACR in diabetic patients with mild to moderate-severe renal dysfunction, independent of serum Ca levels. Long term and relatively large scaled and randomized, placebo-controlled trials are needed to conclusively determine whether vitamin D supplementation reduces renal insufficiency complicated in patients with diabetes.

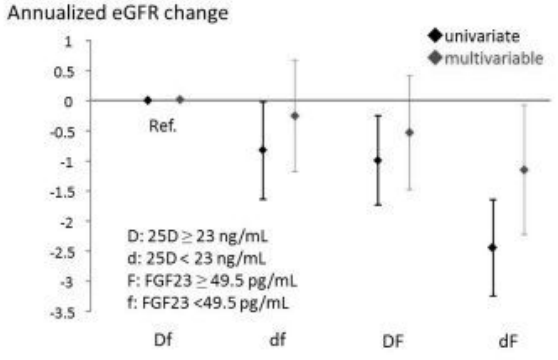
TH-PO939

FGF23 and 25-Hydroxyvitamin D Levels Predict Annualized Estimated GFR Decline Chikako Nakano,[1] Takayuki Hamano,[2] Naohiko Fujii,[3] Isao Matsui,[1] Yoshitsugu Obi,[1] Kazunori Inoue,[1] Akihiro Shimomura,[1] Noriyuki Okada,[4] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Internal Medicine, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan; [4]Nephrology and Hypertension, Osaka General Medical Center, Osaka, Japan.*

Background: We already reported that high fibroblast growth factor 23 (FGF23) and low 25-hydroxyvitamin D (25D) levels in chronic kidney disease predicted renal event (serum creatinine doubling / initiation of dialysis) independent of other mineral bone disorder (MBD)-related factors (CJASN 2012). Our aim was to examine the relationships between these 2 factors and eGFR decline.

Methods: This is a prospective cohort study of 738 predialysis outpatients in 2 nephrology units. The outcome was an annualized eGFR decline calculated by the following formula: (final eGFR – baseline eGFR) / follow-up duration (years). We utilized restricted cubic spline in multiple regression analyses to explore potential non-linear relationships.

Results: At baseline, mean eGFR was 35 mL/min/1.73m^2. During a median follow-up period of 4.4years, 146 patients started maintenance dialysis. In univariate analyses, a non-linear relationship was observed between 25D and eGFR decline (P for linearity: 0.02). The eGFR decline was greater in patients with 25D below 25 ng/mL. Above this threshold, eGFR decline plateaued. As to FGF23, higher levels were related to a greater eGFR decline (p for linearity: 0.43). Similar trends were observed in multiple regression analyses. The analysis treating 25D and FGF23 as categorical variables was shown in Figure 1.



Conclusions: There was a cut-off below which lower 25D levels were associated with steeper eGFR decline.

TH-PO940

Fibroblast Growth Factor 23 in Pediatric Heart Failure Tamara Isakova,[1] Jessica Houston,[1] Laura Santacruz,[2] Eva Schiavenato,[1] Gabe Somarriba,[2] William Harmon,[2] Tracie Miller,[2] Steven Lipshultz,[2] Myles S. Wolf,[1] Paolo Rusconi.[2] *[1]Medicine, UM; [2]Pediatrics, UM.*

Background: FGF23, a bone-derived hormonal regulator of mineral metabolism, has been shown to be elevated in adults with heart failure, in whom it was associated with risks of future hospitalization and mortality. Data on FGF23 levels in pediatric heart failure are lacking.

Methods: We conducted a cross-sectional study of 20 children (mean age 11±6 years; mean eGFR 109.4±56 ml/min/1.73 m^2) with heart failure who underwent echocardiography and the following measurements: FGF23, PTH, phosphate, serum creatinine, and NT-proBNP. Assessment of symptom severity was performed using the NYHA classification system.

Results: Eleven children had dilated cardiomyopathy (DCM); the remaining etiologies included congenital heart disease (n=4), hypertrophic cardiomyopathy (3), failing heart transplant (1), and pulmonary hypertension (1). Loop diuretics were prescribed to 9 patients, and 40% had NYHA class ≥2. Mean serum phosphate and median PTH levels were in the normal range. In contrast, the median FGF23 was elevated (110.9; interquartile range 78.4 – 187.1 RU/ml), and FGF23 levels were higher in patients treated with diuretics compared to untreated patients (P=0.01). FGF23 also correlated significantly with NT-proBNP (r=0.47, P=0.04), and there was a trend for higher levels in patients with higher NYHA class (P=0.05). These associations were independent of eGFR, which was not a significant predictor of FGF23. Among patients with DCM, FGF23 correlated with echocardiographic indicators of hypertrophy and dilation.

Echocardiographic Measures (normalized to age z-score)	FGF23		NT-proBNP	
	r	P Value	r	P Value
Indicators of Systolic Function				
Left ventricular ejection fraction	−0.49	0.12	−0.61	0.046
Left ventricular fractional shortening	−0.53	0.12	−0.53	0.12
Indicators of Hypertrophy				
Left ventricular wall thickness	0.59	0.06	0.02	0.96
Left ventricular septal thickness	0.50	0.12	0.15	0.67
Left ventricular mass	0.61	0.06	0.45	0.19
Indicators of Dilation				
Left ventricular systolic diameter	0.60	0.05	0.59	0.06
Left ventricular diastolic diameter	0.63	0.04	0.57	0.07

Conclusions: FGF23 levels are elevated in children with heart failure and are associated with symptom severity and echocardiographic measures of function and remodeling. Future studies should evaluate the longitudinal relationship between FGF23 and outcomes in children with heart failure.

Funding: NIDDK Support

TH-PO941

Fibroblast Growth Factor 23, High-Sensitivity Cardiac Troponin, and Left Ventricular Hypertrophy in Chronic Kidney Disease Kelsey T. Smith, Christopher deFilippi, Tamara Isakova, Orlando M. Gutierrez, Karen A. Laliberte, Stephen L. Seliger, Walter Kelley, Show-hong Duh, Michael K. Hise, Robert Christenson, Myles S. Wolf, James Januzzi. *The Division of Nephrology and Hypertension, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL.*

Background: Detectable levels of cardiac troponin (cTn) are common among individuals with chronic kidney disease (CKD), even in the absence of symptomatic cardiovascular disease. Abnormal cTn values are associated with coronary artery disease (CAD) and left ventricular hypertrophy (LVH), and predict poor clinical outcomes. Elevated levels of fibroblast growth factor 23 (FGF23) contribute to LVH in CKD. We investigated the association of FGF23 and high-sensitivity (hs) cTnI and cTnT in CKD, and examined the role of LVH in this association.

Methods: In this cross-sectional observational study, we measured hs-cTnI, hs-cTnT and FGF23 in 153 stable outpatients with pre-dialysis CKD and low prevalence of CAD (15%). Left ventricular mass index (LVMI) was assessed by echocardiography and coronary artery calcification (CAC) was measured by computed tomography.

Results: Mean (± standard deviation) age was 64 ± 12 years, mean estimated glomerular filtration rate was 34 ± 11 ml/min/1.73m^2, median (interquartile range) FGF23 was 120 RU/mL (79 - 223 RU/ml), median hs-cTnI was 6.5 pg/ml (3.5 - 14.5 pg/mL), and median hs-cTnT was 16.8 pg/ml (11.1 - 33.9 pg/mL). Concentrations of cTnI and cTnT were >99th percentile of a normal population in 42% and 61% of patients, respectively. In unadjusted and multivariable adjusted analyses, both hs-cTns were significantly associated with FGF23 (β=0.33, *P*=0.003 for hs-cTnI; β=0.24, *P*<0.001 for hs-cTnT in fully adjusted models).

Adjusting for LVMI (β=0.20, *P*=0.07 for hs-cTnI; β=0.20, *P*=0.008 for hs-cTnT), but not CAC (β=0.36, *P*=0.002 for hs-cTnI; β=0.22, *P*=0.002 for hs-cTnT), weakened the association of FGF23 and both hs-cTns.

Conclusions: Minimally elevated cTnI and cTnT, detectable by high-sensitivity assays, are associated with elevated FGF23 levels in stable outpatients with CKD. FGF23-associated LVH may contribute to detectable cTn levels observed in pre-dialysis CKD.

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TH-PO942

Control of Secondary Hyperparathyroidism Using Cinacalcet with/without Active Vitamin D Sterols in Incident Dialysis Patients Serge Cournoyer,[1] Ian Matthew Bridges,[2] Cynthia R. Christiano,[3] Mourad Farouk,[2] Nelson P. Kopyt,[4] Mariano Rodriguez,[5] Pablo A. Urena,[6] Daniel Zehnder,[7] Kerry Cooper.[2] *[1]Hôpital Charles LeMoyne; [2]Amgen; [3]East Carolina University; [4]LehighValley Hospital; [5]Hospital Universitario Reina Sofia, IMIBIC; [6]Clinique Du Landy; [7]Warwick Medical School.*

Background: Initial treatment of SHPT in incident dialysis patients often includes phosphate binders and active vitamin D sterols (vitD). Cinacalcet (Cin) is commonly added when PTH levels are not controlled adequately. In the Incident Dialysis study, Cin with low-dose vitD, if prescribed, more effectively decreased PTH than flexible doses of vitD alone (Urena et al, ASN 2011). We report post hoc results in subgroups of the Cin arm according to use/non use of vitD during the treatment phase.

Methods: Subjects with SHPT (PTH>300 pg/mL) on dialysis for >3–12 mo were randomized 1:1 to Cin (+low-dose vitD, if prescribed) or vitD (if prescribed) alone. VitD was prescribed according to standard treatment guidelines. Treatment duration was 12 mo, with primary endpoint assessed at 6 mo. Of153 Cin-treated subjects, 48 received no vitD at any time during the treatment phase.

Results: There were no obvious differences in baseline characteristics between the vitD and noVitD subgroups. Efficacy and safety were similar in both subgroups. Subject incidence of biochemical hypocalcemia was greater in the vitDthan noVitD subgroup; most incidences occurred in the titration phase.

	Cin arm		
	Overall (N=153)	noVitD (N=48)	VitD (N=105)
Baseline PTH, pg/mL, mean±SD	559±338	556±372	561±324
Achieved ≥30% PTH reduction vs baseline, % subjects			
6 mo*	63	67	61
12 mo	63	65	63
PTH, pg/mL, mean (95% CI)			
6 mo	321 (285, 358)	283 (243, 322)	339 (289, 389)
12 mo	332 (294, 371)	302 (255, 349)	346 (294, 399)
Hypocalcemia (≤7.5 mg/dL), % subjects	33	23	38
Treatment-emergent adverse events, subject incidence (%)†		n=49	n=106
Any event	90	88	91
Serious event	46	43	48

*Primary endpoint; †safety analysis set

Conclusions: In this post hoc analysis, Cin therapy reduced serum PTH similarly both with and without concurrent vitD.

Funding: Pharmaceutical Company Support - Amgen

TH-PO943

Oral Cholecalciferol Decreases Albuminuria and Improves Secondary Hyperparathyroidism in Non-Dialysis Chronic Kidney Disease Patients Pablo Molina,[1,2] Jose L. Gorriz,[2] Sandra Beltrán,[2] Avila Ana,[2] Romero Ramón,[1] Luis M. Pallardo.[2] *[1]Department of Medicine, Universitad Autonoma Barcelona, Spain; [2]Department of Nephrology, Hospital U. DrPeset, Valencia, Spain.*

Background: Beyond its effect on controlling bone metabolism parameters,vitamin D(VD) may have antiproteinuric effects in patients with chronic kidney disease(CKD). This renoprotecive property has been defined in randomized trials using active VD analogs,whereas the antiproteinuric effect of nutritional VD supplementation has been described in only one uncontrolled study.The present study evaluated the effect of cholecalciferol on proteinuira and glomerular filtratrion rate as well as bone metabolism parameters in CKD.

Methods: This controlled,intervention study enrolled 230 non-dialysis CKD patients(3-stage:77%;4-stage:23%;age:75±11y;women:49%)divided into 2 groups according to PTH basal levels.Patients with high PTH levels(n=117;51%) received oral cholecalciferol(666UI/day)whereas patients without hyperparathyroidism(n=113;49%)were considered as control group.Patients previously treated with phosphate binders,calcitriol or paricalcitol were excluded.Mean follow-up:7.2±3.7 months.

Results: A significant increase in calcidiol levels and reduction in PTH levels were observed in the cholecalciferol group.

Evolution of renal function and bone metabolism parameters

	Cholecalciferol (n=117)			Control (n=113)		
	Basal	Post	p	Basal	Post	p
25(OH)D(ng/mL)	16.5±5.9	25.1±7.3	0.000	21.9±6.2	20.4±8.0	0.000
PTHi(pg/mL)	117.9±53.4	98.4±56.3	0.000	59.4±17.2	70.3±30.2	0.000
Caalb(mg/dL)	9.3±0.4	9.4±0.4	0.127	9.3±0.4	9.3±0.4	0.213
P(mg/dL)	3.6±0.5	3.7±0.6	0.000	3.6±0.6	3.7±0.6	0.050
CaxP (mg2/dL2)	33.3±4.6	34.9±5.9	0.000	33.3±5.2	34.5±5.0	0.039
Cr(mg/dL)	1.8±0.5	1.8±0.6	0.119	1.6±0.5	1.7±0.5	0.014
eGFR(mL/min/1.73m2)	38.0±11.0	36.8±12.8	0.127	41.5±10.8	39.7±11.4	0.013
Albuminuria(mg/g)	305.3±551.3	221.1±353.2	0.008	206.9±379.7	262.2±464.6	0.036

In absence of significant changes in blood pressure levels or antihypertensive treatment,stabilization of renal function and reduction in albuminuria were observed only in the cholecalciferol group.

Conclusions: In addition to improving hyperparathyroidism,VD repletion with cholecalciferol had a beneficial effect in decresing albuminuria and delaying the progression of CKD.

TH-PO944

Prospective, Randomized Trial of the Differential Effects of Cholecalciferol versus Calcitriol on Monocytes in CKD Jason R. Stubbs, Shiqin Zhang, Ryan Gillihan, Kerri A. Mcgreal, Alicia Zeiger. *The Kidney Institute, Univ. of Kansas Medical Center.*

Background: Monocytes utilize the local conversion of 25(OH)D to 1,25$(OH)_2$D for autocrine/paracrine functions that result in systemic actions unrelated to mineral metabolism. CKD patients receiving 1,25$(OH)_2$D analogues often exhibit low 25(OH)D levels, but it remains unclear if 25(OH)D repletion provides any benefit to these patients. We therefore performed a prospective trial testing the differential in vivo effects of cholecalciferol and calcitriol on monocytes in patients with CKD.

Methods: A total of 30 CKD patients (eGFR 10-45 ml/min) were randomized to receive cholecalciferol 50,000 U twice weekly or calcitriol 0.25mcg daily for 8 weeks. Monocytes were isolated from patients at baseline and post-therapy and were evaluated by flow cytometry for changes in targets with suspected regulation by vitamin D.

Results: We observed a substantial increase in 25(OH)D levels in the cholecalciferol arm following therapy (16.8 ± 7.8 to 53.3 ± 16.0 ng/ml, P<0.001), with no change in the calcitriol arm (24.3 ± 6.5 to 21.6 ± 6.5 ng/ml, P=NS). Post-treatment flow cytometry analysis of monocytes from patients in the calcitriol arm revealed a significant decrease in total monocyte CD16 expression, a surface marker of "inflammatory" monocytes (from 5441 ± 625.4 to 4214 ± 373.9 MFI, P<0.05). No significant change in CD16 was observed in the cholecalciferol group (4635 ± 618.3 to 5062 ± 622.2 MFI, P=NS). Analysis of monocyte vitamin D receptor expression revealed a 40% increase in monocyte VDR with cholecalciferol therapy (2053 ± 198.2 to 2891 ± 285.6 MFI, P<0.05) and a 50% increase following calcitriol therapy (2376 ± 475.6 to 3579 ± 918.5 MFI, P=NS). We observed no change in monocyte CD14, HLA-DR, Mac-1 or LFA-1a levels with either therapy.

Conclusions: Taken together, our data suggest cholecalciferol and calcitriol to increase monocyte VDR expression, yet have differing effects on monocyte CD16 levels in patients with CKD. This data may provide evidence for unique in vivo roles of these two treatments on immune pathways. Further analysis is ongoing to compare changes in inflammatory cytokines and alterations in monocyte subsets with these therapies.

Funding: Private Foundation Support

TH-PO945

Binding of Circulating Sialylated Angiopoietin-Like-4 (Angptl4) to Its Glomerular Endothelial Receptor Reduces Proteinuria Camille E. Mace, Lionel C. Clement, Sumant S. Chugh. *Medicine / Nephrology, University of Alabama at Birmingham, Birmingham, AL.*

Background: We have previously shown that a sialylated form of Angptl4 is noted in the circulation of patients with different forms of nephrotic syndrome (Nature Medicine 2011, on line supplement). Next, we showed that circulating Angptl4 binds to, and has protective effects on the glomerular endothelium (ASN 2011).

Results: We now show that Angptl4 binds to a transmembrane protein on the endothelial surface (provisionally identified as protein X) in vivo by confocal imaging, and in vitro using plate adhesion assays. Global knockout of gene X in mice preserves glomerular phenotype, and does not result in proteinuria. Induction of proteinuria in gene X knockout and wild type mice using gamma2 NTS results in significantly higher proteinuria in the knockout mice (2-fold difference, P<0.01) 5 to 7 days after induction of proteinuria, at a time when circulating Angptl4 levels are elevated. Adipose tissue specific Angptl4 overexpressing transgenic rats (aP2-Angptl4), that have elevated circulating Angptl4 levels, develop less proteinuria than wild type rats after induction of single intravenous dose puromycin nephrosis (PAN) (P<0.05). However, intravenous injection of antibodies against protein X in aP2-Angptl4 PAN rats increases proteinuria to the level of wild type PAN rats, suggesting that the protective, anti-proteinuric effects of circulating Angptl4 are mediated via protein X. Moreover, injection of antibodies against protein X in wild type rats with PAN beyond the peak of proteinuria delays the recovery of proteinuria compared to PAN rats injected with preimmune serum (P<0.05 to P<0.001).

Conclusions: These studies suggest that the interaction of circulating Angptl4 with protein X on the glomerular endothelial surface stabilize / reduce proteinuria in primary glomerular disease.

Funding: NIDDK Support

TH-PO946

Transcytosis of Intact Albumin from Primary Urine Is FcRn Dependent
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Background: Under physiological conditions, about 1 gr. of albumin passes the glomerular filter per day in humans. Filtered albumin is reabsorbed from the primary filtrate by tubular cells and the cubilin/megalin complex. In our preliminary work in transgenic rats, we have already shown that albumin is retrieved intact from the primary urine into the serum. In the present study, we have confirmed transcytosis of albumin in transgenic inducible mice and examined its molecular mechanism.

Methods: Murine albumin with different charge (anionic versus neutral) was expressed under the Tet-inducible promoter in transgenic mice. To express transgenic albumin specifically in podocytes, mice were crossed with the Pod-rtTA transactivator mice. Finally, these mice were bred into the FcRn knock-out mouse line. FcRn is ubiquitously expressed and retrieves albumin from the early endosome to rescue it from lysosomal degradation.

Results: Transgenic mice expressed both albumins with different charge in an inducible fashion directly into the primary filtrate behind the glomerular filter. Transgenic albumin was retrieved by proximal tubular cells (not by parietal epithelial cells). Both albumins were detectable within the serum independent of their charge. This confirmed our findings in transgenic rats. Backfiltration within the glomerulus was ruled out as a possible mechanism, since both anionic and neutral albumins were retrieved into the circulation. When FcRn was inactivated, transgenic albumin was no longer detectable within the serum.

Conclusions: This study shows that filtered albumin is retrieved intact from primary filtrate back into the serum. FcRn is required for albumin transcytosis.

Funding: Government Support - Non-U.S.

TH-PO947

Proteinuria Triggers Renal Lymphangiogenesis Prior to the Development of Interstitial Fibrosis
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Background: Proteinuria is an important cause of progressive tubulo-interstitial damage. Whether proteinuria could trigger a renal lymphangiogenic response has not been established. Moreover, the temporal relationship between development of fibrosis, inflammation and lymphangiogenesis in chronic progressive kidney disease is not clear. Therefore, we evaluated the time course of lymph vessel (LV) formation in relation to proteinuria and morphological damage in chronic unilateral adriamycin nephrosis.

Methods: Proteinuria and kidneys were evaluated 6, 12, 18, 24 and 30 weeks after induction of nephrosis. LVs were identified by podoplanin/VEGFR3 IF double staining. Myofibroblasts (α-SMA), collagen I and III, macrophages (MQ), focal glomerulosclerosis (FGS) and osteopontin (OPN) were also quantified.

Results: After 6 weeks proteinuria was well-established, without influx of interstitial MQ, α-SMA, collagen I and III deposition, induction of OPN, development of FGS and LV formation. At 12 weeks, a significant increase in cortical LV density was found, gradually increasing over time. This corresponded with a significant increase in tubular osteopontin expression ($p<0.01$) and interstitial myofibroblast numbers ($p<0.05$), whereas collagen deposition and macrophage influx was not yet increased. LV size increased over time as well. VEGF-C was mostly expressed by tubular cells rather than insterstitial MQ and α-SMA. HK-2 cells stimulated with FCS showed a dose-dependent increase in mRNA and protein expression of VEGF-C.

Conclusions: We conclude that established nephrotic range proteinuria up to week 6 was not associated with a renal lymphangiogenic response. However, chronic proteinuria provoked lymphangiogenesis in temporal conjunction with the pro-fibrotic response of tubular activation and influx of myofibroblasts, that preceded deposition of collagen I and III, macrophage influx and development of fibrosis.

TH-PO948

Albuminuria Associated with CD2AP Knockout Mice Is Quantitatively due to Tubular Uptake Dysfunction of Filtered Albumin
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Background: CD2AP knock out mice (CD2AP-/-) die in 5-6 weeks with accompanying massive proteinuria. In order to test that changes in glomerular permeability are responsible for this proteinuria we measured plasma elimination (PE) of fluorescently labelled albumin and dextran mol wt 70,000. Kaysen (1994) previously demonstrated that the increase in PE of albumin in nephrotic states is due to the increase in urinary excretion of albumin. An increase in the PE for dextran in albuminuric states should reflect alterations in glomerular permeability.

Methods: 5 week old mice were maintained in a metabolic cage for 24h prior to being injected with fluorescent labelled polymers in order to determine urine flow rate and albumin excretion rate. Post iv injection, blood samples were taken at 15min, 3h and 24h. The mice were sacrificed and the kidneys examined via microscopy.

Results: CD2AP-/- mice exhibited intact albuminuria without changes in urine flow rate or total protein excretion rate. Their kidneys exhibited nephron occlusion and podocyte effacement. Contrary to what might be expected from a glomerular defect, PE rates actually decreased in CD2AP-/- mice; percentage of material remaining in the plasma at 3h for dextran 70,000 PE for CD2AP+/+ was 28.5±5.9 (n=7) whereas for CD2AP-/- it was 42.4±10.0 (n=8) (P=0.006) and for albumin in CD2AP+/+ was 63.9±8.1 (n=7) whereas for CD2AP-/- it was 74.6±9.3 (n=8) (P=0.03). Albumin peptide excretion in CD2AP-/- mice was completely inhibited.

Conclusions: The massive change in albumin excretion rate in CD2AP-/- mice was not accompanied by an equivalent change in glomerular permeability. The onset of albuminuria could be accounted by inhibition of the degradation pathway in proximal tubules. CD2AP-/- mice do not exhibit the 'classic' nephrotic state where albumin PE is increased but have reduced PE due to occluded nephrons. The progression of these changes in CD2AP-/- mice mimic chronic renal failure with remnant nephrons compensating for hyperfiltration.

Funding: Private Foundation Support

TH-PO949

Immunohistochemical Analyses of Serum Proteins in Puromycin Aminonucleoside Nephrosis of Living Rat Kidneys by In Vivo Cryotechnique
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Background: "In vivo cryotechnique (IVCT)" is a useful method to identify native immunolocalizations of serum proteins in tissues of living animals, by which we have examined dynamic states of serum proteins in each segment of nephron in puromycin aminonucleoside (PAN) nephrosis model rat.

Methods: For PAN nephrosis model, PAN was injected into peritoneal cavity of Sprague-Dawley rats (5, 10 or 15mg/100g BW) once. Their total urine was collected to check proteinuria each day. At day3 or day9 after the injection of PAN, IVCT was performed for anesthetized rat kidneys, followed by freeze-substitution with 2% paraformaldehyde at -80°. Serial sections were stained with hematoxylin-eosin (HE) or immunostained for albumin and IgG1.

Results: The excretion of urinary proteins increased at day3, and exceeded 300mg/day at day9 in 15mg PAN injection group. By plasma protein analyses, PAN nephrosis induced typical hypoalbuminemia and renal dysfunction. By HE staining, no remarkable changes were detected at day3, but congestion of erythrocytes in glomerular capillary loops (GCL) and some casts of renal tubules were observed at day9. At day3, albumin was more immunolocalized in podocytes and reabsorbed into epithelial cells of proximal renal tubules more than the control rats. At day9, immunoreactivity of albumin in the GCL got weak and casts of renal tubules were strongly immunostained. IgG1 was immunolocalized in blood capillaries, but its immunolocalization was also detected in podocytes, and its reabsoaption into epithelial cells of proximal tubules was also detected at day3. At day9, immunoreactivity of IgG1 got weaker in the podocytes, and those casts were strongly immunostained.

Conclusions: Thus, IVCT enabled us to visualize a process of excreting proteins at each segment of nephron and to get the morphofunctional data for analyzing nephrotic kidney disease.

TH-PO950

Identification and Characterization of Urinary Vesicle Proteins Interacting with the Tamm-Horsfall Glycoprotein
Dorota Ewa Tataruch, Luca Musante, Barry Byrne, Brian Shortt, Mayank Sarawat, Harry B. Holthofer. *Centre for BioAnalytical Sciences, Dublin City University, Dublin, Ireland.*

Background: Tamm-Horsfall Protein (THP), also known as uromodulin is the most abundant glycoprotein in urine. It is expressed and secreted into urine in the loop of Henle and early distal convoluted tubule. THP contains the most varied glycans of any human glycoprotein, which suggests a variety of functions including a capacity for adhesion to e.g. urinary exosome vesicles. In fact, electron microscopy analyses have demonstrated that exosome vesicles are entrapped within urinary THP polymers. In the urine THP may precipitate due to many factors of the immediate physico-chemical microenvironment and THP is the main constituent of hyaline urinary casts. The aim of this study was to identify the variety of exosomal proteins interacting with uromodulin. Firstly THP was purified by Diatomaceous Earth Filter (DEF) and then an aliquote was labelled with biotin. In Western blot analysis biotinylated THP was able to recognise several bands in the 18,000 and 200,000g centrifugation pellets, respectively. As a demonstration of its binding ability to vesicle sediment, DEF-extracted THP was assayed in both nanovesicle populations by Surface Plasmon Resonance (SPR). Subsequently, purified THP was bound to cyanogen bromide activated sepharose and an affinity chromatography was carried out to enrich exosome-interacting proteins. In-gel mass spectrometry identification revealed several unique proteins, some of those classified as proteases by a gene ontology (GO) analysis. Finally, purified native THP was shown to interact with bovine pancreatic trypsin. THP underwent a partial proteolysis with the production of a major fragment at 65kDa. Here we describe for the first time the presence of THP interacting proteins in the urinary vesicle fraction. Identification of such targets will allow a better understanding of the role of the interacting vesicles and design of specific inhibitors for a better recovery of vesicles. Finally, THP interaction with bovine pancreatic trypsin and the strong resistance to the enzymatic digestion support an active role as trypsin protease inhibitor.

TH-PO951

Inhibition of β-Catenin Signaling Reverses an Established Proteinuria and Kidney Injury Li Li Zhou,[1,2] Jing Nie,[2] Fan Fan Hou,[2] Youhua Liu.[1] *[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; [2]Division of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China.*

Background: The progression of chronic kidney diseases is believed to be an irreversible process, eventually leading to end-stage renal failure. Once the kidney injury is established, current therapies only slow, but seldom reverse or completely halt the progressive loss of kidney function. In this study, we sought to test whether inhibition of β-catenin signaling by peptidomimetic small molecule ICG-001 can reverse an established proteinuria and kidney injury.

Methods: To assess the therapeutic efficacy of ICG-001, we designed two different treatment protocols. In the withdrawal protocol (ICG-w), ICG-001 was administered at 7 days after adriamycin (ADR) injection, and treatment lasted for 2 weeks, and then stopped. The experiments were terminated at 5 weeks after ADR injection. In the therapeutic protocol (ICG-t), ICG-001 was given at 3 weeks after ADR injection.

Results: In mouse model of ADR nephropathy, either transient therapy (withdrawal protocol) or late administration of ICG-001 was able to abolish an established proteinuria and kidney lesions. ICG-001 restored nephrin, podocin and WT1 expression, and abolished desmin induction, resulting in marked preservation of podocyte integrity. ICG-001 therapy also blunted renal expression of numerous β-catenin target genes such as Snail1, plasminogen activator inhibitor-1, matrix metalloproteinase-7 and fibroblast-specific protein-1, which was accompanied by preservation of renal expression of E-cadherin. ICG-001 therapy inhibited myofibroblast activation; repressed the expression of fibronectin, type I and type III collagens; and reduced renal interstitial fibrosis. Furthermore, inhibition of β-catenin signaling by ICG-001 inhibited pro-inflammatory cytokines expression, reduced renal infiltration of macrophages and repressed NF-κB signaling activation.

Conclusions: These studies indicate that inhibition of β-catenin signaling by ICG-001 reversed an established proteinuria and kidney injury. Therefore, blockade of β-catenin signaling could be an effective therapy for established chronic kidney diseases.

Funding: NIDDK Support, Government Support - Non-U.S.

TH-PO952

Advanced Oxidation Protein Products Induce Podocyte Epithelial-Mesenchymal Transition via Activating Wnt1/β-Catenin Signaling Li Li Zhou,[1,2] Jing Nie,[1] Fan Fan Hou,[1] Youhua Liu.[1,2] *[1]Division of Nephrology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China; [2]Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Podocyte dysfunction plays a crucial role in the pathogenesis of proteinuria and glomerulosclerosis. After injury, podocytes may undergo epithelial-mesenchymal transition (EMT). Recent studies indicate that accumulation of advanced oxidation protein products (AOPPs) is implicated in the pathogenesis of podocyte apoptosis. However, whether AOPPs also induces podocyte EMT remains unknown.

Methods: Mice or podocyte cell line (MPC5) were treated with AOPPs-modified mouse serum albumin (AOPP-MSA). Albuminuria and podocyte phenotypes were assessed. To determine the potential role of Wnt/β-catenin signaling in mediating AOPP action, conditional knockout mice in which β-catenin was selectively ablated in glomerular podocytes were utilized.

Results: Chronic administration of AOPPs induced albuminuria, suppressed nephrin and upregulated the expression of fibronectin, desmin, Snail1 and MMP-9 in podocytes in mice. These pathologic changes were largely abolished by administrating a neutralizing antibody against the receptor for advanced glycation end products (RAGE) in vivo. AOPP also induced Wnt1 and Wnt7a expression and activated β-catenin in glomerular podocytes in mice. In vitro, AOPP triggered podocyte EMT, as shown by loss of nephrin, podocin and ZO-1, and upregulation of fibronectin, desmin, MMP-9 and Snail1. AOPP induced ROS generation in cultured podocytes, and stimulated Wnt1 expression, which was prevented by N-acetyl cysteine (NAC). Furthermore, knockdown of β-catenin almost completely abolished podocyte EMT induced by AOPP in vitro. Finally, in conditional knockout mice in which β-catenin was selectively ablated in podocytes, AOPP failed to induce podocyte dysfunction and proteinuria.

Conclusions: These results demonstrate that chronic exposure to AOPPs induces podocyte EMT and proteinuria. This is attributable to the AOPP/RAGE-mediated oxidative stress, Wnt1 induction and β-catenin activation. Our studies delineate a novel pathway in which oxidative stress triggers Wnt/β-catenin signaling in proteinuria.

Funding: NIDDK Support, Government Support - Non-U.S.

TH-PO953

Opening K_{ATP} Channels with Nicorandil in the Podocytes and Macrophages Improves Chronic Renal Disease in the Rats Yoshifuru Tamura,[1] Katsuyuki Tanabe,[1] Wataru Kitagawa,[1] Shunya Uchida,[2] Richard J. Johnson,[1] Takahiko Nakagawa.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Division of Renal Disease, Teikyo University, Itabashi, Tokyo, Japan.*

Background: Nicorandil causes vasodilatation by opening ATP-dependent potassium channels and donating nitric oxide.

Methods: We evaluated the effect of nicorandil in the rat remnant kidney model (RK) of chronic kidney disease. RK rats were divided into three groups: Sham operated rats (SHAM), Untreated remnant kidney rats (RK), RK rats treated with nicorandil 30 mg/kg/day (NICO).

Results: Blood pressure (tail cuff) was unchanged by a 10 week course of nicorandil. However, nicorandil prevented both albuminuria and histological injury in this model (Table). Oxidative stress, (renal nitrotyrosine level, urine 8-OhdG and heme oxygenase-1) was elevated in this model and was significantly reduced by nicorandil treatment. Consistent with this finding, we found a reduction in podocyte MnSOD and an increase in macrophage xanthine oxidase in the RK model that was prevented by nirocandil. Both podocytes and macrophages express SUR2 which is a binding site of K_{ATP} channels, suggesting that nicorandil might stimulate these cells to open K_{ATP} channels. Indeed, niocrandil increased MnSOD expression in cultured podocytes and blocked angiotensin II-induced xanthine oxidase activation in cultured macrophages. In contrast, this enzyme activity was enhanced by glibenclamide which closes K_{ATP} channel.

Table.

	Serum creatinine	Urinary aibumin	Oxid stress	Glomeruli	Tubulointerstitium
	mg/dl	mg/day	Nitrotyrosine/β-actin	%Glomerulosclerosis/glom.	Collagen III (+) area/field (%)
SHAM (n = 7)	0.32 ± 0.03***	0.6 ± 0.3***	0.07 ± 0.01**	6.7 ± 0.4***	4.9 ± 0.8***
RK (n = 7)	0.71 ± 0.08	16.3 ± 3.3	0.21 ± 0.02	32.9 ± 3.5	14.3 ± 1.5
NICO (n = 7)	0.56 ± 0.03	3.2 ± 1.2**	0.10 ± 0.02*	13.6 ± 1.5***	8.0 ± 0.4**

***P<0.001. **P<0.01. *P<0.05 vs. RK.

Conclusions: In conclusion, nicorandil reduces albuminuria and ameliorates renal injury by upregulating podocyte MnSOD and blocking macrophage oxidative stress in chronic kidney disease. Nicorandil may represent a novel treatment for chronic kidney disease.

TH-PO954

Downregulation of Podocyte Vitamin D Receptor Expression Upregulates HIV Gene Expression in HIV-Associated Nephropathy Partab Rai, Dileep Kumar, Mohammad Husain, Pravin C. Singhal, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: The activation of renin -angiotensin system (RAS) has been demonstrated to play an important role for the progression of HIV-associated nephropathy (HIVAN). Since kidney cell HIV gene expression contribute to the development of HIVAN, we hypothesize that alteration of podocyte VDR expression might be enhancing HIV gene expression through the activation of the RAS.

Methods: Immortalized differentiated human podocytes (IDHP) were transdudced with either empty vector (EV/IDHP) or HIV (NL4-3, HIV/CIDHP) and incubated in media for 72 hours. Immunoblots were probed or VDR and actin. To silence VDR in IDHPs, EV/IDHPs and HIV/IDHPs were transfected with siRNA VDR(siRNAVDR/HIV/IDHPs) or scrambled (Scr-siRNA/HIV/IDHP) siRNA.Immunoblots of IDHP, HIV/IDHPs, siRNAVDR/HIV/IDHP, ScrsiRNA/IDHPs were probed for renin and actin. mRNA expression of HIV genes were quantified by real time PCR. To establish the role of RAS IDHP and HIV/IDHPs were treated with losartan (Ang II blocker) and then assayed for mRNA expression for HIV genes. To activate endogenous RAS, HIVAN mice (Tg26) in control and experimental cells were were bred witn angiotensinogen (Agt) transgenic mice having four Agt copies; this strategy allowed us to develop Tg26 mice with 2, 3 and 4 copies of Agt. Kidneys from 4 weeks and 8 weeks old Tg26 mice with different Agt copies were harvested and analyzed for mRNA expression of HIV genes.

Results: HIV down regulated VDR expression and enhanced renin expression in human podocytes. Podocytes silenced for VDR also displayed activation of the RAS. HV/IDHPs also displayed activation of the RAS and this effect was more pronounced in siRNAVDR/HIV/IDHPs. Silencing of VDR in HIV/IDHPs enhanced expression of Nef, Tat, and Vif. On the other hand, losartan inihbitied expression of HIV genes in HIV/IDHPs. Renal tissues of 8 weeks old Tg26-Agt-4 displayed 2-4 fold increase in mRNA expression of gp120, Vpr, Tat, Nef and Vpu when compared to Tg26 having two copies of Agt.

Conclusions: VDR-induced activation of the RAS contributes to enhanced HIV gene transcription in HIVAN.

Funding: NIDDK Support

TH-PO955

Role of p38 MAPK Activation in the Slit Diaphragm Dysfunction and Proteinuria Caused by a Direct Stimulation to Nephrin Yuichi Takahashi, Mihoko Yamazaki, Aya Takahashi, Asami Takasaki, Yoshiyasu Fukusumi, Masayuki Tomita, Hiroshi Kawachi. *Department of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: p38 MAPK inhibitor, FR167653 is reported to ameliorate proteinuria in PAN- and ADR-nephropathy. It is postulated that FR167653 inhibited the rearrangement of cytoskeleton and ameliorated podocyte injury, since the effacement of foot process was diffusely observed in these models. However, since p38MAPK is postulated to play critical role in producing cytokines and cell survival, the precise pharmacological mechanism of FR167653 is not clear. We have reported that the injection with the antibody against nephrin, a critical component of the slit diaphragm (SD) caused severe proteinuria. Because the effacement of foot process was sparsely observed, we proposed the molecular rearrangement in the SD is important and the rearrangement of cytoskeleton is not essential in the development of proteinuria in this model.

Methods: In this study, we investigated whether p38MAPK in glomeruli is activated in anti-nephrin antibody (ANA) nephropathy, and analyzed the effect of FR167653. ANA nephropathy was induced in rats by an injection of ANA. FR167653 treatment (50 mg/kg BW/day) started 2 days before the disease induction. The expressions of the SD molecules were analyzed with real-time PCR, western blot and confocal microscopy.

Results: Phosphorylation of p38 MAPK in glomeruli was detected already at 1 h. FR167653 ameliorated proteinuria (day 5: 44.3 vs. 15.1 mg/day, p<0.05). Reduced mRNA expression of the SD molecules was inhibited by FR167653 treatment (% to normal control expression, vehicle vs. FR167653 treatment: nephrin, 4.7 vs. 51.8%: podocin, 16.6 vs. 58.7%: NEPH1, 19.0 vs. 55.5%). Redistribution of nephrin, podocin and NEPH1 on day 14 was partially inhibited by FR167653 treatment.

Conclusions: These results indicated p38MAPK was phosphorylated by direct stimulation to nephrin, and the p38MAPK activation is essential for the rearrangement of the SD molecules and the development of proteinuria. p38 MAPK could become a potential target for therapeutic intervention in proteinuric glomerulopathies.

Funding: Government Support - Non-U.S.

TH-PO956

Bisphenol A Induces Podocitopathy with Proteinuria and Hypertension in Mice Ricardo J. Bosch, Nuria Olea, Carmen Muñoz, Rafael Moreno, Marta González-Santander, María I. Arenas. *Physiology, University of Alcalá, Alcalá de Henares, Spain.*

Background: Bisphenol A (BPA) is a chemical used in plastic exposed to humans, which has a significant presence in diabetic patients (JAMA 300:1303, 2008). Recently, BPA has been associated with proteinuria in Chinese adults (Kidney Int. 81:1131, 2012). Herein we explore the renal effects of BPA.

Methods: The effects of BPA were studied in murine podocytes, mesangial cells and tubuloepithelial cells. Cell viability and apoptosis were measured by MTT assay, flow cytometry and DAPI, respectively. Cell hypertrophy was assessed by cell protein content and [H3]-leucine incorporation. Protein expression was analyzed by Western blot. Moreover, mice (n=17) were intraperitoneally injected with BPA at 50mg/Kg Monday through Friday for five weeks. Arterial pressure was determined by the tail-cuff method.

Results: BPA decreased cell viability and induced apoptosis in all cells studied. While these effects were observed in podocytes starting from 50mM, other renal cells were only affected at 200mM. On podocytes, BPA induced -in a dose dependent manner (from 0.5 to 10nM) - hypertrophy and the upregulation of p27kip1, TGF-β and collagen IV, in a fashion similar to that of high glucose -25mM(HG)-, while diminishing the expression of both nephrin and podocin. BPA-injected mice displayed an increase in both urinary albumin excretion (controls © 2,31±1,4 mg/24h vs BPA 5,13±1,5, p<0,05) and creatinine clearance (C 0,05 ml/min vs BPA 0,08, p<0,05). These animals also displayed an increase in both systolic and diastolic blood pressure (C 79, 75±,15 mmHg vs BPA 88,75±14, p<0,05) and (C 65,27±13 mmHg vs BPA 69, 32±14, p<0.05), respectively. Renal histology showed glomerular hypocellularity and mesangial expansion.

Conclusions: On podocytes, BPA is able to induce apoptosis, hypertrophy and the upregulation of p27kip1, TGF-β and collagen IV in a fashion similar to that of HG. BPA-injected animals develop proteinuria, hyperfiltration, glomerular hypocellularity, mesangial expansion and hypertension. Further studies are needed to clarify the potential role of BPA in the pathogenesis of renal diseases, particularly in diabetic patients.

TH-PO957

The Essential Role of Akt2 in Stressed Podocytes Frank Bienaime,[1] Guillaume Canaud,[1] Amandine Viau,[1] Clement Nguyen,[1] Andrea Onetti-muda,[2] Gerard Friedlander,[1] Christophe M. Legendre,[1] Fabiola Terzi.[1] *[1]INSERM U845 - Universite Paris Descartes - APHP; [2]Campus BIo-Medico University.*

Background: In chronic kidney disease (CKD), the loss of functional nephrons results in the overload of the remaining ones. This leads to a metabolic and mechanical stress triggering, in turn, further nephron loss. The molecular pathways engaged to counteract this stress are still unknown. The aim of this study was to evaluate the potential role of AKT proteins in the adaptation of glomerular cells to nephron reduction.

Methods: On that purpose, we combined different models of nephron reduction (subtotal and unilateral nephrectomy, aging nephropathy), glomerular isolation through laser capture microdissection and *Akt1*, *Akt2, Akt2*$^{\Delta pod}$ mutant mice. In addition, we assessed the impact of sirolimus, in terms of AKT pathway activation and adverse effects incidence (proteinuria), in a cohort of renal transplant recipients with different degrees of renal function.

Results: We showed that the activation of the serine/threonine kinase AKT2 plays an essential role in the survival of podocytes and in their adaptation to nephron reduction, without affecting tubular cells. We demonstrated that this function is isoform specific. In fact, glomerular lesions and albuminuria were dramatically increased in *Akt2*$^{-/-}$, but not in *Akt1*$^{-/-}$ mice. Specific gene deletion in podocytes revealed that the protective effect of AKT2 is cell autonomous. Mechanistically, AKT2deficiency acts by preventing the triggering of the compensatory genetic program which involve MDM2, GSK3 and RAC1 functional regulation. This is associated with increased apoptosis and foot processes effacement in *Akt2*$^{-/-}$ podocytes. Remarkably, these data are relevant to human CKD where AKT2 activation by mTORC2 is essential for podocyte survival in patients with severe nephron reduction. More importantly, we provide evidence that AKT2 activation in podocytes could serve as a prognostic marker to predict the deleterious proteinuric effect of sirolimus.

Conclusions: In conclusion, our results disclose a novel function played by AKT2 in podocytes and identify a potential therapeutic target for the maintenance of glomerular function in CKD.

Funding: Government Support - Non-U.S.

TH-PO958

Role of Podocyte Extracellular Sulfatase in Regulation of Glomerular Permeability Yasutoshi Takashima,[1] Hanako Yamashita,[1] Kazuo Sakamoto,[1] Satoshi Hara,[1] Namiko Kobayashi,[1] Toshiharu Ueno,[1] Kazuko Keino-masu,[2] Masayuki Masu,[2] Michio Nagata.[1] *[1]Renal Pathology, University of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Molecular Neurobiology, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: Heparan sulfate proteoglycan(HSPG) plays a pivotal role for maintaining local concentration of growth factors and may be important to explain glomerular pathophysiology. Extracelluar sulfatase(Sulf) 1 and 2 are the core regulator of HSPG and Sulf1 was expressed glomerular podocytes. Since Sulf1 and 2 have been suggested to compensate, Sulf1 and 2 double knockout mice (DKO) are useful to determine the function of Sulf on the glomerular permeability. We suppose that Sulfs alter the bioavailability of signaling molecules transmitting between podocyte foot processes,and examine expression of growth factors and those receptors produced by podocyte.

Methods: DKO were used to examine urinary albumin and glomerular morphology. In addition, mRNA expressions for slit membrane proteins, cytoskeletons, and several growth factors and those receptors by Real time PCR were estimated. Cellular events are analyzed by the immortalized podocyte cell line.

Results: DKO revealed significant increase in albuminuria than wild type. Histology showed frequent mesangiolysis followed by mesangial proliferation in DKO. Ultrastructure showed predominant foot process effacement and increased incidence of out pocket in GBM in DKO than wild type. However presence of anionic sites revealed by PEI stain did not show any difference between DKO and wild type. Kidney lysate in DKO revealed significantly decreased expression of synaptopodin by Real time PCR. Double knock down in Sulf1 and 2 in cultured podocytes revealed down-regulation of podocyte molecules as found DKO *in vivo*, and several growth factors including PDGF, VEGF, FGF. Double knock down in Sulf1 and 2 by siRNA showed induction of PDGFR-β in podocytes *in vitro*.

Conclusions: Endosulfatase may regulate growth factor and receptor expression in podocytes because PDGFR-β is localized in podocytes. Foot process effacement and significant albuminuria in DKO suggests that podocyte sulfatase may participate in glomerular permeability *via* growth factor regulation on podocyte.

Funding: Government Support - Non-U.S.

TH-PO959

Dual Blockade of Angiotensin II and Prorenin Receptors Ameliorates Podocytic Apoptosis in IgA Nephropathy through Inhibition of the Notch Signaling Pathway Joseph C.K. Leung, Ai Ing Lim, Loretta Y.Y. Chan, Sydney C.W. Tang, Kar Neng Lai. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: Glomerulo-podocytic communication plays an important role in podocytic injury in IgA nephropathy (IgAN). We examined the roles of podocytic angiotensin II receptor subtype 1 (ATR1) and prorenin receptor (PRR) in podocytic apoptosis in IgAN.

Methods: Conditioned medium was prepared from growth arrested mesangial cells incubated with polymeric IgA from patients (IgA-medium, n=28) or controls (Ctl-medium, n=25). An immortalized human podocyte cell line was used to study the regulation of ATR1, PRR, TNF-α and TGF-β by IgA-medium. Expression of p53 and cleaved caspase 3 was used as functional readout of podocytic apoptosis.

Results: Podocytic expression of ATR1 and PRR were up-regulated by IgA-HMC medium dose and time dependently (p<0.01). There was increased expression of ATR1 and release of TGF-β, but not PRR, by podocyte cultured with TNF-α (p<0.05). The IgA-medium, TNF-α or TGF-β induced podocytic apoptosis. We further examined the anti-apoptotic effects of mono- or combined blockade of ATR1, PRR, TNF-α and TGF-β; with losartan, PRR siRNA transfection and anti-TNF-α and anti-TGF-β antibodies. Mono-blockade of ATR1, PRR or TNF-α partially reduced podocytic apoptosis. Dual blockade of ATR1 and PRR, or mono-blockade with anti-TGF-β, effectively rescued podocyte from IgA-medium induced apoptosis. Interestingly, IgA-medium or TGF-β up-regulated the expression of Jag1, notch1 and Hes1 by podocyte. The related podocytic apoptosis was abolished by the γ-secretase blocker, suggesting the important role of notch signaling pathway in this apoptotic process. Blockade of PRR or ATR1 decreased the IgA-medium induced TGF-β release and notch1 expression by podocyte, and these TGF-β release and notch1 expression were further reduced through dual blockade of ATR1 and PRR.

Conclusions: Our data suggest that the TGF-β and notch signaling pathway are the key players in podocytic apoptosis induced by glomerulo-podocytic communication in IgAN. Targeting the notch signaling pathway, AT1R and prorenin receptor could be a potential therapeutic option to reduce the podocytic injury in IgAN.

Funding: Other NIH Support - General Research Fund Hong Kong #768910

TH-PO960

Over-Expression of Heme Oxygenase-1 in Rattus Podocytes Antagonizes Puromycin Aminonucleoside Nephrosis Pu Duann,[1] Ling-Mei Chiang,[2] Shing Li.[1] *[1]Medicine / Nephrology Div, Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Paediatric, Chang Gung Medical Center, Keelung, Taiwan.*

Background: Induction of heme oxygenase (HO)-1 is a key defense mechanism against oxidative stress. Compared with tubules, glomeruli are refractory to HO-1 upregulation in response to injury. It may be associated with insufficient production of cytoprotective heme-degradation metabolites. We, therefore, explored whether targeted HO-1 expression

can be achieved in glomeruli without altering their physiological integrity. A transgenic mice was achieved in FVB mice (Am J Physiol. 297[5]:F1476, 2009). But the established glomerulopathy stdies previously done in rat model was unable to be repeat in mice. Mice glomeruli isolation is sophisticated thus prevent mechanism exploration.

Methods: By microinjection Sleeping Beautytransposon vector (*SB10*) with pNeph-FLAG-hHO1expression cassette we obtained transgenic rats with GEC targeted HO-1 overepression. Assessed by linker mediated genomic sequencing, at least five independent gene integration in different chromosomes were confirmed.

Results: The transgene show stable inheritance confirmed by both southern blot analysis and PCR based gene typing. These rats show no apparent changes in weight, eating habits, fur color or motility, infertility or deviated Mendelian ratio. Screen all organs with Western blot analysis, FLAG-tagged transprotein was detected only in the kidney, and furthermore, in isolated glomeruli it show 20 folds high than in crude kidney extract. It was immunolocalized in podocyte overlayed with the nephrin. SB-HO1 transgenic rats show less proteinuria than age- weight-matched wild type control Sprague Dawley rats at 12 hour (UpUc[mg/mg]: 23.12 ± 6.91 vs. 14.81 ± 3.60, p value = 0.013) and 24 hours (UpUc[mg/mg]: 54.14 ± 18.67 vs. 25.15 ± 6.53, p value < 0.01) after tail vein injection of puromycin aminoglycoside (100 mg/Kg BW/100 µl DMSO).

Conclusions: We generated a transgenic rats, SB-HO1, with GEC targeted HO-1 overexpression. The GEC targeted HO-1 overexpression antagonize puromycin aminonucleoside nephrosis and reduce proteinuria. This animal model could help us study the role of renoprotective HO-1 in the GEC pathobiology.

Funding: Other U.S. Government Support

TH-PO961

Inhibition of Notch Signal Reduced Extra-Capillary PEC Lesion and Deteriorated Proteinuria in Mouse Model of Collapsing Focal Segmental Glomerulosclerosis (cFSGS) Toshiharu Ueno,[1] Namiko Kobayashi,[1] Yasutoshi Takashima,[1] Taiji Matsusaka,[2] Michio Nagata.[1] *[1]Department of Renal Pathology, University of Tsukuba, Tsukuba-city, Ibaraki, Japan; [2]Department of Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan.*

Background: Parietal epithelial cell (PEC) hyperplasia is the hallmark of cFSGS. We presented aberrant Notch signals may provoke PEC lesion though Epithelial-Mesenchymal-Transition and migration during the morphogenesis of cFSGS both in mouse model of cFSGS and PEC cell line at ASN 2011. In this study, we aimed to investigate the effect of Notch inhibition in the development of cFSGS in mice.

Methods: NEP25 mice (10-24 weeks of age) indicate podocyte-specific injury upon injection of immunotoxin (LMB2). They were injected LMB2 (4 ng/g B.W, iv) on day 0, treated either with γ-secretase inhibitor (Dibenzazepine; DBZ, 5µg/g B.W, ip) from day 6 to 12 or with vehicle (controls), and sacrificed on day 12 (n=5 per group). Histologically, incidence of PEC lesion (PEC score) and podocyte number (WT-1 positive cell) were analyzed. Proteinuria and area of urinary cast in kidney cortex (cast ratio) were measured.

Results: Controls showed severe proteinuria and histological feature of cFSGS. In light microscopy, number of WT-1 positive cell was similar in both group (2.36±0.09 vs. 2.42±0.07per glomerulus) indicating that same degree of podocyte injury was induced. DBZ treated mice showed significant reduction of PEC lesion represented by PEC score (2.2±0.2 vs. 3.7±0.4;P<0.01). Electron microscopy revealed migration of PEC onto collapsed glomeruli in controls, whereas such lesion was not observed in DBZ treated mice. Podocyte on collapsed glomeruli indicated severe foot process effacement, however, PECs surrounding such collapsed glomeruli showed almost normal morphology in DBZ treated mice. Larger amount of proteinuria was estimated in DBZ treated mice represented by cast ratio compared with controls (24.72±3.06 vs. 8.16±1.07%;P<0.01).

Conclusions: Notch signal is essential for emergence of PEC lesion and inhibition of Notch signal deteriorated proteinuria in mice cFSGS; this implies a potential protective role of PEC for injured filtration barrier accompanied by severe podocyte injury.

TH-PO962

APOL1 Expression and Localization Pattern in Kidney Diseases with and without Association to Risk Genotype John F. O'Toole,[1] Sethu M. Madhavan,[1] Martha Konieczkowski,[1] Santhi Ganesan,[1] Laura M.C. Barisoni,[2] David B. Thomas,[2] Leslie A. Bruggeman,[1] John R. Sedor.[1] *[1]Case Western Reserve University; [2]University of Miami.*

Background: Genetic variants in *APOL1*, encoding apolipoprotein L1 (APOL1), associate with non-diabetic kidney diseases in patients of African ancestry. We have localized APOL1 to podocytes, basolateral domains of proximal tubules, and arteriolar endothelium of normal human kidney. The localization pattern changes in non-diabetic kidney diseases. We hypothesized that APOL1 was synthesized in kidney and variants would alter APOL1 localization and distribution.

Methods: APOL1 localization and expression was examined in cultured cells, normal and diseased human kidney with immunohistology, immunogold electron microscopy (IEM), *in situ* hybridization (ISH), and RT-PCR.

Results: Using ISH and RT-PCR, *APOL1* transcripts were identified in normal human glomeruli but not tubules, despite the presence of APOL1 protein in both locations. IEM localized APOL1 to the cytoplasm of podocytes and the mitochondria of proximal tubules, confirmed by colocalization with the alpha subunit of ATP synthase. In FSGS, HIVAN, HTN, minimal change, diabetes and normal kidney, we used semi-quantitative methods to examine proximity of APOL1 to podocyte markers and its colocalization with mitochondrial markers in tubules. Contrary to our hypothesis, APOL1 distribution patterns, its relationship to podocyte and mitochondrial markers, and APOL1 expression levels did not vary with disease diagnosis or genotype. In cell culture, wild type and variant APOL1 proteins had similar localization under basal conditions and after treatments to mimic ER stress, growth factor-/starvation-induced autophagy and mitochondrial dysfunction.

Conclusions: Podocytes synthesize APOL1, suggesting locally expressed APOL1 regulates non-diabetic CKD pathogenesis. APOL1 in proximal tubules may represent uptake from glomerular filtrate or circulation. Since APOL1 distribution patterns changed concordantly in all diseases and cell culture conditions examined regardless of genotype, we conclude *APOL1* variants do not mediate renal injury by changing APOL1 localization or trafficking, but rather through aberrant protein function.

Funding: NIDDK Support, Clinical Revenue Support

TH-PO963

Podocyte Specific Myh9 Deletion in C57BL/6 Mice Results in Susceptibility to Glomerulopathy in Experimental DOCA-Salt Uninephrectomy Model Philip A. Bondzie, Mostafa Belghasem, Felita Agus, Joseph S. Coppola, Hui Chen, Joel M. Henderson. *Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA.*

Background: Single nucleotide polymorphisms in the gene MYH9 encoding the protein non-muscle myosin heavy chain IIA (NMHCIIA), have been found to be associated with an increased incidence of chronic kidney disease in African Americans. In this study we seek to investigate the role of Myh9 in glomerular response to chronic hypertension.

Methods: C57BL/6 podocyte specific Myh9 knockout (KO) mice and controls were subjected to uninephrectomy and deoxycorticosterone acetate (DOCA)-salt treatment for 6 weeks. Kidney tissue morphology was evaluated by light and electron microscopy, and kidney function was assessed with urine and serum biomarkers. We also profiled gene expression in laser captured glomeruli from these mice.

Results: Podocyte Myh9 KO mice developed widespread juxtamedullary glomerulosclerosis, segmental cellular proliferation within glomeruli, protein casts and mild glomerular ultrastructural changes in normal-appearing glomeruli, as well as significant proteinuria. In contrast, control mice showed milder changes. In podocyte Myh9 KO mice, differential glomerular expression was observed in several genes involved in cell motility.

Conclusions: In summary, Myh9 deficiency in podocytes in C57BL6 mice is associated with susceptibility to glomerulopathy in a DOCA-salt uninephrectomy model. Changes in glomerular expression of genes associated with cell motility may play a role in this process.

TH-PO964

Glomerular Expression of Renin-Angiotensin System Components in Rats with Podocyte Dysfunction Mihoko Yamazaki, Aya Takahashi, Yuichi Takahashi, Yoshiyasu Fukusumi, Masayuki Tomita, Hiroshi Kawachi. *Department of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: The local renin-angiotensin system (RAS) is reported to have essential roles in maintaining the local tissue function. We have previously reported angiotensin II (AII) treatment reduced the expression of the slit diaphragm (SD) components (Am J Pathol 2007). The report indicated that AII is involved in the regulation of the barrier function of the glomerular capillary wall. However, the role of the local RAS in glomeruli remains uncertain.

Methods: In this study, the glomerular expressions of RAS components, angiotensinogen (AGG), renin, ACE, ACE2, aminopeptidase A, neprilysin, AII type1 and type 2 receptors (AT1R, AT2R) were analyzed in rats with puromycin aminonucleoside (PAN) nephropathy and anti-nephrin antibody (ANA)-induced nephropathy. We also analyzed the glomerular expressions of the RAS and the SD components in rats treated with RAS blockers, ARB and angiotensin converting enzyme inhibitor (ACE-I). Then, we investigated the effects of ARB on the expressions of RAS components and on proteinuria in these models. The expressions of RAS components in cultured podocyte were also analyzed.

Results: mRNA expressions of all RAS components were detected in normal rat glomeruli and in cultured podocytes. mRNA expression of AGG was increased in PAN nephropathy(483%). The expression of renin and ACE was downregulated, whereas that of neprilysin converting angiotensin-(1-10) to (1-7) was upregulated. The increase of AGG is also observed in ANA-nephropathy (426%). The expression of ACE was increased in rats treated with ACE-I (224%). The increase of ACE in rats with ARB is milder than that with ACE-I. The expression of AT2R was lowered and that of the SD molecules was increased in both groups. Proteinuria in PAN nephropathy is reduced with ARB treatment (358 vs. 204 mg, p<0.05). The decrease of SD components and the increase of AGG were inhibited by ARB treatment.

Conclusions: Altered expression of local RAS components and the consequent decreased expression of the SD molecules are involved in the development of podocyte dysfunction.

Funding: Government Support - Non-U.S.

TH-PO965

Podocytes Detaching from the GBM in Glomerular Diseases Frequently Adhere to the Parietal Basement Membrane Wilhelm Kriz. *Anatomy, Medical Faculty Mannheim, University of Heidelberg, Germany.*

Background: As known from previous studies of inflammatory glomerular diseases (Le Hir et al. 2001, JASN 12:2060; Moeller et al. 2004, JASN 15:61)podocytes may penetrate in between two parietal epithelial cells of Bowman's capsule and attach to the parietal basement membrane (PBM). Thereby, a cell bridge is established between the tuft

and Bowman's capsule that frequently acts as the nidus for the progression of the injury to complete degeneration of the concerned nephron. It was thought that unknown inflammatory mediators increased the motility of podocytes initiating such contacts with the PBM.

Methods: The interconnections between the tuft and the parietal epithelium were analysed by transmission electron microscopy in several models of non-inflammatory glomerular diseases, including DOCA-salt hypertension in rats, long-term growth stimulation by bFGF in rats, uninephrectomy in young rats, the Fawn-hooded hypertensive rat, the Fa/Fa rat, among others.

Results: These studies revealed that active adhesion of podocytes to the PBM is not restricted to inflammatory diseases but is also seen in degenerative and genetic models of glomerular diseases. It is frequently seen that podocytes undergoing detachment from the GBM fix to the PBM with apical cell portions. Podocytes in danger of detachment from the GBM appear to search for a firm fixation at other sites and may find the PBM. A prerequisite to this kind of migration from the GBM to the PBM appears to consist of a frequently observed surface change of podocytes, called "microvillous transformation". Diseased podocytes (in danger of detachment?) may form countless microvilli that spread into all directions. In case that such a microvillus attaches to some other site (GBM or PBM) it may initiate the migration of the entire cell to this new contact site.

Conclusions: Podocytes in danger of detachment from the GBM seek halt at other sites. In case of a "successful" fixation to the PBM, cell bridges between the tuft and Bowman's capsule may be formed that may lead to a firm adhesion of the tuft to Bowman's capsule, thus to a committed lesion progressing to crescent formation and finally to the loss of the entire nephron.

Funding: Government Support - Non-U.S.

TH-PO966

Amyloid-Like Protein 1: A Biomarker for Podocytes Injury? Zhao-hong Chen, Shaolin Shi, Junnan Wu, Cai-hong Zeng, Ming-chao Zhang, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Background: Amyloid-like protein 1 (APLP1) is a membrane-associated glycoprotein that is cleaved by secretases. This cleavage liberates an intracellular cytoplasmic fragment that may act as a transcriptional activator. APLP1 may play a role in endocytosis, apoptotic process and cell adhesion. APLP1 was found upregulated in the glomeruli of several glomerular injury models. Here we explore if APLP1 is a potential biomarker for podocyte injury.

Methods: 10 cases of biopsy-proven focal segmental glomerulosclerosis were included in this study. Unaffected normal renal tissues from kidneys with cancer (n=5) were used as control. APLP1 mRNA was quantified by qRT-PCR using glomeruli microdessected from renal biopsies. APLP1 protein and localization in glomeruli of FSGS patients were detected by immunohistochemistry (IHC) and immunofluorescence (IF). The effect of TGF-β on APLP1 mRNA and protein expression in podocytes was determinined by qRT-PCR and western blotting.

Results: The results of qRT-PCR showed that APLP1 mRNA was upregulated in glomeruli of FSGS patients as compared to normal controls. Gene expression of APLP1 in kidney biopsies was significantly related (R= 0.468, p= 0.023) to the level of proteinuria. Colocalization studies detected APLP1 protein along with the podocyte marker synaptopodin in glomeruli. No colocalization was observed between APLP1 protein and endothelial (CD31) marker. The results of IHC also showed that APLP1 was expressed in glomerular podocytes and its expression was significantly increased in the patients with FSGS and podocyte disease as compared to normal control (Intensity Score: 6.98±0.8 vs 3.6±0.4, P <0.05). In vitro studies showed that TGF-β upregulated APLP1 mRNA and protein expression in podocytes, which accompanied by podocin decreased expression.

Conclusions: APLP1 is exclusively upregulated in podocytes of FSGS patients, and its upregulation positively correlates with urinary protein level of the patients, thus representing a potential biomarker of podocyte injury. We are determining the role for APLP1 in podocyte injury.

Funding: Government Support - Non-U.S.

TH-PO967

Basement Membrane Changes and Loss of Podocyte-Glomerular Basement Membrane (GBM) Interactions in Adult Mouse Kidney after Deletion of WT1 Sharada Mokkapati,[1] Fei Gao,[2] Weihua Tian,[1] Vicki Huff,[1] Qianghua Hu.[1] *[1]MD Anderson Cancer Center; [2]Chinese Academy of Sciences.*

Background: *Wt1* is a zinc finger transcription factor known to be important for normal kidney function. Previous studies have shown that mutation of this gene results in glomerulosclerosis and end stage renal failure in both humans and in mice. The cellular mechanism by which this mutation leads to the renal phenotype remains to be elucidated in detail.

Methods: Towards this we used two different mouse models in which we ablated *Wt1* in the adult kidney. In the first model, we crossed $Wt1^{fl}$ mice with a podocyte specific Cre transgenic mouse line, *NPHS2-Cre*. At one week of age these mice had no renal pathology but by two weeks these mice developed massive proteinuria with severe glomerulosclerosis and massive podocyte depletion and died between 3-4 weeks of age due to end-stage renal failure. In the second model we ablated *Wt1* in the adult kidney postnatally using the *TM-Cre* transgenic mouse line where the expression of Cre was ubiquitous when induced by tamoxifen treatment.

Results: As early as 10 days after the tamoxifen injection these mice developed proteinuria, which developed into glomerulosclerosis and end-stage renal failure. The phenotype was more dramatic than the mutants with the *NPHS2-Cre*. We further analysed GBM changes in the kidneys after deletion of Wt1 by real time PCR, protein analysis and immunofluorescence. There was a marked increase in the expression of basement membrane components by both real time PCR analysis and by western blotting. Expression of podocyte specific integrins was dramatically reduced in the mutant mice.

Conclusions: Our data, therefore suggests that *Wt1* is critical for podocyte GBM interactions and deletion of *Wt1* leads to loss of this interaction, GBM changes which then lead to glomerulosclerosis and end stage renal failure.

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TH-PO968

Endothelial Ablation of Sirtuin 1 (SIRT1) Leads to Down-Regulation of MMP-14, Spontaneous Interstitial Fibrosis and Exaggerated Fibrotic Response to Injury Jun Chen,[1] Sandhya Xavier,[1] Radovan Vasko,[1] Julien Maizel,[1,2] Jian Cao,[3] Michael S. Goligorsky.[1] *[1]New York Medical College, Valhalla, NY; [2]INSERM, Unit 1088, Jules Verne University of Picardie, Amiens, France; [3]State University of New York, Stony Brook, NY.*

Background: SIRT1 deficiency is a constant companion of aging process and stress-induced premature senescence (SIPS). We have recently demonstrated that various stressors deplete SIRT1 through cathepsin cleavage due to lysosomal membrane permeabilization.

Methods: We used cre-lox strategy to generate mice with truncated SIRT1 gene in Tie-2-expressing endothelial (EC) and endothelial progenitor cells (EPC).

Results: Endo-SIRT1(-/-) mice are fertile, normotensive, thrive normally, but exhibit mild suppression of acetylcholine-induced vasorelaxation and matrigel angiogenesis, elevated numbers of senescent EC and EPC with reduced resistance to stress, as well as interstitial fibrosis, but no proteinuria or elevation of serum creatinine. When challenged with folic acid, mice develop an exaggerated acute loss of function compared to controls, but all recover renal function within 1 month. Three months later, Endo-SIRT1(-/-) mice develop proteinuria and renal insufficiency significantly more severe than their controls. The degree of tubulointerstitial fibrosis is much enhanced in Endo-SIRT1(-/-) mice. To disclose the pathogenetic link between endothelial SIRT1 deficiency and exaggerated renal fibrosis, we examined expression of a master regulator of matrix metalloproteases, MMP-14. qRT-PCR showed down-regulation of MMP-14, confirmed by immunoblot analyses. The promoter region of MMP14 was sequenced and analyzed. It contains consensus binding sites for NF-kB and p53, both controlled by SIRT1 expression. Concanavalin A treatment of cultured EC up-regulated MMP-14 expression regardless of the activity of SIRT1. Sirt inhibitor suppresses angiogenesis of HUVEC and mouse aortic rings in matrigel and ConA restores angiogenesis.

Conclusions: Data obtained in Endo-SIRT1(-/-) mice demonstrate the pathogenetic link between defective SIRT1 expression, SIPS and fibrosis. The latter is a result of the MMP-14 deficiency that develops as a consequence of SIRT1 deficiency.

Funding: NIDDK Support

TH-PO969

PBI-4419, a Novel First-in-Class Anti-Fibrotic Compound, Inhibits CTGF and Collagen Production in Murine and Human Fibroblasts and Reduces Kidney Fibrosis in 5/6-Nephrectomized and Unilateral Ureteral Obstruction Rat Models Martin Leduc, Lilianne Geerts, Brigitte Grouix, François Sarra-Bournet, Liette Gervais, Shaun Abbott, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin, Lyne Gagnon. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: Interstitial fibroblasts are the principal effector cells of organ fibrosis. Transforming growth factor (TGF)-β-stimulated fibroblasts secrete connective tissue growth factor (CTGF) that functions as a downstream mediator of TGF-β action on fibroblastic cell types, and are important in collagen production.

Methods: The aim of this study was to investigate the effect of PBI-4419, a novel first-in-class anti-fibrotic compound, on CTGF and collagen production in TGF-β-stimulated murine (NIH/3T3) and human fibroblasts (normal human dermal fibroblast, NHDF).

Results: In both cell types, TGF-β induced a robust increase of the mRNA expression of CTGF (10 to 25 fold increase) and collagen 1 (2 fold increase). In TGF-β-stimulated NHDF, PBI-4419 reduced CTGF and collagen 1 expression to the control level (untreated). Furthermore, in non-stimulated NHDF, PBI-4419 reduced basal CTGF (80%) and collagen 1 (50%) expression. Modulation of mRNA expression was also translated at the protein level, as TGF-β-induced CTGF production was significantly inhibited by PBI-4419 in NHDF. In NIH/3T3 fibroblasts, TGF-β-induced CTGF and collagen 1 mRNA overexpression were also significantly reduced by PBI-4419. These results correlate with in vivo inhibition of CTGF and collagen 1 mRNA expression observed in different models of kidney fibrosis. In 5/6-nephrectomized rats, oral administration of PBI-4419 significantly decreased the overexpression of CTGF (down to sham level) and collagen 1 (reduction of 60%) mRNA expression in the remnant kidney. In the unilateral ureteral obstruction rat model, a significant dose-dependent inhibition of the expression of CTGF and collagen 1 is also observed in animals treated with PBI-4419.

Conclusions: These results indicate a direct effect of PBI-4419 on fibroblasts as observed by an inhibition of CTGF and collagen 1 mRNA expression and this is translated by a reduction of fibrosis in the kidney.

Funding: Pharmaceutical Company Support - ProMetic BioSciences Inc.

TH-PO970

Exploring Transcriptional Regulation of Renal Fibrosis in the Rat UUO Model Using Next Generation Sequencing Steven M. Weldon,[1] Thomas B. Freeman,[2] Hu Sheng Qian,[1] Damian C. Matera,[1] Tobias Hildebrandt,[3] Ramona Schmid,[3] Detlev Mennerich,[3] Glenn A. Reinhart,[1] William Loging.[2] *[1]CardioMetabolic Disease, Boehringer-Ingelheim Pharm. Inc; [2]Scientific Knowledge Discovery, Boehringer-Ingelheim Pharm. Inc; [3]Target Discovery Research, Boehringer-Ingelheim Pharm. Inc, Ridgefield, CT.*

Background: Tubular interstitial fibrosis (TIF) is a major factor in the progression of chronic kidney disease (CKD). Current therapy for CKD is limited to ACEi and ARBs which provide only modest protection. As such, it is critical to gain a better understanding of the molecular mechanisms modulated by renal injury and CKD.

Methods: We used unilateral ureteral obstruction (UUO) combined with next generation sequencing (NGS) to explore the transcriptional regulation of molecular pathways associated with TIF in rats. SD rats were treated with enalapril (ENA; 3,30,100 mg/kg) or the anti-fibrotic agent BIX-006 (1,10 mg/kg) one day prior + 5 days following UUO. TIF was assessed by multiphoton microscopy using second harmonic signal generation (SHG) for imaging fibrillar collagen. mRNA from renal cortical tissue was subjected to NGS followed by computational analysis to indentify differentially regulated genes.

Results: ENA and BIX treatment resulted in dose-dependent inhibition of TIF, 27-60% and 16-50%, respectively. Effects on mechanistic genes/pathways (Col1, Fn, serpine1, igf1,ren) predicted to be modulated following treatment correlated with fibrosis. A variety of transcript pathways differed between treatment groups. BIX affected fibrotic, whereas ENA affected cell proliferative pathways. These pathways and their component transcripts also displayed dose dependent modulation.

Conclusions: These are the first data to describe the use of NGS to identify and quantitate transcriptional regulation of fibrotic and other selected pathways in the UUO model. Further, we show that changes in the rat transcriptome following UUO correlate to developing TIF and are differentially regulated by enalapril and an anti-fibrotic agent BIX-006. These data offer a novel approach to identifying new target pathways and potential biomarkers of fibrotic renal disease associated with CKD and subsequent renal failure.

Funding: Pharmaceutical Company Support - Boehringer-Ingelheim Pharmaceuticals Inc.

TH-PO971

Brilliant Blue G, a P2X7 Antagonist, Attenuates Renal Inflammation and Fibrosis in Unilateral Ureteral Obstruction in Rats Maurilo Leite,[1] Jose Monteiro Pereira,[3] Andre Barreira,[1] Conrado Rodrigues Gomes,[1] Christina Maeda Takiya.[2] *[1]Division of Nephrology, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil; [2]Biophysics Institute, UFRJ, RJ, Brazil; [3]Division of Urology, UFRJ, RJ, Brazil.*

Background: We have previously documented that purinergic P2X7 receptor (P2X7R) knockout mice, submitted to unilateral ureteral obstruction (UUO), presented less inflammation and interstitial fibrosis compared to wild type mice. The present study investigated the effect of BBG (brilliant blue G), a selective purinergic P2X7R antagonist, on rat kidneys after 14 days UUO.

Methods: Male Wistar rats were submitted to left kidney UUO. Four groups (G) were applied as follows: G1, sham operated and saline infusion; G2, sham and BBG 25mg/kg intravenously (IV); G3, UUO and saline infusion; G4, UUO and BBG 25mg/kg IV. Saline and BBG infusion were given on days 1 and 7. On day 14, the rats were sacrificed, and the left kidneys removed for Sirius red staining, immunohisthochemistry (IH) and PCR studies. For IH, antibodies (Ab) for macrophages (ED-1), myofibroblasts (SMA), PCNA and P2X7 were used. PCR was done for IL-1β.

Results: Sirius red staining revealed higher collagen surface density (SD) on G3 as compared with G1, G2 and G4 (0.017, 0.004, 0.005 and 0.007%, respectively, P<0.0001). ED-1 positive SD was higher on G3 as compared to G1, G2 and G4 (0.015, 0.001, 0.001 and 0.005%, respectively, P<0.0001). Also, SMA positive SD was higher in G3, compared with G1, G2 and G4 (0.046, 0.002, 0.004 and 0.008%, respectively, P<0.0001). However, PCNA tubular proliferative index was higher in G4, as compared to G3, G1, and G2 (32, 8, 10 and 2%, respectively, P<0.0001). G3, but not G4, showed P2X7R Ab positive cells, mainly on tubular epithelium, suggesting some interference of BBG on receptors epitope. PCR study clearly showed weaker appearance of IL-1β mRNA in G4.

Conclusions: In rats submitted to UUO, BBG was able to attenuate renal interstitial inflammation, collagen deposition and promoted tubular cell proliferation. P2X7 antagonism decreased IL-1β mRNA expression. These results constitute evidence that the use of P2X7R antagonists can be a strategic tool for the treatment of progressive kidney disease.

Funding: Government Support - Non-U.S.

TH-PO972

Implication of Connexin37 in Obstructive Nephropathy Ahmed Abed,[1] Ludwig Scheckenbach,[2] Bernard Foglia,[2] Christos Chatziantoniou,[1] Brenda Kwak,[2] Christos E. Chadjichristos.[1] *[1]INSERM UMRS 702, INSERM, Paris, France; [2]Dept. of Pathology and Immunology, University of Geneva, Geneva, Switzerland.*

Background: Chronic kidney disease (CKD) is promoted by a variety of factors that induce chronic inflammation and fibrosis. We have recently demonstrated that altered expression of the gap junction protein connexin37 (Cx37) is an early signal of CKD. The objectives of our study were to characterize the different cell types that express Cx37 in the kidney under normal conditions, and to investigate the role of this Cx in obstructive nephropathy.

Methods: Co-localization studies for Cx37 and appropriate markers of different nephron segments and vascular compartments were performed in renal cortical sections of C57BL/6 mice. In separate experiments, 3 month-old $Cx37^{-/-}$ and wt mice underwent unilateral ureteral obstruction (UUO) for 7 days before tissue collection (n=8 per group).

Results: Cx37 was abundantly expressed in the glomerular and peritubular endothelium (co-localized with MECA-32) of WT mice. Cx37 was also expressed in distal convoluted tubules and collecting ducts as evidenced by its co-localization with NCCT and AQP2, respectively. Seven days after UUO, Cx37 mRNA (qPCR) and protein (immunofluorescence) expressions were dramatically reduced. Interestingly, $Cx37^{-/-}$ mice were protected in terms of tubular dilation (tubular index: 1.0±0.2 for $Cx37^{-/-}$vs. 2.5±0.2 for WT mice, P<0.05), showed less tubulo-interstitial proliferation (Ki67 positive cells/field: 11±1 vs. 20±1 for $Cx37^{-/-}$ and WT, respectively, P<0.05) and lower tubular apoptosis (Tunel positive cells/field: 4.0±0.3 and 8.0±0.5 for $Cx37^{-/-}$ and WT, respectively, P<0.05). At the same time period, F4-80 staining showed reduced monocyte infiltration in the renal cortex of $Cx37^{-/-}$ mice compared to WT littermates (15±2% and 25±2% of cortex surface, respectively, P<0.05). Finally, the expression (western blot) of E-cadherin, a marker of normal renal epithelial cells, was preserved $Cx37^{-/-}$ mice.

Conclusions: Our study highlights the importance of connexins in obstructive nephropathy and suggests that reducing Cx37 may have beneficial effects on the development of renal disease.

TH-PO973

miR-205 Expression Correlates with Severity of Renal Involvement in a Mouse Model of Congenital Obstructive Nephropathy Michael Wilhide,[1] Ashley R. Carpenter,[1] Kirk M. McHugh,[1,2] Susan E. Ingraham.[1,2] *[1]The Research Institute at Nationwide Children's Hospital, Columbus, OH; [2]Department of Pediatrics, Division of Nephrology, College of Medicine at Ohio State University, Columbus, OH.*

Background: Congenital obstructive nephropathy (CON) is a leading cause of chronic kidney disease (CKD) and end stage renal disease (ESRD) in children. CON is a complex disease process involving pathological changes in kidney development and function that occur as a result of obstructed antegrade urine flow beginning *in utero*. We hypothesize that microRNAs (miRs) play important roles in the renal response to urinary obstruction and in regulating gene expression in the pathogenesis of CON. Thus, miRs may represent an important novel class of potential biomarkers and therapeutic targets in CON.

Methods: The megabladder (*mgb-/-)* mouse is an animal model of CON that develops kidney disease secondary to a bladder development defect. Specific miR expression levels were measured by quantitative PCR (qPCR) of kidney samples from wild type and *mgb-/-* mice.

Results: There was increased expression of miR-205 across an unstratified panel of *mgb-/-* kidneys compared to wild-type controls (Relative Quantitation [RQ] =3.49, *P*=0.004). Furthermore, upon stratification of the *mgb-/-* population by severity of kidney involvement, relative miR-205 expression levels rise with worsening grade of hydroureteronephrosis (Mild: RQ=1.60, *P*=0.59; Moderate: RQ=2.73, *P*=0.007; Severe: RQ=6.32, *P*=0.001; all values are relative to wild-type control). This suggests that miR-205 expression correlates with the severity of CON in this animal model. Additional preliminary studies indicate that miRs, including miR-205, can be measured by qPCR from urinary exosomes, including human urine samples.

Conclusions: In summary, our results show that miR-205 correlates with disease severity in a mouse model of CON, and is quantitatively detectable in urine samples. Future studies will explore the target molecules and pathways affected by miR-205 in the *mgb-/-* model of CON, as well as the potential of miR-205 as a non-invasive biomarker of renal injury in congenital urological obstruction.

Funding: NIDDK Support

TH-PO974

A Model of Reversible Unilateral Ureteral Obstruction in Mice Haichun Yang, Agnes B. Fogo. *Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN.*

Background: Unilateral ureteral obstruction (UUO) is widely used to study mechanisms of renal fibrosis. Complete UUO leads to severe hydronephrosis within 2 weeks. Several studies have described reversible UUO model by using ties or clips that were then removed. However, these techniques lead to variable degrees of partial obstruction, and longitudinal measurement of renal function was not possible. Surgical reimplantation of the obstructed ureter into the bladder has also been used, but is technically challenging, and also does not allow distinction of function in obstructed vs nonobstructed kidney. The present study aimed to develop a new reversible UUO model that would be technically reproducible and allow comparison of renal function in obstructed vs nonobstructed kidneys.

Methods: Collagen I-luciferase mice underwent unilateral ureter obstruction (UUO) at day 0. Obstruction was released by inserting a PE20 catheter into the obstructed ureter at day 7, with exteriorization of the catheter. Mice were followed for two additional weeks. Urine was collected from the obstructed side from the catheter, while the urine from the contralateral side was collected from the urethra. Collagen I expression, as a measure of fibrosis, was monitored at intervals by detection of bioluminescence signal of luciferase activity after luciferin i.p. injection.

Results: At day 7, there were more collagen I expression in obstructed kidneys (2.59±0.34 Xe^6) than in the contralateral side (1.33±0.04 Xe^6). At day 10, the collagen I activity continued to increase to 4.95±1.35 Xe^6 in the obstructed side after reversal, while

there was no change in the contralateral side (1.13±0.04 Xe^6). Albuminuria was also detected in the urine from the obstructed kidney (ACR, 888.25±102.23 μg/mg), with no proteinuria from the contralateral side (52.70±10.70μg/mg). In 8 of 10 mice, reestablished urine flow at obstructed side continued till mice were sacrificed.

Conclusions: Our study demonstrates feasibility of a novel reversible UUO model that allows functional assessment of each kidney after reversal of obstruction. This may provide a useful model to test therapeutic strategies for reversing renal fibrosis and restoring renal function.

Funding: NIDDK Support

TH-PO975

Activation of TGF-β/Smad3 Signaling in *Megabladder* Mice with Congenital Obstructive Nephropathy Ashley R. Carpenter, Brian Becknell, Michael Wilhide, Susan E. Ingraham, Kirk M. McHugh. *The Research Institute at Nationwide Children's Hospital and College of Medicine at the Ohio State University, Columbus, OH.*

Background: Congenital obstructive nephropathy (CON) is the leading cause of chronic kidney disease in children. The megabladder (*mgb*) mouse serves as a unique animal model of CON. Mutants develop a functional lower urinary tract obstruction due to a lack of proper detrusor muscle organization *in utero*. This leads to formation of a distended hypomuscular bladder, renal failure and death in early adulthood. To better understand the molecular mechanisms responsible for the pathophysiology of CON, we performed transcriptome analysis of *mgb* kidneys compared to wild type controls.

Methods: Kidneys from age-matched adult $mgb^{-/-}$(mutant) and $mgb^{+/+}$(control) mice were harvested and prepared for evaluation by the Agilent cRNA microarray, qPCR and immunohistochemistry (IHC). ELISA was performed on embryonic day 15 (E15), E18, postnatal day 1-3 (P1-P3), P10, and adult kidney lysates. Gene expression data was analyzed using Ingenuity's IPA software.

Results: Transcriptome analysis of *mgb* kidneys identified a number of changes in genes involved in the canonical TGF-β pathway. Specifically, the expression of receptor-regulated Smad3 and Smad4 (p-value of overlap = 2.31×10^{-9} and 4.42×10^{-5} respectively) were identified as the most transcriptionally active (regulation z-score = 2.896 and 2.108 respectively) based on number of target genes with increased expression. Of the 26 differentially expressed mRNAs regulated by Smad3, 22 were upregulated in mutant kidneys. We confirmed upregulation of Smad3 target genes, Egr1 and Fos by qPCR, and genes implicated in TGF-β-dependent renal fibrosis such as CTGF by IHC. ELISA confirmed a significant increase ($p \leq 0.05$) in TGF-β3 and related TGF-β1 secreted proteins in adult mutant kidneys. Preliminary findings suggest that TGF-β1 may be increased as early as P10.

Conclusions: In the *mgb* mouse model of CON, TGF-β and Smad3/4 profiles are upregulated. The unique progression of renal failure observed in the *mgb* mouse may provide an approach for the investigation of time-point dependent pharmacological inhibition of the molecular pathways responsible for CON.

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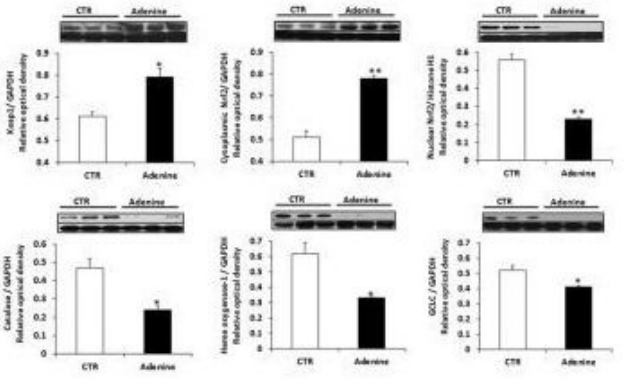
TH-PO976

Oxidative Stress and Inflammation in Chronic Tubulo-Interstitial Nephropathy Are Associated with and, in Part, due to Impaired Nrf2 Activation Mohammad A. Aminzadeh,[1] Susanne B. Nicholas,[2,3] Keith C. Norris,[2,3] Nosratola D. Vaziri.[1] *[1]University of California, Irvine; [2]University of California, Los Angeles; [3]Charles R. Drew University of Medicine and Science, Los Angeles, Los Angeles, CA.*

Background: Consumption of adenine leads to the accumulation of 2, 8-dihydroxyadenine in the renal tubules triggering intense tubulointerstitial nephropathy (TIN) and progressive decline in kidney function. Kidney disease in this model is associated with and largely driven by oxidative stress and inflammation. Oxidative stress and inflammation in the Imai rats with spontaneous focal segmental glomerulosclerosis and in rats with CKD induced by 5/6 nephrectomy is associated with impaired activation of Nrf2 which is the master regulator of genes encoding antioxidant, and cytoprotective enzymes. However, the effect of TIN on Nrf2 pathway and its key target genes is unknown and was investigated here.

Methods: Sprague-Dawley rats were randomized to control and adenine-treated (rat chow containing 0.7% adenine for 2 weeks) groups and followed for 4 weeks.

Results: The adenine-treated animals exhibited marked azotemia, impaired urinary concentrating capacity, intense tubular and glomerular damage, and interstitial inflammation and fibrosis. This was associated with increased expressions of NAD(P)H oxidase, cyclooxygenase-2 and 12-lipoxygenase, and activation of NF-κB which is the master regulator of pro-inflammatory and pro-fibrotic cytokines and other mediators. Oxidative stress and inflammation in the kidney tissue of adenine-treated animals was accompanied by impaired activation of Nrf2 and down-regulation of its target gene products including, catalase, heme oxygenase-1, and glutamate-cysteine ligase.



Conclusions: Adenine-induced TIN is associated with impaired Nrf2 activation which renders the kidney exceedingly vulnerable to the ravages of the prevailing oxidative stress and inflammation.

TH-PO977

CTP-499, a Novel Drug for the Treatment of Chronic Kidney Disease, Ameliorates Renal Fibrosis and Inflammation In Vivo Ara Aslanian, Kristine Hogan, Kara West, Gary W. Bridson, Lijun Wu. *Concert Pharmaceuticals, Lexington, MA.*

Background: Chronic Kidney Disease (CKD) is a complex, multifactorial disease in which renal function is progressively compromised. Decreased glomerular filtration due to dysregulated extracellular matrix (ECM) deposition is a hallmark of progressive CKD. However, oxidative imbalance, inflammation and exaggerated proliferation of fibroblasts and myofibroblasts are now increasingly recognized as major pathogenic mechanisms in CKD. Previously, we have shown that CTP-499, a novel, deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine (HDX), the active M1 metabolite of pentoxifylline, exhibits efficacy in key cellular pathological mechanisms involved in CKD including inhibition of pro-fibrotic gene expression, inflammatory cytokines and oxidative stress.

Results: In the current studies, we demonstrate that CTP-499 ameliorates renal fibrosis *in vivo*. Kidney collagen content was significantly reduced in rats dosed with CTP-499 compared to controls in the unilateral ureteral obstruction (UUO) model. We also extended our previous studies of the anti-inflammatory properties of CTP-499 *in vivo*. The expression of kidney MCP-1 and TNF-α were significantly reduced in CTP-499-treated rats. Also, dosing rats with CTP-499 significantly reduced *ex vivo* LPS-induced expression of MCP-1, TNF-α and IL-6.

To further understand the renoprotective mechanism of CTP-499, we investigated the effects of mitogenic stimuli on kidney cells. CTP-499 prevented the TGF-β-mediated loss of the epithelial marker E-cadherin and induction of the mesenchymal marker vimentin, suggesting CTP-499 may prevent the formation of matrix-forming fibroblasts from proximal tubule cells. Also, CTP-499 significantly inhibited PDGF-induced proliferation of rat mesangial cells.

Conclusions: Taken together, these data show that CTP-499 possesses anti-fibrotic and anti-inflammatory properties *in vivo*. Inhibition of TGF-β-induced kidney cell differentiation and mesangial cell proliferation may contribute to the anti-fibrotic mechanism of CTP-499. These results further support our continued interest in CTP-499 as a novel agent for the potential treatment of CKD.

Funding: Pharmaceutical Company Support - Concert Pharmaceuticals

TH-PO978

Loss of Tubular Hepatocyte Growth Factor Signaling Results in an Exaggerated Epithelial-Mesenchymal Transition in Kidney Fibrosis Dong Zhou, Youhua Liu. *Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Epithelial-mesenchymal transition (EMT) is thought to be a mechanism contributing to renal fibrosis. However, while EMT in vitro is universally accepted, whether EMT does occur in vivo in kidney fibrosis remains highly controversial. EMT is shown to be negatively regulated by hepatocyte growth factor (HGF) signaling. We hypothesize that due to the presence of endogenous antagonists, EMT in vivo probably requires 'two hits', resulting from both activation of pro-EMT program and loss of anti-EMT mechanisms.

Methods: To test this hypothesis, we used conditional knockout mice (Ksp-met-/-) in which c-met, the tyrosine kinase receptor for HGF, was ablated in tubule-specific manner. Ksp-met-/- mice and control littermates were subjected to obstructive injury induced by unilateral ureterl obstruction (UUO). In addition, these mice were also treated with a single dose of folic acid for 2 months to induce chronic kidney disease (CKD).

Results: More severe renal fibrotic lesions and interstitial fibrosis were found in Ksp-met-/- mice after both folic acid and obstructive injury, compared with control littermates, as shown by an increased expression of fibronectin, collagen I, collagen III, α-SMA, PAI-1, Snail1, TGF-β, and connective tissue growth factor (CTGF) in Ksp-met-/- mice. Furthermore, serum creatinine level was higher in Ksp-met-/- mice than that in the controls at 2 months after folic acid injection. Interestingly, using confocal microscopy we demonstrated a marked increase in tubular expression of mesenchymal marker vimentin, a

sign of EMT, in Ksp-met-/- mice. Co-staining revealed that these vimentin-positive tubule cells migrated across the basement membrane and entered the interstitial compartment in Ksp-met-/- kidneys after injury.

Conclusions: These results indicate that tubule-specific loss of HGF/c-met signaling, a negative inhibitor of EMT, resulted in an exaggerated EMT, worsened fibrosis and more severe kidney dysfunction. Our data illustrate that EMT in vivo necessitates both activation of pro-EMT program and loss of anti-EMT mechanisms.

Funding: NIDDK Support

TH-PO979

Characterization of Adenine-Feeding Mice Model of Tubulointerstitial Damage with Renal Dysfuction

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Background: Adenine is produced endogenously as a by-product of the polyamine pathway and is salvaged by adenine phosphoribosyltransferase (APRT:EC 2.4.2.7). APRT deficiency leads to the accumulation of 2,8-dihydroxyadenin(DHA)crystals in human(OMIM:102600).We have reported that mice fed the overload of the adenine-containing diet were mimic to APRT deficiency mice and both were used as human DHA disease model that showed DHA crystals in the tubular lumens with fibrosis and renal dysfunction. The aim of this study is to evaluate the effect of carcitoriol, which have antifibrotic effects,in our established mice model of tubulointerstitial damage (TID) with renal dysfunction induced by adenine feeding.

Methods: Inbred male C57BL/6 mice (10 weeks old) were fed a standard laboratory powder diet containing 0.25% adenine (0.25%Ad) for 7days, then they were fed a standard laboratory diet foraddditional 7days. Mice with subcutaneous injection (s.c) of carcitriol were euthanized on days 3, 7, and 14 (Group 2,4,6), and mice with DMSO s.c. were euthanized on days 3, 7, and 14 (Group 1,3,5) as controls.

Results: Mice fed 0.25%Ad showed nephrolithiasis, extensive tubular dilation, inflammation, necrosis and fibrosis (estimated by Masson's Trichrome stain) with the elevation of serum creatinine (Cr) and TID area (%) in a time-dependent manner (Group 1 vs 3; $p<0.01$). The observation of 0.25%Ad days 7+DMSO and 0.25%Ad days 7 + carcitriol(+) showed that carcitoriol exposure have the protective effectsfor Cr and TID (**$p<0.01$) but this protecitive effect did not prolong for additional 7days observation.

Cr(mg/dl) and TID(%)

	Group1	Group2	Group3	Group4	Group5	Group6
Cr (mg/dl)	0.6±0.045	0.5	0.74±0.05	0.6±0.07**	0.52±0.04	0.44±0.1
TID (%)	1.43±0.73	1.69±0.73	14.5±2.0	5.2±3.2**	13.1±6.5	10.1±5.8

**$p<0.01$

Conclusions: These datum showed that carcitoriol have the prophiractic protective effects in mice fed 0.25%Ad models morphologically and functionally.

Funding: Government Support - Non-U.S.

TH-PO980

The New Immunosuppressive Drug FTY720 Attenuates Tubulointerstitial Inflammation and Fibrosis in 5/6 Nephrectomized Rats

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Background: Tubulointerstitial fibrosis is the common pathway that lead to kidney failure, while persistent tubulointerstitial inflammation is a key event in the development of tubulointerstitial fibrosis. The new immunosuppressive drug FTY720 modifies lymphocyte migration into injured tissues by lymphocyte sequestration to secondary lymphoid organs. This study was designed to explore the effect of FTY720 on tubulointerstitial inflammation and fibrosis in 5/6 nephrectomized rats.

Methods: Seven days after surgery, SD rats were allocated to the following groups: sham, 5⁄6 subtotal nephrectomy(SNX), and SNX+FTY720. Rats were killed on week 12 after surgery and blood, urine and kidneys were collected for analyses. Tubulointerstitial infiltrating inflammatory cells such as T cells ,B cells and macrophages were detected by immunohistochemical staining. Protein expression of pro-inflammatory molecules and pro-fibrotic molecule were analyzed by immunohistochemical staining and western blot. Protein expression of α-SMA and E-cadherin were detected by immunofluorescence analysis and western blot.

Results: FTY720 attenuated the rise in BP, proteinuria, SCr,BUN and NAG in SNX($P<0.01$). Treatment with FTY720 was found to reduce the numbers of peripheral blood Lymphocyte($P<0.01$). Morphological study showed severe tubulointerstitial inflammation and fibrosis in SNX, but the lesions were attenuated in FTY720-treated group ($P<0.01$). Tubulointerstitial infiltrating inflammatory cells expressing CD3, CD4, CD8, CD20, CD68, CD163 and CCR-7 in SNX were attenuated in FTY720-treated group($P<0.01$). FTY720 decreased the expression of pro-inflammatory molecules(IL-6, TNF-α,and MCP-1) and pro-fibrotic molecule(TGF-β1) ($P<0.01$). The protein expression of E-cadherin was down-regulated, while the expression of α-SMA, was up-regulated in kidneys of SNX rats when compared with those in sham group ($P<0.01$). FTY720 administration attenuated these abnormalities($P<0.01$).

Conclusions: FTY720 ameliorates progression of tubulointerstitial injury in SNX by inhibiting tubulointerstitial inflammatory response and tubular epithelial-to-mesenchymal transition.

TH-PO981

Platelet-Derived Growth Factor-C Neutralization Reveals Differential Roles of PDGF Receptors in Liver and Kidney Fibrosis

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Background: Platelet-derived growth factors (PDGFs) are key mediators of organ fibrosis. Nonetheless, the specific role of isoform PDGF-C for kidney and liver fibrosis is not well known.

Methods: We investigated whether PDGF-C -/- mice or mice treated with neutralizing PDGF-C antibodies are protected from bile duct ligation-induced liver fibrosis and compared the effects with those of PDGF-C deficiency or neutralization on kidney fibrosis induced by unilateral ureteral obstruction.

Results: Unexpectedly, and in contrast to kidney fibrosis, PDGF-C deficiency or antagonism did not protect from liver fibrosis or functional liver impairment. Furthermore, the hepatic infiltration of monocytes/macrophages/dendritic cells and chemokine mRNA expression (CCL5, CCL2 and CCR2) remained unchanged. Transcript expression of PDGF ligands increased in both liver and kidney fibrosis and was not affected by neutralization of PDGF-C. In kidney fibrosis, PDGF-C deficiency or antagonism led to reduced expression and signaling of PDGF-receptor-α- and -β-chains. In contrast, in liver fibrosis there was either no difference (PDGF-C -/- mice) or even an upregulation of PDGFR-β and signaling (anti-PDGF-C group). Finally, *in vitro* studies in portal myofibroblasts pointed to a predominant role of PDGF-B and -D signaling in liver fibrosis.

Conclusions: In conclusion, our study revealed significant differences between kidney and liver fibrosis in that PDGF-C mediates kidney fibrosis, whereas antagonism of PDGF-C in liver fibrosis appears to be counteracted by significant upregulation and increased signaling of PDGFR-β. PDGF-C antagonism therefore may not be effective to treat liver fibrosis.

Funding: Government Support - Non-U.S.

TH-PO982

Renal Carcinoma Stem Cells Are Induced by MEK/MAPK/FRA2 Signaling Cascade-Mediated Epithelial-Mesenchymal Transition: Evidence for a Dynamic and Renewable Stem Cell Phenotype

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Background: Cancer stem cells (CSC) are defined by: self-renewal, growth in suspension (spheroids), multi-lineage differentiation, tumorigenesis, and metastasis. One hypothesis states that CSC are derived from genomically unstable adult mesenchymal stem cells. Alternatively, CSC could be continuously generated as a consequence of epithelial-mesenchymal transition (EMT) in response to genomic stress or environmental cues. We reported (Lovett, et al., Carcinogenesis 2011;32:1806) that the MEK1/MAPK signaling cascade induces EMT in MDCK epithelial cells which is dependent on MEK1 and MT1-MMP activities. We directly correlated these to renal cell carcinoma (RCC) tumor grade and invasion. We hypothesized that these features are mediated by EMT-dependent generation of CSC.

Methods: The MEK1 response element (AP1) in the MT1-MMP promoter was mapped and characterized using standard methodology. The molecularly more amenable LLC-MK2 renal epithelial cell line generated, using MEK1 expression, clones spanning the phenotypic range of EMT. Microarray analysis of the ENT clones, spheroid cultures and orthotopic renal injections were performed using standard methodology.

Results: A MEK1 response element in the MT1-MMP promoter was identified as an AP1 site binding FRA2/JUNB heterodimers. FRA2 activity is enhanced by MAPK phosphorylation and FRA2 expression predicts survival in metastatic RCC. FRA2 transfection induced EMT, enhanced MT1-MMP expression and invasive activity. Microarrray identified the CSC-EMT transcription factor TWIST1 as a possible FRA2 target. Mesenchymal LLC-MK2 clones grow as spherules in defined medium and display both mesenchymal-epithelial transition and invasive acivity in the orthotopic model, consistent with a CSC phenotype.

Conclusions: We propose that renal epithelial cells are dynamically converted to CSC through the combined action of MEK1/MAPK/FRA2 signaling and MT1-MMP expression. Inhibition of FRA2 or MT1-MMP could potentially inhibit the generation of renal CSC and affect RCC outcomes.

Funding: NIDDK Support, Veterans Administration Support

TH-PO983

Transforming Growth Factor β Stimulates Profibrotic Epithelial Signaling to Activate Pericyte-Myofibroblast Transition in Obstructive Kidney Fibrosis Szu Yu Pan,[1] Ching-fang Wu,[1] Fan-Chi Chang,[1] Yi-Ting Chen,[1] Wen-Chih Chiang,[1] Yung-ming Chen,[1] Jeremy S. Duffield,[2] Shuei-Liong Lin.[1] [1]*Renal Division, National Taiwan University Hospital, Taipei, Taiwan;* [2]*Renal Division, University of Washington, Seattle, WA.*

Background: Pericytes and perivascular fibroblasts have been identified as the major source of precursors of scar-producing myofibroblasts during kidney fibrosis. The underlying mechanisms triggering pericyte-myofibroblast transition are poorly understood. Transforming growth factor β (TGFβ) is well recognized as a pluripotent cytokine that drives organ fibrosis. Here we show the role of TGFβ in inducing profibrotic signaling from epithelial cells to activate pericyte-myofibroblast transition.

Methods: We studied the *in vivo* effect of activated TGFβ signaling on pericyte-myofibroblast transition and epithelial cell responses using pan anti-TGFβ antibody (1D11) or TGFβ receptor type I inhibitor (SB431542) in collagen I (α1) GFP reporter mice after unilateral ureteral obstruction. Primary cultures of tubular epithelial cells and pericytes were used to study the *in vitro* effect of TGFβ1 and the cross talk between epithelial cells and pericytes.

Results: Increased expression of TGFβ was detected predominantly in injured epithelium after unilateral ureteral obstruction, whereas downstream signaling from the TGFβ receptor increased in both injured epithelium and pericytes. In mice with ureteral obstruction, treated with antibody 1D11 or chemical SB431542, kidney pericyte-myofibroblast transition was blunted. The consequence was marked attenuation of fibrosis. In addition, epithelial cell cycle G2/M arrest and production of profibrotic cytokines were both attenuated. Although TGFβ1 alone did not trigger pericyte proliferation *in vitro*, it robustly induced α smooth muscle actin. In cultured kidney epithelial cells, TGFβ1 stimulated G2/M arrest and production of profibrotic cytokines that had the capacity to stimulate proliferation and transition of pericytes to myofibroblasts.

Conclusions: This study identifies a novel link between injured epithelium and pericyte-myofibroblast transition through TGFβ during kidney fibrosis.

Funding: NIDDK Support, Government Support - Non-U.S.

TH-PO984

A Hepcidin Inhibitor Mobilizes Iron in an Adenine-Induced Renal Failure Model in Rats Valentina Vaja, Chia Chi Sun, Herbert Y. Lin, Jodie L. Babitt. *Program in Membrane Biology, Nephrology Division, Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA.*

Background: Anemia is prevalent in patients with chronic kidney disease (CKD). A key feature of the anemia of CKD is limited iron availability for efficient erythropoiesis despite adequate body iron stores. It is now well established that excess levels of the iron regulatory hormone hepcidin in CKD are responsible for decreasing expression of the iron exporter ferroportin, thereby blocking iron absorption from the diet and iron release from body stores. Adenine treatment in rats has been proposed as an animal model of anemia of CKD with high hepcidin levels that mirrors the condition in patients.

Methods: We developed a modified adenine-induced kidney disease model of anemia in rats by giving a diet supplemented with 0.75% adenine for 3 weeks followed by a normal diet for 3 weeks. We then tested whether the small molecule bone morphogenetic protein (BMP) inhibitor LDN-193189, which has previously been shown to lower hepcidin levels, was able to mobilize iron into the plasma and improve iron-restricted erythropoiesis in adenine-treated rats.

Results: The modified adenine model had a higher survival rate than previously reported models, while maintaining irreversible renal failure and anemia. Adenine-treated rats had increased hepatic hepcidin mRNA, decreased serum iron, increased spleen iron content, low hemoglobin, and inappropriately low EPO levels relative to the degree of anemia. LDN-193189 lowered hepatic hepcidin mRNA and mobilized stored iron into plasma in adenine-treated rats. Moreover, the iron was efficiently incorporated into hemoglobin in reticulocytes. However, LDN-193189 alone did not prevent anemia progression in our model.

Conclusions: Lowering hepcidin improved iron availability, but did not improve anemia in an adenine-induced kidney disease model in rats. Co-administration of hepcidin lowering agents with erythropoiesis stimulating agents (ESAs) may be useful as a combination therapy to correct iron balance and thereby reduce the ESA dose needed to achieve target hemoglobin levels.

TH-PO985

Recombinant Human Soluble Thrombomodulin Attenuates Anti-Glomerular Basement Membrane Glomerulonephritis in Wistar-Kyoto Rats Masayuki Iyoda, Takanori Shibata, Kei Matsumoto, Yukihiro Wada, Yuki Hirai, Yoshihiro Kuno, Tadao Akizawa. *Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.*

Background: Recombinant human soluble thrombomodulin (RH-TM) is newly developed for the treatment of DIC. Since RH-TM has anti-inflammatory properties, the protective effects of RH-TM were examined in nephrotoxic serum nephritis (NTS-N) of Wistar-Kyoto (WKY) rats, in which CD8+ T cells and macrophages are major pathogenetic factors.

Methods: NTS-N (N=26) was induced in WKY rats on day 0. Groups of animals were given either RH-TM (1.5 mg/kg x 2/day, n=13) or vehicle (an equal volume of saline, n=13) daily by intraperitoneal injection from day 0 to day 6; all rats were sacrificed at day 7. Proteinuria, serum creatinine (Cr), and kidney weight were measured at sacrifice. Routine histology, immunohistochemistry for ED1 and CD8, and real-time RT-PCR for cytokines in renal cortex were performed.

Results: There was no significant difference in rabbit IgG, rat IgG, and rat C3 glomerular staining and the levels of serum anti-rabbit IgG antibody between vehicle- and RH-TM-treated rats with NTS-N. Compared to controls, RH-TM-treated rats had less proteinuria (68.6 ± 3.26 vs. 45.15 ± 8.51 mg/day, $p < 0.01$), serum Cr (0.35 ± 0.01 vs. 0.32 ± 0.05 mg/dl,$p< 0.01$) level, and kidney weight (1.49 ± 0.02 vs. 1.25 ± 0.06 g, $p < 0.01$), as well as reduced glomerular tuft area (8572.69 ± 151.21 vs. 7044.46 ± 530.75 mm^2, $p < 0.01$), and percentage of glomeruli with crescent (95.15 ± 1.16 vs. 81.54 ± 3.47 %,$p< 0.001$). Furthermore, RH-TM-treated rats had significantly reduced glomerular macrophage accumulation (ED1 score: 1.51 ± 0.05 vs. 1.25 ± 0.10,$p < 0.01$) as well as reduced renal cortical IL-6 mRNA expression (IL-6/GAPDH mRNA: 1.04 ± 0.09 vs. 0.66 ± 0.16,$p< 0.05$). The CD8+ cell numbers per glomerular cross-section and renal cortical TNF-a, IL-1b, MCP-1, INF-g, IL-4, and IL-17 mRNA expression were identical between the two groups.

Conclusions: RH-TM treatment attenuates the progressionof renal injury in experimental anti-glomerular basement membrane glomerulonephritis through the suppression of macrophages infiltration.

Funding: Private Foundation Support

TH-PO986

Inhibition of p38 MAPK Attenuates Renal Atrophy in a Murine Renovascular Hypertension Model Diping Wang, Ping Yin, Bruce Knudsen, Jingfei Cheng, Gina M. Warner, Karen R. Lien, Catherine Gray, Joseph P. Grande. *Mayo Clinic, Rochester, MN.*

Background: Renovascular hypertension (RVH) is an important cause of chronic renal dysfunction. Recent studies have implicated signaling through CCL2 and other chemokines in the development of chronic renal disease, suggesting that CCL2 may provide a therapeutic target in patients with RVH. Although our *in vitro* studies have demonstrated that p38 MAPK is a necessary intermediate for CCL2 expression, its role in renal disease progression in RVH has not been previously established. We tested the hypothesis that blockade of p38 would ameliorate renal damage in mice with experimental RVH.

Methods: RVH was established using the murine two-kidney, one-clip (2K1C) model. Mice were treated with the p38 inhibitor SB203580 (10 mg/kg/d) or vehicle after surgery. Blood pressure was measured before, 1 and 2 weeks after the surgery. Kidneys were harvested at 2 weeks after surgery. Histologic examination, Western blotting, and real-time PCR were performed to evaluate the impacts of p38 inhibition on renal atrophy and to identify chemokines modulated by SB203580. We also investigated the *in vitro* effects of p38 inhibitor in rat mesangial cells to confirm our observation *in vivo*.

Results: As expected, the clipped kidney developed interstitial fibrosis, tubular atrophy, and interstitial inflammation. SB203580 significantly reduced the level of renal atrophy in stenotic kidneys (70% vehicle vs. 39% SB203580, $p<0.05$) but did not prevent hypertension. SB203580 reduced interstitial fibrosis (6.0% vehicle vs. 2.7% SB203580, $p< 0.01$; trichrome stain). SB203580 prevented induction of *Ccl2*, *Ccr2*, *Tgf*-β, and *Col4a1* mRNA expression. We also observed that SB203580 could block upregulation of CCL2 and COL4A1 expression at protein level (Western blotting, immunohistochemistry). *In vitro*, p38 inhibition prevented TGF-β and TNF-α-induced upregulation of *Ccl2* in rat mesangial cells.

Conclusions: SB203580 prevents CCL2 induction and alleviates renal atrophy and fibrosis in a murine RVH model. We propose that p38 plays a critical role in the development of renal atrophy in 2K1C mice and may provide a therapeutic target to slow progression of RVH.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO987

Ameliorating NO Bioavailability via ROS Reduction by Nicorandil Suppresses the Hypertensive Nephropathy in Salt-Sensitive Dahl Rats Ken Aizawa, Kenji Yogo, Yoshihito Tashiro, Ken-ichi Serizawa, Keigo Yorozu, Michinori Hirata, Nobuhiko Ishizuka, Koichi Endo. *Product Research Department, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan.*

Background: In hypertensive nephropathy, tight blood pressure control is known to be a crucial factor in preventing progression of renal disease, but other factors are undoubtedly involved. Nicorandil, an antianginal drug that has cardioprotective effects, was reported to reduce reactive oxygen species (ROS) and upregulate endothelial nitric oxide synthase (eNOS) expression, suggesting it could be beneficial in treating hypertensive nephropathy under conditions of endothelial dysfunction. In this study, we evaluated the effect of nicorandil on renal failure induced by oxidative stress in salt-sensitive Dahl (DS) rats.

Methods: Male DS rats were divided into three groups; NS (normal diet: 0.3% NaCl), HS (high salt diet: 8% NaCl) and HS + nicorandil (15 mg/kg/day: drinking water). Four weeks after treatment, systolic blood pressure, renal damage parameters in urine and histopathology were determined. The protein level of eNOS and the uncoupling state in glomeruli were quantified with western blotting method.

Results: HS increased proteinuria with hypertension and nicorandil suppressed the proteinuria, without lowering blood pressure. Nicorandil also reduced urine levels of NAG, cystatin-C and 8-hydroxydeoxyguanosine, an oxidative stress marker. Histopathological analysis revealed that nicorandil prevented dilation of renal tubules; the deposition of hyaline casts andperivascular fibrosis. Although both the expression of total eNOS and the coupling state of eNOS (eNOS dimer/monomer ratio) in glomeruli were reduced by salt loading, nicorandil prevented those changes.

Conclusions: Nicorandil may be beneficial in treating hypertensive nephropathy by improving NO bioavailability via the reduction of ROS. Together with cardioprotective effects, the renoprotective effects of nicorandil, achieved by ameliorating endothelial dysfunction, would be efficacious for hypertensive CKD patients with angina.

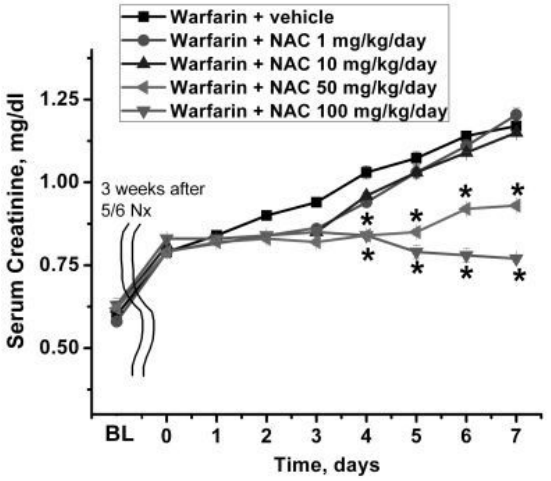
TH-PO988

N-Acetylcysteine (NAC) Prevents Acute Kidney Injury but Not Glomerular Hemorrhage in an Animal Model of Warfarin Related Nephropathy (WRN) Sergey V. Brodsky, Kyle M. Ware, Zahida Qamri, Edward P. Calomeni, Anjali A. Satoskar, Gyongyi Nadasdy, Brad H. Rovin, Lee A. Hebert, Tibor Nadasdy. *Pathology and Medicine, The Ohio State University, Columbus, OH.*

Background: We described earlier that 5/6 nephrectomy (Nx) in rats is an appropriate animal model to study WRN, which we discovered previously in humans. WRN is characterized by glomerular hemorrhage with occlusive tubular red blood cell (RBC) casts, leading to acute kidney injury. The pathogenesis of glomerular hemorrhage is unclear. Herein we tested the hypothesis that oxidative stress plays a significant role in the pathogenesis of WRN.

Methods: 5/6 Nx rats were treated with warfarin (0.04 mg/kg/day) alone and with 4 different doses of NAC per os for 1 week. Prothrombin time (PT) and serum creatinine (SCr) were measured daily. Renal pathology was evaluated after 1 week of treatment.

Results: Warfarin resulted in more than 3-fold PT increase in all experimental groups. SC increased progressively in the animals treated with warfarin + vehicle. However, addition of NAC dose-dependently prevented the increase in SCr starting at 50 mg/kg/day and completely prevented warfarin-induced SCr increase at 100 mg/kg/day (Figure 1).



SCr changes were significantly correlated with PT increase in warfarin and vehicle-treated 5/6 Nx rats (R^2=0.88) with SCr even decreasing in rats treated with warfarin and 100 mg/kg/day of NAC (R^2=0.38). Morphologically, all 5/6 Nx rats treated with warfarin, regardless of NAC dose, showed RBC in the tubules and RBC tubular casts. Acute tubular injury was prominent in warfarin + vehicle treated 5/6 Nx rats, but not in warfarin + 100 mg/kg/day of NAC treated animals.

Conclusions: 1. Oxidative stress plays an important role in the development of acute kidney injury in animals with WRN. 2. NAC does not prevent glomerular hemorrhage seen in WRN, but ameliorates acute tubular injury.

TH-PO989

Glomerular Infiltration of MPO-Positive Cells and Diffuse-Type MPO Deposition on Glomerular Capillary Walls Cause Glomerular Capillary Injury Soko Kawashima, Yoshihiro Arimura, Yoshinori Komagata, Shinya Kaname, Akira Yamada. *Frst Department, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.*

Background: We have previously reported that MPO exists along the glomerular capillary walls near infiltrated MPO-positive neutrophils, which may play some direct roles in the pathogenesis of necrotizing lesion in human MPO-ANCA-associated glomerulonephritis (GN). Here we investigated a possible role of MPO in the pathogenesis of glomerular capillary injury for other types of GN.

Methods: We analyzed 914 renal specimens obtained from 13 patients with IgA nephritis, 12 patients with Henoch-Schoehnlein purpura nephritis (HSPN), 2 patients with post-streptococcal acute glomerulonephritis (PSAGN), 2 patients with anti-GBM GN and compared them with those from 20 patients with MPO-ANCA-associated GN. Glomerular infiltration of MPO-positive cells, deposition of extracellular MPO and endothelial cell injury were analyzed in glomeruli at each stage of the diseases. Colocalization of MPO and CD34 deposition was also examined.

Results: Glomerular infiltration of MPO-positive cells and deposition of extracellular MPO were observed along the glomerular capillary walls in all types of GN especially at an active injury phase. Interestingly, diffuse-type MPO deposition were frequently observed along the glomerular capillaries and seemed to be related to glomerular capillary injuries in anti- GBM GN, IgA GN and HSPN as well as in MPO-ANCA associated GN, whereas it was rarely seen in PSAGN where only limited type deposition without necrosis was observed despite an infiltration of many MPO positive cells.

Conclusions: These results suggest that the diffuse-type MPO deposition with infiltrating MPO-positive cells may be associated with glomerular capillary injuries in various types of GN in addition to MPO-ANCA –associated GN.

TH-PO990

Inactivating Notch3 by Antisense Strategy Alleviates the Development of Glomerulonephritis Fala El Machhour, Laurent Mesnard, Jean-Claude Dussaule, Christos Chatziantoniou. *Inserm UMR 702, Tenon Hospital, Paris, France.*

Background: We have previously shown that Notch3 is involved in the control of renal hemodynamics and function under control conditions. The aim of this work was to study the role of Notch3 receptor in the progression of chronic renal disease, using as model the nephrotoxic serum-induced glomerulonephritis.

Methods: Glomerulonephritis was induced by nephrotoxic serum administration in mice treated daily either with a Notch3 oligo-nucleotide antisense (ODN) or with a scrambled sequence. Mice were sacrificed 10 days after nephrotoxic serum administration, and tissues, urine and plasma samples were obtained and used for subsequent analyses.

Results: Administration of nephrotoxic serum was accompanied by a progressive increase of proteinuria and plasma urea concentration (3.4±0.9 g/mmol and 48±9 mmoL, respectively at day 10, $p<0.001$) associated to histological alterations typical of glomerulonephritis. In parallel, Notch3 expression was induced in glomeruli as evidenced by mRNA and protein expression measurements. The glomerular expression of Notch3 was significantly reduced in mice receiving Notch3 ODN antisense ($p<0.05$), whereas scrambled sequences had a negligible effect. The 2 groups progressed to chronic renal disease, but mice injected with Notch3 ODN antisense were relatively protected compared to scrambled group as evidenced by the values of plasma urea (18±8 vs 41±7 mmoL, $p<0.05$) and proteinuria (0.7±0.2 vs 2.4±0.7 g/mmol $p<0.05$). The improvement of renal function was accompanied by fewer deposits of fibrin within glomeruli, less peritubular and glomerular inflammation and decreased expression of pro-inflammatory mediators ($p<0.01$). Moreover, the inhibition of Notch3 was associated with blunted activation of growth factor signaling pathways well known to be involved in the development of glomerulonephritis such as the PDGF and EGF pathways.

Conclusions: These results show that the activation of Notch3 is a major player in the progression of renal disease by promoting proliferative and pro-inflammatory pathways. Targeting the expression and/or activation of Notch3 could be a novel, promising approach to treat chronic renal disease.

Funding: Government Support - Non-U.S.

TH-PO991

The Best Offense Is a Good Defense: Expression and Function of Beta Defensins in the Infected Kidney and Urinary Tract Brian Becknell,[1] Ashley R. Carpenter,[2] Sheryl S. Justice,[3] Kirk M. McHugh.[4] *[1]Division of Nephrology, Nationwide Children's Hospital; [2]Integrated Biomedical Sciences Program, The Ohio State University; [3]Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital; [4]Center for Molecular and Human Genetics, The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: Beta defensins (BDs) are cationic peptides with immunomodulatory and antimicrobial properties. BD expression and function in the kidney and urinary tract are incompletely defined.

Methods: Tissues were collected from female C57BL/6 mice that remained naïve or were inoculated with uropathogenic *Escherichia coli* (UPEC). BD mRNA and protein expression was evaluated by QRT-PCR and immunoblotting. BD protein and mRNA were localized by immunohistochemistry and *in situ* hybridization. The bactericidal activity of BD peptides was evaluated *in vitro*, and *in vivo* by quantifying the bacterial burden following transurethral challenge of wildtype versus *BD1* knockout mice with UPEC.

Results: *BD1* mRNA was preferentially expressed in kidney, whereas *BD3* and *BD14* mRNAs were enriched in ureter and bladder. BDs displayed unique localization patterns within the kidney and lower urinary tract. *BD1* mRNA was expressed in renal collecting ducts and bladder urothelium. BD3 localized to endothelial cells and papillary collecting ducts of naïve kidneys. BD3 was restricted to the apical surface of bladder umbrella cells, whereas BD14 protein was expressed throughout the urothelium. UPEC decreased bladder expression of *BD1* mRNA, whereas *BD3* mRNA levels increased. BD peptides were detectable in infected urine and demonstrated differential activity toward UPEC *in vitro*. *BD1* knockout and wildtype mice had comparable UPEC colony counts in bladder and kidney homogenates.

Conclusions: BDs are differentially expressed throughout the naïve kidney and urinary tract, and are subject to unique local regulatory changes following UPEC challenge. BDs exhibit microbicidal activity toward UPEC, and further *in vivo* studies are required to delineate their bactericidal and immunomodulatory roles in the infected kidney and urinary tract.

TH-PO992

Pharmacological Inhibition of Plasminogen Activator Inhibitor (PAI)-1 Ameliorates Glomerular Podocyte Injury in Anti-Thy1.1 Glomerulonephritis Hideyasu Kiyomoto,[1,2,3] Kumiko Moriwaki,[4] Sachiko Matsumoto,[3] Atsuhiko Ichimura,[3] Takashi Nakamichi,[2] Takashi Dan,[3] Takefumi Mori,[2] Sadayoshi Ito,[2] Toshio Miyata.[3] *[1]Division of Integrated Nephrology and Telemedicine, Tohoku University, Sendai, Japan; [2]Division of Nephrology, Tohoku University, Sendai, Japan; [3]Department of Molecular Medicine and Therapy, Tohoku University, Sendai, Japan; [4]Department of CardioRenal and CerebroVascular Medicine, Faculty of Medicine, Kagawa University, Kagawa, Japan.*

Background: Plasminogen activator inhibitor (PAI)-1 is involved in glomerular disease processes including thrombosis, fibrosis and inflammation. The specific PAI-1 antagonist, TM5275, was newly synthesized by an *in silico* screening based upon the human PAI-1 tertiary structure. This study is to test the effects of TM5275 on anti-Thy1.1 glomerulonephritis (GN) in rats.

Methods: GN was induced by an injection of ER4G in male SD rats that received daily by gavages either vehicle, clopidogrel (30mg/kg/day) or TM5275 (30mg/kg/day) beginning at 3 days before induction of GN. The rats were sacrificed at day 7, and their perfused kidneys were processed for pathological evaluation. The mRNA expressions in the kidney were estimated by RT-PCR with heat-mapping exhaustive analysis. We also investigated the protective effect of TM5275 in cultured podocyte *in vitro.*

Results: An ER4G injection induced proliferative GN with focal and segmental microaneurysm formation in glomeruli. TM5275 improved the pathological abnormality, i.e. augmented incidence of glomerular microaneurism, macrophage infiltration and thrombi formation in glomeruli concomitant with inhibition of plasma PAI-1 activity, while clopidogrel exerted no protective effects on GN. Interestingly, TM5275 ameliorated the deterioration of podocyte estimated by staining for WT-1 and desmin. The confocal microscopy showed the partial colocalization of synaptopodin and PAI-1 in merge. Moreover, the synaptopodin in cultured podocyte was significantly suppressed by the existence of PAI-1, and this was restored by the presence of TM5275.

Conclusions: Our in vivo and in vitro data suggest that TM5275, a specific PAI-1 inhibitor, may provide potential therapeutic benefits in GN involving the podocyte injury.

TH-PO993

Genetic Analysis of Intracapillary Glomerular Deposits in Aging Mice Gerda A. Noordmans,[1] Yuan Huang,[1] Christina R. Caputo,[2] Marcory van Dijk,[1] Peter Heeringa,[1] Jan-luuk Hillebrands,[1] Ron Korstanje,[2] Harry Van Goor.[1] *[1]Pathology and Medical Biology, University Medical Center Groningen, Netherlands; [2]The Jackson Laboratory, Bar Harbor, ME.*

Background: Decline in renal structure and function is common in aging individuals. Understanding aging related renal damage provides tools for preventive and therapeutic means. To gain insight in pathways involved in aging, we analyzed glomerular intracapillary changes in aged mice. Haplotype association mapping (HAM) was used to identify genes associated with morphological changes.

Methods: Glomeruli from 20-months old male mice from 26 inbred strains were analyzed. Intracapillary PAS-positive deposits were a significant finding in some strains and we quantified the mean severity of deposit formation in 50 glomeruli (max score 400). Affected strains were also analyzed at 10 months. Electron microscopy and immunohistochemistry for apoE, apoB and apoA-IV was performed to characterize the lesion. Additional sections were used to study amyloid (Congo Red) or fibrin (MSB). HAM was used to identify loci associated with glomerular deposits. Strains containing a minimum of two affected mice were considered positive. Associations with a P-value of less than 10^{-4} were considered significant.

Results: Six strains showed PAS-positive deposits, varying in severity: B10 (mean=21 ± 47.5 SD), C57BR (7 ± 24.1), NOD (97 ± 135.7), NZW (41 ± 46.6), C3H (9 ± 19.4) and NON (30 ± 54.8). At 10 months of age only NOD (8 ± 21.4) and NON (33 ± 59.8) already contained glomerular deposits. Intracapillary deposits were strongly positive for apoE and weakly positive for apoB and apoA-IV. They were negative for amyloid and fibrin. HAM showed one peak above 10^{-6} on chromosome 1. The only gene found within this haplotype block is *Esrrg.*

Conclusions: PAS-positive glomerular intracapillary deposits were seen in six aged mouse strains. The lesion mimics human lipoprotein glomerulopathy, which is also characterized by deposition of lipoproteins in glomerular capillaries. Our data indicate that estrogen-related receptor gamma (*Esrrg*) which is highly expressed in the kidney and involved in lipid metabolism, is associated with the development of these glomerular deposits.

Funding: NIDDK Support, Other NIH Support - NIA

TH-PO994

Effects of Dietary Salt Restriction on Renal Tubulointerstitial Injury in Rats with Glomerular Proteinuria Gheun-Ho Kim, Il Hwan Oh, Joon-sung Park. *Internal Medicine, Hanyang University College of Medicine, Seoul, Korea.*

Background: Proteinuria is a major promoter to induce tubulointerstitial injury in glomerulopathy, and dietary salt restriction may reduce proteinuria. Although high salt intake is believed to hasten progression of chronic kidney disease, whether low salt intake may relieve proteinuria-mediated tubulointerstitial injury is not clear. We investigated the effects of dietary salt restriction on rat kidneys using puromycin aminonucleoside (PA) and Adriamycin (ADR) to induce glomerular proteinuria.

Methods: In Protocol I, PA was intraperitoneally administered to male Sprague-Dawley rats at a dose of 150 mg/kg BW at time 0, followed by 50 mg/kg BW on days 28, 35, and 42. Sodium-deficient rodent diet with and without additional NaCl (0.5%) were provided for normal-salt rats and low-salt rats, respectively. On day 63, kidneys were harvested for histopathologic examination and immunohistochemistry. In Protocol II, ADR was intravenously given into the femoral vein as a single bolus (7.5 mg/kg). Five weeks later, kidneys were harvested for histopathologic studies, immunohistochemistry and immunoblot analysis.

Results: Both PA and ADR treatment produced overt proteinuria and renal damage. When the tubulointerstitial injury was semiquantitatively evaluated, it had a positive correlation with proteinuria in both animal experiments. Although dietary salt restriction failed to significantly decrease proteinuria, the increased tubulointerstitial injury score was significantly reversed by low-salt diet in both animal experiments. ED1-positive infiltrating cells and immunostaining for interstitial collagen III were increased in PA- and ADR-treated rat kidneys, and they appeared relieved by dietary salt restriction but did not reach statistical significances. In ADR-treated rat kidneys, however, the increased fibronectin expression was significantly reversed by dietary salt restriction.

Conclusions: Our results suggest that glomerular proteinuria-induced tubulointerstitial injury and its process may potentially be improved by dietary salt restriction. Non-hemodynamic mechanisms induced by low-sodium diet might contribute to renoprotection.

Funding: Government Support - Non-U.S.

TH-PO995

Prevention of Kidney Fibrosis by Regulator of G Protein Signaling 2 (RGS2) Hee-Seong Jang, Jee In Kim, Kwon Moo Park. *Anatomy and BK21 Project, Kyungpook National University School of Medicine, Daegu, Korea.*

Background: Regulator of G protein signaling 2 (RGS2), is a GTPase-accelerating protein that terminates Gq signaling, including angiotensin II (AngII) signaling, which play a critical role in kidney fibrosis. However, the role of RGS2 in kidney fibrosis remains to be defined.

Methods: Here, we investigated the role of RGS2 in kidney fibrosis following ureteral obstruction (UO).

Results: UO significantly increased expression of RGS2 mRNA and protein in the kidney. UO increased the collagen deposition and α-smooth muscle actin in the kidney. These increases in the kidney of RGS2 knock-out ($RGS2^{-/-}$) mice were significantly greater than in the kidney of RGS2 wild-type ($RGS2^{+/+}$) mice. After UO plasminogen activator inhibitor-1, PAI-1, and phosphorylated extracellular signal-regulated kinase, p-ERK, expression in the kidney of $RGS2^{-/-}$ mice, which is mediator of kidney fibrosis, were also greater than $RGS2^{+/+}$ mice. In addition, UO-induced inflammatory responses were greater in $RGS2^{-/-}$ mice than $RGS2^{+/+}$ mice. Increases of phospholylated-STAT3, which is associated with anti-inflammatory signaling, induced by UO were lower in the kidney of $RGS2^{-/-}$ mice than that of $RGS2^{+/+}$ mice. Moreover, UO-induced increase of AngII type 1 receptor (AT1R) was greater in the kidney of $RGS2^{-/-}$ mice than that of $RGS2^{+/+}$ mice. Losartan, a blocker of AT1R, treatment into $RGS2^{-/-}$ mice remarkably reduced kidney fibrosis with reduction of inflammation.

Conclusions: Our findings demonstrate for the first time that RGS2 plays as a negative regulator of kidney fibrosis by inhibition of Gq protein signaling including AngII/AT1R signaling, suggesting that RGS2 could be a useful target for the development of therapeutics of kidney fibrosis.

Funding: Government Support - Non-U.S.

TH-PO996

Proliferation of Bone Marrow-Derived Cells Contributes to the Increase of Fibroblast Population during Kidney Fibrosis Hee-Seong Jang, Jee In Kim, Kwon Moo Park. *Anatomy and BK21 Project, Kyungpook National University School of Medicine, Daegu, Korea.*

Background: An increase in the number of fibroblasts in the kidney interstitium is critical in progression of kidney fibrosis. However, the origin of the increase in the number of fibroblasts remains to be determined.

Methods: Here, we investigated the contribution of bone marrow-derived cells (BMDC) and renal slow-cycling cells, stem- or progenitor-cells, in the kidney fibrosis caused by ureteral obstruction (UO) using eGFP bone marrow-reconstituted chimeric mice.

Results: UO caused dramatic increases in the numbers of interstitial cells and expansion of the interstitium. Most kidney interstitial cells expressed GFP, indicating that these cells are derived from bone marrow. Interstitial BMDCs were to possess fibroblast specific protein-1 and morphological characteristics of fibroblasts. Twenty nine percent of interstitial cells were cells that had proliferated and approximately 89% among them were BMDCs. Proliferation of fibroblasts differentiated from BMDCs significantly occurred in the interstitium of UO-kidney. Treatment with apocynin, an inhibitor of reactive oxygen species production, reduced infiltration of BMDCs into the UO-kidney, leading to reduction of kidney fibrosis. In normal kidney, only a few slow-cycling cells were observed in the interstitium. Even after UO, no change in the number of those cells was observed, indicating that slow-cycling cells in the interstitium may not have, or may have minimal involvement in kidney fibrosis.

Conclusions: BMDCs are a major contributor to kidney fibrosis via infiltration into damaged sites, differentiation to fibroblasts, and subsequent proliferation.

Funding: Government Support - Non-U.S.

TH-PO997

Nitric Oxide Attenuates Renal Fibrosis via Phosphorylation of Beta-Catenin Hajime Nagasu, Minoru Satoh, Hiroyuki Kadoya, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Renal fibrosis is the final common pathway in the development of renal dysfunction. The activation of Wnt/catenin pathway is involved in several renal dysfunction models. Although it is well known that lack of endothelial nitric oxide synthase, an endothelial dysfunction, promotes renal fibrosis, the relationship between the nitric oxide (NO)-protein kinase G (PKG) pathway and the Wnt/catenin pathway in the kidney remains unclear. We hypothesized that PKG activation by NO phosphorylates beta-catenin and downregulates the Wnt/catenin pathway in the development of renal fibrosis.

Methods: We used wild type (WT) and eNOS-deficient (eNOSKO) mice. WT and eNOSKO mice underwent unilateral-ureteral obstruction (UUO). We then used Bay41-2272 as a soluble guanylate cyclase (sGC) stimulator to determine the role of the NO-sGC-PKG pathway on fibrosis process. In some experiment, WT and eNOSKO mice were crossed with BAT-LacZ mice, Wnt reporter mice. Two weeks after surgery, mice were sacrificed to obtain blood and kidney samples.

Results: Increased interstitial renal fibrosis was found in eNOSKO-UUO mice compared with WT-UUO mice. Pro-fibrotic factors (TGF-beta, CTGF, and fibronectin) were also more prevalent in kidney from eNOSKO-UUO compared with WT-UUO mice. The protein expression of beta-catenin and Wnt-1 inducible signaling pathway protein 1 was remarkably increased in eNOSKO-UUO mice. Following crossing of WT and eNOSKO mice with BAT-LacZ mice, increased beta-catenin signaling was detected by beta-galactosidase activity in WT-UUO mice compared with WT-sham mice. Furthermore, this activity was more prevalent in eNOSKO- UUO mice than in WT- UUO mice. Such phenotypic changes in eNOSKO-UUO mice were suppressed by Bay41-2272 treatment.

Conclusions: The present results suggest that the Wnt signaling pathway plays a pivotal role in renal interstitial fibrosis in the UUO model. Furthermore, NO inhibited Wnt signaling via the sGC-PKG pathway. These findings indicate the potential therapeutic utility of NO-sGC-PKG stimulation for the prevention of renal fibrosis.

TH-PO998

Systemic Smad4-siRNA Administration Prevents Interstitial Fibrosis Partially by Inhibiting the Proliferation of Myofibroblasts Derived from Pericytes in Renal Fibrosis Mouse Model Yoshiyuki Morishita, Hiromichi Yoshizawa, Akira Onishi, Minami Watanabe, Eiji Kusano. *Division of Nephrology, Department of Internal Medicine, Jichi Medical University, Shimotsuke-city, Tochigi, Japan.*

Background: The proliferation of myofibroblasts derived from pericytes has been reported to contribute to renal fibrosis (RF). TGFβ_1/Smad4 pathway plays pivotal roles in the development of RF; however, the effects of the regulation of pericytes with systematic siRNA delivery that targets this pathway as a therapeutic approach for RF remain to be determined. In the present study, we investigated the effects of systemic smad4-siRNA administration for treatment of RF and the regulation of pericytes in an RF mouse model.

Methods: RF mouse model was produced by the intraperitoneal injection of folic acid (250 mg/kg) in C57BL/6 mice. Smad4-siRNA (5 nmol) was injected into each mouse via the tail vein three times per week (smad4-siRNA group). RF mice injected with non-targeted siRNA (5 nmol) and no siRNA served as control groups. The mice were sacrificed on the 21st day after folic acid injection. RF was evaluated by histological analysis. The expression levels of smad4, α-smooth muscle actin (αSMA) and pericyte markers such as platelet-derived growth factor receptor-b (PDGFR-b) and NG2 chondroitin sulfate proteoglycan (NG2) in kidney were analyzed by qRT-PCR and/or immunohistochemistry.

Results: The histological analysis showed that RF with increased interstitial myofibroblasts was markedly inhibited in the smad4-siRNA group compared with those in control groups. The qRT-PCR analysis showed that *smad4* expression in kidney was up-regulated and the expression of PDGFR-b, NG2 and αSMA was markedly increased in control groups, and these changes were suppressed in the smad4-siRNA group. The immunohistochemistry showed that double-positive cells with PDGFR-b and αSMA were increased as a part of the increased interstitial myofibroblasts in control groups; however, these cells were markedly fewer in the smad4-siRNA group.

Conclusions: The results of the present study suggested that systemic smad4-siRNA administration prevents RF partially by inhibiting the proliferation of myofibroblasts derived from pericytes *in vivo.*

Funding: Government Support - Non-U.S.

TH-PO999

Lack of Vasohibin-1 Exacerbates Tubulointerstitial Injuries Partly via Activating Smad3 and Excessive Matrix Production in Renal Fibroblasts Hiroyuki Watatani,[1] Yohei Maeshima,[1] Hiroko Yamasaki,[1] Norikazu Hinamoto,[1] Haruyo Ujike,[1] Hitoshi Sugiyama,[1] Yasufumi Sato,[2] Hirofumi Makino.[1] *[1]Dept. of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Okayama, Japan; [2]Dept. of Vascular Biology Institute of Development, Aging, and Cancer, Tohoku Univ, Sendai, Miyagi, Japan.*

Background: Tubulointerstitial injuries are crucial histological alterations predicting deterioration of renal function in chronic kidney disease. We recently reported the protective role of Vasohibin-1(VASH-1), a negative feedback regulator of angiogenesis as well as a maturation factor of neovessels, in diabetic nephropathy, but its role on tubulointerstitial injuries remains to be elucidated. In the present study, we aimed to evaluate the role of endogenous VASH-1 in regulating tubulointerstitial alterations and assessed its role on fibrogenesis and activation of Smad3 in renal fibroblasts.

Methods: Unilateral ureteral obstruction (UUO) was induced in female VASH1+/- or wild-type (VASH1+/+) littermates (C57/BL6J background). Mice were sacrificed on Day 3 or 7 after inducing UUO and the obstructed kidneys (OBK) were obtained. Cultured normal rat kidney fibroblasts (NRK-49F) were transfected with rat VASH1 siRNA prior to stimulation with TGF-beta1. Western blot, immunohistochemistry and real-time PCR were performed.

Results: Interstitial fibrosis, accumulation of type I and III collagen and F4/80$^+$ monocytes/ macrophages in the OBK were significantly exacerbated in VASH1+/- mice compared with VASH1+/+ mice on Day 7 after inducing UUO. Increase in the renal levels of TGF-beta1, phosphorylated Smad 3, CCL2 mRNA and the number of interstitial fibroblast specific protein (FSP)1$^+$ fibroblasts as well as reduction of IL-10 and IL-10ralpha mRNA in the OBK were significantly aggravated in VASH1+/- mice. Knockdown of VASH1 by siRNA led to the increase of pSmad3 and type I/III collagen mRNA in NRK-49F cells.

Conclusions: Taken together, these results suggest the protective role of endogenous VASH1 on tubulointerstitial alterations via regulating inflammation and fibrosis, and the direct anti-fibrotic effects of VASH1 on renal fibroblasts through modulating TGF-beta signaling.

TH-PO1000

Silencing of TGF-b Signaling in the Endothelium Improves Angiogenesis and Blunts Fibrotic Response to Chronic Renal Injury Sandhya Xavier, Radovan Vasko, Robert Chen, Julien Maizel, Praveen N. Chander, Michael S. Goligorsky. *New York Medical College, Valhalla, NY.*

Background: TGF-b is the prototype member of a family of evolutionarily conserved pleiotropic cytokines. Untargeted interference with TGF-b signaling is less informative than its manipulation in distinct cell types.

Methods: To examine the link between TGF-b signaling, fibrosis and endothelial cell (EC) functions we generated mice with deletion of TGF-b receptor type II (TbRII) in the vascular endothelium by mating mice homozygous for floxed TbRII allele with mice heterozygous for Cre-recombinase under the control of Tie2-promoter to obtain Tie2-Cre;TbRII$^{flox/+}$ which were crossed with TbRII$^{flox/flox}$ mice.

Results: Genotyping offsprings identified heterozygous (Tie2-Cre;TbRII$^{flox/+}$) and wild-type pups (TbRII$^{flox/flox}$ or TbRII$^{flox/+}$) while Tie2-Cre;TbRII$^{flox/flox}$ mutant mice were embryonic lethal. TbRII$^{endo+/-}$ mice showed no abnormal renal function at 6 weeks of age. In contrast, TbRII$^{endo+/-}$ mice had accelerated sprouting in aortic ring matrigel assays. In a folic acid nephropathy (FAN) acute injury model, both WT and Het mice showed elevated serum creatinine and urinary albumin:creatinine ratio, with focal mild-to-moderate tubular dilatation and rare tubular necrosis, more pronounced in Het mice. In contrast, during chronic injury (6 weeks of FAN), interstitial fibrosis was much more prominent in TβRII$^{endo+/+}$ than in TβRII$^{endo+/-}$ mice. Consistent with these results expression of collagen I and III was markedly increased in WT+FAN mice, while expression of these genes in Het+FAN mice was ameliorated. Moreover, analysis of capillary density in kidneys revealed better preserved microvasculature in Het+FAN mice compared to WT+FAN group. Ex vivo cultured vessels obtained from TβRII$^{endo+/-}$ mice in the EC had better angiogenic potential after the challenge with FA in comparison with WT mice.

Conclusions: The data collectively document that partial ablation of TbRII in the endothelium supports increased angiogenesis and reduced fibrosis during chronic, but not in acute, kidney injury. These data indicate a critical role of endothelial TGF-b signaling in defective angiogenic response to injury and development of fibrosis.

Funding: NIDDK Support

TH-PO1001

Defect in Runx2 Gene Accelerated Unilateral Ureteral Obstruction-Induced Kidney Fibrosis via Increased TGF-β Signal Jee In Kim, Hee-Seong Jang, Kwon Moo Park. *Anatomy and BK21 Project, Kyungpook National University School of Medicine, Daegu, Korea.*

Background: Runt-related transcription factor x2 (Runx2) regulates osteoblast-specific genes inducing osteoblast differentiation and bone formation. Runx2 has known to negatively regulate TGF-β signal, which involves in the progression of fibrosis of the vascular smooth muscle cells. However, the role of Runx2 gene in the kidney fibrosis is unknown.

Methods: Here, we investigated the effect of partial deletion of Runx2 gene on the progress of kidney fibrosis induced by unilateral ureteral obstruction (UUO) using heterozygous (Runx2 wild type/C-terminus of Runx2 truncated) type of mouse (Runx2+/-).

Results: We observed Runx2 gene decreased by UUO in both Runx2 +/+ and Runx2 +/- mice. Runx2+/- mice revealed higher collagen3 deposition, and higher expression of collagen1 and α-smooth muscle actin (α-SMA) in the kidney indicating exacerbated fibrosis compared to Runx2+/+ mouse kidney. Activation of TGF-β was higher in the Runx2+/- kidney than in the Runx2+/+ kidney, suggesting an inhibitory effect of Runx2 on the TGF-β signal in the kidney fibrosis. Furthermore, overexpression of Runx2 gene using adenoviral vector transfection attenuated TGF-β–induced α-SMA expression in the Mardin Darby canine kidney tubular (MDCK) cells indicating inhibited trans-differentiation of kidney tubule cells into myofibroblasts.

Conclusions: Conclusively, defect in Runx2 gene increased UUO-induced TGF-β signaling and kidney fibrosis, providing Runx2 as a noble target for the protection against kidney fibrosis.

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TH-PO1002

CHOP Deficiency Attenuates UUO-Induced Renal Fibrosis by Reduction of Tubular Injury Kai-ti Chang,[3] Cheng-tien Wu,[1] Ching-chin Yang,[1] Chih-Kang Chiang,[1,2,3] Shing-Hwa Liu.[1] *[1]Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan; [2]Department of Integrated Diagnostics & Therapeutics, National Taiwan University Hospital, Taipei, Taiwan; [3]Department of Internal Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.*

Background: CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) is a key component in endoplasmic reticulum (ER) stress-mediated apoptosis and acts as the chemoattraction of mononuclear cells. The goal of the study was to investigate the role of CHOP in unilateral ureteral obstruction (UUO)-induced kidney injury.

Methods: Tubulointerstitial injury and renal fibrosis were assessed in wild-type (WT) and CHOP-deficient mice following unilateral ureteral ligation. Renal injury and fibrosis was assessed by the H&E stain and Masson's trichrome stain. Immunohistochemistry stain was arranged to evaluate the expression of fibronectin and Ly6G. Renal apoptosis was evaluated by TUNEL staining. Tissue malondialdehyde level and NADPH oxidase 4 (NOX4) expression was conducted for evaluating the severity of oxidative stress. In order to clarify the role of CHOP in innate immunity of UUO-induced fibrosis, we arranged bone marrow transplantation (BMT) of chop knockout to wild type mice.

Results: In WT kidney, UUO induced overexpression of CHOP and Bax, a downstream target in the CHOP-mediated ER stress pathway. Renal fibrosis was attenuated in CHOP-knockout mice. Expression levels of α-smooth muscle actin, extracellular matrix, and plasminogen activator inhibitor-1 were reduced, and apoptotic death was attenuated in CHOP-deficient mice. Furthermore, CHOP deficiency reversed UUO-induced apoptosis via JNK and Bcl2 pathway, ameliorated the Ly6G expression and decreased ROS production, which accompanied by NOX4 suppression. This BMT model did not reverse the tubulointerstitial injury and fibrosis induced by UUO fibrosis model.

Conclusions: In conclusion, UUO induces CHOP-mediated ER stress and triggers renal cell death, and CHOP deficiency attenuates this cell death and subsequent renal fibrosis. The results demonstrate an essential role of CHOP in development of renal fibrosis due to UUO damage.

Funding: Government Support - Non-U.S.

TH-PO1003

Hemizygous Deletion of Connective Tissue Growth Factor (CTGF/CCN2) Does Not Suffice to Prevent Fibrosis of the Severely Injured Kidney Lucas Falke,[1] Amelie Dendooven,[1] Jan Willem Leeuwis,[1] Tri Q. Nguyen,[1] Rob Van Geest,[2] Dionne M. Van der Giezen,[1] Roel Broekhuizen,[1] Karen M. Lyons,[3] Reinout Stoop,[4] Hans Kemperman,[5] Jaap A. Joles,[6] Roel Goldschmeding.[1] *[1]Pathology, UMC, Utrecht, Netherlands; [2]Ophtalmology, AMC, Amsterdam, Netherlands; [3]Molecular & Cell Biology, UCLA, Los Angeles, CA; [4]Metabolic Health Research, TNO, Leiden, Netherlands; [5]Clinical Chemistry & Haematology, UMC, Utrecht, Netherlands; [6]Nephrology & Hypertension, UMC, Utrecht, Netherlands.*

Background: Inhibition of CTGF can prevent development of mild kidney fibrosis, but its influence in severe kidney disease is uncertain. We tested several models of severe kidney disease in mice with hemizygous knockdown for CTGF.

Methods: Both hemizygous CTGF $^{+/-}$ mice and wild type littermates were injected with Streptozotocin or Aristolochic Acid or were unilaterally obstructed at the ureter (UUO). These procedures led to diabetes-induced glomerulosclerosis after 6 months, toxic nephropathy after 25 days and IF/TA after 14days, respectively. The kidneys were analysed by qPCR, Western blot, histochemistry and hydroxyproline measurements.

Results: CTGF$^{+/-}$ mice showed a 50% reduction of both mRNA and protein expression when compared to the appropriate wild type littermate controls ($p<0,05$). This reduction in available CTGF did not lead to a difference in morphology or reduction in fibrosis as measured by scoring PAS/Sirius red stained sections, hydroxyproline content and α-Smooth Muscle Actin expression. Furthermore no reduced inflammatory response as measured by MCP-1 mRNA expression and immunohistological F4-80 positivity was seen.

Conclusions: Unlike previous studies of mild diabetic nephropathy and short-term UUO, scarring of severely and chronically damaged kidneys is not attenuated by a 50% reduction of CTGF. This suggests that CTGF is either redundant in severe and chronic kidney disease, or that it is a limiting factor only at subnormal concentrations requiring further reduction to prevent fibrosis of the severely injured kidney.

Funding: Government Support - Non-U.S.

TH-PO1004

Dot1l-Deficiency Enhances High Na+-Induced Renal Fibrosis Possibly by Upregulating V-ATPase Wenzheng Zhang,[1] Hongyu Wu,[1] Lihe Chen,[1] Xi Zhang,[1] Qiaoling Zhou.[2] *[1]UTHSC, Houston, TX; [2]Central South Univ, Changsha, China.*

Background: Our preliminary studies suggest that Dot1l^{AC} mice, which lack histone H3 K79 methyltransferase Dot1l and thus H3 dimethylation (H3m2K79) in Aqp2^{+} cells, significantly increased intercalated cells (IC) in the expense of principal cells (PC) vs. controls (Wu et al, 2011 ASN abstract # 21278), and developed features of chronic kidney disease (CKD) after chronic Na+ loading (Wu et al. 2011 ASN abstract #20636).

Methods: Mice fed a 8% NaCl diet for 32 days were analyzed by metabolic assay, Masson's trichrome staining, RT-qPCR, IB, IF, IHC, ELISA, and ChIP. Kidney biopsies from 8 minimal change disease (MCD) and 9 CKD patients were stained for AQP2 and V-ATPase subunits B1/B2 (B1B2) as PC and IC markers, respectively.

Results: 1) UAR (mg/g), [BUN] (mg/dl) and blood pH were significantly elevated from 20, 37 and 7.2 in Dot1l$^{f/f}$ to 36, 51 and 7.3 in Dot1l^{AC} mice, respectively. The two groups had similar urine pH; 2) Dot1l^{AC} vs. Dot1l$^{f/f}$ mice significantly increased Masson's trichrome staining and mRNA and protein expression of all fibrotic markers (collagen 1a1, aSMA, TGFb, TIMP1, FSP1, PAI1, and Vimentin) and V-ATPase subunits (A, B1 and B2) examined; 3) Dot1l and H3m2K79 bind V-ATPase B2 promoter in ChIP; 4) Dot1a overexpression significantly decreased a V-ATPase B2 promoter-luciferase reporter in IMCD3 cells; 5) Injection of V-ATPase-specific inhibitor Bafilomycin A1 into Dot1l^{AC} mice significantly improved renal function and decreased expression of the fibrotic markers; 6) PC (AQP2+B1B2-) was decreased from 44% in MCD to 17% in CKD, and IC (AQP2-B1B2+) was increased from 22% in MCD to 43% in CKD. Both groups had 15-22% of AQP2+B1B2+ or AQP2-B1B2- cells; 7) In MCD, only 15% of IC cells lost H3m2K79. This number was significantly increased to 50% in CKD.

Conclusions: Loss of Dot1l-mediated H3m2K79 facilitates derivation of IC from Aqp2+ cells in mouse and possibly in human; Dot1l deletion causes Na+-sensitive renal fibrosis that can be partially blocked by V-ATPase inhibitor Bafilomycin A1. Blockade of V-ATPase may offer a potential therapeutic strategy for some CKD.

Funding: NIDDK Support

TH-PO1005

Thymosin β4 and AcSDKP Ameliorate AngII-Induced Tubulointerstitial Fibrosis in Mice with Deficient TGF-β Activation Sebastian Alexander Potthoff,[1,2] Yiqin Zuo,[2] Haichun Yang,[2] Li-Jun Ma,[2] Agnes B. Fogo.[2] *[1]Department of Internal Medicine / Nephrology, Heinrich-Heine University - Medical Faculty, Düsseldorf, Germany; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN.*

Background: β6 knockout (β6-/-) mice lack αvβ6-integrin dependent TGF-β1 activation and are resistant to tubulointerstitial fibrosis after unilateral ureteral obstruction (UUO). However, in these mice, angiotensin II (AngII) in addition to UUO restores tubulointerstitial fibrosis, along with increased PAI-1 and thymosin β4 (Tβ4) expression. Tβ4 is degraded by prolyl oligopeptidase (POP) to the anti-fibrotic AcSDKP. We previously showed that Tβ4 is necessary for AngII induction of PAI-1 in glomerular endothelial cells, exogenous Tβ4 decreases late UUO fibrosis, and that Tβ4 has been linked to TGF-β. We now investigated whether modulation of the Tβ4-AcSDKP-axis impacts fibrosis in β6-/- mice.

Methods: β6-/- mice (C57/Bl6 background) underwent UUO and AngII infusion (40μg/kgBwt/hr), and additional treatment: no further treatment, AcSDKP (800 μg/kgBwt/day), POP-Inhibitor (40mg/kgBwt/day by gavage) + Tβ4 (150μg i.p. every 3rd day), or Tβ4 alone. Mice were sacrificed on day 5 or14 after UUO (n=5-8 each group).

Results: Targeted genes (collagen I, collagen III, RANTES, POP, Tβ4, TIMP, PAI-1, IL1β) analyzed by qPCR showed no difference amongst groups at day 14. However, on day 5, profibrotic and inflammatory markers (collagen III, Tβ4, POP, TIMP, RANTES, IL1β) were decreased in AcSDKP vs. other groups. AcSDKP significantly reduced fibrosis assessed by Sirius red staining (day 5 and 14). Tβ4 alone also significantly decreased fibrosis at day 14. On day 14, Tβ4 expression assessed by immunohistochemistry was similar in mice with or without AcSDKP (AngII 46.1±6.0; AngII+AcSDKP 43.8±5.4; p=NS). Macrophage infiltration was similar in AcSDKP vs. Tβ4+POP inhibitor.

Conclusions: In summary, AcSDKP reduced fibrosis in β6-/- mice injured with UUO and AngII. Tβ4 administration significantly decreased tubulointerstitial fibrosis at late stage. We conclude that the Tβ4-AcSDKP-axis can modulate fibrosis independent of TGF-β activation.

TH-PO1006

Leukemia Inhibitory Factor Ameliorates Renal Fibrosis via Suppressing Signal Transducers and Activators of Transcription 3 Tyrosine 727 Phosphorylation Chen Yu,[1] Ying Yu,[1] Lunjun Fu,[1] Eugene Chin.[2] *[1]Division of Nephrology, Tongji Hospital, School of Medicine, Tongji University, Shanghai, China; [2]Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences& Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

Background: Leukemia inhibitory factor (LIF) is a pleiotropic glycoprotein belonging to the interleukin-6 family and its function in the kidney under pathophysiologic conditions is largely unknown. The aim of this study was to investigate the effects of LIF on renal fibrosis and the underlying mechanism.

Methods: Rat renal fibroblast cells (NRK-49F) and wild type or STAT3$^{-/-}$ mouse embryo fibroblast-MEF cells were used for in vitro study and unilateral ureter obstruction(UUO) rats for in vivo study.

Results: 1) LIF inhibited AngII-induced expressions of collagen 1 (col1), collagen 3 (col3) and fibronectin (FN1), and reversed AngII-induced reduction of matrix metalloproteinase 9, which led to the depredation of collagens, in NRK-49F cells.2) In the wild type STAT3$^{+/+}$MEF cells, LIF inhibited AngII-induced col1, col3 and FN1 protein and mRNA expressions. However, these effects were not seen in STAT3$^{-/-}$ MEF cells.3) Exposure of NRK-49F cells with AngII markedly increased STAT3 Ser727 phosphorylation, which was followed by STAT3 Tyr705 phosphorylation. LIF treatment however, attenuated AngII-induced STAT3 phosphorylation, more pronouncedly on Ser727.4) Mice were divided into 3 groups: a) sham group, b) UUO group and c) LIF + UUO group (LIF, 25mg/kg/day, daily for 6 days, intraperitoneal) (n=4). LIF treated UUO mice had less severity of renal fibrosis. LIF treatment markedly inhibited the expressions of col1, col3 and FN1 in UUO mice.5) In UUO model, STAT3 phosphorylation on both Tyr705 and Ser727 sites increased

markedly as compared with that in the sham group. However, STAT3 Ser727 and Tyr705 phosphorylation intensity was obviously decreased in LIF treatment group.

Conclusions: Our study uncovered LIF treatment attenuated the severity of renal fibrosis. The renoprotective effect of LIF could be mediated by inhibition of STAT3 Ser727 phosphorylation.

Funding: Government Support - Non-U.S.

TH-PO1007

Angiopoietin-1 Protects against Renal Fibrosis Marie Jeansson,[1] Susan E. Quaggin.[1,2] *[1]Mount Sinai Hospital, Samuel Lunenfeld Research Institute, Toronto, ON, Canada; [2]St Michaels Hospital, University of Toronto, Toronto, ON, Canada.*

Background: The presence of renal tubulointerstitial fibrosis is predictive of progressive decline in kidney function. It is characterized by an increase in alpha-smooth actin-positive (VSMA+) fibroblasts that produce collagen. Identification of factors that regulate the fibrotic response are excellent candidate targets for treatment of kidney diseases. We previously showed that loss of Angiopoietin-1 (Angpt1) in adult mice predisposes to systemic fibrosis in an ear punch model. Angpt1 is expressed in pericytic lineages and acts through the Tie2 tyrosine-kinase receptor expressed on adjacent endothelium. Here, we test the hypothesis that the Angpt1-Tie2 system is protective in the unilateral ureter obstruction (UUO) model of renal fibrosis and loss of Angpt1 will result in a more aggressive fibrotic response.

Methods: To better understand the origin of fibroblast populations in renal fibrosis, we first performed lineage tagging experiments using Z/EG reporter mice that were bred to 4 independent Cre-driver strains and subjected to UUO. This is an important foundation to understand potential mechanisms. These Cre strains mark the tubular epithelium, renal pericytes, renal endothelium and intrinsic renal fibroblasts, respectively (Pax8-rtTA/tetOCre, Pdgfrb-Cre, Tie2-Cre and Tcf21-Cre). Secondly, we performed UUO on Angpt1 conditional knockout mice.

Results: Kidneys were analyzed and co-stained for the lineage tag (eGFP) and VSMA, 10 days post-UUO. We found that only 6.8% and 8.9 % of VSMA+ cells in the tubulointerstitium stained for eGFP, from pericytes and endothelial lineages respectively, suggesting that only a small proportion of interstitial fibroblasts originate from these linages. Studies to determine the contribution of tubular and intrinsic renal fibroblast populations to VSMA+ cells following UUO are currently underway. In adult Angpt1 conditional knockout mice, pilot studies suggest an increase in tubulointerstitial collagen following UUO, more studies are ongoing.

Conclusions: Together, our results support a model whereby Angpt1-Tie2 signaling protects against fibrosis.

Funding: Government Support - Non-U.S.

TH-PO1008

Dependence of Renal Fibrosis on a Cell Cycle Regulator Peter M. Price,[1,2] Judit Megyesi,[1] Adel Tarcsafalvi,[1] Rawad Hodeify,[1] Nang San Hti Lar Seng,[1] Shenyang Li,[1] Didier Portilla.[1,2] *[1]UAMS; [2]VA Med Ctr.*

Background: Expression of a cell cycle regulatory protein, $p21^{WAF1/Cip1}$, is associated with fibrotic changes and progression. Although p21 is an inhibitor of cyclin-dependent kinases-1 and -2 (Cdk1, Cdk2), the mechanism of how its expression contributes to fibrosis is unclear. It is also unclear in which cells of the kidney p21 expression is necessary to cause fibrosis.

Methods: Cultured mouse kidney proximal tubule cells were used *in vitro*, either exposed to 0.5 µg/ml aristolochic acid (AA), or transduced with a p21-expression adenovirus. Also, several mouse models of renal fibrosis were used *in vivo*, either mimicking effects of acute kidney injury or effects of loss of renal mass.

Results: Expression of p21 *in vitro* caused significant elevations of TGFβ1 (5.5 to 8.2 times control) and collagen 1A1 (2.3 to 2.8 X) mRNAs, even greater than AA exposure (1.5 to 2.2 X, and 1.5 to 2.1 X, respectively), a known inducer of these fibrotic markers. A mutant p21 that still inhibited Cdk's did not result in high elevations of these fibrosis-associated mRNAs (1.3 to 1.7 X, and 1.2 to 1.5 X, respectively), so that the gene induction by p21 may be a direct effect of p21 on gene transcription. We reported previously that p21 knock-out mice were protected from renal fibrosis using 5/6 nephrectomy or UUO and release and that transgenic mice over-expressing p21 in proximal tubules had more α-smooth muscle actin 14 days after unilateral ureteral obstruction. We now report that p21 knock-out mice in which p21 could be induced specifically in proximal tubules developed more fibrotic markers than even wild-type mice after either 28 or 42 days following unilateral ischemia or 14 days after UUO that was released after 4 days, showing that proximal tubular cells are a significant source of the signal for induction of fibrosis.

Conclusions: Induction of p21 alone is a causative mechanism for development of fibrotic markers *in vitro* and kidney fibrosis *in vivo*. Whether expression of fibrogenic p21 is required exclusively in proximal tubules or in other kidney cells for development of chronic renal failure will be addressed by similar cell-specific p21 induction in a p21 knock-out background.

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TH-PO1009

Angiotensin II Induces Renal Fibrosis through Upregulation of Cyp4a14 Expression Yunfeng Zhou, Youfei Guan. *Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China.*

Background: Angiotensin II (Ang II) plays an important role in the pathogenesis of hypertension and its associated cardiovascular and renal injury.

Methods: To elucidate the molecular mechanism by which AngII induces renal damage, we measured expression profile of genes involved in arachidonic acid metabolism and its metabolites' function in the kidneys of mice receiving chronic AngII infusion for 14 days.

Results: Cyp4a14 was found to be significantly induced by AngII, which was associated with a marked increase in systolic blood pressure and proteinuria. Real-time PCR, Western blot and immunohistochemistry assays demonstrated that cyp4a14 expression was predominantly upregulated in the proximal tubules of AngII-infused mice. 24-hour urinary excretion of the major cyp4a14 metabolite, 20-HETE, was also significantly increased in the Ang II-treated mice. To determine whether cyp4a14 mediates the effects of AngII, cyp4a14-/- mice were utilized. Compared to wild-type (WT) mice, cyp4a14-/- mice exhibited significantly lower levels of blood pressure and proteinuria following AngII infusion. Histologically, AngII infusion resulted in renal glomerulosclerosis and tubulointerstitial fibrosis in WT mice, which was significantly reduced in $cyp4a14^{-/-}$ mice. Consistently, expression levels of renal collagen I was significantly lower in $cyp4a14^{-/-}$ mice than in WT mice following Ang II treatment. Furthermore, we used a rat proximal tubule cell line (RPTC) to examine the effect of Ang II on cyp4a14 expression and 20-HETE production. Treatment of RPTC cells with Ang II significantly induced cyp4a14 expression, which was associated with an increased level of supernatant 20-HETE. 20-HETE treatment resulted in a marked increase in transforming growth factor β1 and collagen I expression.

Conclusions: Collectively, these findings suggest that Ang II-mediated renal fibrosis may result from increased cyp4a14 expression and 20-HETE levels. Cyp4a14 could be a useful target for the treatment of Ang II-elicited renal damage.

Funding: Government Support - Non-U.S.

TH-PO1010

Pigment Epithelium-Derived Factor Ameliorates Renal Fibrosis Xuemin He, Rui Cheng, Jian-xing Ma. *Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Pigment epithelium-derived factor (PEDF) is a multifunctional protein in the serine proteinase inhibitor family and has been shown to be decreased in the kidney of diabetic animal models, and decreased PEDF levels are believed to contribute to progression of diabetic nephropathy (DN). As DN is characterized by excessive deposition of extracellular matrix in the kidney which leads to fibrosis, the purpose of this study is to establish the anti-fibrosis activity of PEDF in the kidney.

Methods: Human renal proximal tubular cell line (HKC8) was exposed to 4 ng/ml TGF-β1 and treated with different concentrations of purified recombinant human PEDF (rhPEDF) (40, 80, 160 and 320 nM). Loss of tubular epithelial phenotypes was evaluated by detecting E-cadherin, and fibrogenic event was determined by measuring α-smooth muscle actin (α-SMA), fibroblast specific protein 1 (FSP1), and fibronectin (FN) using Western blot analysis. In 8-week-old $PEDF^{-/-}$ mice and aged-matched wild type (WT) mice, sham surgery or unilateral ureteral obstruction (UUO) was operated on the left kidney. Seven days after the surgery, the left kidneys were collected; E-cadherin, α-SMA, FSP1, FN and collagens were determined by Western blot analysis and immunohistochemistry.

Results: In HKC8, TGF-β1 treatment increased the expression of α-SMA and FN dramatically, which was suppressed by rhPEDF in a concentration-dependent manner; in contrast, TGF-β1 decreased E-cadherin levels, while rhPEDF prevented the TGF-β1-induced E-cadherin decrease. UUO induced TGF-β1 and FSP1 expression in the kidneys of WT and $PEDF^{-/-}$ mice, while $PEDF^{-/-}$ UUO mice showed more prominent increase in TGF-β1 levels, compared to that in WT UUO mice. As shown in immunohistochemistry, $PEDF^{-/-}$ UUO mice exhibited more intense staining of collagen IV compared to WT UUO mice.

Conclusions: PEDF exhibits a potent anti-fibrogenic function on renal proximal tubular cells, and knockout of PEDF in the kidney exacerbates renal fibrosis.

TH-PO1011

Kidney Fibrosis: Evidence for a New Treatment Based on Selective Blockade of SnoN Degradation Yuanyuan Shi,[1,2] Yanlin Wang,[1] William E. Mitch,[1] Zhaoyong Hu.[1] *[1]Internal Medicine, Nephrology Division, Baylor College of Medicine, Huston, TX; [2]Renal Section, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, China.*

Background: Several mechanisms causing renal fibrosis have been uncovered. TGFb1 is a major mediator because it stimulates fibrosis by upregulating collagen, fibronectin, smooth muscle actin (SMAa) and other proteins. But, there are few available therapies that will block or suppress TGFb1 and hence, fibrosis. In animal models of fibrosis (e.g., pulmonary fibrosis), thalidomide exerts anti-inflammatory and anti-fibrotic properties by poorly defined mechanisms.

Methods: in mice with unilateral ureteral obstruction (UUO) treated with or without 200 mg/kg thalidomide, we evaluated the degree of kidney fibrosis and inflammation plus TGFb1 expression. We investigated the TGFb1 repressor, SnoN, and how thalidomide might influence SnoN levels to regulate TGFb1 in cultured kidney tubular cells and fibroblast.

Results: UUO stimulated the expression of collagen, fibronectin and SMAa. Thalidomide blocked the induction of collagen I (>50%), fibronectin (>60%) and SMAa (>40%) and suppressed tubulointerstitial fibrosis in kidneys of UUO mice. Thalidomide virtually eliminated the mRNA expression of TGFb1 plus the phosphorylation of Smad3 as

well as UUO-induced TNFa, IL1a, IL6, and bFGF. Thus, thalidomide blocking TGFb1 could be the mechanism induced by thalidomide which suppresses fibrosis mediators. In cultured kidney fibroblasts and tubular cells, we determined how thalidomide suppresses TGFb1 by studying SnoN, a transcription repressor. Thalidomide can selectively inhibit ubiquitin (Ub) conjugation by binding to CBRN-DDP1, an Ub E3 ligase. Indeed, thalidomide decreased Ub conjuagtion to SnoN in cultured fibroblast or mice with UUO. This led to an increase in SnoN to suppression of its degradation in the Ub-proteasome system (UPS). The result was a decrease in TGFb1 and hence, reduced expression of fibrotic proteins.

Conclusions: Thalidomide initiates its anti-fibrotic effects by selectively inhibiting an Ub E3 ligase which regulates the degradation of SnoN. It could become a treatment strategy for kidney fibrosis since this drug is already used to treat leprosy.

Funding: NIDDK Support

TH-PO1012

Rheb/mTORC1 Signaling Activation Promotes Kidney Fibroblast Activation and Interstitial Fibrosis Lei Jiang,[1] Junwei Yang,[2] Chunsun Dai.[3] *[1]Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China; [2]Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu; [3]Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Ras homolog enriched in brain (*Rheb*), a small GTPase that regulates cell growth, differentiation and survival through up-regulating mTORC1 signaling, has been studied in many cell types. However, the role and mechanisms of Rheb/mTORC1 signaling in kidney fibroblast activation and kidney fibrosis remain largely unknown.

Methods: pCAG-Rheb transgenic mice and and sex matched control littermates were used in this study. Rapamycin was given intraperitoneally at a dose of 2.5mg/kg/day for 4 weeks on pCAG-Rheb transgenic mice .pCAG-Rheb mice and control littermates were performed with UUO model.NRK-49F cells treated with TGF-β1 were used for in vitro study.

Results: In this study, we found that Rheb/mTORC1 signaling was activated in the kidney interstitial cells from the fibrotic kidneys. Rheb/mTORC1 signaling was also sharply activated in NRK-49F cells treated with TGFb1. Blocking Rheb/mTORC1 signaling with rapamycin or Rheb siRNA abolished TGFb1-induced fibroblast activation. Ectopic expression of Rheb activated kidney fibroblast. In the Rheb transgenic mice, mTORC1 signaling in both kidney tubular and interstitial cells was activated and progressive kidney interstitial fibrosis was detected. Rapamycin remarkably inhibited mTORC1 signaling activation and fibrosis in the transgenic kidneys. Indeed, mice with fibroblast specific deletion of Tsc1 exhibited mTORC1 signaling activation in kidney interstitial cells and kidney fibrosis.

Conclusions: Together, these results suggest that Rheb/mTORC1 signaling plays an extremely important role for promoting kidney fibroblast activation and interstitial fibrosis, which may act as a potential therapeutic target for kidney fibrosis.

Funding: Government Support - Non-U.S.

TH-PO1013

Assessment of Anti-Fibrotic Effects of Klotho with a High-Throughput Cell Migration Assay Using Scratch Wound Healing Ken Tsuchiya, Hidekazu Sugiura, Takumi Yoshida, Shunji Shiohira, Kosaku Nitta. *Department of Medicine IV, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: The renal expression of the Klotho gene is markedly suppressed in chronic kidney disease (CKD). Acceleration of renal fibrosis is a basic pathophysiology for progression of CKD, and TGF-β1 plays a key role in that process. Previously, we showed that he renal interstitial fibrosis was severer in the kl/+ mice than those in the wild-type mice by UUO treatment. In this study, we clarify the direct relationship between Klotho and TGF-β1 using recombinant protein, siRNA and scratch assay.

Methods: The expression levels of fibrotic marker, such as α-SMA, fibronectin and TGF-β1 was immunohistochemically stained and TGF-β1 levels were assayed. Internal expression of Klotho was modified by siRNA transfection. The growth and migration of cultured cells was quantified by using CL-Quant software to analyze time-lapse images in a Nikon BioStation CT.

Results: TGF-β1 reduced Klotho expression in renal cultured epithelial cells (IMCD and HK2). In addition, in cultured renal fibroblast cells (NRK49F) in which internal Klotho expression was negligible, expression levels of α-SMA and PAI1 were significantly suppressed by addition of recombinant Klotho protein to the medium. These effects were also suppressed by a TGF-β1 receptor inhibitor (ALK5 inhibitor) suggesting that Klotho inhibited TGF-β1 activity. Migration of NRK49F cells was accelerated and expression of fibrotic markers was up-regulated by addition of TGF-β1, in contrast, Klotho addition suppressed the effect. SiRNA reduced the expression of Klotho in epithelial cells, however, Klotho knockdown tended to accelerate cell migration but was not significant.

Conclusions: Taken together, it is likely that there is a cross-link between Klotho and TGF-β1 expression in the progression of renal fibrotic changes. Klotho itself plays a significant role in the fibrotic mechanism was not clear in the present study. Thus, there must be a vicious cycle that suppresses Klotho expression and up-regulates TGF-β1 that aggravates the fibrotic process in damaged kidneys, such as in CKD.

Funding: Government Support - Non-U.S.

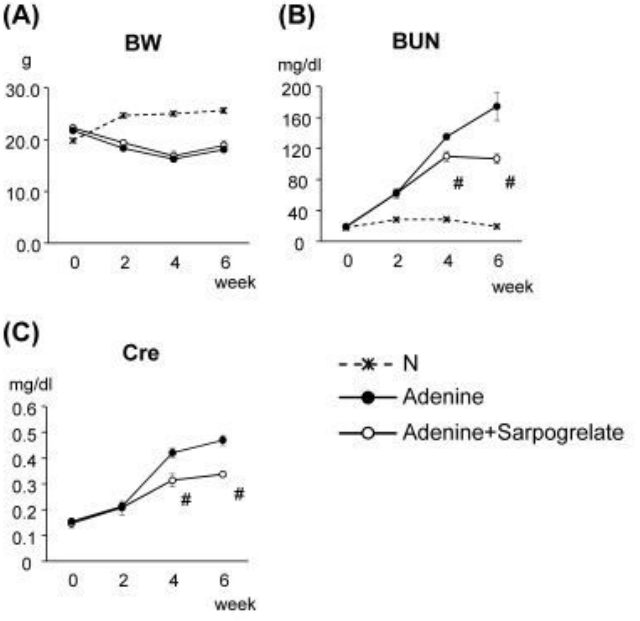
TH-PO1014

A 5-HT Receptor Antagonist Sarpogrelate Ameliorates Renal Tubulointerstitial Fibrosis via Suppressing PAI-1 Yoshifumi Hamasaki, Kent Doi, Masaomi Nangaku, Eisei Noiri. *Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

Background: Chronic hypoxia and subsequent fibrosis in renal tubulointerstitium have been recognized as final common pathway of chronic kidney disease (CKD) progression to end-stage renal disease. Sarpogrelate, a clinically available selective antagonist of 5-hydroxytryptamine (5-HT) 2A receptor, blocks platelet aggregation and causes vasodilatation. We hypothesized that sarpogrelate will reduce renal fibrosis by improving microcirculation in the renal interstitium.

Methods: A mouse renal fibrosis model induced by feeding a 0.2% adenine-containing diet for 6 weeks was used for evaluating whether sarpogrelate would reduce renal interstitial injury. In vitro analysis with mProx cells derived from proximal tubular epithelial cells was conducted to clarify the role of plasminogen activator inhibitor-1 (PAI-1) in the mechanism of action of sapogrelate.

Results: C57BL/6 mice fed a 0.2% adenine-containing diet for 6 weeks developed severe tubulointerstitial fibrosis with kidney dysfunction and sapogrelate treatment (30 mg/kg/day) for 4 weeks significantly improved these changes.



We found that sarpogrelate suppressed fibrinogen deposition, infiltration of F4/80-positive inflammatory cells, and the expression of PAI-1 in the kidney. Intravital microscope revealed that sarpogrelate significantly increased peritubular blood flow in the fibrotic area. In vitro experiments using mProx cells demonstrated that mProx cells incubated with TGF-β1 and 5-HT showed increased expression of PAI-1; sarpogrelate significantly reduced it.

Conclusions: Sarpogrelate contributes to prevent tubulointerstitial fibrosis by multiple pathways; suppressing infiltration of inflammatory cells and fibrin deposition, maintaining peritubular capillary blood flow, and reducing expression of PAI-1.

Funding: Government Support - Non-U.S.

TH-PO1015

Glucosamine Hydrochloride Protects against Unilateral Ureteral Obstruction-Induced Renal Fibrosis by Attenuating TGF-β Signaling Dong Ho Yang,[1] So-young Lee,[1] Yoon Hee Lee,[2] Hoon Jung.[3] *[1]Internal Medicine, Bungdang CHA Medical Center, Seongnam, Gyeonggi-do, Republic of Korea; [2]Pathology, Gangnam CHA Medical Center, Seoul, Republic of Korea; [3]Internal Medicine, Seoul Bukbu Geriatric Hospital, Seoul, Republic of Korea.*

Background: Increased TGF-β signaling is recognized as a key mediator of renal fibrosis, and considerable efforts have focused on targeting TGF-β signaling for antifibrotic therapy. The extracellular domain of the type II TGF-β receptor (TβRII) is modified by N-linked glycoslyation. Recently, we demonstrated that N-linked glycosylation of TβRII was required for its trafficking to the cell surface and increased ligand binding affinity (in press). Glucosamine hydrochloride (GS-HCl) is recognized to inhibit the translational N-glycosylation of specific proteins, including glucose transporter 1, apolipoprotein B-100, ICAM-1, EGFR and COX-2. Inhibition of N-glycosylation decreases secretion, increases protein turnover and/or reduces phosphorylation of affected proteins. Here, we demonstrate that GS-HCl inhibits TGF-β signaling by modifying the N-glycosylation status of TβRII.

Methods: Human proximal tubular epithelial cells (HKC-8) and human kidney proximal tubular cells (HK-2) were used for in vitro experiments. In addition, primary renal epithelial cells were isolated from mouse kidney. The mouse unilateral uretheral obstruction (UUO) model was used for in vivo experiments.

Results: GS-HCl inhibits N-glycosylation of TβRII; this results in inefficient trafficking of TβRII to the cell surface, thus preventing binding to its ligand TGF-β1. In murine UUO kidney cells and renal epithelial cells, GS-HCl exerts an antifibrotic effect by suppressing renal expression of α-smooth muscle actin, collagen I, and fibronectin. Ultimately, suppression of these proteins inhibits renal fibrosis. In addition, UUO- or TGF-β1-induced Smad3 phosphorylation, a downstream transcriptional mediator of TGF-β1 signaling, is significantly reduced in the presence of GS-HCl.

Conclusions: In conclusion, our study demonstrates that GS-HCl may be a promising therapeutic target against TGF-β1-induced renal fibrosis.

TH-PO1016

Peroxisome Proliferator-Activated Receptor Dual Agonist, AZ242, Ameliorates Renal Injury in Unilateral Ureteral Obstruction Model

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Background: Tubulointerstitial injuries are crucial histological alterations that predict the deterioration of renal function in chronic kidney disease. In the present study, we examined the therapeutic efficacies of AZ242, a novel peroxisome proliferator-activated receptor(PPAR) α/γ agonist on tubulointerstitial alterations induced by unilateral ureteral obstruction(UUO).

Methods: Male C57/BL6J mice were divided into three groups: sham-operated, vehicle-treated UUO, and AZ242-treated UUO group. After inducing UUO, AZ242 (1uM/kg/day) and vehicle were treated for 14 days. Animals were sacrificed and blood, urine from bladder and obstructed pelvis, and kidney tissue samples were collected for analyses.

Results: Vehicle-treated UUO group showed marked proteinuria and high blood pressure compared with sham-operated group. As compared with untreated mice, AZ242 treatment showed a little effect on blood pressure, but remarkably attenuated proteinuria in obstructed kidneys. Treatment with AZ242 significantly suppressed the increase of the renal mRNA expressions of Type I collagen, CTGF, MCP-1, TGF-β, PAI-1 in obstructed kidneys. In the histologic study, AZ242 treatment prominently ameliorated renal inflammation and tubulointerstitial fibrosis induced by UUO. These were further confirmed by Sirius red staining and immunohistochemistry with a marked inhibition of tubulointerstitial accumulation of α-SMA+ fibroblasts, F4/80+ macrophage, Type I and Type IV collagen in AZ242-treated mice. Treatment with AZ242 significantly decreased TGFβ1 and p-Smad3 protein expression in UUO kidneys. Additionally, urinary isoprostanes were significantly higher in UUO group and AZ242 treatment decreased urine isoprostane levels.

Conclusions: Taken together, AZ242 treatment attenuated proteinuria and tubulointerstitial fibrosis in UUO mice through reduction of TGFβ /Smad3 signaling and renal oxidative stress.

TH-PO1017

Ecto-5'-Nucleotidase (CD73) Promotes the Development of Renal Fibrosis

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Background: Renal fibrosis develops upon renal injury and is associated with progressive deterioration of kidney function. Ecto-5'-nucleotidase (CD73) catalyzes the extracellular formation of adenosine from AMP, and CD73 has been shown to be involved in the progression of fibrosis in the lung and liver. In this study we addressed the role of CD73 in renal fibrosis using CD73-/- mice.

Methods: To induce renal fibrosis, mice were subjected to unilateral ureter obstruction (UUO, 7d).

Results: UUO induced renal fibrosis in both genotypes, and this was accompanied by an increase in CD73 protein in CD73+/+ kidneys (196±35% of the contralateral kidney; n=6; p=.026). Compared to UUO kidneys from CD73+/+, UUO kidneys from CD73-/- showed lower expression levels of several fibrotic markers like alpha-SMA, fibronectin, TGF-beta, Ki67, MMP-2, MMP-13, MMP-14, monocytes chemotactic protein-1, and of the collagens Col 1a1, Col 1a2, Col 3a1 and Col 4a3, as determined by qRT-PCR, western blotting (p<.01 for CD73+/+ vs. CD73-/-), immunohistochemistry, and Sirius Red/Fast Green staining (p=.008). Extracellular matrix-generating cells were quantified by FACS analysis. In fibrotic kidneys from CD73-/- mice, the number of e.g. fibrocytes (collagen-producing monocytes) was decreased by 57±6% compared to fibrotic CD73+/+ kidneys (n=8; p=.004). The differentiation of CD11b+ monocytes into collagen-producing fibrocytes could be stimulated in vitro by co-culture with CD4+ T-lymphocytes. However, monocytes isolated from CD73-/- showed a reduced differentiation into fibrocytes after co-incubation with CD4+ T-lymphocytes, when compared to CD73+/+ monocytes (-21±1%; p=.01). The differentiation of CD11b+/CD73+/+ monocytes in vitro was similar in the presence of CD4+/CD73+/+ and CD4+/CD73-/- T-lymphocytes, suggesting a critical role of CD73 on monocytes rather than on T-lymphocytes.

Conclusions: In summary, CD73 is induced in the UUO model, and CD73 enhances fibrotic cell recruitment and the development of renal fibrosis. Consequently, CD73 may constitute a therapeutic target for the management of renal fibrosis.

TH-PO1018

Liver X Receptors α and β Differentially Regulate Fibroblast Activity and Renal Fibrosis

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Background: The nuclear receptors LXRα and β are transcriptional regulators of lipid metabolism and innate immunity. Specific natural LXR ligands are generated in inflammatory micromilieu.

Methods: The potential role of LXR signaling in development of renal chronic fibrosing inflammation was investigated.

Results: Early (day 1), subchronic and chronic (day 7 and 21) tubulointerstitial damage in unilateral ureteral obstruction was accentuated in both LXRα and β deficient (-/-) mice in comparison to WT. The number of infiltrating mononuclear cells (F4/80,CD3) remained unchanged. Corresponding to the high expression of LXRβ in tubular epithelial cells, LXRβ-/- kidneys showed a more prominent tubulointerstitial damage and an increase of interstitial myofibroblasts and collagen I/III (αSMA:WT:27.4±1,9; LXRα:35.2±1.5; LXRβ:47.5±1.9 cells/HPF; p<0.001 and p<0.05 LXRβ vs WT and LXRα, respectively, day 21). However, the administration of the LXR specific agonist GW3965 (GW) significantly reduced tubulointerstitial inflammation/fibrosis not only in WT but also in LXRα and β-/- mice, indicating the involvement of both isoforms in renal fibrogenesis (Collagen I/III:p<0.01 with vs without GW in WT, LXRα and LXRβ-/- kidneys, day 21). *In vitro*, without and with bFGF stimulation or when co-cultured with macrophages both LXRα and β-/- dermal fibroblasts showed higher proliferation than WT (25-30%); proliferation was reduced by GW. In addition, in co-culture, pretreatment with GW of macrophages or/and fibroblasts reduced their proinflammatory/profibrotic activity as evidenced by significantly reduced expression of collagen I, MCP-1 and MIP-1β.

Conclusions: The findings suggest that LXR signaling plays an important role in the regulation of fibroblast activation, production of extracellular matrix and development of renal interstitial fibrosis. LXR activation may have therapeutic potential for fibrotic kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO1019

Inhibition of TGF-β1-Receptor Post-Translational Core Fucosylation Attenuates Rat Renal Interstitial Fibrosis

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Background: The vigorous profibrotic cytokine transforming growth factor-beta1(TGF-β1) causes renal interstitial fibrosis (RIF). TGF-β1 can bind to fourteen known receptors, however, it is not clear which of these receptors are responsible for RIF. Studies have indicated that reducing the expression of TGF-β1 can protect against kidney injury; however, this also inhibits the other vital functions of TGF-β1, including cell growth and immune responses. Therefore, we chose the specific "profibrotic receptors" of TGF-β1, TGF-βRII and TGF-βRI (ALK5) as targets for interference. Our previous study showed that core fucosylation was essential for the proper function of both TGF-βRII and ALK5 in HK-2 cells in vitro. However, to date, the blockade of protein function in vivo by inhibiting core fucosylation has not yet been reported in kidney disease.

Methods: Rats in UUO group underwent left unilateral ureteral ligation. We designed and synthesized fluorescently labeled FUT8shRNA adenovirus(Ad-FUT8shRNA) and injected intravenously into the rat tail vein, as 1,6-αFucosyltransferase(FUT8) is known to catalyze core fucosylation. In all experiments, control rats were injected with Ad-GFP.

Results: The expression of activin receptor-like kinase-5 (ALK-5), ALK-7 and transforming growth factor-beta receptor II (TGF-βRII) mRNA significantly increased, and ALK-6 mRNA significantly decreased, in the kidneys of rats with UUO. Core fucosylation plays a crucial role in profibrotic function of the TGF-β1 receptors, and blocking it with FUT8 small hairpin RNA successfully abolished the activation of TGF-β/Smad signaling and attenuated UUO-induced rat renal interstitial fibrosis.

Conclusions: This study demonstrates that inhibition of TGF-βRII and ALK5 core fucosylation successfully suppressed the profibrotic function of these key TGF-β1 receptors, inhibited activation of TGF-β/Smad signaling and prevented rat renal interstitial fibrosis in the UUO kidney. Our results suggest the posttranslational modification of key proteins by core fucosylation could provide an attractive novel approach for treatment of renal fibrotic disease by preventing matrix deposition and tubular EMT.

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TH-PO1020

Nuclear Factor Erythroid Protein 2 (NF-E2) Is a Negative Regulator of Renal Tubular Fibrosis

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Background: TGF-b induced Hsp27 mediates renal fibrosis yet the exact mechanisms are unknown. Hsp27 promotes proteasomal protein degradation and NF-E2/Hsp27 associate in human renal proximal tubular cells (HK-11). We hypothesized TGF-b induced Hsp27 regulates renal fibrosis by regulating NF-E2 expression.

Methods: HK-11 cells were cultured in 10 ng/ml TGF-b or MG132 (proteasome inhibitor) for 24 hrs and immunoblotted with various protein markers. NF-E2 over-expression on TGF-b stimulated CTGF and fibronectin expression was examined. Association of Hsp27/NF-E2 was examined in control and TGF-b treated cell lysates by performing anti-Hsp27 and anti-NF-E2 immunoprecipitation and immunoblotting techiques. NF-E2 expression was examined by immunohistochemistry (IHC) and immunoblot analysis of tissue sections and tissue homogenates from control FVB, and 1,3, and 6 months male STZ-diabetic mice. NF-E2 expression was examined by IHC in control FVB, 6 months OVE26 mice and 3 months OVE26 mice treated with 10 micro g/Kg MG132 for additional 3 months.

Results: TGF-b markedly decreased NF-E2 expression concurrent with increased CTGF and fibronectin expression in HK-11 cells compared to control. Akt phosphorylation was decreased, while pp38 MAPK and pHsp27 levels were markedly increased by TGF-b treatment of HK-1 cells. NF-E2 over-expression inhibited TGF-b induced CTGF and fibronectin expression in HK-11 cells. Additionally, MG132 treatment prevented TGF-b induced NF-E2 degradation and blocked CTGF and fibronectin expression. TGF-b treatment of HK-11 cells enhanced Hsp27/NF-E2 association compared to control. Furthermore, NF-E2 expression was markedly decreased in 6 month STZ mice as documented by western blotting. IHC analysis documented luminal NF-E2 staining in control FVB mice while a punctate pattern was detected in STZ and OVE26 diabetic mice. MG132 treated mice demonstrated a more luminal NF-E2 staining similar to controls.

Conclusions: These results suggest that TGF-b treatment promotes CTGF and fibronectin expression by promoting NF-E2 degradation at the proteasome possibly by binding to Hsp27.

Funding: Other NIH Support - NIAID

TH-PO1021

Functional Role of Serine-204 in the Linker Region of Smad3

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Background: TGF-β/Smad3-mediated fibrogenesis is regulated through complex interactions among signaling pathways. The Smad3 linker region (LR) contains several phosphoacceptor sites (Thr179, Ser204, Ser208 and Ser213) differentially targeted by multiple kinases. We have shown that ERK MAP kinase is required for optimal collagen-I reporter activity; and that an intact Ser204 (S204) is required for collagen-I promoter activation by Smad3. MEK/ERK inhibition (PD98059) blocks both TGF-β-induced S204 phosphorylation (ASN 2011) and collagen induction. Here, we evaluated potential molecular roles for S204.

Methods: Immunoprecipitation was performed to identify Smad3-binding proteins and test if the S204 phospho-site is important for such binding. Smad3-null mouse embryonic fibroblasts were reconstituted with HA-tagged wild-type (WT) or S204A mutant Smad3 constructs. After treatment with vehicle or TGF-β (2ng/ml, 1h) cell lysates were subjected to anti-HA immunoprecipitation for Smad3-binding partners.

Results: Overexpressed WT and S204A Smad3 mutants interacted with ERK, AKT, STAT1 and p300. Surprisingly, all interactions were enhanced by the S204A mutation. The interactions of both Stat1 and p300 with Smad3 (WT and the S204A site mutant) were enhanced by TGF-β. In contrast, TGF-β supressed the binding of AKT with either Smad3 construct.

Conclusions: ERK-Smad3 interaction supports our hypothesis that ERK targets the Smad3LR. The ability of the S204A mutant to bind more ERK than WT-Smad3 suggests that only a transient ERK-Smad3 interaction is needed to support the collagen response, and raises the notion that S204 phosphorylation may be needed to disrupt the interaction to allow the collagen response. AKT is known to counteract TGF-β signaling by directly binding cytoplasmic Smad3. The S204A mutation intensifies this interaction, consistent with our model in which S204 phosphorylation enhances the collagen response to TGF-β. The role of STAT1 as an enhancer or inhibitor of the response remains to be determined. Together, these data suggest potential mechanisms by which S204 phosphorylation of Smad3 may modulate the fibrogenic response to TGF-β.

Funding: NIDDK Support

TH-PO1022

Wnt4 Is Expressed in Proliferating Myofibroblasts during Kidney Fibrosis

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Background: A critical role for Wnt4 during nephrogenesis is well characterized, but the expression pattern and function for Wnt4 in the adult kidney are poorly defined. Since kidney injury recapitulates some aspects of renal development, we hypothesized that Wnt4 would be induced in dedifferentiated epithelia after injury as part of an epithelial to mesenchymal transition.

Methods: To test this hypothesis, we used Wnt4 reporter and conditional knockout models to define the cellular expression and fate of Wnt4+ cells in kidney injury. We employed the unilateral ischemia reperfusion injury model and the unilateral ureteral obstruction injury model. Wnt4 expression was also determined using immunologic detection and qPCR.

Results: Using a Wnt4-GFP knockin, we report that Wnt4 expression is restricted to papillary collecting duct principal cells and urothelium in normal kidney. In two models of chronic kidney injury Wnt4 is upregulated in medullary myofibroblasts, but not cortical myofibroblasts or epithelium. Exogenous Wnt4 drove myofibroblast differentiation of a pericyte cell line, suggesting that Wnt4 may regulate pericyte to myofibroblast transition. To test this possibility we performed genetic fate mapping of Wnt4+ cells using a Wnt4-CreERt2 knockin and also knocked Wnt4 out of FoxD1+ stromal cells using a Wnt4 conditional allele. Fate mapping showed that Wnt4+ medullary myofibroblasts proliferate during fibrosis but do not migrate out of the medulla. We successfully deleted Wnt4 in interstitial cells but this did not reduce myofibroblast proliferation, cell number, or fibrotic gene expression.

Conclusions: Wnt4 is significantly increased in medullary myofibroblasts during fibrosis. These myofibroblasts do not migrate into the cortex and are distinct from cortical myofibroblasts based on Wnt4 expression. Although kidney injury/repair shares certain signaling pathways with nephrogenesis it is not simply a recapitulation of development and must be understood independently.

Funding: NIDDK Support, Private Foundation Support

TH-PO1023

VEGF Overexpression Inhibit TGF-β1 Induced EMT and miR192 Expression of HKC Cells through PI3K/Akt Signal Pathway

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Background: TGF-β1-induced epithelial-mesenchymal transition (EMT) is one of the important processes of renal fibrosis.This study is undertaken to examine the effect of VEGF overexpression on miR-192 in TGF-β1-induced EMT of HKC cells.

Methods: Firstly, human kidney cell lines with stable over-expression of VEGF(HKC-SOEV cells)were successfully established. The normal HKC cells and HKC-SOEV cells were treated with TGFβ1 (5μg/l) or/and LY294002(20μmol/l) for 24H and 48H. LY294002 is a specific inhibitor for PI3K activity. E-cadherin and α-SMA were detected with western blot and laser scanning confocal microscope (LSCM). The expression of miR-192 was determined by real-time PCR. The proteins of P-PI3K, PI3K, P- Akt and Akt were measured with western blot.

Results: a-SMA in HKC-SOEV cells treated with TGF-β1 *was* significantly lower than that in HKC cells treated with TGF-β1 ($p<0.05$) and that in HKC-SOEV cells treated with TGF-β1 and LY294002 ($p<0.05$). E-cadherin in HKC-SOEV cells treated with TGF-β1 *was* significantly higher than that in HKC cells treated with TGF-β1 ($p<0.05$) and that in HKC-SOEV cells treated with TGF-β1 and LY294002 ($p<0.05$). miR-192 expression in HKC-SOEV cells treated with TGF-β1 for 48H was lower than that in HKC cells treated with TGF-β1 ($p<0.05$) and that in HKC-SOEV cells treated with TGF-β1 and LY294002 ($p<0.05$). P-PI3K/PI3K and P-Akt/Akt in HKC-SOEV cells treated with TGF-β1 *was* significantly higher than that in HKC cells treated withTGF-β1 ($p<0.05$) and that in HKC-SOEV cells treated with TGF-β1 and LY294002 ($p<0.05$).

Conclusions: The results demonstrated that overexpression of VEGF may downregulate TGFβ1-induced expression of miR-192 in EMT through PI3K/Akt signal pathway in HKC cells.

Funding: Government Support - Non-U.S.

TH-PO1024

Differential Role of Endoplasmic Reticulum Stress on Epithelial-to-Mesenchymal Transition (EMT) & Apoptosis in Human Peritoneal Mesothelial Cells (HPMC)

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Background: Endoplasmic reticulum (ER) stress is known to be implicated in both apoptosis and EMT of epithelial cells from lung and kidney. The aims of this study were to investigate the role of preconditioning of ER stress as well as ER stress per se in EMT of HPMC. We characterized the pattern of ER stress-induced EMT and apoptosis with an elucidation of mechanisms of protective effect of ER stress preconditioning on TGF-β-induced EMT.

Methods: EMT was evaluated by morphological changes of HPMCs and the expressions of E-cadherin and α-smooth muscle actin after treatment with ER stress inducer tunicamycin (TM) or thapsigargin (TG). Apoptosis was assessed by FACscan. Effect of pretreatment TM (0.01ng/ml) or TG (0.01nM) on TGF-β (1ng/ml)-induced EMT was also evaluated. Mechanisms suggested for peritoneal EMT such as phosphorylation of Smad2/3,snail and nuclear translocation of β-catenin were investigated by WB and ICC. Expression and deglycosylation of TGF-β receptor I and II were studied by WB with IP and lectin.

Results: Lower concentration of TM or TG per se induced a reversible EMT of HPMC at 6 hours with an activation of Smad2/3 and snail whereas prolonged exposure to TM or TG for 48 hours resulted in an apoptosis and irreversible EMT. However, higher concentration of TM or TG induced an apoptosis at 6 hours. Interestingly, preconditioning with low concentration of TMor TG for 6 hours protected HPMC from EMT with an amelioration of Smad2/3 phosphorylation and nuclear translocation of β-catenin in TGF-β-stimulated HPMC. Protective effect of ER stress pre-conditioning on TGF-β-induced EMT was also associated with deglycosylation of TGF-β receptor I and II.

Conclusions: ER stress per se induced EMT and apoptosis in a dose- and time-dependent manner, however adequate preconditioning with low concentration of ER stress inducer for short period of time protected cells from TGF-β-induced EMT via an induction of deglycosylation of TGF-β receptors which blocked the downstream signalingin HPMC exposed to TGF-β. These findings suggest the potential therapeutic implication of ER stress preconditioning in peritoneal fibrosis.

Funding: Government Support - Non-U.S.

TH-PO1025

HIF-TGF-β Interaction in Mesenchymal Cell Type-I Collagen Accumulation Christian Hanna, Susan C. Hubchak, H. William Schnaper. *Pediatrics, Kidney Diseases, Lurie Children's Hospital, Northwestern University, Chicago, IL.*

Background: Cellular adaptation to hypoxia is mediated by hypoxia-inducible factors (HIFs), transcription factors consisting of an oxygen-sensitive α-subunit binding a β-subunit to promote the expression of pro-survival genes. Previously, we showed that transforming growth factor (TGF)-β, a central profibrotic cytokine in progressive kidney disease, increases HIF-1α expression in normoxic as well as hypoxic renal tubular epithelial cells, and promotes fibrosis through functional cooperation with TGF-β. It is unclear whether this interaction depends upon tissue hypoxia. Models of kidney injury leading to glomerular fibrosis may involve less hypoxia than models of renal tubulointerstitial injury, permitting potential evaluation of the role of HIFs in normoxic renal fibrogenesis.

Methods: In normoxia, HIF-1α/2α protein expression was analyzed by western blot in human mesangial cells (HMC) stimulated with TGF-β1, pretreated with vehicle, phosphatidylinositol-3-kinase (PI3K) inhibitor LY-294002 (LY) or the TGF-β1 receptor (TβR1) inhibitor, SB-431542 (SB). To determine the effect on collagen I transcriptional activity, HMC were transfected with COL1A2 or HRE-luciferase reporter constructs, along with empty vectors, mutant HIF-1α constructs, or HIF-1α/2α siRNA before stimulation with TGF-β1. Transfection efficiency was corrected with co-transfected β-Gal.

Results: TGF-β stimulated a 3-fold increase in normoxic HIF-1α/2α expression by 4-6 h. SB blocked TGF-β stimulated HIF-1α/2α expression, indicating that the effect is TGF-β receptor dependent. LY reduced basal and TGF-β-stimulated HIF-1α/2α protein expression, implicating PI3K in normoxic HIF expression. TGF-β induced a 2-fold increase in collagen promoter activity. Expressing a non-degradable HIF-1α increased basal collagen promoter activity by 50%. TGF-β promoter stimulation was increased 4-fold. HIF-1α or -2α siRNA decreased basal and TGF-β-stimulated COL1A2 promoter activity.

Conclusions: HIF-1α and HIF-2α play a significant role in normoxic HMC collagen expression and support our model of a role for TGF-β/Smad3 and HIFs in normoxic kidney fibrogenesis via TβR1 kinase and PI3K pathways.

Funding: NIDDK Support

TH-PO1026

Oncostatin M Inhibits TGF-β1-Induced CTGF Expression via Stat3 in HK-2 Cells Rita Sarkozi, Viktoria Maria Haller, Markus Pirklbauer, Gert J. Mayer, Herbert Schramek. *Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Innsbruck, Austria.*

Background: In kidneys, increasing evidence exists that matricellular proteins are involved in the development of tubulointerstitial fibrogenesis and renal disease progression. Connective tissue growth factor (CTGF), for example, has been implicated in the development of tubulointerstitial lesions in human and experimental diabetic nephropathy. We have recently established oncostatin M (OSM) as a novel inhibitor of basal and TGF-ß1-induced CTGF expression in proximal tubular human kidney-2 (HK-2) cells. In the present study we investigated the molecular mechanism of OSMs inhibitory effect on CTGF expression.

Methods: Cell culture, Western blot, real-time PCR, siRNA transfection.

Results: In HK-2 cells, induction of CTGF mRNA expression by TGF-ß1 (10 ng/ml) started after 15 min, reached a maximum after 2 h and remained elevated for at least 24 h. OSMs inhibitory effect on basal and TGF-ß1-induced CTGF mRNA expression was effective as early as 2 h after ligand administration and lasted for at least 24 h. In contrast to TGF-ß1, OSM (10 ng/ml) led to a rapid phosphorylation of both Stat1 and Stat3, which lasted for at least 6 h and 48 h, respectively. siRNA-mediated knockdown of Stat1 or Stat3 in HK-2 cells resulted in almost complete suppression of the respective mRNA and protein levels. These siRNA treatments did not interfere with TGF-β1-stimulated phosphorylation of Smad2/3 but blocked OSM-induced phosphorylation of the respective Stat isoforms after 1 h of stimulation. Silencing of Stat1 as well as Stat3 gene expression attenuated basal CTGF mRNA expression, suggesting that both Stat1 as well as Stat3 play a role in the regulation of basal CTGF mRNA levels in HK-2 cells. More importantly, siRNA-mediated silencing of Stat3 but not Stat1 abolished the inhibitory effect of OSM on TGF-β1-induced CTGF mRNA expression.

Conclusions: In conclusion, OSM represents a novel and potent inhibitor of TGF-β1-induced CTGF mRNA expression in proximal tubular HK-2 cells. This inhibitory effect is mainly driven by OSM receptor-stimulated Stat3 signaling.

TH-PO1027

Alteration of Hypoxia-Associated Gene Transcripts in Acquired Nephropathies Maja Lindenmeyer,[1,2] David Hoogewijs,[2] Roland H. Wenger,[2] Clemens D. Cohen.[1,2] *[1]Nephrology, University Hospital; [2]Physiology, University, Zurich, Switzerland.*

Background: Most chronic kidney diseases (CKD) are initiated as glomerular damage with loss of glomerular capillaries. The pathogenesis of the glomerular insult can be manifold. The best morphologic indicator of disease progression and development of end-stage renal disease, however, is interstitial fibrosis accompanied by a reduction of capillary density. As hypoxia - a potential consequence of the capillary rarefaction - has been associated with fibrosis the question arises whether renal cells indeed face hypoxia in CKD and respond with a transcriptional program which could lead to progression of renal disease.

Methods: Gene expression profiles from human glomeruli and tubulointerstitium were obtained from more than 160 renal biopsies from patients with different CKD stages using Affymetrix arrays. Proximal tubular cells and podocytes with stable HIF1a and/or HIF2a suppression were generated using a lentiviral approach. Protein levels were studied by Western Blot and immunohistochemistry. Results were validated using qPCR.

Results: Expression of hypoxia-associated genes was assessed in genome-wide expression profiles. From a total of 84 established HIF-target genes 27 correlated with renal function (eGFR) in the tubulointerstitium and 22 in glomerular samples, respectively. These correlations were both positive and negative and in part compartment-specific. Celltype-specific response to hypoxia and the relevance of given hypoxia-induced transcription factors (HIFs) on HIF-target genes were tested by qPCR in the knock-down derivates and revealed specific HIF1/HIF2-dependencies in the different cell lines. To validate the results on protein level we are currently establishing immunohistochemistry of HIF-target genes in human biopsies from patients with a wide range of renal function.

Conclusions: Our gene expression studies do not indicate an over-all hypoxic milieu in acquired kidney diseases. However, the data clearly point to compartment- and celltype-specific dysregulation of hypoxia-associated gene transcripts in CKD. Elucidation of the mechanisms involved may help to understand the pathogenesis of anemia in CKD, interstitial fibrosis, and renal failure.

Funding: Government Support - Non-U.S.

TH-PO1028

Polycystin-1 (PC-1), Ciliary, and Focal Adhesion (FA) Abnormalities Associated with Fibrotic Changes in Autosomal Dominant Polycystic Kidney Disease (ADPKD) Fibroblasts Siobhan M. Moyes, Jill T. Norman, Patricia D. Wilson. *Centre for Nephrology, UCL, London, United Kingdom.*

Background: ADPKD is a very common, monogenic disease where aberrant PC-1 function leads to end-stage renal disease in ~50% of patients with a highly variable age of onset. As ADPKD cyst expansion is associated with variable degrees of pericystic fibrosis we hypothesize that alterations in ADPKD fibroblast function disrupt normal epithelial-fibroblast interactions and play an important role in renal functional decline. In ADPKD cystic epithelia, alterations in PC-1 function, found in primary cilia, cell-cell adherens junctions and cell-matrix FAs are well described, but little is known in ADPKD fibroblasts. Previously we reported a stage-related hyper-proliferative defect in human early-stage (pre-dialysis, E) and end-stage (ES) fibroblasts compared to normal human kidney (NHK) cells.

Methods: Here we used Western blot and immuno-localization techniques *in vivo* and *in vitro* to compare PC-1, fibrotic, ciliary and FA proteins.

Results: Fibrotic markers: interstitial collagen deposition, TGF-β and stress fiber-associated α-SMA increased markedly with disease-stage. PC-1 was detectable in all types of fibroblasts but ADPKD cells were characterized by loss of full-length (~460kD) and ~250kD protein fragments but increased abundance of multiple low molecular weight forms. However, down-regulation of the putative C-terminal (~30kD) fragment appeared to be associated with reduced nuclear localization of PC-1 in ADPKD fibroblasts. Cilia were detected in all fibroblasts, but decreased in length with ADPKD stage (NHK=5.8±1.5μm; E-ADPKD=4.3±0.6μm; ES-ADPKD=3.8±0.3μm). Primary cultures of NHK, E- and ES-ADPKD fibroblasts showed disease stage-related increases in matrix-adhesion and cell spreading associated with increased expression of FAK, paxillin and integrin-linked kinase and altered phosphorylation of these FA proteins; similar to changes seen *in vivo.*

Conclusions: We suggest that altered processing, cellular distribution and function of PC-1 in ADPKD fibroblasts is associated with ciliary and FA functional changes with deleterious impact on epithelial-fibroblast interactions and fibrotic changes, respectively.

TH-PO1029

Anti Phospholipase A2 Receptor Antibodies Diminish Cell Adhesion by Interfering with the Interaction of the Receptor to Collagen Type IV Andrej Skoberne,[1] Astrid Behnert,[2] Kirstin Worthmann,[2] Beina Teng,[2] Hermann G. Haller,[2] Mario Schiffer.[2] *[1]Department of Nephrology, University Medical Centre Ljubljana, Ljubljana, Slovenia; [2]Clinic for Nephrology, Hannover Medical School, Hannover, Germany.*

Background: Recently, antibodies against the phospholipase A2 receptor (PLA2R) have been discovered in patients with primary membranous nephropathy (MN). As of yet, no clear pathogenic role for these antibodies has been established. Fibronectin type II (FNII) domain of the rabbit PLA2R can bind to collagen. The aim of our study was to establish a possible role of human PLA2R in cell attachment to collagen and to establish whether PLA2R antibodies interfere with cell adhesion.

Methods: We constructed a recombinant human PLA2R with a GFP tag. Cell adhesion assays were done in duplicates or triplicates in a 24 well plate coated with human collagen type IV, using wild type and mock transfected HEK cells. During cell incubation we added a commercial PLA2R antibody (cPLA2R) or different patient samples with or without PLA2R antibodies. Cells were counted per power field at a magnitude of 200x, using a DAPI stain.

Results: Attachment of wild type HEK cells was significantly higher than mock transfected cells (mean 316.3 vs. 48.3 cells/power field, p 0.001). Samples with added cPLA2R to wild type cells showed significantly lower cell adhesion as compared to controls (mean 184 vs. 321.2 cells/power field, p 0.019). There was a trend toward a lower cell attachment in patient samples containing PLA2R antibodies as compared to controls. There was no significant difference in cell attachment of wild type cells that had serum from patients without the PLA2R antibodies added to the well as compared to controls.

Conclusions: Our study shows that human PLA2R enhances the ability of HEK cells to attach to collagen IV. Anti PLA2R antibodies diminish cell attachment, possibly by interfering with the PLA2R-collagen interaction, which might have a pathogenic role in MN.

Funding: Government Support - Non-U.S.

TH-PO1030

Matrix Composition Is Important for IgA Nephropathy to Develop Kerstin Ebefors,[1] Peidi Liu,[1] Johannes Elvin,[2] Borje Haraldsson,[2] Jenny C. Nystrom.[1] *[1]Neuroscience and Physiology, University of Gothenburg, Sweden; [2]Molecular and Clinical Medicine, University of Gothenburg, Sweden.*

Background: Depositions of undergalactosylated IgA (uIgA) in the mesangium are one of the key findings in IgA nephropathy (IgAN) together with matrix expansion and mesangial proliferation. However, uIgA can be found in asymptomatic individuals as well. Although many have tried to find a receptor for the IgA on the mesangial cells, there is still no clear answer to how the IgA binds to the cell and how this leads to loss of kidney function. We hypothesize that patients with IgAN have an altered matrix composition which makes the uIgA able to bind to the matrix and cause IgAN.

Methods: To investigate the matrix composition in IgAN, two protocols were used. Firstly, we microdissected biopsies from patients with IgAN (n=14) and kidney donors (n=23). Global gene expression of glomeruli was investigated with focus on matrix-associated genes. Selected genes were analyzed with Q-PCR from another set of patients with IgAN (n=10) and kidney donors (n=10). Secondly, we developed a unique method for culturing mesangial cells from patients with IgAN and healthy controls. These cells were treated with purified IgA1 and the expression of ECM genes was studied.

Results: Array analysis showed that patients with IgAN have increased expression of several ECM genes. The ECM-receptor interaction pathway was the most up-regulated pathway. Q-PCR analysis showed similar results as the array and validated the data. Mesangial cells cultured from patients with IgAN had changed basal gene expression of several ECM genes including: COL1A1, DCN and SDC1 compared to control cells. Treatment of the cells with purified IgA showed a more significant response in the cells from patients compared to control. The difference in the ECM composition may be necessary for the IgA depositions to bind and to be pathological.

Conclusions: These experiments show that the gene expression of matrix genes is affected in IgAN, both on a systemic and cell-specific level, and strengthen the hypothesis that the matrix composition is important for IgAN to develop and may differ between individuals.

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TH-PO1031

Reversibility of AngiotensinII-Induced Early Markers of Renal Damage Anne-Roos Sophie Frenay, Miriam Boersema, Saleh Yazdani, Anne Marijn van der Graaf, Inge Hamming, Femke Waanders, Jacob van den Born, Ruud A. Bank, Gerjan Navis, Harry Van Goor. *University Medical Center Groningen.*

Background: Elucidating the dynamics of renal interstitial damage can provide exciting tools for treatment. We studied whether early interstitial changes induced by AngII are reversible after cessation of AngII. Since salt intake can modify renal fibrosis, rats were given various salt diets after cessation of AngII.

Methods: Rats were infused with AngII (435 ng/kg/min) or saline (VEH) via osmotic pumps (i.p.). At 3 wks pumps were removed and biopsies were taken. Rats were randomized to 3 salt diets (0.05% NaCl (LS), 0.4% NaCl (NS) or 4.0% NaCl (HS)) for 8 wks (n=10/group). VEH rats (n=10) received NS. SBP, creatinine (Cr) and proteinuria (Uprot) were determined. Kidneys were studied for macrophages and fibrosis (α-SMA, collagen I, III), tubular damage (KIM-1) and lymphangiogenesis (LA, Podoplanin).

Results: Compared to VEH, AngII induced hypertension (199 ± 5 vs 146 ± 3 mmHg in VEH), Uprot (51 ± 6 vs 15 ± 2 mg/24hr), raise in plasma Cr (31 ± 2 vs 20 ± 1 μmol/l), inflammation (macs (8.0 ± 0.7 vs 1.3 ± 0.2)), interstitial fibrosis (α-SMA mRNA (10.7 ± 0.9 vs 3.7 ± 0.7) and -protein (169.2 ± 14.0 vs 20.9 ± 2.0); collagen I mRNA (5.7 ± 0.8 vs 1.4 ± 0.2); collagen III mRNA (17 ± 2.7 vs 3.5 ± 0.6) and -protein (169.4 ± 11.3 vs 80.9 ± 13.8)), tubular damage (KIM-1 mRNA (20 ± 4 vs 1.0 ± 0.8) and -protein (0.5 ± 0.1 vs 0.1 ± 0.0)) and LA (Podoplanin (4.4 ± 0.3 vs 2.2 ± 0.4)); all $p<0.01$. After cessation of AngII, SBP and plasma Cr returned to control values in all groups. Uprot returned to control values in LS and NS, but not in HS. Interstitial macrophages, epithelial damage and LA were reduced to control values in all groups ($p<0.01$). Prefibrotic α-SMA protein reduced 10-fold ($p<0.01$) in all groups. Interstitial collagens reduced to control levels at mRNA level ($p<0.01$), but not at protein level.

Conclusions: Clinical and structural early changes induced by AngII are largely reversible over time, depending on sodium consumption. Remaining delicate fibrils of degradation resistant collagen may predispose for future vulnerability to renal events.

TH-PO1032

Hypoxia-Inducible Factor-α Isoforms Are Differentially Regulated in Glomerular Cells, and Modulate Fibrogenesis in Podocyte-Injury Mouse Models Tomoko Hayashida, H. William Schnaper, Susan C. Hubchak, Christian Hanna, Xiaoyan Liang. *Pediatrics, Northwestern University, Chicago, IL.*

Background: Hypoxia-inducible factors (HIF) also have physiological functions in normoxia. We reported that transforming growth factor (TGF)-β upregulates HIF-1α expression in normoxic kidney cell culture and overexpression of non-degradable HIF-1α enhances TGF-β-induced COL1A2 promoter activity (Basu, 2011). Here, we evaluate HIFα isoform expression and their roles in mouse models of kidney fibrosis.

Methods: Human mesangial cells were treated with TGF-β1 in normoxia or hypoxia (1.5%) for 24 hrs to assess HIF target gene induction. NEP25 mice develop FSGS-like lesion by podocyte targeting of a chimeric toxin, LMB2 (Matsuzaka, 2005). The day after LMB2 injection, some mice were subjected to hypoxia (10% O_2). Alternatively, mice with CD2AP haplosufficiency (Kim, 2003), were treated with L-mimosine, a HIF degradation inhibitor, to upregulate HIF in nomoxia. Mice were sacrificed 4 weeks later, and glomeruli were laser-captured from frozen sections for gene expression analyses.

Results: In culture, TGF-β increased normoxic VEGF-A mRNA, a universal HIF target, and was additive to the hypoxic increase (3.6x by TGF-β, 2.6x by hypoxia, 6.1x hypoxia+TGF-β, compared to normoxic control). HIF-1α-specific target genes (BNIP3, HMOX, PGK1) showed a trend similar to VEGF-A, but TGF-β did not further increase HIF-1 specific genes in hypoxia. Conversely, HIF-2α-specific genes (MMP9, PAI1, cyclin D1) were increased by TGF-β to a similar degree in both normoxia and hypoxia, while hypoxia alone did not significantly affect their expression. HIF upregulation by either ambient hypoxia or PHD inhibition aggravated glomerular sclerosis, determined by Jones staining, in NEP25 and CD2AP(+/-) models, and glomerular COL1A2 mRNA expression was enhanced. VEGF-A and HIF2-target genes were increased in NEP25/LMB2 glomeruli even in normoxia housing, which hypoxia enhanced. Interestingly, HIF1-target genes were not significantly increased either in normoxic or hypoxic NEP25 glomeruli.

Conclusions: HIFα isoforms are differentially regulated in normoxia and hypoxia, and they may play different roles in chronic kidney fibrosis.

Funding: NIDDK Support

TH-PO1033

Overexpression of PGC-1α Inhibits Aldosterone-Induced Podocyte Phenotypic Changes and Detachment by Blocking Mitochondrial Dysfunction Aihua Zhang, Yanggang Yuan, Songming Huang, Guixia Ding, Min Su. *Nephrology, Nanjing Children's Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: A growing body of experimental and clinical literature show that podocyte number is a critical determinant for the development of glomerulosclerosis and that podocyte loss leads to progressive renal failure. It has shown that mitochondrial dysfunction (MtD) is associated with many types of kidney disease. This study investigated whether MtD is involved in the pathogenesis of podocyte loss and whether the transcriptional coactivator, peroxisome proliferator activated receptor-g coactivator 1α (PGC-1α), a major regulator of mitochondrial function, prevented podocyte phenotypic changes and detachment by blocking MtD.

Methods: The podocyte cell line MPC-5 was cultured in the presence or absence of aldosterone. The PGC-1α-transgenic (Tg) mice were established. Podocyte phenotypic changes were examined by the expression of podocyte markers nephrin and P-cadherin and mesenchymal cell markers α-smooth muscle actin, desmin and MMP9. MtD was evaluated by several independent parameters: reactive oxygen species (ROS) production; mitochondrial morphology; mitochondrial membrane potential, ATP levels, and mitochondrial DNA copy number.

Results: Aldo dose and time-dependently induced podocyte phenotypic changes, as evidenced by downregulation of nephrin and P-cadherin and upregulation of α-SMA, desmin and MMP9. Meanwhile, Aldo dose and time-dependently induced podocyte detachment, as evidenced by downregulation of α3-integrin and cell adhesion ability. Mitochondrial DNA depletion by ethidium bromide induced MtD, further promoting podocyte phenotypic changes and detachment. Furthermore, overexpression of PGC-1α prevented Aldo-induced MtD and inhibited podocyte phenotypic changes and detachment. Finally, PGC-1α transgenic mouse were resistant to Aldo infusion-induced podocyte loss and MtD.

Conclusions: These findings, which implicate a role for the MtD in podocyte phenotypic changes and detachment and suggest that PGC-1α improve mitochondrial function and prevent podocyte phenotypic changes and detachment, may guide us in therapeutic strategies for podocyte loss.

Funding: Government Support - Non-U.S.

TH-PO1034

Anti-dsDNA Antibodies Induce Epithelial-to-Mesenchymal Transition and Fibrotic Processes in Human Proximal Renal Tubular Epithelial Cells Shirli S.K. Ho, Susan Yung, Kwok Fan Cheung, Wan Wai Tse, Daniel Tak Mao Chan. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: Tubulo-interstitial pathology predicts renal prognosis. We investigated the role of anti-dsDNA antibodies in the pathogenesis of tubulo-interstitial pathology in lupus nephritis, focusing on proximal renal tubular epithelial cells (PTEC) and epithelial-to-mesenchymal transition (EMT).

Methods: Female NZBWF1/J mice at different stages of nephritis were sacrificed and kidneys harvested to assess the tubulo-interstitial changes over time. Growth-arrested primary human PTEC were cultured with human polyclonal anti-dsDNA antibodies or control IgG (10μg/ml for both) for periods up to 48h, and investigated for the synthesis of mediators of fibrosis and EMT.

Results: NZBWF1/J mice showed progressive tubular damage over time, as denoted by tubular atrophy, protein cast deposition, mononuclear cell infiltration and increasing tubulo-interstitial expression of fibronectin, collagen type I and fibroblast specific protein-1 (FSP-1). Anti-dsDNA antibodies significantly induced the synthesis of soluble and insoluble fibronectin in PTEC after 24h incubation ($P<0.01$ for both). Anti-dsDNA antibodies induced FSP-1 and β-catenin synthesis ($P<0.001$ for both), which was accompanied by increased ERK and PKC-α phosphorylation. Inhibition of ERK and PKC-α activation using specific inhibitors reduced anti-dsDNA antibody induced EMT markers and fibronectin synthesis, and preserved normal PTEC phenotype.

Conclusions: Our data demonstrate that anti-dsDNA antibodies contribute to tubulo-interstitial pathology in lupus nephritis via direct effects on PTEC which are mediated through ERK and PKC-α activation.

Funding: Government Support - Non-U.S.

TH-PO1035

CD154 Controls Matrix Metalloproteinase-9 Secretion in Human Podocytes
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Background: Glomerular basement membrane (GBM) matrix remodelling is observed in glomerulosclerosis. CD154, the CD40 ligand, is an inflammatory mediator expressed by activated platelets and controls matrix remodelling in endothelial cells through the synthesis of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs).

We have studied podocyte CD40 expression and whether the CD154/CD40 signalisation was involved in GBM matrix remodelling through the control of MMPs and TIMPs expression by human podocytes.

Methods: CD40 localization and CD40 messenger RNA (mRNA) or protein expression were assessed by immunocytolocalisation (IC) on kidney tissue sections and cells, reverse transcriptase (RT)-PCR and Western blot (WB) on human podocytes exposed to recombinant human CD154 (rhCD154).

The expression and/ or the function of MMPs (MMP-2 and -9) and TIMPs (TIMP-1 and -2) were determined by zymography, WB, ELISA and qRT-PCR.

Results: CD40 was expressed by podocytes as shown in kidney tissue sections (optical and electron microscopy). RT-PCR and WB confirmed CD40 mRNA and protein expression in human cultured podocytes. The CD40 signaling pathways were activated by rhCD154: MAP kinase, NFkB, IkB, STAT3 and JNK. Cell stimulation with rhCD154 significantly induced the pro-MMP-9 secretion and the expression of its mRNA by podocytes. No changes in the secretion and expression of MMP-2, TIMP-1 and TIMP-2 were observed. Platelet-derived CD154 induced an increase of MMP-9 mRNA expression and the secretion of pro-MMP-9 by podocytes.

Conclusions: CD40 is expressed by human podocytes. Its ligand CD154 induces MMP-9 expression by podocytes. In glomerular diseases such as in hypertensive glomerulosclerosis and lupus nephritis, platelet activation and an increase of plasma soluble CD154 are observed. The release of CD154 by activated platelets may play a role in the altered GBM matrix remodelling observed in these diseases through the upregulation of MMP-9 by podocytes.

TH-PO1036

Use of Splicing-Sensitive Fluorescent Reporters for Fibroblast Growth Factor Receptor-2 to Investigate Pathological Mechanisms in Kidney Fibrosis
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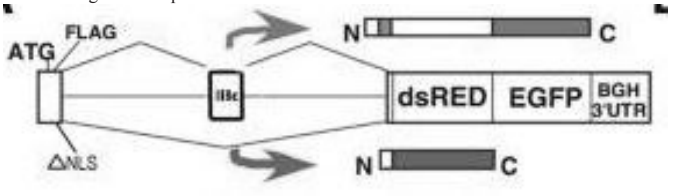
Background: Alternative splicing (AS) of fibroblast growth factor receptor-2 (FGFR2) is cell-type specific with exon 8 (IIIb) present in epithelial cells and exon 9 (IIIc) present in mesenchymal ones. Epithelial splicing regulatory proteins (ESRPs) have been recently reported as main regulators of the AS events associated with epithelial-mesenchymal transitions (EMT), including FGFR-2. We have previously reported the development of splicing-sensitive fluorescent reporters that are able to follow accurately EMT both *in vitro* in single cells and *in vivo* in the whole organism (Clin Exp Met (2008) 25:611, PNAS (2006) 103:14116 and figure). Kidney fibrosis is the main end-point of many renal diseases and a better understanding of this pathological process would be invaluable for development of novel targeted therapies.



Methods: We describe herein the use of FGFR2 SSFR as a tool to investigate molecular mechanisms of kidney fibrosis. Proliferating conditionally immortalized podocytes (PCIPs) and Denys-Drash podocytes (DDS) maintained at a permissive temperature of 33 degrees Celsius were stably transfected with the FGFR2 SSFR. To assess for the expression of ESRPs in podocytes western blotting was performed in PCIPs and DDS podoytes differentiated for 3, 7, 10 and 14 days.

Results: The cells showed GFP fluorescence as expected for proliferating podocytes, which display mesenchymal-like characteristics. Differentiation of podocytes towards an epithelial phenotype (obtained by shifting them to the non-permissive temperature of 37 degrees Celsius) increases RFP fluorescence. Indeed, as expected, ESRPs expression increased progressively during differentiation.

Conclusions: In conclusion, we have constructed a molecular tool useful for investigation of EMT and fibrosis in kidney pathology.

TH-PO1037

Role of Palladin in Activation of Tubular Epithelial Cells
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Background: Chronic hypertension is a leading cause of interstitial fibrosis and ESRD. A genetically engineered mouse line, designated RTG+, displays high renin levels and chronic hypertension. Palladin is an actin-binding protein with a role in both cytoskeletal organization and regulation of gene expression in vascular smooth muscle cells which has been implicated as an early marker of fibrosis in skin, but its role in the kidney has not yet been clarified. Previous studies suggest that palladin expression may be regulated by angiotensin II.

Methods: We used both immunoblot and IHC to explore the expression of palladin in RTG+ mice as well as human kidney. A proximal tubule cell culture model, LLCPK, was treated with TGF-β1 and examined by immunoblot.

Results: Immunoblot of RTG+ kidney lysate detected a ~2-fold increase in palladin compared to wild type litter mates. By IHC, we detected a focal increase in palladin levels in tubular epithelial cells of RTG+ mice, prior to development of overt fibrosis. Furthermore, IHC of human biopsy specimens revealed regions of fibrosis with atrophic and dilated tubules that displayed increased staining for palladin. In both human and mouse, palladin upregulation preceded the development of overt disease. LLCPK cells were treated with TGF-β1 to mimic the pathways that induce renal fibrosis. By day 1 after treatment, palladin levels were significantly increased, reaching a peak by day 4. When siRNA was used to knock down palladin in LLCPK cells, α-smooth muscle actin (α-SMA) was upregulated in control cells treated with TGF-β1 but not in knock down cells.

Conclusions: These results show a correlation between the early stages of tubular epithelial cell injury and an increase in palladin levels. More importantly, they suggest that palladin may play a critical role in regulating the expression of α-SMA and other genes associated with fibrosis, and implicate a palladin-dependent signaling pathway in this process and in the initiation of fibrosis.

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TH-PO1038

Results of the HERAKLES Trial: Efficacy and Safety of Three Different Treatment Regimens in De Novo Renal Transplant Patients
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Background: Comparison of safety and efficacy of 3 immunosuppressive regimens in first year after renal transplantation (Tx).

Methods: 802 patients (pts) were enrolled in 1-year, open-label, randomized, multi-center study. After Basiliximab induction all pts received Cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 mth post tx 499 pts were randomized 1:1:1 to a) standard EC-MPS treatment CsA (100-180 ng/ml) (n=166), b) convert to everolimus calcineurin inhibitor (CNI)-free (EVE; 5-10 ng/ml) and EC-MPS (n=171) c) convert to CNI-low regimen with EVE (3-8 ng/ml) and reduced CsA (50-75 ng/ml) (n=162).

Results: For the randomized study period (M 3-12) BPAR was reported in 13 (8.0%) standard, 16 (9.6%) CNI-free and in 13 (8.1%) CNI-low pts. Patient survival was 98.7% in the standard, 99.4% in the CNI-free and 98.8% in the CNI-low group. One graft loss was observed in either group. The number and proportion of pts discontinued prematurely were 39 (24%) in the standard, 61 (36%) in the CNI-free and 38 (24%) in the CNI-low group. Withdrawal because of an adverse event was the most frequent reason for discontinuation (61.5% standard, 72.1% CNI-free, 71.1% CNI-low).

	Standard N=166 [% of pts]	CNI-free N= 171 [% of pts]	CNI-low N= 162[% of pts]
Serious adverse events	61.4	60.5	55.9
Infections	82.5	87.8	84.5
Serious Infections	30.7	31.4	32.3
CMV	23.5	16.9	14.3
BKV	9.6	4.7	4.3
Anaemia	10.2	14.0	9.9
Leucopenia	17.5	25.0	18.0
Thrombocytopenia	4.2	4.7	3,1
Hyperlipidaemia	5.4	12.2	9.9
Hypertriglyceridaemia	3,0	1,7	5,0
Mouth ulceration	0.6	16.9	6.8
Diarrhoea	25.9	32.0	26.7
Oedema	25.9	16.3	27.3

Renal function cGFR (Nankivell) improved from baseline to Mo 12 by 5.6 mL/min/1.73m² in favor of the CNI-free regimen (63.0 ml/min standard; 68.6 ml/min CNI-free; 63.1 ml/min CNI-low).

Conclusions: The Herakles study showed that immunosuppressive regimens with low dose CNI exposure or without CNI reflect an efficacious and safe therapeutic approach.

Funding: Pharmaceutical Company Support - Novartis Pharma

TH-PO1039

4 Years Follow-Up of the ZEUS Trial: Better Renal Function after Calcineurin Inhibitor Withdrawal by Substitution with an Everolimus/ Enteric-Coated Mycophenolate Sodium Regimen in De Novo Renal Transplant Patients: Petra Reinke,[1] F. Lehner,[1] Oliver Witzke,[1] Wolfgang Arns,[1] Ute Eisenberger,[1] Eva-Maria Paulus,[2] Daniel Baeumer,[2] Christoph May,[2] Klemens Budde,[1] Claudia Sommerer.[1] *[1]ZEUS Study Group; [2]Novartis Pharma.*

Background: Efficacy and safety (from Tx to Mo 48) in de novo allograft kidney recipients after conversion to an Everolimus (EVE)/Enteric Enteric-Coated Mycophenolate Sodium (EC-MPS) regimen after Cyclosporine (CsA) withdrawal.

Methods: In this 12 Mo prospective, open-label, controlled, multi-center study renal allograft patients (pts) were switched at randomization (4.5 Mo post Tx) to either a immunosuppressive regimen consisting of EVE/EC-MPS or CsA/EC-MPS. After completion of the core study at Mo 12, pts were included in an observational follow up study.

Results: At Mo 4.5, 300 pts were randomized to either EVE/EC-MPS (n=155) or CsA/EC-MPS (n=145), 228 (76%) completed the Mo 48 visit. At baseline both groups had a similar renal function measured by cGFR (Nankivell) with an improvement in the ITT population at Mo 48 by 5.0 mL/min/1.73m in favor of the EVE/EC-MPS regimen (ITT: p=0.03) (62.5 ± 17.3 CsA vs. 67.5 ± 22.1 mL/min/1.73m EVE) compared to 10.6 mL at Mo 12. In comparison, all pts who remained on the assigned EVE/EC-MPS therapy (OT=on treatment) had a high improvement at Mo 48 of renal function (OT: 8.4 mL; 71.3 ± 17.8 (n=71) vs. 62.9 ± 17.3 mL, (n=79)) at Mo 48. The observed GFR change in the ITT population from randomization to Mo 48 was +2.9 [95%CI: -2.1, +7.9] for EVE/EC-MPS and -1.1 [-6.2, +3.9] mL/min/1.73m for CsA/EC-MPS pts. BPAR was reported in 22 (14.3%) EVE/EC-MPS-treated vs. 9 (6.2%) CsA/EC-MPS treated pts between M 4.5 and Mo 48 (p=0.02).Two deaths and 4 graft loss were observed in the EVE group vs. 3 deaths and 2 graft loss in the CsA/EC-MPS group. A similar rate of infections (20%) and hospitalisation EVE (21.9%) vs. CsA (26.9%) in both groups was reported.

Conclusions: Conversion to EVE/EC-MPS in *de novo* renal transplant patients after CNI withdrawal early after transplantation reflects a novel therapeutic approach which maintains better renal function over a period of 48 months without compromising efficacy and safety.

Funding: Pharmaceutical Company Support - Novartis Pharma

TH-PO1040

Results of the APOLLO Trial: Renal Function of an Everolimus Based Therapy after 3 Years of Calcineurin Inhibitor Withdrawal in Maintenance Renal Transplant Recipients Petra Reinke,[1] Claudia Sommerer,[1] Wolfgang Arns,[1] Hermann G. Haller,[1] Oliver Witzke,[1] Thomas Rath,[1] Christoph May,[2] Daniel Baeumer,[2] Eva-Maria Paulus,[2] Klemens Budde,[1] Barbara M. Suwelack.[1] *[1]The Apollo Study Group; [2]Novartis Pharma.*

Background: Assessment of renal function, safety and efficacy of an Everolimus (EVE) regimen after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney allograft recipients.

Methods: In an open-label, randomized, controlled, multi-center study 93 patients on a stable immunosuppressive therapy consisting of CNI, Enteric-Coated Mycophenolate Sodium (EC-MPS) with or without corticosteroids were randomized to either continue CNI treatment and EC-MPS or convert to an EVE/EC-MPS based regimen. After completion of the core study at M12, patients were included in a 36 month observational follow-up study.

Results: 93 pts with a mean time of 6.4 years since the most recent transplantation were randomized to either EVE/EC-MPS (n=46) or CNI/EC-MPS (n=47), 74 (79.6%) pts completed the 36 month visit. Trough levels were 98.1 ± 48.8 ng/ml in CsA, 5.4 ± 1.9 ng/ml in Tacrolimus and 6.4 ± 1.9 ng/ml in EVE treated pts. At Month 36 after randomization mean calculated GFR (Nankivell) was higher by 5.12 mL/min/1.73m^2 for the EVE compared to the CNI group (65.66±18.55 vs 60.54±15.49 mL/min/1.73m2; p=n.s.). EVE pts who remained on their assigned treatment (PP) had a better renal function (7.9 min/1.73m^2, 68.0 ± 19.9 (n=25) vs. 60.1 ± 14.7 mL/min/1.73m^2(n=31); p=0.08). The observed GFR change from conversion to month 36 was +5.2 [95% CI: -0.6;+10.9] for EVE/EC-MPS and 0.3 [95% CI: -4.7;+5.2] mL/min/1.73m^2 for CNI/EC-MPS pts. Three deaths and one graft loss were observed in the CNI/EC-MPS group, two death and one graft loss in the EVE/EC-MPS group and no BPAR was observed in either group. The number of patients with infections was 9 pts (19.6%) in the EVE vs. 8 pts (17.0%) in the CsA group. Four (8.5%) malignancies occurred in the CNI group compared to one (4.3%) case in the EVE group.

Conclusions: Late conversion to an EVE/EC-MPS treatment in maintenance renal transplant patients after CNI withdrawal is safe and associated with a tendency towards better renal function after 3 years.

Funding: Pharmaceutical Company Support - Novartis

TH-PO1041

Enteric-Coated Mycophenolate Sodium (EC-MPS) versus Mycophenolate Mofetil (MMF) in De Novo Kidney Transplant Patients: 4-Year Data from the Mycophenolic Acid Observational Renal Transplant (MORE) Registry L. Chan,[1] Fuad S. Shihab,[2] Oleh G. Pankewycz,[3] Cataldo Doria,[4] A. Wiland,[5] Kevin M. Mccague,[5] Anthony J. Langone.[6] *[1]University of Colorado, CO; [2]University of Utah, UT; [3]Buffalo General Hospital, NY; [4]Thomas Jefferson University Hospital, PA; [5]Novartis, NJ; [6]Vanderbilt University, TN.*

Background: Randomized trials of EC-MPS vs MMF are largely restricted to cyclosporine recipients. Mycophenolic acid (MPA) exposure, however, is higher with tacrolimus (TAC).

Methods: Data from the MORE Registry, a prospective, observational study of *de novo* adult renal transplant patients receiving MPA and managed according to local practice at 40 US sites, were interrogated to analyze effectiveness, tolerability and safety of EC-MPS and MMF in TAC-treated patients.

Results: 904 patients (616 EC-MPS, 288 MMF) were followed for up to 4 years. The two groups were similar other than fewer living donor grafts with EC-MPS vs MMF (39% vs 49%, p=0.04). The full recommended dose of MPA (i.e. 1.44g EC-MPS, 2.0g MMF) was maintained in more patients receiving EC-MPS vs MMF at month 1 (79% vs 72%, p=0.02), month 3 (68% vs 57%, p<0.01) and month 6 (53% vs 44%, p=0.03) but was similar thereafter. Mean MPA dose was higher in EC-MPS patients than MMF patients up to month 6 post-transplant. At 4 years, graft survival was 93% vs 95% in the EC-MPS vs MMF groups (log rank p=0.52, Kaplan-Meier estimates); patient survival was 96% vs 94% (p=0.28). Biopsy-proven acute rejection occurred in 14% EC-MPS and 10% MMF patients (p=0.20); mean(SD) serum creatinine was 1.5(1.1)mg/dL and 1.7(1.8)mg/dL, respectively (p=0.25). Adverse events were similar in the EC-MPS and MMF groups, including gastrointestinal complications (73% vs 75%, p=0.57).

Conclusions: In this real-life population, patients in the EC-MPS group received a significantly higher MPA dose vs the MMF group, and were more likely to remain on the full recommended MPA dose, up to month 6 post-transplant. Efficacy outcomes were similar with EC-MPS or MMF to 4 years, despite fewer living donor grafts in the EC-MPS group, and safety profiles were similar despite higher early EC-MPS dosing.

Funding: Pharmaceutical Company Support - Novartis

TH-PO1042

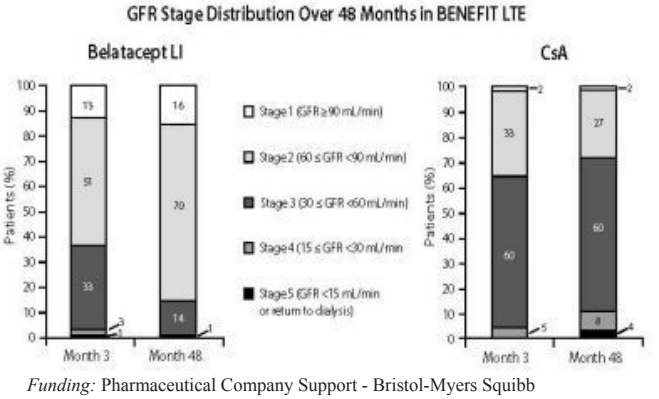
Likelihood of Improving or Sustaining Renal Function over Four Years with Belatacept or CsA: Insights from the BENEFIT Long-Term Extension Study K. Rice,[1] L. Chan,[2] Lionel Rostaing,[3] B. Bresnahan,[4] J.H. Helderman,[5] M.B. Harler,[6] F. Vincenti.[7] *[1]Baylor Univ Med Ctr, US; [2]Univ of CO; [3]Univ Hosp, Toulouse, FR; [4]Univ of WI; [5]Vanderbilt Univ, US; [6]BMS, US; [7]UCSF Transplant Svc, US.*

Background: A consistent safety profile and sustained renal function benefit over 4 years were observed in pts who entered the long-term extension (LTE) of BENEFIT. Changes in cGFR stage of pts in the BENEFIT LTE cohort are reported.

Methods: In BENEFIT, recipients of living or standard-criteria deceased donor kidneys were randomized to a more or less intensive (LI) regimen of belatacept or CsA. Pts who remained on assigned therapy after 3 yrs were eligible to enter the LTE. This posthoc analysis assessed shifts in cGFR staging between Months (Mos) 3 and 48 in the LTE population. cGFR stages were defined per the KDOQI classification of CKD stages. cGFR was imputed as 0 for death or graft loss. 138/166 belatacept LI and 109/136 CsA pts had data available at both Mos 3 and 48.

Results: GFR stage at Mo 3 and 48 in LI and CsA pts are shown in the Figure. Among pts in Stage 2 at Mo 3, GFR stage was sustained or improved through Mo 48 in 68 (96%) LI and 17 (47%) CsA pts. In Stage 3 pts, GFR stage from Mo 3–48 was sustained or improved in 44 (98%) LI and 58 (88%) CsA pts. At Mo 3, 3 LI and 5 CsA pts were in Stage 4; all pts (100%) sustained or improved their GFR stage through Mo 48. One LI pt who was in Stage 5 at Mo 3 improved to Stage 2 at Mo 48; there were no CsA pts who were in Stage 5 at Mo 3 and had cGFR measurements through Mo 48. Trends from Mo 12–48 were similar to those seen from Mo 3–48.

Conclusions: In the BENEFIT LTE cohort at 48 months, a higher percentage of belatacept-treated pts were in Stages 1 and 2 vs CsA pts. Belatacept pts were more likely than CsA pts to experience sustained or improved renal function over 4 yrs.



Funding: Pharmaceutical Company Support - Bristol-Myers Squibb

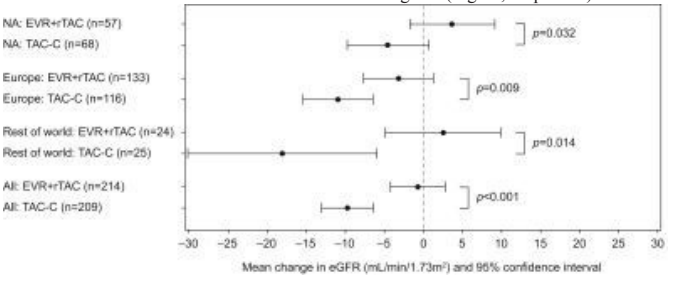
TH-PO1043

Renal-Sparing Effect of Early Everolimus-Facilitated Reduction of Tacrolimus in De Novo Liver Transplant Recipients: 12-Month Data from North America R. S. Brown,[1] B. Koneru,[2] M. A. Huang,[3] G. Junge,[4] A. Wiland,[5] J. M. Hexham,[5] G. Dong,[4] J. Fung.[6] *[1]Columbia U; [2]UMDNJ; [3]Henry Ford Hosp; [4]Novartis AG; [5]Novartis NJ; [6]Cleveland Clinic.*

Background: Study H2304, a large international RCT in de novo liver transplant recipients (LTxR), confirmed that use of early everolimus (EVR) facilitates reduction of tacrolimus (TAC) & significantly reduces TAC-related nephrotoxicity at Month (M) 12. Because of the disparity of preexisting renal dysfunction in LTxR, driven by higher MELD & creatinine at OLT in North America (NA), we report results of the NA subpopulation.

Methods: 24-M, randomized, multicenter, open-label study in 211 NA de novo LTxR comparing EVR (C0 3–8ng/mL)+reduced TAC (C0 3–5ng/mL; EVR+rTAC; n=65) or EVR (C0 6–10ng/mL)+TAC withdrawal (TAC-WD; n=68) to std TAC (C0 6–10ng/mL; TAC-C; n=78); all with steroids. LTxR were randomized 1:1:1 after a 30-day post-LTx run-in period with TAC±MMF. Primary endpoint was composite efficacy failure rate of treated biopsy proven acute rejection (tBPAR), graft loss, or death. Renal function from randomization (RDN) to M12 was based on eGFR (MDRD4).

Results: Enrollment in TAC-WD arm stopped early due to a higher rate of tBPAR. At RDN, mean eGFR (mL/min/1.73m2) was lower in NA vs Europe (EU)/rest of world (ROW); imbalance favoring TAC-C (EVR+rTAC: 69.3 vs 85.9/80.8; TAC-C: 76.4 vs 80.2/79.7); MELD scores were higher in NA vs other regions (EVR+rTAC: 22.8 vs 17.9/17.8; TAC-C: 22.6 vs 16.8/18.9). At M12, the composite efficacy failure rate was lower with EVR+rTAC vs TAC-C in NA (10.9% vs 13.1%) & overall (6.7% vs 9.7%). Mean change in eGFR from RDN to M12 favored EVR+rTAC vs TAC-C in all regions (Figure; all p<0.05).



Conclusions: As seen for the overall population, in patients from NA, early use of EVR at 1M allowed substantial TAC reduction resulting in improved eGFR vs TAC-C while maintaining excellent efficacy.

Funding: Pharmaceutical Company Support - Novartis Pharma AG

TH-PO1044

The HERAKLES Study: Improved Renal Function in an Everolimus-Based Calcineurin Inhibitor Free Regimen Compared to Standard Cyclosporine/ Mycophenolate and Low Cyclosporine/Everolimus Claudia Sommerer,[1] F. Lehner,[1] Martin G. Zeier,[1] Oliver Witzke,[1] Johannes Jacobi,[1] Klemens Budde,[1] Hans-Hellmut Neumayer,[1] Manfred J. Stangl,[1] Christoph May,[2] Daniel Baeumer,[2] Eva-Maria Paulus,[2] Volker Kliem,[1] Wolfgang Arns.[1] *[1]The Herakles Study Group; [2]Novartis Pharma, Nuremberg, Germany.*

Background: Renal function in 3 immunosuppressive regimens with different calcineurin inhibitor (CNI) exposure.

Methods: 802 patients (pts) were enrolled in this 1-year, prospective, open-label, randomized, multi-center study. After Basiliximab induction all pts received Cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 months post tx 499 pts were randomized 1:1:1 to a) standard CsA (100-180 ng/ml) with EC-MPS (n=166), b) convert to CNI-free regimen with everolimus (EVE; 5-10 ng/ml) and EC-MPS (n=171) c) CNI-low regimen with EVE (3-8 ng/ml) and reduced CsA (50-75 ng/ml) (n=162) pts continued on steroids.

Results: CsA trough level at M12, was 118.7 ± 33.3 ng/ml, for standard and 79.5 ± 35.2 ng/ml for CNI-low pts. EVE trough level was 6.8 ± 2.2 ng/ml in CNI-free and 6.1 ± 2.7 ng/ml in CNI low pts. Renal function was similar at Mo 3 post tx with improvement (p<0.0001) by 5.6 mL/min/1.73m [95% CI 2.9; 8.3] in favor of the CNI-free regimen at Mth 12 when compared to standard.

eGFR (Nankivell) [mL/min/1.73 m] Month 12

Treatment Group	N	LS-Mean (95%CI)	Difference vs. standard	p value vs. standard
Standard	159	63.0 (60.6;65.5)	-	-
CNI free	163	68.6 (66.1;71.1)	5.6 (2.9;8.3)	0.0001
CNI low	160	63.1(60.7;65.6)	0.1(-2.7;2.8)	0.9586

Results from ANCOVA model (ITT)

Results of renal function were confirmed by other formulas. 69.5% of CNI-free, 53.8% of CNI low and 54.0% of standard pts had an improvement in renal function. All three groups had a similar rejection rate (8.0% standard CNI, 9.6% CNI-free, 8,1% CNI-low) with an overall comparable safety profile data. Proteinuria was reported in 8.4%, 9.9% and 14.9% of pts (stand., CNI-free and CNI-low).

Conclusions: Results confirm reports of improved renal function after CsA withdrawal with EVE and EC-MPS. However, the profound reduction of CsA in combination with EVE did not result in better renal function compared to standard therapy with EC-MPS.

Funding: Pharmaceutical Company Support - Novartis Pharma

TH-PO1045

Central Fat Distribution Is a Risk Factor for Graft Failure, and Cardiovascular, and All-Cause Mortality in Renal Transplant Recipients Arjan J. Kwakernaak, Steef Jasper Sinkeler, Dorien M. Zelle, Stephan J.L. Bakker, Gerjan Navis. *Dept. of Nephrology, University Medical Center Groningen, Groningen, Netherlands.*

Background: A central body fat distribution is an independent determinant of increased long term renal risk in native kidney disease. Whether a central body fat distribution is a risk factor for renal allograft failure is unknown. In the current study, we therefore prospectively investigated the impact of a central body fat distribution, assessed by waist-to-hip ratio (WHR), on renal allograft failure, and in addition, on cardiovascular and all-cause mortality in renal transplant recipients (RTRs) ≥ 1 year post-transplantation.

Methods: We studied 498 RTRs (mean age 50±12 years, 56% male) with a functioning renal allograft at a median time of 6.0 [2.6 – 12.1] years post-transplantation. Median WHR was 0.97 [0.90-1.04].

Results: At baseline, a higher gender-specific WHR was associated with higher BMI, higher systolic blood pressure despite more antihypertensive medication, a more unfavorable lipid and glucose profile and higher protein excretion. After a median follow-up of 7.0 [6.2 – 7.5] years, 52 death-censored graft losses had occurred, 107 recipients died, with 54 deaths being cardiovascular in origin. In multivariable Cox regression analyses, WHR was associated with death-censored graft loss (HR=2.0 [1.4-2.8], P<0.001), cardiovascular (HR=1.5 [1.1-2.0], P=0.024) and all-cause mortality (HR=1.3 [1.0-1.6] P=0.034), independent of BMI and potential confounders.

Conclusions: In conclusion, a central body fat distribution, assessed by WHR, was a strong and independent predictor of allograft failure, and cardiovascular, and all-cause mortality in RTR late after kidney transplantation. Of note, this association was independent of BMI. Further research is needed to elucidate the mechanisms underlying the association between a central body fat distribution and renal allograft loss.

TH-PO1046

Interventions to Reduce Clinical Inertia in Cardiac Risk Factor Management in Renal Transplant Recipients Dhanashri Kohok, Silvi Shah, Rakesh Kumar, Gregory D. Gudleski, Rocco C. Venuto. *University at Buffalo, Buffalo, NY.*

Background: Cardiovascular disease (CVD) is the leading cause of death in renal transplant recipients (RTRs). Improvement in key risk factors such as hypertension (HTN), dyslipidemia and diabetes reduces the probability of CVD. Clinical inertia (CI) defined as "recognition of the problem, but failure to act" may hinder attaining Kidney Disease Improving Global Outcome (KDIGO) guideline goals. We assessed the effect of educational interventions (EI) in minimizing CI.

Methods: A total of 198 RTRs and 8 physicians enrolled in this interventional study. Knowledge of CVD and its risk factors was assessed in RTRs. Physicians were surveyed for limiting factors in achieving treatment goals. An EI comprised of a one on one session with RTRs and printed material was used to enlighten RTRs about goals for blood pressure (BP) <130/80mmHg, low-density lipoprotein (LDL) < 100mg/dL and hemoglobin A1C (HbA1C) < 7.5%. RTRs were given a card with targets titled 'I need to know' to encourage their participation. Physicians were given 'I should check' card as a reminder to check risk factor control. CI was measured as 'no action' or 'appropriate action' and compared pre and post intervention.

Results: Only 7.4% of RTRs knew that death is the most common cause of graft failure while 32.5% were aware that CVD is their most common cause of death. Physicians recognized non-compliance, time constraints and drug-interactions as limiting factors in achieving goals. Post intervention percentage of patients with 'no clinical action' for BP, LDL and HbA1C control decreased from 10.8% to 3.8% (p=0.02), 28.2% to 11.1% (p=0.008) and 10.3% to 4.5% (p=0.05) respectively while those with 'appropriate action' increased from 66.2% to 83.3% (p<0.001), 68.7% to 79.4% (p=0.008) and 85.1% to 93.2% (p=0.03) respectively.

Conclusions: Interventions to increase awareness about CVD in a post transplant clinic and to encourage active participation by both physicians and RTRs in the care plan seem effective in influencing behavior and overcoming clinical inertia. Sustained effects of such interventions on CI and clinical outcomes need further evaluation.

Funding: Government Support - Non-U.S.

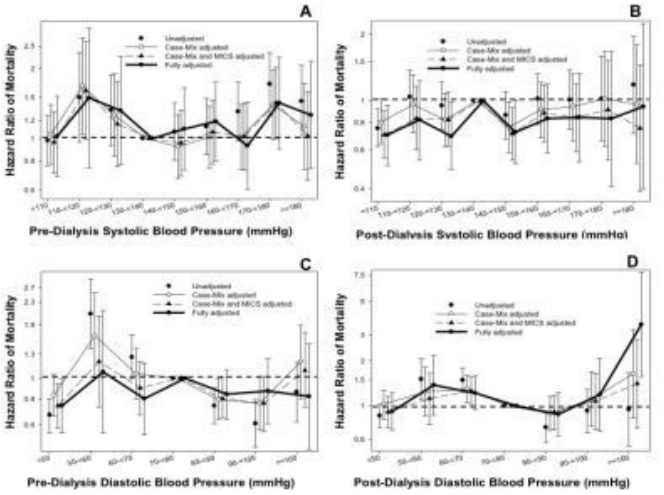
TH-PO1047

Examining the Association of Pre-Transplant Blood Pressure with Post-Transplant Outcomes Miklos Zsolt Molnar,[1] Joni L. Ricks,[1] John J. Sim,[2] Adam Remport,[3] Clarence E. Foster III,[4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center, LABioMed at Harbor-UCLA; [2]Kaiser Permanente; [3]Szent Imre Hospital; [4]University of California, Irvine; [5]University of Tennessee.*

Background: Previous observations in dialysis patients have demonstrated a U-shaped relationship between blood pressure (BP) and mortality. The associations between pre-transplant systolic(SBP) and diastolic BP(DBP) and post transplant outcomes are largely unknown.

Methods: Data of the Scientific Registry of Transplant Recipients were linked to the 5-year cohort of a large dialysis organization in the United States. All patients who received a kidney transplant during this period were identified and categorized groups according to pre-transplant SBP and DBP. Unadjusted and multivariable adjusted predictors of transplant outcomes were examined.

Results: The 13,881 patients were 47±14 years old and included 42% women. There was no association between pre-transplant SBP and mortality, except for a decreased risk in those with low post-dialysis SBP.



Compared to patients with pre-dialysis DBP 70-<80 mmHg, DBP<50mmHg showed a lower risk of death (HR:0.74,95%CI:0.55-0.99). Comparison in post-dialysis DBP groups revealed that patients with DBP≥100 mmHg had higher risk of death (HR:3.50,95%CI:1.57-7.84) compared to 70-<80 mmHg. In addition, the lowest pre-transplant BP's were associated with better graft survival. There was no association between pre-transplant BP and delayed graft function.

Conclusions: Low post-hemodialysis treatment session SBP and low pre-HD treatment DBP were associated with better post-transplant patient survival, whereas very high post-dialysis DBP was associated with higher risk of death after transplantation. Whether targeting different BP ranges during dialysis treatment has implications on post-transplant outcomes needs to be examined in additional studies.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

TH-PO1048

Validation of Pre-Transplant Risk Score for New Onset Diabetes Mellitus after Kidney Transplantation Harini A. Chakkera,[1] Asad Ayub,[2] Thomas A. Gonwa,[2] Yu-hui Chang,[1] E. Jennifer Weil,[3] William Knowler.[3] *[1]Mayo Clinic Arizona; [2]Mayo Clinic Jacksonville; [3]NIDDK- Phoenix.*

Background: New-onset diabetes mellitus after kidney transplantation (NODAT) has adverse clinical and economic implications. A risk score for NODAT could help identify patients for intervention studies. We published results from a single center study describing a pre-transplant predictive risk score for NODAT using 7 simple pre-transplant clinical and laboratory measurements. **Aim:** Validate the pre-transplant risk score in a second cohort.

Methods: All the methods and entry criteria were the same in both the initial and the validation model. The simple risk score was calculated as the sum of points from the 7 variables (age >50 years, BMI >30 kg/m2, fasting glucose>/=126 mg/dL and triglycerides>/=200 mg/dL, and family history of type-2 DM, planned corticosteroid therapy post-transplant, prescription for gout medicine) where each variable was given 1 point. Thus, a score for each patient ranged from 0 to 7.The summary scores were grouped as low (0 or 1), intermediate (2 or 3) or a high risk group (4, 5, 6 or 7). NODAT was defined by hemoglobin A1c ≥6.5%, fasting serum glucose ≥126 mg/dL, or prescribed therapy for diabetes.

Results: Validation cohort included 474 non-diabetic patients who underwent kidney transplantation (March 2001-July 2010). Incidence of NODAT = 27%.The prevalence of some risk factors (planned use of corticosteroids, BMI, fasting glucose, fasting triglycerides, and family history of diabetes) differed significantly between the 2 cohorts. Despite these differences in the 2 groups the predictive value of the score was similar in the 2 groups (Table below)

Comparison of prediction of NODAT by risk group

Risk category	% of patients who develop NODAT in initial sample	% of patients who develop NODAT in validation sample
Low risk	12	11
Intermediate Risk	29	29
High risk	56	51

Areas under receiver operating curves for the initial and validation cohorts were 0.70 and 0.66 (p=0.26) respectively.

Conclusions: A simple risk score computed from 7 pre-transplant variables was validated in a second cohort and can provide the basis to identify risk of NODAT for future clinical intervention studies.

TH-PO1049

Serum Uric Acid Is an Independent Predictor of New-Onset Diabetes after Transplantation (NOADT) in Living-Donor Kidney Transplantation Kentaro Tanaka,[1] Ken Sakai,[1] Masaki Muramatsu,[1] Yoji Hyodo,[1] Yasushi Ohashi,[1] Yoshihide Tanaka,[1] Takeshi Kawamura,[1] Seiichirou Shishido,[1] Sonoo Mizuiri,[1] Akifumi Kushiyama,[2] Atsushi Aikawa.[1] *[1]Department of Nephrology, Toho University Faculty of Medicine, Tokyo, Japan; [2]The Division of Diabetes and Metabolism, The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan.*

Background: We investigated whether serum uric acid (SUA) level before kidney transplantation predicts new-onset diabetes after kidney transplantation (NODAT), and compared with known risk factors for NODAT and type 2 diabetes mellitus(T2DM).

Methods: 191 non-diabetic adult kidney allograft recipients underwent living-donor kidney transplantation between 2001 and 2011. 151(male84, female67) recipients underwent 75gOGTT at pre-transplant were studied. NODAT was defined as FPG≥126mg/dl, RPG≥200mg/dl confirmed by repeated testing on a different day and starting therapy for diabetes after the first two weeks post-transplant. The following data were collected from the electric record: recipient age, gender, BMI, family history of diabetes, A1C, HCV status, medication usage (anti-hyperuricemic, diuretics, induction immunosuppressive agents). SUA and other data were based on before transplant operation. Statistical analysis was used by Cox proportional-hazards models.

Results: 32 recipients (21.2%) developed NODAT. When SUA was stratified into tertile, patients in the highest tertile (8.6mg/dl>for men, >7.7mg/dl for women) had a significantly (log-rank test, p=0.03) higher risk of NODAT than the lower two tertiles presented by survival analysis. In univariate model, SUA was associated with NODAT (Hazard ratio1.27, 95%CI 1.04-1.55, *p*=0.01). In multivariate model 1, SUA (1.33, 1.08-1.64, 0.006) was significant after correction for the factors directly affect SUA value. In model 2, Age (1.04, 1.01-1.08, 0.005) and SUA (1.32, 1.09-1.61, 0.004) was significant after correction for risk factors for the onset of T2DM. In model 3, Age (1.04, 1.01-1.07, 0.01) and SUA (1.31, 1.05-1.62,0.01) was remained significant after correction for previously reported factors for NODAT and model 1 and 2.

Conclusions: SUA is an independent predictor of NODAT in living-donor kidney transplantation.

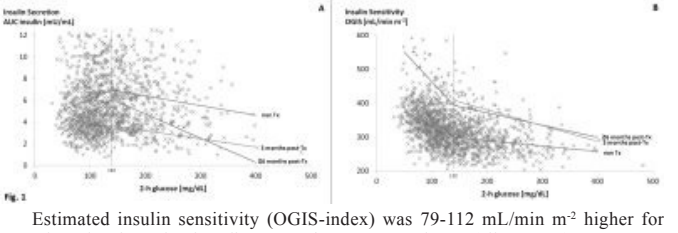
TH-PO1050

Impaired Glucose Metabolism Despite Decreased Insulin Resistance after Renal Transplantation Manfred Hecking,[1] Alexander Kainz,[1] Johannes Werzowa,[1] Michael Haidinger,[1] Angelo Karaboyas,[2] Walter Hoerl,[1] Friedrich K. Port,[2] Marcus Saemann.[1] *[1]Medical University of Vienna, Nephrology, Vienna, Austria; [2]Arbor Research Collaborative for Health, Ann Arbor, MI.*

Background: Current treatment strategies for new-onset diabetes after transplantation (NODAT) follow type 2 diabetes, however the pathophysiology underlying NODAT is still unresolved. The purpose of this study was to determine NODAT prevalence, risk factors, phenotype and pathophysiological mechanisms.

Methods: Demographics and laboratory data were obtained from 1064 stable patients ≥6 months post-transplantation. 307 patients without previously diagnosed type 1 or type 2 diabetes or NODAT were randomly assigned to a 2-hour oral glucose tolerance test (OGTT). Metabolic results were compared to a cross-sectional cohort of 1356 non-transplanted subjects.

Results: Among stable renal transplant patients, 11% had a history of NODAT, and 12% had type 1 and type 2 diabetes. Of all OGTTs, 43% were abnormal (11% diabetic), predominantly in older patients who received tacrolimus. Compared to non-transplanted subjects, basal glucose was lower, glycated hemoglobin higher, insulin secretion worse, and insulin sensitivity superior in stable renal transplant patients, throughout three subgroups of 2-hour glucose. These findings were reinforced in linear spline interpolation models of insulin secretion and sensitivity (all p<0.0007; tx vs non-tx).



Estimated insulin sensitivity (OGIS-index) was 79-112 mL/min m^{-2} higher for transplanted patients despite adjustments for age, sex and BMI (all p<0.001).

Conclusions: Glucose metabolism differs substantially between transplanted and non-transplanted subjects. Because impaired insulin secretion appears to be the predominant pathophysiological feature after renal transplantation, therapeutic regimens that preserve beta cell function are potentially beneficial in this population.

TH-PO1051

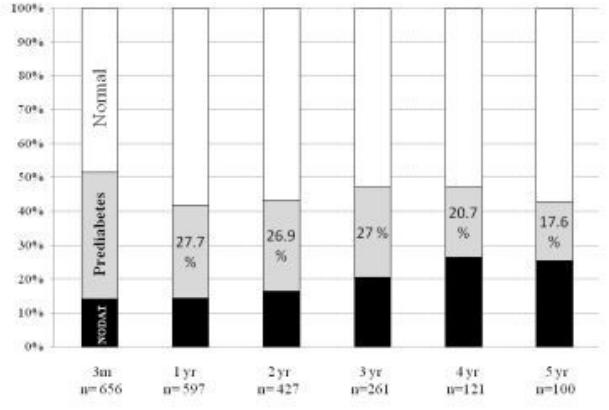
The Natural History of Prediabetes and New Onset Diabetes after Transplantation Esteban Porrini,[1] Irene Silva,[2] Meritxell Ibernon,[2] Benitez Rocio,[3] Joan Manuel Diaz,[2] Patricia Delgado Mallen,[1] Jose Manuel Osorio,[5] Francesc J. Moreso,[4] Armando Torres.[1] *[1]Hospital Universitario de Canarias, Spain; [2]Fundació Puigvert; [3]Hospital de Cruces; [4]Hospital Vall d´Hebron; [5]Hospital Virgen de las Nieves.*

Background: Long-term data on the evolution of pre-diabetes or new-onset diabetes after transplantation (NODAT) are scarce.

Methods: Eight Spanish centers contributed 50-100 non-diabetics scheduled for renal transplantation. Post-operatively, patients underwent oral glucose tolerance test (OGTT) at 3months and annually during 5 years. Patients were categorized in each period as **Normal**, **Prediabetic**: impaired fasting glucose (IFG: glucose ≥100 <126 mg/dL), impaired glucose tolerance (IGT: 2-h glucose ≥140 <200 mg/dL) or **NODAT** (ADA criteria). Prevalence and changes of category were analyzed. Immunosuppresive therapy was CNI+MMF+low dose steroids in 82.9%.

Results: We evaluated 656 patients at 3-m, 597 (1yr), 427 (2yr), 261 (3yr), 121 (4yr) and 100 (5yr). At each period~50% had prediabetes or NODAT. NODAT ranged from 14% (3-m) to 25.3 % (5yr), and prediabetes from 37.4% (3-m) to 17.6% (5yr).

Figure 1



The most frequent prediabetic alteration was IGT: 23.8 % (3-m) to 13.6 % (5yr). Prediabetes evolved into NODAT (16.3%) or normality (37.2%) and 46.5% remained prediabetic (3 year incidence). Most normal and NODAT patients remained stable during follow-up.

Conclusions: NODAT and prediabetes are highly frequent after renal transplantation. The prevalence of prediabetes seems higher than in the general population (6-8% in Spain) and its consequences (cardiovascular disease, evolution to diabetes) deserve further study.

Funding: Government Support - Non-U.S.

TH-PO1052

Cotinine Compared to Self-Reported Smoking Exposure: Dose-Dependent Effect of Smoking on Poor Outcome after Renal Transplantation Merel E. Hellemons, Marc Seelen, Gerjan Navis, Stephan J.L. Bakker. *Internal Medicine, Nephrology, University Medical Center Groningen.*

Background: Smoking exposure is emerging as a risk factor for poor outcome in renal transplant recipients (RTR). It can be assessed by self-report and cotinine measurements. We aimed to investigate (1) whether use of cotinine as a biomarker for smoking exposure can serve as an alternative for self-report and (2) to compare associations of smoking exposure by self-report and cotinine with outcome in RTR and assess potential dose-dependency.

Methods: Firstly, we assessed the agreement of self-reported smoking exposure and smoking exposure according to urinary and plasma cotinine in 603 RTR (age 51.5±12.1 yrs, 55% male). Patients were classified as never, former, light (≤10 cigarettes/day) and heavy smokers (>10 cigarettes/day) according to self-report and analogous categories for urine and plasma cotinine, and compared associations with graft failure (GF) and mortality.

Results: By self-report, 217 (36.0%) RTR were never smokers, 255 (42.3%) former smokers, 64 (10.6%) light smokers and 67 (11.1%) heavy smokers at inclusion. Agreement between self-reported cigarettes/day and cotinine was very strong. The majority of non-smokers had non-detectable cotinine, however, 14 and 13 RTR reporting no active smoking had urine or plasma cotinine consistent with active smoking. Misclassification was 12.2% for urine cotinine, and 11.5% for plasma cotinine.

Heavy smokers had highest risk of GF compared to never- and former smokers, light smokers had intermediate risk. Regarding mortality, both heavy smokers and formers smokers had increased risk compared to never smokers. Importantly, for self-reported smoking there was no sign of a dose-dependent association with poor outcome, whereas there was a dose-dependent association of cotinine levels with both GF and mortality, independent of potential confounders.

Conclusions: There was good agreement of both plasma and urinary cotinine-based measures of smoking exposure with self-reported smoking exposure. Plasma and urinary cotinine were dose-dependently associated with poor outcome in RTR and may serve as a good alternative to self-report.

TH-PO1053

Outcomes after Kidney Transplantation of Patients with Pre-Existing Atrial Fibrillation Colin R. Lenihan, Maria E. Montez-Rath, Wolfgang C. Winkelmayer. *Division of Nephrology, Stanford University School of Medicine, Palo Alto, CA.*

Background: Atrial Fibrillation (AF) is common in the dialysis population and associated with increased mortality. However, little is known about the prevalence and outcomes of patients with pre-existing AF who receive a kidney transplant.

Methods: From the US Renal Data System, we identified all patients who had >1 year of uninterrupted Medicare A and B coverage before receiving their first kidney transplant in 1996-2009. The presence of pre-transplant AF was ascertained from diagnosis codes (1 inpatient or 2 outpatient) in Medicare claims. Detailed demographic and clinical characteristics were extracted for all patients. We studied the post-transplant outcomes of death, all-cause graft failure and death censored graft failure using multivariable Cox regression.

Results: Of 62,706 eligible first kidney transplant recipients studied, 4933 (7.9%) were diagnosed with AF prior to kidney transplant. Patients with AF were older and had a higher burden of co-morbidities. Over a mean follow-up of 4.9 years 41.2% of AF patients and 24.5% without AF died. All cause graft failure and death censored graft failure were 47.6% and 17% respectively in the AF group and 36.2% and 19.5% respectively in those without AF. Table 1 shows the hazard ratios for death, all cause graft loss, and death censored graft loss in patients with AF compared to those without AF.

Table 1

Model Adjustment (all stratified by year)	Demographics	+Co-morbidities	+Transplant Factors
	HR (CI)	HR (CI)	HR (CI)
Death	1.74 (1.66,1.83)	1.51 (1.43,1.58)	1.50 (1.42,1.57)
All-cause Graft Loss	1.63 (1.56,1.70)	1.45 (1.39,1.52)	1.44 (1.38,1.51)
Death Censored Graft Loss	1.41 (1.31,1.52)	1.31 (1.21,1.41)	1.29 (1.20,1.39)

HR - hazard ratio, CI - Confidence Interval

Conclusions: Pre-existing AF is associated with poor post-transplant outcomes. Special attention should be paid to AF in the pre-transplant counseling, evaluation and risk stratification of kidney transplant candidates.

Funding: NIDDK Support, Government Support - Non-U.S.

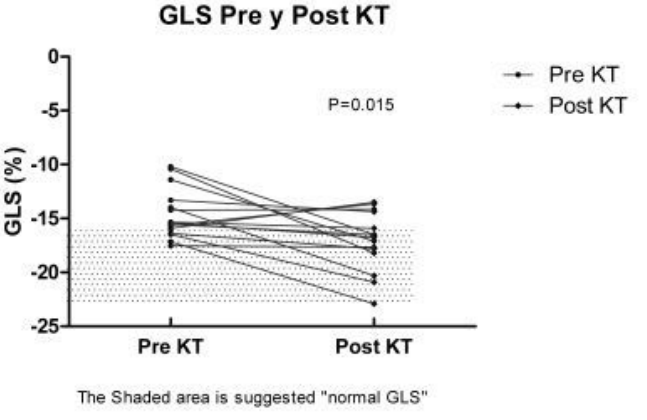
TH-PO1054

Longitudinal Myocardial Deformation in Uremic Cardiomyopathy Had a Excellent Recovery after Kidney Transplantation Omar Israel Salas-Nolasco, J. Pablo Hernandez-Reyes, Fausto E. Barrera- Gomez, Luis E. Morales-Buenrostro. *Nephrology & Mineral Metabolism and Cardiology, Instituto Nacional de Ciencias Medicas y Nutricion "SZ", DF, Mexico.*

Background: Strain imaging allows a more precise characterization of the mechanics of myocardial contraction and relaxation (deformation imaging). Abnormal left ventricular deformation (LVD) is an independent predictor of poor cardiovascular outcome and survival in different cardiomyopathic conditions including end-stage renal disease (ESRD). Usually cardiomyopathy related to uremia is reversible after successful kidney transplant (KT) opposite to ischemic one. The aim of this study is evaluate the impact of kidney transplant on LVD.

Methods: Cohort study of kidney transplantation recipients (KTR). Echocardiography (ECG) was performed at baseline and 6 months after KT. Ischemic heart disease was excluded. Measurement of LVD was performed offline through two-dimensional (2D) Speckle Tracking. Global Longitudinal Strain (GLS) pre and post KT was measured; we compare both measures (paired).

Results: Fifteen KTR were included. Median age was 40 years(18-57 y), 8 were female, cause for ESRD was 33 % unknown, 20 % diabetes, 47 % others. Six were in hemodialysis, 6 were in peritoneal dialysis, 2 were anticipated. Median time on renal replacement therapy was 2.4 years (0.33-9.9 y). Median ejection fraction (EF) pre KT was 67 %(40-74%) and post KT was 69%(48-83%). Standard measures of ECG were abnormal in 46% pre KT and only 6.6% post KT remained whit abnormal ECG. Figure shows GLS pre and post KT.



Conclusions: Pre transplant LVD was abnormal in this cohort and improved after kidney transplantation. It was independent of time in dialysis, structural or functional cardiac alterations. An abnormal GLS in uremic cardiomyopathy shouldn´t be an excluding factor for kidney transplantation by assuming poor cardiovascular outcomes and survival in this population.

Funding: Government Support - Non-U.S.

TH-PO1055

Renal Allograft Dysfunction Is Independently Associated with Subclinical Cardiovascular Disease as Indicated by Left Ventricular Mass and Aortic Pulse Wave Velocity Maggie Kam Man Ma, David Chung Wah Siu, Desmond Y.H. Yap, Susan Yung, Daniel Tak Mao Chan. *Department of Medicine, Queen Mary Hospital, Hong Kong, Hong Kong.*

Background: To investigate the prevalence of and determining factors for subclinical cardiovascular disease (CVD) in stable renal transplant recipients.

Methods: This was a cross-sectional single-center study. Adult renal transplant recipients with no prior history of cardiovascular disease were included. All underwent non-invasive cardiac investigations including treadmill stress test and transthoracic echocardiography. Aortic stiffness was assessed by measuring the aortic pulse wave velocity (aPWV).

Results: 38 stable renal transplant recipients (21 male and 17 female, mean age 51.7±8.8 years) were included. All patients had negative treadmill test. 10 patients (26.3%) had left ventricular (LV) hypertrophy (LV mass >255g for men and >193g for women by 2D echocardiogram with modified Simpson's rule). Serum creatinine (SCr) was associated with LV mass (R=0.541, p=0.00) but not aPWV (R=0.313, p=0.056), systolic function (LV ejection fraction, R=0.264, p=0.109) and diastolic function (mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e') ratio, R=0.143, p=0.399). By multivariant regression analysis, SCr was significantly associated with LV mass (β 0.526, p=0.001) and aPWV (β 0.348, p=0.048), independent of age, gender, hypertension, hyperlipidemia or diabetes mellitus.

Conclusions: The degree of renal allograft dysfunction was associated with subclinical CVD as indicated by LV mass and aPWV, independent of traditional cardiovascular risk factors.

Funding: Government Support - Non-U.S.

TH-PO1056

Fractional Excretion of Creatinine Predicts Risk of Cardiovascular Events and All-Cause Mortality in Renal Transplant Recipients Mohammad Esmaeil Barbati,[1] Steef Jasper Sinkeler,[1] Ali Abbasi,[2,3] Arjan J. Kwakernaak,[1] Stephan J.L. Bakker,[1] Gerjan Navis.[1] *[1]Nephrology, University Medical Center Groningen, Groningen, Netherlands; [2]Epidemiology, University Medical Center Groningen, Groningen, Netherlands; [3]Internal Medicine, University Medical Center Groningen, Groningen, Netherlands.*

Background: Several studies showed that tubular damage markers are associated with adverse outcome in renal patients, including renal transplant recipients (RTRs). The predictive power of markers of tubular functional capacity has not been established. Therefore, we studied whether fractional excretion of creatinine (FE_{creat}), as a marker of tubular excretory capacity, is associated with graft failure, cardiovascular (CV) events and all-cause mortality in RTRs.

Methods: A cohort of 454 RTRs (54% male; age 52±15 years) with a functional graft for at least 1 year were included (2006-10). All patients were on standard immunosuppressives and underwent GFR assessment by ^{125}I-iothalamate. FE_{creat} was calculated as ratio of $(urine/plasma)_{creat}$ to $(urine/plasma)_{^{125}I\text{-}iothalamate}$.

Results: In cross-sectional analyses, FE_{creat} (mean±SD: 110±15) was positively associated with male sex, body surface area and true GFR, but inversely with age (all P<0.001). During median (IQR) follow-up for 7.6 (5.4-12.7) years, 28 RTRs developed graft failure, 77 developed a CV event and 45 died. In multivariable Cox regression analyses, higher FE_{creat} was significantly associated with lower rates of CV events (HR=0.83 [0.70-0.97], P=0.02) and all-cause mortality (HR=0.68 [0.54-0.85], P=0.001), independent of age, sex and cardiovascular and renal risk factors, but not with graft failure (HR=1.23 [0.93-1.63] P=0.14).

Conclusions: FE_{creat} did not predict graft failure, but, remarkably, independently predicted CV events and all-cause mortality in RTRs. These data suggest that adequate tubular excretory function is protective against CV disease, for instance by removal of uremic toxins. Monitoring and preservation of tubular function might help to improve overall outcome in RTRs.

TH-PO1057

Cystatin C in Renal Transplant Recipients: Marker of Cardiovascular Risk Mira T. Keddis, Nick Voskoboev, Andrew D. Rule, Hatem Amer, Walter K. Kremers, John C. Lieske. *Mayo Clinic, Rochester, MN.*

Background: Cystatin C is a powerful predictor of cardiovascular disease and has been shown to be highly correlated with GFR in renal transplant recipients (RTR). In this study, we assess whether cardiovascular risk factors associate with Cystatin C independent of glomerular filtration rate (GFR) in RTR.

Methods: Adult RTR with stable renal function presenting for their annual transplant evaluation at a single center were studied. GFR was measured using iothalamate clearance (mGFR) and a simultaneous serum Cystatin C was assayed using particle-enhanced immunoturbidimetry (PETIA). Cystatin C was regressed on GFR and other cardiovascular risk factors in a multivariable model. All continuous variables were log-transformed.

Results: 492 RTR were studied; mean age was 55 ± 14 years, BMI 30 ± 7 kg/m^2, 58% male and 93% Caucasian. Median time from transplant was 69 months (11,469). DM was present in 35%, 20% had a major cardiovascular event and 62% were on statin therapy. Post-transplant cardiac troponin T (cTNT) at the time of the visit was elevated (>0.01 ng/L) in 6% of patients. Table 1 shows significant associations with Cystatin C adjusted for mGFR and the independent predictors of cystatin C after mGFR adjustment in a multivariable model.

Clinical Associations with Cystatin C

Variables	Parameter estimate, mGFR adjusted	Parameter estimate, Multivariate adjusted
mGFR (ml/min/1.73m^2)	-	-0.59**
BMI (kg/m^2)	0.094**	-
Female gender	-0.019**	-
DM	0.016*	-
Smoking history	0.021**	0.031*
Statin use	-0.017*	-0.034*
HDL (mg/dL)	-0.096**	-0.054*
LDL (mg/dL)	0.04*	-
Triglycerides (mg/dL)	0.058**	0.036*
Albumin (g/dL)	-0.178*	-
Proteinuria (mg/24hrs)	0.031**	0.027**
Elevated cTNT	0.031*	0.08*

*p<0.05 **p<0.01

Conclusions: Independent of GFR, Cystatin C associates with clinical variables that are predictive of cardiovascular events and mortality post-transplant, in particular, proteinuria, elevated cTNT, and dyslipidemia. Cystatin C should be considered both a marker of GFR and of cardiovascular risk in renal transplant recipients.

TH-PO1058

Use of Cystatin C and Creatinine Based Equations to Estimate Glomerular Filtration Rate in Renal Transplant Recipients Mira T. Keddis, Nick Voskoboev, Andrew D. Rule, Hatem Amer, Walter K. Kremers, John C. Lieske. *Mayo Clinic, Rochester, MN.*

Background: Performance of established cystatin C (CysC) based equations for GFR estimation have not been evaluated in a large cohort of renal transplant recipients (RTR) with stable allograft function. In this cross-sectional study we assessed the performance of 3 published CysC-based eGFR equations in a large sample of RTR more than 1 year out from transplant.

Methods: Adult RTR presenting for a routine annual transplant evaluation were actively enrolled. GFR was measured by iothalamate clearance (mGFR). Plasma CysC was analyzed by particle-enhanced turbidimetric immunoassay, while serum creatinine (Cr) was measured with an enzymatic assay, both standardized to international reference materials. eGFR was calculated using CysC-based equations published by Rule (developed in RTR), Flodin and Stevens (both developed in non-RTR) and the widely used Cr-based MDRD equation. The Rule equation was corrected for bias by dividing CysC by a factor of 1.206, determined by remeasurement of CysC in an independent biobanked sample from 2000 when the formula was derived. Overall bias and accuracy for each eGFR equation within 10 and 30% were assessed.

Results: 492 RTR were studied; mean age was 55 ± 14 years, 59% male and 93% Caucasian. Mean+SD mGFR was 57 ± 21 ml/min/1.73^2. The CysC- based equation developed by Rule had the lowest overall bias and classified patients best within 10 or 30% of mGFR.

Cystatin C Equation Performance

eGFR equations	GFR mean, median (range)	Percentage bias overall (95% CI)	Within 10% accuracy, N(%)	Within 30% accuracy, N(%)
MDRD	52.2, 50.1 (13.7, 170.2)	-11 (-24, 2)	156 (31)	411 (83)
Rule	58.7, 56.8 (19.4, 123.2)	-7 (-14, -1)	201 (41)	417 (85)
Stevens	44.0, 42.0 (13.4, 100.2)	-27 (-32, -22)	86 (17)	353 (72)
Flodin	44.6, 42.1 (11.2, 110.2)	-14 (-21, -8)	87 (18)	354 (72)

Conclusions: A CysC-based eGFR equation developed in RTR outperformed 2 CysC equations developed in non-RTR and the Cr-based MDRD equation. Understanding the influence of transplant related variables may further improve performance of CysC-based equations in RTR and other populations.

TH-PO1059

Comparison of Cystatin C, Beta Trace Protein, Urinary Albumin/Creatinine Ratio and 24 Hour Urine Protein Excretion as Predictors of Renal & Cardiovascular Outcomes in Renal Transplant Recipients Hari Manoharlal Talreja, Ayub Akbari, Greg A. Knoll. *Division of Nephrology, Department of Medicine, The Ottawa Hospital, Otatwa, ON, Canada.*

Background: Cardiovascular disease is the leading cause of mortality in kidney transplant recipients [KTR]. Current risk predictors underestimate their cardiovascular risk. Novel risk predictors such as Cystatin C & Beta-trace protein [BTP] have been studied in non-transplant patients. The aim of our study was to compare novel markers, cystatin C and BTP and 24 hour urinary protein excretion & urinary albumin to creatinine ratio [U-ACR] as predictors of renal & cardiovascular outcomes in KTR.

Methods: 200 stable renal transplant recipients from outpatient transplant clinic at The Ottawa Hospital who consented for undergoing DTPA GFR scan with measurements of Cystatin C, BTP, 24 hour urine protein & random urinary ACR in 2004-2005 were included in the study. Follow-up data was available on 125 subjects.

Subjects underwent the baseline tests in 2004-2005, and baseline demographic data was collected, and were followed until October 2011 or until occurrence of any of composite outcomes consisting of renal, cardiovascular outcomes and mortality, whichever was earlier. Renal outcomes were defined as graft loss [need for renal transplant or dialysis] or doubling of serum creatinine; cardiovascular outcomes as acute coronary syndrome, need for intervention, stroke, intervention for peripheral vascular disease; mortality as death due to any cause. Logistic regression was used to estimate Odds Ratio [OR] for any composite outcome using SAS software.

Results: In this retrospective study, out of 125 subjects with mean age of 52.6 ± 12 years, mean corrected GFR 57.9 ± 24.4ml/min/1.73m2], 17 had a composite outcome. OR estimate for any composite outcome for 24 hour urine protein was 0.917 [CI 0.250-1.001], for U-ACR was 1.002 [CI 0.993-1.011], for cystatin C was 0.529 [CI 0.186-1.502] & for BTP was 0.499 [CI 0.145-1.717].

Conclusions: Preliminary data suggests that 24 hour urine protein excretion, U-ACR, cystatin C & BTP are comparable predictors of renal and cardiovascular outcomes in kidney transplant recipients. Complete data will be analyzed soon for final results.

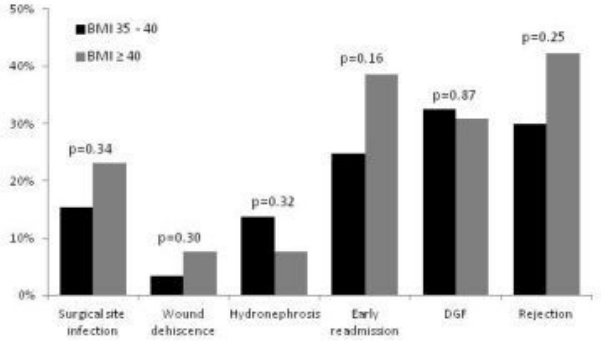
TH-PO1060

Surgical Complications and Outcomes of Renal Transplantation in Morbidly Obese Recipients Yelena Rekhtman, Sumit Mohan, Demetra Tsapepas, Anthony Watkins, Zouhair M. Kabbara, Sowmini Medavaram, David J. Cohen, Lloyd Ratner, Jai Radhakrishnan. *Columbia University.*

Background: Rising obesity rates in the general population are reflected in the rising BMI of prospective renal transplant recipients. Previous studies reporting high rates of surgical complications and poor graft outcomes have discouraged renal transplantation in morbidly obese patients. We report outcomes in morbidly obese patients at our center.

Methods: We performed a retrospective study of adult renal transplant recipients with BMI ≥35 kg/m^2 who received an allograft between January 2004 and April 2012 at our center. Recipients were stratified into two groups, BMI 35 – 40 kg/m^2 and BMI ≥ 40 kg/m^2, and compared with respect to surgical site infection (superficial and deep tissue), wound dehiscence, length of stay, early readmission, and graft outcomes.

Results: Of 143 patients, 117 had BMI between 35 and 40 (mean 37.1, SD 1.4) and 26 had BMI ≥ 40 (mean 43.1, SD 2.3). While morbidly obese patients required longer hospitalizations (7.2 days vs. 11.7 days, p=0.0027), we found no statistically significant differences between the two groups in the incidence of surgical site infection (15.4% vs. 23.1%, p=0.34), wound dehiscence (3.4% vs. 7.7%, p=0.30), hydronephrosis (13.7% vs. 7.7%, p=0.32), or early readmission (24.8% vs. 38.5%, p=0.16). There were no significant differences in delayed graft function (DGF) (32.5% vs. 30.8%, p=0.87), rejection (29.9% vs. 42.3%, p=0.25), rejection-free graft survival (p= 0.079), or overall graft survival (p=0.275).



Conclusions: Surgical complication rates among patients with BMI ≥ 40 are lower than previously reported, and are similar to the rates among patients with BMI between 35 and 40, with similar graft outcomes. Though careful recipient selection is imperative, our results suggest that morbid obesity should not preclude patients from renal transplantation.

TH-PO1061

The Prognostic Significance of Iliac Vessel Calcification in Renal Transplantation Emma L. Aitken, Marc J. Clancy, David Kingsmore. *Department of Renal Surgery, Western Infirmary, Glasgow, United Kingdom.*

Background: Peripheral vascular disease (PVD) is common in patients with ESRF and is associated with considerable morbidity. Nevertheless there is minimal literature on the incidence of amputation following renal transplantation.

Methods: We undertook five year follow-up on the cohort of patients on our renal transplant waiting list in January 2007. All patients to had pelvic x-rays to assess iliac vascular calcification at the time of activation onto the waiting list. Any patients with moderate/ severe calcification were referred for CT angiogram (CTA) of their aorto-iliac vessels. Mortality, transplantation outcomes and amputation rates at five years were correlated with the severity of calcification on pre-operative imaging.

Results: 187 patients were on the waiting list in January 2007 (92 male; mean age 58.3+/-6.2 yrs). By January 2012, 82 patients had a functioning transplant; 45 remained on the waiting list; 40 had died and 20 were alive but no longer on the waiting list. 73 (39.5%) had moderate-severe calcification. Of these, 21.9% had extensive calcification affecting all the iliac vessels and were removed from the waiting list. 50% of patients who developed vascular complications were dead at 5 year follow-up. Mortality and amputation rates were higher in patients with moderate-severe calcification than minimal calcification (30.1% *vs* 16.6% ; p=0.02 and 10.9% *vs* 1.8% p=0.003 respectively). There was no difference in rates of DGF, BPAR or creatinine at 1 year between patients who were transplanted with moderate-severe calcification, however intra-operative vascular complications (26.7% vs. 3%; p<0.001), graft loss (28.1% vs. 3.4%; p=0.01) and death with a functioning transplant (9.7% vs 1.6%; p=0.04) rates were higher in patients with extensive calcification.

Conclusions: Pelvic x-ray is a useful screening tool to identify patients who may require further vascular assessment prior to transplantation. Amputation rates following renal transplantation are low and PVD in isolation should not preclude transplantation. Nevertheless, significant vascular calcification is predictive of mortality both with and without transplantation and graft loss in patients with a renal transplant.

Funding: Government Support - Non-U.S.

TH-PO1062

Risk Factors for Venous Thromboembolism in Renal Transplant Recipients in the Absence of Known Hypercoagulable State Bhavani Adusumilli,[1] Reem Daloul,[1] Dilip Samarapungavan,[2] Steven Cohn,[2] Vandad Raofi,[2] Francis Dumler,[2] Gampala Harish Reddy,[2] Leslie L. Rocher,[2] Alan Koffron,[2] Raviprasenna K. Parasuraman.[2] *[1]Internal Medicine, William Beaumont Hospital; [2]Division of Transplant, William Beaumont Hospital.*

Background: The prevalence of venous thromboembolism (VTE) in renal transplant recipients (RTR) varies between 4-57%, however the prevalence and risk factors in the absence of known hypercoagulable state prior to transplantation is unclear.

Methods: A Retrospective analysis of all adult renal transplants performed at our center between 1/1/2008 -12/31/2009. Patients with prior known hypercoagulable conditions and/or on anticoagulation at the time of transplantation were excluded. The data was analyzed by using Chi-square, Kruskal-Wallis test and Kaplan–Meier methods.

Results: A total of 111/124 patients met the criteria with mean follow up of 30.4±12.9 months. The mean age was 54.1±12.4 yrs, 38.7 % women and 64.4% Caucasians. The prevalence of VTE was 11.7% (13/111), median time 1.7 months (0.1 to 16.8) with 23.1 % occurring in first 30 days, 69.2% < 3 months and 30.7% > 3 months. Among the recipients with VTE, 84.6% (11/13) received deceased donor and 15.4% live donor transplants. The Mean serum creatinine at the time of VTE was 2.4±1.4mg/dL. A total of 22.5% (25/111) had complications (infection, delayed graft function and/or rejection) and 77.5% (86/111) had no complications. VTE was significantly higher in the group with complication at 32% (8/25) vs 5.8%(5/86) in no complication group (p=0.0014) with Odds ratio of 7.64 (95% CI, 2.2-26.2). VTE was higher in those who received anti-thymocyte globulin (rATG) induction at 23.5% (4/17) vs 10.8% (9/83) with IL-2 RA (p=0.37). Only 38.5% (5/13) of patients with VTE had hypercoagulable work-up with 3 showing protein C and S deficiency.

Conclusions: The prevalence of VTE in RTR was 11.7% in this study and it was significantly higher in those with post-transplant complications with Odds ratio of 7.6. Induction with rATG was associated with higher VTE but not significant mostly due to smaller number. Presence of above mentioned complications should increase the index of suspicion for VTE.

TH-PO1063

Risk Factors in Venous Thrombosis of Renal Grafts from Deceased Non Heart-Beating Donors Maria Molina, Esther Gonzalez Monte, Eduardo Gutierrez, Manuel Praga, Amado Andres. *Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

Background: The deceased donor kidney transplant to non heart-beating may have a higher rate of venous thrombosis.The aim of this study is to analyze whether resistance index (RI) high (≥ 0.8), measured by Doppler ultrasound can be a predictor of venous thrombosis. We also analyzed whether early anticoagulation may decrease graft loss associated with venous thrombosis.

Methods: We analyzed 197 patients with renal transplant non heart-beating donor made since 2005-2012. In November 2009 began prophylactic anticoagulation if RI were elevated. Patients were divided in group I(no anticoagulation historical group) and group II(anticoagulated by RI).

Results: The Table compares the Group I to Group II. In univariate analysis cold ischemia time, body mass index of the donor and high RI were factors that were associated with venous thrombosis of the graft.

Table

	Group I (n =87)	Group II (n = 110)	p
RECIPIENT			
Age (years)	45,78 ± 11,16	50,07 ± 12,06	0,01
Men	56,3%	60,9%	ns
First transplant	94,3%	92,7%	ns
Hyperimmunized	1,1%	0,9%	ns
Mismatch	4,20 ± 1,19	4,67 ± 1,00	0,003
Cold ischemia time (minutes)	872,61 ± 304,57	710,25 ± 276,79	< 0,001
DONOR			
Age (years)	38,36 ± 9,81	47,03 ± 10,86	< 0.001
Men	92%	85,5%	ns
Weight (kg)	85,10 ± 13,85	77,48 ± 11,38	0.001
Creatinine (mg / dl)	1,15 ± 0,35	1,24 ± 0,47	ns
EVOLUTION			
Primary graft function	14,9%	13,6%	ns
Acute tubular necrosis (days)	13,64 ± 5,82	13,86 ± 7,43	ns
Resistance indices in the doppler	0,77 ± 0,11	0,78 ± 0,12	ns
High resistance rates	36,8%	44,5%	ns
Loss of graft	10,3%	6,4%	ns
Anticoagulation	0 %	23,6%	<0,001
Venous thrombosis	6,9%	0%	0,007
Biopsy-proven rejection	16,1%	7,3%	0,06
Receptor survival	98,9%	100%	ns

In multivariate analysis the variable that was associated with venous thrombosis was the prolonged cold ischemia time (0.04).We analyzed the subgroup of 79 patients with high RI, 27 patients were anticoagulated, and none had a venous thrombosis compared with 52 patients who received no anticoagulation, of which 6 had vascular thrombosis (0% vs 11,5% p 0, 07).

Conclusions: This study suggests that a careful choice of donor, reduced cold ischemia time, monitoring of the RI and anticoagulation may decrease the rate of venous thrombosis in in these transplants.

Funding: NIDDK Support

TH-PO1064

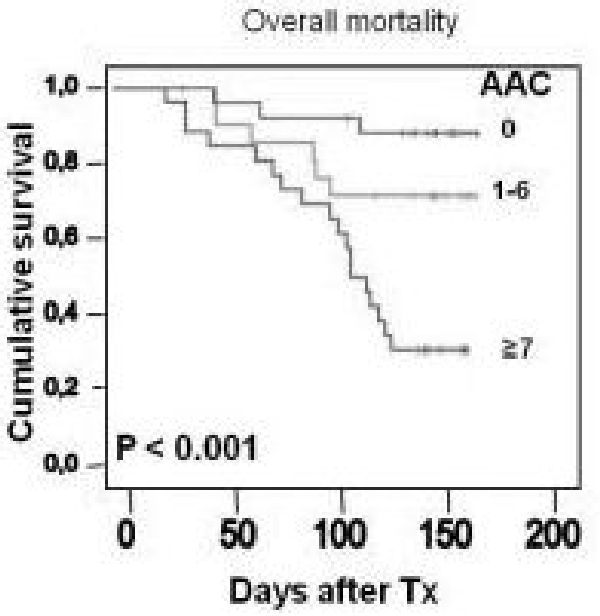
Influence of Abdominal Aortic Calcification on Long-Term Survival in Renal Transplant Recipients

Megan E. Roerink, Henk W. Van Hamersvelt. *464 Nephrology, Radboud University Medical Centre, Nijmegen, Netherlands.*

Background: Despite extensive pretransplant screening, it remains difficult to predict long-term survival in renal transplant recipients. In search for a more accurate method, we tested the predictive value of abdominal aortic calcification (AAC).

Methods: We determined AAC on lateral lumbar radiographs in a cohort of 74 transplant recipients, transplanted between 1997-1999. Radiographs were performed within 3 months of transplantation and were AAC-scores ranging from 0 to 24 using a previously described scoring system. During mean follow-up of 10 years, cardiovascular events (coronary artery disease, cerebrovascular events and peripheral vascular disease) and mortality were recorded.

Results: Mean age in our cohort was 48±13 years. AAC was present in 64% of patients with a mean score of 6. In patients with a AAC-score ≥7 69.2% died compared to 28.6% with a score of 1-6 and 11.1% without calcifications (P=<0.001, figure). Recipients with a high AAC-score had more cardiovascular events (50%) compared to patients with a moderate (29%) or low (26%) AAC-score (P= 0.045). In Cox multivariate analysis, the hazard ratio of each point AAC-score for event-free survival was 1.10 (1.03-1.18, P=0.005). Other factors independently associated with survival were age and diabetes at time of transplantation. The hazard ratio of AAC-score for cardiovascular complications was 1.07(1.01-1.14), with diabetes, cardiovascular medical history and gender as other independent predictors.



Conclusions: The presence of abdominal aortic calcification significantly influences long-term survival and cardiovascular risk in renal transplant recipients and can easily be assessed by lateral lumbar radiography. In future renal transplant recipients, determination of AAC can be used as a tool to predict the risk of long-term complications and survival.

Funding: Clinical Revenue Support

TH-PO1065

Interventional Management of Late Vascular Complications in Simultaneous Pancreas-Kidney Transplantation: A Case Series of 3 Patients

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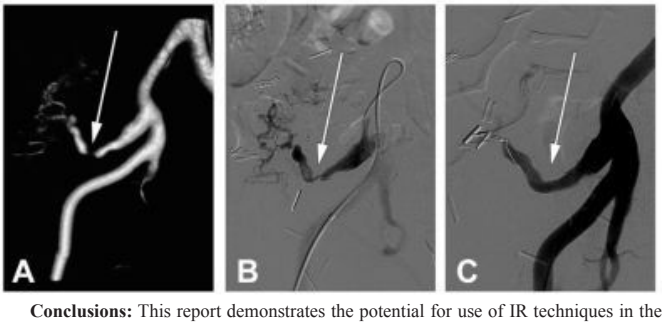
Background: Simultaneous pancreas-kidney (SPK) transplantation is a highly effective therapeutic option for a subset of patients with diabetes mellitus (DM) and chronic kidney disease, allowing for the restoration of normoglycaemic control and consequent improvements in diabetic complications. Much progress has been made in determining the optimal surgical technique for this procedure, and major advances in immunosuppression have reduced the rate of rejection. As a result, long-term outcomes have continued to improve for all categories of pancreas transplantation. It has been reported that technical failure is the most common cause of pancreatic graft loss in SPK transplants, with immune-mediated rejection being the second most common. The majority of cases of technical failure are due to vascular complications, with graft thrombosis accounting for more than 50% of these cases. There are limited data regarding the use of interventional radiology (IR) techniques in order to prevent vascular complications progressing to graft loss.

Methods: We have recently performed interventional procedures for three patients in whom vascular complications had arisen, comprising two cases of transplant arterial stenosis and one pseudoaneurysm.

Results: In all three cases, vascular patency and optimal graft perfusion were maintained without deterioration in graft function. Figure 1 shows a case in which transplant arterial stenosis (seen with magnetic resonance angiography in (A) and intra-arterial angiography in (B)) was successfully treated with balloon angioplasty and stenting (C). In all cases there were no significant intra- or post-procedure complications.



Conclusions: This report demonstrates the potential for use of IR techniques in the management of graft-threatening vascular complications.

TH-PO1066

Dual Kidney Transplantation without Allocation by Preimplantation Biopsy: A Single Center Experience Alexander Weidemann,[1] Marie-Therese Rotter,[1] Johannes Jacobi,[1] Katharina M. Pressmar,[1] Maike Julia Buettner,[2] Kerstin U. Amann,[2] Kai-Uwe Eckardt.[1] *[1]Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Nephropathology, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Kidney transplants from expanded criteria donors (ECD) have become generally accepted due to the increasing organ shortage. Dual kidney transplantation (DKT) of marginal kidneys not suitable for single kidney transplantation (SKT) is a possibility to further expand the ECD kidney pool. Still, DKT remains underused and potentially eligible kidneys are often discarded. In order to evaluate whether DKT can be performed savely without allocation by histological scores, we analysed outcomes of DKT in our center and compared them to SKT and to an ideal kidney transplantation (IKT), a living donor kidney transplantation with old donor and recipient.

Methods: In a retrospective analysis 10 DKT and 16 SKT were matched by recipient age, HLA and ischemia time. 13 pts. were in the IKT group. Statistical analyses were performed by one way ANOVA, Chi^2 and student's t-tests.

Results: Donor age and creatinine was significantly higher in DKT compared to SKT and IKT. Patient survival was 100% in all groups. No graft was lost during follow-up in the IKT and DKT group, but one in SKT. No difference in renal function at discharge was observed between DKT and SKT, with a trend towards more delayed graft function in DKT. Best-creatinine in the follow-up period was not significantly different in all three groups. DKT patients had significantly higher incidence of lymphocele and blood transfusions compared to SKT and IKT. However, the duration of hospitalization was not significantly longer. Rejection episodes were lower in DKT compared to IKT and SKT.

Conclusions: Two marginal kidneys allocated by eGFR and careful recipient selection but not histological scores can be performed safely and successfully. Although surgical complications are increased, best creatinine levels in the follow up are not different compared to SKT and to IKT. Although a larger cohort of patients and longer follow up is needed, our results suggest that DKT can be used to transplant kidneys which otherwise might have been discarded.

Funding: Government Support - Non-U.S.

TH-PO1067

The Integration of Donor Kidney Function into the Assessment of Post-Transplant Function Identifies Differences between Kidney Transplants from Living versus Deceased Donors Otherwise Not Detectable Riyadh Nasir Alsehli,[1] Scott O. Grebe,[2] Valerie A. Luyckx,[1] Thomas F. Mueller.[1] *[1]Medicine, University of Alberta, Edmonton, AB, Canada; [2]Medicine, University of Witten-Herdecke, Helios Kliniken, Wuppertal, Germany.*

Background: Donor nephron supply has a major impact on post-transplant function. However, measurements of transplant kidney function rely exclusively on recipient creatinine or GFR measurements. We have developed a formula integrating recipient (R) metabolic demand and donor (D) nephron mass to predict the expected post-transplant creatinine (exp R-Cr). Here we compare this formula to the standard measures of kidney function in recipients of deceased (DD) vs. living donor (LD) kidneys, latter known to have better function and outcome.

Methods: We calculated the exp R-Cr based on D and R age, weight, gender, D crea and renal adaptive capacity for recipients of 79 LD and 67 DD kidneys. The exp R-Cr was compared to the observed kidney function as measured by serum creatinine (obs R-Cr) or estimated GFR (Cockcroft Gault, MDRD, CKD-Epi) during the post-transplant course.

Results: The mean period of post-transplant follow-up was 66 months. D and R demographics were similar between the LD and DD groups at time of transplantation. The 4 year graft survival rates were 96% and 88% for the recipients of LD vs. DD kidneys, resp. Transplant function measured by Cr, CG, MDRD or CKD-Epi was similar between LD and DD recipients. However, calculating the exp R-Cr vs. the obs R-Cr demonstrated a significant difference between LD and DD kidney function (p<0.01). As shown in table 1 DD organs function about 40 % less than expected compared to about 10 % less function in LD organs.

Transplant function in LD vs. DD kidneys

	D-Cr [umol/L]	R-Cr y1 [umol/L]	R-Cr y4 [umol/L]	exp R-Cr [umol/L]	exp-obs R-Cr y1 [%]	exp-obs R-Cr y4 [%]
LD [n=79]	72	127	122	120	-14	-11
DD [n=69]	68	120	126	99	-41	-47
p-value	n.s.	n.s.	n.s.	0.004	0.004	0.002

Conclusions: The integration of donor kidney information into the assessment of post-transplant function detects poorer function of DD organs that is missed by the conventional measurements.

Funding: Government Support - Non-U.S.

TH-PO1068

Acceptable Patient and Allograft Outcomes Can Be Achieved Using Expanded Criteria Donors for Re-Transplantation Janani Rangaswami,[1] Po Nan Chang,[2] Mahbub Jamil,[1] Campos Stalin,[1] Kamran Khanmoradi,[1] Radi Zaki,[1] Afshin Parsikia,[1] Jorge Ortiz.[1] *[1]AEMC; [2]Drexel University.*

Background: Extended criteria donors (ECD) are an important means of expanding the donor pool. Data on outcomes using ECD kidneys in patients with a history of a failed allograft are limited.

Methods: We compared three groups. 1) patients who were transplanted with ECD kidneys after a history of failed allograft (ECD re-transplant), 2) patients who were transplanted with Standard criteria donor (SCD) kidney after failed allograft (SCD re-transplant). 3)First time ECD transplant recipients. We generated Kaplan Meier curves for patient and allograft survival at 1 and 3 years using the log rank test. We ran a Cox Proportional Hazards regression model to evaluate the effects of delayed graft function(DGF), BMI, HTN, DM, donor age, and cold ischemia time(CIT) on survival.

Results: There were 19 ECD re- transplants, 95 SCD re- transplants and 169 first- time ECD transplants. 1 year patient survival was 88.6%, 97.5%, 87.8%,in, re-ECD, re-SCD, first ECD groups. 3 year patient survival was 73%, 95%, 74%. 1 and 3 years patient survival between the 3 groups was statistically different (P-value=0.0265and 0.0006 respectively). 1 year graft survival was 94.5%, 94.6%, 88% for the re-ECD, re-SCD, first ECD group respectively. 3 year allograft survival was 79.4% and 79.6%, 75%. Graft survival at 1 and 3 years was not statistically different (P-value=0.1952and 0.6228 respectively). One and 3year patient and allograft survival was not statistically significant between first ECD vs re ECD. The Cox regression model showed that differences in DGF, BMI, race, DM, HTN, donor age and CIT, were not statistically significant (p-value > 0.05).

Conclusions: 1 and 3 years patient survival was best in the re-SCD group. There was no difference in allograft survivals between the three groups.ECD patient and allograft survivals are similar to established norms. Re-ECD patient and allograft survivals are statistically similar to ECD transplants. Therefore, re-ECD represents an appropriate option for patients awaiting a second transplant.

TH-PO1069

Role of Donor Glomerula Filtration Rate & Recipient Body Mass Index on Outcomes of Cadaver Renal Transplantation Kadiyala V. Ravindra, William Irish, Anthony W. Castleberry, Deepak S. Vikraman, Matthew Jay Ellis, Debra L. Sudan. *Surgery, Duke Univerisity, Durham, NC.*

Background: There is scant data on role of recipient BMI (rBMI) on outcomes of deceased donor renal transplants. This has particular relevance with increased use of ECD organs. Aim: To assess role of donor GFR & recipient BMI on graft survival in cadaver kidneys using OPTN/UNOS registry.

Methods: Adult recipients of primary, solitary, kidney from cadaver donor (2002-'10) were studied. GFR was calculated with MDRD equation. The groups were: eGFR: >90, 60-90 and 30-59 & rBMI: <25, 25 – 29, 30-34 and ≥35. Graft survival was estimated by Kaplan-Meier method. Impact of donor eGFR & recipient BMI on graft failure was assessed with Cox's proportional hazards model. Hazard ratios and 95% confidence intervals were used as measures of association and precision. Effect modification was assessed by incorporating relevant two-way interactions into model.

Results: Study patients (n=54,600) were mean age 53 years, 61% male, 32% Black, median follow-up of 4 years. Of these, 22% received ECD organs. Graft survival at 4-years was 78 ± 0.2% in SCD and 66 ± 0.5% in ECD. Multivariable results of donor eGFR and rBMI on risk of graft failure are in Table 1. The impact of donor eGFR was independent of recipient and donor BMI (p=0.591 and p=0.897) and ECD (p=0.483). Also, impact of rBMI was independent of donor BMI (p=0.458) and ECD (p=0.199).

Table 1: Impact of donor eGFR and recipient BMI on graft failure and evaluation of effect modification

Effect	HR*	95% CI	p-value
Donor eGFR Group(ref=>90)			
60-90	1.08	1.12	<0.0001
30-59	1.17	1.12-1.22	<0.0001
Recipient BMI(ref=<25)			
25-29	1.07	1.03-1.12	0.0008
30-34	1.15	1.10-1.20	<0.0001
>=35	1.27	1.19-1.34	<0.0001

* Adjusted for donor BMI, ECD vs. SCD kidneys and peak PRA & immunosuppression at discharge

Conclusions: Recipient BMI and donor eGFR are both independently&significantly associated with risk of graft failure following deceased donor kidney transplantation. This has implications for emphasizing weight reduction prior to deceased donor renal transplantation. Matching donor eGFR to recipient may help to improve long-term prognosis of kidney transplant recipients.

TH-PO1070

Donor Age >45 Years Is Associated with Poor Outcomes Following Donation after Cardiac Death (DCD) Kidney Transplantation Hani Wadei,[1] Michael Heckman,[2] Bhupendra Rawal,[2] Martin L. Mai,[1] Waleed Farahat,[1] Cynthia Leaphart,[1] Mary B. Prendergast,[1] Thomas A. Gonwa.[1] *[1]Transplantation, Mayo Clinic, Jacksonville, FL; [2]Biostatistics, Mayo Clinic, Jacksonville, FL.*

Background: Kidney transplantation (tx) from DCD donors confers similar patient and graft survivals compared to tx from brain dead donors (DBD). However, information regarding kidney function in DCD tx is lacking.

Methods: Included were 64 DCD and 248 DBD kidney tx performed between 11/2000 and 9/2008. Living donor, extended criteria tx, multi-organ and dual kidney tx were excluded. Recipients, donors and tx characteristics were retrospectively collected and compared between DCD and DBD. Iothalamate GFR was measured annually. The primary outcome was the cumulative incidence of poor graft function defined as graft loss, death, or 2 consecutive GFR<50 ml/min within 5 years from tx.

Results: Patients' characteristics are presented in **Table 1**

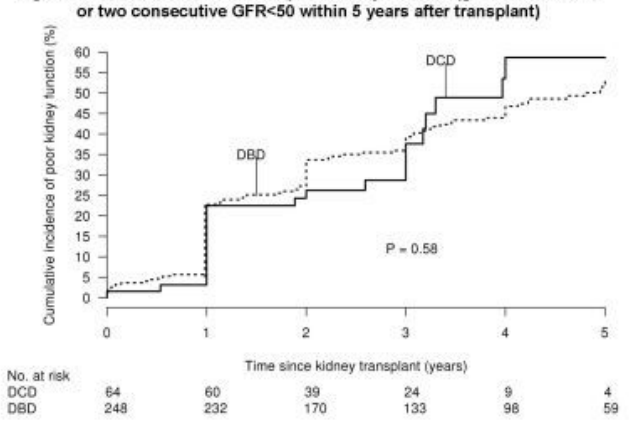
Table 1:Recipient, donor and transplant characteristics in DCD and DBD

	DCD (N=64)	DBD (N=248)	P
Median age at tx (yrs)	56	57	1
% male	62	57	0.5
% Pre-tx diabetes	19	27	0.3
% Preemptive tx	16	10	0.3
Median CIT (hr)	16	19	0.002
Median WIT (min)	43	44	0.3
Median donor age	30	36	1
% DGF	45	28	0.01
% rejection	33	25	0.3

CIT: cold ischemia time; WIT: warm ischemia time; DGF: delayed graft function

142 (46%) experienced the primary outcome. There was no difference between DCD and DBD regarding the primary outcome (RR: 1.14,CI:0.7–1.8,P=0.58) **Figure1**

Figure 1: Cumulative incidence of poor kidney function (graft loss, death, or two consecutive GFR<50 within 5 years after transplant)



Of the 64 DCD tx, 25 (39%) experienced the primary outcome. On multivariate analysis, donor age was the only factor that related to the outcome in DCD tx (RR:1.64 (per 10-yr increase),CI:1.2-2.3,P=0.005) with the highest risk observed for donor age >45 years (RR:5.27,CI:2.2–12.5, P<0.001).

Conclusions: 1) Outcomes are similar between DCD and DBD kidney tx. 2) Donor age >45 yrs is associated with poor graft function in DCD kidney tx.

TH-PO1071

Validation of the CKiD Bedside Formula as eGFR in Children Post Renal Transplant

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[1]Pediatric Nephrology, UT Southwetern Medical Center, Dallas, TX; [2]Pediatric Nephrology, Children's Medical Center, Dallas, TX.

Background: Serum creatinine (S_{Cr}) and S_{Cr} based formulae are used as markers to estimate glomerular filtration rate (eGFR) following renal transplantation. It has been recognized that such formulae are inaccurate. Recently the Chronic Kidney Disease in Children (CKiD) study reported a "bedside formula" to eGFR in non-transplanted children (CKID-BS= 0.412 x ht/S_{Cr}). The objective of our study is to validate the CKiD-BS to eGFR in children post-renal transplant.

Methods: Plasma disappearance of ^{125}I-iothalamate was used to measure GFR (C_{IO}). Data collected included: patient demographics, diagnosis, height, weight, S_{Cr}, serum albumin (S_{alb}) and BUN. Multiplicative models of C_{IO} were fit by regressing log C_{IO} on log-transformed covariates using mixed linear regression to account for correlation among repeated measurements within patients. Sensitivity and specificity of estimates for predicting C_{IO} < 90 mL/min /1.73 m^2 were evaluated at the nominal level.

Results: 220 C_{IO} studies were performed on 123 post-transplant renal patients. Mean age at C_{IO} was 13.8 ± 4.6 (age range: 2.7-20.8 years). CKiD-BS underestimates C_{IO} in our cohort by an average of 11% with underestimates exceeding 20% at high GFR. K in the CKiD-BS was recalibrated (eGFR= 0.461 x ht/S_{Cr}) in post-transplant children (R-Bedside). We also developed a GFR model = 10.73 x (ht)$^{0.51}$ /(S_{Cr})$^{0.90}$ x (BUN)$^{0.23}$ (GFR-M). Incorporating gender, ethnicity and S_{alb} did not affect the predictive value of our GFR model. In eGFR in <90 ml/min/1.73m^2 range, our GFR-M shows the highest specificity at 55.6% compared to 22.2% for CKiD-BS formula. Sensitivities of both estimates were similar (98.9% and 94.9% respectively).

Conclusions: Our R-Bedside formula, although more accurate than CKiD-BS, has the same diagnostic performance, the two differing only by a multiplicative factor. Our GFR-M did outperform the CKiD-BS by achieving higher specificity. Despite continued refinement, formulae to eGFR remain inaccurate and complex for clinical use. GFR measurement remains the gold standard in estimating renal function in children with renal transplant.

TH-PO1072

CKD-EPI GFR Estimating Equations in Kidney Transplantation: Which Added Value for the New Cystatin C-Based Equation?

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[1]Renal Dpt, University Hospital, Saint-Etienne, France; [2]Dialysis Dpt, University Hospital, Liege, Belgium; [3]Transplantation Dpt, University Hospital Toulouse, Toulouse, France.

Background: The absence of a standardized measurement for serum cystatin C (Scyst) has hindered a thorough evaluation of Scyst to predict GFR in renal transplantation. We took advantage from the recent validation of a reference calibrator for Scyst and from the re-expression of the CKD-EPI Scyst-based equations to reassess its role as a biomarker of renal graft function.

Methods: We studied (i) the relationship of Scyst and Screat with inulin clearances performed in 670 stable transplant patients, (ii) the influence of non-GFR determinants upon Scyst concentration, and (iii) compared the performance of the CKD-EPI Scyst-based equations with that of the CKD-EPI serum creatinine (Screat) equation.

Results: Scyst level alone was significantly superior to Screat to discriminate patients at different GFR thresholds with ROC AUC superior to 0.90. The non-GFR determinants independently associated to Scyst were BMI, gender, proteinuria and CRP. As compared to the CKD-EPI Screat equation, the Scyst CKD-EPI equations performed better. However they did not significantly ameliorate the classification of patients across CKD stages nor the detection of patients with severe dysfunction defined as an inulin < 20.

Our data do not support implementation of Scyst for the evaluation of renal graft function in clinical practice. However, given (i) that Scyst remains a better endogenous GFR marker than Screat in transplant patients and (ii) that the non GFR determinants of Scyst are also well-defined cardiovascular risk factors, Scyst might be a surrogate for cardiovascular complications after transplantation.

Conclusions: In conclusion, while offering a better GFR estimation, Scyst equations have limited clinical added value. The possibility of using Scyst not solely as a GFR predictor but rather as a predictor of patient outcome should however stimulate further research in renal transplantation.

TH-PO1073

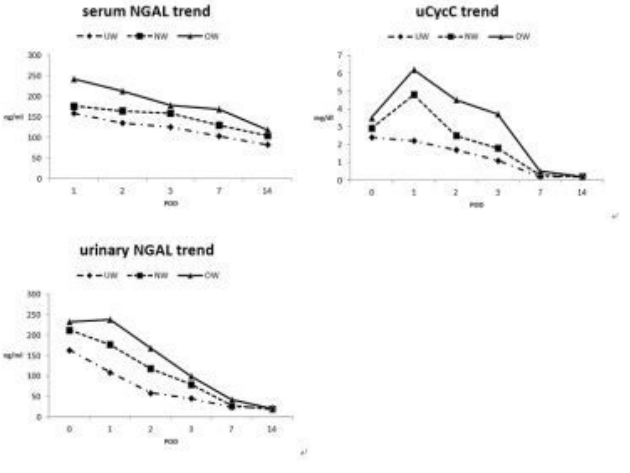
NGAL and Cystatin C Elevations Were Associated with Short-Term Graft Function in Over Weight Recipients after Kidney Transplantation

Junko Kohei, Shunji Shiohira, Ken Tsuchiya, Kosaku Nitta. *Fourth Department of Internal Medicine, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.*

Background: Recent studies have shown that pre-transplant over weight (OW) and obesity has no significant impact on long-term post-transplant outcomes comparing with normal weight (NW) recipients. On the other hand, it still has been reported opposite results that obesity has influenced on short-term graft function, graft survival and mortality. We studied that whether pretransplant body mass index (BMI) influenced short-term graft function or not immediately after kidney transplantation by measuring biomarkers.

Methods: This study was prospective, single-center study of living-donor kidney transplant patients. We collected serial urine and blood specimens first 6 hours and 1,2,3,7,14 postoperative days and examined concentration of serum and urinary neutrophil gelatinase-associated lipocalin (NGAL), urinary interleukin 18 (IL-18), urinary liver-type fatty acid binding protein (L-FABP), urinary cystatin C(uCysC), urinary N-acetyl-β-D-glucosaminidase (NAG).Patients were divided into three groups, UW (Under Weight:BMI<20kg/m^2), NW(BMI20-24.9kg/m^2) and OW(BMI>25 kg/m^2) by pretransplant BMI.

Results: In the results, 35 patients were in UW, 37 were in NW, and 19 were OW group, only two cases in OW were slow graft function. UCysC, Serum and Urinary NGAL in OWhad remained the highest for over a week, it suggested that association with short-term graft function. Other biomarkers did not show any significant differences among the three groups.



Conclusions: This is a first report showing an correlation of BMI and short-term graft function.Kidney injury immediately after kidney transplantation is difficult to diagnose with only serum creatinine, therefore this research has a high possibility of helping to diagnose delayed graft function in the postoperative early stage.

TH-PO1074

Predictive Value of Contrast Enhanced Sonography in the Early Period after Kidney Allograft Transplantation Lars Kihm, Joerg Seckinger, Christian Morath, Claudia Sommerer, Martin G. Zeier, Vedat Schwenger. *Nephrology, University of Heidelberg, Heidelberg, Germany.*

Background: Real-time contrast-enhanced sonography (CES) can assess microvascular tissue perfusion using gas-filled microbubbles. The study was performed to evaluate the feasibility of CES in predicting early allograft function in comparison to color Doppler ultrasonography (CDUS).

Methods: A total of 50 consecutive renal transplant recipients were prospectively studied using CES and conventional CDUS investigation at day 7 post transplant. Transplant tissue perfusion imaging was performed by low-power imaging during intravenous administration of the sonocontrast Sonovue™. Renal tissue perfusion was assessed quantitatively using flash replenishment kinetics of microbubbles to estimate renal blood flow A *ß (A = peak signal intensity, ß= slope of signal intensity rise). The obtained sonography values were correlated with clinical selected data on day 7, 90 and 365 in the post-transplant period.

Results: In contrast to conventional CDUS resistance and pulsatility indices, renal blood flow estimated by CES at day 7 post transplant was significantly related to kidney allograft function at day 90 ($r = 0.81$, $p < 0.001$) and day 365 ($r = 0.74$, $p < 0.001$). An estimated renal blood flow of A *ß > 11.2 dB/s was able to predict a GFR of above 45ml/min one year after kidney transplantation with high specifity and sensitivity. Determination of renal blood flow by CES revealed a significant correlation to donor age ($r = -0.55$, $p < 0.001$) but not recipient age, whereas conventional CDUS resistance index was significantly related to recipient age ($r = 0.49$, $p < 0.001$) but not donor age.

Conclusions: This is the first prospective study demonstrating the prognostic value of CES early after kidney transplantation. In contrast to CDUS, CES reveals information of kidney allograft perfusion independent of the recipients vasculature.

TH-PO1075

Early-Onset Anemia after Transplantation Is an Independent Risk Factor for Graft Loss in Renal Recipients Julio Pascual,[1] Manuel Arias,[2] Josep Maria Campistol Plana,[3] J. Grinyo,[4] Domingo Hernández,[5] J. Morales,[6] Luis M. Pallardo,[7] Daniel Seron.[8] *[1]H.Mar, Barcelona, Spain; [2]H.U.Marqués Valdecilla, Santander, Spain; [3]H.Clínic, Barcelona, Spain; [4]Ciutat Sanit. Bellvitge, Hospitalet, Spain; [5]H.Carlos Haya, Málaga, Spain; [6]H.12 Octubre, Madrid, Spain; [7]H.U.Dr. Peset, Valencia, Spain; [8]H.Vall Hebron, Barcelona, Spain.*

Background: Our aim was to explore the relationship between post-transplant anemia and graft survival after renal transplantation(RT).

Methods: Multicenter, retrospective, observational study in patients(pts) with RT in 2007 in Spain who were followed up until 3 years(y), graft loss or death. We used multivariate Cox regression to predict death-censored graft survival.

Results: We included 675 pts (65% male, mean(SD) age 52(13)y, 7% PRA>10%, donor age 51(15)y and 57% of donors with stroke); 17% suffered acute rejection(AR) and 10% lost their graft. The prevalence of post-RT anemia (hemoglobin[Hb]<11 g/dL and/or treatment with erythropoietin[EPO] and/or transfusion) over time was: 84% at 7 days(d), 76% 14d, 59% 1 month(m), 41% 2m, 34% 3m, 26% 6m, 16% 12m, 14% 2y, 18% 3y. Anemia at 1m was the most strongly associated to evolution of graft function. MDRD-GFR at 14d in subgroups defined by anemia at 1m was: 22(15)ml/min in untreated anemics (Hb<11g/dl without EPO,n=60), 25(18)ml/min in anemia non-responders (Hb<11g/dl with EPO,n=134), 35(19)ml/min in anemia responders (Hb≥11 g/dl with EPO,n=176) and 45(19) ml/min in non-anemics (Hb≥11g/dl without EPO,n=253). By 2m, pts with Hb<11g/dl at 1m (with or without EPO) had still a lower renal function than non-anemics. After adjusting by change in MDRD-GFR between 14d-2m (hazard ratio[HR](95%CI) 0.97(0.95-0.99) for each +1ml/min,p=0.02), untreated or non-responding anemia at 1m remained as an independent predictor of graft loss (HRs 4.6(0.98-21.8) and 3.9(1.1-13.6) for Hb<11 without or with EPO, respectively vs Hb≥11 without EPO[ref.];p=0.05/0.03), along with a PRA>10% (HR 3.3(1.1-10.0),p=0.04), a donor with stroke (HR 3.4(1.1-10.5),p=0.04) and >1 AR (HR 6.6(1.2-35.4) vs no AR,p=0.03).

Conclusions: In RT recipients, untreated or non-responding anemia at 1m seems to be an independent risk factor for graft failure.

Funding: Pharmaceutical Company Support - Amgen, S.A.

TH-PO1076

Independent Risk Factors for the Development of Urinary Tract Infection (UTI) in 1175 Renal Transplant Recipients and the Relationship between UTI with Bacteremia, Acute Cellular Rejection, and Allograft Function in the First Year of Transplantation John R. Lee, Thangamani Muthukumar, Manikkam Suthanthiran. *Nephrology and Hypertension, Weill Cornell Medical College, New York, NY.*

Background: UTI is the most common infection in renal transplant recipients and earlier studies have been limited in size and scope. Herein, we examined risk factors for the development of UTI and its impact in 1175 consecutive renal transplant recipients.

Methods: We reviewed the records of 1175 transplant recipients who underwent renal transplantation from 2005 to 2010 for the development of UTIs, defined as greater than 10^4 cfu/mL, within the first three months of transplantation. We collected demographical data and performed logistic regression analyses to identify independent risk factors for the development of UTIs. We also evaluated the following outcomes: bacteremia within 3 months, acute cellular rejection (ACR) within 1 year, and eGFR at 1 year.

Results: 583 of 1175 transplant recipients (49.6%) developed an episode of UTI. In a multivariable logistic regression, the independent risk factors for development of UTIs were: female gender (Odds Ratio [OR]:4.9,P<0.001), age > 65 years (OR:1.9,P<.001), stent placement (OR:1.5,P=.002), and lack of bactrim prophylaxis (OR:2.1,P=.030). Type of intra-operative antibiotic prophylaxis, type of induction therapy, and steroid utilization were not significantly associated with UTI development. Bacteremia within 3 months was strongly associated with UTIs (7.2%[UTI group] vs. 2.2%[no UTI group], p<.001). At 1 year, ACR and eGFR were not significantly different between transplant recipients with UTI and those without UTI (ACR: 6.3%[UTI] vs. 5.9%[no UTI],p=.112) (eGFR: 51.0 mL/min/1.73m^2[UTI] vs. 51.6 mL/min/1.73 m^2[no UTI],P=.636).

Conclusions: Independent risk factors for the development of UTI include female gender, age > 65 years, stent placement, and lack of bactrim prophylaxis. UTI was associated with bacteremia within 3 months but not associated with ACR or decline in renal function at 1 year. Prevention of bacteremia, a serious transplant complication, may warrant further studies on antibiotic prophylaxis targeting a population at high risk for UTI.

TH-PO1077

The Impact of Early and Late Acute Rejection on Graft Survival in Renal Transplantation Min Su Kim,[1] Eun Hee Koo,[1] Hye Ryoun Jang,[1] Jung Eun Lee,[2] Yoon-Goo Kim,[1] Dae Joong Kim,[1] Ha Young Oh,[1] Wooseong Huh.[1] *[1]Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Joslin Diabetes Center, Boston, MA.*

Background: Previous studies investigated whether the timing of acute rejection (AR) episode influences long-term graft survival after kidney transplantation and showed that late acute rejection (LAR) has a detrimental impact on long-term graft survival compared to early acute rejection (EAR). We evaluated whether EAR or LAR influences graft survival or patient survival, and analyzed risk factors for EAR and LAR.

Methods: We performed a retrospective cohort study including 1126 patients who received kidney transplantation between Feb 1995 and Dec 2009 at Samsung Medical Center. Patients who were under 18 years of age or had multiple organ transplants (e.g. kidney and liver or kidney and pancreas) or had primary non-functioning allografts were excluded. All patients were divided into 3 groups: no rejection, EAR, or LAR. EAR and LAR were defined as rejection before 3 months and after 3months, respectively. Differences in graft survival and patient survival among the 3groups were analyzed.

Results: Of the total 1067 patients, 278 patients (26.1%) had biopsy- proven AR. EAR and LAR developed in 142 patients (51.1%)and 136 patients (48.9%), respectively. Further, 789 patients had no rejection episode (73.9%). Initial immunosuppression regimen was different between EAR and no rejection group. Donor recipient relationship was different from EAR and LAR group as compared to no rejection group. Recipients with EAR were more likely to be male compared to the no rejection group (P= 0.000). EAR and LAR primarily had older donors than no rejection group (P=0.000, P=0.002 respectively). There was no difference in patient survival among the 3 groups. And there was no difference between EAR and LAR in overall graft survival (P= 0.207) and patients survival (P=0.972).

Conclusions: There were no differences in graft survival and patient survival between EAR and LAR. AR, regardless of its timing, significantly worsened graft survival. A treatment targeted at reducing the incidence of AR and improving the prognosis is needed.

TH-PO1078

Long-Term Graft Outcome in a Large Cohort of Renal Transplant Recipients with Protocol Biopsies Wilfried Gwinner,[1] Irina Scheffner,[1] Tanja Abeling,[1] Jan U. Becker,[1] Verena Broecker,[3] Michael Mengel,[2] Hans H. Kreipe,[1] Anke Schwarz,[1] Hermann G. Haller.[1] *[1]Hannover Medical School, Germany; [2]University of Alberta, Canada; [3]University of Cambridge, United Kingdom.*

Background: This study examines renal graft losses over a period of up to 10 years in patients (pts) with protocol biopsies (pBx) (total observation: 4805 pts-years).

Methods: 529 male and 363 female pts with a kidney (or kidney+other solid organ) transplantation (Tx) between 2000-2007 were included. pBx were taken at 6 weeks (n=738), 3 (n=776) and 6 months (n=737) post-Tx. 862 biopsies for cause were taken within the first year and 262 thereafter. Lost-to follow-up was negligible (n=15).

Results: Patient and graft survival was 80% at 5 years and 68% at 10 years. Graft survival was 88% at 5 years and 82% at 10 years. Graft loss occurred in 119 pts and was related to acute rejection (26%), chronic rejection (5%), non-specified function decline (19%), infection (8%), recurrent GN (3%), primary marginal function (14%). Graft losses were less frequent in living-donor or combined kidney/pancreas Tx and more frequent in extended donor criteria-Tx. After correcting for potential confounding by these factors, the following variables were related to graft loss: higher donor's age & S-creatinine, pre-formed antibodies, delayed graft function, lower GFR within first 6 weeks, cardiovascular disease and higher body mass index. In pBx, BANFF IA/B rejections were more frequent in pts with graft loss compared to pts without graft loss (0.31 vs 0.17/patient). In biopsies for cause, frequencies for borderline rejections (0.36 vs. 0.19), BANFF IA/B rejections (0.39 vs. 0.13) and BANFF IIA/B rejections (0.32 vs. 0.05) were higher in pts with graft loss, as well as signs for humoral rejection (glomerulitis/±c4d: 0.40 vs. 0.06, peritubular capillaritis/±c4d 0.16 vs. 0.05).

Conclusions: Donor-derived organ quality, recipient's cardiovascular disease and immune-mediated injury remain as important challenges to improve the long-term outcome of renal Tx pts. Immune-mediated injury/inflammation is clearly increased in protocol biopsies from pts with graft failure.

Funding: Government Support - Non-U.S.

TH-PO1079

Long-Term Patient Outcome in a Large Cohort of Renal Transplant Recipients with Protocol Biopsies Wilfried Gwinner,[1] Tanja Abeling,[1] Irina Scheffner,[1] Jan U. Becker,[1] Verena Broecker,[2] Michael Mengel,[3] Hans H. Kreipe,[1] Anke Schwarz,[1] Hermann G. Haller.[1] *[1]Hannover Medical School, Germany; [2]Cambridge University, United Kingdom; [3]University of Alberta, Canada.*

Background: This study examines the deaths over a period of up to 10 years in renal transplant (Tx) patients (pts) with protocol biopsies (pBx) (total observation: 4805 patient-years).

Methods: 529 male and 363 female pts with a kidney Tx (or kidney+other solid organ) between 2000-2007 are included. pBx were taken at 6 weeks (n=738), 3 (n=776) & 6 months (n=737) post-Tx. 862 biopsies for cause were taken in the 1st year and 262 thereafter. Lost-to follow-up was negligible (n=15). Subclinical & clinical acute rejections and clinical borderline rejections in pBx were treated.

Results: Patient and graft survival was 80% at 5 years and 68% at 10 years. Patient survival was 92% at 5 years and 82% at 10 years. 98 deaths occurred, relating to infection (24%), cardiovascular disease (15%), malignancy (16%), other specified causes (8%); 37% were unclear. Deaths were less in living-donor and kidney/pancreas Tx and more frequent for extended donor criteria-Tx. After correcting for potential confounding by these factors, the following variables related to death: higher age, prolonged cold ischemia time, delayed graft function, lower GFR in the first 6 weeks, HLA-DR mismatch, cardiovascular disease, diabetes mellitus. In pts who died, cellular and humoral rejections in pBx were as frequent as in survivors. In biopsies for cause, BANFF IA/B acute rejections were more frequent in pts who died, with 0.18 rejections/patient compared to 0.13 in survivors. Signs for humoral rejection (glomerulitis/peritubular capillaritis, ±c4d) were more often seen in pts who died (0.16 vs. 0.11). The proportion of anti-rejection treatments/increase in the maintenance immunosuppression was not higher in pts who died from infections or malignancies, compared to pts dying from other causes.

Conclusions: Inferior graft function is related to death, besides the classical risk factors age, cardiovascular disease and diabetes mellitus. Increased treatment of rejections detected in pBx apparently does not lead to fatal malignancy or infectious complications.

Funding: Government Support - Non-U.S.

TH-PO1080

Long Term Outcomes of Kidney Transplantation in Patients above Age 70: A Single Center Experience Samia Sheikh, Tim E. Taber, Muhammad Ahmad Mujtaba, Dennis P. Mishler, William Goggins, Asif A. Sharfuddin, Muhammad S. Yaqub. *Indiana University School of Medicine/ IU Health, Indianapolis, IN.*

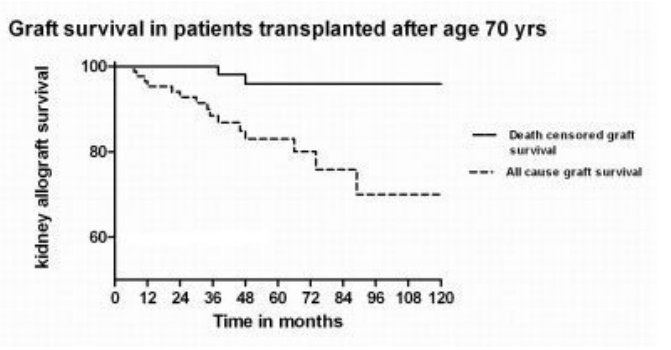
Background: Over the last decade elderly patients (70 yr and older) are the fastest growing ESRD group requiring renal replacement therapy. With its increasing success, kidney transplantation has been offered to a growing number of elderly patients.

Methods: To evaluate our institutional outcomes of geriatric renal transplants over a 10 year period and examine graft and patients survival rates with respect to both living and deceased donor kidney transplants in patients aged more than 70 years. Retrospective chart review from clinical database from 2001- 2010 of recipients who were age 70 or above at the time of kidney transplant. Baseline characteristics are in table 1.

Baseline Charecteristics n= 86

Mean age	76.1±3.6
Age 70-75	55 (64%)
Age > 75	33 (36%)
Male	53 (61%)
White	71 (82%)
Blacks	9 (10%)
Deceased Donor	67 (77%)
ESRD Cause	
HTN	29 (33%)
DM	17 (19%)
GN	19 (22%)

Results: Mean age was 76.1± 3.1. Mean follow up was 54± 29.7 months. Death with functioning graft was the most common reason for graft loss. 1 and 5 yr graft survival were 95 and 83% respectively. 1 and 5 yr patient survival were 95 and 89% respectively. Rejection rate was 8% at 1 year. SCr was 1.334± 0.48 at 1 yr and 1.26 ± .48 at 5 years. There was no significant difference in patient and graft survival in recipients between age 70 to 75 and above 75 years.



Conclusions: With careful selection elderly recipients above age 70 have excellent patient and graft survival.

TH-PO1081

Very Long Term Survivors of Kidney Transplantation Carol A. Traynor, Patrick O'Kelly, Peter J. Conlon. *Nephrology and Transplantation, Beaumont Hospital, Dublin 9, Ireland.*

Background: There have been few studies of patients with renal allografts functioning for more than 30 years. We sought to identify clinical factors associated with 30 year renal allograft survival and to determine the prevalence of comorbidities in these patients.

Methods: The National Renal Transplant Database was retrospectively analysed. All kidney transplants (n=349) between 1st January 1970 and 31st August 1981 were included. Data extracted included baseline recipient and donor demographic data, and occurrence of acute rejection. Follow up analysis was until August 1, 2011. Patients charts were reviewed and data regarding comorbidities was recorded.

Results: During the study period, there were 349 transplants in 324 patients. Of these, 44 patients (12.6%) had a renal transplant functioning for in excess of 30 years. Table 1 compares the characteristics between those who had a functioning kidney transplant for in excess of 30 years (survivors) to those whose transplant functioned for less than 30 years (non survivors). Those with a transplant functioning for in excess of 30 years were more likely to be younger, have fewer HLA mismatches and have a living related donor. Acute rejection occurred in 60.6% of non survivors and 48.7% of survivors (p=0.157).

Median creatinine was 102 µmol/L (range 66-386µmol/L).

The prevalence of ischaemic heart disease, skin malignancy and other malignancy was 13.6%, 45.4% and 18% respectively.

Baseline Demographics of Patients

Variable	Non Survivors (n=280)	Survivors (n=44)	p value
Recipient age (years)	35.0+/-11.6	28.4+/-10.1	<0.001
Donor age (years)	27.8+/-15.3	25.7+/-10.1	0.940
Recipient gender (male)	67.2%	63.6%	0.638
Donor gender (male)	62.9%	51.3%	0.160
Cold ischaemia time(hours)	11.1+/-5.0	11.1+/-6.1	0.794
No. of HLA mismatches(mean)	1.36+/-1.2	0.26+/-0.75	<0.001
Living donor	12.5%	52.3%	<0.001

survivors=single functioning transplant for 30 years

Conclusions: We identified important clinical factors associated with very long term transplant survival. These results highlight the importance of promoting the use of a living donor. We also identified a high incidence of cancer emphasising the need for continued close surveillance of these patients following their prolonged exposure to immunosuppression.

Funding: Private Foundation Support

TH-PO1082

Rejection Is Comparable in Matched African- and Caucasian-American Recipients of Kidney Transplants with Lupus Gabriel Contreras, Hua Li, Roque A. Diaz-Wong, Tamara Isakova, Leonardo Tamariz, Adela D. Mattiazzi, Giselle Guerra, Amna Ilahe, Yoel Brito, Warren L. Kupin, David Roth, Myles S. Wolf. *Medicine, University of Miami Miller School of Medicine/Jackson Memorial Hospital, Miami, FL.*

Background: African-American recipients of a kidney allograft with lupus have high prevalence of predictors for rejection, which can explain their poor outcomes.

Methods: Of 1627 African- and 1869 Caucasian-Americans who received kidney transplants between 1987 and 2006 with complete UNOS records, a cohort of 780 pairs of recipients were matched in 16 baseline predictors employing a predicted probability of group membership based on observed predictors obtained from a multivariable logistic regression model. Primary outcome was rejection. Secondary outcomes were delayed graft function, mortality, and composite of allograft failure or mortality.

Results: The matched pairs were predominantly female recipients (82.6%) with a mean age of 39.2±11.3 years. 91.4% recipients received kidneys from deceased donors with 23.3% from expanded criteria donors. African- and Caucasian-Americans matched well (P>0.05): recipient age, gender, education and insurance; donor age, gender and race; and the predictors of need of dialysis prior to transplant, kidney from expanded criteria donor, cold ischemia time, history of prior kidney transplant, panel reactive antibodies, HLAs mismatch, ABO blood type compatibility and transplant Era. Only donor type was significantly different between both groups (deceased donor 89.9% vs. 93%, p=0.03).

Contrary to the unmatched groups with significantly higher rejection events in African-compared to Caucasian-Americans (39.9% vs. 34.4%, p=0.0007), matched pairs had similar rejection events (38.3% vs. 36.8%, p=0.53). Matched pairs had similar delayed graft function (23.1% vs. 20.6%, p=0.24), and mortality (13.3% vs. 15.8%, p=0.17), but different composite event of allograft failure or mortality (43.5% vs. 38.5%, p=0.04).

Conclusions: In lupus recipients of kidney allograft, African- and Caucasian-Americans have similar incidence of rejection when predictors are matched between groups.

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TH-PO1083

Renal Phosphate Handling and Long-Term Outcome after Kidney Transplantation Leandro Cunha Baia,[1] Stephan J.L. Bakker,[1] Marc G. Vervloet,[2] Gerjan Navis,[1] Martin H. De Borst.[1] *[1]Nephrology, University Medical Center Groningen; [2]Nephrology, VU-MC, Amsterdam, Netherlands, for NIGRAM.*

Background: In the first year after kidney transplantation (Tx) a renal phosphate leak frequently develops due to persistently elevated FGF23 and PTH levels. Whether this leak persists >1 year after Tx for a Western population and whether this has consequences for patient or graft survival is unknown.

Methods: Serum phosphate, fractional phosphate excretion (PE) and TmP-GFR were determined in 598 stable kidney transplant recipients (KTR) >1 yr (7.9±6.4 yrs) after Tx and in 146 kidney donors as healthy controls. Both groups were compared by t-test or Mann Whitney. In KTR we subsequently assessed the association of serum phosphate, PTH, FGF23, TmP-GFR and 24h-urinary PE with the composite endpoint of all-cause mortality and graft failure by multivariable Cox regression with adjustment for age, gender, creatinine clearance (CrCl), proteinuria, 24h-urea excretion, traditional cardiovascular risk factors and graft vintage. Data are presented as mean±SD or median[interquartile range] where appropriate.

Results: Serum phosphate was similar between KTR and controls (3.3±0.7 vs 3.3±0.6 mg/dl), but TmP-GFR (2.4±0.7 vs 2.8±0.7 mg/dl, p<0.05) was lower in KTR and fractional PE was higher in KTR (29±11 vs 12±5, p<0.05), suggesting phosphate leak. KTR with CrCl >60 ml/min had lower serum phosphate (3.1±0.6 mg/dl), but KTR with CrCl <30 ml/min had higher (4.1±0.8 mg/dl) serum phosphate than controls (both p<0.05). At follow-up of 6.4±1.7 yrs, 134 (22%) KTR had died and 54 (9%) had lost their graft. Higher FGF23 (full model hazard ratio 1.68[95% CI 1.30-2.18] per 1 SD, p<0.001) and serum phosphate (1.36[1.04-1.77] per 1 mg/dl, p=0.02) were associated with an increased risk of the composite endpoint. TmP-GFR (1.24[0.93-1.65], p=0.15) and PTH (1.25[0.94-1.66], p=0.13) showed similar trends. Higher PE protected against graft loss (0.61 (0.47-0.81) per 1 g/d, p=0.001).

Conclusions: Our data suggest that renal phosphate leak persists >1 year after kidney Tx, but it is overruled by a CrCl <60 ml/min. Elevated FGF23 and serum phosphate are associated with mortality and graft failure after Tx, independent of traditional risk factors.

Funding: Government Support - Non-U.S.

TH-PO1084

25-Hydroxy Vitamin D Status and the Incidence of Acute Cellular Rejection in Kidney Transplant Recipients Amanda Ingemi,[1] Megan Rech,[1] James Fleming,[1] Nicole R. Pinelli,[2] Carol Moore.[1] *[1]Henry Ford Hospital, Detroit, MI; [2]College of Pharmacy, UNC, Chapel Hill, NC.*

Background: Vitamin D is a steroidal hormone that can elicit immunomodulatory properties. Limited human and animal data have shown that active 1,25-OH vitamin D may improve renal transplant outcomes; however, the influence of its precursor, 25-OH vitamin D, on renal transplant outcomes is unknown.

Methods: This retrospective cohort study included patients transplanted from 1/1/04-8/30/10 who were ≥18 years, had at least one 25-OH vitamin D level following transplantation, and had 1 year follow-up. The patients were divided into 2 groups based on their mean 25-OH vitamin D levels taken from their first year post-transplant or until first rejection: vitamin D insufficient (VDI) ≤20ng/mL and vitamin D sufficient (VDS) >20ng/mL. The primary outcome was treated or biopsy proven acute cellular rejection. The secondary outcomes were BK viremia, CMV infection, delayed graft function (DGF) (defined as HD within 7 days of transplant), and graft loss. A multivariate logistic regression model was built with backward selection.

Results: A total of 669 patients were screened; of those, 203 met the inclusion criteria (53% VDI group, 53±13 years, 58% male, 51% African American, 9% with a previous kidney transplant, and 82% received dialysis) were included in the analysis. Baseline demographics were comparable between groups except for additional extended criteria donations (ECD) in the VDI group (19% vs. 7%, p=0.04). Compared to the VDS group, acute rejection occurred more often in the VDI group (22% vs. 8%, p<0.01). On multivariate analysis, after adjusting for ECD, panel reactive antibody >20%, and deceased donors, the VDI group was independently associated with rejection (OR 3.1; 95%CI 1.3-7.3). The incidence of developing BK viremia (21% vs. 18%, p=0.54), CMV infection (14% vs. 20%, p=0.25), and graft loss (7% vs. 4%, p=0.45) was comparable between groups. In contrast, DGF occurred more often in the VDI group (34% vs. 18%, p=0.01).

Conclusions: This data suggests low vitamin D levels are associated with an increased incidence of acute cellular rejection. Future prospective studies are needed for validation and to examine supplementation.

TH-PO1085

Prediction of Kidney Failure after Liver Transplantation Tobias J. Weismüller,[1] Christian Lerch,[2] Eleni Evangelidou,[3] Christian P. Strassburg,[1] Hermann G. Haller,[3] Mario Schiffer.[3] *[1]Gastroenterology, Hannover Medical School; [2]Pediatrics, Hannover Medical School; [3]Nephrology, Hannover Medical School.*

Background: Kidney failure after orthotopic liver transplantation (OLT) is a serious concern and currently no concepts exist to predict GFR development in these patients. We performed a retrospective analysis in 320 patients after OLT and followed their kidney function for 3 years.

Methods: We developed models for predicting CKD stages at 1 and 3 years after liver transplantation. Models were fitted for (i) covariables available before Tx, (ii) peri- and postoperative factors and (iii) immunosuppression. We checked discrimination, calibration and internal validity of the models.

Results: We detected within the group of patients 3 subgroups with a distinct pattern for their GFR development. We identified patients starting with a GFR (˜73ml/min) that developed a significant loss of GFR within the first year (GFR ˜36ml/min) after transplantation with no improvement over the time period of 3 years. A second group of patients starting with a GFR (˜69 ml/min) developed a significant loss of kidney function at year 1 after transplantation (GFR ˜51ml/min) and showed a significant improvement of kidney funktion at year 3 with a GFR of ˜63 ml/min. A 3rd group started with a GFR of ˜61ml/min and showed an improvement of kidney function at year 1 (GFR ˜69ml/min) that was maintained over the 3 years. Comparing baseline characteristics of the respective groups, short CIT, lower age and short ICU stay were associated with a better development of kidney function. In a clinical prediction model we found predicted probabilities for GFR very close to observed probabilities for development of CKD 3 or worse. However, prediction of CKD 4 or 5 was less stable.

Conclusions: Imputing the classical risk factors in a prediction model leads to a good prediction of loss of kidney function, but cannot predict whether a patient would lateron require dialysis. The patterns that we recognized further underline that OLT patients are a very heterogeneous patient group and that even if kidney function deteriorates in the first post transplant year we cannot foresee if the patients kidney function recovers or not.

Funding: Government Support - Non-U.S.

TH-PO1086

Liver Transplantation for Hereditary Renal Amyloidosis: the United Kingdom Experience Arie J. Stangou, Bridget Gunson, Peter Ashcroft, Paolo Muiesan. *Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Background: The liver is the exclusive source of the amyloidogenic protein in hereditary fibrinogen α-chain amyloidosis (AFib) and responsible for production of 50% of the variant apolipoprotein in ApoAI amyloidosis (AApoAI). Variant lysozyme production is extrahepatic but liver and kidneys can be targets for amyloid deposition in hereditary lysozyme amyloidosis (ALys). We evaluated the role of liver transplantation (LT) in the hereditary renal amyloidoses and present the long-term outcomes in AFib, AApoAI and ALys amyloidosis.

Methods: Between 1993-2011, fifteen patients with hereditary renal amyloidosis received LT in the UK. Ten patients (median age 58 years) with AFib amyloidosis and renal failure had combined liver/kidney transplant (LKT). Three patients aged 50, 52 and 56 years received LKT for AApoAI amyloidosis with renal and liver failure. Two siblings with ALys amyloidosis and extensive hepatic amyloid received LT as emergency at 15 and 24 years old for spontaneous liver rupture.

Results: At median posttransplant fol-up of 77 (range 12-175) months, 7/10 AFib patients are alive with no amyloid progression after LKT (cumulative survival 70%). Two AFib patients transplanted before dialysis, retain stable native kidney function at 8 and 7 years post-LKT. Three fatal outcomes occurred in long-term dialysis cases and were due to vascular or biliary complications. All 3 patients with AApoAI amyloidosis maintain normal dual graft function with no amyloid progression at 36, 109 and 152 months post-LKT. The first of the 2 ALys patients who received urgent LT died at 12 years with massive haemorrhage related to intestinal amyloidosis, while the second is alive at 8 years with good graft function.

Conclusions: 1. Combined LKT for end-stage renal failure in AFib is curative, but incurs significant risks. Preemptive isolated LT at early stages of amyloid nephropathy may halt renal amyloid progression. 2. The addition of LT to kidney transplant is curative in AApoAI amyloidosis, however, the indication of LT in AApoAI is reserved for liver failure. 3. LT is life-saving, albeit not curative, in ALys patients with amyloid liver failure and posttransplant life expectancy is acceptable.

TH-PO1087

The Impact of Preoperative Renal Function and Proteinuria in Patients Following Heart Transplantation Lutfi Alkorbi, Mamdouh N. Albaqumi. *Medicine, KFSHRC.*

Background: Preoperative conditions play a major role in solid organ transplantation outcome. The purpose of this study is to assess the clinical impact of preoperative impaired renal function and proteinuria in heart transplant recipients.

Methods: Patients with heart transplant from 2003 until 2010 were included. Clinical and laboratory data were collected. Based on their preoperative eGFR, they were divided into two groups: patients with impaired renal function (GFR <60 ml/min/1.73m^2) and patients

with normal renal function (GFR ≥ 60 ml/min1.73m^2). Outcome was measured as acute kidney injury using AKI Network Definition, the need for renal replacement therapy, and mortality at both 7 and 30 days.

Results: A total of 92 patients were identified. There was no difference between patients with impaired renal function and those with normal renal function in terms of acute kidney injury or the need for renal replacement therapy in the first 7 days. However, patients with impaired renal function had a higher mortality rate in the first 7 days compared to patients with normal renal function (15.4% vs. 3%, P=0.008). Mortality rate at 30 days was also significant in patients with impaired renal function (27% vs. 3%, P=0.008) as well as the need for renal replacement therapy (18.2% vs.3.1%, P=0.017) at 30 days but with no difference in AKI between the two groups. When we used preoperative proteinuria to divide the patients into two groups, we found that patients with proteinuria had a higher mortality rate (12.9% vs. 1.7%, P=0.024) and higher need for renal replacement therapy in the first 7 days (13.8% vs. 1.7%, P=0.020) than those without proteinuria. This difference disappeared at 30 days post heart transplant. Patients with both proteinuria and impaired renal function have a significant mortality rate at 30 days (57%).

Conclusions: Impaired renal function has a significant impact on mortality and the need for renal replacement therapy at 30 days. Proteinuria alone has no significant impact in the first 30 days, but when combined with renal impairment, it has a detrimental impact on 30 days mortality and need for renal replacement therapy.

TH-PO1088

Treatment of Cirrhosis-Associated Hyponatremia Refractory to Vasopressin 2-Receptor Antagonist Ajay Yadlapati, Minhtri K. Nguyen. *Nephrology, UCLA, Los Angeles, CA.*

Background: Hyponatremia is a common laboratory finding and reason for admission in patients with end stage liver disease (ESLD). In cirrhotic patients whose hyponatremia fails to correct with free water restriction, vasopressin 2 receptor antagonist (V2RA) is an effective alternative therapy. In this case report, we describe a patient with hepatorenal syndrome (HRS) whose hyponatremia failed to improve with tolvaptan and conivaptan but subsequently corrected with treatment with midodrine and octreotide.

Methods: A 51-year-old female with history significant for ESLD was admitted for a serum sodium of 118mmol/L. The patient's hyponatremia failed to improve with free water restriction, so tolvaptan was started. Despite adequate up-titration, her serum sodium level remained unchanged. Due to the potential concern for inadequate GI absorption she was started on IV conivaptan, which did not help. On day 13, she was started on midodrine and octreotide for treatment of acute kidney injury thought to be due to HRS, which slowly brought the patient's serum sodium level from 122 to 132mmol/L over a 5 day course coinciding with the resolution of the HRS. Repeat laboratory values revealed a stable serum sodium level of 135mmol/L nearly 2 weeks after initiation of the drugs.

Conclusions: Hyponatremia is a common medical finding in patients with cirrhosis, and is a reflection of the severity of the underlying liver disease as well as a predictor of morbidity and mortality. Free water restriction and V2RAs are the main therapeutic option, which failed in our patient. The lack of response to the V2RAs in our patient was likely due to avid renal proximal fluid reabsorption due to decreased effective circulatory volume, thereby resulting in diminished fluid delivery to the collecting tubule. The improvement in her hyponatremia with midodrine and octreotide was likely due to increased distal fluid delivery to the collecting tubule as reflected in the simultaneous increase in urinary output and relatively constant urinary osmolality. To date, this is the first case report of a cirrhotic patient with hyponatremia refractory to V2RAs which resolved with treatment with midodrine and octreotide.

TH-PO1089

Triiodothyronine: An Iatrogenic Cause of Thyrotoxic Hypokalemic Periodic Paralysis Ekamol Tantisattamo, Chuong Dinh, Alexander L. Pan. *Medicine, University of Hawaii John A. Burns School of Medicine, Honolulu, HI.*

Background: Being a potential life-threatening condition, thyrotoxic hypokalemic periodic paralysis (THPP) is curative if early recognized and treated. Exogenous stimuli can trigger severe hypokalemia and muscle weakness. We report a case of a man who presented with THPP from triiodothyronine use.

Methods: A 30-year-old Japanese man presented with acute bilateral lower extremity weakness which was worse until he was unable to stand up. He denied prior episode of this symptom, family history of muscle weakness, vomiting, diarrhea, vigorous exercise, or high carbohydrate intake. His heart rate was 107 beats/minute and BP was 156/75 mmHg. BMI was 34.95 kg/m^2. Neurological exam revealed motor power of grade 2/5 in the proximal and distal muscles both upper and lower extremities. Deep tendon reflexes were diminished throughout. Laboratory data revealed serum K of 1.7 mmol/l, CK of 86 IU/L, TSH of <0.07 uIU/ml, FT4 of 0.5 ng/dl, and total T3 of 486 ng/dl. EKG was normal. He reported a 50-pound intentional weight loss over 1 year from taking triiodothyronine (T3) which he bought online. He adjusted the T3 dose between 50 to 150 µg depending on his heart rate. He was taking 150 µg of T3 daily and denied diuretic use. T3 was discontinued and KCl was replaced for a total of 130 mEq. Serum K increased to 3.8 mmol/l in 10 hours. His muscle strength gradually improved and returned to normal within 24 hours. No rebound hyperkalemia occurred.

Conclusions: Acute proximal muscle weakness in our patient resulted from severe hypokalemia and rapidly resolved after KCl replacement. Evidences of hyperthyroidism including tachycardia and systolic hypertension with a wide pulse pressure were consistent with his abnormal thyroid function tests. These features support THPP, an acquired form of periodic paralysis. T3 intake increases the activity of the Na^+/K^+-ATPase causing intracellular shift of K. Our patient from Asian ethnicity may be susceptible to THPP during the hyperthyroid state. Thyroid function tests and a history of exogenous thyroid hormone use are crucial to approach hypokalemic periodic paralysis and differentiate other causes of periodic paralysis.

TH-PO1090

Oncogenic Osteomalacia: A Case of Phosphaturic Invasive Pleomorphic Spindle Cell Sarcoma Presenting with Upper Airway Compromise Ekamol Tantisattamo,[1] Wichit Sae-Ow,[2] Kristi Adachi,[3] Roland C.K. Ng.[1] *[1]Medicine; [2]Pathology; [3]Otolaryngology, University of Hawaii.*

Background: Oncogenic osteomalacia (OOM), also known as tumor-induced osteomalacia, is a rare paraneoplastic syndrome commonly caused by benign mesenchymal tumors of mixed connective tissue in soft tissue. OOM-related malignant tumors occur in only 10% of cases. We report one case of a man presented with a malignant sarcoma of the neck complicated with upper airway compromise and isolated renal phosphate wasting.

Methods: A 36-year-old man presented with a progressively enlarging right-sided neck mass for 1 month. He had voice change and odynophagia but no difficulty breathing. Physical exam revealed muffled voice but clear lungs. SpO_2 was 100% on ambient air. Chest x-ray showed trachea deviation to the left side. Neck CT scan revealed 6-cm complex solid and cystic mass in the right neck. He was emergently intubated and underwent tumor removal at the right parapharyngeal space with total right parotidectomy. Postoperatively, he did well and airway was secured by tracheostomy tube. Pathology revealed high grade pleomorphic spindle cell sarcoma involving the right parapharyngeal, masseteric spaces, and the right parotid gland. He also developed persistent hypophosphatemia with the lowest serum phosphorus level at 1.1 mg/dl despite phosphorus replacement. His serum phosphorus returned to normal on postoperative day 4. TMP/GFR was 0.775 µmol/L (normal range 0.96–1.44 µmol/L). Fibroblast growth factor (FGF) 23 was elevated to 17.7 pg/ml (normal range ≤6.5 pg/ml). One month later, serum phosphorus remained normal and followup FGF 23 has normalized.

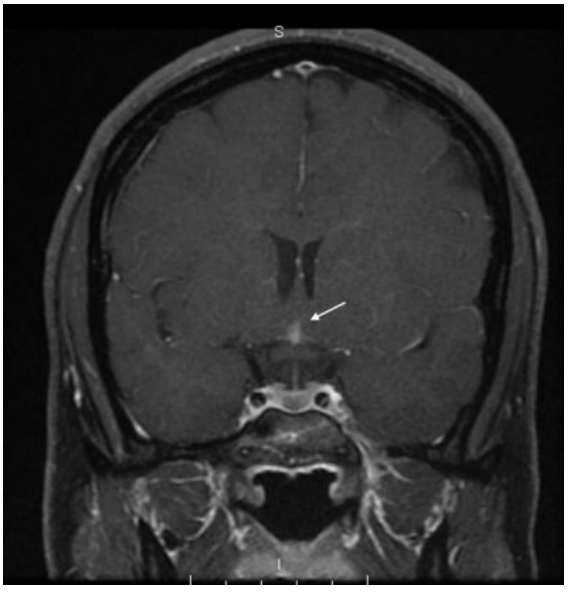
Conclusions: Our patient had persistent hypophosphatemia and low TMP/GFR which supports renal phosphate wasting. The elevated FGF 23 level and normalized serum phosphorus after tumor removal confirm OOM. Even though benign mesenchymal tumor is the most common tumor causing OOM, malignant tumors like rapidly growing high grade pleomorphic spindle cell sarcoma causing upper airway compromise in our patient is unique. OOM can occur in almost any tumor, more often benign but also malignant. In both types of tumor, FGF 23 is elevated, and the mechanism appears to be the same.

TH-PO1091

An Unusual Case of Hypernatremia Gearoid M. McMahon, Jennifer M. Joe, David J.R. Steele. *Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Serum osmolality is tightly regulated by both ADH and the stimulation or suppression of thirst. Defects in thirst sensation are often accompanied by diabetes insipidus (DI) because the thirst center is located near the site of hypothalamic ADH production. We present a case of a patient who developed hypernatremia and adipsia due to a hypothalamic lesion.

Methods: A 41 year old woman presented with a 3-month history of fatigue, myalgias and dyspnea. Serum chemistries were normal, but computed tomography (CT) of the thorax revealed hilar lymphadenopathy and a subsequent biopsy found confluent granulomas consistent with sarcoidosis. Her symptoms improved spontaneously and she was not treated with steroids. One year later, she presented with recurrent symptoms. A repeat CT thorax was unchanged. Serum chemistries were significant for Na 159mmol/L, Cl 123mmol/L, creatinine 0.93mg/dl and osmolality 321mosm/kg. She reported drinking 3-6 glasses of water daily and denied polydipsia and polyuria. Examination was significant for normotension, tachycardia (107 bpm), and euvolemia. Urine chemistries were significant for Na 146mmol/L, K 76mmol/L Cl 189mmol/L and osmolality 1103mosm/kg. The presence of a maximally concentrated urine indicated preservation of ADH secretion and suggested the possibility of a lesion in her thirst center. A T1 post-contrast brain MRI revealed focal hypothalamic enhancement consistent with sarcoidosis. She was prescribed regularly scheduled free water and her Na normalized.



Conclusions: Adipsia is an unusual cause of asymptomatic hypernatremia. It is usually related to surgery although sarcoidosis is a recognized cause. Patients who do not have associated DI can be treated with free water alone and the utility of steroids remains unclear.

TH-PO1092

Hypokalemic Hypertension with Hypercalcaemia Husham Rasheed, Calum Neil Ross, Gary Campbell. *Renal Medicine, Norfolk & Norwich University Hospital, Norwich, Norfolk, United Kingdom.*

Background: We present the case of a teenage girl diagnosed with two rare and unrelated autosomal dominant conditions during investigation for secondary hypertension. Liddle's syndrome (hyper-function of the epithelial sodium channel - ENaC) was confirmed genetically whilst a clinical diagnosis of Familial Hypocalciuric Hypercalcaemia (FHH) was predicated on the presence of hypercalcaemia, non-suppressed parathyroid hormone and a low urinary calcium/creatinine clearance ratio.

Methods: A 17 year old girl was referred with hypertension. This was incidentally noted aged 14 and was confirmed on 24 hour ABPM: at the time she was hypokalemic, [K+] 3.1 mmol/L (3.5-5.0) with a normal [HCO3-] of 26 mmol/L (21-28). Secondary causes of hypertension were not investigated by paediatricians. Subsequent interval BP readings were noted to be increased to 160/100. Random renin and aldosterone levels were low - renin 4 mU/L (5.4-60) and aldosterone <65 pmol/L (100-450). Initial (post-sampling) therapy with ramipril was changed to amiloride (ENaC antagonist) with effect. Genetic analysis confirmed Liddle's syndrome. Relatives have been referred for genetic screening: there is a family history of hypertension praecox diagnosed in her father in his late twenties. The serum [Ca]c was increased at 2.81 mmol/L (2.2-2.62) with a non-suppressed [PTH] at 3.4 pmol/L (1.6-6.9). A 24 hour urinary calcium/creatinine ratio was extremely low at 0.0058 (<0.01), confirming FHH: both the father and sister were found to have increased [Ca] and [PTH] establishing autosomal dominant inheritance. Genetic analysis is pending.

Conclusions: This is the first reported case of a patient with both Liddle's syndrome and FHH, two rare and unrelated autosomal dominant conditions. This case demonstrates the importance of methodical investigation of hypertensive patients with biochemical abnormalities, particularly in the context of a relevant family history. The co-inheritance of two chromosomally discrete autosomal dominant conditions is a medical curiosity with no, apparent, pre-reproduction lethality. Genetic counselling will be complex.

TH-PO1093

A Challenging Case of Hypomagnesaemia in an Alcoholic Patient Rabih Nasr, Chadi Saifan, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Hypomagnesaemia has been reported to cause cardiac dysrhythmias especially torsade de pointe. The diagnosis and treatment are crucial to prevent complications and mortality. Chronic alcoholism causes tubular dysfunction leading to persistent hypomagnesemia.

Methods: 53 year old man with a history of alcohol abuse presented with dizziness and palpitations. In the ED, he was found to have afib, that was treated with cardizem. His hospital course was complicated with cardiac arrest and acute respiratory failure. On admission, his BUN was 11mg/dL, creatinine 0.68mg/dL, Na 136meq/L, K 4.6mmol/L, Ca 9mg/dL, Mg 1.5mg/dL and P 4.3mg/dL. U/A showed 1+ protein, glucose, and blood with RBC's 6-12/hpf. On cardiac monitoring, he developed tachyarrhythmia, torsade de pointe requiring several shocks. His repeated magnesium level revealed persistent low magnesium even after 4 weeks of hospitalization requiring numerous repletion.

table1

Mg repletion rate	Mg oxide PO and Mg sulfate IV	Mg oxide PO and Mg sulfate IV	Mg oxide PO	Mg oxide PO and Mg sulfate drip	Mg oxide PO	Mg oxide PO	Mg oxide PO
Serum Mg level	1.8	1.6	1.7	2.3	1.7	1.7	1.7

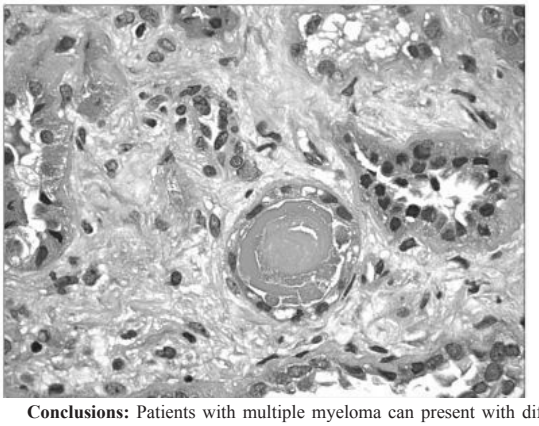
A 24-hour urine collection showed creatinine 1623.38 mg, volume 2925 ml, urine magnesium 394.88 mg, urine calcium 225.23 mg. The calculated fractional excretion of magnesium was 9% consistent with renal magnesium wasting.

Conclusions: The fractional excretion of magnesium is an important tool to differentiate between renal and extra renal causes. Hypomagnesaemia usually occurs in association with hypokalemia and hypocalcaemia. A study about chronic alcoholism shows renal magnesium wasting due to tubular dysfunction as evidenced by glycosuria and hypercalciuria "Renal Tubular Dysfunction in Chronic Alcohol Abuse -- Effects of Abstinence NEJM 1993". Magnesium level may remain low for up to 4 weeks after abstinence. Treatment requires frequent administration of magnesium and careful monitoring of the level. If possible, oral route is the preferred method of supplementation. In our case, we touch on the aspect of hypomagnesaemia in the setting of chronic alcoholism and the relevance of frequent measurement of the level to avoid subsequent increase in mortality and morbidity.

TH-PO1094

Myeloma Cast and Light Chain Nephropathy: A Challenging Diagnosis Rabih Nasr, Chadi Saifan, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Methods: A 70 year old man with history of hypertension, diabetes, hyperlipidemia, and kidney stones, presented to the hospital for a creatinine of 5 mg/dl on routine blood work. Two weeks ago, he was seen for right sided flank pain and his creatinine was 3.5 mg/dl. His physical examination was unremarkable. His blood test showed Na: 138 mg/dl, K:4 mg/dl, Cr: 5.28 mg/dl, BUN: 55 mg/dl, and Hb: 11.6 g/dl. His U/A showed 30 mg/dl protein with no cells or casts. The UTP/Cr was 0.72. The kidney ultrasound was normal. Additional blood work was normal including the SPEP with immunofixation. A kidney biopsy was performed. (Refer to pictures). A diagnosis of light chain deposition disease, kappa type and focal atypical casts suspicious of myeloma cast nephropathy was made. He was started on bortezomib and corticosteorids. His creatinine improved and reached a level of 3.8 mg/dl. The rest of his blood work showed a urine immunofixation positive for free kappa light chain, a free kappa level in the serum of 3120 mg/l, and a free kappa/lambda ratio of 234.59.



Conclusions: Patients with multiple myeloma can present with different forms of renal manifestations. Four forms are encountered: primary amyloidosis, myeloma cast nephropathy, monoclonal immunoglobulin deposition disease, and rarely proliferative glomerulonephritis. The presence of two or more forms is rarely described. Qian Q et al. reported in 2010 a coexistence of myeloma cast nephropathy, light chain deposition disease, and nonamyloid fibrils. Our patient had myeloma cast nephropathy and kappa light chain deposition disease with minimal proteinuria and showed a good response to treatment. Therefore, clinical presentation can not predict the pathologic findings in patients with multiple myeloma, despite the fact that it is essential for treatment, management and prognosis.

TH-PO1095

Modified Continuous Veno-Venous Haemodiafiltration (CVVHDF) to Correct Life Threatening Hyponatremia Elanchezzian Balakumar, Mangalakumar Veerasamy, Andrew Gratrix. *Hull Royal Infirmary, United Kingdom.*

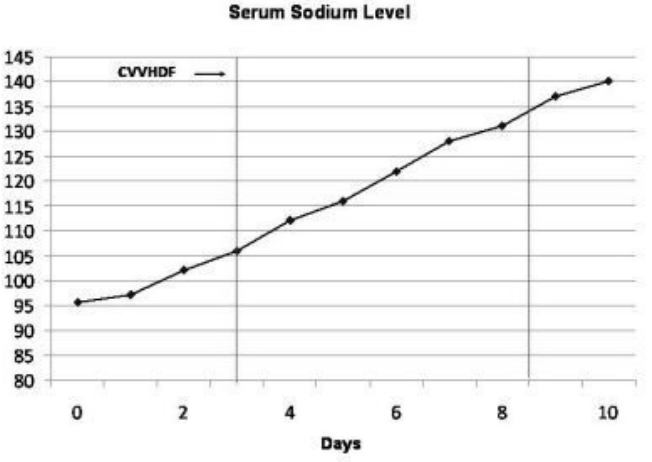
Background: Rapid correction of severe hyponatremia can lead to osmotic demyelination syndrome(ODS). We describe the management of a patient admitted with life threatening hyponatremia (Serum Sodium 95.6mmol/L) and acute kidney injury (AKI), who was successfully managed with a ***modified*** CVVHDF.

Methods: 55 year old male was admitted with confusion and vomiting. He was suffering with flu like illness in the preceding week. His oral intake was poor and was managing only water. Past medical history included ethanol abuse. On admission GCS was 14/15 without any focal neurology; jugular venous pressure was low and was in type I respiratory failure due to left lower lobe pneumonia. ECG was consistent with hyperkalaemia.

Laboratory results on admission

pH	7.44
Bicarbonate	22.1mmol/L
Na	95mmo/L
K	6.8mmol/L
Chloride	55mmol/L
Urea	43.6mmol/L
Craetinine	1628micromol/L
ALT	146IU/L
CRP	98mg%
WCC	15100
Hb	11.3gm%
Platelet	186000

Patient was treated with 0.9% saline resuscitation, antibiotics and multiorgan support. Since he was non-oliguric and there was a risk of ODS due to rapid correction of hyponatraemia, hyperkalaemia was managed conservatively rather than by renal replacement therapy (RRT). Inspite of optimal fluid therapy sodium level remained low and he developed new metabolic acidosis. Hence we instituted modified CVVHDF on day 3, which resulted in gradual correction of sodium over 96 hours without any permanent neurological deficit.



Conclusions: Patients with AKI and severe metabolic derangement require RRT. The dialysate fluid marketed in UK for CVVHDF has a sodium concentration of 140mmol/L. Use of this in patients with AKI and hyponatraemia would result in rapid correction of sodium and ODS. Hence, we customised the dialysate fluid sodium concentration by adding sterile water (Ostermann M *et al, 2010)*. The latter allows safe and effective correction of hyponatraemia.

TH-PO1096

A Case of Familial Pseudohyperkalemia Naomi Matsuo, Taku Miyoshi, Kimio Tomita, Kenichiro Kitamura. *Department of Nephrology, Kumamoto University School of Medicine, Kumamoto, Japan.*

Background: Hyperkalemia has been reported to occur in 1.0% to 10% of all hospitalized patients. The cause of hyperkalemia is multifactorial, but reduced renal function, medications, and hyperglycemia are the most frequent contributing factors.

Methods: A previously healthy 84-year-old woman was referred to our hospital because of severe hyperkalemia. Her serum potassium (K^+) level was 10.9 mEq/L at a physician's office where she was seen for lumbago. She had no past medical history and was not taking medications. Physical examination found no abnormalities and ECG showed no signs of hyperkalemia. Laboratory examination revealed a serum K^+ level of 4.4 mEq/L with euglycemia. Reduced renal function, hematological abnormalities such as hemolysis, leukocytosis, thrombocytosis, or morphological abnormalities of red blood cells (RBC) were not found. Therefore, we considered the possibility of pseudohyperkalemia. Her plasma K^+ level was markedly elevated after 6 hr incubation of blood at 4 °C without hemolysis, but not at 37 °C, indicating that hyperkalemia was caused by abnormal leakage of K^+ from RBC at lower temperatures without coagulation. We also confirmed the same phenotype in her two daughters, suggesting hereditability of this disorder. Finally we reached the diagnosis of familial pseudohyperkalemia (FP).

Conclusions: FP is an autosomal dominant disorder characterized by fragility of erythrocyte membranes, which causes abnormal leakage of K^+ from RBC at low temperature (below 20 °C). The leakage of K^+ persists when the Na-K ATPase or Na-K-2Cl cotransporter is inhibited, indicating passive leakage of K^+. Several families from Europe and one from Japan with FP were reported and three types of FP are described based on the leak–temperature dependency curve, 1) FP Edinburgh and FP Lille, 2) FP Chiswick and FP Falkirk, and 3) FP Cardiff. This case closely resembles FP Edinburgh and FP Lille. Microsatellite analysis is currently being performed to confirm a genetic basis for this trait. This is a rare disorder, but offers valuable insight for electrolyte pathophysiology. In addition, to our knowledge, this is the second Japanese case of FP.

Funding: Government Support - Non-U.S.

TH-PO1097

A Case of Light Chain Deposition Disease Associated with Multiple Myeloma Diagnosed by LC-MS/MS Analysis Yoshimi Nakashima,[1] Keiko Tajiri,[1] Taku Miyoshi,[1] Hiroyuki Hata,[2] Masayoshi Tasaki,[3] Konen Obayashi,[4] Yukio Ando,[3] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Kumamoto University School of Medicine, Kumamoto, Japan; [2]Department of Hematology, Kumamoto University School of Medicine, Kumamoto, Japan; [3]Department of Neurology, Kumamoto University School of Medicine, Kumamoto, Japan; [4]Diagnostic Unit for Amyloidosis, Department of Laboratory Medicine, Kumamoto University School of Medicine, Kumamoto, Japan.*

Background: Renal impairment is common and often associated with multiple myeloma (MM). Although the diagnosis of these light chain deposition disease (LCDD) is usually made by the immunohistochemical demonstration of immunoglobulin (Ig) light chain (LC) deposits, studies may be falsely negative due to the variable reactivity of the antibodies against LC deposits. Therefore, we tried to diagnose LCDD by LC-MS/MS analysis in our patient.

Methods: A 70-year-old Japanese woman was referred to our hospital for renal impairment (Cr 1.8mg/dL) and heavy proteinuria (3g/day). Laboratory examination revealed a suppression of all serum Ig classes (IgG, IgA, IgM, IgE, and IgD), but no monoclonal protein was detected by serum or urine immunoelectrophoresis. Serum free LC analysis demonstrated substantially increased free Igκ LCs (5870ng/mL) and κ/λ ratio, and bone marrow aspiration showed a significant increase in plasma cells (28.8%). These findings indicated a diagnosis of Bence-Jones protein-type MM. Renal biopsy showed membranoproliferative glomerulonephritis-like features and electron microscopy demonstrated amorphous deposits in the mesangium and in the subendothelial space. However, Congo red staining and immunostaining against Igs including κ and λ LCs were all negative, resulting in no definitive diagnosis for the renal lesions. Therefore, we analyzed the LC deposits from kidney biopsy specimens by LC-MS/MS and found large amounts of Igκ LC. Consequently, the patient was diagnosed as LCDD associated with MM.

Conclusions: Our case shows that LC-MS/MS analysis of biopsy specimens should be considered for patients who are suspected of having LCDD but who have no evidence of immunostaining for LC deposits in the affected organs.

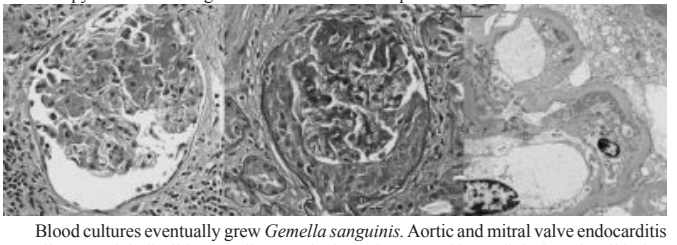
Funding: Government Support - Non-U.S.

TH-PO1098

Gemella Sanguinis Endocarditis with Anti-PR3/c-ANCA-Associated Immune Complex Necrotizing Glomerulonephritis with Full House Pattern on Immunofluorescence Mathieu Rousseau-Gagnon,[1] Julie Riopel,[4] Anne Desjardins,[3] Daniel Garceau,[2] Mohsen Agharazii,[1] Simon Desmeules.[1] *[1]Nephrology, CHUQ, Quebec, QC, Canada; [2]Nephrology, IUCPQ, Quebec, QC, Canada; [3]Infectious Diseases, CHUQ, Quebec, QC, Canada; [4]Pathology, CHUQ, Quebec, QC, Canada.*

Background: Kidney manifestations of bacterial endocarditis include septic emboli and proliferative glomerulonephritis. Recently, cases of bacterial endocarditis have been associated with c-ANCA/anti-PR3 necrotizing glomerulonephritis. We report the first case of c-ANCA/anti-PR3 positive immune complex glomerulonephritis with "full house" immunofluorescence pattern due to bacterial endocarditis.

Methods: A 67 year-old man was referred for progressive renal failure. He had lost 30 lbs in the last month and had been treated for lower back pain. On admission, he was afebrile and had a creatinine of 10.3 mg/dL. C-ANCA/anti-PR3 were positive. A kidney biopsy was performed, revealing focal necrotizing glomerulonephritis with crescents and immune complex deposits with a "full house" pattern on immunofluorescence. Electron microscopy showed mesangial and subendothelial deposits.



Blood cultures eventually grew *Gemella sanguinis*. Aortic and mitral valve endocarditis as well as spondylodisciitis were diagnosed. The patient started hemodialysis and antibiotics. Prednisone was added for 14 days due to dialysis requirement in the presence of necrotizing glomerulonephritis. Aortic and mitral valve were replaced on day 17 and renal function recovered completely on day 93.

Conclusions: This first case of *Gemella sanguinis* endocarditis-associated necrotizing glomerulonephritis with anti-PR3/c-ANCA and immune complex deposits with "full house" pattern underscores the importance to consider subacute bacterial endocarditis in the differential diagnosis of ANCA vasculitis and lupus nephritis. This is crucial as these conditions involve different treatment strategies.

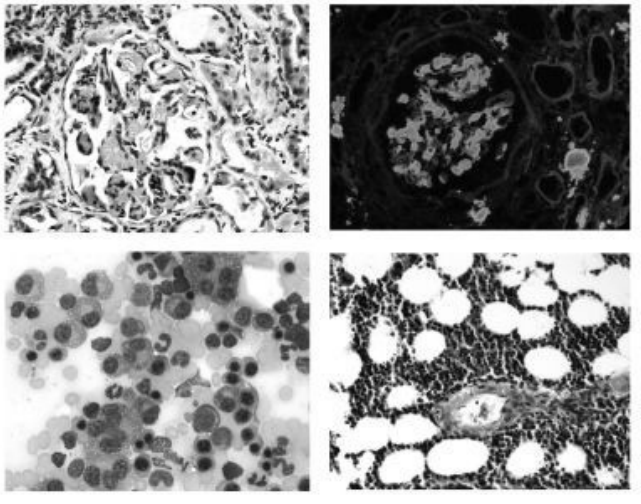
TH-PO1099

Plasma Cell Myeloma with Amyloidosis Presenting as Hypotension, Nephrotic Syndrome and Renal Failure Omkar U. Vaidya,[1] Sara A. Monaghan,[2] Xin J. Zhou,[2] Robert D. Toto.[1] *[1]Nephrology, UT Southwestern, Dallas, TX; [2]Pathology, UT Southwestern, Dallas, TX.*

Background: Plasma cell myeloma without amyloidosis and primary amyloidosis without plasma cell myeloma can independently present both nephrotic syndrome and renal failure.

Methods: Case report: A 45yr old Hispanic male with a medical history of diabetes presented with a 3-week history of fatigue and lower extremity edema. Review of his systems was unremarkable. He was found to have asymptomatic hypotension with a blood pressure of 95/60 and a pulse rate of 90 /min. Physical exam was unremarkable except for edema and macroglossia. Laboratory revealed creatinine 3.83 mg/dl and 24 hour urine protein of 18 g; urinalysis revealed microhematuria and pyuruia; urine free light chain excretion 144.37 mg/24 hr, serum IgG level 2101 mg/dl (700-160 mg/dl), Ig kappa free light chain 2.94(0.33-1.94mg/dl) and Ig lambda free light chain- 31.2(0.57- 2.63 mg/dl). An EKG showed low voltage QRS complex. Two-dimensional echocardiogram showed biventricular hypertrophy, ejection fraction of 62 % and grade II diastolic dysfunction. Skeletal survey was negative for lytic lesions. Congo red stain of a renal biopsy showed apple green birefringence under polarized light involving the mesangium and segmentally involving capillary walls. Immunofluorescence staining revealed extensive deposition of lambda light chain in the mesangium and segmentally in capillary walls with scattered interstitial positivity. Staining for kappa light chain was negative. A bone marrow biopsy revealed increased atypical plasma cells, accounting for 15% of the cellularity, throughout the interstitium and in focal dense collections. Congo red stain confirmed amyloid deposition in vessel walls. The patient was started on bortezomib and dexamethasone. However, on the 14th day of admission he had ventricular arrhythmia and failed to regain pulse.

Conclusions: Plasma cell myeloma presenting initially with amyloidosis is uncommon.



TH-PO1100

Clinical Utility of Mass Spectrometry-Based Proteomics in the Diagnosis of Monoclonal Gammopathy-Associated MembranoProliferative Glomerulonephritis Deepika Jain,[1] Khaled Abdel-Kader,[1] Robert H. Yenchek,[1] Sanjeev Sethi,[2] Jamie Green.[1] *[1]The Renal-Electrolyte DIvision, University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Background: Monoclonal gammopathy is increasingly being recognized as a common cause of membranoproliferative glomerulonephritis (MPGN), however establishing the diagnosis may sometimes be challenging.

Methods: We present the case of a 58 year old asymptomatic woman with a history of longstanding hypertension who presented with 5 gram proteinuria and microscopic hematuria. Renal biopsy was consistent with MPGN pattern of injury. Immunofluorescence (IF) studies showed staining for IgM and C3, suggesting immune-complex mediated MPGN. Electron microscopy showed subendothelial, subepithelial and mesangial electron dense deposits. The work up was negative for any infectious causes or autoimmune disease; however an IgG kappa monoclonal protein was identified in the serum at 0.4 mg/dL. Serum free light chain kappa to lambda ratio was elevated at 2.96. Bone marrow biopsy revealed 8% plasma cells, and and there was no evidence of any lytic lesions, hypercalcemia, anemia, or renal dysfunction. Due to the discordance between the serum monoclonal protein (IgG kappa) and IF findings (IgM, no light chain restriction), it was unclear if the the patient's MPGN was related to the monoclonal gammopathy. Therefore, the renal biopsy specimen was analysed further with laser microdissection and mass spectrometry at Mayo Clinic (Rochester, MN) which identified the deposits as monoclonal IgG kappa. Hence, the diagnosis of monoclonal gammopathy-associated MPGN was made.

Conclusions: This case emphasizes the importance of searching for an underlying etiology causing MPGN before labelling it as idiopathic. Laser microdissection and mass spectrometry provides a good ancillary technique for the diagnosis of monoclonal protein related glomerulonephritis when IF studies are inconclusive.

TH-PO1101

A Case of Membranoproliferative Glomerulonephritis with Cryoglobulinemia due to Both Hepatitis C Infection, and B-Cell Lymphoma Amarpreet S. Sandhu, Helbert Rondon-Berrios. *Nephrology, University of New Mexico School of Medicine, Albuquerque, NM.*

Background: Membranoproliferative glomerulonephritis (MPGN) is characterized by mesangial cell proliferation and thickening of the capillary walls due to subendothelial extension of the mesangium. It can be subdivided into idiopathic and secondary forms. Secondary forms are due to infectious diseases, systemic immune complex diseases, neoplasms, liver diseases, and miscellaneous. We encounter a patient who had MPGN with cyroglobulinemia from hepatitis C and B cell lymphoma, both seen on kidney biopsy.

Methods: A 60-year-old man with no significant past medical history presented with mild dyspnea and lower extremity edema. Initially diagnosed with new-onset congestive heart failure but later found to have nephrotic syndrome with a urine protein-to-creatinine ratio of 4.3. His serum creatinine on admission was 1.4 mg/dL and during his hospitalization it ranged from 1.4 to 2 mg/dL. His urinalysis showed dysmorphic RBCs but no acanthocytes or RBC casts. His work up revealed elevated WBC count with variant lymphocytes, a new diagnosis of hepatitis C infection with a viral load of 1.8 million IU/mL, serum protein electrophoresis with immunofixation demonstrated an IgM kappa monoclonal protein. Other important findings were: extremely elevated rheumatoid factor titer of 120,000 IU/mL, high C4 and normal C3 levels, a cryocrit of 6%, elevated kappa light chain with and elevated kappa-to-lambda ratio. The kidney biopsy showed cryoglobulinemic glomerulonephritis with an MPGN pattern of injury and immune deposits suggestive of an IgM-kappa paraprotein along with interstitial aggregates of atypical lymphoid cells. Flow cytometry of whole blood showed a kappa light chain restricted B-cell population and the bone marrow biopsy revealed a low grade B-cell lymphoma compatible with a splenic marginal zone lymphoma.

Conclusions: In previously reported MPGN cases with cryoglobulinemia were either associated with hepatitis C infection or B- cell lymphoma. However, our patient had findings of both, making his treatment choice difficult. Given low intensity B-cell lymphoma, decision was made to treat his hepatitis C first and then the malignancy.

TH-PO1102

Pseudoleukocytosis in an Immunosuppressed Patient with Essential Mixed Cryoglobulinemia (EMC) and Glomerulonephritis: Case Report Andrea C.E.P. Valenca, Edmir R.B. Dias, Marclebio M.C. Dourado, Maria Carolina N.R. Neves, Luis H.B.C. Sette, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil.*

Background: EMC is an immune complex-mediated vasculitis that involves small-medium-size vessels. Renal involvement is associated with a poor prognosis. Treatment depends on the severity of symptoms and includes: steroids, cyclophosphamide and plasma exchange. Commonly these patients develop infections associated with immunosuppression. Here we describe a case of pseudoleukocytosis associated with EMC and glomerulonephritis.

Methods: A 46-year-old male had 1 year history of arthritis of large joints, fatigue and palpable purpura in lower extremities. He had a skin biopsy consistent with vasculitis leucocytoclastic and was followed by a rheumatologist using azathioprine and prednisone for the last 6 months. One month before admission he developed edema, weight gain and hypertension. He was referred to nephrology consultation when laboratory tests showed sCr 1.6mg/dL (last 0.8mg/dL), serum albumin of 2.0g/dL and anemia. Urinalysis revealed hematuria and proteinuria (10g/24h). His purpura became painful and fever was reported. Blood and urine cultures were negative. His white blood cell count (WBC) was 43,000 cells/mL. Complement C3 level was normal and C4 was low. Cryoglubulins were positive. ANA, serology for hepatitis B, C (fourth-generation ELISA), syphilis and HIV were negative. Kidney biopsy showed a membranoproliferative glomerulonephritis secondary to EMC type II. Manual leukocyte counting showed 6,500 cells/mL. The patient was started on plasmapheresis and prednisone. After 6 sessions his kidney function improved and WBC count was 10,300 cells/mL.The leukocytosis in our patient was secondary to the cryoglobulins. Patients with EMC may have a temperature increase in leukocyte counts due to various sizes of precipitated cryoglobulin particles. Therefore the counter "recognize" cryoglobulin particles as WBC.

Conclusions: We reported a case of pseudoleukocytosis due to EMC and glomerulonephritis. This is a differiencal diagnosis in immunosuppressed and febrile patients that could have either active disease or infection.

TH-PO1103

A Case Report of Secondary Hemosiderosis on Renal Biopsy in a Patient with a Left Ventricular Assist Device Jennifer C. Rodrigues,[1] Ahsan Alam,[1] Chantal Bernard,[2] Tiina Podymow.[1] *[1]Nephrology, McGill University, Montreal, QC, Canada; [2]Pathology, McGill University, Montreal, QC, Canada.*

Background: Continuous flow left ventricular assist devices (LVADs) are used both as a bridge to transplantation and as destination therapy. Moderate to severe renal dysfunction may persist chronically in some LVAD recipients; the etiology is unknown and there are no renal biopsy data.

Methods: We report a 19 year old previously healthy female requiring LVAD implantation post viral cardiomyopathy. She developed compartment syndrome and rhabdomyolysis with acute kidney injury (AKI) requiring continuous renal replacement for 3 weeks, intermittent hemodialysis for 8 weeks, then partial recovery with a new baseline creatinine of 140 mmol/L (1.5 mg/dL). Urine analysis throughout her hospitalization showed trace to large amounts of blood. A hemolysis work up as part of her chronic anemia demonstrated elevated lactate dehydrogenase values between 569-1562 U/L (reference 110-210 U/L) and decreased levels of haptoglobin 0.06 U/L (reference 0.69-1.69 U/L). The patient's renal function continued to deteriorate and her GFR by nuclear medicine scan was 23 mL/min. Transjugular renal biopsy was performed for dual cardiac/renal transplant work and revealed acute tubular necrosis (ATN) with marked iron/hemosiderin deposition. Mild tubular atrophy and interstitial fibrosis were also noted; glomeruli were unremarkable, with no thrombi or sclerosis, and there were no immune deposits. Renal biopsy was repeated 6 months later due to clinical deterioration and demonstrated similar pathology with glomerulosclerosis in 15% of glomeruli.

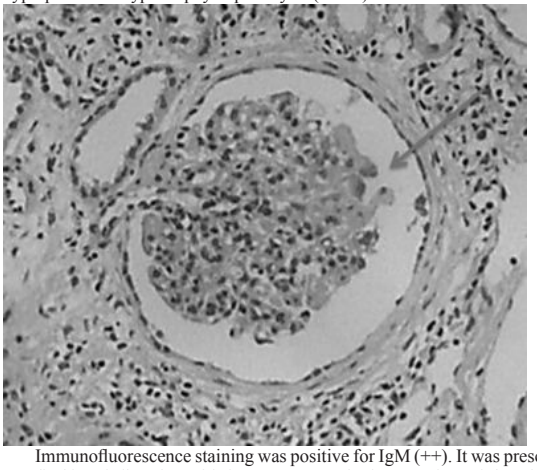
Conclusions: We report ATN, hemosiderosis, and renal dysfunction in a patient with LVAD. The etiology of renal dysfunction post LVAD is unknown and could include ATN secondary to toxicity from hemolysis and iron deposition or ATN due to a chronic "low flow state" with LVAD use. Hemosiderosis on renal biopsy secondary to hemolysis has been shown post-valvular repair. This is, to our knowledge, the first reported renal biopsy in a patient with a LVAD. Secondary hemosiderosis with acute tubular necrosis should be considered in patients with LVAD and unexplained kidney disease.

TH-PO1104

Collapsing Glomerulopathy in a Patient with Lymphangioleiomyomatosis: Case Report Maria Carolina N.R. Neves, Andrea C.E.P. Valenca, Luis H.B.C. Sette, Edmir R.B. Dias, Marclebio M.C. Dourado, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas da Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Background: Collapsing Glomerulopathy (CG) is a glomerular injury that can occur in association with various etiologic factors. It also leads to a poor renal prognosis. We describe a case of CG in a patient with the diagnosis of Lymphangioleiomyomatosis (LAM). This is an uncommon disease characterized by smooth muscle cell infiltration and cystic destruction of the lung. The association between this two pathologies had never been descrbed.

Methods: A 27-year-old female reported sudden onset of edema, dyspnea, nausea and vomiting, for the past month. She had no significant past medical history and was not taking any medications. Physical exam revealed periorbital swelling and bilateral lower extremity edema.Urinalysis showed no microscopic hematuria and 24-h urine protein 6,1g. Blood evaluation showed normal blood count, albumin 1,4mg/dl, Cr 2,5mg/dl; negative ANA, normal complement, serology for hepatitis B, C and HIV were negative. Chest x-ray was normal. Kidney biopsy was performed [figure 1] and revealed 10 collapsed glomeruli with hyperplasia and hypertrophy of podocytes (arrow) and moderate tubule-interstitial damage.



Immunofluorescence staining was positive for IgM (++). It was prescribed prednisone 1mg/kg/d and diuretics with improvement of edema and renal function. Last sCr was 1.4mg/dl. Two months later, she complained of sudden dyspnea. CT scan revealed bilateral pneumothorax and diffuse cystic changes on both lungs. She underwent to a thoracic drainage and an open lung biopsy that showed proliferation of spindle and epithelioid cells consistent with LAM.

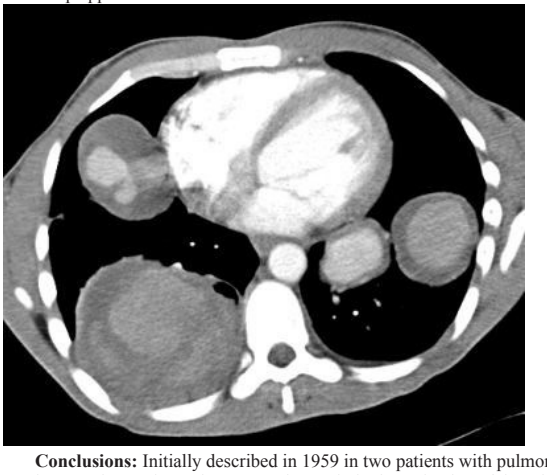
Conclusions: This is a report describing a patient with LAM and CG.

TH-PO1105

An Uncommon Cause of Hemoptysis Jason M. Kidd, JulieAnne G. McGregor, Ronald J. Falk. *Division of Nephrology and Hypertension, University of North Carolina Kidney Center, Chapel Hill, NC.*

Background: Hemoptysis is a common symptom leading to medical evaluation. Nephrologists are often involved in the care of patients with pulmonary-renal syndromes. In this case, we used our expertise in the treatment of vasculitis for the management of a pulmonary limited process.

Methods: A 28 year old male presented to a local emergency room with teaspoon sized hemoptysis. Medications included warfarin for a deep venous thromboembolism in his right leg diagnosed 3 months prior to the onset of hemoptysis. He noted one month of dyspnea, cough, fevers and weight loss. Chest radiography revealed bilateral rounded pulmonary masses. Computed tomography of his chest showed seven large pulmonary artery aneurysms; the largest in the right lower lobe (Figure). The patient was transferred to our institution for further management. The diagnosis of Hughes-Stovin Syndrome was made. Our patient underwent right lower lobectomy. Pathology showed no evidence of active vasculitis. Azathioprine and prednisone were started. Repeat imaging 1 month later showed decreased size of the remaining aneurysms. Unfortunately, patient has not attended follow up appointments. Chest CT.



Conclusions: Initially described in 1959 in two patients with pulmonary aneurysms, Hughes-Stovin Syndrome is a rare entity. Pathologic evaluation of the original aneurysms noted a high degree of inflammatory cellular infiltrate.[1] It has been characterized as a subcategory of Behcet's disease.[2] Untreated disease results in fatal hemoptysis and death. Our case illustrates the use of immunosuppression in the treatment of this disease.

[1] Hughes JP, Stovin PGJ. Segmental pulmonary artery aneurysms with peripheral venous thrombosis. Br J Dis Chest 1959;53: 19-27.

[2] Emad Y et al. Hughes-Stovin Syndrome: is it incomplete Behcet's? Report of two cases and review of the literature. Clin Rheumatol 2007;26: 1993-1996.

TH-PO1106

A Non-Glomerular Cause of Dysmorphic Hematuria Jason M. Kidd, Lindsay Kruska, Gerald A. Hladik. *Department of Nephrology and Hypertension, University of North Carolina Kidney Center, Chapel Hill, NC.*

Background: In their seminal manuscript[1], Fairley and Birch suggested that glomerular bleeding is differentiated from other causes of hematuria based on the presence of dysmorphic red blood cells on urine microscopy. This case illustrates a unique, non-glomerular cause for dysmorphic hematuria.

Methods: A 59 year old white male with a history of deceased donor kidney transplant presented for follow up. His initial kidney disease was due to autosomal dominant polycystic kidney disease. He noted a 1 week history of gross hematuria. Urine microscopy revealed 15 red cells per high powered field. Scattered acanthocytes were present. Three months prior, renal allograft biopsy was performed and was significant only for moderate arterionephrosclerosis. He had no fevers, cough, abdominal pain or skin rash.

Estimated glomerular filtration rate was 33.6 ml/min/1.73m^2, which was stable from prior tests, and he had a urine protein to creatinine ratio of 0.206. Cystoscopy was normal. The presence of dysmorphic hematuria was concerning for glomerulonephritis.

Renal biopsy was planned, but due to his history of polycystic kidney disease, ruptured cyst was also considered. The patient underwent magnetic resonance imaging without contrast, which showed multiple cysts containing internal hemorrhage. Hematuria resolved several weeks later.

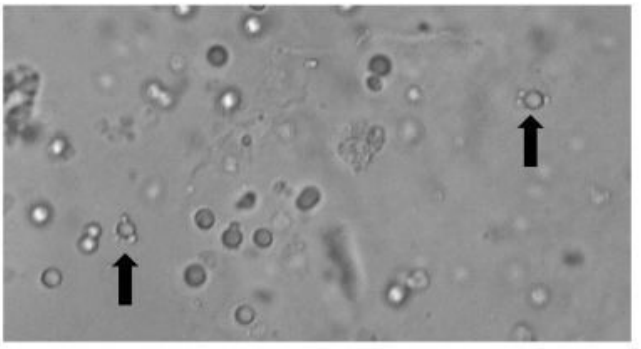


Figure 1: Dysmorphic red blood cells (arrows) due to ruptured cysts in a patient with polycystic kidney disease

Figure 1: Dysmorphic red blood cells (arrows) due to ruptured cysts in a patient with polycystic kidney disease.

Conclusions: While highly sensitive for glomerular lesions, the presence of dysmorphic erythrocytes on microscopy should not preclude investigation of other causes of hematuria. This case illustrates a common cause of hematuria presenting with unique findings on urine microscopy.

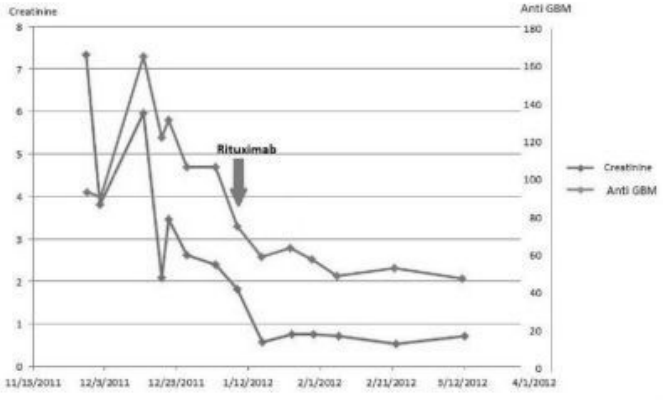
[1] Fairley KF, Birch DF. Hematuria: A simple method for identifying glomerular bleeding. Kidney International, Vol 21(1982),105-108.

TH-PO1107

Rituximab for a Refractory Case of Simultaneous Anti-GBM and Membranous Nephropathy Ghassan Bandak, Bruce A. Jones, Jian Li, Kausik Umanath. *Henry Ford Hospital, Detroit, MI.*

Background: Anti-GBM antibody disease is a rare autoimmune disorder causing rapidly progressive glomerulonephritis. Membranous nephropathy is a disease manifesting as nephrotic syndrome. Their co-existence is rare.

Methods: A 24 y.o. male smoker presented with hemoptysis and gross hematuria of 2 weeks' duration. The serum creatinine (SCr) increased to 4.3 mg/dL in one week, in conjunction with an ACR of 1000 mg/g and red cell casts in the urine sediment. Kidney biopsy showed crescenteric glomerulonephritis with linear immunofluorescence of the basement membrane staining by IgG and epimembranous electron-dense deposits, when the anti-GBM titer was 165 units. Intravenous methylprednisolone, oral cylcophosphamide (CTX) and plasma exchange (PLEX) were initiated, with a modest response to treatment, SCr ~4 mg/dL. Kidney function deteriorated with decreasing PLEX frequency resulting in dialysis dependence. Daily PLEX was reinstituted, with renal improvement and discontinuation of dialysis. However, after 8 wks of PLEX and CTX, anti-GBM titers were maintained at ~50 units, prompting additional treatment by rituximab. This agent permitted discontinuation of PLEX and a reduction of anti-GBM titer to < 20 units and SCr to 1.76 mg/dL.



Conclusions: Untreated anti-GBM disease portends a poor prognosis with a high probability of end-stage renal disease. The dual diagnosis of membranous nephropathy and anti-GBM disease is rare but has been documented previously. The response rate to traditional therapy of CTX, PLEX and steroids is nearly 50%. This patient manifested an extremely high antibody titer that proved resistant to traditional therapy. Given the pathophysiology of this disorder, directed B-cell depletion with rituximab was attempted and proved efficacious, with rapid anti-GBM titer reduction, and discontinuation of PLEX. Rituximab is a potential, alternative therapy for anti-GBM disease.

TH-PO1108

Outcome of Severe Thrombotic Microangiopathy with Cortical Necrosis in IgA Nephropathy Rapeepat Lekkham,[1] Talerngsak Kanjanabuch.[2] *[1]Medicine, Albert Einstein Medical Center, Philadelphia, PA; [2]Medicine, Chulalongkorn University, Bangkok, Patumwan, Thailand.*

Background: There are several studies on the clinical and pathological features that aid in predicting outcome of IgA nephropathy. In addition to the known clinical variables, the new Oxford classification also provides the pathological features that help in predicting renal outcome. However, thrombotic microangiopathy (TMA) is not included in the classification, so the clinical significance of TMA in this setting is not well understood.

Methods: A healthy 25-year-old Asian man who reported an episode of productive cough and oligoarthritis 6 months earlier, presented with fever with chills, productive cough, severe vomiting and blurred vision. He found to have acute renal failure (Cr of 14.7mg/dl, normal 2 months earlier) with nephrotic-range proteinuria (8.5 gm/day), severe hypertension (BP190/120), volume overload, respiratory failure required a mechanical ventilation and an emergent hemodialysis. Blood tests showed mild anemia, leukocytosis, and normal platelets. Urinalysis showed red blood cells with board coarse granular casts. Complement levels were normal. ANA, p-ANCA, c-ANCA,cryoglobulin and viral hepatitis profiles were negative. Kidney ultrasonography showed bilateral renal parenchymal disease. A renal biopsy was consistent with the mesangial IgA nephropathy;M1 E1 S1 T2 (by Oxford IgA classification) with severe thrombotic microangiopathy, necrotizing glomerulitis, focal cortical necrosis and hemorrhage. Patient received several hemodialysis sessions, intravenous steroid and plasmapheresis were tried. He was not improved hence intravenous immunoglobulin was initiated. His condition was later improved. He was discharged with oral corticosteroids and mycophenolate mofetil but need to be continued on long-term hemodialysis as outpatient.

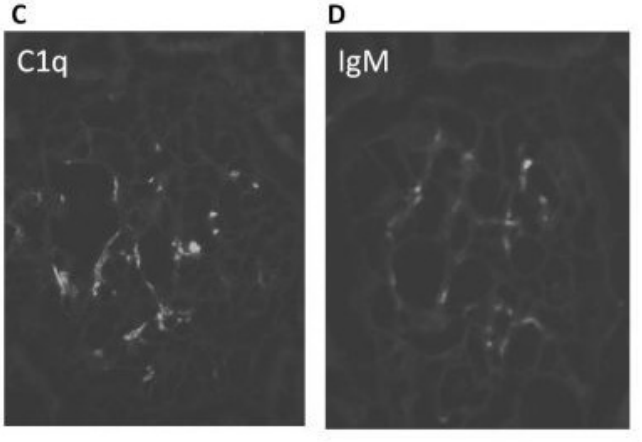
Conclusions: According to our patient who had evidence of TMA and cortical necrosis developed the worst renal outcome required long-term renal replacement therapy. Hence, we suggest that even rare but once presents in IgA nephropathy, TMA is likely to be the useful factor to predict worse renal outcome in addition to standard Oxford classification and clinical factors.

TH-PO1109

C1 q Nephropathy and BK Virus Replication in a Renal Transplant Recipient: Occam's Razor versus Hickam's Dictum Purva D. Sharma,[1] Serena M. Bagnasco,[2] Duvuru Geetha.[1] *[1]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: C1 q nephropathy is a rare glomerular disease characterized by dominant mesangial C1q deposits in the absence of SLE.We report the first case of a deceased donor (DD) kidney transplant recipient who developed proteinuria in the setting of rising plasma BK virus (BKV) titers and had biopsy proven de novo C1q nephropathy.

Methods: A 44 year old male received a DD kidney transplant in Feb 2007. His course in August 2007 was complicated by BKV nephropathy.Mycophenolate mofetil (MMF) was stopped and he was treated with leflunomide (LEF).LEF was stopped after 5 months due to transaminitis and he was restarted on low dose MMF. His serum creatinine remained stable with low level BK viremia.In 2012,he was treated with steroids for gout. His plasma BKV titer rose from 700 copies/ml to 2500 copies/ml and he developed new onset proteinuria. Allograft biopsy revealed mild glomerulitis and focal mesangial expansion with mild focal tubulitis.The interstitium showed edema and inflammatory infiltrate of lymphocytes and plasma cells. Immunostaining for SV 40 was negative.Indirect immunofluorescence revealed fine granular staining, segmental for IgG2+, IgM2+,C1q 2-3+.



Electron microscopy revealed focal mesangial deposits.One month after stopping steroids,his plasma BKV titer fell to 500 copies/ml and proteinuria resolved.

Conclusions: The use of steroids in our patient increased BKV replication. Although serum creatinine was stable, the proteinuria was new and was evaluated with allograft biopsy which revealed C1q nephropathy. After stopping the steroids, the BKV titer decreased with resolution of proteinuria. The temporal relationship of BKV replication and proteinuria suggests that C1q nephropathy was triggered by BKV.

TH-PO1110

Complement C4 Deficiency: Juvenile Central Eastic Arteries after 28 Years of Renal Replacement Therapy Emanuel Zitt,[1,3] Florian Knoll,[1] Dennis Intemann,[2] Karl Lhotta.[1,3] *[1]Department of Nephrology and Dialysis, LKH Feldkirch, Feldkirch, Austria; [2]Department of Internal Medicine, Clinical Institution of Angiology, LKH Felkdirch, Feldkirch, Austria; [3]Vorarlberg Institute for Vascular Investigation and Treatment, LKH Feldkirch, Feldkirch, Austria.*

Background: Complement activation products are present in atherosclerotic plaques. Recently, binding of complement to elastin and collagen in the aortic wall has been demonstrated, suggesting a role of complement in the development of aortic stiffness and atherosclerosis. The definitive role of complement in arteriosclerosis and atherosclerosis, however, remains unclear.

Methods: We here describe a patient with hereditary complete deficiency of complement C4 suffering from Henoch-Schoenlein purpura who has been on renal replacement therapy for twenty-eight years now. The patient has experienced the full range of risk factors for vascular damage such as hypertension, volume overload, hyperphosphatemia and hyperparathyroidism. Despite that, his carotid artery intima media thickness was below the normal range and his aortic pulse wave velocity was normal. In contrast, the patient's peripheral muscular arteries were found to be heavily calcified.

Conclusions: This case supports the hypothesis that complement plays an important role in the development of arterio- and atherosclerosis of central elastic arteries. We speculate that inability to activate complement by the classical or lectin pathways protected the patient from arteriosclerosis with stiffening and calcification of the aorta and atherosclerotic plaque formation of the carotid arteries. This idea is supported by recent experimental data that found complement C3 and C4 accumulation in the external elastic lamina of healthy old mice and extending complement accumulation into the internal elastic lamina and intimal plaques in atherosclerosis-prone ApoE(-/-) mice (Shields K. et al, Clin Transl Science 2011). Inhibition of complement activation may be a potential target for prophylactic and therapeutic interventions.

TH-PO1111

Renal Ascites: Lymph Weep from Allograft Associated with Homozygous NPHP1 Deletion Tomoki Tsukahara, Christie P. Thomas. *Nephrology, University of Iowa, Iowa City, IA.*

Background: NPHP1 gene encodes nephrocystin-1 and its homozygous deletion causes juvenile nephronophthisis (NPHP). NPHP often leads to ESRD and NPHP transplant recipients are thought to have excellent outcomes. We report 2 cases with NPHP1 deletion that presented with intractable ascites from late post-transplant renal lymph leakage.

Methods: The first case is a 15-year-old man who developed an enlarged edematous allograft with ascites 14 month after his third transplant. Kidney biopsy showed acute cellular rejection and hypertrophic tubules. Laparotomy revealed clear fluid weeping from the surface of the intraperitoneal kidney. Transplant nephrectomy was performed because of intractable ascites and failure to thrive. He received his fourth transplant but developed a massively enlarged allograft 54 months later with a perinephric fluid collection. His graft function deteriorated slowly and kidney biopsy showed chronic vascular rejection and hypertrophied remnant tubules. The second case is a 24-year-old woman who developed an enlarged edematous renal allograft with ascites 19 month after her third transplant. Laparoscopy revealed clear fluid weeping from the surface of the intraperitoneal kidney. Kidney biopsy showed positive C4d in peritubular capillaries and interstitial edema, though her graft function is excellent. Her ascites is controlled by a peritoneo-venous shunt and paracentesis as needed.

Conclusions: In both cases serum-ascites albumin gradient was high with ascitic fluid chemistry consistent with lymph. There was no evidence of cirrhosis, portal hypertension, right heart failure, urine leak, peritonitis, or malignancy. We suspect that ascites was caused by lymph seeping from allograft. Obstruction to hilar lymphatic drainage may lead to a weeping of lymph via the superficial capsular network. Renal lymph leakage may be immune-mediated since both patients were highly sensitized and had the same gene deletion. When transplanted, such patients may develop antibodies against nephrocystin or an interacting protein that was previously shielded from the immune system. Further studies are needed to elucidate the mechanisms of post-transplant 'renal ascites'.

TH-PO1112

The Use of CRRT for Removal of Dabigatran in an Anuric Patient with Life-Threatening Bleeding Tomoki Tsukahara, Lisa M. Antes. *Nephrology, University of Iowa Hospitals and Clinics, Iowa City, IA.*

Background: Dabigatran is an oral direct-thrombin inhibitor which has been approved for stroke prevention for non-valvular atrial fibrillation. While it has been associated with at least 260 fatal bleeding events worldwide, no specific antidote exists for dabigatran reversal.

Methods: A 71-year-old woman with atrial fibrillation on dabigatran 75 mg twice daily presented with hemorrhagic shock due to right hemothorax after sustaining a fall. Lab data on presentation included: hemoglobin 4.1 g/dl, creatinine 4.4 mg/dl, PT-INR 5.3 and aPTT 70 seconds. She underwent 2 emergent thoracotomiesand bled an estimated 9.2 liters. In spite of extensive coagulation factor and blood product support, her PT-INR remained 1.7 and aPTT 49 seconds.

Continuous renal replacement therapy (CRRT) was initiated on day 2 when she became anuric. She remained oligo-anuric for 6 hours after CRRT. Vasopressors were discontinued on day 3. She became independent of blood products on day 4. PT-INR and aPTT gradually improved, normalizing on day 5 when CRRT was discontinued. She was discharged home on day 13 with a GFR of 23 ml/min.

Conclusions: Hemodialysis has been proposed as a treatment in cases of severe bleeding with dabigatran, as it is dialyzable based on its properties of low protein binding (35%), volume of distribution (50-70L), and relatively small molecular weight (471 daltons). The fraction of the drug removed by intermittent hemodialysis is 68% in 4 hours. No studies have examined the efficacy of CRRT in removing dabigatran.

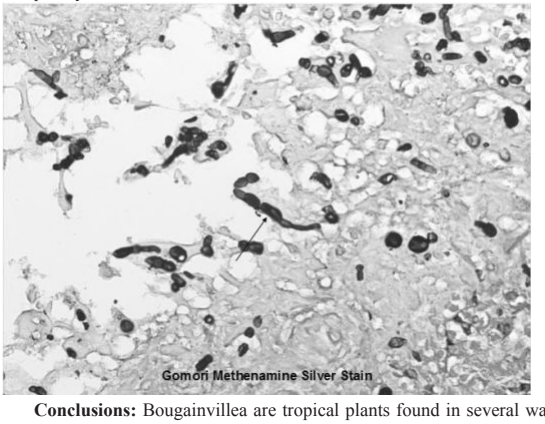
In our case we believe normalization of PT and PTT was achieved more quickly with CRRT. Even a single administered 150-mg dose of dabigatran, leading to moderate INR elevation, will take 4 days to be eliminated if the GFR is < 30 ml/min. The magnitude of the initial coagulation studies in our patient suggest dabigatran accumulated to a much greater degree and it would have taken longer to be eliminated without CRRT. With the more widespread use of dabigatran, it will be important to consider the potential role of CRRT to aid in dabigatran removal in unstable anuric patients with life-threatening bleeding.

TH-PO1113

Alternaria Phaeohyphomycosis from Exposure to Bougainvillea in Kidney Transplant Recipient Jaudat H. Khan,[1] Ashfaq S. Balla,[1] Venu Velagapudi,[1] Sonia Nagy Chimienti.[2] *[1]Renal Medicine, University of Massachusetts Medical School, Worcester, MA; [2]Infectious Disease, University of Massachusetts Medical School, Worcester, MA.*

Background: Alternaria is a cosmopolitan fungus isolated from plants and soil that rarely causes disease in healthy humans but can cause opportunistic infection in immunocompromised individuals. We hereby present a case of a kidney transplant recipient with cutaneous alternaria infection from exposure to Bougainvillea bushes.

Methods: A 69 year old Kenyan male received a living related donor kidney transplant done for diabetic nephropathy. He was induced with alemtuzumab and steroids. His maintenance immuno-suppression regimen consisted of Mycophenolate Mofetil, Tacrolimus, and Prednisone. His post-transplant course was uncomplicated with stable allograft function and acceptable Tacrolimus levels. Seven months after transplantation, the patient visited Kenya for an extended vacation where he regularly tended to Bougainvillea plants. Tacrolimus levels during overseas stay were not available. He developed non-tender papulo-nodular violaceous skin lesions on his upper and lower extremities at 12 months post-transplant with no other systemic symptoms. Biopsy of the skin lesions and culture revealed Alternaria species-septate fungal hyphal elements. The patient was initiated on itraconazole and immunosuppression was reduced. His lesions began to slowly improve and he was also referred for surgical excision as antifungal treatment alone may not completely cure lesions.



Conclusions: Bougainvillea are tropical plants found in several warm states in US and immunosuppressed patients should be counseled about using protection barrier during exposure.

TH-PO1114

Disseminated Bartonella Henselae Infection Inducing Hemophagocytic Lymphohistiocytosis in a Kidney Transplant Recipient Atul Poudel,[1] Vikas R. Dharnidharka.[2] *[1]Department of Pediatric Nephrology, University of Florida, College of Medicine, Gainesville, FL; [2]Department of Pediatric Nephrology, Washington University School of Medicine, St Louis, MO.*

Background: Bartonella Henselae (BH) is a bacterium that lives under the nails of cats and can cause infection in human. Presentations include lymphadenopathy and fever, but can include more systemic lesions in the liver, spleen, bone, eyes and/or brain. Hemophagocytic Lymphohistiocytosis (HLH) can present with similar systemic symptoms with pathognomonic findings of raised ferritin levels and hemophagocytic activity in the bone marrow.

Methods: A 14 year old Caucasian girl presented at 13 months post-kidney transplant with 10 day history of intermittent fever, diarrhea and weight loss. Examination was notable for pallor and dehydration.Laboratory tests showed marked drop in hemoglobin, thrombocytopenia, elevated serum creatinine, elevated LDH, high haptoglobin. Abdominal ultrasound showed hypodense lesions in spleen, ring-enhancing by CT scan. She had recently obtained a kitten, so azithromycin was started for putative BH infection. Soon after presentation, she developed septic shock, acute lung injury requiring mechanical ventilation, and acute kidney injury. All blood and urine cultures were negative for bacteria and fungi, CMV and EBV PCR were undetectable, Toxoplasma IgM was negative. BH IgM was strongly positive at 1:128 and doxycycline was added. In the presence of

worsening clinical picture and hemolytic anemia and thrombocytopenia, continuing fever and high serum ferritin level of 5025 ng/ml, HLH was investigated. Bone marrow aspirate confirmed hemophagocytic activity. Intravenous steroids were instituted and the patient's condition improved. She recovered from septic shock, was extubated, acute kidney injury, hemolytic anemia and thrombocytopenia resolved. Ferritin levels dropped to 1221 prior to hospital discharge.

Conclusions: Review of literature revealed that among a series of 17 cases of HLH in renal transplant patients, a solitary case was secondary to BH. Treatment in these cases has been with 2 agents and for several months.

BH infection is rare but can induce HLH in transplant recipients.

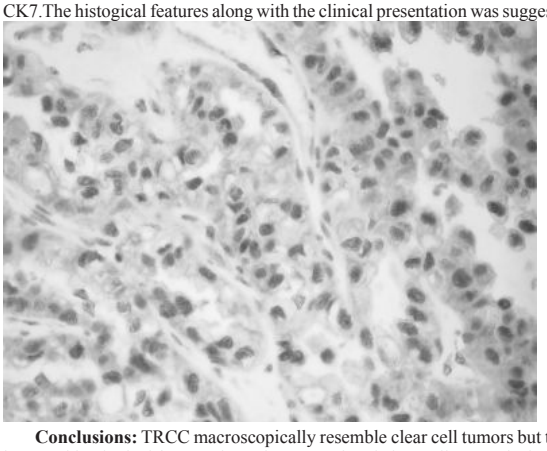
TH-PO1115

All that Grows in Pregnancy Is Not "BABIES": A Rare Case of Translocation Renal Cell Carcinoma in Pregnancy and Review of Literature

Bhavani Adusumilli, Murali Veluru. *Internal Medicine, William Beaumont Hospital.*

Background: Renal cell carcinomas (RCC) associated with Xp11.2 translocations are rarely seen in pregnancy. To the best of our knowledge, the case herein presented is the second report of Xp11.2 translocation RCC (TRCC) occurring during pregnancy.

Methods: A 30-year-old pregnant lady with past history significant for hypertension and RCC treated with radical nephrectomy 4 years ago presented with difficult to control hypertension. Labs including cell counts, serum creatinine, electrolytes, hepatic function panel, renin and cortisol levels and 24 hr urine catecholamines were all within normal limits. Blood pressure was adequately controlled by adjusting her oral regimen. The patient delivered a healthy child at 34 weeks and subsequently underwent a screening MRI 2 weeks later for follow up of RCC which showed a 10 cm x 5.6 cm x 6.1 cm non-homogeneously enhancing mass in the right renal fossa consistent with RCC for which she underwent resection. Microscopy revealed a carcinoma with papillary and clear cell features. The tumor cells stained positive for TFE3(transcription factor gene fusions), but negative for CK7.The histogical features along with the clinical presentation was suggestive of a TRCC.



Conclusions: TRCC macroscopically resemble clear cell tumors but the most distinct immunohistological feature absent in conventional clear cell RCCs, is the nuclear staining for the chimeric (mutant) TFE3 protein .Surgical resection is the preferred therapy and may be performed in pregnant women. There is some evidence that hormonal changes and increased immune tolerance during pregnancy predispose pregnant women toTRCC. They are inherently more aggressive in adults, thus early diagnosis and treatment plays a vital role in the management of these patients.

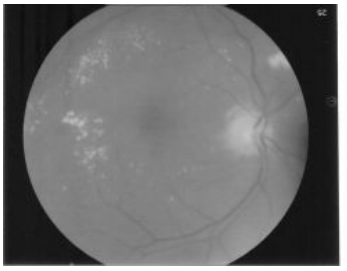
TH-PO1116

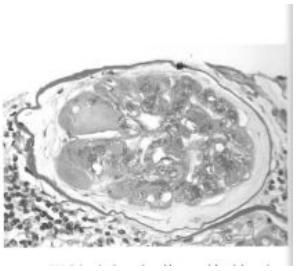
Nodular Glomerulosclerosis Preceding Overt Diabetes

Iqbal Masood, Alejandro Diez. *Nephrology, Lankenau Medical Center & Lankenau Institute for Medical Research, Wynnewood, PA.*

Background: Chronic diabetes leads to alterations in glomerular architecture (mesangial expansion, glomerular basement membrane thickening and glomeruloscelrosis) leading to albuminuria and progressive kidney disease. Current dogma states these changes are seen in patients with 10 to 15 years of overt disease. We are presenting a case of a patient with retinopathy and nodular glomeruloscelrosis on renal biopsy characteristic of diabetes, with impaired glucose tolerance (IGT) as the only manifestation of diabetes.

Methods: A 60 year old obese white female with past medical history of newly diagnosed hypertension and retinopathy was referred for proteinuria (2.7 grams/day). Renal work-up for proteinuria was unrevealing: creatinine, Hemoglobin A1c, infectious and autoimmune serology, and ultrasound were all negative or normal. A renal biopsy showed diffuse and nodular glomerulosclerosis and basement membrane thickening consistent with Kimmelsteil-Wilson syndrome.





Based on renal biopsy and fundoscopy the most likely etiology for proteinuria was diabetes; despite having normal hemoglobin A1c and non-diabetic range fasting blood sugars on multiple occasions. A 2-hour oral glucose tolerance test revealed impaired glucose tolerance(IGT). Initiation of Lisinopril reduced the proteinuria to 400mg/day within three months.

Conclusions: Idiopathic Nodular golermuloscelrosis has been linked to IGT, smoking and long standing hypertension. IGT and smoking promotes formation of advanced glycation end products, which alters the extracellular matrix, especially in the presence of pre-existing pro-sclerotic insult like hypertension. Classically, changes in glomerular architecture caused by diabetes are sine qua non of long-standing overt diabetes. This case presents an uncommon presentation for a common disease in which renal histological changes are seen in the absence of overt underlying disease.

TH-PO1117

A Case of Type B Lactic Acidosis in a Patient with Large Cell Lymphoma

Iqbal Masood, Geoffrey S. Teehan. *Nephrology, Lankenau Medical Center & Lankenau Institute for Medical Research, Wynnewood, PA.*

Background: Lactic acidosis most commonly occurs during tissue hypoperfusion, but can occur in its absence, and is called either Type B or D lactic acidosis. We present a case of Type B lactic acidosis refractory to hemodialysis due to large cell lymphoma.

Methods: An 81 year old Caucasian male with baseline creatinine of 1.6mg/dl and Chronic Lymphocytic leukemia(CLL) for 5 years, not on treatment, presented to the hospital feeling weak, found to be in ATN and profound lactic acidosis with lactate of 12.3 mmol/L, BUN 95, Creatinine 9.2 and PH of 7.10. No hypotension or sepsis was present. Physical exam was essentially normal. CT scan of abdomen and pelvis revealed a 4.4 cm infrarenal aneurysm.

Patient was started on IV fluids, but lactate levels remained high, requiring hemodialysis which had little effect on his lactic acid levels.

He underwent CO2 angiogram which ruled out aortic and renal artery dissection. A diagnostic laparoscopy showed no evidence of mesenteric ischemia. Liver function test were normal as was a liver biopsy which did not show lymphomatous infiltration. Volatile acid screen was negative. WBC count was 17200 with 70% lymphocytes. There was no evidence of heavy CLL burden as there was no significant adenopathy or evidence of bone marrow involvement. A course of steroids was unsuccessful. Patient was administered thiamine without effect and Riboflavin levels were elevated. Patient underwent bone marrow biopsy which showed large B cell lymphoma. Due to deteriorating condition, patient was made hospice and died.

Conclusions: Tissue hypoperfusion is the most common cause of lactic acidosis. In type B lactic acidosis there is no evidence of tissue hypoperfusion. Usual causes include alcoholism, Metformin, HIV, Malignancy including leukemia, lymphoma and solid tumors. The mechanism for lactic acidosis in malignancy is unclear. An anaerobic mechanism due to dense cluster of tumor cells, and or metastatic replacement of liver parenchyma by tumor cells has been proposed. Alternatively, lactic acid may be produced by tumor cell. Removal of the tumor by surgery, chemotherapy or radiation can correct lactic acidosis.

TH-PO1118

Acute Kidney Injury with Bluish Green Urine,Highest Possible FENa and Rapid Recovery after Methylene Blue Infusion

Iqbal Masood, Paul Robbins. *Nephrology, Lankenau Medical Center, Wynnewood, PA.*

Background: Methylene blue is used as a tissue marker,for the treatment of methemoglobinemia and refractory vasodilatory shock after cardiac surgery.It leads to bluish green discoloration of the urine .We are presenting a case of acute kidney injury in a patient who received Methylene blue for refractory shock with a rather quick recovery along with interference in urine creatinine assay leading to a very high FENa.

Methods: 77 Year old white male with CKD II/CHF, EF 10% underwent insertion of left ventricular assist device complicated by refractory hypotension due to bleeding requiring Methylene blue infusion with improvement. Patient became oliguric with creatinine of 4mg/dl and was noted to have bluish green discoloration of the urine and skin along with edema. U/A consistent with ATN with urine creatinine noted to be less than 10, in spite of having urine urea nitrogen of 108 and urine Na of 130 on 2 occasions ,giving a very high variable FENa between 39-350%.Urinary creatinine started to rise once urine color cleared up in 3 days. Jaffe method was used to check creatinine assay and manufacturer did not mention methylene blue affecting the assay, but based on urine creatinine it probably did. Patient's creatinine came down to 0.9mg/dl in few days with good urine output without needing dialysis.

Conclusions: Methylene blue has been shown to cause a decrease in Nitric Oxide (which is associated with proximal tubular injury in sepsis) and attenuation of the urinary excretion of renal tubular injury markers in refractory septic shock. Our patient in spite of having a lots of co-morbidities had good renal recovery, unclear whether methylene blue played a role in recovery or not.In our patient, a possible interference with urinary creatinine assay was noted, therefore in patients who receive methylene blue, urinary creatinine may not reflect true creatinine clearance.

TH-PO1119

Double Trouble: The Importance of Examining Nonneoplastic Tissue in Nephrectomy Specimens with Neoplasia Suzanne Wilhelmus,[1] Ingeborg M. Bajema,[1] Natascha Goemaere,[2] Marinus A. Van Den Dorpel,[3] Reinout M. Swart.[3] *[1]Pathology, Leiden University Medical Center, Leiden; [2]Stichting Pathan, Rotterdam; [3]Nephrology, Maasstad Hospital, Rotterdam, Netherlands.*

Background: A nephrectomy for an epithelial neoplasia not only yields tumor but also normal tissue. Often this tissue is glanced at, but not looked at as one would look at a renal biopsy. This case demonstates the importance of careful examination of nonneoplastic tissue in resection specimens.

Methods: A 63 year old woman was diagnosed with right sided renal cell carcinoma after she was referred with non-specific complaints and an increased ESR. A nephrectomy was performed. The first postoperative day her creatinine increased to 358 µmol/l (from 96 µmol/l one month earlier). The deterioration of renal function could not be attributed solely to reduction in glomerular number. Further tests excluded pre- or postrenal causes. Urine analysis showed erythrocyturia and subnephrotic range proteinuria. With a differential diagnosis of tubulointerstitial nephritis or a glomerulonephritis the nonneoplastic renal tissue was re-examined showing a necrotizing crescentic glomerulonephritis, mixed class. Frozen tissue for immunofluorescence was not available. Electron microscopy showed no deposits or podocyte lesions, consistent with a pauci-immune glomerulonephritis. ANCA-testing revealed positivity for MPO-ANCA, although at a very low level. ANCA-associated vasculitis (limited to the kidney) was diagnosed and treatment with steroids and cyclophosphamide started. An initial response was seen, but treatment was discontinued early because of a severe pulmonary infection.

Conclusions: In the initial pathology work-up, only a small amount of nonneoplastic tissue was examined in the HE-staining. During the re-examination, extra tissue was embedded and silver and PAS stainings were performed, by which the glomerulonephritis became apparent. This case demonstrates the importance of careful examination of nonneoplastic renal tissue of nephrectomy specimens as others have also recognized (Henriksen et al. Arch Pathol Lab Med 2009). It gives the opportunity to detect and treat early in the course of incidental kidney disease, thereby improving outcome.

TH-PO1120

Urothelical Carcinoma of the Renal Pelvis Mimicking Acute Pyelonephritis with Abscess Jinuk Jeong, Kitae Bang, Jongho Shin. *Department of Internal Medicine, Eulji University of Medicine, Eulji Medical Center, Daejeon, Korea.*

Background: Primary tumors of the renal pelvis and collecting system are relatively uncommon. Their clinical presentation is nonspecific and variable, and they can be mistaken for acute pyleonephritis or renal abscesses. This report presents an unusual case of urothelial carcinoma in which was at first mimicking acute pyelonephritis and presented as renal abscess several months later.

Methods: A 75-year-old man presented with a week history of left flank pain.His urinalysis did not show protein and RBC. Uine cytology revealed no atypical cells.Computed tomography showed left renal pelvic and ureteral wall thickening with enhancement, mild hydronephrosis with multifocal wedge shaped decreased enhancing lesions on Lt renal lower pole (Figure 1). Oral treatment with ciprofloxacin was given for 2-weeks period, And clinical improvement was observed.3 months later patients developed abdominal pain on Lt upper quadrant again. Rechecked abdominal CT revealed 8*8cm lobulated abscess with septation in the lower pole of the left kidney (Figure 2). But irregular thickend abscess wall with enlarged enhanced peripheral rimmed central necrotic paraaortic lymph nodes and absense of fever and elevated immflamatory markers were thought to be malignancy or tuberculosis. So percutaneous nephrostomy was done and about 200 ml/day of hemorrhagic fluid were drained.The microbiologic features including tuberculosis were sterile.Cytology revealed no malignancy. So the patient underwent laparoscopic excisional biopsy of paraaortic lymph nodes. The pathologic diagnosis was urothelical carcinoma (Figure 3).

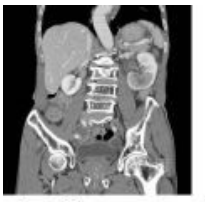


Figure 1-1

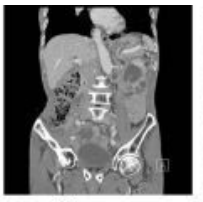


Figure 1-2

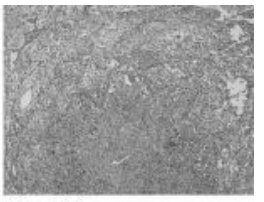


Figure 1-3

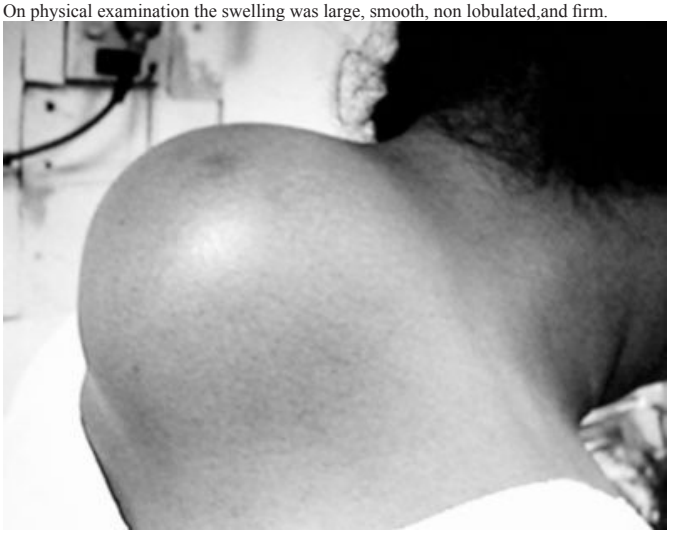
Conclusions: Urothelial carcinoma present in a variety of ways mimicking acute pyelonephritis or renal abscesses.We should suspect malignancy if there is no inflammatory sign or symptoms in the finding of acute pyelonephritis or abscess in various imaging including CT.

TH-PO1121

Unusual Complication in Chronic Renal Failure: Cervical Tumoral Calcinosis with Severe Secondary Hyperparathyroidism Sham Sunder, Himanshu Verma, Venkataramanan K. *Nephrology, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, Delhi, India.*

Background: Cervical tumoral calcinosis due to severe secondary hyperparathyroidism is an uncommon and severe complication of chronic renal failure.

Methods: A 50 year-old female on thrice weekly maintenance hemodialysis for the last three years presented with a solitary, tumour-like swelling over the nape of the neck. On physical examination the swelling was large, smooth, non lobulated,and firm.



The patient was investigated and was found to be having high serum calcium x phosphate product with high serum iPTH, CT Scan of the cervical spine showed a well defined calcified mass along the spinus process, radionucleide parathyroid scan suggested parathyroid hyperplasia ,and biopsy from the mass confirmed the diagnosis of tumoral calcinosis.The patient was treated with vitamin D withdrawl, strict control of serum phosphate levels with sevelamer, and sub-total parathyroidectomy. Postoperatively the neck swelling gradually decreased but again started increasing in size after two years of parathyroidectomy. The patient was prescribed cinacalcet along with sevelamer and the swelling again decreased in size with improvement of biochemical parameters.

	Pre Parathyroidectomy	6 months post Parathyroidectomy	2 years post parathyroidectomy	After Cinacalcet therapy
S. Calcium	10.10 mg/dl	8.9 mg/dl	10.30 mg/dl	9.5 mg/dl
S. Phosphate	8.1 mg/dl	7 mg/dl	8.4 mg/dl	7.40 mg/dl
S. iPTH	1324 pg/cc	253 pg/cc	551 pg/cc	123.20 pg/cc

Conclusions: The prevalence of tumoral calcinosis in hemodialysis patients is 0.5% to 1.2%. Uremic tumoral calcinosis usually occurs after long duration of hemodialysis (approximately 10 years). In our patient tumoral calcinosis occurred after 3 years of maintenance hemodialysis. The metastatic calcification occurring in the patient was most likely related to high calcium x phosphate product with secondary hyperparathyroidism.

TH-PO1122

Life Threatening Hypocalcemic Tetany Following the Use of Sodium Phosphate Enemas Elizabeth I. Anyaegbu. *Pediatric Nephrology, Washington University in St Louis, St Louis, MO.*

Background: The clinical spectrum of hypocalcemia ranges from a dearth of symptoms to life threatening tetany. Common causes of hypocalcemia include renal insufficiency, tumor lysis syndrome, hypoparathyroidism, rhadbomyolysis and exogenous administration of phosphorus-containing compounds. With excessive phosphate administration, calcium phosphate precipitation occurs in the skeleton and microvasculature, resulting in hypocalcemia.

Methods: A 6-year-old female with a history chronic constipation presented to the ED with altered mental status, muscle rigidity and tetany. Her home bowel regimen consisted of daily polyethylene glycol and 2 sodium phosphate enemas administered every other day. She complained of abdominal pain attributed to constipation, the day of presentation. She received 3 adult enemas within 12 hours to achieve an explosive passage of stool and became unresponsive within a few hours. An EKG showed sinus tachcardia with non-specific ST and T wave abnormalities. Results included serum phosphorus of 45.2mg/dl, potassium 1.8 mmol/L, bicarbonate 20 mmol/L, total calcium 5.1 mg/dl, ionized calcium 1.8 mg/dl, albumin 4.5mg/dl, magnesium 1.5 mg/dl, and serum creatinine of 0.8 mg/dl, increased from 0.3 mg/dl 8 months prior. She received normal saline and a calcium gluconate infusion was commenced. She improved dramatically with a decrease in serum phosphorus to 7.3 mg/dl within 8 hours and increase in ionized calcium to 2.8 mg/dl. By hospital day 2, her mental status and electrolyte disturbances normalized. Her serum creatinine was 0.4 mg/dl at discharge.

Conclusions: Sodium phosphate enemas deliver a massive exogenous phosphate load which can produce fatal acute hyperphosphatemia. Increasing urinary phosphate excretion is essential; therefore aggressive intravenous hydration is the first line of treatment. Symptomatic hypocalcemia requires prompt treatment with intravenous calcium salts. Coexisting hypomagnesemia should always be corrected. Acute hyperphosphatemia usually resolves quickly in patients with normal renal function. Hemodialysis may be necessary if renal function is impaired. The indication for hemodialysis is oliguria or anuria, rather than absolute serum phosphorus levels.

TH-PO1123

Hypercalcaemia and Renal Failure: A Diagnostic Conundrum Louise E. Ross, Konstantinos Koutroutsos, Debasish Banerjee. *Renal Department and Transplantation Unit, St George's Healthcare NHS Trust, London, United Kingdom.*

Background: Hypercalcaemia and renal failure is not an uncommon clinical problem for a nephrologist. When associated with a serum calcium of >3mmol/L the differential diagnoses are few and initial blood tests, including phosphate, parathyroid hormone and serum electrophoresis narrows the differential to even fewer. However, we describe a case which required a multidisciplinary approach, therapeutic trial and several investigations before the diagnosis was made.

Methods: A 50-year-old Caucasian man presented with a two week history of nausea and constipation. Examination was normal.

Biochemical Investigations

Investigation	Result	Reference Range
Creatinine	387	60-110 umol/L
Adjusted Calcium	3.71	2.2-2.6 mmol/L
Phosphate	1.6	0.8-1.5 mmol/L
PTH	2.5	1.1-6.9 pmol/L
PTHrP	<0.7	<2 pmol/L
ALP	52	30-130 U/L
ACE	38	20-70 U/L
25 hydroxy vitamin D	137	75-200 nmol/L
1,25 dihydroxy vitamin D	102	40-150 nmol/L
Urine Calcium	18.7	2.5-7.5 mmol/24hrs

A renal ultrasound, chest X-ray, bone scan, and myeloma screen showed no abnormality. A CT chest, abdomen, pelvis reported bilateral small pulmonary nodules of uncertain significance. A renal biopsy showed tubulointerstitial scarring and tubular dystrophic calcification in keeping with nephrocalcinosis. An MRI of the neck suggested an enlarged parathyroid gland and hence a parathyroidectomy was performed but the serum calcium remained elevated. To investigate further a PET scan highlighted a single nodule in the right lower lobe of the lung. The biopsy of the nodule demonstrated non-caeseating granulomas establishing a diagnosis of sarcoidosis. Treatment with high dose corticosteroid improved the serum calcium and partially improved the renal function.

Conclusions: This case shows how a relatively common presentation of hypercalcaemia with renal failure, which was due to sarcoidosis can be difficult to diagnose. The patient underwent a kidney biopsy and parathyroidectomy but the diagnostic tissue biopsy was after a PET scan. It highlights the importance of PET scan to search for sites of inflammation which cause hypercalcaemia. Every effort should be made in such cases as the diagnosis may be difficult to make but easy and satisfying to treat.

TH-PO1124

Severe Hypophosphatemia due to Oncogenic Osteomalacia Associated with Metastatic Colon Adenocarcinoma David E. Leaf,[1] Harald Jüppner.[2] *[1]Renal Medicine, Brigham and Women's Hospital, Boston, MA; [2]Pediatric Endocrinology, Massachusetts General Hospital, Boston, MA.*

Background: Oncogenic osteomalacia, also known as tumor-induced osteomalacia, is a paraneoplastic syndrome associated with elevated levels of the phosphaturic hormone Fibroblast Growth Factor 23 (FGF23). It is usually associated with benign mesenchymal tumors of bone or soft tissue, however, 10% of cases are due to malignant tumors. To date, no cases of oncogenic osteomalacia associated with colon cancer have been described.

Methods: An 80-year old woman with recently diagnosed stage IV adenocarcinoma of the cecum with liver metastases was referred to nephrology for evaluation of severe symptomatic hypophosphatemia. She had several days of fatigue and muscle weakness and labs revealed a serum phosphate of 0.4 mg/dl. She had no personal or family history of hypophosphatemia. Initial serum and urine studies are shown in the table.

Laboratory Values

	Reference Range	Patient's Value
Calcium (mg/dl)	8.5-10.5	9.2
Phosphate (mg/dl)	2.6-4.5	0.4
Creatinine (mg/dl)	0.6-1.5	0.7
Fractional Excretion of PO4 (%)	<5	34
25(OH)D (ng/ml)	33-100	33
1,25(OH)2D (pg/ml)	18-78	13
PTH (pg/ml)	10-60	36
PTH-rP (pmol/L)	<2.0	1.1
c-terminal FGF-23 (RU/ml)	7-71	674

Fractional excretion of phosphate was inappropriately elevated, suggesting renal phosphate wasting as the etiology of her hypophosphatemia. Elevated levels of FGF23 and decreased levels of 1,25-dihydroxyvitamin D confirmed the diagnosis of oncogenic osteomalacia. Tissue obtained from biopsy of a metastatic liver lesion stained positively for a highly specific FGF23 antibody. She was treated with palliative chemotherapy (Fluorouracil and Oxaliplatin) as well as supplements containing 750mg phosphorus three times daily, and her serum phosphate levels normalized.

Conclusions: Reported malignant tumors associated with oncogenic osteomalacia include prostate adenocarcinoma, small cell lung carcinoma, and multiple myeloma. We report the first case of colon adenocarcinoma associated with oncogenic osteomalacia, a paraneoplastic syndrome which should be considered in patients with malignancy, hypophosphatemia, and renal phosphate wasting.

TH-PO1125

Idiopathic Hypercalciuric Nephrotlithiasis with 1,25 Dihydroxy D_3 Hypervitaminosis, Growth Retardation and Osteopenia: Testing for Renal Epithelial Calcium Channel TRPV5 and Long Term Response to Hydrochlorothiazide and Growth Hormone Swati Arora,[1] Anke Lameris,[2] Joost G. Hoenderop,[2] Barbara A. Clark.[1] *[1]Nephrology, Allegheny General Hospital, Pittsburgh, PA; [2]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Physiology, Radboud University Nijmegen Medical Centre, Njmegen, Netherlands; [4]Nephrology, Allegheny General Hospital, Pittsburgh, PA.*

Background: Idiopathic hypercalciuric nephrolithiasis has varying phenotype with no specific gene yet identified. It is not well rcognized that excess endogenous 1,25 $(OH_2)D_3$ could be the cause or that the initial presentation may be bone/ growth problems. The role of the renal epithelial calcium channel, TRPV5, in this disorder is unknown.

Methods: A short stature 17yo WM with fam hx of stones in F and PGF presented with his first episode of nephrolithiasis. Workup revealed hypercalciuria (457 mg/day); (PO_4, citrate, oxalate wnl); pH 6; sCa 9.9 mg/dL; PO_4 3.8 mg/dL; sCr and HCO_3 wnl; iPTH < 3 pg/ml; low 25 OH vitamin D (14 ng/ml); but elevated 1,25 (OH_2) D_3 at 160 pg/mL and osteopenia (bone age 14; Z score -3.8; 15th % ht). Because of phenotypic similarity to the hypercalciuria model of knockout mice deficient in TRPV5 (also increased 1,25 $(OH_2)D_3$ due to over expression of 1,25 alpha hydroxylase), his genomic DNA was sequenced for TRPV5. No mutations were discovered. He was treated with hydrochlorothiazide (HCTZ) and Norditropin (hrGH). Teriparatide was considered but deferred due to uncertain longterm side effects at his age. At age 21 he has not had any recurrent symptomatic stones. Repeat calcium excretion has been as low as 238 mg/day; latest Z score is -2.6 and height is now 50th%.

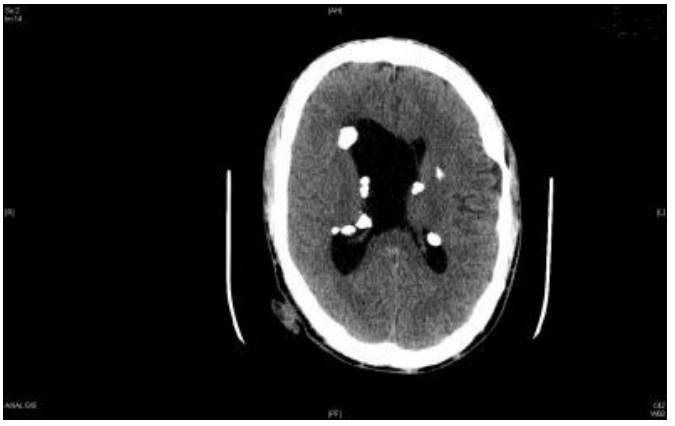
Conclusions: Some cases of idiopathic hypercalciuria have significant elevations of 1,25 (OH_2)Vitamin D_3 that can contribute to the hypercalciuria. Initial presentation may be bone/growth problems even before symptomatic stones. Suppressed PTH levels may be a clue. The genetic defect does not appear to involve TRPV5 in this patient. Response is good to HCTZ. In adolescents with associated growth retardation and bone demineralization hrGH may help. Use of PTH might be considered in older individuals.

TH-PO1126

Tuberous Sclerosis and Polycystic Kidney Disease: A Are Finding Chadi Saifan, Rabih Nasr, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Polycystic kidney disease is a cause of chronic kidney disease. The association with tuberous sclerosis is linked to a genetic defect because of the proximity of genes: TSC1 and TSC2. It is known by tuberous sclerosis complex.

Methods: 30 y/o man presented for a 1-day history of right flank pain and vomiting. Past medical history was significant for tuberous sclerosis, seizure disorder, and hypertension. Family history was irrelevant. Physical examination revealed speech and hearing impairment, multiple faical angiofibromas, and hypomelanotic macules on bilateral upper extremities. Lab tests revealed BUN of 22 mg/dl and Cr of 3.59 mg /dl. CT of the abdomen and pelvis showed innumerable cysts within the both kidneys. Fatty soft tissue masses were also seen in the liver and the right kidney suggestive of angiomyolipoma. Multiple calcified tubers were detected on a CT of the head within the areas lining the ventricles, a feature consistent with tuberous sclerosis.



Patient was started on IV fluids and improved clinically. He significantly felt better over three days and his kidney function mildly recovered with BUN of 18 mg /dl and creatinine of 2.13 mg/dl.

Conclusions: Tuberous sclerosis is a rare genetic disease. PKD is a multisystemic and progressive genetic disorder characterized by the formation and enlargement of cysts in the kidney and other organ. Clinical features usually begin in the third to fourth decade of life, but cysts may be detectable in childhood and in utero. Early imaging study can detect the APKD in the tuberous sclerosis patient which can subsequently help assess and manage the deterioration in kidney function.

TH-PO1127

A Novel *UMOD* Mutation in a Family with Medullary Cystic Kidney Disease Type 2 Masafumi Kamijo,[1] Masahito Tamura,[1] Nana Ishimatsu,[1] Tetsu Miyamoto,[1] Ryota Serino,[1] Narutoshi Kabashima,[1] Kaori Kanegae,[1] Yumi Furuno,[1] Kenichiro Bando,[1] Junichi Nakamata,[1] Akihiro Kuma,[1] Shingo Ishimori,[2] Naoya Morisada,[2] Kazumoto Iijima,[2] Yutaka Otsuji.[1] *[1]Department of Cardiology and Nephrology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan; [2]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan.*

Background: Mutations in the *UMOD* gene cause medullary cystic kidney disease (MCKD) type 2, which is an autosomal dominant tubulointerstitial kidney disease. We report the case of a family with MCKD type 2 characterized by a novel mutation in the *UMOD* gene.

Methods: A 31-year-old woman developed moderate kidney dysfunction (estimated glomerular filtration rate, 59.7 mL/min/1.73 m^2) without any abnormality in urinalysis. She had mild hyperuricemia and had never experienced gout attacks. Her grandfather, father, and aunt developed end-stage kidney failure, and were introduced to maintenance hemodialysis when they were in their forties. Histological analysis revealed diffuse tubulointerstitial fibrosis with moderate inflammatory cell infiltration and tubular atrophy. Renal cysts were not detected. Peripheral blood mononuclear cells were isolated from the patient and her father, DNA was extracted from these cells, and a mutation analysis of the *UMOD* gene was conducted. This analysis revealed a novel missense C135G mutation in exon 3. We did not detect any mutation in 100 healthy volunteers.

Conclusions: MCKD type 2 is a rare disorder, and about 50 *UMOD* mutations other than the one detected in the present cases have been reported thus far. Similar to the mutation in the present cases, most of the *UMOD* mutations are missense changes in one of the conserved cysteine residues, and the mutations are clustered in exons 3 and 4 encoding the N-terminal half of the protein. In patients with MCKD type 2, chronic renal failure generally occurs between the second and fourth decade of life. There is no specific therapy for this condition other than the correction of water and electrolyte imbalance that may occur. Genetic testing is necessary for definite diagnosis of MCKD type 2, and accumulation of data on such cases is required.

TH-PO1128

Cystic Kidney Disease in a Patient with Systemic Toxicity from Long-Term D-Penicillamine Use Farrukh M. Koraishy, Neera K. Dahl. *Internal Medicine/ Nephrology, Yale University School of Medicine, New Haven, CT.*

Background: D- Penicillamine (PCA) has been extensively used in the management of cystinuria. It forms a polymer with cysteine to make it more soluble and hence reduces stone formation. PCA has also been known to cause impaired collagen deposition and dysfunction in the elastic fibers resulting numerous systemic toxicities. Renal disease associated with PCA therapy has been reported to be at the glomerular level; typically a membranous glomerulonephritis pattern with proteinuria.

Methods: Here we describe a patient with severe bilateral cystic kidney disease associated with long-term PCA use (one gram per day for over twenty years) for cystinuria. The cysts in the kidneys were noted during imaging as part of the work up of a new onset of renal dysfunction. A pancreatic cyst was also noted. This was associated with the subsequent development of other systemic toxicities of PCA including cutis laxa (elastolysis). All potential causes of bilateral kidney cysts were ruled out. Of note, the patient had no evidence of cysts on renal imaging three year prior when his kidney function was also normal. Over the next two years, the size/ number of his kidney cysts continued to progress and his renal function declined. At the same time, the PCA-related systemic toxicities also progressed. PCA was consequently stopped and replaced with Captopril. The progression of cystic disease in the kidney halted since the discontinuation of PCA therapy and the renal function became stable. Other systemic toxicities (including the pancreatic cyst) have also remained stable.

Conclusions: To our knowledge, this is the first report of cystic kidney disease associated with PCA therapy in the setting of known systemic toxicities from PCA. The etiology of cyst formation due to PCA is not clear. The possible mechanism is the malfunction of the elastic fiber proteins and extra-cellular matrix of the kidney leading to tubular dilatation and cyst formation. Clinicians should be alerted to the potential of cystic renal disease in association with systemic Penicillamine therapy.

TH-PO1129

Vancomycin Causing Acute Allergic Interstitial Nephritis as a Part of Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) Syndrome Dhwanil Vyas, Anuja Vyas, Mamta Shah, Nancy Day Adams. *University of Connecticut.*

Background: Vancomycin causes nephrotoxicity at a frequency of 5-35%. The most common mechanism of injury is increased oxidative stress in renal tubules leading to reversible ischemic injury. However, since its first description by Eisenberg et al. in 1981, only a few cases of Acute Interstitial Nephritis (AIN) due to Vancomycin have been reported. Recently, Vancomycin has also been associated with DRESS Syndrome. We describe a case of Vancomycin induced AIN as a part of DRESS Syndrome improving dramatically with glucocorticoid therapy.

Methods: A 50 year-old man with stage IV Squamous Cell Cancer of the left tonsil developed neutropenia and MRSA sepsis after induction chemotherapy. After 4 days in the hospital, he was discharged home on IV Vancomycin. Vancomycin trough upon discharge was 18 ug/ml. 2 weeks later, the patient was readmitted with fever, transaminitis, acute kidney injury with creatinine of 3.4 mg/dl and random Vancomycin level of 68 ug/ml. Over next few days, along with worsening liver and kidney function, he developed a confluent erythematous rash and leukocytosis with prominent eosinophilia (35%). Due to these findings, the diagnosis of DRESS Syndrome was considered. The kidney biopsy was consistent with AIN pattern with a predominantly eosinophilic infiltration. The patient was promptly started on 60 mg/day of IV Methylprednisolone (1mg/kg/day of Prednisone equivalent). At the time of discharge, after 6 days of glucocorticoid therapy, his rash had disappeared and the fever, leukocytosis, eosinophilia, liver and kidney dysfunction had resolved.

Conclusions: A thorough literature search yielded less than 10 cases of biopsy proven AIN and even fewer cases of DRESS syndrome attributable to Vancomycin. However, these entities are possibly missed often due to a low index of suspicion. Clinico-pathologically, they represent a spectrum of allergic reactions, so glucocorticoid therapy is highly effective. Vancomycin induced AIN and DRESS Syndrome, albeit rare, should be considered in appropriate clinical settings as significant morbidity can be avoided by prompt therapeutic interventions, as depicted in this case.

TH-PO1130

Rasburicase Therapy for Acute Urate Nephropathy in an Adult Patient with Lesch-Nyhan Syndrome Kalanie Mendis, John A. Walker. *Medicine, UMDNJ - Robert Wood Johnson Medical School, New Brunswick, NJ.*

Background: Lesch-Nyhan Syndrome (LNS) is an X-linked recessive disorder due to mutations in the *HPRT1* gene, resulting in a deficiency of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase 1. The resultant overproduction of uric acid leads to hyperuricemia, hyperuricosuria, nephrolithiasis, and gout. Children with LNS present typically with impaired motor development, dyskinesias, and compulsive self-mutilation. Acute kidney injury (AKI) due to acute urate nephropathy (AUN) may complicate LNS.

Methods: A 28 year old man with LNS treated chronically with allopurinol (300 mg PO daily) was admitted with shortness of breath and fever. Baseline laboratory values included a serum creatinine of 0.7 mg/dL and a serum uric acid (UA) of 6.1 mg/dL. Sputum culture was positive for Klebsiella sp. and the patient was treated with levofloxacin. On hospital day 19 the patient pulled out his nasogastric tube and for the next 3 days did not receive allopurinol. During this 3 day period his serum creatinine and UA increased to 3.5 mg/dL and 20.9 mg/dL, respectively, and he became oliguric. His urine sediment contained many uric acid crystals, and urine fractional excretion of sodium and uric acid were 2.9% and 47.02% respectively. A presumptive diagnosis of AUN was made. Nasogastric intubation was reestablished and enteral allopurinol was resumed, accompanied by intravenous isotonic sodium bicarbonate and one intravenous dose of rasburicase (0.2 mg/kg). 2 days later, serum creatinine and UA had declined to 2.1 mg/dL and 6.1 mg/dL respectively, and non-oliguria ensued.

Conclusions: In AUN, kidney injury results from intratubular deposition of uric acid crystals with attendant tubular obstruction and granulomatous inflammation accompanied by macrophage and T-cell infiltration. Rasburicase is a recombinant urate oxidase used in the prophylaxis or treatment of hyperuricemia associated with tumor lysis syndrome. There has been one report of a neonate with LNS and AUN treated successfully with rasburicase. To our knowledge this is the first report of the safe and successful use of rasburicase in the treatment of acute hyperuricemia and presumed AUN in an adult patient with LNS.

TH-PO1131

Maintenance of Residual Renal Function with Low Dose Immunosuppressive Therapy in Peritoneal Dialysis Patient with Allograft Failure Nadear A. Elmahi, Tibor Fulop, Kenneth E. Kokko. *Department of Medicine, Division of Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: Many patients with failed allograft progress to end stage renal disease (ESRD) and require dialysis. Immunosuppressive therapy is often withdrawn in those patients and this can lead to an accelerated loss of residual renal function (RRF). While maintenance of RRF appears to provide a survival benefit to peritoneal dialysis (PD) patients, it is not clear whether this benefit of maintaining RRF in failed allograft patients returning to PD outweigh the risks of maintaining immunosuppression. This case report addresses the role of minimal dose of immunosuppression in maintaining RRF in PD patient with failed allograft.

Methods: A 49 year-old Caucasian male developed progressive allograft failure nine years after living-donor renal transplantation (LDRT). Hemodialysis was initiated via tunneled dialysis catheter (TDC) and immunosuppression was gradually withdrawn. Two weeks after the withdrawal of the immunosuppressive therapy he developed a febrile illness, which necessitate removal of the TDC and conversion to PD. He was maintained on small dose of tacrolimus (1 mg daily) and prednisone (5 mg daily). Currently (1 year later) he is doing exceedingly well on cycler-assisted PD. Residual urine output ranges between 600-1200 mL/day. Total weekly Kt/V achieved 1.82.

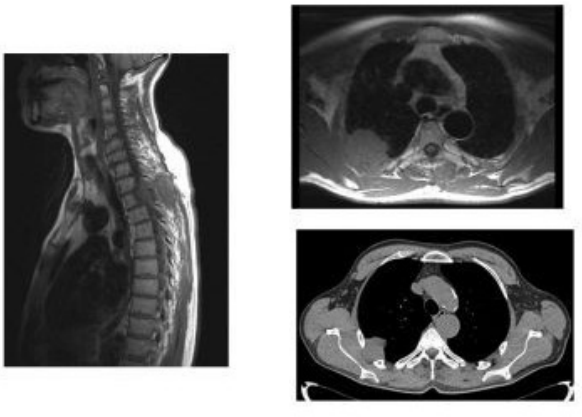
Conclusions: When a renal transplant fails, the nephrologists will be challenged by two major decisions: optimal management of immunosuppression and whether or not to perform graft nephrectomy. The use of low dose immunosuppressive medications in failed renal allograft is the most reasonable approach. There is no clear evidence to support the superiority of hemodialysis or peritoneal dialysis in the treatment of patients with failed allograft who progress to ESRD. Our patient failed renal transplant and was declared ESRD. Peritoneal dialysis was initiated and he was maintained on minimal immunosuppressive regimen, which helped to maintain good RRF. This strategy will need further study in well-defined cohorts of PD patients with failed allografts and residual renal function to determine efficacy and safety.

TH-PO1132

A Rare Case of Multiple Solitary Plasmocytoma in a Peritoneal Dialysis Patient Pietro Claudio Dattolo, Alma Mehmetaj, Stefano Michelassi, Giulia Sansavini, Sergio Sisca, Francesco Pizzarelli. *Nephrology, S M A Hospital, Florence, Italy.*

Background: Extramedullary plasmacytoma, solitary plasmacytoma of bone and multiple solitary plasmacytoma are rare clinical entities, different from multiple myeloma. We report a case of multiple solitary plasmacytoma extended to the surrounding soft tissues. The clinical picture case is remarkable because of the scarcity of similar cases in literature.

Methods: A 57 year old male started peritoneal dialysis in July 2011. In september 2011 a rapidly-growing swelling interesting soft tissues appeared in the median region of the back and physical examination showed ataxic gait. Imaging studies (chest HRCT, MRI of spine, contrast CT) found pleural-extrapleural solid tissue associated with lytic changes of DII and DIII and a mass lesion substituting most of the vertebral body of DIII, extended to the vertebral canal and dislocating the dural sac.



A biopsy of the soft tissue swelling revealed monotypic lambda plasma cells infiltrating both skin and pleura. A bone-marrow biopsy from the posterior iliac crest was not significant (< 10 % interstitial plasmacytosis). Serum calcium levels were in normal range. Serum and urine tests were negative for monoclonal immunoglobulins and free light chains. A multifocal solitary plasmacytoma was diagnosed.

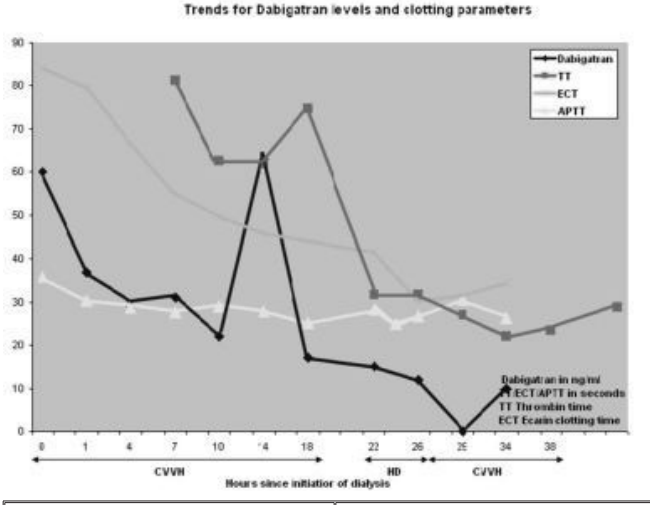
Conclusions: Solitary plasmacytoma is defined as the association of monoclonal plasma cell infiltrate in a "single" lytic lesion of bone which may eventually spread to surrounding soft tissues, no lytic changes of other bones, no pathological plasma cell proliferation on random bone biopsy, no systemic abnormalities possibly due to myeloma (anaemia, hypercalcemia, renal impairment, serum or urine monoclonal protein). All these fetaures perfectly fitted to our patient but one, that is more than one bone were affected. So, the case falls within the rare entity of solitary multiple plasmacytoma.

TH-PO1133

Role of Dialysis in Dabigatran Related Bleeding Ashish Verma, Reenu Nathan, Konstantin Abramov, Matthew J. Trainor, Jeffrey S. Stoff. *Renal Medicine, University of Massachusetts Medical School, Worcester, MA.*

Background: Dabigatran (DG), an anticoagulant for stroke prophylaxis in patients with non-valvular atrial fibrillation was the leading drug reported to FDA for serious reactions including hemorrhage, kidney failure and death. Dialysis is recommended for reversal of its anticoagulant effect when life threatening bleeding occurs. DG has a molecular weight of 628g/mol and volume of distribution is 50 - 70liters. Thus, it should be cleared by dialysis. We report the impact of 36 hours of renal replacement therapy (RRT) on DG's half-life (t1/2) and clotting parameters in a patient who developed spontaneous intracranial hemorrhage on DG.

Methods: 72 year old male had AF and CKD III (GFR 40ml/minute). He took the last dose of DG 150mg, 14 hours earlier (t1/2 12-17 hours). He presented with severe headache and was somnolent, dysarthric and had nystagmus. Imaging revealed cerebellar and intraventricular bleeding. Fresh-frozen plasma, factor VII and DDAVP were administered. CVVH was begun 3 hours after presentation and 17 hours after DG dose. After 19 hours of CVVH, 4 hours of hemodialysis and then another 12 hours of CVVH were performed. He continued to deteriorate despite clot evacuation and died 4 weeks later. DG levels, clotting parameters and t1/2 trends are shown below.



CVVH duration (hours)	Half-life (hours)
0	15
0-9	2.3
0-19	4

Conclusions: CVVH was chosen to minimize hemodynamic instability and has not been reported in this scenario. As intrinsic kidney function was unchanged, the significantly improved t1/2 is attributable to CVVH. A third of the drug is protein-bound and that may necessitate prolonged treatment for sustained clearance of the drug and reversal of anticoagulant effect. Ecarin clotting time (ECT) and Thrombin time (TT) are reliable markers of DG's anticoagulant effect but are not readily available. APTT is not suitable for monitoring.

TH-PO1134

The Spectrum of Acute Kidney Injury in Bath Salts Intoxication Jonathan McNeely, Samir Parikh, Nabil J. Haddad, Christopher Valentine, Ganesh B. Shidham, Brad H. Rovin, Anil K. Agarwal. *Internal Medicine, Division of Nephrology, The Ohio State University Medical Center, Columbus, OH.*

Background: Bath salts are a substance of abuse with negative toxicology screening. Although illegal in several U.S. states, they remain easily obtainable elsewhere and via the Internet. Detection of novel toxins that cause acute kidney injury (AKI) is a diagnostic challenge, particularly if the manifestations of the toxin mimic those of well established mechanisms of AKI.

Methods: A 29-year-old white male was observed wandering the streets, behaving erratically. In the local emergency department he reported recent use of bath salts, confirmed by family members. His past medical history included hepatitis C, polysubstance abuse, and tobacco use. His blood pressure was 91/52 mm Hg, pulse rate 93 bpm, and temperature 107 degrees F. He was agitated, but had no focal neurologic deficits. The remainder of the exam was unremarkable. Laboratory data showed creatine kinase 201,410 U/L, creatinine 2.28 mg/dL, potassium 8.1 mmol/L, and uric acid 21.2 mg/dL. Serum toxicology was negative. Urine toxicology detected lorazepam and cotinine, but was negative for >80 other substances. Within 24 hours, in addition to rhabdomyolysis, he developed shock, lactic acidosis, disseminated intravascular coagulation (DIC), and nSTEMI. He received intravenous fluids, vasopressor support, empiric antibiotic coverage, dantrolene, and rasburicase. Within two days of presentation he was oliguric; two days later, he was dialysis-dependent.

Conclusions: Bath salts are synthetic stimulants, reported to include mephedrone and MDPV. Methods for detecting mephedrone and MDPV are published, but are not yet commonly available. The spectrum of AKI due to bath salt abuse has not previously

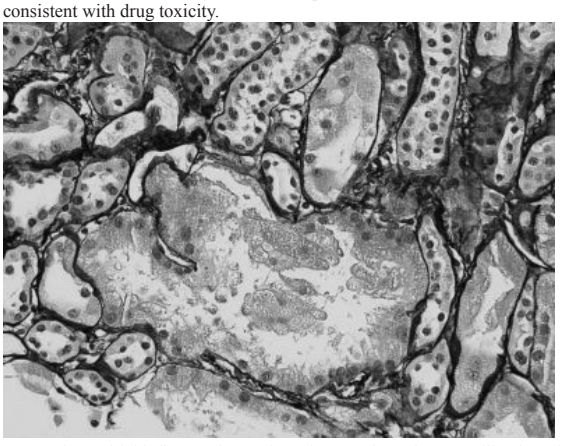
been described. We present a case of AKI and dialysis-dependence after self-reported bath salt abuse and negative toxicology results. The clinical course was marked by severe hyperthermia, rhabdomyolysis, DIC, oliguria, shock, lactic acidosis, and nSTEMI. Our patient demonstrates clinical presentation, difficulty in definitive diagnosis, differential diagnoses, and multiple potential mechanisms of AKI related to bath salt toxicity.

TH-PO1135

Deferasirox-Induced Acute Kidney Injury and Fanconi's Syndrome
Mohsen Elramah, Micah R. Chan, Hemender Singh Vats. *Department of Nephrology, UW Hospital and Clinics, Madison, WI.*

Background: a 21-year-old male survivor of Ewing sarcoma who had progressive decline in renal function and Fanconi's syndrome after being treated with Deferasirox for a ferritin level of 1502ng/ml and an MRI showing iron deposition in multiple organs. Hemochromatosis gene mutations were negative,iron overload was thought to be due to multiple transfusions.

Results: The patient was started on Deferasirox in April 2011 ;his serum creatinine was 1.0mg/dl.The patient's renal function declined with a creatinine of 1.25mg/dl in 08/2011 and 1.5mg/dl in 01/2012.During the months he received deferasirox,his urinalyses showed 3+ proteinuria and 3+ glucosuria.In 03/2012,the patient was admitted with pancreatitis and had a creatinine of 2.5mg/dl, bicarbonate of 16mmol/L, potassium of 2.7mmol/L, proteinuria (spot protein /creatinine ratio of 1.9),and glucosuria.Urine sediment was bland. Serum protein electrophoresis was normal.Kidney biopsy revealed nromal glomeruli. Tubules showed severe injury.Tubular epithelial cells showed isometric vacuolization consistent with drug toxicity.



No interstitial inflammation and no electron-dense immune-like deposits.Deferasirox was stopped;electrolytes were supplemented.Eleven days later,his creatinine improved to 1.5mg/dl and bicarbonate was 24mmol/L.sixty days later his creatinine remained at 1.5 mg/dl,the rest of electrolytes normalized.

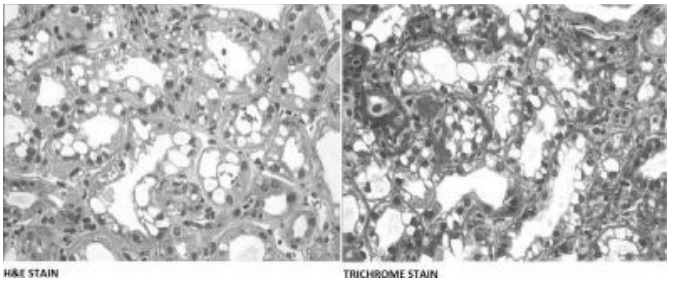
Conclusions: Our literature review revealed few cases of acute kidney injury secondary to Deferasirox .reports include Fanconi's syndrome, acute interstitial nephritis, and acute kidney injury.In all cases,after cessation of drug,kidney function returned to baseline.To our knowledge, this is the first biopsy-documented case of Deferasirox-induced Fanconi's syndrome and AKI.This case documents an often unrecognized cause of drug-induced renal failure which may not be totaly reversible and should be used cautiously.

TH-PO1136

Acute Kidney Injury Secondary to Bedbug Insecticide
Babar Bashir,[1] Shree G. Sharma,[2] Harold D. Stein,[1] Robert A. Sirota,[1] Vivette D. D'Agati.[2] *[1]Medicine, Abington Memorial Hospital, Abington, PA; [2]Pathology, Columbia University, New York, NY.*

Background: Bedbug infestation is becoming a world-wide epidemic due to the emergence of insecticide-resistant strains. Pyrethroids are approved by the EPA for use against bedbugs and are considered minimally toxic to humans, with known respiratory, neurologic and gastrointestinal effects.

Methods: We present the first case of pyrethroids-induced toxic acute tubular necrosis (ATN). A 65 year old healthy female on no potentially nephrotoxic medications presented to the ER with extreme weakness, decreased urine output and acute kidney injury. Over the preceeding 2 weeks, she had administered several applications to her bedroom of a bedbug spray (permethrin) and a fogger (pyrethrin), exceeding the manufacturer's recommended amounts. She was found to be in non-oliguric renal failure with features of type 1 renal tubular acidosis. Laboratory findings included BUN 91 mg/dL, creatinine 13 mg/dL, blood pH 7.18, serum bicarbonate <5 meq/L, anion gap (AG) 28 meq/L, serum potassium 1.8 meq/L, transtubular potassium gradient 9.4 mmol/L and positive urine AG. Urinalysis showed 100 mg/dL protein and scattered granular casts. Biopsy revealed toxic ATN with extensive tubular degenerative changes and cytoplasmic vacuolization. She was treated with fluid and electrolyte support followed by renal functional recovery 2 weeks later.



Literature review uncovered no prior report of ATN in humans induced by pyrethroid-based insecticides. However, 2-week exposure to pyrethroid causes similar ATN with tubular vacuolation in rats (Sakr, SA et al. 2001).

Conclusions: Bedbug insecticides containing pyrethroids should be used with caution due to potential development of toxic ATN from excessive exposure.

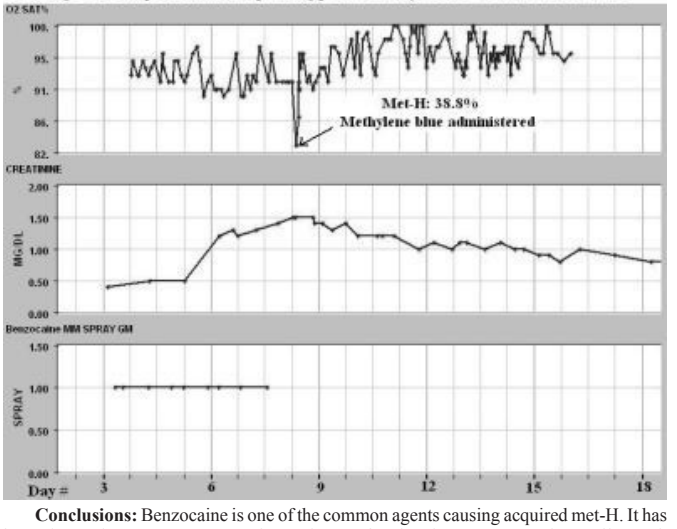
TH-PO1137

Benzocaine Induced Methemoglobinemia and Acute Tubular Necrosis: A Case Report
Wei Chen, Jason Matos, Maria Coco. *Department of Nephrology, Albert Einstein Montefiore Medical Center, Bronx, NY.*

Background: Methemoglobinemia (met-H) occurs when hemoglobin is oxidized to form methemoglobin (metHb). MetHb impedes oxygen delivery to tissue and may lead to tissue hypoxia. We describe a case of benzocaine induced acute met-H with concurrent acute tubular necrosis (ATN).

Methods: A 46 year old woman with hypertension and morbid obesity was admitted for anastomotic leak status post gastric bypass. On day 6, the patient began to develop non-oliguric acute kidney injury with peak creatinine (Cr) of 1.5 mg/dl on day 8. She had no signs or symptoms of hypovolemia. Medications include vancomycin (without high serum level), piperacillin/tazobactam (given until day 10), albuterol, ipratropium, pantoprazole, hydromorphone, ketorolac (received only one dose on day 1) and the benzocaine spray (prescribed on day 3). No contrast study was performed. There was no peripheral eosinophilia and no evidence of hemolysis or rhabdomyolylsis. On day 8, she became cyanotic with pulse oximeter saturation (SpO2) of 83%. An ABG was done, and revealed metHb level of 38.8%. Urine sediment showed many muddy brown casts, suggestive of ATN. Methylene blue was administered with the resolution of met-H and increase in oxygenation, immediately followed by a significant improvement in renal function, which returned to baseline on day 18.

Figure 1. Temporal relationship among pulse oximetry, creatinine and benzocaine use



Conclusions: Benzocaine is one of the common agents causing acquired met-H. It has been shown that SpO2 can overestimate the true fractional oxygen saturation until metHb reaches about 35%, so the degree of tissue hypoxia in this patient was more profound than that being detected by SpO2. Furthermore, the temporal relationship among SpO2, Cr, benzocaine use and metHb level implies a cause and effect relationship. Therefore, we hypothesize that benzocaine induced met-H caused ATN in this case, likely from prolonged severe renal hypoxia.

TH-PO1138

Focal Segmental Glomerulosclerosis (FSGS) in Association with Neurofibromatosis Type 1 (NF1): Proposal of Molecular Pathways
Farsad Afshinnia, Paul D. Killen, Matthias Kretzler, Virginia Vega-warner, Friedhelm Hildebrandt. *University of Michigan, Ann Arbor, MI.*

Background: NF1 is a rare genetic disorder. FSGS is also a rare glomerulopathy. In this abstract clinical course of a patient with NF1 and FSGS is presented and the molecular pathways which may potentially explain this association are discussed.

Methods: Case Report: Patient is a 42 years old female with history of NF, referred for evaluation of nephrotic range proteinuria diagnosed 6 months earlier. In her clinic visit she was asymptomatic. Family history was remarkable for NF in her maternal grandmother, mother and her daughter. She denied history of smoking, or drug abuse. Physical examination reveled normal vital signs. She had no developmental delay. Examination of heart, lung, an abdomen was unremarkable, but she had multiple neurofibromas in chest, abdomen, and posterior trunk. She also had multiple café-au-lait spots. Her serum creatinine was 0.5 mg/dL. Protein-creatinine ratio was > 11 g/mg. Kidney biopsy showed FSGS. DNA analysis confirmed a heterozygous truncating mutation in NF1 gene on chromosome 17. All 8 exones of NPHS2 gene were negative for podocine mutation. Lisinopril 20 mg once a day was started and after 10 months she had a gradual decrease of protein-creatinine ratio to 0.33 g/mg.

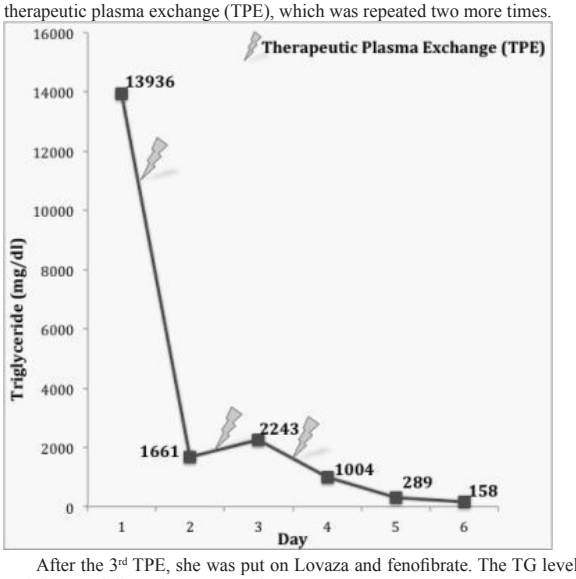
Conclusions: Discussion: NF gene locates on chromosome 17 and its product is neurofibromin. Neurfobromin belongs to a family of GTPase activating proteins (GAPs) which downregulates cellular proto-oncogene p21-ras. Ras activates Stem Cell Factor (SCF)/c-kit, mTOR and MAPK signaling pathways. Activation of MAPK and mTOR are linked to podocyte injury and focal segmental sclerosis in animal models. In other model systems, mutation at NF1 gene resulted in reduction of the number of glomeruli in metanephrons, hypoplasia and developmental delay of kidney. We hypothesize that mechanisms for development of FSGS in NF1 may include activation of MAPK, and mTOR signaling pathways, in association with possible reduced nephron numbers and developmental delay of the kidney. MTOR signaling pathway is partially mediated through angiotensin-II activation, therefore ACE inhibition may serve as an effective treatment.

TH-PO1139

Extreme, Refractory Hypertriglyceridemia and Plasma Exchange: Poorly Defined Endpoints Sasan Raeissi,[1] Hamid Emamekhoo,[1] Angel Mena,[1] Amir Izhar.[2] [1]*Internal Medicine, Good Samaritan Hospital, Cincinnati, OH;* [2]*Nephrology, Good Samaritan Hospital, Cincinnati, OH.*

Background: Therapeutic plasma exchange (TPE) is used for treatment of hypertriglyceridemia predominantly in cases of pancreatitis. Literature recommends TPE prophylactically in asymptomatic cases of triglycerides (TG) >2000 mg/dL, although case reports are rare, and recommendations are not clearly defined.

Methods: A 39-year-old Hispanic female known case of Familial Hypertriglyceridemia (FHTG) and history of pancreatitis 9 years ago, presents with throat pain. She reported a spontaneous abortion 2 months ago at 12 weeks gestation. Her mother and two sisters also have FHTG. She was compliant with her diet and medicines, which included colesevelam. Vital signs and physical exam were normal, with no symptoms of pancreatitis. BMP, CBC, amylase, and lipase were unremarkable. Her last serum TG level 5 months before admission, was 89 mg/dl. Lipid profile revealed a TG level of 13936 mg/dl and total cholesterol 1004 mg/dl, which were both confirmed on repeat testing. The patient underwent urgent therapeutic plasma exchange (TPE), which was repeated two more times.



After the 3rd TPE, she was put on Lovaza and fenofibrate. The TG level normalized and she was followed as an outpatient.

Conclusions: Our patient underwent TPE without evidence of pancreatitis because of her extraordinarily high TG. The patient had a normal TG level five months before admission and she was compliant with her medications. The short time course over which her TG level rose makes the lost pregnancy a possible instigating factor. Very rarely, repetitive use of TPE is reported. Distinctively, our patient required multiple sessions of TPE to adequately reduce TG burden, although the goal TG is unclear in an asymptomatic patient. We applied centrifugal TPE with Albumin 5% and Anticoagulant Citrate Dextrose (ACD-A).

TH-PO1140

Use of ACTH Gel in a Patient with Progressive Nephrotic Syndrome Secondary to Transplant Glomerulopathy (TG) Ankita B. Patel, Mariana S. Markell. *Nephrology, SUNY Downstate, Brooklyn, NY.*

Background: TG represents a severe form of chronic allograft nephropathy(CAN) that leads to morbidity from nephrotic syndrome(NS). ACTH gel has been used in resistant native kidney NS. We report a case where ACTH gel led to a significant decrease in proteinuria in a woman with TG.

Methods: A 44 year old white woman with history of ESRD due to medullary cystic disease received a living related donor (LRD) kidney transplant from her mother in 1995. She developed non-Hodgkin's lymphoma associated with de novo EBV infection in 1998 which was successfully treated with CHOP.Her allograft failed in 2005 and she received a second LRD kidney transplant from her sister. In Mar '11, she complained of bilateral swelling of her feet.She had 8 g/day of proteinuria.Her creatinine was 1.82.She underwent a renal biopsy which revealed chronic transplant glomerulopathy.Despite addition of valsartan and Mycophenolate to her tacrolimus regimen, her proteinuria continued to rise, reaching 12g by Nov '11. She started ACTHAR(Repository corticotropin injection) gel 80 mg twice weekly in Dec '11. By Mar '12, her proteinuria decreased to 6.1 g/day where it has remained for 3 months. Her serum albumin rose from 2.8 g/dl to 3.5 g/dl with resolution of edema. Her latest creatinine is 1.48 mg/dl. She has no evidence of recurrent lymphoma.

Conclusions: In the last 2 decades, adrenocorticotropic hormone (ACTH) has re-emerged as a potentially effective therapy for nephrotic syndrome, particularly for patients who have failed more conventional therapies.ACTH stimulates the adrenal cortex to secrete corticosteroids through melanocortin-2 receptor.It has shown to improve glomerular morphology, podocyte ultrastructure, and tubulointerstitial fibrosis in multiple animal models of nephrotic diseases.ACTH performed comparably to steroids and alkylating agents in a randomized study of idiopathic membranous nephropathy patients.In an observational study of 21 patients with NS, 11 of 21 patients achieved complete or partial remission of which 4 achieved a complete remission.ACTH thus may be a viable treatment option for treatment of transplant glomerulopathy, and appears to have produced a partial remission in our patient.

TH-PO1141

Collapsing Glomerulopathy Associated Lupus in a Black Female with Homozygous APOL1 Mutation Tomek Kofman,[1] Celine Narjoz,[2,3] Gary S. Hill,[1] Eric Thervet.[2,4] [1]*Anatomopathologie, APHP, HEGP, Paris;* [2]*INSERM UMR-S 775, Université Paris Descartes, Paris;* [3]*Biochimie, Pharmacogénétique et Oncologie Moléculaire, APHP, HEGP, Paris;* [4]*Néphrologie, APHP, HEGP, Paris.*

Background: We present the first case of collapsing glomerulopathy (CG) and systemic lupus erythematosus (SLE) in a young Afro-American (AA) female carrier of a homozygous mutation of APOL1.

Methods: The diagnosis of SLE was made in 2008 because of nonerosive polyarthritis, fever, pancytopenia and positive antinuclear antibodies (ANA) and anti-double strand DNA antibodies (ds-DNA). Despite proteinuria, no renal biopsy was performed. An association of prednisone at 0.5 mg/kg/d and hydroxyquinine induced a clinical and laboratory improvement. In April 2011, she was admitted for polyarthralgia, a nephrotic syndrome (albumin 16.4 g/l, nonselective proteinuria at 4.6 g/d) and an acute renal failure (creatinine 676 µmol/l). There was no hematuria but leucocyturia was positive. ANA and ds-DNA were strongly positive. She also presented a general seizure. Beside, symptomatic treatment, she received since admission high doses of methyl prednisolone (1 g/d for 3 days). A transjugular renal biopsy was performed 12 days after admission.

Most glomeruli on the renal biopsy displayed segmental or global collapse with sclerosis without proliferative lesion corresponding to CG. Widespread tubular atrophy and interstitial fibrosis were present. Direct immunofluorescence showed strong granular staining of glomeruli and in a vascular and tubulointerstitial pattern with predominant staining with polyclonal anti IgG, anti-C1q and anti-C3. There was no risk factor associated with CG. We performed genotyping of APOL1 in our patient and found that she is was a homozygous carrier for the G2 allele of APOL1.

Immunosuppressive treatment associating cyclophosphamide and plasma exchange was started. Her neurologic symptomatology improved, but she remains on chronic dialysis.

Conclusions: We describe the first case of CG in the context of a flare of lupus in a patient carrier of a homozygous mutation of APOL1. This case report suggests that this pattern of glomerular injury is related to aggression of the podocytes carrying this homozygous mutation.

TH-PO1142

Nephrotic Syndrome with Compound *NPHS1* Heterozygous Mutations Kirsten A. Kusumi, Stephen D. Cha, Andrew L. Schwaderer. *Pediatrics, Ohio State University/ Nationwide Children's Hospital, Columbus, OH.*

Background: Although classical dogma has taught that mutations of *NPHS1* gene were exclusively found in congenital nephrotic syndrome (NS), we now know that there exists greater variability in the genotype of mutations with subsequent variability in phenotype. Of note, patients were found to have a mean age of onset of NS of 3.0 yr (6 mo to 8 yr) and thus represented a wide departure from the previous understanding of *NPHS1* disease presenting at than 3 months of age. These patients were universally resistant to corticosteroid treatment. While a variety of treatments were undertaken, approximately 45% progressed to ESRD.

Methods: *NPHS1* Gene sequencing in a 2 year old female with steroid resistant nephrotic syndrome followed by clinical case review with literature search.

Results: Genetic testing revealed a compound heterozygote for *NPHS1* mutations despite a negative family history and unremarkable urine dipstick evaluation of the parents. A frame shift mutation at 45_46 resulting in two base pair duplication of GG as well as a point mutation at nucleotide position 2928 leading to a transversion of G > T were identified.

Conclusions: Previously, the identified point mutation has been associated with phenotypically mild disease. With phenotypically unaffected parents, the compound heterozygous *NPSH1* mutations raises the possibility that nephrotic syndrome may result from a "2 hit" phenomenom with one mutation inherited from each parent. Ongoing mutation analysis of the parents will lend further insight into the genetic etiology of *NPHS1* associated nephrotic syndrome with absent family history.

TH-PO1143

Dent's Disease Presenting as Global Glomerulosclerosis Alfonso Eirin, Maria V. Irazabal, John C. Lieske, Samih H. Nasr, Sanjeev Sethi, Fernando C. Fervenza. *Div. of Nephrology and Hypertension and Laboratory Medicine and Pathology, MN.*

Methods: An 18-year-old Caucasian presented with fever, proteinuria (1.5g/24h), and serum creatinine 2.0mg/dL. Kidney biopsy showed focal global sclerosis and minimal interstitial fibrosis. IF was negative. EM not done. A presumptive diagnosis of minimal change disease was made and treatment with prednisone (1mg/kg) was initiated, without proteinuria response. He was switched to Cyclosporine (125 mg bid) for a presumptive diagnosis of FSGS, but remained proteinuric and was referred to our institution. Blood pressure = 96/62 mmHg. Family history was positive for unspecified chronic kidney disease (CKD) in a maternal grandfather. Repeat biopsy demonstrated mild global glomerulosclerosis and interstitial fibrosis. Cortical tubules were plugged with von Kossa-positive crystals. A 24-hour urine collection showed albuminuria (193 mg) but very high levels of the low molecular weight (LMW) proteins, retinol binding protein (212,573 mcg; normal <163 mcg) and α-1 microglobulin (656 mg, nl <19 mg). There was no glucosuria or aminoaciduria. These findings raised suspicion of Dent's Disease. Genetic analysis confirmed a (novel frame-shifting change of CLCN5, c.92delA, p.Val31fs15X in hemizygosity). The patient has been on a thiazide diuretic to treat the hypercalciuria (24-h calciuria 169 mg on therapy), and an ACE inhibitor.

Conclusions: Dent's disease is a rare X-linked recessive disorder characterized by LMW proteinuria, hypercalciuria, and CKD. Some but not all patients also suffer from kidney stones and bone disease. Mutations in the CLCN5 gene, which encodes for a chloride channel found within endocytic vesicles in proximal tubular cells and elsewhere are causative. Marked increase in urinary excretion of LMW is diagnostic, and points to the tubular (rather than glomerular) source of the proteinuria. Dent Disease should be considered in all male patients that present with unexplained CKD and mild to moderate proteinuria. Establishing the correct diagnosis avoids unnecessary and potentially harmful treatments, such as immunosuppressive agents.

TH-PO1144

Subacute Progressive Chronic Renal Failure: A Clinical Presentation of IgG4 Related Systemic Disease Jiwan K. Thapa, Amr El Toukhy, Saul Nurko. *Nephrology and Hypertension, Cleveland Clinic, OH.*

Background: IgG4 related systemic disease (IgG4-SD) is a newly described entity which encompasses autoimmune multi-organ involvement which shares same pathologic and clinical features. Kidney is one of the many target organs. Herein, we present a case of IgG4-SD diagnosed based on progressive kidney insufficiency in a patient with remote pancreatic disease.

Methods: Our patient is a 76 year old white male who developed pancreatic insufficiency (late onset DM and steatorrhea) 4 years before presentation.

Now he came with worsening fatigue concordant with rise in Serum Creatinine (S.Cr-2 to 4.3 over 6 weeks), swollen & tender b/l submandibular glands, dry mouth & dry eyes. Examination was remarkable only for enlarged, tender submandibular nodes.

Lab work revealed - +ve ANA with –ve SSA & SSB. Serum immunofixation showed abnormal IgM and Kappa bands. Knees, spine, chest & sinus imaging were negative. Renal USG showed 10.4 and 11.9 cm kidneys with simple cysts. There was no proteinuria and urine sediment had few granular casts.

Bone Marrow biopsy and Flow cytometric analysis showed no evidence of abnormal cell population. Given progressive renal failure, renal biopsy was done which showed – severe plasma cell-rich interstitial inflammation, severe tubulointerstitial atrophy and fibrosis, Immunofluorescence revealed positive tubular basement membrane (TBM) staining for C3C. Immunohistochemistry showed marked increase in IgG4 (+) plasma cells with ratio between IgG4 to IgG>50%. Electron microscopy showed TBM electron dense deposits.

Diagnosis of IgG4-SD and chronic interstitial nephritis was made. Treatment was initiated with prednisone 40mg qday which led to resolution of submandibular swelling and gradual downtrend in S.Cr.

Conclusions: IgG4 related kidney disease involves Tubulointerstitial Nephritis, glomerular disease, and obstructive uropathy related to retroperitoneal fibrosis. Our patient had AIP and subacute renal failure spaced in time with kidney biopsy clinching the diagnosis. IgG4-SD should be suspected when there is multi-organ involvement which is not easily explained otherwise. Subacute renal failure is a rare but unique clinical presentation of IgG4 related systemic disease.

TH-PO1145

Lupus-Like Glomerulonephritis in a Patient Treated with Interferon-alpha for Hepatitis C Subhasish Bose,[1] Avrum Gillespie.[1] *[1]Department of Nephrology, Temple University Hospital, Philadelphia, PA; [2]Department of Pathology, Temple University School of Medicine, Philadelphia, PA.*

Background: Introduction:

Chronic hepatitis C virus (HCV) infection and anti-viral treatment with interferon-alpha (INF-a) has been associated with the development of autoimmune diseases like systemic lupus erythematosus (SLE) and Sjogren's syndrome.

Methods: Case Description:

51 year old male with background history of hepatitis C (on treatment with Ribavirin and INF-a for 1 year), presented with 1 week history of fever, malaise and generalized weakness. On exam: temperature 101F, BP 150/90, basal crackles in lungs and bilateral pitting pedal edema. He had patchy erythematous lesions on his legs. On initial labs, hemoglobin was 4.9. He was also noted to have acute renal failure (BUN 58, creatinine 7.6) with nephrotic range proteinuria (urine protein : urine creatinine = 4). Microscopy of urine sediment was notable for RBC and WBC cast and presence of tubular cells.

Serological tests such as ANA, anti-dsDNA antibody, anti-proteinase3 antibody, anti-myeloperoxidase antibody, HIV 1&2 antibody and P24 antigen, hepatitis B surface antigen & core antibody and rheumatoid factor were all negative/normal. Serum C3 and C4 were low and cryoglobulin was positive. Anti-RNP antibody and anti-SSA antibody were positive.

Patient underwent skin rash biopsy which was inconclusive. His kidney biopsy showed immune complex glomerulonephritis with subepithelial, subendothelial and mesangial deposits suggestive of lupus-like collagen vascular disease. There were also presence of organized glomerular capillary immune deposits and "fingerprint" type subendothelial deposits that was suggestive of cryoglobulins.

Conclusions: Given the clinical history of interferon therapy for hepatitis C virus infection in this patient, he was treated as a therapy-induced autoimmune disease. The treatment included Cyclophosphamide and plasmapharesis. He needed intermittent hemodialysis for a duration of 3 weeks and was discharged home with close follow up. His serum creatinine on 3 month follow up is stable at around 3.0 mg/dl.

Funding: Clinical Revenue Support

TH-PO1146

Calcineurin Inhibitor Associated Posterior Reversible Encephalopathy Syndrome: Case Report in a Child with Minimal Change Nephrotic Syndrome Xamayta Negroni- Balasquide, Joseph R. Angelo, Joshua A. Samuels. *Pediatric Nephrology and Hypertension, UT Houston Medical School, Houston, TX.*

Background: Posterior reversible encephalopathy syndrome (PRES) is a clinical and neuroradiological diagnosis presenting with headache, visual changes, altered level of consciousness and seizures. PRES is commonly described in association with hypertension but infrequently with calcineurin inhibitors (CNI).

Methods: We describe a case of a 5 year old Caucasian male with minimal change nephrotic syndrome (NS) who achieved remission on steroid therapy shortly following initial diagnosis. However, three months after initial presentation he had his first relapse which was followed by multiple subsequent relapses. Due to frequently relapsing NS, Tacrolimus (Tac) 1.5mg/kg/dose twice a day was added to his steroid and ACE inhibitor therapy.

Five days after starting treatment with Tac, pt presented with headache, emesis and blood pressures of 140/90; and subsequently, tonic clonic seizure activity. He was admitted to the pediatric intensive care unit; electrolytes were within normal limits and workup for infectious etiology was negative. Tac levels were 14. CT scan of brain was negative and EEG was without definitive abnormality. MRI of brain showed focal regions of FLAIR hyper-intensity within the subcortical white matter of bilateral occipital lobes, changes consistent with PRES. Tac was held and HTN was treated. Subsequently, he was re-started on Tac at a lower dose of 1mg/kg/dose twice a day. He had no further seizures during admission. Pt was discharged on Lisinpril, prednisone and adjusted dose of Tac.

Conclusions: There have been few cases in pediatric literature describing PRES in association with CNI, and most of these are in the setting of solid organ transplantation. Increasingly, the use of Tac is expanding outside of the realm of transplantation. This case demonstrates the importance of maintaining a high index of suspicion for PRES in pts treated with CNI; particularly in those pts with concurrent risk factors such as underlying HTN and kidney disease.

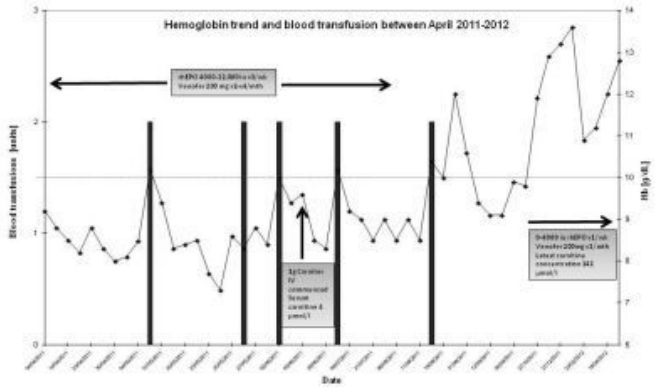
TH-PO1147

Carnitine Deficiency in Poorly Responsive Anemia of Renal Disease Naushad Ali Junglee, Siva Shrikanth. *Ysbyty Gwynedd, Bangor, United Kingdom.*

Background: Reaching target hemoglobin (Hb) in hemodialysis (HD) patients may involve excessive use of erythropoetin (rhEPO) and blood transfusions. We describe a 25 year old HD patient with hepatosplenomegaly who was not reaching target Hb. Following a detailed screen for apparent EPO resistance, marrow trephine and liver biopsy suggested ineffective erythropoesis. His serum carnitine levels was low and so L-cartinine was instituted after each HD session. Within a few months, his Hb normalized with reduced use of rhEPO and blood.

Methods: Over several months, a 25 year old male HD patient was requiring very high rhEPO (Eprex) dosing of upto 12000 iu thrice weekly with iron and periodic blood transfusion to maintain Hb > 10 g/dl. Compliance with HD and treatment of renal bone disease was satisfactory (av. Kt/V 1.4, PTH 22 pmol/l). Clinically, he exhibited marked

hepatosplenomegaly - confirmed on CT imaging. A liver biopsy supported extramedullary hematopoesis and marrow trephine suggested ineffective erythropoesis but was otherwise normal. Extensive investigations including hematinics, gastrointestinal endoscopy, extended infection screens, aluminium levels and rhEPO antibodies were all unremarkable. Serum carnitine was 4 µmol/l (normal range 15-153 µmol/l) and thus he was started on Carnitor 1 mg IV after each dialysis. This resulted in normalisation of Hb with lower rhEPO, iron and blood transfusion requirements.



Conclusions: The features of ineffective erythropoesis prompted conisderation of cartinine deficiency. Due to a lack of randomised-controlled trials, use of L-carnitine is uncommon despite deficiency being well-recognised in HD patients. Nevertheless, our case illustrates that 1) carnitine deficiency can blunt response to anemia treatment; and 2) correction can avoid copious investigations, excessive rhEPO and blood transfusion - all of which are costly, potentially harmful and may jeopardize future transplantation.

TH-PO1148

Hemolytic Uremic Syndrome Associated with Clostridium Difficile Colitis

Anthony Alvarado,[1] Sergey V. Brodsky,[2] Tibor Nadasdy,[2] Todd E. Pesavento,[1] Neeraj Singh.[1] *[1]Division of Nephrology, Ohio State University, Columbus, OH; [2]Department of Pathology, Ohio State University, Columbus, OH.*

Background: We report three cases of clostridium difficile associated hemolytic uremic syndrome (HUS) with biopsy proven renal thrombotic microangiopathy(TMA). HUS is reported to be cause by Shiga-like toxin produced by E. coli O157: H7 and shigella dysenteriae. Clostridium difficile associated HUS is rare; with few cases in children and two in adults.

Methods: Two patients received kidney transplant whereas the third developed acute renal failure (ARF) in the native kidneys. Presentation was preceded by acute diarrhea and stool clostridium difficile toxin was detected in all cases.

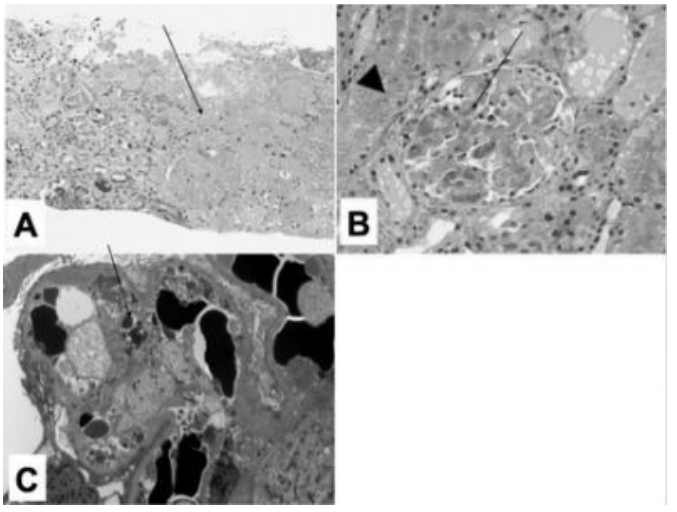
Differences between patients

	Case #1	Case #2	Case #3
Age in years	29	52	33
Gender	Female	Female	Female
Cause of ESRD	Diabetes	Hypertension	N/A
Renal transplant	LRRT	DDRT	N/A
Hemoglobin gr/dl	9.3	10.3	7.6
Platelets(150-400K/uL)	188	40	60
LDH 100-190U/L)	529	590	1992
Schistocytes	1+	2+	2+
Haptoglobin(27-139mg/dl)	12	6	10
Stool C.diff toxin by PCR	+	+	+
Treatment	Oral Vancomycin	Oral Metronidazole	Oral Vancomycin
Outcome	Complete recovery	Allograft Failure	HD for 3 months
ADAMTS-13	Low normal	Low normal	Low
Plasmapheresis	No	Yes	Yes

LRRT(live related renal donor), DDRT (deceased donor renal transplant)

Clostridium difficile associated HUS was suspected due to thrombocytopenia, microangiopathic hemolytic anemia, and biopsy proven renal TMA. Two patients were treated with oral vancomycin and one with oral metronidazole. Two underwent plasmapheresis. One patient had complete recovery in 4 weeks, one remained dialysis dependent for few months, and the third never recover renal function.

Conclusions: This case series shows that clostridium difficile is a rarely recognized but an important cause of ARF due to renal TMA, renal biopsies were not performed in the prior case reports.



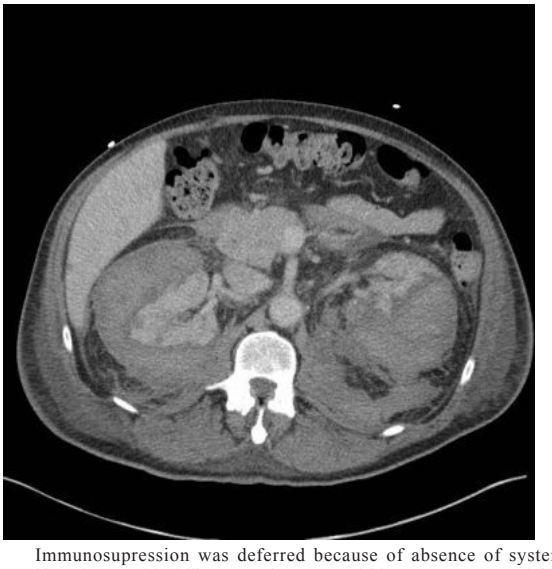
TH-PO1149

Spontaneous Bilateral Renal Sub-Capsular Hematoma: Secondary to Microscopic Polyangiitis

Srinivasa Iskapalli,[1] Deepthi Panjam,[2] Raviprasenna K. Parasuraman.[3] *[1]Internal Medicine, Beaumont Health System, Royal Oak, MI; [2]Internal Medicine, McLaren Regional Medical Center; [3]Nephrology and Transplantation, Beaumont Health System, Royal Oak, MI.*

Background: We report a rare case of spontaneous bilateral renal hematoma and renal failure as the presenting manifestation of microscopic polyangiitis (MPA); an anti-neutrophil cytoplasmic antibody (ANCA) mediated vasculitis.

Methods: A 69y-old male presented with acute back pain and severe acute kidney injury with blood urea nitrogen of 115 mg/dL and serum creatinine of 8.93 mg/dL. Ultrasound showed large globular kidneys and CT scan revealed massive bilateral sub-capsular renal hematoma and relevant investigations were negative except for perinuclear-ANCA with a titer of 1:80.



Immunosupression was deferred because of absence of systemic vasculitis manifestations and inability to perform a renal biopsy. He presented five months later with severe peripheral neuropathy and bilateral hemorrhagic pleural effusion and P-ANCA titer was elevated at 1:320. Open lung biopsy was significant for alveolar hemorrhage, necrotizing capillaritis, and organizing arterial thromboemboli. A diagnosis of MPA was made and started on 150mg of cyclophosphamide and 60mg of prednisone daily along with trimethoprim-sulfamethoxazole. Patient had complete resolution of symptoms but no recovery of renal function and P-ANCA titer decreased from 1:320 to less than 1:20.

Conclusions: This is the first reported case of spontaneous,massive, bilateral renal hematoma as the presenting manifestation of MPA.Spontaneous renal hematoma can occur from renal tumors, vascular disease, infections and vascular disease accounted for 17% of cases in a meta-analysis by Zhang et al. ANCA-positive vasculitis despite low titer should be considered in the initial differential diagnosis of spontaneous renal hematoma so that early treatment can be initiated to avoid complications.

TH-PO1150

Is Idiopathic Nodular Glomerulosclerosis Idiopathic? Syed Muhammad Mohsin Raza, Lakshminarayanan Nandagopal, Unnikrishnan Ponnamma Kunjan Pillai, Xu Zeng, Atul Singh. *Nephrology, Wayne State University/ Detroit Medical Center, Detroit, MI.*

Background: Idiopathic Nodular glomerulosclerosis(ING) is associated with hypertension(HTN) and smoking.

We present a case of 56 year old woman with HTN and nephrotic proteinuria with ING.

Methods: A 56-year-old African American woman with hypertension was evaluated for nephrotic syndrome. The patient has never smoked and has no known history of diabetes mellitus(DM). Her examination was remarkable only for bilateral pitting pedal edema. Serum creatinine was elevated at 3.7 mg/dL with estimated Glomerular Filtration Rate [eGFR] of 16 ml/min/1.73 m2. She had a normal two hour glucose tolerance test and a hemoglobin A1c (HbA1c) 5.4%. Serologies for hepatitides, antinuclear antibody, and human immunodeficiency virus were negative. Complement levels were normal, and serum immunofixation was negative. Urine exam was unremarkable other than random urine protein-creatinine ratio of 4.2.

Renal histopathology revealed focal nodular glomerulosclerosis, moderate tubular atrophy, interstitial fibrosis and severe hyalinization of the arterioles with negative congo red stain.

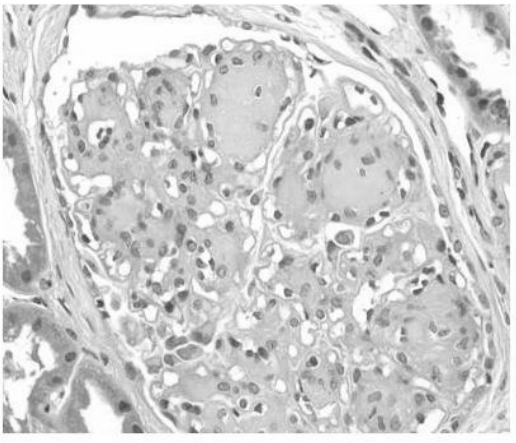


Figure 1

Conclusions: ING has been reported in patients with history of HTN and smoking. Pathogenesis is unclear, advanced glycation end products (AGE) and activation of Renin-Angiotensin System (RAS) may play a role. AGE binds with receptors for AGE (RAGE) in podocytes, activating inflammatory cascade, leading to extracellular matrix deposition as in diabetic nephropathy. Hypertension has also shown to up regulate RAGE expression in a study on rats. The coexistence of nodular glomerulosclerosis with the histological changes associated with HTN, as in our patient raises the possibility for a causative association between HTN and ING. While glomerular sclerosis is well described in HTN, whether ING represents one end of the spectrum remains to be answered.

TH-PO1151

An Immune Reaction to an Oocyte Donor Pregnancy: A Novel Trigger for Preeclampsia Randy L. Luciano,[1] Gilbert W. Moeckel,[2] Ursula C. Brewster.[1] *[1]Section of Nephrology, Dept of Internal Medicine, Yale University School of Medicine, New Haven, CT; [2]Department of Pathology, Yale University School of Medicine.*

Background: A 33-year old woman with an in vitro fertilization pregnancy presented with acute onset of proteinuria at 18 weeks gestation.

Methods: This pregnancy was conceived with an anonymous egg donor and her husband's sperm. She had one previously successful pregnancy using her own egg and her husband's sperm 4 years prior. Urine was positive for protein at 18 weeks +3days and by 20 weeks increased to 4.2 grams. By 21 weeks she was edematous, severely hypertensive and had 22 grams on a spot P/Cr ratio. Serologic workup (Table 1) was unrevealing and several ANAs returned borderline positive with negative ENA and dsDNA. Kidney biopsy revealed severe glomerular endotheliosis and immune complex deposits. No TRIs were seen. Donor Specific Antibody testing showed an abnormal antibody in the patient, not directed to self. Five days after D&C, HTN resolved and proteinuria dramatically improved without further therapy. Repeat ANA was negative. By 12 weeks post-partum, urine P/Cr ratio was 0.5 mg/mg Cr. A repeat biopsy showed resolving glomerular endotheliosis and no residual immune complex deposits. Full HLA studies of fetal tissue pending and will be available by the time of the ASN meeting.

Laboratory Data

Lab Test	4 Days Prior to Delivery	1 week postpartum	Normal Range
Serum Creatinine	0.7 mg/dl	0.6 mg/dL	0.5-1.2 mg/dl
C3	99mg/dL	113 mg/dL	80-145 mg/dL
C4	14 mg/dL	14 mg/dL	16-43 mg/dL
Albumin	1.9 g/dL	2.8 g/dL	3.5-5.0 g/dL
ANA	Positive 1:40	Negative	Negative
dsDNA	Negative	Negative	Negative
Extractable Nuclear Antigen-Antibody Screen (ENA)	Negative	Negative	Negative
Urine Prot:Creat Ratio	22.6 mg/mgCr	4.5 mg/mgCr	<0.1 mg/mgCr

Conclusions: We propose the following pathogenesis of the immune complex formation and early, severe preeclampsia: Egg donor placentas have been shown to induce an immune response at the placental-uterine interface. In this case, an immune reaction staged by the foreign pregnancy led to immune complex deposition in the kidney and glomerular injury. This process resulted in a severe and early pre-eclamptic syndrome that resolved upon termination of the pregnancy.

TH-PO1152

Strongyloides Stercoralis Transmission by Renal Transplantation in Two Recipients from a Common Donor D.A. Roseman, Dima Kabbani, Joann Kwah, Dorothy Bird, Robin Ingalls, Amitabh Gautam, Matthew Nuhn, Jean M. Francis. *Boston University Medical Center, Boston, MA.*

Background: S. stercoralis is an infectious disease with a high mortality rate. Clinical symptoms may be vague and non-specific including pulmonary and gastrointestinal complaints. Immunocompromised patients are the most vulnerable population at risk of developing a life-threatening hyperinfection syndrome characterized by disseminated strongyloidiasis and sepsis. Risk factors include steroid use and solid organ transplantation. Donor-derived S. stercoralis by renal transplantation is an uncommon diagnosis and is difficult to prove.

Methods: We report two cases of renal allograft recipients who developed strongyloidiasis early after transplant from the same deceased donor who was treated with high-dose steroids prior to organ procurement. The first recipient presented with abdominal pain and then a small bowel obstruction with CT findings of proximal bowel wall thickening. Larvae were demonstrated on a duodenal biopsy and isolated from gastric, pulmonary, and stool samples. The patient was seronegative for S. stercoralis when tested locally however the same sample tested positive at the CDC. The patient had a protracted hospital course complicated by a hyperinfection syndrome requiring subcutaneous ivermectin due to an ileus. Subcutaneous ivermectin is not approved for human use and required emergency FDA and IRB approval on a compassionate use basis. The patient survived but the renal allograft was lost. The second recipient had larvae detected in stool samples after complaints of diarrhea and this was treated with oral ivermectin. The donor was seropositive on retrospective testing at the CDC.

Conclusions: These cases add to the limited data of confirmed donor-derived S. stercoralis in renal transplantation and highlight classic presentation features. This report also adds more evidence to the safety of subcutaneous ivermectin in humans which was well tolerated and should be considered in the setting of malabsorption. Finally, there is testing center variability for strongyloidiasis serologies and the CDC is available for help with high risk patients.

Funding: NIDDK Support

TH-PO1153

Reactivation of Hepatitis B: A Dilemna in Immunosuppressive Therapy but Preventable Kashaf A. Rasheed, Zohreh S. Soltani. *Nephrology and Hypertension, Louisiana Health Science Center, New Orleans, LA.*

Methods: This is a case of a 38 year old male who presented to the hospital with bilateral lower extremity edema, twenty five pound weight gain, and fatigue. His chemistry panel revealed that he was in acute renal failure with BUN of 53 and Creatinine of 7.1 mg/dL. He has no past medical history.

He was found to have proteinuria of 25 gram/day, hyperlipidemia, and hypoalbuminemia of 2.4 mg/dL, which is consistent with a diagnosis of nephrotic syndrome. Renal US showed mildly enlarged kidneys and increased cortical echogenicity. The remainder of workup was unremarkable except his Hepatitis B serology was consistent with chronic persistent hepatitis with no evidence for cirrhosis. HBV DNA did reveal 3,317 copies.

Kidney biopsy pointed to a diagnosis of Minimal Change Disease. Patient posed a treatment dilemma. Patient was started on prednisone because of the severity of the proteinuria and renal failure. He never required renal replacement therapy during the hospital course. He was promptly referred to GI and started on antiviral therapy which closely coincides with initiation of Prednisone therapy. Patient has had significant positive response within 2 weeks to steroid therapy with improvement of proteinuria to 3 grams/day and serum creatinine to 1.9 mg/dL. His liver function has been normal in follow-up visit.

Conclusions: This case is an excellent example of the consideration that we as clinicians should have when starting a patient on immunosuppressive medications who has chronic or past HBV infection (even carrier state). A systematic review of 14 studies shows that in patients who did not receive prophylactic antiviral therapy before initiation of immunosuppressive therapy, 36.8% had HBV reactivation, 33.4% had HBV-related hepatitis, 13% had liver failure, and 5.5% died. If immunosuppression is to be used in treatment of renal disease or any condition, it is imperative that any chronic Hepatitis B be treated with antiviral therapy prophylactically to prevent reactivation and also antiviral therapy is less effective once hepatitis ensues.

TH-PO1154

Antibody Mediated Rejection Associated with CFHR1 Deficiency Successfully Treated with Eculizumab Damien Gerard Noone,[1] Kathryn J. Tinckam,[2] Paul S. Thorner,[1] Walter H. Kahr,[1] Diane Hebert,[1] Christoph Licht.[1]
[1]The Hospital for Sick Children, Toronto, ON, Canada; [2]University Health Network - Toronto General Hospital, Toronto, ON, Canada.

Background: Antibody mediated rejection (AMR) can be accompanied by thrombotic microangiopathy (TMA). TMA post transplant can represent recurrence of a preexisting aHUS disease or it can occur de novo, in association with AMR or other factors known to induce endothelial damage such as drugs (CNIs) or ischemia-reperfusion injury. Eculizumab, a monoclonal C5 antibody blocks the terminal complement cascade and has recently emerged as a promising therapy for AMR.

Methods: A highly sensitized 13 y/o girl with ESKD secondary to spina bifida and reflux nephropathy developed severe steroid-, ATG- and plasmapheresis-resistant AMR with TMA 1 week post 2nd kidney transplant despite previous desensitization therapy. Eculizumab rescue therapy resulted in a complete biochemical (C3; creatinine) and hematological (platelets) recovery within 6 days. The patient was deficient in CFHR1, a plasma protein that regulates the complement cascade and has been involved in the pathogenesis of aHUS caused by CFH autoantibodies (DEAP HUS).

Results: CFHR1 is a regulator of the alternative pathway (AP) C5 convertase and terminal complement cascade. The CFHR1 deficiency may have contributed to the clinical course of our patient (i) by contributing to the severity of her sensitization, as CFHR1 deficiency is a susceptibility factor for autoantibody development (DEAP-HUS), and (ii) by having allowed for faster, more efficient C5-conversion. The rapid response to treatment with Eculizumab supports a crucial role of the complement cascade for the manifestation and/or course of AMR in our patient and further confirms recent evidence for the efficacy of Eculizumab.

Conclusions: We conclude that AP dysregulation may have an accentuating role in AMR and should be considered in severe cases of AMR particularly when accompanied by TMA. Eculizumab may not only be an effective therapy in patients with AMR, but may also prove useful in the prevention of rejection in other organ transplant rejection.

FR-PO001

Renalase Protects against Ischemic Acute Kidney Injury in Mice H. Thomas Lee,[1] Mihwa Kim,[1] Gary V. Desir.[2] *[1]Anesthesiology, Columbia University, New York, NY; [2]Medicine, Yale University, New Haven, CT.*

Background: Acute kidney injury (AKI) is a devastating clinical problem without effective therapy and renal ischemia reperfusion (IR) injury is a major cause of AKI. Renalase is a novel, renal proximal tubule secreted flavin adenine dinucleotide dependent amine oxidase. Renalase degrades circulating catecholamines, regulates blood pressure and its deficiency markedly aggravates ischemic myocardial necrosis.

Methods: Here, we tested the hypothesis that renalase protects against ischemic AKI in mice. After Columbia University IACUC approval, mice deficient in renalase (KO) and their littermate wild type (WT) mice were subjected to 30 min renal ischemia and 24 hr reperfusion or to sham-operation.

Results: Renalase KO mice subjected to renal IR developed exacerbated renal injury (Cr=2.8±0.1 mg/dL, N=6) compared to renalase WT mice (Cr=2.3±0.1 mg/dL, N=6, p<0.05). Compared to renalase WT mice, ischemic AKI in renalase KO mice resulted in drastically worse renal tubular inflammation, necrosis and apoptosis. Furthermore, renalase WT mice subjected to renal IR had reduced kidney (>60%) and plasma (>90%) renalase levels compared to sham-operated mice. Administration of recombinant renalase (1.5 mg/kg given s.c. 10 min before renal ischemia) ameliorated ischemic AKI in renalase WT mice (Cr=1.4±0.2 mg/dL, N=4, p<0.01). In cultured human proximal tubule epithelial (HK-2) cells, recombinant renalase (10 mg/ml) significantly reduced necrosis induced with 2 mM H_2O_2 (LDH released at 6 hr=28+2%, N=3) compared to vehicle-treated HK-2 cells subjected to H_2O_2 necrosis (LDH=47±3%, N=3, p<0.01).

Conclusions: Taken together, our data show that renalase serves to protect against ischemic AKI by reducing renal tubular necrosis, apoptosis and inflammation. In addition, as plasma renalase dramatically fall after ischemic AKI, it may serve as a novel and sensitive biomarker for the detection of AKI. Finally, recombinant renalase therapy may provide a novel therapeutic approach for the prevention and treatment of AKI.

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FR-PO002

Endogenous Toll Like Receptor 9 Regulates Acute Kidney Injury Shaun A. Summers, Sharon Lee Ford, Joanna Ghali, A. Richard Kitching, Stephen R. Holdsworth. *Department of Medicine and Nephrology, Monash Medical Centre and Monash University, Melbourne, Victoria, Australia.*

Background: Acute Kidney Injury (AKI) is a major cause of morbidity and mortality. The severity of cisplatin induced AKI is influenced by leukocytes, including regulatory T cells (Tregs). Toll like Receptors (TLRs) are innate pattern recognition receptors which recognise environmental 'danger signals' and then recruit leukocytes and promote host immunity. Endogenous TLR9 has both pro-inflammatory and protective properties. We sought to define the role of TLR9 in cisplatin induced AKI.

Methods: We administered cisplatin (20mg/kg) to C57BL/6 wild type (WT) and $TLR9^{-/-}$ mice (BL/6 background). We measured kidney inflammation, histological injury and blood urea nitrogen (BUN). CD25 negative(-) splenocytes (effector cells) were isolated by magnetic bead selection. For Treg depletion we used monoclonal anti-CD25 antibodies.

Results: Kidney injury and leukocyte recruitment were enhanced in the absence of TLR9. Functional (BUN: WT 43.6±11.1 vs. $TLR9^{-/-}$ 98.9±15.0mmol/l, P<0.01) and histological injury (WT 2.8±0.1 vs. $TLR9^{-/-}$ 3.4±0.1, Score 0-4, P<0.001) were increased in $TLR9^{-/-}$ mice. Interstitial neutrophil recruitment and kidney mRNA CXCL1 and CCL2 expression were significantly increased in $TLR9^{-/-}$ mice, while FoxP3 expression was decreased. Recombinant gene activation-1 $(RAG\text{-}1)^{-/-}$ mice (lacking adaptive immunity) reconstituted with effectors (CD25- splenocytes) from WT and $TLR9^{-/-}$ mice showed no difference in functional (BUN: WT reconstituted 43.5±6.4 vs. $TLR9^{-/-}$ reconstituted 39.2±7.9mmol/l, P=n/s) or histological injury between groups after cisplatin. These results proved that CD25- effector capacity was similar in WT and $TLR9^{-/-}$ mice. Treg depletion prior to cisplatin treatment in WT and $TLR9^{-/-}$ mice showed no difference in functional (WT 56.5±8.9 vs. $TLR9^{-/-}$ 50.0±10.7mmol, P=n/s) or histological injury, experiments ended early due to severe disease. These results suggested that Tregs require TLR9 for their maximal protective effects in cisplatin induced AKI.

Conclusions: Endogenous TLR9 is protective in experimental AKI, an effect mediated by regulatory T cells.

Funding: Government Support - Non-U.S.

FR-PO003

HMGB1 in Renal Ischemic Injury May M. Rabadi, Savneek S. Chugh, Michael S. Goligorsky, Brian B. Ratliff. *New York Medical College, Valhalla, NY.*

Background: Factors that initiate cellular damage and trigger the inflammatory response cascade and renal injury are incompletely understood in AKI. HMGB1 is a DAMP molecule that in stress situations is released into the circulation, where it acts as a danger signal.

Methods: Immunohistochemical and Western blot analysis were used to identify time course HMGB1 nuclear-cytoplasmic translocation (NCT) and release from renal cells in vivo during increased duration of ischemia. In vitro, HUVEC were transfected with HMGB1-GFP plasmid and analyzed, using time-lapse videomicroscopy, for HMGB1 translocation during treatment with H_2O_2 to simulate oxidative stress. The effects of ethyl pyruvate (EP) inhibition of HMGB1 redistribution were evaluated in vivo by assessing serum creatinine, albuminuria, cyto-/chemokine release and long-term residual fibrosis.

Results: HMGB1 release into the venous circulation progressively increased in parallel with increased duration of ischemia. Time-lapse videomicroscopy of HMGB1-GFP-transfected cells showed that H_2O_2 induced HMGB1 NCT and release from HUVEC within 3 hours of treatment. This effect was significantly blocked by EP. In vivo, EP resulted in nuclear retention and significant blunting of HMGB1 release into the circulation after ischemia and improved parameters of acute (serum creatinine, albuminuria, cyto-/chemokine release) and chronic injury (albuminuria and fibrosis.) The renoprotective effect of EP was abolished by intravenous injection of exogenous HMGB1 suggesting that EP's therapeutic efficacy is mediated by blocking HMGB1 NCT. Exogenous HMGB1 administered to healthy animals at a pathophysiologically relevant dose induced a rapid surge in systemic circulation of cyto-/chemokines (TNFα, eotaxin, G-CSF, IFNγ, IL-10, IL-1α, IL-6, IP-10, and KC) and led to mobilization of bone marrow CD34+Flk1+ cells into the circulation.

Conclusions: Our results indicate that increased ischemic duration and oxidative stress causes progressively enhanced HMGB1 release into the circulation triggering damage/repair signaling. This effect is inhibited by EP because of its ability to block HMGB1 nuclear-cytoplasmic translocation.

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FR-PO004

Pharmacological Targeting of Sphingosine-1-Phosphate Receptor 1 Improves Peritubular Capillary Function during Sepsis in the Mouse Philip R. Mayeux, Zhen Wang. *Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: Microcirculatory failure and vascular leakage are hallmarks of sepsis in humans. Studies in rodents indicate that renal microvascular failure and increased microvascular permeability occur early during the course of sepsis and contribute to the development of AKI. There is growing evidence suggesting that sphingosine-1-phosphate (S1P) signaling pathways regulate vascular permeability. Stimulation of S1P receptor 1 (S1PR1) reduces permeability whereas stimulation of receptor 2 (S1PR2) enhances permeability.

Methods: To investigate the mechanism of microvascular leakage and its role in peritubular capillary hypoperfusion, we used the cecal ligation and puncture (CLP) model of sepsis in male 40 wk C57BL/6 mice and the S1PR1 agonist SEW2871 and S1PR2 antagonist JTE-013. Evans blue dye leakage was used to measure renal microvascular permeability and intravital video microscopy was used to quantitate renal peritubular capillary perfusion.

Results: CLP caused a rapid increase in capillary permeability beginning at 2h (prior to capillary hypoperfusion) and was sustained through 18h. SEW2871 at doses of 1, 3, 10 or 30 mg/kg ip or JTE-013 at doses of 0.1, 1, or 10 mg/kg, ip administered at the time of CLP partially reduced to a similar extent renal capillary leakage but neither restored capillary perfusion. However, when administered 6h post CLP, only SEW2871 reduced capillary leakage at 18h. SEW2871 also restored capillary perfusion at 18h and improved renal function as assessed by reduced levels of serum BUN and creatinine.

Conclusions: Overall, our studies suggest that in CLP-induced septic mice, S1PR1 and S1PR2 signaling pathways do not regulate the early decline in renal capillary perfusion. However, later in the course of sepsis, pharmacological stimulation of S1PR1 signaling, even after injury has occurred, not only reduces capillary leakage but also restores capillary perfusion and renal function. These findings also suggest that pharmacological stimulation of S1PR1 signaling should be tested further as an adjunct therapy for sepsis-induced AKI.

Funding: NIDDK Support, Private Foundation Support

FR-PO005

Delayed Restoration of Renal Blood Flow and Peritubular Capillary Perfusion by Inhibition of Phosphodiesterase 4 Partially Protects against Sepsis-Induced AKI in Mice Philip R. Mayeux, Zhen Wang, Joseph H. Holthoff. *Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: In animal models of septic AKI, renal dysfunction is correlated with microcirculatory failure that has been attributed to several pathological insults including reactive nitrogen species (RNS) and a decline in renal blood flow (RBF). Pharmacological agents that increase endothelial cyclic AMP levels have been shown to improve the microcirculation. Thus, we hypothesized that administration of the phosphodiesterase 4 (PDE4) inhibitor, rolipram, would improve the renal microcirculation and protect against septic AKI.

Methods: To test our hypothesis, sepsis was induced in C57/BL6 male mice aged 38-40 weeks using cecal ligation and puncture (CLP). RBF was measured by Doppler flow and peritubular capillary perfusion, cellular redox stress and RNS generation were measured by intravital video microscopy (IVVM).

Results: Rolipram (0.3, 1, 3, or 10 mg/kg i.p.) or vehicle was administered at 5.5 h post CLP and the renal microcirculation was evaluated using IVVM. Rolipram acutely restored the renal cortical microcirculation in a dose-dependent manner. Furthermore, rolipram (1 mg/kg) acutely raised RBF. At 6h post CLP, RNS generation is associated with capillary hypoperfusion, both of which likely play a role in the development of AKI. To evaluate the potential clinical efficacy to protect against AKI, rolipram (1 mg/kg) was administered at 6h post CLP (after the onset of sepsis) and mice were examined at 18h. Rolipram significantly improved the renal microcirculation and reduced cellular redox stress. Interestingly, rolipram did not reduce the generation of RNS measured by rhodamine fluorescence but delayed therapy did reduced serum levels of creatinine although not urea nitrogen.

Conclusions: These data establish that PDE4 inhibition can acutely increase RBF and restore the microcirculation in septic mice. Importantly, even delayed therapy

targeting PDE4 partially improved renal function suggesting that improving the renal microcirculation can seemingly offset the damaging effects of RNS generation in the peritubular microenvironment.

Funding: NIDDK Support

FR-PO006

Circulating, but Not Pulmonary, IL-6 Mediates Lung Injury via CXCL1 Production after Acute Kidney Injury (AKI) in Mice Rhea Bhargava, Nilesh Ahuja, Ana Andres-hernando, Chris Altmann, Zhibin He, Sarah Faubel. *Renal Diseases and Hypertension, CU Denver, Aurora, CO.*

Background: Inhibition of IL-6 in mice with AKI reduces lung injury that is associated with a reduction in the chemokine CXCL1 and lung neutrophils. Whether circulating IL-6 or locally produced lung IL-6 mediates lung injury after AKI is unknown; whether CXCL1 plays a role in AKI-mediated lung injury is also unknown.

Methods: Ischemic AKI was induced by 22 minutes of bilateral renal pedicle clamping in adult male C57Bl/6 mice. IL-6 and CXCL1 was determined by ELISA.

Results: To examine the role of pulmonary IL-6 in AKI-mediated lung injury, we first administered intratracheal (IT) IL-6 to normal mice and found that lung MPO activity (a marker of lung neutrophils), BAL fluid CXCL1, lung CXCL1, and serum CXCL1 were not different versus vehicle. Next, IT IL-6 was administered to wild type mice with AKI and lung CXCL1 production and MPO activity were not increased, demonstrating that excess IL-6 in the lung does not exacerbate AKI-mediated-lung injury. Finally, IT IL-6 was administered to IL-6 deficient mice with AKI; although BAL fluid CXCL1 was increased, no increase in whole lung CXCL1 or lung MPO activity occurred. These data suggest that lung IL-6, and specifically, intra-alveolar IL-6, does not contribute to lung injury after AKI. To test the role of circulating IL-6 in AKI-mediated lung injury, recombinant murine IL-6 was administered intravenously to IL-6 deficient mice with AKI which increased serum CXCL1, lung CXCL1, and lung MPO activity versus vehicle. To test the role of CXCL1, CXCR2 deficient and CXCL1 antibody treated mice with AKI were studied and both had reduced lung neutrophil content versus wild type/vehicle treated (CXCR2 is the receptor for CXCL1).

Conclusions: In summary, we demonstrate for the first time that *circulating* IL-6 is a mediator of lung injury after AKI via CXCL1 production. Serum IL-6 is increased in patients with AKI and predicts prolonged mechanical ventilation and increased mortality; thus, our data suggest that serum IL-6 is not simply a biomarker of poor outcomes but a pathogenic mediator of lung injury.

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FR-PO007

Macrophage IL-4/JAK3/STAT6 Pathway Is Involved in Recovery from Acute Kidney Injury Bing Yao, Shilin Yang, Raymond C. Harris, Ming-Zhi Zhang. *Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.*

Background: Acute kidney injury (AKI) is characterized by abrupt and reversible kidney dysfunction. We have developed transgenic mice selectively expressing the human diphtheria toxin receptor in renal proximal tubule epithelial cells (DTR mice) and have shown that a single injection of DT induces reversible and reproducible proximal tubule injury in these mice. We have previously shown an important role for M2-polarized renal monocytes in the recovery from AKI.

Methods: Male DTR mice were administered DT (100 ng/kg) to induce AKI, with or without the JAK3 inhibitor CP-690550 (15 mg/kg/day via osmotic minipump). In addition, IL-4 null mice crossed with DTR mice were studied.

Results: Following DT-induced injury, there was an early increase in renal expression of interleukin-4 receptor α (IL-4Rα), a common receptor subunit for both IL-4 and IL-13, and increases in the expression of STAT6 and p-STAT6. These increases were blunted by macrophage/dendritic cell depletion with clodronate, indicating activation of the macrophage/dendritic cell IL-4 and/or IL-13/JAK3/STAT6 pathway. Pharmacologic inhibition of this pathway with CP-690550 significantly inhibited p-STAT6 expression, markedly delayed functional recovery and led to increased tubulointerstitial fibrosis and albuminuria. To investigate further the role of IL-4 in recovery from acute kidney injury after DT, DTR mice/IL4 null mice were studied. Similar to CP-690550 institution, deletion of IL-4 led to delayed functional and structural recovery from acute kidney injury after DT injection. Neither pharmacologic or genetic inhibition of the IL-4/JAK3/STAT6 pathway decreased the number of renal F4/80+ monocytes.

Conclusions: These studies demonstrate that IL-4 plays an important role in renal tubule regeneration following acute kidney injury induced by DT. The mechanism by which IL-4 mediates recovery is under investigation.

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FR-PO008

Differential Gene Expression in Macrophage Subpopulations Following Renal Injury Meghan Clements, Michael Gershenovich, Christopher Chaber, Juanita Campos Rivera, Pan Du, Mindy Zhang, Steven R. Ledbetter, Anna Zuk. *Genzyme R&D.*

Background: Macrophages are a heterogeneous cell type implicated in injury, repair and fibrosis following acute kidney injury (AKI).

Methods: In this study, we used a renal bilateral ischemia-reperfusion injury model in C57BL6 mice to explore different macrophage subpopulations and functions by microarray analysis.

Results: Three distinct subpopulations were identified by differential expression of Ly6C. The $CD11b^{+}/Ly6C^{high}$ population is associated with a robust burst of tissue pro-inflammatory cytokines shortly after reperfusion and onset of injury while the $CD11b^{+}/Ly6C^{intermediate}$ population peaks during repair of the tubular epithelium. The $CD11b^{+}/Ly6C^{low}$ population emerges when numerous genes associated with fibrosis are upregulated in the kidney which include: TGF-b, collagen I and III. Each subpopulation was sorted and whole genome mRNA profiling was performed with Affymetrix mouse 430 2.0 array. Comparative analysis ($p<0.001$) identifies gene signatures unique to each subpopulation of cells. The $Ly6C^{low}$ subpopulation has the greatest number of unique transcripts, 740, while the $Ly6C^{int}$ and $Ly6C^{low}$ have 102 and 72, respectively. Interestingly, similar gene signatures could be identified in each subpopulation in the sham and ischemic mice indicating these populations are present in the uninjured kidney. Correlation analysis using the NextBio database identifies similarities between the $Ly6C^{int}$ signature and activated macrophages as well as studies linked to tissue repair. Genes known to be reparative or anti-inflammatory were identified in this signature including: chitinase-3-like-1 and-3, matrix metalloproteinase-8, lipocalin-2 and plasminogen activator, urokinase receptor. In the $Ly6C^{low}$ population, gene correlations were found with a mouse model of pulmonary fibrosis and kidney transplant patients with interstitial fibrosis suggesting a potential role in developing fibrosis following AKI. Using linguamatic I2E software, genes upregulated in the $Ly6C^{low}$ population were linked to kidney and fibrotic diseases in patients.

Conclusions: Ongoing studies are evaluating the interrelationships between these populations.

Funding: Pharmaceutical Company Support - Sanofi

FR-PO009

Assessment of Renal Morphology and Hemodynamics in Acute and Chronic Kidney Failure with Advanced Unenhanced Magnetic Resonance Imaging (MRI) Techniques Zohar Milman, Nathalie Corchia (Nachmansson), Jonathan H. Axelrod, Samuel N. Heyman, Rinat Abramovitch. *Hadassah Hebrew Univrtsity Hospitals, Jerusalem, Israel.*

Background: Contrast-enhanced imaging techniques are limited in the presence of renal dysfunction due to nephrotoxicity or to the risk of developing nephrogenic systemic fibrosis. We have recently demonstrated the sensitivity of Hemodynamic Response Imaging (HRI), a functional blood oxygenated level (BOLD) magnetic resonance imaging (MRI) method combined with transient hypercapnia and hyperoxia (Barash, Radiology 2007), for the evaluation of renal vascular reactivity without the use of contrast agents (ISMRM 2011). True-FISP is another unenhanced MRI method, providing high-resolution anatomical images in short acquisition times. The aim of the present study was to establish the use of these MRI methods for the non-invasive evaluation of changes in renal hemodynamics and morphology during renal impairment, without the need for contrast-agents.

Methods: Renal-HRI maps during hypercapnia and hyperoxia and True-FISP images were repeatedly acquired in two models of kidney injury in mice: along 4 weeks in adenine-induced progressive CKD model, and over 3 weeks during the induction and recovery from rhabdomyolysis-induced AKI. Contrast enhanced (CE)-MRI was used in parallel experiments for comparison.

Results: In intact animals renal HRI maps show profound changes (ΔS) during hypercapnia and with subsequent hyperoxia. By contreast, ΔS response was markedly blunted during changes in inspired gases with evolving kidney dysfunction in the two models, reflecting hampered renal vascular reactivity and perfusion. True-FISP images showed high sensitivity to renal morphological changes, comparable to CE-MRI, with different patterns characterizing each model. Calculated data obtained from HRI and True-FISP maps during the evolution of renal failure and upon recovery, closely correlated with the degree of renal impairment.

Conclusions: This study illustrates the potential utilization of HRI combined with True-FISP for the non-invasive assessment of renal dysfunction, without the potential risk associated with the administration of contrast-agents.

Funding: Government Support - Non-U.S.

FR-PO010

Identification of Novel Biomarkers of Cardio-Renal Syndrome in Adult Cardiac Surgery Patients Michael Haase,[1] Rinaldo Bellomo,[2] Anja Haase-Fielitz.[1] *[1]Nephrology, Otto von Guericke University, Magdeburg, Germany; [2]Intensive Care, Austin Health, Melbourne, Australia.*

Background: Novel technologies are being advanced for the purpose of identification and validation of new renal biomarkers.

Methods: In a nested cohort study of 100 adult cardiac surgical patients, we assessed the value of biomarkers of cardiorenal syndrome for the prediction of acute kidney injury (RIFLE-AKI), acute dialysis and mortality. A proteomic approach for antibody-free targeted protein quantitation based on high-end mass spectrometry was used to measure serum concentrations of the LG3 fragment of perlecan, latent transforming growth factor binding protein 2 (LTBP2) and cathepsin L at baseline, on arrival in the intensive care unit (ICU) and at 24 hours postoperatively. We calculated AUC-ROC values for all biomarkers including serum creatinine, cystatin C, plasma/urine NGAL.

Results: On arrival in ICU all *serum* biomarkers were not useful for prediction of AKI, N=21 (all AUC-ROC ≤0.70). *Urine* NGAL had an AUC of 0.74 [95% CI 0.62-0.86] for AKI prediction. The predictive value of uNGAL increased to AUC 0.83 [0.69-0.98] for *severe* AKI (RIFLE I+F) whereas *serum* biomarkers had AUC ≤0.70 at this timepoint. On ICU arrival, in patients without preoperative chronic kidney disease (CKD), uNGAL

predicted acute dialysis (AUC 0.80 [0.55-0.99]) but not in patients with CKD. At 24hrs postoperatively in all patients, *serum* LG3 (AUC 0.89 [0.81-0.96]) and *serum* LTBP2 (AUC 0.88 [0.80-0.96]) had very good AUC values for dialysis prediction. In patients with normal preoperative renal function uNGAL at 24 hours was the best predictor for mortality with AUC-ROC 0.95 [0.89-0.99]. At 24hrs postoperatively, Cathepsin L had the highest AUC-ROC (0.88 [95% CI 0.79-0.96]) for AKI prediction. After exclusion of patients with preoperative renal impairment (N=40), the predictive performance of Cathepsin L further increased. Cathepsin L levels showed a good correlation with mean arterial pressure (r^2=0.36)and lactate (r^2=0.24).

Conclusions: Novel serum markers including LG3, LTBP2 and Cathepsin L can precisely predict acute dialysis and mortality at 24hrs postoperatively and uNGAL predicts AKI and acute dialysis (without preoperative CKD) at ICU arrival.

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FR-PO011

Partial Recovery from AKI, Followed by Sepsis, Worsens Kidney Injury and Mortality, but Protects Other Organs Takayuki Tsuji,[1,2] Ana Carolina Souza,[1] Xuzhen Hu,[1] Peter S.T. Yuen,[1] Robert A. Star.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]1st Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.*

Background: The Program to Improve Care in Acute Renal Disease (PICARD) observational study found that 40% of hospitalized patients who were not septic at the time of AKI diagnosis subsequently developed sepsis. The in-hospital mortality of late onset sepsis patients was 20% higher than patients remaining sepsis-free. Thus, sepsis frequently develops after AKI and portends a poor prognosis. Based on our prior work with CKD before sepsis, we hypothesized that AKI before sepsis might amplify the susceptibility to multiple organ failure and hence worsen prognosis. We investigated the acute impact of partial recovery from AKI on sepsis.

Methods: We used 10-12 week old male C57BL/6 mice. We performed sham surgery or 40 min bilateral renal ischemia/reperfusion (I/R), waited 48 hrs, then induced polymicrobial sepsis by cecal ligation and puncture (CLP) surgery. We measured outcomes 24 hrs after CLP, and performed a 4-day survival study.

Results: As expected, I/R significantly intensified renal injury after sepsis [I/R->CLP: BUN 189±20.0 and serum creatinine (Scr) 1.40±0.58 vs. sham->CLP: BUN 117.7±7.4 mg/dl ($p<0.05$) and Scr 0.82±0.37 mg/dl ($p<0.05$)]. Death occurred significantly faster in I/R->CLP than sham->CLP (40 vs 68 hrs). In contrast, AST, LDH, CPK, and spleen apoptosis (by active caspase 3 immunohistochemistry) were significantly lower in I/R->CLP than sham->CLP. Systemic inflammatory cytokines (HMGB-1, TNF-α, IL-10 and IL-6) in I/R->CLP were modestly (but not significantly) lower in I/R->CLP than sham->CLP.

Conclusions: Partial recovery from AKI, followed by sepsis, worsened kidney function and survival compared to sepsis alone, although there was less liver, muscle, and spleen damage. Thus, partial recovery from AKI unexpectedly dissociates the renal and systemic effects of sepsis. Mortality follows the kidney injury--not other organ injury--in this acute-on-acute model. The uncoupling of multiple organ failures from each other requires further investigation, but highlights the difficulty of substituting organ-specific endpoints for mortality in sepsis trials.

Funding: NIDDK Support

FR-PO012

Sepsis Inhibits Mitochondrial Biogenesis While Stimulating Mitochondrial Oxygen Consumption in Acute Kidney Injury L. Jay Stallons, Ryan Whitaker, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC.*

Background: Most cases of acute kidney injury (AKI) result from sepsis, renal ischemia, or acute drug or toxicant exposure, affecting up to 5% of all long-term hospital patients. Mortality from AKI remains near 50%, and the added comorbidity of sepsis increases mortality of AKI to 70%. Because AKI and sepsis are both known to have mitochondrial pathologies, we examined renal mitochondrial dysfunction in a mouse model of sepsis-induced AKI.

Methods: Male C57BL/6 mice were injected with 10 mg/kg lipopolysaccharide (LPS) and 18 h later with 10 ml/kg saline. Renal function was evaluated using serum creatinine and urine volume. Renal mitochondrial biogenesis and protein were measured using RT-qPCR and immunoblot analysis for PGC-1α, NDUFB8, COXI, and ATP synthase β (ATPSβ). ATP levels were measured using a bioluminescence assay. Mitochondria were isolated from the kidney and assayed on a Seahorse Bioscience XF96 Analyzer to measure state 2 and 3 respiration.

Results: Renal function was impaired 42 h after LPS injection as shown by increased serum creatinine and reduced urine volume. Mitochondrial biogenesis (PGC-1α) and protein (NDUFB8, COX1, ATPSb) markers exhibited reduced transcript levels at 18 h and reduced protein levels at 42 h. Renal ATP was reduced at both 18 and 42 h. Isolated renal mitochondria displayed normal state 2 respiration 18 h after LPS injection, but state 3 respiration increased and remained increased at 42 h.

Conclusions: We have shown decreased mitochondrial biogenesis and ATP in a model of LPS-induced AKI. Transcript levels of mitochondrial genes were reduced at 18 h in advance of the development of AKI at 42 h. At 42 h, mitochondrial proteins were depleted. Renal mitochondrial deficiency is supported by reduced ATP 18 and 42 h after LPS injection. Remarkably, we found that isolated renal mitochondria have increased state 3 respiration at 18 and 42 h after LPS, while the state 2 respiration was unchanged. We hypothesize that while renal mitochondrial biogenesis is suppressed in endotoxic AKI, an alternative pathway is activated to directly stimulate electron transport chain (ETC) activity and ATP synthesis.

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FR-PO013

Complement and AKT Pathway Regulate Endothelial to Mesenchymal Transition (EndMT) in Renal Ischemia Reperfusion (I/R) Injury G. Castellano,[1] Claudia Curci,[1] Antonia Loverre,[1] Alessandra Stasi,[1] S. Simone,[1] M. Cariello,[1] Vincenzo Montinaro,[1] P. Ditonno,[1] M. Battaglia,[1] Antonio Crovace,[1] F. Staffieri,[1] Beatrijs D. Oortwijn,[2] Edwin S. Van Amersfoort,[2] Francesco Paolo Schena,[1] Loreto Gesualdo,[1] Giuseppe Grandaliano.[3] *[1]DETO, Univ of Bari, Italy; [2]Pharming Group NV, Leiden, Netherlands; [3]Medical and Surgical Sciences, Univ of Foggia, Italy.*

Background: EndMT may significantly contribute to the development of tissue fibrosis. However, the pathogenic factors and signaling pathways regulating EndMT are poorly understood.

Methods: In an experimental model of I/R, 10 pigs underwent 30 min of renal warm I and 24h of R. Just before R, recombinant human C1-inhibitor (C1INH) was administered in 5 animals. CD31, αSMA and FSP1 protein expression were investigated by confocal microscopy and Western blot.

Results: Renal I/R injury reduced the density of peritubular capillaries (t24h 1.2±.4; t0 2.4±.8 CD31 pixel$^+$/total area ratio, p=.03). We observed also an increased interstitial expression of the myofibroblast marker αSMA (t24h 5.19±.58; t0 1.75±.13, p=.04) and the appearance of CD31$^+$/FSP1$^+$ renal endothelial cells (t24h 18.9±4.4; t0 8.2±3.0, p=0.04), suggesting EndMT. After C1INH administration, CD31$^+$/FSP1$^+$ endothelial cells were significantly reduced in the renal interstitium (t24h C1INH 7.7±1.1, p=.03 vs t24h ctr), peritubular capillary density was preserved (t24h C1-INH 2.15±.2, p=0.04 vs t24h ctr)and α-SMA staining disappeared.Activation of cultured endothelial cells by C3a led to EndMT, as shown by a decrease in von Willebrand Factor (C3a 4.6±.8, basal:7.3±.6, p=.04) and an increase of αSMA expression (C3a .9±.1; basal .3±.1, p=.003) along with a significant increase in AKT phosphorylation. The increase of αSMA was significantly reduced by inhibiting AKT in vitro (C3a .6±.1, C3a+AKT inh .3±.1, p=.03). Accordingly, C1INH infusion in vivo abrogated AKT phosphorylation in renal endothelial cells.

Conclusions: In conclusion, our data demonstrate a critical role for Complement in the acute induction of the EndMT process via the AKT pathway. Therapeutic inhibition of these systems may be essential to prevent the development of vascular-derived tissue fibrosis.

Funding: Pharmaceutical Company Support - Pharming Group NV

FR-PO014

Coupled Plasma Filtration Adsorption Prevents Renal Fibrosis by Inhibition of Endothelial to Mesenchymal Transition in a Swine Model of Sepsis-Induced Acute Kidney Injury G. Castellano,[1] Alessandra Stasi,[1] Anna Maria Di Palma,[1] Giuseppe Stefano Netti,[2] Claudia Curci,[1] Angelica Intini,[1] C. Divella,[1] Enrico Fiaccadori,[3] Clelia Prattichizzo,[2] Giovanni Pertosa,[1] Giuseppe Grandaliano,[2] Loreto Gesualdo.[1] *[1]Nephrology Unit, DETO, Univ of Bari, Italy; [2]Dept of Medical and Surgical Science, Univ of Foggia, Italy; [3]Internal Medicine and Nephrology Dept, Univ of Parma, Italy.*

Background: Activation of endothelial cells (EC) plays a key role in acute kidney injury (AKI). Aim of our study was to investigate the contribution of EC on renal fibrosis and to test the efficacy of CPFA (Coupled Plasma Filtration Adsorption) in modulating EC activation in a swine model of sepsis-induced AKI.

Methods: After 3h from LPS infusion (300μg/Kg), 8 pigs were treated with CPFA for 6h; 8 control pigs received no treatment. Renal biopsies were performed before (T0) and 9h (T9) after LPS infusion.

Results: In septic pigs, Masson's trichrome staining revealed extensive collagen deposition at the interstitial level and diffuse glomerular thrombi at T9. By immunofluorescence, we observed an interstitial increase of the myofibroblast marker α-SMA at T9 compared to T0 in septic pigs(7.58±0.49fold change, p=0.001). Immunohistochemical analysis for Caspase-3 showed rare apoptotic peritubular and glomerular EC in sepsis, while double immunofluorescence analysis revealed CD31$^+$/Ki-67$^+$ proliferating EC(T9 12.5±0.7fold change vsT0, p=0.01). CD31$^+$EC expressed the fibroblast marker FSP1(T9: 14.14±0.97fold change vsT0,p=0.02) and α-SMA in sepsis, indicating the occurrence of Endothelial to Mesenchymal Transition (EndMT) at tubulointerstitial level. In vitro, LPS induced EC proliferation and EndMT, confirming the in vivo data. Septic pigs treated with CPFA showed a significant reduction in collagen deposits and glomerular thrombi compared to untreated animals. α-SMA expression(2.43±0.31fold change,p=0.001), as well as EndMT(CD31$^+$/FSP1$^+$3.73±1.02fold change,p=0.001)was strongly reduced by CPFA treatment.

Conclusions: Our data demonstrate a critical role of EndMT in sepsis-induced renal fibrosis. CPFA treatment seems to counteract the activation of EC, thereby inhibiting the vascular-derived tissue fibrosis.

FR-PO015

Iodinated Contrast Media Decrease Nitric Oxide Bioavailability and Induce Oxidative Stress in Afferent Arterioles of Mice Zhi Zhao Liu, Andrea Perlewitz, Mauricio Michalak Sendeski, Andreas Patzak. *Institute of Vegetative Physiology, Charite - Universitaetsmedizin Berlin, Berlin, Germany.*

Background: Contrast induced acute kidney injury (CI-AKI) is a common hospital-acquired case of renal failure. We showed recently that contrast media constrictafferent arterioles of mice. Here we investigate the influence of contrast media on nitric oxide bioavailability and superoxide in afferent arterioles to understand the underlying mechanism of reduced renal perfusion in CI-AKI.

Methods: Afferent arterioles from C57Bl/6 mice were perfused with either vehicle solution or contrast media (ioxaglate or iodixanol, 23mg iodine/ml), followed by angiotensin II administration. The fluorescent dyes DAF-FM and dihydroethidium (DHE) were used for quantification of nitric oxide bioavailability and superoxide concentration in the afferent arteriolar wall, respectively.

Results: Compared to control group, ioxaglate and iodixanol reduced afferent arteriole diameters significantly and similarly by 10% within 20min and enhanced the response to angiotensin II. DAF-FM fluorescence decreased during iodixanol treatment (-7.4%) and by nitric oxide synthase inhibition (L-NAME, -7.5%), whilst it increased by 11.0% in the control group. This indicates an impaired nitric oxide bioavailability. Further, iodixanol increased DHE fluorescence ratio by 13.8%, implying oxidative stress in the arteriolar wall. The superoxide dismutase mimic tempol blunted the increase of DHE ratio during iodixanol administration.

Conclusions: The study shows that contrast media with different physicochemical properties exert a similar impact on afferent arteriolar function *in vitro*. Decreased nitric oxide bioavailability and increased superoxide concentration may play a role in mediating the constrictive effects of contrast media. The findings indicate an endothelial dysfunction by contrast media which is combined with oxidative stress in preglomerular vessels.

FR-PO016

Glomerular Endothelial and Permeability Barrier Dysfunction Induced in Early Bacterial Sepsis Ruben M. Sandoval,[1] George Rhodes,[1] Silvia B. Campos-bilderback,[1] Sarah E. Wean,[1] Bruce A. Molitoris.[1,2] *[1]Medicine/ Division of Nephrology, IU School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN.*

Background: Sepsis remains the leading cause of acute kidney injury (AKI) characterized by hemodynamic effects and a reduction in GFR.

Methods: To determine if there is glomerular involvement we studied surface glomeruli in Munich Wistar Frömter rats using intravital 2-photon microscopy in a cecal ligation and puncture (CLP) model of sepsis at 0, 4, 12 and 24hrs post injury.

Results: The permeability of a 150kDa dextran, as determined by glomerular sieving coefficient (GSC), increased progressively ($P \leq 0.05$) from control values (0.0068±0.0030) to 0.0203±0.0072, 0.0407±0.0085, and 0.0968±0.0462 at 4, 12 and 24hrs post CLP, respectively. A dramatic reduction ($P \leq 0.05$) in speed of flowing RBC's within capillary loops from control values (1,771±467 μm/sec) to 910±414, 182±50, and 576±327 μm/sec was seen at 4, 12 and 24hrs post CLP, respectively. Since activated white blood cells (WBC's) are known to occlude flow within capillary loops we measured adherence of WBC's and found an increase ($P \leq 0.05$) from control values (0.4±0.3 per standardized volume) to 3.4±1.1, 3.7±1.0, and 7.3±5.8 at 4, 12 and 24hrs post CLP, respectively. The presence of Rouleaux formations was scored (values of 0-4 progressively worsening), and appeared at 12hrs and remained present at 24 hrs post CLP. Serum creatinine values did not elevate above control values (0.2±0.1 mg/dL) until 24hrs post CLP (0.8±0.2, $P \leq 0.05$). Mean blood pressure values became progressively more variable between rats.

Conclusions: These results indicate the reduction in renal function may be due in part to interactions of RBC's and WBC's to reduce flow, and along with podocyte injury; cause an increase in glomerular permeability associated with proteinuria.

Funding: Other NIH Support - P30DK079312

FR-PO017

Total B Cells (Expressing CD19) Prostaglandin I_2 Attenuates Initial Renal Injury Following Warm Ischemia-Reperfusion Injury in Mice Hye Ryoun Jang,[1] Minsu Kim,[1] Jung Eun Lee,[1] Dae Joong Kim,[1] Wooseong Huh,[1] Kwon Wook Joo,[2] Jin Suk Han,[2] Ha Young Oh,[1] Yoon-Goo Kim.[1] *[1]Medicine, Sunkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; [2]Medicine, Seoul National University Hospital, Seoul, Korea.*

Background: Inflammation is a major pathophysiologic process during kidney ischemia reperfusion injury (IRI). Prostaglandin I2 (PGI2) was reported to have an anti-inflammatory function. We tested the hypothesis that PGI2 attenuates initial renal injury following warm IRI in mice.

Methods: C57BL/6 mice were randomly allocated into PGI_2-sham, control, and PGI_2-IRI groups. PGI_2 was diluted with DMSO and 10 mg/kg was injected into peritoneal cavity right before reperfusion. Serum creatinine was measured for 48 hours. The expression of cytokines and major sodium transporters were measured at 48 hours after IRI with bioplex array and western blotting, respectively. Immunofluorescent staining for F4/80 was performed for comparing the monocyte infiltration.

Results: Early renal injury was functionally and structurally attenuated in the PGI_2-IRI group compared with the control (serum creatinine, Day 0: 0.37±0.014 in PGI_2-sham, 0.52±.035 in control-IRI, 0.35±0.033 in PGI_2-IRI, Day 1: 0.52±0.053 in PGI_2-sham, 2.72±0.167 in control-IRI, 1.40±0.206 in PGI_2-IRI, Day 2: 0.44±0.051 in PGI_2-sham, 2.33±0.427 in control-IRI, 0.73±0.114 in PGI_2-IRI). The expression of IFN-γ was augmented in the postischemic kidney, but this augmentation was significantly attenuated by PGI_2 treatment (pg/mg of whole kidney protein extract, 95.25±8.95 in PGI_2-sham, 115.65±.7.56 in control-IRI, 68.05±10.72 in PGI_2-IRI). However, there was no difference in the expression of IL-10 among 3 groups (pg/mg of whole kidney protein extract, 179.3±9.45 in PGI_2-sham, 139.3±.16.11 in control-IRI, 121.0±18.18 in PGI_2-IRI). The down-regulation of Na^+-K^+-$2Cl^-$ cotransporter and Na^+-Cl^- cotransporter was attenuated in PGI_2-IRI group. There was no difference in the infiltration of F4/80 positive mononuclear cells into the post-ischemic kidney between groups.

Conclusions: PGI_2 treatment attenuated early renal injury by down-regulation of IFN-γ through humoral effects and prevented down-regulation of NKCC2 and NCC following IRI.

Funding: Private Foundation Support

FR-PO018

Renal Peritubular Capillary Injury with Impaired Renal Microcirculation in Hepatic Acute Kidney Injury after Severe Hepatic Failure in Rats Akira Shimizu,[1] Yukinari Masuda,[1] Ayako Sato,[2] Tetsuo Morioka.[2] *[1]Department of Pathology, Nippon Medical School, Tokyo, Japan; [2]Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: Acute kidney injury (AKI) is a common complication in the acute liver dysfunction. However, the mechanisms of AKI during the development of acute liver dysfunction are still uncertain. In the present study, we characterize a rat model of AKI during the development of acute liver dysfunction following liver transplantation.

Methods: Acute hepatic failure was induced in rat by liver transplantation from DA (RT1a) to Lewis (RT1l) rats without immunosspression. Acute rejection progressed, and rats were dead around day 11 with severe acute liver dysfunction. We examined the clinical and laboratory data and pathological characteristics of kidneis at day 5, day 7, and day 9 to 11, focusing on the tubular and peritubular capillary (PTC) injury. In addition, hemodynamic events in PTCs in vivo were evaluated functionally and quantitatively by the use of a real-time confocal laser-scanning microscope (CLSM) system (Kidney Int 59: 252-259, 2001).

Results: During the progression of rejection in hepatic graft, acute liver dysfunction (T-Bil 7.9±1.8; AST 711.3±98.2, $p<0.01$) and acute kidney injury (BUN 112.0±22.5; Cr 0.6±0.1, $p<0.05$) developed by day 11. During the development of AKI, renal tubular degeneration with bile pigment accumulation, mitochondrial degeneration, KIM-1 expression, and severe disruption of f-actin. TUNEL+ dead cells were noted in tubules and PTCs with increased expression of caspase-3. In addition, endothelial dysfunction in PTCs developed with decreased expression of eNOS, vascular endothelial growth factor (VEGF), angiopoietin-1 (ang-1), and ang-2. Indeed, marked reduced blood flow of PTCs (320±122 μm/sec at days 9 to 11 vs 860±145 μm/sec in control, $p<0.001$) was noted in the kidney in hepatic AKI. Interstitial edema also occurred with CD68+ macrophage infiltration.

Conclusions: AKI developed in rats during the development of acute liver dysfunction and was characterized by renal tubular injury as well as endothelial dysfunction in PTCs with marked impaired microcirculation.

Funding: Private Foundation Support

FR-PO019

Serum microRNAs Are Accurate Biomarkers of Acute Kidney Injury in ICU Patients Elia Aguado Fraile,[1] Edurne Ramos,[1] Elisa Conde,[1] Esperanza Macarena Rodriguez Serrano,[1] Aurora Lietor,[2] Maria Teresa Tenorio,[3] Angel M. Candela-Toha,[2] Fernando Liano,[3] Laura Garcia-Bermejo.[1] *[1]Pathology Dpt., Hospital Ramon y Cajal, IRYCIS, Madrid, Spain; [2]Critical Care Dpt., Hospital Ramon y Cajal, IRYCIS, Madrid, Spain; [3]Nephrology Dpt., Hospital Ramon y Cajal, IRYCIS, Madrid, Spain.*

Background: microRNAs are small endogenous RNA molecules which regulate every cellular process. Recent studies have demonstrated that these molecules are secreted to the extracellular environment and they could be detected in body fluids, such as serum. Serum microRNAs profiles have been associated to physiological and pathological conditions, becoming accurate diagnostic biomarkers of several diseases, including kidney diseases.

Our aim to identify and validate a serum microRNA profile for Acute Kidney Injury (AKI) precise diagnosis in Intensive Care Units patients.

Methods: Total RNA was extracted from ICU patient serum using an optimized protocol. Previous work of our lab identified, by massive screening experiments, a set of serum microRNAs which are modulated in AKI patients. We have studied if these microRNAs are AKI biomarkers in an ICU setting. microRNAs were detected by qRT-PCR in 32 ICU patients with AKI (20 Acute Tubular Necrosis (ATN) and 12 transient AKI) and compared with those found in 20 healthy people. In the ICU-AKI patients blood samples for measuring microRNAs and serum creatinine levels were withdrawn from Day 0 (diagnosis) to day 7.

Results: Our results demonstrate that miR-101-1, miR-210, miR-26b, miR-146a and miR-10a are diagnostic biomarkers of AKI in ICU patients, compared to healthy control, showing areas under the curve values higher than 0.95 in ROC analysis. Moreover, miR-210 and miR-146a can significantly discriminate between AKI and transient AKI patients. Remarkably, serum levels of these microRNAs also correlate with AKI severity estimated by AKIN classification.

Conclusions: In summary, the serum microRNA profile here identified has a diagnostic value of AKI in ICU population. Moreover, these accurate biomarkers also indicate AKI severity. Both features could have important implications in ICU patients management allowing more precise and early therapeutic intervention.

Funding: Government Support - Non-U.S.

FR-PO020

Renal Hemodynnamics and Metabolism in Septic AKI Prabhleen Singh,[1,2] Hai Pham,[2] Scott C. Thomson,[1,2] Francis B. Gabbai,[1,2] Roland C. Blantz.[1,2] *[1]UCSD; [2]VASDHS.*

Background: AKI in the setting of sepsis is frequently observed. The traditional archetype of septic AKI caused by renal vasoconstriction has been challenged by recent experimental data demonstrating reduction in GFR even with preserved renal blood flow (RBF) in some models of sepsis. Hence, the underlying pathophysiology of septic AKI remains unclear and is a significant barrier to progress in this field. We aimed to to characterize in-vivo renal hemodynamics, metabolism, and glomerular hemodynamics at 24 hours after injury to explain the underlying pathophysiology.

Methods: We used the cecal ligation and puncture (CLP) model of sepsis in rats. Experiments included measurement of blood pressure, renal blood flow (RBF) by flowprobe, renal oxygen consumption (QO2), GFR by 3-H inulin clearance and single nephron GFR (SNGFR) and glomerular hemodynamics by micropuncture.Data presented as mean±sem.

Results: GFR was significantly lower in CLP vs. shams (1.9±0.4 vs. 3.7±0.9 ml/min, p=0.04). Blood pressure was not different between CLP and shams (108 vs. 105 mm Hg) nor was the RBF (5.7±0.3 vs. 5±0.8 ml/min). The filtration fraction was significantly lowered in CLP vs. shams,0.25 vs. 0.44, p<0.001. Renal oxygen extraction and QO2 factored for GFR (an estimate of filtered load) were both significantly higher in CLP compared to shams, p<0.001 and p=0.004 respectively. Micropuncture data showed a proportional decrease in SNGFR in CLP (42.5±3.7 vs. 20±1.4, p<0.0001). Glomeular hemodynamic measurements revealed higher ΔP in CLP 37.4 vs. 30.6 mmHg, lower single nephron plasma flow 89.7 vs. 141.5 nl/min, and higher afferent (0.32 vs. 0.24) and efferent resistances (0.25 vs. 0.14).

Conclusions: These data demonstrate increased renal oxygen utilization in CLP rats in the setting of lower reabsorptive load, thus suggesting either inefficiency in oxygen utilization for sodium transport or diversion of oxygen for non-transport processes. This may contribute to tubular injury. Glomerular hemodynamics in CLP reveal increased vascular resistances with decreased nephron plasma flow with increased effective filtration pressure, suggesting a reduction in ultrafiltration co-efficient to explain the reduction in SNGFR.

Funding: NIDDK Support, Other NIH Support - Pilot and Feasibility Grant P30DK079337, Veterans Administration Support

FR-PO021

TNF Increases Kidney Glomerular Endothelial Permeability via Modification of Glomerular Endothelial Glycocalyx Chang Xu, Bradley K. Hack, Michael T. Eadon, Patrick Cunningham. *Medicine, University of Chicago, Chicago, IL.*

Background: TNF released in conditions such as sepsis or ischemia increases endothelial permeability to macromolecules in pulmonary and brain microvascular endothelial cells (ECs). Whether TNF induces an increase in permeability to macromolecules in renal glomerular ECs and the relevance of this phenomenon to renal function such as sepsis-induced albuminuria have not been well studied. In this study, we analyze the effect of TNF on the endothelial barrier in mouse renal and human kidney glomerular ECs.

Results: TNF treatment reduced trans-endothelial electrical resistance in mouse renal ECs by 45%, increased albumin passage across the mouse renal EC monolayers by 81%, and human kidney glomerular EC monolayers by 41%. The TNF-induced increase in endothelial permeability was significantly reduced by inhibitors of Rho kinase and myosin light chain kinase (MLCK), but was not associated with disruption of tight junction proteins ZO-1, or claudins -5, -12, or -15, as determined by real time PCR or immunofluorescence. We confirmed the existence of fenestrae in these ECs, via scanning EM and stimulated emission depletion (STED) nanoscopy. We hypothesized that a layer of glycocalyx covering the fenestral domains of the glomerular EC surface is responsible for the endothelial permeability, and we then examined the effect of TNF on the glomerular endothelial glycocalyx, whose main constituents are heparan sulfates. Endothelial glycocalyx was visualized by confocal immunofluorescence of heparan sulfate and by wheat germ agglutinin labeling. TNF disrupted the endothelial surface glycocalyx and induced the endothelial cells to secrete heparanase, which degrades heparan sulfate. TNF treatment increased heparanase expression by 100% in mouse renal ECs by immunoblot.

Conclusions: These results show that TNF increases glomerular endothelial cell heparanase expression and thus causes disruption of the glomerular endothelial glycocalyx, which contributes to the increased endothelial permeability induced by TNF. This study suggests that during sepsis, TNF may disrupt the function of glycocalyx in the restriction to protein passage and contribute to albuminuria.

Funding: NIDDK Support

FR-PO022

Formation of Endothelial Microparticles in Ischemic Acute Kidney Injury Dylan Burger,[1] Jonathan G. Boucher,[1] Andreea Slatculescu,[2] Todd Fairhead.[1,2] *[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]University of Ottawa, Ottawa, ON, Canada.*

Background: Acute kidney injury (AKI) occurs in about 5% of hospitalized patients with a 50% mortality. Endothelial injury in AKI is characterized by changes in microvascular bloodflow, coagulation, and permeability and contributes to tubular epithelial injury and subsequent chronic kidney disease. Microparticles (MPs) are small, anuclear fragments shed from the cell membranes under conditions of stress/damage. MPs have procoagulant activity and may promote crosstalk between cell types. Endothelial MPs are found at low concentrations in the plasma of healthy subjects and at increased concentrations in conditions of chronic vascular injury such as vasculitis and chronic kidney disease. However, it is not known whether MP levels are increased in AKI. We hypothesized that endothelial MP levels would be increased in AKI and would contribute to inflammation.

Methods: We examined MP formation in male C57BL6 mice subjected to bilateral renal ischemia-reperfusion (I/R) for 45 minutes. Blood was collected 2 hours after reperfusion and MPs were isolated from platelet-free plasma by differential centrifugation. MPs were then quantified by flow cytometry and distinguished as VE-Cadherin^{+ve} (Endothelial MPs) and/or Annexin V^{+ve} (Total MPs) events ranging between 100-1000 nm in size.

Results: Compared with sham-operated mice, mice subjected to I/R displayed significantly higher plasma levels of endothelial microparticles (3149±902 MPs/ml vs.1059 ±152, P<0.05). Conversely, the total number of circulating microparticles was not significantly altered (6009±2126 vs. 6804±2126, P<0.05). To assess whether endothelial MPs could activate neighboring endothelial cells, MPs collected from human microvascular endothelial cells (HMVECs) were incubated with healthy HMVECs in culture. After 12 and 24 hours, no difference in endothelial expression of CD31 (PECAM), or CD144 (VE-Cadherin) was observed.

Conclusions: Our results show that endothelial microparticles are increased early after reperfusion in an animal model of ischemic AKI. Such increases may be indicative of underlying vascular injury, and could serve as an early biomarker of AKI.

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO023

In Vivo Imaging of the Host-Pathogen Interaction in the Urinary System with the Biomarker NGAL Neal A. Paragas,[1] Ritwij Kulkarni,[2] Jonathan M. Barasch.[1] *[1]Medicine, Columbia University, New York, NY; [2]Pediatrics, Columbia University, New York, NY.*

Background: NGAL is a critical component of innate immunity because it binds catecholate-siderophores which constitute the dominant iron capture mechanism of urinary pathogenic *E. coli* (UPEC). Urinary(u) NGAL is maximally expressed at mg/L levels after either septic or aseptic injuries of the kidney. It has two potential functions, epithelial growth or iron scavenging leading to bacteriostasis. Here we determined the dominant role of uNGAL as an inhibitor of urinary tract infections (UTI).

Methods: (1) We designed a conditional allele of NGAL. (2) We created a bioluminescent mouse that expresses Luciferase and mCherry when the NGAL locus is activated. (3) We developed a model of bacterial cystitis.

Results: In mouse cystitis, the intensity and timing of urinary CFUs (uCFUs) were mirrored by uNGAL levels, including their onset, their peak and their resolution. To determine uNGAL's physiological role, we constructed a global NGAL KO which poorly defended itself from UPEC. In vitro we found NGAL significantly inhibited UPEC growth, due to reversible depletion of bacterial iron. To visualize NGAL expression *in vivo*, we developed a NGAL reporter mouse (NGAL-*Luc2/mC*). UPECs introduced into the bladder induced NGAL-*Luc2/mC* distantly in the kidneys. Reporter expression was consistent with kidney *Ngal* expression measured by QPCR, *in situ* hybridization and IHC which located *Ngal* to alpha intercalated cell.To determine whether UPEC signaling directly activated kidney epithelia, we developed *in vitro* assays using explants from the reporter mouse. These cells express Ngal-*Luc2/mC* in response to co-culture with UPECs. An intercalated cell line expressed NGAL in the presence of LPS. TLR4 was the critical local sensor of infection.

Conclusions: uNGAL is essential for clearance of a UPEC in a model of cystitis. The kidney responds to infections localized to the bladder by secreting NGAL. These findings provide an explanation for the intensive expression of NGAL in the kidney in both septic and aseptic diseases, demonstrating that the kidney defends the urinary system via exocrine delivery of NGAL.

Funding: NIDDK Support

FR-PO024

Low Dose Paclitaxel Ameliorates Endotoxemic Kidney Injury by Binding MD-2 to Block TLR4-Mediated NF-κB Signaling and Inflammation Dongshan Zhang, Zheng Dong. *Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center.*

Background: Paclitaxel is used for cancer therapy. Recent research has suggested that it may have other potential therapeutic effects.

Methods: This study examined the effect of low-dosage paclitaxel on lipopolysaccharide (LPS)-induced acute kidney injury (AKI) in mice.

Results: It was shown that paclitaxel significantly prevented LPS-induced AKI and improved animal survival. The beneficial effects of paclitaxel were accompanied by the inhibition of NF-κB-p65 expression, normalization of F4/80 and MCP-1 expression, and down-regulation of serum TNF-a,IL-1,IL-6 and HMGB-1. In cultured renal tubular HK2 cells, paclitaxel decreased the expression of TLR4, MCP-1 and HMGB-1 during LPS treatment, inhibited the degradation of IkBa, and blocked the expression and activation of NF-κB-p65 and NF-κB DNA binding activity. In addition, paclitaxel reduced LPS-induced interaction of MD-2 with TLR 4 in co-immunoprecipitation analysis. Paclitaxel also bound to recombinant MD-2, and siRNA knockdown of MD-2 significantly decreased TNF-a,IL-1,IL-6,MCP-1 and HMGB-1 levels and NF-κB DNA binding activity during LPS treatment. Furthermore, paclitaxel also significantly reduced HMGB-1-induced cytokines expression, and the interaction of HMGB1 with TLR4. Anti-HMGB-1 antibody ameliorated renal dysfunction by down-regulating cytokines levels *in vitro and vivo*.

Conclusions: These resultsprovide evidence that paclitaxel at low-doses has an anti-inflammatory effect via inhibiting NF-κB activity by binding MD-2 and down-regulating cytokines and HMGB1 expression.

Funding: NIDDK Support, Veterans Administration Support

FR-PO025

Novel Mouse Model of Albumin Overload with Enhanced Renal Damage and Proteinuria Shipra Agrawal,[1] Melinda Chanley,[1] David B. Thomas,[3] Ruma Pengal,[1] Rainer Benndorf,[1,2] William E. Smoyer.[1,2] *[1]Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; [2]Department of Pediatrics, The Ohio State University, Columbus, OH; [3]Department of Pathology, University of Miami Hospital, Miami, FL.*

Background: Inducible models of glomerular disease in mice are limited by generally poor susceptibility to injuring agents. Moreover, current models are strain-dependent, limiting their use in many knockout and transgenic mice. We hypothesized that glomerular disease resistance in mice can be overcome by combining PAN or adriamycin (ADR) with albumin overload.

Methods: 129/SvJ male mice (16-18 wk) received either low-endotoxin bovine serum albumin (BSA) I.P. for 4 days (D1-D4) at 400 mg/d, or saline control. PAN or ADR was injected I.V. on D1 at 150mg/kg and 10mg/kg, respectively, alone or with BSA. Albuminuria was analyzed by SDS-PAGE of urine samples on D0-D5, and BUN was measured on D0, D3 and D5. At D5, kidneys were processed for histology and western blotting.

Results: BSA-treated mice developed a moderate albuminuria at D2-D5, which was greatly enhanced with combination treatments (i.e. ADR+BSA; PAN+BSA). In contrast, singly (ADR or PAN) treated mice exhibited only a minor or no albuminuria. Serum BUN was notably higher in combination treatments (50-96 mg/dl) vs. controls or single treatments (30-40 mg/dl). Both BSA alone and combination treatments induced diffuse acute tubular epithelial cell injury, with tubular dilatation slightly greater in combination vs. BSA alone treatment. No renal injury was seen in controls or after PAN or ADR treatment alone. Stress response proteins (Hsp25; Hsp70i) were induced in renal cortices after BSA alone or combination treatments, but not in controls. In contrast, GRP78 expression was decreased after BSA alone and further decreased after combination treatments.

Conclusions: The combination of albumin overload with PAN or ADR treatment appears to enhance susceptibility of mice to renal damage compared to PAN, ADR, or BSA alone. This approach may have potential utility for induction of glomerular disease in otherwise resistant mouse strains commonly used for genetic manipulations.

FR-PO026

Endotoxemia Differentially Regulates Renal Ca^{2+}-Transport Proteins in Mice Klaus Höcherl. *Institute of Physiology, University of Regensburg, Regensburg, Germany.*

Background: Hypocalcemia is frequently reported in critically ill patients, most commonly in association with sepsis syndrome, and is associated with increased mortality in septic patients. The kidney is an important organ for the maintenance of the body Ca^{2+} homeostasis through Ca^{2+} reabsorption along the nephron. The influence of sepsis on renal calcium transport proteins has yet to be defined. Therefore, we examined the effects of endotoxemia on renal calcium excretion and on the regulation of major calcium transport proteins in mice.

Methods: Male C57BL/6 mice, 8-10 wk old weighing 22-25g, were used. Endotoxemia was induced by the administration of a single dose of lipopolysaccharide (LPS; 3 mg/kg; i.p.). The mRNA levels were measured by quantitative real-time RT-PCR. Immunoblotting and immunoflourescence studies were performed for the quantification of protein levels. Calcium and creatinine levels were measured in urine and blood samples by commercially available assay kits.

Results: Administration of LPS (3 mg/kg; i.p.) caused a time-dependent hypocalciemia and an increase in the urinary calcium-to-creatinine-ratio 16 hours after injection of LPS. The renal mRNA expression of the epithelial calcium channels TRPV5 and TRPV6 were increased 2.3- and 4.5-fold, respectively, 16h after the administration of LPS. Further, LPS decreased the expression of the calcium binding protein calbindin-D_{28K} to about 40% of control values, whereas the abundance of calbindin-D_{9K} was unchanged. In addition, the basolateral natrium/calcium exchanger NCX1 was decreased to about 20% of control levels, whereas the abundance of the plasma membrane ATPase type 1b (PMCA1b) was unaltered in response to LPS. Semiquantitative immunoblotting and immunohistochemistry confirmed changes in renal protein abundance of TRPV5, NCX1 and calbindin-D_{28K}.

Conclusions: We conclude that LPS decreases renal calcium reabsorption mainly via the downregulation of calbindin-D_{28K} and of the natrium/calcium exchanger NCX1. This may contribute in part to the development of hypocalcemia in response to endotoxemia.

FR-PO027

Serotonin (5-HT) 1F Receptor Agonism as a Potential Treatment for Acceleration of Recovery from Acute Kidney Injury Sara M. Garrett, Rick G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Mitochondrial dysfunction is common in acute kidney injury (AKI) and chronic kidney disease. We have proposed that stimulation of mitochondrial biogenesis (MB) may accelerate cellular repair and recovery from AKI. Unfortunately, very few chemicals/drugs have been identified that induce MB and we have initiated a drug discovery program to identify such agents. The goal of these experiments was to explore 5-HT receptors in MB.

Methods: A MB assay incorporating FCCP-induced uncoupled oxygen consumption rate (OCR) in a Seahorse Biosciences analyzer was used (Beeson et al., 2010). PGC-1a, the master regulator of MB, 5-HT1F, and mitochondrial proteins Cox1, ATP synthase β, and NDUFB8 were measured by real time RT-PCR and immunoblot analysis on rabbit renal proximal tubule cells (RPTC) and murine tissues. Mitochondrial copy number was determined using real-time RT-PCR. siRNA knockdown of 5-HT1F was used in RPTC.

Results: Immunoblot and qPCR analyses revealed 5-HT1F receptor expression in RPTC. Two 5-HT1F receptor agonists, LY334370 and LY344864, increased uncoupled OCR at 1nM-100nM. Both agonists increased the nuclear-encoded mitochondrial proteins ATP synthase β and NDUFB8, and mitochondrial-encoded Cox1. Knockdown of 5-HT1F receptor with siRNA decreased 5-HT1F receptor protein levels and agonist-induced up-regulation of MB proteins. In renal cortical tissue of naïve mice, PGC1α, Cox1, and NDUFB8 transcript levels increased in response to LY334370 and LY344864. Both agonists also increased mitochondrial copy number, PGC1α, Cox1, and NDUFB8 transcript levels in the hippocampus and liver. Sumatriptan and related drugs, 5-HT1F receptor agonists used for the treatment of migraines, also increased uncoupled OCR in RPTC (1-3 nM).

Conclusions: In summary, these studies reveal the novel observation that 5-HT1F receptors are linked to MB and that 5-HT1F receptor agonists induce MB in renal cells and in the kidney. Based on our results, we suggest that a treatment potential exists for the acceleration of recovery from AKI via 5-HT1F agonist-mediated MB in the kidney.

FR-PO028

Early Endothelial Outgrowth Cells (eEOCs) and BMP-5 in Acute Ischemic Kidney Injury (AKI) Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Background: Early Endothelial Outgrowth Cells (eEOCs) protect mice from acute ischemic kidney injury. Bone morphogenetic protein-5 has been shown to act antifibrotic in chronic hypertensive nephropathy. Aim of the study was to evaluate modulatory effects of BMP-5 in the setting of an eEOC-based therapy of AKI.

Methods: Male, 8-12 weeks C57/Bl6N mice were subjected to bilateral renal ischemia (40 minutes) with subsequent systemic injection of either untreated or BMP-5 pretreated syngeneic murine eEOCs (stimulation concentration 100 ng/ml, incubation time 1 hour). Cultured murine eEOCs were analyzed for migratory activity and production / release of proangiogenic / proinflammatory mediators.

Results: Postischemic mice with administration of untreated eEOCs showed significantly impaired renal function (Creatinine post-ischemia: 1.09 ±0.23 mg/dl and post-ischemia + untreated eEOCs: 1.26 ±0.21 mg/dl vs. Controls: 0.26 ±0.1 mg/dl, p=0.02 und p=0.005). Pretreatment of eEOCs with BMP-5 dramatically increased renoprotective effects of the cells (Creatinine post-ischemia + eEOCs + BMP-5: 0.2 ±0.05 vs. Creatinine post-ischemia: + eEOCs 1.26 ±0.21 mg/dl, p=0.0006 and Creatinine post-ischemia + eEOCs + BMP-5: 0.2 ±0.05 vs. Controls: 0.26 ±0.1 mg/dl, p=0.6). BMP-5 did not stimulate cellular production / release of IL-6, TGF-b, or VEGF, but migratory activity of cultured eEOCs was significantly increased (wound area reduction: BMP-5 33 ±0.9% vs. Controls 8.2 ±6.1%, p=0.01).

Conclusions: In summary, BMP-5 could be established as very potent eEOC agonist in AKI. Cellular activation is reflected by increased migratory activity of eEOC, paracrinic actions are not affected.

FR-PO029

Phosphodiesterase Inhibitors Stimulate Mitochondrial Biogenesis: A Potential Therapy for AKI Ryan Whitaker, Lauren P. Wills, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Background: Our laboratory has demonstrated persistent mitochondrial dysfunction and depletion of mitochondrial proteins following renal ischemia/reperfusion (I/R) injury in rodents that correlates with sustained renal tubular dysfunction. These results support the hypothesis that restoration of mitochondrial function through stimulation of mitochondrial biogenesis (MB) may be an effective therapeutic strategy for accelerating recovery from AKI.

Methods: We tested PDE3, 4 and 5 inhibitors for MB due to the established role of cAMP and cGMP in the regulation of PGC-1α. PDE3 inhibitors increase cGMP and cAMP, PDE4 inhibitors increase cAMP, and PDE5 inhibitors increase cGMP. FCCP-uncoupled respiration was measured in primary cultures of renal proximal tubule cells (RPTC) using the Seahorse Biosciences Analyzer as a marker of MB. In addition, C57BL/6 mice were treated with cilostamide, trequinsin, sildenafil or vehicle control (IP) and euthanized 24 hr later. Kidneys were collected for RT-PCR and immunoblot analysis.

Results: PDE3 inhibitors anagrelide, cilostamide, enoximone and trequinsin, and the PDE5 inhibitor sildenafil stimulated MB as assessed by increases in FCCP-uncoupled respiration in RPTC following a 24 hr treatment. Additionally, mRNA expression of PGC-1α and complex I proteins ND6 and NDUFB8 increased. In contrast PDE4 inhibitors did not stimulate MB. Exposure of RPTC to 8-Br-cGMP stimulated MB, while 8-Br-cAMP did not. These data suggest cGMP serves as a primary mediator of MB in RPTC. Cilostamide, trequinsin and sildenafil increased mRNA expression of PGC-1α, NDUFB8, ATPβ, COX1 and ND1 and increased mitochondrial DNA copy number in the kidney cortex.

Conclusions: These data demonstrate that classes of PDE inhibitors that increase cGMP stimulate MB in RPTC and kidney, and suggest that these compounds may serve as viable pharmacological agents to stimulate MB in the treatment of AKI.

Funding: Other NIH Support - GM084147, Veterans Administration Support

FR-PO030

Y-Box Binding Protein Regulates Innate Immune Response *In Vivo*

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Background: The Y-box binding protein (YB)-1 is involved in multiple inflammatory processes. To understand the molecular mechanisms of YB-1 during acute inflammation, we studied a model of endotoxemia, mimicking sepsis-induced acute kidney injury in wildtype (WT) and heterozygous YB-$1^{+/d}$ knockout mice, which exhibit half maximal YB-1 levels.

Methods: Hepatic and renal expression of YB-1, inflammatory receptors and chemokines in YB-$1^{+/d}$ *versus* WT mice were studied 6h and 48h following i.p. injection of lipopolysaccharide (LPS; 1.5 mg/kg). Bone marrow derived granulocytes were assessed for chemotaxis, and livers and kidneys for immune cell infiltration. Renal tubular damage was assessed via kidney injury molecule (KIM)-1 expression. Cylosporine A treatment (CsA; 30 mg/kg; s.c.) was performed following a low-sodium diet.

Results: Following LPS injection, enhanced YB-1 expression and activation temporarily occurred in immune cells, liver and kidney and secreted YB-1 was detectable in the abdominal cavity and urine. In YB-$1^{+/d}$ mice we noted a decreased influx of immune cells, which coincided with a significantly reduced expression of toll-like receptor (TLR)-4 on bone-marrow derived immune cells and a reduced CCR5 expression in the kidney. In addition, a markedly altered expression of chemokines (e.g. CCL5, CCL2) in infiltrating cells, kidneys and livers of YB-$1^{+/d}$ mice was detected. Consistent with this, granulocytes derived from YB-$1^{+/d}$ mice exhibited a reduced migration capability towards CCL5. Upregulation of KIM-1 was reduced in YB-$1^{+/d}$ mice. Thus, an impaired immune response occurred in YB-$1^{+/d}$ mice. This resulted in 50% mortality coinciding with an enhanced bacterial infection when the immunosuppressant CsA was administered.

Conclusions: In conclusion, our data identify YB-1 as an important factor in sepsis-induced acute kidney injury and in the innate immune response.

Funding: Government Support - Non-U.S.

FR-PO031

Spironolactone Administration after Severe Acute Kidney Injury Prevents Chronic Kidney Disease Development

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Background: Acute Kidney Injury (AKI) has been recognized as a risk factor to promote Chronic Kidney Disease (CKD). We previously demonstrated that prophylactic spironolactone (Sp) administration prevents CKD induced by an episode of renal ischemia (I). Because, in many cases AKI cannot be predicted, this study was designed to determine if Sp administration after an ischemic insult could protect against CKD development.

Methods: Thirty-five male Wistar rats were divided in: sham-operated, rats underwent 45 min of bilateral I, rats receiving a low dose of Sp (20 mg/kg) immediately, 1.5 or 3 hours after I, and rats receiving a high dose of Sp (80 mg/kg) immediately or 1.5 hours after I. All groups were followed throughout 90 days. Proteinuria (UProt) was evaluated every 30 days. At the end, creatinine clearance (CrC) and renal blood flow (RBF) were measured. Right kidney was used for molecular studies and the left for histopathological analysis.

Results: Rats underwent to I developed CKD characterized by a progressive increase in UProtV, together with a moderate reduction in CrC and RBF. Glomerular hypertrophy, tubular dilation and tubule-interstitial fibrosis were also observed after 90 days of inducing I. These alterations were associated with an up-regulation of MCP-1, αSMA, TGFβ and pSmad3. In contrast, in all Sp-treated groups, Uprot was prevented and the renal dysfunction was not observed. In addition, glomerular hypertrophy was partially prevented in the rats treated with the lower Sp dose and totally prevented in rats receiving the higher dose. Renal architecture preservation in the rats treated with the higher dose was associated with prevention of αSMA, MCP-1 and TGFβ up-regulation.

Conclusions: We show for the first time that Sp administration even after the renal insult has occurred is a novel strategy to prevent CKD induced by a single episode of AKI. The Sp renoprotective mechanisms included prevention of glomerular and tubular hypertrophy, as well as activation of fibrotic and inflammatory processes.

Funding: Government Support - Non-U.S.

FR-PO032

Peroxisomal and Lysosomal Dysfunction in LPS-Induced AKI

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Background: In a model of defective lysosomal autophagy, Lyst-mice, we have previously demonstrated that LPS treatment results in a more severe renal injury than in wt-type mice. We implicated impaired autophagy of peroxisomes (POS), pexophagy, as important contributor to injury by LPS.

Methods: We used in vitro and in vivo approaches to study POS function in LPS-induced AKI, and the impact of defective lysosomal autophagy.

Results: POS harbor the highest cellular content of catalase, their crucial antioxidant enzyme. In vivo, wt-mice had lower kidney catalase abundance but higher enzymatic activity than Lyst-mice under basal conditions. This difference was further aggravated after treatment with LPS. Ex vivo, lysosome-defective cells also exhibited decreased catalase activities and higher reactive oxygen species under basal conditions, with further increase and longer persistence after LPS. Our time-lapse microscopy studies with POS-targeting-sequence (PTS1)-GFP revealed decreased peroxisomal protein import after LPS, which additionally compromised peroxisomal balance of catalase possessing an atypical weaker PTS1. Renal expression of PPARα, primarily involved in activation of POS, was higher in Lyst- than wt-mice, reflecting an adaptive response to stimulate impaired POS. Endothelial cells isolated from Lyst-mice exhibited enhanced basal and LPS-induced inflammatory responses, with higher levels of IL-6, IL-8, G-CSF, GM-CSF and MCP-1. Cytokine profiles of cell secretomes matched those obtained from sera of experimental animals. LPS decreased PPARα and ACOX1 (the first enzyme in POS β-oxidation) in wt-mice; opposite occurred in lysosomal dysfunction, where LPS caused increase of PPARα, ACOX1 and hence hydrogen peroxide (byproduct of POS β-oxidation), resulting in redox imbalance due to impaired catalase.

Conclusions: Impaired pexophagy results in a worsening of LPS-induced renal injury. We identified 2 mechanisms governing this phenomenon: 1) imbalance between increased H_2O_2 generation by activated POS oxidases and concomitantly decreased antioxidative defense due to catalase deficiency; and 2) exaggerated pro-inflammatory endothelial secretome potentially orchestrating a systemic inflammatory response.

Funding: NIDDK Support

FR-PO033

Circulating Micro-RNAs in Acute Kidney Injury after Liver Transplantation: Early Observations

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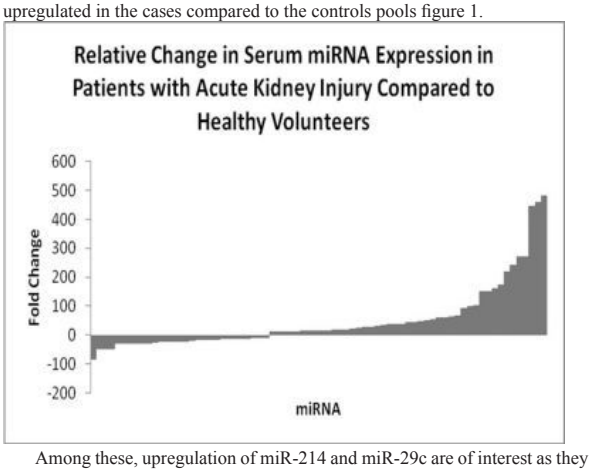
Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and are present in the blood in a stable form. We tested whether circulating miRNAs in the serum of acute kidney injury (AKI) patients post-liver transplant are deregulated when compared to healthy controls.

Methods: We performed miRNA profiles using RNA isolated from n=10 healthy volunteers (controls) and n=7 subjects (cases) who developed AKI after liver transplantation. AKI was defined using the Acute Kidney Injury Network (AKIN) definition.miRNA profiles were analyzed using a custom quantitative PCR array containing 372 miRNAs previously determined to be readily detectable in serum (Qiagen, Frederick, MD). Arrays were analyzed as 1 case pool and 3 control pools.

Results: We identified 10 healthy volunteers (M/F: 7/3) with a mean (SD) age of 58 ± 2 yrs who had ideal cardiovascular health. AKI patients (M/F: 4/3) had a mean (SD) age of 59 ± 4 yrs. Based on a cut off of $C_t \geq 35$ cycles and all miRNA that demonstrated ≥ 10 fold difference, 29 miRNAs were significantly downregulated and 46 miRNAs were upregulated in the cases compared to the controls pools figure 1.



Among these, upregulation of miR-214 and miR-29c are of interest as they have been associated with kidney injury in rodent models.

Conclusions: Expression of miR-214 and miR-29c were upregulated in AKI patients post-liver transplant compared with healthy subjects, consistent with a miRNA profile of kidney damage. The utility of this profile for prediction of AKI will need validation and testing in a larger population.

Funding: Pharmaceutical Company Support - Qiagen, Private Foundation Support

FR-PO034

Renal Proximal Tubule Epithelial Cells Regulate Immunological Danger through Expression of Kidney Injury Molecule-1

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Background: Phagocytosis of apoptotic cells is vital to preventing inflammation. Uncleared apoptotic cells can undergo secondary necrosis and release endogenous danger signals such as high mobility group box protein 1 (HMGB1) into the extracellular milieu. The existing pool of professional phagocytes can be overwhelmed during acute tissue

injury. Kidney injury molecule-1 (KIM-1) is a scavenger receptor for apoptotic cells that is upregulated on kidney proximal tubule epithelial cells (PTECs) following acute kidney injury, transforming surviving PTECs into avid phagocytes. We hypothesized that clearance of apoptotic cells by KIM-1-expressing PTECs prevents release of HMGB1 and activation of innate immune responses.

Methods: We added varying doses of apoptotic cells to cultures of PTECs expressing or not expressing KIM-1. After 24hrs incubation, the resultant conditioned cell media and lysates were analyzed for HMGB1 by Western blot. Next, conditioned cell media from both cell types were added to cultures of dendritic cells. We performed flow cytometry after 24hrs incubation to assess dendritic cell activation as a marker of innate immunity.

Results: Compared to that of control cells not expressing KIM-1, we observed significantly more intracellular HMGB1 in the lysates and less HMGB1 in the conditioned media of KIM-1-expressing cells that were exposed to apoptotic cells. Further, dendritic cells exposed to conditioned media from KIM-1-expressing cells showed less activation, compared to dendritic cells exposed to media from cells not expressing KIM-1. This suggests that there were more uncleared apoptotic cells in the conditioned media of cells not expressing KIM-1.

Conclusions: KIM-1-expressing PTECs may regulate innate immunity through clearance of apoptotic cells. We propose that upregulation of KIM-1 during acute kidney injury is a host defense mechanism that curtails inflammation through this mechanism.

Funding: Government Support - Non-U.S.

FR-PO035

Mechanisms by Which Chronic Nicotine (NIC) Suppresses Heme Oxygenase-1 (HO-1) and Exacerbates Ischemia-Reperfusion-Induced Acute Kidney Injury (I/R-AKI) Istvan Arany,[1] Dustin Reed,[1] Robert Kampen,[1,2] Luis A. Juncos.[2] *[1]Pediatrics, University of Mississippi Medical Center, Jackson, MS; [2]Medicine/Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: HO-1 constitutes a potent protective mechanism against IR-AKI; its deficiency promotes inflammation, fibrosis and injury. Chronic NIC exacerbates pro-inflammatory and pro-fibrotic signaling in a mouse model of IR-AKI in part via attenuation of HO-1 induction.

Methods: In vivo: The impact of chronic NIC (4 weeks) on expression of renal HO-1, phosphorylation of STAT3 and ERK, and I/R-AKI were determined in the kidneys of mice exposed to 18-minutes of bilateral warm renal ischemia followed by 24 hours of reperfusion. In vitro: Cultured renal proximal tubule cells (LLC-PK1) were treated with 200 mM NIC for 24 hours aftetr which cell injury was induced by H_2O_2 (400 mM). The roles of tyrosine phosphorylation of STAT3, serine/threonine phosphorylation of ERK (pERK), and HO-1 were evaluated by manipulating their expression or activity using pharmacological and genetic means (AG490, Y705F-STAT3 for STAT3; U0126, dnMEK, for ERK; and CoPP and SnPP for inducing and inhibiting HO, respectively).

Results: Chronic NIC exposure attenuated IR-AKI-induced increases in tyrosine phosphorylation of STAT3 and also HO-1, but exacerbated I/R-AKI-induced increases in pERK.

In vitro, H_2O_2-mediated activation of the HO-1 promoter was dependent on tyrosine phosphorylation of STAT3 and the antioxidant response element (ARE). NIC reduced this induction of the HO-1 promoter through a pERK-dependent attenuation of STAT3 binding element (SBE) and ARE.

Conclusions: Our data demonstrates that I/R-AKI/oxidative stress induces HO-1 through SBE and ARE, protecting renal proximal tubules. Chronic NIC, via sustained activation of ERK- attenuates HO-1 transcription by inhibiting both SBE and ARE activation. Manipulation of those pathways may present therapeutic means to ameliorate adverse effects of chronic NIC/smoking.

Funding: Other NIH Support - 5RO1DK073401-05S1 for Luis A. Juncos

FR-PO036

Vitamin D Deficiency Is a Potential Risk Factor for the Progression of Chronic Kidney Disease after Ischemia/Reperfusion-Acute Kidney Injury Janaina Garcia Gonçalves, Ana C. de Bragança, Daniele Canale, Maria Heloisa M. Shimizu, Lucia Andrade, Antonio C. Seguro, Rosa M.A. Moyses, Rildo A. Volpini. *Nephrology, University of Sao Paulo School of Medicine, SP, Sao Paulo, Brazil.*

Background: Vitamin D deficiency (VDD) is highly prevalent in chronic kidney disease (CKD) patients. Ischemia/reperfusion-Acute Kidney Injury (IR-AKI) is considered a risk factor for CKD progression. VDD is known to be associated with increased metabolic syndrome (MetS) risk; serum cholesterol and triglyceride levels; and insulin resistance. There is an association between CKD and MetS, and decreased Klotho expression is an early marker of stage 1 CKD. We hypothesized that VDD-induced MetS is a pathway linking AKI and CKD.

Methods: For 90 days, Wistar rats were fed a standard diet (control [C] and IR-AKI groups, n=8 each) or a 25-hydroxyvitamin D [25(OH)D]-free diet (VDD and VDD+IR-AKI groups, n=8 each). On day 28, IR-AKI and VDD+IR-AKI rats were submitted to 45-min clamping of both renal arteries. On day 90, we measured inulin clearance (Cin); proteinuria, glycosuria; serum 25(OH)D, triglycerides and total cholesterol. We estimated fibrosis by fractional interstitial area (FIA) and immunoblotted renal tissue for Klotho. Data are mean±SEM.

Results:

Variable	C	VDD	IR-AKI	VDD+IR-AKI
Cin (ml/min/100 g)	0.64±0.03	0.56±0.04	0.58±0.04	0.59±0.03
25(OH)D (ng/ml)	15.4±1.0	<1.5*	15.0±0.6	<1.5*
Proteinuria (mg/day)	23.5±2.0	22.8±2.6	26.1±2.4	33.2±1.0[a,b,c]
Glycosuria (mg/dl)	10.5±1.1	17.6±1.2[a]	12.4±0.7[b]	21.6±2.1[a,c]
Total cholesterol (mg/dl)	41.0±4.7	66.7±4.6[a]	60.0±3.4[a]	61.7±4.5[a]
Triglycerides (mg/dl)	10.0±2.8	171.5±32.6[a]	47.5±7.4[b]	87.2±18.1[a,b]
FIA (%)	7.3±0.5	17.2±0.8[a]	24.4±2.9[a,b]	34.9±0.55[a,b,c]
Klotho† (%)	98.7±0.7	47.5±6.0[d]	53.3±7.6[d]	46.7±2.5[d]

*undetectable; †protein expression; [a]p<0.05 vs. C; [b]p<0.05 vs. VDD; [c]p<0.05 vs. IR-AKI; [d]p<0.001 vs. C

Conclusions: The increased proteinuria/fibrosis in VDD+IR-AKI rats suggests progressive renal injury. MetS-related lipidic metabolism and glycosuria could explain progressive renal injury after IR and progression to CKD. Downregulation of Klotho is a possible marker of chronification. The mechanistic role of VDD-induced MetS in progressive CKD merits further study.

Funding: Government Support - Non-U.S.

FR-PO037

Natural Triggering of p21 Upregulation Is Decreased in Vitamin D-Depleted Rats with Ischemic Acute Kidney Injury Ana C. de Bragança, Daniele Canale, Janaina Garcia Gonçalves, Rildo A. Volpini, Maria Heloisa M. Shimizu, Rosa M.A. Moyses, Antonio C. Seguro, Lucia Andrade. *Nephrology, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.*

Background: Prior vitamin D deficiency (VDD) is a major predictor of mortality in critically ill patients. Renal ischemia/reperfusion injury (IRI), activates pathways of cell proliferation and cell death. Acute kidney injury (AKI) induces cell cycle inhibitors, including the cyclin-dependent kinase inhibitor p21, which protects against AKI. In many cell types, p21 is a genomic target of 25-hydroxyvitamin D [25(OH)D], which, acting via vitamin D receptors (VDRs), has potent immunomodulatory and antiproliferative effects, suggesting that it plays a role in the pathophysiology of renal disease.

Methods: Wistar rats were fed 25(OH)D-depleted or normal diets for 30 days. On day 28, some rats were induced to IRI by 45-min clamping of both renal arteries. We studied four groups: control (C), VDD, IRI and VDD+IRI. At 48 h after IRI, we measured inulin clearance (Cin); proteinuria; and serum 25(OH)D, Ca, and P. In renal tissue, we immunoblotted for p21, VDR and klotho. Data are means±SEM.

Results:

	Cin (ml/min/100g)	Proteinuria (mg/day)	Renal weight/body weight	25(OH)D (ng/ml)	p21* (%)	VDR* (%)	klotho* (%)
C (n=7)	0.93±0.05	21±1.1	0.4±0.02	14.8±0.08	99±6.0	99.5±0.5	98±1.2
VDD (n=6)	0.76±0.03[a]	33±2.1	0.41±0.01	4.1±0.8[a]	100±27	57.5±8.5[a]	76±2.4[b]
IRI (n=7)	0.43±0.04[a,c]	33±6.1	0.52±0.01[a,c]	13±1.0[c]	290±4.8[d,e]	198.5±0.7[d,e]	22±6.0[d,e]
VDD+IRI (n=8)	0.29±0.03[a,c,f]	48±6.5[a]	0.58±0.02[a,c,f]	3.8±0.23[a,f]	182±29[a,c,g]	172.7±10.9[c,e,f]	16.7±2.4[d,e]

* protein expression; [a] p<0.05 vs. C; [b]p<0.01 vs. C; [c]p<0.05 vs. VDD; [d]p<0.001 vs. C; [e]p<0.001 vs. VDD; [f]p<0.05 vs. IRI; [g]p<0.01 vs. IRI

VDD, IRI and VDD+IRI rats showed higher renal vascular resistance, higher urinary volume, lower urinary osmolality and lower serum P and Ca than did controls.

Conclusions: We speculate that, by altering the levels of p21 in IRI AKI, via VDRs, 25(OH)D controls renal inflammation, proliferation and cell injury. Further studies are needed to determine whether correction of VDD provides clinical benefits in AKI.

FAPESP, CNPq.

Funding: Government Support - Non-U.S.

FR-PO038

Critical Role of Interleukin-11 (IL-11) Signaling in A_1 Adenosine Receptor Mediated Renal Protection Joo Yun Kim,[1] Ahrom Ham,[1] Mihwa Kim,[1] H. Thomas Lee,[1] George N. Cox.[2] *[1]Anesthesiology, Columbia University, New York, NY; [2]Bolder BioTechnology, Inc., Boulder, CO.*

Background: Acute kidney injury (AKI) occurs in ~20% of all hospitalized patients and renal ischemia reperfusion (IR) injury is a major cause of AKI. We previously demonstrated that renal A_1 adenosine receptor (ARs) activation ameliorated renal IR injury by attenuating multiple pathways of cell death including necrosis, apoptosis and inflammation. However, extra-renal side effects (bradycardia and hypotension) may limit the A_1AR agonist therapy for ischemic AKI. IL-11 is a multi-functional hematopoietic cytokine clinically approved to treat chemotherapy-induced thrombocytopenia.

Methods: Here, we show that selective A_1AR activation protects against IR injury by direct induction of renal tubular IL-11 synthesis.

Results: In cultured human proximal tubule epithelial (HK-2) cells, a selective A_1AR agonist 2-chlorocyclopentyladenosine (CCPA) dose-dependently induced IL-11 mRNA and protein expression. CCPA also induced IL-11 mRNA and protein synthesis in mouse kidney. CCPA-mediated induction of IL-11 was significantly attenuated by a specific inhibitor of ERK MAPK (PD98059) but not by an inhibitor of Akt (wortmannin). In addition, IL-11 receptor (IL-11R) wild type mice subjected to 30 min of renal ischemia and 24 h of reperfusion developed severe AKI (Cr=2.3±0.2mg/dL, N=6). Supporting a critical role of IL-11 in A_1AR-mediated renal protection, A_1AR agonist CCPA protected IL-11R wild type mice against ischemic AKI (Cr=1.4±0.2mg/dL, N=6, P<0.01) but failed to protect IL-11R deficient mice (Cr=2.3±0.2mg/dL, N=6). In addition, IL-11 neutralizing antibody completely abolished the renal protection provided by CCPA (Cr=2.4±0.2mg/dL,

N=5). Finally, exogenous administration of recombinant human IL-11 protected against ischemic AKI in mice (Cr=1.5±0.2 mg/dL, N=5) mimicking the renal protective effects A_1AR activation.

Conclusions: Taken together, we show that induction of renal tubule IL-11 is critical for A_1AR-mediated renal protection. Selective renal tubular induction of IL-11 or exogenous administration of IL-11 may provide a novel therapeutic approach for the prevention and treatment of ischemic AKI.

Funding: NIDDK Support

FR-PO039

The Inflammasome in Cisplatin-Induced AKI Hyun-Jung Kim, Dong Won Lee, Zhibin He, Danica Ljubanovic, Charles L. Edelstein. *Univ. of Col. Denver.*

Background: We have demonstrated that there is increased caspase-1, IL-1α, IL-1β and IL-18 in cisplatin (Cis)-induced AKI (CIA). As IL-1α, IL-1β and IL-18 are activated by caspase-1 in the inflammasome, the aim of the study was to further investigate the inflammasome in CIA. The NALP3 inflammasomes is a cytosolic complex consisting NALP3 protein, ASC protein and caspase-5. BID is a pro-apoptotic protein that is secreted by the inflammasome.

Methods: Mice were injected with Cis (25 mg/kg) and developed AKI on day 3. ATN was graded as to the number of tubules with histological features of ATN.

Results: On immunoblot of whole kidney, there were a 1.3-fold increase in NALP3 on day 2 (P=0.05, n=9) and a 2.6-fold increase in NALP1 (P<0.05, n=9) on day 3 of CIA. On immunoblot, there were a 2-fold increase in ASC on day 2 (P<0.05, n=10), a 3-fold increase in caspase-5 protein on day 2 (P<0.05, n=10), a 2-fold increase in BID on day 2 (P<0.05, n=10), and a 1.2-fold increase in caspase-1 (P<0.05, n=9). As the increase in NALP3 occurred on day 2 at the same time as the increase in ASC, caspase-5, BID and caspase-1, and before the AKI, we considered that inhibition of NALP3 may play an injurious role. To determine whether the increase in NALP3 plays an injurious role in CIA, $NALP3^{-/-}$ mice were studied. Serum creatinine (mg/dL) was 0.22 in vehicle (Veh)-treated, 2.0 in Cis-treated wild type (P< 0.001 vs. Veh, n=10) and 1.2 in Cis-treated $NALP3^{-/-}$ mice (NS vs. wild type, n=9). BUN (mg/dL) was 23 in Veh-treated, 209 in Cis-treated wild type (P< 0.001 vs. Veh, n=10) and 201 in Cis-treated $NALP3^{-/-}$ mice (NS vs. wild type, n=9). ATN score was 1.8 in Cis-treated wild type and 1.5 in Cis-treated $NALP3^{-/-}$ mice (NS vs. wild type, n=5). Apoptosis score (apoptotic cells per 10 HPF) was 8.4 in Cis-treated wild type and 1.4 in Cis-treated $NALP3^{-/-}$ mice (NS vs. wild type, n=5).

Conclusions: Inflammasome proteins NALP3, caspase-1, caspase-5, ASC and BID are increased in whole kidneys on day 2 of CIA. However, $NALP3^{-/-}$ mice are not functionally or histologically protected suggesting that the NALP3 inflammasome does not play an injurious role in CIA. Further investigation of the NALP1 imflammasome is warranted.

FR-PO040

Upregulation of Hypoxia Inducible Factor-1 Prevents Development of Hepatorenal Syndrome Meghana Awad, Siddhartha S. Ghosh, Todd W. Gehr, Arun Sanyal, Daniel E. Carl. *Internal Medicine, Commonwealth University of Virginia, Richmond, VA.*

Background: The pathophysiology of hepatorenal syndrome (HRS) is not clearly understood due to lack of suitable animal models. Carbon tetrachloride (CCl4) is used in mice to induce cirrhosis. Hif-1 is a well known factor produced in tissues in response to ischemia. The potential role of Hif-1 in HRS is not known. We hypothesized that failure to upregulate Hif-1 would be associated with functional renal failure in our model of HRS. **AIMS: 1) To evaluate expression of Hif-1 in our mouse model of HRS, 2) to evaluate the effect of dimethyloxalylglycine (DMOG), which upregulates Hif-1.**

Methods: Cirrhosis in C57bl mice was induced with CCl4 (1 ml/kg) given for 6 weeks. Mice were divided into (n=5 in each):Vehicle (corn oil), CCl4(1mg/kg) for 6 weeks, CCl4+ LPS, CCl4+DMOG, CCl4 +DMOG +LPS. DMOG (100mg/kg/day)was administered from day 38 to sacrifice. Lipopolysaccharide (LPS) was administered (1.5 mg/Kg) intraperitoneally and mice were sacrificed 16 hours later.

Results: There was no change in urine volume, urine sodium (UNa) and serum creatinine (SCr) between controls and controls+LPS. The table below highlights the changes in urine volume, UNa, and SCr between groups. There was a 30% decrease in kidney Hif-1 expression in CCl4 +LPS mice. Administration of DMOG abrogated this response and was associated with preserved renal function after CCl4 + LPS. Animals receiving CCl4 + DMOG had a modest rise in Hif-1 without changes in renal function. There were no changes in urine sediment. Liver histology confirmed cirrhosis and renal histology revealed intact renal tubules and glomeruli.

	Vehicle	LPS	CCl4 +LPS	CCl4+ DMOG	CCl4/LPS/ DMOG
Urine volume (ml)	1.32±0.06	1.7±0.7	0.85±0.38	1.37±0.73	1.36±0.4
SCr (mg/dL)	0.25±0.04	0.54±0.1	0.97±0.35	0.41±0.03	0.33±0.02
UNa (mmol/hr)	118.4±21.6	136.9±26.3	45.7±12	119.8 ±19.8	86.2±20.1
HIF1α	100	96	71	114	90

Conclusions: A mouse model of CCL4 treatment followed by LPS produces functional renal failure and mimics type 1 HRS. In this model, onset of renal failure is associated with decreased Hif-1 expression; enhanced Hif-1 expression prevents development of renal failure. These data suggest Hif-1 as a novel therapeutic target for type 1 HRS.

FR-PO041

Antiapoptotic Effect of Paricalcitol in Gentamicin-Induced Kidney Injury Sang Heon Suh,[1] Koeun Lee,[1,2] Chang Seong Kim,[1] Joon Seok Choi,[1] Eun Hui Bae,[1] Soo Wan Kim.[1] *[1]Internal Medicine, Chonnam National University Hospital, Gwangju, Korea; [2]Physiology, Chonnam National University Medical School, Gwangju, Korea.*

Background: Beneficial effects of paricalcitol in gentamicin (GM)-induced kidney injury were previously reported. It is, however, not yet clear whether paricalcitol attenuates the apoptosis, which mainly contributes to the development GM nephropathy. We investigated the effect of paricalcitol on apoptotic pathways in rat kidneys damaged by GM.

Methods: Rats were randomly divided into three groups: 1) Control group (n=5), where only vehicle was delivered, 2) GM group (n=5), where rats were treated with GM (100 mg/kg/day) intramuscularly for 14 days, 3) PARI group (n=5), where rats were co-treated with paricalcitol (0.3 μg/kg/day) and GM for 14 days.

Results: Paricalcitol attenuated renal dysfunction by GM administration in biochemical profiles. In TUNEL staining, increased apoptosis was observed in GM group, which was reversed by paricalcitol co-treatment. Immunoblotting using protein samples from rat cortex/outer stripe of outer medulla showed increased Bax/Bcl-2 ratio and cleaved form of caspase-3 in GM group, both of which were reversed by paricalcitol. The phospholyation of phosphatidylinositol 3-kinase (PI3K) and Akt (protein kinase B) was enhanced in PARI group compared with GM group. The phosphorylated JNK (Jun-N-terminal kinase) expression was increase in GM, which was inhibited by paricalcitol.

Conclusions: Conclusively, paricalcitol protects GM-induced renal injury by antiapoptotic mechanisms, including inhibition of intrinsic apoptosis pathway and JNK, and activation of PI3K/Akt pathway.

FR-PO042

Hepatic Sulfotransferase Inhibitors Prevent Progression of Ischemia/ Reperfusion-Induced Acute Kidney Injury in Rats by Suppressing Indoxyl Sulfate Accumulation Misato Yoshimura,[1] Masataka Sagata,[1] Megumi Komori,[1] Chika Saigo,[1] Yuko Yamamoto,[1] Yui Nomura,[1] Go Yoneda,[1] Kazuhiko Nishi,[3] Hirofumi Jono,[2] Hideyuki Saito.[2] *[1]Department of Clinical Pharmaceutical Sciences, Kumamoto University School of Pharmacy, Kumamoto, Japan; [2]Department of Pharmacy, Kumamoto University Hospital, Kumamoto, Japan; [3]Department of Hemodialysis and Apheresis, Kumamoto University Hospital, Kumamoto, Japan.*

Background: Ischemia/reperfusion (IR)-induced AKI is evoked by diverse pathophysiological and/or surgical situations in association independently with increased morbidity and mortality. Despite early detection of AKI followed by prompt treatments are required, adequate therapeutic management has not yet been established. In this study, we evaluated renal preventive effects of AST-120, oral spherical adsorptive carbon (Kremezin), and phytochemical sulfotransferase (SULT) inhibitors, which hamper hepatic IS generation in IR-AKI model rats.

Methods: AKI was evoked by clamping isolated arteries of right and left kidneys for 30 min, followed by reperfusion of the kidneys. AST-120 or SULT inhibitors was orally administered to rats at -24, -1 h before and 24 h after IR.

Results: IR of the kidney caused renal injury with the increases in Scr (12-fold vs sham-operated rats) and BUN (13-fold), in association with the significant accumulation of serum IS (23-fold), and urinary excretion of Kim-1. Oral administration of AST-120 to IR rats resulted in the restoration of Scr and BUN levels with the suppression of serum IS and urine Kim-1 levels. Administration of quercetin and resveratrol, potent SULT inhibitors, appeared to lower serum IS level, accompanied with a significant amelioration of renal injury. The expression of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), an antioxidant protein inducer, in the renal nuclear fractions was markedly elevated 48 h after IR, but was suppressed by the treatment with AST-120 or SULT inhibitors.

Conclusions: These results suggested that the IS accumulation contributed to renal generation of oxidative stress and the following progression of kidney injury, and suppression of hepatic IS generation could be a novel strategic approach for AKI treatment.

Funding: Government Support - Non-U.S.

FR-PO043

A Triphasic Model of Kidney Injury Induced by Adenine in the Rat Amnon Gil,[1] Vera Brod,[2] Victor Frajewicki.[1] *[1]Department of Nephrology and Hypertension, Carmel Medical Center, Haifa, Israel; [2]Ischemia-Shock Research Laboratory, Carmel Medical Center, Haifa, Israel.*

Background: Adenine is a nephrotoxic agent which induces tubule- interstitial injury. Aim: investigate the dynamic characteristics of the injury caused by adenine to the kidneys of rats.

Methods: The study group (Group A) was composed of 6 rats, which were fed by an adenine enriched diet for 4 weeks and switched to regular chow for an additional period of 4 weeks. In the control group (Group B) were included 6 rats, which were on a normal diet during the 8 weeks. Blood samples for chemistry analysis, were drawn from each rat, at the beginning of the study, and after 1, 3, 4, 5, 6, 7, 8 weeks. Urea and creatinine values of the study group were compared in each interval to baseline and control group.

Results: Urea and creatinine in the study group were significantly higher than in the control group at each time interval, except for the baseline values, which were similar. These parameters in the study group were also significantly higher at each interval, compared to the baseline values. Blood urea level increased gradually from a baseline level of 37±6mg%

to a maximal value of 249±77mg% after 4 weeks of adenine diet, compared to 35±7mg% and 40±3mg% simultaneously in the control group. After switching from adenine to normal diet, urea gradually decreased to a new plateau, higher than the baseline (134±60mg%) after 8 weeks, while in the control group it was 42±5mg%. Creatinine values followed the same pattern: 0.3±0.07mg% at the start of the experiment, 1.87±0.7mg% after 4 weeks of adenine diet and 1±0.33mg% after 8 weeks. The values were significantly different from that of the control group.

Conclusions: Adenine induced kidney injury in the rat may be used as a triphasic model for acute injury- while on adenine diet, partial recovery of kidney function- after switching to normal diet and chronic kidney disease- after stabilization of renal function. The simplicity and avoidance of traumatic insult of this model may be an advantage over the traditional surgical/ traumatic models of kidney damage.

FR-PO044

Comparison of Serum Cystatin C, Serum Creatinine and Blood Urea Nitrogen as Predictors of Mortality after Sepsis-Induced Acute Kidney Injury in Mice Ana Carolina Souza,[1] Asada Leelahavanichkul,[1,2] Richard Chen,[1] Robert A. Star,[1] Peter S.T. Yuen.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]Department of Microbiology, Chulalongkorn University, Thailand.*

Background: Mortality from sepsis is increased when complicated by acute kidney injury (AKI), and early detection of AKI is crucial in septic patients. Serum creatinine (Scr) has several limitations that delay recognition of AKI. We have found that after cecal ligation and puncture (CLP)-induced sepsis, serum cystatin C (SCysC) peaks more rapidly, and is better correlated with FITC-inulin, than Scr or blood urea nitrogen (BUN). The aim of this study was to compare the performance of SCysC, Scr and BUN on predicting mortality after CLP-induced sepsis.

Methods: 12-16 week old male CD-1 mice (35-50g) were used (N=35). Six hours after CLP, 50 µl of blood was collected via retro-orbital sinus and SCysC (ELISA), Scr (HPLC) and BUN were measured. Mice received fluids and buprenorphine immediately after CLP and were given fluids, antibiotic, and buprenorphine at 6 h after surgery and every 12 hours until death. Morbidly ill mice were euthanized per protocol.

Results: The higher values of all biomarkers (above the 50th percentile) were associated with decreased survival (median values: SCys C 1.19 µg/ml, SCr 0.24 mg/dl and BUN 54.6 mg/dl). After simple regression analysis, both SCysC and Scr but not BUN correlated with time to death ($p < 0.001$, R^2 0.352 and 0.339, respectively). Adding mouse body weight into a multiple regression analysis only slightly improved the results ($p < 0.001$, R^2 0.401 and 0.419, respectively). Receiver Operating Characteristics analysis was performed between high (top 50th percentile) vs. low (bottom 50th percentile) values for each biomarker as predictors of time of death. The Area Under the Curve was calculated for each biomarker at different times after sepsis induction, but SCysC and Scr were not statistically different.

Conclusions: SCysC did not outperform Scr on predicting mortality after CLP-induced sepsis. BUN did not predict mortality. Both correlations of SCysC or Scr with time to death have $R^2 < 0.7$, probably because dysfunction of non-renal organs also contribute to mortality from sepsis.

Funding: NIDDK Support

FR-PO045

Dexamethasone Attenuates Renal Ischemia Reperfusion Injury through Inhibiting HMGB1 Secretion Junhua Li, Jiong Zhang, Lili Liu, Gang Xu. *Tongji Hospital.*

Background: Our previous research showed that blockage of HMGB1, an inflammatory mediator released in renal ischemia reperfusion injury (IRI), could protect kidney from IRI. In this study, we demonstrated HMGB1 in renal IRI might be regulated by dexamethasone (DM).

Methods: Renal IRI was induced by clamping the left pedicle for 60 minutes in uninephrectomized C57BL/6 mice. DM (4mg/kg) was administrated intravenously 1 hour before renal ischemia. Mice treated with saline or without clamping pedicle were used as controls. 24 hours after reperfusion, sera and renal tissues were collected for renal function and pathologic analysis. Expression of HMGB1, p-p38, p-ERK, HIF and VEGF-C in kidney was detected by Western blot. Furthermore, expression of HMGB1 was analyzed by immunohistochemistry.

Results: Compared with control subjects, levels of BUN and serum creatinine in mice treated with DM were significantly decreased Pathological examination demonstrated that tissue damage caused by IRI was markedly reduced by DM treatment. Through immunohistochemistry and western blot, the expression of HMGB1 was higher in DM treated renal cells which may be maintained intra-cellular compared with IRI kidney cells. It demonstrated that some of HMGB1 might be released from renal cells in blood circulation through IRI. In comparison to control group, western blot showed a significant up-regulation p-p38, p-ERK, HIF and VEGF-C in DM treated mice. DM intervention significantly attenuated mice lethal kidney ischemia injury in survival experiment.

Conclusions: These data suggest that DM could inhibit HMGB1 secretion, which could reduce inflammatory effect of HMGB1. Reduced secretion of HMGB1 could induce rapid phosphorylation of ERK and p38, increase HIF and VEGF-C expression, therefore inhibit apoptosis and infiltration of inflammatory cells, finally DM attenuates kidney ischemia reperfusion injury.

FR-PO046

CD44-Deficiency Delays LPS-Induced Acute Renal Injury Elena Rampanelli, Nike Claessen, Gwendoline J.D. Teske, Jaklien Leemans, Sandrine Florquin. *Dept. Pathology, AMC, Amsterdam, Netherlands.*

Background: Sepsis is a severe and dysregulated inflammatory response to infection. Acute kidney injury (AKI) is a frequent complication of sepsis as it occurs in 20-50% of septic patients. CD44 is a cell-surface transmembrane receptor expressed by many cell types including inflammatory, epithelial and endothelial cells. CD44 plays a role in proinflammatory cytokines production, TLR signalling regulation, leukocytes migration and adhesion.

Methods: To study the role of CD44 in sepsis-induced AKI, Wt and CD44 KO mice (n=8/group), received an intraperitoneal injection of 10 µg/g body weight of LPS. Two, 4, and 24 hours after injection, mice were sacrificed; kidneys and blood were collected for analysis.

Results: At 2 and 4 hours after LPS injection, the plasma and renal levels of the pro-inflammatory cytokines MCP-1, TNF-α, IL-1β were lower in CD44 KO mice in respect to the Wt, and the opposite was found for the anti-inflammatory cytokines IL-10 and HGF. Furthermore, TLR4 gene expression in the CD44 KO kidneys was induced later and to a lesser extent than in the Wt; while the mRNA expression of the protective HO-1 (heme oxygenase-1) was higher in the CD44 KO kidneys than in the Wt. This could explain the cytokines pattern of the CD44 KO mice, as TLR4 signaling leads to secretion of pro-inflammatory molecules and HO-1 controls the balance pro-/anti-inflammatory cytokines. This was associated with preserved renal function in CD44KO compared to Wt at 2 and 4 hours after LPS, decreased T cells influx and apoptosis rate of tubular and interstitial cells in the CD44 KO kidneys.

Conclusions: In conclusion, these data suggest that the lack of CD44 protects against sepsis-induced AKI by impairing the leukocytes influx in the kidney parenchyma, the pro-inflammatory cytokines secretion via HO-1 induction and delayed TLR4 expression and therefore maintaining a better renal function.

Funding: Private Foundation Support

FR-PO047

Rats with an Inactivating Mutation of CD26 (Dipeptidyl Peptidase 4) Have Lower Serum SDF-1α/CXCL12 Levels and Increased Susceptibility to Acute Kidney Injury Jon D. Ahlstrom,[1] Anna Gooch,[1] Elizabeth Phillips,[1] Erin Birch,[1] Christof Westenfelder.[1,2] *[1]Medicine, University of Utah and VA Medical Centers, Salt Lake City, UT; [2]Physiology, University of Utah, Salt Lake City, UT.*

Background: We demonstrated that the chemokine SDF-1α/CXCL12 (SDF-1) and its receptor CXCR4 are dramatically upregulated in mouse kidneys post IRI AKI (Kidney Int 2005), and that this response mediates the recruitment to the kidney of CXCR4-expressing cells, such as infused Mesenchymal Stem Cells (MSC), which are renoprotective (Am J Physiol Renal 2005). F344/CD26mut rats (F344/DuCRlCrlj, Charles River Japan) have an inactivating mutation of the CD26/Dipeptidyl Peptidase 4 gene (DPP4), which leads to the loss of DPP4 activity on the surface of most cells. DPP4 inhibits SDF-1 activity by removal of the two N-terminal amino acids.

Methods: We tested in F344/CD26mut the hypothesis that reduced inactivation of SDF-1 in the kidney should result in improved outcome post-AKI (activation of its receptors, increased recruitment of MSCs).

Results: However, contrary to our hypothesis, we discovered that F344/CD26mut rats have significantly lower plasma SDF-1 levels than sex and age-matched wt F344 rats with normal CD26 function (F344/DuCrl, Charles River). Furthermore, bilateral renal pedicle clamping x 38-40 min resulted at 24 hrs in drastically higher animal mortality in F344/CD26mut rats (0% survival), while an identical IRI insult in age and sex matched wt F344 rats was well tolerated (100% survival). It has previously been shown that decreasing renal SDF-1 expression in mice increases renal damage following AKI (Nephrol Dial Transplant 2010 25:3852).

Conclusions: We conclude that F344/CD26mut rats have increased sensitivity to IRI AKI due to reduced serum levels of SDF-1. Although unknown, these findings may have implications for type 2 diabetic patients taking CD26/DPP4 inhibitors such as sitagliptin, which, if it were to similarly lower serum SDF-1 levels, it could potentially increase these patients' risk for AKI. Furthermore, the F344/CD26mut model will be useful to elucidate the role that CD26 may have in the physiological regulation of circulating SDF-1 levels.

Funding: Veterans Administration Support, Private Foundation Support

FR-PO048

MCP-1, a Target of Meprin A during Acute Kidney Injury? Christian Herzog, Sudhir V. Shah, Gur P. Kaushal. *Medicine, UAMS, Little Rock, AR.*

Background: Meprin A, an astacin like protease, is highly enriched in the brush border membrane (BBM) of kidney proximal tubular cells. As a heterotetramer of α and β subunits it is bound by its β subunit to the BBM. During acute kidney injury (AKI) meprin A is redistributed within the proximal tubular environment and may become damaging to the kidney. Meprin A has been shown to activate proinflammatory cytokines proIL-1β and proIL-18. In this study we examined its effect on osteopontin (OPN) and the chemokine MCP-1, both of which accumulate during tubular interstitial injury.

Methods: Meprin A was purified from rat kidney by differential centrifugation, DEAE chromatography and dialysis. The recombinant promeprins α and β were overexpressed in HEK-cells and purified by affinity chromatography and characterized by Western blot and enzymatic activity with fluorogenic substrate OCK^{+}. OPN and MCP-1 were digested with

meprin A and the activated promeprins α and β. Digestions were visualized by Western blot and cleavage sites of MCP-1 determined by peptide sequencing. Urinary excretion of MCP-1 was monitored by ELISA following cisplatin-induced AKI. Double immunofluorescence staining was used to monitor redistribution of meprin A in kidneys of mice after ischemia/reperfusion injury (IR) or cisplatin treatment (CP).

Results: Mice subjected to CP or IR injury showed altered localisation of meprin A from the BBM towards the cytosol and the basolateral membrane. Meprin α, β and A cleaved MCP-1 to products of different molecular sizes. MCP-1 was cleaved by meprin α and meprin A to a product of 10 kDA (N-terminally of ^{7}Ala and C-terminally most likely after ^{73}Arg) whereas meprin β cleaved only C-terminally after ^{74}Ser as determined by LC/MS/MS. OPN was degraded rapidly by meprin β, albeit slower by meprin α and A.

Conclusions: Meprin α, β and A cleave MCP-1 into defined peptide fragments with yet unknown biological function whereas OPN is degraded completely. The altered localisation of meprin A following AKI and its capability to process proinflammatory cytokines and chemokines indicate a regulatory function of meprin A during inflammation. Meprin A therefore presents a valuable target for pharmaceutical intervention.

Funding: NIDDK Support, Veterans Administration Support

FR-PO049

NGAL Lacks Specificity for Detection of Renal Damage in Ischemia-Induced Acute Kidney Injury Anja H. Bienholz,[1] Philipp Ickerott,[1] Oliver Witzke,[1] Herbert De Groot,[2] Frank Petrat,[2] Andreas Kribben,[1] Thorsten Feldkamp.[1] *[1]Department of Nephrology, University Duisburg-Essen, Essen, Germany; [2]Institute of Physiological Chemistry, University Duisburg-Essen, Essen, Germany.*

Background: Early and specific biomarkers for the detection of acute kidney injury (AKI) are urgently needed to enable the development and early application of therapeutic interventions and improve patient outcome. Therefore, the time course of the appearance and the specificity for renal damage of Neutrophil gelatinase-associated lipocalin (NGAL) and Kidney injury molecule-1 (KIM-1) were evaluated in a rat model of ischemic AKI.

Methods: Ischemia (I) was induced in male Sprague Dawley rats by clamping both renal pedicles for 40 min, followed by reperfusion (R). Plasma creatinine, the appearance and mRNA expression of NGAL and KIM-1 in rat kidneys were measured after 45 min and 4 h of reperfusion by immunhistochemistry (IHC) and quantitative real-time PCR.

Results: Plasma creatinine was significantly increased after 45 min and 4 h of R. After 45 min of R NGAL was already detectable by IHC in the kidneys compared to sham controls and persisted at 4 h. KIM-1 was not detectable by IHC after 45 min of R, but was also clearly detectable after 4 h of R. Surprisingly, I did not significantly induce renal mRNA expression for NGAL either after 45 min or 4 h of R. Inline with IHC, renal mRNA expression was significantly increased for KIM-1 after 4 h of R (*p = 0.028; n = 4). In contrast to the strong staining effects, mRNA expression was only 4-fold increased (n. s. vs. control) for NGAL while the increase was 27-fold for KIM-1 compared to controls after 4 h of R.

Conclusions: The time course and intensity of IHC staining for NGAL was not paralleled by renal mRNA expression. Therefore, most NGAL detected in the kidney is probably not of renal origin. These data show that NGAL is no specific biomarker for renal damage in ischemia-induced AKI.

Funding: Private Foundation Support

FR-PO050

Inflammation Is Present 7 Days after Ischemic Acute Kidney Injury in Mice Ana Andres-hernando,[1] Chris Altmann,[1] Rhea Bhargava,[1] Zhibin He,[1] Sarah Faubel.[1] *[1]University of Colorado Denver; [2]Clinical Hospital Dubrava.*

Background: Increased mortality occurs in patients with AKI that may be due to systemic complications. AKI is associated with lung, liver, brain, and gut injury that occur within 48 hours in animal models. To date, systemic complications of AKI have not been examined beyond 48 hours after AKI. Given the association between AKI and systemic complications in patients, we hypothesized that lung injury and other systemic complications would be present 1 week after AKI in mice.

Methods: Surgery for sham operation or ischemic AKI (22 minutes of renal pedicle clamping) was performed and lung inflammation (lung MPO activity [a marker of neutrophils] and lung neutrophils by flow cytometry), lung CXCL1 (a neutrophil chemokine), kidney injury (serum creatinine and BUN), serum proinflammatory cytokines, serum AST and serum glutamate, were determined at day 7. Weight was determined daily. Urine NGAL was measured on days 1, 4, and 7.

Results: Urine NGAL was significantly higher in AKI at days 1, 4 and 7 compared to sham. Serum creatinine (mg/dL) was 0.34±0.02 in sham and 0.43±0.1 (P=NS), BUN (mg/dL) was 22.5±0.8 in sham and 79.7±20 in AKI (P=0.02). Serum pro-inflammatory cytokines IL-6 and CXCL1 were significantly higher in AKI versus sham. Lung MPO activity was 0.69±0.16 in sham and 1.36±0.2 in AKI (P<0.02). Lung neutrophil by flow cytometry was (% of CD45+ cells) 12.9±3.2 in sham and 36.6±5.1 in AKI (P<0.02). Lung CXCL1 (pg/mg of protein) was 53±14 in sham and 167±44 in AKI (P<0.04). All mice lost weight 24 hours post procedure, but sham operated mice returned to their baseline weight by day 7; mice with AKI were 11% below baseline body weight by day 7. Serum AST (a marker of liver injury) was (mU/mL) 46±3 in sham and 62±4 in AKI (P<0.05).Serum glutamate, a marker of muscle wasting, was higher in AKI mice 35±5mM versus sham 20±3mM (P<0.02).

Conclusions: In summary, these data demonstrate that lung injury and other systemic complications are present up to 1 week post-AKI. These data lend further support to the notion that AKI is a multisystem disease. Interventions that target these later systemic complications of AKI may be the key to reducing mortality in patients with AKI.

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FR-PO051

Clinical Significance of Plasma Cell-Free DNA in Hemodialysis Patients Ju-Young Moon, Eun Young Kim, Wha-young Suk, Joo Hee Cho, Hong Joo Lee, Jungkook Wi, Young Wook Choi, Kyung-hwan Jeong, Tae-won Lee, Chun-Gyoo Ihm, Sang-Ho Lee. *Department of Internal Medicine, Division of Nephrology, Kyung Hee University, Seoul, Korea.*

Background: Plasma levels of cell-free DNA (cfDNA) are elevated in various proinflammatory or apoptotic conditions. Previously, increased cfDNA were reported during hemodialysis. However, there is limited data regarding its clinical relevance in HD patients. The aim of this study was to investigate the clinical significance of cfDNA in patients on maintenance HD.

Methods: We measured the pre-dialysis plasma levels of cfDNA and evaluate its clinical significance in 95 HD patients. cfDNA levels were determined using real-time EIF2C1 gene sequence amplification and analyzed the association with clinical parameters and hsCRP before 6, 3, 0 and after 3 months of cfDNA measurement.

Results: Plasma level of cfDNA was significantly elevated in HD patients compared to 15 healthy controls (3884 ± 407 vs. 1420 ± 121 GE/mL, respectively, $p < 0.05$). In HD patients, cfDNA levels were significantly higher in patients with diabetes compared to non-diabetic patients (4612 ± 640 vs. 2858 ± 358 GE/mL, respectively, $p < 0.01$). Intriguingly, cfDNA was significantly correlated with current HbA1c ($r = 0.38$, $p < 0.01$), but not with those of other time points. Patients with history of cerebrovascular or cardiovascular disease had also higher cfDNA. Among clinical parameters, systolic blood pressure was correlated with the levels of cfDNA. Current WBC counts was significantly associated with higher level of cfDNA, but there was no association between cfDNA and inflammatory parameters of different time points. Interestingly, the concentration of cfDNA was negatively correlated with the dose of erythropoietin stimulating agent. Other parameters of dialysis adequacy including KT/V, nPCR and iPTH, were not associated with the plasma levels of cfDNA.

Conclusions: Our results indicate cfDNA could be a potential biomarker of proinflammatory or apoptotic milieu in maintenance HD patients. However, the underlying reasons for the release of cfDNA and its functional role in uremic condition should be further defined for its clinical application.

Funding: Private Foundation Support

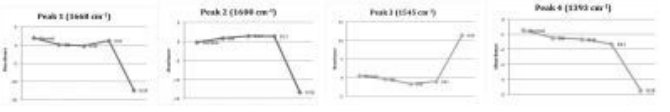
FR-PO052

Detection of Urine Renal Biomarkers Using Fourier Transform Infrared Spectroscopy Mei-Ching Yu,[1] Peter R. Rich,[2] Amandine Marechal,[2] Robert J. Unwin,[3] Frederick W.K. Tam.[1] *[1]Kidney and Transplant Institute, Imperial College London; [2]Structural and Molecular Biology, University College London; [3]Centre of Nephrology, University College London, United Kingdom.*

Background: More reliable biomarkers are desirable to improve early detection of asymptomatic patients with renal injury. Infrared (IR) spectroscopy has been widely used in biomedical research but its application in renal disease remains limited. Particularly useful is the 1800-900 cm^{-1} mid-IR. Most molecules have characteristic absorption bands in this range that can be used as molecular fingerprint. An advantage of this technique is spectra of potential markers may be obtained quickly and without subjecting biological materials to chemical manipulations.In this work,FTIR spectroscopy has been employed to analyse urine collected from a rodent model of GN with the aim of identifying potential renal biomarkers.

Methods: Nephrotoxic nephritis was induced in Wistar Kyoto rats. Urine samples from nephritic rats on day8 (n=7), day14 (n=5), day21 (n=7) and day28 (n=7) were compared with normal rats (n=20).Urine was analysed by FTIR. All original spectra were recorded in between 4000 to 900 cm^{-1} and analysed with Bruker OPUS 6.5 software. For detailed analyses, the second derivatives of absorption spectra were generated and components of interest were quantitated by integration analysis. The peak intensities of interest were normalized to the intensity of the 1450 cm^{-1} peak of urea.

Results: Bands at 1668,1660,1545 and 1393 cm^{-1} normalized with the urea peak, all appeared to change in concert with renal progression.



Conclusions: Several urinary biomarkers relevant to progression of experimental GN have been identified by FTIR spectra. Future work includes identification of the biochemical species responsible and extension of analyses to FTIR microscopy of renal tissues to identify the cellular origins of these biomarkers.

Funding: Clinical Revenue Support

FR-PO053

Patterns of Gene and Metabolite Define the Effects of Extracellular Osmolality on Kidney Collecting Duct Hyo-jung Choi, Tae-Hwan Kwon. *Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, Korea.*

Background: A variety of different extracellular microenvironments are formed in the tubular lumen and interstitium, to which renal tubular epithelial cells and interstitial cells are directly exposed. We hypothesized that altered extracellular osmolality *per se* could change a broad spectrum of cellular processes by affecting transcriptomic and metabolomic

profiles in IMCD cells, and hence it could produce the changes in renal tubular function, particularly in water and sodium reabsorption.

Methods: This was directly investigated by exploiting an integrated omics analysis of the primary cultured IMCD cells of rat kidney. We generated the metabolomic profiles through ^{1}H-NMR-based metabolomic analysis and identified the differentially expressed metabolites (DEMs) by the changes of extracellular osmolality. Gene expression profiles were generated via transcriptomic analysis and identified differentially expressed genes (DEGs) by the changes of extracellular osmolality. Cellular processes affected by the changes of extracellular osmolality were then identified through functional enrichment analysis of the DEGs. Quantitative real-time RT-PCR analysis and immunoblotting analysis were performed to confirm the changes of mRNA expression of genes and their protein abundance in the IMCD cells.

Results: Integrated analysis revealed that decreased extracellular osmolality was associated with decreased levels of organic osmolytes, glucose, intermediates of citric acid cycle, and branched-chain amino acids in the IMCD cells, along with significantly decreased gene expression and protein abundance of P-type transporters (ATP1B1), ABC transporters (ABCC5 and ABCG1) and insulin signaling pathways (IRS2). Quantitative real-time RT-PCR and semi-quantitative immunoblotting confirmed the changes of transcript levels of differentially expressed genes and protein levels.

Conclusions: Taken together, integrated analysis of omics data demonstrated that changes of extracellular osmolality per se could contribute to altered water and sodium reabsorption in the kidney collecting duct cells by affecting transcriptome and metabolome.

Funding: Government Support - Non-U.S.

FR-PO054

Ontology for Fluid and Solute Transport Processes in Peritoneal Dialysis (PD)

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Background: Computational models, formulated as a set of mathematical equations, are used for studying the dynamic behavior of physiological processes. The standard formats of their representation, as Systems Biology Markup Language, allow for expressing the mathematical structure of models, but they lack the means for describing the model semantics (meaning) that still requires human interpretation. However, semantic information on physiological models can be addressed using ontology, defined as the formal, explicit specification of a domain of the knowledge. Our objective was to formulate the Fluid and Solute Transport Processes in PD Ontology (FSTPPD-O) that can be read and processed by computers and contains a reusable standard for the description of models of peritoneal transport.

Methods: The ontology was built using the Methontology methodology and was formalized in OWL-DL sublanguage using Protégé-2000 ontology editor.

Results: FSTPPD-O has normalized, modular structure and includes 210 classes and 88 properties. It contains standardized, formal, semantic knowledge underlying mathematical models of fluid and solute transport that are used in clinical and experimental PD research such as: thermodynamic model, three-pore model for the capillary wall, three pore model for peritoneal dialysis and two layer model. The knowledge associated with the models is divided into two components: semantic and procedural. The semantic component describes the transport phenomena, transport barriers, model parameters and their interpretation and relationships, solute features, and patient population characteristics. The procedural part contains selected characteristics of model equations.

Conclusions: FTSPPD-O provides formalized semantic knowledge underlying models of fluid and solute transport in PD. Its modular structure is transparent for human interpreters and supports the model reuse and maintainability. The potential users of the ontology are researchers who create and/or apply transport models in their studies.

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FR-PO055

Long-Term Maintenance of Bioartificial Renal Epithelial Cell System (BRECS) in a Hemodialysis (HD) Circuit

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Background: Clinical experience has demonstrated that renal cell therapy incorporated into conventional CRRT circuit has provided therapeutic efficacy in acute renal failure (ARF). Treatment has been limited to 72 hours. However, hospitalization due to these acute events lasts approximately 2 weeks with ICU stays closer to 1 week. Methods to ensure patency and continued sterility of the HD circuit through cartridge exchanges, as well as the retention of cell viability within the BRECS was determined over the projected 7 day time course for clinical therapy.

Methods: Hemofiltration was established in 4 sheep with incorporation of the BRECS into an immune-isolated ultrafiltrate loop, allowing for maintenance of cell viability and communication between BRECS and host. The experiment was designed to represent the potential manipulations projected for clinical handling, during which the circuit must be interrupted daily to allow for hemofilter replacement and for clinical procedures not compatible with HD. Sheep underwent HD daily for 7 days, with the BRECS maintained in a secondary perfusion loop during and between HD sessions. Animal general health and hemodynamic stability was monitored. To investigate infection risk, blood cultures were taken. BRECS viability was monitored daily by O_2 consumption and glutathione (GSH) metabolism, followed by post-study verification via a fluorescein diacetate based viable dye. Preservation of differentiated phenotype of human renal epithelial cells was confirmed using immunohistochemistry.

Results: The BRECS maintained O_2 consumption and GSH metabolism remained stable through the seven day time-course without compromising the animal health. The immunoisolating filter design of the circuit was demonstrated to protect from bacterial transmission to the animal.

Conclusions: BRECS maintained viability and functionality in extracorporeal UF circuit for over 7 days, demonstrating feasibility and safety of clinical application in patients with ARF.

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FR-PO056

Development of a Neo-Kidney Augment Product Intended to Prevent or Delay the Need for Dialysis or Transplantation in CKD Patients

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Background: Chronic Kidney Disease (CKD) affects over 26 million in the US alone. Disease progression typically leads to dialysis and eventually a kidney transplant. Current standard of care is to treat the underlying conditions of CKD to slow down progression of disease. Tengion is developing regenerative medicine solutions to CKD with a Neo-Kidney Augment (NKA) product intended to catalyze kidney tissue regeneration to prevent or delay the need for dialysis or transplantation in this growing patient population.

Methods: NKA is composed of autologous, homologous Selected Renal Cells (SRC) that are primarily of tubular epithelial phenotype, and a gelatin-based hydrogel. Renal cells are obtained from a biopsy of the patient's kidney, isolated using enzyme digestion and expanded in culture. SRC are obtained by density gradient separation of harvested cells after a brief exposure to hypoxia. Cells are characterized with multiple markers to ensure tubular phenotype. SRC are formulated with the hydrogel to produce NKA. Biomaterial addition to SRC provides cell stability, enhanced shelf life and targeted delivery. Cell viability and function in NKA product was evaluated by testing for ability to metabolize Presto Blue, uptake albumin and presence of LAP and GGT enzyme activity.

Results: *In vivo* response to NKA implantation was evaluated by direct injection into the kidney cortex in animal models of CKD. NKA treatment was well tolerated with no morphological alterations observed in the tubular or glomerular compartments and histology confirmed reduction of kidney disease. NKA is packaged for implantation in the clinic via a product delivery device. The delivery system utilizes a syringe and needle compatible for NKA delivery. The system is designed to enable distribution of the product at multiple sites in the patient's kidney via a laparoscopic procedure.

Conclusions: Taken together, these data suggest that NKA implantation into the kidney has the potential to catalyze regeneration of kidney tissue and provide a much needed treatment of CKD.

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FR-PO057

Evaluation of Bioartificial Renal Tubule Device Prepared with Human Renal Proximal Tubular Epithelial Cells Cultured in Serum-Free Medium

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Background: Acute kidney injury (AKI), accompanied by the development of systemic inflammatory response syndrome and multiorgan dysfunction syndrome, is associated with a high risk of death. Bioartificial renal tubule device (BTD) is a cell therapy that improves the conditions common to artificial kidney recipients treated for kidney diseases. We previously reported significant improvements of conditions of AKI animals by treatment with BTD which was produced with lifespan-extended human renal proximal tubular cells (RPTEC). However, when the BTD is used for human patients, the major obstacle is the safety of BTD, because the RPTEC have been cultured in medium containing fetal calf serum.

Methods: In this study, we evaluated BTD produced with lifespan-extended human RPTEC cultured in available serum-free medium (RELAR, Nipro, Osaka), comparing to BTD peoduced with same cells cultured in serum-containing medium (REGM, LONZA, Basel).

Results: Lifespan-extended human RPTEC cultured in serum-free medium can proliferate as in serum-containing medium. Comparison of leakage and reabsorption of small molecules in BTD produced with RPTEC cultured in serum-free medium to those in BTD produced with RPTEC cultured in serum-containing medium showed almost equal abilities of transportation in these two types of BTDs. When AKI goats were treated with these BTDs individually for 24hrs, almost same extents of extension of lifespan and suppression of cytokine expression in blood cells were observed with comparison to sham-BTD.

Conclusions: All these data indicated that lifespan-extended human RPTEC cultured in serum-free medium is functionally equal to that cultured in serum-containing medium, and that the safety of BTD for human patients could be elevated without loss of functional ability by being produced with RPTEC cultured in serum-free medium.

FR-PO058

Contribution of Structural Domains to Ribonuclease 7's Gram-Positive and Gram-Negative Activity against Uropathogens David S. Hains,[1,2] Huanyu Wang,[2] John David Spencer,[1,2] Andrew L. Schwaderer.[1,2] *[1]Department of Pediatrics/Division of Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: Ribonuclease 7 (RNase 7) is a 14.5 kDa peptide that possesses potent antimicrobial properties against Gram-negative and Gram-positive bacteria and is expressed in a variety of epithelial tissues. Little is known about its mechanisms of action and the determinants of its antimicrobial properties. The objective of this study is to identify the intrinsic functional domains of RNase 7 that influence its activity against uropathogenic Gram-negative and Gram-positive bacteria.

Methods: In this study, a series of RNase 7 fragments were generated that contained different numbers of its secondary motifs starting from both N-terminus and C-terminus of RNase 7. We determined antimicrobial properties of each fragment against both Gram-positive and Gram-negative uropathogenic bacteria.

Results: RNase 7 fragments displayed significant differences in their antimicrobial activity profiles. Compared to N-terminal fragments, C-terminal fragments showed uniformly decreased activity against *Escherichia coli* and *Staphylococcus saprophyticus*. In addition, the fragments that lack β-sheets 1, 3 and 4 demonstrated significantly decreased activities. We have also identified one fragment with at least four-fold increased potency against both *E. coli* and *S. saprophyticus* compared to full-length peptide. We have also identified distinct regions of the peptide that are independently responsible for Gram-negative and Gram-positive activity.

Conclusions: Our results suggest that RNase 7 has antimicrobial activity against both Gram-positive and Gram-negative bacteria but with distinct mechanisms. We also have identified a peptide fragment with increased activity compared to the naturally occurring peptide. These findings serve as the foundation to design future novel antimicrobial and therapeutic agents.

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FR-PO059

Peritoneal Cell Sheet Engineering Suppress Abdominal Adhesion and Inflammatory Angiogenesis in Rat Model Kunio Kawanishi,[1] Ryoichi Sakiyama,[2] Kazuho Honda,[1] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan; [2]Department of Clinical Engineering, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: Surgical enterolysis or noble plication for Encapsulating peritoneal sclerosis (EPS) improved the survival rate. However, the recurrence or re-surgery for EPS still remains a huge clinical and economical problem. Aim of this study is developing a new therapeutic method to prevent intra-abdominal adhesions by cell sheet engineering.

Methods: Primary mesothelial cells and fibroblasts were isolated from parietal peritoneum of 8-week old male Lewis rats. Mesothelial cells were seeded over the confluent fibroblast layer on temperature-responsive culture dishes at 37°C. Transplantable peritoneal cell sheets were harvested by incubation at 20°C. Eight weeks old female Lewis rats were anesthetized and the cecum were isolated and cauterized. Then peritoneal cells sheets from male were transplanted without suturing. Thirty female rats were divided into sham group (n = 10), transplantation group (n = 10), and control laparotomy group (n = 10) received open abdominal surgery without cauterization. Seven days after operations, they were sacrificed and examined by adhesion score, Y-FISH, immunohistochemistry of cytokeratin, vimentin, fibrin, CD31, podoplanin,CD68 and CD163.

Results: Peritoneal cell sheet transplantation resulted in adhesion prevention. Y-FISH analysis or immunohistochemistry revealed that transplanted male cell sheets were engrafted successfully to female in the transplantation group. Fibrin deposition, CD31 or podoplanin positive vessel increasing and CD68 or CD163 positive macrophages infiltration were significant restricted in the transplantation group than in the sham.

Conclusions: We developed peritoneal cell sheets composed of mesothelial cells and fibroblasts with temperature-responsive culture dishes. Transplantation of peritoneal cell sheets prevented abdominal adhesion, fibrin deposition, angiogenesis, lymphangiogenesis and macrophage infiltration in rat cecal cauterization adhesion model. This novel technique would be useful to prevent postoperative tissue adhesion in clinical settings.

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FR-PO060

Systems Biology in Diabetic Nephropathy: Building a Useful Model from Multiple Markers and Profiles Gert J. Mayer,[1] Paul Mayer,[2] Bernd Mayer.[2] *[1]Department of Internal Medicine IV (Nephrology and Hypertension), Medical University Innsbruck, Innsbruck, Austria; [2]Emergentec Biodevelopment GmbH, Vienna, Austria.*

Background: The pathophysiology of diabetic nephropathy (DN) is driven by a complex, multi-facetted interplay of numerous molecular processes (protective as well as damaging) and the balance between these, rather than the activity of a single pathway determines the clinical presentation and outcome. The goal of this study is to derive a biomarker panel, which characterizes the different molecular processes involved.

Methods: We used a hybrid gene/protein interaction network that holds ample information on molecular features (nodes) and their relations (edges), by this providing a basic structure for navigation in molecular content and context being identified as relevant in DN.

Results: Extensive literature search on omics studies in DN provided a molecular feature lists mapping to a total of 2,175 unique protein coding genes (13 from SNPs, 12 as targets from relevant miRNAs, 1,583 from transcriptomics, 5 from proteomics, and 53 from metabolomics via linking to enzymes; 509 features are identified from multiple sources). Further data were derived from searching NCBI Pubmed (utilizing MeSH and gene-to-pubmed) for human protein coding genes associated with DN, resulting in 287 such genes. Text mining of patents and clinical trial descriptors in the context of DN adds further about 1,000 features. These data were used to label the respective nodes in the interaction network, by this obtaining a DN specific subgraph. Using a segmentation algorithm applied on this subgraph allowed identification of DN specific units, each unit characterizing a cluster of genes/proteins with a high internal functional association. We interpret each unit as a functionally relevant molecular process contributing to the presentation of DN, and the total set of such units as a molecular model of DN.

Conclusions: We propose that selecting appropriate biomarkers from each unit might allow describing a patient specific "type" of DN, ultimately leading to a better stratification of patients regarding progression risk and optimal interventional approach.

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FR-PO061

Effects of Pressure and Electrical Charge on Macromolecular Transport across Bovine Lens Basement Membrane: Implications for Glomerular Permeability Nicholas J. Ferrell,[1] Kathleen O. Cameron,[1] Joseph J. Groszek,[1] Ross A. Smith,[1] Andrew L. Zydney,[2] William Fissell.[1,3] *[1]Biomedical Engineering, Cleveland Clinic, Cleveland, OH; [2]Chemical Engineering, Pennsylvania State University, State College, PA; [3]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: The mechanisms by which the glomerulus retains proteins in plasma are still not fully understood despite implications for renal physiology and disease. Evaluating glomerular permselectivity *in vivo* can be challenging due to the complex nature of the capillary wall. We set out to study the effects of pressure and charge on the filtration properties of collagen IV rich extracellular matrix using the lens capsule of the bovine eye as a model experimental system.

Methods: We measured sieving of fluorescently tagged Ficoll across lens basement membrane (LBM) at pressures ranging from 1-3 psi. We used mathematical transport modeling to evaluate the relative role of diffusion, convection, and altered membrane permeability in determining the sieving properties of the membrane. The effect of electrical charge on filtration was evaluated by comparing the sieving of negatively charged and neutral Ficoll and by measuring the streaming potential generated by electrolye flow through the membrane.

Results: A decrease in the sieving coefficient was observed with increased pressure for a range of molecular radii. Based on transport modeling, the altered sieving properties could be accounted for, at least in part, by changes in Peclet number (ratio of convective to diffusive transport). No evidence was found for pressure dependent changes in the structure of the membrane (i.e. membrane compression) based on consistent Darcy permeability. A slight, but consistent increased passage of negatively charged Ficoll was observed relative to neutral Ficoll. While this is counter to the traditional model of increased retention of negatively charged solutes, it was consistent with our measured streaming potential.

Conclusions: LBM provides a convenient model to evaluate macromolecular transport across biological basement membrane and may provide additional insights into the mechanisms of glomerular permselectivity.

Funding: NIDDK Support

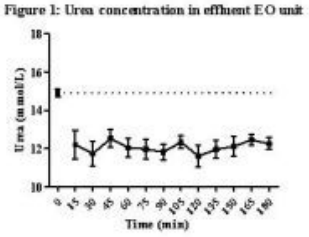
FR-PO062

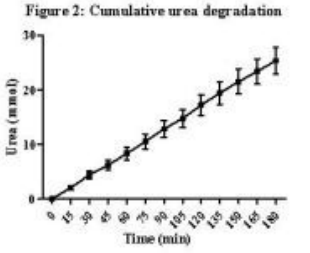
Removal of Urea via Electro-Oxidation and Elimination of Resulting Chloramines in a Prototype Wearable Artificial Kidney Device Maarten Wester,[1] Frank Simonis,[2] Jeroen Kooman,[3] Walther H. Boer,[1] Jaap A. Joles.[1] *[1]Nephrology, UMC Utrecht, Utrecht, Netherlands; [2]Nanodialysis BV, Oirschot, Netherlands; [3]MUMC, Maastricht, Netherlands.*

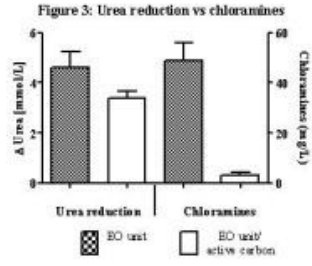
Background: In the consortia iNephron & NEPHRON+ we are developing a wearable artificial kidney device (WAKD). For detoxification we propose to combine adsorption (e.g. removal of potassium and phosphate) with electro-oxidation (EO), which degrades nitrogen-containing toxins like urea and creatinine. This offers the possibility to regenerate dialysate and thus miniaturize the WAKD. Chloramines are byproducts of EO which can cause hemolysis. We studied the feasibility of urea degradation by EO combined with chloramine removal by active carbon plus vitamin C, a potent reducing agent.

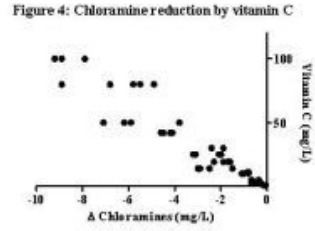
Methods: A urea solution (15mmol/l) was pumped through an EO unit (50ml/min) in a single pass and effluent urea and chloramine concentrations were measured for 3 hrs (n=3). This was repeated with active carbon incorporated in the EO unit. Finally, we evaluated whether vitamin C further reduced chloramines.

Results: EO reduced urea from 15 mmol/l to 12.1±0.8 mmol/l after a single pass for 3 hrs (Fig 1). The cumulative urea removal by the device in 3 hours was 25.4±4.2 mmol (Fig 2). Active carbon in the EO unit substantially lowered chloramines in the effluent ($p<0.05$) without compromising urea removal (Fig 3). Vitamin C dose-dependently reduced chloramines (Fig 4).









Conclusions: EO is a promising technique for prolonged urea removal allowing effective recycling of dialysate in the WAKD. Incorporation of active carbon in the EO unit combined with vitamin C infusion in the effluent can effectively reduce toxic chloramines offering the perspective of in vivo tests.

Funding: Government Support - Non-U.S.

FR-PO063

The Efficiency of Microdialyzer System Using Nanoporous Polyethersulfone Membrane Yoshihiko Kanno,[1] Hikaru Ito,[2] Matsuhiko Hayashi,[1] Gunawan Setia Prihandana,[2] Norihisa Miki.[2] *[1]Apheresis and Dialysis Center, School of Medicine, Keio University, Tokyo, Japan; [2]Department of Mechanical Engineering, School of Science and Technology, Keio University, Yokohama, Japan.*

Background: Over 300 thousand patients who need renal replacement therapy in our country still struggled from daily life restriction and complications by receiving dialysis therapy. They have to spend 3 half day a week for hemodialysis therapy which account for 95% of renal replacement therapy in Japan. Although the complications in hemodialysis patients are almost came from the shortage of dialysis quantity, we could not improve the usual system of hemodialysis therapy using extracorporeal circulation. Recently, portable dialysis system was clinically investigated,and small devices are demanded. We have developed a microdialyzer containing polyethersulfone (PES) membranes, which could remove urea nitrogen and electrolytes from the dissolved solution.

Methods: Microdialyzer system was consisted of 24x24 mm^2 laminable PES membrane sandwiched between 2 titan made thin layers made by deep wet etching. The membrane was made by wet phase inverse method and estimated pore sizes were 2-5 nanometer. 50ml of whole cattle blood containing 100 mg/dl of urea nitrogen and 6.0 mEq/L of potassium was dialyzed with single layer membrane (38.3 mm^2) using in closed circuit system for up to 48hours. Blood flow rate was 3 mL/min.

Results: Blood concentrations of urea nitrogen and potassium were slowly decreased with dialysis as in table.

	Urea Nitrogen (mg/dl)		Potassium (mEq/L)	
Time(hours)	Blood	Dialyzate	Blood	Dialyzate
0	140	0	8.4	2.1
1	142	0	8.3	2.1
4	132	11	7.8	2.5
24	73	47	5.8	3.7
48	55	61	5.4	3.8

Occlusion in the circuit system and hemolysis were not observed during the dialysis session. Any thrombus on the PES membrane was not observed after 48 hour dialysis.From the present results, it is expected to increase the diffusion efficiency to support human life by multiplies PES membrane layer to 60 sheets.

Conclusions: Diffusing function and durablility of the microdialyzer were investigated. Thus our new dialysis device could remove enough urea and potassium from whole blood to use it in vivo.

Funding: Private Foundation Support

FR-PO064

Extractable and Leachable Residues from Extended Use of Hemodialysis Blood Treatment Set, Dialysate Circuit and Dialyzer Bruce F. Culleton,[1] Matthew R. Muller,[2] Angelito A. Bernardo.[1] *[1]Clinical/Medical Affairs, Baxter Healthcare, Deerfield, IL; [2]Research & Development, Baxter, Roundlake, IL.*

Background: Small quantities of chemicals are released by components of dialysis circuits (blood tubing, dialysate tubing, and dialyzers) during routine hemodialysis. The aim of this study was to characterize chemical residues from a hemodialysis system (consisting of blood treatment set, dialysate circuit and dialyzer) after initial citric acid disinfection and after multiple hot water disinfections. We hypothesized that any chemical residues measured on first use would progressively dissipate with multiple disinfections.

Methods: Toxicological analysis was conducted per ISO 10993-1. Samples for "worse case" (T1A) were drawn after the dialysis circuit was treated with hot citric acid (5%-6%) followed by hot water disinfection (85°±5° C, 60 min). The T1B sample was obtained after hot water disinfection only (before any treatment would be initiated). Samples for extended use cycles were done after 15 (T15) and 30 (T30) hot water disinfections. Analyses of extractable and leachable chemicals were done by gas chromatography, mass spectrophotometry and total organic carbon.

Results: Concentrations of acetone, 2-butanone (methyl ethyl ketone), cyclohexanone, methylene chloride, and trimethylsilanone were present at baseline (T1A and T1B). At T15, only acetone and methylene chloride were present. Minute amounts of acetone were detected at T30.

Average of three replicates of leachable chemicals (μg/L) at baseline and after 15 and 30 hot water disinfections

Chemical	T1A	T1B	T15	T30
Acetone	92.6	58.7	26.9	22.7
Methyl-ethyl ketone	17.8	3.73	BDL	BDL
Cyclohexanone	0.441	0.314	BDL	BDL
Methylene chloride	108	66.8	BDL	BDL
Trimethylsilanol	29.9	7.8	BDL	BDL

BDL- Below Detection Limit

Conclusions: The amount of volatile organic residues progressively declined after multiple hot water disinfection cycles of the same dialysis circuit. Although the clinical relevance of this finding is uncertain, limiting exposure to chemical residues in chronic HD patients may confer a safety benefit over single-use systems.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO065

Non-Invasive Detection of Glomerulonephritis by MRI-Based Molecular Imaging Natalie J. Serkova,[1] Kendra Huber,[1] Brandon Renner,[2] Matthew C. Pickering,[3] Joshua M. Thurman.[2] *[1]Anesthesia, University of Colorado Denver School of Medicine, Aurora, CO; [2]Medicine, University of Colorado Denver School of Medicine, Aurora, CO; [3]Medicine, Imperial College, London.*

Background: The diagnosis of glomerulonephritis requires a renal biopsy, but biopsies are susceptible to sample error, and the risk of complications limits their use.

Methods: We have developed a molecular imaging contrast agent that targets tissue-bound complement activation fragments (iC3b and C3d). The contrast agent is comprised of a C3-targeting protein attached to superparamagnetic iron-oxide (SPIO) nanoparticles. The SPIO nanoparticles decrease T2 relaxation time and negatively enhance (i.e. darken) T2-weighted magnetic resonance images (MRI) in proportion to the abundance of the target molecule. We tested this contrast agent in mice with targeted deletion of the gene for the complement regulator factor H ($fH^{-/-}$ mice). These mice develop glomerulonephritis characterized by glomerular deposits of iC3b and C3d. We injected $fH^{-/-}$ mice with targeted SPIO nanoparticles and performed T2-weighted MRI of the kidneys 4, 24, 48, and 72 hours after injection.

Results: The T2-relaxation times in the cortex of the $fH^{-/-}$ mice were significantly reduced with tmax at 48 hours after injection with the targeted nanoparticles (-13.35±1.44 msec in T2 values, $n = 6$, $P < 0.001$). Mice injected with untargeted (control) SPIO nanoparticles did not demonstrate significant changes at any time-point (-1.00±2.58 msec T2-decrease at 48 hrs, $n = 5$, $P = NS$). We also tested $fH^{-/-}fB^{-/-}$ mice, which do not develop glomerular C3 deposits. No changes in the kidneys were seen by MRI after injecting the $fH^{-/-}fB^{-/-}$ mice with targeted nanoparticles (-1.53±1.76 msec T2-decrease, $n = 4$, $P = NS$), confirming that the reduced T2-relaxation times were due to the C3 deposits.

Conclusions: Molecular imaging of the kidneys using targeted SPIO by T2-weighted MRI may provide a quantitative, non-invasive alternative for monitoring disease activity in patients with glomerulonephritis.

Funding: Private Foundation Support

FR-PO066

Evaluation of UUO Kidney Injury Using High Resolution Magnetization Transfer Ratio Mapping Feng Wang,[1] Keiko Takahashi,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

Background: Magnetic resonance imaging (MRI) complements the physiological information obtained from conventional assays of kidney function and facilitates our understanding of pathological mechanisms in kidney disease. Magnetization transfer ratio (MTR) provides indirect information about the macromolecular component in tissues. In this study, we optimized MRI methods for MTR mapping in mouse kidneys and assessed its application in studying progressive kidney injury using the unilateral ureteral obstruction (UUO) mouse model.

Methods: MRI protocols were optimized on Agilent 7T MRI. Rapid acquisition and respiration gating were applied to minimize motion artifacts. MTR was measured based on a 2D RF-spoiled gradient echo sequence(α=7°,TR=24ms,TE=3.3ms,FOV=25 .6×25.6mm^2,matrix size=128×128,thickness=0.5mm,81accumulations). Off-resonant RF irradiation was accomplished with use of Gaussian RF pulses(6000Hz,12ms). To calculate MTR, additional acquisitions were performed without MT pulses. Each measurement was preceded by a 20s period of dummy scans to drive the spin system into steady state. MTR maps were obtained for UUO mice on day 1, 3, and 6 after obstruction. Sham-operated mice were used as a control.

Results: MT imaging detected fine structural changes (size, shape, contrast, thickness) of renal compartments in UUO kidney as early as day 1 post obstruction. The overall MTR of the UUO kidney declined as the disease progressed (day 1: 27%, day 3: 34.0%, day 6: 52%) and its reduction was pronounced in renal medulla, especially in inner medulla and papilla (>64%, day 3), perhaps due to cell apoptosis. In contrast to renal medulla, MTR changes were minimal (<10%) in UUO renal cortex. Through the duration of the study, structural features and MTR were unchanged in the contralateral kidney and sham kidney.

Conclusions: MTR mapping detects progressive UUO renal injury, especially in renal medulla. This MRI method could be used for the assessment of kidney injury associated with the change of macromolecular components(apoptosis/fibrosis).

Funding: NIDDK Support

FR-PO067

Automated Renal Scintigraphy Modeling Mohammed Noor Tantawy,[1] Dana Zemel,[2] Todd E. Peterson,[1] Keiko Takahashi,[2] Raymond C. Harris,[2] Christopher Chad Quarles,[1] Takamune Takahashi.[2] *[1]Vanderbilt University Institute of Imaging Science, Nashville, TN; [2]Vanderbilt O'Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.*

Background: Renal scintigraphy using ^{99m}Tc-MAG3 is widely used in human and animal studies. We previously reported a method to extract biological parameters from the time-activity curves (TACs), including peak activity (renal blood volume), time-to-peak (TTP, renal perfusion), and the slope of the initial influx (RBF', renal blood flow). However, extracting these parameters is tedious and time consuming especially when there is a large influx of data. To automate the parametric analysis, this study was aimed to identify a highly flexible, non-linear arbitrary analytical model that fits a heterogeneous range of MAG3 TACs.

Methods: ^{99m}Tc-MAG3 was injected to mice and TACs of each kidney were generated by planar dynamic acquisition on NanoSPECT. The analytical model was developed using the formula (Muzic & Cornelus 2001) with some modification: $f(t)=(at-b-c)e^{-gt}+be^{-hk+l}+ce^{-lt}$ [a, b, c, g, h, k, and l are constants]. The fit was assessed by both X^2 and visual inspection. MATLAB was used to carry out the fit. The t (TTP) was calculated by taking the derivative of the formula and setting it equal to zero, then substituted in the original formula to determine the peak activity. The t^* at which the peak begins was calculated by setting the third derivative of the formula to zero, then df(t)/dt (at $t = t^*$)= RBF' was determined. The model was validated using both normal and UUO (day1,3,6) mice.

Results: The correlation between the manually extracted parameters and those obtained via the formula was ~0.99 in normal mice. In UUO mice, similar correlation was obtained for peak(plateau) and RBF'. The TAC's plateaued in UUO kidney due to no secretion of urine. Determining the time-to-plateau visually may include many errors. However, using the formula we were able to determine an exact time-to-plateau.

Conclusions: We have developed the formula that can be used for the parametric analysis of MAG3 TACs. Further evaluation of the formula and the development of executable software should provide a standardized protocol of MAG3 TACs analysis.

Funding: NIDDK Support

FR-PO068

Relationship between Hemodynamics and Later Lumen Changes in a Porcine Arteriovenous Graft Model Yan-Ting E. Shiu,[1] Daniel B. Pike,[2] Christi M. Terry,[1] Yong He,[3] Huan Li,[1] Ilya S. Zhuplatov,[1] Alfred K. Cheung.[1,4] *[1]Medicine, Univ of UT, Salt Lake City, UT; [2]Bioengineering, Univ of UT, Salt Lake City, UT; [3]Surgery, Univ of FL, Gainesville, FL; [4]Medicine, VASLCHCS, Salt Lake City, UT.*

Background: Hemodialysis arteriovenous grafts (AVGs) often fail due to neointimal hyperplasia (NH) at the venous anastomosis (VA), resulting in stenosis and clotting. Aberrant hemodynamics may play a role in the focal nature of NH formation, but a clear correlation has not yet been demonstrated. We have developed an advanced and efficient technological pipeline to investigate this correlation in a porcine model of AVG stenosis.

Methods: AVG was placed between the common carotid artery and the external jugular vein in swine. At 1, 2, 3 or 6 weeks (wk) (n=3 at each wk), the AVG lumen geometry and blood flow at the in- and out-flow sites were obtained by black-blood and cine phase-contrast MRI, respectively, and used as input for computational fluid dynamics analyses to model hemodynamic parameters, including the oscillatory shear index (OSI) (a measure of change in flow direction and value over a cardiac cycle). OSI was co-registered with later lumen areas using the VA as an anatomic landmark.

Results: Disturbed flow patterns (e.g., spiral and retrograde flow) occurred early and were common particularly at the VA. On average, early (1, 2 wk) OSI at the VA region was higher than that in proximal (downstream) vein segments. The venous flow and lumen changes were asymmetrical across upstream and downstream regions of the VA. In one pig monitored serially, the VA distal (upstream) region OSI increased early and remained high, but OSI at the VA downstream region increased early then dropped to almost control vein levels by 6 wk. Of note, the distal vein had the largest lumen increase and the least amount of change in OSI compared to proximal vein.

Conclusions: We have developed a robust pipeline of techniques to assess hemodynamic parameters with later lumen area changes in porcine AVG. Further serial analyses in larger studies will illuminate the relationship between early hemodynamics and subsequent focal NH development, with the objective of improving grafts and surgical techniques that lead to AVG longevity.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support

FR-PO069

Process Management Reengineering in Healthcare: Why Old and New Paradigms Have Failed: The Roles of Critical Healthcare-Specific Complex Unidentified Bottlenecks in a Complex Adaptive System: Do We Need More MD MBAs? Macaulay A. Onuigbo,[1,2] Mark Onuigbo,[3] Nnonyelum T. Onuigbo,[4] Obi Egbuniwe.[5] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic; [3]Aeronautics Engineering, University of Michigan; [4]Information Technology, NTEC Solutions LLC; [5]Information Technology, Nurture Healthcare Service LLC.*

Background: The limitations of the straight juxtaposition of process management (PM) theories and practices with proven effectiveness in manufacturing into the healthcare service industry, often without adequate analysis and strategization, and the consequent poor outcomes is well known to healthcare managers. The introduction of the EMR/EHR to limit medical errors while increasing productivity has often led to the exact opposite outcomes. Early hospital discharge strategies have failed. Strategies to improve access have not materialized.

Methods: Literature Review.

Results: Attempts at simply juxtaposing manufacturing PM paradigms into healthcare have failed to achieve desired goals. The healthcare system is a complex adaptive system (CAS) that demands a far better understanding of its function, before solutions are proffered. A constraint is something that limits the performance of a process or system in achieving its goals. In PM, one often overlooked constraint is a bottleneck, leading to unbalanced systems. Bottleneck management is therefore an important operational strategy to increase effective capacity or productivity, and therefore throughput. In healthcare, due to the unfamiliarity of the managers (IT, HR) with the system, very often, unique and unidentified bottlenecks are neglected in PM strategization, leading to failed solutions. Such bottlenecks include Cognitive Drift (EMRs), excessive workaround (EMRs), EMRs out of sync with workflow, and patients preferring to have lunch before hospital discharge.

Conclusions: Current PM changes introduced into healthcare to solve problems and accelerate throughput and productivity have often failed due to unfamiliarity of the PM experts with the CAS. New better informed approaches are warranted - Either a far-more integrated collaborative stratagem between physicians and managers or guess what? We need more MD MBAs!!!

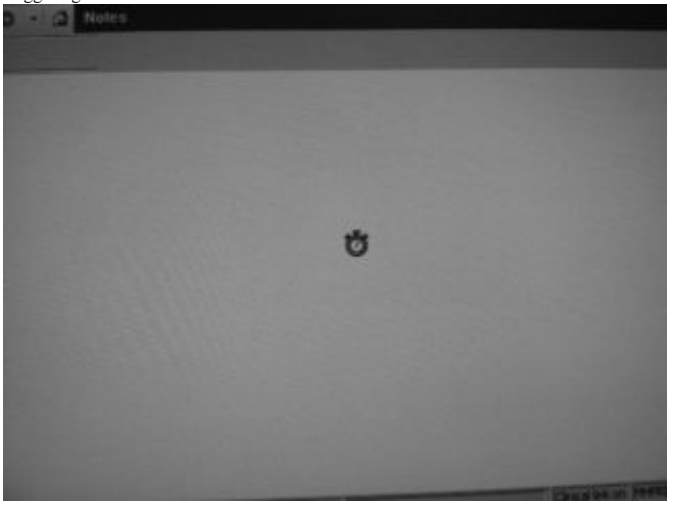
FR-PO070

Cognitive Drift in the Electronic Medical Record System: An Unrecognized Source of Physician Stress and a Silent Cause of Medication Errors: Results of a Small Physician Survey Macaulay A. Onuigbo,[1,2] Nnonyelum T. Onuigbo.[3] *[1]Nephrology, Mayo Clinic Health System; [2]Medicine, Mayo Clinic; [3]IT, NTEC Solutions.*

Background: Medications represent the most common intervention in healthcare but lead to an estimated 1.5 million adverse drug events and tens of thousands of hospital admissions annually in the USA. The 98,000 deaths/year and many more injuries resulting from medical errors have propelled patient safety as top priority. Many authorities had hoped that many medication errors - the most common cause of preventable injuries in hospitals - can be prevented by computerized physician order entry (CPOE), a component of the electronic medical record (EMR). EMRs have become ubiquitous in the US, mandated by the Affordable Care Act. According to Levitt and Dubner, (Superfreakonomics), a person using a computer experiences "cognitive drift" (CD) if more than one second elapses between clicking the mouse and seeing new data on the screen. If ten seconds elapsed, the person's mind is somewhere else entirely and this leads to medical errors. For our survey, we defined CD as elapsed time >10 seconds.

Methods: Survey - Ten randomly selected ICU physicians in 2012.

Results: 10 of 10 (100%) of the ICU physicians reported experiencing CD several times a day. All physicians cited CD as a source of significant frustration, stress and possible burnout. A definition of CD as >1 second would have produced even more staggering statistics.



Conclusions: Cognitive drift is a common and unrecognized source of physician stress. CD causes physician distraction, CPOE and medication errors. EMRs must deploy more robust and faster servers, networks and work stations to eliminate these consequences. Our other study of stress in the healthcare workplace identified EMR-induced stress (EIS) as a major cause of employee stress/burnout; CD was a major driver of EIS. CD warrants further study.

FR-PO071

Multicopy Crystallographic & Biophysical Analyses of the N-Terminal Domain of NBCe1-A: Illumination of the Human R298S Mutational Defect Harry S. Gill. *Medicine, The Medical Faculty Associates & the George Washington University, Washington, DC.*

Background: NBCe1-A membrane-embedded macromolecules cotransport sodium and bicarbonate ions across the bilayer that serve to maintain acid-base homeostasis throughout the body. Defects are linked to a number of disorders, including proximal renal tubule acidosis, mental retardation, dental defects, and cataracts. Previously, we demonstrated the N-terminal domain of NBCe1-A (Nt) is in a pH-sensitive monomer-dimer equilibrium. At neutral pH, bicarbonate ions bind the Nt, stabilizing dimerization and intermolecular self-associations of dimers.

Methods: We determine and analyze the X-ray crystal structure of the Nt as a dimer at 2.4-Å resolution using molecular-replacement methods, and a multicopy crystallographic structure of the monomer using 5 atomic models and strict 4-fold NCS constraints in refinement procedures. We measure the pH-sensitivity of a truncated Nt mutant by light-scattering techniques, and bicarbonate, bisulfite, mutant self-association bindings by surface plasmon resonance techniques.

Results: The structures reveal that R298 implicated in the disorders is part of a putative conduit that transverses the Nt. The conduit opens to the transmembrane domain (TMD) on one end and an apparent foyer entrance on the opposite end. The naturally occurring mutation R298S disrupts an electrostatic pocket within the conduit that disables substrate binding. We also report similar conducts in family member AE1 (Band 3) when exploring its crystal structure. Further, we identify by biophysical analyses on a truncated Nt that the autoregulatory domain (ARD) at the N-terminus of the Nt is responsible for self-associations.

Conclusions: The Nt responds to changes in pH or bicarbonate fluctuations. In proximal tubule cells, we propose a model where the ARD is a gate for the foyer. When self-associated, the foyer entrance is accessible, allowing substrate entry into the conduit. During acid loads, the gates close entry into the foyer, preventing bicarbonate from leaving the cell. The R298S defect similarly prevents bicarbonate ions from being transported to blood, giving rise to metabolic acidosis that results from the renal tubule acidosis.

Funding: NIDDK Support

FR-PO072

Sulfatides Are Required for Renal Adaptation to Metabolic Acidosis Hermann-Josef Groene,[1] Paula Stettner,[1] Soline Bourgeois,[2] Carsten A. Wagner.[2] *[1]Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [2]Institute of Physiology, University of Zurich, Zurich, Switzerland.*

Background: Urinary ammonia excretion by the kidney is an essential process to maintain blood pH in the physiological range. A key step in renal ammonia handling is the generation of a steep cortico-papillary ammonia gradient providing the driving force for ammonia secretion by the collecting duct into urine. In this study we demonstrate that sulfatides i.e. anionic glycolipids of the outer plasma membrane are important for papillary ammonium retention and increased urinary acid elimination during metabolic acidosis.

Methods: Sulfatide synthesis was inhibited by tubular epithelial-specific, genetic disruption of cerebroside sulfotransferase (Cst) in the mouse kidney (Pax8 cre); compensatory synthesis of other negatively charged glycosphingolipids was inhibited by simultaneous deletion of glucosylsceramide synthase (Ugcg) activity.

Results: Renal Ugcg-, Cst-, and Ugcg/Cst-deficient mice had an abnormal acidic urinary pH which was - similar to type II diabetics - accompanied by higher urinary net acid- but lower ammonia excretion as compared to control littermates (pH: 5.95 ± 0.03 vs 5.35 ± 0.02). Upon acid diet, renal Ugcg/Cst-deficient mice were not able to increase urinary ammonia adequately (123 ± 2 vs 176 ± 8 mM/mM creatinine) and developed chronic metabolic acidosis (day 9 pH: 7.02 ± 0.04 vs 7.21 ± 0.04). Systematic analyses including transporter expression studies, tissue ammonia measurement, and in vitro microperfusion with ammonia transport study of collecting ducts of acid-loaded Ugcg/Cst-deficient mice indicated normal transepithelial NH_3 transport, but decreased ammonia retention in the papilla resulting in a blunted axial ammonia gradient.

Conclusions: We therefore propose that renal sulfatides, by their negative extracellular charge, act as counterions for ammonia binding and storage in the papillary interstitium. This study highlights an instrumental role of sulfatides in renal ammonia handling, urinary acidification, and acid-base homeostasis.

Funding: Government Support - Non-U.S.

FR-PO073

Upregulation of Mice Carbonic Anhydrase XII in the α-Intercalated Cell of Outer Medullary Collecting Duct during Acidosis Yukiko Yasuoka,[1,2] Yuichi Sato,[3] Hiroshi Nonoguchi,[4] Katsumasa Kawahara.[1,2] *[1]Physiol., Kitasato U. Sch. of Med, Sagamihara, Japan; [2]Cell & Mol. Physiol., Kitasato U. Grad. Sch. of Med. Sci., Sagamihara, Japan; [3]Mol. Diag., Kitasato U. Grad. Sch. of Med. Sci., Sagamihara, Japan; [4]Internal Med., Kitasato U. KMC Hospital, Kitamoto, Japan.*

Background: In mammalian kidneys, various types of carbonic anhydrase (CA) play important role for net HCO_3^- reabsorption at the proximal tubule and for HCO_3^- formation and reabsorption through the distal nephron. We investigated regulation of mice CA isozymes mRNAs along the nephron in response to NH_4Cl loading.

Methods: Localization and expression of both CAs (CAII, CAIV, CAXII, and CAXIV) and anion exchanger (AE1) mRNAs were assessed by a quantitative in situ hybridization (ISH) technique along the mouse (C57BL/6J, male, 10 weeks) nephron, in proximal convoluted and straight tubules (PCT and PST), descending and ascending thin limbs (DTL and ATL), medullary and cortical TAL (MTAL and CTAL), distal convoluted tubule (DCT), and cortical, outer medullary, and inner medullary collecting ducts (CCD, OMCD, and IMCD). Under the microscope, abundance of CAs and AE1 mRNAs (brown dots) was counted and evaluated during normal condition and acidosis (0.28 M NH_4Cl solution drinking, 6 days in metabolic cages).

Results: In normal condition, localization and expression of CAII mRNA was at PCT, CCD, and OMCD > DTL, MTAL, CTAL, and IMCD. Similarly, CAIV was at PCT and MTAL > CTAL and intercalated cell (IC) of OMCD, CAXII was at CCD-IC and OMCD-IC > PCT, CAXIV was at DTL > PCT. There was no expression of CAs at PST and DTL and ATL of inner medulla. Moreover, AE1 mRNA expressed at CCD-IC and OMCD-IC. During NH_4Cl loading, the CAII mRNA expression increased only at PCT. Similarly, CAIV increased at PCT and MTAL and CAXII increased at PCT, CCD-IC, and OMCD-IC. AE1 mRNA increased at OMCD-IC. However, there was no change in the CAXIV mRNA expression. Interestingly, cell height of the CAXII and AE1 mRNA-positive cell (α-IC of OMCD) increased specifically and significantly ($P < 0.005$) by 39.1% during acidosis.

Conclusions: Induction of CAXII may contribute to formation and reabsorption of HCO_3^- in α-IC of mice kidney OMCD, especially during acidosis.

Funding: Government Support - Non-U.S.

FR-PO074

Effect of Metabolic Acidosis on miRNA Expression in Rat Kidney Cortex and Isolated Proximal Convoluted Tubules Norman P. Curthoys, Lynn Taylor. *Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO.*

Background: Computational predictions and genome wide identification of targets suggest that expression of at least half of the human transcriptome is regulated by miRNAs. In mammalian cells, miRNAs inhibit gene expression primarily by enhancing the rate of degradation of specific mRNAs. Proteomic analysis identified >100 proteins that are increased in the rat proximal convoluted tubule during onset of metabolic acidosis. Many of the corresponding mRNAs contain AU-rich elements that have >85% sequence identity to the pH-response element that mediates stabilization of glutaminase mRNA during acidosis.

Methods: To investigate their possible role in this adaptation, miRNAs were isolated from renal cortex and proximal convoluted tubules derived from control and 4-h acidotic rats. Acidosis was induced by stomach loading with 20 mmol NH_4Cl/100g bd.wt. This protocol produced pronounced decreases in arterial blood pH (7.18 ± 0.03) and HCO_3^- (12.6 ± 0.3 mM). The miRNAs were screened using a Rat miFinder PCR Array (Qiagen).

Results: Of the 84 miRNAs profiled on this array, only miR-22, miR144 and miR-182, were decreased significantly (3- to 4-fold) during acute acidosis. miR-182 is derived from a pre-miRNA that also encodes miR-96, miR-183 and miR-3553. Therefore, specific miScript miRNA qPCR assays were used to quantify the temporal changes in the 4 miRNAs from this cluster. As a control, miR-23a and miR-23b were also assayed. The individual qPCR assays established that within this cluster only miR-96 and miR-182 were decreased 2.5-fold in the 4-h acidotic samples. miR-96 was also decreased 3-fold in the samples prepared from isolated proximal convoluted tubules. However, the level of miR-182 was unchanged, suggesting that the decrease observed in cortex occurs in some other segment of the nephron. Array analysis of miRNAs obtained from isolated proximal convoluted tubules indicated that miR-22, -210, -32, -144, -let-7b, and RNU 6-2 were also decreased >3-fold.

Conclusions: The observed decreases in specific miRNAs may contribute to the stabilization of mRNAs that occur following onset of acidosis.

Funding: NIDDK Support

FR-PO075

Renal Adaptation to Metabolic Alkalosis in Mice Nilufar Mohebbi,[1,2] Alessandro Genini,[1] Carla Bettoni,[1] Carsten A. Wagner.[1] *[1]Institute of Physiology, University of Zurich, Zurich, Switzerland; [2]Division of Nephrology, University Hospital Zurich, Zurich, Switzerland.*

Background: In the collecting duct, the fine-tuning of salt and acid-base homeostasis is regulated by transport proteins located in at least two different cell types, intercalated and principal cells. The Cl^-/HCO_3^- exchanger AE1 expressed on the basolateral membrane characterizes acid-secretory type A intercalated cells while the apical anion exchanger pendrin is localized to bicarbonate secretory type B intercalated cells. AQP2 identifies principal cells. During acidosis, acid-secretory intercalated cells proliferate which may

contribute to the remodelling of the collecting duct. Several transcription factors such as Foxi1, GDF-15, and CP2L1 have been also reported to be involved in intercalated cell proliferation and differentiation.

Methods: This study aimed to investigate acute and chronic adaptive responses in the collecting duct to metabolic alkalosis. Mice were subjected to either tap water (control), 0.28 mmol/L NaCl, or 0.28 mmol/L $NaHCO_3$ in drinking water for either 12 hours, 24 hours, 2 days, or 7 days, respectively. Quantitative real-time rt-PCR was performed for different cell markers (AE1, pendrin, AQP2) and (transcription) factors implied in intercalated cells differentiation (Foxi1, GDF-15, and CP2L1). Western blotting was performed for AE1, pendrin, and AQP2.

Results: Pendrin mRNA was significantly increased after 24 hours of alkali load. In contrast, AE1 mRNA levels were diminished after 12 hours in $NaHCO_3$ treated mice. AQP2 mRNA was markedly increased in the $NaHCO_3$ group compared to control and NaCl treated animals. Notably, protein abundance of AE1 was decreased after 24 hours, pendrin protein expression was not different between groups, and AQP2 protein levels were significantly elevated after 24 hours, 2 and 7 days. Foxi1 mRNA levels were increased after 24 hours and 2 days, and GDF-15 after 2 days alkali load.

Conclusions: In conclusion, during alkalosis key acid base transport proteins in the collecting duct are regulated. Factors implied in regulating intercalated cell proliferation and differentiation show transient and distinct patterns of regulation and may have an impact on this adaptive response.

Funding: Government Support - Non-U.S.

FR-PO076

An Evidence of Transdifferentiation of Principal Cells Into Intercalated Cells in Hypokalemic *AQP2-cre;ROSA Mouse Kidney* Sun-ah Nam, Jules Kun Hyoe Rhoo, Yumi Kim, Jin Kim, Wan-Young Kim. *Anatomy and Cell Death Disease Research Center, The Catholic University of Korea, Seoul, Republic of Korea.*

Background: In K^+-depleted animals, there was a reversible increase in number of intercalated cells (ICs) in the inner stripe part of outer medullary collecting duct (OMCD), but the cell proliferation had been mainly detected in the principal cells (PCs). A suggested mechanism to explain the increase of ICs in K^+-depleted animal was a direct or indirect conversion of PCs to ICs. The purpose of this study was to propose the possible transdifferentiation of PCs to ICs using a kidney of hypokalemic *AQP2-cre;ROSA mouse.*

Methods: Adult male transgenic mice *AQP2-cre;ROSA)* were used in the study and the experimental group was fed with K^+-depleted diet for two weeks. Each Ics and PCs were immunolabeled with specific markers for H^+-ATPase and AQP2, respectively.

Results: As a result, in the control group, ROSA-positive signal was mainly evidenced in PCs (35.4% of H^+-ATPase-negative/AQP2-positive PCs), but a few ICs showed ROSA-positive signal (5.5% of H^+-ATPase-positive/AQP2-negative ICs) even in the normal kidney. In hypokalemic kidney, the proportion of ROSA-positive cells in ICs was significantly increased to 16.2% but the proportion of ROSA-positive cells in PCs (37.0% of H^+-ATPase-negative/AQP2-positive PCs) was comparable to the control group.

Conclusions: In conclusion, we propose a mechanism of transdifferentiation of the ICs from AQP2-ROSA-positive PCs in OMCD, causing the increase in number and proportion of ICs at least in hypokalemic mice.

Funding: Other U.S. Government Support

FR-PO077

Double Knockout of Carbonic Anhydrase II (CAII) and Na-Cl Ctransporter (NCC) Causes Robust Salt Wasting, Volume Depletion and Kidney Failure in Mice Jie Xu,[1,2] Sharon Barone,[1,2] Kamyar A. Zahedi,[1,2] Manoocher Soleimani.[1,2] *[1]Research Services, Veterans Administration Hospital; [2]Medicine, University of Cincinnati.*

Background: The Cl^-/HCO_3^- exchanger pendrin and the NaCl cotransporter NCC are expressed in the distal nephron and mediate salt absorption. NCC KO mice display no salt wasting under baseline conditions but demonstrate upregulation of pendrin in their kidneys (JASN 2007). CAII is abundantly expressed in intercalated cells and plays an important role in acid base transport in intercalated cells. CA II KO (Car2 null) mice display significant reduction in the number of intercalated cells and pendrin expression (AJP:Renal 1995 and Cell Physiol Biochem 2008).

Methods: We hypothesized that pendrin plays an essential role in distal tubule salt absorption in the setting of NCC inactivation. To test our hypothesis, CAII KO (Car2 null) mice which show significant pendrin downregulation were crossed with NCC KO mice to generate double NCC/CAII KO mice.

Results: Northern and western blots showed that the kidney expression of pendrin is reduced by 80% in CAII KO KO or NCC/CAII double KO mice Vs. WT mice ($P<0.01$). Balanced studies demonstrate the presence of significant salt and water wasting in NCC/CAII double KO mice, with urine output of 3.5 ± 0.3 ml/24 hr in double KO mice Vs. 0.8-1.2 ml/day in WT or single KO mice ($p<0.01$). Excretion of sodium and chloride increased by 40 and 60%, respectively, in CAII/NCC double KO mice Vs. other genotypes ($p<0.01$). Western blots demonstrated that the expression of renin increased by ~300% in kidneys of double CAII/NCC KO mice Vs. other genotypes. BUN levels increased in double CAII/NCC KO mice, consistent with prerenal failure. High salt diet for 7 days significantly improved the expression of renin and reduced BUN levels in double CAII/NCC KO mice.

Conclusions: We conclude that pendrin upregulation is essential for the prevention of salt wasting in NCC KO mice and its downregulation in the setting of NCC inactivation will lead to salt wasting and volume depletion. We propose that the combined inhibition of pendrin and NCC can be a novel and potent diuretic regimen for the treatment of fluid overloaded states, such as CHF.

Funding: NIDDK Support, Veterans Administration Support

FR-PO078

The Carbonic Anhydrase Inhibitor Acetazolamide (ACTZ) Downregulates Pendrin in the Kidney and in Conjunction with Hydrochlothiazide (HCTZ) Causes Massive Diuresis, Salt Wasting, and Volume Depletion Sharon Barone,[1,2] Jie Xu,[1,2] Kamyar A. Zahedi,[1,2] Manoocher Soleimani.[1,2] *[1]Department of Medicine, University of Cincinnati, Cincinnati, OH; [2]Research Services, Veterans Administration Hospital, Cincinnati, OH.*

Background: The Cl^-/HCO_3^- exchanger pendrin and the thiazide-sensitive Na-Cl cotransporter NCC are expressed in the distal nephron and mediate salt absorption. Treatment with ACTZ for 2 weeks decreases the number of B-intercalated cells in rat kidney (Bagnis et al, AJP:Renal 2001). Hypothesis: ACTZ downregulates pendrin and primes the kidney for salt wasting when HCTZ is added, subsequent to combined NCC/pendrin inactivation.

Results: Male Sprague Dawley rats (150-200 gm) were treated with daily injection of ACTZ for two weeks. Urine output increased from 11 ± 1 to 27 ± 3 ml/day after 14 days of ACTZ injection ($p<0.01$, $n=5$). Northern and western blots showed ~80% reduction in pendrin expression and immunofluorescence labeling indicated significant downregulation of pendrin labeling in kidneys of ACTZ-treated rats. In separate studies, daily injection of rats with HCTZ alone for 4 days mildly increased the urine output, from 11.5 ± 1 to 13.7 ± 2 ml/day. However, treatment of rats that were pretreated with ACTZ for 14 days with daily HCTZ injection increased the urine output from 27 ± 3 to 59 ± 8 ml/day on day 4 of HCTZ injection ($p<0.01$, $n=5$). Animals treated with ACTZ plus HCTZ displayed significant salt wasting and developed severe volume depletion, as determined by a 3-fold increase in renin mRNA and protein abundance in their kidneys. BUN levels increased significantly in ACTZ plus HCTZ group Vs. other groups ($p<0.01$).

Conclusions: 1. ACTZ downregulates the expression of pendrin and primes the kidneys for profound salt and water wasting when thiazide is added. 2. The thiazide sensitive Na-Cl cotransporter plays a major role in the maintenance of vascular volume in the setting of pendrin inactivation and increased delivery of salt from proximal tubule caused by ACTZ. 3. Despite being considered mild agents individually, we propose that the combination of ACTZ and HCTZ is a powerful diuretic regimen for the treatment of fluid overloaded states.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO079

Localization, Regulation, and Relevance of the (Pro)renin Receptor/Atp6ap2 for H^+-ATPases in Kidney Arezoo Daryadel,[1] Marta Figueiredo,[1] Soline Bourgeois,[1] Nicole Beate Kampik,[1] Nilufar Mohebbi,[1] Marcel Meima,[2] Alexander H. Danser,[2] Carsten A. Wagner.[1] *[1]Inst of Physiology, Univ of Zurich, Zurich, Switzerland; [2]Depart of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.*

Background: The (Pro)renin receptor (PRR) has been identified as a cell surface protein capable of binding and non-proteolytically activate prorenin. Surprisingly, PRR is associated with H^+-ATPases and alternative functions in H^+-ATPase regulation as well as in Wnt signalling have been suggested. The kidneys express very high levels of H^+-ATPase which are involved in multiple functions in endocytosis, membrane protein recycling as well as urinary acidification and salt absorption.

Methods: In this study we investigated the expression pattern of PRR in kidney and its possible role in H^+-ATPase function using real-time PCR, immunoblotting, immunohistochemistry, animal and cell culture studies, and micropferfusionof isolated collecting ducts.

Results: qPCR and immunohistochemistry demonstrated expression of the PRR along the entire mouse and rat nephron with highest levels in the collecting system coinciding with H^+-ATPases. Further experiments demonstrated expression in all types of intercalated cells colocalizing with H^+-ATPases. In mice treated with NH4Cl, NaHCO3, KHCO3, NaCl, or DOCA for 7 days, PRR and the B1 H^+-ATPase subunit were increased following the NaHCO3 treatment in both cortex and medulla at protein and mRNA levels whereas NH4Cl reduced PRR and B1 expressions only in cortex. Lastly, microperfusion experiments of isolated cortical collecting duct and application of 20 nM prorenin did not stimulate H^+-ATPase activity.

Conclusions: Our results suggest that the PRR may form a complex with H^+-ATPases in renal intercalated cells but that prorenin has no direct or acute effect on H^+-ATPase activity.

Funding: Government Support - Non-U.S.

FR-PO080

Expression of Rh B Glycoprotein (RhBG) in Human Kidney Hyun-Wook Lee,[1] Ki-Hwan Han,[2] Florence M. Whitehill,[1] Jill W. Verlander,[1] Mary E. Handlogten,[1] Jesse M. Bishop,[1] Byron P. Croker,[3] William L. Clapp,[3] I. David Weiner.[1,4] *[1]Renal Division, University of Florida, Gainesville, FL; [2]Department of Anatomy, Ewha Womans University, Seoul, Korea; [3]Pathology Department, University of Florida, Gainesville, FL; [4]Renal Section, NF/SGVHS, Gainesville, FL.*

Background: Rh B Glycoprotein is critical for renal ammonia metabolism in mice, but whether it is expressed in human kidney is controversial. This study's purpose was to reassess human kidney RhBG expression.

Methods: We used standard gene sequencing, site-directed mutagenesis, cell-expression, immunoblot and immunohistochemistry methods. Human mRNA and protein homogenates were from commercial sources. Tumor-free portions of kidneys removed for treatment of renal cancer were used for immunohistochemistry.

Results: We identified reports of two distinct human RhBG mRNA sequences encoding different carboxy termini. Sequencing human kidney and liver mRNA in the region of difference showed 8 consecutive cytosines beginning at nt 1537, not 7 as has been reported, and was otherwise identical to both published sequences. Genomic DNA confirmed these mRNA findings. Sequencing the commonly used GFP-RhBG vector showed only 7 cytosines, indicating it encodes a frame-shift mutation of human RhBG. We mutated the RhBG vector to the correct sequence and expressed it in HEK-293 cells. An antibody against the first extracellular loop labeled non-glycosylated and glycosylated RhBG protein from RhBG-transfected cells, but not protein from mock- or human RhCG-transfected cells. Immunoblot analysis of human kidney showed RhBG protein expression. Immunohistochemistry identified basolateral RhBG immunolabel in the connecting segment and the cortical and outer medullary collecting ducts. Double-immunolabel of RhBG with H^+-ATPase or pendrin identified RhBG expression in CNT cells and A-type and non-AB intercalated cells; Type B intercalated cells and principal cells did not express detectable RhBG.

Conclusions: We show for the first time that RhBG protein is expressed in the human kidney in CNT cells and both A-type and non-AB intercalated cells, where it is ideally located to mediate an important role in human renal ammonia transport.

Funding: NIDDK Support, Veterans Administration Support

FR-PO081

Colonic α-H^+,K^+-ATPase and B1-H^+-ATPase mRNA Expression Declines in GPR4-/- Mice with Aging Juan Codina,[1] Snezana Petrovic,[2] Xuming Sun,[2] Raymond B. Penn,[3] Thomas D. DuBose.[1] *[1]Sections on Nephrology and Molecular Medicine, Departments of Internal Medicine and Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Section of Nephrology and Molecular Medicine, Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC; [3]Department of Medicine, University of Maryland, Baltimore, MD.*

Background: The H^+,K^+-ATPase and H^+-ATPase participate importantly in systemic acid-base homeostasis to defend against metabolic acidosis. We have shown that plasma membrane expression of the colonic α H^+,K^+-ATPase ($HK\alpha_2$) is increased in response to a reduction in both pH_a and extracellular $[K^+]$. Furthermore we have shown that pH-dependent regulation of $HK\alpha_2$ is dependent on an increase in phosphorylation mediated by PKA to promote maturation and plasma membrane expression. Co-expression of GPR4, a proton-sensing Gs coupled receptor, increases $HK\alpha_2$ protein abundance in HEK-293 cells stably transfected with $HK\alpha_2$.

Methods: In the present study, we applied quantitative real time RT PCR to determine mRNA expression of $HK\alpha_2$ and the B1-subunit of H^+-ATPase in whole kidney of a mouse model of genetic ablation of GPR4 (GPR4-/-) in which the phenotype includes spontaneous metabolic acidosis.

Results: Our study demonstrates that while the mRNA expression of $HK\alpha_2$ and the B1-subunit of H^+-ATPase are similar in young GPR4+/+ and GPR4-/- mice (2.5±0.8 vs. 2.17±0.5 for $HK\alpha_2$, and 2.5±0.3 vs. 2.42±0.2, N=5, for B1). However, expression is dramatically decreased in age-matched older GPR4+/+ vs. GPR4-/- (2.42±0.5 vs. 0.83±0.1 units, N=7 and 13, respectively, $p<0.001$, for $HK\alpha_2$, and 1.75±0.2 vs. 1.1±0.1, N=7 and 13, respectively, $p<0.05$) for B1.

Conclusions: In summary these data demonstrate that GPR4 ablation is associated with a marked decrease in $HK\alpha_2$ and B1- H^+-ATPase subunit mRNA expression in older mice. Therefore, $HK\alpha_2$ and B1- H^+-ATPase mRNA expression in aging rats may be dependent on the presence of GPR4, a putative pH sensor in the kidney.

FR-PO082

Microarray Analysis of Gene Expression in Kidneys of Proton Receptor Knockout Mice Doris P. Molina,[1] Xuming Sun,[1] Snezana Petrovic.[1,2] *[1]Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Internal Medicine Section of Nephrology, Wake Forest University School of Medicine, Winston-Salem, NC.*

Background: GPR4 is a GPCR activated by protons. It couples with Gs and stimulates cAMP/PKA signaling. GPR4-/- develop dRTA, suggesting that GPR4 may function as a pH sensor required for acid-base homeostasis.

Methods: To further examine GPR4's role in the kidney, we performed microarray analysis of gene expression in the kidney tissue from GPR4-/- and +/+, using Operon Array-Ready Mouse Genome Oligo Set from Qiagen (38,784 optimized 70-mer oligos used for microarrays). Genes with $p<0.01$ were further analyzed with IPA, Ingenuity Systems Software.

Results: We found that deletion of GPR4 upregulated 509 and downregulated 362 genes. Changes in several genes involved in response to acidosis like the a4 subunit of H^+-ATPase (ATP6V0A4) and ammonium transporter Rhcg and the PKA signaling pathway (AKAP12, PKAr, filaminA) corroborated GPR4 involvement in acid-base regulation and its Gs coupling. Phosphate transporter Slc17a2 was increased 3.6x, consistent with lower urine phosphate excretion in GPR4-/- vs. GPR4+/+. A number of genes involved in salt and volume homeostasis were upregulated: SGK1, aENaC, renin, angiotensin converting enzyme (ACE), and Cyp4a14. Another significantly modulated group of genes was linked to the metabolism of arachidonic acid included gluthathione peroxidase 6 and several microsomal monooxygenases.Genes involved in tissue remodeling and fibrosis were also affected: adiponectin, erythropoetin, hexokinase, hypoxia-inducible factor 3α, renin, ACE were upregulated, NF kB transcription factor was activated, and Kim1, uric acid transporter (URAT1) and xanthine dehydrogenase were downregulated. Significant changes were also found among cancer related gene networks (including p21 and IGF2), consistent with reports showing that GPR4 influences tumor growth and angiogenesis.

Conclusions: In summary, the analysis of the kidney transcriptome of GPR4-/- vs. +/+ suggests that GPR4 may mediate pH dependent regulation of genes involved in salt and volume homeostasis, cancerous transformation, and tissue remodeling and fibrosis.

Funding: Private Foundation Support

FR-PO083

Basolateral P2X Receptors Trigger a Marked Alkalinization of Mouse Thick Ascending Limb Pauline I.A. De Bruijn, Thomas Pudlarz, Rita D. Marques, Helle A. Praetorius, Jens G. Leipziger. *Department of Biomedicine, Aarhus University, Aarhus, Denmark.*

Background: Extracellular ATP is an important regulator of renal tubular transport. Recently, we found that basolateral ATP markedly inhibits NaCl absorption in mouse medullary thick ascending limb (mTAL) via a P2X receptor. However, the underlying mechanism of this ATP-dependent transport inhibition in mTAL remains unclear.

Methods: In this study, we investigated basolateral ATP-induced alterations of pH_i in single perfused mouse mTALs using the pH indicator dye BCECF.

Results: Interestingly, basolateral ATP (100 μM) caused a prominent and reversible alkalinization of mTAL, with an average pH_i increase of 0.14 ± 0.01 (n=12). This effect was completely abolished in presence of the P2X receptor antagonist oxidized-ATP (50 μM). Typically, G-protein coupled receptors that trigger an increase of $[Ca^{2+}]_i$ cause a significant acidification of tubular epithelial cells. This was also observed in this study, when $P2Y_2$ receptors were stimulated with 100 μM UTP. The pH_i decrease was 0.04 ± 0.01 (n=6). When the CaSR was stimulated with 5 mM Ca^{2+}, a similar acidification was observed.

Conclusions: This study describes the surprising finding of a basolateral P2X receptor-triggered alkalinization in isolated mouse mTAL. These data may provide the basis to understand the signaling mechanism responsible for the pronounced ATP-induced transport-inhibition in mouse mTAL.

FR-PO084

Aurora Kinase A (AURKA) Regulates the Vacuolar H^+-ATPase (V-ATPase) in Human Kidney Carcinoma Cells via a Subunit Phosphorylation Mohammad M. Al-bataineh, Rodrigo Alzamora, Fan Gong, Allison L. Marciszyn, Nuria M. Pastor-Soler. *Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: The V-ATPase mediates ATP-driven transport of H^+ and is present at the apical membrane of some kidney proton secreting cells. The V-ATPase has been implicated in the process of metastasis. Phosphorylation of the V-ATPase A subunit at S175 is important for PKA-mediated V-ATPase activity in intercalated cells. We hypothesized that AURKA, a kinase that when overexpressed leads to aggressive carcinomas, regulates the V-ATPase in human kidney carcinoma cells (Caki2) by phosphorylating S175.

Methods: In vitro and in vivo phosphorylation assays of the V-ATPase WT and S175AA subunits by AURKA were performed. Wound healing assays of Caki2 cells were performed in the presence/absence of an AURKA activator. V-ATPase distribution was examined by immunofluroescence labeling.

Results: AURKA is abnormally expressed in the cytoplasm of Caki2 cells. AURKA phosphorylates the V-ATPase A subunit at S175 in vitro. AURKA activation increased WT A subunit phosphorylation in Caki2 cells, but failed to increase phosphorylation levels of the S175A mutant. Immunoprecipitation assays of Caki2 cells transfected with A subunit constructs were performed followed by immunoblots for anti-phospho-S175. Higher levels of phospho-S175 were detected from Caki2 cells transfected with WT A subunit and treated with an AURKA activator, compared to untreated cells, and compared to cells expressing the S175A mutant. Immunofluorescence labeling of Caki2 cells revealed that the AURKA activator induced a more marked V-ATPase membrane accumulation in cell projections after wounding compared to untreated cells. Furthermore, AURKA activation enhanced the rate wound healing in Caki2 cells compared to untreated cells.

Conclusions: Overexpressed cytoplasmic AURKA in kidney carcinoma cells phosphorylates a key V-ATPase residue that induces V-ATPase membrane accumulation. The AURKA-mediated V-ATPase regulation may have a role in the metastatic potential of kidney carcinomas and may represent a potential therapeutic target (supported by NIH/NIDDK).

Funding: NIDDK Support, Pharmaceutical Company Support - DCI

FR-PO085

Hypoxia-Inducible Factor (HIF)-3α Is a HIF-1 Target Gene and Counteracts Migration of Proximal Tubular Cells in Hypoxia Tetsuhiro Tanaka,[1] Kumi Shoji,[2] Junna Yamaguchi,[2] Masaomi Nangaku.[2] *[1]Division for Health Service Promotion, University of Tokyo, Tokyo, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan.*

Background: Tubulointerstitial hypoxia is a common mediator in progressive renal disease. The expression of two major isoforms of HIF-α, HIF-1α and HIF-2α, is determined by the posttranslational escape from prolyl hydroxylation and proteasomal degradation, and they display preferential spatial distribution in the tubular (HIF-1α) and interstitial (HIF-2α) compartments of the kidney. On the other hand, the expression of the HIF-3α gene seems to undergo transcriptional regulation, and the spatial distribution as well as the functional role of HIF-3 in the kidney has not been investigated in depth.

Methods: mRNA of HIF-3α splicing variants was quantified by real-time PCR. Localization of HIF-3α protein was immunohistochemically characterized in kidneys subjected to experimental infarction. Hypoxia-responsive enhancer in the HIF-3α promoter was identified by luciferase reporter assays and chromatin immunoprecipitation (ChIP) analyses. Migration of tubular epithelial cells was evaluated by scratch assays.

Results: Out of multiple splicing variants of mouse HIF-3α mRNA, the expression of splicing variant 2 (sv2) and the inhibitory PAS domain protein (IPAS) was observed in mouse cortical tubular cells (MCT), in a hypoxia-inducible manner. In vivo, systemic hypoxia in mice led to 2.7±0.4 and 23.0±9.3 fold increases in renal HIF-3α sv2 and IPAS mRNA, respectively. Immunohistochemical analysis of the infarcted kidney identified immunoreactive HIF-3α protein in HIF-1α- positive, hypoxic tubules. Promoter analysis of the mouse HIF-3α gene identified a novel hypoxia-responsive element at 709 nucleotides upstream of the IPAS translational start site. ChIP assays confirmed recruitment of HIF-1α to the identified enhancer. Functionally, stable overexpression of HIF-3α in human proximal tubular cells (HK-2) resulted in suppression of cell migration under hypoxia.

Conclusions: Results of these studies signify the hypoxic, HIF-1-mediated induction of HIF-3α mRNA and suggest a possible involvement of HIF-3 in the pathogenesis of hypoxic renal disorders.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO086

Indoxyl Sulfate Induces Cbp/p300-Interacting Transactivator, with Glu/Asp-Rich Carboxy-Terminal Domain, 2 (CITED2), via the Mitogen-Activated Protein Kinase Cascade and Impairs Hypoxia Response of Tubular Epithelial Cells Tetsuhiro Tanaka,[1] Junna Yamaguchi,[2] Masaomi Nangaku.[2] *[1]Division for Health Service Promotion, University of Tokyo, Tokyo, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan.*

Background: Chronic hypoxia in the tubulointerstitium serves as a final common pathway in progressive renal disease. Hypoxia-inducible factor (HIF)-1α is expressed in the ischemic tubular epithelial cells, but the functional operation of HIF-1 may be influenced in a CKD milieu, and we hypothesized that indoxyl sulfate (IS), a representative uremic toxin, might impair cellular hypoxia response by HIF-1.

Methods: In human proximal tubular cells (HK-2), the expression of HIF-1α, CITED2 and the hypoxic induction of HIF-1-target genes were measured by immunoblotting and real-time PCR. Binding of HIF-1 to the target gene promoter was evaluated by chromatin immunoprecipitation (ChIP). Association of the cofactor p300 with HIF-1α was assessed by mammalian two-hybrid assays. mRNA stability of CITED2 was measured by actinomycin D treatment. The role of MAP kinase pathways was evaluated by using specific inhibitors.

Results: IS reduced the hypoxic induction of HIF-1-target gene mRNA and protein. This effect was not accompanied by a parallel quantitative change in the HIF-1α protein, but was associated with the functional impairment of the HIF-1α C-terminal transactivator domain (CTAD). Out of candidate factors which impede the recruitment of transcriptional cofactors to the HIF-1αCTAD, CITED2 protein was markedly upregulated by IS. The induction of CITED2 was mediated by posttranscriptional mRNA stabilization, which was blunted by treatment with an inhibitor of extracellular signal-regulated kinase (ERK)1/2, U0126. A series of luciferase assays using various CITED2 mRNA fragments as a synthetic 3'untranslated region (3'UTR) of the luciferase gene revealed that the entire coding region of CITED2 was required for the IS-mediated mRNA stabilization.

Conclusions: Results of these studies uncover a novel role of IS in modulating the transcriptional response by HIF-1, which is mediated through activation of the MAP kinase signaling and stabilization of CITED2 mRNA.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO087

Dipeptidyl Peptidase (DPP) IV Inhibitor Attenuates Kidney Injury in Rat Remnant Kidney Shin-young Ahn,[1] Sejoong Kim,[1] Ho Jun Chin,[1] Kwon Wook Joo,[2] Dong Wan Chae,[1] Ki Young Na.[1] *[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: DPP IV inhibitor has been reported to reduce renal damage in streptozotocin induced diabetic rat. Forkhead box O (FoxO) transcriptional factors regulate glucose metabolism, cell cycle, and detoxification of reactive oxygen species. The aim of this study was to investigate whether DPP IV inhibitor, sitagliptin, could attenuate kidney injury and to evaluate the status of FoxO in the rat remnant kidney.

Methods: After a subtotal (5/6) nephrectomy (Nx), rats were randomly assigned to control or treatment group. Rats in treatment group received food mixed with 200mg/kg/day of sitagliptin for 8 weeks. We measured physiological data, evaluated histological renal injury, performed immunohistochemical staining with ED-1, and assessed apoptosis via TUNEL assay. We examined the expression level of glucagon-like peptide-1 receptor (GLP-1R) and the phosphorylation status of FoxO3a.

Results: Sitagliptin ameliorated renal dysfunction (sCr and CrCl, Nx vs Nx + sitagliptin: 0.9±0.03 vs 0.7±0.07 mg/dl, p=0.021 & 0.3±0.01 vs 0.4±0.04 ml/min/100g, p=0.037). It also attenuated the extent of glomerulosclerosis (score: 3.2±0.6 vs 1.9±0.8, p=0.004) and tubulointerstitial injury (score: 2.7±0.5 vs 1.9±0.6, p=0.019). GLP-1R expression was increased (p=0.016) and the level of phosphorylated FoxO3a was decreased in the treatment group (p=0.013). Consequently, the expression of catalase, anti-oxidant, was increased (p=0.047), the number of apoptotic cells were reduced (absolute No.: 57.4±12.9 vs 17.6±4.6, p<0.001), and the expression levels of cleaved caspase-3, -9 and Bax were also decreased in this group (p=0.009, 0.016, & 0.009 respectively). As well the number of ED-1 positive cells was decreased (p=0.003) and the TGF-ß expression tended to decrease but it was statistically insignificant (p=0.172).

Conclusions: In rat remnant kidneys, DPP IV inhibitor increased the expression of GLP-1R and attenuated kidney injury by anti-oxidative, anti-apoptotic, and anti-inflammatory action through FoxO3a pathway.

FR-PO088

Regulation of Tubule Cell Nrf2 Phosphorylation by Diabetes and High Protein Concentrations Michelle T. Barati, Susan M. Isaacs, Jason R. Parks, Abuhusnain S. Khundmiri, Jon B. Klein. *Nephrology, University of Louisville, Louisville, KY.*

Background: Tubule cell dysfunction is significant to diabetic nephropathy pathogenesis. Tubule cell exposure to high glucose and protein concentrations in diabetes increases reactive oxygen species (ROS). Activation of nuclear factor erythroid-derived 2-related factor 2 (Nrf2) is a compensatory mechanism to alleviate oxidative stress, accomplished in part by transcriptional induction of enzymes which synthesize glutathione, and NAD(P) dehydrogenase, quinone-1 (NQO1). Activated Nrf2 undergoes Ser-40 phosphorylation and nuclear translocation and regulation of this process by diabetes specifically in renal tubule cells, is not defined. This study addressed the hypothesis that Nrf2 phosphorylation would be altered in tubules of diabetic mice and proximal tubule (PT) cells exposed to high protein concentrations, mimicking proteinuria, conditions known to induce oxidative stress.

Methods: Phospho-Ser-40 Nrf2 (P-Nrf2) was analyzed by immunohistochemistry of kidney sections from 3 and 7 months old OVE26 diabetic and FVB control mice, followed by quantification of staining in cortical regions. For *in vitro* studies, human PT cells (HK2) were treated with 1mg/ml human serum albumin (HSA). Total, cytosol, and nuclear extracts were analyzed for Nrf2, P-Nrf2, and the Nrf2 target NQO1.

Results: P-Nrf2 was decreased in cortical tubules of 7 month old mice in both groups, compared to 3 month old mice. Furthermore, cortical tubules of 7 month old diabetic mice had >50% less nuclear P-Nrf2 compared to age-matched non-diabetic mice. Exposure of HK2 cells to HSA increased P-Nrf2, its nuclear translocation, and NQO1 expression, demonstrating Nrf2 activation.

Conclusions: Decreased Nrf2 phosphorylation in cortical tubules of older mice suggests dysregulation of this compensatory pathway with age and further attenuation with diabetes may exacerbate oxidative stress. Activation of Nrf2 and induction of NQO1 expression in HK2 cells following exposure to high protein concentrations suggests that the diabetic milieu alters tubule cell compensatory responses to known inducers of oxidative stress, such as proteinuria.

Funding: NIDDK Support

FR-PO089

Signaling Downstream of p38 MAPK Is Channeled towards HSPB1 and HSPB5 Dimers in Podocytes and Mesangial Cells Ruma Pengal,[1] Adam J. Guess,[1] Rainer Benndorf,[1,2] William E. Smoyer.[1,2] *[1]Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; [2]Department of Pediatrics, The Ohio State University, Columbus, OH.*

Background: Cells respond to unfavorable conditions with a complex signaling pattern that constitutes the stress response. Activation of p38 MAPK signaling is part of this stress response, and is transduced downstream largely via the protein kinase MK2. MK2 itself can phosphorylate ~25 known effector molecules, including the small heat shock proteins (sHSP) HSPB1 and HSPB5. In nephrotic syndrome (NS), increased p38 MAPK signaling in podocytes was reported to be associated with injury, whereas its inhibition protected cells from injury and also ameliorated proteinuria in animal models. However, the molecular pathways downstream of MK2 that are involved in cell injury and protection are not understood.

Methods: *In vitro* cultured podocytes and mesangial cells were exposed to various stress conditions (osmotic, oxidative and metal toxicant stress) relevant to kidney physiology. Activation of p38 MAPK→MK2 signaling was determined via phosphorylation of MK2 substrates, including HSPB1, HSPB5 and others. To determine the intracellular spatial distribution of p38 MAPK→MK2 signaling, the subcellular distribution of phosphorylated vs. total HSPB1 was determined by immunofluorescence microscopy.

Results: The applied stress treatments greatly activated p38 MAPK→MK2 signaling in both podocytes and mesangial cells. Surprisingly, the signal was transduced primarily towards HSPB1 and covalently-linked HSPB5 dimers (a novel species of this sHSP), while

other tested MK2 substrates were not notably phosphorylated in response to these stress treatments. In podocytes, phosphorylated HSPB1 appeared enriched in the perinuclear space, whereas total HSPB1 was evenly distributed throughout the cytoplasm.

Conclusions: Activation of p38 MAPK→MK2 signaling in podocytes and mesangial cells is channeled primarily towards HSPB1 and HSPB5 dimers, rather than other MK2 substrates. This suggests that phosphorylation of HSPB1 and/or HSPB5 dimers may play a pathophysiologic role in cell injury in NS.

FR-PO090

The Molecular Basis of the Renal and Vascular Consequences of Uremia

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Background: Chronic renal failure is characterized by progressive renal scarring and accelerated cardiovascular disease. In animal models, this has been attributed to non-dialyzable uremic toxins – small, protein-bound molecules normally secreted via Organic Anion Transporters (OATs) in the proximal renal tubule rather than filtered at the glomerulus. The best studied of these is indoxyl sulfate (IS).

Methods: We examined global gene expression in cultured normal human renal tubular cells (Innovative BioTherapies) incubated with control plasma (n=5) or pre- and post-dialysis uremic plasma (n=10). After 24 hours of exposure, total cellular RNA was extracted and analyzed with Affymetrix cDNA microarrays.

Results: Incubation with uremic plasma significantly altered expression of 2016 genes. The expression of 537 genes normalized in post-dialysis plasma, suggesting removal of low molecular weight uremic toxins by dialysis. The expression of the majority of dysregulated genes (1479) was not normalized in post-dialysis plasma. These likely represent the effects of substances such as IS, not effectively removed by dialysis (protein-bound uremic toxins). Addition of IS to control plasma simulated most effects (81.1%) of uremic plasma; the addition of probenecid, an OAT inhibitor, to uremic plasma reversed most changes in gene expression. Analysis of molecular programs with the NIH-curated DAVID database, confirmed by Gene Set Enrichment Analysis (GSEA), revealed patterns common to IS-treated control plasma, pre-dialysis, and post-dialysis uremic plasma; these included increased cell turnover, pro-inflammatory, and pro-fibrotic molecular programs, particularly effectors and targets of the TGF-β pathway.

Conclusions: Changes in the gene expression profile were observed in human renal cortical cells cultured with plasma from uremic patients: most were not corrected by dialysis. The emergent patterns confirm that inflammatory and pro-fibrotic programs are active and there is increased cellular proliferation. These findings suggest an important role of non-dialyzable, protein-bound uremic toxins in the pathogenesis of renal scarring and uremic vasculopathy.

Funding: Private Foundation Support

FR-PO091

Erythropoietin Activates NAD(P)H Oxidase via Erythropoietin Receptor and Beta Common Receptor on Vascular Endothelial Cells

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Background: Recent clinical trials have demonstrated that erythropoietin (EPO) is not always beneficial in preventing cardiovascular diseases. Nevertheless, most of experimental studies performed in animal models, higher doses are usually used, indicated that EPO exerts vascular-protective activities through non-hematopoietic actions. Thus, it is still controversial whether clinically applicable doses of EPO are beneficial for cardiovascular protection. There are two putative receptors for EPO. One is the homo-dimeric EPO receptor (EPO-R) responsible for erythropoiesis. Another is a hetero-dimeric receptor that consists of the EPO-R and the beta common receptor (bcR). We investigated whether administration of therapeutic doses of EPO could accentuate oxidative stress and modulate endothelial function.

Methods: In-vivo experiments, normal male Sprague–Dawley (SD) rats were treated with either EPO (20 IU•kg-1•week-1 subcutaneously) 3 times a week or darbepoetin (D-EPO; 0.1 μg•kg-1•week-1 subcutaneously) once a week for 4 weeks. Endothelial-dependent vasodilatory response, NADPH oxidase activity, and gene expression of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) were assessed. Human umbilical vein endothelial cells were stimulated with EPO to assess NADPH oxidase activity. Possible involvement of either EPO-R or bcR in signal transduction of EPO was identified in small interfering RNA knockdown experiments.

Results: The acetylcholine-dependent vasodilatory response was impaired significantly in both EPO and D-EPO treatment groups. NAD(P)H oxidase activity and aortic gene expression of ICAM-1 and MCP-1 augmented in both groups. We confirmed EPO-mediated superoxide production and extracellular signal-regulated kinase (ERK) activation in vitro. Both EPO-R and bcR were essential for ERK activation by EPO in endothelial cells.

Conclusions: Administration of EPO and D-EPO increased oxidative stress and impaired endothelial function in normal rats and in human endothelial cells.

FR-PO092

IL-6 Increases Production of Galactose-Deficient IgA1 by IgA1-Secreting Cells from IgA Nephropathy Patients through STAT3 Signaling Pathways

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Background: IL-6 is a B-cell differentiation factor that regulates IgA1 production through STAT3 and STAT1. We have shown that IL-6 increases production of galactose-deficient IgA1 (Gd-IgA1) in IgA1-secreting cells from IgA nephropathy (IgAN) patients but not in those from healthy controls (HC). Here, we analyzed the regulatory pathways involved in IL-6-induced production of Gd-IgA1 in IgAN.

Methods: EBV-immortalized IgA1-secreting cells derived from the circulation of IgAN patients and HC were stimulated with IL-6. Levels of IgA1 and Gd-IgA1 were determined by ELISA. STAT3 and STAT1 phosphorylation was analyzed using SDS-PAGE and Western blotting and confirmed by using specific inhibitors of signaling.

Results: IL-6 increased production of IgA1 by 45% in cells from HC and by 15% in cells from IgAN. Moreover, IL-6 increased the production of Gd-IgA1 in the cells only from IgAN patients, by 33%. Phospho-STAT3 was up-regulated by IL-6 in a dose-dependent manner, and to a higher degree (2.5 fold) in the cells from IgAN patients compared to those from HC. Phospho-STAT1 was down-regulated by IL-6 in IgAN and HC cells. Inhibitors of STAT signaling prevented IL-6-induced increase in production of IgA1 and Gd-IgA1 in a dose-dependent manner. One of the inhibitors of STAT signaling blocked IL-6-induced increase of IgA1 by up to 112% in HC cells and up to 68% in IgAN cells, and reduced production of Gd-IgA1 in IgAN cells by up to 93%. This blockade was associated with down-regulation of STAT3 phosphorylation.

Conclusions: IL-6 increased production of Gd-IgA1 in IgAN through STAT3 activation. Thus, blockade of STAT3 signaling may be considered as a possible future therapeutical approach in IgAN.

Funding: NIDDK Support, Other NIH Support - GM098539

FR-PO093

Signaling Pathways that Stimulate Production of Galactose-Deficient IgA1 in Tonsillar Cells from Patients with IgA Nephropathy

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Background: IgA1-producing cells from tonsils of IgA nephropathy (IgAN) patients secrete galactose-deficient IgA1 (Gd-IgA1), one of the key factors in the pathogenesis of this disease. The pathways involved in the production of Gd-IgA1 are not well understood. Here, we characterized cytokine-induced signaling pathways leading to overproduction of Gd-IgA1 in tonsillar cells.

Methods: We assessed the effects IL-6 on IgA1 production and *O*-glycosylation using EBV-immortalized IgA1-secreting cell lines derived from tonsils (IgAN-TC) and the peripheral blood (IgAN-PB) of IgAN patients, tonsils of controls with sleep apnea syndrome (HC-TC), and the peripheral blood (HC-PB) of healthy controls. STAT3 and STAT1 phosphorylation was determined by SDS-PAGE and Western blotting and validated by using specific inhibitors of signaling.

Results: IL-6 increased production of Gd-IgA1 in IgAN-TC and IgAN-PB, but not in HC-PB or HC-TC. This effect was mediated by STAT3 signaling. STAT3 phosphorylation was up-regulated and that of STAT1 down-regulated by IL-6 in a dose-dependent manner, and to a higher degree in IgAN-TC compared to HC-TC and HC-PB. Inhibitors of STAT3 signaling prevented, in a dose-dependent manner, IL-6-induced increase in production of IgA1 in all cell lines and that of Gd-IgA1 in IgAN-TC and IgAN-PB. One inhibitor that blocked IL-6-induced phosphorylation of STAT3 in all cell lines up-regulated phosphorylation of STAT1 in cells from the circulation but not in cells from tonsils, suggesting differences between IgAN-TC and IgAN-PB.

Conclusions: IL-6 enhances production of Gd-IgA1 in IgA1-secreting cells from tonsils and the circulation of patients with IgAN. This process is mediated by STAT3/STAT1 signaling and inhibitors of the signaling pathways may be considered for future therapeutic approaches. These findings may be relevant to the consideration of tonsillectomy in some patients with IgAN.

Funding: NIDDK Support, Other NIH Support - GM098539

FR-PO094

Paricalcitol Attenuates 4-Hydroxy-2-Hexenal-Induced Inflammation and Fibrosis in Human Renal Proximal Tubular Epithelial Cells

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Background: 4-hydroxy-2-hexenal (HHE), which is the aldehyde product of lipid peroxidation, might be responsible for the pathogenesis of renal mitochondrial dysfunction and apoptosis. Recently, paricalcitol (19-nor-1, 25-dihydroxyvitamin D2) is renoprotective in various experimental nephropathy models through its anti-inflammatory and anti-fibrotic

actions. We investigated the effects of paricalcitol on inflammation and fibrosis after HHE-induced renal tubular epithelial cell injury.

Methods: To investigate the underlying molecular mechanisms of HHE-induced tubule cell injury, we examined the nuclear factor-κB, mitogen-activated protein kinase (MAPK), β-catenin signaling, and the expression of the iNOS, COX-2, CTGF and fibronectin in human proximal tubular epithelial (HK-2) cells by semiquantitative immunoblotting.

Results: In HHE-treated HK-2 cells, paricalcitol attenuated the increases of the expression of p38 MAPK, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), and also prevented the activation of nuclear factor-κB. The expression of the inflammatory protein iNOS and COX-2 were attenuated by paricalcitol treatment. In addition, HHE increased the expression of the TGF-β1 and fibrotic protein such as fibronectin and CTGF, which were attenuated by the treatment of paricalcitol. Treatment of HHE results in the activation of β-catenin signaling, whereas paricalcitol reduced the expression of β-catenin in HHE-treated cells. Inhibitor of β-catenin (ICG-001) decreased the expression of TGF-β1 and attenuated HHE-induced fibrosis.

Conclusions: Paricalcitol appears to attenuate HHE-induced renal tubular cell injury by suppression of inflammation and fibrosis through inhibition of the nuclear factor-κB, MAPK and β-catenin signaling pathways.

FR-PO095

Regulation of Fibronectin in Diabetes: Phosphorylation of CREB Induces mRNA Expression and Promoter Activity of Fibronectin in Renal Proximal Tubular Cells Samy L. Habib,[1,2] Sitai Liang,[2] Shaza Tizani,[2] Anthony J. Valente.[3] *[1]Geriatric Research, Education, and Clinical Center, South Texas Veterans Healthcare System, San Antonio, TX; [2]Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX; [3]Medicine, University of Texas Health Science Center, San Antonio, TX.*

Background: Diabetic nephropathy is characterized by excessive deposition of extracellular matrix in the kidney, leading to tubulointerstitial fibrosis. The rate of deterioration of kidney function is highly correlated with the degree of tubular fibrosis and accumulation of cell matrix proteins. The mechanisms of renal cell matrix expansion in the diabetic milieu are incompletely characterized.

Methods: Mouse proximal tubular cells were grown in normal glucose (NG, 5mM) or treated with high glucose (HG, 25mM) for 24h. Nuclear and cytoplasmic proteins, and RNA from cells treated with HG or HG+rapamycin were extracted. Cells were infected with *Ad-CREB* or transfected with siRNA against CREB then treated with HG or HG+rapamycin.

Results: HG induced CREB phosphorylation and led to increased expression of fibronectin mRNA, suggesting transcriptional regulation. We have cloned a section of the fibronectin promoter region and identified two potential putative CREB binding sites. Using EMSA assay, we found that HG significantly enhanced the binding of CREB in the cell nuclear extracts to the putative fibronectin-CREB sites. Further, HG significantly increased the promoter activity of fibronectin while rapamycin reversed HG effects to NG levels. In addition, down-regulation of CREB by siRNA or expression of dominant negative CREB in HG-treated cells significantly reduced fibronectin mRNA and promoter activity to the NG levels.

Conclusions: We have identified novel putative CREB binding sites in the fibronectin promoter that regulate the transcriptional activity of fibronectin. Downregulation of CREB by siRNA or Ad-CREB resulted in a significant decrease in cell matrix protein accumulation in cells exposed to HG. These data identify CREB, as a major transcription factor involved in the regulation of cell matrix protein in diabetic patients, and a potential therapeutic target.

Funding: Veterans Administration Support

FR-PO096

Angiotensin II (AngII) Activation of NF-kB Is Mediated by beta-Arrestin Dependent AT1a Receptor (AT1aR) Internalization in Rat Aorta Vascular Smooth Muscle Cells (RASMC) Thomas Morinelli,[1] Mi-hye Lee,[1] Ryan T. Kendall,[1] Louis Luttrell,[1,2] Linda Walker,[1] Michael E. Ullian.[1,2] *[1]Medical University of South Carolina, Charleston, SC; [2]Ralph H. Johnson VA Hospital, Charleston, SC; [3]Medical University of South Carolina.*

Background: AngII, a key hormone in reno-vascular homeostasis, also contributes to renal and blood vessel inflammation and fibrosis. Activation of the AngII $AT_{1A}R$ in RASMC increases synthesis of the pro-inflammatory enzyme cyclooxygenase 2 (COX-2). We have previously shown that nuclear localization of internalized $AT_{1A}R$ activates transcription of the gene for COX-2, PTGS-2. Others have suggested that AngII stimulation of COX-2 protein synthesis is mediated by NF-κB. The purpose of the present study was to examine the interrelationship between $AT_{1A}R$, β-arrestin and NF-κB in the ability of AngII to increase COX-2 protein synthesis in RASMC.

Methods: We utilized RASMC, inhibitors of the NF-κB pathway, β-arrestin knockdown, radioligand binding, immunoblotting and immunofluorescence to characterize the roles of $AT_{1A}R$ internalization, NF-κB activation and β-arrestins in AngII-induced COX-2 synthesis.

Results: The NF-κB inhibitors Ro 106-9920 or parthenolide, agents that inhibit the initial steps of NF-κB activation, blocked AngII-induced p65 NF-κB nuclear localization, COX-2 protein expression, β-arrestin recruitment to the $AT_{1a}R$ and $AT_{1A}R$ internalization, without inhibiting AngII-induced p42/44 ERK activation. The AT1R biased (non-G protein-mediated) agonist SII-AngII, also promoted p65 NF-κB nuclear localization. Curcumin, an inhibitor of NF-κB induced transcription, blocked AngII-induced COX-2 protein expression but without altering $AT_{1A}R$ internalization, AngII-induced p65 NF-κB nuclear localization or p42/44 ERK activation. SiRNA-induced knockdown of β-arrestin 1 and 2 inhibited AngII-induced p65 NF-κB nuclear localization.

Conclusions: Therefore, in vascular smooth muscle cells, internalization of the activated $AT_{1A}R$ mediated by β-arrestins activates the NF-κB pathway, producing nuclear localization of the transcription factor and initiation of COX-2 protein synthesis; thereby linking the internalization of the receptor with the NF-κB pathway.

Funding: Clinical Revenue Support

FR-PO097

Blockade of Smad Signaling by 3'-Deoxyadenosine: A Mechanism for the Anti-Fibrotic Potential Liubao Gu, Hisashi Johno, Shotaro Nakajima, Hironori Kato, Masanori Kitamura. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: *Cordyceps militaris* has been used in Eastern countries for the treatment of various diseases including chronic kidney diseases (renal fibrosis). However, there are no reports that identified its active entities and molecular mechanisms underlying its therapeutic usefulness. 3'-Deoxyadenosine is a major nucleoside isolated from *Cordyceps militaris*. In the present report, we investigated whether and how 3'-deoxyadenosine interferes with fibrogenic processes in the kidney.

Methods: Effects of 3'-deoxyadenosine on the expression of collagens and activity of Smad signaling were tested *in vitro* and *in vivo*. Northern and Western blot analyses, reporter assays and immunohistochemical analyses were utilized for this purpose. Unilateral ureteral obstruction (UUO) in mice was used as a model of renal fibrosis.

Results: 3'-Deoxyadenosine suppressed expression of collagens induced by TGF-β1 and BMP-4 in renal tubular cells. This suppression occurred at the transcriptional level and was correlated with blunted activation of the CAGA box and the BMP-responsive element. The suppressive effects were mediated by adenosine transporter and A_3 adenosine receptor, but not A_1/A_2 adenosine receptor. 3'-Deoxyadenosine reduced levels of both phosphorylated and total Smad proteins (Smad1, 2, 3). It was mainly ascribed to transcriptional suppression, but not enhanced protein degradation or eIF2α-mediated translational suppression. Consistently, *in vivo* administration with 3'-deoxyadenosine reduced the levels of phosphorylated and total Smad proteins as well as the levels of Smad mRNAs in the kidney subjected to UUO. It was associated with blunted induction of type I collagen and α-smooth muscle actin, the markers for fibrogenesis and downstream targets of Smad pathways.

Conclusions: Our data suggest that 3'-deoxyadenosine interferes with the TGF-β and BMP signaling via down-regulation of Smads, which may underlie the anti-fibrotic effect of this agent. 3'-Deoxyadenosine may be useful for therapeutic intervention in TGF-β-related fibrotic disorders.

Funding: Government Support - Non-U.S.

FR-PO098

The miRNA Landscape of a Murine Inner Medullary Collecting Duct Cell Mollie E. Jacobs,[1] Amanda Welch,[2,3] Charles S. Wingo,[2,3] Brian D. Cain.[1] *[1]Biochemistry and Molecular Biology, University of Florida, Gainesville, FL; [2]Medicine, University of Florida, Gainesville, FL; [3]North Florida/South Georgia VA Medical Center, Gainesville, FL.*

Background: MicroRNAs (miRNAs) are a family of small (~22 nt) noncoding RNAs. They have been shown to play a crucial role in post-transcriptional gene regulation by blocking protein translation or inducing degradation of specifically targeted messenger RNAs. Dysregulation of miRNA levels has been shown to be involved in the pathogenesis of many diseases including renal fibrosis, diabetic nephropathy, and hypertension-related renal disease. However, miRNAs also play important roles in the regulation of metabolism in the healthy cell. Aldosterone is the major known steroid hormone responsible for sodium retention in the kidney collecting duct. To date, the role of aldosterone to modulate miRNA expression in a clonal cell line has not been examined. Our working hypothesis is that aldosterone alters the miRNA content in the inner medullary collecting duct which results in increased translation of mRNAs that are involved in sodium reabsorption and urinary acidification.

Methods: To look for miRNAs subject to aldosterone regulation, an miRNA microarray analysis was performed. Murine inner medullary collecting duct cells (mIMCD-3) were plated in transwell inserts to induce polarity. Cells were treated with either 100nM aldosterone or vehicle for 24 hours. Total RNA was extracted using Trizol, and RNA integrity was determined using the Agilent 2100 Bioanalyzer. Ten independently prepared RNA samples, five aldosterone treated samples and five vehicle treated samples, were used in a Toray 3D-Gene miRNA microarray analysis. The microarray chip contained probes for 1080 mature miRNAs.

Results: We identified five miRNAs whose abundance increased significantly with aldosterone treatment and several miRNAs that were down regulated by aldosterone.

Conclusions: These results demonstrate a novel and heretofore unknown mechanism for aldosterone to modulate gene expression. In addition, the results provide a database of those miRNAs that are expressed in mIMCD-3 cells and potential pathways that may be coordinately regulated by aldosterone.

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FR-PO099

Altered Mitochondrial Homeostasis, p62, and PPARγ and α Contribute to Oxidative Stress-Induced Kidney Injury David M. Small,[1] Nigel C. Bennett,[1] Jeff S. Coombes,[1,2] David W. Johnson,[1,3] Glenda C. Gobe.[1] *[1]Centre for Kidney Disease Research, Sch of Medicine, Univ Queensland; [2]Sch of Human Movement Studies, Univ Queensland; [3]Dpt of Nephrology, PA Hospital, Brisbane, Queensland, Australia.*

Background: Oxidative stress deregulates mitochondrial genes and has a key role in the development of many kidney diseases. Balancing degradation of defective mitochondria with renewal of healthy mitochondria is vital for renal cell health. p62 (indicates faulty mitochondria) and nuclear transcription factors peroxisome proliferator-activated receptor (PPAR) γ and α (oxidative stress-responsive, mitochondrial biogenesis) need investigation. The aim was to investigate mitochondrial homeostasis, p62 and PPARγ and α expression and activation in oxidative stress-induced renal proximal tubular (PT) epithelial injury.

Methods: HK-2 PT cells were treated with hydrogen peroxide (H_2O_2; 0.2, 0.4, 0.6, 0.8, 1.0mM) for 2h and 18h. p62, phospho-PPARγ/PPARγ, PPARα and LC3-II (autophagy) (Western blot; densitometry), and cellular ATP (luciferase-based assay) were analyzed. Mitochondrial biogenesis was quantified (MitoTracker Red; analysis software). PPARγ agonists and antagonists were used to determine protective or cytotoxic effects.

Results: Following 2h H_2O_2 exposure, p-PPARγ/PPARγ, p62 and PPARα decreased ($p<0.05$), along with ATP and MitoTracker Red ($p<0.001$, $p<0.05$, respectively). These results indicate early mitochondrial dysfunction and degradation. PPARγ modulation had no effect. Following 18h H_2O_2 exposure, p62 and LC3-II expression increased ($p<0.05$), p-PPARγ/PPARγ was normal, and ATP and MitoTracker Red remained low ($p<0.001$, $p<0.05$, respectively). Apoptosis increased progressively with H_2O_2 in a concentration and temporal manner. Results suggest mitochondrial biogenesis is impeded by degradation and autophagy resulting in progressive loss of renal cells after oxidative stress.

Conclusions: Oxidative stress promotes mitochondrial destabilisation in HK-2 cells by increasing p62, and perhaps by early loss of PPARγ activation. Failure to remove damaged mitochondria via autophagy, or defective p62, may lead to a spiralling cycle of oxidative stress, with progressive deterioration of tubular function in kidney disease.

Funding: Government Support - Non-U.S.

FR-PO100

Stanniocalcin-1-Mediated Endothelium-to-Epithelium Cross Talk Promotes Kidney Epithelial Cell Survival Luping Huang,[1] Tatiana Belousova,[1] Jie Du,[3] Pumin Zhang,[2] David Sheikh-Hamad.[1] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX; [3]Laboratory of Remodeling-Related Cardiovascular Diseases, Institute of Heart Lung and Blood Vessel Diseases, Beijing, China.*

Background: Stanniocalcin-1 (STC1) is an extracellular signaling protein; it binds to the cell surface, internalized to the mitochondria and diminishes superoxide generation through induction of uncoupling proteins (intracrine signaling). The cellular distribution of STC1 mRNA and protein in the kidney does not parallel; the mRNA is observed in blood vessels, cortical and medullary collecting ducts, and is absent in PT cells; but the protein is detected in blood vessels and along the entire nephron (albeit, at varying levels), suggesting that STC1 is produced by one cell and is picked up by neighboring cells. STC1 Tg mice which display preferential expression of the transgene in endothelial cells are resistant to ischemia/reperfusion kidney injury (KI, 2012); kidney epithelial cells express high levels of UCP2 and produce less superoxide. Therefore, we hypothesized that endothelium-derived STC1 is picked up by neighboring epithelial cells, where it promotes cell survival.

Methods: We generated STC1 shRNA Tg mice that express STC1 siRNA upon removal of floxed reporter stuffer (PGK-driven EGFP), bringing the H1 promoter ahead of the siRNA cassette. Using ultrasound microbubble-mediated delivery of Tie2-Cre to the kidney we knocked down the expression of STC1 in kidney endothelial cells in STC1 shRNA Tg mice (within 4-5 days), but not in scrambled shRNA Tg mice.

Results: This reveled diminished staining for STC1 in epithelial cells of STC1 shRNA Tg kidneys, accompanied by tubular epithelial cell injury [vacuolization, cell sloughing, lower expression of UCP2 and greater generation of superoxide (MitoSox fluorescence)].

Conclusions: These novel observations suggest STC1-mediated endothelium-to-epithelium cross-talk. Endothelium-derived STC1 is picked up by neighboring epithelial cells; where, it drives the expression of UCP2, diminishing superoxide generation - promoting epithelial cell survival.

Funding: NIDDK Support

FR-PO101

Bidirectional Regulation of miR-29c on Inhibiting Renal Fibrosis Chen Yu,[1] Ying Yu,[1] Lunjun Fu,[1] Eugene Chin.[2] *[1]Division of Nephrology, Tongji Hospital, School of Medicine, Tongji University, Shanghai, China; [2]Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences& Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

Background: Strong antifibrotic effects of miR-29s have been demonstrated in heart, kidney, and other organs. Leukemia inhibitory factor (LIF) is a pleiotropic glycoprotein belonging to the interleukin-6 family of cytokines. We previously showed that LIF ameliorated the progression of heart and renal fibrosis. The databases of TargetScan, miRBase and miRNA.Org show there is binding site for STAT3, the downstream transcription factor of LIF signaling pathway, in miR-29c promoter region and it is highly conserved among different species. The aim of this study was to explore the role of miR-29cc in LIF anti-renal fibrosis processes.

Methods: Rat renal fibroblast cells (NRK-49F) were used for in vitro study and unilateral ureter obstruction(UUO) rats for in vivo study.

Results: Our results showed that: **1)** LIF inhibited TGF-β and AngII-induced expressions of type 1 collagen (col1) and type 3 collagen (col3) protein and mRNA, in a dose dependent manner, in NRK-49F cells and mice UUO model. **2)** MiR-29c enhanced the expression of LIF and LIFR protein and mRNA, and slightly increased the expression of gp130 (the homo-receptor of LIF) protein and mRNA. **3)** MiR-29c increased phosphorylation of STAT3 on Tyr705 and on Ser727, especially on the former. **4)** Using luciferase assay tests, it was seen that miR-29c down-regulated fluorescence intensities of luciferase reporter containing col1- and col3-3'UTR. **5)** Over-expression miR-29c decreased col1 and col3 expressions in NRK-49F cells, in a dose-dependent manner.

Conclusions: Our data uncovered firstly that miR-29c exerted anti-renal fibrosis effects via bidirectional regulation: on one hand, miR-29c down-played directly the production of collagen; on the other hand, miR-29c enhanced the expression LIF and LIFR, which in turn inhibited TGF-β and AngII-induced production of collagen.

Funding: Government Support - Non-U.S.

FR-PO102

Connexin43 Hemichannel-Mediated Release of ATP Suppresses AMPK Activation in Renal Tubular Epithelial Cells Yuan Chi, Kun Gao, Hironori Kato, Shotaro Nakajima, Masanori Kitamura, Jian Yao. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: ATP/P2 receptor system regulates multiple renal functions. However, the molecular mechanisms underlying ATP release and its actions are still poorly understood. Here we tested the possible involvement of connexin (Cx) hemichannels in ATP release and explored the potential influence of the released ATP on AMP-activated kinase (AMPK), a key regulator of multiple channel activities.

Methods: AMPK activation was evaluated by phosphorylation levels of AMPK at Thr-172. ATP was measured using a luciferin/luciferase bioluminescence assay kit. Cx43 channels was interrupted with various blockers or through specific siRNA. Cell membrane permeability was detected by measurement of the fluorescent intensity of the preloaded calcein.

Results: 1) Activation of Cx-hemichannels in renal epithelial NRK-E52 cells by lowering the extracellular calcium concentration resulted in a rapid efflux of ATP, which was significantly prevented by hemichannel blocker heptanol and lindane. 2) Inhibition of the hemichannels with various blockers or downregulation of Cx43 using siRNA caused an unexpected activation of AMPK under calcium- or glucose-deprivation. This effect was similarly achieved by degradation of ATP with apyrase or inhibition of P2-purinergic receptor with suramin and PPADS, but abolished by the exogenous ATP. 3) ATP induced a P2-receptor-dependent activation of AKT. Inhibition of PI3K/AKT with specific inhibitors reproduced, whereas transfection of cells with myr-Akt, a constitutively active form of Akt, abrogated the activation of AMPK. 4) Exposure of NRK-E52 cells to hypotonic condition elicited an abrupt release of ATP, which was followed by membrane destabilization. Inhibition of P2-receptor with suramin or activation of AMPK with AICAR significantly prevented the destabilization.

Conclusions: Our results indicate that Cx hemichannel-mediated release of ATP suppresses AMPK activation through P2-purinergic receptor-triggered activation of AKT. Our findings thus provide novel insights into the mechanisms of Cx hemichannels and ATP in the regulation of renal cell functions.

Funding: Government Support - Non-U.S.

FR-PO103

Receptor Activator of NF-kappaB and Its Ligand Is a Novel Receptor-Ligand Complex for Survival Response during Podocyte Injury Shuangxin Liu, Wei Shi, Xinling Liang, Wenjian Wang, Zhiming Ye, Jianchao Ma, Yunfeng Xia, Lixia Xu. *Division of Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: Glomerulosclerosis correlates with a reduction in podocytes number that occurs through mechanisms that include apoptosis. Podocyte injury or podocyte loss in the renal glomerulus has been proposed as the crucial mechanism in the development of glomerulosclerosis. However, it is poorly understood how podocytes respond to injury. Whether ligand of receptor activator of NF-kappaB (RANKL), a TNF-related molecule that is essential for osteoclast survival and activation through interaction with its receptor activator of NF-kappaB (RANK), is a factor for injured podocytes was investigated.

Methods: In this study, RANKL and RANK was examined in human podocyte diseases and rat model of puromycin aminonucleoside nephrosis (PAN).

Results: Compared with control, RANK was increased in human podocyte diseases and rat PAN model, and double immunofluorescence staining revealed that RANK protein expression was mainly attributed to podocytes. Immunoelectron microscopy showed that RANK was localized predominantly at the top of the foot process membrane and the cytoplasm of podocytes of rat. In addition, RANK was upregulated in mouse podocytes in vitro after injury induced by puromycin aminonucleoside (PA). Knockdown of RANK expression by small interference RNA (siRNA) exacerbated podocytes apoptosis induced by PA. However, RANKL inhibited significantly the apoptosis of podocytes induced by PA.

Conclusions: These findings suggest the increase of RANK expression is a response to podocyte injury, and RANK -RANKL may be a novel receptor-ligand complex for survival response during podocyte injury.

Funding: Government Support - Non-U.S.

FR-PO104

Cyclooxygenase-2 Is Induced by Oxidative Stress in a ROS and MAPK-Dependent Signaling Pathway in Renal Medullary Interstitial Cells
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Background: Oxidative stress resulting from unilateral ureteral obstruction (UUO) may be aggravated by increased production of reactive oxygen species (ROS) from the mitochondria. Our previous studies have documented increased COX-2 expression in renal medullary interstitial cells (RMIC) in response to UUO. We here investigated the role of ROS in the induction of COX-2 in RMIC subjected to oxidative stress.

Methods: Rats were subjected to 3dUUO and treated with inhibitors of the mitochondrial metabolism (complex I inhibitor rotenone (ROT) and NADPH oxidase inhibitor diphenyleneiodonium (DPI)). Furthermore, RMIC were subjected to oxidative stress by administration of H_2O_2 and treated with ROT and DPI in order to study specific cellular pathways involved in the induction of COX-2. Using immunoblot analysis, we examined the regulation of COX-2, nitrotyrosin, ERK1/2 and p38. The fluorescent probe 2',7'-dichloroflourescein deacetate was used to measure intracellular ROS levels. Apoptosis was analyzed using the ratio of Bax/Bcl-2.

Results: The oxidative stress marker nitrotyrosin was induced after UUO in renal medulla. COX-2 was increased in response to UUO in renal medulla and this increase was attenuated by ROT and DPI treatment. Oxidative stress treatment (75 μM H_2O_2) for 6 h induced a 5-fold increase in COX-2 expression and comparable increases in prostaglandin E_2 release in RMIC, both of which were reduced by ROT and DPI. ROS levels were increased following exposure of RMIC to H_2O_2 and this was significantly reduced by DPI administration, but not by ROT. Cell viability and apoptosis were not affected by administration of H_2O_2 or the mitochondrial inhibitors. The increases in phosphorylation of ERK1/2 and p38 were detected 6 h following H_2O_2 treatment and were both prevented by DPI. Blocking the ERK/p38 signaling pathways by pharmacological inhibitors attenuated the H_2O_2 induced COX-2 expression.

Conclusions: Taken together, these results demonstrated that oxidative stress induces COX-2 and this induction might be mediated through NADPH oxidase-derived ROS-dependent ERK/p38 pathways.

Funding: Government Support - Non-U.S.

FR-PO105

Cold Shock Protein YB-1 Triggers Akt and Erk Kinase Signalling via Notch-3 Receptors in Monocytic Cells
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Background: Y-box (YB) binding protein-1 is the prototypic member of the cold shock protein family with pleiotropic functions relating to transcription and translation, mediating cell proliferation, matrix synthesis and chemotaxis in kidney diseases. Recent findings indicate YB-1 secretion *via* a non-classical pathway and profound extracellular effects mediated by Notch-3 receptors. Here, we determined intracellular signalling following extracellular stimulation with recombinant YB-1 protein (rec.YB-1), abbreviated YB-1 proteins and peptides corresponding to YB-1 protein domains in monocytic (THP-1), mesangial as well as tubular cells.

Methods: Cell lines of different origin were stimulated with purified rec.YB-1 protein. Activation of the PI3K/Akt- and MAPK-signalling pathways was assessed by Western blot analysis.

Results: Extracellular rec.YB-1 (1 μg/ml) resulted in a time- and dose-dependent phosphorylation and activation of Akt as well as MAP (Erk) kinases. Phosphorylation of Akt-S473 was present from 4 to 8h following incubation, phosphorylation at Akt-T308 was not observed at any time point. Erk1/2 activation at T202/Y204 was present from 7' to 8h following protein addition. YB-1 deletion constructs revealed that Akt-S473 phosphorylation is dependent on the ancestral cold shock and adjacent N-terminal domains. Similar results were obtained with synthetic peptides corresponding to YB-1 protein subdomains. Extracellular blockade of Notch-3 receptor signalling in THP-1 cells completely abrogated YB-1 -dependent Akt and Erk1/2 phosphorylation, highlighting the importance of the YB-1:Notch-3 receptor interaction.

Conclusions: Akt as well as MAP (Erk) kinases are involved in apoptosis, cell proliferation, motility and cell growth. Notch-3 receptor signalling activates these two pathways. Thus extracellular YB-1 initiates promitogenic effects in THP-1 cells by Akt and MAP kinase pathways.

FR-PO106

ERK5 Activation in Human Renal Cells under Diabetic Stimuli
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Background: Diabetic nephropathy is the primary factor causing end-stage renal disease worldwide and is particularly characterised by podocytopaenia and tubulointerstital fibrosis. ERK5 is an atypical MAP kinase distinct from its siblings through the possession of a unique C-terminus. Current literature has implicated ERK5 in mediating proliferation and survival pathways in cancer; its effects in podocytes and proximal tubule epithelial cells (PTECs) remain largely unknown. The aim of this study was to explore the expression and role of ERK5 in human podocytes and PTECs.

Methods: Conditionally immortalised human podocytes, terminally differentiated after 14 days at 37°C, and the HKC-8 human PTEC cell line were subjected to growth factor stimulation (10 ng/ml EGF, 2.5 ng/ml TGF-β1), diabetic stimuli (15 mM D-glucose v 5 mM D- + 10 mM L-glucose, 30 mM D- v 5 mM D- + 25 mM L-glucose; 50 μg/ml AGE-BSA v 50 μg/ml control BSA) and inhibition of ERK5 activation with 10 μM of the inhibitor BIX02188 directed to the upstream MEK5. ERK5 protein expression and phosphorylation(p-ERK5), E-cadherin (a marker of PTEC phenotype) were measured by Western blotting.

Results: Podocytes and HKC-8 cells expressed ERK5 and it was phosphorylated in response to EGF and TGF-β1; inhibition of MEK5 attenuated ERK5 phosphorylation. EGF induced distinct morphological changes in podocytes and additional MEK5 inhibition resulted in increased cell death along with de-differentiation as evinced by a loss of synaptopodin. Inhibition of MEK5 in HKC-8 cells rescued E-cadherin expression, following TGF-β1 treatment thereby preventing loss of PTEC phenotype, in contrast to the apparent effect of knock down of total Erk5 expression by siRNA. AGE-BSA treatment of podocytes decreased p-ERK5 whereas glucose appeared to have a biphasic effect; 15mM D-glucose causedan acute reduction whilst 30 mM D-glucose increased p-ERK5.

Conclusions: This work demonstrates that the atypical MAP kinase ERK5 is involved in signalling cascades activated by a number of pathological stimuli involved in renal disease, however its role appears to be complex regulating diverse outcomes.

Funding: Private Foundation Support

FR-PO107

The Role and Mechanism of T-Brachyury in TGF-β1 Induced EMT of Renal Tubular Epithelial Cells
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Background: Epithelial-to-mesenchymal transition (EMT) which is recognized as losing of epithelial characteristics and associated with a mesenchymal phenoptye plays important roles in the progression of fibrosis in the kidney.Recent study have revealed that T-brachyury, an evolutionarily conserved transcription factor, promoted EMT involved in cancer progression and metastasis by repressed E-cadherin transcription, leading to loss of E-cadherin-mediated cell–cell adhesion, activation of EMT regulator which might play important roles in mediating the invasion, migration, and metastatic activity of different carcinoma cell, possibly resulting in renal fibrosis.

Methods: To understand the molecular mechanism in this process, and to identify the critical genes in the initial phase of the TGF-β1–mediated EMT, a high-throughput gene expression microarray analysis was used. To investigate the role and mechanism of T-brachyury in TGF-β1 induced EMT of renal tubular epithelial cells many other biochemical analyses were used.T-brachyury was found to be induced prominently in TGF-β1-treated human proximal tubular epithelial (HK2) cells.

Results: Brachyury overexpression in HK2 cells induced changes characteristic of EMT. Brachyury induction also repressed E-cadherin transcription; an effect partially mediated by Slug, whereas knockdown Brachyury and Slugby short interfering RNA (siRNA) effectively reduced TGF–β1–mediated EMT and partially restored E–cadherin. E-cadherin promoter contains Brachyury -binding sites and increased transcription was showed in Brachyury-overexpressing cells which transfected of reporter constructs using the promoter. Chromatin immunoprecipitation assays identified the presence function of Brachyury -binding sitewithin the E-cadherin gene promoter. In rat model of obstructive nephropathy and IgA nephropathy biopsies, expression of T-brachyury was induced, suggesting that it may play roles in EMT and renal fibrosis *in vivo*.

Conclusions: This studies confirm that T-brachyury play important roles in regulating TGF-β1–mediated EMT and could be an attractive target for progression of renal disease therapies.

FR-PO108

Genetic Inactivation and Pharmacological Inhibition of Glycogen-Synthase Kinase 3 beta (GSK-3β) in Madin-Darby Canine Kidney Cells Increase Epithelial Resistance
Francois Jouret, Jingshing Wu, Vanathy Rajendran, Michael J. Caplan. *Cellular and Molecular Physiology, Yale Medical School, New Haven, CT.*

Background: Glycogen-synthase kinase 3-beta (GSK-3β) is a ubiquitous serine-threonine kinase involved in distinct biological processes. Pharmacological inhibition of GSK-3β by lithium or SB216763 compound leads to the assembly of tight junction (TJ) components in epithelial cells, even in the absence of extracellular Ca^{2+} (Ca^{2+}_e).

Methods: First, we stably knocked-down (KD) the expression of GSK-3β in Madin-Darby Canine Kidney (MDCK) epithelial cells using shRNA. Next, we quantified the expression of TJ components in GSK-3β KD versus control cells at steady-state by real-time RT-PCR and immunoblotting. Finally, we investigated the effects of GSK-3β inactivation or pharmacological inhibition on TJ formation and disruption.

Results: In GSK-3β KD cells, the expression of claudin 1, claudin 4, occludin and ZO-1 is significantly increased, with no change in claudin 2 and β-catenin abundance. Steady-state transepithelial electrical resistance (TEER) is significantly higher in GSK-3β KD (763.4 ± 54.5 ohm.cm^2) and SB216763-treated MDCK cells (801.4 ± 32.4 ohm.cm^2) than in untreated cells (332.6 ± 38.6 ohm.cm^2). Following Ca^{2+} switch from low (5μM) to normal (1.8mM) Ca^{2+}_e, the relocation of ZO-1 to cell-cell contacts is significantly faster in GSK-3β KD versus control cells. Conversely, Ca^{2+}_e deprivation in control cells for 2 hours induces an internalization of the GSK-3β substrate, β-catenin, with a loss of measurable TEER. In similar conditions, β-catenin mostly stays at the basolateral membrane in GSK-3β KD cells and in MDCK cells pre-treated with SB216763 [20μM] for 60 minutes, and TEER remains measurable (197.1 ± 23.9 ohm.cm^2).

Conclusions: These results support that GSK-3β activity modulates TJ assembly and disruption in epithelial cells.

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FR-PO109

The Ubiquitin-Like Protein FAT10 Inhibits Autophagy in Renal Tubular Epithelial Cells Michael J. Ross, Bin Wang, Jeremy S. Leventhal, Pengfei Gong. *Division of Nephrology, Mt Sinai School of Medicine, New York, NY.*

Background: Autophagy is the process by which superfluous or damaged macromolecules or organelles are degraded by the lysosome. Pharmacological and genetic evidence indicate that autophagy plays pleiotropic functions in cellular homeostasis, development, survival and differentiation. FAT10, as a small ubiquitin-like modifier, plays an important role in various cellular processes, including mitosis, immune response, and apoptosis.

Methods: In these studies, we investigated the role of FAT10 in autophagy. We used a lentiviral short hairpin RNA vector directed against FAT10 or control shRNA vector to transduce cells. Tubular cells were grown from kidneys of FAT10$^{-/-}$ and wild type mice according to standard techniques.

Results: We found that over-expression of FAT10 suppressed autophagy in human proximal tubular (HK2) cells as detected by decreased LC3B II/I protein ratio. Knockdown of FAT10 expression in HK2 and 293T cells using FAT10 shRNA increased the LC3B II/I ratio. Moreover, the LC3B II/I ratio was increased in primary renal tubular epithelial cells (RTEC) isolated from FAT10$^{-/-}$ mice was also increased as compared to RTEC from wild type mice. We also detected a marked increased in the abundance of intracellular vesicles staining for acridine orange in FAT10$^{-/-}$ RTECs and in 293T cells expressing FAT10 shRNA and a significant increase in the abundance of fluorescent punctae of 293T cells expressing LC3-mCherry-GFP after transduction with FAT10 shRNA. Finally, increased pAMPK/AMPK was also observed in RTECs from the FAT10 knockout mice and 293T cells expressing FAT10 shRNA.

Conclusions: Together, these data suggest that the ubiquitin-like protein FAT10 inhibits RTEC autophagy and this effect may be mediated via AMPK activation.

Funding: NIDDK Support

FR-PO110

Peroxidasin Forms Sulfilimine Bonds in Basement Membranes Using Hypohalous Acids Gautam B. Bhave, Roberto M. Vanacore, Vadim Pedchenko, Billy G. Hudson. *Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN.*

Background: Collagen IV is the predominant protein network of basement membranes, a specialized extracellular matrix, which underlie epithelia and endothelia. These networks assemble through oligomerization and covalent cross-linking to endow mechanical strength and shape cell behavior through interactions with cell surface receptors. A novel sulfilimine (S=N) bond between a methionine sulfur and hydroxylysine nitrogen reinforces the collagen IV network.

Methods: We used a combination of biochemistry, mass spectrometry, and genetics to delineate the mechanism of collagen IV sulfilimine cross-link formation and its role in basement membrane and tissue integrity.

Results: We demonstrate that peroxidasin, an enzyme found in basement membranes, catalyzes formation of the sulfilimine bond. Drosophila peroxidasin mutants exhibit disorganized collagen IV networks and torn visceral muscle basement membranes pointing to a critical role for the enzyme in tissue biogenesis. Peroxidasin generates hypohalous acids as reaction intermediates suggesting a paradoxically anabolic role for these usually destructive oxidants.

Conclusions: This work highlights sulfilimine bond formation as the first known physiologic function for peroxidasin and a role for hypohalous oxidants in tissue biogenesis. Since the sulfilimine cross-link determines autoantibody reactivity in Goodpasture's disease and promotes stability of collagen IV networks, our work implicates a role for peroxidasin in the pathogenesis Goodpasture's disease, glomerular matrix expansion as seen in diabetic nephropathy, and vascular matrix remodeling and atherosclerosis.

Funding: NIDDK Support

FR-PO111

Loss of E-Cadherin in Renal Proximal Tubular Cells Promotes Epithelial-Mesenchymal Transition and Kidney Fibrosis through α3 Integrin in Murine Model of Unilateral Ureteral Obstruction Guoping Zheng,[1] So Ra Lee,[1] Jianlin Zhang,[1,2] Hong Zhao,[1,2] Tim Tzu-ting Hsu,[1] Thian Kui Tan,[1] Ye Zhao,[1] David A.F. Loebel,[3] Isabelle Rubera,[4] Michel Tauc,[4] Yiping Wang,[1] Ya Wang,[1] Qi Cao,[1] Changqi Wang,[1] Vincent W.S. Lee,[1] Patrick P.l. Tam,[3] David C. Harris.[1] *[1]CTRR, WMI, University of Sydney, Sydney, New South Wales, Australia; [2]Dept. of Biochem & Mol Biol, Shanxi Medical University, Taiyuan, Shanxi, China; [3]CMRI, University of Sydney, Sydney, New South Wales, Australia; [4]UMR 6097, University of Nice-Sophia, Nice, France.*

Background: Our previous studies in murine models of kidney fibrosis showed that disruption of E-cadherin by MMPs caused EMT whereby E-cadherin passes on extracellular signals to the nucleus through β-catenin and slug. This study examined the role of E-cadherin in renal tubular EMT using a novel conditional knockout of E-cadherin in mouse renal proximal tubular cells.

Methods: Conditional knockout of E-cadherin in renal tubular cells was generated by crossing cdh1flox/flox mice sequentially with CMV Cre, Sglt2Cre mice and then backcrossing to cdh1flox/flox mice. Primary culture of proximal tubular cells from knockout mice was treated with TGF-β1 (3ng/ml) to examine the role of E-cadherin in EMT *in vitro*. Kidney fibrosis was assessed by unilateral ureteral obstruction (UUO) in the E-cadherin -/- and littermate control mice.

Results: Depletion of E-cadherin in proximal tubular cells resulted in a transcriptional upregulation of α3β1 integrin. Forced expression of E-cadherin in E-cadherin-depleted tubular epithelial cells reversed the upregulation of α3β1 integrin. TGF-β1-induced EMT was significantly up-regulated in proximal tubular cells from E-cadherin -/- mice compared to littermate controls. siRNA silencing of α3, but not β1 integrin reduced the upregulation of EMT in proximal tubular E-cadherin -/- cells. Kidney fibrosis after UUO was worse in proximal tubule E-cadherin -/- mice compared to control mice, as demonstrated by Gomori Trichrome and Sirius red staining, and by immunohistological staining of α-SMA.

Conclusions: Conditional depletion of E-cadherin in proximal tubule aggravated kidney fibrosis in UUO through α3 integrin-dependent upregulation of EMT.

Funding: Government Support - Non-U.S.

FR-PO112

Targeting Alternative Splicing of Fibronectin Rescues TGFβ1-Induced Phenotypic Change in Human Tubule Cells Felicia Heidebrecht,[1] Mysore Keshavmurthy Phanish,[1] Susan M. Freier,[2] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]Isis Pharmaceuticals, Carlsbad, CA.*

Background: Fibrosis is characterised by changes in the quantity and quality of extracellular matrix (ECM). Renal fibrosis is associated with a change in the profile of collagen and de novo expression of alternative splice variant of fibronectin EDA+ (FN EDA+). Evidence suggests that these changes may play a critical role in the loss of cell phenotype and function associated with progressive fibrogenesis. This study investigates the role of FN EDA+ in the loss of phenotype of human proximal tubule cells (PTEC) initiated by TGFβ1 and identifies a potential therapeutic intervention to limit progressive fibrosis.

Methods: 20 RNase-H independent antisense oligonucleotides (ASO) targeting the proximal and distal region of the EDA exon were screened in transformed and primary PTEC to identify a sequence that would selectively inhibit splicing of the EDA exon without altering total fibronectin expression or the alternative splicing of the EDB exon. Pro-fibrotic changes were induced by treatment with 2.5 ng/mlTGFβ1; a concentration causing wide ranging alternations of cell phenotype. Subsequently, cells were treated with active and negative control ASO.

Results: TGFβ1 caused pronounced changes in cell phenotype: cadherin expression was altered, α Smooth Muscle Actin (αSMA) and connexin 43 (Cx 43) were induced, less cortical f-actin localisation was observed and MMP2 & 9 secretion was increased. Prevention of EDA inclusion by ASO treatment resulted in significant reduction in αSMA, a reduction in secretion of MMP2 & 9 and an almost complete inhibition of Cx43. There was a moderate increase in f-actin cortical localization and interestingly at this time point the ASO had no discernible effect on TGFβ1-mediated cadherin loss.

Conclusions: These results demonstrate that selective targeting of FN EDA+ with ASO can limit the pro-fibrotic effects of TGFβ1 on PTEC. Importantly, this effect was observed when the ASO was delivered after the initial TGFβ1 challenge. The target sequence on the pre-mRNA would appear to be very specific as striking differences were observed between ASO with a >70% overlap.

FR-PO113

Mechanisms of Extracellular Matrix Modulation in Interstitial Fibrosis: Role for Collagen Crosslinking? Miriam Boersema,[1] Anne-Roos Sophie Frenay,[2] Harry Van Goor,[2] Ruud A. Bank.[1] *[1]Medical Biology, UMCG, Groningen, Netherlands; [2]Pathology, UMCG, Groningen, Netherlands.*

Background: The net accumulation of extracellular matrix (ECM) in renal fibrosis depends, in part, on the delicate balance between the production and degradation of collagens. Crosslinking of collagen fibers by specific enzymes determines the stiffness and degradability of the ECM. We investigated genes involved in production, degradation and cross-linking of extracellular matrix using specific arrays. Furthermore, using *in situ* digestion of collagens with MMP-1 and-2, we measured the degradability of ECM in human and experimental interstitial fibrosis.

Methods: Human interstitial fibrotic tissue (IF/TA) was processed for mRNA using a specific array containing mRNA primers for proteins involved in ECM degradation, production and cross-linking. Cross-linking of collagen may inhibit the capacity of MMPs to degrade collagen. Human (IF/TA) and experimental (AngII infusion) interstitial fibrotic tissue sections were subjected to *in situ* digestion with active MMP-1 and -2 to evaluate the degradability of the ECM, followed by a collagen I immunostaining.

Results: Gene expression of collagen I, III, IV and V was increased (2-10 fold) in fibrotic tissue compared to control kidney tissue. Several members of the lysyl oxidase and lysyl hydroxylase family (collagen crosslink enzymes) were upregulated (2-14 fold), as well as prolyl hydroxylases and other collagen-modifying enzymes. In human IF/TA, MMP-1 digested the smaller fragments of interstitial collagen I, while larger fibrils were unaffected. MMP-2 did not digest collagen I in human IF/TA. In experimental interstitial fibrosis, MMP-1 was able to digest all interstitial collagen I. MMP-2 digested about 80% of all interstitial collagen I.

Conclusions: Gene expression of specific collagen crosslinking enzymes is increased in human IF/TA. Using an *in situ* collagen digestion assay we demonstrated that MMP-1 and -2 did not digest all interstitial collagen I in human IF/TA and experimental interstitial fibrosis. Cross-linking of collagens may inhibit the capacity of MMPs to degrade interstitial collagens and thereby enhance the progression of interstitial fibrosis.

FR-PO114

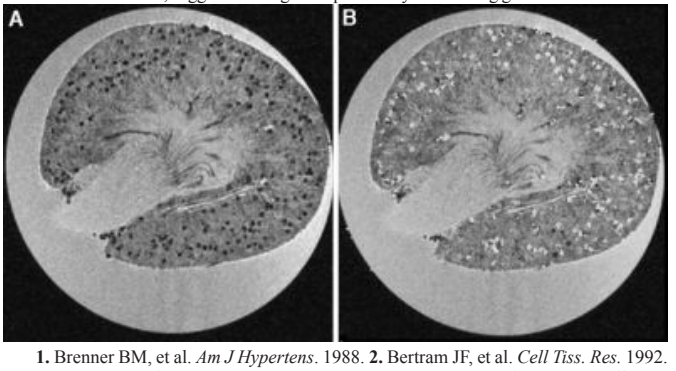
Counting Glomeruli in Mouse Kidneys Using MRI Scott Charles Beeman,[1] John F. Bertram,[2] Min Zhang,[1] Teresa Wu,[1] Jennifer Richardson Charlton,[3] Kevin Bennett.[1] *[1]Arizona State University; [2]Monash University; [3]University of Virginia.*

Background: Glomerular number plays an important role in a wide range of diseases[1-2], though the techniques currently employed to count glomeruli are estimates based on histological sections. A method for counting every glomerulus would be important to research and clinical diagnostics. It has been shown that all glomeruli in the rat kidney can be counted ex vivo using cationized ferritin nanoparticles (CF) and magnetic resonance imaging (MRI)[3-5]. With the huge library of transgenic mouse models employed in kidney research, such a technique, adapted to murine kidneys, would prove vital to basic kidney research. Here, we demonstrate the first steps towards visualizing and counting all glomeruli in the mouse kidney.

Methods: Three male C57BL/6 mice were given a 5.75 mg/100g dose of CF and one remained naive. Mice were perfused with PBS and formalin. Kidneys were imaged ex vivo at 19T using T_2^*-weighted 3D MRI. Glomeruli were counted in the MRI volumes using custom software.

Results: MRI imaging of kidneys resected from mice injected with CF revealed punctate spots of signal darkening (Fig. 1A). Each dark spot represents a single glomerulus. MRI-based counting (Fig. 1B) of kidneys from mice injected with CF yielded 12,084 ± 1,896 glomeruli per kidney (n=3). Histological validation is pending, though our numbers are consistent with those reported in the literature[6].

Conclusions: This result, along with previous in vivo detection of glomeruli and counts made in the rat, suggest the long-term possibility of counting glomeruli in the clinic.



1. Brenner BM, et al. *Am J Hypertens*. 1988. **2.** Bertram JF, et al. *Cell Tiss. Res.* 1992. **3.** Bennett KM, et al. *Magn Reson Med*. 2008. **4.** Beeman SC, et al. 2011. **5.** Heilmann MM, et al. *Nephrology Dialysis Transplantation*. 2012. **6.** Cullen-McEwen LA, et al. *Hypertension*. 2003.

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FR-PO115

Dual-Linked Role of Integrin αV Mediates TGF-β Activation and Fibronectin Fibrillogenesis to Regulate Laminin-332-Mediated Repair in MDCK Epithelial Cells Jose V. Moyano,[1] Joshua O. Hendin,[1] Patricia Gonzalez-Greciano,[1] Terhi Piritta Teräväinen,[2] Satu Marja Myllymäki,[2] Aki Manninen,[2] Karl S. Matlin.[1] *[1]University of Chicago, Chicago, IL; [2]University of Oulu, Oulu, Finland.*

Background: TGF-β is secreted by cells in an inactive form (*latent TGF*-β) through its association with Latency-Associated Protein (LAP). Activation is believed to involve binding of LAP by αV integrins, but this has not been clearly demonstrated in kidney epithelial cells. TGF-β has been linked by us and others to epithelial repair, but it also plays a role in fibrosis. Thus, understanding the TGF-β activation machinery in renal epithelial cells is of great importance in epithelial repair after injury.

Methods: Integrin αV expression was suppressed by shRNA. Localization of proteins was assessed by confocal microscopy. Interaction between Integrin αV and LAP was established by cell adhesion assays. TGF-β activation was measured by ELISA, and LM-332 synthesis was assessed by metabolic labeling. Smad signaling was determined by Western blot.

Results: MDCK cells with integrin αV knocked down (αVKD) show a reduction in TGF-β activation, Smad2-mediated laminin-332 production and spreading. Addition of active TGF-β restored normal levels of LM-332 and cell spreading. Control, but not αVKD cells, adhered to LAP, suggesting that LAP is a ligand for integrin αV. In control cells, integrin αVβ3 focally colocalized with LAP in the lamellipodium, but were segregated towards the cell body, suggesting its dissociation. Interestingly, TGF-β accumulated in discrete foci towards the dorsal surface in control cells. However, αVKD resulted in almost perfect colocalization of TGF-β with LAP. This suggests that, in the absence of integrin αV, TGF-β cannot dissociate from LAP, possibly due to lack of mechanical tension. Such mechanical tension might be provided by fibronectin (FN). In fact, αVKD cells were unable to assemble FN. FN assembly is dependent on ROCK, and blocking its activity resulted in reduced TGF-β activation.

Conclusions: Mechanical tension provided by integrin αV-mediated FN assembly during cell migration allows integrin αV to also bind to LAP and activate TGF-β to induce LM-332 expression and epithelial repair.

FR-PO116

Interleukin-1-beta Induces Hyaluronan/CD44-Dependent Cell Protrusions that Facilitate Monocyte-Fibroblast Binding Soma Meran, John Martin, Robert Steadman, Aled O. Phillips. *Institute of Nephrology, Cardiff University, Cardiff, United Kingdom.*

Background: Persistent inflammation is a determinant of progressive tissue fibrosis however the reasons for this remain unclear. In the kidney, fibrosis is the hallmark of progressive disease and is preceded by macrophage infiltration and characterised by matrix deposition and fibroblast expansion. There is evidence indicating the role of the cytokine IL-1b in profibrotic responses; and IL-1b staining has been strongly associated with severity of interstitial lesions, proteinuria and renal impairment. In our previous studies we have shown that fibroblasts stimulated with IL-1b increased their generation of the matrix polysaccharide hyaluronan (HA) and greatly increased their expression of the HA Synthase enzyme (HAS2). Objectives: The aim of this study was to determine the role of IL-1b induced changes in HA and HAS2 in the regulation of fibroblast function.

Methods: HA generation was assessed by radiolabelling and chromatography. HAS2 CD44 ICAM1 TSG6 & CD45 expression was assessed by RT-QPCR. HA, CD44 & ICAM1 cell localisation was identified by immunocytochemistry. siRNA technology was used to inhibit HAS2, CD44 & TSG6 expression.

Results: Stimulation of fibroblasts with IL-1b resulted in relocalisation of HA associated with the cell to the outer cell membrane, where it protruded from the cell in a linear manner. Furthermore IL-1b resulted in the formation of HAS2 and CD44-dependent cell membrane protrusions from which the cell-associated HA projected. CD44 was concentrated within these membrane protrusions where it colocalised with the intracellular adhesion molecule, ICAM1, and enhanced IL-1b-dependent fibroblast-monocyte binding. Whilst previous data has shown the importance of the HA binding protein TSG6 in maintaining the TGF-b1-dependent HA coat; TSG6 was not essential for the formation of the IL-1b-dependent HA protrusions thus identifying it as the key difference between IL-1b and TGF-b1 dependent HA matrices.

Conclusions: IL-1b dependent HA and CD44 play a role in fibroblast immune activation leading to sequestration of monocytes within inflamed tissue and providing a possible mechanism for perpetual inflammation and progressive fibrosis.

FR-PO117

Unilateral Nephrectomy in PEXTKO Mice Leads to the Development of Podocyte Hypertrophy Kevin J. McCarthy,[1] Yingjian Wang,[2] Kaitlin Marie McCarthy,[1] Deborah J. McCarthy.[1] *[1]Pathology, LSU Health Sciences Center, Shreveport, LA; [2]Surgery, LSU Health Sciences Center, Shreveport, LA.*

Background: PEXTKO(podocyte-specific EXT1 knockout)mouse podocytes (podos) cannot assemble heparan sulfate (HS) chains on HS core proteins. Previous reports from our laboratory showed that HS- podos develop significant foot process effacement after birth but PEXTKO mice do not develop significant proteinuria. *In vitro* studies of HS- podos show abnormal cytoskeletal organization, cell-matrix adhesion, and cell migration, due to loss of HS associated with cell surface proteoglycans. Because of the latter *in vitro* functional abnormalities, the *in vivo* performance of PEXTKO podos subjected to a physiologic stressor (hyperfiltration) was evaluated.

Methods: Six month old $Ext1^{+/+}$(control) or PEXTKO mice were either unilaterally nephrectomized (UNX) or subjected to sham surgery(SS). After three months, animals from all groups were sacrificed and the tissues processed for light (LM)and electron microscopy (EM). LM tissue sections were analyzed using morphometric methods to measure differences in glomerular hypertrophy and mesangial expansion. EM was used to to evaluate alterations in the nature of podos-GBM interactions.

Results: Morphometry of PAS stained sections showed that both PEXTKO groups had significant increase in glomerular area compared to EXT1 animals (SS/UNX). UNX-PEXTKO glomeruli had a significant increase in glomerular area compared to SS-PEXTKO glomeruli($1.26x10^5 pixels^2$ vs $1.03\ x10^5 pixels^2$, $p<0.0002$). EM studies showed that the podos morphology from UNX-PEXTKO glomeruli differed significantly from those in the other groups by: 1. Primary attachment by direct processes from the cell body to the GBM rather than the usual 1°→2°→pedicel process attachment seen in control animals; 2. the increase in binucleated podos in tissue sections; 3 the extreme crowding of intercapillary spaces with podos cell bodies/processes.

Conclusions: The added stressor (UNX) on HS- podos results in additive glomerular hypertrophy with concomitant change in podos attachment/organization and podo hypertrophy, a potential compensatory response not seen in the EXT1 SS/UNX or PEXTKO SS.

Funding: NIDDK Support

FR-PO118

A New Approach to Targeting the RAS System: Identification of Regulators of AngII-Induced TGFalpha Cleavage, a Novel Risk Factor for CKD Progression Andreas Herrlich. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Despite dual RAS blockade, residual AngII-AT1R-dependent signals accelerate progression of chronic kidney disease (CKD) and 15-20% of treated patients still reach dialysis. AngII-induced metalloprotease cleavage of TGFalpha in the kdiney is critical in the development of CKD lesions in mice and likely humans. Targeting this pathway downstream of the AT1R would circumvent problems of current therapies. Metalloprotease inhibitors have failed clinically due to their broad-spectrum inhibition of substrate cleavage. Our study identifies novel regulators of AngII-induced TGFalpha release that regulate TGFalpha cleavage independent of affecting protease activity itself, a significant advantage over metalloprotease inhibitors.

Methods: To identify novel genes that regulate ectodomain shedding of substrates downstream of PKC activation, we carried out a lentiviral shRNA gene knockdown screen targeting most human kinases and phosphatases, focusing our attention on phorbol ester-induced cleavage of TGFalpha, a classical ADAM17 substrate. Phorbol ester mimics DAG in the activation of PKC. Cleavage of TGFalpha was measured by FACS (fluorescent-activated cell sorting) (Dang et al., 2011; Herrlich et al., 2008) and western blots. Identified regulators were retested in HEK293T cells expressing the AT1R.

Results: We identify here, for the first time, pathway components that distinguish substrates of ADAM17. Downregulation of protein-kinase-C (PKC) alpha or of the PKC-regulated PP1-inhibitor PPP1R14D block AngII-induced cleavage of TGFalpha, HB-EGF and Amphiregulin, but not of Neuregulin, TNFR-1 or the ADAM10 substrate c-Met. This regulation is conferred by inhibition of PP1alpha. NRG cleavage, in contrast to TGFalpha, is regulated by a PKCdelta-dependent C-terminal serine phosphorylation. Using Proteolytic Activity Matrix Analysis, we show that none of the cleavage regulators affects ADAM17 protease activity.

Conclusions: Our study identifies novel regulators of AngII-induced TGFalpha cleavage and shows that regulation of enzyme activity is clearly separated from substrate selection, an important mechanism that offers itself for application in disease.

Funding: NIDDK Support

FR-PO119

Healthy Lifestyle and Risk of Kidney Disease Progression, Cardiovascular Events and Death in the Chronic Renal Insufficiency Cohort (CRIC) Study Ana C. Ricardo,[1,2] Cheryl A. Anderson,[2] Xiaoming Zhang,[2] Michael J. Fischer,[1,2] Martha L. Daviglus,[1] Laura M. Dember,[2] Jeffrey C. Fink,[2] Anne Frydrych,[1,2] Nancy Gail Jensvold,[2] Eva Lustigova,[2] Lisa C. Nessel,[2] Anna C. Porter,[1,2] Mahboob Rahman,[2] Julie A. Wright,[2] Wei (Peter) Yang,[2] James P. Lash.[1,2] *[1]U. Illinois; [2]CRIC Study Group.*

Background: A healthy lifestyle that includes not smoking, regular exercise and low dietary sodium is associated with favorable health outcomes in the general population. The effect of such lifestyle on clinical outcomes in individuals with chronic kidney disease (CKD) is understudied.

Methods: A healthy lifestyle score (HLS) was calculated at baseline for 3,670 CRIC enrollees by allocating 1 point for each of the following 3 factors: not currently smoking; moderate exercise (≥150 min/wk), vigorous (≥75 min/wk) or both (≥150 min/wk); and urinary sodium <100mEq/day. HLS range was 0-3, with 3 indicating the healthiest lifestyle. Cox proportional hazards models were used to evaluate the association of HLS with the following outcomes ascertained over 4 yrs: CKD progression [≥50% eGFR loss or end-stage renal disease], and cardiovascular (CV) events (myocardial infarction, stroke, heart failure or peripheral arterial disease) or death.

Results: Most participants adhered to 1 or 2 healthy lifestyle factors (Table). Women, non-Hispanic Whites and college graduates were more likely to have a HLS of 3 (p<0.01). Adherence to a healthy lifestyle was associated with a significant lower risk of CV events or death. The association of HLS with CKD progression was less definitive (Table).

		CKD Progression		CV Event or Death	
Healthy Lifestyle Score	N (%)	Event Rate*	HR, 95% CI**	Event Rate*	HR, 95% CI**
0	201 (6)	109	Ref	122	Ref
1	1415 (39)	67	0.70, 0.53-0.93	70	0.65, 0.50-0.83
2	1726 (47)	52	0.76, 0.57-1.01	49	0.55, 0.42-0.71
3	328 (9)	41	0.93, 0.62-1.38	39	0.56, 0.38-0.81

*Per 1000 person-year. **Adjusted for baseline demographic and clinical variables

Conclusions: Findings from this cohort suggest that adherence to a healthy lifestyle should be promoted in patients with CKD because it may be associated with more favorable CV and possibly kidney outcomes.

Funding: NIDDK Support, Veterans Administration Support

FR-PO120

Low Health Related Quality of Life Is Associated with Poor Health Outcomes among Participants in the Chronic Renal Insufficiency Cohort (CRIC) Study Anna C. Porter,[1] James P. Lash,[1] Claudia M. Lora,[1] Ana C. Ricardo,[1] Jennifer Lynn Deluca,[3] Radhika Kanthety,[3] John W. Kusek,[2] Lisa C. Nessel,[3] Qiang Pan,[3] Martin J. Schreiber,[3] Julie A. Wright,[3] Dawei Xie,[3] Michael J. Fischer.[1] *[1]Medicine, U. Illinois/Jesse Brown VA, Chicago, IL; [2]NIH/NIDDK; [3]CRIC Study Group.*

Background: Low health-related quality of life (HRQOL) is associated with higher mortality in end-stage renal disease (ESRD), but the association between HRQOL and outcomes is poorly described in adults with stage II-IV chronic kidney disease (CKD).

Methods: We conducted a prospective analysis of CRIC subjects to assess the association between HRQOL and CKD progression (50% eGFR loss or ESRD), incident cardiovascular disease (myocardial infarction, congestive heart failure, stroke, or peripheral arterial disease), and death. HRQOL was assessed with the Kidney Disease Quality of Life-36 (KDQOL-36), which has 5 subscales: mental component summary (MCS), physical component summary (PCS), burden of kidney disease (burden), effects of kidney disease (effects), and symptoms and problems of kidney disease (symptoms). We examined the association between low baseline HRQOL scores (>1 standard deviation below mean) and outcomes with Cox proportional hazards models.

Results: Among 3939 subjects with a mean of 3.9 years of follow up, lower baseline KDQOL subscale scores were associated with non-white race, income <$20,000/yr, diabetes, and urine albumin/Cr >300 mg/g. Crude rates of these outcomes were significantly higher in those with low PCS, effects, and symptom scores compared to those without the low subscale scores (p<0.05). Regression results adjusted for study site, demographics, comorbidity, eGFR and proteinuria are in Table 1.

KDQOL Subscale	Outcome		
	Hazard Ratio for low HRQOL score		
	CKD progression	Death	Incident CV disease
PCS	1.21*	1.61*	1.64*
MCS	1.06	1.24	1.26*
Burden	1.09	1.22	1.25*
Effects	1.07	1.40*	1.46*
Symptoms	0.99	1.33*	1.45*

*p<0.05

Conclusions: In this cohort, many low baseline KDQOL subscale scores were independently associated with an increased risk for worsening CKD, incident CV disease, and death. PCS, effects, and burden subscales had the largest and most consistent association with the outcomes.

Funding: NIDDK Support

FR-PO121

Dietary Patterns and Kidney Disease: A Longitudinal Analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) Julie Lin,[1] Ronit Katz,[2] Jennifer A. Nettleton,[3] Holly J. Kramer,[4] David R. Jacobs,[5] Michael Shlipak,[6] Ian H. de Boer.[2] *[1]Brigham and Women's Hospital, Boston, MA; [2]University of Washington, Seattle, WA; [3]University of Texas Health Science Center, Houston, TX; [4]Loyola Medical Center, Maywood, IL; [5]University of Minnesota School of Public Health, Minneapolis, MN; [6]San Francisco VA, San Francisco, CA.*

Background: Diet may influence kidney disease progression but longitudinal data on this relationship are limited. We examined dietary patterns and change in estimated glomerular filtration rate (eGFR) and urine albumin-creatinine ratio (UACR).

Methods: This is a cohort study of 5,405 MESA participants who had baseline dietary data from a 120-item food frequency questionnaire and at least 2 measures of serum creatinine (sCr), cystatin C (cys C), or UACR. We used principal components analysis to derive 4 dietary patterns (named by highest loading food groups): (1) "fats and processed meats" (similar to a 'Western' pattern); 2) "beans, tomatoes, and refined grains"; 3) "vegetables and fish"; and 4) "whole grains and fruit" (similar to a 'prudent' pattern), previously associated with baseline UACR as well as incident DM and CV disease in MESA. EGFR using sCr or cys C was calculated, and rapid eGFR decline was defined as ≥3 ml/min/1.73 m^2 per year decrease over 5 years of follow up. We also examined change in UACR. We tested associations using logistic regression and linear mixed models.

Results: Mean age was 62 yrs, 52% female, 41% White, 25% Black, 21% Hispanic, 13% Asian, 11% had DM, median baseline eGFR 93 (by cysC) and 78 (by sCr) ml/min/1.73 m^2, and UACR was 5.1 mg/gm. Adherence to each of the 4 dietary patterns varied markedly by race/ethnicity. However, we observed no significant associations between any dietary pattern with either eGFR decline or change in ACR in unadjusted or adjusted models, which included demographics, BMI, HTN, DM, lipids, and physical activity as covariates.

Conclusions: In a large, longitudinal analysis of racially and ethnically diverse adults, we did not observe associations of dietary patterns with change in eGFR or urine ACR over 5 years of follow-up.

Funding: Other NIH Support - NHLBI

FR-PO122

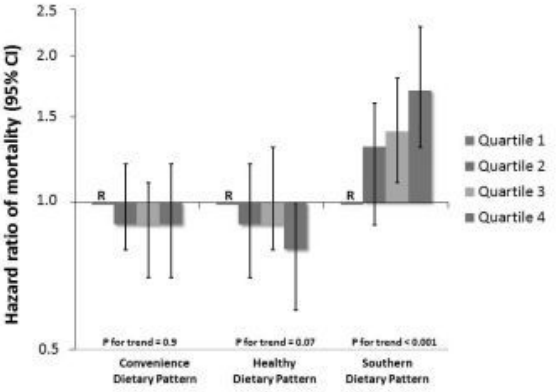
Dietary Patterns and Mortality in Chronic Kidney Disease Orlando M. Gutierrez,[1] Paul Muntner,[1] Dana Rizk,[1] William M. McClellan,[2] David G. Warnock,[1] Suzanne E. Judd.[1] *[1]UAB; [2]Emory.*

Background: Dietary patterns are associated with clinical outcomes in the general population, but few data exist in individuals with chronic kidney disease (CKD).

Methods: We examined the associations of dietary patterns with estimated glomerular filtration rate (eGFR), urinary albumin to creatinine ratio (ACR), c-reactive protein (CRP), and risk of death in 3,971 persons with CKD from the REasons for Geographic and Racial Differences in Stroke (REGARDS) study, a national cohort of black and white adults ≥45 years of age. Principal component analysis was used to empirically derive 5 dietary patterns based on 56 food groups from the Block 98 food frequency questionnaire. We focused on 3 patterns: "convenience," characterized by high intake of Chinese and Mexican foods, pizza, pasta and other mixed dishes; "healthy," by high intake of fruits and vegetables; and "southern," by high intake of fried foods, organ meats, sweetened beverages and by disproportionately high residence in Southern US states.

Results: Convenience pattern scores were not associated with eGFR, ACR, or CRP. In multivariable models adjusted for demographic and clinical factors, higher healthy pattern scores were associated with higher eGFR and lower CRP levels, whereas higher southern pattern scores were associated with lower eGFR, higher ACR, and higher CRP (P for trend<0.05 for all comparisons). There were 611 deaths over a median of 5 years of follow-up. There were no statistically significant associations of convenience or healthy pattern scores with mortality in multivariable-adjusted Cox regression models (figure). In contrast, higher southern pattern scores were associated with higher risk of death in the multivariable model (P for trend<0.001).

Figure: Multivariable-adjusted* hazard ratio (95% CI) of death according to quartiles of convenience, healthy and southern dietary pattern scores



*Adjusted for age, race, gender, geographic region of residence, energy intake, smoking, physical activity, education, income, hypertension, heart disease, urinary ACR, eGFR

Conclusions: A southern dietary pattern rich in fried foods, organ meats and sweetened beverages was independently associated with higher risk of death in persons with CKD.

Funding: NIDDK Support, Other NIH Support - NINDS, Pharmaceutical Company Support - Amgen Corporation

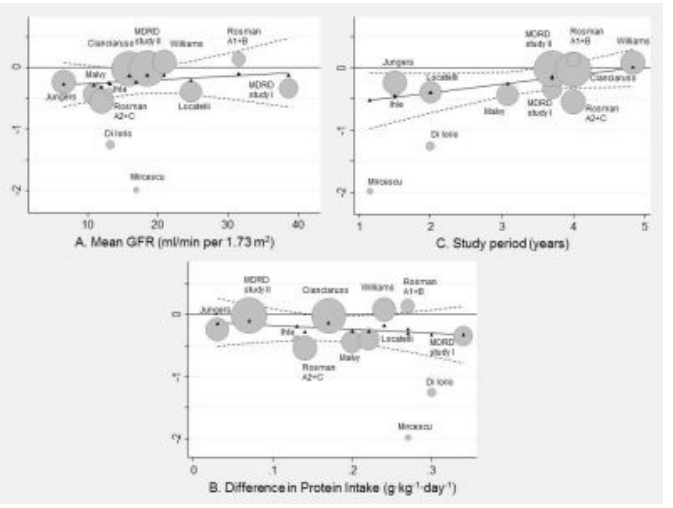
FR-PO123

Examining Heterogeneity in the Effect of Protein Restriction for Chronic Kidney Disease: A Meta-Analysis and Meta-Regression of Randomized Controlled Trials Yoshitsugu Obi,[1] Yoshiki Suzuki,[2] Yoshitaka Isaka,[1] Toshiki Moriyama.[3] *[1]Department of Geriatric Medicine & Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Health Administration Center, Niigata University, Niigata, Japan; [3]Health Care Center, Osaka University, Toyonaka, Osaka, Japan.*

Background: Protein restriction offers metabolic benefits to patients with advanced chronic kidney disease (CKD). However, its effect on reducing death or end-stage renal disease (ESRD) remains inconclusive.

Methods: We performed a meta-analysis on the efficacy of protein restriction in reducing the incidence of death or ESRD among adult patients with moderate to severe CKD. We included 10 randomized controlled trials (RCTs) (n = 1,128) comparing 2 protein intake level with ≥1 year of follow-up. To elucidate the heterogeneity in the results of RCTs, we also conducted meta-regressions using the influential characteristics of each trial.

Results: The pooled relative risk (RR) was 0.80 (95% CI, 0.77-0.96), but with obvious "small study effects" favoring protein restriction. Although insignificant due to low statistical power, meta-regressions showed that the lower mean kidney function of the participants and the larger difference of protein intake between study groups tended to be associated with the larger effect. Conversely, the effect was smaller in trials with longer follow-up period, and lost its significance with >3 years of follow-up.



We also found that protein restriction did not show any significant renoprotective effects in RCTs where metabolic acidosis was appropriately corrected in both study groups. Moreover, many RCTs did not even report the serum bicarbonate concentration.

Conclusions: Protein restriction delays the development of ESRD; however, the effecacy under the current standard treatment would be overestimated based on information available from published studies.

FR-PO124

Role of Potential Renal Acid Load (PRAL) in the Relation of Food Insecurity with Kidney Damage Tanushree Banerjee,[1] Deidra C. Crews,[2] Neil R. Powe.[1] *[1]University of California, San Francisco; [2]Johns Hopkins University, Baltimore.*

Background: Food insecurity refers to the inability to afford nutritionally adequate and safe foods, and has been shown to have an association with chronic diseases, including CKD. We hypothesized that food insecurity may influence dietary intake, as can be estimated by calculating dietary Potential Renal Acid Load (PRAL), and thus kidney damage associated with food insecurity could be mediated by PRAL.

Methods: Our analysis included 10,825 participants from the National Health and Nutrition Examination Survey 2001-2006, aged >20 years with a household income ≤400% of the federal poverty level. We considered a participant food insecure if ≥3 items in the 18-item questionnaire were answered affirmatively. We calculated PRAL based on 1-day dietary recall and body surface area, where the highest quintile of PRAL was the least desirable. Kidney damage was defined as microalbuminuria (30-300 mg/g creatinine) or macroalbuminuria (>300 mg/g). Multivariable logistic regression was used to estimate the association between food insecurity, PRAL, and kidney damage, with adjustment for demographics (age, gender, race/ethnicity), income, education, hypertension, diabetes and obesity.

Results: 1,253 (11.6%) participants were food insecure. The median value of PRAL in the food insecure group was greater, -2155.89 mEq/100gm, than in the food secure group, -2461.64 mEq/100gm. Food insecurity showed significant association with the highest quintile of PRAL compared to the lowest quintile both in unadjusted and adjusted analysis (OR [95% CI: 1.69 [1.13, 2.55]). Food insecurity was also associated with macroalbuminuria in unadjusted analyses and after adjustment for demographics (OR [95% CI]: 1.88 [1.16, 3.06]), however inclusion of PRAL to the model fully attenuated the relationship of food insecurity and macroalbuminuria to non-significance (OR [95%CI]: 0.78 [0.38, 1.59]).

Conclusions: Food insecurity is associated with increased dietary acid load which may explain the relationship of food insecurity with macroalbuminuria. Further studies are needed to elucidate the role of high dietary acid load in socioeconomic disparities in CKD.

FR-PO125

Association between Dietary Potential Renal Acid Load (PRAL) and Hyperfiltration Tanushree Banerjee,[1] Deidra C. Crews,[2] Donald E. Wesson,[3] Rajiv Saran,[4] Anca Tilea,[4] Meda E. Pavkov,[5] Nilka Rios Burrows,[5] Neil R. Powe.[1] *[1]University of California, San Francisco; [2]Johns Hopkins University, Baltimore; [3]Texas A&M College of Medicine; [4]University of Michigan, Ann Arbor; [5]Centers for Disease Control and Prevention.*

Background: Hyperfiltration is the result of intraglomerular hypertension, possibly leading to microalbuminuria, and may be influenced by dietary habits. We undertook this study to examine the association of dietary habits, which we quantify as Potential Renal Acid Load (PRAL), with hyperfiltration and with microalbuminuria in the hyperfiltering group.

Methods: We considered 15,903 U.S. adults aged >20 years in NHANES 1999-2004 as our sample. Hyperfiltration was defined as eGFR≥135 ml/min/1.73m^2 which was at least 2 standard deviations above the mean GFR (107 ml/min/1.73m^2) in the reference population with eGFR 80-130 ml/min/1.73m^2. eGFR was calculated using CKD-EPI equation. We calculated PRAL based on 1-day dietary recall and body surface area where the highest quintile of PRAL was the least desirable value. Variables included in logistic regression models were demographics (age, gender, race/ethnicity), poverty status, hypertension, diabetes and obesity.

Results: 6,364 (40%) participants had hyperfiltration. There was a significant linear trend across quintiles of PRAL and hyperfiltration (p=0.003). Fully, 491 (8%) participants with hyperfiltration had microalbuminuria. Significant differences were seen in PRAL values across the normoalbuminuria and microalbuminuria groups (p=0.0002). In unadjusted analyses, a greater PRAL level was associated with greater prevalence of microalbuminuria in the hyperfiltering group. After adjustment, the highest quintile of PRAL was associated with significantly greater odds of microalbuminuria [OR (95% CI) = 3.77 (1.69, 8.43)] compared with the lowest quintile. A significant trend was observed across quintiles of PRAL and microalbuminuria for hypertensives only (p=0.045).

Conclusions: Dietary acid load are associated with microalbuminuria in hyperfiltering individuals. These findings have implications for dietary modification as a potential intervention to reduce high PRAL related albuminuria.

Funding: Other U.S. Government Support

FR-PO126

High Sodium and Low Potassium Dietary Intake May Be Linked to Obesity Risk Nishank Jain,[1,2] Abu T.M. Minhajuddin,[2] Essam F. Elsayed,[1,2] Robert F. Reilly,[1,2] Susan Hedayati.[1,2] *[1]VA North Texas Health Care System; [2]UT Southwestern Medical Center.*

Background: Previous studies that reported an association of dietary sodium (Na) with metabolic syndrome and type 2 diabetes mellitus were limited by use of imperfect measures of Na intake such as dietary recall. To our knowledge, no studies have investigated the association of dietary potassium (K) with obesity. We hypothesized that high dietary Na and low K measured by first void morning urinary Na-to-K ratio ($U_{Na/K}$) is independently associated with markers of obesity in 3,303 participants of the population-based multi-ethnic Dallas Heart Study.

Methods: Robust linear regression was used to explore the association of $U_{Na/K}$ with body mass index (BMI) and total body percent fat (TBPF, measured by dual X-ray absorptiometry scan and expressed in percent), which mitigates the violations of linear regression assumptions when $U_{Na/K}$ distribution is right-skewed. Multivariable models were adjusted for age, race, gender, systolic and diastolic blood pressure and diabetes mellitus.

Results: Of the cohort, 52% were African American, 56% female, 17% Hispanic, 12% diabetic, and 36% hypertensive. Mean (SD) age was 44 (10) years, BMI 30 (7) kg/m^2, TBPF 32 (10) % and $U_{Na/K}$ 4.2 (2.8). In the unadjusted model, for each SD (3 units) increase in $U_{Na/K}$, BMI increased by 0.6 kg/m^2, 95% CI (0.4, 0.9), and TBPF by 0.8% (0.3, 1.3), p <0.0001 and 0.003, respectively. This association remained significant even after adjusting for confounders. TBPF increase was higher in Hispanics and Caucasians than African Americans per 3-unit increase in $U_{Na/K}$, interaction p = 0.02.

Association of Urinary Sodium-to-Potassium Ratio with Body Mass Index (BMI) and Total Body Percent Fat (TBPF)

Univariate Model	Change (95% CI), per 3-unit increase in UNa/K
BMI (kg/m2)	0.63 (0.38, 0.87)
TBPF(%)	0.75 (0.25, 1.25)
Multivariate Model	
BMI (kg/m2)	0.40 (0.17, 0.63)
TBPF(%)	0.45 (0.16, 0.73)

Conclusions: Dietary sodium excess and potassium deficiency may be associated with obesity, independent of other cardiovascular risk factors. Future clinical trials should explore whether modification of dietary Na and K could be used as an interventional strategy to reduce the prevalence of obesity.

Funding: Veterans Administration Support

FR-PO127

Relationships between Osteoprotegerin Level, Vascular Stiffness and Patient Survival in CKD James Ritchie,[1] Lakhvir Assi,[2] Richa Sinha,[1] Darren Green,[1] Philip A. Kalra.[1] *[1]Vascular Research Group, University of Manchester; [2]The Binding Site Group, Birmingham, United Kingdom.*

Background: Osteoprotegerin (OPG), produced by osteoblasts and vascular endothelium, is implicated in the development of vascular calcification in patients with CKD. Elevated levels have been associated with increased mortality in advanced CKD. However, little data exist for earlier CKD stages. This study aims to:

1 Assess the relationship between OPG and mortality in moderate CKD.

2 Explore the relationship between OPG level and vascular stiffness.

Methods: 499 patients with all cause CKD were identified within the Chronic Renal Insufficiency Standards Implementation Study (CRISIS). Vascular stiffness was assessed with pulse wave analysis (augmentation index corrected for heart rate, AIx). OPG levels were measured from stored serum samples, taken on the same day as AIx measurements. Patients were divided by OPG quartile. Survival differences (all cause mortality) between quartiles were assessed using Cox regression. Differences in Aix between quartiles were calculated using a linear regression model. Analyses were adjusted for age, gender, blood pressure, renal function, previous cardiovascular events, smoking, use of angiotensin blockade/statin therapy. The lowest quartile provided the referent group.

Results: Mean age was 65, eGFR 36ml/min/1.73m^2. Hazard ratio (HR) for death significantly increased with each quartile of OPG (quartile 2,3,4 HR for death: 4.0; 5.8; 8.7 respectively, p<0.001 for all). This finding was consistent when the model was adjusted for AIx, but not when also adjusted for other vascular risk factors (quartile 2, 3 and 4 adjusted HR for death: 1.75(p=0.2); 2.65(p=0.03); 2.17(p=0.09)). A significant increase in Aix for higher OPG levels was observed. The increase in Aix peaked in the 3rd quartile of OPG level (23% higher Aix in quartile 3 vs. quartile 1, p=0.002), and was less elevated for the 4th quartile (12% higher Aix in quartile 4 vs. quartile 1, p=0.02).

Conclusions: In moderate CKD, increased OPG levels are associated with increased vascular stiffness. An association is also observed for risk of death, though this becomes less significant when traditional risk factors are taken into account.

FR-PO128

Hypertensive Pregnancy Disorders as a Risk Factor for Future Renal Disease: A Population-Based Study Andrea G. Kattah,[1] Catherine M. Brown,[2] Slavica Katusic,[3] Cynthia L. Leibson,[3] Jeanine Ransom,[3] Amy L. Weaver,[3] Virginia Miller,[4] Veronique L. Roger,[4] Vesna D. Garovic.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Beaumont Hospital, Dublin, Ireland; [3]Health Sciences Research, Mayo Clinic, Rochester, MN; [4]Mayo Clinic, Rochester, MN.*

Background: The long-term effects of hypertensive pregnancy disorders (HPD), including preeclampsia, on kidney function later in life remain unclear. While early reports suggested that there is no increased risk of chronic kidney disease (CKD) after an episode of preeclampsia, more recent case-control and registry-based cohort studies indicate that there is an increased risk of microalbuminuria, need for renal biopsy, and CKD in the affected women later in life. We aimed to confirm the association between HPD and future renal disease in a population-based study.

Methods: Using the unique population-based longitudinal resources of the Rochester Epidemiology Project (REP), we identified all female residents of Olmsted County, MN who delivered between 1976 and 1982. Our exposure definition included any diagnostic code suggestive of HPD and related complications. Outcomes were defined based on codes for renal disease (including albuminuria, renal failure, and CKD) occurring at any time after index pregnancy.

Results: A total of 10,295 births occurred in Olmsted County from 1976-1982, which identified 8100 mothers, of which 7582 had REP records (93%). A total of 1023 mothers had at least one live or stillbirth with codes suggestive of HPD. The median age of women still living in Olmsted County was 52 years. Looking at the entire cohort, 652 (8.6%) mothers had a renal outcome of interest. Women with a history of HPD, compared to those without HPD, had a significantly higher risk for renal disease: 149 of 1023 (15%) versus 503 of 6559 (8%), p<0.0001. The relative risk of having renal disease was 2.05 (CI 1.69-2.50) for women with, versus those without, diagnostic codes suggestive of HPD.

Conclusions: Women with a history of HPD, including preeclampsia, may be at increased risk of renal disease later in life. Our data suggest that the renal function of the affected women should be closely monitored after their pregnancies.

FR-PO129

Kidney Disease Is an Independent Risk Factor for Maternal Mortality in Pregnancy Shailendra Sharma,[1] John R. Holmen,[2] M. Chonchol,[1] Gerard John Smits,[1] Jessica B. Kendrick.[1] *[1]University of Colorado School of Medicine, Aurora, CO; [2]Intermountain Health Care, Salt Lake City, UT.*

Background: Presence of kidney disease is recognized to adversely affect maternal outcomes during pregnancy. The objective of this study is to provide a contemporary assessment of the risk of kidney disease during pregnancy on important maternal outcomes including death.

Methods: We used data from an integrated healthcare system between 2000 and 2011. Of 317,990 pregnancies, 646 pregnancies from women with kidney disease were identified and 62,757 pregnancies from women without kidney disease were randomly selected for comparison. Kidney disease was defined by ICD9 code. Adverse maternal outcomes included pre-term delivery, delivery via cesarean section, length of stay in the hospital and maternal death. Multivariate logistic regression analysis was used to examine the association between kidney disease and adverse maternal outcomes adjusted for important confounders.

Results: In the whole study population there were 4,068 (25%) preterm deliveries, 12,025 (52%) cesarean sections, 113 (1.1%) maternal deaths and the mean (SD) length of stay in the hospital was 2 ± 1.8 days. Women with kidney disease had a mean (SD) age and gestational age at delivery of 28 ± 5 years and 37 ± 3 weeks, respectively. Women with kidney disease were more likely to have comorbid conditions including a history of chronic hypertension (n=177; 27%) and diabetes (n=77; 12%). Compared to women without kidney disease, those with kidney disease had a three-fold increased risk of death after adjusting for age, race, history of diabetes, chronic hypertension, liver disease and connective tissue disorders (OR 3.38, 95% CI 1.40 to 8.12). Furthermore, women with kidney disease had an increased risk of preterm delivery (OR 1.95, 95% CI 1.37 to 2.77), delivery via cesarean section (OR 1.38, 95% CI 1.04 to 1.82) and longer length of stay in the hospital (OR 1.39, 95% CI 1.04 to 1.86).

Conclusions: Pregnant women with kidney disease have an increased risk of adverse maternal outcomes including maternal mortality independent of underlying comorbid conditions that can occur with kidney disease.

Funding: NIDDK Support

FR-PO130

Smoking and Risk of End-Stage Kidney Failure in the Singapore Chinese Health Study Tazeen H. Jafar,[1] Jin Ai Zhen,[2] Woon-puay Koh,[3] Khuan Yew Chow.[2] *[1]Duke-NUS Graduate Medical School, Singapore; [2]Health Promotion Board, Singapore; [3]National University of Singapore.*

Background: Studies in European-origin populations suggest potential association between smoking and chronic kidney disease. However, the relationship between smoking and risk of end-stage kidney failure (ESKF) in people of Chinese origin is not clear. We

analyzed data from the Singapore Chinese Health Study to investigate whether smoking increases the risk of ESKF.

Methods: The Singapore Chinese Health Study is a population-based cohort of 63,257 Chinese adults enrolled between 1993 and 1998. Information on smoking status was collected at baseline. Incidence of ESKF was identified via record linkage with the nationwide Singapore Renal Registry until 2008. ESKF was defined by one of the following: 1) serum creatinine ≥5.7 mg/dl, 2) estimated glomerular filtration rate < 15 ml/min/1.73m^2, 3) hemodialysis or peritoneal dialysis, 4) kidney transplantation. Multivariable model was built. Cox proportional hazard regression analysis was performed for the outcome of ESKF after adjusting for age, education, dialect, herbal medications, body mass index, sex, physician-diagnosed hypertension and diabetes.

Results: The mean age of subjects was 55.6 years, 44% were men, and 30.6% were smokers (current or former) at baseline. A total of 674 incident cases of ESKF occurred during a median follow-up of 13.3 years (774,434 person years). Among men, smokers had a significant increase in the risk of ESKF [hazard ratio (HR): 1.29; 95% CI: 1.02-1.64] compared to never smokers. There was a strong dose-dependent association (p for trend=0.011 for duration, and p for trend =0.02 for never, light, and heavy smoker defined by quanity and duration of smoking). The risk of ESKF decreased after prolonged cessation (≥10 years since baseline). Among women, the direction of the relationships between smoking and ESKF were similar however there were too few smokers for conclusive results.

Conclusions: Cigarette smoking is associated with an increased risk of ESKF among Chinese men. The risk appears to be dose and duration dependent, and modifiable after long duration of cessation.

Funding: Other NIH Support - National Cancer Institute

FR-PO131

Serum Cholesterol-Ester and Adipose Tissue Fatty Acid Composition as Biomarkers of Habitual Dietary Fat Intake in Older Men with Chronic Kidney Disease Xiaoyan Huang,[1] Per Sjogren,[2] Tommy Cederholm,[2] Johan Arnlov,[2,3] Bengt Lindholm,[1] Ulf Risérus,[2] Juan Jesus Carrero.[1,4] *[1]Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden; [2]Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden; [3]School of Health and Social Studies, Dalarna University, Falun, Sweden; [4]Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.*

Background: Fatty acid (FA) composition in serum cholesterol esters (CE) and adipose tissue (AT) can provide an accurate measure of long-term FA intake. The objective was to identify which CE and AT fatty acids were suitable biomarkers of habitual FA intake in individuals with chronic kidney disease (CKD).

Methods: Cross-sectional analysis in 506 men of 70 years of age and with serum cystatin C-based glomerular filtration rate (GFR) <60 ml/min per 1.73 m^2 from the Uppsala Longitudinal Study of Adult Men cohort. Dietary habits were evaluated with a 7-day dietary record. FA compositions of CE and AT were analyzed by gas-liquid chromatography in two random subsamples of 248 and 318 individuals, respectively.

Results: Both in CE and AT, linoleic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were strongly associated with their corresponding intake, after adjustments for non-dietary factors. CE and AT proportions of palmitic and palmitoleic acid moderately correlated with dietary intake, while correlations for other FA were weaker or absent. Proportions of EPA and DHA in CE and AT were positively associated with total energy-adjusted fish intake. Results were confirmed in adequate reporters as identified by the Goldberg cutoff method. These relationships held constant, regardless of GFR above or below 45 ml/min per 1.73 m^2 or the prevalence of microalbuminuria.

Conclusions: Linoleic acid, EPA, and DHA in serum CE and AT are fairly adequate indicators of habitual dietary FA intake in older CKD men. Dietary fish intake may serve as a surrogate marker of intake of n-3 polyunsaturated FA of marine origin.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

FR-PO132

Provider and Pre-ESRD Care Characteristics Associated with eGFR at Dialysis Initiation Yelena Slinin,[1] Haifeng Guo,[2] Suying Li,[2] Jiannong Liu,[2] David T. Gilbertson,[2] Allan J. Collins,[2] Areef Ishani.[1,2] *[1]Veterans Administration Health Care System, Minneapolis, MN; [2]Chronic Disease Research Group, Minneapolis, MN.*

Background: There is a trend in the United States toward initiation of chronic dialysis at higher levels of estimated glomerular filtration rate (eGFR). Provider and medical care characteristics associated with timing of dialysis initiation as assessed by eGFR are unknown. We aimed to determine whether provider characteristics and pre-ESRD care patterns are independently associated with higher eGFR at dialysis initiation.

Methods: Using United States Renal Data System data for patients who initiated dialysis during 2006 (N=83,621), we defined patient characteristics, eGFR, and identified provider at dialysis initiation. Provider data were obtained from the AMA. Provider/patient ratio (per 100 ESRD patients) in each Health Service Area in 2006 was calculated, expressed as quintiles, and presented by HSA of patients' residence. We determined associations between provider characteristics and eGFR at dialysis initiation (eGFR>10 ml/min – early initiation) using logistic regression.

Results: Of the 83,621 patients included into the cohort, 47.9% initiated dialysis at eGFR > 10 ml/min, 16.2% at eGFR ≥15ml/min.

Provider and Medical Care Characteristics	Logistic model eGFR > 10 vs. < 10 ml/min, AOR (95% CI)
Years in Practice	
0 - 8	1
9- 21	0.92(0.89-0.95)**
22+	0.86(0.83-0.90)**
Foreign medical graduate (yes)	1.16(1.12-1.19)**
Nephrology provider density per 100 / ESRD patients (ratio)	
0	1.00(0.92-1.08)
>0-<=0.6	0.90(0.85-0.96)**
>0.60-<=1.0	0.96(0.92-0.99)*
>1.0-<=1.5	1
>1.5	0.91(0.88-0.95)**
Pre-ESRD Nephrology Care	
None	1
<12 months	1.17(1.13-1.22)**
>=12 months	1.05(1.01-1.10)*
Access at Dialysis Initiation	
Catheter	1
Fistula	1.04(1.00-1.09)*
AV Graft	1.16(1.08-1.24)**

*P<0.5; **P<0.001;aAdjusted for patient age, race, ethnicity, primary cause of ESRD, SES, eligibility for Medicaid, urban status, comorbidities, inability to ambulate or transfer, hemoglobin, albumin, BMI, provider's gender, and variables in the table.

Conclusions: Several provider and pre-ESRD care characteristics were independently associated with early dialysis initiation.

Funding: NIDDK Support

FR-PO133

Association of Duration of Residence in the Southeastern United States with Chronic Kidney Disease (CKD) Differs by Race: The Reasons for Geographic and Racial Differences in Stroke Study Laura Plantinga,[1] Virginia J. Howard,[2] Suzanne E. Judd,[2] Paul Muntner,[2] Rikki M. Tanner,[2] Dana Rizk,[2] Daniel T. Lackland,[3] David G. Warnock,[2] George Howard,[2] William M. McClellan.[1] *[1]Emory University, Atlanta, GA; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Medical University of South Carolina, Charleston, SC.*

Background: Longer duration of residence in the southeastern United States is associated with higher prevalence of hypertension. We explored whether a similar association exists for CKD within black and white populations.

Methods: In a national population-based cohort study of 30,239 adults (≥45 years; 42% black/58% white) enrolled 2003-2007, lifetime southeastern residence duration was calculated and categorized [none (0%), less than half (>0-<50%), half or more (≥50-<100%), all (100%)]. Prevalent albuminuria (urine albumin:creatinine ratio≥30 mg/g) and reduced kidney function (eGFR<60 ml/min/1.73 m^2) were defined at enrollment. Incident end-stage renal disease (ESRD) was determined through linked United States Renal Data System records.

Results: White and black participants most often reported living their entire lives outside (35.7% and 27.0%) or inside (27.9% and 33.8%, respectively) the southeastern United States. Albuminuria prevalence was not associated with southeastern residence duration, except for half or more vs. none among blacks only (OR=0.74, 95% CI, 0.62-0.89). There was no statistical interaction of race with southeastern residence duration, after adjustment for age, sex, education, income, diabetes, and hypertension ($P_{raceXduration}$=0.27). ESRD incidence was not associated with 100% vs. 0% southeastern residence duration (HR=0.50, 95% CI, 0.22-1.14) among whites but was associated with an increased risk among blacks (HR=1.63, 95% CI, 1.02-2.63; $P_{raceXduration}$=0.011). Reduced kidney function was not associated with southeastern residence duration in whites or blacks.

Conclusions: Estimates suggest that blacks who live in the Southeast their entire lives, relative to never having lived there, are at increased risk of ESRD. These results could help generate further hypotheses regarding the effects of region of residence on the prevalence and incidence of CKD.

Funding: Other NIH Support - NINDS, Pharmaceutical Company Support - Amgen

FR-PO134

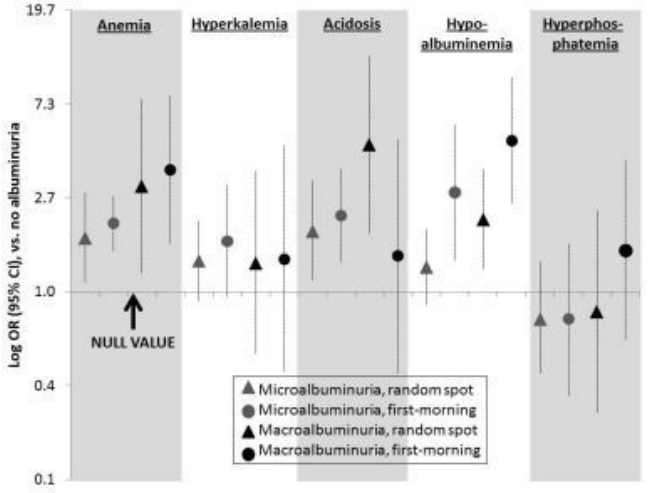
Estimates of Effect of Albuminuria on Common Kidney Complications May Be Underestimated by Random Albuminuria Measurement Laura Plantinga,[1] Vanessa Grubbs,[2] Jerry Yee,[3] William M. McClellan,[1] Nilka Rios Burrows,[4] Mark Eberhardt,[5] Neil R. Powe.[2] *[1]Emory University, Atlanta, GA; [2]University of California, San Francisco, CA; [3]Henry Ford Hospital, Detroit, MI; [4]Centers for Disease Control and Prevention, Atlanta, GA; [5]National Center for Health Statistics, Hyattsville, MD.*

Background: Single urine albumin collection may result in misclassification of albuminuria and bias in risk estimates, which may be reduced by first-morning vs. casual (random) specimen collection. We estimated the associations of albuminuria with common CKD complications using both collection methods and compared the results.

Methods: Using 2009-2010 National Health and Nutrition Examination Survey data, we estimated the urine albumin:creatinine ratio (UACR) of 4,938 non-pregnant adults (≥20 years) with casual urine and first-morning specimens (within 10 days). We used logistic regression to estimate odds ratios (ORs) and 95% CIs by method of urine collection and microalbuminuria (30-299 mg/g) and macroalbuminuria (≥300 mg/g) vs. no albuminuria for anemia (hemoglobin <13/<12 mg/dl for males/females), hyperkalemia (potassium >4.5

mmol/l), acidosis (bicarbonate <23 mmol/l), hypoalbuminemia (albumin <4.0 g/dl), and hyperphosphatemia (phosphate >4.5 mg/dl), adjusting for age, race/ethnicity, sex, eGFR, diabetes, and hypertension.

Results: Generally, ORs were farther from the null when albuminuria was assessed by first-morning vs. casual urine. Precision (width of confidence interval on the log scale) with first-morning vs. casual specimens was better for anemia (64% smaller) but worse for hyperkalemia, acidosis, hypoalbuminemia, and hyperphosphatemia (38% larger, on average).



Conclusions: Assessment of albuminuria obtained casually vs. first-morning produced ORs for the association of albuminuria with CKD complications that were closer to the null.

Funding: Other U.S. Government Support

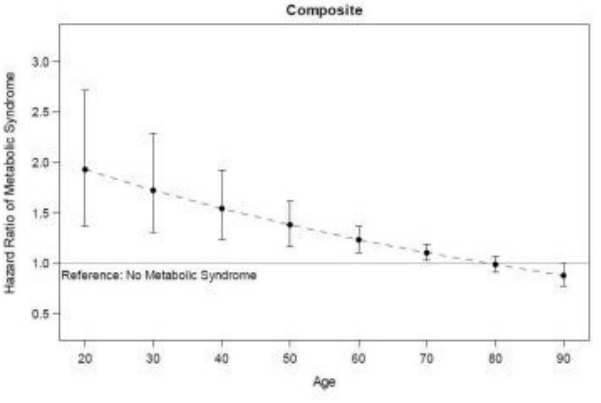
FR-PO135

Metabolic Syndrome, Kidney Disease Progression and Death Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Anne S. Tang,[2] George Thomas,[1] Martin J. Schreiber,[1] Emilio D. Poggio,[1] S. Beddhu,[3] Joseph V. Nally.[1] *[1]Nephrology, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Nephrology, University of Utah.*

Background: Previous studies reported an association between metabolic syndrome (MetS) and incident chronic kidney disease. We examined the associations between MetS and its components with kidney disease progression and death among those with stage 3 and 4 CKD.

Methods: 25,882 patients with stage 3 and stage 4 CKD who had relevant data relating to MetS components after the diagnosis of CKD were included. Cox proportional hazards models were used to assess the associations between MetS, its components and the composite end-point (all-cause mortality and End Stage Renal Disease[ESRD]) while adjusting for relevant confounding variables. Two-way interactions between MetS and age, gender, race and eGFR were examined.

Results: Forty-two percent of the study population (n=10,934) had MetS. During a median follow-up of 2.4 years, 3691 participants died or reached ESRD. In the cox proportional hazards model, presence of MetS was not associated with the composite end-point (HR 1.06; 95% CI 0.99,1.14). However, age modified these associations with younger patients with MetS having a higher risk for reaching the composite end-point (p for interaction <0.001).



Among the individual components of MetS, diabetes (HR 1.32; 95% CI 1.22, 1.43), elevated triglycerides (HR 1.07; 95% CI 1.00, 1.15) and low HDL levels (HR 1.17; 95% CI 1.09, 1.26) were associated with the composite end-point. No significant association between hypertension and obesity with the composite end-point was noted.

Conclusions: Presence of MetS in younger stage 3 and stage 4 CKD patients are associated with all-cause mortality and ESRD. The associations between individual components of MetS and the composite end-point seem to vary with diabetes and dyslipidemia having pronounced associations.

Funding: NIDDK Support, Pharmaceutical Company Support - Development of the CCF CKD Registry Was Funded by an Unrestricted Educational Fund to the Department of Nephrology and Hypertension, Cleveland Clinic

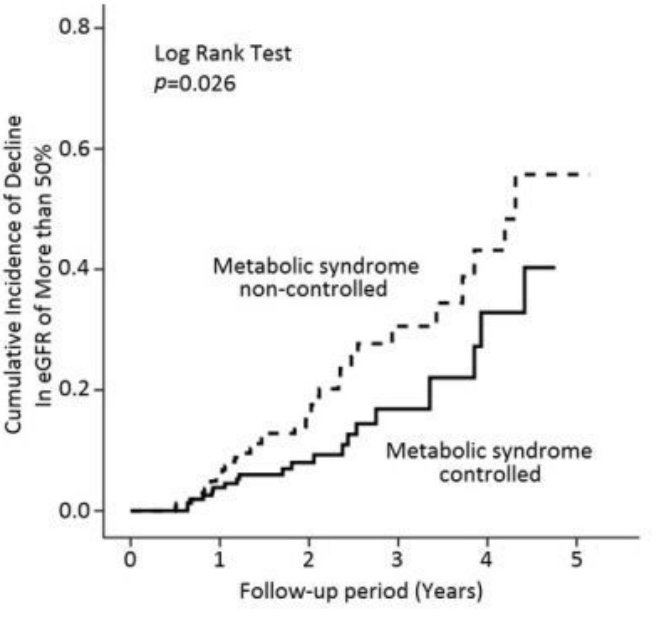
FR-PO136

Treating Metabolic Syndrome Improves Progression of Chronic Kidney Disease Yuan-chun Chiang,[1,2] Chih-Ching Lin,[1,2] Der-Cherng Tarng,[1,2] Yao-ping Lin,[1,2] Jinn-Yang Chen,[1,2] Wu-Chang Yang.[1,2] *[1]Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan; [2]School of Medicine, National Yang-Ming University, Taipei, Taiwan.*

Background: Metabolic syndrome (MetS) is a well-known risk factor for development and progression of chronic kidney disease (CKD). However no data is available to evaluate whether treating MetS components can ameliorate renal function deterioration in such patients with CKD, which prompted this study in a single medical institute.

Methods: A longitudinal retrospective cohort study enrolled 323 patients with incident CKD (eGFR<60ml/min/1.73m2) and MetS who had been followed for ≥12 months. Diagnosis of MetS was defined as modified National Cholesterol Education Program (NCEP), in which the waist circumference>90cm was substituted with body mass index ≥24. After 3 months treatment, patients were divided into 2 groups: MetS controlled (MetS components<3, group 1) and MetS non-controlled (MetS components≥3, group 2). The primary endpoint is defined as reduction in eGFR of > 50%.

Results: The baseline eGFR was comparable between these two groups (27.9±12.1 vs. 28.1±14.1 ml/min/1.73m2, p=0.88), but group 2 had significantly more female, type 2 diabetics and Mets component numbers. During a 4.5 years follow-up group 1 patients had lower cumulative incidence of reduction in eGFR>50%.



Moreover, the more MetS components were controlled, the better preservation of renal function was achieved (p=0.025, figure not shown).

Conclusions: MetS and more MetS components are associated with higher incidence of CKD. This study demonstrated that treating metabolic syndrome and its components has reno-protection effect to delay CKD progression.

Funding: Government Support - Non-U.S.

FR-PO137

Non-Steroidal Anti-Inflammatory Drugs and Chronic Kidney Disease Progression: A Systematic Review and Meta-Analysis Paul Nderitu, Lucy Doos, Peter W. Jones, Simon J. Davies, Umesh Kadam. *Health Service Research Unit, Institute of Science and Technology in Medicine, United Kingdom.*

Background: Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are widely regarded as one of the risk factors which influence Chronic Kidney Disease (CKD) progression. However, previous literature reviews have not quantified the risk in moderate to severe CKD patients. Our systematic review aims to estimate the strength of association between chronic NSAID use and CKD progression using meta-analysis, by identifying general population studies.

Methods: Searched electronic databases were MEDLINE, EMBASE, Cochrane, AMED, BNI, and CINAHL until September 31st 2011 without date or language restrictions. Searches also included the reference lists of relevant identified studies, WEB of KNOWLEDGE, openSIGLE, specific journals, the British Library and expert networks.

For relevant studies, random effects meta-analysis was used to estimate the association between NSAID use and accelerated CKD progression (defined as a Glomerular Filtration Rate (GFR) decline of ≥15ml/min/1.73m^2).

Results: From a possible 768 articles, after screening and selection, seven studies were identified (5 cohort, 1 case-control and 1 cross-sectional). Using 3 cohort studies (total sample size, n=54,663), regular dose NSAID use did not significantly affect the risk of accelerated CKD progression; pooled Odds Ratio (OR)= 0.96 (95%CI; 0.86 to 1.07), but high dose NSAID use significantly increased the risk of accelerated CKD progression; pooled OR= 1.26 (95%CI; 1.06 to 1.50).

Conclusions: The avoidance of NSAIDs in the medium term is unnecessary in patients with moderate to severe CKD, if not otherwise contraindicated. As the definition of high dose use remains unclear, the lowest effective dose of NSAIDs should be prescribed where indicated.

Funding: Private Foundation Support

FR-PO138

Non-Steroidal Anti-Inflammatory Drug Prescription and Significant Chronic Kidney Disease Progression: A Clinical Linkage Study from General Practice

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Background: The association between Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Chronic Kidney Disease (CKD) progression is unclear. However, NSAIDs are commonly used by patients with inflammatory musculoskeletal conditions. This study aims to quantify the risk of medium term NSAID prescription on CKD progression.

Methods: A historical cohort was constructed from two population-based general practices in England (UK) linking diagnostic, prescribing and routine clinical data. This dataset included all subjects aged 40 years and over with 2 or more estimated Glomerular Filtration Rate (eGFR) measurements spaced at least 90 days apart between January 1st 2009 and December 31st 2010 (n=4,145). Cumulative NSAID prescriptions given before the last eGFR test were standardised using the Defined Daily Dose (DDD) and subjects were categorised into non-user (0 DDDs), normal dose (DDDs <85th percentile) and high dose (DDDs ≥85th percentile) groups. Logistic regression methods were used to explore the associations between NSAID prescription and the outcome of significant CKD progression (defined as eGFR decline rate >5ml/min/1.73m^2 per year) adjusting for age, gender, deprivation, diabetes, cardiovascular disease, Angiotensin Converting Enzyme inhibitor or Angiotensin Receptor Blockers or Renin inhibitor prescription, Aspirin or Paracetamol prescription and baseline CKD stage.

Results: The prevalence of NSAID prescribing was 17.2% (n=711) and 16.1% (n=667) of the study cohort had stage 3-5 CKD. Significant CKD progression occurred in 928/3434 (27.0%) non-users, 149/605 (24.6%) normal dose and 22/106 (20.8%) high dose users. There was no significant association between normal dose NSAID prescribing (odds ratio (OR)= 1.02, 95%CI: 0.83-1.25) or high dose NSAID prescribing (OR= 0.83, 95%CI: 0.51-1.35) and significant CKD progression.

Conclusions: NSAID prescription over two years does not lead to significant CKD progression. However, the effects of long term NSAID prescription on CKD progression remain uncertain.

Funding: Private Foundation Support

FR-PO139

Association of Multidisciplinary Chronic Kidney Disease Care on Patient Outcomes: A Population Based Analysis

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Background: Multidisciplinary chronic kidney disease (MCKD) care may delay and improve transition to renal replacement therapy (RRT), and modify risk factors for both progression of chronic kidney disease (CKD) and cardiovascular disease. The evidence on the effectiveness of MCKD care is based on small studies with limited follow up.

Methods: All incident stable CKD patients with eGFR< 45 between 2002-2007 were identified from a population based cohort in Alberta using laboratory and administrative data. We evaluated the association of MCKD care on outcomes at 5 years using adjusted Cox proportional hazards models with MCKD care as a time-varying exposure. Subgroup analyses were done on patients who did and did not initiate RRT. Sensitivity analyses were done using a 2:1 propensity score matched cohort.

Results: 1,116 patients received MCKD care from a total of 39,102 CKD patients. Overall, MCKD care was associated with lower mortality at 5 years (HR 0.73 95% confidence interval, 0.66-0.81). MCKD care patients were more likely to receive RRT (HR 3.11; 2.75-3.50), transplantation (HR 3.03; 2.24-4.09), and were associated with a lower serious cardiovascular event (acute MI or stroke requiring hospitalization) risk (0.74; 0.61-0.90). In patients who initiated RRT, MCKD care was not associated with mortality benefit (0.82, 0.66-1.01), but was associated with a lower cardiovascular event risk (0.67; 0.49-0.91). Of patients who did not initiate RRT, MCKD care was associated with lower mortality (0.73, 0.64-0.82) and lower cardiovascular event risk (0.62, 0.48-0.81). Propensity score matched analyses show similar results.

Conclusions: Multidisciplinary CKD care is associated with lower mortality, increased transition to renal replacement therapy, and lower risk of serious cardiovascular events. Mortality benefits associated with CKD care may be related to cardiovascular risk factor reduction. While this analysis does not account for unmeasured confounders, results were similar in rigorous propensity score matched analyses.

FR-PO140

Pain Management in a Nephrology Department: Final Results of the PAIN Study

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Background: Pain (P) remains underestimated and undertreated. In renal insufficiency (RI) patients, handling drugs is difficult due to drug dosage adjustment (DDA) and especially for analgesics and opioids. The PAIN study (Protocole Analgesia by Service ICAR in Nephrology) aimed at evaluating P, its features, its consequences on the emotional status and its medical management of RI patients admitted in our department of nephrology.

Methods: PAIN is a French prospective monocentric study based on a 4-sections' patient form filled by the patient with the help of medical students from 02-2011 to 07-2011. This form evaluated nociceptive P, neuropathic P, care related P and emotional status. Glomerular filtration rate (GFR) was estimated using abbreviated Modification of Diet in Renal Disease (aMDRD). Pain killers (PK), antidepressant drugs (AD) and anxiolytics (AX) were studied with regard to dosage adjustment.

Results: 76 patients included, 60.5% males; median age, 58 years. 13 patients hemodialyzed and 15 transplanted. Median GFR was 32.5 ml/min/1.73 m^2. 42% and 34% referred at least one pain location for more than 3 months and for less than 3 months respectively. 60.5% received a PK. 19.7% had neuropathic P, 80% were not treated. 69.7% of patients suffered from care related P and 47.2% received a preventive treatment. Anxiety and depression occurred in 40.8% and 22.4 % respectively. 23.5% of depressive patients and 19.4% of anxious patients received an appropriate treatment. Of the 76 PK, AD and AX prescribed, 90.8% required dosage adjustment for RI. Of the 42 patients treated with PK, AD or AX, 59.5% received at least one such drug.

Conclusions: There is a high prevalence of P, anxiety and depression in our RI patients, and DDA is often necessary. P should be better evaluated by a P specialist in coordination with nephrologists for a better handling of those drugs that are used for psychological and physical pain management.

FR-PO141

Association of Mental Illness with Emergency Re-Hospitalization in People with Chronic Kidney Disease

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Background: Chronic kidney disease **(**CKD) is associated with high risk for repeated hospitalization. Serious mental illness (SMI) is also associated with increased risk of hospital admission. The primary objective of this study was to determine if SMI further increases risk for re-hospitalization, particularly via the emergency department (ED), among people with CKD.

Methods: People hospitalized in the State of Washington from April 2006 to Dec 2008 were included in the study. Those with index diagnoses of CKD (N=31,166), SMI (defined by schizophrenia and/or mood disorder; N=20,167), and combined CKD and SMI (N=717) were separated into three cohorts for comparisons to the reference group without CKD or SMI (N=548,532). Main outcomes were re-hospitalization for conditions other than mental illness: 1) via ED; 2) any admission; and 3) admission resulting in death. Cox regression was used to analyze time to main outcomes controlling for multiple pre-specified covariates.

Results: Risk of re-hospitalization via the ED was increased for CKD (HR=1.28, 95%CI:1.25-1.31, p<.001) and SMI (HR=1.39, 95%CI:1.34-1.44, p<.001) cohorts, but was significantly greater in the combined cohort (HR=1.65, 95%CI:1.49-1.82, p<.001) relative to any other group. Risk of any re-hospitalization was increased for CKD (HR=1.22, 95%CI:1.19-1.25, p<.001) and SMI (HR=1.15, 95%CI:1.12-1.19, p<.001) cohorts, while significantly greater risk was observed for the combined cohort (HR=1.38, 95%CI:1.26-1.51, p<.001) compared to all other groups. Risk of re-hospitalization resulting in death was increased for CKD (HR=1.47, 95%CI:1.35-1.55, p<.001) and SMI (HR=1.07, 95%CI:1.00-1.15, p=.041) cohorts, but not in the combined group (HR=1.25, 95%CI:0.98-1.59, p=.075).

Conclusions: SMI increased risk of CKD patients experiencing hospitalization; particularly via the ED. Addressing SMI may be an important strategy for reducing emergency admissions in the CKD population.

Funding: Other U.S. Government Support

FR-PO142

Depressive Symptoms and Health Outcomes among Patients with Chronic Kidney Disease: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study

Michael J. Fischer,[1] Ana C. Ricardo,[1] Neil Jordan,[3] Clarissa Jonas Diamantidis,[3] Dawei Xie,[3] Valerie L. Teal,[3] Amanda Hyre Anderson,[3] Lydia Bazzano,[3] Jing Chen,[3] John W. Kusek,[2] Kristine Yaffe,[3] Neil R. Powe,[3] James P. Lash.[1] *[1]Jesse Brown VAMC/U. Illinois; [2]NIH/NIDDK; [3]CRIC Study Group.*

Background: Depression is associated with increased morbidity and mortality in adults with chronic kidney disease (CKD). However, the impact of depression on a broad range of adverse clinical outcomes in diverse populations with CKD has not been well studied.

Methods: We assessed the relationship between baseline elevated depressive symptoms (DS) in CRIC Study participants and the following outcomes over a 5 year period: CKD progression [50% eGFR loss or end-stage renal disease (ESRD)], incident cardiovascular disease (CVD: myocardial infarction, congestive heart failure, stroke, or peripheral arterial disease), and all-cause death. DS were assessed by the Beck Depression Inventory (BDI) and defined by a BDI score >= 11. Cox proportional hazards models were used to determine the association between baseline DS and outcomes.

Results: Of 3,853 participants at study enrollment, mean age was 58 years, 45% were female, 42% white, 42% black, and 13% Hispanic. Twenty-seven percent had evidence of DS at baseline. During follow-up, rates (per 1000 person-years) of CKD progression (87.2 v. 52.7), incident cardiovascular disease (59.1 v. 39.3), and death (29.7 v. 22.3) were significantly higher in those with DS than in those without DS ($p<0.01$). After adjustment for site, demographics, and clinical factors including eGFR and urine protein, DS were significantly associated with an increased risk of CKD progression (HR 1.21; 95% CI: 1.03-1.43) and incident CVD (HR 1.21; 95% CI: 1.01-1.46) but not all-cause death (HR 1.21; 95% CI: 0.95-1.53).

Conclusions: In a large diverse CKD cohort, DS were independently associated with an increased risk of CKD progression and incident cardiovascular disease but not all-cause death.

Funding: NIDDK Support, Veterans Administration Support

FR-PO143

Depression Is Associated with Declining Kidney Functioning in Patients with Chronic Kidney Disease Daniel Cukor,[1] Yvette Fruchter,[1] Ankita B. Patel,[2] Subodh J. Saggi.[2] *[1]Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY; [2]Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: The incidence and prevalence of Chronic Kidney Disease (CKD) is increasing with 17% of the US having CKD. Understanding the factors associated with declining renal function is of clinical significance. Current study surveyed CKD clinic patients with varying stages of GFR. Our main goal was to identify variables that could predict decline in GFR over time.

Methods: Seventy CKD patients completed psychological questionnaires and medical variables were extracted from the medical charts, by an investigator blinded to the patients' questionnaire responses. Follow-up Glomerular Filtration Rate (GFR) was collected 6 months later. CKD patients with elevated depression scores were compared to patients with sub-clinical depression on medical other psychological variables. The impact of depressive affect on variation in GFR scores was examined.

Results: Average Beck Depression Inventory (BDI) score was a 10.0 ± 7.8, placing the mean below the cut-off for clinical elevation. GFR was significantly different for the two groups (w/o depression = 40.0 ±11.3, with elevated depression 29.6 ± 8.9 ($p<.05$)). Similarly, patients with elevated depression scores reported lower quality of life (SF-36, $p<.05$) inferior social support (ISEL, $p<.05$), increased body image sensitivity (BIS, $p<.05$), and worse community integration (CIQ, $p<.05$). There were no between group differences on other medical variables ($p>.05$, all cases). Utilizing a regression, with a model correcting for baseline GFR, the depression explained 10% of the variance in declining GFR score ($t=2.0$, $p<.05$).

Conclusions: Increased levels of pre-existing depression were associated with inferior quality of life, social support and kidney functioning in CKD patients. Depression score explained a significant amount of variance in GFR scores at 6 months even when corrected for baseline variability. Elevated depression scores are prevalent in CKD populations and further research on the impact of depression intervention is warranted, with particular emphasis on improving adherence to both medical appointments and recommendations.

FR-PO144

Depression in People with Chronic Kidney Disease: A Meta-Analysis of Prevalence and Associations with Clinical Outcomes Suetonia Palmer,[1] Mariacristina Vecchio,[2] Valeria Maria Saglimbene,[2] Marinella Ruospo,[3] Fabio Pellegrini,[2] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Consorzio Mario Negri Sud; [3]Diaverum Medical-Scientific Office.*

Background: Depression is associated with mortality and cardiovascular events in the general population.

Studies evaluating depression in people with chronic kidney disease (CKD) provide variable estimates of prevalence and associations with clinical outcomes. We summarize the evidence on prevalence of and outcomes associated with depression in people with CKD and explore the effects of specific patient and study characteristics on these.

Methods: We conducted a systematic review and meta-analysis of observational studies reporting prevalence of or clinical outcomes related to depression in people with CKD. Studies were identified from systematic searching MEDLINE. Estimates of prevalence and associations with clinical outcomes were summarized using random-effects model. The effects of participant and study characteristics on prevalence and associations with outcomes were explored using meta-regressions.

Results: 201 studies (263 cohorts) including 121 648 people with CKD were analyzed. In 163 cohorts (112 577 participants) the prevalence of depression measured by any tool was 35% (95% confidence interval [CI] 32-39%). Stage of chronic kidney disease was an effect modifier; people on dialysis experienced a higher prevalence of depression (40% [CI 36-44%]), compared to those with earlier stages of CKD (27% [CI 17-40%]) and kidney transplant recipients (23% [CI 16-31%]). People with CKD and depression experienced higher risks of mortality (15 studies, 82,240 participants; risk ratio (RR)1.68 [CI 1.39-2.04]) and hospitalization (6 studies, 16 662 participants; RR 1.17 [CI 1.06-1.30]) compared to people with CKD not depressed. Subgroup analysis incompletely explained differences between studies.

Conclusions: Approximately one-third of people with CKD may be depressed. Prevalence of depression may be higher in people on dialysis and lower in people with earlier stages of CKD. Depression is associated with adverse clinical outcomes in observational analyses.

FR-PO145

Depression Associated with Higher Mortality in the Renal Research Institute Chronic Kidney Disease (CKD) Study Linton Cuff,[1] Anca Tilea,[1] Brenda W. Gillespie,[1] Fredric O. Finkelstein,[3] Margaret A. Kiser,[4] Aleksandar Milovanovic,[1] George Eisele,[5] Peter Kotanko,[2] Rajiv Saran.[1] *[1]Univ. of Michigan; [2]Renal Research Institute; [3]Metabolism Associates; [4]Univ. of North Carolina; [5]Med. College of Albany.*

Background: Depression is common in patients with end stage renal disease (ESRD) and predicts mortality in that setting. However, there is limited information on the prevalence and significance of depression as it relates to health outcomes in patients with CKD .

Methods: This prospective observational study enrolled patients in CKD stages 3-5. Demographic, medical, quality of life (QoL) and medication data were collected at baseline and at follow-up visits. The presence of a diagnosis of clinically documented depression (CDD) was verified by chart review. Patient-reported depressive symptoms (PRD) were inferred from responses to 2 specific questions pertaining to depression in the Kidney Disease QOL SF-36, as previously reported, using the 3 lowest out of 6 ordinal responses. Association between outcomes of death and ESRD with baseline PRD and CDD was assessed by Cox regression.

Results: Of 2,182 enrolled in the study, 1,414 patients completed the KDQoL at enrollment; 192 (14%) with PRD and 158 (7%) with CDD. Mean (±SD) age was 62±15 years, 53% were males and mean eGFR 25±10 ml/min/1.73m^2. In both unadjusted and adjusted models, depression (by either definition) was associated with significantly higher risk of mortality.

		Outcome:Death		Outcome:ESRD	
PRD	*Unadjusted*	1.66	0.06	1.18	0.30
	*Adjusted**	1.69	0.07	1.16	0.36
CDD	*Unadjusted*	1.78	0.03	0.96	0.84
	*Adjusted**	2.43	0.01	1.28	0.19
PRD or CDD	*Unadjusted*	1.65	0.04	1.11	0.44
	*Adjusted**	1.87	0.01	1.21	0.21

* Adjusted for baseline age, gender, race, eGFR, diabetes, and hypertension

Conclusions: A significant proportion (7-14%) of patients in this CKD cohort reported depressive symptoms or had a clinical diagnosis of depression. Depression by either definition was associated with higher risk of mortality. Systematic monitoring and management of depression in this patient population should therefore receive greater attention in the CKD population.

Funding: Pharmaceutical Company Support - Renal Research Institute

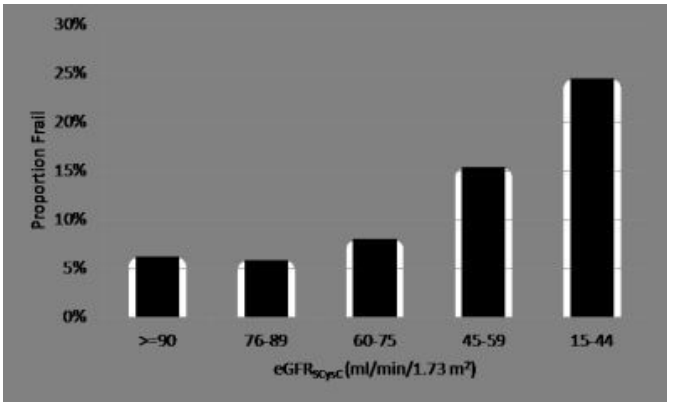
FR-PO146

Kidney Function and Frailty in Older Adults Lorien S. Dalrymple,[1] Ronit Katz,[2] Dena E. Rifkin,[3] Mark J. Sarnak,[4] David Siscovick,[2] Anne B. Newman,[5] Linda F. Fried,[5,6] Michael Shlipak.[7,8] *[1]UC Davis; [2]University of Washington; [3]UC San Diego; [4]Tufts Medical Center; [5]University of Pittsburgh; [6]VA Pittsburgh Healthcare System; [7]VA Medical Center San Francisco; [8]UC San Francisco.*

Background: Frailty is characterized by loss of physiologic reserve and increased vulnerability. The aim of this study was to examine whether lower levels of kidney function, estimated by serum cystatin C ($eGFR_{SCysC}$), were associated with a higher risk of prevalent and incident frailty.

Methods: The Cardiovascular Health Study is a prospective cohort study of adults ≥65. The exposure was $eGFR_{SCysC}$ (ml/min/1.73m^2) and outcomes were prevalent and incident frailty. Frailty was defined by weight loss, exhaustion, and physical limitations using validated criteria.[1] Logistic regression and Cox proportional hazards models estimated the risk of prevalent and incident frailty, respectively. Multivariable models included $eGFR_{SCysC}$, age, sex, race, diabetes, hypertension, heart disease, heart failure, body mass index, and tobacco use.

Results: Analyses of prevalent frailty included 4,150 participants with mean age of 75, 41% male, 17% black, and median $eGFR_{SCysC}$ 73. Prevalence of frailty differed by kidney function.



In multivariable analysis, eGFR$_{SCysC}$ 45-59 and 15-44 were associated with an 80% (OR 1.80;95%CI 1.17-2.75) and 187% (OR 2.87;95%CI 1.72-4.77) higher odds of frailty, respectively, when compared to eGFR$_{SCysC}$≥90. To examine incident frailty, the cohort was restricted to 3,459 participants followed for a median of 4 years. In multivariable analysis, eGFR$_{SCysC}$ was not associated with incident frailty (HR 1.03, 95%CI 0.94-1.13).

Conclusions: Lower level of kidney function is associated with a higher prevalence but not incidence of frailty. Ref: 1. Fried LP et al. Frailty in Older Adults: Evidence for a Phenotype. *J of Gerontology 56(3):M146-156, 2001.*

Funding: Other NIH Support - The research reported in this article was supported by contracts HHSN268201200036C, N01-HC-85239, N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, and Grant HL080295 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG-023629, AG-15928, AG-20098, AG-027058, AG-027002 from the National Institute on Aging (NIA), and UL1 RR024146 from the National Center for Research

FR-PO147

Cystatin C and Risk of Frailty and Mortality in Older Men Allyson Hart,[1] Areef Ishani,[1] Misti L. Paudel,[1] Brent C. Taylor,[1] Eric Orwoll,[2] Peggy M. Cawthon,[3] Kristine E. Ensrud.[1] *[1]University of Minnesota/Minneapolis VA, Minneapolis, MN; [2]Oregon Health Sciences Center, Portland, OR; [3]California Pacific Medical Center Research Institute, San Francisco, CA.*

Background: Declining kidney function and frailty are common with aging. A prior cross-sectional study reported that higher cystatin C (cysC) was associated with greater prevalent frailty status, but longitudinal associations of cysC with incident frailty and death at follow up are uncertain.

Methods: Serum cysC and creatinine (Cr) were measured at baseline among 1242 non-frail community dwelling men aged ≥65 yrs (mean age 73.8 yrs). Repeat frailty status assessment was performed an average of 4.6 years later. Frailty status (comprised of shrinking, weakness, exhaustion, slowness and low physical activity) was defined as an ordinal outcome of robust, intermediate stage, and frail based on the number of frailty components present (0, 1-2, or ≥ 3 respectively). Vital status was assessed every 4 months. CysC, Cr, and eGFR$_{Cr}$ were expressed in quartiles. Multinomial logistic regression analyses were adjusted for age, race, clinical site, and baseline frailty status (intermediate vs robust).

Results: At follow up, 546 men (44%) were intermediate stage, 113 (9.1%) were frail and 129 (10.4%) died in the interim. Higher cysC was associated in a graded manner with increased odds of intermediate stage (vs robust) and frail (vs robust), and also associated with a higher risk of mortality (vs robust).

Quartile of Baseline Cys.C[a]	Odds Ratio (95% CI)[b]		
	Intermediate (Pre-frail) vs. robust	Frail vs. robust	Dead vs. robust
Q1 (referent)	1.0	1.0	1.0
Q2	1.44 (1.00 – 2.08)	1.38 (0.69 – 2.77)	0.70 (0.33 – 1.51)
Q3	1.55 (1.05 – 2.29)	1.52 (0.75 – 3.09)	1.63 (0.85 – 3.15)
Q4	1.41 (0.93 – 2.15)	1.90 (0.94 – 3.81)	2.67 (1.40 – 5.11)
p for linear trend	0.08	0.05	<0.001

[a] Quartile cutpoints 0.80, 0.90, 1.03
[b] Adjusted for age, race, clinical site and baseline frailty status

In contrast, higher serum Cr and lower eGFR$_{Cr}$ were not related to frailty status and death at follow up.

Conclusions: Among non-frail older men, higher cysC, but not serum Cr or eGFR$_{Cr}$ was associated with a higher odds of greater frailty status and an increased risk of mortality at follow up. These results suggest that cystatin C may be a promising biomarker for identification of older adults at increased risk of adverse health outcomes.

Funding: NIDDK Support, Other NIH Support - The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute on Aging (NIA), the National Center for Research Resources (NCRR), and NIH Roadmap for Medical Research under the following Grant numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647, U01 AR45654, U01 AR45583, U01 AG18197, U01-AG027810, and UL1 RR024140. A. Hart is Supported by T32 Grant Number 2T32DK007784-11

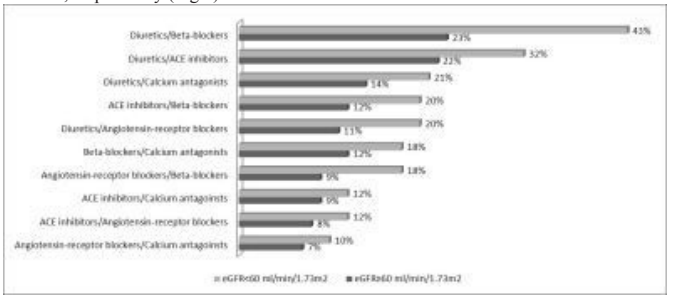
FR-PO148

Antihypertensive Medication and Kidney Function in Individuals at Age ≥70, the Berlin Initiative Study Natalie Ebert,[1] Olga Jakob,[2] Reinhold Kreutz,[3] Peter Martus,[4] Elke Schaeffner.[1] *[1]Nephrology, Charité, Berlin, Germany; [2]Biostatistics, Charité, Berlin, Germany; [3]Clinical Pharmacology, Charité, Berlin, Germany; [4]Biostatistics, Eberhard Karls University, Tübingen, Germany.*

Background: High blood pressure (BP) is one of the most prevalent risk factors for cardiovascular disease, especially at age ≥70. Prevalence data of hypertension in people ≥70 yrs in combination with data on kidney insufficiency are scarce.

Methods: Data are from baseline visit of the Berlin Initiative Study, a population based prospective cohort of 2070 individuals, mean age 80 yrs. A complete list of medication was electronically linked to a German "Rote Liste" reference coding system to identify and classify antihypertensive medication. Office BP was measured twice electronically and eGFR was determined using the CKD-Epi estimating equation.

Results: Overall 61% were hypertensive with 34, 18, and 9% presenting with stage I, II, and III, respectively. Mean BP was 146/81 mmHg. 78% of 2070 participants were on antihypertensive medication. Among those 21, 22, and 19% were receiving 1, 2 and 3 classes of antihypertensives, respectively. Age- and sex-specific means of BP did not significantly differ by eGFR ≥ or <60 ml/min/1.73m^2 (t-test). Participants were prescribed a median of 4 drugs of which 2 were antihypertensives. Most common substances were diuretics (48%), beta-blockers (46%), and ACE inhibitors (27%). Most common combinations were diuretics/beta-blockers and diuretics/ACE inhibitors in individuals with eGFR ≥60 and <60, respectively (Fig 1).



Conclusions: In our elderly cohort we saw a high prevalence of hypertension and antihypertensive treatment. Moreover, mean BP was 146/81 mmHg and thus comparable to target BP of the HYVET trial in octogenarians. No significant differences of BP means were observed when eGFR was ≥ or <60, probably due to effective treatment of participants with decreased GFR.

Funding: Private Foundation Support

FR-PO149

Octogenarians with Advanced Chronic Kidney Disease (CKD): A Longitudinal Follow-Up Study Ulka M. Desai,[1,2] Nicole Piero,[2] Loretta Simbartl,[2] Christine Edie,[2] Kristen Schmitt,[2] Charuhas V. Thakar.[1,2] *[1]Nephrology and Hypertension, University of Cincinnati, Cincinnati, OH; [2]Renal Section, Cincinnati VA, Cincinnati, OH.*

Background: Octogenarians represent 28% of older US adults and have high prevalence of CKD. Several studies have evaluated outcomes of older dialysis patients. We conducted a longitudinal follow-up of patients older than 80 years (yrs) with advanced CKD and measured parameters of resource utilization and clinical outcomes.

Methods: Between 1/1/06 and 12/31/08 there were 398 patients seen at a Veterans Affairs healthcare system that were > 80 yrs of age and had one outpatient measure of estimated glomerular filtration rate (eGFR) of < 30 ml/min/1.73m2. 78 were excluded (missing data/dialysis at baseline), and 320 were followed until 12/31/2011. Variables included demographics, co-morbidities, resource utilization parameters (renal clinic visits, medical/surgical hospitalizations), and lab parameters (creatinine, eGFR, overt proteinuria). Probability of dialysis and death before dialysis was estimated after accounting for other patient characteristics.

Results: The cohort was 97.5% male (mean age - 84.5 yrs). Median eGFR at the beginning of the study was 26 ml/min/1.73m2 (q1, 22, q3, 28). 44% had diabetes, 94% had hypertension, 86% had heart disease, 57% had proteinuria when assessed. 54% of patients sought nephrology care (renal visits 1-15) during study period. 165 (51%) patients requried hospitalization (15% - primary diagnosis was altered mental status). 18% underwent major/minor surgery. 23% of hospitalizations were associated with falls/trauma. The adjusted probability of dialysis was 7% [95% confidence limit (CI) 4.9% - 11%], and it was 69% (95% CI, 64% - 74%) for death before dialysis. From the beginning of the study period, median time to death and dialysis was 1.4 yrs (q1, 0.5, q3, 2.4) and 1.7 yrs (q1, 1.1, q3, 2.7) respectively. 75% dialysis patients died (median time to death 3.3 months; q1, 1.1, q3, 15.2).

Conclusions: Octogenarians with advanced CKD are 9-times more likely to die than start dialysis; survival after dialysis remains poor. Individualized care may be more appropriate in this age group rather than "guideline" based CKD management.

Funding: Veterans Administration Support, Clinical Revenue Support

FR-PO150

Loss of Renal Function in the Elderly: Physiology or Pathology?
Ciro Esposito,[1] Marta Arazzi,[1] Massimo Torreggiani,[1] Nicoletta Serpieri,[2] Maria Lucia Scaramuzzi,[2] Alessandra Manini,[2] Davide Catucci,[1] Edoardo La Porta,[1] Antonino Dal Canton.[2] *[1]Nephrology, Fondazione IRCCS S. Maugeri, University Pavia, Pavia, Italy; [2]Nephrology, Fondazione IRCCS San Matteo, University Pavia, Pavia, Italy.*

Background: In 1950 Davies and Shock showed that GFR decreases at a rate of 1 ml/min/year after the third decade of age. Nowadays it seems that CKD is outbreaking, mostly in the elderly. The aim of our study was to assess the progression of CKD in different ages.

Methods: We conducted a monocentric, retrospective, observational study enrolling 116 patients afferent to our outpatient clinic. Inclusion criteria: age>18 years, follow-up≥5 years, eGFR<60ml/min/1,73m^2 and/or diagnosed renal disease and/or presence of renal damage. We excluded all the patients without at least one control a year and those who presented with acute renal failure at the first visit. Patients were divided into four groups according to their age: 25-55 years (n=27), 56-65 (25), 66-75 (42), 76-87 (22). eGFR was calculated using the MDRD and the CKD-EPI formulas.

Results: Younger patients had a significantly longer follow-up and less comorbidities, evaluated by the CIRS score, compared to the other groups. There was no difference between creatinine at baseline and at the end-of-follow-up period among the groups. Even though renal function significantly decreased in all groups, we noticed a slower progression as the age increased and the difference between basal and end-of-follow-up eGFR was minimal in the group of patients aged 76-87 years. Analyzing the eGFR of every ambulatory control plotted against the year of follow-up, we showed a more rapid loss of filtrate in the younger group. Instead, loss of renal function decreased as the age of patients increased.

Conclusions: Renal function loss progresses more slowly in the elderly than in young patients. Therefore, these patients should not be considered as CKD patients but they should be followed closely by their family physician as they are at greater risk of developing acute renal failure or suffer from cardiovascular complications.

Funding: Government Support - Non-U.S.

FR-PO151

Rehospitalizations among Elderly Chronic Kidney Disease Patients
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Background: The reduction of rehospitalizations among Medicare beneficiaries remains a current public policy objective. Previous studies have shown high rehospitalization rates among general Medicare and hemodialysis patients: respectively, nearly one fifth and over one third of hospital discharges were followed by a 30-day rehospitalization. However, current rehospitalization rates among the chronic kidney disease (CKD) population have not been analyzed.

Methods: Rehospitalization rates were calculated among prevalent Medicare CKD patients on January 1, 2010, age 66 and older, using the 5% Medicare sample. During 2009, CKD stage was defined and patients were continuously enrolled in Medicare. ESRD patients were analyzed separately. Live all-cause hospital discharges were included from January 1 to December 1, 2010, and patients with at least one discharge were included. Data excluded rehabilitation claims, transfers, and discharges with a same-day admission to long-term care and critical access hospitals. Events were first rehospitalization and/or mortality. Rates indicated the percent of live discharges with an event within 30 days. Annual adjusted rehospitalization rates were computed using direct adjustment.

Results: Results included 63,031 discharges from 34,862 CKD patients. Rehospitalization rates within 30 days among CKD patients were 24% compared to 18 and 34% for non-CKD and ESRD, respectively, and those for death or rehospitalization were 30% compared to 22 and 39%. Rates increased with CKD severity: rates in CKD stage 4-5 were 26% compared to 23% in stage 1-2 CKD. Among CKD patients, rates were highest among non-white races (27-28%). Adjusted rehospitalization rates among CKD patients decreased only slightly from 27% in 2002 to 24% in 2010.

Conclusions: Nearly one in four hospital discharges among CKD patients were followed by a 30-day rehospitalization, and rates improved minimally in the last decade. Findings support rehospitalization reduction efforts among CKD in addition to ESRD populations. Awareness of high-risk subgroups, including non-white and late-stage patients, could guide reduction efforts.

Funding: NIDDK Support

FR-PO152

A Prospective Study of Serum Uric Acid and Albuminuria in Older Adults: The Rancho Bernardo Study
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Background: Serum uric acid is associated with albuminuria in those with diabetes, but the association in healthy older adults without diabetes and whether sex differences exist in this association has not been described. We investigated the cross-sectional and longitudinal association between uric acid and albuminuria by urine albumin/creatinine ratio (ACR) in a population of older community-dwelling men and women.

Methods: Uric acid and ACR were measured in 1070 women and 694 men, aged 30-97 (mean 71) years at a baseline visit in 1992-1996 and again in 657 women and 402 men who returned in 1997-99 (mean 3.5 years later). Multivariable linear regression was used to evaluate the association between baseline uric acid and logACR at baseline and follow-up and logistic regression to assess for an association between baseline uric acid and ACR>30mg/g at baseline and follow-up, before and after stratifying by diabetes status and gender.

Results: At baseline, mean uric acid was 4.8mg/dL and median ACR was 12mg/g. In multivariable analyses adjusted for baseline age, sex, body mass index, systolic blood pressure, fasting plasma glucose, estimated glomerular filtration rate and diuretic use, baseline uric acid was not associated with logACR in cross-sectional analyses in any group. In longitudinal analyses, baseline uric acid was associated with follow-up logACR in the entire cohort (β=0.056, p=0.045). In stratified analyses, this association persisted in those without diabetes (β=0.075, p=0.010) but not those with diabetes, and in men (β=0.167, p=0.002) but not in women. In categorical analyses, there was a significant association between baseline uric acid and ACR>30mg/g at the follow-up only in men (OR 1.53, 95%CI 1.13-2.07, p=0.006).

Conclusions: In a cohort of community-dwelling older adults, baseline uric acid was independently associated with future albuminuria among those without diabetes and in men but not women.

Funding: NIDDK Support, Other NIH Support - National Institute on Aging (AG07181); and National Institutes of Health and the National Institute on Aging (R01AG028507), Veterans Administration Support

FR-PO153

Inaccuracy of Morning Spot PC Ratio to Estimate 24-h Proteinuria (Pr) Magnitude in Individual Patients with Non-Diabetic Chronic Kidney Disease (CKD) or Lupus Nephritis (LN)
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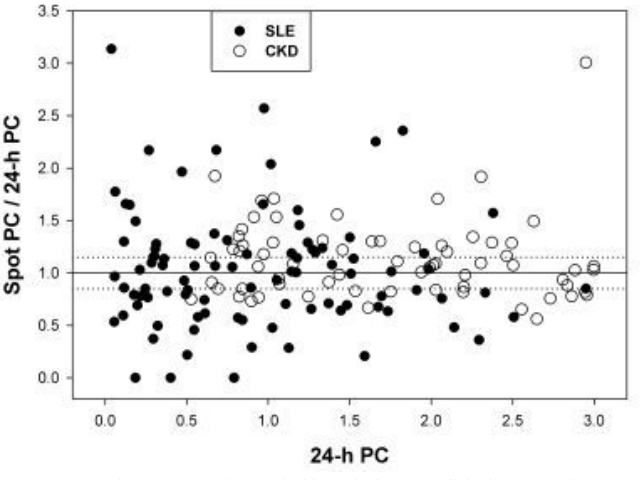
Background: Spot PC ratio is used clinically to estimate Pr magnitude. However, urine PC ratio shows considerable diurnal variability. Short collections (e.g. spot) reveal this variability. Long collections (e.g.≥ 50% of a complete 24-h collection) conceal this variability. So, the PC ratio of a long collection is a more accurate estimate of the 24-h urine PC ratio than the PC ratio of a short collection. Despite this limitation of short collections, spot PC ratio has performed well in cohort studies where it strongly correlates with 24-h Pr, and predicts CKD progression better than 24-h Pr. However, in cohort studies of spot PC ratio variability is offset by averaging. By contrast, patient management, decisions are often based on a single spot PC ratio. Here, spot PC ratio variability becomes a liability, as we and others have found in LN.



Methods: The present work extends this analysis to non-diabetic CKD patients (REIN trial, 98 pts) and compares it by correlation coefficient and calibration plot to an expanded LN cohort (81pts).

Results: Similar to previous reports, we found a strong statistical association between spot PC ratio and 24 Pr (e.g. P<0.001) however r^2 is weak. (e.g. nephrotic range r^2 =0.164 (CKD) and 0.361 (LN). The figure shows the calibration plot for the subnephrotic range. The dotted lines show the expected limits of agreement if spot PC was an aliquot of the 24hr collection.

Conclusions: Spot PC can be substantially inaccurate in estimating 24hr PC ratio in both CKD and LN. The impact of this inaccuracy on patient management needs further study.

Funding: NIDDK Support

FR-PO154

Influence of Urine Creatinine on the Relation of Albumin to Creatinine Ratio with Cardiovascular Disease Events: The Multi-Ethnic Study of Atherosclerosis Caitlin E. Carter,[1] Ronit Katz,[2] Holly J. Kramer,[3] Ian H. de Boer,[2] Carmen A. Peralta,[4] David Siscovick,[2] Mark J. Sarnak,[5] Andrew S. Levey,[5] Lesley Stevens Inker,[5] Matthew Allison,[1] Michael H. Criqui,[1] Michael Shlipak,[4] Joachim H. Ix.[1] *[1]UC San Diego; [2]University of Washington; [3]Loyola Medical Center; [4]UC San Francisco; [5]Tufts Medical Center.*

Background: Urine creatinine (UCr) correlates with muscle mass and low muscle mass is associated with cardiovascular disease (CVD) events. Whether muscle mass, as reflected by urine creatinine, influences the association between modest elevations in albumin/creatinine ratio (ACR) and CVD events remains poorly explored.

Methods: Among a multi-ethnic cohort of 6,770 without CVD, ACR was measured and participants were followed for median 7.1 years for CVD events (MI, stroke, cardiac arrest, and fatal CVD or stroke). Cox regression evaluated associations of doubling of 1/UCr, urine albumin (UA), and ACR with CVD events in an unadjusted model and models adjusted for demographics and CVD risk factors. Associations of ACR≥10mg/g v. lower with CVD events were evaluated in strata by age (≥ 65 years v younger), sex, race, and weight (tertiles).

Results: Female, older, white or Chinese, and lower weight participants had higher 1/UCr (p<0.001 for all). 1/UCr was not significantly associated with CVD events, whereas higher UA and ACR were. (Table 1)

Table 1. Association of Spot Urinary Indices with CVD Events in MESA.

	HR* (95%CI)
1/UCr	1.04 (0.94, 1.16)
UA	1.21 (1.16, 1.28)†
ACR	1.26(1.20, 1.33)†

*Hazard Ratio (95% CI) per doubling; †p<0.001

Results were similar in adjusted models. ACR≥10mg/g was more strongly associated with CVD in the lowest v. highest weight tertiles (HR 4.34 v. 1.97; p-interaction=0.006). No effect modification was seen by age, gender, or race (p-interactions all > 0.362).

Conclusions: Females, and older, lower weight, and non-black participants have lower UCr. The association of ACR≥10 mg/g with CVD events was stronger in those with low body weight. If confirmed in future studies, the ACR may be biased towards stronger associations with CVD in low weight individuals.

Funding: Other NIH Support - Carter: T32 HL007261

FR-PO155

Longitudinal eGFR Trajectories in Patients with Type 2 Diabetes and Nephropathy Hiddo Jan Lambers Heerspink,[1] Liang Li,[4] Paul Smink,[1] Tom Greene,[2] Hans-Henrik Parving,[3] Dick de Zeeuw.[1] *[1]Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands; [2]University of Utah, Salt Lake City, UT; [3]Medical Endocrinology, University Hospital of Copenhagen, Copenhagen, Denmark; [4]Cleveland Clinic, Cleveland, OH.*

Background: In contrast to the traditional paradigm of a linear glomerular filtration rate (GFR) decline, a recent study in 846 Afro-Americans with hypertensive nephrosclerosis documented that 42% of patients exhibit a > 90% probability of a nonlinear GFR trajectory or a prolonged period of non-progression. We here describe the estimated GFR (eGFR) trajectories of subjects with type 2 diabetes, nephropathy, and proteinuria.

Methods: Data from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial was used for analysis. RENAAL participants had albuminuria >300 mg/g and serum creatinine values between 1.3 and 3.0 mg/dL. eGFR was calculated every 3 months during an average of 3.4 years follow-up. A total of 1086 subjects (72%) with more than 2 years follow-up and available eGFR measurements were included in order to estimate accurate eGFR trajectories. The probability of a nonlinear trajectory and the probability of a period of non-progression were calculated for each patient from a Bayesian model of individual eGFR trajectories as described earlier.[1]

Results: 101 (9%) patients showed a >90% probability of having a nonlinear trajectory or prolonged period of non-progression; in 419 (39%) the probability was > 0.5. Higher baseline eGFR (β=0.0014; p=0.049) and lower baseline albuminuria (β=0.030; p=0.001) were associated with a higher probability of a nonlinear eGFR decline or period of nonprogression.

Conclusions: The majority of patients with type 2 diabetes, nephropathy and proteinuria have a progressively linear eGFR trajectory in 3 to 4 years follow-up, unlike Afro-Americans with hypertensive nephrosclerosis and minimal proteinuria in ~10 years follow-up. These data support the paradigm that in type 2 diabetes and nephropathy renal function loss progresses linearly over time.

1 Li L. et.al. Longitudinal Progression Trajectory of GFR among patients with CKD. Am J. Kidney Dis 2012 Apr;59(4):504-12.

Funding: Pharmaceutical Company Support - Merck

FR-PO156

The Classification Tree Methodology: A New Way to Estimate the Risk and the Predictors of Progression of Chronic Kidney Disease Marcora Mandreoli,[1] Paola Rucci,[2] Dino Gibertoni,[2] Mattia Corradini,[3] Antonio Santoro.[1] *[1]Dept. of Nephrology; Dialysis and Hypertension, Policlinico S. Orsola-Malpighi, Bologna, Italy; [2]Dept. of Public Health and Statistic, University of Bologna, Bologna, Italy; [3]Dept. of Nephrology and Dialysis, Arcispedale S. Maria Nuovo, Reggio Emilia, Italy.*

Background: The measurement of eGFR alone does not give any information as to the rate of CKD progression which is relevant from a clinical point of view. In a large cohort of patients participating in a Regional Program of Prevention of Progressive Renal Insufficiency (PIRP project) we have evaluated the rate of decline of GFR and the factors influencing that decline.

Methods: We analyzed data of 2109 outpatients entering in the PIRP Registry of Emilia Romagna Region, who had at least 4 assessments of GFR to evaluate the annual variation of their GFR using a classification tree analysis (CTA). This methodology was used to define a predictive model of CKD progression using gender, age, BMI, comorbidities, smoke, baseline creatinine and other laboratory variables as independent variables.

Results: The mean age was 70.5± 8.9 years; Female 34.7%. The prevalence of the major comorbid conditions was 32.5% for DM, 55.6% for cardiovascular diseases. The average estimated annual decline in GFR was -1.5 ml/min/1.73 ± 4.3 m^2. The final CTA identified three age-groups with a significantly different decline in GFR: **i) group 1** aged ≤53 years, having the highest annual decline (-3.5 ml/min/1.73 m^2); **ii) Group 2** aged 54-67 years, with moderate decline in GFR (-2.19). In this group diabetic patients and patients with a serum phosphate level more than 3.7 mg/dL, had a faster progression of their CKD. **iii) Group 3** aged >67 years with a slight decline (-1.06). The presence of a proteinuria in this group was an acceleration factor in the GFR decline.

Conclusions: CTA allows for the identification of patients with a different rate of decline in kidney function. Having this information may be important from the clinical point of view because it allows for an intensification in the surveillance and the clinical controls for patients classified as 'fast progressors' and instead reduce it for those who can be considered 'slow progressors.'

FR-PO157

Is Renal Replacement Therapy a Precise Hard Renal Endpoint in Drug Trials? Misghina Tekeste Weldegiorgis,[1] Hiddo Jan Lambers Heerspink,[1] Dick de Zeeuw,[1] Ron T. Gansevoort,[1] Julia Lewis,[2] Hans-Henrik Parving,[3] Vlado Perkovic.[4] *[1]Clinical Pharmacology, University Med Center Groningen, Groningen, Netherlands; [2]Nephrology, Vanderbilt University, Nashville, TN; [3]Med. Endocrinology, University of Copenhagen, Copenhagen, Denmark; [4]George Institute, Sydney, Australia.*

Background: Renoprotective drugs are registered for preventing end-stage-renal-disease. Renal replacement therapy (RRT; chronic dialysis or renal transplantation) is used as a hard clinically relevant endpoint in drug trials, since it reflects the failure of proper kidney function. However, RRT initiation may be based on many factors beyond GFR such as patient wellbeing, doctor habits, and local guidelines. We assessed whether RRT initiation is driven by changes in serum creatinine (eGFR) or whether it is influenced by other factors.

Methods: We performed a post-hoc analysis of the RENAAL and IDNT trials. We used within patient linear regression to estimate the time to reach eGFR 11 ml/min/1.73m^2 and compared this with the actual time to reach RRT as recorded in the trial. Logistic regression was performed to identify factors that were associated with RRT onset before reaching eGFR 11.

Results: In RENAAL 341 patients started RRT. Of these 100 patients started RRT before and 241 after reaching eGFR 11. In univariate regression various characteristics were associated with RRT initiation before eGFR 11 such as male gender, body weight, blood pressure, hemoglobin (Hb), and proteinuria (UP). Independent determinants of RRT initiation before eGFR 11 were male gender [OR 2.4, (95% CI 0.9-6.4)], Hb [OR 0.7, (0.5-0.9)], and UP [OR 2.1, (1.3-3.3)]. A large variability was observed between the time to reach eGFR 11 and RRT: only 34% of subjects reached eGFR 11 within 90 days of the actual RRT decision. Similar results were found in the IDNT trial.

Conclusions: The initiation of RRT may be driven by many parameters beyond serum creatinine alone. These factors may vary among patients and physicians. Interventions could treat a symptom of uremia and delay RRT but not slow the rate of eGFR decline. In this instance using RRT as clinical trial endpoint may be testing drug effects beyond the drug's ability to prevent GFR decline.

Funding: Pharmaceutical Company Support - Merck; Bristo Meyer Squibb

FR-PO158

Systematic Analysis of Nephrology Studies in ClinicalTrials.gov Jula K. Inrig,[1,2] Robert M. Califf,[2] Asba Tasneem,[2] Karen Chiswell,[2] Uptal D. Patel.[2] *[1]UT Southwestern, Dallas, TX; [2]Duke Clinical Research Institute, Durham, NC.*

Background: The quality and quantity of clinical trials in nephrology may be lower than other medical specialties, however, the overall profile of recent trials following mandatory registration is not known. Thus, we performed a systematic analysis of nephrology studies using data from ClinicalTrials.gov.

Methods: Between October 2007-September 2010, 40,970 interventional studies were registered with ClinicalTrials.gov. Physician reviewers annotated Medical Subject Heading terms and Conditions by disease areas & studies were individually reviewed to validate classification by clinical specialty. Trial characteristics within and between specialties were compared using chi-square tests.

Results: Of 40,970 trials overall, 1,054 were classified as nephrology (2.6%). The majority of nephrology trials were for treatment (75.4%) or prevention (15.7%) with very few diagnostic, screening, or health services research studies (2.2%, 0.3%, and 1.4%, respectively). Compared to 2,264 cardiology trials (5.5% overall), nephrology trials were more likely to be smaller (64% enrolling <100 patients vs 48% in cardiology), to be phase I-II (29% vs 20%), to have a crossover study design (9.4% vs 5.4%), to have more than 2 study arms (18% vs 13%), and to be unblinded (66% vs 53%; p<0.05 for all). Although not significant during this limited time period, analyses of trends suggest improvements over time in use of more rigorous trial design features in nephrology studies. When compared to cardiology, nephrology trials were also more likely to include a drug intervention (72.4% vs 41.9%), have an endpoint classified as pharmacokinetic, pharmacodynamics or both (9.7% vs 3.3%), and include US sites only (38% vs 32%; p<0.05 for all). Finally there were few registered trials funded by the NIH during this time period (3.3% for nephrology and 4.2% for cardiology).

Conclusions: Critical differences remain between clinical trials in nephrology and other specialties such as cardiology. Improving care for patients with kidney disease will require a concerted effort between the NIH, industry, academia, and key stakeholders to increase the scope, quality, and quantity of clinical trials within nephrology.

Funding: Other NIH Support - K23 HL092297, Other U.S. Government Support

FR-PO159

Methodological Considerations in the Study of Kidney Function Decline

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Background: Change in kidney function is an important and increasingly common outcome in clinical research studies. The standard approach to modeling change in estimated glomerular filtration rate (eGFR) is to estimate the subject-specific slope between time and eGFR. However, linear slope is often strongly associated with baseline eGFR, creating potential bias and complicating the analysis and interpretation.

Methods: We created a relative change model by estimating the subject-specific slope between time and log-transformed eGFR in 4,436 participants in the Cardiovascular Health Study (CHS), a community-based cohort of older adults, and in 380 participants in the Seattle Kidney Study (SKS), a clinic-based cohort with chronic kidney disease (CKD). We estimated GFR using the CKD-EPI equation.

Results: The number of eGFR measurements range from 2 to 3 in CHS and from 2 to 6 in SKS. Mean relative change (SD) in eGFR was -3.7% (3.9%) in the CHS and -7.4% (20.6%) in SKS. In CHS the Pearson correlation between baseline and change in eGFR was 0.001 in the relative change model compared to -0.22 in the linear change model. In SKS, the correlation between baseline and change in eGFR was 0.06 in the relative change model compared to -0.26 in the linear change model. The relative change model yielded stronger and more precise estimates for associations of known risk factors with kidney function decline.

Conclusions: In two observational cohorts, the use of a relative decline eGFR model dissociates longitudinal change in eGFR with baseline eGFR, more precisely models associations for known kidney disease risk factors, and yields coefficients that are easily interpretable as proportionate decline in eGFR over time. This method should be considered for evaluating changes in eGFR over time in observational studies. Further evaluation is warranted in other study populations and different study designs.

Funding: NIDDK Support, Other NIH Support - NHLBI

FR-PO160

Evaluating Four Methods of Assessing Protein Excretion and Their Relationship with Chronic Kidney Disease Progression

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Background: 24-hour urine protein excretion rate (24UPE) is most predictive of chronic kidney disease (CKD) progression or glomerular filtration rate (GFR) decline. Spot urine protein-creatinine ratio (UPCR) and albumin-creatinine ratio (UACR) estimations of 24-hr urine protein excretion (24UPE) and 24-hour urine albumin excretion (24UAE), respectively, are used in clinical practice. But the established accuracy of these ratios for estimating 24-hour urine collections predated the standardization of creatinine assays. Moreover, the reliability of these ratios for predicting GFR decline is unclear. We evaluated 4 methods of assessing urine protein excretion: UPCR, UACR, 24UPE and 24UAE to GFR decline in a multi-ethnic Asian population with a variety of CKD.

Methods: The Asian Kidney Disease Study prospectively collected 24-hour urine followed by early morning spot urine, which were analyzed for protein (pyrogallol-based assay), albumin (turbidimetry), and creatinine (enzymatic assay). 225/232 participants had a follow-up serum creatinine. We estimated GFR using the CKD-EPI equation. To compare the predictive performance of urine protein assessment methods with GFR decline, we developed models using stepwise linear regression. The urine protein assessment method in the best model was substituted in turn for three additional models. Variables with p-values ≤0.05, age, gender, and ethnicity were included in the final models.

Results: All models had good predictive performance, with the best performance in the model that included 24UPE, followed in order by 24UAE, UPCR, and UACR.

Models predicting GFR decline*

Model	R2	Model p-value	Proteinuria assessment	P-value	AIC	BIC
1	0.1224	<0.001	24UPE	<0.001	514	547
2	0.1084	<0.001	24UAE	<0.001	520	550
3	0.1009	0.0032	UPCR	0.0015	522	555
4	0.0758	0.0156	UACR	0.0019	528	558

Includes age, sex, ethnicity

Conclusions: 24-hour urine protein excretion rate is the best predictor of kidney disease progression. However, all the 4 methods correlated well with GFR decline, and any method can be used in routine clinical practice.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO161

The NKFI KidneyMobile: A Mobile Resource for Community Based Screenings of Chronic Kidney Disease and Its Risk Factors

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Background: Early detection and treatment of chronic kidney disease (CKD) and its risk factors improves health outcomes; however, many high-risk individuals do not have access to routine health care or screenings. The National Kidney Foundation of Illinois (NKFI) developed the KidneyMobile (KM) to conduct community based screenings, education, and follow-up for diabetes, hypertension, and CKD in Illinois.

Methods: We conducted a cross-sectional analysis of NKFI KM participants between 2005 and 2011 throughout Illinois. Working with community partners, the NKFI selected KM sites designed to reach high-risk and vulnerable communities. Sociodemographics, medical history, vital signs, anthropometric measures, and laboratory tests were assessed at the screening visit. Consensus definitions based on these data were used to define the presence of hypertension (HTN) or diabetes (DM). CKD was defined by eGFR < 60 ml/min/1.73m^2 or urine albumin/creatinine >= 30mg/g. Telephone interviews were conducted post-screenings with participants with abnormal findings to determine follow-up activities. Descriptive statistics were used to characterize all findings.

Results: Among 23,166 adults, mean age was 54 years, 68% were female, 51% were African-American or Hispanic, 24% primarily spoke Spanish, and over 30% lacked health insurance. At screenings, 65% of adults not reporting HTN and 10% not reporting DM were found to have these conditions, while 24% of participants not reporting kidney disease met criteria for CKD. In adults with known HTN, 42% had blood pressure >= 140/90 mmHg, while in those with known DM, 44% had HbA1c > 8%. Among 4,937 participants with abnormal findings and post-screening interview, 75% had a physician appointment and further evaluation.

Conclusions: A high-risk disadvantaged population with limited resources is being reached by the NKFI KidneyMobile's community based screenings and being connected to appropriate healthcare services. A significant proportion of these individuals were either given a new diagnosis of DM, HTN, and CKD or informed that their DM and HTN were poorly controlled.

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FR-PO162

The CKD Express©: A New Effective, Efficient and Cost-Saving IT-Based Program to Expedite CKD Care among Large Populations: Results of a Pre-Design CKD Survey

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Background: Over 15% of the US population has CKD. Despite increased calls for referrals of later stage CKD patients to nephrologists, it remains unclear whether this model of care is indeed cost-effective. Also, the natural history of CKD in the general population is uncertain. Furthermore, the escalating cost of the US healthcare system is unsustainable; healthcare costs will increase from $2.5 trillion in 2009 to over $4.6 trillion by 2020. Besides, quality outcomes are suboptimal, and care is fragmented and poorly coordinated. My UW Consortium MBA class developed a new IT-based program to optimize population-based CKD care in a resourceful and yet cost-cutting manner.

Methods: We completed a market survey of CKD patients, with family members/medical staff serving as surrogate patients, to assess the level of satisfaction with current CKD care. We subsequently carried out a market analysis and competitive analysis of the new product.

Results: There were 80 respondents, 49F:31M. 50% ranked cost of care as the most important attribute. Overall, affordability and easy access to care ranked highest. Face-to-face meeting with physician was not as important. We therefore designed a new self-contained IT system with the capability to remotely track and monitor serum creatinine and eGFR of CKD patients, utilizing artificial intelligence and decision support systems tools, and able to prompt the supervising Nurse Practitioner to repeat blood tests, refer to the Emergency Department/nephrologist, and so on. This new system is called The CKD Express © and trial runs have been informally completed in my office.

Conclusions: We must bend the healthcare curve through innovative re-engineering methodologies and the incorporation of economic analysis to healthcare paradigms. The CKD Express © is one such paradigm change waiting to happen. It will make CKD care affordable, more convenient and able to reach large populations all over the US. A patent application for The CKD Express © is in progress.

FR-PO163

A Randomized, Controlled Clinical Effectiveness Trial of Real-Time, Wireless Blood Pressure Monitoring for Older Patients with Kidney Disease and Hypertension Dena E. Rifkin,[1] Joseph A. Abdelmalek,[1] Cynthia Miracle,[1] Chai L. Low,[2] Phil Rios,[1] Carl Stepnowsky,[1] Zia Agha.[1] *[1]UC San Diego; [2]Medicine, VA San Diego.*

Background: Older adults with chronic kidney disease (CKD) have a high rate of uncontrolled hypertension. Home monitoring of blood pressure (BP) is an integral part of management, but requires that patients bring records to clinic visits. Telemonitoring interventions however have not targeted older, less technologically skilled populations.

Methods: Veterans with CKD stage 3 or greater and uncontrolled hypertension were randomized to a novel telemonitoring device pairing a Bluetooth-enabled BP cuff with an Internet-enabled hub which automatically transmitted readings in real time (n=28), or usual care (n=15). Participants did not have to have a computer and no Internet skills were required. Transmitted home recordings were reviewed weekly by the study pharmacist and physician team, and telemonitoring participants were contacted if BP was above goal. Primary endpoints were improved data exchange and device acceptability. Secondary endpoint was BP change.

Results: 43 participants (average age 68, 75% white) completed the 6-month study. Average start-of-study BP was 147/78. Those in the telemonitoring arm transmitted a median of 29 [22,53] BP readings per month, with 78% continuing to use the device regularly, while only 20% of those in the usual care group brought readings to in-person visits. The median number of telephone contacts triggered by telemonitoring was 2[1,4] per patient over the course of the study. Both groups had a significant improvement in systolic BP ($p < 0.05$ for both changes); systolic BP fell a median 13 mmHg in telemonitored participants vs. 8.5 mmHg in usual care participants (p for comparison 0.31). The device was rated as highly acceptable by participants, with 27/28 (96%) stating that they would like to continue using the system.

Conclusions: This low-cost, low-impact telemonitoring solution was rated as highly acceptable by an older population, led to greater sharing of data between patients and clinic, and produced a trend toward improvements in BP control over usual care at 6 months.

Funding: Other NIH Support - UCSD CTRI Innovative Technology Pilot Grant

FR-PO164

Improving Chronic Kidney Disease Co-Management among Primary Care and Nephrology Practitioners William E. Haley,[1] Amy L. Beckrich,[2] Judy J. Sayre,[3] Rebecca B. Mcneil,[4] Peter Fumo,[5] Vijaykumar M. Rao,[6] Edgar V. Lerma.[6] *[1]Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL; [2]Renal Physicians Association, Rockville, MD; [3]Mayo Clinic, Jacksonville, FL; [4]Biostatistics, Duke University, Durham, NC; [5]Delaware Valley Nephrology, Philadelphia, PA; [6]Associates in Nephrology, Chicago, IL.*

Background: Improving co-management is an important strategy to enhance outcomes in chronic kidney disease (CKD). Objectives were to improve CKD identification, referral, communication and co-management among primary care physicians (PCP) and nephrologists (Neph).

Methods: Six Neph and 9 PCP practices participated over 12-15 months, testing tools provided to improve CKD identification, appropriate referral, communication, and co-management. Practice patterns, perceptions, and tool-use data were obtained from taped interviews of physician leaders and site champions, monthly telephone conferences, questionnaires and surveys. 252 eligible patient charts were audited before and 235 after tool implementation, using instruments pre-specified for PCP and Neph practices; the former focused on CKD identification and the latter on advanced CKD guideline adherence.

Results: Qualitative analysis revealed key themes: 1) Enhanced awareness and identification of CKD 2) Increased and improved communication and co-management 3) Increased awareness of recommended guidelines and changes in CKD referral patterns and care 4) Variation in practice patterns, and barriers to tools use 5) Enhanced satisfaction levels. PCP and Neph patients shared similar demographics. PCP audits revealed significant improvement in CKD identification ($p = 0.0115$); Neph audits demonstrated improved BP control ($p = 0.018$). Improvement in clinical endpoints and guideline adherence rates were denoted in those practices affirming most consistent tool usage.

Conclusions: Engendering co-management and improving processes, facilitated by specific tools, led to enhanced awareness and identification of CKD; increased and enhanced communication among PCPs, Neph, and respective staff; changes in CKD referral patterns; and increased awareness of recommended clinical guidelines. Ameliorating CKD co-management offers promise to improve clinical outcomes.

FR-PO165

The British Columbia Nephrologists' Access Study: Reducing Wait Times for Outpatient Nephrology Consultations Michael Schachter, Alexandra Romann, Ognjenka Djurdjev, Adeera Levin, Monica C. Beaulieu. *BC Provincial Renal Agency, Vancouver, BC, Canada.*

Background: Waiting time (WT) guidelines for outpatient nephrology consultations (ONC) are lacking. Automatic eGFR reporting increased referrals in Canada, and elsewhere. National recommendations suggest development of WT targets (WTT). We sought to describe WT for ONC in British Columbia (BC). Using a modified Delphi process, WTT were developed. WT was then re-measured to determine the impact of target setting.

Methods: Data collection occurred in 2 phases: 1) Baseline description (Jan 18-28, 2010) and b) Post Target-Introduction (Jan 16-27, 2012). WT was defined as the interval from receipt of referral letters to assessment. Nephrologists and Family Physicians developed WTT for commonly referred conditions through meetings and surveys. Targets consider comorbidities, eGFR, BP and albuminuria. Referred conditions were assigned priority scores of 1-4.

Results: In 2010 and 2012, 43/52 (83%) and 46/57 (81%) of BC nephrologists participated. Table 1 describes nephrologists and pts. WT decreased from 98(IQR44,157) to 64(IQR21,120) d from 2010 to 2012 (p=x), despite no change in referral eGFR, demographics, nor number of office hrs/wk. WT improved most in high priority patients (Fig 1).

Nephrologist and Patient Characteristics

Group, characteristic	2010	2012	p value
Nephrologists	n=43	n=46	
Age, yr, number (%)			
<40		17 (37)	
41-50		15(33)	
51-60		6(13)	
>60		8(17)	
Practice Size, number (%)			
<300	4 (10)	16 (37)	
301-500	7 (16)	15 (35)	<0.001
>500	33 (75)	12 (28)	
Office hrs/wk	8.5 (6)	8(6)	
Patients			
Age, yr, number (%)			
<50	78 (15)	63 (16)	
50-64	146 (28)	115 (29)	0.889
65-79	185 (35)	148 (37)	
>80	106 (21)	74 (19)	
sex, female (%)	51	49	0.555
Referral eGFR, ml/min per 1.73m2 (%)			
<30	85 (18)	57 (16)	
30-60	292 (65)	217 (61)	0.044
>60	75 (17)	84 (23)	

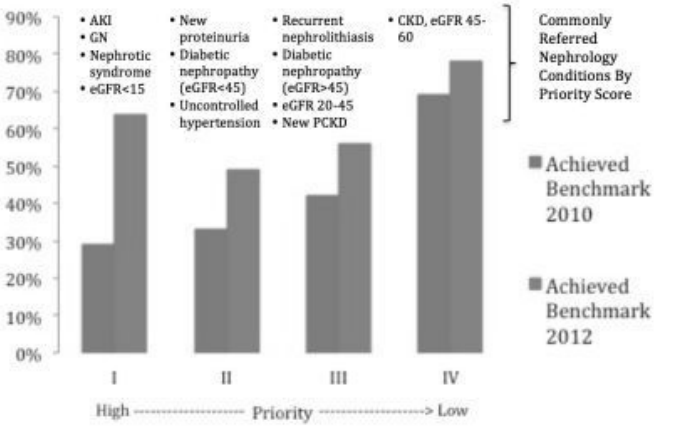

Figure 1| The proportion of patients seen within recommended wait time targets increased the most for high priority patients. Representative commonly referred conditions are shown above bars by priority score.

Conclusions: Development of *WTT* for ONC resulted in marked reductions in WT. Sustainability of the improvement will be monitored.

Funding: Government Support - Non-U.S.

FR-PO166

Improving Care of Patients with CKD: A Randomized Controlled Trial of a Pharmacist/Registry Based Intervention Paul E. Drawz, Adam T. Perzynski, Yang Liu, Brook Watts, R. Tyler Miller. *Case Western Reserve University.*

Background: The objective of this study was to evaluate whether a pharmacist based intervention utilizing a chronic kidney disease (CKD) registry improves blood pressure (BP) control and quality of care for patients with CKD.

Methods: We conducted a randomized controlled trial at the Louis Stokes Cleveland VAMC. Patients with advanced CKD (estimated glomerular filtration rate < 45 mL/min/1.73m^2) were randomized to the intervention or usual care. The pharmacist based intervention utilized a CKD registry to identify patients not receiving or achieving guideline based recommendations. During the one year study, two pharmacists called intervention subjects within two weeks of a primary care visit and evaluated medication adherence, provided education regarding CKD and hypertension, ordered guideline recommended labs, and communicated with primary providers via the electronic medical record. The primary clinical outcome was the percent of subjects with a baseline BP >130/80mmHg whose last study BP was at goal. The primary process outcome was the percent of patients with at least one PTH measured during the study period.

Results: 1070 subjects were randomized to the intervention and 1129 to usual care. Among those with poorly controlled BP at baseline, there was no difference in the last recorded blood pressure or the percent whose last study BP was at goal (41.7% vs 40.2% in the control arm, *P*=0.7). Subjects in the intervention arm were more likely to have a PTH

measured during the study period (46.9% vs 16.1%, *P* <0.001) and were on more classes of antihypertensive medications at the end of the study (2.7 vs 2.5, *P*=0.05). There was no difference in medication adherence or the rate of ESRD (1.8% vs 2.4% in the control arm, *P*=0.3) but the number of deaths in the intervention arm was slightly lower than the control arm (4.7% vs 6.6%, *P*=0.06).

Conclusions: A pharmacist based intervention utilizing a CKD registry, patient education, and collaboration with primary care physicians did not improve BP control but did improve guideline adherence and increased the number of antihypertensive medications prescribed to subjects with poorly controlled BP.

Funding: NIDDK Support

FR-PO167

Budget Impact Model of Medicare Bundled End-Stage Renal Disease Prospective Payment System: A Dialysis Organization Perspective

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Background: In 2014, oral medications, including phosphate binders (PBs), will be included in the new Medicare bundled reimbursement scheme. We developed a budget impact model (BIM), which is capable of evaluating different scenarios, in order to estimate the incremental budget impact associated with a bundled prospective payment system (PPS) in Medicare beneficiaries on dialysis receiving PBs. The impact on budget was estimated from the perspective of a large dialysis organization in the United States (US).

Methods: The BIM estimated the potential annual budget impact of the new bundled PPS associated with the inclusion of PBs (including calcium-based binders, lanthanum carbonate [LC], sevelamer hydrochloride [SH], and sevelamer carbonate [SC]). Treatment efficacy associated with PBs (in terms of reduced risks of hospital days and mortality rates), market share, costs and any assumptions were based on published literature, market research data, and US Renal Data System reports. Scenario and sensitivity analyses were conducted. Costs were reported in US dollars.

Results: In total, 139,935 patients per year were predicted to be treated by a large dialysis provider and to receive PBs. Current (2012) utilization-weighted Medicare Part D cost per dialysis session for PBs was estimated at $23.52. The predicted base case for the 2014 total bundled rate of $278 per dialysis treatment, included $243 for dialysis-related services and a base case of $35 for oral medications. Future scenarios based on current PB market share utilization estimates may also allow dialysis organizations to remain within the bundled rate. In one such scenario, moving 10% market share from SH and SC to LC remained within the predicted PB bundle reimbursement rate. The BIM also depicts the potential impact of other scenarios and dosing levels in relation to the bundled rate.

Conclusions: This model suggests that PB costs may be manageable with the new PPS bundling rate. However, given the assumptions made and the variety of scenarios, there is uncertainty around the base case forecast. Further research to estimate the budget impact of the new bundled PPS is warranted.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

FR-PO168

Impact of a Multidisciplinary Low Clearance Clinic on the Management of Predialysis Chronic Kidney Disease Patients

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Background: A multidisciplinary low clearance clinic (LCC) run by a renal physician, clinical pharmacist and coordinator was set up in National University Hospital, Singapore. This study aimed to assess LCC's impact on hypertension (HTN), anemia and mineral and bone disorders (MBD) management in predialysis chronic kidney disease (CKD) patients, compared to standard care. Interventions made by the LCC pharmacist and physician acceptance rates were also determined.

Methods: This retrospective cohort study included stage 4-5 predialysis CKD patients referred to LCC or standard care (non-LCC). Blood pressure (BP), hemoglobin (Hgb), serum phosphate (P), calcium (Ca) and intact parathyroid hormone (i-PTH) concentrations, as well as proportion of patients who achieved KDOQI goals for these parameters were compared between both groups at baseline, and approximately 6 and 12 months thereafter. LCC patient charts were reviewed for therapeutic recommendations made by the pharmacist and their acceptance rates calculated.

Results: Fifty-three LCC and 96 non-LCC patients were evaluated. At 6 and 12 months, LCC patients had lower systolic BP (SBP) and higher Hgb than baseline (baseline vs. 12-month SBP and Hgb: 160±24 vs. 149±24 mmHg, and 10.6±1.7 vs. 11.2±1.5 g/dL, respectively; P<0.05). Serum P and Ca were similar over the study period. Non-LCC patients had lower serum Ca at 6 and 12 months (2.30±0.12 and 2.32±0.14 mmol/L, respectively) compared to baseline (2.34±0.11 mmol/L) (P<0.05). The proportion of patients who achieved KDOQI goals for all parameters was similar for both groups. The LCC pharmacist identified 411 drug-related problems during the study period and the overall acceptance rate of therapeutic recommendations was 61.3%.

Conclusions: Compared to standard care, 1-year follow-up at the multidisciplinary LCC was associated with improved HTN control but not anemia and MBD management. The overall acceptance rate of pharmacist recommendations was 61.3%. More studies are needed to confirm the long-term impact of multidisciplinary care on clinical outcomes of predialysis CKD patients.

FR-PO169

Assessing Perceived versus Actual Knowledge and Quality of Life in Predialysis Chronic Kidney Disease Patients

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Background: Educating patients about chronic kidney disease (CKD) and its complications and management is essential as adequate patient knowledge has been linked to improved clinical outcomes. However, little is known about the difference in perceived vs. actual disease knowledge and the impact of such knowledge on health-related quality of life (HRQoL) in CKD patients. This study aimed to assess perceived and actual CKD knowledge, HRQoL, and examine the association between knowledge and HRQoL in our local predialysis CKD patients.

Methods: A survey was developed to assess perceived and actual CKD knowledge in this cross-sectional study involving predialysis CKD patients from the outpatient renal clinic in National University Hospital, Singapore. The Kidney Disease Quality of Life-Short Form (KDQoL-SF™) version 1.3 and EuroQoL-5 dimensions (EQ-5D) questionnaires were administered to assess HRQoL. Knowledge scores were compared using independent t-test. Univariate analyses and multiple linear regression were performed to determine associations between knowledge and HRQoL.

Results: A total of 95 patients were included in the analysis. Patients who perceived they had "At least some knowledge" had higher actual knowledge scores (58.2±21.2%) than those who perceived they had "No knowledge" (52.5±21.0%) (p=0.22). HRQoL of our local predialysis CKD patients is poorer than that of the U.S. general population but was better than patients with other chronic disease states in Singapore. Patients with better disease knowledge reported better HRQoL in only one scale: symptom/problem list (mean±SD scores for moderate vs. poor knowledge: 92.7±9.1 vs. 82.1±16.0 respectively; β-coefficient = -0.331, p=0.030).

Conclusions: This study showed that the self-reported knowledge level and actual knowledge scores of our local predialysis CKD patients were consistent and that CKD knowledge has little impact on actual HRQoL. Further studies need to be done to determine other correlates of HRQoL in predialysis CKD patients.

FR-PO170

Health Literacy and Disease Management Self-Efficacy: The Explanatory Role of Disease Knowledge

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Background: This investigation examined if disease knowledge was mediating the relationship between health literacy and disease management self-efficacy.

Methods: People between the ages of 12 to 29 with chronic kidney disease stages ≥ 4 were eligible to participate. The six IRB-approved measures included: 1) The TR$_x$ANSITION Scale - transition knowledge/ self-activation (Ferris et.al. 2012) 2) A 10-item disease knowledge questionnaire 3) The Newest Vital Sign - health numeracy (Weiss et al., 2005) 4) The REALM (Davis et al., 1993) - health literacy 5) The STAR$_x$ to assess transition self-management and disease management self-efficacy 6) A demographics questionnaire.

Results: To date, 25 participants (12 transplant recipients) have been enrolled. Their demographic characteristics were as follows: 60% female, 44% Caucasian; 32% African-American, 16% Hispanic. The mean age was 21.56 (± 4.7) and the mean age at diagnosis was 9.47 (±8.46). Disease knowledge was a significant positive predictor of self-management (β= .40, p= .08), disease management self-efficacy (β= .45, p= .05), and knowledge/ self-activation (β= .49, p= .03). Additionally, both health literacy (β= .79, p= .00) and health numeracy (β= .72, p= .00) were significant positive predictors of disease knowledge. The Baron and Kenney (1986) procedure was applied to determine that disease knowledge was mediating the relationship between health literacy and disease management self-efficacy (β= .52, p= .01).

Conclusions: Adolescents and young adults with higher disease knowledge have more transition self-management and disease management self-efficacy. This suggests it is important for healthcare providers to provide ongoing patient education. Additionally, results indicate that disease knowledge mediates or is the mechanism through which health literacy contributes to disease management self-efficacy. This suggests the importance of providing patient education at a literacy appropriate level.

FR-PO171

Health Literacy and Numeracy as Predictors of Transition Readiness/Self-Management and Depressive Symptoms among Adolescents and Young Adults with Chronic Kidney Disease

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Background: Adolescents and young adults with chronic kidney disease (CKD) have great survival and must either undergo a transition process from pediatric to adult-focused health care or learn how to self-manage their disease effectively to decrease morbidity. We examined the roles of health literacy and numeracy in predicting transition readiness and/ or self-management and depressive symptoms.

Methods: We recruited English-speaking patients ages 14-29 with CKD stage ≥ 4 from the UNC Kidney Center pediatric and adult nephrology clinics. Participants completed

three provider-administered scales: (1) Rapid Estimates of Adult Literacy in Medicine (REALM) to assess health literacy, (2) Newest Vital Sign, a food label to assess literacy/numeracy, (3) TR$_x$ANSITION Scale to assess transition readiness/self-management, and a self-administered web-based scale: (4) CES-D to assess depressive symptoms.

Results: To date, we have enrolled 25 patients with CKD stage ≥ 4 (including 20 transplant patients) with the following characteristics: 60% Female; 50% White, 34% Black, 13% Hispanic; Mean age 21.6 (±4.7); Mean age at diagnosis 9.5 (±8.5). Nine participants were managed by pediatric nephrology and 16 by adult nephrology. *Health literacy* – The REALM score was a significant negative predictor of depressive symptoms (β= -.38, *p*=.09) and a significant positive predictor for overall transition readiness/self-management (β= .64, *p*=.00). *Health numeracy* – The Newest Vital Sign score was a significant negative predictor of depressive symptoms (β= -.41, *p*= .07) and a significant positive predictor for overall transition readiness/self-management (β= .38, *p*= .02).

Conclusions: Higher health numeracy and literacy independently predict fewer depressive symptoms and higher overall transition readiness/self-management among youth with CKD. Without adequate health literacy and numeracy skills, adolescents and young adults with chronic health conditions are ill-equipped for disease self-management and have poorer mental health. Enrollment continues underway.

Funding: Private Foundation Support

FR-PO172

OrganJet: Overcoming Geographical Disparities in Access to Deceased Donor Kidneys in the United States Anton I. Skaro. *Feinberg School of Medicine, Comprehensive Transplant Center, Evanston, IL.*

Background: There is a huge imbalance between the supply of and the demand for deceased donor kidneys. Under the current UNOS allocation policy, the vast majority of organs are allocated locally. There are significant disparities in waiting times and access to kidney transplant based on geography. However, there exist substantial ethical and logistical obstacles to the successful reform of organ allocation policy, which limit a more equitable distribution of kidneys. An operational solution (OrganJet) for patients on the transplant list is proposed which facilitates multiple-listing at transplant centers in distant donor service areas (DSA) of their choosing.

Methods: We modeled patient decisions of the location(s) to multiple-list as a selfish routing game in which each patient tries to minimize their "congestion cost" (i.e. maximize their life expectancy). Waiting times in each DSA were modeled through an overloaded fluid model. Using a stylized game-theoretic model, we studied the impact of multiple-listing on patients' accept/reject decisions of organ offers. (Co-authors: B. Ata, S. Tayur).

Results: OrganJet significantly remedies current disparities provided a small fraction (15%) of patients choose to multiple list, resulting in more uniform waiting times for kidney transplant in the US.

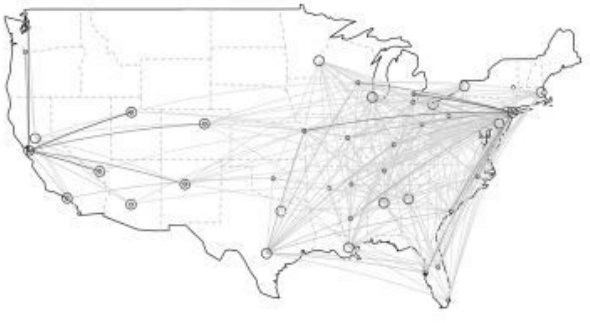


Figure 1: Equilibrium flow rates on a US map for $\pi = 1$. Each line corresponds to a particular flow; and for each flow, the large circle indicates the fly-out DSA whereas the small circle corresponds to the associated fly-in DSA.

The variation in waiting times across DSAs goes down to 13% from 23% as measured by the coefficient of variation. Similarly variation in access to transplant in different DSAs goes down to 21% from 38%. Also, when the time-to-death distribution has an increasing hazard rate, multiple-listing may leads to fewer deaths even if there is no increase in the number of kidneys procured. We also observed that under multiple-listing, organ discard may decrease.

Conclusions: Broader multiple-listing significantly reduced the geographic disparities in access to kidney transplantation in the US.

FR-PO173

Focus Group Evaluation of New CKD Educational Material from the Increasing Kidney Awareness Network (IKAN) Transplant Project Bessie A. Young,[1,2,3] Clarence Spigner,[3] Georgia Galvin.[1] *[1]VA Puget Sound Health Care System, Nephrology, Health Services Research and Development, Seattle, WA; [2]Kidney Research Institute, University of Washington, Seattle, WA; [3]Health Services, School of Public Health, University of Washington, Seattle, WA.*

Background: Although many CKD educational materials are available for kidney replacement therapy (KRT) education, few were developed to be culturally responsive to ethinic minorities.

Methods: We conducted focus groups among subjects with CKD or CKD connections to evaluate familiarity with educational materials and to determine responses to new educational material. Based on prior key informant interviews, a focus group guide was developed after IRB approval. Subjects were recruited from dialysis, transplant recipients, living kidney donors, potential living kidney donors, and pre-dialysis patients. All focus groups were digitally recorded and transcribed verbatim, and then read and re-read for content analysis. Transcripts were coded to develop subject content to develop emerging themes and categories until saturation was acheived.

Results: Two focus groups were held with 12 participants. Of participants 100% were African American, 66% were male, 66% had some college or were single, 22% had a kidney transplant, 33% were pre-dialysis, 22% were on dialysis, and 11% were potential kidney donors. Major themes from the focus groups included: 1) Altruism on the part of the potential recipient; 2) Difficulty with asking a relative for a kidney; 3) Financial Issues; 4) Emotional Issues, and; 5) Lack of Education regarding available therapies. Subjects in mixed focus groups felt they had better support and understanding of issues than they would have in separated focus groups. All groups found the educational material useful and better targeted towards people of color.

Conclusions: Culturally targeted CKD educational increases awareness of specific options for KRT, which may not have been offered previously. Testing of newly developed CKD educational material is needed to determine if interest in and preparation for kidney transplant or home dialysis therapies are increased.

Funding: NIDDK Support, Veterans Administration Support

FR-PO174

Evaluation of the Effect of a Health Care Model for CKD Patients on Lifestyle and Renal Function Alberto Barajas,[1] Laura Cortes-sanabria,[2] Guillermo G. Garcia,[1] Marcello Tonelli.[3] *[1]Nephrology, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico; [2]UIMER, CMNO IMSS, Guadalajara, Jal, Mexico; [3]Medicine, University of Alberta, Edmonton, AL, Canada.*

Background: Negative lifestyle habits are risk factors for progression of kidney disease and a barrier to achiev treatment goals. We evaluated the effect of multidisciplinary care on lifestyle and renal function in patients with stage 3 CKD.

Methods: A prospective cohort of patients received educational intervention guided by a multidisciplinary team (nurse, dietitian, physical) at our CKD Prevention Clinic. A lifestyle questionnaire was applied at baseline and at the end of follow-up (6 months); the questionnaire evaluated diet (maximum value=36), exercise (12), tobacco use (8), alcohol consumption (8), management of emotions (12), knowledge of disease (8) and treatment adherence (16); the higher the score, the healthier the behavior. Relevant clinical, biochemical, and renal function data were recorded. ANOVA, Kruskal-Wallis, and χ^2 were used when appropriate. A p value <0.05 was considered significant.

Results: 61 patients, age 56 ± 17 y, were included; female gender 56%; 57% were diabetics. Results are shown in table 1.

Lyfestyle Questionnaire (max score)	Baseline	3 mo	6 mo
Diet (36)	20 (10-32)	27 (10-36)*	29 (12-36)*
Exercise (12)	6 (0-10)	6 (0-12)	6 (0-12)
Tobacco (8)	8 (0-8)	8 (0-8)	8 (0-8)
Alcohol (8)	8 (0-8)	8 (0-8)	8 (0-8)
Knowledge of Dis (8)	0 (0-6)	0 (0-6)	0 (0-6)
Emotion mgment (12)	6 (0-12)	6 (0-12)	8 (0-12)*≠
Treatment Adherence (16)	12 (2-16)	16 (4-16)*	16 (4-16)*
Total (100)	60 (40-80)	68 (34-92)*	70 (50-92)*≠
Clinical Data			
SystBP mmHg	143±31	137±27	134±23*
DiastBP mmHg	80±15	73±11*	73±11*
BMI	27±5	27±5	26±4
Hgb g/dl	12.4±1.8	12.0±1.7	12.3±1.3
Glucose mg/dl	121±69	117±38	115±40
Urea mg/dl	63±24	74±35*	75±34*
Cholesterol mg/dl	180±59	177±50	171±50*≠
eGFR ml/min/1.73m²†	43(37-40)	41 (31-50)	41(27-47)
Proteinuria mg/day	122(30-500)	146 (30-300)	283 (25-930)

*p=<0.05 vs baseline; ≠ vs 3 months; †MDRD

Conclusions: We conclude that multidisciplinary care provided by a nurse-lead CKD prevention clinic: 1)reduces significantly negative lifestyle behavior, especially diet, emotion management and treatment adherence;2) improves BP and lipid control; and 3) preserves kidney function.

FR-PO175

Barriers to Kidney Transplantation: A Qualitative Study of Perceptions from Dialysis Patients and Transplant Recipients Clarence Spigner,[1] Courtney R. Lyles,[4] Georgia Galvin,[2] Bessie A. Young.[1,2,3] *[1]Health Services, University of Washington, Seattle, WA; [2]VA Puget Sound Health Care, Nephrology, Seattle, WA; [3]Kidney Research Institute, University of Washington, Seattle, WA; [4]GIM, University of California, San Francisco, San Francisco, CA.*

Background: Racial and ethnic disparities in kidney transplantation persist. More research is needed to generate theories and/or explanations as to why these racial/ethnic differences exist, and from the perspective of those most affected by the disease.

Methods: We recruited 58 kidney patients of which 30 were on dialysis and 28 had a kidney transplant. Semi-structure questions were designed to solicit opinions about kidney disease and transplantation. Phone interviews were conducted by a single investigator (GG). Sessions were audio recorded, and transcribed verbatim to hard-copy for content

analysis. Open coding was employed by using each respondent s' exact words from which to form categories. Saturation was reached when no new information was forthcoming. A core phenomenon emerged. Validity was assured by having two team members (CL, BY) independently employ the same coding scheme to systematically assess coding. Where disagreement occurred, discussion ensued until consensus was reached.

Results: The central element to emerge was fear; among both the dialysis respondents facing the transplant procedure and as recalled by the post-transplant respondents. Several propositions directionally led to the concept of fear, which related mainly to overall quality of life. Dialysis respondents were more focused on personal factors that qualified or unqualified them for a transplant, while transplanted respondents indicated more knowledge of institutional and societal requirements for having obtained an organ.

Conclusions: Our findings support studies about apprehension of the unknown surgical outcome. Fear of transplantation stemmed mainly from lack of education about the procedure. This can address in part by post-transplant patients who well understand that fear. What emerged is an aspect of Social Learning Theory where the post-operative patients might be the best teachers of pre-operative patients.

Funding: NIDDK Support, Veterans Administration Support

FR-PO176

Cost-Utility Analysis of Sodium Polystyrene Sulfonate versus Potential Alternatives for Chronic Hyperkalemia Dustin J. Little, Robert Nee, Kevin C. Abbott, Christina M. Yuan. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Hyperkalemia is common during renin-angiotensin-aldosterone system (RAAS) inhibitor treatment, and may prevent optimum dosing. Limited treatment options include sodium polystyrene sulfonate (SPS) potassium binding resins, but concerns have been raised regarding their efficacy and safety; including associated colonic necrosis (CN), with an incidence of 0.14% (95% CI 0.03– 0.40%) reported recently. Alternative agents have been studied, but their cost-utility has not been estimated.

Methods: A computer-based decision model was developed using TreeAge Pro software (Williamstown, MA) to represent the costs and effectiveness of SPS compared to a theoretical "Drug X" binding resin for treatment of chronic hyperkalemia. Model inputs were obtained from literature review to include a cost of $10.65 per 30gm SPS dose, and baseline quality-adjusted year of life (QALY) utility indices were obtained from the Tufts Medical Center Cost-Effectiveness Analysis Registry. Model life-span was one year. The primary endpoint was the incremental cost-effectiveness ratio in dollars per QALY gained. A willingness-to-pay threshold of $50,000 per additional QALY determined cost effectiveness.

Results: Drug X could cost no more than $10.77 per dose to be cost-effective, and at a cost of $40.00 per dose achieved an incremental cost-effectiveness ratio of $26,088,369.00 per QALY gained. SPS was more cost-effective than Drug X in 100% of model iterations using Monte Carlo probabilistic sensitivity analysis, and the expected value of perfect information (EVPI) was $0.00 at Drug X costs of ≥$21.00 per dose. One-way sensitivity analysis showed SPS to be the cost-effective option for CN incidences of ≤19.9%, over 10-fold greater than the highest estimates of SPS-associated CN.

Conclusions: Our analysis suggests that SPS alternatives may not be cost effective unless priced similarly to SPS. This cost-utility analysis may help to guide decisions regarding adoption of alternative agents for control of chronic hyperkalemia as they enter clinical use, and also suggests that SPS be used as an active control in clinical trials of these agents.

Funding: Other U.S. Government Support

FR-PO177

Cost-Effectiveness of Lowering LDL-Cholesterol in Chronic Kidney Disease: Results from the Study of Heart and Renal Protection (SHARP) Borislava N. Mihaylova,[1] Jingky P. Lozano-kuehne,[1] Iryna Schlackow,[1] William G. Herrington.[2] *[1]On Behalf of the SHARP Collaborative Group, Department of Public Health; [2]Clinical Trial Service Unit, University of Oxford, United Kingdom.*

Background: The SHARP study showed that simvastatin 20mg plus ezetimibe 10mg daily safely reduced the rates of major atherosclerotic events (MAEs: non-fatal myocardial infarction, coronary death, non-haemorrhagic stroke or arterial revascularisation) in patients with advanced chronic kidney disease (CKD). Within-trial cost-effectiveness is reported.

Methods: 9,270 individuals with advanced CKD (3,023 on dialysis) were randomized to ezetimibe/simvastatin or matching placebo for a median follow-up of 4.9 years. Hospital admissions and visits in SHARP were mapped into UK-based Healthcare Resource Groups and hospital costs were derived from NHS costs (2011); ezetimibe/simvastatin cost of £1.19 per day was based on Prescription Cost Data for England (2011). The treatment costs minus the hospital and concomitant lipid lowering drug cost savings per MAE avoided were estimated for all participants (with costs and outcomes discounted at 3.5% per annum). To allow for the different LDL cholesterol (LDL-C) reductions, analyses for participants not on dialysis and on dialysis at baseline are reported using overall study relative effects on MAE and vascular hospitalisation costs per 1mmol/L LDL-C reduction.

Results: Allocation to ezetimibe/simvastatin yielded an average LDL-C difference of 0·85 mmol/L and was associated with a 17% (95% confidence interval 5-28%) proportional reduction in all MAEs (38 MAEs prevented per 1000 treated). The cost of ezetimibe/simvastatin was ca. £1,400 per participant. The reduction in vascular-related hospital cost was 15% (4-25%) (£177 per participant). Costs for hospitalisation for other reasons were not affected. The additional cost per MAE prevented was £34,300. The cost of preventing each MAE per 1mmol/L lower LDL-C was £30,700 for those on dialysis and £37,700 for those not.

Conclusions: The costs of preventing MAEs with ezetimibe/simvastatin in patients with advanced CKD are comparable to those observed for proprietary LDL-C lowering regimens in other patients at high risk of coronary heart disease.

Funding: Pharmaceutical Company Support - The SHARP Study Was Funded Mainly by Merck/Schering-Plough Pharmaceuticals (North Wales, PA, USA), Government Support - Non-U.S.

FR-PO178

Trends in Predialysis Anemia Care in Older Patients Approaching ESRD in the U.S. (1995-2009) Wolfgang C. Winkelmayer, Aya Alice Mitani, Benjamin A. Goldstein. *Stanford University School of Medicine, Palo Alto, CA.*

Background: Anemia is common in patients with advanced CKD approaching ESRD. Little is known about patterns and trends in the anemia care patients receive prior to initiating chronic renal replacement therapy (RRT) in the U.S.

Methods: From USRDS files, we studied all patients aged 67+ yrs on their ESRD date who had Medicare A+B as their primary payer in >2 yrs prior to start of RRT. From billing claims, we identified the earliest administration of an erythropoiesis-stimulating agent (ESA) as well as the reported hematocrit on this index claim. We tabulated temporal trends of ESA initiation (proportion receiving any ESA prior to start of RRT; timing of ESA initiation relative to first ESRD date; mean hematocrit reported with these ESA initiation claims) and the receipt of any blood transfusions.

Results: We studied 440,924 older patients initiating RRT between 1995 and 2009. The proportion of patients receiving any ESA in the 2 years prior to RRT increased from 3.2% in 1995 to 40.9% in 2007; thereafter ESA use declined to 36.4% in 2009. The median time of ESA initiation relative to the RRT start date increased from 99 days (1995) to 470 days (2009). The mean reported hematocrit at first ESA use was 26.7% in 1995, exceeded 31% in all years from 1999-2007 and then declined to 30.2% in 2009. The proportion of patients receiving any blood transfusions prior to start of RRT increased monotonically from 20.8% in 1995 to 38.6% in 2009.

Conclusions: Between 1995 and 2009, the intensity of anemia care in older adults prior to their start of RRT increased considerably. We observed a slight reversal of this trend in patients initiating RRT after 2007, possibly as a consequence of safety concerns reported in the CHOIR trial in late 2006 and subsequent FDA-mandated boxed warning. Despite ESA treatment being initiated earlier and in more patients over time, the proportion of patients receiving blood transfusions prior to initiation of RRT has almost doubled. While ESAs were first approved for transfusion avoidance, this benefit of increased ESA use prior to RRT is invisible on a population level, likely a consequence of general trends towards more lenient transfusion thresholds in U.S. practice.

Funding: NIDDK Support

FR-PO179

Characterization of the Clinical Context for the Administration of Red Blood Cell (RBC) Transfusions in Patients with Chronic Kidney Disease (CKD) Requiring Chronic Dialysis Jason Jones,[1] Carly J. Paoli,[2] Felicia Bixler,[1] Joseph Cha,[1] Susan V. Yue,[2] Matthew Gitlin,[2] Victoria A. Kumar.[1] *[1]Kaiser Permanente Southern California; [2]Amgen, Inc.*

Background: The objective of this study is to characterize the clinical context surrounding the administration of RBC transfusions among CKD patients requiring dialysis. Understanding the clinical context may suggest ways to improve anemia management and reduce the potential for RBC transfusion events in these patients.

Methods: Two nephrologists reviewed medical records from Kaiser Southern California for CKD on dialysis who received a RBC transfusion in 2009 or 2010; 150 were randomly selected for review; here, we report interim results for 86 patients. Data was collected surrounding the RBC transfusion event and classified into 4 clinical context categories: Hb level/ESA response, anemia symptoms, medical condition, and surgery. The decision to transfuse is likely multifaceted thus patients could have one or more categories supporting the need for a RBC transfusion. The nephrologists then subjectively prioritized the categories for each patient. For patients who had Hb level selected as a clinical context category, the Hb level that supported the order for the RBC transfusion was recorded.

Results: Of the 86 patients identified, the mean age was 67.5 and 52% were female. Table 1 provides the clinical context surrounding the RBC transfusions events. For patients who had Hb level selected as a clinical context category, the median Hb value indicated on the order was 7.8g/dL (95%CI: 7.6 - 8.2); n=71.

Table 1: Clinical Context Categories for RBC Transfusion (n = 86)*

	Clinical Context Categories	Ranked as Priority #1
Hb Level/ESA Response	83% (CI:73-90%); n=71	65% (CI:54-75%); n=56
Lack of ESA Response	8% (CI:3-16%); n=7	-
Medical Diagnosis	64% (CI:53-74%); n=55	20% (CI:12-30%); n=17
Top Diagnosis: Acute Blood Loss (<72 hours)	36% (CI:26-47%); n=31	-
Anemia Symptoms	40% (CI:29-51%); n=34	14% (CI:7-23%); n=12
Top Symptom: Shortness of Breath	15% (CI:8-24%); n=13	-
Surgery	12% (CI:6-20%); n=10	0%; n=0
Top Surgery Type: Elective Surgery	10% (CI:5-19%); n=9	-
No Clinical Context Found in Chart	n=1	n/a

* All percentages are calculated based on total n=86. Descriptive statistics and 95% confidence intervals are provided.

Conclusions: This study is the first in characterizing the clinical context surrounding the need for RBC transfusions in CKD patients on chronic dialysis. We demonstrate that the clinical context for the majority of RBC transfusions is a complex set of conditions including Hb level, medical conditions and anemia symptoms.

Funding: Pharmaceutical Company Support - Amgen, Inc.

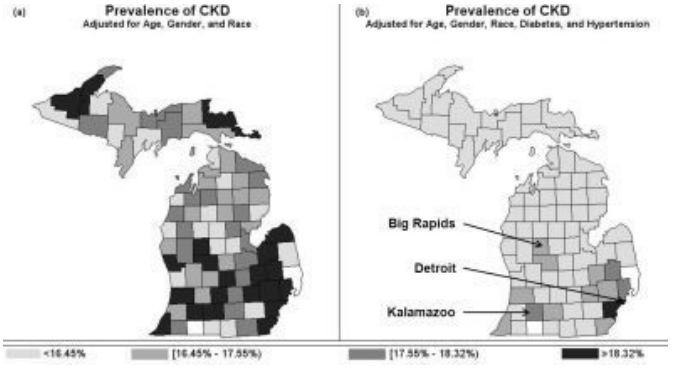
FR-PO180

Utility of Administrative Health Data for Chronic Kidney Disease Surveillance at the County Level Elizabeth Hedgeman,[1] Hal Morgenstern,[1] Deidra C. Crews,[2] Meda E. Pavkov,[3] Neil R. Powe,[4] Rajiv Saran.[1] *[1]University of Michigan; [2]Johns Hopkins University; [3]Centers for Disease Control and Prevention; [4]University of California San Francisco.*

Background: Population prevalence of chronic kidney disease (CKD) depends on individual and macro-level factors; identification of CKD depends on healthcare access, awareness, and testing of at-risk individuals. We postulated that healthcare claims could be used for county-level CKD surveillance to identify priority areas for state and county public-health authorities.

Methods: Focusing on Michigan, we examined 2010 Medicare claims 5% sample data. 49,209 adults met the eligibility criteria: age 65+ years, MI resident, continuous enrollment in parts A and B, no part C, no ESRD. County of residence, age, sex and race were identified from the denominator file; CKD, diabetes and hypertension were identified by ICD-9-CM diagnosis claims. County prevalences of diagnosed CKD, standardized to the covariate distribution of the total study sample, were estimated by generalized linear mixed model treating counties as random effects.

Results: The crude prevalence of diagnosed CKD in the sample was 19.3% (95% CI: 18.9-19.6%). County prevalence estimates are displayed in the Figure, adjusting for demographic variables without and with adjustment for diabetes and hypertension. Adjustment for risk factors explained most of the county variation. High CKD prevalence clustered around three areas.



Conclusions: We have shown the feasibility of using claims data to generate county-specific estimates of diagnosed CKD prevalence. This method depends on data completeness and accuracy, and population representativeness. We will expand this approach, incorporating county-level contextual variables (e.g., poverty, rurality) to further explain geographic differences in CKD, thereby informing public policy.

Funding: Other U.S. Government Support

FR-PO181

Hemodialysis Quality-of-Care Indicators and Patient Ability to Work: Data from a Current US Renal Data System Study Nancy G. Kutner,[1] Kirsten L. Johansen,[2] Dana Dacosta,[1] Julie W. Doyle,[2] Rebecca H. Zhang.[1] *[1]USRDS Rehab/QOL SSC, Emory Univ, Atlanta, GA; [2]USRDS Nutrition SSC, UCSF, San Francisco, CA.*

Background: Restoring patients' ability to work was a key rationale for establishing the ESRD Program of Medicare. An association has been shown between incremental achievement of hemodialysis (HD) quality indicator (QI) goals and patient morbidity and quality of life (Lacson et al. 2009), but whether patient-assessed ability to work varies as a function of QI goals is unknown.

Methods: Patients on HD treatment for ≥3 months were enrolled 2009-2011 for a USRDS study ("ACTIVE-ADIPOSE") in 7 San Francisco, CA outpatient clinics and 7 Atlanta, GA outpatient clinics. 509 patients aged 18-64 responded to the interview question "Are you now able to work for pay (full-time or part-time)?" HD QI goals considered were serum albumin ≥ 4.0 g/dl, hemoglobin 10-12 g/dl, kt/V ≥1.2, serum phosphorus 3.5-5.5 mg/dl, and catheter absence. Likelihood of reported ability to work by achievement of QI goals was examined in logistic regression models adjusted for age, sex, race, educational level, diabetes, congestive heart failure, depressive symptomatology (CES-D score), vintage, and facility clustering.

Results: 36% of the cohort (181/509) reported ability to work. Mean (S.D.) number of HD QI goals met was 3.31 (1.07); median = 3.0. The proportion of the cohort meeting a specific goal ranged from 49% for serum phosphorus 3.5-5.5 mg/dl to 91% for kt/V ≥ 1.2. With number of QI goals met (0-5) as a continuous variable, patients' likelihood of reporting ability to work increased with an increasing number of QI goals met (OR 1.28 [95% CI 1.04-1.57]; p = 0.02). Age less than 55 and lower CES-D score were additional independent predictors of reported ability to work.

Conclusions: Meeting more HD QI goals was associated with greater likelihood of reporting ability to work. Ability to work is a concept that cannot be directly measured; self-reported ability to work and employment status both provide only indirect measures (AHRQ Evidence Report 2000). However, the data we summarize suggest an important link between meeting HD QI goals and vocational rehabilitation potential in the HD population.

Funding: NIDDK Support

FR-PO182

Inpatient Healthcare Utilization among US Children with Nephrotic Syndrome Cheryl L. Tran,[1] Debbie S. Gipson,[1] Kassandra L. Messer,[1] Joyce P. Samuel,[2] Emily G. Herreshoff,[1] Susan F. Massengill,[3] David T. Selewski.[1] *[1]Pediatric Nephrology, University of Michigan, Ann Arbor, MI; [2]Pediatric Nephrology, University of Texas-Houston, Houston, TX; [3]Pediatric Nephrology, Levine Children's Hospital, Charlotte, NC.*

Background: There is little data describing the inpatient healthcare utilization in children with nephrotic syndrome (NS) and related complications. Our goals were to define the frequency, charges and length of hospitalizations among children with NS, the frequency of complications and the characteristics associated with inpatient costs.

Methods: Kids' Inpatient Database (HCUP-KID) data was obtained for the 2006 and 2009 cohort years. HCUP-KID is a database of US hospital discharges for children compiled every 3 years with sponsorship from the Agency for Healthcare Research and Quality (AHRQ). We identified patients by searching for NS ICD-9-CM diagnosis codes.

Results: There were 6308 hospitalization discharges in children with a primary or secondary diagnosis of NS reported by 38 and 44 participating states in 2006 and 2009 respectively and representing 9934 discharges nationally. Total charges attributed to NS related hospitalizations summed to nearly $259 million. The mean charge per hospitalization was approximately $26,500 (sd $1,100) and length of stay was 4.9 days (SE 0.1). Sixteen percent of discharges for NS had a diagnosis code for at least one severe complication including thromboembolism (3.6%), septicemia (3.8%), peritonitis (2.6%), pneumonia (5.4%), or diabetes (2.4%). Mulitvariate analysis showed age > 14 years, race, higher socioeconomic status, acute renal failure, thromboembolic disease, hypertension, and infections predicted higher mean hospitalization costs.

Conclusions: We present the first systematic description of inpatient health care utilization in children with NS. NS and associated complications resulted in 9,900 hospitalizations, an estimated 48,700 inpatient days, and $259 million in inpatient charges in children. Furthermore the complications of NS including thromboembolism, infection, and hypertension contribute significantly to these costs.

FR-PO183

Blood Pressure Control among CKD Patients in a Public Health System Delphine S. Tuot,[1] Charles E. McCulloch,[2] Chi-yuan Hsu,[1] Tanushree Banerjee,[1] Margaret Handley,[1,2] Dean Schillinger,[1] Neil R. Powe.[1] *[1]Medicine, UCSF, San Francisco, CA; [2]Biostatistics and Epidemiology, UCSF, San Francisco, CA.*

Background: Blood pressure (BP) control is suboptimal among patients with chronic kidney disease (CKD). Little is known about BP control among ethnically diverse CKD patients who receive care in public health settings, which disproportionately care for patients at higher risk for CKD progression.

Methods: We examined uncontrolled BP (systolic BP >140 or diastolic BP >90) using 18,864 clinical BP measurements from 6618 adults (23% White, 34% Black, 18% Hispanic, 21% Asian) with CKD (defined by 2 values of either eGFR <60 ml/min/1.73m^2 or abnormal dipstick albuminuria) who received primary care in 2010-2012 in the Community Health Network (CHN), the integrated delivery system serving San Francisco's uninsured & publically insured residents. Generalized estimating equations adjusting for age, gender, language, insurance, and primary care clinic were used to estimate the prevalence and odds of uncontrolled BP by race/ethnicity overall, and stratified by CKD stage. Results were compared to weighted and similarly adjusted estimates of uncontrolled BP among 2404 NHANES participants with CKD (defined by single values of eGFR <60 or albuminuria > 30mg/g) who had seen a doctor in the prior year.

Results: Adjusted prevalence of uncontrolled BP in the CHN was 25.3%, with differences across race/ethnic groups overall (p<0.001) and within each CKD stage (p<0.004 for each stage). Compared to Whites, overall odds of uncontrolled BP were higher among Blacks (adjusted odds ratio (AOR)=1.11, 95%CI 1.08-1.13), Hispanics (AOR=1.05, 1.02-1.08) and Asians (AOR=1.04, 1.02-1.08). Adjusted prevalence of uncontrolled BP among NHANES participants was 21.2%; compared to Whites, odds of uncontrolled BP were higher among Blacks (AOR=2.00, 1.62-2.46) but not Hispanics (AOR=1.0, 0.67-1.50).

Conclusions: BP control among patients with CKD in a public health setting is nearly 20% higher than national estimates with smaller, though still significant, disparities between Black and White patients. Continued efforts at improving BP control should target integrated public delivery systems.

Funding: NIDDK Support, Other NIH Support - KL2RR024130

FR-PO184

Variation of Pre-ESRD Care and Black-White Difference across Urban-Rural Counties Guofen Yan,[1] Jennie Z. Ma,[1] Alfred K. Cheung,[2] Tom Greene,[2] Mohammed Norman Oliver,[1] Wei Yu.[1] *[1]University of Virginia; [2]University of Utah.*

Background: Access to pre-ESRD care has varied greatly among region, but the variation between urban and rural areas in the U.S. is unknown. Using USRDS, we examined the extent of variation in pre-ESRD care and black-white disparity across urban-rural counties.

Methods: We identified 372,434 black or white patients aged >18 years who initiated first dialysis between 2005 and 2009 across 3081 counties, grouped into large-metropolitan (LM), non-large metropolitan (NLM), suburban (S), and rural (R). Four pre-ESRD care indicators included receipt of nephrologist care at least 12 months prior to ESRD, fistula (AVF) at the first dialysis, receipt of dietitian care, and use of erythropoietin (ESA). Data were analyzed using logistic regression with generalized estimating equation.

Results: LM and NLM counties had the worst and best rates of nephrologist care (24% and 30%), while S and R counties had rates in between (27% and 26%). Among patients who received nephrologist care, those living in metro counties were more likely to receive dietician care (19%) than in S and R (14%). Rates of ESA use were also higher in metro counties (56%) than other areas (50%) while rates of AVF were similar across four areas. Black patients received less care than whites in all areas, especially dietitian care in R (table). The results were largely unchanged after adjusting for various patient factors.

Rates of receiving pre-ESRD care[a]

County category	Neph. Care	AVF	Dietitian	ESA
(#patients/#counties)	black vs. white	black vs. white	black vs. white	black vs. white
LM (193,623/412)	20.9 vs. 26.0**	19.9 vs. 22.3**	20.1 vs. 20.3	53.3 vs. 58.0**
NLM (110,822/676)	29.2 vs. 30.5	20.6 vs. 22.4*	15.8 vs.18.2*	51.5 vs. 56.5**
S (46,409/1,051)	25.6 vs. 27.9*	18.1 vs. 22.7**	10.8 vs. 15.5**	44.5 vs. 51.2**
R (21,580/942)	23.1 vs. 27.0*	21.9 vs. 22.8	7.8 vs. 16.6**	48.8 vs. 51.4

[a] *p < 0.05, **p < 0.001.

Conclusions: Access to nephrologist care appeared to be worst in LM whereas receipt of dietitian care was particularly low in rural counties. Blacks received less care in all areas. Further study to delineate the factors associated with these urban-rural differences will likely improve care for CKD patients.

Funding: NIDDK Support

FR-PO185

Racial/Ethnic Disparities in Mortality among Individuals with Chronic Kidney Disease Rajnish Mehrotra,[2,3] Dulcie Kermah,[1] Keith C. Norris.[1,3] *[1]Research, Charles R. Drew University of Medicine and Science, Lynwood, CA; [2]Research, Harborview Medical Center, University of Washington, Seattle, WA; [3]School of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Black individuals have a significantly higher risk for premature mortality than whites in the general population but have a survival advantage in the ESRD population, particularly among older patients. The association between race/ethnicity and mortality in people with earlier stages of chronic kidney disease (CKD) is less clear.

Methods: We evaluated racial/ethnic differences in mortality among 2185 adult participants (> 20 years) with CKD from the National Health and Nutrition Examination Survey (NHANES 1999-2004), defined as an estimated GFR <60 ml/min per 1.73 m^2 or by the presence of albuminuria (not on dialysis).

Results: Adjusting for age, gender and race in the overall cohort, the hazard ratio (HR) for all-cause mortality in the CKD group compared to No CKD was 2.41 (95% CI - 2.08-2.87; P <0.001). In the cohort with CKD, the HR for all-cause mortality between races after controlling for age, gender, did not differ among those less than 65 years .After further adjustment for cardiovascular risk factors, socioeconomic and CKD stage we found no difference between racial/ethnic groups among those less than 65 years. In the 65-75 year group, blacks had a significantly high risk for mortality with a fully adjusted HR of 1.8 (95% CI - 1.2-2.7; p=0.008) compared to Whites, and in the 75 and older age group, blacks had a trend toward increased mortality risk with an adjusted HR of 1.7 (95% CI - 0.96-2.8; P= 0.06) compared to Whites. Similar findings were noted when we restricted the outcomes to cardiovascular related mortality.

Conclusions: In conclusion, these data demonstrate racial/ethnic disparities in mortality among individuals with CKD. The survival advantages for blacks compared to whites in the ESRD population do not extend fully to the non ESRD CKD population.

Funding: Other NIH Support - NIH-NIMHD Grant U54MD007598 (Formerly U54RR026138); P20MD00182; U54RR022762

FR-PO186

A Mexican Government-Sponsored Chronic Kidney Disease (CKD) Screening Demonstration Project (DP) Based on KEEP (Kidney Early Evaluation Program) Methodology Gregorio T. Obrador,[1] Nadia Olvera,[2] Veronica Gutierrez,[2] Antonio Villa,[1] Patricia D. Lopez Perez,[3] Miguel Angel Mejia,[4] Santiago Lastiri,[5] Juan A. Tamayo-orozco.[5] *[1]Universidad Panamericana School of Medicine; [2]Fundacion Mexicana del Rinon; [3]Secretaria de Salud, Estado de Jalisco; [4]Instituto Nacional de Salud Publica; [5]Sistema de Proteccion Social en Salud.*

Background: KEEP is a CKD screening program aimed at adult individuals with diabetes (DM), hypertension (HTN), and/or family history of DM, HTN, or CKD. KEEP was originally developed by the US-NKF and in 2008 was adapted for use in Mexico by the Mexican Kidney Foundation. The Mexican government planned a CKD screening DP based on KEEP methodology directed to diabetic patients who were already attending primary care clinics in the Jalisco State. The objectives of the study were 1) to adapt the KEEP methodology for rapid and massive CKD screening, and 2) to compare the CKD prevalence between participants in the DP and in KEEP.

Methods: Seven teams comprised of 27 health professionals each were trained. CKD screening was planned to be conducted during 5 working days for 6 consecutive weeks at 6 sites. SCr and urine ACR were measured with the point-of-care testing devices iSTAT and Clinitek, respectively. iSTAT-SCr was aligned to IDMS-SCr with a correction factor obtained in a previous validation study.

Results: A total of 7689 diabetic patients were screened for CKD within a 6-week period. Their mean age was 58±12 years and 72% were women. Forty-five percent of patients had DM and 55% had DM+HTN, with mean disease duration for each of 8 years. The overall CKD prevalence was 44%. Of these, 22% had CKD stage 1, 13% stage 2, 8% stage 3, and 1% stages 4-5. CKD prevalence was 40% for patients with DM and 47% for those with DM+HTN. There were no statistically significant differences in overall CKD prevalence between diabetic participants in the DP and in KEEP (N=1658).

Conclusions: It is possible to successfully conduct rapid and massive CKD screening based on KEEP methodology. CKD prevalence among diabetic patients is high. Most patients have early CKD stages, which allows for implementation of interventions to delay CKD progression.

Funding: Government Support - Non-U.S.

FR-PO187

Assessing Awareness of Risk Factors for Cardiovascular and Chronic Kidney Disease among Undergraduate Students at a Single Institution Caitlin Thys, Courtland Winborne, Hsiao L. Lai. *IM, ECU, Greenville, NC.*

Background: A preliminary study was conducted from Jun to Oct 2010 to assess cardiovascular (CV) risk among undergraduate students. Of the 525 participants screened 30% had two or more risk factors, 12% had three or more risk factors for CV disease. This group shows a high prevalence of CV risk factors and would be a promising group for targeted risk modification. It is essential to expand this population cohort to further confirm the prevalence of CV risk in this young adult population. It has been well established that CV risk factors such as obesity, hypertension, diabetes, and smoking increase risk for chronic kidney disease (CKD). CKD has a high prevalence in adults, therefore early awareness of CKD risk factors may be an effective prevention strategy.

Methods: From Nov to Dec 2011, 209 undergraduate students participated in cardiovascular screening. Prior to the screening, informed consent was collected. Participants completed a 10-question survey that assessed physical activity, perceived health status, smoking and tobacco use, salt, dairy, and alcohol consumption, and sun exposure. Height, weight, blood pressure, lipid, and glucose measurements were obtained.

Results: The mean age was 19 yo with the youngest being 18 and the oldest being 26. Fifty-one percent are female; 79% are white; 11% are black. Thirty percent of participants were reported to have two or more cardiovascular risk factors, 5.4% had 3 or more risk factors. Predominant risk factors included BMI > 25 (11.5%), hypertension (44.2%), low HDL (52.9%), regular smokers (7.2 %), and sedentary lifestyles (34.6%). Only 5.8% of participants felt their health was worse than other people.

Conclusions: Undergraduate students have a high prevalence of multiple risk factors with a low prevalence of established disease. Risk modification is essential at a young age to decrease the potential for developing chronic disease later in life. This demonstrates that risk factors can be detected early for chronic disease prevention and intervention could be promising. The next phase of the study will focus on risk modification for those with multiple risk factors through an educational intervention.

FR-PO188

Readmission after Hospitalization for Heart Failure among Patients with CKD: A Prediction Model Robert M. Perkins, Ion D. Bucaloiu, Evan Norfolk, William DiFilippo, James E. Hartle, H. Lester Kirchner. *Geisinger Clinic.*

Background: 30-day readmission rates after hospitalization for congestive heart failure (CHF) approach 25% in the general population, and patients with CKD are disproportionately represented.

Methods: We aimed to develop a prediction tool for 30-day readmission after hospitalization for CHF among those with CKD. Geisinger Clinic primary care patients with stage 3-5 CKD (without history of renal replacement therapy) hospitalized with a primary discharge diagnosis of CHF at least once during the period July 1, 2004 through February 28, 2010 were eligible for cohort inclusion, and followed through March 31, 2010 for readmission. Multivariate logistic regression was employed to build models from predictors of 30-day readmission, drawn from demographic, clinical, laboratory, and pharmaceutical variables in the electronic health record. These variables were obtained during the year prior to admission and during the index hospitalization itself. Models were assessed for optimization of the AUC of the ROC curve for readmission within 30-days. Variables were manually removed to achieve satisfactory goodness of fit and parsimony while maximizing AUC. Internal model validation was performed using the bootstrap resampling method with 1000 samples to provide a bias-corrected AUC.

Results: 607 patients with CKD were admitted for CHF during the study period and constituted the study population; 116 (19.1%) were readmitted within 30 days. A model incorporating 22 variables across domains of medical history, active outpatient pharmaceuticals, vital signs, laboratory tests, and recent inpatient and outpatient resource utilization yielded an AUC (95% CI) of 0.792 (0.759-0.848). The bias-corrected AUC was 0.743, suggesting good internal validity. At an estimated readmission probability of 20%, the model correctly classifies readmission status for 73% of the population, with a sensitivity of 69% and a specificity of 73%.

Conclusions: Electronic health records allow for the development of reasonably accurate readmission risk tools which incorporate a large number of predictor variables. Robust IT infrastructure may allow for the clinical application of well-validated prediction tools.

FR-PO189

Establishment of a Method to Detect Microalbuminuria by Measuring the Total Urinary Protein-to-Creatinine Ratio in Patients with Risk Factors for Cardiovascular Disease Kyoko Yamamoto,[1,2] Yasuhiro Komatsu,[2] Hiroyuki Yamamoto,[2] Yutaro Nishi,[3] Koichiro Niwa,[3] Chiyohiko Shindoh.[1] *[1]Laboratory Medicine and Clinical Science, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Nephrology, St Luke's International Hospital, Tokyo, Japan; [3]Cardiology, St Luke's International Hospital, Tokyo, Japan.*

Background: The latest KDIGO CKD guidelines recommend that CKD is classified based on cause, GFR category and albuminuria category. The American Heart Association recommends combined screening for low eGFR and microalbuminuria in patients with risk factors for cardiovascular disease (CVD). Although albuminuria is preferred marker than total urinary protein, its measurement may be limited in some countries including Japan, due to its higher cost and reimbursement by health insurance system. The aim of the present study is to establish a method that predicts the presence of microalbuminuria by measuring the total urinary protein-to-creatinine ratio (TPCR) among patients with CVD risks.

Methods: Spot urine samples were obtained from 609 adult patients with CVD risks visiting cardiovascular clinics of St. Luke's International Hospital in Tokyo from February 1 to April 30 2012. The TPCR and urinary albumin-to-creatinine ratio (ACR) of spot urine samples were determined.

Results: There was a strong positive correlation between TPCR and ACR (R^2=0.878, p<0.0001). A receive-operating characteristic curve analysis for the TPCR had a sensitivity of 94.8%, specificity of 84.1%, a positive predictive value of 80.5% and a negative predictive value of 95.9%, respectively, for the detection of albuminuria (both micro- and maroalbuminuria) and a cutoff value of 0.084g/g creatinine.

Conclusions: These results suggest that the detection of the TPCR can be used to screen for the presence of microalbuminuria among patients with CVD risks. This simple and inexpensive method can facilitate wider application, which can lead to earlier intervention and public benefit.

Funding: Private Foundation Support

FR-PO190

The Role of Body Composition Analysis for Predicting the Presence of Metabolic Syndrome and Renal Dysfunction Jung-ho Shin, Youn-su Park, Min-jee Han, Su Hyun Kim, Suk-hee Yu. *Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea.*

Background: Metabolic syndrome (MetS) is a complex of metabolic abnormalities that confers increased risk factors regarding renal injury. We investigated if fat contents derived from a bioimpedance analysis (BIA) were associated with MetS and kidney function in a healthy population.

Methods: We retrospectively reviewed 4,841 adults selected from the Health Promotion Center at Chung-Ang University Hospital. The Chronic Kidney Disease Epidemiology Collaboration creatinine equation (CKD-EPI) was used to obtain the estimated glomerular filtration rate (eGFR). The definition of MetS used followed the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria. The Inbody 720 Body Composition Analyzer was used for the BIA, and the visceral fat area (VFA) and percentage of body fat (PBF) were derived accordingly.

Results: We recruited 2,604 men (53.8%) and 2,237 women (46.2%). We found a significant relationship between MetS and CKD (p<0.001). VFA and PBF were measured to be 95.5±34.8cm^2 and 26.5±6.7% in men, and 96.1±33.8cm^2 and 26.6±6.6% in women, respectively. In men, eGFR in adults without MetS was significantly higher than those with MetS (p=0.025). However, the VFA and PBF measurements were not different. Also, the VFA and PBF were not correlated with the CKD stage. In women, we could confirm that the presence of MetS was significantly related with eGFR and VFA, but not related to PBF (p<0.001, p=0.032 and 0.077). VFA in women increased according to the progression of the CKD stage; 93.3±33.6cm^2 in CKD stage 1, 98.1±33.7cm^2 in CKD stage 2 and 104.5±35.9cm^2 in CKD stage 3-5 (p=0.002). PBF was also correlated with the CKD stage in women (p=0.024). In a linear regression analysis, eGFR was associated not with PBF but with VFA in women (p=0.575 and 0.015).

Conclusions: We could confirm that metabolic syndrome and chronic kidney disease were significantly related to each other using a bioimpedance analysis. A body composition assessment by the bioimpedance analysis can be simply used to predict the presence of metabolic syndrome and renal dysfunction in healthy women.

FR-PO191

The Effect of Thyroid Hormone Replacement Therapy on Renal Function in Chronic Kidney Disease Patients with Subclinical Hypothyroidism Dong Ho Shin,[1] Dae-Suk Han,[1] Mi Jung Lee,[1] Hye-young Kang,[2] Seong Hun Kim,[2] Shin-Wook Kang.[1,2] *[1]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei University, Seoul, Republic of Korea.*

Background: Subclinical hypothyroidism (SH) is not a rare condition in female, the elderly, and patients with chronic kidney disease (CKD). Even though previous studies demonstrated that thyroid hormone replacement therapy (THRT) improved cardiac function and dyslipidemia in patients with subclinical hypothyroidism, it has never been explored whether THRT has a beneficial effect on renal function in CKD patients with SH. This study was undertaken to investigate the impact of THRT on the changes in estimated glomerular filtration rate (eGFR) in CKD patients with SH.

Methods: A total of 124 CKD patients with SH, who were treated with L-thyroxine and had available eGFR for at least 24 months both before and after THRT, were enrolled between January 2005 and December 2011. The slopes of the decline in renal function before and after THRT were calculated by linear regression analysis of serial eGFR for each patient; the slope was expressed as the regression coefficient (mL/min/year/1.73m^2). In addition, a linear mixed model was used to compare the changes in eGFR before and after THRT.

Results: The mean age was 60.6 ± 12.4 years, and 62 (50.0%) patients were male. The mean follow-up durations before and after THRT were 28.6±8.5 and 30.6±6.4 months. THRT significantly reduced TSH levels (6.85 ± 1.43 to1.59 ± 1.29 μIU/mL, p<0.001). In contrast, there were no significant changes in T3 and fT4 concentrations by THRT. Serum cholesterol, triglyceride, albumin, calcium, and phosphate levels were also comparable after THRT. The rates of decline in eGFR before and after THRT were -4.64±0.90 and -1.49±0.72 mL/min/year/1.73 m^2, respectively, and a linear mixed model revealed that there was a significant difference in the rates of eGFR decline before and after THRT [coefficient (-0.18, -0.01), P < 0.01].

Conclusions: Thyroid hormone replacement therapy may have a beneficial effect on renal function in CKD patients with SH.

FR-PO192

Dose Dependent Effect of Weight Loss on Hyperfiltration as a Result of Bariatric Surgery Philip D. Evans,[1] Tim J. James,[2] Garry Tan,[1] Chris W. McIntyre.[1,3] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Department of Clinical Biochemistry, Oxford Radcliffe Hospitals, Oxford, United Kingdom; [3]School of Graduate Entry Medicine and Health, University of Nottingham, Derby, United Kingdom.*

Background: Obesity rates and the associated morbidity burden are increasing Worldwide. Obesity is an established risk factor for chronic kidney disease (CKD) but the underlying mechanisms, and impact of weight loss, are yet to be fully defined.

Methods: 14 patients listed for bariatric surgery were recruited from a single secondary care centre. Each participant underwent clinical assessment, including anthropomorphic measurements, iohexol glomerular filtration rate (iGFR) and urine and serum biochemistry tests within the 4 week period preceding their operation. These assessments were then repeated at 3 months post-surgery.

Results: The mean age (SD) was 48 ± 12yrs, 64% were male and 14% had a diagnosis of diabetes. At baseline the mean weight (SD) was 155.9 ± 23.6kg, mean iGFR(SD) 128.79 ± 28.2mL/min and median urinary albumin:creatinine ratio, ACR, [IQR] 0.65 [0.2-2.58] mg/mmol. At 3 months post-surgery there was a significant decrease in mean weight (135.2 ± 20.0kg; p = <0.001) and iGFR (114.3 ± 24.6mL/min; p = 0.009). There was no significant decrease in ACR. Univariable linear regression analysis revealed a significant correlation between the change in iGFR and change in weight (r^2 = 0.488; p = 0.036).

Conclusions: Glomerular hyperfiltration in obese subjects improves significantly within the first 3 months following bariatric surgery. There appears to be a linear relationship between change in iGFR and change in weight. Weight loss may therefore be a significant factor in reducing future renal risk.

Funding: Clinical Revenue Support

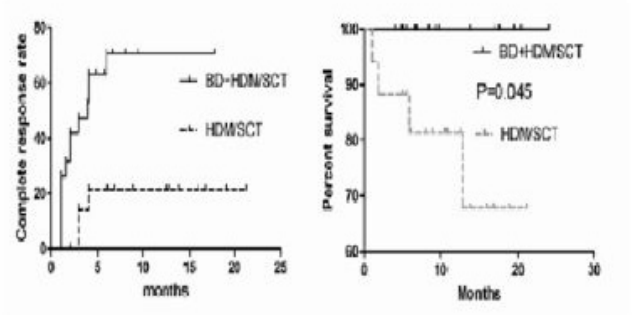
FR-PO193

Induction Chemotherapy with Bortezomib and Dexamethasone Followed by Autologous Stem Cell Transplantation for AL Amyloidosis Xiang-hua Huang, Qingwen Wang, Mingjun Shi, Wencui Chen, Cai-hong Zeng, Zhao-hong Chen, Dehua Gong, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu.*

Background: The use of bortezomib alone and in combination with steroids has shown efficacy in primary systemic amyloidosis (AL amyloidosis); however, its role in combination with HDM/SCT is unknown. In this study, we evaluated bortezomib in combination with dexamethasone (BD) for induction chemotherapy prior to HDM/SCT.

Methods: We conducted a prospective study comparing induction therapy consisting of two BD cycles followed by HDM/SCT (Group 1) with HDM/SCT alone (Group 2) in the treatment of patients with naive AL amyloidosis. The efficacy and toxicity of each treatment was evaluated.

Results: Thirty-three patients were enrolled in this study: sixteen in Group 1 and seventeen in Group 2. The patients presented with comparable disease severity and time from diagnosis to transplantation in the two treatment groups. The overall hematological response rates (ORR) were obtained for fifteen (93.8%) group 1 and nine (52.9%) group 2 patients (HR=3.41, *P*=0.004), and the complete hematological response rates were 68.8% (11/15) for group 1 and 17.6% (3/17) for group 2 (HR=4.95, *P*=0.016) (Figure 1A). Group 1 patients also achieved a better renal response (87.5 vs. 35.3%, HR=3.74, *P*=0.008) and a better heart response (40 vs. 20%) without major adverse events related to BD therapy. After a median follow-up of 12 months (range: 4-24 months), overall survival was significantly higher in Group 1 (*P*=0.045).



Conclusions: Our preliminary data suggest that induction chemotherapy with BD can significantly improve both the hematologic and organ response rates in patients with AL amyloidosis who undergo the HDM/SCT procedure; however, confirming the long-term benefits of this treatment will require further observation.

Funding: Government Support - Non-U.S.

FR-PO194

Effects of Atorvastatin on Renal Function in Patients with Dyslipidemia and Chronic Kidney Disease: Design of the ASsessment of Clinical Usefulness in CKD Patients with Atorvastatin (ASUCA) Trial Masato Kasahara,[1] Kenji Ueshima,[1] Daisuke Koya,[2] Tetsuya Babazono,[3] Tosiya Sato,[6] Miyuki Imamoto,[4] Shinji Yasuno,[1] Akira Fujimoto,[1] Genjiro Kimura,[6] Kazuwa Nakao.[1] *[1]EBM Research Center, Kyoto University, Japan; [2]Diabetes and Endocrinology, Kanazawa Medical University, Japan; [3]Nephrology and Hypertension, Diabetes Center, Tokyo Women's Medical University School of Medicine, Japan; [4]Food and Nutritional Science, Kobe Women's Junior College, Japan; [5]Biostatistics, Kyoto University School of Public Health, Japan; [6]Cardio-Renal Medicine and Hypertension, Nagoya City University.*

Background: The ASUCA trial was designed to consider whether the kidney function of Japanese CKD patients with dyslipidemia improves by atorvastatin.

Methods: We have decided to carry out a prospective multi-center, open-labeled, randomized trial to compare the reno-protective effects between diet therapy alone and atorvastatin plus diet therapy in patients with dyslipidemia and CKD (eGFR < 60 mL/min/1.73m^2).The primary endpoint is the change in eGFR after a 2-year treatment.

Results: A total of 334 patients (213 male and 121 female) were randomly assigned to either diet therapy alone or atorvastatin plus diet therapy and included in an intent-to-treatment population. In the atorvastatin and the control groups, mean ages were 63.2 and 63.1 years-old, mean eGFR were 55.9 and 54.0 mL/min/1.73m^2, and median urinary albumin /creatinine ratios were 24.9 and 29.1 mg/g, respectively.

Conclusions: While renal disorder due to metabolic syndrome increases recently, neither ACE inhibitor nor ARB is necessarily effective. The reno-protective effect of statin is suggested in the meta-analyses of large-scale clinical trials of Europeans and Americans. Since Japanese BMI is well known to be low as compared with BMI of Europeans and Americans, our study focusing on mild metabolic patients will lead to a clarification of a direct and/or pleiotropic effect of statin. While CKD patients don't tend to decrease so readily, it's important to establish a kidney disease remedy besides the RAS system restraint medicine; and for that reason alone, we expect that the outcome of our study will be all the more significant.

Funding: Pharmaceutical Company Support - Pfizer Japan Inc.

FR-PO195

Cardiac Resynchronization Therapy in Chronic Kidney Disease: A Systematic Review and Meta-Analysis Neha Garg, Sankar D. Navaneethan, George Thomas. *Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Congestive heart failure is highly prevalent among chronic kidney disease (CKD) patients. Cardiac resynchronization therapy (CRT) confers mortality benefits to heart failure patients by remodeling of cardiac structure and function. We conducted a meta-analysis to examine the effects of CRT therapy (with or without defibrillator) in CKD population.

Methods: We searched MEDLINE, Web of Science and Scopus (1946 till December 2011) and abstracts from conference proceedings (2005 till 2012) for relevant studies. Studies that compared outcomes of a) CKD patients with CRT to non-CKD patients with CRT and b) CKD patients with CRT to CKD patients without CRT were included. Mortality, eGFR and left ventricular ejection fraction (EF) data reported in individual studies were extracted and pooled when appropriate using random effects model.

Results: Twelve studies (8 observational studies and 4 randomized trials, 8210 patients) were included. In most observational studies the improvement in EF with CRT was found to be similar across CKD and non-CKD populations. However survival outcomes after CRT were inferior in the CKD group as compared to the non-CKD group (3 observational studies, OR 1.89, 95% CI 1.33, 2.69). In contrast, the subgroup-analysis of the RCTs reported no significant mortality difference between the CKD and non-CKD groups after CRT therapy (2 RCTs, HR 0.67, 95% CI 0.50, 0.89). Amongst the CKD population, four studies reported stabilization or improvement in eGFR after CRT compared to those without CRT. One study reported a significantly favorable survival in CKD patients with CRT compared to CKD patients who received other heart failure therapy (HR 2.19%, 95% CI 1.31, 3.65, p= 0.002).

Conclusions: CRT improves EF and eGFR in CKD population with heart failure and whether these improvements translate into a survival benefit remains unclear. Given the increasing number of cardiac device placement in CKD population, further studies examining the effects of CRT in those with CKD on mortality and renal function are warranted.

FR-PO196

Impact of ß-Blocker Therapy on All-Cause Mortality and Initiation of Chronic Dialysis in Patients with Chronic Kidney Disease Not Yet on Dialysis: A Propensity Score Analysis Anna Jeanette Jovanovich,[1] M. Chonchol,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom Greene,[2,4] Jessica B. Kendrick.[1] *[1]University of Colorado; [2]VASLCHCS; [3]VA Boston Healthcare System; [4]University of Utah.*

Background: Patients with advanced chronic kidney disease (CKD) not yet on dialysis have an elevated risk of all-cause mortality and kidney disease progression. Sympathetic over-activity is commonly seen in CKD and appears to be an important contributor to increasing the risk of death and kidney disease progression. Little evidence exists on the efficacy of β-blockers for the prevention of death or progression to dialysis initiation in patients with advanced CKD.

Methods: We studied the effects of β-blockers in 1099 patients with advanced CKD not yet on dialysis who participated in the Homocysteine in Kidney and End Stage Renal Disease (HOST) study. Outcome measures were all-cause mortality and initiation of chronic dialysis. Cox proportional hazards models were used to control for important risk factors for cardiovascular disease and kidney disease progression, including propensity analysis for β-blocker use.

Results: Patients had a mean age 69 ± 11 years, 98% were male, 26% were African American, 96% had a history of hypertension, 55% had diabetes, and 82% had a history of cardiovascular disease. The mean estimated Modified Diet Renal Disease-glomerular filtration rate was 18 ± 6 ml/min/1.73m^2, and 653 (59%) were on ß-blocker therapy. Over a mean follow-up of 3 years, 453 (41%) patients died from any cause and 615 (56%) patients initiated chronic dialysis. Baseline β-blocker use was not associated in univariate or multivariate analyses with lower risk of all-cause mortality (adjusted HR of 0.91; 95% CI, 0.75-1.11; p=0.35) or lower risk of initiation of chronic dialysis (adjusted HR of 0.90; 95% CI, 0.76-1.06; p=0.20).

Conclusions: In patients with advanced CKD and a high burden of cardiovascular disease, β-blocker use was not associated with a lower risk of all-cause mortality or initiation of chronic dialysis.

Funding: NIDDK Support

FR-PO197

Prevention, Detection, and Management of Early Chronic Kidney Disease: A Systematic Review of Clinical Practice Guidelines Pamela Andrea Lopez-Vargas,[1,2] Allison Tong,[1,2] Premala Sureshkumar,[1,2] David W. Johnson,[3] Jonathan C. Craig.[1,2] *[1]Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia; [2]Centre for Kidney Research, the Children's Hospital at Westmead, Sydney, NSW, Australia; [3]Department of Nephrology, University of Queensland at Princess Alexandra Hospital, Brisbane, Queensland, Australia.*

Background: Chronic kidney disease (CKD) is rapidly increasing worldwide. Clinical practice guidelines have been developed to manage and prevent its progression. This study aims to compare the scope, content and consistency of international guidelines on CKD Stages 1-3.

Methods: Guideline databases, electronic databases, and nephrology societies' websites were searched to November 2011. The Appraisal of Guidelines for Research and Evaluation (AGREE) II instrument and textual synthesis was used to appraise and compare recommendations.

Results: Fifteen guidelines and one consensus statement were included. Guideline methodological rigour was variable.Inconsistencies were evident in a number of recommendations. For detection of CKD, all guidelines recommended eGFR measurement and serum creatinine, but some also recommended dipstick urinalysis and 24 hr urine. The recommended PCR and ACR thresholds varied as did the proteinuria (150-300 mg/day, >300 mg/day and >500 mg/day) values. Blood pressure thresholds varied from 125/75 to 140/90 mmHg. ACEi and ARBs were recommended as first line treatment for hypertension, however recommendations were conflicting regarding separate or combined administration. Dietary protein intake recommendations were inconsistent (no restriction or 0.75g/kg/day -1.0g/kg/day). Salt intake of 6g/day was recommended by most. Psychosocial support and education were recommended but specific strategies were not provided.

Conclusions: CKD guidelines are variable with respect to their methodologic quality, stakeholder involvement, coverage, specific recommendations and applicability. To promote effective primary and secondary prevention of CKD, guideline recommendations that are based on the best available evidence and augmented with healthcare context-specific strategies and action plans for implementation are warranted.

Funding: Government Support - Non-U.S.

FR-PO198

Characteristics and Outcomes of CKD Patients with Implantable Cardioverter Defibrillator Sadaf S. Khan,[1] Jesse D. Schold,[2] Anne S. Tang,[2] Victoria Konig,[2] Jennifer Lyons,[3] Stacey Jolly,[1] Joseph V. Nally,[3] Sankar D. Navaneethan.[3] *[1]Medicine, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Nephrology, Cleveland Clinic.*

Background: Chronic kidney disease (CKD) is associated with increased cardiovascular morbidity and sudden cardiac death. Implantable cardioverter defibrillator (ICD) placement has been shown to improve mortality rates in certain populations. We examined the characteristics of CKD patients with ICD and its associations with all-cause mortality.

Methods: 43828 patients with non-dialysis dependent CKD (eGFR ≤ 60 ml/min/1.72m^2) in the Cleveland Clinic CKD registry were included. Logistic regression analyses and cox proportional hazards models were used to examine the characteristics of CKD patients with ICD and the associations between ICD and all-cause mortality after adjusting for relevant confounding variables.

Results: Among the 43828 CKD patients, 1939 (4.4%) had ICD. CKD patients with ICD were more likely to be male (OR 2.35, 95% CI 2.08, 2.65), have a diagnosis of heart failure (OR 11.98, 95% CI 10.65, 13.47), coronary artery disease, coronary revascularization procedures, and ventricular arrhythmia. During a median follow-up of 2.3 years, 375 participants died. Among those with CKD and ICD, each 5 ml/min/1.73 m^2 decrease in eGFR was associated with a 2% increased hazard for death (HR 1.02, 95% CI 1.01, 1.03). Compared to stage 3a CKD patients with ICD, stage 4 CKD patients with ICD had a higher hazard for death (HR 2.00, 95% CI 1.47, 2.72). Other independent predictors of mortality in this population included male sex, heart failure, cerebrovascular disease, and ventricular arrhythmia.

Conclusions: Among CKD patients with ICD, advanced kidney disease was associated with an increased mortality. Whether mortality differs among patients with similar comorbid disease burden but without ICD merits further studies to understand the role of ICD in this high-risk population.

Funding: Pharmaceutical Company Support - Development of the CCF CKD Registry Was Supported by an Unrestricted Educational Fund to the Nephrology and Hypertension Department by Amgen

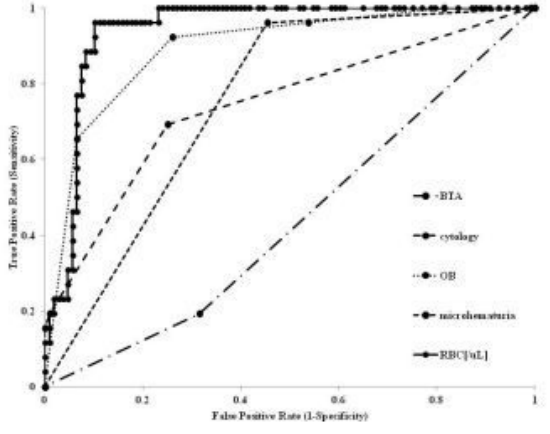
FR-PO199

Screening for Bladder Cancer with the Automated Analyzer UF-1000i® Urine Flow Cytometer Satoru Muto, Akiko Nakajima, Akira Horiuchi, Toshiyuki China, Hisamitsu Ide, Shigeo Horie. *Urology, Teikyo University, Tokyo, Japan.*

Background: We evaluated the efficacy of the automated urine analyzer UF 1000i® to diagnose bladder cancer from the morphology of red blood cells (RBC) in urine.

Methods: The Sysmex UF 1000i® data of 264 samples were analyzed. After excluding the patients with other disease that causes of hematuria, we included 134 patients with any one of positive occult blood in urine (OB), microhematuria, positive urine cytology, or positive bladder tumor antigen (BTA) test in this prospective study. We compared the bladder tumor markers such as the morphology of RBC in urine detected by UF 1000i®, urine cytology, BTA test, OB, microhematuria, between bladder cancer group and non-cancer group. RBC in urine were classified into glomerular type (dysmorphic) and non-glomerular type (isomorphic) by using the Sysmex UF 1000i®.

Results: Urine cytology, OB, microhematuria showed the significant differences between cancer group and non-cancer group (cytology; P=0.00, χ^2=28.88, OB; p=0.00, χ^2=56.35, microhematuria; p=0.0000, χ^2=21.86). As the reference for bladder cancer, the area under the curve for UF1000i® RBC counts is 0.94, which is higher than that for BTA (0.44), urine cytology (0.74), OB (0.89), and microhematuria (0.75).



We could detect the isomorphic type of RBC in urine of all 26 patients with bladder cancer without exception (p=0.00, χ^2=91.25). Multiple logistic regression analysis showed the morphology of RBC in urine were significant predictors to detect bladder cancer (P=0.00). Analytic parameters such as sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of isomorphic RBC in urine to detect bladder cancer were 100.0%, 91.7%, 74.3%, 100.0%, 93.3%, respectively.

Conclusions: Our results demonstrated the efficacy of screening for bladder cancer with the automated analyzer UF1000i® Urine Flow Cytometer.

FR-PO200

Serum IgA/C3 Ratio Predicts Progression of IgA Nephropathy Jun Zhang, Cheng Wang, Hui Peng, Zengchun Ye, Tan-qi Lou. *Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou.*

Background: The serum IgA/C3 ratio has been shown to be a good predictor of histological lesions and prognosis for patients with IgA nephropathy (IgAN) in Japanese. But its validity in Chinese population is unclear. We sought to explore the long-term outcomes of IgAN, its clinical and histopathological predictors in Chinese patients. In particular, the role of serum IgA/C3 ratio in course of IgAN was addressed.

Methods: A total of 217 biopsy-diagnosed IgAN patients were recruited into this prospective cohort with a mean follow-up of 36.10±23.51 months. Sociodemographics, serum IgA/C3 level, other clinical examinations, and Lee's histological grade were measured. Receiver operating curve (ROC) of serum IgA/C3 ratio was employed to determine the optimal cut-off in predicting endpoint event. Serum IgA/C3 ratio together with other clinical, pathology parameters, using univariate and multivariate Cox regression analysis, with halving of baseline eGFR or received renal replacement therapy as the endpoint event. Renal survival and the relationships between clinical, pathologyparameters and renal outcomes were assessed.

Results: The optimal cut-off value of serum IgA/C3 ratio was 3.32 in the prediction of renal end point of IgAN. In multivariate analysis, renal end point of IgAN was significantly predicted by Proteinuria ≥1 g/day (RR = 2.65, 95% CI 1.01-7.68), hypertension (RR = 3.15, 95% CI 1.07-9.29), and higher Lee's histological grade (RR = 4.67, 95% CI 1.43-15.25) and serum IgA/C3 ratio≥3.32 (RR = 4.31, 95% CI 1.33-13.96).

Conclusions: A proportion of patients with IgAN developed end stage renal disease in Chinese. In addition to some traditional risk factors, we also confirmed that serum IgA/C3 ratio is a useful predictor of poor outcomes of IgAN in Chinese patients.

Funding: Government Support - Non-U.S.

FR-PO201

Kidney Damage Markers and Strenuous Labor among Western Nicaraguan Workers in a Region of Epidemic CKD Michael Mcclean,[1] Juan Jose Amador,[1] Oriana Ramirez-rubio,[1] Rebecca L. Laws,[1] James S. Kaufman,[2] Daniel E. Weiner,[3] Marcel Sanchez,[4] Daniel R. Brooks.[1] *[1]Boston University School of Public Health; [2]Boston University School of Medicine; [3]Tufts Medical Center; [4]Ministry of Health, Managua, Nicaragua.*

Background: An epidemic of non-proteinuric CKD is occurring across Central America, with over 20,000 deaths, mainly among younger men. Given the extreme heat in the region, occupational heat stress and recurrent acute kidney injury in the setting of strenuous work is one hypothesized cause of CKD.

Methods: Workers in multiple jobs within the sugarcane industry in Western Nicaragua were tested at the beginning and during the latter part of the 2010-2011 sugar cane harvest to evaluate whether more strenuous jobs, such as cane cutting, were associated with markers of kidney injury, including serum creatinine (to estimate GFR) and urine albumin, NGAL, NAG, and IL-18.

Results: In 284 sugarcane workers, eGFR declined minimally but significantly over the growing season in those engaged in the most strenuous work tasks, while urine ACR remained low in all groups. Significant increases from the start to end of the season were seen in NGAL, NAG, and IL-18 and were most notable among workers engaged in the most intensive tasks. Using factory workers as a reference, the changes from start to end of the season in both NGAL (Table) and IL-18 were significantly greater for cane cutters, while the change in NAG was significantly greater for cane cutters, seed cutters, irrigators and agrichemical appliers.

Conclusions: These results suggest that tubular kidney damage may occur during the working season and may be more common among workers engaged in more strenuous physical labor. If acute kidney damage is on the causal pathway to CKD, heat or other work-related exposures may be contributing to the regional epidemic.

Job	N	Pre-Season NGAL	End-Season NGAL	Mean Change (µg/mg)
Cane Cutters	51	7.6	19	+24
Seed Cutters	26	16	15	-3.2
Irrigators	50	7.2	14	+8.9
Drivers	39	6.8	7.7	0.7
Seeders	28	23	20	-3.2
Chemical Appliers	28	7.0	6.9	+3.0
Factory Workers	59	7.2	7.2	+3.5

Pre- and end-season values are geometric mean urine NGAL levels (µg/mg creatinine)

Funding: Pharmaceutical Company Support - The Office of the Compliance Advisor/Ombudsman, The World Bank Group

FR-PO202

Assessment of Renal Reserve in Young Adults by Iohexol Plasma Clearance
Dana F. Work, George J. Schwartz. *Pediatric Nephrology, University of Rochester, Rochester, NY.*

Background: Renal reserve (RR) is a concept used to explain the kidney's ability to increase its filtration rate in response to a stimulus. There are no published studies on the use of iohexol for the determination of RR. We sought to find if using plasma iohexol disappearance and a protein load (protein shake or hamburger) to stimulate GFR could be used to measure RR in healthy young adults.

Methods: Serum cystatin C (cysC), a chem. 8, and an iohexol blank were obtained and 10 ml of iohexol was given to adults ages 20-30. CysC, a rapidly responsive renal biomarker, was measured by immunonephelometry and iohexol concentrations by HPLC. Blood samples for iohexol analysis were obtained at 120,180,240,330,390 and 450 min after injection; a second cysC was also obtained 390 min after the meat meal. Subjects drank a 60 gm protein shake or ate a hamburger containing 60 gms of protein 245-260 min after iohexol injection. GFR was calculated from the iohexol dose and slope disappearance and also estimated from cys C (Schwartz,et al,KI in press 2012) before and after the protein load; the difference in GFR was considered the RR.

Results: For 6 subjects who received the protein shake, the mean baseline GFR was 105.8 ± 12.6 ml/min per 1.73 m^2 and the mean change in GFR was -9.0 ± 14.5 (p=0.188 by paired t test). For 6 subjects who ate the hamburger, the mean baseline GFR was 106.3 ± 8.2 and the mean change in GFR was 8.4 ± 7.6 (p=0.044 by paired t test). The average baseline calculated GFR by serum cysC was 102.3 ± 8.6 with a mean change in GFR of 9 ± 4 (p=0.008 by paired t test).

Conclusions: There was no significant increase in GFR with the protein shake but there was with the hamburger. A more significant increase in GFR was computed from cysC changes. The increase in iGFR in response to meat was not consistent between subjects despite a consistent decrease in serum cysC. We speculate that the slope iohexol clearance does not respond quickly enough to the protein load and may underestimate RR. It is likely that repetitive measurement of serum cysC may be adequate to characterize RR in young adults. Further studies on RR utilizing renal clearance methods will help to establish if cysC can serve as a surrogate measurement of RR.

Funding: Other NIH Support - T32HD057821

FR-PO203

Metabolic Syndrome and Risk of Progression of Chronic Kidney Disease: A Single Center Cohort Study
Kosaku Nitta. *Department of Medicine, Kidney Center, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: Metabolic syndrome (MetS) is a risk factor for the development of diabetes and cardiovascular disease, and recently was linked to incident chronic kidney disease (CKD). The purpose of this study is to examine whether MetS is associated with CKD progression in Japanese at a single center.

Methods: Outcome variables were a decrease in estimated glomerular filtration rate (eGFR) of 50% or 25 ml/min/1.73 m^2, end-stage renal disease (ESRD), death, or a composite outcome of all 3. There were 213 subjects of the analysis, and **40.4% of them met the criteria for MetS.**

Results: The group of subjects with MetS had higher urinary albumin-to-creatinine (UACR) levels. Survival curves stratified by MetS status showed early separation of the curves and a significantly higher survival rate in the group without MetS (P=0.0086). Comparisons with normoalbuminuria and microalbuminuria showed that macroalbuminuria was equally associated with predicted composite outcome (GFR, ESRD, or death) both in the presence and absence of MetS. Multivariate analyses for all covariates showed that eGFR (HR 8.286, 95% CI 2.360-28.044, P=0.0012) and the UACR (HR 2.338, 95% CI 1.442-3.861, P=0.0005) at baseline were independently associated with the composite outcomes.

Conclusions: MetS was associated with albuminuria in a cohort of Japanese CKD patients, and both MetS and albuminuria were independently associated with CKD progression.

FR-PO204

Management of Metabolic Bone Disease in Patients with Chronic Kidney Disease and Congestive Heart Failure
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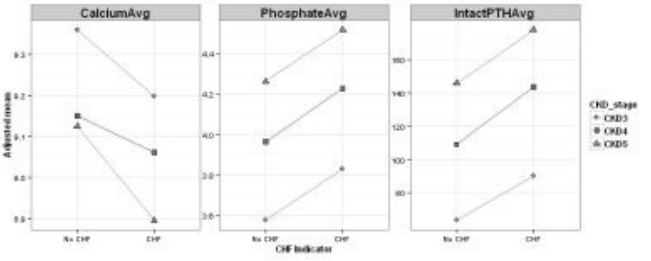
Background: Chronic kidney disease (CKD) is frequent in patients with congestive heart failure (CHF). The prevalence of metabolic bone disease (MBD) increases with advancing CKD. Whether the management of CKD-associated MBD differs between patients with and without CHF is unknown.

Methods: A cohort of patients followed in a Nephrology practice between 2000 and 2010 was evaluated. Adult patients with CKD stage 3 and above were included. Dialysis and Transplant patients were excluded. Average calcium, phosphorus and iPTH were calculated for each patient. Multilinear regressions were used to determine the effect of CKD and CHF on Calcium, Phosphorus and iPTH after controlling for age, race, gender. Because none of the dependent variables were normally distributed, we log-transformed iPTH, and used Box-Cox transformation for Phosphorus and Calcium. The interaction between CHF and baseline CKD stage was explored for each model. The adjusted means were back transformed.

Results: A total of 11,883 patients were included. Mean followup was 4 years; 24.3% had CHF; 75.8% had CKD stage 3, 21.7% CKD stage 4 and 2.5% CKD stage 5 at baseline. Mean age was 68.8 ± 12.5 in patients with CHF versus 65.6 ± 13.7 in those without. As shown in figure 1, the adjusted mean for calcium was lower in patients with CHF at each CKD stage (p<0.0005). The interaction between CKD and CHF was significant (p=0.0053). The adjusted means for phosphorus and iPTH were higher in patients with CHF at each CKD stage (p<0.05) whereas the interactions between CKD and CHF were not significant.



Conclusions: The control of calcium, phophorus and iPTH is poorer in patients with CHF at each CKD stage. Better management of MBD is warranted in these patients and might contribute to lower morbidity and better quality of life.

Funding: Other NIH Support - Partly INBRE Funding

FR-PO205

The Impact of Chronic Psychological Stress in CKD Progression
Vasiliki Tsarpali,[1] Efharis Panagopoulou,[2] Aikaterini Michalaki,[3] Olga Nikitidou,[3] Dorothea Kapoukranidou,[4] Myrto Kostopoulou,[3] Michail Pazarloglou,[1] Konstantinos Leivaditis,[3] Vassilios Liakopoulos,[3] Nicholas V. Dombros.[3] *[1]Nephrology Department, Agios Pavlos-Panagia Hospital; [2]Department of Biological Sciences and Preventive Medicine, Medical School, Aristotle University of Thessaloniki; [3]Renal Unit, 1st Department of Medicine, AHEPA Hospital, Thessaloniki, Greece; [4]Physiology Department, Medical School, Aristotle University of Thessaloniki, Greece.*

Background: The association of psychological stress with adverse health outcomes like hypertension and cardiovascular disease (CVD) has been well described. Social groups with known psychosocial stressors have an augmented rate of decline of renal function. The direct impact of psychological stress on the progression of CKD has not yet been investigated.

Methods: A retrospective study was conducted in 41 patients with CKD stage 2-4 (age 73±6 years), with controlled levels of blood pressure and plasma glucose receiving optimal therapy. Estimated GFR (MDRD formula) was calculated one year before and at the time of the psychological evaluation. Stress and depression were assessed by Hospital Anxiety Depression Scale (HADS). Salivary cortisol levels were measured after morning awakening and late in the afternoon.

Results: An elevated rate of CKD progression was observed for patients with a high HADS score (decline in eGFR 1.1±1.7 vs. increase 5.4±1.7, p=0.01). Measurement of stress in HADS scale was positively correlated with the rate of CKD progression (r=0.54, p<0.001). After multivariate adjustment for age, sex, CVD, depression, diabetes and hypertension, stress continued to be strongly associated with renal function decline. Morning and afternoon salivary cortisol levels were elevated in all participants and the diurnal rhythm of cortisol secretion was blunted, with higher afternoon values.

Conclusions: The pattern of cortisol secretion resembled the one observed in chronic stress, indicating that CKD itself is an independent stressor that contributes to psychological stress. These results suggest a possible relationship between chronic stress and CKD progression.Further investigation is warranted for factors mediating this relationship and its potential clinical consequences.

FR-PO206

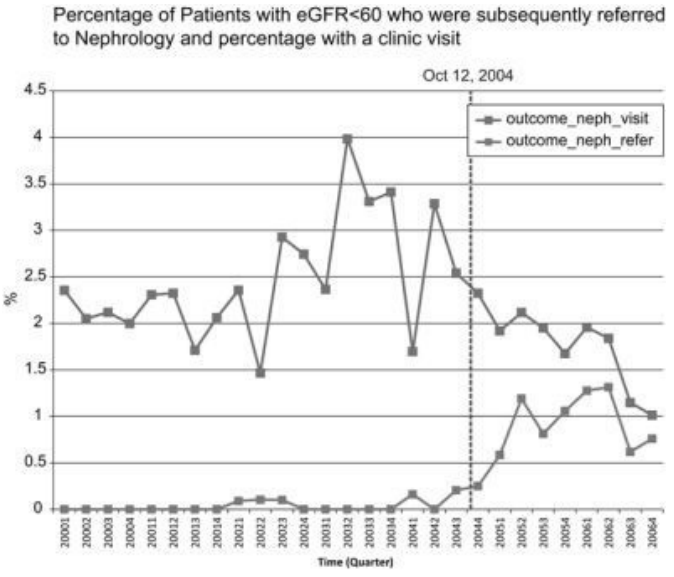
Does Automated Reporting of eGFR Affect Primary Care Practice Patterns?
Micah L. Thorp,[1] David Smith,[1] Nancy A. Perrin,[1] Jessica W. Weiss,[2] Suma Vupputuri,[3] Amanda F. Petrik,[1] Xiuhai Yang.[1] *[1]CHR, Kaiser Permanente Northwest; [2]Oregon Health and Science University; [3]CHR, Kaiser Permanente Georgia.*

Background: Experts have recommended automated calculation and reporting of estimated glomerular filtration (eGFR) rates among all patients who have a serum creatinine measured. Few studies have assessed changes in clinical practice patterns in response to this new initiative. We conducted a time series analysis assessing the rate of nephrology referrals, visits and follow up laboratory testing before and after automated reporting was implemented.

Methods: We conducted a retrospective cohort study of patients who had incident eGFR levels<60 measured before and after implementation of eGFR reporting at an HMO. We compared rates of subsequent evidence of clinical recognition including nephrology referral, repeat serum creatinine and proteinuria testing before and after implementation of eGFR reporting. Logistic models were used to compare change in clinical recognition rates controlling for baseline trends, and determine if the change in rates is related to clinician characteristics.

Results: We found 21,612 adults who had an eGFR<60, and did not have a diagnosis of CKD. The number of referrals increased after the eGFR by 1.3 referrals/month (p=.05). However, the trend in monthly referral slowed after eGFR by .59 per month in comparison to the baseline trend (p = .02). Difference in the change in likelihood of referral after eGFR were found for age (p=.01), amount of FTE (p=.04), and type of practice (p=.01). Slope changes in subsequent orders for other testing (i.e. proteinuria) were not significant.

Conclusions: Following implementation of eGFR reporting, the likelihood of referral to nephrologists increased though the number of nephrology clinic visits did not. Clinicians who were younger, family medicine, and full time were more likely to increase referrals after eGFR.



Funding: Other U.S. Government Support

FR-PO207

Highly Efficient Differentiation of Human Embryonic and Induced Pluripotent Stem Cells into Targeted Mesendoderm, Endoderm, and Mesoderm Cell Fates Albert Q. Lam,[1,2] Benjamin S. Freedman,[1] Joseph V. Bonventre.[1,2] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Harvard Stem Cell Institute, Cambridge, MA.*

Background: Human pluripotent stem cells (hPSCs) have the potential to generate the wide diversity of human cell types. Here we report a highly efficient system to induce mesendoderm, mesoderm, and endoderm differentiation in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) using simple, chemically defined monolayer culture conditions.

Methods: For all differentiation experiments, hESCs or hiPSCs were plated as single cells onto Geltrex-coated wells in mTeSR1 medium and cultured for 2-3 days until they reached 50% confluence. To induce mesendoderm differentiation, cells were treated with serum-free media supplemented with a small molecule GSK-3β inhibitor (GSK3i). Differentiation into endodermal or mesodermal cell types was achieved by first inducing mesendoderm with GSK3i, then adding specific combinations of growth factors or small molecules at precise time intervals.

Results: Treatment of hPSCs with GSK3i rapidly induced BRACHYURY$^+$MIXL1$^+$ mesendoderm differentiation with nearly 100% efficiency in a manner which recapitulated mesendoderm formation during embryonic development. GSK3i-induced mesendodermal cells could be robustly differentiated into endodermal or mesodermal lineages with the precisely-timed addition of Activin A, BMP, and FGF signals. The combination of GSK3i and Activin A robustly generated SOX17+ definitive endoderm, which could subsequently be differentiated into albumin-expressing hepatocytes, hormone-producing pancreatic endocrine cells, and the precursors to lung, thyroid, and intestinal tissue. Optimization of GSK3i exposure times and the addition of BMP4 and FGF2 resulted in mesoderm differentiation. Importantly, we discovered a novel combination of signals which differentiated hPSCs into PAX2$^+$LHX1$^+$ intermediate mesoderm cells, precursors to human kidney tissue, which have not been previously attainable.

Conclusions: Our findings establish a system whereby hPSCs can be flexibly and robustly differentiated into various endodermal or mesodermal lineages, including kidney progenitors, for regenerative medicine.

Funding: NIDDK Support, Private Foundation Support

FR-PO208

Rescue of Chronic Diabetic-Ischemic Nephropathy by Intravenous Renal Cell Transplantation Katherine J. Kelly,[1] Jesus H. Dominguez.[2] *[1]Medicine, IUMC, Indianapolis, IN; [2]Medicine, VAMC, Indianapolis, IN.*

Background: Acute kidney injury (AKI) complicates chronic kidney disease (CKD and it is the most frequent cause of progression to end stage renal disease (ESRD) from diabetic nephropathy (DN). Unfortunately, only a fraction of ESRD patients eventually receive a kidney transplant.

Methods: We developed intravenous renal cell transplantation (IRCT) using primary kidney cells expressing the tubulogenic protein SAA1. We now tested in obese/diabetic female F_1 hybrid Zucker diabetic/SHHF (ZS) rats the hypothesis that unrelenting renal decline in ischemic/diabetic CKD can be abrogated by IRCT.

Results: We studied four groups, diabetic ischemic (subjected to bilateral renal ischemia for 20 min) and transplanted with cells expressing an empty vector (ADI, n = 9), diabetic ischemic transplanted with cells transfected with a SAA expressing vector (BDI, n = 9), sham operated diabetic transplanted with cells transfected with an empty vector (ADS, n = 6), and diabetic sham transplanted with renal cells transfected with a SAA expressing vector (BDS, n = 6). ZS rats were subjected to ischemia or sham surgery at 10 weeks of age, and given two doses of IRCT (1.5 X 10^6 cells per injection) at 15 and 20 weeks of age (after nephropathy was firmly established). The rats were terminated at 34 weeks of age. ADI had severe renal failure with creatinine clearance (CC) of 0.8 ± 0.1ml/min, which improved to 1.5 ±0.2, in BDI (p = 0.009), and unchanged in ADS 1.5 ± 0.2, and BDS 1.3 ± 0.3. These corresponded to a mean serum creatinine of 0.51 ± 0.3 in ADI, 0.37 ± 0.1in BDI (p=0.03), 0.45 ± 0.02 in ADS and 0.35 ± 0.01 in BDS. Kidneys were larger in ADI 3.4 ± 0.2 mg/g body weight, smaller in BDI 2.8 ± 0.06 (p = 0.03), and unchanged in ADS 3.0 ± 0.09, and BDS 2.9 ± 0.08. Fractional interstitial fibrosis (IF) was 25 ± 2 % in ADI, and much lower, 8 ± 1 in BDI (p <0.001). In ADS, IF was 12 ± 1 and reduced to 7 ± 1 in BDS, (p<0.01). IRCT did not affect any of the metabolic syndrome abnormalities.

Conclusions: We conclude IRCT with adult primary cells effectively stops renal decline in ischemic DN, supporting a valuable role for renal regeneration with adult renal cells given by peripheral vein infusions.

Funding: NIDDK Support, Veterans Administration Support

FR-PO209

In Vitro Branching of Ureteric Bud Tubes Generated by Micropatterned Mold Peter V. Hauser,[1,2] Masaki Nishikawa,[1,2] Hiroshi Kimura,[3] Teruo Fujii,[3] Norimoto Yanagawa.[1,2] *[1]David Geffen School of Medicine, UCLA, Los Angeles, CA; [2]Renal Regeneration Laboratory, VAGLAHS at Sepulveda, North Hills, CA; [3]Institute of Industrial Science, University of Tokyo, Tokyo, Japan.*

Background: Kidney development is initiated by the interaction of the ureteric bud (UB) with the metanephric mesenchyme (MM). Using a micropatterned gel we generated a tubular structure from dispersed UB-derived cells. The aim of this study was to induce budding and branching of the tissue engineered UB tubes *in vitro* using growth factors and aggregation with primary MM cells.

Methods: Tubular structures were formed by seeding dispersed mouse UB (CMUB-1) or collecting duct cells (mIMCD) in collagen I (2.4%) into a micropatterned 3% agarose gel (5.10^5cells/mold) by centrifugation (1200rpm,10min), followed by culture in DMEM (10%FCS+P/S) at 37C, 5% CO_2. Tubes thus formed were harvested after 24h. *In vitro* budding was induced by exposing the generated tubes to growth media with 125ng/ml glial cell-derived neurotropic factor (GDNF) or 125ng/ml fibroblast growth factor 7 (FGF7) for 5 to 7 days, while edogenous Activin A was inhibited by by follistatin-soaked beads (500ng/ml) placed in close proximity to the tubes. To induce branching, the tubes were embedded in aggregates of MM cells harvested from mouse embryos at E11.5 or E13.5. Aggregates were formed by centrifugation of 1 tube with 8.10^4 MM cells (800g, 3min), and cultured in DMEM. To inihibit tubular branching TGF-ß (2ng/ml) was added to the growth media.

Results: Tubular structures generated from dispersed UB cells exhibit budding next to the follistatin soaked beads when cultured in GDNF or FGF7 containing growth media. The generated ureteric tubes further showed strong branching when embedded in primary MM aggregates, which could be inhibited by TGF-ß to the media.

Conclusions: We demonstrate that budding and branching can be induced from the tubular structures generated from dispersed UB-derived cells in micropatterned gels *in vitro*. The branching tubes can be used for tissue engineering attempts to generate renal tissue with functional draining system. Current research is underway to optimize the combination of MM and generated tubes.

Funding: Private Foundation Support

FR-PO210

Vasculogenesis by Mouse Kidney Progenitor Cells in Three-Dimensional Co-Culture: Role of PDGF-BB Chakradhar Velagapudi,[1] Myung-ja Lee,[2] Hannah Burns,[2] Brent Wagner,[1] Veronique Barnes,[2] Hanna E. Abboud,[1] Jeffrey L. Barnes.[1,2] *[1]Dep of Medicine/ Nephrology, University of Texas Health Science Center, San Antonio, TX; [2]Probetex, Inc, San Antonio, TX.*

Background: PDGF-BB plays an important role in vasculogenesis (VG) during development. We previously showed that mouse kidney metanephric mesenchymal (MM) and ureteric bud (UB) cells undergo reciprocal induction of simple organogenesis when combined in three-dimensional (3-D) matrigel co-culture in SCID mice. We also showed that MM cells proliferate and migrate in response to PDGF-BB chain in 2-D culture. Here we explored contributory roles of metanephric mesenchymal (MM) and uretric bud (UB) cells in PDGF-BB-induced VG in 3-D co-culture.

Methods: Employing a 3-D culture assay using PDGF-BB impregnated agarose beads as surrogates of UB cells to assess MM migration; Interference of a PDGF-BB chemotactic gradient using a neutralizing antibody or excess growth factor; and by interference of PDGFR-ß using the chemical inhibitor, imatinib mesylate (IM), or PDGFR-ß-null MM cells. The effects were examined by bright-field, immunofluorescence, and electron microscopy.

Results: UB cells synthesized PDGF-BB chain, but not its receptor (PDGFR-ß). Conversely, MM cells synthesized PDGFR-ß, but not PDGF-BB chain, indicating that the two cell types have the machinery for directional induction via this growth factor. VG in 3-D co-culture was inhibited using a neutralizing antibody to PDGF-BB or by including PDGF-BB in the matrix presumably disrupting a chemotactic gradient. PDGF-BB impregnated agarose beads in place of UB cells, resulted in the attraction of MM cells and development of rudimentary vasculogenic structures at the bead surface. Inclusion of IM with MM and UB cells inhibited VG. Similarly, PDGFR-ß-null MM cells grown with UB cells in 3-D co-culture failed to undergo VG.

Conclusions: These studies verify that UB cells provide a paracrine source of PDGF-BB that induces MM cell VL in 3-D co-culture that can be inhibited by blocking the growth factor or its receptor. This system provides a new organotypic model to investigate VG under controlled conditions *ex vivo*.

Funding: Other NIH Support - NIH STTR R41 Grant

FR-PO211

Differentiation of Human Mesenchymal Stem Cells into the Ureteric Bud in Chicken Embryos Akira Fukui, Takashi Yokoo, Nobuo Tsuboi, Yoichi Miyazaki, Yasunori Utsunomiya, Tatsuo Hosoya. *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

Background: We previously demonstrated that human mesenchymal stem cells (hMSCs) transplanted into the metanephric mesenchyme (MM) region of rodent embryos at embryonic day (E) 11.5 resulted in their differentiation into nephrons, derivatives of the MM. However, they were unable to differentiate into collecting duct system, a derivative of the ureteric bud (UB). This suggests that for differentiating hMSCs into collecting duct system, hMSC-derived UB must be generated by their transplantation at earlier stage. So, we focused on using chicken embryos that are easier to be manipulated and cultured than mammals. We already succeeded in having chicken Pax2-expressing hMSCs differentiate into the Wolffian duct (WD) by their transplantation into chicken embryos at E1.5. However, hMSC-derived cells in chimeric WD were few in number and they stopped migrating on their way to elongating WD before reaching the UB. Thus, the aim of this study is to generate hMSC-derived UB in chicken embryos.

Methods: hMSCs were lipofected with chicken Pax2 or Lim1 and they were co-transfected with GFP as a tracer. hMSCs were transplanted *in ovo* into the intermediate mesoderm region adjacent to the most caudal somite at each stage with a mouth pipette. Using a fluorescent dissecting scope, GFP-expressing hMSCs were followed to determine their migration and location at later stage.

Results: We found that chicken Lim1-expressing hMSCs differentiate into the WD more efficiently and that they can differentiate into the WD even by their transplantation at later stage, when the site of transplantation is closer to the UB. As expected, when chicken Lim1-expressing hMSCs were transplanted at E2.5, we found that hMSC-derived cells can migrate into the UB epithelia 3-5 days after transplantation.

Conclusions: We succeeded in having chicken Lim1-expressing hMSCs integrated into the UB epithelia by their transplantation into chicken embryos. We are now examining whether they differentiate properly into the UB and they can differentiate into the ureters and collecting duct after further development.

Funding: Government Support - Non-U.S.

FR-PO212

Proteinuria Impairs Renal Progenitors Differentiation into Podocyte by Sequestering Retinoic Acid Elena Lazzeri,[1] Anna Julie Peired,[1] Maria Lucia Angelotti,[1] Elisa Ronconi,[1] Benedetta Mazzinghi,[1] Alessandro Sisti,[1] Laura Lasagni,[1] Paola Romagnani.[1,2] *[1]Excellence Center DENOTHE, University of Florence, Florence, Italy; [2]Nephrology Unit, Meyer Children's Hospital, Florence, Italy.*

Background: Podocyte loss and proteinuria predict disease progression and renal outcome. Lowering proteinuria retards progression and even induces regression of glomerulosclerosis, which associates with generation of novel podocytes. A population of renal progenitor cells (RPC) localized at Bowman's capsule can differentiate into podocytes and replace lost ones.

Methods: In vitro podocyte differentiation of RPC was performed in VRAD medium and with retinoic acid (RA). RA uptake was performed with [3H]RA and HSA. In vivo experiments were performed in a model of adriamycin nephropathy induced in RA Response Element (RARE)-lacZ transgenic mice treated with injections of RA. RA levels in urine of mice were assessed by mass spectrometry.

Results: Exposure of RPC to HSA impaired their differentiation into podocytes induced by RA. In vitro experiments of RA uptake demonstrated that HSA sequestered RA through specific binding, and blocked its entry within the cell impairing podocyte-specific gene transcription. RA levels were assessed in urine of RARE-lacZ transgenic mice after induction of adriamycin nephropathy. Although RA was not measurable in the urine of healthy mice, it became detectable, following podocyte injury, only in mice with low levels of proteinuria, and disappeared as soon as proteinuria increased. Consistently, activation of RARE-LacZ activity was shown within RPC following the podocyte injury. However, RARE-lacZ signal disappeared as soon as proteinuria increased. Treatment with RA allowed the persistence of RARE-lacZ signal in RPC, which associated with reduction of proteinuria and with an increased generation of novel podocytes, as demonstrated in renal biopsies performed before and after RA treatment.

Conclusions: This study suggests that albumin lost during glomerular disorders sequesters RA in Bowman's space and impairs podocyte regeneration by RPC. These results explain why reducing proteinuria delays progression of chronic kidney disease and can even induces regression of glomerular disorders.

FR-PO213

Identifying Genetic Modulators of Cell Senescence in Human Renal Epithelium via Genome-Wide RNA-Interference Sophie Domhan,[1,2] Maoyun Sun,[1] Lili Ma,[1] Orfeas Liangos,[1] Martin G. Zeier,[2] Lynn Hlatky,[1] Amir Abdollahi.[1,2] *[1]Tufts University School of Medicine; [2]University of Heidelberg Medical School.*

Background: Replicative senescence in the aging kidney or stress-induced premature senescence leads to permanent and irreversible cell growth arrest and plays a central role in renal pathophysiology by decreasing the renal regenerative capacity. We aimed to systematically investigate the molecular mechanisms governing replicative senescence in human Renal Proximal Tubule Epithelial Cells (RPTEC) by an *in-vitro* genome-wide loss of function study.

Methods: A library of 200K shRNA constructs against ~47K transcripts was generated using lentiviral vectors. RPTEC were stably transduced with the shRNA library and cultured *in-vitro* (RPTEC-RNAi). Quantitative enrichment of shRNA constructs was determined using a microarray based DNA-barcode readout technology. Telomerase activity, senescence-associated β-galactosidase and expression profile of p16 and ARF were longitudinally detected.

Results: human RPTEC underwent replicative senescence after 11-13 in-vitro passages. In contrast, the life span of RPTEC-RNAi was markedly expanded to 22 passages (p22). The expression levels of p16 and ARF followed a U-shaped curve with max. at p11/12 and a gradual decrease thereafter. Hence, a substantial fraction of gene knock-downs does not surpass the natural p16/ARF senescence barrier in RPTEC. The complexity of the RPTEC-RNAi was significantly reduced in p20 indicating that cell senescence exerted a strong selection pressure. Telomerase activity was not affected in RPTEC-RNAi and p22 cells failed to form tumors in nude mice. Loss of function of ~150 genes delayed the entry of RPTEC into replicative senescence but not immortalized or transformed them providing an attractive therapeutic strategy to improve the regenerative potential of the kidney.

Conclusions: The evolutionary fitness landscape of a fundamental cellular program –senescence- is investigated on a genome-wide scale. Knockdown of the here identified candidate regulators of RPTEC senescence may elicit beneficial effects in modulating aging-related kidney disease.

Funding: Other U.S. Government Support

FR-PO214

A New Mechanism of Chronic Kidney Disease-Induced Muscle Atrophy: Akirin1 Regulates Muscle Progenitor Cells Yanjun Dong, Yanlan Dong, William E. Mitch, Liping Zhang. *Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Background: CKD stimulates glucocorticoid (GC) production that is necessary but not sufficient to cause muscle wasting. We have found that CKD impairs muscle progenitor cell (satellite cells or muscle stem cell) functions that contribute to muscle wasting (JASN, 2010). It is not known if GC participates in the CKD-induced impairment of satellite cell function.

Methods: Mice were infused with dexamethasone (Dex,2 μg/100 g/day) for 10 days. Tibialis anterior (TA) muscles were injured by cardiotoxin (CTX) to study satellite cell function *in vivo*.

Results: Dex reduced body and muscle weights ($P<0.05$) vs mice treated with PBS. In gastrocnemius muscles of Dex-treated mice, the expression of myogenic genes (MyoD, myogenin and Myf-5) were low, indicating impaired satellite cell function. There also was an increase in muscle myostatin ($p<0.05$) but a decrease in Akirin-1, a promyogenic factor regulating muscle regeneration. Dex suppressed ($p<0.05$) isolated satellite cells in both their proliferation and differentiation as well as the expression of MyoD, myogenin and Akirin1. To identify the role of Akirin1, we infected satellite cells with an Akirin1 adenovirus and found it blocked Dex-induced defects in satellite cell functions. At 3 days after muscle injury, Dex administration suppressed Brdu incorporation and myogenin expression in muscle of mice (i.e., impairment of proliferation and differentiation). At days 5 or 7 after injury, Dex reduced newly formed myofibers (recognized by central nuclei) in injured muscle. Dex also increased myostatin and decreased Akirin1 in injured muscles. These responses are consistent with the report that myostatin down-regulates Akirin1. To verify that Dex impairs satellite cell function by raising myostatin to decrease Akirin1, we inhibited myostatin with an anti-myostatin peptibody in Dex-infused mice and found improved ($p<0.05$) regeneration and increased levels of Akirin1 and other myogenic genes.

Conclusions: CKD-induced GC production upregulates myostatin which downregulates Akirin1. This new, "feed forward" pathway impairs satellite cell function and ultimately, increases the severity of muscle wasting in CKD.

Funding: NIDDK Support, Other U.S. Government Support, Private Foundation Support

FR-PO215

Adipose-Derived Mesenchymal Stem Cells Regulate miRNA Expression in Renovascular Disease and Decrease Apoptosis and Renal Injury Xiang-Yang Zhu,[1] Behzad Ebrahimi,[1] Alfonso Eirin,[1] Kyra L. Jordan,[1] James Krier,[1] Joseph P. Grande,[3] Amir Lerman,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]Cardiovascular Diseases, Mayo Clinic, Rochester, MN; [3]Pathology, Mayo Clinic, Rochester, MN.*

Background: Mesenchymal stem cells (MSC) promote tissue repair, but the mechanisms involved are not fully understood. Micro-RNAs (miR) are post-transcriptional regulators, of which miR-26a inhibits cell apoptosis. We hypothesized that MSC-induced

kidney repair in experimental renal artery stenosis (RAS) is partly mediated by increasing miR-26a expression.

Methods: Single-kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were assessed using multi-detector CT, and tubular function (response to IV furosemide) by blood-oxygen-level-dependent (BOLD) MRI in pigs with a 10-wk RAS, RAS 4 wks after intra-renal infusion of autologous adipose-derived MSC ($2.5x10^5$ cells/kg), and controls. Renal expression of miR-26a was measured in kidney tissue using both plate-based and fluorescent in-situ hybridization, and tubular cell apoptosis by TUNEL staining and expressions of apoptosis inducible factor (AIF) and cleaved caspase-3.

Results: The post-stenotic kidney showed decreased miR-26a expression, mainly in tubular cells as compared to normal, which was restored in MSC-treated pigs.

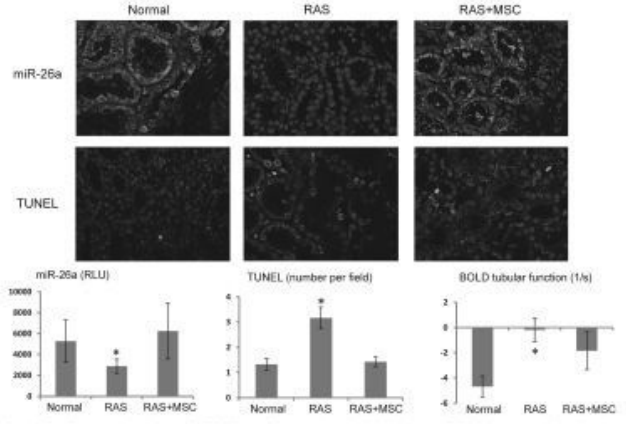


Figure 1. Top: Representative images of miR-26 (green, fluorescent in situ hybridization) and TUNEL (green, nuclei) staining in kidneys of normal, renal artery stenosis (RAS), and RAS treated with mesenchymal stem cells (MSC). Bottom: quantitative results of miR-26a (plate-based hybridization), TUNEL, and kidney tubular function evaluated using MRI BOLD method. *p<0.05 vs. Normal

MSC also attenuated tubular cell apoptosis, downregulated AIF and caspase-3 expressions, and decreased stenotic kidney fibrosis. Furthermore, MSC improved RBF, GFR, and tubular function, which were all reduced in RAS.

Conclusions: Intra renal delivery of autologous MSC restored miR-26a expression, attenuated tubular cell apoptosis, and improved stenotic kidney function. MiR-26a expression might mediate beneficial effects of MSC and represent a novel therapeutic target in renovascular diseases.

Funding: NIDDK Support, Other NIH Support - HL77131, and HL085307

FR-PO216

Effect of Adipose-Derived Stem Cell Transplantation on Acute Kidney Injury Induced by Cisplatin Weiwei Wang, Wei Wang, Yan Jiang, Jinyuan Zhang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: Adipose-derived mesenchymal stem cells (ADSCs) have been reported as a new source of stem cells for use in restoring renal tubular structure and improving renal function in acute kidney injury (AKI). We found that ADSCs could promote the cisplatin-induced HK-2 cells in vitro by ameliorating cytoskeletal damage and decreasing the number of apoptotic cells. In this research, we explore the effect of ADSCs transplantation on acute kidney injury induced by cisplatin in animal model.

Methods: ADSCs were labelled by enhanced-green fluorescent protein(EGFP) and the cells expressing EGFP were screened out by FACS. AKI model was established by intraperitoneal injection of cisplatin in mice. Then ADSCs(labelled by EGFP) were administrated by tail vein($1x10^5$). After 3 days, serum creatinine (Scr), blood urea nitrogen (BUN) levels and renal histopathology were examined. Apoptosis of renal cells were tested by TUNEL. The expression of RANTES, TNF-α and IL-10 were detected by immunohistochemical method and ADSCs distribution observed by fluoroscope in renal tissue. Immunofluorescence was performed to detect the expression of cytokeratin-18(phenotype character) in tubular cells. And anti-cytokeratin18 IgG was combined with Cy3(red fluorescence).

Results: After ADSCs intervention, Scr and BUN levels were decreased, and the damage of renal histopathological(such as vacuolar degeneration and disappear of brush border in tubular cells and inflammatory cells infiltration) were lessened. Apoptosis of renal tubular epithelial cells were reduced significantly compared with the model group. And also the expression of inflammatory cytokines(RANTES and TNF-α) in renal tissue were reduced. The immunofluorescence results showed that a small quantity of green fluorescent protein (from ADSCs) were present in renal tissue, but they cannot be observed transformatation to the renal tubular epithelial cells obviously.

Conclusions: ADSCs transplantation can improve the damage of the kidney structure and function in acute kidney injury. The mechanism may not be related to the transformation into tubular epithelial cells and more likely to the effect by the paracrine role in ADSCs.

Funding: Government Support - Non-U.S.

FR-PO217

Therapeutic Effects of Mesenchymal Stem Cell Derived Microparticles in Progressive Chronic Renal Failure Hyeong Cheon Park, Hoon Young Choi, Sung-kuk Kim, Sun Hee Ahn, Sung-Kyu Ha. *Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea.*

Background: Microparticles (MP) shed from bone marrow mesenchymal stem cells (MSC) conferred protective effects against acute tubular injury via transfer of messenger RNA and microRNA. We recently demonstrated that in vitro expanded kidney derived-MSC (KMSC, Kidney Int 2008; 74:879-889) protected peritubular capillary endothelial cells in acute ischemic reperfusion injury.

Methods: KMSC were cultured in hypoxic chamber in serum deprived MEM with hydrogen peroxide (200 μM) for 24 hours to mimic hypoxic microenvironment. MP were isolated from supernatants by differential ultrafiltration (2,000x g, 10 min, 50,000x g, 2hr, twice) for flowcytometric characteriaztion and MP RNA was extracted using ExoMir kits. Isolated MP were co-cultured with human umbilical vein endothelial cells (HUVEC) to assess their biologic effects on endeothelial cells. Mice subjected to 7 day unilateral ureteral obstruction (UUO) were treated with PKH26 stained MP ($1x10^6$/mice) via tail vein to assess its antifibrotic and peritubular capillary sparing effects. Renal tubulointerstitial damage was examined with Masson's trichrome, F4/80 and alpha-SMA staining and peritubular capillary rarefaction index was determined by CD31 staining.

Results: Flow cytometric analysis of MP demonstrated presence of adhesion molecules shown to be expressed on KMSC membrane such as CD29, CD44, CD73, alpha4- and alpha 6 integrins. Quantitative real time PCR confirmed the presence of 3 splicing variants of VEGF-A (120, 164, 188) and IGF-1 in isolated MP. MP dose dependently improved in vitro HUVEC viability and promoted endothelial tube formation. Indoxyl sulfate (IS: 250ug/mL) or TGF-β (10ng/mL) induced endothelial-mesenchymal transition (EMT) in HUVEC, and pretreatment of HUVEC with MPs ameliorated IS or TGF-β induced EMT. Furthermore, injection of MP significantly improved peritubular capillary rarefaction score and ameliorated tubulointerstital inflammation (F4/80 postive) and fibrosis in UUO mice.

Conclusions: Our results suggest that KMSC-derived MP may confer renoprotective effects via ameliorating tubulointerstitial scarrng.

Funding: Government Support - Non-U.S.

FR-PO218

KSP-Positive Cells Derived from Mouse Embryonic Stem Cells Serve as Progenitors of Renal Tubule Cells Ryuji Morizane, Toshiaki Monkawa, Shizuka Fujii, Hiroshi Itoh. *Internal Medicine, Keio University School of Medicine, Tokyo, Japan.*

Background: We have previously reported that Activin enhances the expression of kidney specific protein (KSP) of mouse embryonic stem (ES) cells and of induced pluripotent stem (iPS) cells, and that KSP-positive cells derived from mouse ES cells form tubule-like structures resembling renal tubule cells. In this study, we characterized KSP-positive cells derived from mouse ES cells in more depth, and examined effects of Wnt4 on them.

Methods: Mouse ES cells were differentiated with Activin and insulin-like growth factor (IGF) for 18 days, and KSP-positive cells were purified by use of our original mouse monoclonal anti-KSP antibody. To characterize KSP-positive cells derived from ES cells, gene expressions of KSP-positive cells were analyzed by microarray in two independent experiments. After cell selection using anti-KSP antibody, KSP-positive cells were co-cultured with NIH3T3-Wnt4 which ubiquitously expressed Wnt4.

Results: Gene ontology analysis revealed that KSP-positive cells significantly expressed genes related to kidney development and urogenital system development more than KSP-negative cells. Interestingly, mesenchymal genes such as Osr1 were up-regulated in KSP-positive cells compared to KSP-negative cells. These results suggest that KSP-positive cells have relatively immature characteristics rather than mature renal tubule cells. KSP-positive cells derived from ES cells could form tubule-like structures in Matrigel ®, however reproducibility was less than 50%. To promote differentiation and tubular formation, KSP-positive cells were co-cultured with NIH3T3-Wnt4. After one day of co-culture with NIH3T3-Wnt4, abundant tubular formation was observed. PCR showed up-regulation of each segment-specific gene of renal tubule cells such as Megalin, Uromodulin, Slc12A3, AQP1, AQP2, AQP3 and Podocalyxin. Immunohistochemistry also revealed that Megalin, AQP1, AQP2 and Podocalyxin were expressed in tubule-like structures.

Conclusions: In conclusion, KSP-positive cells derived from mouse ES cells serve as progenitors of renal tubule cells, and we could induce renal tubule cells from mouse ES cells with cell selection using anti-KSP antibody and Wnt4.

FR-PO219

A Novel Source of Cultured Podocytes for In Vitro Studies Stefano Da Sacco,[1] Ilenia Zanusso,[1] Sargis Sedrakyan,[1] Astgik Petrosyan,[1] Kevin V. Lemley,[1] Janos Peti-Peterdi,[2] Roger E. De Filippo,[1] Laura Perin.[1] *[1]Children's Hospital Los Angeles, Los Angeles, CA; [2]University of Southern California.*

Background: The podocyte is the pivotal cell maintaining normal structure and function of the glomerulus. Podocytes present a very limited ability to replicate and their loss is associated with progression of kidney disease. Various aspects of podocyte biology have been studied using in vitro systems. The current state-of-the-art cultured podocyte is conditionally immortalized, which replicates only some features of this highly specialized cell. Herein we describe a novel cell population of human amniotic fluid kidney progenitor cells (AKPC) that can be cultured and differentiated toward mature and functional podocytes without immortalization.

Methods: AKPC were differentiated into podocytes and compared to human immortalized podocyte cells (hPOD). Differentiation efficiency was assessed by FACS. Morphology was evaluated by light and electronic microscopy. Microarray, qPCR and Western Blotting analysis were performed to confirm expression of mature podocyte and GBM markers. Contractile ability was assessed by Angiotensin II stimulation and calcium intake assay. AKPC were seeded onto mouse kidney extracellular matrix (ECM) and their structure evaluated by SEM.

Results: AKPC can be fully differentiated into mature podocytes, acquiring an arborized morphology, formation of foot processes and rearrangement of F-actin fibers. Expression of podocyte markers including WT1, nephrin, synaptopodin was confirmed. AKPC showed the ability to vigorously contract upon stimulation. AKPC showed superior traits when compared to hPOD, in particular in the higher expression of podocyte genes confirmed by microarray, in the secretion of collagen IV alpha 3-4-5, the major constituent of the GBM and the ability to attach, replicate, differentiate and develop mature foot processes when seeded onto decellularized ECM.

Conclusions: In conclusion, for the first time we showed in a comprehensive and broad manner that extra-renal cells can be differentiated into fully mature and functional podocytes in vitro and established a culture system that will allow a better understanding of podocyte biology than conventional hPOD lines.

FR-PO220

Microvesicles Derived from CD133$^+$ Human Renal Progenitors Protect from Kidney Injury: A Role for Hypoxia Benedetta Bussolati, Aldo Moggio, Giovanni Camussi. *Dept of Internal Medicine, University of Torino, Torino, Italy.*

Background: Increasing evidences show that stem cells are involved in tissue regeneration through a paracrine release of factors, including exosomes/microvesicles (MVs). MVs released from stem cells, beside proteins and bioactive lipids, may mediate a horizontal transfer of genetic information involved in reprogramming of the target cells. We recently characterized a population of CD133$^+$ epithelial progenitors in the renal papilla and we found that hypoxia promotes their stem/progenitor properties (Bussolati et al., Am J Physiol-Renal Physiol 2012). The aim of the present study was to evaluate the effect of MVs from CD133+ progenitors in renal repair and the possible modulation of hypoxia.

Methods: MVs released from cultured renal CD133$^+$ progenitor cells in normoxia and in hypoxic condition (1% O_2) were isolated by ultracentrifugation and characterized using the NanoSight system and cytofluorimetric analysis using latex beads. The effect of MVs on renal repair was tested in a murine model of glycerol-induced acute kidney injury. MicroRNA content of the normoxic and hypoxic CD133$^+$ MVs was evaluated by the Applied Biosystems TaqMan® MicroRNA Assay and confirmed by deep sequencing.

Results: The CD133$^+$ MVs showed a size of approximately 130 nm and expressed exosome surface markers (CD24, CD63). In vitro, only hypoxic but not normoxic CD133$^+$ MVs stimulated the proliferation of starved epithelial tubular cells. Moreover, RNase treatment reverted this proliferative effect. In vivo, a single injection of hypoxic but not normoxic CD133$^+$ MVs improved renal function evaluated as serum creatinine, nitrogen urea and morphological tissue analysis. Immunofluorescent analysis showed a rapid uptake by tubular cells of labelled MVs. In addition, MV showed the presence of several miRNAs involved in cell proliferation, differentiation and inhibition of fibrotic pathways.

Conclusions: In conclusion, we show the ability of CD133$^+$ renal papillary progenitor cells to induce repair in the injured kidney through the delivery of MVs. This mechanism could be locally involved in cell-to-cell communication in pathological conditions of renal injury and could be exploited for therapeutic purposes.

Funding: Government Support - Non-U.S.

FR-PO221

Kidney-Targeted Gene Delivery Using AAV: Model of Cystinosis Celine Rocca,[1] Frank Harrison,[1] Brian Yeagy,[1] Corinne Antignac,[2] Richard Samulski,[3] Stephanie Cherqui.[1] *[1]Molecular and Experimental Medecine, The Scripps Research Institute, La Jolla, CA; [2]INSERM U983, Hôpital Necker-Enfants Malades, Paris, France; [3]UNC Gene Therapy Center, University of North Carolina, Chapel Hill, NC.*

Background: A wide range of monogenic kidney disorders has been identified and so far no gene therapy has been developed. The main goal of our project is to develop an efficient kidney-targeted gene delivery system using recombinant Adeno-Associated Viruses (rAAV). As a proof of concept, we are using the cystinosis mouse model. Cystinosis is an autosomal recessive metabolic disease characterized by intracellular accumulation of cystine. The defective gene is *CTNS* encoding the lysosomal cystine transporter, cystinosin. Affected individuals typically present with proximal tubulopathy before one year of age and progressive loss of glomerular function and finally progress to end-stage renal failure. The *Ctns*$^{-/-}$ mice develop renal dysfunction similar to the patients. Thus, they represent an excellent model for chronic kidney diseases.

Methods: Our goal is to optimize kidney-targeted gene delivery via retrograde renal vein injection by testing several rAAV serotypes that have the potential of transducing a wide range of renal cells. We performed injections of rAAV5, 6, 8 and 9 coding for either the luciferase or the green fluorescent protein (GFP).

Results: Our preliminary results show that rAAV6 is the most suitable serotype to target specifically the kidney. Indeed, using the IVIS live imaging system, we observed that only rAAV6 leads to a strong luciferase expression specifically localized in the kidney. This has been confirmed by confocal GFP observation and GFP-specific RT-qPCR quantification. We are also testing different promoters that could lead to a more specific and efficient renal cells transduction such as the Parathyroid Hormone Receptor (PTHR) kidney specific promoter. Once the optimal conditions will be defined, we propose to test this approach based on renal vein injection of rAAV-CTNS as a minimally invasive procedure for treating the renal dysfunction in cystinosis.

Conclusions: If successful, this strategy may be used in many monogenic hereditary nephropathies.

Funding: NIDDK Support, Private Foundation Support

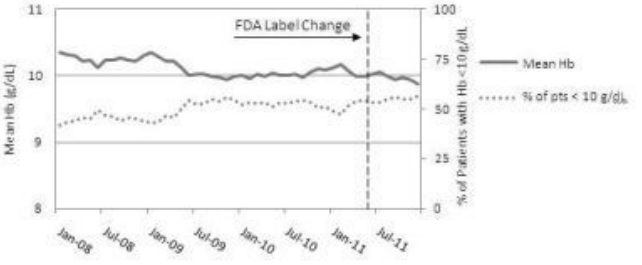
FR-PO222

Hemoglobin Trends in Incident End-Stage Renal Disease Patients from 2008-2011 Joe Weldon,[1] Scott Sibbel,[1] Rhoda Silva Brown,[1] Allen R. Nissenson,[2] Mahesh Krishnan.[1] *[1]DaVita Clinical Research, Minneapolis, MN; [2]DaVita Inc., Denver, CO.*

Background: Major changes to erythropoiesis-stimulating agent (ESA) product labeling in 2011 have impacted anemia management as recently noted in the prevalent end-stage renal disease (ESRD) population (Collins, NKF 2012). However, changes in pre-ESRD care are also affecting incident ESRD hemoglobin (Hb) levels. We sought to describe the mean and distributional changes in Hb among incident ESRD patients (pts).

Methods: In a retrospective analysis, we identified all incident pts at a large dialysis organization from 1/1/08–12/31/11 and used the first Hb measurement on dialysis as a surrogate for pre-ESRD anemia management. Mean values for the first Hb test after starting dialysis (incident pts) were used. Population mean and percent of pts with Hb < 10 g/dL were plotted over time. Hb differences in the ±2 months surrounding the changes in FDA guidelines for ESAs were tested using ANOVA models.

Results: Mean Hb levels at the first test after starting dialysis demonstrated a modest decline over the 4 years analyzed from 10.3 ± 1.4 g/dL (mean ± SD) in Jan 2008 to 9.9 ± 1.3 g/dL in Dec 2011 (Figure). The percent of pts with Hb < 10 g/dL showed a concurrent increase (42.0%–56.6%). Initial Hb levels appeared to plateau after June 2009 and a slight decline was observed after the FDA label change in late June 2011.



Conclusions: Incident pt Hb levels declined from 2008 to 2011, suggesting that ESA treatment prior to initiation of dialysis has become more conservative. Over 50% of patients currently begin dialysis with sub 10 g/dL Hb levels. Pts are starting dialysis with lower Hb, reflecting changes in CKD treatment within the nephrology community. The observed declines in Hb levels corresponded temporally with the implementation of the revised FDA guidelines and label changes for ESAs.

Funding: Pharmaceutical Company Support - DaVita Inc.

FR-PO223

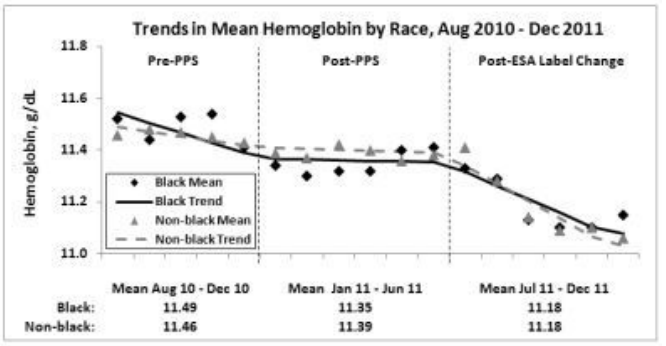
Potential Racial Disparities in Anemia Treatment: Impact of Recent Payment and Regulatory Changes in the US Marc Turenne,[1] Brett Lantz,[1] Douglas S. Fuller,[1] Diane Steffick,[2] Ronald L. Pisoni,[1] Claudia Dahlerus,[1] Jeffrey Pearson,[1] Brian Bieber,[1] Bruce M. Robinson.[1] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]U of Michigan, Ann Arbor, MI.*

Background: Changes to the Medicare ESRD prospective payment system (PPS) in Jan 2011 and dosing guidelines for erythropoiesis-stimulating agents (ESAs) in Jun 2011 removed previous incentives and indications for ESA use in dialysis patients. Given historically higher ESA use among black patients, we studied the potential impact on racial disparities in anemia treatment.

Methods: We defined pre-PPS (Aug-Dec 2010), post-PPS (Jan-Jun 2011), and post-ESA label change (Jul-Dec 2011) periods. Analyses used data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Practice Monitor, a sample of over 100 facilities. Survey-weighted regressions were used to model mean Hgb and prescribed weekly IV epoetin (EPO) dose using linear splines for time with knots at each policy change and an interaction with race (black/non-black).

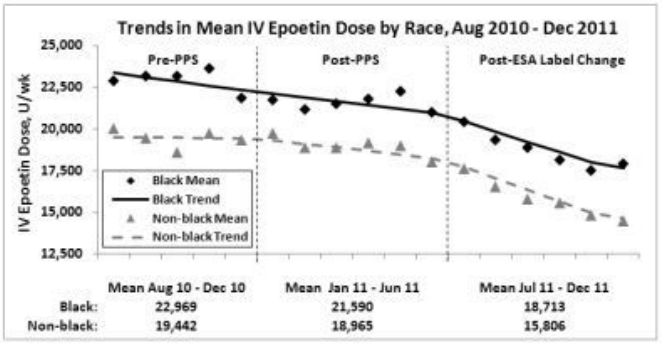
Results: From Aug 2010-Dec 2011, mean Hgb declined from 11.5 to 11.1 g/dL ($p<0.001$), mean EPO dose declined from 20872 to 15578 U/wk ($p<0.001$), and % Hgb<10 g/dL increased (Hgb<9: 3.0 to 5.2%, $p=0.008$; Hgb 9-9.9: 5.7 to 10.5%, $p=0.001$). Declines in mean Hgb and EPO dose and increases in % Hgb<10 also accelerated post label change. Rates of change in Hgb and EPO dose did not differ by race, and mean EPO dose remained higher in blacks (Figure). Overall, % Hgb<10 was more common in blacks, peaking for both races post label change (Hgb<9: 4.7% vs 3.4%, $p=0.03$; Hgb 9-9.9: 9.7% vs 9.2%, $p=0.60$).

Hemoglobin Trend (1 month, continuous)



Mean N (black) = 1097 / month; Mean N (non-black) 2383 / month

Prescribed ESA dose (1 month, continuous)



Mean N (black) = 846 / month; Mean N (non-black) = 1762 / month

Conclusions: Recent US dialysis payment and regulatory changes were associated with similar changes in EPO dosing and Hgb for blacks and non-blacks. Differences by race in prevalence of low Hgb indicate a need to examine possible racial disparities in blood transfusions and other anemia-related clinical outcomes.

Funding: Other NIH Support - National Institute on Minority Health and Health Disparities, National Institutes of Health, Pharmaceutical Company Support - The DOPPS Is Administered by Arbor Research Collaborative for Health and Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), Abbott (Since 2009), Baxter (Since 2011), and Vifor Fresenius Renal Pharma (Since 2011), without Restrictions on Publications

FR-PO224

Change in Erythropoiesis-Stimulating Agent Dosage in For-Profit versus Non-Profit Dialysis Facilities after a Black Box Warning

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Background: Erythropoiesis-stimulating agents (ESAs) are a significant component of Medicare's expenditures on dialysis patients, and higher ESA dosing has been observed in for-profit than non-profit dialysis facilities. It is unknown whether ESA use changed to the same extent in for-profit versus non-profit dialysis facilities and if the discrepancy in ESA use persisted after the FDA's black box warning in March 2007 that advised using the minimum necessary ESA dose.

Methods: From the USRDS, we identified 275,291 Medicare-covered adults who were routinely receiving in-center hemodialysis in February 2007, February 2008, or both time periods. Mixed models, accounting for clustering of data by dialysis chain and facility, were used to study the association between year, dialysis facility profit status and mean weekly ESA (epoetin and darbepoetin) dose.

Results: For-profit facilities had a smaller proportional decrease in ESA dose from 2007 to 2008 than non-profit facilities (7.3% vs. 9.8%, p-value 0.001) after adjustment for demographic and clinical characteristics. In 2008, compared with non-profit facilities, for-profit facilities used higher ESA doses in every hematocrit (Hct) category (<30, 30-<33, 33-<36, 36-<39, ≥39%, p-value for all comparisons <0.0001) and had a greater percentage of patients receiving ESA with a Hct ≥39% (13.7% vs. 9.8%), a range far exceeding the upper hemoglobin limit of 12 g/dL (i.e. Hct 36%) recommended by the black box warning. From 2007 to 2008, ESA dose increased by 41.5% in patients who switched from a non-profit to for-profit facility and decreased by 32.6% in patients who switched from a for-profit to non-profit facility.

Conclusions: For-profit facilities decreased their ESA use to a lesser extent after the black box warning and were still prescribing more ESA than non-profit facilities in 2008, even after adjustment for case-mix and regardless of degree of anemia. The persistence of higher ESA usage in for-profit facilities suggests that for-profit facilities may have failed to maximally respond to the FDA's safety directive to use the minimum necessary ESA dose.

Funding: NIDDK Support

FR-PO225

Patterns of Anemia and Mineral Bone Disease Management in Hemodialysis Patients in China: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS)

Xueqing Yu,[1] Mia Wang,[2] Brian Bieber,[2] Shuchi Anand,[3] Mei Wang,[1] Li Zuo,[1] Ronald L. Pisoni,[2] Xiao Yang,[1] Jia Qi Qian,[1] Nan Chen,[1] Yucheng Yan,[1] Bruce M. Robinson,[2] Sylvia Paz B. Ramirez.[2] *[1]China DOPPS Country Investigator; [2]Arbor Research; [3]Stanford U.*

Background: Limited data exist on management of anemia and mineral bone disorder in Chinese hemodialysis (HD) patients (pts). We previously described differences in hemoglobin (Hb) and PTH levels in Chinese pts as compared to other DOPPS countries. This analysis examines whether practices of medical directors for frequency of measurement and target levels contribute to these differences.

Methods: 1379 pts were randomly sampled from a stratified, random sample of 45 HD units in Beijing, Guangzhou and Shanghai in 2011. Medical directors reported data on practice patterns concurrent with the pt data. Results for China are compared to other DOPPS regions from 2009-11.

Results: Hb levels in China (10.5 g/dL) were comparable to Japan (10.4) but lower than EUR/ANZ and North America (NA) (11.5). Nearly all pts were prescribed an erythropoietic stimulating agent (ESA) in China. Hb targets were higher but Hb measurement frequency was lower in China, compared with other DOPPS regions. After a change in ESA dose, Hb level was checked monthly or less often in 66% of Chinese facilities whereas labs were measured more often in other DOPPS regions (>57% of facilities in other regions measured at least every 2 weeks).

Consistent with targets, mean PTH levels in China (386 pg/mL) were higher than Japan (162), but comparable to EUR/ANZ (315) and NA (374). Vit D was less frequently prescribed in China (54%) and cinacalcet was used by <1% of pts. PTH measurement was less frequent in China-75% measured every 3 mos or more often (> 87% in EUR/ANZ and NA); 25% measured bi-annually or less often (similar to Japan).

Conclusions: Despite Hb targets of 11-12 or higher, mean Hb levels in China were lower, potentially due to less frequent Hb monitoring. Mean PTH levels were more consistent with target levels but monitoring was infrequent compared to NA and EUR/ANZ. The long term impact of these practice patterns will be studied prospectively in the current phase of China DOPPS.

Funding: Pharmaceutical Company Support - The Initial Cross-Sectional DOPPS Study in China Was Supported by Abbott Laboratories without Restrictions on Publications. The International DOPPS Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), and Abbott Laboratories (Since 2009)

FR-PO226

Changing Practice Trends in Erythropoietic Stimulating Agent (ESA) Use Prior to Initiation of Dialysis

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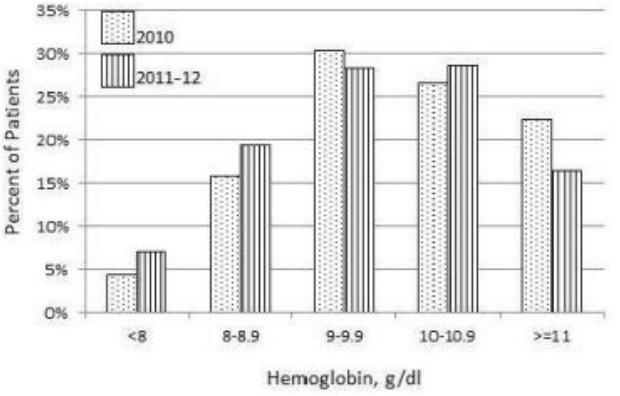
Background: TREAT, CHOIR and recent FDA label changes for ESA have had major impact on their use in the ESRD and CKD population. Introduction of bundling in 2011 has further augmented awareness of ESA exposure risk.

Methods: To assess impact of these developments on physician practice, ESA treatment and hemoglobin (Hb) before initiation of dialysis, we examined data from Form 2728 submissions for new patients initiating ESRD therapy at Northwest Kidney Centers from 1/2010-3/2012, spanning before and after most recent ESA label changes and initiation of the bundle's QIP program (n for 2010, 2011, 2012 = 371,395,103 respectively). Patients returning to dialysis due to transplant failure or other causes were excluded.

Results: Despite comparable percentages of patients seen by nephrologists prior to initiation of dialysis, compared to 2010, a decrease in Hb was seen in 2011 and 2012; average Hb 9.99 g/dL, 9.83 and 9.60 in 2010, 2011 and 2012, respectively (p=0.008, 2010 vs 2012). Similarly, the percent of patients with low Hb (<9.0 gm) increased, and with higher Hb (≥11.0 gm) decreased significantly (p<0.05).



Conclusions: While causality for this finding cannot be identified from these data directly, change in practice pattern is suggested. Possible contributing factors may include later inception of ESA therapy or inception at lower dosages, and/or effects of label changes

and published studies on physician behavior. Such change in delivery of CKD care could predispose new patients to need for transfusion with intercurrent illness, hospitalization, or surgery early in the course of ESRD. Further investigation of the underlying causes of this apparent change in physician practice is needed.

FR-PO227

Treatment of Renal Anemia with Erythropoiesis Stimulating Agents in Predialysis Period Influences Hemoglobin Variability in First Months of Hemodialyis Therapy Michal P. Nowicki, Bartosz Orlowski. *Dept. Nephrology, Hypertension and Kidney Transplantation, Medical University, Lodz, Poland.*

Background: Treatment of CKD-related anemia with ESA may affect variability of hemoglobin (Hb) concentration. Greater hemoglobin variability is associated with increased risk of cardiac events. We tested the hypothesis that introduction of ESA to the treatment of renal anemia before the start of chronic hemodialysis therapy (HD) may allow maintaining more stable Hb levels after start of HD compared to patients who started ESA at beginning of HD.

Methods: Study population comprised 102 patients who were receiving ESA for at least 12 months before HD and continued the therapy after its commencement. The reference group consisted of 70 patients who did not start ESA before HD but were under a care of nephrologists in that period. Analysis of the level of anemia correction and Hb variability was based on monthly blood cell counts performed over a period of first six months of HD. Standard deviation (SD) of Hb values, residual SD and fluctuations across thresholds were calculated.

Results: At start and after six months of HD mean Hb was 8.9 vs. 9.5 ($p<0.05$) and 10.9 vs 10.7 g/dL (ns), respectively in patients untreated and treated with ESA in the pre-HD period. Although mean Hb was maintained within target range of 10 to 12 g/dl from month 3 to 6 of HD in both groups of patients, 17.6% patients in the predialysis ESA treatment group and 48.6% of patients in the reference group showed high amplitude Hb fluctuations ($p<0.01$). Mean residual SD was higher in the group untreated with ESA in predialysis stage than in ESA treated (0.92±0.48 vs 0.73±0.39, respectively, $p<0.05$). Hb fluctuations, SD and residual SD were not different between patients treated with short-acting ESA (epoetin beta, n=43) and long-acting ESA in pre-HD period (darbepoetin alfa and pegylated epoetin beta, n=59) (residual SD 0.69 vs 0,76).

Conclusions: Introduction of anemia therapy with ESA ahead of the start of HD may result in more stable Hb levels after commencement of HD. The choice between short- or long-acting ESA in that period does not have any significant influence on the stability of hemoglobin level after the start of HD.

Funding: Government Support - Non-U.S.

FR-PO228

Predictors of Red Cell Transfusion in Hemodialysis Patients Scott Sibbel,[1] Tracy Jack Mayne,[1] Mahesh Krishnan,[1] David B. Van Wyck.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]DaVita, Denver, CO.*

Background: USRDS has recently reported that transfusions increased in 2011 while mean epoetin dose and hemoglobin (Hb) levels declined in US dialysis patients. We undertook the current study to evaluate whether iron status measures predict transfusion in hemodialysis patients.

Methods: We compared maintenance hemodialysis patients receiving transfusion (n=2,252) to patients without transfusion (n=6,646) [1/2/2001-5/31/2011]. We compared demographic variables, dialysis characteristics, and comorbidities to identify a parsimonious risk factor model. After assessment of the largest unadjusted effects for each covariate, we used multivariate logistic models to simultaneously adjust each risk factor and identify the independent effects of covariate on transfusion risk.

Results: The table shows odds ratios (OR) for transfusion for Hb, ferritin, and IV iron dose, unadjusted and adjusted for race, gender, body mass, age, infection, vascular access, osteomyelitis, adequacy, gastrointestinal bleed, and hematologic disease.

Covariate	Unadjusted OR	Adjusted Cumulative Dose Model*
Hemoglobin (g/dL)		
<10	4.457 (3.485-4.718)	3.844 (3.337-4.429)
10-12	REF	REF
> 12	0.786 (0.680-0.909)	0.769 (0.657-0.900)
Ferritin (ng/mL)		
<500	1.102 (0.956-1.271)	1.044 (0.794-1.148)
500-800	REF	REF
800-1,200	1.062 (0.899-1.255)	0.948 (0.784-1.146)
>1,200	2.027 (1.656-2.481)	1.358 (1.071-1.720)
Iron Sucrose (Cumulative 3-month dose)		
0	1.284 (1.084-1.521)	1.145 (0.944-1.391)
0-150	REF	REF
300-500	0.858 (0.768-1.020)	0.993 (0.849-1.161)
500-1,500	1.329 (1.121-1.576)	1.483 (1.229-1.790)
>1,500	2.263 (1.796-2.851)	1.796 (1.378-2.340)

Conclusions: Lower Hb levels, higher serum ferritin levels, and greater IV iron sucrose doses are each independent risk factors for red cell transfusion. These findings call into question the efficacy of high IV iron administration in anemic patients with hyperferritinemia.

Funding: Pharmaceutical Company Support - Keryx Biopharmaceuticals

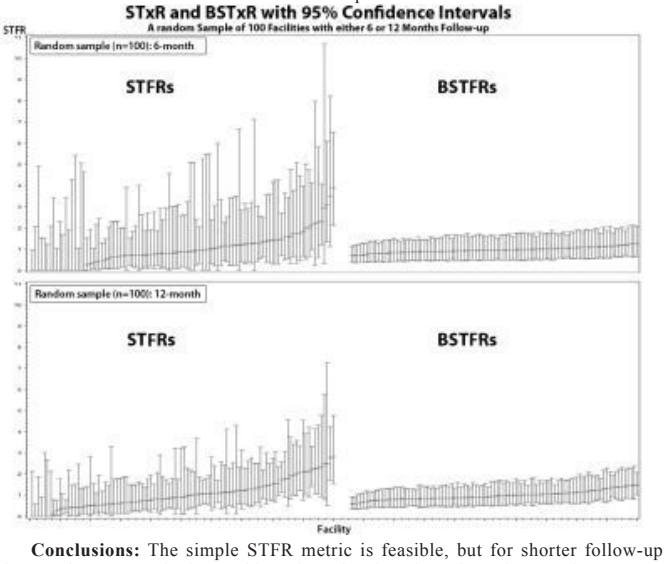
FR-PO229

Development of a Facility-Level Transfusion Quality of Care Metric Jiannong Liu,[1] Suying Li,[1] Keri Monda,[2] Tom Arneson,[1] Brian D. Bradbury,[2] David T. Gilbertson,[1] Allan J. Collins.[1,3] *[1]Chronic Disease Research Group, MMRF, Minneapolis, MN; [2]Amgen, Thousand Oaks, CA; [3]University of Minnesota, Minneapolis, MN.*

Background: Recent evidence has shown hemoglobin levels have declined and transfusions (TF) have increased among dialysis patients (pts) following implementation of the prospective payment system and changes in the FDA label. These changes suggest a quality incentive program metric assessing TF may be warranted.

Methods: Using Medicare data, we constructed a metric to evaluate dialysis facility (DF)-level TF. All US DFs with ≥11 Medicare pts in 2008 were included. Pts were assigned to a DF if they had Medicare as primary payer (MPP) with ≥90 days on dialysis on Jan 1, 2008 and contributed at least 120 days within the DF during the calendar year. Pts were followed from Jan 1 to the earliest of death, transplant, loss of MPP, or 1 year. Inpatient and outpatient TFs were derived from Medicare claims. 6- and 12-month standardized transfusion ratio (STFR, observed divided by expected # of TFs) were estimated for each DF adjusting for pt age, race, sex, ESRD cause, dialysis vintage, and comorbidities. Expected # of TFs was estimated from a Poisson model. The estimate of the STFR may be less stable for small facilities, thus we evaluated estimates from a Bayesian model (BSTFR).

Results: 4,677 DFs and 226,488 pts were included. Mean (SD), range were as follows: 6-month STFR=1.00 (0.83), 0-19.58; 12-month STFR=1.00 (0.60), 0-4.85; 6-month BSTFR=1.00 (0.19), 0.60-4.04; 12-month BSTFR=1.00 (0.23), 0.45-3.52. STFRs/BSTFRs and 95% confidence intervals for a random sample of 100 DFs are shown below.



Conclusions: The simple STFR metric is feasible, but for shorter follow-up times estimates may be less stable and precise than those from the complex BSTFR. Implementation will require balancing timing, precision, and simple vs. more complex methods.

Funding: Pharmaceutical Company Support - Amgen, Inc., Private Foundation Support

FR-PO230

Outpatient (OP) Red Blood Cell (RBC) Transfusion Payments among Medicare End Stage Renal Disease (ESRD) Patients on Dialysis Andrew Lee,[3] Allyson Kats,[2] Matthew Gitlin,[1] Tom Arneson,[2] Jesse Fishman,[1] Stephan C. Dunning,[2] David T. Gilbertson.[2] *[1]Amgen Inc, Thousand Oaks, CA; [2]Chronic Disease Research Group, Minneapolis, MN; [3]Previously with Amgen.*

Background: Estimate per OP RBC transfusion and associated procedure payments among Medicare ESRD patients.

Methods: Retrospective economic analysis based on 2010 Medicare paid costs per OP RBC transfusion, in dialysis patients, using Medicare Parts A & B data. The study included the US ESRD registry dataset, pts on dialysis, with ≥1 OP RBC transfusion, & ≥ 6 months continuous Medicare primary coverage prior to initial transfusion. Only the first eligible transfusion per patient was used for these analyses. The sum of payments per OP RBC transfusion included pre/post screening/monitoring (+/-3d), blood acquisition/administration (0 to +2d) & associated acute complications (0 to +3d), (eg circulatory overload, acute lung injury, hyperkalemia, hemolytic, non-hemolytic/allergic reactions). Subgroup analyses were run by various patient characteristics.

Results: A total of 5,287 patients with transfusion were included in the analysis, with a mean age of 64.1 (SD 15.4), 54% men, & 64.6% white, 31.5% African American, 3.8% other. Mean OP RBC transfusion payments overall were $662.77 & were highest for those <45 years old, white & with dialysis vintage >5 years; $705.48, $699.27, $758.44, respectively. Acute complications (n=251) were primarily responsible for the variability of the highest transfusion costs which ranged up to $49,486.70 per patient.

OP RBC Tranfusion Payments

	Mean ($)	Median ($)	SD ($)
Per Episode per Patient ($) (N=5287)			
Pre-Transfusion screening/Monitoring	25.03	0	48.45
Post Transfusion Screening/Monitoring	71.01	54.74	71.70
Blood Acquisition/ Administration	390.25	403.52	219.45
Acute Complications	176.47	0	1740.25
Total	662.77	509.72	1756.95

Conclusions: Results show overall mean payments for OP RBC transfusions are driven by blood acquisition/administration (58.9%), followed by acute complications (26.6%). However, acute complications explained some of the variation in payments within subgroups. Although infrequent, transfusion complications have potential to increase costs substantially when they occur.

Funding: Pharmaceutical Company Support - Amgen Inc

FR-PO231

Accuracy of Non-Invasive Hemoglobin Measurements during Hemodialysis Yanna Dou,[1,2] Georges Ouellet,[1,2,3] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York, NY; [2]Beth Israel Medical Center, New York, NY; [3]Maisonneuve-Rosemont Hospital, Quebec, Canada.*

Background: Hemoglobin (Hgb) is one of the most frequently ordered laboratory tests in hemodialysis (HD) patients.Hgb measurements have traditionally required a blood draw. Non-invasive blood volume monitoring devicesare increasingly used to measure relative blood volume (RBV) continuously during HD. The Crit-Line ® device(Hema Metrics), in addition to measuring RBV, also provides a Hgb readout. The objective of this study was to compare the accuracy of Hgb readings obtained with this non-invasive device to routine laboratory Hgb measurements.

Methods: Chronic HD patients from a single U.S. dialysis center were enrolled and studied during one HD treatment each.Crit-Line recordings were performed continuously throughout each treatment,and the Hgb values (Hgb_CL) recorded at the start of HD, at 1h and 2h into the treatment and at the end of HD. At the same time points, blood was drawn for standard laboratory Hgb measurements. These served as the reference values (Hgb_REF).For each time point, correlation analyses between the methods and Bland-Altman analyses were performed.

Results: 19 patients (age 60.7±15.0 years, 7 females) were enrolled in this study.The Hgb values (mean±SD) for each method and time pointwere shown in table 1.

Comparison of Hgb values between Hgb_CL and Hgb_REF during hemodialysis

	start	1 hour	2 hour	end
Hgb_REF[g/dL]	11.18±1.02	10.99±1.02	11.18±1.06	11.76±1.38
Hgb_CL[g/dL]	10.57±0.99	11.06±1.03	11.28±1.08	11.68±1.31
P value	<0.05	NS	NS	NS

At the start of dialysis, Hgb_CL underestimated the reference value by 0.62±0.42 g/dL. At all subsequent time points, however, the bias was consistently less than 0.1 g/dL. Bland-Altman analysis showed no signs of heteroscedasticity or systematic trend in bias.

Conclusions: Aside from the relative blood volume data provided by Crit-Line, the monitor may be used to non-invasively obtain Hgb measurements which could be used in addition to the routinelaboratory measurements when required.

Funding: Private Foundation Support

FR-PO232

Red Blood Cell Lifespan Is Associated with Erythropoietin Resistance Index and Its Variability in Hemodialysis Patients Yanna Dou,[1,2] Georges Ouellet,[1,2,3] Stephan Thijssen,[1,2] Nathan W. Levin,[1,2] Peter Kotanko.[1,2] *[1]Renal Research Institute, New York, NY; [2]Beth Israel Medical Center, New York, NY; [3]Maisonneuve-Rosemont Hospital, Canada.*

Background: Increased erythropoietin resistance index (ERI) is correlated with allcause mortality and cardiovascular events. In many patients, inflammation contributes to the hyporesponsiveness to erythropoiesis stimulating agent (ESA) therapy.We aimed to study the association between red blood cell life span (RBCLS) and ERI in hemodialysis (HD) patients.

Methods: Hemoglobin (Hgb), high-sensitivity CRP (hsCRP), serum iron, TSAT and RBCLS were measured at baseline. RBCLS was estimated from alveolar carbon monoxide concentration and Hgb (Strocchi, 1992). Demographics and weekly epoetin alfa (EPO) dose were recorded. ERI was defined as weekly EPO dose per kilogram divided by Hgb. Patients were stratified by RBCLS (<60 and ≥60 days). Following RBCLS measurement, monthly Hgb, EPO dose, and post-HD weight were collected for 6 months. Over these 6 months, mean and standard deviation of ERI (ERImean_6M; ERISD_6M), and slope of ERI (ERIslope_6M) were calculated. Associations between these three variables and RBCLS were assessed by linear correlation analysis.

Results: Forty-five HD patients (31 males, age 58±15 years, dialysis vintage 46±46 months, 21 diabetics) were enrolled. ERImean_6M and ERISD_6M were significantly lower in patients with RBCLS≥60 days, and there was no significant difference in other parameters.

Baseline characteristics of two RBCLS groups

	RBCLS<60 (N=16)	RBCLS≥60 (N=29)
Hgb (g/dL)	11.1±1.4	11.6 ±1.2
hsCRP (mg/L)	8.2 (5.3, 18.6)	4.9 (1.3, 9.7)
Iron (mcg/dL)	71.4±35.2	76.0±26.6
TSAT (%)	31.8±14.9	32.3±9.6
RBCLS(days)	46.4±5.9	82.2±15.5
ERImean_6M(U •dL)/ (week•kg•g) *	20.3 (8.7, 28.1)	8.6 (3.4, 16.1)
ERISD_6M*	8.9 (2.7, 13.5)	2.5 (1.6, 7.5)

Data are presented as mean ± SD or median (quartiles), * means p<0.05

An inverse correlation was found between RBCLS and ERImean_6M (r=-0.38, P<0.05), and RBCLS and ERISD_6M (r=-0.35, P<0.05).

Conclusions: RBCLS is inversely related to mean and SD of ERI over at least 6 months. Considering RBCLS is important when interpreting responsiveness to EPO.

Funding: Private Foundation Support

FR-PO233

Personalized Anemia Management Improves Hemoglobin Variability in Hemodialysis Patients Adam E. Gaweda,[1] George R. Aronoff,[1] Alfred A. Jacobs,[1] Michael E. Brier.[1,2] *[1]Medicine / Nephrology, University of Louisville, Louisville, KY; [2]Robley Rex VA Medical Center, Louisville, KY.*

Background: Erythropoiesis Stimulating Agents (ESA) are dosed per protocols based on the combination of product label and national guidelines. Recent changes to the label recommend individualization of ESA dose to reduce the need for RBC transfusions. We developed a Clinical Decision Support System for personalized ESA dosing, called "Smart Anemia Manager" (SAM), and performed a double blind randomized controlled trial to test the hypothesis that it improves hemoglobin (Hb) maintenance within 10-12 g/dL over a standard protocol.

Methods: We enrolled 62 subjects receiving hemodialysis at the University of Louisville (KDP) as of April 1, 2011 and used equal allocation to Control (KDP protocol) and Treatment (SAM) arms. Subjects and physicians were blinded to group assignment. Dose recommendations for both groups were generated by computer and validated by physicians, who could override the recommended dose if necessary. Subjects were followed for 12 months. ESA (Epoetin alfa) dose adjustment was performed monthly. The primary endpoint was the percentage of Hb observations within 10-12 g/dL over the follow-up period, calculated using monthly Hb measurements as per standard practice, using last value of the month.

Results: Average Hb achieved was 10.6±1.5 g/dL (Control) vs. 11.0±1.2 g/dL (Treatment). Table 1 presents the percentages of Hb below, within, and above the target range. Absolute difference in primary endpoint between the groups is 11.8%. Hb fell below 10 g/dl in fewer than half as many patients in the Treatment group compared to Controls. Median ESA dose for both groups was 2,000 IU/wk. There were 4 dose overrides in the Control group vs. none in the Treatment group.

Percentage Hb below, within, above target (C-control, T-treatment)

	C	T
% Hb < 10	25.7	11.7*
% 10 < Hb < 12	60.8	72.6*
% Hb > 12	13.5	15.6

* difference significant at p < 0.05

Conclusions: Personalized ESA dosing with SAM resulted in more Hb's within the target range, fewer below 10 g/dl, and not more above 12 g/dl despite a 0.4 g/dL difference in mean Hb. SAM represents a significant step toward satisfying the new ESA label.

Funding: NIDDK Support

FR-PO234

Use of the Mayo Clinic Anemia Management System (MCAMS) Decreases Hemoglobin (Hgb) Variation in Chronic Hemodialysis (HD) Patients Receiving Darbepoetin James T. McCarthy,[1] Robert C. Albright,[1] Craig L. Hocum,[1] James Lee Rogers,[3] Edward J. Gallaher,[3] John J. Dillon,[1] LaTonya J. Hickson,[1] Amy W. Williams,[1] David Dingli.[2] *[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Division of Hematology, Mayo Clinic, Rochester, MN; [3]Advance Management Group, LLC, Rochester, MN.*

Background: MCAMS is a proprietary software program that uses Biomedical System Dynamics to simulate erythropoiesis in HD pts receiving an Erythropoietic Stimulating Agent (ESA). It predicts Hgb values in an individual HD pt in response to an ESA. Five physiologic parameters that determine erythropoiesis in the presence of an ESA are included in the model: the average daily rate of production of erythroid blast-forming units (BFU-E); the average survival of erythroid colony forming units (CFU-E), the average daily rate of reticulocyte maturation & survival, the individual response of the erythropoietin receptor (EPOR) to the ESA; & the lifespan of red blood cells (RBC).

Methods: MCAMS was implemented in the HD facilities of the Mayo Clinic Dialysis System in 2009 in place of a protocol-based system that adjusted ESA dose based on the last Hgb value. To determine if MCAMS led to less variation in Hgb values, we analyzed data for all 61 stable HD pts in 2008-2010 meeting these criteria: iron replete; no hospitalization of more than1 day in the time frame of analysis; a minimum of 3 continuous months of Hgb values & darbepoetin doses using our pre-MCAMS protocol; a minimum of 3 continuous months immediately after initiation of MCAMS; administration of at least 90% of darbepoetin doses recommended by MCAMS. The target Hgb value was defined as the midpoint of the desired Hgb range. The mean values for the descriptive statistics pre- and Post MCAMS were compared.

Results:

Table 1

	Pre-MCAMS	Post-MCAMS	P value
Hemoglobin (g/dL)	11.82 ± 0.75	11.36 ± 0.30	< 0.0002
Coefficient of Variation	0.072 ± 0.036	0.053 ± 0.024	< 0.0006
Standard Deviation	0.85 ± 0.42	0.60 ± 0.26	< 0.0001
[Actual - Target]			
Hemoglobin (g/dL)	0.98 ± 0.46	0.67 ± 0.26	< 0.0001

Mean ± SD; Student's t-test with 2 tails and unequal variances

Conclusions: Use of MCAMS was associated with less variation in Hgb values in stable HD pts.

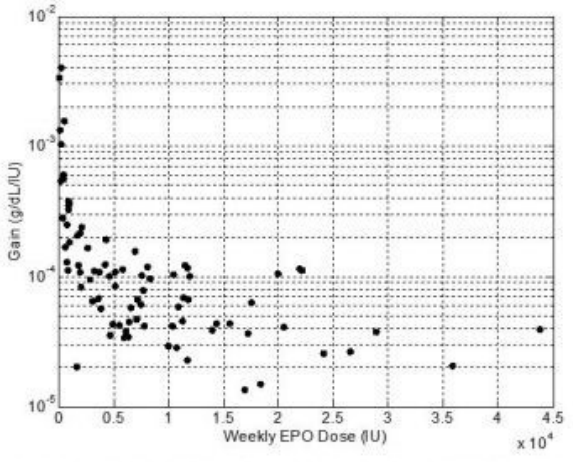
FR-PO235

A Measure of Patient Responsiveness for Effective Anemia Management Protocols Michael J. Germain,[2] Christopher V. Hollot,[1] Joseph Horowitz,[1] Rajiv P. Shrestha,[1] Yossi Chait.[1] *[1]University of Massachusetts; [2]Western New England Renal & Transplant Associates, PC.*

Background: Identifying end-stage renal disease (ESRD) patients' hemoglobin (Hgb) responses to erythropoiesis stimulating agents (ESAs) is central to successful individualization of anemia management. We describe a patient's response using "gain", an important concept in feedback control theory. The main benefit of feedback controllers, of which anemia management protocols (AMPs) are examples, is in dealing with uncertainty. Recent clinical experience indicates that inter-patient gain uncertainty may be too large for a single, fixed AMP to work satisfactorily for all individuals.

Methods: The patient's gain is defined as the ratio of the long-term Hgb response based on a fixed ESA dose to that dose. The gains for 44 ESRD patients were estimated from retrospective Hgb data collected over some 200 weeks. We designed patient-specific AMPs using model-based feedback control and are currently applying them in a small pilot study.

Results: The 44 patients' gains ranged from 1.35×10^{-5} to 4.07×10^{-3} g/dL/IU (a factor of 300) with mean 2.56×10^{-4} and standard deviation 6.05×10^{-4} (see figure). For patients receiving up to 10000 IU/week, the associated factor of gain variation is almost 200. We will report prospective results from the pilot study.



The gains (on log scale) of the 44 ESRD patients are plotted against the weekly EPO doses estimated to produce long-term Hgb level of 11.25 g/dL.

Conclusions: The factor of gain variation 300 is too large to expect a single, fixed AMP to cope with. Moreover, patients receiving up to 10000 IU/week are typically classified as hyper-responsive, which conveys the notion that they are all equally responsive. But the situation is more complicated than that; in fact, the associated factor of gain variation is almost 200. This prompts us to advocate that patients' Hgb responsiveness be classified by their gains rather than by administered ESA doses, and that individualized AMPs be designed in accordance with that classification.

FR-PO236

Persistent Increases in ESA Utilization Following Hospitalization of End-Stage Renal Disease Patients T. Christopher Bond,[1] Steven Wang,[1] Jaime Rubin,[1] Alex Yang.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Affymax Inc, Palo Alto, CA.*

Background: Hemodialysis (HD) patients are frequently hospitalized. The combination of the interruption of normal dialysis and 3x/weekly ESA treatments and the underlying reasons for hospitalization often lead to lowered hemoglobin (Hb) levels and increased utilization of ESAs in the post-hospitalization period.

Methods: In a retrospective database analysis, epoetin alfa utilization before and after hospitalization in 138,762 adult (≥18 yrs old) HD patients receiving in-center dialysis from 01/01/09-12/31/10 was assessed. A total of 181,595 hospitalizations preceded by >30 hospital-free days were included in the analysis. Mean per-session epoetin alfa dose for the time periods 15 and 30 days pre/post-hospitalization, as well as mean monthly epoetin alfa doses for the 2 months before and 6 months after hospitalization were calculated.

Results: Analysis of epoetin alfa use during the pre/post-hospitalization period showed increases in dose which persisted over time. After minimal change in the first immediate post-hospitalization dose (versus last pre-hospitalization dose), a 15-day pre/post analysis showed 53.3% of hospitalizations associated with a rise in mean per-session epoetin alfa dose (13.8% with no change, 32.6% with a drop). In a 30-day pre/post analysis, 60.8% of hospitalizations were followed by an increase in mean per-session epoetin alfa dose (4.9% with no change, 34.3% with a drop). Mean per-session dose increases (for all hospitalizations) were 843 U and 1,322 U (SD; 4,320 and 5,161) for the 15 day and 30 day analyses, respectively. Mean total monthly dose rose starting in the month before hospitalization and continued to increase during the 2 months after hospitalization. Recovery to the mean monthly dose from 2 months before hospitalization did not occur until 4 months after hospitalization.

Conclusions: This analysis suggests that hospitalizations frequently lead to increases in ESA dose, which persist for several months post-hospitalization. Strategies for better management of anemia during hospitalization and in the post-hospitalization period should be assessed.

Funding: Pharmaceutical Company Support - Affymax Inc and Takeda Pharmaceutical Company

FR-PO237

Missed Dialysis Sessions Result in Increased Erythropoiesis-Stimulating Agent (ESA) Use T. Christopher Bond,[1] Steven Wang,[1] Jaime Rubin,[1] Alex Yang.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Affymax Inc, Palo Alto, CA.*

Background: Patients who miss dialysis sessions may experience adverse clinical outcomes (Saran et al, 2003). In addition to missing dialysis itself, patients fail to receive medications (such as ESAs) dosed at each session. We characterized missed sessions and ESA utilization to better understand underlying patterns.

Methods: In a retrospective analysis, we assessed 903,179 patient-months of data from 105,519 adult (≥18 yrs old), hemodialysis patients receiving in-center dialysis. Missed sessions for all reasons (including hospitalization) were included. Consecutive missed sessions were considered part of a missed session "episode". Differences in per-session epoetin alfa utilization were calculated for the periods 14, 30 and 31-60 days before and after missed sessions, stratified by length of episode. In a second analysis, total monthly epoetin alfa dose was tracked for all calendar months by number of missed sessions/month.

Results: Per-session ESA use increased following a missed session episode: longer missed session episodes resulted in greater increases in per-session ESA use on return to the clinic. Increases ranged from 134–1,512 U/session, 1,835–4,093 U/session, and 63–2,778 U/session for the 14, 30 and 31-60 day time periods before and after missed session episodes, respectively, with larger increases tied to longer episodes. Total monthly dose was increased in months where patients missed ≤6 sessions, compared to those in which all sessions were attended.

Missed Sessions (per patient-month)	N (patient-months)	Change in Total Monthly ESA Dose (U)
0	613,049	-
1	105,093	+ 8,707
2	53,739	+ 14,130
3	32,358	+ 15,125
4	24,156	+ 9,122
5	16,191	+ 5,651
6	10,279	+ 1,803
7	7,218	- 7,797
8	5,796	- 17,281

Conclusions: The results suggest missed dialysis sessions result in increased epoetin alfa use on a per-session basis in the post-miss period, but also when including missed sessions in calculations of total monthly use. This finding is consistent with the pharmacology and biology of short-acting ESA. Patients missing ≤6 sessions/month use more total ESA than patients attending every session. This may have economic implications for dialysis centers.

Funding: Pharmaceutical Company Support - Affymax Inc and Takeda Pharmaceutical Company

FR-PO238

Increasing Erythropoiesis Stimulating Agent Dosage in Hospitalized Hemodialysis Patients Increases Risk of Overshooting Target Hemoglobin and Does Not Reduce Transfusion Risk Ben C. Wong,[1] Pietro Ravani,[1] Braden J. Manns,[1] Xin Zhang,[1] Rick Chin,[1] Brenda Hemmelgarn,[1] Marcello Tonelli,[2] Matthew J. Oliver,[3] Robert R. Quinn.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada; [3]University of Toronto, Toronto, ON, Canada.*

Background: Doses of erythropoiesis stimulating agents (ESA) are often increased in hospitalized hemodialysis (HD) patients in an attempt to restore hemoglobin levels to target. The impact of these changes is not known. We conducted a retrospective cohort study to determine if changes in ESA dose at hospital admission influenced the risk of normalization of hemoglobin, blood transfusions, cardiovascular outcomes, or death.

Methods: Linked administrative, lab, and blood transfusion data were used to identify HD patients hospitalized in the Calgary Health Region between 2004 and 2008. Change in ESA dose was determined by comparing the average weekly dose over the preceding 6 weeks to the dose administered during the first 14 days following admission (expressed as an equivalent darbepoietin alfa dose in micrograms per week). Cox proportional hazards models, adjusted for baseline characteristics, were used to study the impact of change in ESA dose on the risks of normalization of hemoglobin (>130g/L), blood transfusion, cardiovascular outcomes, and death.

Results: A total of 700 hospitalizations in 448 patients (mean hemoglobin 113 g/L) were identified. There was a significant and graded increase in the risk of normalization

of hemoglobin as the ESA dose was increased by more than 40 mcg/week above baseline. However, higher ESA doses were not associated with a reduced need for blood transfusion. Change in ESA dose was not associated with the risk of cardiovascular endpoints or death.

Conclusions: Increasing ESA dose at hospitalization in HD patients is associated with a higher risk of hemoglobin normalization, but does not reduce the need for transfusion. A randomized trial testing different ESA dosing strategies in hospitalized HD patients is needed.

FR-PO239

Impact of Missed Treatments on Epoetin-Alpha to Maintain In-Center Patients in a Target Hemoglobin Range Kirk Finchem. *Clinical Operations, Renal Advantage, Inc, Franklin, TN.*

Background: Clinicians seek to manage ESRD-patient anemia (Hb levels) within a target range (9 to 11 g/dL, for the present study). Individual patients may be hypo-, normal-, or hyper-responsive to epoetin-alpha; the differences may be chronic (due to systemic biological factors), or acute (due to acute conditions).

Methods: Patients (n=5,245) had been on dialysis for more than 90 days, and were treated in-center at one or more of 155 dialysis centers operating in 22 states within the United States, during each month October 2011 thru January 2012.

Table 1: Patient Demographics and Characteristics

	Hyper-responsive	Normal-responsive	Hypo-responsive	Total
Patients	1,314	2,619	1,312	5,245
Men	694	1,285	682	2,661
Women	620	1,334	630	2,584
Age	65.3	63.4	60.2	63.1
Weight (kg)	77.9	79.8	80.5	78.5
Epoetin-alpha Dose (Units)	10,633	31,050	91,734	41,115
Treatments (count)	12.6	12.6	12.4	12.5
Hemoglobin (g/dL)	10.6	10.3	10.0	10.3
Fistula Patients	886	1,635	751	3,272
Graft Patients	289	645	337	1,271
CVC Patients	139	339	224	702

Monthly values for hemoglobin (Hb in g/dL), Albumin (AL in g/dL), treatments, and weight (kg) were averaged. Patient age (as of January 1, 2012), sex, and vascular access type was included. Hyper- and Hypo-Responsive levels were chosen to assign the smallest and largest 20 percent of period-average (monthly) epoetin-alpha doses to each group, respectively; the balance of patients were assigned to the Normal-responsive group.

Results: A linear regression model was developed to estimate the benefit (or penalty) of predictor variables to the dose of epoetin-alpha required to maintain the patient within the target hemoglobin range. The model included hemoglobin, albumin, monthly treatments, weight, access type, responsiveness, and sex. The final model achieved an R^2 of 0.7104, with all predictor variables showing significance at the 95% level.

Conclusions: Higher hemoglobin and albumin values were associated with lower epoetin-alpha doses, as were AV fistulas. The mean penalty (i.e., increased epoetin-alpha dose) for missing a single monthly treatment was 278.2 units per treatment (95%CI: 242.0, 314.3), similar to the penalty of CVCs compared to AV fistulas of 258.7 (95%CI: 114.6, 402.8).

FR-PO240

Reticulocyte-Based Estimation of Red Blood Cell Lifespan Wojciech Krzyzanski,[1] Adam E. Gaweda,[2] Michael E. Brier.[2,3] *[1]University at Buffalo, Buffalo, NY; [2]University of Louisville, Louisville, KY; [3]Robley Rex VA Medical Center, Louisville, KY.*

Background: Decreased red blood cell (RBC) production and increased RBC turnover are the main factors contributing to the anemia of ESRD. To facilitate optimal anemia management, the contribution of both processes should be known.

Methods: We measured the RBC lifespan in 5 hemodialysis dependent end-stage renal disease patients. RBC lifespan is determined by measuring the RBC count using standard techniques and determining the RBC production rate from the reticulocyte residual RNA content. Given these two measures one can simply calculate the RBC production rate. The RNA degradation half-life is determined for each subject on day one over 12 hours using thiazole orange and flow cytometry. Reticulocyte age distribution is then measured weekly for 2 months yielding the RBC production rate time course. The RBC lifespan is estimated by fitting the integrated RBC production rate over a time interval to the RBC count. All calculations were performed using MATLAB.

Results: Results are shown in the following table for RBC Lifespan, RNA half-life, average weekly EPO dose, and mean Hb achieved during 2 month follow up.

Subject	Lifespan (days)	tRNA1/2 (days)	EPO Dose (week)	Hb achieved
1	72	0.71	0	13.67
2	61	0.83	20,000	10.38
3	108	0.74	12,000	10.73
4	74	0.73	4,000	8.75
5	67	0.55	4,000	8.68

We performed an analysis of variance on the data with hemoglobin achieved as the dependent variable. Both EPO (p=0.03) and Lifespan (p=0.05) were statistically significant and positively correlated to hemoglobin.

Conclusions: Despite the small study size, we were able to demonstrate a relationship between EPO dose and RBC lifespan and hemoglobin achieved using a new technique. Subjects with a higher EPO dose and longer RBC lifespan had higher achieved hemoglobin. One subject was EPO naive and despite abnormal RBC lifespan had adequate hemoglobin. We conclude that the achieved hemoglobin is a result of a complex interaction of many variables including EPO dose and RBC lifespan. Determining RBC lifespan using this simple technique can help us better understand the anemia of chronic renal disease and potentially improve patient care and warrants further work.

Funding: NIDDK Support, Veterans Administration Support

FR-PO241

Associations among Epoetin Therapy, Inflammation, Nutritional Status and Mortality in Patients on Hemodialysis Hirokazu Honda,[1] Naoki Kimata,[2] Kenji Wakai,[3] Tadao Akizawa.[1] *[1]Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan; [2]Kidney Center, Division of Blood Purification, Tokyo Women's Medical University, Tokyo, Japan; [3]Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Inflammation is a key factor for increased mortality and associated with abnormal body composition in patients under dialysis. Association among these factors in those patients is quite complex. Inflammation and nutritional status possibly lead to erythropoiesis-stimulating agents (ESA) hyporesponsiveness by close interaction. Thus, the aim of the study was to assess impact of inflammation and nutritional status on mortality considering ESA therapy in hemodialysis (HD) patients.

Methods: We assessed associations among doses of epoetin (EPO), inflammation and nutritional status and estimated the impact of EPO dose on mortality in a cohort of prevalent HD patients (n = 37,003) using data from the Japanese Dialysis Registry (2005–2006). Patients were categorized according to tertiles of ESA responsiveness index (ERI, EPO dose (IU/week)/body weight (kg)/hemoglobin (g/dL). Nutritional index (body mass index (BMI)) and C-reactive protein (CRP) levels were measured and one-year all-cause and cardiovascular mortality were estimated.

Results: CRP levels were lower in the lower ERI tertile than in the other groups. Bimodal peaks indicated associations between CRP and BMI in each group. Hazard ratio (HR) curves of CRP according to BMI in the upper ERI tertile particularly with diabetes mellitus (DM) were U-shaped, whereas the increased HRs were associated with low BMI in the other groups. Survival was worse in the upper ERI tertile, than in the other groups. However, mortality was better in the lower ERI tertile group without DM, than in the EPO-free group. These associations were confirmed by multivariate Cox hazard models in a propensity score-matched population.

Conclusions: The findings indicate that the risk association between CRP and risk of death is changed by nutritional status in patients who require a higher dose of EPO especially those with DM, and a low dose of EPO was effective in patients without DM.

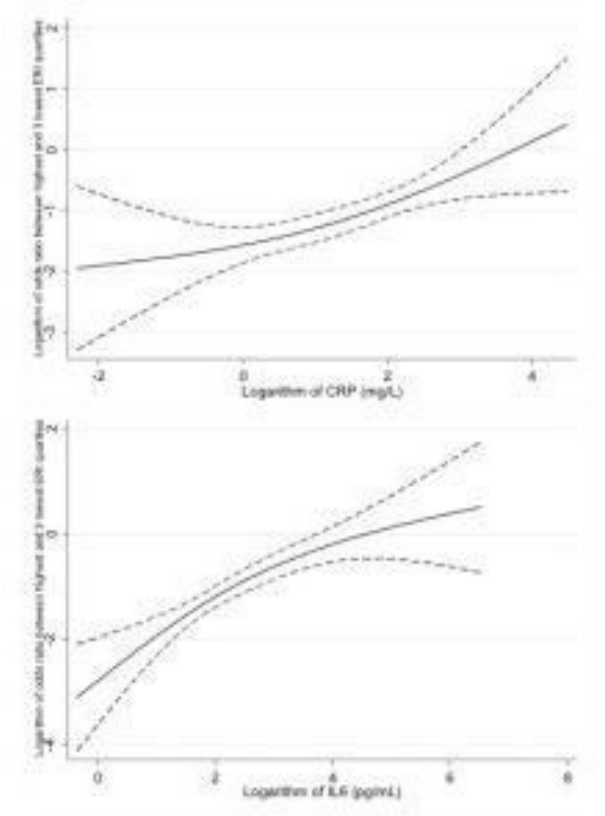
FR-PO242

Association of Malnutrition-Inflammation Complex and Responsiveness to Erythropoiesis Stimulating Agents in Long-Term Hemodialysis Patients Manoch Rattanasompattikul,[1] Miklos Zsolt Molnar,[1,5] Joshua Zaritsky,[2] Parta Hatamizadeh,[1] Jennie Jing,[1] Keith C. Norris,[3] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,2] *[1]Harold Simmons Center, LABioMed at Harbor-UCLA, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Charles R. Drew University; [4]Nephrology, University of Tennessee, Memphis, TN; [5]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary.*

Background: Protein-energy wasting, inflammation and refractory anemia are common in long-term hemodialysis patients. A decreased responsiveness to erythropoiesis stimulating agents (ESA) is often the cause of the refractory anemia. We hypothesized that malnutrition-inflammation complex is an independent predictor of decreased responsiveness to ESAs in hemodialysis patients.

Methods: In a 6-year prospective cohort study of 754 hemodialysis patients, we examined for association between inflammatory and nutritional markers including the malnutrition-inflammation score (MIS) and responsiveness to ESA (ERI).

Results: Mean age of patients was 54±15 years, 53% were diabetic and 49% Hispanic. Both C-reactive protein (Log CRP) (β=0.19) and interleukin-6 (Log IL-6) (β=0.32) were strong and independent predictors of ERI using multivariate linear regression. Each 1SD higher MIS, higher CRP and lower albumin were associated with 86%, 44% and 97% higher likelihood of highest vs. 3 lowest ERI quartile in fully adjusted models (odds ratio [95% CI] of 1.86 [1.31-2.85], 1.44 [1.08-1.92], and 1.97 [1.41-2.78]), respectively. Cubic splines illustrated continuous and incremental association of Log CRP and Log IL-6 with ERI.



Conclusions: Malnutrition-inflammation complex is a significant and independent predictor of decreased responsiveness to ESAs in hemodialysis patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK078012

FR-PO243

Protein Carbamylation, Erythropoietin Resistance, and Mortality in End Stage Kidney Disease Sahir Kalim,[1] Hector Tamez,[1] Elizabeth D. Ankers,[1] Joseph James Deferio,[1] Anders H. Berg,[2] S. Ananth Karumanchi,[3] Ravi I. Thadhani.[1] *[1]Department of Medicine, Division of Nephrology, Massachusetts General Hospital, Boston, MA; [2]Department of Pathology, Division of Clinical Chemistry, Beth Israel Deaconess Medical Center, Boston, MA; [3]Department of Medicine, Division of Nephrology and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Resistance to recombinant human erythropoietin (EPO) leads to an increased risk of adverse outcomes but the biological mechanism is not fully understood. Carbamylation is a non-enzymatic reaction which occurs through uremic and inflammatory pathways and can alter the charge, structure, and function of proteins such as erythropoietin.

Methods: In this study, we characterize the relationship between protein carbamylation and EPO resistance index (ERI) in 158 hemodialysis patients using carbamylated albumin levels measured at day 90 of dialysis initiation.

Results: The highest quartile of carbamylated albumin was positively associated with ERI compared to the lowest quartile (P= 0.03; P for trend = 0.002) with similar findings in a multivariate model (highest vs. lowest quartile P= 0.01, P for trend = 0.02). In logistic regression models, ERI was predictive of one year all cause mortality (univariate OR 1.43, [95% confidence interval [CI] 1.02–2.02], P= 0.04; multivariate OR 1.62, [95% CI 1.05–2.50], P=0.03). However, the association between ERI and mortality was mitigated when carbamylation was added to the multivariate analysis (highest vs. lowest quartile carbamylation OR 5.47, [95% CI 1.48–20.21], P=0.01; compared to ERI OR 1.19, [95% CI 0.74–1.93], P=0.47).

Conclusions: Carbamylation burden was associated with ERI in incident dialysis patients and was a better predictor of mortality than ERI.

Funding: NIDDK Support

FR-PO244

Erythropoiesis-Stimulating Agent (ESA) Responsiveness in a European Haemodialysis Cohort: Case-Crossover Study Iain C. Macdougall,[1] Iain A. Gillespie,[2] Marc Froissart,[3] Sharon Richards,[2] Vincent Jones,[2] Kai-Uwe Eckardt.[4] *[1]King's College Hospital, London, United Kingdom; [2]Amgen Ltd, Uxbridge, United Kingdom; [3]Amgen Europe GmbH, Zug, Switzerland; [4]University of Erlangen, Nuremberg, Germany.*

Background: ESA hyporesponsiveness (hR) is associated with increased morbidity and mortality in chronic kidney disease patients. Previous studies, focussing on baseline predictors of hR, may be biased by confounding by indication and may be unable to examine transient exposures. Accordingly, a case-crossover study was performed to examine factors associated with the transition to a hyporesponsive state in a European haemodialysis (HD) cohort. Case-crossover studies are useful for studying the effect of transient exposures on outcomes.

Methods: Patients' follow up time was split into 90 day periods of weight-adjusted weekly ESA exposure. Haemoglobin (Hb) response was evaluated for the last 30 days of one period and the first 30 days of the next. Each period was defined as responsive, hyporesponsive, under-treated or missing based on a median ESA dose (0.404 µg/kg/wk) and a 10g/dL Hb threshold. Where a patient's first hyporesponsive period was preceded by a responsive period, these periods were coded as 'case' and 'control' periods respectively and hospitalisation, HD and laboratory data accompanying the periods were compared using conditional logistic regression.

Results: Patients on ESAs (n=6645) contributed 38,427 ninety-day analysis periods of ESA exposure. Most ESA exposure periods (75.7%) were periods of Hb response. Almost five percent (4.7%) resulted in hR periods. 672 patients experienced hR periods with preceding response periods. Periods of hR were more likely to follow periods of hospitalization or changes in vascular access, and were marked by a preceding increase in ferritin and CRP. The findings were largely insensitive to alternative ESA dose and Hb thresholds.

Conclusions: Transition to ESA hR may be preceded by events requiring hospitalization, vascular access issues and signs of biological inflammation. These events should alert clinicians to the potential occurrence of an ESA hR-related risk.

Funding: Pharmaceutical Company Support - Amgen Europe GmbH

FR-PO245

Influence of Hemoglobin Levels, Hypo-Responsiveness or Hemoglobin Variability on the Survival in Incident Hemodialysis Patients Receiving Erythropoiesis Stimulating Agent Tadao Akizawa.[1] *[1]Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; [2]JET Study Group.*

Background: It has been reported that hypo-responsiveness or large variability in hemoglobin (Hb) levels under erythropoiesis stimulating agent (ESA) therapy are associated with a poor prognosis. In this study, we evaluated the maintained Hb levels and mortality in incident hemodialysis (HD) patients receiving ESA therapy and compared them between patients with hypo-responsiveness (Gr. A) or large Hb variability (Gr. B) and the remaining patients (Gr. C).

Methods: Patients who had newly-initiated HD and received epoetin beta (EPO) therapy in Japan were stratified by their Hb levels at 6 months after the start of EPO therapy to evaluate their mortality during 3 years. Gr. A and Gr. B were defined as those who had a Hb level <10.0 g/dL and also were receiving EPO at a dose ≥9000 IU/week and who had more than one of up/down-excursion of over 1.0 g/dL across 10.0 to 11.0 g/dL of Hb during 6 months, respectively. Analysis was performed using a Cox regression analysis and hazard ratios (HRs) were calculated.

Results: The analysis included 6631 patients. The mean Hb levels were 8.2 g/dL and 10.2 g/dL at baseline and 6 months of EPO therapy, respectively. The patients were stratified by Hb level at 6 months after the start of EPO therapy as follows: 11.4% in Gr. 1(<9.0 g/dL), 28.5% in Gr. 2(≥9.0 and <10.0 g/dL), 35.9% in Gr. 3(≥10.0 and <11.0 g/dL), 19.3% in Gr. 4(≥11.0 and <12.0 g/dL) and 4.9% in Gr. 5(≥12.0 g/dL). The adjusted HR to Gr. 3 was 2.08, 1.17, 0.91 and 1.32 for Gr. 1, 2, 4 and 5, respectively.

Of the 6631 patients, 4.4%, 14.9% and 80.7% were identified as Gr. A, B and C, respectively. Analysis of Gr. C showed the adjusted HR to Gr. 3 was 2.18, 1.23, 0.83 and 1.38 for Gr. 1, 2, 4 and 5, respectively. Gr. A and Gr. B had an adjusted HR of 1.71 and 1.23, respectively, compared with Gr. 3 in Gr. C.

Conclusions: Based on the prognosis during 3 years of EPO therapy, it is associated good survival to maintain the Hb level at 10.0 to 12.0 g/dL in incident HD patients. This study also suggests the presence of ESA hypo-responsiveness or large Hb variability is associated with an increased mortality risk.

Funding: Pharmaceutical Company Support - Chugai Pharmaceutical Co., Ltd

FR-PO246

Association of Neutrophil-to-Lymphocyte Ratio with Inflammation-Malnutrition Syndrome and EPO Resistance in Dialysis Jerome Pineault, Michel Vallee, Vincent Pichette, Martine Leblanc, Georges Ouellet, Jean-Philippe Lafrance, Robert Zoël Bell. *Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Neutrophil-to-lymphocyte ratio (NLR) has been widely studied in different medical and surgical specialties as a pronostic marker and is yet to be used or validated in nephrology.

Methods: (Single-center retrospective study). We included patients ≥18 y.o. who were on dialysis for ≥3 months and excluded those on chemotherapy, not on EPO or in whom weight was unavailable. We evaluated the relation between NLR and biomarkers of inflammation-malnutrition syndrome: CRP and albumin. We also evaluated the association between EPO resistance and NLR, as it increases in inflammatory states. We made direct correlation analyses between NLR, CRP and albumin. We measured mean values of NLR, CRP and albumin when patients were acutely ill (on antibiotics or hospitalized) and compared them to mean values of the same patients when they are not acutely ill. Among the patients who died during the observation period, we observed the mean NLR monthly during the 6 months preceding death. For the EPO resistance, we calculated EPO resistance index (ERI) monthly for each patient and we calculated a mean NLR corresponding to this value.

Results: A total of 529 patients were analyzed. We found a positive, linear and moderate correlation between NLR and CRP and the exact opposite (as expected) between NLR and albumin that are all statistically significant (SS). Values of NLR with CRP (N=123, r=0.48, B=0.018, P < 0.001) and NLR with albumin (N=123, r=-0.53, B=-0.454, P < 0.001) corresponded well with our reference value that was CRP with albumin (N=123, r=-0.45, B=-0.021, P < 0.001). When comparing values of acutely ill patients vs. not ill, NLR was SS higher among ill patients (5.21 vs. 4.56, P < 0.001). All results mentioned above are still SS among HD patients only and PD patients only. NLR was also rising constantly in the months preceding death. When we divided ERI in quartiles, we also observed that as the ERI rises, NLR is higher.

Conclusions: NLR seems to be a valuable, inexpensive and reproducible marker of the inflammatory state in dialysis. A cut-off of clinically significant inflammation is still needed as well as a normal value of NLR.

FR-PO247

Association between Decrease Rate of Hematocrit due to Renal Anemia and Resistance to Erythropoietin Stimulating Agents Therapy in Hemodialysis Patients Tadashi Kuji,[1,2] Tetsuya Fujikawa,[2] Midori Kakimoto-Shino,[2] Yoshiyuki Toya,[2] Satoshi Umemura.[2] *[1]Yokodai Central Clinic; [2]Cardiorenal Medicine, Yokohama City University, Yokohama, Kanagawa, Japan.*

Background: In treatment of renal anemia of hemodialysis (HD) patients, there is fluctuation of hematocrit (Hct) where Hct basically decreases due to renal anemia by discontinuation of erythropoietin stimulating agents (ESA) and rises by ESA dosing; however, it is unknown how resistance to ESA therapy is associated with the decrease and increase rates of Hct. To help elucidate it, we examined the association of ESA resistance index (ERI) with changes of Hct per week with a simple protocol using fixed-dose ESA in HD patients.

Methods: A total of 120 HD patients, 68.5 ± 11.5 years (mean ± SD), in our units were studied for 26 weeks. Hct was checked weekly. Fixed-dose epoetin beta (EPO) 9000 IU per week were prescribed when Hct level was less than 32%. EPO were discontinued when Hct was 32 % or more. Change rate of Hct (%/week) with EPO administration was defined as the average change rate of Hct per week after restart of EPO therapy. Change rate of Hct (%/week) without EPO administration was defined as the average change rate of Hct per week after discontinuation of EPO therapy. ERI was calculated as weekly weight-adjusted dose of EPO divided by Hct level during the observation.

Results: The mean value of ERI was 2.98 ± 1.50 IU/kg/week/% for 26 weeks. Hct level achieved was 31.8 ± 1.11% during the observation period. The mean change rate of Hct with EPO was 0.738 ± 0.589 %/week, and the mean change rate of Hct without EPO was -0.660 ± 0.750 %/week. The change rate of Hct without EPO administration showed a significant inverse correlation with ERI (r = -0.460, p < 0.001), although the change rate of Hct with EPO administration was not.

Conclusions: The decrease rate of Hct per week after discontinuation of EPO was correlated with ERI, while the increase rate of Hct after restart of EPO was not. Our finding suggested that large decline rate in Hct levels after discontinuation of ESA is a more important factor to deteriorate responsiveness to ESA, compared to increase in Hct with ESA administration. Further study is needed to confirm the association.

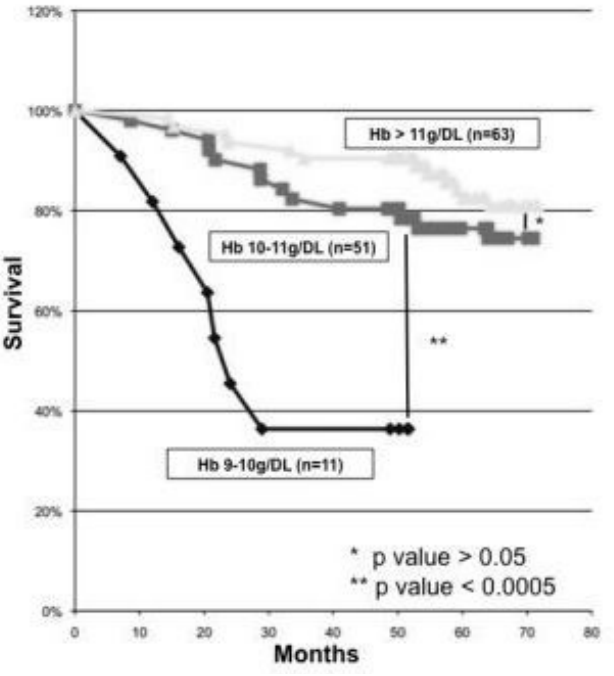
FR-PO248

The Effects of Mean Haemoglobin on the Survival of Patients Requiring Chronic Haemodialysis for End Stage Renal Failure: A 6 Year Retrospective Study Adam Sharp, Subash Somalanka, Maggi Steele, Pinnaduwage Vipula De Silva, David Makanjuola. *St Helier Hospital, Carshalton, United Kingdom.*

Background: Treating & maintaining haemoglobin [Hb] in patients undergoing chronic haemodialysis [HD] is a controversial subject with regard to what the optimal target levels are. We looked at our patient population to investigate the association of mean Hb on the survival of patients requiring chronic HD for ESRF.

Methods: All patients who were started on chronic HD in our renal centre in 2006 & 2007 were eligible for the study. A total of 124 patients were included & followed up over a 6 year period. Their baseline characteristics & comorbidities were recorded. Mean Hb for each patient was calculated from all measurements taken whilst on HD. Patients were grouped according to their mean Hb (9-10g/dl, 10-11g/dl & >11g/dl). A comparison between mean Hb & survival (all cause mortality) was made, correcting for baseline characteristics & co-morbidities.

Results: Of the 124 patients in the study there was no demonstrable difference in their baseline characteristics and comorbidities. Patients with a mean Hb on chronic HD of >11g/dl had no significant survival advantage when compared to those patients with a mean Hb between 10g/dl & 11g/dl (p>0.05) for all cause mortality. However, both groups had a significant survival advantage over patients with a mean Hb between 9 - 10g/dl (p=0.0005) for all cause mortality.



Conclusions: In our patient population there was no survival advantage between chronic HD patients that have mean Hb between 10g/dl & 11g/dl compared to those with mean Hb of >11g/dl. However, both these groups have a significant survival advantage from all cause mortality compared to those patients with Hb between 9 - 10g/dl. Interestingly, our findings lend weight to the current guidelines of the UK renal association, which recommend a target lower limit Hb of 10g/dl.

FR-PO249

Association of All-Cause Mortality with Hemoglobin Variability in Patients Undergoing Peritoneal Dialysis Li Zuo,[1] Li Liu,[1] Nayyar Saleem,[2] Rong Xu.[1] *[1]Institute of Nephrology, Peking University; [2]Shaikh Zayed Hospital Lahore.*

Background: Patients with chronic kidney disease exhibit significant hemoglobin variability, especially with the use of erythropoiesis stimulating agents and iron. Analyses of different dialysis cohorts produced conflicting results regarding association of Hb variability with patient outcomes. Moreover, very few studies were conducted in peritoneal dialysis (PD) patients. Purpose of this study was to assess the association of hemoglobin variability with all-cause mortality in our PD center.

Methods: Totally 219 incident PD patients (59±14 years, males in 41.6%, diabetes in 39.3%)were followed for 12 to 102 months from 2002 Dec to until 2011 Jun. Mean Hb of every three months in the first year after initiation of dialysis were calculated for each patient. Intrapatient hemoglobin variability was defined by two methods, standard deviation (SD) and residual SD, calculated from Hb measured in the first year. The association between Hb variability (represented by SD or residual SD) and mortality were analyzed by univariate logistic regression. Two Cox regression models, including either SD or residual SD, were used to analyze the relation between survival and Hb variability, after age, gender and diabetes were adjusted.

Results: Hb variability SD and residual SD was 10.94 and 7.84 g/L, respectively. There were 90 deaths (41.1% of all patients). In univariate logistic regression analysis, residual SD was associated with mortality (OR=1.076, p=0.013), but SD was not (OR=1.044, p=0.080). By Cox regression analysis with SD, age (older than 65 years, HR=3.019 for SD model, p<0.001; HR=2.934 for residual SD model, p<0.001) and diabetes (HR=1.805 for SD model, p=0.007; HR=1.823 for residual SD model, p=0.006) were associated with mortality, while either SD or residual SD was not (HR=1.010, p=0.565; HR=1.021, p=0.278).

Conclusions: Although hemoglobin variability measured as residual standard deviation was associated with mortality in PD patients, it cannot predict the mortality independently after age and diabetes were adjusted.

FR-PO250

Native Vitamin D Therapy in Hemodialysis Patients Results in Reduced Erythropoietin (EPO) Requirements-A Cluster Randomised Study Tarun Kaushik, Mark Blunden, Martin J. Raftery, Ravindra Rajakariar, Magdi Yaqoob. *Nephrology, The Royal London Hospital, London, United Kingdom.*

Background: Haemodialysis patients have low measured 25-OH Vitamin D levels.Native vitamin D supplementation in dialysis patients has been shown to reduce inflammation by lowering pro inflammatory cytokine.Sterile inflammation in dialysis patients is associated with EPO resistance resulting in higher dosage and increased morbidity and mortality.We therefore hypothesized that Vitamin D2 supplementation leads to reduced EPO requirements.

Methods: We conducted a cluster randomised study where half patients in a satellite unit were given oral ergocalciferol (n = 118) whilst remaining patients did not receive the treatment (n= 117) as per clinical preference of two physicians.In both groups combined,median 25 OH-Vitamin D level at baseline was 33 nmol/L (range 39.7– 48.4). The treated group (n= 118) received supervised 50,000 units weekly for 4 weeks followed by once monthly for 5 months.There was no difference in demographics, dialysis vintage, use of activated vitamin D and Cinacalcet in both groups.

Results: With treatment vitamin D levels rose from median of 32 to 78 nmol/L ($p<0.0001$) which remained unchanged in untreated group.In the treatment group, there was a significant reduction in EPO requirement as assessed by Hb index (weekly EPO dose in units/Hb g/dl) from a mean of 671 to 580 ($p<0.001$) but remained unchanged in the untreated group from mean of 795.0 to 743.1 ($p=0.12$) at 6 months.Furthermore, in the treated group the mean EPO dose per kg body weight per week reduced from 104 to 86 ($p=0.015$) but tended to increase non-significantly in untreated group (120 baseline to 160 at 6 months; $p=0.21$).Serum ferritin, calcium, phosphate, PTH and non hs-CRP were similar in both groups during the six month study period. Ergocalciferol was well tolerated with no adverse effects.

Conclusions: We conclude that native vitamin D2 in physiological doses leads to reduction in EPO requirement by an unknown mechanism.This simple and cheap strategy is both safe and well tolerated.The benefit of this therapeutic approach may potentially translate into significant cost saving and better patient outcomes.

FR-PO251

Effect of Ergocalciferol (D_2) Supplementation on Anemia and Mineral Metabolism Management in Hemodialysis Patients Shweta Bansal,[1] Ruby Naldoza,[2] Jonathan Vidola,[2] Ken Williams,[1] Paolo Fanti.[1] *[1]Division of Nephrology, University of Texas Health Sciences Center at San Antonio, San Antonio, TX; [2]Renal Dialysis Unit, University Health System, San Antonio, TX.*

Background: Vitamin D deficiency is prevalent in chronic kidney disease patients but literature on its supplementation is scarce. The aim of this study was to evaluate the effect of D_2 supplementation on erythropoietin (EPO) requirement and mineral metabolism in hemodialysis (HD) patients.

Methods: Medical records of 53 hemodialysis patients were examined retrospectively for a 6-month period, after initiation of a protocol to assess serum 25(OH)D levels and implement treatment with ergocalciferol if the levels were <35 ng/mL. Fifty patients (94%) had 25(OH)D levels <35 ng/ml and 47 were included for the analysis. Parameters of erythropoiesis, iron, and mineral metabolism and monthly doses of erythropoietin, intravenous iron and paricalcitol were assessed before and after D_2 supplementation.

Results: Mean 25(OH)D level was 17.15 ± 8 ng/ml and increased to 33.66 ± 10.3 ng/ml after 6 months of D_2 supplementation per protocol. Mean EPO use decreased by 15% (from 35,451 ± 26,192 IU/month to 29,960 ± 22,395 IU/month; $p=0.03$), and paricalcitol dose by 16% (from 26.15 ± 17.4 µg/month to 21.85 ± 14.1 µg/month; $p=0.039$) after 6 months of D_2 supplementation without any increase, rather a non significant downtrend in intact parathyroid hormone level. Serum calcium increased from 9 ± 0.5 mg/dl to 9.4 ± 0.7 mg/dl ($p=0.0008$), and bone-specific alkaline phosphatase from 19.4 ± 13.2 IU/ml to 25.6 ± 30.2 IU/ml ($p=0.04$). Monthly hemoglobin, ferritin, iron saturation, albumin, and phosphorus levels were not different before and after D_2 supplementation. No significant change was observed in dosage of phosphate binders and cinacalcet. On multivariate analysis the decrease in EPO dose was partly explained by decrease in paricalcitol dose.

Conclusions: Oral ergocalciferol supplementation in HD patients seems to be a cost-effective therapeutic measure. It allows reduced dosing of erythropoiesis-stimulating agents and active vitamin D analgoue, both of which are associated with their inherited adverse effects.

FR-PO252

Hemodialysis System Using Dissolved Dihydrogen(H2) Produced by Water Electrolysis Reduced the Darbepoetin Dose Noritomo Itami, Kazushi Tsuneyama, Susumu Uemura. *Kidney Center, Nikko Memorial Hospital, Muroran, Japan.*

Background: High levels of pro-inflammatory cytokines and increased oxidative stress are common features contributing to malnutrition, recombinant human erythropoietin(rHuEPO) resistance, and atherosclerosis in ESRD patients. The administration of H2, an inert gas with no known side effects, dissolved in water is reported to suppress oxidative or inflammatory injury. We changed dialysate from standard water to water with high levels of dissolved H2. We report the effect of HD with high levels of dissolved H2 on anemia and darbepoetin alfa(DA).

Methods: Dialysate with high levels of dissolved H2(average of 60 ppb) was produced by mixing dialysate concentrates and reverse osmosis water containing dissolved H2 generated by the HD-24K water electrolysis system(Nihon Trim, Osaka Japan) details are reported in(Nephrol Dial Transplant 25;3026,2010). Thirty-nine stable patients on standard HD(S-HD), (M/F ratio 21:18, Mean Age: 64±10.3yr, Vintage: 11.9±8.5yr, original diseases: 12CGN, 9DM, 4PCK, others 14) were switched to HD with water with high levels of dissolved H2(HD-H2) after obtaining informed consent. DA dose was altered biweekly according to our algorithm considering hemoglobin(Hb) level, Hb change gradient and iron parameters. Other anemia treatment was carried out as usual. Average DA dose and clinical parameters were examined.

Results: Hb was unchanged, S-HD for 12 months the mean Hb was 11.1g±1.0/dL and in HD-H2 for 11 months the mean Hb was 11.0±0.9g/dL. The number of patients taking iron treatment in S-HD was a mean of 15.4±2.8 patients/month and in HD-H2 a mean of 12.9±2.3 patients/month(n.s). Serum ferritin was significantly increased from a mean of 156.3±113.7ng/mL in S-HD to a mean of 184.9±121.2ng/mL in HD-H2. The average TSAT was unchanged, 29.9±11.5% in S-HD VS 28.0±11.2% in HD-H2. Average DA dose was significantly reduced from 15.6±11.0µg/week in S-HD to 14.0±12µg/week in HD-H2($p<0.01$). The average C-reacting Protein was unchanged, 0.20±.34mg/dl in S-HD VS 0.22±0.42mg/dl in HD-H2.

Conclusions: HD-H2 reduced the dose of DA in the same range of Hb although further multicenter studies are needed.

FR-PO253

Comparison between Short Acting Erythropoiesis Stimulating Agents Epotein Alfa, and Long Acting Darbepoietin Alfa, in Hemodialysis Patients: Target Hemoglobin, Variability, Conversion Factor, Outcome and Cost Bassam O. Bernieh,[1] Samra Abouchacra,[1] Yousef Boobes,[1] Mohamed Raafat Al Hakim,[1] Ahmed Chaaban,[1] Faiz Abayechi,[1] Hanan Eljack,[1] Qutaiba Hussain Daoud,[1] Imran Khan,[1] Nicole Gebran,[2] Hanan Al Omary.[1] *[1]Nephrology, Tawam Hospital, Al Ain, United Arab Emirates; [2]Pharmacy, Tawam Hospital, Al Ain, United Arab Emirates.*

Background: Maintaining target hemoglobin (Hb) with minimal hemoglobin variability is a difficult challenge in anemia management of hemodilaysis (HD) patients affecting their outcome. The aim of this study is to compare the long acting ESA Aranesp, and the short acting Eprex,in achieving target Hb, variability, outcome, and cost.

Methods: Randomized,prospective, open labeled study of 24 weeks of 2 phases, titration and evaluation, including stable patients on HD>3 months,age>18 years,and on Eprex for>3 months.Patients randomized in two groups: A-(Aranesp Group):HD patients on Eprex Q TIW or BIW were converted to Aranesp Q weekly,by using the recommended conversion factor of 200:1;and those on Eprex Q weekly to Aranesp Q 2 weeks; B-(Eprex Group):patients continued on Eprex treatment.Hemoglobin target set at (105 – 125 g/l). Primary end points: percentage of patients achieving target Hb,hemoglobin variability, and number of dose changes in each group.

Results: 140 HD patients 72 in the Aranesp,and 68 in the Eprex, Mean (SD) age 54(16.2) year,78(56%) male. 46% were diabetic. Target Hb achieved in 64.8% of the Aranesp, and 59.7% in the Eprex ($p=0.006$). Hb variability was less frequent in the Aranesp at the target Hb (105-125) [$p=0.2$], with significant difference [$p=0.04$] at Hb of (110-130). Mean number of dose changes in the evaluation phase was 1.3 (0.87) in the Aranesp, and 2 (1.2) in the Eprex ($p<0.001$). The average conversion factor was 268 ($p<0.001$). The average weekly cost/kg was 1.4 USD for Aranesp, and 0.7 for Eprex ($p<0.01$).There was 1 vascular access thrombosis in the Aranesp, and 8 in the Eprex ($p<0.001$).There was no difference in hospitalization and death number between the 2 groups.

Conclusions: Aranesp Q weekly or every 2 weeks, is more efficient in achieving target Hb, with less dose changes,Hb variability,and minor vascular access complications.

FR-PO254

Occasional Assist by Epoetin in Addition to Fixed Dosage of Darbepoetin on Anemia Management in Hemodialysis Patients Kazumasa Shimamatsu. *Hemodialysis Unit, Shimamatsu Naika Iin, Chikushino City, Fukuoka, Japan.*

Background: Taking advantage of characteristics of both long- and short-acting drugs (darbepoetin, DA and epoetin, EPO) might be reasonable for anemia management to avoid a possible overshoot in hemoglobin (Hb) rise and to stabilize a Hb cycling. The halves of weekly doses of EPO monotherapy were converted to DA in combination with the remaining half doses of EPO. Thereafter, EPO assisted with on-and-off fashion fixed dosage of DA depending upon Hb levels. The effect of DA therapy assisted by EPO on Hb control was retrospectively evaluated in comparison with that of EPO monotherapy.

Methods: Twenty-six hemodialysis (HD) patients whose annual mean Hb values were available both in EPO monotherapy period and in DA/EPO combination period were selected. The mean age of the patients (15 male, 11 female) was 60.6±13.9 yr (mean±SD). The mean HD duration was 9.1±7.1 years. The DA doses (µg) close to 1/200 of the halves of weekly EPO doses (IU) were given on the second HD day of a week. The remaining half doses of EPO were given dividedly on the first and the third HD day of a week. The target Hb value was 11 g/dl. After introduction of DA therapy when the Hb levels rise over 12 g/dl, EPO was eliminated. When the Hb levels go down under 10 g/dl, EPO was added again. The standard deviation (SD) of annual mean Hb from 26 HD patients in DA/EPO period was compared with that in EPO period. Also, the difference (d-Hb) between the annual mean Hb of 26 patients and the target Hb of 11 g/dl in DA/EPO period was compared with that in EPO period. Paired *t*-test was used for analysis.

Results: The SD of annual mean Hb in DA/EPO period was significantly smaller than that in EPO period (11.2±0.25 vs. 11.0±0.50 g/dl, respectively, $p<0.001$). Also, the d-Hb of annual mean Hb in DA/EPO period was significantly smaller than that in EPO period (0.22±0.21 vs. 0.38±0.31 g/dl, respectively, $p<0.03$). The mean dose of EPO was 3656±2107 IU/week in EPO period and the mean dose of DA was 13.6±7.5 g/week with mean EPO dose of 284±436 IU/week in DA/EPO period.

Conclusions: The occasional assist by EPO in addition to fixed dosage of DA may be useful for controlling Hb levels within narrow range of the target.

FR-PO255

Factors Influencing Hyperviscosity in Maintenance Hemodialysis Patients

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Background: Many factors also could affect plasma viscosity as white blood cell, platelet count, iron deficiency and drugs in maintenance hemodialysis (MHD) patients. Increased risk for thrombosis is the main factor limiting the use of erythropoietin stimulating agents (ESA). In this present study, we investigated the clinical and laboratory factors influencing plasma viscosity in MHD patients.

Methods: 84 MHD patients (30 female, age; 54.7 ± 13.7 years) (TSAT ratio>30; mean ferritin:553,39 ng/ml) were included. Patients with iron deficiency, chronic inflammatory disease and malignancy were excluded. Plasma viscosities of all subjects were measured at 37°C in a Brookfield DV– II + Cone Plate Viscometer (Brookfield, Stoughton, MA, USA). Laboratory data and monthly ESA requirements were retrospectively collected. Patients were grouped according to plasma viscosities as upper and lower halves. Patients were also grouped according to their ESA requirements as no (n: 21), low (n: 31), high (n: 32) ESA requiring patients.

Results: MHD patients had high plasma viscosity compared to healthy subjects (range 1.6-3.9m Pas, 2.52 ± 0.65 mPas vs 1.10-1.30 mPa.s.). Iron parameters, hemoglobin, CRP levels were similar. However we found that subjects in high viscosity group used higher ESA dose in previous year (224.4 ± 237.8 260.4 ± 340.5 U/kg/month, p: 0.038). 68.8% of the high ESA requirement group intersected with higher viscosity group while this was 38.7% in the low ESA group (p: 0.001) despite similar hemoglobin levels. Linear regression analysis revealed that EPO resistance and HD duration were the major determinants of hyperviscosity. HD duration of patients in "no rHuEPO" was the longest; low rHuEPO group had the shortest.

Conclusions: According to our findings, majority of MHD patients have increased plasma viscosity compared to previously reported healthy subjects. We think that high dose ESA usage might increase plasma viscosity despite similar hemoglobin levels achieved when iron deficiency is excluded.

FR-PO256

Darbepoetin-alpha Does Not Increase the Risk for Thrombotic Events in Hemodialysis Patients: Role of TAFI-Dependent Thrombosis and Inflammation

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Background: Cardiovascular disease is a leading cause of death in hemodialysis (HD) patients. One of the potential side-effects of erythropoietin (EPO) therapy is an increase in thrombotic events. Thrombin activatable fibrinolysis inhibitor (TAFI) is a proenzyme, which potently inhibits fibrinolysis. There are no data about effects of eritropoetic agents on serum TAFI levels in HD patients. Our aim was to assess TAFI levels and its relationship with other parameters in HD patients in which epoetin alfa (EA) was switched to darbopoetin alfa (DA) treatment.

Methods: Thirty-five stable HD patients receiving EA for 6 months were switched to DA. Blood samples were taken at the beginning and 6 months after the start of DA. The control group consisted of HD patients not on EPO treatment.

Results: TAFI and mean platelet volume (MPV) levels were significantly lower after DA treatment than EA treatment and controls. After DA treatment CRP levels were also lower and a positive correlation was detected between TAFI and CRP levels.

Laboratory Parameters in Patients and Controls

	Control (n=35)	EA Group (n=35)	DA Group (n=35)
Hemoglobin (g/dL)	12±0.3	11.06±1.31	10.88±1.5
Platelet count (x109)	251000±123000	248310±100079	267000±154976
MPV (fL)	8.13±0.75(a)	8.62±0.85(b)	7.99±0.68
TAFI antigen (%)	77.12±35.23(c)	79.63±37.44(d)	48.15±18.68
CRP (mg/dl)	2.23±1.87(e)	2.11±2.06(f)	0.80±0.65

a p=0.001 Control versus DA; b p=0.001 EA versus DA; c p=0.001 Control versus DA; d p=0.002 EA versus DA; e p=0.018 Control versus DA; f p=0.011 EA versus DA.

Conclusions: We found that TAFI antigen, CRP and MPV levels were significantly decreased in HD patients on DA therapy. TAFI is expressed as an acute phase protein and causes inflammation and coagulation response. Significant correlation between TAFI level and CRP strenghten this hypothesis. Decreased TAFI levels after DA can be considered as a factor to explain the lower thrombotic tendency in these patients. Lower MPV after DA treatment could be an additional factor for decreased thrombosis as larger platelets can contribute to thrombosis.

FR-PO257

FG-4592, a Novel Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor (HIF-PHI), Maintains Hemoglobin Levels and Lowers Cholesterol in Hemodialysis Patients: Phase 2 Comparison with Epoetin Alfa

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Background: Dyslipidemia is a major factor associated with increased cardiovascular risk in advanced CKD. We evaluated effects of FG-4592 on hemoglobin (Hb) and total cholesterol (TC) in hemodialysis (HD) patients switched from epoetin alfa (rhEPO).

Methods: In an ongoing open-label phase 2 study, 159 HD patients taking rhEPO 3X weekly (TIW) were randomized to FG-4592 TIW or continued rhEPO for 6 or 19 wks. FG-4592 doses were adjusted every 4 wks to maintain Hb 11-13 g/dL. Intravenous (IV) iron supplementation was disallowed. There were no protocol-specified restrictions on lipid medication use or thresholds for medication changes.

Results: Hb data for patients treated 6 wks have been reported (Provenzano 2011). Mean±SD baseline (BL) Hb levels for patients treated 19 wks with FG-4592 (N=54) and rhEPO (N=19) were 11.3±0.7 g/dL and 11.4±0.9 g/dL, respectively. The corresponding mean±SD Hb changes from BL in the last 4 treatment wks were -0.2±1.4 g/dL and -0.6±1.3 g/dL, respectively; changes were not significantly different between the 2 groups.

FG-4592 patients (N=91) had a Week 6 mean±SD 20%±15% TC reduction from BL, and rhEPO patients (N=32) had a mean±SD 4%±16% TC increase from BL ($p<0.0001$); the difference remained significant at Week 19 ($p<0.0001$). A substudy showed lower plasma erythropoietin levels with FG-4592 treatment compared with rhEPO treatment at screening. FG-4592 was well tolerated, with an adverse event profile consistent with the patient population.

Conclusions: Oral FG-4592 TIW for 19 weeks maintained corrected Hb levels in HD patients without IV iron supplementation. The HIF-PHI mechanism of action appeared to reduce TC and plasma erythropoietin in patients given FG-4592 vs rhEPO.

Funding: Pharmaceutical Company Support - FibroGen, Inc.

FR-PO258

Virdaglin, an Inhibitor of Dipeptidyl Peptidases 4 (DPPI-4), Can Improve Anemia in Diabetic Hemodialysis (HD) Patients

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Background: Several reports demonstrate hematopoietic effects of DPP-4 via CD26pathways for regulating hematopoietic growth factors (Jones B, et al. Blood 2003). We hereby evaluate the effects of DPP-4 on improvement of anemia in HD patients when converted from sulfonyl urea or glinede analogues.

Methods: From September to December 2010, 15 HD outpatients with diabetes were enrolled in this study after appropriate IC, and were monitored by various parameters including fasting plasma glucose (FPG), HbA1c, Hb, albumin or body mass index (BMI). Erythropoietin (EPO) doses had stayed the same.

Results: As shown in Table 1, FPG and HbA1c level had been stabilized after dose adjustment of virdaglin. There was significant increase in Hb level (10.8±1.49 to 11.6±1.08, p=0.018) though EPO doses had been unchanged. Other nutritional parameters stayed the same.

Effects of virdaglin on improving anemia in HD patients (n=15)

	sulfonyl urea / glinede analogues	virdaglin	
FPG (mg/dL)	138±49	119±34	ns
HbA1c (%)	6.8±1.9	7.1±1.1	ns
Albumin (g/dL)	3.2±0.8	3.3±0.4	ns
BMI	24.0±4.3	26.6±3.9	ns
Hb (g/dL)	10.4±1.3	11.8±1.2	p<0.05

Conclusions: Virdaglin can potentially improve anemiain hemodialysis HD patients independently of nutritional status. Whether virdaglin improves the survival of ESRD patients remains to be investigated with further studies.

Funding: Private Foundation Support

FR-PO259

Novel Sustained Delivery of Erythropoietin in Hemodialysis Patients for Safer Anemia Control Using EPODURE Biopumps: Autologous Dermal Tissue Samples Secreting Erythropoietin

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Background: EPODURE Biopumps are autologous dermis tissue explants (30mm x 2mm) converted into units producing sustained erythropoietin (EPO) by introducing the EPO gene using a Helper Dependent Adenoviral vector. Increased incidence of cardiovascular

and cerebrovascular complications in hemodialysis patients treated with EPO has been hypothesized to be due to transient supra-physiological serum levels of EPO produced by IV or SC administration. In contrast, EPO levels in patients treated by EPODURE Biopumps remain within physiologic range, as reported in patients with Stage 3-4 CKD (ASN 2011). The current study aims to examine the feasibility of using EPODURE Biopumps in ESRD patients on dialysis, to maintain Hb levels in the target range without the need for exogenous ESA injections.

Methods: Following on the CKD study, where a single implantation of EPODURE Biopumps maintained Hb in the 10-12g/dl range for 3-30 months without any ESA injections and without serum EPO levels exceeding 70 mU/ml, we have commenced a PH2 open label, single center study in up to 20 ESRD hemodialysis patients in Israel. EPODURE Biopumps are implanted to replace ESA injections, with dose based on the patient's weight and historical EPO administered pre-enrollment with baseline Hb levels of 10-12 g/dl.

Results: First ESRD patient received EPODURE Biopumps May 24, 2012, elevating serum EPO to peak of 42 mU/ml, absolute reticulocyte levels above baseline, with Hb maintained in baseline range of 11.0-11.4 g/dl as of abstract submission. Additional patients are being enrolled.

Conclusions: Early results in the first ESRD hemodialysis patient are consistent with prior CKD study results. Enrollment continues and additional results will be obtained to further test the hypothesis that Hb can be maintained within target range by sustained EPO delivery without causing supra-physiologic EPO levels, to potentially improve safety.

Funding: Government Support - Non-U.S.

FR-PO260

Hemoglobin Control and Dose Alterations with Peginesatide versus Epoetin for Hemodialysis Patients Bruce S. Spinowitz,[1] Steven Zeig,[1] Helen Tang,[2] Sandra Tong,[2] Nina Oestreicher,[2] Alex Yang,[2] Wadi N. Suki.[1] *[1]AFX01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: Peginesatide (OMONTYS®) is a PEGylated, peptide-based ESA indicated for treatment of anemia due to CKD in adult patients (pts) on dialysis. Peginesatide demonstrated noninferiority to epoetin in maintaining Hb in hemodialysis pts in two Phase 3 randomized, active-controlled, open-label trials (EMERALD 1, 2; Schiller et al. ASN 2010). This post hoc analysis compared dose adjustments and postponements for peginesatide vs epoetin and achievement of target hemoglobin (Hb).

Methods: Data were pooled from the 2 trials: peginesatide (Q4W; n=1066) vs epoetin (1-3x weekly; n=542). Dose adjustments (defined as change >±20% from last dose) were not to be made more frequently than Q4W to maintain Hb 10-12 g/dL (per guidelines in effect at time of trial). Hb measurements were Q2W, except weekly during evaluation period or dose postponements (defined as >35 d after last dose of peginesatide or >4, 6, or 9 d after last dose epoetin TIW, BIW, or QW, respectively). Frequency of dose alterations was calculated per pt-year of exposure.

Results: Dose adjustments and postponements by study period are below.

Study Period	Peginesatide	Epoetin	Ratio: Peginesatide/ Epoetin
Adjustments/pt-year			
TP	3.80	10.18	0.37
EP	3.84	10.75	0.36
LTSE	3.39	9.81	0.35
Postponements/pt-year			
TP	0.60	4.64	0.13
EP	0.58	4.75	0.12
LTSE	0.66	5.37	0.12

TP, titration period Wks 0-28; EP, evaluation period Wks 29-36; LTSE, long-term safety & efficacy Wks 37-52

Through end of treatment, 22% of patients on peginesatide and 20% on epoetin had confirmed Hb excursions (2 consecutive values above 13 g/dL), with median 22 and 20 days per excursion, respectively. Confirmed Hb excursions below 10 g/dL occurred in 54% of patients on peginesatide and 51% on epoetin; median duration per excursion was 38 and 39 days, respectively.

Conclusions: There was a higher frequency of dose adjustments and postponements for epoetin, compared with peginesatide. Frequency and duration of Hb excursions were also similar between study arms. These results suggest that once monthly peginesatide achieves similar Hb outcomes with fewer dose adjustments and postponements.

Funding: Pharmaceutical Company Support - Affymax, Inc., and Takeda Pharmaceuticals

FR-PO261

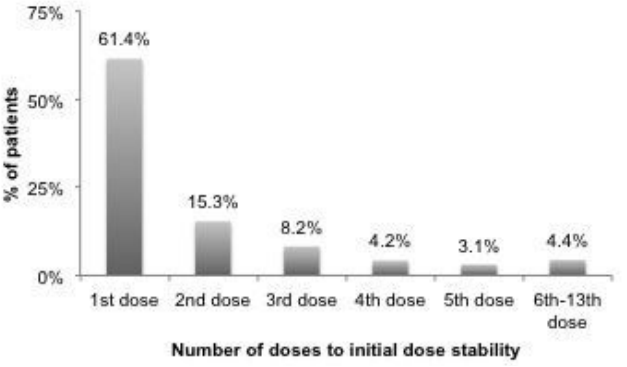
Initial Dose Stability after Conversion to Peginesatide in HD Patients Brigitte Schiller- Moran,[1] Rebecca J. Schmidt,[1] Anjay Rastogi,[1] Whedy Wang,[2] Sandra Tong,[2] Alex Yang,[2] Nina Oestreicher,[2] Mark Kaplan.[1] *[1]AFX01-12 and -14 Peginesatide Study Groups; [2]Affymax, Inc., Palo Alto, CA.*

Background: Peginesatide (OMONTYS®) is a PEGylated, peptide-based ESA indicated for treatment of anemia due to CKD in adult patients (pts) on dialysis. Peginesatide demonstrated noninferiority to epoetin in maintaining hemoglobin (Hb) in hemodialysis (HD) pts in two Phase 3 randomized, active-controlled, open-label trials (EMERALD 1, 2; Schiller et al. ASN 2010). It was previously shown that pts on epoetin had more frequent dose adjustments than peginesatide for maintaining similar Hb (Sharma et al. Renal Nutr Week 2012). This post hoc analysis evaluated the number of doses required to reach initial dose stability following conversion from stable epoetin to peginesatide.

Methods: Data were pooled from the two trials assessing safety and efficacy of peginesatide (Q4W; n=1066) vs epoetin (1-3x weekly; n=542) in HD pts. Dose adjustments were to be made no more frequently than Q4W (unless required for safety purposes) to maintain Hb 10-12 g/dL (per guidelines in effect at time of trial). Hb was measured Q2W (or QW during evaluation period or dose postponements). Initial dose stability was established if (1) the subsequent dose was within 20% of prior dose (21-35d between doses), and (2) at least 1 Hb was within target between doses. The evaluable population included all patients who received ≥2 doses (n=1034).

Results: 61.4% of pts reached initial dose stability after the first dose, 76.7% by the second dose, 85% by the third dose. Only 4.4% required 6 or more doses.

Distribution of Doses to Initial Dose Stability.



Conclusions: Conversion to peginesatide in HD pts was successful, with the majority of peginesatide pts who were previously on stable dose of epoetin reaching initial dose stability within 1-2 doses after conversion to peginesatide.

Funding: Pharmaceutical Company Support - Affmax, Inc. and Takeda Pharmaceuticals

FR-PO262

Peginesatide Immunogenicity in Clinical Studies of CKD Patients Peter J. Schatz, Sandra Tong, Vandana S. Mathur, Minjia Chen, Karen Leu, Richard B. Mortensen, Rezi Zawadzki, Daniel S. Cooper, Richard B. Stead, Martha Mayo, Krishna R. Polu, Anne-Marie Duliege. *Affymax, Inc, Palo Alto, CA.*

Background: Peginesatide is a synthetic, pegylated, peptide-based erythropoiesis-stimulating agent (ESA) that activates the erythropoietin (EPO) receptor. Peginesatide has no sequence similarity to EPO and is being evaluated in treatment of CKD patients (pts) with anti-EPO antibody (Ab)-mediated pure red cell aplasia (PRCA) (Macdougall, 2009, NEJM). Peginesatide is indicated for the treatment of anemia due to CKD in adult pts on dialysis, with once monthly dosing.

Methods: Peginesatide's immunogenicity was systematically evaluated in Phase 2 & 3 clinical studies in CKD pts receiving peginesatide mostly for up to 2 years, some for up to ~4 years. Serum samples were evaluated by ELISA for peginesatide-specific binding Abs (BAb). Positive samples were tested for in vitro peginesatide-neutralizing Abs (NAb) using a cell-based assay and for Abs cross-reactive with recombinant human (rHu) EPO using a radioimmunoprecipitation assay. Clinical consequences of Abs on safety and efficacy were evaluated.

Results: 29 of 2357 pts tested (1.2%) had detectable BAb levels. NAb were detected in 21 of these pts (0.9%). No pt developed de novo Abs to EPO after exposure to peginesatide, and no new cases of PRCA were reported, suggesting lack of immunological cross-reactivity between peginesatide and EPO. The Abs were specific for the peptide rather than the PEG component of peginesatide. IgG4, which does not fix complement or form immune complexes, was the immunodominant isotype. Allergic, hypersensitivity, or anaphylactic reactions were not observed in BAb^+ pts. In approximately half of the BAb^+ pts, the presence of Abs was temporally (ie, within ±90 days of the Ab detection period) associated with declining hemoglobin levels, increased peginesatide doses, and/or transfusions.

Conclusions: Abs to peginesatide were uncommon (1.2%) and not associated with allergic-type reactions. Although evidence of reduced efficacy was temporally-associated in approximately half of the BAb^+ pts, Abs to peginesatide did not cross-react with rHuEPO, suggesting that peginesatide is unlikely to cause PRCA.

Funding: Pharmaceutical Company Support - Affymax, Inc. and Takeda Pharmaceuticals

FR-PO263

A Comparative Analysis of Transfusion Trends between Types of Providers of Dialysis Services Using Medicare Claims Data Mahesh Krishnan,[1] Allen R. Nissenson,[1] Rachel Feldman,[2] Mark Desmarais,[2] Lianna Weissblum.[2] *[1]DaVita Inc., Denver, CO; [2]The Moran Company, Arlington, VA.*

Background: The question of practice patterns and impact on transfusions is currently being debated in light of the significant ESA label changes in June of 2011. However, little is known about how these practice patterns varied amongst provider types prior to the label change. We used Medicare data to compare transfusion trends in for-profit (FP) vs non-profit (NP) dialysis facilities in the two years prior to the ESA label change.

Methods: Medicare Standard Analytic File Claims data from 2009 and 2010 were analyzed at the facility level using the Dialysis Facility Compare provider-type data to create comparator groups. Inpatient hospital and dialysis facility blood transfusions were identified using the presence of blood product revenue codes. Outpatient hospital blood transfusions were identified using the presence of blood product revenue codes and transfusion procedure codes.

Results:

Prevalent ESRD Patients with ±1 Type of Product Administrations, %	Inpatient Blood		Outpatient Blood		Blood Product on a Dialysis Claim	
	2009	2010	2009	2010	2009	2010
All Dialysis Facilities	25.8	25.3	6.1	5.9	1.2	1.1
Non-Profit Facilities	25.7	25.3	8.0	7.8	2.8	2.5
For-Profit Facilities	25.9	25.3	5.5	5.4	0.4	0.3
Hospital-based Units	29.3	28.3	12.1	12.0	5.7	5.5
Other Freestanding Facilities	27.2	26.8	6.8	6.8	1.3	1.2

Conclusions: Overall, most transfusions appear to occur in the hospital setting, and there was a slight decrease in use of transfusions in 2010 compared with 2009. Transfusions in the dialysis facility appear to be very rare, but do appear to occur in non-profit and some free-standing facilities. Patients in hospital-based and non-profit facilities had the highest rates of transfusions compared with those in for-profit facilities in 2009 and 2010. As these data reflect anemia management practice patterns prior to June 2011 ESA label changes, they can serve as a baseline to future work reflective of the current treatment paradigms.

Funding: Pharmaceutical Company Support - DaVita Inc.

FR-PO264

The First Report of the Latin American Register of Anemia Management among Chronic Hemodialysis Patients Raul G. Carlini,[1] Gregorio T. Obrador,[2] Ricardo Correa-Rotter,[3] Liliana Andrade,[4] Alberto Locatelli.[5] *[1]Hospital Universitario de Caracas, Venezuela; [2]Universidad Panamericana School of Medicine, Mexico; [3]Instituto Nacional de la Nutricion, Mexico; [4]Hospital Complejo Medico Policial Churruca-Visca, Argentina; [5]Nefronosa S.A., Argentina.*

Background: Anemia almost invariably occurs in patients with chronic kidney disease (CKD). Limited data are available regarding anemia management in Latin American (LA) hemodialysis (HD) patients. We report the first results of the Anemia Register of the LA Society of Nephrology and Hypertension (SLANH).

Methods: A survey was sent to independent, non-chain owned HD units via the nephrology society members of SLANH to collect cross-sectional anemia management data between 09/2009 and 03/2010. The following parameters were analyzed: age, sex, time on HD, dry weight, etiology of CKD, levels of Hb, ferritin, and transferrin saturation (TSAT), iron, and ESAs doses.

Results: A total of 134 nephrologists from 16 LA countries responded to the survey and provided information on 9,054 patients. Their mean age was 57.4±15.7 years, 58% were men and had been on chronic HD for 5.0±3.7 years. Dry weight was 65.1±14.9 kg. The causes of CKD were hypertension (41%), diabetes mellitus (30%), glomerulonephritis (80%) and others (11%). The mean Hb level was 10.5±1.9 g/dL, ferritin 556±496 mg/dL and TSAT 29.8±15%. In 46.2% of the patients, the Hb level was <10.5g/dL, 32.6% between 10.5 and 12 g/dL and >12.0 g/dL in 23%. Only 32.6% of patients were within the SLANH's recommended anemia guidelines Hb target of 10.5-12.0 g/dL (46.2% were below and 21.3% above). ESAs were given subcutaneously in (SQ) 83.9% of patients. The majority (84.7%) were receiving an ESAs, mostly erythropoietin-α (72.9%) at a median weekly dose of 6,000 IU SQ and 12,000 IU IV. Most patients (69.3%) were receiving IV iron, preferentially iron sucrose (56.3%) at a median monthly dose of 200 mg.

Conclusions: Despite frequent ESAs and IV iron use, nearly half of LA chronic HD patients had Hb levels below the recommended target of 10.5 g/dl. Further studies are needed to determine factors and interventions that can improve the quality of anemia management.

FR-PO265

Intensity of Care at Initiation of Chronic Dialysis and Long-Term Outcomes in Older Adults with End-Stage Renal Disease Susan P.Y. Wong, William Kreuter, Ann M. O'Hare. *University of Washington.*

Background: Prognostication in older adults initiating chronic dialysis can be challenging, particularly when dialysis is begun in the setting of acute illness. We hypothesized that measures of illness severity around the time of dialysis initiation may be useful prognostic markers in this population.

Methods: Using data from the United States Renal Data System, we performed a retrospective study on 585,291 Medicare beneficiaries aged ≥67 years who began chronic dialysis between 1/1/1995 and 12/31/2008. We examined illness severity at dialysis initiation as reflected in the following measures of healthcare intensity: whether dialysis was initiated during a hospitalization for acute illness, the length of hospital stay, and whether an intensive procedure (mechanical ventilation, cardiopulmonary resuscitation, and feeding tube placement) was performed during the index hospitalization. We measured the association between intensity of care experienced at dialysis onset with survival and remaining lifetime spent hospitalized.

Results: One in two older patients (53.9%) initiated chronic dialysis in the inpatient setting. Of these, 36.3% were hospitalized for ≥2 weeks, and 7.4% underwent one or more intensive procedures. Patients who received the highest level of intensity of care at dialysis initiation (hospitalized ≥2 weeks, and received one or more intensive procedures) had a shorter life expectancy (median 0.7 v 2.1 years; HR 1.42, 95% CI 1.39-1.45) and spent a greater percentage of their remaining lifetime in the hospital (median 26.6% v. 1.2%; HR 1.85, 95% CI 1.81-1.89) as compared with those who received the lowest level of intensity of care (initiation as an outpatient). Among patients who received the highest level of intensity of care, median survival was approximately 8 months of which 2 months were spent hospitalized.

Conclusions: The decision to initiate chronic dialysis is often made in the less than ideal circumstance of acute illness. Simple, objective measures of healthcare intensity around this time may have prognostic value to older adults facing the prospect of chronic dialysis.

Funding: Other NIH Support - National Institute of Aging, Veterans Administration Support

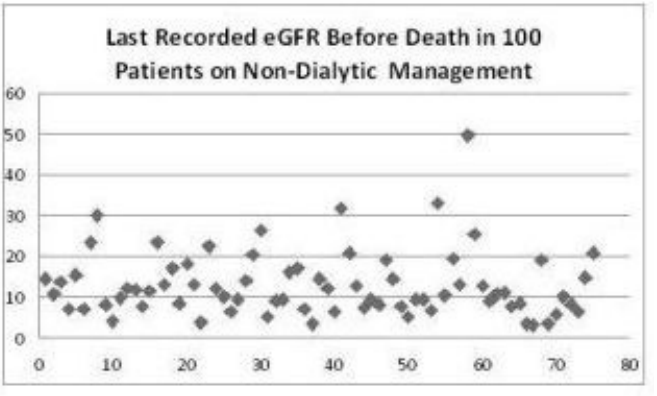
FR-PO266

What Can Elderly Frail Patients with Advanced CKD Expect If They Elect Not to Undergo Dialysis? Thomas D. Hughes, Helen Alston, Aine Burns. *Centre for Nephrology, UCL, London, United Kingdom.*

Background: The growing aged CKD population in first world countries presents many challenges. In the last decade evidence has emerged that hospital free survival amongst elderly patients with multiple co-morbidities who undergo dialysis may not differ substantially from those having non-dialytic or maximum conservative management (MCM).

Methods: We searched the center's database for patients who followed our MCM care pathway and who had died between January 1st 2002 and December 12th 2011 identifying 284 patients. We examined in detail the electronic demographic, medical records, biochemical and haematological data on 100 of these patients selected as they had died most recently. Charlson co-morbidity scores were calculated. Post-codes were used to assess deprivation scores.

Results: The mean age for the population was 82.4 (median 84, range 40 to 98) 55% were male. Amongst the 100 selected for detailed evaluation the Co-morbidity scores were high as expected. Mean last recorded eGFR (MDRD) prior to death was 12.83mls/min (median 10.8, range 2.89 to 49.69).



Mean and median last recorded haemoglobin estimations were 10.5 & 10.3, (range 7 to 14.5). All expected ethnic, religious and social groups were represented in proportion to that found in the local population.

Conclusions: We have described a unique group of elderly patients with CKD who have died having received renal supportive care only. We have determined that a proportion is likely to have died from inter-current illnesses rather than renal failure per se. Many however, survived until eGFRs were well into single figures. This work is important as it enhances understanding of the final journeys of such patients and can be used to help them, their relatives, nephrologists and policy makers to balanced decisions regarding care. It also questions the optimum time to start dialysis in elderly patients with multiple co-morbidities.

FR-PO267

Cognitive Impairement in CKD5D Patients: A Cross-Sectional Study Anne Kathrin Malecki,[1] Sabrina Schneider,[1] Hermann G. Haller,[2] Jan T. Kielstein.[2] *[1]Institute of Psychology, Martin-Luther-University, Halle-Wittenberg, Germany; [2]Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany.*

Background: Severel studies could show significant impairement in CKD patients the worsens in parallel with the decline in renal function. Assessment of cognitive function is cumbersome and routine bedside test like the mini mental state exam fail to reflect the changes observed in CKD5D. The current study assesses the congitive function in CKD5D patients using large neuropsychological testbattery and valid test procedures.

Methods: We examined 28 (16 M; 12 F) medically stable CKD5D patients aged 55±11 years. Memory, working memory, attention and concentration, executive functioning, psychomotor speed by using the following tests: Rivermead behavior memory test (RBMT), Rey Cpmplexe Figure Test (RCFT), Trail Making Test A+B (TMT), Wechsler Memory Scale (WMS- R), Behavior Assessment of Dysexecutive Syndrome (BADS), Regensburger Wortflüssigkeits- Test (RWT), Testbatterie zur Aufmerksamkeitsprüfung (TAP).

Results: As compared to matched controls CKD5D patients show a signficant impairment in TMT A / B (CKD5D:47.5s / 110.5s vs 40s / 98s), WMS- R (CKD5D: 5 digits vs cntrols:6 digits), TAP / RWT (CKD5D:25 / 15 words vs controls 30 / 17 words). Moreover there was a trend in deficits of the logical memory span (CKD5D: 7.3 / 6 digits vs. controls:9.5 and 8 digits).

Conclusions: Our data demonstrate cognitive impairement in CKD5D when comparing these patients with their apropriate age group. Like previous studies we could find deficits in executive functioning like visual exploring, cognitive shifting and working memory. Moreover we found deficits in concentration and attention regarding the speed of reaction. In contrast to previous studies we could also find mnestic deficits. These findings should be considered regarding everey day communication as well as in the situation of obtaining consent for procedures or investigating non-adherence to medication.

FR-PO268

Detailed Analysis of Cognitive Function in Patients on Hemodialysis (HD) Anne B. Froment, Virginia M. Newton, Susan L. Hogan, Yichun Hu, Aysenil Belger, Patrick H. Nachman. *University of North Carolina, Chapel Hill, NC.*

Background: Cognitive impairment is common among patients on HD. We aimed to identify risk factors for impairment by performing a longitudinal analysis of change in cognitive function. We report on baseline evaluation and analysis of association with dialysis-related variables.

Methods: Patients underwent tests for each of attention/concentration, visual learning/memory, verbal learning/memory and executive function domains. Each test score was converted to a *t* score adjusted for age ± education according to published norms (the mean ± SD *t* score for a healthy population is 50 ± 10). An average *t* score was calculated for each cognitive function domain. Spearman correlation analysis was performed to explore influences of age, sex, dialysis vintage, ekdrt/v, PTH, blood pressure, and dialytic change in blood pressure and serum Na with each of the 4 domains.

Results: 33 patients (39% males, 65.0 ± 8.4 yo, 61% AA, 63% with diabetes, 40% college educated, median time on dialysis 29.3 months (IQR 12.7-62.5) underwent neurocognitive testing. The mean ± SD (IQR) *t* scores were 41.9±7.5 (35-48) for attention/concentration, 42.7±11.1 (34-48) for verbal learning/memory, 43.6±9.5 (37-49) for visual learning/memory and 40.4±10.1 (32-49) for executive function. 27% of tested patients had impairment in attention, 25% in verbal memory, 22% in visual memory and 45% in executive function. Verbal and visual learning/memory were negatively correlated with dry weight (r= -0.37, p=0.03 and r=-0.37, p=0.04 respectively). Executive function correlated negatively with post dialysis standing systolic pressure (r=-0.55, p=0.005) and correlated positively with time on dialysis among for patients on dialysis > 25 months (n=15) (r=0.82, P <0.0001), but not for those on dialysis ≤ 25 months (n=17) (r=-0.33, P =0.2).

Conclusions: These tests provide a detailed analysis of cognitive function in patients on HD. Decreased executive function is the most commonly detected impairment and is associated with post-dialysis hypertension. The association between decreased executive function and long vintage on HD may reflect a selection bias based on patient survival or willingness to participate in the study.

Funding: Other NIH Support - CTSA UL1RR025747

FR-PO269

Longitudinal Study of Change in Cognitive Function in Patients on Hemodialysis (HD) Anne B. Froment, Virginia M. Newton, Yichun Hu, Aysenil Belger, Susan L. Hogan, Patrick H. Nachman. *University of North Carolina, Chapel Hill, NC.*

Background: Cognitive impairment is common among patients on HD. We aimed to identify dialysis-related risk factors for cognitive impairment in patients (age 50-80 yrs) on HD by performing a longitudinal analysis of change in cognitive function. We describe changes in cognitive function over 1 year of HD.

Methods: Patients underwent tests of attention/concentration, visual learning/memory, verbal learning/memory and executive function at baseline and repeated after 1 year. Test scores were converted to *t* scores, adjusted for age ± education according to published norms (the mean ± SD *t* score for a healthy population is 50 ± 10). p values for change in average *t* score for each cognitive domain were calculated by paired signed rank test. When significant change in a cognitive function domain was detected, Spearman correlation analysis was performed to explore influences of age, sex, dialysis vintage, and baseline ekdrt/v, PTH, blood pressure, and dialytic change in serum Na with change in function.

Results: 22 patients underwent baseline and repeat testing after 1 yr (59% female, 65.86±8.77 y.o.; 63% AA; 59% with diabetes; 36% college educated). No significant change was detected in attention/concentration or executive function. There was a small increase in verbal learning/memory, but a significant decrease in visual learning/memory. The latter was not associated with any of the baseline variables.

	Baseline	Change T2-T1*	P value**
Attention/Concentration	42.1±8.8	-1.0±5.4	0.24
Verbal Learning Memory	47.5±11.1	3.7±6.9	0.016
Visual Learning Memory	36.8±10.1	-5.2±7.8	0.008
Executive Function	42.8±9.1	-2.0±8.0	0.52

*paired difference. **p values were calculated by paired signed rank test.

Conclusions: These tests allow for a detailed analysis of change in cognitive function in patients on HD. The small increase in verbal memory may be attributable to learning from repeated testing. The decline in visual learning/memory was most marked, and was not previously reported. Repeat testing after longer follow up, a larger cohort and additional tests of visual memory are needed to detect the impact – if any- of dialysis-related factors on progressive decline in cognitive function.

Funding: Other NIH Support - CTSA UL1RR025747

FR-PO270

Cognitive Performance before and during Hemodialysis: A Randomized Crossover Trial David A. Drew, Hocine Tighiouart, Tammy Scott, Saeed K. Shaffi, Daniel E. Weiner, Mark J. Sarnak. *Tufts Medical Center, Boston, MA.*

Background: Dialysis patients are educated and counseled during the hemodialysis (HD) procedure. There are few data assessing whether cognitive performance varies with testing during dialysis.

Methods: 40 patients from 6 Boston area HD units were randomly assigned using a cross-over design to detailed cognitive testing. Sequences were as follows: Testing 1 hour before dialysis followed by repeat testing during the 1st hour of dialysis (n=21) and testing during the 1st hour of dialysis followed by 1 hour before dialysis (n=19). To limit learning there was a wash-out period of 1 month before crossing over for the second cognitive test. Mixed models were used to evaluate the effect of timing (difference between testing before vs during dialysis) and learning (difference between 1st and 2nd tests).

Results: Mean age was 58.1 years; 60% were female, 23% African American, and 56% had some college education. While a learning effect was detected in 4 of 16 tests (predominantly memory tasks), cognitive assessment before vs during HD had no effect on test performance(Table 1).

Table 1. Effect of Learning and Timing of Testing on Cognitive Performance

Test	Learning Effect β	p	Timing Effect β	p
Mini-Mental State Exam	-0.279	0.43	0.089	0.80
Verbal Intelligence	-0.146	0.79	-0.881	0.23
Delayed Recall	**-0.907**	**0.003**	0.145	0.61
Short Delayed Recall	**-0.813**	**0.005**	-0.234	0.40
Total Recall	**-2.914**	**0.0001**	-0.229	0.74
Recognition	-0.120	0.78	0.406	0.35
Blocks Design	-1.587	0.09	0.026	0.89
Digits Forward	0.287	0.28	-0.239	0.36
Digits Backward	0.032	0.91	0.111	0.71
Digits Total	0.353	0.31	-0.183	0.64
Digit Symbol Substitution	**-3.269**	**0.01**	1.889	0.14
Trails A†	2.385	0.53	-1.194	0.76
Trails B†	1.813	0.84	-8.766	0.29
Mental Alternation	-0.464	0.60	0.787	0.36
COWAT Animal	-0.868	0.30	0.470	0.59
COWAT Supermarket	0.654	0.57	0.917	0.42

Learning effect = Difference in scores for 1st time taking test and 2nd time taking test
Timing effect = Difference in scores for testing before and during dialysis
For all tests except Trails A and B, a negative β coefficient is consistent with learning, while for Trails A and B a positive β coefficient indicates learning
Similarly, for all tests except Trails A and B, a positive timing β coefficient suggests before performance is superior

Conclusions: We found no difference in cognitive performance depending on the time of testing, suggesting that cognitive tests performed during dialysis are a valid assessment of consistent performance.

Funding: NIDDK Support, Other NIH Support - T32 Training Grant

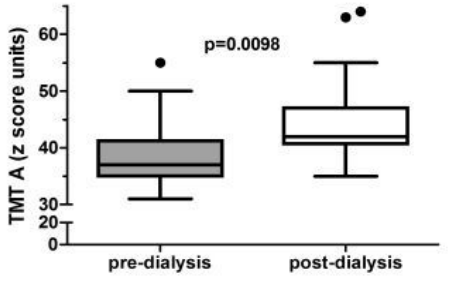
FR-PO271

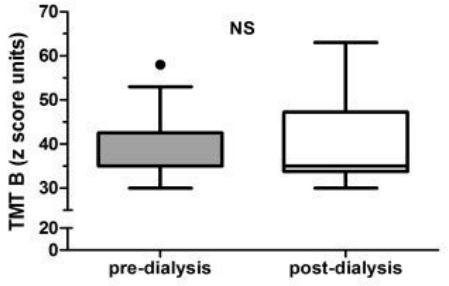
Effect of a Single Dialysis Session on Cognitive Function in CKD5 Patients: A Prospective Clinical Study Sabrina Schneider, Anne Kathrin Malecki, Hermann G. Haller, Olaf Boenisch, Jan T. Kielstein. *Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany.*

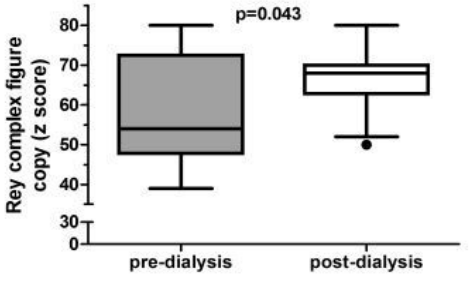
Background: Cognitive function is impaired in CKD5D patients. The potential effect of a single dialysis function on cognitive function reamins still ellusive. im of the study was to assess cognitive function using a wide test battery and avoiding exclusing effects of circadian variations.

Methods: Twentyfive (11 female) CKD5D patients (54 ±12 years, dialysis vintage 4.3±5.7 years) were enrolled. Cognitive testing was performed 1 h prior to dialysis as well as 24 h thereafter including assessment of memory, attention and concentration, executive functioning and psychomotor speed by using the following tests: Rivermead behavior memory test (RBMT), Rey Cpmplexe Figure Test (RCFT), Trail Making Test A+B (TMT), Wechsler Memory Scale (WMS- R), Behavior Assessment of Dysexecutive Syndrome (BADS), Regensburger word fluency test (RWT) and test battery for attention (TAP).

Results: A single dialysis session lead to a significant improvement in logical and visual memory (RBMT [pre: 7.5 and 6 digits/ post: 8.5 and 8 digits] and RCFT [pre:32.5 digits/ post:48 digits]) psychomotor speed and concentration (TMT A), while task switching (TMT B) did not improve.







TESTS	PRE- dialysis (median ± SD)	POST- dialysis (median ± SD)	p
Story RBMT (words)			
recall	7.5 (2.6)	8.5 (3.8)	0.002*
delay	6 (2.5)	8 (3.6)	0.001*
Wechsler Digit Span			
foreward	8 (1.4)	8 (1.9)	0.055
backward	6 (2.0)	6 (1.6)	0.266
Trail Making Test (seconds)			
Trail A	46 (13.7)	40 (12.1)	0.002*
Trail B	108 (38.2)	93 (44.9)	0.726
RWT (words)			
lexical	12 (4.2)	17 (5.8)	0.000*
lexical - change	16 (4.4)	17 (5.9)	0.764
semantic	28 (9.0)	31 (8.4)	0.272

Conclusions: Our data demonstrate improvements in memory functions, executive functions and psychomotor abilities after a single dialysis session, pointing to a reversible component of cognitive impairment in CKD5D.

FR-PO272

Cognitive Impairment Is Common among Malaysian Hemodialysis Patients

Li Ping Tan,[1] Nur Syazwani Jamhuri,[1] Cathie Wu,[1] Soo Kun Lim,[1] Kok Peng Ng,[1] Tee Chau Keng,[1] Yip-Boon Chong,[1] Wai Yew Kong,[1] Chew Ming Wong,[1] Wan Ahmad Hafiz Wan Md Adnan,[1] Mun Hoe Wong,[1] Abdul Hafidz Muhammad Iqbal,[2] Li Han Lim,[1] Maisarah Jalalonmuhali.[1] *[1]Medicine, University of Malaya Medical Center, Kuala Lumpur, Wilayah Persekutuan, Malaysia; [2]Medicine, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.*

Background: Cognitive impairment is common among hemodialysis patients with a reported frequency between 16%-37%. Little is known about the factors that contribute to cognitive impairment. Limited asian data exist with most studies being in western populations. The Montreal Cognitive Assessment test (MOCA) is a 30 point cognitive screening test that has been validated for the detection of cognitive impairment, is easy to administer and available in many languages.

Methods: All chronic hemodialysis patients from 5 dialysis centers in the Klang Valley were considered for inclusion. Patients were excluded if they were not able to communicate fluently, had dementia or confusion. All patients were administered the MOCA test by a single trained individual. MOCA scores below 26 were considered as positive for cognitive impairment. Medical charts were reviewed and most recent clinical data was extracted. Data was analyzed with SPSS (version 19.0).

Results: 48 patients were recruited. Mean age was 54.33±10.2 years. 56.3% were male. Ethnicity was mixed with 54.2% Malay, 33.1% Chinese and 10.4% Indian. Etiology of kidney disease was predominantly diabetes (47.9%). 47.9% had only 12 years of schooling. Mean duration of dialysis was 5.4±3.72 years. the MOCA identified 85.4% as having cognitive impairment. Mean scores for the entire cohort was 21.5±4.2. Serum sodium, hemoglobin, calcium, phosphate, iPTH and dialysis duration did not differ amongst those with and without cognitive impairment. Measurement of dialysis adequacy did trend towards significance with kt/v of 1.43±0.45 in those with cognitive impairment while those without had kt/v measurements of 1.92±1.0 (p=0.05).

Conclusions: Cognitive impairment is common amongst hemodialysis patients in Malaysia. Increasing kt/v may result in improved cognitive function. Further studies are needed to confirm this finding.

FR-PO273

Determining Patient Factors Influencing the Switch from Conservative to Dialysis Therapy

Christopher Cheang Han Leo, Tsun Gun Ng. *Renal Medicine, Tan Tock Seng Hospital, Singapore.*

Background: Although not reported in the literature, there appears to be an emerging trend of End Stage Renal Disease (ESRD) patients initially declining dialysis, but subsequently opting for dialysis after referral to Palliative Care Services (PCS). We sought to determine factors that may have contributed to their initial apprehension for renal replacement therapy and to identify aspects of pre-dialysis assessments that may improve the decision making process.

Methods: 273 ESRD patients in a single centre (mean age 73.0±12.0 years, 76% Chinese, 57% Female) who declined dialysis and were referred to PCS from January 2006 to April 2010 were studied. Patients who subsequently underwent dialysis were identified and data extracted from their medical records.

Results: 26 (9.5%) patients on follow-up with PCS subsequently agreed for long-term dialysis (mean age 63.0±7.3 years, 69% Chinese, 58% Male). Most were married (85%) with children (81%) and lived with their families (92%). 80% were ADL independent, 62% were community ambulant without aid and mean Charlson Comorbidity Index score was 5±1, at the time of PCS referral. 35% had consulted a nephrologist <6 months before the PCS referral. All patients were offered dialysis initially but 32% declined formal dialysis counselling. The mean serum creatinine (umol/L)/ eGFR (ml/min/1.73m^2) was 814.4±390.2/5.7±4.1 at the time of PCS referral and 1071.1±352.8/4.5±1.7 at dialysis initiation. 70% of them were initiated on dialysis within 3 months of a PCS consult. Reasons for declining dialysis initially were financial concerns (41%), personal choice (37%) and fear of needling/procedures (15%). Reasons for opting for dialysis subsequently were worsening symptoms (65%) and persuasion by family members (15%). 6-months survival post dialysis initiation was 100%.

Conclusions: Younger ambulant patients with moderate co-morbidities and good family support overcame initial apprehension for dialysis once they became more symptomatic. These unplanned dialysis initiations could be avoided by employing a multidisciplinary approach including early access to a nephrologist, dialysis counsellor, social worker, patient support groups and active family involvement.

FR-PO274

Attitudes and Perceptions of Dialysis Patients to Advance Care Planning

Maria Da Silva-gane,[1] David Wellsted,[2] Ken Farrington.[1] *[1]Renal, Lister Hospital, Stevenage, Hertfordshire, United Kingdom; [2]Centre for Lifespan & Chronic Illness Research, University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.*

Background: The needs of HD patients who are approaching the end of their life are often unaddressed. We wished to understand the attitudes of patients, perceived as 'failing despite dialysis', to dialysis and to the concept of advance care planning (ACP).

Methods: 20 HD patients previously identified, by use of the "surprise question" were interviewed. Interviews were carried out using a semi-structured schedule and took place in the patient's home. They were audio taped and transcribed, before analysis using Grounded Theory.

Results: Mean age 68years (range 47-88). 12 were male. Dialysis vintage 1-19 years (mean 6). 16 were considered to have severe co-morbidity.

Themes identified.

Complex relationship with dialysis. Despite the negative impact, sense of loss and the limited life experienced on dialysis, the treatment had become such an integral part of their life that it had almost been absorbed into their being. Most had never considered withdrawal from treatment as a future option.

Incongruous perception of prognosis.There a lack of insight into their situation. Most did not perceive themselves as terminally ill. There was an assumption that HD would keep them alive indefinitely, in spite of experiencing increasing physical deterioration. The degree to which patients deployed 'non-engagement' as a coping mechanism was not clear.

Words can be barriers. Many interpreted matters relating to 'end of life' as pertaining to private decisions about wills and funeral arrangements. Having overcome these barriers, many regarded ACP in relation to life goals and future options as being potential beneficial.

Conclusions: Patients approaching end of life have a complex relationship to their treatment. Many professed to have little insight of their prognosis, and few had considered withdrawal. Most articulated the wish for sensitive realistic communication including appropriate and individualised prognostic information. ACP discussions were perceived as being useful if set in the context of current health status, realistic treatment and life goals.

FR-PO275

Withdrawal of Care in Patients Receiving Continuous Renal Replacement Therapy (CRRT) in Intensive Care Units (ICU) Emily S. Kenner,[1] Veronika Glushets,[1] Karen Allard,[1] Anthony Leonard,[1] Charuhas V. Thakar.[1,2] *[1]University of Cincinnati; [2]Cincinnati VA.*

Background: Up to 20% of Americans spend their last days in ICU. In ICU patients receiving CRRT, deliberations regarding prognosis/end of life (EOL) care should occur based on disease severity and evidence of futility of further treatment. No studies have examined patient factors/physician practices associated with withdrawal of care (WOC) in CRRT patients in ICU.

Methods: From a tertiary care academic center, we report data (part of a 5-year project) examining EOL care practices in CRRT patients in ICU. We extracted 64 variables (details of ICU admission, CRRT and events before death/WOC). Characteristics were compared in those who died, by WOC status, using Chi-square and Wilcoxon rank-sum tests.

Results: In 2011, 83/206 (40%) patients receiving CRRT died. 12 were excluded (transplants/missing data), 71 analyzed. 69% were admitted to Medical and 31% to Surgical ICU; infection the diagnosis in 32%. Sample was 62% male, 50% White with mean age 56.8 years [standard deviation (SD) 15]. Co-morbidities included diabetes (45%), end stage renal disease (21%), cirrhosis (17%) and malignancy (13%); 93% required mechanical ventilation. Mean ICU stay - 18.2 days (SD 26), mean dialysis days - 6.7 (SD 6); 28% received both CRRT and intermittent dialysis. Of those who died, futility was documented in 47 (68%), EOL discussion occurred in 59 (83%) and care withdrawn in 52 patients (73%). Of the 19 without WOC, 10 had futility noted and EOL discussions. From the initial documentation of futility, time to death was 2.1 days (SD 5.2). EOL discussions occurred an average of 1.2 days (SD 4.2) before WOC. WOC patients received 87 cumulative days of dialysis after EOL discussion. 28% of patients had a catastrophic event (e.g. cardiac arrest) within 48 hrs of death. Only 18% had palliative care team involved. Patient characteristics were similar when compared by WOC status.

Conclusions: WOC resulting in death was common in CRRT patients and was preceded by a catastrophic event in 28% of subjects. Better predictors of mortality in dialysis subjects and appropriate involvement of palliative care in ICU may avoid unnecessary suffering and impact costs of care.

Funding: Clinical Revenue Support

FR-PO276

Should We Still Focus that Much on Cardiovascular Mortality in End Stage Renal Disease Patients? The CONvective TRAnsport STudy (CONTRAST) Claire H. Den Hoedt,[1,2] Michiel Bots,[3] Muriel Grooteman,[4] Albert H. Mazairac,[2] Erik L. Penne,[4] Neelke C. Van Der Weerd,[4] Pieter M. Ter Wee,[4] Menso Jan Nube,[4] Renee Levesque,[5] Peter J. Blankestijn,[2] Marinus A. Van Den Dorpel.[1] *[1]Maasstad Hospital, Rotterdam; [2]UMCU, Utrecht; [3]UMCU, Utrecht; [4]VU MC, Amsterdam, Netherlands; [5]Centre Hospitalier de l'Université de Montréal, Montréal, Canada.*

Background: We studied the distribution of causes of death in the CONTRAST cohort and compared the proportion of cardiovascular (CV) deaths with other populations to answer the question whether CV mortality is still the principal cause of death in end stage renal disease (ESRD). In addition, we compared patients who died from CVD with those who died from non-CVD. Finally, we aimed to clarify reasons for dialysis withdrawal.

Methods: We used data from CONTRAST, a randomized controlled trial in 714 chronic hemodialysis (HD) patients (age 64 ±14, 62% men, median vintage 2 yrs, 25% DM) on the effects of online hemodiafiltration versus low-flux HD. Causes of death were adjudicated. The distribution of causes of death was compared to that of the Dutch dialysis registry and of the Dutch general population.

Results: In CONTRAST, 231 patients died on treatment. 32% died from CVD, 22% due to infection and 23% because of dialysis withdrawal. These proportions were similar to those in the Dutch dialysis registry and the proportional CV mortality was similar to that of the Dutch general population. CV death was more common in patients <60 years. Patients who withdrew were older, had more co-morbidity and a lower mental quality of life at baseline. Patients usually withdrew from dialysis for a combination of disease conditions. 46% died within 5 days after the last dialysis session.

Conclusions: Although the absolute risk of death is much higher, the proportion of CV deaths in an ESRD population is similar to that of the general population. In older HD patients CV and non-CV death risk are equally important. Withdrawal from dialysis was much more frequent than anticipated. In view of the importance of withdrawal from dialysis in daily practice, well-defined criteria for the registration of dialysis withdrawal are warranted. More insight in the incidence and risk factors for dialysis withdrawal is urgently needed.

Funding: Pharmaceutical Company Support - Fresenius Medical Care (The Netherlands) and Gambro Lundia AB (Sweden). Roche Netherlands; the International Society of Nephrology/Baxter Extramural Grant Program

FR-PO277

Transplant Professionals' Views on the Participation of Compatible Pairs in Kidney Exchange Programs Marie-chantal Fortin,[1,2] Céline Durand.[2] *[1]Nephrology and Transplant Division, CHUM, Montréal, QC, Canada; [2]Research Center of CHUM, Montréal, QC, Canada.*

Background: Kidney exchange programs (KEPs) facilitate living kidney transplantation for incompatible pairs. While such programs can increase living organ donation, they also pose challenges in terms of distributive justice. Kidney transplant recipients (R) in the O blood group are at a disadvantage when it comes to KEPs, because they can only receive organs from O donors (D) whereas the latter are universal D. A way to remedy this situation is through altruistic unbalanced paired kidney exchange (AUPKE), in which a compatible pair consisting of an O blood group D and a non-O R is invited to participate in a KEP. The aim of this study was to gather empirical data on how transplant professionals view AUPKE.

Methods: 19 transplant professionals working in 4 Canadian transplant programs took part in semi-structured interviews between 11/2011 and 05/2012. The content of these interviews was analyzed using the qualitative data analysis method.

Results: 89% of the transplant professionals were willing to propose AUPKE to compatible pairs. They noted the societal benefits of AUPKE (solution for O blood group R; transplantation of more patients; reduction of costs associated with dialysis), as well as risks for the patient and D (travel of D for procurement; transplantation delayed while waiting for a compatible R, etc.), and disadvantages for medical professionals (more work and logistical issues). All of the transplant professionals (except for two) felt comfortable about discussing the possibility of AUPKE with their patients. However, they also stressed the importance of not pressuring patients and providing compatible pairs with comprehensive information in order to secure their informed consent.

Conclusions: AUPKE appeared to be an acceptable option for the Canadian transplant professionals who participated in this study, since it is a way to increase the number of transplantations and make more organs available for O blood group R. However, the systematic recruitment of compatible pairs did not seem appropriate, since it would not benefit all compatible pairs, and there is also a risk of medical teams pressuring patients to participate.

Funding: Government Support - Non-U.S.

FR-PO278

The Experiences of Commercial Kidney Donors: Thematic Synthesis of Qualitative Research Allison Tong,[1,2] Jeremy Chapman,[3] Germaine Wong,[3] Nick Cross,[4] Pikli Batabyal,[2] Jonathan C. Craig.[1,2] *[1]Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, NSW, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, NSW, Australia; [4]Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.*

Background: Commercial transplantation has expanded because of the major shortage of kidneys for transplantation, such that waiting times can exceed many suitable patients' life expectancy. This study aims to synthesize qualitative studies on the experiences and perspectives of commercial kidney donors.

Methods: We conducted a comprehensive literature search in electronic databases to April 2011, and consulted experts in the field of commercial transplantation. Thematic synthesis was used to analyse the findings.

Results: Seven studies involving over 676 commercial kidney donors were included. We identified three major themes: desperation, the participants' decision to sell their kidney was forced by poverty or the need to repay a debt or to fulfil a family obligation; despair (destroyed body integrity, shame and secrecy, dehumanised and dispirited, loss of livelihood, heightened sense of vulnerability, disappointment and regret); and debasement (deception by brokers and recipients, victimised by the hospital, stigmatised by community, and rejected by family).

Conclusions: Commercial kidney transplantation can result in severe and often unexpected ramifications on the donors' mental, physical, and social well-being. Donors feel victimised, live in constant fear of their future health, and bear an overwhelming and pervasive sense of emptiness, shame, guilt, regret and depression. Effective implementation of both the WHO guiding principles and legislated regulation in almost all countries is urgently required to deter potential recipients and healthcare providers from pursuing commercial transplantation; to protect the unwitting, vulnerable and impoverished communities which are known sources for commercial transplantation.

Funding: Government Support - Non-U.S.

FR-PO279

Literacy Rates among Hemodialysis and Kidney Transplant Patients Zeeshan Perveze,[1] Amit M. Patel,[1] Hari Garapati,[1] Robert Podolsky,[1] Laura L. Mulloy.[1] *[1]Medicine, Georgia Health Sciences University, Augusta, GA; [2]Medicine, Georgia Health Sciences University, Augusta, GA; [3]Medicine, Georgia Health Sciences University, Augusta, GA.*

Background: ESRD is a life-long health problem commonly treated with hemodialysis (HD) or kidney transplantation (KT). The decision to pursue a KT depends in part on patient (pt) understanding of the causes, treatment and prognosis of both modes of therapy. Pt comprehension and understanding of these issues may influence decisions about the pursuit of KT. Information relevant to this issue is many times dependent on written material provided to pts. Given the necessity to comprehend a high volume of complicated issues

surrounding KT, often presented in writing, we theorized that transplant recipients would exhibit a higher rate of literacy than patients on HD. To address this question we surveyed HD and KT pts and assessed their reading and comprehension skills.

Methods: HD pts in the Augusta area and KT recipients from GHSU were studied. Reading comprehension was assessed using a 66-word recognition test which provides an estimate of reading skills in less than 3 minutes (Rapid Estimate of Adult Literacy in Medicine - REALM).

Results: 50 HD and 50 KT pts completed the study. All HD pts were from 3 HD units in Augusta. There were 27 dialysis units of origin represented in 43/50 KT pts with 22% from Augusta. Seven KT pts were from unknown dialysis units. When compared to KT, HD pts were older (55±13 vs 49±15 mean±SD years for HD vs KT, respectively, $p < 0.05$) and primarily African American (AA) (88% vs 48% for AA vs Caucasian, respectively, $p < 0.05$). There were no differences in the gender distribution between the two groups. KT pts scored significantly higher on the REALM test when compared to HD (96±6% vs 87±14% correct for KT vs HD, respectively, $p < 0.05$). These data indicate that literacy rates are higher among KT pts when compared to HD, and that HD pts tended to be AA.

Conclusions: KT pts have higher literacy rates than HD, and tend to be Caucasian. Socioeconomic, geographic, and/or educational factors may influence these results. Literacy programs focused on HD pts may improve the rate of KT.

FR-PO280

Fertility Preservation in Children Treated for Nephrotic Syndrome or Vasculitis (NS/V) with Cyclophosphamide (CPO) Steven D. Miller,[1] Yimei Li,[2] Kevin E.C. Meyers,[3] Jill P. Ginsberg,[2] Arthur Caplan,[4] Victoria A. Miller,[2] Tuua Ruutiainen,[4] Lindsay M. Griffin.[3] *[1]Pediatrics, Johns Hopkins University, Baltimore, MD; [2]Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; [3]Nephrology, Children's Hospital of Philadelphia, Philadelphia, PA; [4]Penn Center for Bioethics, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA.*

Background: Fertility preservation for children at risk for infertility from treatment with alkylating agents has become standard practice in pediatric oncology. Within pediatric nephrology, there are no similar standards when CPO is used for NS/V. This study explores current practice patterns among pediatric nephrologists.

Methods: Physicians on the ASPN listserv were sent a survey via email using REDCap. The survey queried participants about demographics, dosing of CPO, counseling, referral, and gamete banking as well as physician attitudes and reported barriers to practice. Descriptive statistics and Chi-squared analyses were employed to analyze the data using SAS.

Results: Of 579 ASPN members invited, N=186 completed the survey (32% response rate). CPO was dosed in mg/kg 23% of the time, g/m^2 40% of the time, and both 37% of the time. 80% agreed that pubertal M should be offered a fertility referral prior to treatment, while 53% report that they actually refer pts >=1% of the time. Those who never refer M pts are less likely to cite infertility as a concern for the doctor (p=0.029), more likely to cite 'lack of training' as a barrier (p=0.045), more likely to cite 'no place to refer' as a barrier (p<0.0001), and more likely to cite 'stays under limit of toxicity' as a barrier (p=0.003). Results were similar for F.

Conclusions: Survey results demonstrate variability in CPO dosing, referral and sperm banking of pts treated with CPO. This points to a divide in the field of pediatric nephrology between those who never preserve the fertility of their pts and those who do. Further research into long-term fertility effects of current dosing regimens of CPO and discussion of standard practice for fertility preservation in pediatric nephrology is needed.

Funding: Private Foundation Support

FR-PO281

Severe Cognitive Impairment Is Not a Contraindication to Pediatric Renal Transplantation Ashton Chen, Jen-Jar Lin. *Pediatrics, Wake Forest Baptist Health, Winston-Salem, NC.*

Background: Renal transplantation in patients with mental retardation and cognitive impairment is controversial. There are limited outcomes data in pediatric renal transplant recipients with severe cognitive impairment.

Methods: A retrospective chart review was performed of all children who underwent renal transplantation between January 1, 2002 and May 15, 2012. Patients were divided into two groups, those with severe intellectual or cognitive impairment (CI) prior to transplantation and those without. Demographic characteristics were compared between the two groups using Chi-square test. Patient survival and graft survival were compared between the two groups.

Results: There were N=67 patients who received a renal transplant during the study period. There were n=9 patients who received a renal transplant with CI and n=58 renal transplant recipients without CI. Patient characteristics of the CI group are shown in Table 1.

Table 1 Characteristics of Patients with Cognitive Impairment

Patient #	Etiology of Renal Disease	Etiology of CI
1	Congenital polycystic kidney disease	CVA/Temporal lobectomy
2	Echogenic small kidneys	Joubert Syndrome
3	Sepsis-related ATN	Hypoxic Ischemic Encephalopathy
4	Renal dysplasia	Uncertain
5	HUS	Bacterial meningitis & CVA
6	Renal dysplasia	Periventricular leukomalacia
7	FSGS	CVA
8	PUV	Down syndrome
9	FSGS	Dyskeratosis congenita

The majority of CI patients were non-verbal and required a full-time caregiver. All CI patients were on peritoneal dialysis prior to transplantation and 7 out of 9 patients received a deceased donor transplant. Patient survival at 1 year post-transplant was 100% in both the CI and non-CI groups. Graft survival at 1 year post-transplant was 100% in the CI group and 88% in the non-CI group (P=NS).

Conclusions: There is no contraindication to renal transplantation in pediatric patients with severe CI. These children can be considered as transplant candidates.

FR-PO282

Report of the Hyponatremia Registry, a Multi-Center, International Study of Current Treatment Practices for Hyponatremia Arthur Greenberg,[1] Joseph G. Verbalis,[2] Alpesh Amin,[3] Esteban Poch,[4] Joseph Dasta,[5] Keith Friend,[6] Volker Rolf Burst.[7] *[1]Nephrology, Duke University, Durham, NC; [2]Endocrinology and Metabolism, Georgetown University, Washington, DC; [3]Hospitalist Program, University of CA, Irvine, Orange, CA; [4]Hospital Clinic, Barcelona, Spain; [5]UT College of Pharmacy, Hutto, TX; [6]Otsuka America Pharmaceutical, Inc., Princeton, NJ; [7]Internal Medicine, Renal Division, University of Cologne, Cologne, Germany.*

Background: Hyponatremia (HN) is the most common electrolyte disorder of hospitalized patients (pts) and is an independent predictor of increased mortality. This registry documents currently used HN therapies, characterizes their efficacies, and assesses their impact on hospital resource utilization.

Methods: After informed consent or waiver, records of pts meeting entry criteria, principally adults with euvolemic or hypervolemic HN (serum sodium ([Na]) ≤130 mmol/L), were abstracted. Accrual to date approximates 90% of planned enrollment. Any pt with anomalous data undergoes adjudication to confirm that all inclusion and exclusion criteria were met.

Results: From September 2010 to May 2012, 3,157 pts were enrolled at 149 US and 89 EU sites; 1,838 had sufficient data for analysis. The mean±SD entry and discharge [Na] values were 126.3±7.0 and 131.7±5.1 mmol/L, respectively. The average length of stay was 10.3±9.4 days.

Demographics, Etiologies, Chronicity and Outcomes

Baseline Characteristics	*All Patients with evaluable data (n=1,838)*		*Patients who completed or did not require adjudication (n=482)*
	%		%
Male	46.90		52.70
Age ≥ 65 years	49.13		44.19
Etiology of HN			
SIADH	**40.49**		**33.82**
• Tumor		20.97	18.40
• CNS		8.53	8.59
• Drug Induced		18.67	22.70
• Pulmonary		16.51	15.95
• Other		18.81	21.47
• Unknown or Idiopathic		13.80	14.72
Heart failure	**32.00**		**44.61**
Cirrhosis	**14.81**		**23.44**
Nephrotic syndrome	**3.16**		**1.24**
More than one hypervolemia dx	**2.67**		**3.53**
Onset/Duration of HN			
Chronic HN[1]	41.19		45.85
HN as the admitting dx	24.48		28.42
HN developed during admission	76.82		82.74
HN on a prior admission	25.91		30.08
HN at discharge	41.29		52.28

Single therapy only - all patients (n=1,838)	*Mean rate of [Na] change (mmol/L per day of treatment)*	*Median LOS[2] (days)*	*% of pts whose correction was overly rapid[3]*
			%
No treatment (n= 535, 29%)	0.90	-	2
Any pharmacological agent[4] (n=77, 4%)	2.39	5	2
Vaptan only (n=28, 2%)	3.26	3	4
Any pharmacological agent, excluding vaptans (n=49, 3%)	1.62	5	0
Fluid restriction (n=348, 19%)	1.30	5	1
Normal saline (n=248, 13%)	2.92	4.5	4
Hypertonic saline (n=14, 1%)	9.36	4.5	29

[1]HN of more than 48 hours duration; [2]Length of Stay from the day treatment began; [3]Overly rapid defined as an increase in [Na] exceeding 12 mmol/L/24 h; [4]Conivaptan, tolvaptan, demeclocycline, urea, loop diuretic.

Conclusions: HN pts received no treatment 29% of the time; 19% received fluid restriction only. More than 40% still had HN at discharge. Among pts who received a single therapy, only 4% received a drug targeted at HN. Pts treated with hypertonic saline were most likely to experience overly rapid correction (29%). This registry is the largest study of HN to date, which will allow assessment of multiple aspects of currently employed therapies of hospitalized patients with HN.

Funding: Pharmaceutical Company Support - Otsuka America Pharmaceutical, Inc.

FR-PO283

Validity of Hospital-Wide Hyponatremia Treatment Protocol: A Quality Improvement Program in a Teaching Hospital Kumiko Shimasaki,[1] Masahiko Nagahama,[1] Fumika Taki,[1] Kenichiro Koitabashi,[1] Keita Hirano,[1] Yuki Heath,[1] Sachiko Ohde,[2] Yasuhiro Komatsu.[1] *[1]Nephrology, St.Luke's International Hospital, Tokyo, Japan; [2]Clinical Epidemiology, St. Luke's Life Science Institute, Tokyo, Japan.*

Background: Hyponatremia is the most common electrolyte disorder and is associated with increases in morbidity, mortality and length of hospital stay. There exits wide variation in clinical practice of hyponatremia management due to lack of specific guidance. Hospital wide systematic approach for the treatment of hyponatremia can be an excellent Quality Improvement (QI) Program, since it will improve patient outcome and lessen unnecessary workload. The aim of the present study is to assess and report our hyponatremia treatment QI program.

Methods: Panel of nephrologists, critical care physicians, and residents developed hyponatremia treatment protocol which specifies timing of nephrology consultation, initial physical and laboratory tests, intravenous fluid prescription (selection and rate), monitoring, and prevention of overcorrection.To evaluate the effectiveness of the QI program, quality indicators were measured, which include nephrology consultation rate, recovery time, and hospital stay, etc. We analyzed clinical and biochemical parameters of all patients admitted to St.Luke's International Hospital in Tokyo in 2012 (QI group). The same medical information in all admitted patients in 2007 was collected as historical comparison (Comparison group).

Results: There were 9,428 patients in Comparison group and 10,233 patients in QI group. Incidence of moderate hyponatremia (120≤serum Na≤135mEq/L) was 13.5% in comparison group and 11.2% in QI group, and incidence of severe hyponatremia (serum Na<120mEq/L) was 0.59% and 0.52%, respectively. Nephrology consultation rate for severe hyponatremia was 25.5% in Comparison group and 37.7% in QI group (P<0.05). Length of hospital stay was 33.9±30.3 (mean+/-S.D.) days in Comparison group and 16.5±15.3 days in QI group (P<0.05).

Conclusions: Our structured program shows safety and feasibility in managing hyponatremia. This underscores the importance of developing QI program, which can improve the quality of practice as well as education in a teaching hospital.

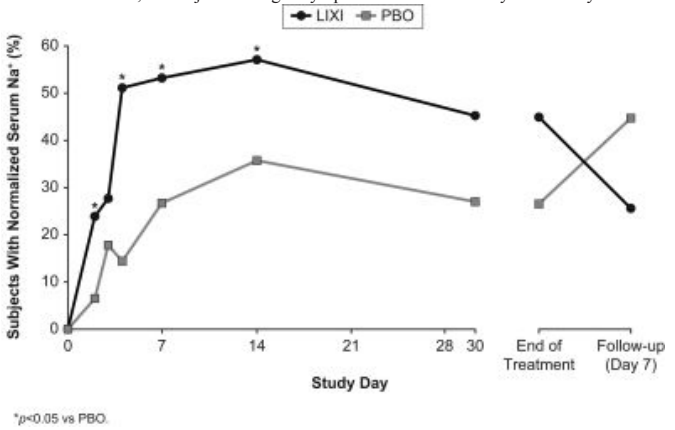
FR-PO284

Effect of Lixivaptan on Serum Sodium Levels in Subjects Initially Hospitalized with Euvolemic Hyponatremia William Abraham,[1] Johannes Hensen,[2] Peter Gross,[3] Daniel G. Bichet,[4] Richard Josiassen,[5] Deodatta Chafekar,[6] Cesare Orlandi.[7] *[1]The Ohio State Univ; [2]Klinikum Hannover-Nordstadt; [3]Uni.Klinikum C.G. Carus; [4]Université de Montréal; Hôpital du Sacré-Coeur; [5]Drexel Univ Col of Med; [6]Shri Samarth Hosp; [7]Cardiokine Biopharma, Inc.*

Background: Lixivaptan (LIXI) was previously shown to increase serum Na^+ levels in subjects with euvolemic hyponatremia (EH) (ASN 2010). We further assessed the rapidity, degree, predictability, and safety of this increase in hospitalized subjects with EH.

Methods: In this multicenter, randomized, double-blind study (LIBRA), subjects received oral LIXI 50mg (n=54) or placebo (PBO; n=52) once daily, with subsequent dose titration (25-100mg) based on serum Na^+ levels. Fluid restriction was implemented at the investigator's discretion. Treatment period: 30 days. Initial titration was in an inpatient setting; subjects were then treated as outpatients.

Results: Mean baseline serum Na^+ was 127.6 (LIXI) and 126.1mmol/L (PBO). Least-squares mean±SE changes from baseline to Day 7 (primary endpoint): 6.7±0.7 and 4.5±0.8mmol/L, resp. (p=0.034). The benefit of LIXI was consistent across gender/age groups; the magnitude of change in serum Na^+ increased with increasing baseline severity of EH. Normalization rates (serum Na^+ levels 135-145mmol/L) were greater with LIXI than PBO (53.2% vs 26.7% on Day 7, p<0.05; Figure). Kaplan-Meier analysis of time to first serum Na^+ level ≥135mmol/L favored LIXI (p=0.004); >50% of subjects achieved this Na^+ level by Day 4 (LIXI) or Day 30 (PBO). Few subjects had serum Na^+ levels >145mmol/L (4.0% LIXI, 2.0% PBO) or a rapid rise during titration (4.0% LIXI, 5.9% PBO). LIXI was well tolerated; no subject had signs/symptoms of osmotic demyelination syndrome.



Conclusions: LIXI normalizes serum Na^+ within 4 days in a majority of subjects with EH, with tolerability similar to PBO.

Funding: Pharmaceutical Company Support - Cardiokine Biopharma, Inc

FR-PO285

Hyponatremia Associated Hip Fracture Allen I. Arieff, Juan Carlos Ayus. *[1]Medicine, Univeresity of California, San Francisco, CA; [2]Nephrology, Renal Consultants of Houston, Houston, TX.*

Background: Hyponatremia is often associated with orthopedic injury (JAMA 281; 1999:2299-04). Hip fracture is a worldwide and frequently disabling orthopedic injury. The association with hyponatremia is unclear.

Methods: Hyponatremia as a possible cause of hip fracture was investigated by studying 76 patients who suffered hip fracture and were admitted to two tertiary medical centers. A) This group consisted of 25 acute cases of hip fracture who had hyponatremia (plasma Na < 130 mmol/L) and suffered hip fracture due to impaired balance and falls; B) This group consisted of 51 adult patients who were part of a retrospective analysis of 5 years of adult inpatient surgery. The data in Group B were generated by computer search of the hospital records using the SAS database to evaluate all surgical patients whose preoperative plasma sodium was below 130 mmol/L.

Results: There were 44,225 consecutive adult inpatient surgeries, of whom 7181 had measurement of preoperative plasma Na. Among these were 708 cases of hip fracture (9.9%). Fifty-one patients with hip fracture (7.2% of all hip fractures) had preoperative hyponatremia (plasma Na = 127±7 mmol/L) but this was present in less than 0.1% of all other types of surgery. Seventy - four of 76 hyponatremic hip fracture patients were female (age = 74±12 yrs). Among the 25 Group A patients, the plasma Na was 120±7 mol/L, all had normal renal function, 44% had osteoporosis, 44% had vitamin D deficiency and 31% were taking anti-depressants. The three-month all cause mortality among all 7181 postoperative patients was 1.8%, but among the 76 with hip fracture and hyponatremia, it was 5.8% (p < 0.01).

Conclusions: 1) Hyponatremia can lead to weakness, dizziness and falls with resulting hip fracture; 2) Data from 708 patients with hip fracture suggest that 7.2% of all hip fractures are associated with hyponatremia; 3) The all cause mortality among patients with hip fracture and hyponatremia is triple that of all other postoperative patients.

Funding: NIDDK Support

FR-PO286

The Incidence and Risk Factors for Terlipressin-Induced Hyponatremia in Liver Cirrhosis Patients Dong Jun Park, Yeojin Kang, Se-Ho Chang, Dae-hong Jeon, Hyejung Ha. *Internal Medicine, Nephrology, Gyeongsang National University Hospital, Jinju, Gyeong Sang Province, Korea.*

Background: As a prodrug of vasopressin, terlipressin has agonistic effects on the V1 receptor and partial agonistic effects on renal vasopressin V2 receptors. However, its effects on the serum sodium concentration are controversial.

Methods: This study retrospectively investigated 127 liver cirrhosis patients treated with terlipressin to examine the changes of the serum sodium level.

Results: Terlipressin was prescribed for the purpose of bleeding control (99) and management of hepatorenal syndrome (28). In all of the patients, theserum sodium level decreased from 134.04 ± 6.52 mmol/L to 130.39 ± 6.22 mmol/L during or after the terlipressin treatment (*P < 0.001)*. In 45 patients (35.4%), the serum sodium concentration decreased by more than 5 mmol/L from 137.853.62 mmol/L to 128.49 4.16 mmol/L: in 29 patients (22.8%), by 5 to 10 mmol/L; and in 16 patients (12.6%), by greater than 10 mmol/L. In the latter group, 5 patients showed neurologic manifestations. After the withdrawal of terlipressin, the serum sodium level improved spontaneously to be within the normal range with the restoration of neurologic manifestations. In the univariate analysis it was also found that patients with the normal or near-normal baseline serum sodium level were at high risk of hyponatremia and the initial sodium level and hepatic functions represented with the MELD and Child-Pugh score were significantly associated with the terlipressin-induced reduction of serum sodium. In multivariate analysis, it was found that the initial sodium level was the most powerful predictor of the terlipressin-induced reduction of serum sodium.

Conclusions: An acute reduction of the serum sodium concentration is not uncommon during terlipressin treatment, and the baseline serum sodium level is closely related to the reduction of the serum sodium concentration.

FR-PO287

Use of Serum Albumin (ALB) to Identify Colloid-Related Artifact of Serum Sodium (Na) Measured by the Indirect Ion-Selective Electrode (ISE) Method: A Non-Linear Effect Isabelle Ayoub,[1,2] Reisha T. Browne,[1] Robert H. Barth,[1] Philip Goldwasser.[1] *[1]VA NY Harbor Healthcare System; [2]SUNY Downstate, Bkly, NY.*

Background: When the serum colloid level is lower than normal, the measurement of Na by the usual indirect ISE method (iNa) tends to be artifactually high—compared with direct ISE (dNa) used in gas panels—while above normal serum colloid results in the opposite. One study reported that the iNa artifact can be estimated with a linear regression equation based on ALB, a routinely measured colloid (Story '07), while another found the ALB effect to be nonlinear (Dimeski '05).

Methods: To test this, we (i) paired chemistry and gas panels obtained < 20 minutes apart in a retrospective cohort of all patients (pts) admitted to our critical care units during a 1 year period, (ii) calculated iNa - dNa (NaDIFF) and the inter-panel glucose difference

(GluDiff), and (iii) examined the influence of ALB on NaDIFF, adjusting for GluDiff. Limiting each pt to one pairing and excluding hemolyzed or turbid samples left 190 iNa/dNa pairs.

Results: Significant inter-panel differences were found (NaDIFF [mean±sd]: 1.9±2.0 mM, $p<10^{-28}$; GluDiff: -7.2±25 mg/dL, $p=10^{-4}$) implying calibration bias. NaDIFF correlated weakly with ALB (r=-0.14 p<.06) and GluDiff (r=-0.13 p<.09), and not at all with iNa + dNa (r=0.05). A non-linear effect of ALB was suggested by the trend of NaDIFF means versus ALB grouped into categories (TABLE).

ALB Category	N	ALB, g/dL	NaDiff, mM (t-test)
A (< 2 g/dL)	4	1.7±0.1	4.5±1.3 (p≤01 vs. B & C; P<.001 vs. E)
B (2-2.9 g/dL)	31	2.5±0.2	1.8±1.8
C (3-3.9 g/dL)	71	3.5±0.3	2.0±1.9
D (4-4.5 g/dL)	59	4.2±0.2	2.2±1.9
E (> 4.5 g/dL)	25	4.8±0.2	0.6±2.1 (p≤001 vs. A, C, & D)

ANCOVA testing of ALB category, modeled as a polynomial, revealed significant (p<.001) linear and cubic components, adjusting for GluDiff (slope= -1.2 mM per 100 mg/dL; p<.04).

Conclusions: In summary, three sources of discordance between iNa and dNa were detected: calibration bias; GluDiff; and colloid-related artifact, for which a nonlinear function of ALB may be a useful estimate. When faced extreme values of ALB, confirming iNa with dNa is prudent.

Funding: Veterans Administration Support

FR-PO288

A G2 Cell Cycle Arrest of Proliferating Principal Cells May Explain the Loss of These Cells in Lithium-Induced Nephrogenic Diabetes Insipidus

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Background: Vasopressin-induced plasma membrane expression of the Aquaporin-2 (AQP2) water channel is essential for urine concentration. Lithium, the drug of choice for the treatment of bipolar disorders, affects the urine concentrating ability, resulting in ~20% of patients with nephrogenic diabetes insipidus (NDI), a disorder characterized by polyuria and polydipsia. Studies with rats demonstrated that lithium-induced NDI is caused by downregulation of AQP2 on short term (< 5 days) and loss of principal cells on long term (1-4 weeks). Surprisingly, lithium treatment increased the number of principal cells positive for the proliferation marker PCNA. The aim of our study was to identify how lithium-induced principal cell proliferation can lead to its loss.

Methods: To this end mice were treated for 0, 4, 7 and 10 days with lithium and housed in metabolic cages to analyze urinary parameters. Kidneys were analyzed using immunoblotting and immunohistochemistry.

Results: In line with earlier data, lithium treatment resulted in a significantly increased urine volume, decreased urine osmolality, and diminished AQP2 abundance. Analysis of urine, collected during treatment, did not reveal principal cell debris, indicating that principal cells are not shed and lost via the urine. PCNA immunostaining demonstrated that lithium induced proliferation of mainly principal cells in the papilla base starting at 4 days and being most prominent at 7 and 10 days. Additional immunostaining revealed that at 7 days of lithium treatment, around 35% of the proliferating principal cells exhibited a foci-like staining for pHiston-H3, indicative for late G2 phase. As this is significantly higher than the number of pHiston-H3 positive cells during normal proliferation (< 20%), our data indicate that lithium arrests principal cells in the G2 cell cycle phase.

Conclusions: In conclusion, our data indicate that lithium treatment initiates proliferation of renal principal cells, but that these are arrested in the G2 cell cycle phase, thereby halting further proliferation.

FR-PO289

Pseudohyperkalemia with Fist Clenching: A Graded Phenomenon

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Background: Pseudohyperkalemia is a false elevation of serum potassium concentration (S_K). Unrecognized, it can lead to potentially harmful treatment. Fist clenching with phlebotomy can cause pseudohyperkalemia, but the determinants of its magnitude are unknown. We hypothesized that the increase in S_K is proportional to the force of muscle contraction.

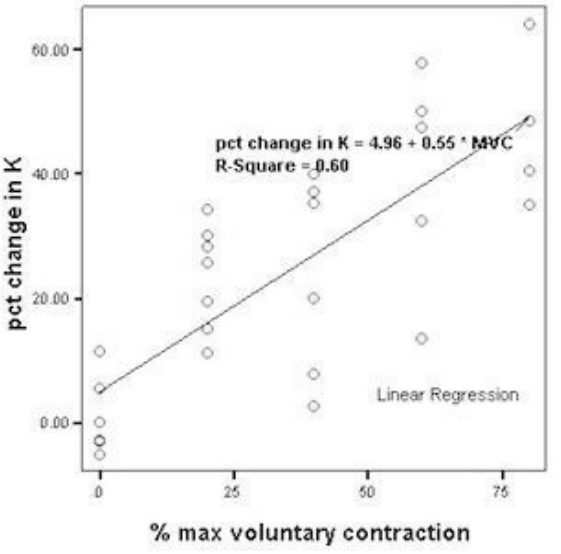
Methods: We studied 8 healthy adult subjects. Maximum voluntary contraction (MVC) was determined by squeezing a dynamometer with maximum force for one minute. We measured antecubital vein S_K at baseline and after one minute, under five conditions: tourniquet only; 20%, 40%, 60%, and 80% of MVC, with a one-minute washout in between. Serum was analyzed for potassium, sodium and bicarbonate using clinical laboratory methods. We analyzed differences with paired t-test; change over MVC, with one-way ANOVA and linear regression.

Results: Results of S_K (mEq/L, mean ± SD) are shown as baseline *vs.* final, by condition.

Serum K (mean±SD) baseline vs final

Condition	Baseline	Final	Difference	p-value
Tourniquet only	3.7 ± .2	3.6 ± .2	0 ± .2	.73
20% MVC	3.7 ± .3	4.5 ± .4	.9 ± .3	<.001
40% MVC	3.8 ± .3	4.6 ± .6	.9 ± .5	<.01
60% MVC	3.6 ± .2	5.1 ± .7	1.5 ± .7	<.01
80% MVC	3.8 ± .2	5.6 ± .6	1.5 ± .8	<.01

There was a linear increase in %-change S_K with increasing force of contraction (R^2 = 0.60; p <.001), which was not seen with serum sodium (R^2 = .01; p = 0.31) or bicarbonate concentration (R^2 = .01; p = 0.45).



Conclusions: We noted a clinically important graded rise in S_K with force of fist clenching. S_K rose ~25% with moderate magnitude of fist clenching (20-40% MVC).

Future studies will estimate the prevalence of significant pseudohyperkalemia, by assessing the strength of fist clenching during routine phlebotomy.

Funding: Clinical Revenue Support

FR-PO290

Endemic Severe Hypokalemia (HK) with Chronic Kidney Disease (CKD) in a Rural South Indian Population

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Background: Severe hypokalemic paralysis is rare; however we have observed this condition frequently at our hospital's emergency dept & report preliminary findings on a case series from our center in South India.

Methods: We describe the clinical and biochemical features of 90 consecutive patients (pts) from 2010-12. A case report form was used to collect data prospectively with pts followed through their hospital stay.

Results: Mean age (±SD) at presentation was 40±10 yrs (n=90,24 F). Presenting complaints were flaccid quadriparesis in 70, paraparesis in 4, muscle cramps in 15 & myalgia in 1. Nocturia was present in 50 & respiratory paralysis in 8. Mean MDRD eGFR was 79±59 ml/min/1.72m^2; 36 had eGFR <60ml/min/1.72m^2. 44% reported similar attacks in past. 1 pt died. Mean BP was 112±16 mm/Hg. Mean serum K+ at presentation was 2.5±0.05 mEq/L. Mean urinary K+ loss was 15±11 mEq/L. ABG analysis revealed metabolic acidosis in 10; 18 pts had severe metabolic alkalosis & developed carpopedal spasm following K+ correction. Almost half (48%) were agricultural laborers, 34% reported exposure to pesticides, 56% reported excessive sweating at work, and 13% had loose stool; all but 1 pt denied intake of drugs known to produce HK. Our center is located in South India with a tropical climate with average temperatures up to 39^{0}C from March to August. All pts lived in rural settings & were of low socioeconomic status. Rice was their staple diet, consumed thrice daily with low consumption of high K+ foods (meat, vegetables & fruits).

Conclusions: We report, for the first time from rural south India, a high incidence of hypokalemic paralysis possibly related to very low dietary K+ coupled with excessive loss in sweat. We hypothesize that extremely low dietary K+ coupled with excessive loss of K+ in sweat resulted in severe HK±CKD (likely from hypokalemic nephropathy). Further investigation into the epidemiology and etiology of this possible endemic is planned.

Funding: Government Support - Non-U.S.

FR-PO291

Low Dietary Potassium Intake Is Associated with an Increased Risk of Metabolic Syndrome in US Adults

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Background: High dietary potassium intake decreases blood pressure levels and decreases the risk of cardiovascular disease but the relationship between potassium intake and metabolic syndrome (MetS) has not been examined.

Methods: We performed a cross-sectional study using the National Health and Nutrition Examination Survey (1999-2007). A total of 25,754 US adult participants with dietary data were included in the analysis. Dietary potassium intake was calculated from 24-hour dietary recall obtained by trained interviewers. Potassium intake was examined

in quartiles 1-4 (≤1701, 1702-2415, 2416-3290, >3290 mg/day). The primary outcome was MetS defined according to recent guidelines from the National Cholesterol Education Program. Multivariate logistic regression models were used to examine the association between potassium intake and MetS.

Results: The mean (SE) age of participants was 45 (0.3) years. The mean (SE) potassium intake was 2718 (18) mg/day. Participants in the lowest quartile of potassium intake were more likely to be female, to be black, and to have lower years of education than subjects in the higher quartiles of potassium intake. 6,118 (23.8%) participants met the diagnostic criteria for MetS. Subjects with MetS were older, had higher systolic blood pressure and lower estimated glomerular filtration rate (eGFR) than subjects without MetS. After adjustment for age, sex, race, smoking status, body mass index, eGFR, and serum albumin, subjects in the first quartile of potassium intake had a 35% increased risk of MetS compared to subjects in the fourth quartile (OR 1.35, 95% Confidence Interval [CI] 1.16 to 1.57). Subjects in the second quartile of potassium intake also had an increased risk of MetS compared to subjects in the fourth quartile (OR 1.22, 95% CI 1.06 to 1.40).

Conclusions: Low dietary potassium intake is associated with an increased risk of metabolic syndrome in US adults. Interventional studies are needed to determine if increasing dietary potassium intake reduces the risk of metabolic syndrome.

Funding: NIDDK Support

FR-PO292

Dietary Potassium Intake and Risk of Metabolic Syndrome Hajeong Lee,[1] Jeonghwan Lee,[1] Nam Ju Heo,[2] Jin Suk Han.[1] *[1]Department of Internal Medicine, Seoul National University Hospital, Korea; [2]Department of Internal Medicine, Healthcare System Gangnam Center, Seoul National University Hospital, Korea.*

Background: Higher potassium intake is related to lower blood pressure and subsequent cardiovascular outcomes. Recent studies revealed that lower dietary potassium was associated with new onset diabetes. However, only few studies have investigated the effect of potassium intake on metabolic syndrome (MS).

Methods: To investigate dietary potassium intake in relation to risk of MS, the Korean National Health and Nutritional Examination Survey data from 2008 to 2010 were obtained. Among 29,235 participants, 19,193 adults who performed dietary survey were included. MS was defined as ATP III guideline. To estimate the insulin resistance (IR), homeostasis model assessment indices were calculated and their highest quartile was defined as IR group.

Results: Individuals with higher potassium intake showed lower risk of MS (*P=0.002*) in women, but not in men (*P=0.161*). After multivariate adjustment such as age, body mass index, smoking and alcohol intake, physical activity, education, income, frequencies of vegetables and fruit intake, and carbohydrate energy ratio, highest quartile group of potassium intake was associated with lower risk of MS (odds ratio [OR] 0.739; 95% confidence interval [CI] 0.626-0.873, *P<0.001*) in women. Among the 5 components of MS, this association was maintained only in the hypertriglyceridemia (OR 0.710, 95% CI 0.612-0.823, *P<0.001*). Especially, women without both diabetes and hypertension (OR 0.566, 95% CI 0.439-0.730, *P<0.001*) or with postmenopausal state (OR 0.728, 95% CI 0.597-0.886, *P=0.002*) showed more protective effect of potassium on MS. Furthermore, highest potassium quartile group was an independent protective factor for IR (OR 0.804, 95% CI 0.693-0.933, *P=0.004*). In men, potassium intake was not associated with MS or IR.

Conclusions: Our findings suggest that higher potassium intake is significantly associated with lower risk of MS in women of general population and IR is thought to be participated in the relationship. These results support the recommendations for higher consumption of potassium rich foods to prevent cardiovascular diseases in another aspect.

FR-PO293

Ion Selective Electrode and the Anion Gap (AG): What Should Be the Normal Gap? Rendell E. Manalo, Seyed-Ali Sadjadi, Navin Jaipaul, James I. McMillan. *Nephrology Section, Jerry L Pettis VA Medical Center, Loma Linda, CA.*

Background: For more than 30 years, US labs have used ion selective electrode (ISE) for measurement of serum electrolytes and it is well known that use of ISE is associated with higher serum chloride and lower serum AG. We and others reported on this issue over 20 years ago that the range of the gap is much narrower with ISE than with flame photometery technique. However major journals and textbooks continue to quote the old value of 12±4 meq/L for normal AG.

Methods: We retrospectively reviewed hospital records of 409 patients with eGFR ≥60 mL/min/1.73 m^2 BSA, 68 patients with ESRD, and 299 patients with serum lactate level ≥4 mmol/L in a single medical center. Data were collected and analysed on age, serum electrolytes, BUN, creatinine, albumin, and comorbidites of these patients.

Results: In patients with normal renal function and serum albumin, the mean serum AG was 7.2±2.0 meq/L, with 95% confidence interval (CI) of 3-11 meq/L. Assuming that the negative charge of albumin contributes 75% of the AG, every gram of serum albumin accounted for 1.35 meq/L of the gap. The mean anion gap was 12.4±3.2 meq/L in ESRD patients with mean serum albumin of 3.4 g/dL, and 12.8± 9.5 meq/L in patients with lactic acidosis and a mean serum albumin of 2.4 g/dL. We queried a few hospital labs and found that many of them do not have an established AG reference range of their own and use the manufacturer recommended AG of 10 to 20 meq/L.

AG in patients with normal kidney function, ESRD, and lactic acidosis

Condition	Number of patients	AG± SD, meq/L	Albumin, g/dL	AG albumin corrected, meq/L
Normal function	409	7.2±2.0	≥4.0	
ESRD	68	12.4±3.2	3.4	13.2
Lactic acidosis	299	12.8±9.5	2.4	15.0

Conclusions: 1) Mean serum AG in patients with normal kidney function is 7.2±2 meq/L with 95% CI of 3-11 meq/L. In ESRD and lactic acidosis, it is 12.4±3.2 meq/L and and 12.8±9.5 meq/L, respectively. 2) Every gram of albumin accounts for 1.35 meq/L of the gap, much lower than the current standard of 2.5 meq/L. 3) Many labs do not have an established range for AG. 4) Current levels for AG are too high and need to be lowered to alert clinicians to the presence of acid/base disorders.

Funding: Veterans Administration Support

FR-PO294

Detection of Novel Interstitial Large Deletions in the ATP6V0A4 Gene and Mutations in the ATP6V1B1 Gene in Patients with Distal Renal Tubular Acidosis Ken-ichiro Miura,[1] Takashi Sekine,[1] Yutaka Harita,[1] Junko Takita,[1] Kazuhiro Takahashi,[1] Masayuki Ishihara,[2] Masataka Hisano,[3] Takashi Igarashi.[1] *[1]Pediatrics, University of Tokyo, Tokyo, Japan; [2]Pediatrics, Kochi Medical School, Nankoku, Kochi, Japan; [3]Pediatric Nephrology, Chiba Children's Hospital, Chiba, Japan.*

Background: Mutations in the ATP6V1B1 and the ATP6V0A4 genes, most of which are nonsense or missense mutations, have been reported to cause primary autosomal recessive distal renal tubular acidosis (dRTA). However, interstitial large deletions of either gene in patients with dRTA have not been described thus far.

Methods: A total of 11 Japanese patients with primary dRTA from 9 unrelated kindreds were enrolled in this study. Exon and exon-intron boundaries of the ATP6V1B1 and the ATP6V0A4 genes were analyzed by direct DNA sequencing and quantitative PCR using genomic DNA purified from peripheral blood leukocytes. Clinical features were also analyzed.

Results: Three novel mutations in the ATP6V1B1 gene were identified in 2 kindreds, including frameshift, in-frame insertion, and nonsense mutations. Interstitial large deletions in the ATP6V0A4 gene were identified in 2 kindreds; in one patient, genomic PCR product of exon 15 was not amplified, and PCR covering wide region around exon 15 confirmed compound heterozygous deletions of 3.7 kb and 6.9 kb nucleotides including whole exon 15. In the other patient, only one heterozygous frameshift mutation was identified by direct DNA sequencing. Subsequent quantitative PCR indicated that the region from N-terminus to exon 8 was deleted in the other ATP6V0A4 allele. It should be noted that 4 out of 6 patients whose data were available presented with hyperammonemia at onset.

Conclusions: We described the first cases with interstitial large deletions involving whole one or more exons in the ATP6V0A4 gene in patients with dRTA. Long genomic PCR as well as quantitative genomic PCR analyses should be considered to detect such large deletions. It also should be noted that hyperammonemia is a frequent manifestation in dRTA at onset.

Funding: Government Support - Non-U.S.

FR-PO295

Effects of Dietary Sodium Citrate on Expression of Sodium Transporter and Fibrosis in CRF Rat Kidneys Sejoong Kim,[1] Jinyoung Yang,[1] Shin-young Ahn,[1] Jeonghwan Lee,[2] Nam Ju Heo,[2] Ki Young Na,[1] Jin Suk Han.[2] *[1]Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

Background: Metabolic acidosis is associated with the progression of renal disease. Citrates as alkaline agents may affect renal acid-base transporters. However, there are few reports on the role of dietary sodium citrate on expression of those transporters in chronic renal failure rat kidneys.

Methods: Sprague-Dawley rats consumed dietary sodium citrate (NaCitrate) or sodium chloride (NaCl) with 20% casein after a 5/6 nephrectomy, and were sacrificed at week 4 and week 8.

Results: After undergoing the casein diet, the NaCitrate-treated group had higher levels of serum bicarbonate than the control group. At week 4, the glomerular filtration rate in the NaCitrate group was higher than that in the NaCl group. Tubulointerstitial damage at week 4 and in the NaCitrate-treated group were less severe, and the difference became prominent at week 8, compared to controls. Urinary sodium excretion rate in the NaCitrate-treated group was faster than in the control group. Sodium/hydrogen exchanger type 3 (NHE3) expression in the NaCitrate-treated group was significantly decreased at week 4 and week 8. However, H-ATPase and sodium/bicarbonate cotransporter expression was not different between the two groups. Endothelin-1 levels in the kidney were also decreased in the NaCitrate-treated group, compared to the control group.

Conclusions: We found that dietary sodium citrate may correct metabolic acidosis, enhance urinary sodium excretion rate, and reduce the renal expression of endothelin-1, which may be associated with the altered expression of NHE3 in the remaining kidney.

FR-PO296

Clinical Characteristics and Mutational Pattern of Genetically-Proven Gitelman's Syndrome Takeshi Ninchoji,[1] Hiroshi Kaito,[1] Kandai Nozu,[1] Koichi Nakanishi,[2] Norishige Yoshikawa,[2] Kazumoto Iijima.[1] *[1]Pediatrics, Kobe University Hospital, Hyogo, Japan; [2]Pediatrics, Wakayama Medical University, Wakayama, Japan.*

Background: Gitelman's syndrome (GS) is inherited salt-losing tubulopathy (SLT) associated with inactivating mutations in *SLC12A3*. Hypomagnesemia, hypocalciuria and normal growth are common symptoms which allow differentiation between GS and another

SLT. However, clinical characteristics of patients with genetically-proven GS have not been fully-clarified, and pathophysiological processes responsible for these symptoms remain to be completely elucidated.

Methods: We retrospectively investigated clinical data from genetically-proven GS patients, These clinical data were compared with those of age-matched control (pseudo-GS), who is present with symptoms clinically identical to GS but no mutation was found in every disease-causing genes of SLT(*SLC12A1*, *KCNJ1*, *BSND*, *CLCNKA*, *CLCNKB,* and *SLC12A3*).

Results: Thirty-one (13 male and 18 female) patients were enrolled in this study. All the patients showed typical findings of diuretic test. Serum magnesium level (sMg) was 1.6±0.1 mg/dl and 13 (42%) patients presented completely normal value. Male patients showed significantly higher sMg than female (1.69±0.07 vs 1.46±0.07; $p<0.05$). Their height was -1.0±0.2 standard deviation (SD) and 29% of them exhibit medically short stature as below -2SD. Seven (54%) of 13 patients with normal s-Mg have the same mutation as L849H, which is located near the C-terminal tail. In contrast, there are no common genetical features about growth impairment. Pseudo-GS patients have significantly lower BMI (16.0±0.8 vs 20.8±0.7 kg/m^2; $p < 0.05$) and lower eGFR (55.6±12.5 vs 121.0±7.9 ml/min/1.73m^2; $p < 0.05$) than GS.

Conclusions: Our study first revealed that it is not unusual for GS patients to show severe growth impairment. Furthermore, it is of special interest that many patients had no overt hypomagnesemia, despite of the physiological confirmation of failure in the function of the transporter responsible for GS. Gender and mutation site may affect serum magnesium level of GS. Our results also indicated it is of much use to check BMI and renal function for exact differentiation between GS and pseudo-GS.

FR-PO297

Dipeptidyl Peptidase-4 Inhibitor Ameliorates PPARγ Agonist-Induced Body Weight Gain via Reduction in Body Fat Mass, but Not Fluid Volume

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Background: Peroxisome proliferator-activated receptor γ (PPARγ) agonists, like pioglitazone (PGZ), are anti-diabetic drugs used to treat type 2 diabetes, but they can induce fluid retention and body weight (BW) gain. Dipeptidyl peptidase-4 (DPP-4) inhibitors are new anti-diabetic drugs that can enhance renal fluid and Na^+ excretion. Therefore, we examined whether the DPP-4 inhibitor alogliptin (ALG) ameliorates PGZ-induced BW gain.

Methods: Male SV129 mice were treated with vehicle (VEH; repelleted diet), PGZ (220 mg/kg diet), ALG (400 mg/kg diet) or combination of PGZ and ALG (PGZ+ALG) (n=8-10 per group). After 14 days treatment, BW, plasma DPP-4 activity, hematocrit (Hct) and fluid content of abdominal fat pads were measured. Body fluid distribution [total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF)] and body fat mass (FM) were measured by bioimpedance spectroscopy. * P<0.05 vs VEH; # P<0.05 vs PGZ alone.

Results: Both ALG and PGZ+ALG decreased plasma DPP-4 activity by about 80% vs VEH (each *) while PGZ alone had no effect. PGZ increased, and ALG lowered BW vs VEH (delta BW: 4.2±0.8*, -0.1±0.5*, and 1.7±0.3 %). ALG+PGZ (0.8±0.8 % #) prevented the increase in BW observed in PGZ alone. ALG+PGZ decreased Hct to a greater degree than PGZ alone (46.9±0.9 # vs 49.9±0.3 %; each *) while ALG alone had no effect vs VEH (50.4±0.3 vs 50.8±0.3 %). PGZ tended to increase and ALG+PGZ significantly increased fluid content of abdominal fat pads vs VEH (9.1±0.9, 9.5±0.6*, 7.4±1.0 %). ALG+PGZ increased TBW, ECF and ICF to a similar degree as PGZ alone (in absolute terms and related to BW; each by 10-20% vs VEH; each *). ALG alone had no effect on FM vs VEH (FM/BW: 22.4±1.4 vs 20.5±1.9 %). However, ALG+PGZ decreased FM to lower levels than PGZ alone (7.6±1.0 # vs 13.7±2.4 %; each *; similar results were obtained for absolute values).

Conclusions: Our data suggest that the DPP-4 inhibitor alogliptin ameliorated the BW gain induced by the PPARγ agonist pioglitazone via a reduction in fat mass, but not in fluid retention.

Funding: Veterans Administration Support, Pharmaceutical Company Support - Takeda Pharmaceuticals U.S.A., Inc.

FR-PO298

Chemical Library Screening for Direct SPAK Inhibitors by a Newly Developed ELISA System

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Background: Pseudohypoaldosteronism type II (PHAII) is an autosomal dominant disease characterized by hypertension, hyperkalemia, and metabolic acidosis. We found that PHAII caused by a WNK4 mutation was caused by the constitutive activation of WNK-OSR1/SPAK-NaCl cotransporter (NCC) signal cascade. In addition to NCC, OSR1/SPAK kinases phosphorylate and activate other Slc12a transporters such as NKCC1 and NKCC2. In fact, the arteries of SPAK knockout mice showed the reduced response to phenylephrine due to the decreased level of NKCC1 phosphorylation. Thus, the inhibition of SAPK kinase is expected to show anti-hypertensive effect at least by dual actions, i.e., NaCl diuresis and vasodilation. The purpose of this study was to develop an efficient system to screen chemical libraries for direct SPAK inhibitors.

Methods: We sought to develop a new screening system of ELISA for SPAK inhibitors. Indirect ELISA system was used to detect NKCC2 phosphorylation as a substrate of SPAK. The amino terminal portion of NKCC2 fused with GST was first coated on ELISA plate. Then, the kinase reaction was optimized using GST-fused whole SPAK and MO25α. The degree of NKCC2 phosphorylation was determined by anti-phosphorylated NKCC2 antibody.

Results: As a result of initial screening of 18,000 compounds owned by Tokyo Medical and Dental University Chemical Biology Screening Center, we could identify several different primary candidates of SPAK inhibitors with reproducibility.

Conclusions: The ELISA assay we established in this study was easy, efficient, and reproducible. The compounds we identified in this screening could be promising seeds for a new type of antihypertensive drug.

Funding: Government Support - Non-U.S.

FR-PO299

Development of Sandwich Enzyme Linked Immunosorbent Assay for Measurement of Urinary Total and Phosphorylated Na-Cl Cotransporter Protein

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Background: Thiazide-sensitive sodium chloride cotransporter (NCC) localizes to the apical membrane of the distal convoluted tubule (DCT) in kidney, and was responsible for reabsorbing 5–10% of the filtered load of sodium chloride. Pseudohypoaldosteronism type II (PHAII) is an autosomal-dominant disorder characterized by hyperkalemia and hypertension. By analyzing the pathogenesis of PHAII, we discovered that WNK-OSR1/SPAK kinase cascade is a powerful regulator of NCC. NCC phosphorylated by OSR1/SPAK kinases was shown to be functionally active and increased on the apical plasma membranes of DCT. Since NCC protein is known to be excreted in urine, we hypothesized that NCC excretion in urine could be a biomarker for estimating NCC activity in vivo.

Methods: In order to investigate this hypothesis, sensitive, reproducible, and quantitative methods measuring urinary total NCC (tNCC) and phosphorylated NCC (pNCC) are necessary. In this study, we developed sandwich ELISA methods to measure as low as 1 pmol/ml and 0.1 pmol/ml of tNCC and pNCC in human urine, respectively.

Results: The amount of tNCC and pNCC measured by the sandwich ELISA was well correlated with the data obtained by the immunoblots ($r=0.94$, $p<0.0001$, $r=0.95$, $p<0.0001$, respectively). We also found that pNCC concentration in spot urine samples remained constant within a day when they were corrected by respective creatinine concentration, suggesting that single spot urine can be used to estimate total excretion of pNCC for 24 hours. Urinary tNCC and pNCC from normal healthy volunteer is 241.8±29.2 fmol/mgCr and 50.1±5.3 fmol/mgCr, respectively (n=13, mean±S.E.M). We also confirmed that urine NCC excretion varied according to different salt intake. Finally, we could also detect the increased tNCC and pNCC urinary excretion in the *Wnk4*$^{D561A/+}$ mice, a mouse model of PHAII.

Conclusions: These results suggest the utility of this methods to estimate the in vivo activity of NCC.

Funding: Government Support - Non-U.S.

FR-PO300

Post-Operative Outcomes in Dialysis Patients Undergoing Elective or Emergent Non-Cardiac Surgery

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Background: Renal failure puts patients at a high risk of complications from noncardiac surgery. Existing literature is scant about post operative risks and mortality in dialysis-dependent patients.

Methods: We used the 2007-08 American College of Surgeons-National Surgical Quality Improvement Program to determine the 30 day mortality and post-operative event rates in chronic dialysis patients who underwent abdominal, major vascular and limb amputation surgery. Low risk and dialysis access surgeries were excluded. We also calculated elective vs. emergent surgical outcomes.

Results: 5178 dialysis patients from 189 hospitals were included. 3872 surgeries were elective and 1306 emergent. 87% of the surgeries used general anesthesia. 30 day mortality was 14.3% (elective: 9.7%; emergent: 27.8%). 30% patients had at least one complication. Respiratory failure, surgical site infection/ wound disruption and sepsis were common complications.

30 day post-operative outcomes

	Abdominal		Major Vascular		Amputation		Overall
	Elective	Emergent	Elective	Emergent	Elective	Emergent	
Patients	1316	830	1431	340	1125	136	5178
30 day mortality	124(9.4)	282(34)	109(7.6)	52(15.3)	143(12.7)	29(21.3)	739(14.3)
Respiratory failure	185(14.1)	320(38.6)	104(7.3)	55(16.2)	89(8)	31(22.8)	784(15.1)
Surgical site complication	146(11.1)	106(12.8)	97(6.8)	26(7.7)	65(5.8)	9(6.6)	449(8.7)
Sepsis/septic shock	102(7.8)	79(9.5)	73(5.1)	33(9.7)	80(7.1)	16(11.8)	383(7.4)
Pneumonia	72(5.5)	107(12.9)	40(2.8)	17(5)	63(5.6)	12(8.8)	311(6)
Venous thromboembolism	23(1.8)	38(4.6)	26(1.8)	7(2.1)	17(1.5)	2(1.5)	113(2.2)
Stroke	7(0.5)	18(2.2)	15(1.1)	4(1.2)	11(1)	0	55(1.1)
Cardiac arrest	42(3.2)	56(6.8)	54(3.8)	10(2.9)	50(4.4)	8(5.9)	220(4.3)
Myocardial infarction	10(0.8)	12(1.5)	13(0.9)	2(0.6)	7(0.6)	0	44(0.9)
Transfusion required	23(1.8)	66(8)	30(2.1)	12(3.5)	8(0.7)	5(3.7)	144(2.8)
Mean length of stay(days)	15.4	21.9	10.7	16.6	17.1	22.6	

Parentheses: Incidence (%) within subgroup

Conclusions: Dialysis patients are at an extremely high risk for morbidity/mortality after intermediate and high risk non-cardiac surgeries; more so in emergent surgeries. Our results should help guide informed decision making about surgery in this patient population.

FR-PO301

Hospital Admissions Following Long and Short Interdialytic Intervals among Hemodialysis Patients Tricia L. Roberts,[1] Robert N. Foley,[1,2] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of Minnesota, Minneapolis, MN.*

Background: An increased risk of cardiovascular (CV) hospital admissions after the long (2-day) interdialytic interval among hemodialysis (HD) patients has recently been reported. We assessed this association among a large, current cohort of Medicare HD patients, with additional analysis of all-cause admissions, infectious admissions, and the short (1-day) interdialytic interval.

Methods: Data included 162,672 U.S. Medicare adult prevalent HD patients on January 1, 2010, alive on January 31, and receiving HD three times weekly on a Monday/Wednesday/Friday or Tuesday/Thursday/Saturday schedule. HD schedule was determined from Medicare claims from January 18 to 31, 2010. Follow-up for hospital admissions began on February 1 and continued until censoring at the latest on December 31, 2010. Patients with a bridge hospitalization spanning follow-up were excluded. Infectious and CV admissions were determined by principal ICD-9-CM diagnosis codes. Admission rates by day of the dialysis week were adjusted for age, gender, race, Hispanic ethnicity, and primary diagnosis using the Poisson model and direct adjustment.

Results: For all-cause, CV, and infectious admissions, the highest adjusted rates occurred on the day after the long interdialytic interval (respectively, 2,101, 682, and 501 admissions per 1,000 patient years). The all-cause admission rate on the day after the long interdialytic interval was 1.5 times the rate on days after the short interdialytic intervals (1,412) and 1.9 times the rate on days without HD (1,093). All-cause, CV, and infectious admissions by day of the HD week produced a sawtooth pattern with higher rates on the days with an HD treatment after the long and short interdialytic intervals than on the preceding and following days.

Conclusions: The days after the long and short interdialytic intervals among HD patients were associated with elevated all-cause and infectious admissions in addition to CV. Results suggest a potential need to further evaluate the U.S. standard frequency of HD treatments.

Funding: NIDDK Support

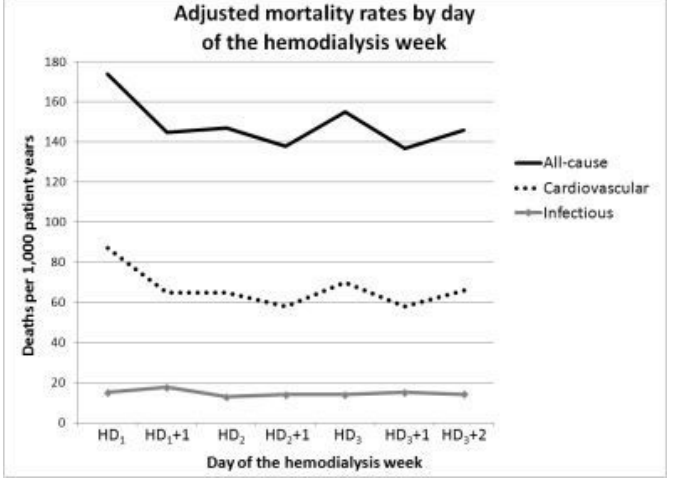
FR-PO302

Mortality Following Long and Short Interdialytic Intervals among Hemodialysis Patients Robert N. Foley,[1,2] Tricia L. Roberts,[1] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Medicine, University of Minnesota, Minneapolis, MN.*

Background: Increased mortality risk following the long (2-day) interdialytic interval has recently been reported among thrice-weekly hemodialysis (HD) participants in the End-Stage Renal Disease Clinical Performance Measures Project. Associations between mortality and long and short (1-day) interdialytic intervals have not yet been assessed in the most recent Medicare cohort.

Methods: We studied 162,679 U.S. Medicare adult prevalent HD patients on January 1, 2010, alive on January 31, and receiving HD three times weekly on Monday/Wednesday/Friday or Tuesday/Thursday/Saturday. Medicare claims from January 18 to 31, 2010, determined the schedule. Follow-up began on February 1, 2010, and was censored at modality change, end of Medicare payer status, recovery of renal function, deviation from HD schedule, loss to follow-up, or December 31, 2010. All-cause, infectious, and CV mortality rates were computed by days of the HD week: HD_1, HD_2, and HD_3 were the first, second, and third HD sessions; HD_1 denoted the day after the long interdialytic interval; and HD_2 and HD_3 were the days after the short intervals. Rates were adjusted for age, gender, race, Hispanic ethnicity, and primary diagnosis with the Poisson model and direct adjustment.

Results: All-cause mortality rates were highest on HD_1 (174 deaths per 1,000 patient years), followed by the days after the short interdialytic intervals (151) and without HD (142). CV mortality also peaked at HD_1 (87), while infectious mortality was highest on the day after the first HD session (18). Patterns of all-cause mortality mirrored CV with generally higher rates on days with HD than days before and after.



Conclusions: The days after the long and short interdialytic intervals were associated with increased all-cause and CV mortality rates.

Funding: NIDDK Support

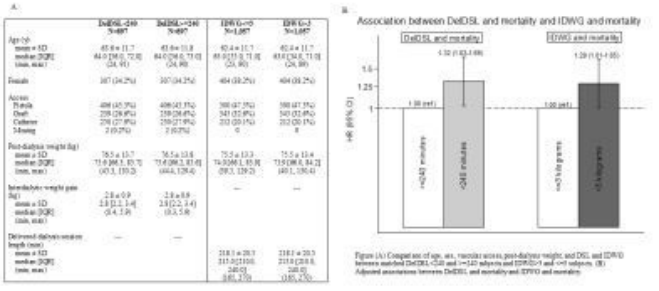
FR-PO303

Disentangling the Ultrafiltration Rate (UFR): Mortality Association: The Roles of Session Length and Weight Gain Jennifer E. Flythe, Steven M. Brunelli. *Brigham and Women's Hospital, Boston, MA.*

Background: Rapid UFRs are associated with increased mortality among hemodialysis (HD) patients. UFR is determined by both interdialytic weight gain (IDWG) and dialysis session length (DSL). Both IDWG and DSL have been linked to mortality, but the relationship of each to mortality, independently of the other, is not defined. This study was designed to evaluate whether shorter DSL independent of IDWG and larger IDWG independent of DSL are associated with mortality.

Methods: Data were taken from a nationally-representative cohort of 11,834 patients undergoing thrice-weekly in-center HD at one large dialysis organization who had adequate urea clearance (URR>=65%). Patients with delivered session length (DelDSL) >=240 and <240 minutes were pair-matched on IDWG (+/- 1.5 kg), and patients with IDWG <=3 and >3 kilograms were pair-matched on DelDSL (+/- 5 minutes), as well as on age, sex, vascular access type, and post-dialysis weight, resulting in near perfect balance (Figure A).

Results: DelDSL<240 was associated with increased mortality: adjusted HR (95% CI) 1.32 (1.03-1.69) when compared to DelDSL >=240 minutes; IDWG >3 was associated with increased mortality: 1.29 (1.01-1.65) compared to IDWG <=3 kilograms (Figure B). All associations were consistent across strata of age, sex, post-dialysis weight; the IDWG--mortality association was similar across DelDSL strata (p-interaction=0.17); the DelDSL--mortality association was similar across IDWG strata (p-interaction=0.56).



Secondary analyses indicated dose-response trends in the associations of DelDSL and IDWG with mortality (not shown).

Conclusions: Among patients with adequate urea clearance, shorter DelDSL and greater IDWG are associated with increased mortality. Both DelDSL and IDWG play important and independent roles in mediating the UFR--mortality relationship.

Funding: NIDDK Support

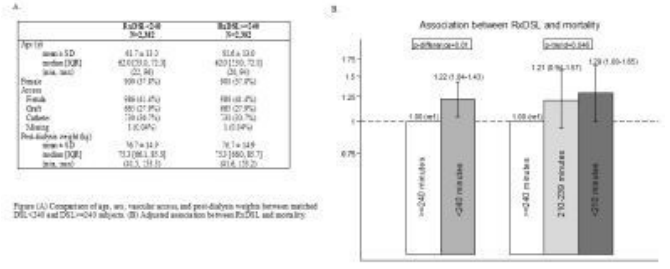
FR-PO304

Shorter Prescribed Dialysis Session Length (RxDSL) Is Associated with Increased Mortality Independent of Body Weight Jennifer E. Flythe, Steven M. Brunelli. *Brigham and Women's Hospital, Boston, MA.*

Background: Prior studies have demonstrated that shorter RxDSL is associated with greater mortality. However, these observations may have been confounded by body size differences among patients with longer and shorter RxDSL. This study was designed to test the hypothesis that shorter RxDSL is associated with greater all-cause mortality independently of body weight.

Methods: Data were taken from a nationally-representative cohort of 10,571 patients undergoing thrice-weekly in-center hemodialysis (HD) at one large dialysis organization who had adequate urea clearance (URR≥65%). Patients with RxDSL ≥240 and <240 minutes were pair-matched on post-dialysis weight (±1 kg), as well as on age, sex, and vascular access type, resulting in near-perfect balance (Figure A).

Results: RxDSL < 240 was associated with greater mortality: adjusted HR (95% CI) 1.22 (1.04-1.43; p=0.01) when compared to RxDSL ≥ 240 minutes (Figure B). A similar trend was observed when RxDSL was considered in categories of < 210, 210-239 and ≥ 240 minutes (p-trend=0.046; Figure B). Restriction subgroup analyses demonstrated that the association between RxDSL and mortality was similar regardless of age (p-interaction=0.53), sex (p-interaction=0.80), race (p-interaction=0.79), and post-dialysis weight (p-interaction=0.89).



Conclusions: RxDSL<240 minutes is associated with increased mortality in chronic HD patients with adequate urea clearance independently of body size. This association follows a dose-response pattern: incrementally shorter sessions are associated with higher hazard.

Funding: NIDDK Support

FR-PO305

Left Atrial Volume Is an Independent Predictor of All-Cause Mortality in Chronic Hemodialysis Patients Kosaku Nitta. *Department of Medicine, Kidney Center, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: An enlarged left atrium (LA) has recently been identified as a risk factor for adverse cardiovascular outcomes in various pathologic conditions. However, few studies have valuated its prognostic value in hemodialysis (HD) patients.

Methods: We conducted an observational study to investigate whether an enlarged LA predicted all-cause mortality in 174 HD patients. Patients were stratified into two groups based on the LA volume index (LAVI) value of 32 mL/m^2.

Results: An increased left atrial volume index (LAVI >32 mL/m^2) was present in 28 (16.1%) of the HD patients. During the follow-up period (50.1 ±22.4 months), 77 patients (44.3%) died. A Kaplan–Meier analysis revealed that the 7-year survival rate was significantly lower in the group whose LAVI was >32 mL/m^2 than in the group whose LAVI was ≤32 mL/m^2 (*P =0.0033*). Multivariate analyses adjusted for echocardiographic parameters and clinical and laboratory data showed that increased LAVI was an independent predictor of all-cause mortality (hazard ratio 1.030, 95% confidence interval 1.004–1.056,*P =0.0260*). Moreover, increased LAVI had a higher predictive value for all-cause mortality (area under the receiver operating characteristic curve =0.612, *P = 0.0059*) among the measured echocardiographic parameters.

Conclusions: The results of the present study suggested that measurement of LAVI may be helpful in the risk stratification of HD patients and in providing therapeutic direction for their management.

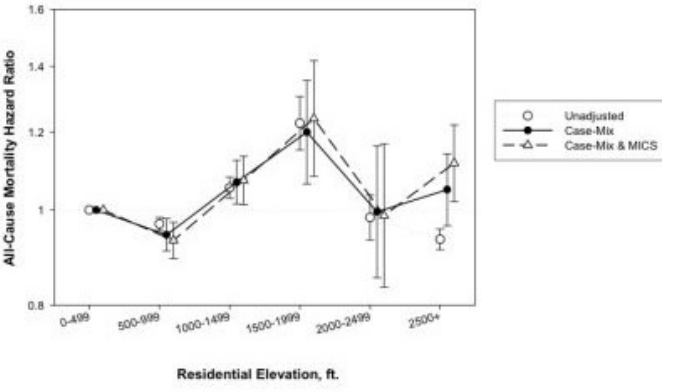
FR-PO306

Association between Altitude and All-Cause Mortality in Maintenance Dialysis Patients Bryan B. Shapiro,[1] Elani Streja,[1] Miklos Zsolt Molnar,[1,2] Joel D. Kopple,[3] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [3]Nephrology, Harbor-UCLA, Torrance, CA.*

Background: Higher residential altitude has previously been reported to be associated with a reduction in all-cause mortality in incident dialysis patients, believed to be attributable to enhanced availability of iron due to hypoxia at high altitudes.

Methods: Using 5-year patient data of a large dialysis organization, we linked all-cause mortality according to patient zip codes to a database containing average residential altitudes for 43,191 zip codes in the United States. We identified 153,334 hemodialysis (HD) patients. Mortality risks were estimated by Cox regression (hazard ratio [HR]).

Results: Patients were 48±14 years old and included 39% women and 35% diabetics. Relative to patients living in low-altitudes (<500 ft), patients residing in the highest altitude sextile were associated with a 12% (HR:1.12[1.02-1.22]) increase in case-mix and malnutrition-information complex (MICS) adjusted mortality risk. However, no clear trend was found between higher altitudes and variation in all-cause mortality at baseline or when adjusted.



Conclusions: No clear association exists between altitude and all-cause mortality in dialysis patients. These data seems to contradict earlier findings indicating that high altitudes are associated with reduced mortality in incident dialysis patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO307

Increased Mortality Risk of Serum Alkaline Phosphatase: Relation to Changes in Body Composition in Hemodialysis Patients Neal Mittman, Brinda Desiraju, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, S.U.N.Y. Downstate Medical Center UHB at Long Island College Hospital, Brooklyn, NY.*

Background: Higher levels of alkaline phosphatase (AlkP) have been associated with increased mortality in hemodialysis (HD) patients (pts). We examined the associations of AlkP with clinical and biochemical status in our HD pts, including body composition.

Methods: Sixty-four HD patients were enrolled in this study beginning in 2000. On enrollment, demographics, clinical data and biochemical data were recorded. Patients were followed up to the end of 2011. Body composition parameters were determined by bioimpedance analysis (BIA).

Results: The mean age was 62 years. Fifty-eight percent were female, and the majority (76%) were of African descent. Forty percent were diabetic. Mean and median AlkP were 122 U/L and 88.5 U/L, respectively, with a very wide range. Thirty-nine percent of patients had elevated levels. Levels of AlkP were not influenced by age, race, gender or diabetic status. Correlations of AlkP with biochemical and body composition variables are shown in Table1.

Correlations of Serum AlkP with Biochemical and Body Composition Parameters

Variable	Correlation coefficient	p-value
Albumin-Corrected Calcium (mg/dL)	0.31	0.02
Intact Parathyroid Hormone (pg/mL)	0.42	0.004
Body Mass Index (Lbs/inch^2)	0.32	0.039
Total Body Fat (Lbs)	0.44	0.004
Extracellular Water (Lbs)	0.39	0.014

Not surprisingly, AlkP correlated directly with serum intact parathyroid hormone and with albumin-corrected calcium. In addition, serum AlkP was directly correlated with extracellular water (ECW), body mass index (BMI), and body fat. In Cox's multivariate analysis, AlkP, above vs. below mean value, was a strong independent predictor of mortality risk (RR: 13.6, p=0.016), imparting a 14-fold greater risk. ECW was also an independent mortality predictor (RR: 1.141, p=0.004), imparting 14% increased risk per lb increase.

Conclusions: Higher AlkP in these HD pts, with increased body fat relative to lean body mass and increased extracellular water, may be partly explained by hepatic congestion related to volume overload or cardiac dysfunction, or both, which may be factors in the associated mortality risk.

FR-PO308

Association of Physical Activity with Survival among Ambulatory Patients on Dialysis: The Comprehensive Dialysis Study Kirsten L. Johansen, George A. Kaysen, Lorien S. Dalrymple, Barbara A. Grimes, Glenn M. Chertow. *USRDS Nutrition Special Studies Center, San Francisco, CA.*

Background: Despite high mortality and low levels of physical activity among patients starting dialysis, the link between low physical activity and mortality has not been carefully evaluated in this population.

Methods: The Comprehensive Dialysis Study was a cohort study performed by the United States Renal Data System Nutrition and Rehabiliation/QOL Special Studies Centers. 1678 patients were enrolled from a random sample of 297 dialysis facilities in the US during June, 2005 through June, 2007, of whom 1554 ambulatory patients were included in this analysis. To determine the association between physical activity and mortality, we estimated physical activity using the Human Activity Profile (HAP) and used multivariable

Cox proportional hazards modeling. We followed patients until death or September 30, 2009. Outcome measures were all-cause mortality and death or first hospitalization.

Results: The average age of participants was 59.8 (14.2) years; 55% were male, 28% African American, and 56% had diabetes mellitus. The majority of participants (57.3%) were estimated to have low fitness based on HAP score. The median follow-up was 2.6 (interquartile range 2.2 to 3.1) years. The association between physical activity and mortality was linear across the range of scores (1 to 94). After adjustment for age, sex, race, dialysis modality, and comorbid conditions, lower Adjusted Activity Score on the HAP was associated with higher mortality (HR 1.30, 95% CI 1.23 to 1.39 per 10 points). Patients in the lowest level of fitness experienced a 3.5-fold (95% CI 2.54 to 4.89) increase in risk of death compared to those with average or above fitness. Less active patients were also at significantly increased risk of death or first hospitalization.

Conclusions: Low levels of physical activity are strongly associated with mortality among patients new to dialysis. Interventions aimed to preserve or enhance physical activity should be prospectively tested.

Funding: NIDDK Support

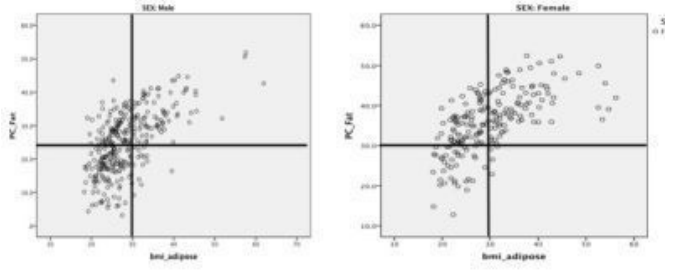
FR-PO309

Comparison of Prevalence of Obesity Based on BMI and Body Fat among HD Patients Kirsten L. Johansen, Glenn M. Chertow, George A. Kaysen, Lorien S. Dalrymple. *USRDS Nutrition Special Studies Center, San Francisco, CA.*

Background: Although the association of obesity with outcomes has been intensely studied among patients on HD, the extent to which the standard BMI-based definition of obesity derived in healthy populations is applicable to dialysis patients has not been addressed.

Methods: The USRDS Analyses Designed to Investigate the Paradox of Obesity in ESRD (ADIPOSE) is a prospective cohort study that enrolled prevalent patients on HD from the San Francisco and Atlanta areas from 6/09 to 8/11. Height, weight, and waist circumference were measured and bioelectrical impedance spectroscopy (BIS) performed before a midweek dialysis session. Designation of obesity by BMI (≥30 kg/m^2) was compared to obesity based on percent body fat by BIS (≥25% for men, 30% for women); waist circumference (>40 in for men, 35 in for women) was also compared to % body fat.

Results: The mean (sd) age was 56 (14) years (n=520), 40% were women, 58% black. Overall, 36.5% were obese by BMI, 62.5% by % fat, and 54.8% by waist circumference; women were more likely to meet all three criteria (45% vs. 31% for BMI; 80% vs. 51% by % fat; 74% vs. 57% by waist). More women were misclassified by BMI (39% vs. 30% of men, p=0.04); both sexes were more likely to be underidentified as obese by BMI (upper left quadrant) than overidentified (lower right quadrant) (Figure).



Misclassification was less using waist circumference (women 26%, men 24%). Misclassification by BMI differed by age and was greater among older (>65) compared with younger (47% vs. 29%, p<0.0001) but did not differ statistically by race.

Conclusions: BMI identified considerably fewer patients as obese than criteria based on estimation of body fat, particularly among women and older patients, 80% and 74% of whom were obese based on body fat, respectively. Waist circumference performed slightly better than BMI.

Funding: NIDDK Support

FR-PO310

Differences in Mortality between Hispanic and Non-Hispanic Whites Initiating Dialysis in the United States Maria Cristina Arce,[1] Benjamin A. Goldstein,[2] Aya Alice Mitani,[2] Wolfgang C. Winkelmayer.[1] *[1]Division of Nephrology, Stanford University School of Medicine, Palo Alto, CA; [2]Division of General Medical Disciplines, Stanford University School of Medicine, Palo Alto, CA.*

Background: Hispanic patients undergoing chronic dialysis experience better survival compared with non-Hispanics. It is unknown, however, whether this association differs by age, has changed over time, or is due to differential access to kidney transplantation.

Methods: Using the U.S Renal Data System, we identified 615,618 Caucasians who initiated dialysis between 1/1/1995 and 12/31/2007 and investigated the temporal trends in relative mortality, stratified by age, between Hispanics and non-Hispanic whites initiating dialysis. All-cause mortality was analyzed using proportional hazards regression and estimating unadjusted and adjusted cause-specific hazard ratios (HR_{CS}) and subdistribution (competing risk analysis) hazard ratios (HR_{SD}) with corresponding 95% confidence intervals (CI).

Results: We found that Hispanics initiating dialysis experienced a lower mortality but age modified this association (P<0.001). Compared with non-Hispanics, mortality among Hispanics was 35% lower at ages 18-39 (HR_{CS}=0.65; 95%CI, 0.62-0.69) and 40-59 (HR_{CS}=0.65; 95%CI, 0.64-0.67), and 20% lower at ages 60-79 (HR_{CS}=0.80; 95%CI, 0.79-0.81), and 7% lower at age ≥80 years (HR_{CS}=0.93; 95%CI, 0.91-0.96). When accounting for the differential rates of transplantation, the associations were markedly attenuated in the younger age strata—the survival benefit for Hispanics was reduced from 35% to 14% at ages 18 to 39 years (HR_{SD}=0.86; 95% CI, 0.82-0.91) and from 35% to 21% among those aged 40 to 59 years (HR_{SD}=0.79; 95% CI, 0.77-0.80).

Conclusions: Overall, Hispanics experienced a lower mortality but the association decreased with older age. Interestingly, competing risk analyses attenuated these findings suggesting that differential access to kidney transplantation was responsible for much of the apparent survival benefit among Hispanics.

Funding: Other NIH Support - Dr. Arce is Supported by an Underrepresented Minority Supplement (URM) to Grant T32 DK007357-26

FR-PO311

Weakness of Propensity Score Matching in the Comparison of Mortality on Hemodialysis versus Peritoneal Dialysis from Registry Data Maurizio Nordio, Gianpaolo Amici, Mariano Feriani, Barbara Rossi, Nicola Tessitore. *Veneto Dialysis and Transplantation Registry, Padua, Italy.*

Background: The only RCT comparing hemodialysis (HD) and peritoneal dialysis (PD) survival was inconsistent for unsuccessful recruitment. Recently many observational studies using propensity score as causal inference methodology were performed. Their results were heterogeneous. The aim of the study was to investigate this heterogeneity.

Methods: We considered the cohort of incident dialysis patients from 1/1/1998 to 31/12/2010 in Veneto Region with demographic characteristics, prevalent dialysis modality, primary renal disease and comorbidities. A propensity score matching was performed using nearest neighborg. Since 32% of PD patients and 13% of HD patients were transplanted, a competing risk (death and transplantation) survival analysis was performed to avoid informative censoring. The treatment effect was compared by Gray's test and competing risks regression. The Rosenbaum approach was used to perform sensitivity analysis.

Results: 6962 patients were recruited in the considered period, 77.5% were on HD and 22.5% on PD. 1070 subjects on PD and 1998 on HD were matched. The common support covered patients achieving a probability from 2.4% to 51.3% to be offered PD. The cumulative incidence of death was significantly different with a mortality of 68% on HD and 48% on PD at 10 years, but the effect became non significant (SHR = 0.92, CI 95% : 0.78 -1.03) after adjustment. The patients out of the common support showed fewer comorbid conditions and a female excess. Sensitivity analysis showed that the study was sensitive to a hidden bias increasing only by 20% the odds.

Conclusions: Dialysis modality does not affect survival. Sensitivity analysis shows that the propensity score is not robust to hidden bias. The effect may significantly change simply considering different populations or other factors. This behavior may be due to the presence of important unknown confounders not included in propensity score as well as to a weak treatment effect. The heterogeneity of patients out of common support undermine the external validity of this approach, underlining the necessity of a RCT.

FR-PO312

Predictors of Mortality in the First 90 Days of Dialysis in an Asian Dialysis Cohort Sabrina Haroon,[1] Horng Ruey Chua,[1] Nan Luo,[2] Boon Wee Teo,[1] Evan J.C. Lee,[1] Titus W. Lau.[1] *[1]Division of Nephrology, National University Health System, Singapore; [2]School of Public Health, National University of Singapore, Singapore.*

Background: Dialysis served as life saving therapy for end stage renal failure (ESRF) patients. However, it is resource intense and appropriate counseling with regards to prognosis is essential and ethical. Knowing the various predictors of early mortality will help guide risk counseling in starting dialysis.

Methods: This is a retrospective study of all incident ESRF patients that were started on dialysis from Jan 1, 2005 to Dec 31, 2010. SAS was used for all statistical analyses. All plausible mortality risk factors were included in the final model to determine predictors of early mortality (90-day mortality) using logistic regression analysis.

Results: A total of 870 patients were included in this study. There were a total of 92 deaths (10.6%) within 90 days of starting dialysis. The predictors of interest included were age (< 45 years being the reference; 45-<65; >=65); diabetic status; gender; presence of various co-morbiodities - ischemic heart disease, stroke, periphery vascular disease with and without amputation; hemoglobin level; serum albumin level; serum phosphate level and if patient was initiated on dialysis with a ready access (AV fistula/graft or Tenkhoff catheter). Results of our analysis showed that older age (> 65 years) (OR 6.68 95% CI 1.93-23.16, p = 0.0027) and periphery vascular disease with amputation (OR 3.12 95% CI 1.41-6.93, p = 0.0051) were independently associated with higher risk of first 90 days mortality after initiating dialysis. Co-morbidities such as diabetes, history of ischemic heart disease, stroke or periphery vascular disease without amputation did not independently predict early mortality. Starting dialysis with a planned access reduces the risk of early mortality significantly (OR 0.27 95% CI 0.13-0.55, p = 0.0003).

Conclusions: Any patient above age 65 years with amputation due to periphery vascular disease has a high risk of early mortality on dialysis and should take this into consideration when deciding for maintenance dialysis. This risk is mitigated by planning an access early and initiating dialysis with a planned access.

FR-PO313

Characteristics and Mortality among Young Adults with Incident End-Stage Renal Disease (ESRD) in the United States Brett W. Plattner, Debbie S. Gipson, Hal Morgenstern, Rajiv Saran, Yi Li, Ying Qian, Valarie B. Ashby. *University of Michigan.*

Background: Despite evidence for heterogeneity of the US ESRD population by age, young adult patients (ages 19-35) have received little attention by researchers. Our aims were to describe this population, compare it with patients diagnosed at other ages, and identify risk factors for mortality.

Methods: 85,839 young adult ESRD patients diagnosed from 1995 to 2011 were identified from the CMS national data. Cox regression was used to examine associations between demographic and clinical characteristics and all-cause mortality between diagnosis and 12/31/11.

Results: 42% of the patients were black, compared with 28% of patients diagnosed before age 19 and 28% diagnosed after age 35. Glomerulonephritis (26%), diabetes (25%), and HTN (20%) were the leading causes of ESRD. The majority (87%) utilized hemodialysis (HD), compared to 56% of patients diagnosed before age 19 and 92% diagnosed after age 35. The adjusted hazard ratio (HR) was greatest for blacks, a diabetes etiology, HD patients, and those with cardiovascular or drug-dependency comorbidities.

Demographics and Adjusted HR for ESRD Pts Ages 19-35

Predictor	N	%	Adjusted HR* (95%CI)
Race			
White	42344	49	1 (ref)
Black	36179	42	1.14(1.11,1.18)
Asian	3982	5	0.48(0.44,0.56)
Native American	1186	1	0.81(0.72,0.89)
Sex			
Male	48284	56	1 (ref)
Female	37555	44	1.11(1.08,1.14)
Etiology of ESRD			
Diabetes	21303	25	1(ref)
HTN	17451	20	0.4(0.39,0.42)
Lupus	6307	7	0.57(0.54,0.61)
GN(except Lupus)	22582	26	0.51(0.49,0.53)
Modality			
HD	74825	87	1(ref)
PD	10724	12	0.86(0.82,0.90)
CHF	2629	10	1.27(1.23,1.32)
Atherosclerotic Heart Disease	2015	2	1.19(1.11,1.28)
Other Cardiac	26292	3	1.27(1.19,1.36)
PVD	2240	3	1.22(1.14,1.29)
Drug Abuse	2580	3	1.49(1.40,1.58)

*HR adjusted for age,BMI, Hispanic ethnicity, access type, comorbidities, incidence year and the predictors above. p<.0001 for each predictor

Conclusions: This analysis focuses on young adult dialysis patients. Unexpected findings include low PD utilization, a high prevalence of congestive heart failure, and a higher mortality rate among blacks. Future research should further explore distinct features of young adult ESRD cases to improve the healthcare of this understudied population.

Funding: Other NIH Support - T-32 Training Grant Award Number 5-T32-DK-007378-32

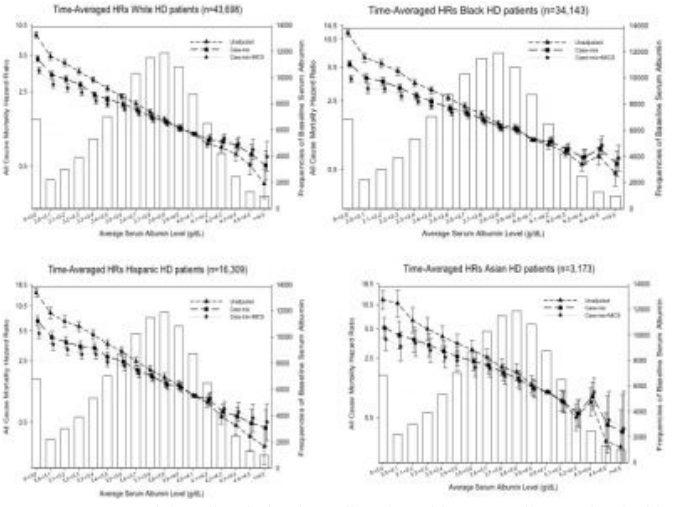
FR-PO314

Association of Serum Albumin and Mortality in Four Races in Hemodialysis Patients Alla Victoroff,[1] Miklos Zsolt Molnar,[1,2] Jennie Jing,[1] Elani Streja,[1] Vanessa A. Ravel,[1] Csaba P. Kovesdy,[3] Joel D. Kopple,[4] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LABioMed at Harbor-UCLA, Torrance, CA; [2]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [3]Nephrology, University of Tennessee, Memphis, TN; [4]David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Low serum albumin level is the strongest mortality-predictor in maintenance hemodialysis (MHD) patients. It is not entirely known whether this association is consistent across all races.

Methods: We examined the association of time-averaged serum albumin and all-cause mortality in 105,523 MHD patients across different race groups using time-dependent Cox models to estimate death hazard ratios for time-averaged serum albumin increments controlled for case-mix, comorbidity, dialysis dose, and available markers of malnutrition-inflammation-complex syndrome (MICS) in all race groups.

Results: The study population consisted of 105,523 patients with mean age of 60±16 years old and included 45% women, 33% African Americans and 59% diabetic patients. Compared to white patients with time-averaged serum albumin 4.0-<4.1 g/dL, white patients with serum albumin ≥4.5, 3.4-<3.5, and <3.0 g/dL had 39% (HR: 0.61, 95% CI: 0.45-0.85) lower; 90% (HR: 1.90, 95% CI: 1.78-2.03), and 292% (HR: 3.92, 95% CI: 3.65-4.21) higher all-cause mortality in fully adjusted models, respectively (see Figure). Similar trend was seen in African-Americans, Hispanic and Asian patients.



Conclusions: Higher albumin levels are linearly and incrementally associated with greater survival in all major racial and ethnic groups of MHD patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

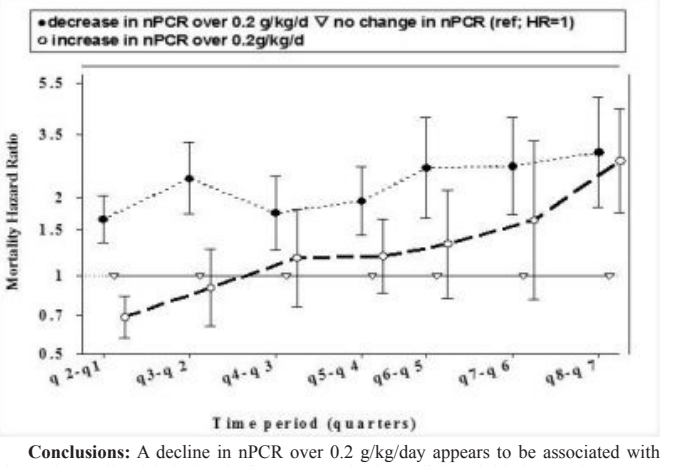
FR-PO315

Associations between Changes in Protein Catabolic Rate and Mortality in Incident Hemodialysis Patients during First Two Years after Hemodialysis Initiation Lilia R. Lukowsky,[1,3] Leeka I. Kheifets,[3] Onyebuchi A. Arah,[3] Allen R. Nissenson,[4] Kamyar Kalantar-Zadeh.[1,2,3] *[1]Harold Simmons Center, LABioMed at Harbor UCLA; [2]Harbor-UCLA; [3]UCLA Fielding School of Public Health; [4]DaVita, Inc.*

Background: Urea dynamic calculated protein catabolic rate (nPCR) is assumed to represent dietary protein intake normalized for body weight. It is recommended that maintenance hemodialysis (MHD) patients have a high daily protein intake of 1.2g/kg/day. Some studies suggest that nPCR >1g/kg/day is associated with better survival in dialysis patients. However, the role of nPCR in early dialysis mortality is not clear.

Methods: Over a five-year period, we identified 17,445 incident hemodialysis patients from a large dialysis organization. We examined the mortality impact on change in serum levels of nPCR across the first 8 consecutive calendar quarters following the hemodialysis therapy initiation. MHD patients with no change in nPCR (an increase or a decrease within ±0.2 g/kg/day) served as the reference category. Change in nPCR was defined as change above ±0.2g/kg/day from a previous quarter.

Results: Patients were 64±15 years old (mean±SD) and included 45% women, 24% African Americans, and 58% diabetics. A decline in nPCR over time was associated with elevated all-cause mortality. An increase in nPCR was associated with better survival during the first several months of dialysis treatment. However, further increase in nPCR during subsequent calendar quarters was associated with a paradoxical increase in the risk of death by more than two folds, especially towards the end of 2nd year.



Conclusions: A decline in nPCR over 0.2 g/kg/day appears to be associated with elevated mortality risk during the first 2 years after hemodialysis initiation. An increase in nPCR has varying associations with mortality, from lower mortality in the first 3 months to higher mortality in Year 2.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO316

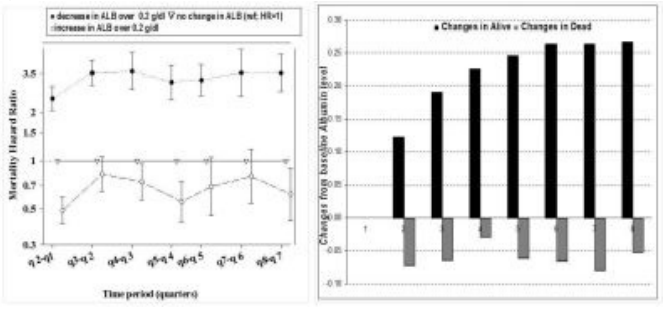
Associations between Quarterly Changes in Serum Albumin and Mortality in the First 24 Months after Initiation of Hemodialysis

Lilia R. Lukowsky,[1,3] Leeka I. Kheifets,[3] Onyebuchi A. Arah,[3,4] Allen R. Nissenson,[2,5] Kamyar Kalantar-Zadeh.[1,2,3] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor UCLA, Torrance, CA; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]Department of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, CA; [4]Department of Public Health, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [5]DaVita, Inc., Denver, CO.*

Background: Low serum albumin level is associated with lower survival. The role of change in serum levels from one quarter to the next is less clear in clinical outcomes of the first several months of hemodialysis therapy.

Methods: Over a five-year period (7/2001-6/2006), we examined the association between mortality and changes in serum levels of albumin from the prior quarter for the first eight quarters after dialysis initiation in 17,445 incident hemodialysis patients. We used a group with no change (an increase or a decrease no higher than ±0.2 g/dl) as a reference category and compared them to those who had changes greater than ±0.2g/dl.

Results: Patients were 64±15 years old (mean±SD) and included 45% women, 24% African Americans, and 58% diabetics. We observed a negative change in the mean serum levels of albumin from the prior quarter among patients who died but an increase or no change from the previous quarter in mean serum levels of albumin among the survivors. A decrease in serum level of albumin was associated with elevated all-cause mortality by over 3 fold.



Conclusions: Decrease in quarterly serum levels of albumin over 0.2 g/dl was associated with elevated mortality risk during all eight calendar quarters of the first 2 years after dialysis initiation in incident hemodialysis patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO317

Associations of Serum Albumin Level and Dietary Protein Intake Estimate with Patient Mortality in the First 24-Months of Incident Hemodialysis

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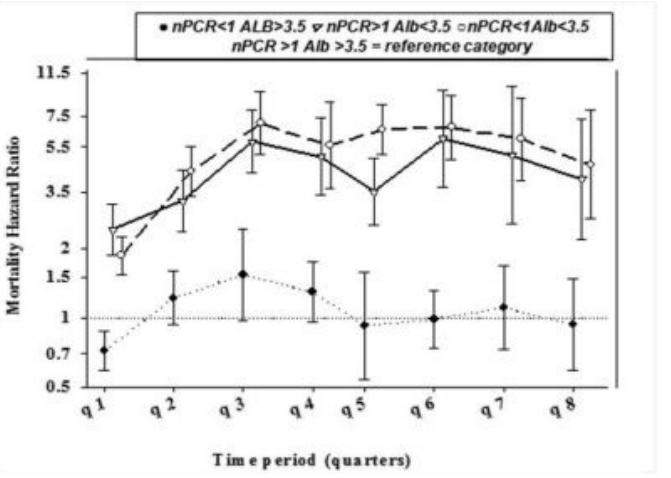
Background: Low serum albumin and low dietary protein intake are known to correlate with higher mortality in maintenance hemodialysis (HD) patients. The role of these nutritional markers is less clear in clinical outcomes of the first several months of hemodialysis therapy, when mortality is exceptionally high.

Methods: Over a five-year period, we identified 17,445 incident HD patients whose first week of dialysis treatment started in an outpatient dialysis clinic or a large dialysis organization. We examined the association between mortality and combined categories (alb<3.5 g/dl vs. higher, and nPCR <1d/kg/day vs. higher; leading to 2x2=4 groups) of quarterly averaged values for albumin and normalized protein catabolic rate (nPCR) for the first eight calendar quarters after hemodialysis initiation.

Results: Patients were 64±15 years old and included 45% women, 24% African Americans, and 58% diabetics. Correlation coefficients between albumin and nPCR varied from +0.18 to +0.25. Compared to the concordant group of alb ≥3.5g/dl & nPCR ≥1g/kg/day, both categories with low alb <3.5g/dl were persistently associated with higher mortality for all quarters regardless of nPCR levels (increase in death hazard ratio over 3-folds after the 3rd quarter). Contrasting the original hypothesis, nPCR of<1g/kg/day was significantly associated with greater survival during the first 3 months although this association reversed thereafter.



Conclusions: Whereas low albumin <3.5g/dl is a robust and persistent predictor of high mortality in new HD patients, the nPCR-death association varies over the first 24 months. The paradoxical association of lower nPCR <1g/kg/day with greater survival in the first 3 months warrants additional studies.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO318

The Association of Serum Phosphorus Levels with Mortality in Elderly and Non-Elderly Hemodialysis Patients

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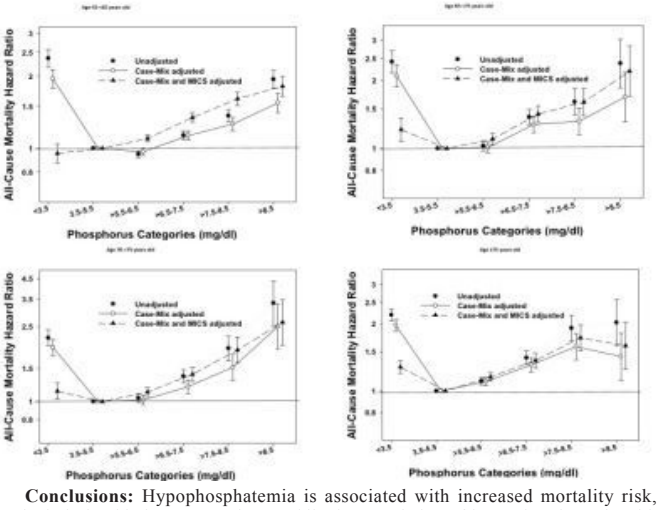
Background: Hypo- and hyperphosphatemia are associated with increased mortality in maintenance hemodialysis (MHD) patients. The correlations between serum phosphorus levels and mortality risks have not been investigated across different age groups of MHD patients.

Methods: We divided 88,635 MHD patients (aged ≥ 45 years old) treated in a large dialysis organization into 4 age groups (45-<65, 65-<70, 70-<75 and ≥75 years old). We examined the relationships of serum phosphorus levels with mortality risk in 4 incremental age categories using Cox proportional hazards models with adjustment for case-mix and surrogates of malnutrition and inflammation complex syndrome (MICS).

Results: Patients were 66±11 years old and included 46% women, 32% Blacks and 63% diabetics. The median follow-up time for the cohort was 761 days (408-1274 days). In the fully adjusted model hypophosphatemia (P<3.5 mg/dL) was significantly associated with increased death risks only in the older age groups (aged ≥65 years old), but not in the younger group (aged 45-<65 years old). Hyperphosphatemia was associated with increased mortality risk in all age categories.



Conclusions: Hypophosphatemia is associated with increased mortality risk, particularly in elderly MHD patients, while the association of hyperphosphatemia with increased death risk is similar across different ages.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO319

Physical Activity and Its Association with Mortality and Quality of Life in a Large Cohort of Hemodialysis (HD) Patients in the DOPPS

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Background: Physical activity is associated with better outcomes in the general population. We examined associations of types and levels of activity with outcomes in 5677 ambulatory chronic HD patients (pts) from 12 countries in the Dialysis Outcomes and Practice Patterns Study (DOPPS 4 2009-2011).

Methods: Self-reported activities (aerobic, muscle strength and flexibility) were assessed with the Rapid Assessment of Physical Activity instrument. The KDQOL and CES-D were used to assess quality of life (QOL) and depression, respectively. Adjusted associations were estimated by using Cox models for mortality, linear models for continuous outcomes, and ordinal logistic models for categories of HD recovery time.

Results: The distribution of aerobic activity was 29% sedentary, 10% infrequent, 17% light, 24% moderate, and 20% active. Frequencies of muscle activities were 4% strength, 9% flexibility, 7% both, and 80% neither. Pts at the 43% of facilities offering exercise programs reported higher levels of aerobic activity ($p<0.001$). Aerobic activity was inversely associated with mortality, recovery time, and depression, and positively associated with QOL (Table 1). Results for strength and flexibility were inconsistent (Table 2).

Table 1: Aerobic Activity

Adjusted Association* (95% CI)	Sedentary (N=1,654)	Infrequent (N=590)	Light (N=958)	Moderate (N=1,363)	Active (N=1,167)
Hazard Ratio for Death	1 (ref)	0.81 (0.64, 1.02)	**0.79 (0.65, 0.97)**	**0.77 (0.64, 0.92)**	**0.57 (0.45, 0.72)**
Difference in Physical HRQOL Summary Score (Higher is Better)	0 (ref)	**2.22 (1.31, 3.13)**	**4.17 (3.29, 5.05)**	**4.52 (3.72, 5.31)**	**7.52 (6.64, 8.41)**
Difference in Mental HRQOL Summary Score (Higher is Better)	0 (ref)	**2.64 (1.50, 3.77)**	**3.79 (2.84, 4.75)**	**3.23 (2.31, 4.15)**	**4.22 (3.18, 5.26)**
Difference in Kidney Disease Burden Score (Higher is Better)	0 (ref)	1.49 (-0.79, 3.76)	**7.76 (5.42, 10.11)**	**6.36 (4.52, 8.40)**	**10.36 (8.20, 12.52)**
Difference in Depression Symptoms Score (Lower is Better)	0 (ref)	**-1.11 (-1.71, -0.51)**	**-2.37 (-2.86,-1.88)**	**-2.09 (-2.54, -1.64)**	**-2.74 (-3.18, -2.31)**
Odds Ratio of Longer Recovery Time After HD Session (Lower is Better)	1 (ref)	**0.68 (0.48, 0.96)**	**0.65 (0.50, 0.85)**	**0.63 (0.48, 0.82)**	**0.41 (0.31, 0.55)**

Table 2: Muscle Strength or Flexibility

Adjusted Association* (95% CI)	None (N=4,542)	Strength (N=248)	Flexibility (N=530)	Both (N=392)
Hazard Ratio for Death	1 (ref)	0.84 (0.56, 1.26)	0.76 (0.57, 1.02)	1.22 (0.93, 1.61)
Difference in Physical HRQOL Summary Score (Higher is Better)	0 (ref)	-0.49 (-1.92, 0.95)	0.48 (-0.47, 1.42)	-0.18 (-1.31, 0.95)
Difference in Mental HRQOL Summary Score (Higher is Better)	0 (ref)	0.99 (-0.40, 2.39)	0.81 (-0.24, 1.85)	0.65 (-0.66, 1.95)
Difference in Kidney Disease Burden Score (Higher is Better)	0 (ref)	**-4.90 (-7.91, -1.89)**	2.08 (-0.16, 4.33)	-1.45 (-4.23, 1.34)
Difference in Depression Symptoms Score (Lower is Better)	0 (ref)	-0.11 (-0.76, 0.53)	-0.50 (-1.04, 0.04)	-0.05 (-0.56, 0.46)
Odds Ratio of Longer Recovery Time After HD Session (Lower is Better)	1 (ref)	1.06 (0.72, 1.57)	0.91 (0.68, 1.22)	**0.66 (0.46, 0.96)**

*Adjusted for sociodemographics, vintage, BMI, 14 comorbidities, lab variables, Kt/V, catheter use, geographic region and accounting for facility-level clustering. **Bold** values indicate $p<0.05$. Sample excludes non-ambulatory patients and those with missing RAPA data. For published RAPA scoring methods, the highest self reported activity level activity was used to classify patients. Infrequent and moderate refer to "under-active" and "under-active regular" in RAPA.

Conclusions: Our findings support the possibility that aerobic activity improves survival and QOL in ambulatory HD pts. Clinical trials to investigate the effectiveness of physical activity interventions are indicated.

Funding: Pharmaceutical Company Support - The DOPPS Is Administered by Arbor Research Collaborative for Health and Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), Abbott (Since 2009), Baxter (Since 2011), and Vifor Fresenius Renal Pharma (Since 2011), without Restrictions on Publications

FR-PO320

Independent and Joint Associations of Handgrip Strength and Malnutrition-Inflammation Score with Mortality Risk in Men and Women on Hemodialysis in the PROHEMO

Antonio Alberto Lopes,[1] Luciana Ferreira Silva,[2] Larissa Moura Silva,[1] Cacia Mendes Matos,[3] Gildete Barreto Lopes.[1] *[1]Universidade Federal da Bahia; [2]Universidade do Estado da Bahia; [3]INED, Salvador, Brazil.*

Background: Higher malnutrition-inflammation score (MIS) is associated with higher risk of death in maintenance hemodialysis (MHD) patients. We have shown that lower handgrip strength (HGS) correlates with higher MIS in MHD patients. We investigated independent and joint associations of HGS and MIS with mortality risk, separately for men and women on MHD.

Methods: The data were from 863 adult patients (340 women, 523 men, mean age 48.6±14.3 yr) from the first phase of the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEMO), a prospective cohort developed at four dialysis clinics in Salvador, Brazil (2005-2011). Collection of HGS was initiated in 2007 using a dynamometer. Data on MIS, HGS and both MIS and HGS were available for 813, 493 and 443 patients, respectively. Cox regression was used to estimate hazard ratio (HR) of death adjusted for sociodemographics, vintage, Kt/V and comorbid conditions. Interaction between MIS and HGS was evaluated under the additive risk model.

Results: The observed death rates for the combination MIS≥6 and HGS≤median (Table) were higher than the expected under additivity by 6% in men (observed 14.1, expected 13.3) and 44% in women (observed 11.7, expected 6.6). The reduction in the adjusted hazard of death per 5-point increase in HGS was 31% (HR= 0.69, 95% confidence interval (CI)= 0.50-0.94, P=0.020) in women and 27% (HR=0.73, 95% CI=0.62-0.85, P<0.001) in men. The increase in the adjusted hazard of death per 5-point increase in MIS was 33% (HR=1.33, 95% CI=1.01-1.73, P=0.039) in women and 47% (HR=1.47, 95% CI=1.18-1.83, P=0.001) in men.

Death Rate by 100 Person-Years in Men and Women

MEN			
	HGS>Median	**HGS≤Median**	**Rate Difference**
MIS<6	1.4	9.3	7.9
MIS≥6	5.4	14.1	8.7
WOMEN			
MIS<6	3.7	4.3	0.6
MIS≥6	6.0	11.7	5.7

HGS=Handgrip Strength, MIS=Malnutrion-Inflammation Score, Median HGS was 18.5 kg for women and 28.8 kg for men

Conclusions: The results support the evaluation of MHD patients with both HGS and MIS to identify those who may need special care to improve survival.

FR-PO321

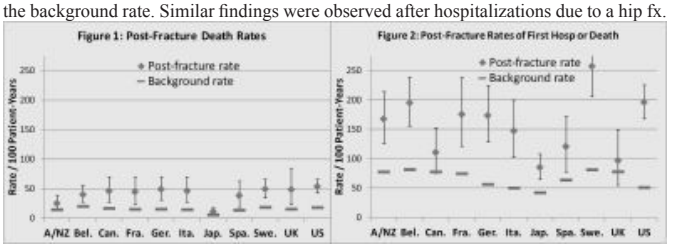
Higher Rates of Death and Hospitalization Following a Fracture among Participants in the Dialysis Outcomes and Practice Patterns Study (DOPPS)

Francesca Tentori,[1,2] Keith McCullough,[1] Ryan D. Kilpatrick,[3] Peter G. Kerr,[4] Brian D. Bradbury,[3] Bruce M. Robinson,[1,5] Ronald L. Pisoni.[1] *[1]Arbor Research Collaborative for Health, Ann Arbor, MI; [2]Vanderbilt University Medical Center; [3]Center for Observational Research, Amgen, Inc.; [4]Monash Medical Center; [5]University of Michigan.*

Background: Altered bone structure increases the risk of fracture (fx) in patients with end-stage renal disease. Though it is likely that major fx events adversely affect overall health, this has not been tested in a multi-national study. We estimated death and hospitalization (hosp) rates following a fx-related hosp in the international DOPPS cohort.

Methods: Rates of first hosp due to fx (length of stay ≥1 day) were collected from 34,083 hemodialysis (HD) patients from 12 DOPPS countries (2002-11). Within each country, death rates were estimated (1) over a 1-yr follow-up period among participants who had experienced any fx (n=1,183) and a hip fracture (n=509) ("post-fx") and (2) among all participants ("background"). Country-specific rates of the composite event of death or first hosp were also calculated.

Results: Fx incidence after DOPPS enrollment varied across countries (range: 1.2 [Japan] - 4.4 per 100 patient-years [Sweden]). Post-fx mortality rates were higher than background rates across the countries (Figures 1,2). Rates of the composite hosp/death events after a bone fx also varied across countries and ranged from 1.2-3.8 times higher than the background rate. Similar findings were observed after hospitalizations due to a hip fx.



Conclusions: Fx events can have serious implications for HD patients. After a fx, the morbidity and mortality burden is substantially elevated compared to the risk in the general dialysis population. Thus prevention of fxs is of great importance. Future studies should investigate post-fx healthcare resource utilization to more fully quantify their burden.

FR-PO322

Performance of Health Literacy Assessment Methods in Chronic Hemodialysis

Kerri L. Cavanaugh, Kenneth A. Wallston, Natalia E. Plotnikova, Francesca Tentori, T. Alp Ikizler. *Vanderbilt University.*

Background: Limited health literacy (HL) is a risk for poor outcomes, including mortality, in patients receiving hemodialysis therapy. Current methods commonly used to evaluate health literacy are not practical for clinical applications. We examined the performance of three brief health literacy screening questions in a sample of prevalent maintenance hemodialysis (MHD) patients.

Methods: In a cross sectional study, we enrolled adults receiving MHD therapy. We administered previously validated HL measures, REALM and TOFHLA, and 3 brief HL screening questions: 1). How confident are you filling out forms; 2). How often do you have problems learning about your medical condition; and 3). How often do you have someone help you read hospital materials. Adequate and inadequate HL was defined for each survey and the C-index and test characteristics were compared. In addition, associations between the brief HL items and patient outcomes was analyzed using Chi-squared or Student's t-tests.

Results: In 150 patients, mean (SD) age 52 (14) years, 50% male, 75% non-white, vintage 4.7 (5.4) years the prevalence of limited HL was 27%, 15%, 14% by REALM, TOFHLA, and brief questions, respectively. Overall, 30% reported being confident with forms "somewhat" or less. These questions showed similar performance for detecting limited HL when compared to the REALM (C-index=0.64, (0.52-0.75)) or the TOFHLA (C-index=0.64, (0.48-0.81)). Limited HL by brief items was associated with older age, non-white race, and years education. Limited HL was also associated with lower dialysis knowledge (53% vs. 69%, p=0.003) and less optimal phosphorus control (76% vs. 54%, p=0.06).

Conclusions: Limited health literacy is common among patients receiving hemodialysis. The brief 3 HL items discriminated between those with inadequate and adequate literacy compared to other measures and were related to important patient outcomes. HL may be a factor to consider in risk stratification and as an intervention target to improve outcomes in hemodialysis for both researchers and clinicians.

Funding: NIDDK Support, Private Foundation Support

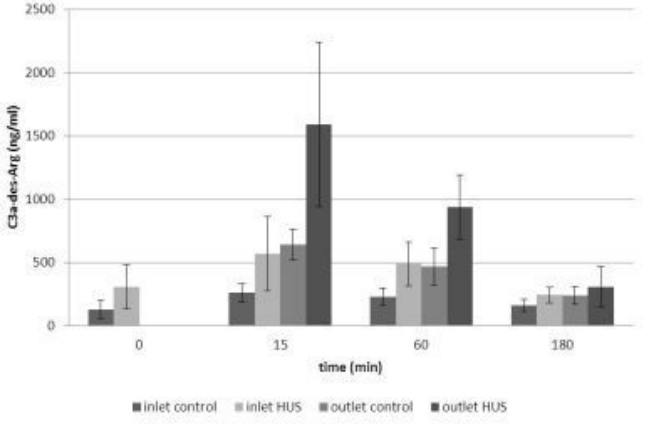
FR-PO323

Complement Activation during Hemodialysis in Patients with Atypical Hemolytic Uremic Syndrome Rogier Caluwé,[1] Annemieke Dhondt,[2] Eric Goffin,[3] Steven Van Laecke,[2] Ignace M. Becaus,[1] Bruno Van Vlem,[1] Raymond C. Vanholder.[2] *[1]Nephrology, OLV Hospital, Aalst, Belgium; [2]Nephrology, University Hospital, Gent, Belgium; [3]Nephrology, UCL Hospital, Brussels, Belgium.*

Background: Atypical hemolytic uremic syndrome (aHUS) is often caused by a defect in one of the regulatory proteins of the alternative complement system pathway. This case-control study evaluates whether aHUS patients have more intense complement system activation during hemodialysis.

Methods: During 2 hemodialysis sessions complement system activation was evaluated in 4 patients with aHUS (proven factor H mutation) and 14 controls. C3a-des-Arg was measured at the start of dialysis and after 15, 60 and 180 minutes at both the helixone dialyzer inlet and outlet. Factor B, factor Bb and the soluble terminal complement complex (sTCC) were measured at the start of dialysis and after 180 minutes at the dialyzer inlet. The Mann-Whitney-U test was used for statistical analysis.

Results: C3a-des-Arg levels were significantly higher in aHUS patients at baseline (109±66 vs. 267±317, p=0.015) and at every measured timepoint at the dialyzer inlet as visualized in the figure. After passing the dialyzer membrane C3a-des-Arg levels increased at every timepoint. This increase was significantly higher in aHUS patients at 15 (353±143 vs. 916±684, p=0.003) and 60 (207±118 vs. 434±151, p=0.015) minutes. Factor B levels did not differ between groups. The increase in Factor Bb levels was higher in aHUS patients (1561±821 vs. 2269±1194, p=0.026). sTCC levels increased but did not differ between groups.



Conclusions: aHUS patients have a more pronounced activation of the alternative complement pathway during hemodialysis when compared to controls. In view of the poor prognosis of aHUS patients on hemodialysis these findings are of importance as they might play a role in sustaining a deleterious pro-inflammatory tendency.

Funding: Clinical Revenue Support

FR-PO324

Body Composition Is Associated with Quality of Life in Hemodialysis Patients Nestor E. Almeida,[1] T. Alp Ikizler,[3] Y. Zhang,[1] R. Filipowicz,[1] Mary B. Sundell,[3] G. Morrell,[1] G. Wei,[1] J. Abraham,[1] A. N. Habib,[1] T. S. Bjordahl,[1] Tom Greene,[1] Alfred K. Cheung,[1,2] S. Beddhu.[1,2] *[1]Univ of Utah, Salt Lake City, UT; [2]VA, Salt Lake City, UT; [3]Vanderbilt Univ, Nashville, TN.*

Background: Even though, higher body size has been linked to increase survival in hemodialysis (HD) pts, it is not clear whether muscle mass or fat mass or both are associated with better quality of life (QOL). Therefore we examined the associations of physical composite score (PCS) and mental composite score (MCS) of SF-12, a validated survey for QOL with body size, body mass index (BMI) and body composition, MRI measurement of mid-thigh muscle area (MTMA) and intra-abdominal fat area (IAFA).

Methods: 110 pts in the Protein Intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) study who underwent SF-12 survey and MRI were included. Generalized estimating equations (GEE) were used to relate the associations of the PCS and MCS with MTMA and IAFA areas, and BMI.

Results: Mean age was 51 ± 17 years, 57% were men, 78% were Caucasians and 42% had diabetes. Follow-up MRI data were available in 82 and 46 pts at 6 and 18 months visits, respectively. Mean MTMA and standard deviation (SD) at baseline, 6 and 18 months were 108 ± 29, 107 ± 27, and 102 ± 26 cm^2, respectively, and IAFA were 128 ± 73, 132 ± 80, and 141 ± 81 cm^2, respectively. The associations of BMI and body composition with QOL are summaryzed in Table 1.

Table 1. Associations of mid-thigh muscle, intra-abdominal fat areas, and BMI with PCS and MCS scores of the SF-12 survey

	PCS β, (95% CI), p value	MCS β, (95% CI), p value
Each SD ↑ in MTMA (cm^2)	2.0 (-0.03 to 4.0), 0.054	1.0 (-1.1 to 3.1), 0.37
Each SD ↑ in IAFA (cm^2)	-1.5 (-3 to -0.02), 0.05	0.2 (-1.4 to 1.8), 0.82
Each SD ↑ in BMI (kg/m^2)	-0.05 (-1.5 to 1.4), 0.94	0.2 (-1.2 to 1.6), 0.74

Adjusted for age, gender, race, ESRD duration, vascular access type and study site

Conclusions: Higher muscle area and lower fat area are associated with better PCS. Body composition is not associated with MCS. Interventions that increase muscle mass and decrease fat mass may improve physical well being of patients undergoing HD.

Funding: NIDDK Support

FR-PO325

Pregnancy in Patients on Dialysis: Experience over 40 Years in Australia and New Zealand Shilpa Jesudason,[1] Blair S. Grace.[2] *[1]Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia; [2]ANZDATA Registry, RAH, Adelaide, Australia.*

Background: The management of the pregnant woman requiring dialysis is a rare but challenging scenario. Recent case series from single centres suggest outcomes in these high-risk pregnancies are improving. This study reports for the first time outcomes regarding women receiving dialysis in pregnancy across Australia and New Zealand over 40 years.

Methods: The ANZDATA Registry records annual incident data from all Renal units in Australia and New Zealand. Prior to 2001, pregnancy was recorded only when reported; after 2001 detailed pregnancy-related data including conception date was collected. We reviewed all reported data for pregnancies in women who recieved intra-pregnancy dialysis from 1963 – 2010 (n=104).

Results: From 1963-2000, 33 women had dialysis during 38 pregnancies - 35 were conceived on dialysis, 3 conceived with a transplant then required dialysis. Almost all were managed with haemodialysis. There were 9 live births, 9 surgical terminations, 14 spontaneous abortions < 20 weeks gestation and 2 stillbirths > 20 weeks. From 2001-2010, there were 64 pregnancies in 61 women. 45 of these pregnancies were conceived on dialysis and 19 conceived before dialysis started. 56 were managed with haemodialysis, and 8 with peritoneal dialysis. There were 41 live births, 11 surgical terminations, 6 spontaneous abortions < 20 weeks and 6 stillbirths > 20 weeks. In those who did not terminate the pregnancy, the live birth rate was 77 %. Live births had median gestational age 33.6 weeks (IQR 30.4 – 38.6) and median birth weight 1780 g (IQR 1186 – 2675). The 28 day neonatal survival for live births was 98%. There were no maternal deaths.

Conclusions: This is one of the largest series reported and the only nation-wide, multi-centre report. Pregnancy in this cohort resulted in a high live birth rate, advanced gestational age and excellent neonatal survival, which is important to report given the high risk nature of these pregnancies and previous pessimism regarding outcomes. Further analyses will address the relevance of timing of conception, rate of complications and aspects of dialysis delivery.

Funding: Government Support - Non-U.S.

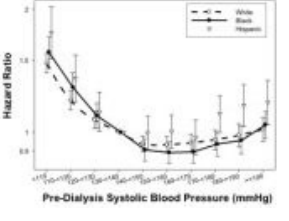
FR-PO326

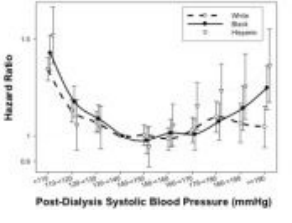
Racial-Ethnic Differences in the Impact of Systolic Blood Pressure on Mortality in Hemodialysis Patients Joni L. Ricks,[1] John J. Sim,[2] Miklos Zsolt Molnar,[1] Csaba P. Kovesdy,[3] Kamyar Kalantar-Zadeh.[1,4] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LABioMed at Harbor-UCLA, Torrance, CA; [2]Kaiser Permanente; [3]Nephrology, University of Tennessee, Memphis, TN; [4]Nephrology, Harbor-UCLA, Torrance, CA.*

Background: Previous research has demonstrated that low systolic blood pressure (SBP) is associated with decreased survival among maintenance hemodialysis patients (MHD). Empirical research examining this link by race has indicated that black patients demonstrate a survival advantage compared to whites across all SBP groups. However, additional racial-ethnic groups have not been examined.

Methods: We examined the survival impact of baseline SBP in a 5-year (7/2001-6/2006) cohort of 47,813 white, 35,874 black, and 16,180 Hispanic outpatients in DaVita dialysis clinics using Cox models adjusted for case-mix and surrogates of Malnutrition-Inflammation Complex.

Results: Among whites, blacks and Hispanics, the mean age (mean±SD) was 65±15, 57±15, and 58±15, yrs old and included 42%, 48% and 45% women; and 54%, 56% and 69% diabetics, respectively. Across 10 a priori selected SBP groups from <110 to ≥190, both low Pre-Dialysis and Post-Dialysis SBP were associated with higher mortality in whites, blacks and Hispanics. Moreover, the two highest Pre-Dialysis SBP groups were not associated with higher mortality among blacks and whites; whereas the two highest Pre-dialysis SBP groups were associated with higher mortality in Hispanics.





Conclusions: Our findings evaluate the differential impact of SBP among racial-ethnic groups. Among MHD patients, all races show worsened survival in the lowest SBP groups; however, only Hispanics demonstrated an increased mortality with high pre and post-dialysis SBP.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO327

Secular Trends and Outcomes in Lower Extremity Amputations in End Stage Renal Disease Patients

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Background: The nontraumatic lower extremity amputation (LEA) rate attributable to diabetes mellitus and peripheral vascular disease has been high in ESRD patients. LEA rates increased during the 1990s, but ESRD patient survival has since improved. We present trends in LEA in Medicare ESRD patients between 1985 and 2007, to identify risk factors for and rates and levels of LEA, and associations between LEA and survival.

Methods: Using the United States Renal Data System (USRDS), rates for LEA among ESRD patients were calculated from 1985-2007, stratified by demographic factors, diabetes, and amputation level. Kaplan-Meier analysis was used to estimate patient survival after first LEA, stratified by amputation level. Cox regression analysis identified associations between patient characteristics and mortality.

Results: From 1985-2007, 173,612 (9.4%) of the 1,851,652 patients in the ESRD program had a LEA. ESRD amputees were more likely to be older, male, black, Native American, and have diabetes than nonamputees. The LEA rate among diabetic ESRD patients fell from 13/100 person years in 1996 to 7.9/100 in 2008, with a similar trend among nondiabetic ESRD patients. 1-year mortality among ESRD patients undergoing amputation was 47.2%. Above the knee amputees had a 69.8% 1-year mortality compared to 34.9% among toe amputees. ESRD amputees experienced poorer survival than nonamputees. In multivariate analysis, increasing age, female gender, white race, absence of diabetes, and proximal sites of amputation were all associated with a higher mortality after LEA.

Conclusions: LEA rates in both diabetic and nondiabetic ESRD patients are very high, but have dramatically decreased from 1996-2008, perhaps related to advances in vascular, radiologic, microbiologic, and multidisciplinary diagnosis and care in patients at risk for lower limb ischemia. Level of amputation was associated with differential 1-year mortality in univariate and multivariate analysis. Programs to improve care for lower extremities in ESRD patients may improve survival.

Funding: NIDDK Support

FR-PO328

Post-Fracture Outcomes among U.S. Medicare Hemodialysis Patients from 2000-2008

Anne C. Beaubrun,[1] Ryan D. Kilpatrick,[2] Janet K. Freburger,[1] Brian D. Bradbury,[2] Lily Wang,[1] M. Alan Brookhart.[1] *[1]University of North Carolina at Chapel Hill; [2]Center for Observational Research, Amgen, Inc.*

Background: Dialysis patients (pts) are at increased risk of fracture (Fx), a contributor to morbidity and mortality. Data on post-Fx consequences in this population are limited.

Methods: A retrospective study was conducted using U.S. Medicare claims of adult hemodialysis pts from Jan. 1, 2000-Dec. 31, 2008. Index hospitalizations (hosp) for 7 Fx categories [vertebral, pelvis/hip, femur, lower leg, ribs/sternum, shoulder/upper arm, forearm/wrist] were identified. Median hosp length of stay (LOS), mortality, and discharge destination were determined. Age, sex, and race adjusted mortality, hosp and skilled nursing facility (SNF) admissions, hosp and SNF days were calculated over 1-year of follow-up.

Results: Primary hosp LOS was shortest for forearm/wrist Fxs (5 days, 95% CI 3-9) and longest for femur Fxs (8 days, CI 5-13). 28% (ribs/sternum) to 56% (pelvis/hip) of patients were discharged to a SNF or other post-acute care facility. Re-hosp rates ranged from 3.3-4.4/PY and pts spent a median of 16-28 days in the hosp and/or 0-25 days in a SNF. Death rates ranged from 0.4 to 0.6/PY; highest for vertebral, pelvis/hip and femur Fxs.

Post-Fx Consequences by Fx Type, 2000-2008

	Primary Hosp		Post-Discharge (1yr)				
Fx Type (N)	LOS§	Discharged to SNF(%)	Re-hosp Rate†	Re-hosp Days§	SNF Admission Rate†	SNF Days§	Death Rate†
Pelvis/Hip (15,423)	7 (5-12)	56	3.7 (3.6-3.7)	25 (6-70)	1.0 (1.0-1.0)	25 (0-99)	0.5 (0.5-0.5)
Vertebral (6536)	7 (4-13)	33	4.4 (4.3-4.5)	28 (6-74)	0.9 (0.9-1.0)	0 (0-79)	0.6 (0.6-0.6)
Lower leg (4747)	6 (4-10)	48	3.5 (3.4-3.6)	20 (4-57)	0.9 (0.9-0.9)	11 (0-83)	0.4 (0.4-0.4)
Shoulder/ Upper arm (3223)	6 (3-10)	41	4.0 (3.9-4.1)	22 (4-61)	0.8 (0.8-0.9)	9 (0-89)	0.5 (0.5-0.6)
Femur (2627)	8 (5-13)	53	3.9 (3.8-4.0)	27 (6-77)	1.1 (1.1-1.2)	22 (0-99)	0.6 (0.5-0.6)
Ribs/ Sternum (2308)	6 (3-10)	28	4.1 (4.0-4.2)	20 (4-55)	0.7 (0.7-0.8)	0 (0-45)	0.5 (0.5-0.5)
Forearm/ Wrist (1693)	5 (3-9)	38	3.3 (3.2-3.4)	16 (3-47)	0.7 (0.6-0.7)	0 (0-66)	0.4 (0.4-0.4)

*With follow-up,†per person-year(CI),§median(Q1-Q3)

Conclusions: The burden of Fxs in the dialysis population continues well beyond the inpatient admission. Efforts to prevent Fxs in dialysis pts are warranted.

Funding: Other U.S. Government Support, Pharmaceutical Company Support - Amgen, Inc.

FR-PO329

Hip Fracture Trends in US Medicare Hemodialysis Population, 1993:2010

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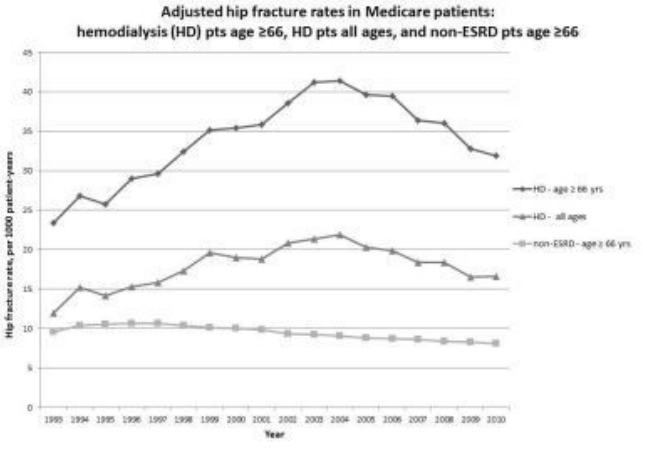
Background: Hip fracture (Fx) is the most common fracture type resulting in hospitalization among hemodialysis (HD) patients (pts), causing significant morbidity and increased mortality. We aimed to estimate current hip Fx rate and characterize historical rates to describe changes which may have resulted from medication management strategies or changing HD population characteristics.

Methods: We studied annual cohorts of point prevalent Medicare (MC) HD pts from 1993-2010 for first hip Fx, defined by hospitalization with a diagnosis code for hip Fx. Pts were followed to earliest of Dec. 31 of index yr, hip Fx, kidney transplant, change of MC Parts A/B enrollment or death. Rates were adjusted for age, race, gender, dialysis vintage, ESRD cause, number of hospitalized days and comorbidities in prior year. To distinguish trends specific to dialysis pts, hip Fx rates in the subset of pts ≥66 yrs old (50-53%) were compared to annual cohorts of non-ESRD pts, in MC 5% sample, adjusting for age, gender and race.

Results: Both unadjusted and adjusted hip Fx rates in MC HD pts increased from 1993 to 2004 and decreased from 2004 to 2010. Linear spline with node at 2004 showed a significant change in the trend before and after 2004 (p<.0001). The trend for the ≥66 HD subset was similar to the trend for the full HD cohort, but differed markedly in magnitude and pattern for ≥66 non-ESRD MC pts, which was always substantially lower with a slowly decreasing rate since 1996.



Conclusions: Hip fracture rates among MC HD pts increased from 1993-2004 and since 2004 have been decreasing, in contrast to non-ESRD MC pts where hip fracture rate has steadily declined since 1996. Further research is needed to define factors associated with observed trends.

Funding: Pharmaceutical Company Support - Amgen, Inc., Private Foundation Support

FR-PO330

Administration of IL-1ra Improves Adiponectin (ADPN) Levels in Chronic Hemodialysis (CHD) Patients

Chutatip Limkunakul,[2] Edward D. Siew,[1,2] Cindy Booker,[1,2] Charles D. Ellis,[2] Ayumi Shintani,[3] T. Alp Ikizler,[1,2] Adriana Hung.[1,2] *[1]CSR&D, Veterans Administration TVHS, Nashville, TN; [2]Nephrology, Vanderbilt University, Nashville, TN; [3]Biostatistics, Vanderbilt University, Nashville, TN.*

Background: Adiponectin (ADPN), an adipose tissue-derived hormone, is known to be insulin sensitizing, anti-inflammatory, and anti-atherogenic in the general population. However ADPN secretion is suppressed by inflammation. Interleukin 1 receptor antagonist (IL-1ra) administration is an effective anti-inflammatory intervention in CHD patients. In this study, we evaluated the effects of IL-1ra administration on adipokines (ADPN and Leptin) in 14 stable CHD patients. We also examined the association between adipokines and insulin sensitivity at baseline.

Methods: Data was derived from a pilot RCT of the administration of IL-1ra in CHD patients with hsCRP > 5 mg/dL for 3 consecutive months. Patients were randomized to Anakinra 100 mg subcutaneous injection or placebo at each dialysis session for 4 weeks. Analysis of covariance was used to compare percent change from baseline to 4 weeks.

Results: Twenty CHD patients were randomized and 14 completed the trial (mean age 49 ± 13 years, 71% African American, 71% males and 21% had diabetes). At baseline, the median values for ADPN, Leptin, Leptin-Adiponectin ratio (LAR) and HOMA were 11.5 µg/mL (IQR 9, 28.5), 17.8 ng/mL (3.9, 50.0), 2.20 (0.13, 3.98) and 2.8 (2.0, 3.6), respectively. HOMA was associated with ADPN (p=0.004), Leptin (p=0.01) and LAR (p=0.0006). IL-6 was associated with HOMA (p=0.03) and borderline with LAR (p=0.06), but not with ADPN or leptin alone. Patients in the intervention arm had a mean percent

increase in serum ADPN of 22% vs. 14% decrease in the placebo arm (p=0.003). Leptin, LAR or HOMA did not change with intervention.

Conclusions: Administration of IL-1ra significantly increased adiponectin (ADPN) levels while concomitantly reducing inflammation among CHD patients. There were no obvious improvements in insulin sensitivity with the intervention. This study demonstrates that anti-inflammatory interventions significantly impact adiponectin physiology in ESRD population.

Funding: NIDDK Support, Veterans Administration Support

FR-PO331

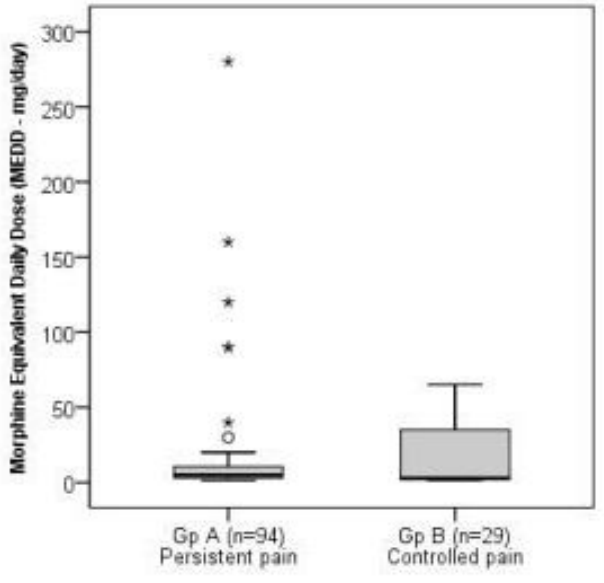
Factors Associated with Persistent Pain in Hemodialysis Patients

Ahraaz Wyne, Jessica M. Sontrop, Sharon L. Baker, William F. Clark, Rita Suri. *Western University, London, Canada.*

Background: Persistent pain is highly prevalent in hemodialysis (HD) patients. We hypothesized that inadequate pain control in HD patients is associated with psychiatric illness and analgesic under-prescription.

Methods: We surveyed a random sample of 189 HD patients from 4 Canadian academic HD units for pain, comorbidities, medications, preferences, anxiety (Spielberger State Trait Anxiety Inventory), depression (Beck Depression Inventory) and sleep (MOS Sleep Scale). To eliminate indication bias, we excluded 65 patients not taking analgesics (41 no pain, 25 with pain). Of the remaining 123, we compared 94 (76%) subjects with persistent pain despite analgesic use (GpA), to 29 (24%) controls whose pain was completely controlled on analgesics (GpB).

Results: Demographics and comorbidities were similar between groups, except for trend to more males in GpA (59 vs 35%, p=0.06). Compared to GpB, GpA had less anxiety (STAI=21 vs 24, p=0.02), but similar depression and sleep. There was no difference in type of analgesics used (GpA, GpB: opioids 44, 48%; NSAIDs 12, 7%; acetaminophen 72, 69%; all p=NS). Opioid doses were similar (median morphine equivalent daily dose: GpA 5mg/d, GpB 3mg/d, p=0.4), but GpA had a narrower interquartile range. Interestingly, most in GpA (76%) felt their physicians were addressing their pain, but only 31% would accept more analgesics if offered. Reasons for declining more analgesics included fears of: too many medications (38%), side effects (15%) and addiction (10%).



Conclusions: We found that inadequate pain control was not associated with depression, insomnia, more anxiety, or physician inattention. Rather, most HD patients with persistent pain did not want more analgesics due to fear of polypharmacy and addiction. There is a need to educate patients about acceptability regarding analgesics and explore non-pharmacologic pain control strategies in HD patients.

Funding: Government Support - Non-U.S.

FR-PO332

The Burden of Drug Dependence among Hemodialysis Patients

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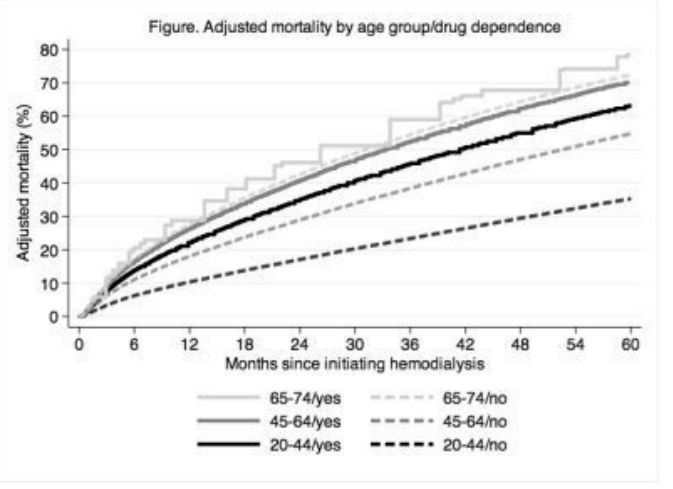
Background: Illicit drug use is known to cause and exacerbate a wide spectrum of kidney disease. We examined the prevalence and associated mortality of illicit drug dependence among the hemodialysis population.

Methods: From the U.S. Renal Data System 2000-2009, we examined the prevalence of illicit drug dependence (current or within the last 10 years) checked off as a comorbidity on the Medical Evidence Form 2728 among 931,280 incident hemodialysis patients aged 20-90 years. Among the 95,750 patients initiating hemodialysis in 2005, we used Cox proportional hazard models to estimate the probability of death by age group and drug dependence adjusted for gender, race, and primary cause of end-stage renal disease.

Results: The prevalence of drug dependence was 1.3% overall, but increased over time from 1.0% to 1.6% (p<0.001 by chi-square test for linear trend) and was 4.1% among 20-44 year olds and 2.0% among 45-64 year olds. Nearly all (95.9%) drug dependence was among patients under age 65 years. Adjusted mortality varied by drug dependence and age group (Figure, $p_{interaction}$ <0.001). Dependent 20-44 year olds had higher likelihood of mortality than their non-dependent counterparts (HR 2.30, 95% CI 2.03-2.60) and non-dependent 45-64 year olds (HR 1.26, 95% CI 1.12-1.42). Likelihood of mortality among dependent 45-64 year olds was higher than their non-dependent counterparts (HR 1.54, 95% CI 1.39-1.69) and similar to that of non-dependent 65-74 year olds (HR 0.94, 95% CI 0.86-1.04). Likelihood of mortality was similar among 65-74 and 75-90 year olds regardless of drug dependence (p>0.1).



Conclusions: Illicit drug dependence among the youngest adult hemodialysis patients is common and is associated with a higher likelihood of death than non-dependent middle-aged patients.

Funding: Other U.S. Government Support, Private Foundation Support

FR-PO333

Clinical Parameters in Dialysis Patients Show Different Circannual Patterns in Different Regions of the World

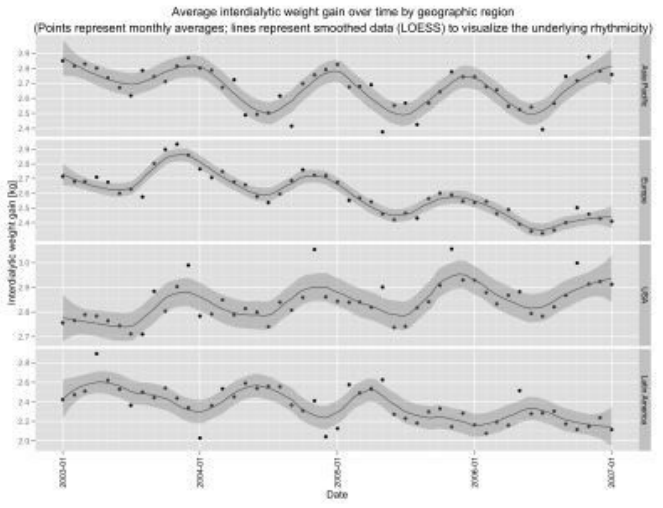
Stephan Thijssen,[1] Len A. Usvyat,[1] Inga Bayh,[4] Michael Etter,[3] Aileen Grassmann,[4] Adrian Marcos Guinsburg,[5] Jeroen Kooman,[6] Nathan W. Levin,[1] Eric Liu,[3] Daniele Marcelli,[4] Cristina Marelli,[5] Frank van der Sande,[6] Laura Scatizzi,[4] Adam Tashman,[1] Edwin B. Toffelmire,[7] Yuedong Wang,[2] Peter Kotanko.[1] *[1]Renal Research Institute, New York, NY; [2]University of California, Santa Barbara, CA; [3]Fresenius Medical Care, Hong Kong, Hong Kong; [4]Fresenius Medical Care, Bad Homburg, Germany; [5]Fresenius Medical Care, Buenos Aires, Argentina; [6]University Hospital Maastricht, Maastricht, Netherlands; [7]Fresenius Medical Care, Toronto, Canada.*

Background: Seasonal variability in clinical parameters has been described in hemodialysis (HD) patients. No systematic comparison of these patterns has been performed between different regions of the world.

Methods: The MONitoring Dialysis Outcomes (MONDO) consortium consists of HD databases from Renal Research Institute clinics (US), Fresenius Medical Care clinics in Europe, Asia, Latin America and Canada, Maastricht University (Netherlands), and KfH clinics in Germany (latter three not used here). Monthly data (up to 20,000 pat. records) on serum albumin, pre-HD systolic BP (SBP), pre-HD weight and interdialytic weight gain (IDWG) were analyzed between 01/2003 and 01/2007 for the US, Asia Pacific (5 countries excl. Australia), Latin America (Argentina), and Europe (16 countries).

Results: IDWG, SBP, and body weight showed a circannual rhythm with highest values in winter and lowest values in summer. With Argentina being in the southern hemisphere, the pattern was shifted by about 6 months compared to the other regions (see Fig. 1 for IDWG; shaded area shows 95% conf. band). There was no clear seasonal pattern for albumin in any region.

Conclusions: SBP, weight and IDWG show clear circannual patterns, and our data suggest the primacy of climatic factors in determining these patterns.



FR-PO334

Prediction of Serum Bicarbonate Levels in Chronic Hemodialysis Patients: Results from a Retrospective Cohort Study Jochen G. Raimann, Len A. Usvyat, Stephan Thijssen, Peter Kotanko. *Renal Research Institute.*

Background: Chronic hemodialysis (HD) patients have varying levels of acid generation which mainly derive from dietary proteins. Dialysate bicarbonate (DBic) counteracts the resulting metabolic acidosis. This analysis aims to investigate the feasibility to predict post-HD serum bicarbonate levels (post-HD SBic).

Methods: We studied chronic HD patients treated between 7/2005 and 11/2011 in Renal Research Institute HD clinics in whom pre- and post-HD SBic were available. Pre-HD DBic to SBic gradient (GBic) was defined as DBicminus pre-HD SBic. Linear regression analysis was employed to predict post-HD SBic.

Results: Three-hundred ninety-five HD patients were included (pre-HD SBic24.4±3.2 mEq/L; post-HD SBic30.1±3.1 mEq/L; pre-HD GBic 12.3±3.4 mEq/L; treatment time 209±29 minutes; blood flow rate 407±57 mL/min; dialysate flow rate 648±87 mL/min). Pre-HD SBic and GBic were significant predictors of post-HD SBic (R^2=0.24; P<0.01).

Conclusions: In multiple regression analysis post-HD SBic is significantly correlated with pre-HD SBic and GBic. The low predictive power of the developed model suggests that a mathematical model including inter– and intradialytic acid-base balance, intradialytic bicarbonate mass transfer as being necessary to individualize DBic prescription.

FR-PO335

Prediction of Monthly Serum Bicarbonate Levels in Chronic Hemodialysis Patients Jochen G. Raimann, Len A. Usvyat, Stephan Thijssen, Peter Kotanko. *Renal Research Institute.*

Background: In chronic hemodialysis (HD) patients both high (>28 mmol/L) and low (< 20 mmol/L) pre-HD serum bicarbonate levels (SBic) have been associated with mortality. We aimed to identify predictors of monthly SBic.

Methods: We studied chronic HD patients treated between 11/2011 and 3/2012 in Renal Research Institute HD clinics. Multiple linear regression models with backward elimination were constructed with SBic in 3/2012 as the dependent variable and laboratory and clinical predictors assessed in the 4 preceding months. Models were developed in a randomly selected derivation cohort and tested in an independent validation cohort.

Results: We enrolled 994 chronic HD patients (age 62±15 years, 49% Blacks, 50% diabetes; SBic 24.6±3.0 mmol/L). In the derivation cohort (N=364) SBic was predicted by SBic levels of the 3 preceding months, dialysate concentrations of acetate and chloride, enPCR, and serum sodium concentration (all P<0.001); serum levels of albumin, creatinine, and chloride were marginally significant predictors (P-values between 0.02 and 0.05). Current and historical dialysate bicarbonate concentrations were excluded from all models (all P>0.1). Model evaluation in the validation cohort (N=630) showed a high correlation between predicted and observed Sbic (adjusted R^2 0.393; P<0.001); the mean difference between predicted and observed Sbic was 0.41 mmol/L (95%CI 0.19 to 0.63).

Conclusions: Next month's SBic can be predicted with clinically acceptable accuracy with SBic levels of the preceding 3 months, protein intake (enPCR), acetate and chloride levels in the dialysate and serum sodium as the key determinants.

FR-PO336

Quality of Life among Hemodialysis Patients in China: Results from the China Dialysis Outcomes and Practice Patterns Study (DOPPS) Jia Qi Qian,[2] Brian Bieber,[1] Mia Wang,[1] Mei Wang,[3] Li Zuo,[3] Xueqing Yu,[3] Xiao Yang,[3] Nan Chen,[3] Yucheng Yan,[3] Ronald L. Pisoni,[1] Sylvia Paz B. Ramirez.[2] *[1]Arbor Research; [2]Co-First Author, China DOPPS Country Investigator; [3]China DOPPS Country Investigators.*

Background: Differences in Quality of Life (QoL) for Asian vs non-Asian pts with chronic medical conditions have been reported. However, published literature among Chinese hemodialysis (HD) pts compared to other racial/ethnic groups is limited. To understand practice patterns of HD care and pt QoL in China, an initial cross-sectional study using the DOPPS protocol was conducted in Beijing, Guangzhou and Shanghai.

Methods: We present analyses of self-reported QoL and depression (self-reported and physician diagnosed) for 1090 pts in China from 2011 compared to pts in the other DOPPS 4 countries (2009-11). QoL was captured via pt responses to the SF-12. Self-reported depression was defined by a CES-D (Center for Epidemiological Studies Depression Screening Index) score of ≥ 10.

Results: The mean MCS score in China (44) was, similar to other DOPPS countries (range, 41-48). Mean PCS score in China (36) was lower than Japan (42) but slightly higher than other DOPPS countries (range, 34-36). A relatively high percentage (51%) of self-reported depression was seen in China as compared to other countries (range, 34-64). Only 6% of Chinese pts had a history of physician-diagnosed depression in their medical record, similar to Japan(5%) but less frequently diagnosed than in other DOPPS countries (range, 11%-22%).

Conclusions: The SF-12 has previously been utilized in the Chinese non-ESRD population. Assuming that the SF-12 is valid for Chinese HD pts, similar MCS and PCS scores were observed as pts in other DOPPS countries. Mean MCS scores were similar in Japanese and Chinese pts, but mean PCS scores were higher among Japanese HD pts. The discrepancy observed between self-reported and physician-diagnosed depression in China, suggests that depression is potentially under-diagnosed. Determinants of these pt-reported outcomes, as well as their relationship with clinical outcomes, will further be explored in an ongoing longitudinal DOPPS study in China.

Funding: Pharmaceutical Company Support - The Initial Cross-Sectional DOPPS Study in China Was Supported by Abbott Laboratories without Restrictions on Publications. The International DOPPS Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), and Abbott Laboratories (Since 2009)

FR-PO337

Associations of Epworth Sleepiness Scale and Cognitive Function in Hemodialysis Population Neelakania A. Dadi,[1,2] G. Wei,[1] G. Chelune,[1] R. Filipowicz,[1] S. Boddeda,[1] Y. Zhang,[1] Nestor E. Almeida,[1] Manjula Kurella Tamura,[3] Mark L. Unruh,[4] Tom Greene,[1] T. Alp Ikizler,[5] S. Beddhu.[1,2] *[1]Univ of Utah, Salt Lake City, UT; [2]VHASLC, Salt Lake City, UT; [3]Stanford Univ, Palo Alto, CA; [4]Univ of Pittsburgh, Pittsburgh, PA; [5]Vanderbilt Univ, Nashville, TN.*

Background: Hypoxic episodes associated with sleep disordered breathing might result in ↑ sleepiness and ↓ cognition. Therefore, we examined the associations of ↑ sleepiness on Epworth Sleepiness Scale (ESS) with cognitive function assessed by Montreal Cognitive Assessment (MOCA) in 121 hemodialysis (HD) patients (pts).

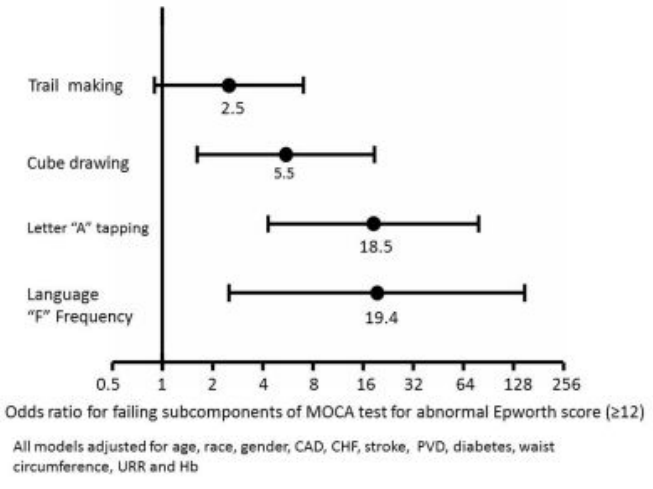
Methods: Separate multiple linear regression and logistic regression models were used to relate the total MOCA score and each of the 30 MOCA sub-components to an abnormal ESS (score≥12) controlling for comorbidity and demographics.

Results: Clinical characteristics in those with low and high ESS scores are summarized in Table 1.

Clinical characteristics by low and high ESS scores

	Low (0-12)	High (12-24)	P value
Age (yrs)	61 ± 17	62 ± 15	0.58
Male (%)	58	65	0.44
White (%)	90	83	0.25
CAD (%)	32	54	0.01
CHF (%)	23	44	0.02
Stroke (%)	23	19	0.55
PVD (%)	26	31	0.53
Diabetes (%)	56	48	0.37
Waist circumference(cm)	112 ± 18	105 ± 17	0.04
Hemoglobin (g/dl)	11.5 ± 1.5	12.8 ± 9.4	0.24
URR	0.7 ± 0.1	0.7 ± 0.04	0.86
Serum Albumin(mg/dl)	3.8 ± 0.6	3.8 ± 0.4	0.96
MOCA score	20 ± 6	19 ± 5	0.66

In the linear regression model, compared to the low ESS group, the high ESS group did not have significantly different MOCA scores (beta -1.4, p=0.18). However, subject to multiple comparisons, the odds of failing some of the subcomponents of MOCA appeared to differ between the 2 groups as shown in Figure 1.



Conclusions: Prevalence of moderate to severe cognitive impairment is common in HD pts. The overall cognitive scores were not different between those with low and high ESS scores. The association of ESS with executive function, attention and language fluency warrants further investigation.

Funding: NIDDK Support

FR-PO338

Depression as a Risk Factor for Mortality in Dialysis Patients: A Systematic Review and Meta-Analysis Farhat Farrokhi,[1] Neda Abedi,[2] Joseph Beyene,[3] Sarbjit Vanita Jassal.[1,4] *[1]Institute of Health Policy, Management & Evaluation, University of Toronto, Toronto, ON, Canada; [2]Department of Psychiatry, University of Saskatchewan, Saskatoon, SK, Canada; [3]Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, Canada; [4]Division of Nephrology, Department of Medicine, University Health Network, University of Toronto, Toronto, ON, Canada.*

Background: We aimed to systematically review and analyze the association of depression with survival of patients on chronic maintenance dialysis.

Methods: Searching MEDLINE, EMBASE, PsychINFO, we identified studies examining the association of depression, measured as depressive symptoms or clinical diagnosis. Data extraction were carried out by two reviewers. Appraisal of the studies was done using the Newcastle-Ottawa Scale. The inverse variance method and random effects model were used to summarize the effect sizes, and the trim-and-fill method to adjust for potential publication bias.

Results: Fifteen of 31 included studies showed a significant link between depression and mortality, including 5 of 6 studies with >6000 participants. A significant link was established between presence of depressive symptoms and mortality (HR=1.51; 95%CI=1.35-1.69; I^2=40%), based on 12 studies reporting depressive symptoms using depression scales (n=21055). After adjusting for publication bias, depressive symptoms remained a significant predictor of mortality (HR=1.45; 95%CI=1.27-1.65). Combining across 6 studies reporting depression scores (n=7857) resulted in a similarly significant effect (HR=1.04 per score; 95%CI=1.01-1.06; I^2=74%). Based on 3 retrospective studies reporting odds ratios, the association of depression (diagnosis in medical charts) with mortality was non-significant.

Conclusions: There is considerable between-study heterogeneity in the reports of depressive symptoms among dialysis patients, especially because of utilization of variable measurement methods of depression. However, the overall significant independent effect of depressive affect on survival of dialysis patients warrants studying the underlying mechanisms of this relationship and the potential benefits of interventions to improve depression on the outcomes.

FR-PO339

Statin Use, and Calciphylaxis: A Case Control Study Sagar U. Nigwekar, Ishir Bhan, Ravi I. Thadhani. *Massachusetts General Hospital.*

Background: Calciphylaxis, described as cutaneous analogue of myocardial infarction, is a highly fatal condition seen in hemodialysis (HD) patients. Key features of calciphylaxis include cutaneous vascular calcification and intense inflammation. Statins have anti-inflammatory and anti-calcification properties; however, association between statin use and calciphylaxis has not been investigated.

Methods: Cases (n=62) comprised HD subjects with skin-biopsy confirmed calciphylaxis diagnosed between 2000 and 2011. Controls (n=124) were HD subjects without calciphylaxis matched to cases by gender and calendar year. Covariates were compared between cases and controls and between statin users and non-users. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using unadjusted and adjusted logistic regression models.

Results: Comparison between cases and controls is summarized below-

Comparison of calciphylaxis cases and controls

Characteristic	Cases (n=62)	Controls (n=124)	P
Age, yr	58.3	58.1	0.94
Females, %	67.7	67.7	NA
Caucasian, %	58.9	63.8	0.62
HD vintage, days	312	365	0.71
Diabetes, %	43.5	41.9	0.88
Obesity, %	37.1	34.7	0.75
Macrovascular disease, %	38.3	40.1	0.99
Calcium, mg/dL	8.6	8.4	0.52
Phosphorous, mg/dL	5.4	4.9	0.07
Alkaline phosphatase, U/L	123	120	0.11
PTH,pg/mL	297	285	0.60
Albumin, g/dL	2.9	3.6	<0.001
LDL, mg/dL	89	90	0.29
Statin, %	12.9	25.8	0.04
Active vitamin D therapy, %	50.0	40.3	0.22
Calcium based phosphate binders, %	32.3	33.1	0.91
Warfarin, %	43.6	18.5	<0.001

Statin use was significantly lower in calciphylaxis cases compared to controls (p=0.04). Statin users had lower LDL (p= 0.04), higher prevalence of diabetes (p=0.02) and obesity (p<0.001) compared to non-users. In unadjusted analyses, statin use was associated with reduced odds of calciphylaxis (OR 0.43, 95% CI 0.18-0.99) whereas elevated alkaline phosphatase, hypoalbuminemia, and warfarin use were associated with increased odds. In adjusted analyses, statin use continued to be associated with reduced odds of calciphylaxis (OR 0.23, 95% CI 0.07-0.77).

Conclusions: Statin use might confer protection against calciphylaxis development. Larger prospective studies are needed to confirm this association.

FR-PO340

Renal Recovery Function Trends and Dispositions Shu-cheng Chen,[1] James P. Ebben,[1] Robert N. Foley,[1,2] Allan J. Collins.[1,2] *[1]USRDS Coordinating Center, MMRF, Mpls, MN; [2]Medicine, University of MN, Mpls, MN.*

Background: Generally, a very small percent of ESRD patients are expected to recover renal function after ESRD initiation. The recent increase of recovered renal function (RRF) noted by CMS and the renal research community, however, has raised concerns regarding the ESRD certification process and the accuracy of RRF ascertainment at dialysis facilities. We proposed to analyze trends of RRF, post-RRF dispositions, and the RRF coding system.

Methods: Data were derived from the USRDS database and CMS's Standard Information Management System (SIMS) with 98,761 total patients in the study population as of 9/30/2011. CMS and ESRD Networks identify RRF with an event code in the SIMS Patient Event File. In order to strengthen the definition, USRDS applies additional RRF criteria: < 180 days of first ESRD service date, >= 90 days before the next event, and must not be contradicted by other USRDS data sources.

Results: Using CMS/ESRD Networks definition, nearly 73% of the RRF events occurred in the first 6 months of ESRD. Many of the events seemed implausible: RRFs followed by death and RRFs occurring a year or more after initiation. In examining post-RRF events within a 90-day window, 72% were restart dialysis and another 25% death. These findings give evidence for a more robust RRF definition. Using this enhanced definition, we found a steady escalation of RRF rates among incident dialysis patients, from 1.6% in 1990 to 5.2% in 2010, a 3.25 times increase in the last 20 years. Regionally, similar rising trends were observed across Networks with Network 3 having the lowest RRF rate of 2.0% and Network 7 the highest at 8.1% in 2010. Among these USRDS defined renal recovered patients, 11% restarted dialysis and 25% died within a year. This one-year death rate is almost 1/3 higher than the 1-year crude death rate of dialysis patients of 19%.

Conclusions: Increases in RRF in recent years suggest that the criteria for ESRD certification have changed. The renal community should adopt a consistent and more accurate definition of ESRD for certification. More studies are needed to assess factors associated with rising RRF rates and evaluate the reasons behind post-RRF death and return to ESRD rates.

Funding: NIDDK Support

FR-PO341

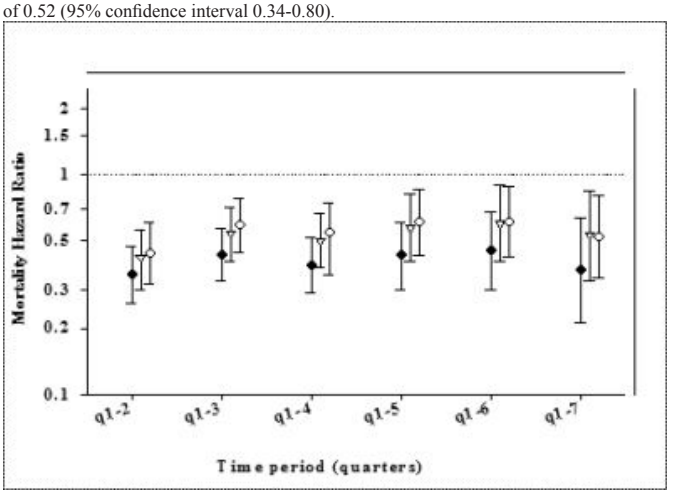
Marginal Structural Modeling of Dialysis Modality and Survival in Incident Dialysis Patients during the First Two Years Lilia R. Lukowsky,[1,3] Leeka I. Kheifets,[3] Onyebuchi A. Arah,[3] Allen R. Nissenson,[2,4] Kamyar Kalantar-Zadeh.[1,2,3] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]David Geffen School of Medicine at UCLA; [3]UCLA Fielding School of Public Health; [4]DaVita, Inc.*

Background: Previous research indicated survival differences between hemodialysis (HD) and peritoneal dialysis (PD) modalities. Given the challenges of conducting randomized trials, differential rates of modality switch and transplantation, and time-varying confounding in cohort data during early dialysis treatment, use of novel analytical techniques in contemporary observational cohorts can help examine the PD versus HD survival discrepancy.

Methods: Over a three-year period (7/2001-6/2004), we identified 22,360 incident hemodialysis (HD) and 1,358 peritoneal dialysis (PD) patients from a large contemporary cohort. We used casual models known as marginal structural models (MSM) fitted using

inverse probability weights (IPW) to examine survival differences between PD and HD over the first 24 months. Our methods accounted for modality change, differential transplantation rates, and detailed time-varying laboratory measurements in incident dialysis patients.

Results: Incident PD patients were younger, had less co-morbidity and were 9-times more likely to switch dialysis modality and 3-times more likely to receive kidney transplantation over the 2 year period, compared to HD patients. In MSM analyses, PD offered persistently greater survival independent of the known confounders including dialysis modality switch or transplant censorship with a PD-versus-HD death hazard ratio of 0.52 (95% confidence interval 0.34-0.80).



Conclusions: PD was associated with 48% lower mortality over the first 2 years of therapy.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO342

Brain Swelling during Dialysis: A Comparison of Low Flux Hemodialysis with Pre-Dilution Hemodiafiltration Niels Johansen,[1 3] Krista Dybtved Kjaergaard,[1 3] Christian D. Peters,[1 3] Michael Pedersen,[2 3] Jens Dam Jensen.[1 3]
[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark; [2]MR Research Centre, Aarhus University Hospital, Aarhus, Denmark; [3]Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark.

Background: Previous studies have shown that hemodialysis (HD) is associated with subsequent brain swelling. No studies have compared low flux HD and pre-dilution hemodiafiltration (pre-HDF) with respect to brain swelling. Our aim was to measure the effect of HD and pre-HDF on brain swelling after dialysis, and their impact on ventricular, grey and white matter volumes.

Methods: 12 patients (9 males/3 females), dialysis vintage (47 months), age (58 years) were treated with HD and pre-HDF on separate days in a randomized controlled crossover study. Session length (4 hours), ultrafiltration (2.7±1.3 L), blood flow (300 mL/min), and dialysate flow (500 mL/min) was equal in both sessions. Pre-HDF substitution fluid flow was 65 L/session. Dialysate sodium concentration was matched to the spontaneous sodium concentration in plasma. Brain volumetric MRI was performed before and after HD/pre-HDF. MRI data was processed in the neuroanalysis software SIENA v2.6, allowing for accurate automatic segmentation of the brain and estimation of volume changes. 5 healthy controls underwent a 4-hour resting period instead of dialysis in order to test measurement precision.

Results: The mean increase in brain volume was 1.8±1.7% (18.7 mL) during HD and 2.0±0.9% (22.3 mL) during pre-HDF. It was significantly higher ($p = 0.001$) than the control group, -0.1±0.3%. The difference between HD and pre-HDF was -0.2±1.4% (NS). The differences in mean ventricular (HD -3.8 mL, pre-HDF -3.6 mL), grey (HD 19.3 mL, pre-HDF 21.2 mL) and white matter volumes (HD 7.4 mL, pre-HDF 5.7 mL) were NS when comparing HD and pre-HDF. Urea reduction ratio was 68±2% in HD and 69.7±2% in pre-HDF (NS).β2-microglobulin reduction ratio was -14±4% in HD and 67±3% in pre-HDF ($P < 0.001$).

Conclusions: Both HD and pre-HDF caused brain swelling. No significant differences were observed between mean HD and pre-HDF brain volumes. Results from the control group indicate that changes in brain volume can be accurately assessed using MRI.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO343

Oral Hygiene Habits in People on Hemodialysis: A Multinational Prospective Cohort Study (Oral-D) Suetonia Palmer,[1] Marinella Ruospo,[2] Patrizia Natale,[2] Letizia Gargano,[2] Mariacristina Vecchio,[3] Valeria Maria Saglimbene,[3] Fabio Pellegrini,[3] Jonathan C. Craig,[4] Paul Stroumza,[2] Eduardo Jorge Celia,[2] Michela Sciancalepore,[2] Jorgen B.A. Hegbrant,[2] Giovanni F.M. Strippoli.[2,3,4]
[1]University of Otago; [2]Diaverum Medical Scientific Office; [3]Mario Negri Sud Consortium; [4]University of Sydney.

Background: Data describing oral hygiene habits in people with end end-stage kidney disease on hemodialysis as sparse. We prospectively surveyed global oral hygiene habits in a large outpatient hemodialysis population.

Methods: ORAL-D is an ongoing multinational prospective cohort study of oral diseases in people on hemodialysis. We consecutively enrolled adults on hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Europe and South America. We assessed oral hygiene habits using standard self-administered patient questionnaires. We summarized data using descriptive statistics.

Results: 4720 hemodialysis patients in the participating clinics from Italy, Hungary, Poland, Argentina, Portugal, France and Spain responded to the questionnaire. Of these, 2388 (52%) did not remember when they had last visited a dentist or reported they did not have a regular dental practitioner. 1264 (27%) reported their first dental visit at 30 years or older, 533 (12%) never brushed their teeth, 1722 (37%) used mouthwash and only 327 (7%) used dental floss. 1510 (33%) participants changed their toothbrush as needed, and only 1492 (35%) spent more than 2 minutes on daily oral hygiene cares.

Conclusions: Using validated instruments to evaluate oral hygiene, nearly half of adults on hemodialysis do not regularly visit a dental practitioner and many have poor oral hygiene habits. Additional study of the effectiveness of dental intervention and education on dental and clinical outcomes may be warranted.

Other authors: David Johnson; Marietta Torok; Luc Frantzen; Miguel Leal; Jan Dulawa; Ruben Gelfman; Charlotta Wollheim; Anna Bednarek (missing disclosure affidavit).

FR-PO344

Plasma Gelsolin and Survival in Chronic Hemodialysis Patients Laura Rosales,[1] Georges Ouellet,[3] Len A. Usvyat,[1] Rebecca H. Apruzzese,[1] Mary Carter,[1] Stephan Thijssen,[1,2] Nathan W. Levin,[1] Peter Kotanko.[1,2]
[1]Renal Research Institute, New York, NY; [2]Beth Israel Medical Center, New York, NY; [3]Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.

Background: Low levels of circulating plasma gelsolin (pGSN) which is an actin-binding protein, have been associated with poor outcomes in critically ill and in hemodialysis (HD) patients [Lee JASN 2009; Jin Y Crit Care 2012]. In a prospective study we investigated the relationship between pGSN levels and all-cause mortality in chronic HD patients.

Methods: We determined pGSN levels (normal range between 3600 and 9750 mU/mL) on one occasion with the 2C4 pGSN ELISA kit (Critical Biologics, Cambridge, MA) in maintenance HD patients from 3 inner-city HD centers; demographics (age, vintage, race, gender, body mass index (BMI), vascular access) and co-morbidities (diabetes, cardiovascular disease, HIV status, hepatitis) were recorded. Patients were stratified into two groups by the median pGSN levels. Survival was assessed by Kaplan-Meier analysis and Cox proportional hazards models with adjustment for age, race, gender, albumin, body mass index, diabetes, vintage, and access type.

Results: We studied 153 patients (age 61±15 years; vintage 3.9±3.7 (range 0.2-20.0) years; 52% male; 42% Blacks, 52% diabetes). Median follow-up time was 390 days (interquartile range: 388 to 415). Median pGSN level was 6353 mU/mL (interquartile range 5244 to 7640). Kaplan-Meier analysis revealed no significant difference in group survival ($P=0.570$). Cox proportional hazards analysis indicated that pGSN was not a significant predictor of all-cause mortality ($P=0.181$).

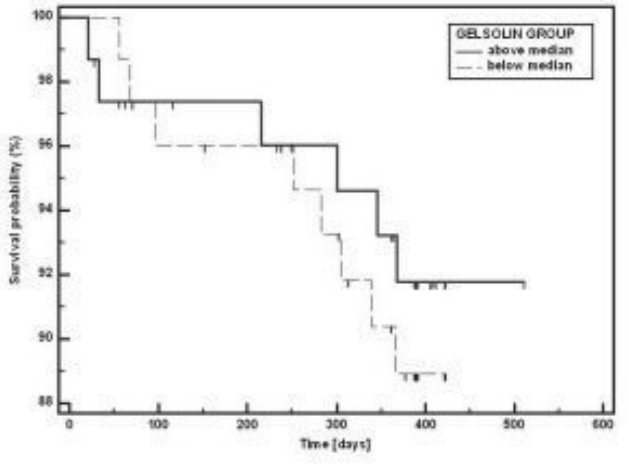


Fig 1. Kaplan-Meier curves showing survival stratified by above groups of high and low levels of pGSN.

Conclusions: This study in chronic HD patients indicates that pGSN is not related to all-cause mortality.

FR-PO345

Dialysis Adequacy and Solute Removal: Effect of Intradialytic Exercise Danielle Kirkman,[1,2] Mahdi Jibani,[2] Jamie Hugo Macdonald.[1] *[1]College of Health and Behavioural Sciences, Bangor University, Wales, United Kingdom; [2]Betsi Cadwaladr University Health Board, Wales, United Kingdom.*

Background: Dialysis adequacy is a significant predictor of hospitalization and mortality in maintenance hemodialysis (HD) patients. Whether intradialytic exercise training can enhance dialysis adequacy is unclear.

Methods: In a single blind, controlled, randomized crossover study, 11 HD patients (mean (SD) age: 58(13) years) completed three trial arms: normal routine care (CONT); increased HD time of 30 minutes (TIME); and intradialytic exercise (EXER), consisting of 60 minutes of cycling exercise at 95% of the lactate threshold in the last 90 minutes of HD. Each trial was completed twice, with all treatment parameters standardized between trials. The primary outcome was equilibrated Kt/V_{urea}. Secondary outcomes included reduction and rebound ratios of urea, creatinine, phosphate and $beta_2$-microglobulin. Data were analyzed by repeated measures analysis of variance.

Results: Increased HD time, but not exercise, significantly increased equilibrated Kt/V_{urea} compared to the control trial (TIME:1.48(0.28); EXER:1.36(0.24); CONT:1.33(0.27); p=0.02). Increased time also improved reduction ratios of urea (TIME:77(5)%; EXER:73(5)%; CONT:73(4)%; p<0.01) and creatinine (TIME:69(6)%; EXER:68(6)%; CONT:66(5)%; p=0.02), and tended to improve reduction ratio of $beta_2$-microglobulin (TIME:58(12)%; EXER:53(7)%; CONT:50(11)%; p=0.09). In contrast, phosphate reduction ratio was enhanced with exercise compared to both extra time and control trials (TIME:55(11)%; EXER:59(10)%; CONT:50(18)%; p=0.03).

Conclusions: This is the first study to compare intradialytic exercise and longer HD session time for dialysis adequacy. As expected, an extra 30 minutes of HD time significantly enhanced small and middle molecule clearance. Although exercise enhanced phosphate clearance, exercise did not enhance dialysis adequacy, as determined by the primary outcome of equilibrated Kt/V_{urea}. Thus intradialytic exercise cannot replace the traditional prescription of increased HD time, but may be a useful adjunctive therapy for serum phosphate control. *Registered clinical trial NCT01481688. Funding:* Unrestricted grant from B Braun Avitum AG.

Funding: Pharmaceutical Company Support - B Braun Avitum AG, Germany

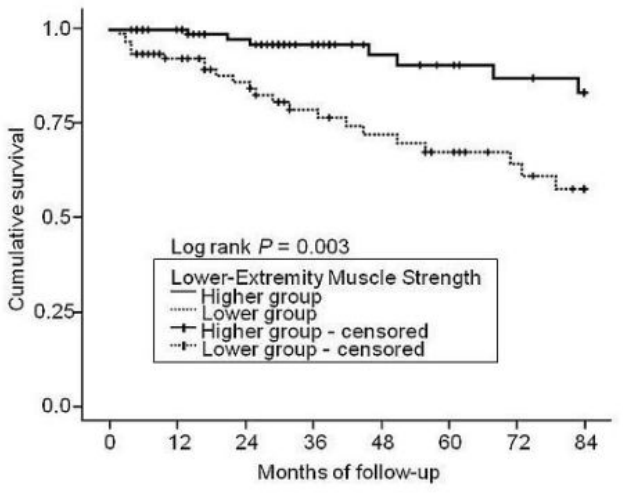
FR-PO346

Lower-Extremity Muscle Strength and Survival in Hemodialysis Patients: A Prospective Cohort Study Ryota Matsuzawa,[1] Atsuhiko Matsunaga,[1] Akira Ishii,[1] Yoshifumi Abe,[1] Toshiya Minowa,[1] Kei Yoneki,[1] Yutaka Takagi,[2] Atsushi Yoshida,[2] Kouju Kamata,[1] Naonobu Takahira.[1] *[1]Kitasato University; [2]Sagami Junkanki Clinic.*

Background: Decreased muscle strength is closely associated with physical inactivity and adverse health-related events such as fall-related injuries or walking disabilities in both the general population and those with chronic diseases. In this study, we investigated the prognostic significance of lower-extremity muscle strength on 7-year survival in a cohort of clinically stable hemodialysis (HD) patients.

Methods: A total of 190 outpatients (age 65±10, 46.8% male) who were undergoing maintenance HD therapy 3 times a week at a HD center were monitored for 7 years. Clinical characteristics including age, sex, body mass index, time on HD, comorbid conditions and serum albumin and C-reactive protein levels were obtained at baseline. In addition, maximum voluntary isokinetic knee extensor strength (leg strength), which reflects lower-extremity muscle strength, was measured using a handheld dynamometer (μTas MT-1; Anima, Japan). The median value of leg strength was used to divide patients into higher and lower groups. A Kaplan-Meier estimate of survival and a Cox proportional hazard regression were used to assess the contribution of leg strength to all-cause mortality.

Results: During the follow-up period (median 45±30 months), 30 patients died. The 7-year cumulative survival rate was 92.6% in the higher group and 75.8% in the lower group.



After adjusting for the effects of clinical characteristics, leg strength was a significant predictor of all-cause mortality. The hazard ratio of the lower group to the higher was 3.96 (95% confidence interval, 1.50-10.42; p=0.005).

Conclusions: Decreased lower-extremity muscle strength was strongly associated with survival in clinically stable HD patients.

FR-PO347

Determinants of Decreased Walking Speed among Patients Undergoing Ambulatory Hemodialysis Yoshifumi Abe, Atsuhiko Matsunaga, Ryota Matsuzawa, Akira Ishii, Toshiya Minowa, Kei Yoneki, Toshiki Kutsuna, Kyo Shigeta, Kouju Kamata. *Kitasato University, Sagmihara-shi, Kanagawa-ken, Japan.*

Background: Walking speed among patients undergoing hemodialysis (HD) reportedly decreases by 60% compared to healthy people, suggesting that patients undergoing HD may be at risk for adverse health-related events such as physical inactivity, limited mobility, and hospitalization. However, factors that decrease walking speed among these patients remain unclear. The purpose of this study was to identify factors associated with decreased walking speed based on clinical data of patients undergoing ambulatory HD.

Methods: A total of 118 Japanese outpatients undergoing maintenance HD three times per week at a HD center, who did not require assistance for walking and did not have dementia, low vision, or paralysis due to stroke, were eligible for inclusion in the study. We measured clinical characteristics (age, sex, HD duration, serum albumin and hematocrit, and history of fall-induced injury), walking speed, motor function (leg strength, lower extremity flexibility, and standing balance), and comorbid conditions (peripheral neuropathy and vascular diseases). HD patients were divided into two groups based on maximum walking speed: the slow speed group (women, ≤1.35 m/s; men, ≤1.50 m/s for men) and the fast speed group. Multivariable logistic regression analysis was performed to identify factors that predict slow and fast speed.

Results: Mean (SD) age, HD duration, and maximum walking speed were 68 (9) years, 8.6 (8.6) years, and 1.51 (0.36) m/s, respectively; 48% of subjects were women and 41% belonged to the slow speed group. The slow speed group was significantly associated with leg strength (odds ratio [OR] = 0.94, 95% confidence interval [CI] = 0.89 - 0.99, P = 0.014), standing balance (OR = 0.97, 95% CI = 0.95 - 1.00, P = 0.02), age (OR = 1.07, 95% CI = 1.01 - 1.14, P = 0.03), and a history of fall-induced injury (OR = 6.23, 95% CI = 1.31 - 29.52, P=0.02).

Conclusions: These results suggest that periodic evaluation and management of both motor function and falls are necessary to implement effective measures to prevent decreases in walking speed among clinically stable patients undergoing HD.

FR-PO348

Effect of Electron Beam Radiation Sterilized Polyethersulfone Dialyzers on Platelets during Hemodialysis Detlef H. Krieter,[1] Horst-Dieter Lemke,[2,3] Karin Merget,[2] Christoph Wanner.[1] *[1]Div. of Nephrology, University Hospital, Würzburg, Germany; [2]eXcorLab GmbH, Obernburg, Germany; [3]R & D, Membrana GmbH, Wuppertal, Germany.*

Background: In a substantial number of maintenance dialysis patients, e-beam sterilized polysulfone (ePSu) dialyzers are associated with significant thrombocytopenia following HD. The present study investigated if e-beam sterilized polyethersulfone (ePES) dialyzers induce a similar effect.

Methods: The platelet count (PC) of 63 patients subjected to maintenance HD with ePES dialyzers (PUREMA® H, 1.9 m^2) was measured pre- and postdialysis. Those patients with a significant PC drop (≥25% decrease) were enrolled in a prospective randomized controlled trial (RCT) comparing platelet activation (PC and P-selectin (CD62P)) at 0, 10, 60, and 240 min during HD with ePES, ePSu (Optiflux® Advanced Fresenius Polysulfone®, 1.8 m^2), and g-sterilized PES (gPES; PUREMA® H, 1.8 m^2).

Results: In 3 of 63 patients on ePES, a significant, but non-hazardous PC reduction to 65% (Patient #1; 181 vs. 118 x 10^3/μl), 45% (#2; 239 vs. 107 x 10^3/μl), and 54% (#3; 320 vs. 174 x 10^3/μl) of baseline was identified. In the RCT, the postdialysis PC with ePES was 67, 89, and 95% of baseline for #1, #2, and #3, resp. (mean±SD 225±70/195±85 x 10^3/μl pre/post). HD with gPES resulted in a PC decrease to 54, 89, and 96% (231±78/196±107 x 10^3/μl pre/post). With ePSu, the PC at 240 min was 64, 77, and 97% of baseline for #1, #2, and #3, resp. (246±794/205±119 x 10^3/μl pre/post). The PC with ePES and gPES was lowest at 240 min. With ePSu, a steep and early nadir at 10 min was observed in all patients. CD62P increased to between 132 % (ePES #3) and 210 % (ePSu #1) after 10 min and declined to baseline levels at 240 min. Interestingly, patient #2, differing in a clopidogrel comedication, had 2 to 3 times higher baseline CD62P and reached its maxima at 60 min.

Conclusions: Like with other sterilization modes, also with ePES, we found a minority of patients with reduced postdialysis PC. Comedications (e.g. GP IIb/IIIa antagonists) may impact on platelet activation. Screening for postdialysis PC drop should become a standard in maintenance HD.

Funding: Pharmaceutical Company Support - Membrana GmbH

FR-PO349

U Shaped Cost Curve in an ESRD Value-Based Integrated Care System
Robert C. Albright, Jeffrey Sigrist, John J. Dillon, James T. McCarthy, Stephen F. Gudgell, Kathryn Zavaleta, Paul Klugherz, Michelle C. Hedin, Bradley D. Wick, Amy W. Williams. *Division of Neph/HTN, Mayo Clinic, Rochester, MN.*

Background: Provision of value-based, patient-centric care to patients with ESRD demands maximizing meaningful longevity, patient satisfaction and transplantation rates, while minimizing hospitalizations, preventable complications and cost. The aim of this study was to determine the actual costs in the 6-month initiation phase of ESRD, the intervening months and the last 6 months of receiving ESRD care.

Methods: We conducted a prospective query of a decision support system of an integrated care system, which captures all costs for patients who initiated and/or received chronic ESRD care from 2006 through 2010. All patients whose care was delivered entirely at this center's hospital, outpatient multispecialty clinics and dialysis units were included. All costs were captured for this cohort, comparing the initial 6 months, the interval months and the last 6 months of ESRD care. Care was captured as dialysis days, which were defined as days in the system's care.

Results: 781 unique patients with 29,615 encounters, and 283,812 dialysis days were included. Cost data was normalized to the middle period, and then segregated into the 3 groups specified: initial 6 months of ESRD care, last 6 months of ESRD care, interval middle time. Despite only 12.8 avg days spent in the hospital/year on dialysis, ~50% of all costs were due to hospital care.

Costs

COSTS	Initial 6 months	Middle HD career	Last 6 months
GLOBAL*	1.17 ± 0.11	1.0 ± 0.15	1.55 ± 0.15
N	718	789	253
Hospital**	1.93 ± 0.23	1.0± 0.16	2.41 ± 0.12
N	220	414	196
[Mean + SD] costs of middle time = 1.00			
*p=0.06 Initial vs Middle; p=0.002 Middle vs Last			
**p=0.002 First vs Middle; p<0.0001 Middle vs Last			

Conclusions: A U shaped cost curve illustrates an increase in costs during the first and last 6 months of ESRD care. A major contribution was the higher hospital costs during these two phases of ESRD care. Emphasizing integrated care processes that avoid initiation of ESRD care in the hospital, and fostering compassionate care team engagement for end of life issues should lead to enhanced value of care for ESRD patients.

Funding: Clinical Revenue Support

FR-PO350

Mortality Risk for Women on Hemodialysis Differs by Age
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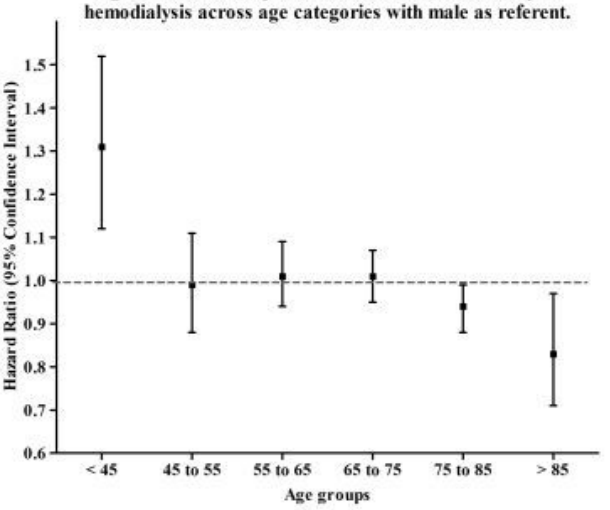
Background: Previous reports have demonstrated similar survival for men and women on hemodialysis, despite women's increased survival in the general population. To examine this paradox, we investigated the effect of age on mortality in women undergoing hemodialysis.

Methods: We identified 28,971 (Women 11,792 (40.7%), Men 17,179 (59.3%)) adult patients who initiated hemodialysis between Jan. 2000 and Dec. 2009 from the Canadian Organ Replacement Registry (CORR) and survived > 90 days. Multiple imputation was used for missing data. Crude and Adjusted rates were calculated for men and women. Cox proportional hazards and competing risks models were employed to determine the independent association between gender, age and likelihood of mortality or renal transplantation.

Results: Overall there was no mortality difference between the gender however, the age X gender interaction was highly significant (p<0.0001). Younger women experienced a considerable increase in mortality (Age < 45: aHR 1.31(1.12-1.52), p<0.0001) whereas elderly women experience improved mortality (Age 75-85: aHR 0.94 95%CI 0.88-0.99, Age > 85: aHR 0.83 95%CI 0.71-0.97).

Figure 1: Female adjusted hazard ratio for mortality on hemodialysis across age categories with male as referent.



Adjusted for demographics, co-morbidities, BMI, cause of ESRD, region, pre-dialysis care, distance, serum albumin and hemoglobin, vascular access and transition to PD by 90 days.

This relationship persisted after accounting for the competing risk of transplantation. Predictors of mortality in women < 45 included increasing age, co-morbidity (cardiac disease, pulmonary edema, diabetes, any serious illness), lower albumin and lack of an AVF.

Conclusions: Female survival on chronic hemodialysis varies by age compared to men with a significant increase in mortality in women < 45. AVF creation in this age group may represent a modifiable risk factor for mortality.

FR-PO351

Aboriginal Mortality Differs Based on Dialysis Modality: A Canadian National Study
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Background: Previous studies have shown that Aboriginals and Caucasians experience similar outcome on dialysis in Canada. Utilizing the Canadian Organ Replacement Registry (CORR), we examined whether dialysis modality (peritoneal or hemodialysis) impacted mortality in Aboriginal patients.

Methods: We identified 30,601 adult patients (Hemodialysis: Aboriginal 1,897, Caucasian 22,116, Peritoneal dialysis: Aboriginal 463, Caucasian 6125) who initiated dialysis between Jan. 2000 and Dec. 2010. Aboriginal status was identified by self report. Dialysis modality was determined 90 days after dialysis initiation. Multivariate Cox proportional hazards and competing risk models were constructed to determine the association between race and mortality by dialysis modality.

Results: During the study period, 962 (50.7%) of Aboriginals and 12,193 (55.1%) of Caucasians initiating hemodialysis died while, 140 (30.2%) and 1,842 (30.1%) respectively initiating peritoneal dialysis died. In comparison to Caucasians, Aboriginals on hemodialysis had a comparable risk of mortality (adjusted HR 1.04 95% CI 0.96-1.11, p=0.4). However on peritoneal dialysis, Aboriginals experienced a higher risk of mortality (aHR 1.29 95% CI 1.06-1.57, p<0.0001) and technique failure (aHR 1.29 95%CI 1.03-1.60, p=0.03) than Caucasians. The risk of technique failure varied by patient age, with younger Aboriginals (<50 years old) more likely to develop technique failure than Caucasians (aHR 1.76 95% CI 1.23-2.52, p=0.002).

Conclusions: Aboriginals on peritoneal dialysis experience higher mortality and technique failure relative to Caucasians. Reasons for this race disparity in peritoneal dialysis outcomes are unclear.

FR-PO352

Impact of a Multidisciplinary Intensive Management Clinic on Asian Incident Hemodialysis Patients
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Background: Morbidity/mortality rates are high in the early months after hemodialysis (HD) initiation. The Hemodialysis Initiation and Transition (HIT) Clinic run by a renal physician, clinical pharmacist and coordinator was set up to provide multidisciplinary, intensive and consistent care to patients in the first 3-4 months after HD initiation. This longitudinal study aimed to compare lab, clinical (morbidity/mortality) and economic outcomes between patients of HIT clinic vs. conventional management.

Methods: Adult incident HD patients referred to HIT clinic or outpatient renal clinic prior to set up of HIT clinic (control group) were included. Patient demographics, medical/medication histories, lab data [hemoglobin (Hgb), transferrin saturation (TSAT), ferritin,

serum calcium (Ca), phosphorus (P), intact-parathyroid hormone (i-PTH), albumin (alb), and hemoglobin A1c (HbA1c)], hospitalizations, 90/120-day mortality and medication costs were compared between HIT and control groups at the patients' first (baseline) and third clinic visits.

Results: Eighty-five HIT and 78 control patients were included. Mean lab values of both groups at baseline and third visit were similar. More HIT patients met KDOQI goals for Hgb (61.2% vs. 33.8%; P<0.05) at the third visit compared to control group, and achieved KDOQI targets for TSAT, ferritin, CaxP, alb and HbA1c at third visit compared to baseline. Conversely, fewer control patients achieved KDOQI targets for P and i-PTH at third visit. More HIT patients had reduction in monthly medication costs (77.1% vs. 62.8%) at the third visit, received influenza (72.9% vs. 15.4%) and pneumococcal (72.9% vs. 17.9%) vaccines and had permanent access created within 6 months of HD initiation (92.9% vs. 80.8%) (P<0.05). Hospitalization and mortality rates between groups were similar.

Conclusions: A multidisciplinary intensive management clinic helps incident HD patients achieve goal therapeutic targets, create permanent vascular access and may result in costs savings for patients. Further studies are needed to determine if such interventions help reduce morbidity and mortality in these patients.

FR-PO353

Comparison between Interferon Gamma Release Assay and Tuberculin Skin Tests for the Diagnosis of Latent Tuberculosis in Patients on Hemodialysis in India, a Tuberculosis Endemic Area Sanjay K. Agarwal,[1] Sabahat Husain Zaidi,[3] Urvashi B. Singh,[2] Sanjay Gupta.[1] *[1]Department of Nephrology, AllMS, New Delhi, India; [2]Department of Microbiology, AllMS, New Delhi, India; [3]Department of Biochemistry, JN Medical College & Hospital, Aligarh, UP, India.*

Background: Tuberculosis (TB) is common in maintenance hemodialysis (MHD). Majority have reactivation of past infection. Diagnosis of latent tuberculosis (LTB) is important. Interferon Gamma Release Assay (IGRA) is reported to be better than tuberculin skin test (TST) in this setting. However, there is no data from an endemic area of TB like India.

Methods: Patients on MHD were included. Active TB patient excluded. Patients were subjected to TST using 0.1 ml solution (5 tuberculin units). After 72 hours, an induration of ≥10 mm was taken as positive. All patients were subjected to IGRA, using QuantiFERRON gold tube test (Cellestis Limited, Australia), which included purified protein derivative from *M. tuberculosis,* ESAT-6 & CFP-10 and avian sensitin. Positivity criteria for IGRA were as per CDC recommendation. TST and IGRA were compared for diagnosis of LTB. Study was funded by Indian Council of Medical Research.

Results: Of 185 patients, 69.7% were males; mean age 36.7 ± 12.3 years. History of TB was in 9.9% and 72.4% had BCG vaccination scar. IGRA was positive in 66 (35.6%), TST in 32 (17.2%) and both in 13 (7%) patients. Of the 66 patients positive with IGRA, 13 (19.6%) were TST positive. Of the 32 TST positive, 13 (40.6%) were IGRA positive. 100 (54%) were negative for both and 85 (45.9%) were positive for either of two tests. Of the 153 TST negative, IGRA was positive in 53 (34.6%). Agreement between two tests was 61.08% (kappa = 0.0421, St. Error = 0.0656). In Logistic regression, Odds of positive IGRA with BCG vaccination was 1.23 and with history of TB 0.99, (insignificant). Odds of positive TST in patients with BCG vaccination was 1.04 and with history of TB 0.99(Insignificant).

Conclusions: In conclusion, in India, on MHD, more patients (35.6%) were positive for IGRA than with tuberculin skin test (17.2%). There is agreement of 61.08% between the two tests for diagnosis of LTB. There is no effect of BCG vaccination and history of TB on both tests.

Funding: Government Support - Non-U.S.

FR-PO354

Successful Elimination of Hemodialysis Related Bacteremia and Vascular Access Infection Jafar Al-Said, Aimee Pagaduan, Soni Murdeshwar. *Nephrology and Internal Medicine, Bahrain Specialist Hospital.*

Background: Hemodialysis related Bacteremia and dialysis induced infection are one of the major mortality and morbidity factors for patients with end stage renal disease.

Methods: Since the start of our hemodialysis service, 8 years ago, we have been following tight infection control steps to monitor, control and prevent infection. In this retrospective study we wanted to identify the results of our tight infection control protocol on the prevalence of hemodialysis related Bacteremia and vascular access infection. All the hemodialysis sessions performed from Jan. 2004 till the end of Dec 2011 were reviewed. Patients demographics were collected. Vascular access type, the presence of DM and HTN were recorded too. Hemodialysis related Bacteremia was confirmed by the combined presence of any of the clinical signs such as: fever, chills, generalized weakness or hypotension with a positive blood or dialysis catheter culture.

Results: Over 108 months we had 6161 hemodialysis sessions performed on 118 patients. over 8 years we had only 15 episodes of dialysis related Bacteremia in 9 patients. Three of them required admission for hemodialysis related bacteremia. The blood culture in one patient was MRSE and the other 14 patients had Sphingomonas paucilomobilis. The source of Sphingomonas organism was found to be the main dialysis water tank and machines stagnant tubing water. Infection protocol adjustments were implemented and this had successfully eliminated all infections since then. All the 9 patients recovered from the infection with Antibiotic course.

Hemodialysis Related Bactermia Outcome Results

Prevalence of HD related Bacteremia /100 patients month	0.0198
Average rate of HD Bacteremia per monthly HD sessions	0.001
Prevalence of admission for HD related Bactermia/1000 patient year	0.79
Mortality rate secondary to HD related Bacteremia	Zero

Conclusions: As compared to published figures, our infection control protocol have successfully reduced hemodialysis related Bacteremia, vascular access infection and related admissions. We would like to discuss and share our successful infection control experience with nephrologist from different parts of the world. We believe it would make a difference.

FR-PO355

Potassium Binders: "Old & Tried"… but Are They Useful in Hemodialysis Patients? Samra Abouchacra,[1] Ahmed Chaaban,[1] Nicole Gebran,[2] Faiz Abayechi,[1] Qutaiba Hussain Daoud,[1] Noura Saif Al Nuaimi.[1] *[1]Medicine, Tawam Hospital, Al-Ain, United Arab Emirates; [2]Pharmacy, Tawam Hospital, Al-Ain, United Arab Emirates.*

Background: There is insufficient evidence for the utility of potassium binding resins in dialysis patients especially given their poor tolerability. Our objective was to assess the efficacy and tolerability of the binder calcium resonium (CaR) and investigate the impact of patient education (Ed) on adherence and treatment response.

Methods: Adult hemodialysis (HD) patients receiving CaR were enrolled with control group not on this agent. Adherence and adverse effects were recorded in adherent (A), non-adherent (NA), and control (C) groups. An educational intervention was undertaken and serum potassium levels (SK) were evaluated for 3 months before and after. Inter-and intra group results were analyzed by ANOVA and paired student t- test respectively.

Results: SK levels were much lower in controls (p<0.001) and there was a significant difference in baseline SK between A and NA (p<0.003) but not post Ed (p 0.71). This was due to worsening control in the former, because of GI- related noncompliance, and not due to an improvement in NA. Dietary indiscretions and lack of cathartics use may have contributed but no difference in dialysis adequacy was noted among groups, although the impact of residual renal function was not assessed. Of note, no within group effect was observed for patient counseling.

Table 1

	NA (n= 42)	A (n=28)	Control (n=30)
Age (yrs)	61.8 ± 16.8	54.5 ± 17.9	48.9 ± 18.7
F:M (%)	55:45	61:39	48:52
Diabetics (%)	58	46	47
HD Duration (mo)	62.3 ± 47	88.5 ± 81.7	35.6 ± 33.4
HD : HDF (%)	41: 59	36 : 64	41:59
permacath (%)	31	21	40
Access Flow (ml/min)	1268 ± 857	1319.4 ± 934	1583.3 ± 973.5
Kt/V	1.2 ± 0.2	1.3 ± 0.3	1.3 ± 0.2
SK pre Ed (mmol/l)	5.56 ± 0.58	5.17 ± 0.42*	5.08 ± 0.69
SK post Ed (mmol/l)	‡5.48 ± 0.65	‡5.42 ± 0.74**	

Ed effect: intergroup * p<0.003 ** p NS intragroup ‡ NS

Conclusions: These findings raise concern regarding the cost-efficacy of CaR and support investing in traditional antihyperkalemic measures; namely dietary compliance and adequate dialysis. Long-term trials are awaited to better define the role of CaR in the dialysis setting.

FR-PO356

Ramadan Fasting in Hemodialysis Patients Wan Ahmad Hafiz Wan Md Adnan, Mun Hoe Wong, Abdul Hafidz Muhammad Iqbal, Yip-Boon Chong, Tee Chau Keng, Kok Peng Ng, Li Ping Tan, Soo Kun Lim. *Nephrology Unit, Department of Medicine, University Malaya Medical Centre, Kuala Lumpur, Malaysia.*

Background: Muslims are required to fast during the month of Ramadan except for those who are sick. Despite the uncertainty of the risks involved in patients undergoing haemodialysis, many Muslim patients voluntarily fast during Ramadan. The purpose of the study was to determine the effect of voluntary Ramadan fasting in patients undergoing haemodialysis.

Methods: This prospective cohort study was performed in 3 haemodialysis centres in Kuala Lumpur from 15th July 2011 to 29th August 2011. All patients who managed to fast for any number of days were included in this study (n=35, 54% female, mean age 54 ± 11 years). 89% of patients fasted for more than 15 days, 89% have hypertension and 49% have diabetes. 43% of patients have diabetes as the primary cause of end-stage kidney disease. Blood tests were taken in the last week prior to Ramadan and the last week of Ramadan for comparison. Paired t-test was used to examine the differences in dialysis parameters and biochemical values pre and post Ramadan using SPSS 19 statistical software.

Results: Both pre and post dialysis weight were significantly decreased during Ramadan fasting by 0.8±1.0 kg and 0.7±0.9 kg, respectively (p=<0.001), compared to the month prior. There was a significant decrease in the amount of ultrafitration by 0.3±0.5 litre (p=0.002). Mean urea reduction ratio (URR) pre- and during Ramadan were 67.2% and 66.5%, respectively (p=0.53). There were no significant differences in dry weight, inter-dialytic weight gain or blood pressure measurement at the end of Ramadan compared to the month prior. Biochemically, there was a significant increase in serum albumin level (1.0±2.0 g/L, p=0.006) and a significant decrease in serum phosphate level (0.2±0.4 mmol/L, p=0.02) at the end of Ramadan. There were also trends towards significant decreases in serum urea (1.2±3.4 mmol/L, p=0.051) and serum haemoglobin (0.3±0.9 g/dL, p=0.066) as well as an increase in serum calcium (0.06±0.16 mmol/L, p=0.053).

Conclusions: Ramadan fasting is associated with reduced weight, improved serum albumin and phosphate level in haemodialysis patients.

Funding: Private Foundation Support

FR-PO357

Patients with Diabetic Kidney Disease Have a Worse Survival than Patients with Diabetes as Co-Morbidity in Chronic Hemodialysis Patients

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Background: Diabetic kidney disease is a major cause of end-stage renal disease (ESRD). It is not clear if the survival of patients with diabetic kidney disease (DKD) associated ESRD is different from patients with diabetes as a co-morbidity. The aim of this study is comparing the survival of DKD patients and patients with diabetes as co-morbidity in chronic hemodialysis (HD) patients.

Methods: An observational cohort analysis based on database of Taiwan Renal Registry. Patients: Adult patients (n=46596) on chronic HD for at least 90 days at 450 facilities in Taiwan from 1995 to 2005. Survival status was observed until Dec 31, 2008. All patients have complete observation of study factors - age, gender, primary renal disease, co-morbidity, blood glucose, hematocrit and serum levels of albumin, calcium, phosphate and i-PTH. Statistics: Patients' survival and hazard ratio for death were determined using Kaplan-Meier analysis and Cox proportional-hazard models.

Results: A total of 20,489 (44%) patients were identified from 46,596 hemodialysis patients. Among them, 15430 patients had DKD and 5059 patients had DM as co-morbidity. They were followed for an average of 4.9 ±2.5 years. The mortality rate was 68.9% for patients with DKD and 39.7% for patients with diabetes as co-morbidity ($p<0.001$). The survivals for patients with DKD associated ESRD was significantly worse than patients with DM as co-morbidity ($p<0.001$, log-rank test). The hazard ratio for death of DKD associated ESRD patients was 1.95 (95% CI: 1.849 to 2.056) in Cox proportional regression with case-mix-and multiple covariant adjustment.

Conclusions: In chronic hemodialysis patients, those with diabetic kidney disease as primary renal disease are linked to a much higher mortality risk than patients with diabetes as co-morbidity.

Funding: Government Support - Non-U.S.

FR-PO358

The Risks of Long-Term Hospitalization at the Initiation of Hemodialysis

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Background: Long-term hospitalization at the initiation of hemodialysis(HD) increases the cost of hospitalization and reduces the patients' quality of life. In order to prevent those, we have to elucidate the positive and negative.

Methods: In our study group, there were 2,120 HD initiations from 2006 to 2010. We selected the patients with whom the necessary data were available. Those items were age, sex, early referral to a nephrologist (ER), the incidence of diabetes mellitus, the modality of vascular access at the initiation of HD (AVF), average blood pressure (ABP), hemoglobin (Hb), Albumin (Alb), C-reactive protein (CRP), estimated glomerular filtration rate (eGFR), and blood urea nitrogen (BUN), and the medication before the initiation such as recombinant erythropoietin (EPO), angiotensin receptor blocker (ARB), angiotensin converting enzyme inhibitor (ACEI), calcium channel blocker (CCB), and loop diuretics. We defined a long hospital stay as longer than three weeks in a hospital and an early referral to a nephrologist as longer than three months. We applied binominal logistic regression. The dependant variable was a long hospital stay and the independent variables were those items mentioned above.

Results: The number of subjects was 1.351. Average age was 66.9±13.5. Male and female ratio was 895/456. The duration from the first referral to nephrologist to the initiation of HD, 25% 59 days, median 407 days, 75% 1,123 days. Significant ($P<0.05$) independent variable were age, EPO, ARB, CCB, AVF, ABP, BUN, Alb, CRP. The values of their odds ratio were 1.013, 0.673, 0.653, 0.730, 0.160, 0.986, 1.006, 0.551, 1.101 respectively.

Conclusions: The factors that increase the risk of a long hospital stay were age, BUN, CRP. The factors that contribute to a shorter hospital stay were EPO, ARB, AVF, ABP, Alb. Except age all factors are controllable to some extent by nephrologists. Therefore a referral to a nephrologist contributes directly and indirectly to a shorter hospital stay at the initiation of HD.

FR-PO359

Differences in Predicting Mental Health among Taiwanese and American Dialysis Patients

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Background: Explore differences in predicting mental health by comparing QOL data from dialysis patients in Taiwan and the US.

Methods: KDQOL surveys collected from patients treated in Renal Research Institute (US, 4349) and NephroCare clinics (Taiwan, 294). US surveys collected routinely; only first survey per patient was used. Taiwanese data was collected for academic research on QOL predictors across cultures. Predictor variables: disease burden, symptoms, effects subscales. Control variables: gender; age; relationship, vocational, socio-economic status; ethnicity; cause of renal failure; dialysis vintage. Outcome measure: mental composite score (MCS).

Results: Incomplete questionnaires were excluded. Refer to Fig.1 for significant associations (r) with MCS.

	US (n=4103)			Taiwan (n=258)		
	α	β	r	α	β	r
Burden	0.83	0.31**	0.52**	0.86	0.20**	0.45**
Symptoms	0.58	n/a	n/a	0.88	n.s.	0.41**
Factor 1	0.78	0.17**	0.46**	n/a	n/a	n/a
Factor 2	0.76	0.07**	0.38**	n/a	n/a	n/a
Effects	0.84	0.19**	0.50**	0.83	0.48**	0.60**
Employment	n/a	0.02	0.02	n/a	0.14**	0.25**
SES	n/a	0.01	0.01	n/a	0.09*	0.30**

**: p<0.01; *: p<0.1; n.s.: not significant; n/a: not applicable; SES: socio-economic status

For Taiwan, hierarchical multiple regression model explained 44% of adjusted variance in MCS ($F(5,252) = 41.10$, $p<0.01$). Demographic variables explained 11% (block 1, $\Delta R^2=0.11$, $F(2,255)=15.43$, $p<0.01$), disease variables 34% (block 2, $\Delta R^2=0.34$, $F(3,252) = 52.04$, $p<0.01$). Disease effects were most significant independent predictor (β). For US, internal consistency (α) of symptoms scale could only be improved to acceptable standards by separating into two factors. Regression model explained 36% of adj. variance ($F(4,4201)=587.93$, $p<0.01$).

Conclusions: Mental health in Taiwanese patients appears less affected by burden and symptoms of dialysis than by its effects on daily life that cannot be mitigated through internal mental processing. Future research should analyze why internal consistency in symptoms scale was low in the US, and contribution of disease symptoms not significant in Taiwan, e.g. because of different disease perception. New tools may need to be developed to measure mental health for dialysis patients across cultures.

FR-PO360

Generalized Anxiety Disorder in Prevalent Patients on Hemodialysis

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Background: The prevalence of the Generalized Anxiety Disorder (GAD) in Spain is up to 13.7%. GAD is characterized by excessive anxiety symptoms, worrying, nervous feeling, irritability, concentration difficulties, sleep disturbances and fatigue syndrome. Generalized Anxiety Disorder questionnaire (GAD-7) is a validated tool to identify potential patients suffering from GAD. Aims: To describe the prevalence of GAD in patients on hemodialysis (HD) and its impact on several clinical and laboratory parameters.

Methods: We studied 156 patients on HD between January and March in 2011, the mean age was 62.0 ± 12.9 years, 67.9% were male, on HD for a median time of 29.5 months (IQR: 16-53 months), and 47.4% were diabetic. It was used the following scoring scale of the GAD-7: a) grade 1 (0 to 4 points). b) grade 2 (5 to 9 points). c) grade 3 (10 to 14 points). d) grade 4 (15 to 21 points). We divided the population into 2 groups considering that a cut-off value > 9 points defines moderate-severe GAD. We compared clinical and laboratory parameters between both groups.

Results: 20.5% out of the studied patients exhibited a clinically relevant anxiety disorder. Compared with patients not suffering from anxiety disorder, we observed an increased use of psychiatric drugs (62.5% vs 41.9%, $p = 0.037$) and the prevalence in women was higher (34% vs 14%, $p = 0.004$). Furthermore, we observed a negative correlation between the GAD-7 score and the age (OR: -0.24, $p <0.001$) as well as with the time on dialysis (OR: -0.18, $p = 0.016$). We either found no correlation GAD-7 score with the related- bone-mineral metabolism, and biochemical parameters, weight gained between dialysis sessions and the absolute number of drugs taken by these patients.

Conclusions: The GAD-7 is a simple and easy to use tool for the screening of GAD in patients on HD. GAD was associated with increased use of psychiatric drugs and was more common in women. The prevalence of GAD was higher in younger patients and in those with less time on HD, which was possibly related to their adaptation to a chronic treatment.

Funding: Clinical Revenue Support

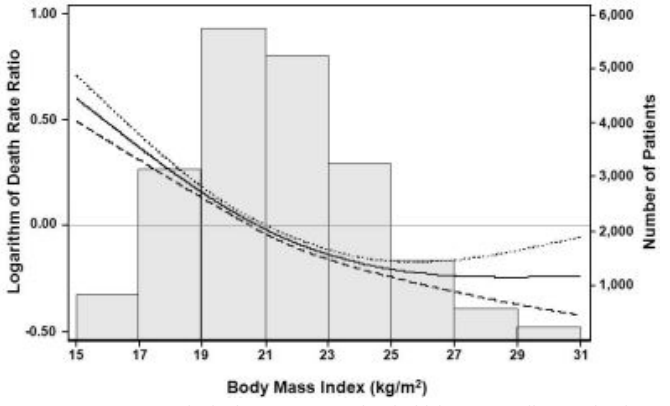
FR-PO361

Mortality Prediction of Body Mass Index in South Korean Hemodialysis Patients Jongha Park,[1,2] Dong Chan Jin,[3] Jong Soo Lee,[2] Hyun Chul Chung,[2] Jennie Jing,[1] Miklos Zsolt Molnar,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Ulsan University Hospital, Ulsan, Republic of Korea; [3]St. Vincent Hospital, the Catholic University of Korea, Suwon, Republic of Korea.*

Background: In hemodialysis (HD) patients, lower body mass index (BMI) has been show to correlate with higher mortality rates, a phenomenon referred to as the obesity paradox or reverse epidemiology. However, this association has not been firmly proven in East Asian dialysis populations who tend to have smaller average body size than non-Asian dialysis patients.

Methods: The association of baseline BMI with all-cause mortality was evaluated in 20,818 HD patients registered on ESRD Registry Program of Korean Society of Nephrology from February 2001 to June 2009 (with follow up to February 2012). Survival analyses included Cox proportional hazard and spline models with adjusting for case-mix and other surrogates of nutritional status.

Results: The patients were 54±14 (mean±SD) years old and included 43% women and 39% diabetics. Median BMI was 21.2 kg/m^2 (1st to 99th percentiles: 15.6-30.5). Patients were divided into BMI quartiles: 1st; <19.4, 2nd; 19.5-21.2, 3rd; 21.3-23.2 and 4th; >23.2 kg/m^2. With 2nd quartile as the reference, patients in 1st quartile reported 26% (HR: 1.26, 95%CI: 1.18-1.36) higher risk of death; while patients in 3rd and 4th quartiles reported 9% (HR: 0.91, 95%CI: 0.85-0.99) and 21% (HR: 0.79, 95%CI: 0.73-0.86) lower risk of death, respectively. The spline models confirmed that higher BMI exhibited a monotonic association with lower mortality rates.



Conclusions: Larger body size appears associated with lower mortality rates in a large cohort of Korean HD patients, suggesting that the obesity paradox previously reported for the US and European HD populations also hold in East Asian HD patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

FR-PO362

Iron Deficiency without the Presence of Anemia Is Associated with Intradialytic Hypotension: Role of Iron beyond Erythropoiesis Kenichiro Koitabashi,[1] Masahiko Nagahama,[1] Kumiko Shimasaki,[1] Keita Hirano,[1] Yuki Heath,[1] Fumika Taki,[1] Sachiko Ohde,[2] Yasuhiro Komatsu.[1] *[1]Nephrology, St Luke's International Hospital, Tokyo, Japan; [2]Clinical Epidemiology, St Luke's International Hospital, Tokyo, Japan.*

Background: Intradialytic hypotension (IDH) is a relatively common problem, being related to high morbidity and mortality as well as increased patients care in hemodialysis (HD) unit. Several potential risk factors for IDH have been addressed, such as old age, female sex, presence of diabetes, low cardiac ejection fraction (EF) and hypoalbuminemia. Although several studies suggested that iron deficiency is related to hemodynamic instability through reduced vascular tone, the relation of iron deficiency and IDH has not been elucidated. The aim of the present study is to clarify the association of body iron store and IDH.

Methods: We analyzed clinical and biochemical parameters of 99 patients on maintenance HD at St Luke's International Hospital in 2012. IDH was diagnosed with a decrease in systolic BP≥30mmHg associated with clinical events and need for nursing interventions. Risk factors for IDH were sought using multivariate logistic regression.

Results: Among 99 patients, 44 (44.4%) patients were diagnosed with IDH. The mean age of the patients was 66±13 (mean+/-S.D.) yr, the male/female ratio was 26/18, duration of HD was 8.9±6.6 years and the Kt/V was 1.4±0.2. The mean Hb level was 10.7±1.2 g/dl, blood ferritin level (FRN) was 39.8±36.9 ng/ml and transferrin saturation (TSAT) was 18.1±11.8%. On univariate analysis, patients with IDH had more interdialytic weight gain (P=0.004), more likely to be iron deficient, TSAT <20% (P<0.01) and FRN<100 ng/ml (P=0.02). Multivariate logistic regression analysis identified TSAT<20% (odds ratio = 3.00, P=0.034) and higher Hb (odds ratio = 1.80, P=0.034) as independent risk factors for IDH.

Conclusions: The present study shows that iron deficiency without presence of anemia is related to IDH. This implies importance of iron role beyond erythropoiesis, being involved in oxidative metabolism and cellular energetics. Given the effects of IDH on HD patients, our study underscores the importance of treating iron deficiency even without the presence of anemia.

Funding: Private Foundation Support

FR-PO363

The Survival of Elderly Hemodialysis Patients Misaki Moriishi,[1] Hideki Kawanishi.[2] *[1]Internal Medicine, Tsuchiya General Hospital, Hiroshima, Japan; [2]Surgery, Tsuchiya General Hospital, Hiroshima, Japan.*

Background: Recently, the numbers of elderly dialysis patients are increasing, but they are poorly prognosis on dialysis therapy. We studied risk factors of death in dialysis patients aged over 80 years.

Methods: In this single center, 146 dialysis patients aged over 80 years who started hemodialysis from 2005 through 2010, were investigated age, gender, primary ESRD cause, e-GFR, serum albumin, hemoglobin, comorbidity disease, planned dialysis, and non-planned dialysis, associated with survival. Date a was analyzed using multivariable stepwise Cox analysis, Kaplan- Meier analysis, and Student's t-test.

Results: For 6 years, 87 patients out of all patients were died. 1 year survival rate was 70%, 2 years survival rate was 57%. In multivariable stepwise Cox analysis, independent predictors of death were non-planned dialysis and >1.8mg/dL CRP. HR was 2.1 (95%CI, 1.51-4.02) for non-planned dialysis, and 2.3 (95%CI, 1.76 -4.64) for >1.8mg/dL CRP, respectively. For 19 patients who died < 90 dialysis days, their serum albumin value was significantly lower compared to surviving patients. (3±0.6 mg/dL vs. 3.3±0.5 mg/dL, p<0.001). Based on their serum albumin value of the 146 patents, the three groups can be formed, which is 1) < 3 mg/dL, 2) 3-3.4 mg/dL and 3) >3.5 mg/dL. Applying the Kaplan- Meier analysis, the survival rate of the group 1) was significantly lower than the other groups. The 40% of the group 1) died within 180 dialysis days.

Conclusions: The expected benefit of RRT should be considered and discussed for the elderly patients with low serum albumin and high CRP, at the time of the application of the hemodialysis.

FR-PO364

Outcomes Associated with Years of Hemodialysis Therapy in the HEMO Study Chi-Ting Su,[1,2] Jonathan Yabes,[1] Mark L. Unruh.[3] *[1]GSPH, University of Pittsburgh; [2]Nephrology, National Taiwan University Hospital, Yun-Lin; [3]Renal-Electrolyte Division, University of Pittsburgh Medical Center.*

Background: The factors associated with long-term survivorship on maintenance thrice weekly hemodialysis (HD)are unclear.This study examines the extent to which comorbid conditions, nutrition,life quality,and HD parameters are associated with long-term survivorship.

Methods: This report takes advantage of the HEMO Study,which provides a well-characterized cohort of prevalent HD patients.HD years, Index of coexistent diseases score, baseline demographics, fluid removal, membrane flux, dialysis dose, vascular access and anthropometric parameters were selected as covariates.Adjusted Cox-regression model was used to examine patient survival predictors.We characterized patients on dialysis >3.7 years as long-term survivors.

Results: Compared to patients on HD for <1 year and >3.7 years, the history of peripheral vascular diseases (PVD), congestive heart failure (CHF) admission, vascular access, sleep quality, and midarm circumference(MAC) were different predictors.Among those on HD >3.7 years, patients with a history of CHF admission(HR1.7, p=.003) had worse all-cause mortality and those with better sleep quality(HR0.96)and higher MAC(HR0.93) tended to have better survival.Diagnosis of PVD(HR1.6,p=.014) and vascular access(AVG *vs* AVF:HR1.8,p=.008) were factors for higher mortality in patients on dialysis <1 year but not in those who survived longer on HD(p=.231).

Conclusions: There were a number of modifiable factors associated with long-term survivorship including avoiding overhydrated, sleep quality, nutrition status, and vascular access. Among long-term survivors, history of CHF admission, poor quality of sleep and less MAC was associated higher mortality in the subgroup of patients with >3.7 HD years but not in those with<1 year.

Comparison Predictors for all-cause mortality

Predictors	<1 year(n=403)	>3.7 years(n=933)
PVD	79.2% HR1.6	80.9% N/A
CHF	44.2% HR1.6	36.7% HR1.32
CHF admission	13.6% N/A	10.0% HR1.67
AVG vs AVF	HR1.8	N/A
MAC(2cm)	30.1 ± 5.,07 N/A	29.9 ± 5.08 HR= 0.93
Sleep quality 0-10 (poor-good)	6.4 N/A	6.1 HR0.96

Categorical covariates: % in each subgroup; HR: adjusted Cox-regression model

FR-PO365

Blood Pressure Control without Antihypertensive Medications Is a Good Predictor of Dry Weight in Dialysis Pre Transplant Patients Sonia C. Rivera Gonzalez, Nadia Saavedra, Magdalena Madero. *National Heart Institute, Mexico City.*

Background: Hypertension in renal replacement therapy (RRT) is usually related to volume expansion. Adequate blood pressure (BP) control in RRT suggests an appropriate dry weight. There are limited data regarding the utility of pre transplant RRR weight comparing the use of antihypertensive medications (HTNM) as a marker of dry weight. The aim of this study was to compare pre transplant RRT weight with and without HTNM with post transplant weight.

Methods: This was a retrospective study that included patients undergoing living kidney transplant (LKT) between 2008-2012. Weight and mean blood pressure (MBP) were analyzed at the time they started RRT (B), 1 week before undergoing LKT (PRE) and 1 week after the LKT (W1). W1 was considered the dry weight. Other variables included number of pre LKT HTNM, ecocardiographic parameters (left ventricular septum and

posterior wall thickness) and urine output (UO) in the first 24 hrs post LKT. We calculated the percent change in body weight PRE and W1.

Results: The study included 100 patients that were divided according to the use of HTNM. Mean age was 28±9 and 67% were male. Patients in the HTNM group had an average of 1.7±1 BP meds. Compared to the group with HTNM, patients in the group without HTNM did not have a significant difference in weight when PRE and W1 were compared. In addition MBP, urine output and left ventricular hypertrophy parameters were lower in the no HTNN group.

Patient characteristics according to the use of HTNM

	B , PRE, W1 (weight/kg)	p value W1/B, pW1/PRE	Delta W1/ PRE (%)	MBP mmHg	UO 24 h post LKT (L)	LV septum (mm)	Posterior wall thickness (mm)
BP with HTNM (n=60)	60±13, 62±14, 58±14	0.01, 0.01	0.4±6	105±15	5.5±2	11.4±2	11.4±2
BPcontrol no HTNM(n=40)	60±14, 57±15,56±14	0.01, NS	-6.5±7	88.9±13*	3.7±1.7*	10.4±2*	10.7±2*

p<0.05

Conclusions: BP control without HTNM is a precise method of assessing dry weight.

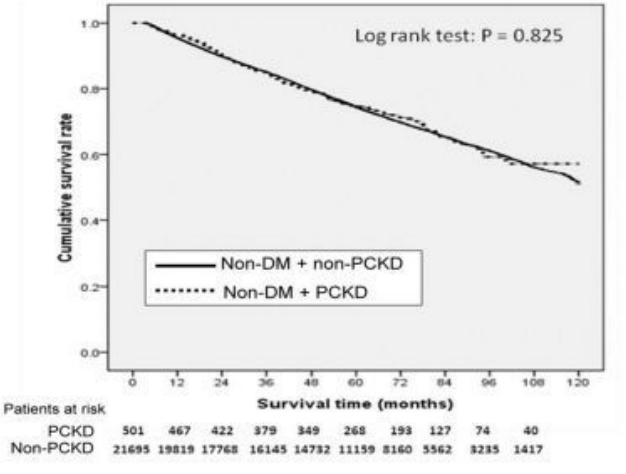
FR-PO366

Epidemiology and Mortality among Dialysis Patients with and without Polycystic Kidney Disease: A National Cohort Study in Taiwan Chih-Ching Lin.[1,2] *[1]Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; [2]School of Medicine, National Yang-Ming University, Taipei, Taiwan.*

Background: Polycystic kidney disease (PCKD) is one of the most common inherited disorders in end-stage renal disease (ESRD) patients. It is generally thought that survival of PCKD patients on dialysis surpasses that of general dialysis patients, but the cause of better survival is not well defined.

Methods: Using Taiwan's National Health Insurance claim data, we performed a longitudinal cohort study to investigate the survival and impact of comorbidities on mortality in dialysis patients with and without PCKD. Further analysis among non-diabetic mellitus (DM) patients were performed after excluding DM patients. A Cox proportional hazards model was used to identify the risk factors for all-cause mortality. Overall patient survival was described using the Kaplan-Meier method.

Results: Of 22298 non-diabetic incident dialysis patients, 501 patients (2.25%) had PCKD. There was no significant difference in survival rates between the two groups. Male gender, age ≥ 65 years old, congestive heart failure, and cerebrovascular accident were independent predictors of mortality among PCKD dialysis patients.



Conclusions: The proportion of PCKD as the underlying cause of ESRD in Taiwan is lower than those in Western countries. The long-term survival outcome was similar between non-diabetic dialysis patients with and without PCKD in Taiwan.

Funding: Government Support - Non-U.S.

FR-PO367

Descriptive Analysis of a Single-Center 40-Year Home Hemodialysis (HHD) Experience Eric Goffin,[1] Tony Goovaerts,[1] Krystel Carlier,[2] Veronique Chapalain,[3] Michel Y. Jadoul.[1] *[1]Nephrology, Université Catholique de Louvain, Brussels, Belgium; [2]Keyrus Biopharma, Lasnes, Belgium; [3]Keyrus Biopharma, Paris, France.*

Background: There is worldwide renewed interest in HHD, a dialysis modality chosen by a significant proportion of our ESRD patients over the last four decades.

Methods: We here present the patients' and dialysis modality characteristics, trends over time and outcomes of the HD patients from our institution who started HHD as their first RRT modality over the last 40 years.

Results: Between 1970 and 12/2011, 246 [169 males; 181 (73.6 %) being professionally active] patients started HHD as first RRT. Median follow-up time on HHD was 2.4 (IC 95%: 2.1-3.0) years.The trends over those 4 decades are presented in the table.

	<1980	1980-89	1990-99	2000-11	Overall	p
N (patients)	49	65	38	94	246	
Age at first HHD (range)	46 (19-67)	44 (21-69)	42 (16-71)	42 (15-79)	43 (15-79)	NS
Solo-HHD : N (%)	0 (0)	2 (3.1)	5 (13.2)	48 (51.1)	55 (23.4)	<0.001
Charlson comorbidity*	2 (2-8)	2 (2-7)	2 (2-7)	2 (2-10)	2 (2-10)	NS
AV Fistula (%)	100	96.9	100	81.9	92.2	<0.001
Catheter ** (%)	2.1	1.5	2.6	23.4	10.2	<0.001
Training time (days)*	81 (21-250)	73 (17-205)	56 (7-120)	36 (10-149)	60 (7-250)	<0.001
Dialysis modality *** (%)						<0.001
Conventional (3x4 hrs)	73.4	92.3	84.2	41.5	67.9	
Daily	0	3.1	10.5	7.4	5.3	
Nocturnal	4.1	1.5	0	11.7	5.7	
Other	22.4	3.1	5.3	39.4	21.1	

* median (range), ** transient or permanent, *** at last follow-up. Hospitalization incidence (all causes) averaged 3.21 (3.15-3.27) cases/100 patients-months. At last follow-up, 123, 49, 33 and 41 patients have been transplanted, changed their mode of dialysis, died (one only in relation to a HHD technical problem) or are still on HHD, respectively.

Conclusions: HHD is a safe and convenient modality for ESRD patients. Over time, there has been i. a significant increase in the proportion of patients doing solo-HD, ii. with a transient or tunnelled catheter and iii. doing more intense (daily or nocturnal) HHD while iv. training time significantly decreased.

Funding: Pharmaceutical Company Support - Baxter Healthcare, Amgen, Shire, Gambro, Fresenius

FR-PO368

Improvement in Left Ventricular Hypertrophy after Nocturnal Home Hemodialysis with an Alternate Night Schedule Hon-lok Tang,[1] Ho Sing Joseph Wong,[3] Clara Poon,[1] Culen Lau,[2] William Lee,[1] Au Cheuk,[1] Ka Fai Yim,[1] Ka-foon Chau,[3] Samuel K.S. Fung,[1] Matthew K.L. Tong.[1] *[1]Division of Nephrology, Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong, China; [2]Cardiology Unit, Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong, China; [3]Renal Unit, Department of Medicine, Queen Elizabeth Hospital, Hong Kong, China.*

Background: Left ventricular hypertrophy (LVH) is an independent risk factor for mortality in dialysis patients. LVH in end-stage renal disease (ESRD) patients has been attributed to factors including hypertension, anemia and uremia. In Hong Kong, nocturnal home hemodialysis (NHHD) is performed with alternate night schedule. This study aims to investigate the effect of alternate night NHHD on LVH and its contributing factors.

Methods: Sixty-two consecutive patients were started on NHHD in 2 dialysis centers between 8/2006 and 12/2011 with an alternate night schedule (3.5x/week) for 6-9 hours. Left ventricular mass index (LVMI) measured by echocardiogram, systolic and diastolic blood pressure (BP), number of anti-hypertensive medications, hemoglobin (Hb), erythropoietin (EPO) dosage and weekly spKt/V at baseline before NHHD and at 24 months were retrospectively reviewed.

Results: Twenty-three patients had completed NHHD for 24 months. After 2 years of NHHD, LVMI decreased from 214.4±72.5 at baseline to 191.7±82.2 g/m^2 (P<0.05). Systolic BP decreased from 146±18 to 135±15 (P<0.05) and diastolic BP decreased from 90±9 to 84±9 (P<0.05). The number of anti-hypertensive medications also significantly reduced from 2.6±1.2 to 1.3±1.5 (P<0.01) with 10 patients (43%) able to stop all anti-hypertensives. Hb increased from 9.4±1.4 g/dL to 11.3±2.4 g/dL (P<0.01) despite a reduction in EPO dose requirement from 113.3±44.5 to 51.8±50.7 U/kg/week (P<0.01). Weekly spKt/V during conventional HD was 3.67±0.98 while that during NHHD was 2.7 times higher at 9.85±4.04 (P<0.001).

Conclusions: NHHD with an alternate night schedule improves left ventricular hypertrophy in patients with ESRD. It alleviates the contributing factors: reduces BP and the need for anti-hypertensive medications, improves anemia control, and enhances uremia clearance.

FR-PO369

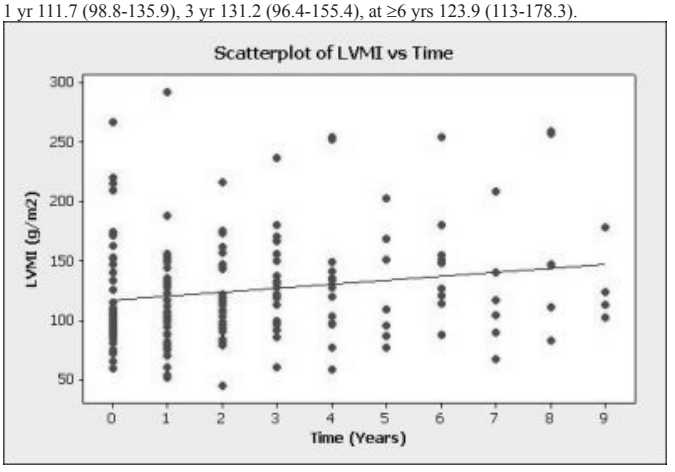
Long Term Effects of Nocturnal Home Hemodialysis on Left Ventricular Mass Index Holly L. Hutton,[1] Yu Zhang,[2] John W.M. Agar,[1] Christine A. Somerville,[1] Anthony D. Perkins.[1] *[1]Nephrology, Barwon Health, Geelong, Victoria, Australia; [2]Medicine, University of Melbourne, Melbourne, Victoria.*

Background: Trials have shown decreases in left ventricular mass index (LVMI) in pts treated with Nocturnal Hemodialysis (NHD) compared to conventional HD. However there is a paucity of data about the long term effects of NHD on LVMI. We studied changes in LVMI in a cohort of NHD pts, focusing on a subgroup on NHD ≥6 years.

Methods: Yearly transthoracic echocardiograms (TTE), comorbidities, cardiac events, duration of previous RRT, BP and laboratory parameters were obtained by retrospective analysis of records. Correlation was done using regression analysis. A sign test was used to calculate average LVMI, expressed as median and interquartile range(g/m^2).

Results: 39/44 pts had at least a baseline and 1 yr TTE. Median baseline LVMI was 108.05 (92.63-146.72); 1 yr 106.81(88.18-132.1). 11 pts with data at 1, 3 and ≥6 years were

studied with most recent TTE as the final reading(mean 7.8 yrs). At ≥6 yrs, 4 patients had a reduction and 7 had an increase in LVMI. Median LVMI at baseline was 115 (75.5-152.7), 1 yr 111.7 (98.8-135.9), 3 yr 131.2 (96.4-155.4), at ≥6 yrs 123.9 (113-178.3).



Changes in LVMI did not correlate with Hb, CRP, PTH, Ca/PO4 product, homocysteine, BP, or cardiac events on regression analysis. At 3 yrs, longer duration of previous conventional HD was associated with less of an increment in LVMI (0.62 g/m^2 per mth, p=0.001).

Conclusions: Decreases in LVMI seen in the first year of NHD were not sustained after 3 yrs, and changes during the first year were not indicative of long term trends. A previous study in conventional HD showed decrements in LVMI over 5 yrs; whilst this was not shown in our small cohort, baseline and final LVMI were comparatively lower. The lack of correlation between changes in LVMI and the studied parameters may be attributed to relative stability of these parameters.

FR-PO370

Dialysate Recirculation Significantly Reduces Dialysate Volume James G. Heaf,[1] Mette K.M. Axelsen,[2] Robert Smith Pedersen.[3] *[1]Nephrology B, Herlev Hospital, University of Copenhagen, Herlev, Denmark; [2]Institute of Public Health, Aarhus University, Aaarhus, Denmark; [3]Flexdialysis A/S, Rungsted Kyst, Denmark.*

Background: The use of single pass (SP) hæmodialysis treatment requires the production of large volumes of dialysate. An in vitro investigation has shown that dialysate recirculation leads to a better utilization of dialysate. We tested a new dialysis principle in vivo, using minimal dialysate volumes, continuously recycled during treatment (multipass hemodialysis, MPHD). Theoretical calculations suggest that MPHD performed 6 times weekly for 8 hours/night, using a dialysate bath containing 50% of the calculated body water, will achieve urea clearances equivalent to conventional HD 4 hours thrice weekly, and a substantially higher middle molecule clearance.

Methods: Ten stable HD patients were dialyzed for 4 hours using standard SPHD (dialysate flow 500 ml/min). Used dialysate was collected. One week later an 8-hour MPHD was performed. The dialysate volume was 50% of the calculated water volume. The dialysate inflow was 500 ml/min – 0.5 x ultrafiltration/minute and the outflow 500 ml/min + 0.5 x ultrafiltration/minute. Elimination rates of urea and β2-microglobulin (B2M) determined hourly.In addition to calculation the water volume (TBW) was measured directly using bioimpedance.

Results: Eight hours of MPHD removed 63% of the amount of urea removed by 4 hours of SPHD. The corresponding figure for B2M was 111%. The dialyate volume was 22.9 ± 4.8 liter. If TBW determined by bioimpedance had been used, only 19.9 liters on average would have been required.

Conclusions: The clearance of both small and (even more so) middle molecules using MPHD will exceed traditional HD. The limited dialysate requirement permits the delivery of centrally produced dialysate delivered to the patient's home or any other destination. This modality will increase patients' freedom of movement compared to traditional home HD. The principle can also be used in the intensive care unit and for automated peritoneal dialysis (APD).

Funding: Pharmaceutical Company Support - Flexdialysis A/S

FR-PO371

Sufficient Phosphate Elimination Can Be Achieved Using a Multipass Batch System James G. Heaf,[1] Mette K.M. Axelsen,[2] Robert Smith Pedersen.[3] *[1]Nephrology B, Copenhagen University Hospital at Herlev, Herlev, Denmark; [2]Institute of Public Health, Aarhus University, Aarhus, Denmark; [3]Flexdialysis A/S, Rungsted Kyst, Denmark.*

Background: The phosphate content of protein is 12-16 mg/gram. A dialysis patient, weighing 70 kg, and eating 1.2 gram protein/day consumes therefore approx. 1 g phosphate/day, of which 60% is absorbed. The absorbed phosphate is thus 4.2 g/week. Only about 1 g/treatment is removed using conventional single pass dialysis (SPHD) for 4 hours x3/week. Most HD patients therefore require phosphate binders. We investigated phosphate removal using a minimized dialysate volume, continually recirculated during dialysis, multipass hemodialysis (MPHD). Theoretical calculations suggest that MPHD performed 6 times weekly for 8 hours/night, using a dialysate bath containing 50% of the calculated body water, will achieve substantially higher phosphate clearance than SPHD.

Methods: Ten stable HD patients were dialyzed for four hours using standard SPHD (dialysate flow 500 ml/min). Used dialysate was collected. One week later an 8-hour MPHD was performed. The dialysate volume was 50% of the calculated body water. The dialysate inflow was 500 ml/min – 0.5 x ultrafiltration/min and the outflow 500 ml/min + 0.5 x ultrafiltration/min. Elimination rates of phosphate and dialysate saturation were determined hourly.

Results: Three hours of MPHD removed 51% (~ 496 mg) of the amount of phosphate removed by 4 hours of SPHD (~ 972 mg), and eight hours removed 77% (~ 753 mg). 34% of the total phosphate removal occurred during the last 5 hours of dialysis. Substantial compartment effects were observed, with mobilization of phosphate from the deep compartments after 3 hours. SPHD was calculated to remove 2.9 g/week and MPHD 4.5 g/week.

Conclusions: The amount of phosphate removed using MPHD will be sufficient to maintain a normal serum phosphate without the need for phosphate binders, even in anuric patients.

Funding: Pharmaceutical Company Support - Flexdialysis A/S

FR-PO372

Dialysate Volume Requirements for High Dose Hemodialysis Based on Urea and Phosphorus Kinetics J. Ken Leypoldt,[1] Alp Akonur,[1] Angelito A. Bernardo,[1] Hieronymus Henricus Vincent,[2] Bruce F. Culleton.[1] *[1]Medical Products R&D (Renal), Baxter Healthcare Corporation, Deerfield, IL; [2]Internal Medicine, St. Antonius Ziekenhuis, Nieuwegen, Netherlands.*

Background: The Frequent Hemodialysis Network Daily Trial showed that daily hemodialysis (HD) improves patient outcomes; approximately 90% of the patients treated in this trial by daily HD had urea stdKt/V > 3.0. Serum phosphorus (P) levels were reduced by daily HD in this trial; however, the levels achieved were not generally in the normal range.

Methods: We used kinetic modeling for urea and P using conventional 2-compartment and pseudo 1-compartment models, respectively, to assess the effect of a frequent HD prescription on urea stdKt/V and serum P levels. Patient differences in P kinetics were characterized by the rate of P mobilization (K_M). Frequent HD (4, 5 or 6 treatments or Tx per week) included: short daily HD (SDHD) for Tx times of 2, 3 and 4 hours and nocturnal HD (NHD) for a Tx time of 8 hours. Using dialysate volumes per Tx of 30, 45, 60, 90, and 120 L, we determined whether such therapies could achieve high dose HD targets, defined as urea stdKt/V > 3.0 and serum P < 7.0 mg/dL without oral phosphate binders. Patients of normal and large body size and with normal and high P dietary intake were considered.

Results: 1) For NHD with 5 & 6 Tx per week, 45 L of dialysate achieved high dose HD targets, except for patients with low K_M and high P intake who required 60 L when treated 5 times per week. 2) For NHD with all HD prescriptions, 90 L of dialysate achieved urea stdKt/V > 3.0 in all patients but at least 5 Tx per week were needed to achieve serum P < 7.0 mg/dL for patients with low K_M and high P intake. 3) For SDHD, 90 L of dialysate achieved urea stdKt/V > 3.0 in all patients, but 6 Tx per week for 3 and 4 hours are generally necessary to achieve serum P < 7.0 mg/dL for patients with normal and high P intake, respectively.

Conclusions: Achievement of high dose HD targets during frequent HD requires individualized prescriptions. NHD requires less dialysate volume than SDHD. To achieve high dose HD targets during both NHD and SDHD in a large fraction of patients, a dialysate volume of at least 90 L is necessary.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO373

Intradialytic Hypotension in 6x Weekly In-Center Hemodialysis: Results from the Randomized Frequent Hemodialysis Network Daily Trial Peter Kotanko,[1] John B. Stokes,[2] Amit X. Garg,[3] Thomas A. Depner,[4] Andreas Pierratos,[5] Christopher T. Chan,[6] Nathan W. Levin,[1] Tom Greene,[7] Brett Larive,[7] Gerald J. Beck,[7] Jennifer J. Gassman,[7] Alan S. Kliger,[8] The FHN Trial Group.[9] *[1]Renal Research Institute, NY; [2]Univ of Iowa, IA; [3]Western Univ, ON, Canada; [4]Univ of California Davis; [5]Univ of Toronto, ON, Canada; [6]Univ Health Network, ON, Canada; [7]Cleveland Clinic Foundation, OH; [8]Yale Univ School of Medicine, CT; [9]NIDDK.*

Background: Intradialytic hypotensive episodes (IDHE) are common complications during hemodialysis (HD) and an independent risk factor for mortality. It is unknown if 6x weekly in-center HD affects the frequency of IDHE.

Methods: The Frequent Hemodialysis Network (FHN) Daily Trial randomized 245 patients to receive 12 months of 6x vs. 3x weekly in-center HD. We sampled HD sessions during one week periods for each month of follow-up. We defined IDHE as those HD sessions (IDHE sessions) during which hypotensive symptoms led to either solely lowering the ultrafiltration rate or to saline administration. We analyzed relative rates as well as the absolute counts of IDHE sessions. Treatment-based differences were tested using generalized estimating equations.

Results: As a percentage of the total number of HD sessions sampled, the trend was for a lower rate of IDHE sessions among the 6x weekly group (10.9%) compared to the 3x weekly group (13.6%, P=0.056). However, as there were more HD sessions in the 6x weekly group, the absolute number of IDHE sessions involving saline administration was significantly greater among the 6x weekly subjects (Table 1).

Conclusions: Frequent in-center HD is associated with an increased relative risk for number of IDHE sessions with saline administration. The clinical significance of this finding is unclear.

Table 1

Event	Count of Sampled Dialysis Sessions		Relative Risk (95% CI)	P-value
	3x weekly Group	6x weekly Group		
No IDHE	2973	5945		
IDHE sessions without saline administration	218	293	1.26 (0.89, 1.77)	0.18
IDHE sessions with saline administration	249	429	1.53 (1.11, 2.09)	0.0086

Funding: NIDDK Support

FR-PO374

Interdialytic Weight Gain in Hemodialysis Patients Is Influenced by Dialysis Time and Frequency, Pre-Dialysis Serum and Dialysate Sodium Concentrations Plus Sex and Serum Albumin Benjamin Ka Thomson,[1] Shih-Han S. Huang,[1] Christopher T. Chan,[2] Rita Suri,[1] Robert M. Lindsay.[1] *[1]Medicine/Nephrology, Western University, London, ON, Canada; [2]Medicine/Nephrology, University of Toronto, Toronto, ON, Canada.*

Background: Increased interdialytic weight gain (IDWG) is associated with hypertension, left ventricular hypertrophy and may influence cardiac morbidity and mortality. Reductions in dialysate sodium have been shown to reduce IDWG and improve morbidity and perhaps mortality. The dialysis time and frequency will influence patient sodium gain where a diffusive sodium gradient exists but this has previously been overlooked as an influence on IDWG.

Methods: We performed a retrospective Cox multivariate regression analysis of 86 patients in the Southwestern Ontario home hemodialysis program where patients are treated by frequent nocturnal, short hours daily, or intermittent conventional hemodialysis, to determine the factors involved in predicting IDWG. Age, diabetic status and residual renal function did not correlate to IDWG.

Results: Using factors that significantly correlate to IDWG, we created an equation that predicted IDWG on the basis of serum albumin, patient sex, patient minus dialysate sodium gradient, and dialysis time and frequency ($R2 = 0.275$, $p<0.001$). **IDWG (Liters)** = 5.0244 – 0.0963 (dialysis frequency) – 0.19014 (if female) – 0.03439 (albumin in g/L) + 0.00014153 (Dialysate – predialysis serum Na+)(dialysis time in minutes).

Conclusions: This equation identifies factors that may be modified to reduce IDWG. This equation will be validated in a prospective study involving the same patient cohort.

FR-PO375

Treatment Effect of Frequent Hemodialysis (HD) on Interdialytic Weight Gain (IDWG) and Extracellular Volume (ECV) and Their Relation to Changes (Δ) in Left Ventricular Mass (LVM): Frequent Hemodialysis Network (FHN) Daily Trial Jochen G. Raimann,[1] John T. Daugirdas,[2] Frank A. Gotch,[1] Tom Greene,[3] George A. Kaysen,[4] Alan S. Kliger,[5] Robert M. Lindsay,[6] Nathan W. Levin,[1] The FHN Trial Group.[7] *[1]Renal Research Institute; [2]University of Illinois College of Medicine; [3]University of Utah; [4]UC Davis; [5]Hospital of Saint Raphael; [6]London Health Sciences Centre; [7]NIDDK.*

Background: Frequent dialysis reduces LVM particularly in patients (pts) with low daily urine volume (UV; Chan 2012). Effects on ECV have been reported previously (Kaysen 2012). We analyzed treatment effects on IDWG and bioimpedance-measured ECV and the relation to ΔLVM.

Methods: Treatment effects on IDWG and ECV were analyzed in subgroups (UV≤ and >100 mL/day) of subjects randomized to daily (6x/wk) or conventional (3x/wk) HD, using mixed models. Predictors of ΔLVM from baseline to month 12, focusing on ΔIDWG and ΔECV were analyzed using linear regression (LR). Data reported as mean (95% CI).

Results: Frequent dialysis reduced IDWG by -0.98 (-1.16 to -0.81) kg in 234 pts with available data. The effect in pts with UV≤100 mL/day was -1.00 (-1.22 to -0.79) kg and in those with UV >100 mL/day -0.90 (-1.21 to -0.60) kg, interaction P=0.16. Frequent dialysis also lowered ECV, but the treatment effect did not differ significantly {UV≤100 mL/day [-0.6 (-1.5 to +0.3) L; UV > 100 mL/day [-2.1 (-3.4 to -0.9) L], interaction P=0.33}. ΔECV was a significant independent predictor of ΔLVM in pts with UV≤100mL/day only (Table 1).

ΔLVM [g] as a function of ΔIDWG [L] and ΔECV [L].

	All Pts	All Pts	Pts with UV≤100 mL/day	Pts with UV≤100 mL/day
Parameter	Reg. Coeff. (SE)	P	Reg. Coeff. (SE)	P
ΔIDWG [L]	3.3 (4.0)	0.42	8.1 (5.3)	0.13
ΔECV [L]	2.0 (1.2)	0.09	3.3 (1.5)	0.03

LR Model with ΔLVM as dependent variable, controlling for baseline ECV/Total body water, baseline IDWG, age, diabetes, and randomized treatment assignment. Regression coefficients (Reg. Coeff.) show the magnitude of the association.

Conclusions: In addition to previously described associations between ΔLVM and Δblood pressure, ΔECV is involved in pts with low UV. A similar but non-significant association was found in pts with higher UV.

Funding: NIDDK Support, Other NIH Support - Support Was Received from CMS and the NIH Foundation

FR-PO376

The Impact of Frequent Hemodialysis on Sleep Quality: Frequent Hemodialysis Network (FHN) Trials Jocelyn Kim, Mark L. Unruh, Brett Larive, Paul W. Eggers, Amit X. Garg, Jennifer J. Gassman, Fredric O. Finkelstein, Paul L. Kimmel, Glenn M. Chertow. *FHN Trials Group, NIDDK.*

Background: Patients undergoing maintenance HD have an impaired quality of sleep. Previous studies suggest frequent HD improves sleep quality, which is a strong motivation for some patients to undertake the treatment. We studied the effects of frequent in-center and nocturnal HD on self-reported sleep quality in the FHN randomized trials.

Methods: A total of 332 patients were randomly allocated to frequent (six times per week) or conventional (three times per week) HD in the Frequent Hemodialysis Network Daily (n=245) and Nocturnal (n=87) Trials. We used the Medical Outcomes Study Sleep Problems Index (SPI), a validated and reliable instrument in the ESRD population, to measure self-reported sleep quality. The SPI is scored from a value of 0 to 100 with a lower value indicating better sleep quality. The primary sleep outcome was the change over 12 months on the SPI score.

Results: The results for the SPI in the FHN Trials are show in the Table. In the Daily Trial, after adjustment for baseline SPI, subjects randomized to frequent as compared with conventional in-center hemodialysis experienced a 4.2 (95% CI 0.4 to 8.0) greater adjusted mean decline in SPI at 4 months, and a 2.6 (-2.3 to 7.5) greater decline at 12 months. In the Nocturnal Trial, subjects randomized to frequent as compared with conventional home hemodialysis experienced 2.9 (-3.4 to 9.3) and 4.5 (-3.2 to 12.2) greater declines at months 4 and 12, respectively. None of the treatment differences approached statistical significance with the exception of the Daily Trial Month 4 comparison ($p < 0.05$).

FHN: Comparison of changes for frequent (6x-per-week) vs. conventional (3x-per-week) hemodialysis

Trial	**Trt.**	**Sleep Symptoms & Problems Index (Mean ± SD)**		
		Baseline	**F4**	**F12**
Daily	3X	35.5 ± 21.0	35.7 ± 19.3	34.8 ± 22.8
	6X	36.2 ± 21.2	31.7 ± 19.4	31.2 ± 22.3
Noct.	3X	32.3 ± 17.9	33.8 ± 18.7	33.0 ± 23.1
	6X	32.3 ± 18.3	31.5 ± 18.1	29.1 ± 18.6

Conclusions: Although a trend suggesting a possible benefit of daily in-center HD was observed at 4 months, the FHN Trials were unable to demonstrate significant improvements in sleep quality at 12 months.

Funding: NIDDK Support, Other NIH Support - T35 Training Grant

FR-PO377

Effect of Daily Hemodialysis (DHD) on Anemia Parameters: Interim Results from the FREEDOM Study Bertrand L. Jaber,[1] George R. Aronoff,[2] Lori Lyn Price,[1] Amy W. Williams,[3] Janice P. Lea,[4] Isaac Teitelbaum,[5] Michael S. Gersch,[6] Troy J. Plumb,[7] James E. Novak.[8] *[1]Tufts, MA; [2]U Louisville, Louisville, KY; [3]Mayo, MN; [4]Emory, GA; [5]U Colorado, CO; [6]ANRA, AR; [7]U Nebraska, NE; [8]HFHS, MI.*

Background: The FREEDOM Study, an ongoing prospective cohort study investigating the benefits of DHD, has demonstrated improvements in several health-related quality of life measures.

Methods: This interim analysis presents the trends in anemia parameters during the first year of DHD (for patients enrolled by June 30, 2010), including changes in levels of hemoglobin (Hgb), hematocrit (Hct), ferritin, and transferrin saturation (TSat), as well as trends in prescribed erythropoietin-stimulating agents (ESAs) and oral/intravenous iron preparations.

Results: Of 336 enrolled pts, 171 completed 1 year follow-up, constituting the as-treated cohort. Mean age was 53 yrs, 65% male, 70% white, 59% used a fistula, 46% had diabetes, and 26% had heart failure. Mean (SD) estimates of the as-treated cohort are shown below:

Anemia Parameters of the As-Treated Cohort

Anemia Parameter	Baseline	Month-4	Month-12	Global P value[1]
Hgb (g/dL)	11.8 (2.3)	11.3 (1.6)	11.2 (1.7)	0.008[1]
Hct (%)	35.4 (3.9)	34.5 (5.1)	33.8 (5.1)	0.004[1]
Ferritin (ng/mL)	538 (344)	506 (342)	470 (353)	0.02[1]
TSat (%)	29 (12)	27 (13)	26 (11)	0.03[1]
% Prescribed ESAs	66	80	77	0.003[2]
% Prescribed Iron	36	46	50	0.01[2]
% in-range Hgb (11-12 g/dL)[3]	36	35	36	0.93[2]
% in-range Hgb (10-11 g/dL)[4]	22	18	26	0.23[2]

[1]by repeated measures ANOVA; [2]by GEE model; [3]In accordance with 2007 KDOQI update on anemia management in CKD; [4]In accordance with clinical practice following 2011 FDA Drug Safety Communication on ESA use

Conclusions: In summary, at 1 year, pts initiating DHD had a significant decrease in mean Hgb, Hct and TSat, accompanied by a significant increase in the proportion of pts prescribed ESAs and iron. However, these trends did not result in a significant shift in the proportion of pts maintaining in-range Hgb levels during the same period. Factors that may confound these results include changes in Hgb target ranges and ESA prescribing guidance, reliance on prescribed vs. self-administered ESA data, variability of timing and dosing of iron, and center therapy practices.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

FR-PO378

Catheter-Related Bacteremia in Nocturnal Home versus In-Center Hemodialysis Patients Hui Xue,[1] Eduardo K. Lacson,[2] Nien-chen Li,[2] Steven M. Brunelli,[1] Franklin W. Maddux,[2] Robert S. Lockridge.[3] *[1]Brigham and Womens Hospital, Boston, MA; [2]Fresenius Medical Care North America, Waltham, MA; [3]Lynchburg Nephrology Physicians, Lynchburg, VA.*

Background: The CDC reported catheter-related bacteremia (CRB) rate of 3.2/100 pt-months for hemodialysis patients. We hypothesized that patients with central venous catheters (CVC) on nocturnal home hemodialysis (NHHD) will have lower CRB rates than conventional in-center hemodialysis patients (IHD).

Methods: 63 of 64 NHHD patients with CVC from Lynchburg Nephrology Dialysis (VA) were matched at a ratio of ~1:2 by age, gender, race, vintage, and diabetes to 121 IHD patients, from a pool of 6,285 admitted to Fresenius Medical Care North America facilities using a CVC in Virginia and surrounding states, from January 1, 2007 to December 31, 2010. CRB events (positive blood cultures or hospitalization for sepsis) were followed for up to 20 months or until censoring (e.g. death, removal of the catheter, etc.). Cox models were used to compare time to CRB.

Results: Both NHHD and IHD study cohorts were younger and had less DM than the general IHD population, but were similar to each other in terms of age (52.8 vs. 53.8 years), vintage (38.5 vs. 37.1 months), sex (57.1% vs. 57.9% males), race (57.1% vs. 57.0% white), and DM (20.6% vs. 21.5%), respectively. Mean duration for the first CVC was longer for NHHD than IHD (16.3 vs. 6.4 months). CRB-rates were similar when only the first catheters (1.77/100 vs. 1.95/100 pt-months, p=ns) were considered and with inclusion of subsequent catheters (1.51/100 vs. 2.01/100 pt-months, p=ns). The corresponding hazard ratios for NHHD were 1.01 (unadjusted) and 1.03 (adjusted) for first catheters; and 0.80 (unadjusted) and 0.91 (adjusted) including subsequent catheters, all p=ns.

Conclusions: CRB rates were not significantly different between matched NHHD and IHD patients. However, CRB rates were lower in both cohorts than published data. Patient selection (e.g. healthier subjects), small sample size, and failure to capture all CRB events (like hospital drawn cultures for IHD) may help explain these findings.

FR-PO379

Racial Differences in Initial Dialysis Access and Hospitalizations among Home Hemodialysis Patients Luis A. Bent-Shaw,[1,2] Margaret K. Yu,[1,2] Bessie A. Young.[1,2,3] *[1]Nephrology, University of Washington, Seattle, WA; [2]Kidney Research Institute, University of Washington, Seattle, WA; [3]Veterans Affairs Puget Sound Health Care System, Seattle Epidemiology Research and Information Center, Seattle, WA.*

Background: Home hemodialysis (HH) is a rapidly growing modality, however, less is known regarding differences in initial access among patient initiating home hemodialysis in a large urban program.

Methods: Data from all HH patients treated in the Northwest Kidney Center's HH unit were abstracted from 2001-2010. Patients received 3Xweek HD, 2.5-4 hours SD on the NxStage machine, or ND defined as greater than 6-8 hours of dialysis at night. Categorical and continuous data were analyzed using student t-test and chi squared. Logistic regression models were used to analyze odds of tunneled catheter or graft compared to arterial-venous fistula (AVF).

Results: Of the 174 patients who were scheduled to initiate training in the HH unit, 42 (24.1%) initiated CHD, 108 (62.1%) SD, 11 (6.3%) ND, and 13 (7.5%) did not complete training. The majority of patients were white (59.9%), while blacks (21.3%), Asians (14.4%), and others (7.4%)) made up the remainder of patients. Diabetes was the main cause of kidney failure for 42.8% of patients. The majority of patients had arterial venous fistulas (69.5%), while 1.7% had grafts and 28% had catheters. African American patients were 2.6-fold (95% CI 1.01-6.6) more likely to start without a fistula compared to whites after adjusting for age, diabetes, smoking, sex, modality, and hospitalizations. There was no difference in the number of hospitalizations between African American and Asian American patients compared to whites.

Conclusions: African Americans are more likely to start an outpatient home hemodialysis program without a fistula compared to whites; however, no racial difference in hospitalizations was found. Efforts should be continued for fistula first in all patients.

Funding: NIDDK Support, Veterans Administration Support

FR-PO380

Distress Screening for Dialysis Patients: The Distress Assessment Response Tool (DART) Marta Novak,[1,2] Joanne M. Dell,[1] Christopher T. Chan,[3] Joanne M. Bargman,[3] Sarbjit Vanita Jassal,[3] Madeline Li.[1] *[1]Dept. of Psychiatry, University Health Network, Toronto, ON, Canada; [2]Inst. of Behavioral Sciences, Semmelweis University, Budapest, Hungary; [3]Division of Nephrology, University Health Network, Toronto, ON, Canada.*

Background: DART is a tool currently used to assess distress and the needs of patients living with cancer. We aimed to test the utility and feasibility of integrating the DART system in the clinical care of patients receiving home dialysis therapy.

Methods: Fourty patients on home dialysis therapy were approached to complete the self-report questionnaires of the DART: the Edmonton Symptom Assessment System (ESAS), the Patient Health Questionnaire (PHQ-9), the General Anxiety Disorder-7 (GAD-7) and the Social Difficulties Inventory (SDI-21). The DART protocol was used to examine the proportion of patients that would be referred for further assessment.

Results: 39/40 patients (22 women, 17 men; mean [+/-SD] age 58 [17] yrs) completed the DART. Among them, 20 received PD (12 male and 8 female, average age 65.0, SD = 16.9) and 19 received HHD (10 male and 9 female, average age 50.5, SD = 15.0). Gender and dialysis vintage did not differ between the PD and HHD groups, but HHD patients were significantly younger.

26 patients were flagged for further assessment: 8 (20%) for depression, 5 (13%) for anxiety, and 17 (44%) for social difficulties. Nineteen participants (49%) received at least one flag for further assessment, while 7 (19%) were flagged in two or more areas. HHD patients experienced significantly greater levels of symptoms than the PD group on the GAD-7, PHQ-9, and SDI-21, as well as on the pain, depression, and drowsiness subscales of the ESAS and this remained significant when controlling for age. Significantly more HHD vs PD patients (68 vs 30%, p=0.016) were flagged for further assessment or referral.

Conclusions: The DART tool identified a significant proportion of patients on home dialysis who would likely benefit from further assessment and intervention. The DART tool, after further validation of the protocol, could be an important clinical instrument to identify patients at risk to improve their quality of life and their coping with the burden of dialysis.

Funding: Clinical Revenue Support

FR-PO381

Predictors of Patient Selection to Home Dialysis Patrik Finne,[1] Eero Honkanen,[2] Carola Gronhagen-Riska.[2] *[1]Finnish Registry for Kidney Diseases, Helsinki, Finland; [2]Department of Nephrology, Helsinki University Central Hospital, Helsinki, Finland.*

Background: The use of home dialysis, i.e. peritoneal dialysis and home hemodialysis, varies greatly internationally from less than 10% in the United States to more than 50% of all dialysis patients in New Zealand. The very large variability indicates that there is a potential for increasing home dialysis in many places. Our aim was to explore which factors predict selection of patients to home dialysis.

Methods: The Finnish Registry for Kidney Diseases provided information on all patients who had entered RRT aged 20 years or older in 2000 to 2010 in Finland and who were still on dialysis at 91 days after dialysis start (n=4984). The registry contains information on patients' basic characteristics, kidney disease diagnosis, comorbidities, and laboratory variables. Using forward stepwise selection of these explanatory variables, we established a multivariable logistic regression model with the binary outcome variable home dialysis or centre hemodialysis at 91 days from start of dialysis treatment. Based on this model, probability of home dialysis could be calculated for each patient.

Results: At 91 days from dialysis start 1463 (29%) patients were on home dialysis, and of these 92% were on peritoneal dialysis. The final multivariate model included twelve variables of which the most significant predictors of home dialysis were a body mass index lower than 35 kg/m^2, high concentration of hemoglobin, and high diastolic blood pressure. Age decreased probability of home dialysis. Of kidney disease diagnoses, polycystic kidney degeneration and amyloidosis were connected to the lowest, and type 1 diabetes to the highest probability of home dialysis.

Conclusions: Multiple factors predict home dialysis. Our multivariable model produced individual probabilities of home dialysis, and thus it was possible to identify centre hemodialysis patients with high calculated probability of home dialysis (indicating typical characteristics of home dialysis patients). In the next step of this research project we will examine this group of patients closer, in order to estimate whether there is a potential to increase the proportion of patients on home dialysis.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO382

Determinants of Home Hemodialysis Training Outcomes Michael Schachter, Karthik K. Tennankore, Christopher T. Chan. *University of Toronto, Canada.*

Background: Home hemodialysis (HHD) has clinical and economic advantages over in-center therapy. Many health systems wish to broaden the population to which HHD can be successfully offered. The purpose of this study was to examine HHD training outcomes.

Methods: Characteristics of patients (pts) initiating HHD in our center were collected from 2003-2011. We compared those who received HHD for at least 365 days, or received a renal transplant ("HHD/Tx>365"), to those who failed training or experienced technique failure (TF) before 1 year ("HHD<365").

Results: 177 pts trained for HHD. In HHD<365 pts (n=38), 24 did not finish training, 8 had TF, and 6 died. Table 1 shows demographic, comorbidity, social and dialysis factors. The most common reasons for modality end <365 days (n=38) were death (16%), medical deterioration (16%), inability to cope with HHD (13%), inadequate family support (8%) and non-compliant pt (8%). Reasons for not finishing training are shown in Table 2. In HHD/Tx>365 pts, the most common outcomes were: remain on NHD (47%), renal transplant (35%), or expire (11%). Median modality survival was 3.3 (2.0,5.2) yrs.

Table 1| Characteristics of Patients who initiated home hemodialysis training during the era 2003-2010

Characteristic	HHD <365 (n=32)	HHD > 365 or transplant (N=139)	P-value
Demographics			
Age	49 (16)	45 (13.5)	0.13
Percent male	56	63	0.46
Race (%)			
Caucasian	49	57	0.367
Non-Caucasian	51	43	
Education (%):			
High school or below	32	39	0.452
College/University or higher	68	61	
Comorbidities			
Charlson comorbidity Index	4.6 (2.1)	3.5 (1.8)	0.003
Prior renal transplant (%)	19	27	0.38
Social Factors			
Owns Home (%)	62	75	0.152
Living alone (%)	28	18	0.221
Distance from HHD training unit (km)	18 (8,29)	22 (11,44)	0.48
Nephrology management before start of HHD (%)	82	98	0.001
Dialysis Factors			
RRT Vintage (years)	0.83 (0,10.5)	6.8 (0.61,14.8)	0.013
Initiating HHD with CVC (%)	72	47	0.007

Table 2| Reasons for Failure to Graduate from HHD Training (n=24)

Rank	Reason	Proportion of Patients (%)
1	Home Inappropriate	16.7
2	Can't cope with burden of HHD	12.5
2	Pt unable to work constructively with team	12.5
2	Failed Training Tests	12.5
3	Insurmountable language barrier	8.3
3	Inadequate Family support	8.3
3	Imminent renal transplant, decided to forego training	8.3
3	Financial burden	8.3
4	Anxiety/nervousnes about HHD	4.2
4	Care-giver anxiety about HHD (dependent pt)	4.2
4	Manual dexterity	4.2
4	Visual impairement	3.1

Conclusions: Pts who failed training had greater co-morbidity and were more likely to be unprepared for renal replacement therapy. Given these challenges, enhanced supports or a customized education strategy could be considered to further promote adoption of HHD in vulnerable pts.

Funding: Clinical Revenue Support

FR-PO383

Present Status of Home Haemodialysis in Japan: from the Japanese Home Haemodialysis Registry Ikuto Masakane,[1] Norio Hanafusa,[2] Shigeru Nakai,[3] Kanenori Maeda,[4] Hiromichi Suzuki.[5] *[1]Yabuki Shima Clinic, Yamagata, Japan; [2]University of Tokyo Hospital, Tokyo, Japan; [3]Fujita Health University School of Health Scieneces, Toyoake, Japan; [4]Maeda Clinic, Shimabara, Japan; [5]Saitama Medical University, Iruma, Japan.*

Background: In recent two decades home haemodialysis (HHD) has been promoted throughout the world because it has dramatically improved the prognosis and the QOL of choric dialysis patients. HHD started in 1968 in Japan but the number of HHD patients has been still very small, 327 at the end of 2011 as 0.1% of all dialysis patients. The Japanese Society for Home Haemodialysis (JSHHD) established a patient registry system (JHHDR) on 2011 to grasp the present status of HHD and promote more utilization of HHD throughout Japan.

Methods: Clinical parameters about HHD patients as of 31st Dec 2011 were collected using questionnaire. The parameters were patient's basal characters, results of blood chemistry, therapeutic options for renal anaemia and CKD-MBD, dialysis prescriptions and outcome parameters.The questionnaires have been still collected, and 142 patients' data from 22 dialysis facilities were collected by the end of April 2012. Some of data were compared with the past data of the Patient Registration of the Japanese Society for Dialysis Therapy (JSDT).

Results: The interim analysis showed that the mean age of the patients was 51.7 years old and 15 years younger than JSDT, and male was dominant (76.1%). The mean dialysis vintage was longer than JSDT (10.8 yrs. vs. 7.05 yrs.) The mean frequency of dialysis session in HHD was 4.45 per week and haemodialysis products (HDP) were greater than 72 in 60 % of the patients. The mean systolic blood pressure was significantly lower than JSDT (141.2 mmHg vs. 153.5mmHg) and the percentage of the patients who didn't need antihypertensive agents was greater than JSDT (47.9% vs. 37.3%).

Conclusions: It is a very interesting issue how much impact HHD has on the survival rate of chronic dialysis patients in Japan, which has been still one of the best results in the world. The findings from JHHDR could give us much useful information about better dialysis prescriptions for HHD.

FR-PO384

An Overview of a 8-Year-Old Provincially Coordinated Independent Hemodialysis Program in British Columbia Canada Lee Er,[1] Donna Murphy-burke,[1] Ognjenka Djurdjev,[1] Adeera Levin.[1,2] *[1]BC Provincial Renal Agency, Vancouver, BC, Canada; [2]Division of Nephrology, University of British Columbia, Vancouver, BC, Canada.*

Background: BC has the only coordinated provincial program for independent hemodialysis (IHD) in Canada. Established in 2004, the program has been actively involved in enhancing and fostering excellence in the delivery of IHD throughout BC. The objectives are to describe the program growth experience over 8 years, and to identify trends and the potential implications for further program growth.

Methods: Observational cohort study of all patients who started IHD between 2004 and 2011, using data obtained from the provincial centralized patient registry known as PROMIS. Outcomes of interest include technique survival, pt survival, and annual program growth.

Results: A total of 390 pts received IHD training of which 364 (93%) pts started IHD. Fig 1 indicates a fast growth in the first 4 yrs but on a gradual decline since settling at 5% prevalent rate. The IHD technique survival at 1yr is 80% and median survival time is 33 mos (Fig 2). The in-take is declining over time after the peak at 2nd yr (Fig 3); the attrition rate is increasing over time (Fig 4). Transplantation, deceased and medical reasons (e.g. hospitalization, inadequate dialysis etc) are the top 3 attrition reasons. The potential barriers for expansion identified are: 1) high turn-over rate on supporting staff like trainers, 2) lack of pt education during pre-dialysis, and 3) lack of respite-care and home support.

Conclusions: Despite provincial funding and support of IHD growth appears to have plateaued. Systematic evaluation of causes, and development of strategies to a) look at barriers to the uptake of new patients, and b) look at ways to minimize attrition rate within a provincial framework is important to ensure sustainability of this modality.

Figure 1: Prevalent Rate of IHD Over Time

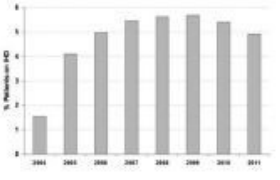


Figure 2: IHD Technique Survival Curve

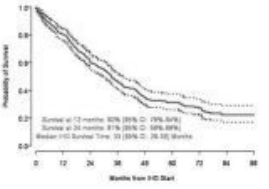


Figure 3: %IHD In-Take by Training Year

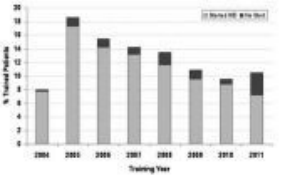
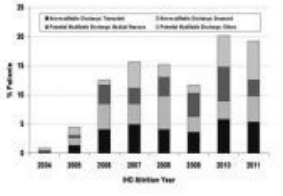


Figure 4: IHD Attrition Reasons by Attrition Year



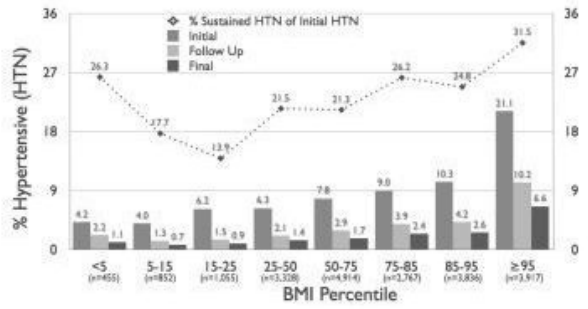
FR-PO385

Blood Pressure and BMI in School Aged Children Joshua A. Samuels,[1] Karen Mcniece Redwine,[2] Tim Poffenbarger,[1] Joyce P. Samuel,[1] Cynthia S. Bell.[1] *[1]Pediatric Nephrology & Hypertension, UT Houston Medical School, Houston, TX; [2]Pediatric Nephrology & Hypertension, UAMS/Arkansas Children's Hospital, Little Rock, AR.*

Background: Obesity is the leading risk factor for essential hypertension in adolescents. We hypothesize that overweight and obese children have a higher prevalence of hypertension at initial and follow-up BP measurement screenings.

Methods: We performed school-based BP screening in Houston area children from 2000 to 2012. At each screening visit, height and weight were measured to determine BMI and BP was obtained up to 4 times with the average used to determine final BP status. Children whose BP was initially elevated underwent confirmation visits on 2 subsequent occasions to diagnose sustained hypertension according to Working Group criteria.

Results: Over 21,000 children aged 10-19 years (mean 13.3±1.7) have been screened. Although 13% of all children are hypertensive at the initial screening, the final prevalence of sustained hypertension is 2.7%. Hypertension rates are markedly increased in children with higher BMI percentiles at all screenings (Figure). Among children who are hypertensive at the initial screening, those with a high BMI percentile are more likely to have sustained HTN after 3 screenings (OR=1.09 for each additional 10 BMI percentiles, p<.001).



Conclusions: Though initial screening identifies significantly more abnormal blood pressure than final diagnosis, elevated BMI is an important factor associated with higher rates of initial and sustained hypertension in school aged children. Additionally, hypertensive children with a normal BMI are more likely to normalize their BP at follow-up screenings.

Funding: NIDDK Support

FR-PO386

High Blood Pressure in Children with Chronic Kidney Disease (CKD) Correlates with 1,25(OH)2 Vitamin D and Not 25(OH) Vitamin D

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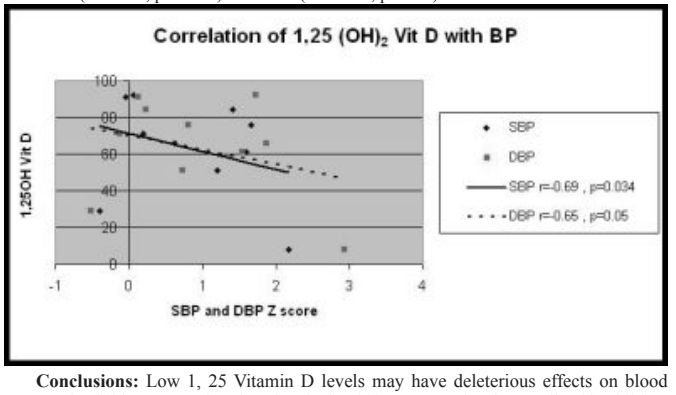
Background: Vitamin D deficiency is well known for its musculoskeletal complications in children and adults. In vitro studies have suggested 1, 25(OH)2Vit D (1,25 Vit D) as a vascular protective agent by its effect on the endothelium. However, limited studies are available looking at the effect of Vitamin D on blood pressure in children.

Methods: We enrolled 46 patients (25 patients with CKD, 9 primary hypertension (PH), and 12 Control (C). We collected data on age, sex, race, cause of kidney disease, eGFR, Ht, Wt, BMI, BP percentiles and z scores, phosphorus, calcium, 25 vit D, 1,25 vit D, PTH. Spearman coefficient was used to determine the correlation between Vitamin D, SBP/DBP z score, GFR, and PTH using SPSS version 2.0.

Results: SBP %'ile was significantly higher in PH (87.7 ± 17.6) and CKD (75.4 ± 27.6) than C (43.5 ± 26.9) and DBP%'ile significantly higher in PH (90 ± 10.6) and CKD (77 ± 24.6) than C (46.4 ± 18.9).eGFR (ml/min/1.73m2) was significantly lower in patients with CKD (70.2 ± 37.6) than C (106 ± 27.7). 86% patients had Vitamin D level <30 ng/ml. Mean 25 Vit D level was 14 ± 5.8 ng/dl. There was no correlation of 25 Vit D with SBP(r=+ 0.041, p=0.42) and DBP(r= - 0.16, p= 0.23)z-score. 25 Vit D did not correlate with eGFR (r= + 0.290, P=0.134) or PTH (r= - 0.260, P=0.370). 1,25 Vit D level correlated negatively with SBP (r= -0.69, p=0.034) and DBP (r= - 0.65, p=0.05) z scores.



Conclusions: Low 1, 25 Vitamin D levels may have deleterious effects on blood pressure in children. 1,25 Vitamin D levels should be measured and normalized in children with hypertension.

FR-PO387

Implementation of a Home Blood Pressure Telemonitoring System in Children with Hypertension

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Background: Successful chronic disease management requires a coordinated effort including adherence to clinical practice guidelines and treatment plans and comprehensive patient-provider communication.Major barriers to successful hypertension (HTN) management include medication non-adherence and failure to monitor/communicate BP readings.Development of successful HTN monitoring and treatment programs in children,such as the home telemonitoring system (HTMS) in our study,is vitally important given the high prevalence of pediatric HTN and concern for associated HTN-related morbidity/mortality in adults.

Methods: A study member provided training on the HTMS to children ≤21 years of age with essential HTN.The HTMS was used to complete a symptom diary,assess medication side effects,obtain BP and complete HTN education modules.Acceptance of the HTMS was assessed by attitudinal survey and semi-structured qualitative interview.

Results: 8 children participated:62.5% male,75% Caucasian,mean age 12 yrs,average time with HTN 2.3 yrs. 63% reported their BP as stable and BP symptoms as none;50% reported their HTN knowledge to be good; 63% reported daily home computer use. Attitudinal survey results: 88% reported that working with the computer was not difficult;100% reported that self-testing procedures,BP monitor,symptom diary and medication side effect questions were not complicated;75% felt the self-testing procedure took little time and did not interfere with their usual activities;63% felt slightly to significantly safer while monitored by the HTMS;63% felt it was important to know that the self-testing results can be immediately reviewed in the medical center after the test;88% would like to use the HTMS in the future.Qualitative interview results showed consistently positive comments for content,interface and process components and provided suggestions for improvements.

Conclusions: The HTMS was positively accepted, reported as easy to use and participants found the components to be helpful.This system demonstrates a new method to monitor BP,BP symptoms,and provide HTN education in the home setting to children with HTN.

Funding: Private Foundation Support

FR-PO388

Association of Blood Pressure and Sleep Complaints in Children Evaluated for Hypertension by Ambulatory Blood Pressure Monitoring (ABPM)

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Background: Understanding the risk factors for hypertension (HTN) in childhood is a key to preventing serious long term outcomes such as cardiovascular and end stage renal disease as HTN has its antecedents in childhood. Sleep problems are associated with development of HTN in adults but there is very little information about this association in children. Our aim was to study the association between sleep complaints and blood pressure (BP) in a pediatric population.

Methods: Consecutive patients between age 13-19 yrs referred to pediatric nephrology between 2009-2012 who had ABPM for evaluation of HTN and who completed a sleep questionnaire were included. Of the 66 patients undergoing ABPM, 39 were included in final data analysis. Medical records were reviewed to obtain demographics and BMI. Data are presented as mean ± SD. Student t-test was used to compare continuous data.

Results: Characteristics for the 39 children were : M:F ratio 2:1, Age 15.9 ± 1.6 years, BMI 29.4 ± 6.6 kg/m^2, week day sleep 516.3 ± 111.7 minutes, week end sleep 592.2 ± 86.5 minutes. Prevalence of sleep related complaints were: ever snore 41%, habitual snoring (>50% of time) 15%, wake up not refreshed 30%, daytime sleepiness 36%, morning headache 24%. Stages of hypertension were: prehypertension 6 (15%), stage I - 22 (56%), stage II - 11 (28%). Habitual snorers had significantly higher average daytime diastolic BP (89.2 ± 16.6 vs. 79.2 ± 8.0 mmHg, p =.026) and a trend towards higher average daytime systolic BP (145.5 ± 11.3 vs. 139.6 ± 6.6 mmHg, p =.082). Children reporting daytime sleepiness had higher average night diastolic BP (69.6 ± 10.8 vs. 64.0 ± 5.7 mmHg, p =.040). As expected, children with weekday sleep duration ≤ 8 hours reported more daytime sleepiness (67% vs. 18%).

Conclusions: There is a high prevalence of sleep related complaints in pediatric patients referred for evaluation of HTN. Habitual snoring and daytime sleepiness are associated with higher BP parameters on ABPM.

FR-PO389

Etiology of Hypertension and Prevalence of Target Organ Damage in a Referral Sample of Icelandic Children

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Background: Limited information exists on the prevalence and extent of target organ damage (TOD) in children with hypertension. The aim of the study was to investigate the causes of hypertension and prevalence of TOD in a group of hypertensive Icelandic children.

Methods: This was a retrospective study of all patients below 18 years of age who received care at the Pediatric Hypertension Clinic of Landspitali - The National University Hospital of Iceland during the years 2003-2008. This institution is a referral center for the whole nation. Medical records of all identified pediatric subjects were retrospectively reviewed for the etiology of hypertension, echocardiographic changes consistent with hypertensive heart disease and microalbuminuria.

Results: Of 93 children identified with hypertension during the study period, 50 (54%) were male and the median age was 12 (0-17) years. Twenty-three children (25%) had sustained essential hypertension, 66 (71%) had secondary hypertension and 4 (4%) were considered to have white coat hypertension. Causes of secondary hypertension included renal parenchymal disease in 23 children (25%), congenital anomalies of the kidney and the urinary tract in 20 (22%), medications known to elevate blood pressure in 7 (8%), coarctation of the aorta in 2 (2%) and miscellaneous etiology in 10 (11%). Echocardiography was carried out in 59 children (63%) and showed left ventricular hypertrophy in 11 (19%) and decreased ejection fraction or dilated left ventricle in 2 (3%). Microalbuminuria was noted

in 18 children, 6 of whom had no evidence for underlying renal parenchymal disease. Of those, 3 had secondary hypertension and 3 essential hypertension. Thus, TOD was noted in 16 (17%) study subjects.

Conclusions: Two-thirds of this referral cohort of hypertensive children had secondary hypertension. Evidence for TOD was present in a significant proportion of study subjects.

FR-PO390

Increased Provider Recognition of Elevated Blood Pressure in Children Tammy M. Brady, Alicia M. Neu, George Siberry, Barry Solomon. *Pediatrics, Johns Hopkins University, Baltimore, MD.*

Background: High blood pressure (BP) in children is often unrecognized by providers. We evaluated the effect of a real-time electronic medical record (EMR) alert on recognition of high BP in a pediatric clinic.

Methods: Cross-sectional review of well and acute care visits from 1/1/09-6/30/09 of children 3-21 yrs with a high BP in triage and no history of hypertension (HTN). All high BPs (≥90th%ile or ≥120/80) generated an EMR alert. High BP was "recognized" by providers if the clinic note documented: high BP or HTN; provider repeated BP; plan to repeat BP or evaluate high BP. Prevalence of recognition was compared pre- and post-EMR alert implementation. Continuous and categorical variables were compared with t-tests and chi-squared analyses. Prevalence ratios of recognition were obtained by univariate log-binomial regression.

Results: Post-EMR alert, 42% (568/1368) of high BP were recognized (vs. 13% pre-alert; p<0.001). Children with recognized BP were more likely older, non-African American (non-AA), male, overweight/obese, with a family history of cardiovascular disease (CVD), a systolic BP≥120 and seen for well care.

Characteristics of 1368 Children with Elevated Blood Pressure and Prevalence Ratios of Recognition

Characteristic (Mean (SD) or % (N))	Overall	BP Recognized	BP Unrecognized	p-value		Prevalence Ratio (95% Confidence Interval)	p-value
Age (yrs)	12.4 (5.8)	12.9 (5.7)	12.1 (5.8)	0.02		1.01 (1.00, 1.02)	0.02
AA (vs. non-AA)	93% (1275)	92% (520)	94% (755)	0.049		0.8 (0.64, 0.97)	0.03
Male	42% (568)	46% (259)	39% (309)	0.01		1.2 (1.04, 1.34)	0.009
Overweight/obese*	61% (530/870)	65% (270/416)	57% (260/454)	0.02		1.19 (1.02, 1.38)	0.02
Acute visit (vs. well visit)	54% (439/808)	40% (132/334)	65% (307/474)	<0.001		0.55 (0.46, 0.65)	<0.001
Family history CVD	11% (146)	15% (86)	8% (60)	<0.001		1.49 (1.28, 1.74)	<0.001
SBP >=120 mmHg	64% (872)	70% (398)	59% (474)	<0.001		1.3 (1.16, 1.53)	<0.001
DBP >=80 mmHg	8% (105)	9% (50)	7% (55)	0.9		1.16 (0.94, 1.43)	0.2

*Body Mass Index >=85%ile

Conclusions: EMR alerts increase recognition of high BP in a pediatric clinic setting; however, younger, female and AA children, and those without obvious CV risk factors are less likely to be recognized. Increased provider awareness is needed to improve recognition.

Funding: Private Foundation Support

FR-PO391

Vitamin D Deficiency and Cardiovascular and Renal Risk Factors among Children from Various Ethnic Groups in the United States Gangadarshni Chandramohan,[1] Sheena Cecille Marie T. Go,[1] Keith C. Norris,[2] Kamyar Kalantar-Zadeh.[1] *[1]Pediatric Nephrology, LA Bio-Medical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; [2]Charles R. Drew University, Lynwood.*

Background: According to recent studies vitamin D (VitD) deficiency has become highly prevalent among children of every ethnicity, particularly, among the African American (AA) population. Over 80% of AA children has either vitamin D insufficiency (vitDinsuf) or vitamin D deficiency (vitDdef) in the United States. Vitamin D deficiency is known to be associated with cardiovascular/renal risk factors. There are only a few studies that have looked at the association between vitDdef and CV/renal risk factors in children of different ethnicities. Thus, in this study we determined the prevalence of vitDinsuf and vitDdef in children from different ethnicities and assessed the OR for CV risks among those who had vitDdef, utilizing the National Health and Nutrition Survey 2001-2004.

Methods: CV/renal risk factors were defined according to standard guidelines. VitDdef defined as <15ug/ml and vitDinsuf <30ug/ml.

Results: We identified 4849 children between the ages of 6 – 17 years, Whites 28%, AAs 36% and Hispanics 36%, males 50%. African American children had a significantly higher prevalence of vitDdef compared to Whites (31% vs. 2%, p=<.001). Similarly, Hispanicv children had a higher prevalence of vitDdef compared to Whites (7% vs 2%, p=<.001). Association between vitDdef and high BP and fasting blood sugar (FBS) were observed among Hispanic children. However the odds ratio for vitDdef and obesity, high BP, abnormal lipid profile and abnormal FBS were not significant in White and Hispanic children. Female gender carried a higher risk of vitDdef across all ethnic groups. When obesity and high BP were clustered, there was a strong association observed with vitDdef in AA and Hispanic children.

Conclusions: Vitamin D deficiency is highly prevalent among minority children compared to White children. The association between vitDdef and high BP and obesity combined, and abnormal FBS raises a concern regarding long term CV/renal outcome in this population. A longitudinal study is warranted to confirm this findings.

Funding: NIDDK Support

FR-PO392

The Significance of Combined Biomarkers Test for Renal Injury in Preeclampsia Yong Gu,[1,2] Jing Xiao,[1] Jianying Niu,[1] Qianqian Yu,[1] Xianwu Ye,[1] Chuan-Ming Hao.[2] *[1]Nephrology, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai, China; [2]Nephrology, Huashan Hospital, Fudan University, Shanghai, China.*

Background: Preeclampsia is a pregnancy-specific disorder, It is characterized by hypertension and proteinuria. Rapid diagnosis and early treatment are vital to preserve kidney function and slow down renal injury, while traditional markers for diagnosing the injury are insensitive. The aim of our study was to determine whether combined biomarkers detecting renal injury in preeclampsia was more sensitive.

Methods: Three groups were included in our study, they are preeclampsia, gestational hypertension and normal pregnant women and each group included 25 people. ELISA kits were used to measure the level of serum(s)Cystatin C, retinol-binding protein(RBP), neutrophil gelatinase-associated lipocalin(NGAL)and Interleukin-18 (IL-18), urinary (u) kidney injury molecule-1(KIM-1), NGAL, RBP, and IL-18 were also tested.

Results: Compared with the normal pregnancy group, the level of sCystatin C, uRBP, uNGAL, and uKIM-1 were significantly higher in preeclampsia group (P <0.05). The area under the ROC curve of above-mentioned biomarkers is 0.769,0.831,0.830 and 0.875. When biomarkers combined, the sensitivity and specificity was increased. The sensitivity and specificity of sCystatin C combined with uRBP, sCystatin C with uNGAL, sCystatin C with uKIM-1, uRBP with uNGAL, uRBP with uKIM-1, uNGAL with uKIM1 were 96%/51.2%, 96% /49.9%, 94%/57.6%, 94%/70.2%, 96%/62.4% and 94%/72% respectively.When three biomarkers combined, the sensitivity and specificity was as follows:sCystatin C,uRBP anduKIM-1 was 92%/88%; sCystatin C,uNGAL anduKIM-1 was 94%/80%; sCystatin C,uRBP and uNGAL was 90%/72%; uRBP,uNGAL and uKIM-1 was 94% /94%;when sCystatin C,uNGAL,uRBP and uKIM-1 combined, thesensitivity and specificity was 100%/98.20% .

Conclusions: The level of sCystatin C,uRBP, uNGAL and uKIM-1 were significantly increased in patients with preeclampsia. uKIM-1,with the highest sensitivity and specificity, was the most potential one and may serve as a useful early biomarker for renal injury in preeclampsia. We also showed that the more biomarkers combined, the better sensitivity and specificity.

FR-PO393

Circulating Endothelial Cell Number and Markers of Endothelium in Previously Preeclamptic Women Zeyneb Tuzcu, Ebru Asicioglu, Mehmet Koc. *Division of Nephrology, Marmara University, Istanbul, Turkey.*

Background: Patients with preeclampsia (PE) have an increased risk of mortality. Recent studies suggest that the endothelium plays a key role in the development of atherosclerosis. Circulating endothelial cells (CEC) may reflect the state of the endothelium since CEC number is markedly increased in conditions associated with a high degree of endothelial cell activation/injury. We hypothesized that the number of CEC is increased in previously PE women reflecting ongoing endothelial cell activation/injury.

Methods: We studied 21 healthy women with ongoing normal pregnancy, 24 healthy currently non-pregnant women with a history of normal pregnancy, 17 women with currently active PE and 16 currently non-pregnant women with a history of PE.

Results: Blood samples from women with active PE (mean age 29 ± 6 years) had higher CEC (9.9 ± 7.9 per ml) compared to healthy pregnant women (3.0 ± 4.1 per ml, p<0.001) or healthy non-pregnant women (3.4 ± 4.0 per ml, p<0.001) and women with a history of PE (2.4 ± 2.0 per ml, p<0.001). The number of CECs were similar between women with a history of PE and healthy non-pregnant women with a history of normal pregnancy. Patients with active PE had significantly higher sVCAM-1, sE-selectin and soluble vascular endothelial receptor-1 (sVEGFR1) and urinary albumin/creatinine ratio than healthy pregnant women. sVCAM-1, sE-selectin, urinary albumin/creatinine ratio were similar in women with a history of PE and healthy non-pregnant women with a history of normal pregnancy. However, women with a history of PE had higher sVEGFR1 levels than women with a history of normal pregnancy (p<0.05).

Conclusions: Our results suggest that the endothelium is activated in PE but after the term its activation status is similar to the age matched non pregnant women with a a history of normal pregnancy. In addition, sVEGFR-1 levels remains higher in women with a history of PE compared to women without a history of PE. Further studies are needed to evaluate the significance of higher sVEGFR-1 levels in women with a history of PE.

Funding: Government Support - Non-U.S.

FR-PO394

Postpartum Evaluation of Blood Pressure and Kidney Function in Preeclamptic Women Antoinette Pechere-Bertschi,[1] Michel Burnier.[2] *[1]Hypertension Unit, University Hospital, Geneva, Switzerland; [2]Nephrology and Hypertension Unit, CHUV, Lausanne, Switzerland.*

Background: It has been shown that preeclampsia is not just a disease of the pregnancy that resolves with the delivery. Preeclampsia may be considered a risk marker for later-life diseases, including cardiovascular and renal diseases.

Methods: In a longitudinal prospective cohort study, we examined women having suffered from a preeclampsia defined as de novo hypertension (BP ≥140/90mmHg) and proteinuria (≥0.3g/d or ++ urine dipstick) >20we of gestation. Office BP and 24hABPM, plasma creatinine, electrolytes, CRP, hormonal profile and genetics data bank were obtained. Urine spot and 24h collect were obtained in order to establish renal sodium handling.

Results: We evaluated 127 women, mean age 32.1±5.8, 69% were Caucasian, 14% Hispanic, 12% Black and 5% from Asia. Mean BMI was 29.4 ±5.7. Mean duration of pregnancy was 36 weeks. Thirty % suffered from severe preeclampsia or HELLP, 21% were previously hypertensive, 10% were active smokers, and 10% suffered from gestational diabetes. At 6 weeks postpartum the prevalence of office hypertension defined by BP≥140/90 mmHg or ongoing antihypertensive treatment was 36%, and the prevalence of 24hambulatory hypertension was 39%. Mean day time ambulatory BP was 122/86 ±16/11mmHg, and nigh time 111/75 ±20/11mmHg. We observed a renal hyperfiltration when measured by Gault et Cokroft formula,and a high prevalence of microalbuminuria. eGFR measured with MDRD formula was lower, especially for black people.

Us CRP (mg/ml)	Normal <2	6.9 ± 9.7
GFR ml/min/1.73m2	GaultCokroft	160 ± 23
eGFRml/min/1.73m2	MDRD	106± 12
MAU/creat. ratio (mg/ml/ mmol/ml)	Normal < 3.5	13.0±6.5
24h Urine albumin mmol/d	Normal <20/d	225±52
Urine 24h Na excretion (mmol/ day)	Normal<100mmol/d	204±48
% ≥ 135/85 mmHg mean ABPM		39%
% nocturnal hypertension (non-dippers)		10%

Conclusions: Preeclamptic women do not normalize their BP and renal fucntion in the post-partum. We have to better identify women in whom early prevention may reduce their risk and need scientific data in order to establish an efficient prevention and to impact throughout the postpartum course.

FR-PO395

Preeclampsia and Prevalence of Microalbuminuria 10 Years Later Bjorn Egil Vikse,[1,2] Miriam Kristine Sandvik,[2] Stein I. Hallan,[3,4] Einar Svarstad.[1,2] *[1]Department of Medicine, Haukeland University Hospital, Bergen, Norway; [2]Renal Research Group, Institute of Medicine, University of Bergen, Bergen, Norway; [3]Department of Medicine, St.Olav University Hospital, Trondheim, Norway; [4]Department of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, Trondheim, Norway.*

Background: Previous studies indicate increased risk of microalbuminuria after a preeclamptic pregnancy. These studies have however been small, and more data is needed.

Methods: We used data from the Medical Birth Registry in Norway and identified women who had preeclampsia in their first pregnancy 9 to 11 years previously, excluding women with diabetes, rheumatic disease, essential hypertension or renal disease before first pregnancy, and/or preeclampsia in later pregnancies. 180 women with preeclampsia and 180 women without preeclampsia were invited (matched on age, year of first birth and municipality). We measured blood pressure, body mass index (BMI), serum creatinine and albumin/creatinine ratio (ACR) in three morning urine samples.

Results: A total of 89 women with preeclampsia and 69 women without preeclampsia participated. Only one woman had urinary albumin/creatinine ratio above 2.5 mg/mmol in two of three urine samples, she belonged to the preeclampsia group. Median ACR was 0.53 mg/mmol and 0.50 mg/mmol in women with and without preeclampsia, respectively. There was also no difference in mean estimated glomerular filtration rate (107.9 vs. 104.9 ml/min/1.73m^2, p=0.1) or BMI (26.7 vs 26.0 kg/m^2, p=0.4). Preeclampsia, low birth weight offspring, preterm birth, higher blood pressure and body mass index was not associated with higher risk of having a urinary ACR above the 75th percentile (0.70 mg/mmol).

Conclusions: No increased risk of microalbuminuria was observed 10 years after pregnancy complicated with preeclampsia.

Funding: Government Support - Non-U.S.

FR-PO396

Pre-Partum Proteinuria Is a Risk Factor for Short-Term High Blood Pressure Persistence after Delivery Ciro Esposito,[1] Maria Lucia Scaramuzzi,[2] Alessandra Manini,[2] Massimo Torreggiani,[1] Fausta Beneventi,[3] Andrea Spinillo,[3] Fabrizio Grosjean,[1] Gianluca Fasoli,[2] Antonino Dal Canton.[2] *[1]Nephrology, Fondazione S. Maugeri, University of Pavia, Pavia, Italy; [2]Nephrology, Policlinico San Matteo, Pavia, Italy; [3]Gynecology, Policlinico San Matteo, Pavia, Italy.*

Background: Gestational hypertension complicates 10-15% of pregnancies, being of new onset in 70% of cases. A small percentage of women maintains chronic elevated blood pressure even after delivery. Several evidences suggest that gestational hypertension is associated with a greater risk of future cardiovascular disease. We aimed to identify risk factors predisposing to the persistence of high blood pressure after delivery in patients who developed gestational hypertension.

Methods: We conducted a prospective observational study enrolling 38 patients with gestational hypertension or preeclampsia. We recorded anthropometric, clinical and biohumoral parameters before and every 15 days after delivery. We divided patients into two groups: women whose blood pressure normalized in 15 days after delivery (group 1) and women who still had high blood pressure values 15 days after delivery (group 2). We defined hypertension as the need to assume medications to maintain blood pressure below 130/80 mmHg.

Results: Patients were comparable for age, BMI, primiparity and comorbidities. We did not notice significant differences in the intragravidic weight gain or in the newborn's weight. Hypertension was diagnosed earlier in group 2 (17 days) than group 1. Pre-partum proteinuria greater than 300mg/24hours was observed more frequently in group 2 (84% vs. 32%). Pre-partum proteinuria was associated with an 11 fold greater risk of maintaining elevated blood pressure 15 days after delivery (OR=11.56, p<0.01). Moreover, both systolic and diastolic blood pressure were higher in group 2 than 1 and not completely controlled by the therapy (132 vs. 122 mmHg, p<0.05; 88 vs 77 mmHg, p<0.001).

Conclusions: This study demonstrates a strong association between the presence of pre-partum proteinuria and the risk of maintaining hypertension after delivery. Thus, it is mandatory to follow proteinuric patients closely even after delivery.

Funding: Government Support - Non-U.S.

FR-PO397

Etiology and Outcome of Neonatal Hypertension Shefali Vyas, Isabel Roberti. *Pediatric Nephrology and Transplantation, Saint Barnabas Medical Center, Livingston, NJ.*

Background: Hypertension (HTN) in the NICU setting is often multifactorial. Its follow-up data has been rarely reported. Overall, HTN is rare in the normal neonate with a reported incidence of 0.2-3%.

Methods: We reviewed NICU consults for the past 5 yrs for HTN. We identified 70 babies with HTN among a total of 138 NICU consults. HTN definition: SBP and/or DBP>95 centile for age/gender, confirmed in multiple evaluations when child asleep. Chart review included: Pre-natal history, birth weight, gestational data, apgar, canulation of umbilical vessels, renal sonogram with doppler, echocardiogram, blood chemistries, UA, thyroid panel, PRA and aldosterone. Co-morbidities and medications were noted. Clinical data was compared between neonates with "early HTN" (< 2 mos of age) vs "late HTN" (>2 mos).

Results: We found complete records in 47 of the 70 NICU consults for HTN. Overall 29 (62%) were males, 33 (70%) AA; age at presentation: 1 to 245 days (median 56); Birth wt: 400 to 3820g (median=1190); gestational age: 24 to 40 weeks (median=30); Catheterization of umbilical vessels in 27 (57%). Seven were symptomatic: 1CHF, 3 ALTE, 1 seizures, 2 irritability. Thirteen had **LVH** (28%). **Etiology**:2 arterial thrombosis, 5 persistent pulmonary hypertension (PPH) (3 ECMO), 31 BPD, 2 drug induced, 2 renal infarcts, 1 ARPKD, 1 renal dysplasia, 1 thyrotoxicosis. **Outcome**: **Anti-HTN meds:** 38 (81%). 39 (81%) resolved (median time:4 weeks), 1 ESRD, 1 deaths, 6 lost f/u. **Comparison between "early HTN" (n=23) vs "late HTN" (N=24):** the "late HTN" group had significantly lower gestational age and birth weight, more umbilical lines (p=0.01). At the time of first consultation 54% were on diuretics (p<0.01 vs "early HTN") and 21 (91%) had BPD/PPH (p<0.01). However there were no differences regarding need for medications, time to resolution or outcome.

Conclusions: Neonatal hypertension has been reported as uncommon but represented 50% of the renal consults in the NICU in our institution. Most required treatment for the HTN which typically resolved in 4 weeks. Its diagnosis was done at a later age in prematures and often associated with BPD. **The prognosis was excellent despite high rate of LVH and serious symptoms.**

FR-PO398

Renal Histology in Preeclampsia, Normal Pregnancy and in Young Women with Chronic Hypertension: A Nationwide Autopsy Study Maria Elisabeth Penning,[1] Kitty Bloemenkamp,[2] Jan A. Bruijn,[1] Ingeborg M. Bajema,[1] Hans J. Baelde.[1] *[1]Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Obstetrics, Leiden University Medical Center, Leiden, Netherlands.*

Background: Knowledge on the histopathological changes in the kidney during preeclampsia (PE) is mostly derived from biopsy studies and small group autopsy studies. Endotheliosis and vascular changes due to anti-angiogenic factors seem to be salient features of PE, but it is unknown to what extent these changes also occur in normal pregnancies and/or chronic hypertension. Renal autopsy materials of these groups were compared in this study.

Methods: A search for tissue specimens derived from autopsies was performed using a nationwide computerized database (PALGA). Renal tissue samples were studied from 9 women who died during pregnancy with PE; from 16 women who died during pregnancy due to non-hypertensive causes and from 8 non-pregnant women with chronic hypertension.

Results: Women with PE had MPGN-like lesions with varying amounts of tram tracking, endotheliosis and podocyte damage. Women with normal pregnancies only had minor lesions in the kidneys: glomeruli were normal although 2 cases had endotheliosis and only 2 cases showed hyalinosis of arterioles. Young women with chronic hypertension had severe ischaemic glomerular lesions, 2 had hyalinosis of arterioles, and 2 had FSGS. In this group all women had intimal thickening of arteries, in 1 case with onion shapes. Positive intraglomerular staining for Ki-67 was occasionally found in all groups, and was regarded non-specific. Mean glomerular size measurements showed no significant differences between all groups.

Conclusions: In this study, all women with PE had MPGN-like lesions in their kidneys, which were absent in the other groups and therefore regarded characteristic for PE. Endotheliosis was, although not exclusively, most prominent in PE. Major large vessel lesions were only found in women with chronic hypertension, and were not an important feature of PE. As VEGF downregulation leads to glomerular endotheliosis in animal studies, we hypothesize inhibition of VEGF by sFlt-1 might play a crucial role in the pathogenesis of renal lesions in PE.

FR-PO399

Angiotensin Converting Enzyme 2 Plasma Activity Is Lower in Female Hemodialysis Patients and Associations Differ between Males and Females

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Background: Angiotensin converting enzyme 2 (ACE2) is a novel regulator of the renin-angiotensin system that counteracts the adverse effects of angiotensin II. The gene for ACE2 is located on the X chromosome. Plasma ACE2 is an independent predictor of adverse events and elevated levels are associated with greater severity of myocardial dysfunction in patients with heart failure. To date there have been no studies examining plasma ACE2 activity in patients with kidney disease, who have a substantial risk of cardiovascular disease (CVD).

Methods: Patients groups included (a) chronic kidney disease Stage III/IV (CKD), (b) patients treated with hemodialysis (HD), and (c) kidney transplant recipients (KTR). Plasma ACE2 enzyme activity was measured (serum stored at -80ºC) using a fluorescent substrate assay. Linear regression was performed in males and females separately to determine covariates associated with log-transformed ACE2.

Results: The median (inter-quartile range) plasma ACE2 activity in pmol/minute/mL was 15.9 (8.4-24.2) in CKD (n=57), 9.2 (3.9-18.2) in HD (n=100) and 13.1 (5.7-21.9) in KTR (n=80; p<0.01). Males on HD had levels of 12.1 (6.8-19.6) compared to 4.4 (2.5-10.3) in females undergoing HD (p<0.01). Log-transformed ACE2 plasma activity was associated with the post-HD systolic blood pressure (SBP) in females (β-coefficient 0.04, 95% confidence interval 0.01-0.06, p=0.006), but not log-transformed B-type Natriuretic Peptide (BNP). In males, log-transformed ACE2 plasma activity was associated with BNP (0.39, 0.19-0.60, p<0.001), and inversely associated with diabetes (-1.01, -1.73 to -0.28, p=0.007) and log-transformed time (years) on dialysis (-0.29, -0.53 to -0.05, p=0.018). No independent association with SBP was demonstrated in males.

Conclusions: Plasma ACE2 activity is reduced in HD patients compared to CKD patients, and in female HD patients compared to male. In HD patients, the association of plasma ACE2 activity with BNP in males and SBP in females indicates that the role of ACE2 in CVD may differ by gender.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO400

UMOD Gene in Salt Sensitivity: A New Pathogenetic Mechanism

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Background: Uromodulin, codified by UMOD gene, is the most abundant urinary protein and is produced only by epithelial cells of the thick ascending limb of Henle's loop (TAL). Even if its function is not completely known, two recent studies showed that its co-expression with ROMK e NKCC2 induces an increment of the activity of these two carriers of the apical membrane, which are involved in the renal reabsorption of sodium and chloride in the TAL. This suggests a role for uromodulin in the saline homeostasis in the kidney. Genome wide scan identified polymorphisms in UMOD gene associated to chronic kidney disease and hypertension.

Methods: To verify the associated functional aspect we colleced AMBP and tested the response to oral furosemide 25 mg in a group of 233 essential hypertensive patients.

Results: The results show that carriers of risk variants of UMOD have an increase of diastolic blood pressure in basal condition (day-time DBP at ABPM: TT n=163, 96.6±0.7 mmHg vs CT+CC n=70, 94.0±1.0 mmHg; p = 0.036). A greater reduction of blood pressure, increased excretion of sodium and water after administration of furosemide (TT n=118 ΔSBP: -4.32 ± 0.95 mmHg vs CT+CC n=47 -0.88 ± 1.61 mmHg with p = 0.006; ΔDBP: TT -2.03 ± 0.64 vs CT+CC 0.47 ± 1.00 mmHg with p = 0.037; UNa: TT 532.2 ± 18.6 µEq/min vs CT+CC 454.76 ± 28.50 µEq/min with p = 0.030; urinary volume: TT 1746.5 ± 46 ml vs CT+CC 1529.0 ± 71 ml with p = 0.013) was observed.

Conclusions: These data suggest a new mechanism of hypertension dependent by the modulation of sodium homeostasis in TAL caused by overexpression of uromodulin.

FR-PO401

Hyponatremia in Malignant Hypertension: Predictor of Long-Term Renal Outcome

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Background: Hyponatremia is associated with malignant hypertension (MHT) and might reflect the vicious cycle of severe hypertension, pressure natriuresis and renal ischemia. We assessed whether hyponatremia predicts renal outcome in these patients.

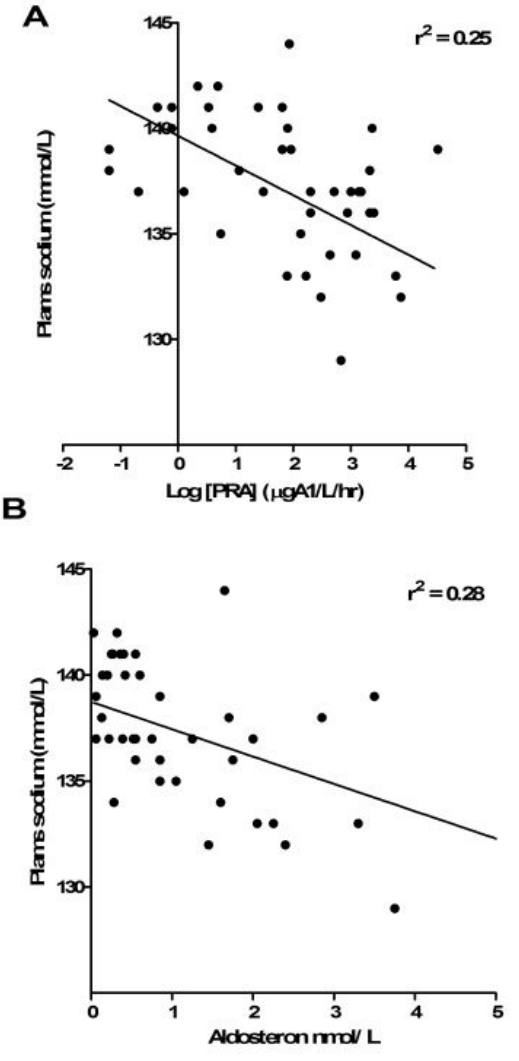
Methods: Retrospective analysis.

Results: 120 patients with MHT were included. Thirty-five (29%) patients had a plasma sodium < 136 mmol/L and were classified as having hyponatremic-hypertensive syndrome (HHS). Patient characteristics are listed in Table 1. Median serum creatinine was higher in patients with HHS ($P < 0.01$) and these patients more often had macroalbuminuria ($P < 0.05$) and Thrombotic microangiopathy ($P < 0.01$). Plamsa renin activity and plasma aldosterone were correlated with plasma sodium ($P < 0.01$ for both), Figure 1.

After 67 months, 14 (40%) patients with and 15 (18%) patients without HHS developed end-stage renal disease (ESRD), $P = 0.02$. The likelihood ratio for ESRD in patients with MHT and hyponatremia was 4.8 ($P = 0.03$).

Conclusions: Hyponatremia is common in MHT and is associated with more severe renal insufficiency. Plasma sodium is inversely correlated with RAAS activation suggesting that pressure natriuresis and subsequent volume depletion further activate RAAS. After 5 years, patients with hyponatremia have a higher risk of developing ESRD.

Clinical characteristics	Plasma sodium < 136 mmol/L	Plasma sodium >=136 mmol/L	P-value
Patients, n (%)	35 (25%)	90 (75%)	
Age, years (mean ± SD)	40 ± 9	45 ± 13	< 0.05
Male, n (%)	22 (73%)	61 (68%)	0.57
Black, n (%)	21 (70%)	36 (40%)	< 0.01
Systolic BP, mmHg (mean ± SD)	233 ± 25	229 ± 22	0.47
Diastolic BP, mmHg (mean ± SD)	148 ± 15	144 ± 16	0.28
Serum creatinine, µmol/L (median, [IQR])	402 [176-664]	155 [96-264]	< 0.01
Macroalbuminuria, n (%)	27 (75%)	39 (46%)	< 0.05
Trombotic microangiopathy, n (%)	22 (61%)	14 (17%)	< 0.01



FR-PO402

Chlorthalidone Alters the Serum Potassium Set-Point in African Americans

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Background: In the African American population, hypertension occurs at a younger age and can lead to more severe target organ damage. Chlorthalidone is a thiazide diuretic that has been shown to have beneficial effects in lowering blood pressure and to reduce cardiovascular events in African Americans, however, its use may be limited by hypokalemia.

Methods: We used the data from the Uric Acid in Hypertension in African Americans Trial to investigate the level of potassium losses in individuals on chlorthalidone to provide guidelines for supplementation. 121 African-American men or women between the ages of

18 and 50 years with stage I hypertension were started on chlorthalidone (25 mg/day) and potassium chloride (20 mEq/day) for 4 weeks. At 4 weeks, if blood work demonstrated hypokalemia they were given oral potassium supplementation 25-100 mEq up to three times a day for 3 days and their potassium chloride supplementation was increased to 40 mEq/day. Due to persistent hypokalemia, the protocol was changed to potassium chloride at 40mEq/day and at 4 weeks if hypokalemia was detected, we increased the potassium supplementation to 50 mEq/day. The levels of serum potassium was determined at weeks 5 and 13 and at week 5, 24 hr urinary potassium excretion was determined.

Results: Multivariate mixed effects linear model with repeated measurements showed that the serum potassium was significantly different between baseline and week 4 (p-value 0.0007) independent of the level of initial potassium supplementation. Unexpectedly, there was no significant change in serum potassium noted between week 4 and week 5 (p-value 0.7603), and week 4 and week 13 (p-value 0.6823). Interestingly the only significant difference in those subjects initiated on 20 mEq or 40 mEq potassium chloride a day was the urinary potassium excretion (p-value 0.0037).

Conclusions: This study suggests that in African Americans increasing potassium chloride supplementation in patients on chlorthalidone who are hypokalemic does not improve serum potassium, suggesting that there is a set-point for serum potassium that cannot be overcome with further potassium supplementation.

Funding: NIDDK Support

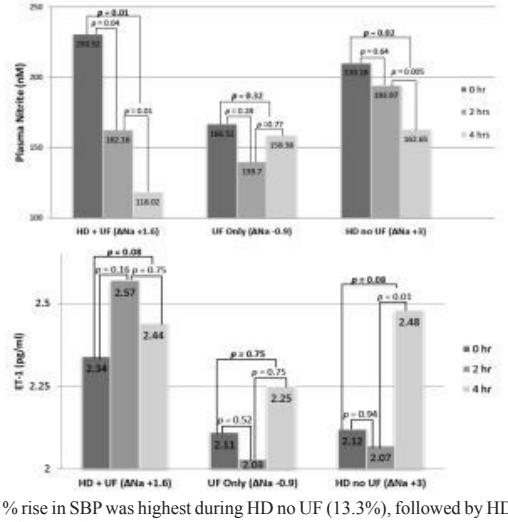
FR-PO403

Dialysate Sodium Promotes Endothelial Cell Dysfunction in Patients with Intradialytic Hypertension Bohyun C. Kim, Peter N. Van Buren, Natasha Klimas, Robert D. Toto, Jula K. Inrig. *University of Texas at Southwestern.*

Background: Intradialytic hypertension (HTN) is associated with endothelial cell (EC) dysfunction, but the cause is unknown. In EC culture, exposure to high sodium (Na^+) concentration promotes EC stiffness and imbalances in vasoconstrictors (endothelin-1 [ET-1]) and vasodilators (nitric oxide [NO]). We hypothesized that, among patients with intradialytic HTN, exposure to standard dialysate Na^+ (140 mEq/L) would lead to decreases in NO, increases in ET-1, and increases in systolic blood pressure (SBP) during hemodialysis (HD).

Methods: In a 6-week crossover study of 13 HD patients with intradialytic HTN, changes in nitrite, ET-1, and SBP were measured during 3 different midweek HD treatments consisting of: 1) regular HD with standard dialysate (Na^+ 140) and ultrafiltration (HD+UF); 2) UF only without dialysate exposure (UF only); and 3) HD (Na^+ 140) without UF (HD no UF). Changes between treatments were analyzed using mixed linear models.

Results: Among 13 subjects, the average age was 60.4 years, 85% were male, 46% AA, 46% Hispanic, 54% diabetic. The average pre-HD serum Na^+ was 137.4 mEq/L. Serum Na^+ levels rose during HD+UF (+1.6) and HD no UF (+3), but fell during UF only treatments (-0.9). Plasma nitrite levels fell with all treatments, but significant changes were seen only in treatments with dialysate exposure (HD+UF and HD no UF, Figure). There was a trend toward greater increases in ET-1 with dialysate exposure (HD+UF and HD no UF, Figure)



The % rise in SBP was highest during HD no UF (13.3%), followed by HD+UF (6.9%) and UF only (5.7%).

Conclusions: Among patients with intradialytic HTN, there was an association between dialysate Na^+ exposure and decreases in NO, increases in ET-1, and increases in SBP during HD. We propose that high dialysate-to-plasma Na^+ gradient may promote EC dysfunction and contribute to intradialytic HTN.

Funding: NIDDK Support, Other NIH Support - K23 HL092297

FR-PO404

Effects of Dietary Potassium on Blood Pressure in Stage 3 Chronic Kidney Disease: A Randomized, Controlled, Feeding Trial Sharon Turban, Edgar R. Miller, Cheryl A. Anderson, Carol B. Thompson, Lawrence J. Appel. *Johns Hopkins University, Baltimore, MD.*

Background: In the general population, potassium (K) lowers blood pressure (BP) and may help prevent cardiovascular disease. K has renoprotective effects in animals. Patients with chronic kidney disease (CKD) may therefore benefit from higher K intake, but due to concerns of hyperkalemia, the currently recommended K intake in stage 3 CKD is 51-102 mEq K/d, lower than for the general population.

Methods: Randomized, controlled feeding trial with 2-period crossover to test the effects of a diet containing 100 vs 40 mEq K/d on BP in adults with stage 3 CKD and prehypertension or stage 1 hypertension. A 3-4 week washout separated the 4-week periods. Generalized linear models with generalized estimating equations were used.

Results: Of the 29 randomized participants, 2 were withdrawn because repeated serum K > 5.5 mEq/L. The 24 participants who finished both periods (38% male and 29% White) had a mean screening: estimated glomerular filtration rate 52 ml/min/1.73 m^2, age 69 yrs, SBP 126 mmHg, and DBP 71 mmHg. Effects of dietary K depended on K order; P value for interaction of K intake and K order: 0.03 (SBP) and 0.001 (DBP). In participants who received the lower K diet first, the higher K diet significantly lowered SBP and DBP (see table). In those who received the higher K diet first, there was no significant BP difference, suggesting a possible carryover effect.

Mean SBP, DBP (mmHg), urinary K (UrK; mEq/d), & serum K (SK; mEq/L) ± SD, adjusted Δ *, & P values by K level and K order

K order	Outcome	Lower K	Higher K	Adj Δ* (lower K - higher K)	P value
L to H (N=12)	SBP	123±12	116±13	7	< 0.001
	DBP	65±11	62±8	2	0.003
	UrK	36±12	80±35	-44	<0.001
	SK	4.2±0.4	4.5±0.3	-0.3	0.002
H to L (N=12)	SBP	121±6	120±11	2	0.3
	DBP	67±6	68±5	-1	0.1
	UrK	41±18	83±34	-41	<0.001
	SK	4.1±0.4	4.3±0.4	-0.1	0.2

*Model includes K level, K order, & their interaction and is adjusted for age, race, sex, & outcome at baseline.

Conclusions: These results highlight the potential benefit of a higher K diet in lowering BP in stage 3 CKD, but also the need for monitoring serum K given the risk of hyperkalemia. Longer-term trials evaluating the effects of K intake on BP and other clinical outcomes are warranted.

Funding: Private Foundation Support

FR-PO405

Long WNK1 Correlates with Blood Pressure Increase in Response to Low Dietary Potassium Susan Hedayati,[1,2] Masoud Afshar,[2] Beverley Adams-huet,[2] Jian Xie,[2] Orson W. Moe,[2] Chou-Long Huang.[2] *[1]Dallas VA; [2]Univ of TX Southwestern, Dallas, TX.*

Background: Mechanisms mediating salt-sensitive hypertension are poorly understood. Diet K^+ restriction in rats increased whole-kidney WNK1 mRNA. Upregulation of WNK1 in humans causes hypertension in type 2 pseudohypoaldosteronism. We investigated whether low dietary potassium (K) correlates with increased long WNK1 mRNA (using white blood cells as surrogate tissue) and higher BP, and if this BP increase is mediated by renal sodium (Na) retention.

Methods: In a cross-over double-blind study, 10 healthy subjects were randomized to 70 mEq of oral KCl or placebo for 4 weeks and consumed a diet with 150 mEq/d of Na and 30 mEq/d of K. After a 21-day washout of random diets, subjects crossed over to the other drug for 4 weeks. WNK1 mRNA, 24h urine Na and K, and BP response to 2 L IV saline were measured after each 4 weeks and compared as a paired t-test or Wilcoxon Signed Rank for each subject. WNK1 was log-transformed.

Results: Baseline mean (SD) systolic and diastolic BP were 106 (21) and 74 (8) mmHg. Mean urinary K was 33 (4) mEq/24h during the placebo and 92 (15) mEq/24h during the KCl phase, p <.0001, indicating adequate adherence. Urinary Na excretion was not statistically different between the 2 phases at 141 (48) and 147 (59) mEq/24h. WNK1 mRNA was lower during the KCl vs. placebo phase, geometric means (95% CI) 0.78 (0.61, 1.10) and 1.19 (0.92, 1.53) respectively, p =.03. Both systolic and diastolic BP were lower following the saline load during the KCl as compared to the placebo phase (p =.003 for systolic and .03 for diastolic; Figure 1). Urinary Na excretion rate following saline increased during the KCl as compared to the placebo phase, suggesting impaired natriuresis during placebo (AUC =33, p =.03, Figure 2).

Conclusions: Dietary K may exert a BP-lowering effect by increasing renal Na excretion mediated by WNK1.

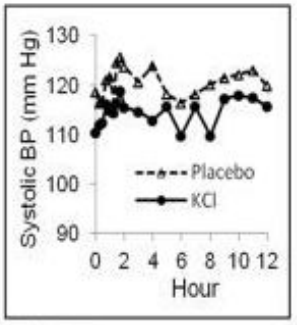


Figure 1

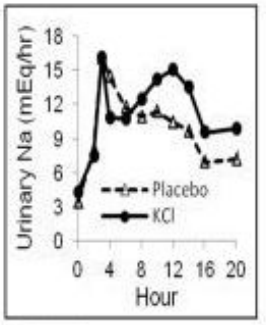


Figure 2

Funding: NIDDK Support, Other NIH Support - UL1RR024982

FR-PO406

Utility of Renin/Aldosterone Profiling in Complex Hypertension Debbie L. Cohen, Danny Haddad. *Medicine, University of Pennsylvania, Philadelphia, PA.*

Background: Despite multiple pharmacotherapies BP remains poorly controlled. Renin/aldosterone (R/A) profiling helps identify categories of hypertension (HTN). Purpose of this study was to determine if R/A profiling would aid in control of HTN with tailored therapy.

Methods: Retrospective study was performed of all new patients (pts) seen at the UPenn HTN clinic by one HTN specialist from 2005-2010. Pts were screened with renin and aldosterone levels. Pts with known secondary causes of HTN such as pheo were excluded. Data was reviewed at initial and subsequent visits for 1 year. The pts were categorized as: normal, salt sensitive (SS) (renin <1, aldo <15), hyperaldo (renin <1, aldo ≥ 15) and high renin (renin ≥ 10). Meds were then tailored to the individual profile.

Results: 158 patients with a mean age of 52.9 ± 1.25 years were included, 47% males, 53% females, 75% white, 21% black, and 4% other. Mean SBP/DBP at baseline for the entire cohort was 149/87 ± 2/1 mm Hg and was decreased by mean of 20/9 ± 2/1 mm Hg at 1 year (p=0.000). No change in the overall number of meds at baseline and 1 year despite improved BP control (p = 0.770).

	Normal	SS	Hyperaldo	High Renin
Patients n (%)	56 (36)	43 (27)	40 (25)	19 (12)
Renin (ng/ml)	4.0 ± 0.37	0.4 ± 0.04	0.4 ± 0.05	33.3 ± 4.2
Aldosterone (ng/dl)	12.8 ± 1.5	7.3 ± 0.59	38.5 ± 7.3	33.3 ± 3.5
SBP/DBP baseline (mmHg)	150±3/88±2	154±4/87±2	149±4/90±4	**138±4/76±3***
SBP/DBP at 1 year (mmHg)	131±3/79±2	130±3/78±2	123±3/77±2	132±5/77±4
Average Change in SBP/DBP (mmHg)	**19±3/8±2 ****	**25±4/9±3****	25±4/13±2 **	5±5/-2 ± 3
# of Meds Time 1 - Time 2	-0.14 ± 0.2 (ns)	**-0.46 ± 0.2 *****	**0.55 ± 0.2 ******	0.52 ± 0.2 (ns)

Values expressed as mean ± SEM. Values considered significant if p < 0.005. *SBP only significantly lower when compared to SS group, DBP significantly lower than all other groups, ** p≤ 0.001; ***p=0.003; **** p=0.026 ; ns: non-significant

Conclusions: R/A profiling in pts with severe HTN helps tailor therapy resulting in significantly improved BP control without an overall increase in number of meds. Most patients required at least 3 meds to achieve BP control. R/A profiling is a helpful tool to improve BP control in a referred population with severe HTN.

FR-PO407

Marinobufagenin and Dietary Sodium Restriction: Influences on Blood Pressure and Large Elastic Artery Stiffness Kristen L. Jablonski,[1] Olga Fedorova,[2] M. Chonchol,[1] Matthew Racine,[3] Candace Geolfos,[3] Bradley S. Fleenor,[3] Edward G. Lakatta,[2] Alexei Bagrov,[2] Douglas R. Seals.[3] *[1]Medicine/ Renal, University of Colorado Denver, Aurora, CO; [2]Intramural Research Program, NIA, NIH, Baltimore, MD; [3]Integrative Physiology, University of Colorado Boulder, Boulder, CO.*

Background: Systolic blood pressure [SBP] and large elastic artery stiffness both increase with age, and dietary sodium restriction reduces SBP and large elastic artery stiffness, as measured by aortic pulse-wave velocity [aPWV]. Production of the natriuretic hormone marinobufagenin (MBG), an endogenous α1 Na+,K+-ATPase inhibitor, is increased in salt-sensitive hypertension (i.e. contributes to the rise in SBP following high sodium load).

Methods: We hypothesized that dietary sodium restriction would reduce SBP and aPWV in middle-aged and older adults with moderately elevated SBP (139±2/83±2 mmHg, mean±S.E.) in part by reducing MBG production.

Results: SBP and aPWV were reduced (-10±3 mmHg and -143±25 cm/sec) following a 4 week low sodium (LS; 77±9 mmol/day) vs. normal sodium (NS; 144±7 mmol/day) diet (p<0.05; randomized, cross-over design; 8M/3F; 62±1 yrs). Urinary MBG excretion was lower during the LS vs. NS condition (range for weekly measurements: 24.0±4.2–28.3±4.6 [LS] vs. 27.8±5.7-32.4±3.7 [NS] pmol/kg/day, p<0.05) and was positively correlated with both SBP (r= 0.39, p<0.001) and urinary sodium excretion (r= 0.46, p<0.001). When each sodium condition was analyzed separately, these relations were evident during the NS (SBP: r= 0.49, p<0.001; sodium excretion: r=0.44, p<0.005) but not the LS condition (SBP: r=0.16, p=0.24, sodium excretion: r=0.20, p=0.14). As measured following each 4 week condition, MBG excretion was also positively correlated with aPWV (r=0.51, p<0.05), and plasma MBG levels were inversely related to endothelial cell protein expression of the antioxidant enzyme manganese superoxide dismutase (r=-0.51, p<0.05).

Conclusions: These results demonstrate that dietary sodium restriction reduces urinary MBG excretion and suggest that changes in MBG may contribute to reductions in SBP and large elastic artery stiffness, possibly via a reduction in oxidative stress.

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FR-PO408

Impact of Fructose and Sodium Restriction on Mitochondrial Content and Oxidative Stress in Hypertensive and Overweight Subjects Magdalena Madero,[1] David Cruz-robles,[1] Sergio Hernández-Estrada,[1] Silvia Hernández-Lobato,[1] Maria Villalobos-Martín,[1] Richard J. Johnson,[2] Roman Hernandez,[1] Francisco E. Rodríguez Castellanos,[1] L. Gabriela Sanchez-Lozada.[1] *[1]INCIC; [2]U of Colorado.*

Background: Fructose and sodium intake have been associated with hypertension and metabolic syndrome (MS). This association may be mediated in part by mitochondrial dysfunction and systemic oxidative stress (SOS).There are no data regarding whether fructose and sodium restriction have an effect on SOS and mitochondrial DNA (mtDNA) content in overweight and hypertensive subjects. The objective of this study was to compare the effect of two isocaloric diets, Na restricted and Na-fructose restricted, on SOS and mtDNA.

Methods: This study is an exploratory analysis of a randomized controlled trial aiming to determine the impact of the low Na, low Na-fructose isocaloric diets (with respect to baseline diets), on blood pressure (BP) over 8 weeks. The present study included 36 overweight subjects with prehypertension or stage 1 hypertension selected randomly from the trial. 24hr ambulatory BP, anthropometric measures, laboratory data, mtDNA by Real-time PCR, malondialdehyde(MDA) and 2,4-dinitrophenylhydrazine(DNPH) as markers of SOS were determined at baseline, weeks 4 and 8.Markers of SOS were measured in plasma and mtDNA was determined from peripheral white cells.

Results: Weight loss was not achieved in any group. Both diets resulted in a significant decrease in DNPH and an increase in mtDNA, effect that was more marked at week 8. At week 8 MtDNA levels were higher in the low Na-Fructose group compared to the low Na group although this was not significant.

Table

Diet	Low Na n=21	Low Na-Fructose n=15	Low Na	Low Na-Fructose	Low Na	Low Na-Fructose
	Baseline		Week 4		Week 8	
BMI (kg/m²)	32±4	34±4	32±4	32±4	32±4	32±4
SBP (mmHg)	136±10	134±12	134±14	132±14	131±11	128±10
MDA (nmol)	2.2±2.3	1.9±2	1.8±1.7	1.1±1.3	1.4±1.8	2±2.6
DNPH (nmol)	4.6±2.2	5.8±2.2	3.4±1.7α	4.8±3.1	2.6±0.8β	2.2±1.9β
mtDNA (copies X10³)	2±6	1±4	3±6α	1±0	13±25β	140±420β

α p<.05 within group week 4 vs baseline, β within group p<.05 week 8 vs 4

Conclusions: Both diets had a beneficial effect on SOS and mtDNA content.These effects were independent of weight loss.

Funding: Government Support - Non-U.S.

FR-PO409

Promoter Polymorphism of the IL-6 (C-174G) Gene and the Estimation of Metabolic Syndrome Occurrence among a Cohort of Patients with Hypertension Andrei Alkmim Teixeira,[1] Maria Dalboni,[1] Beata Marie Redublo Quinto,[1] MArcelo Costa Batista.[1,2] *[1]Medicine/ Nephrology Division, UNIFESP; [2]Hospital Israelita Albert Einstein.*

Background: Visceral obesity, the central core of Metabolic Syndrome (MS) is conceived as the pathogenic basis of increasing cardiovascular burden of syndrome and is related with higher cytokines levels. In observance of the modern concept of MS as an inflammatory disease we investigated whether IL-6 (C-174G) gene polymorphism is associated with MS prevalence in a cohort of hypertensive patients.

Methods: It was included 664 patients currently followed in Hypertension and Diabetes Clinic where we concomitantly analyzed lipid profile apolipoprotein A and B, CRP and albumin. Patients were stratified by the presence of MS according to International Diabetes Federation criteria for MS. Framingham risk score was used to evaluate the CV risk. The IL-6 –174 (C/G) genotyping was performed by polymerase chain reaction with sequence-specific priming using the SSP DNA Typing kit.

Results: MS prevalence was 52,6 % (n= 349) and 34% were men. Smoking habit was observed in 11,1% of patients. Cardiovascular disease (CVD) was present in 10,1% n=62) of the cohort and 27,4% of patients (n=168) had positive familiar history for CVD. It was identified 45 % of "C" carriers genotype among MS patients. Characteristics of "C" carriers and non-carriers patients were respectively: BMI(30.1±5.4 *vs* 28.9±5.1;p<0.05 waist(97.1±12.2 *vs* 95.0±12.3,p<0.04), CRP(0.67±0.73 *vs* 0.5±0.6,p<0.005), VLDL-C(30.5±13.4 *vs* 27.1±13.1,p<0.0002); HDL-C (55.4±15.3 *vs* 58.7±15.4;p<0.01) and apoA (137±25.4 *vs* 143±25.4;p<0.008). On binary logistic regression it was observed that the presence of IL-6 single nucleotide polymorphism 174 C/Gwas independently

associated with MS occurrence. This association remained significant after adjusting for covariates**:** sex, age, race, CVD, LDL-C and Framingham score covariates (variation of HR 1.47 – 1.68, 95% CI).

Conclusions: The C-allele at the -174 locus of human IL-6 gene is independently associated with occurrence of MS pinpointing the importance of inflammatory genetic background as the basis of visceral obesity and related cardiovascular burden.

FR-PO410

Influence of Blood Pressure Levels on Urinary Albumin Excretion in Patients with Chronic Renin-Angiotensin System Suppression Julian Segura, Cesar Cerezo, Jose A. Garcia-donaire, Diego Marquez, Manuel Praga, Luis M. Ruilope. *Hypertension Unit. Department of Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

Background: An adequate blood pressure (BP) control and renin-angiotensin system (RAS) suppression are both useful tools to prevent or delay the development of micro or macroalbuminuria. However, there are evidences that renal and cardiovascular damage may still develop under chronic RAS suppresion.

Methods: We analyzed the effect of BP control on urinary albumin excretion in hypertensive patients treated with an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB), alone or in combination with other antihypertensive drugs. We included 1141 patients (mean age 59.7±12.5 years, male 48.7%), all presenting normoalbuminuria at baseline. New-onset microalbuminuria was defined as albumin-to-creatinine ratio between 20–200 mg/g in men or 30–300 mg/g in women confirmed in at least a second occasion among the 6-monthly determinations performed in three samples of early morning urine. Patients received during the follow-up the highest available dose of an ACEi or an ARB, accompanied by a diuretic or a calcium channel blocker, or both, if needed.

Results: Percentage of patients developing microalbuminuria increased progressively during the follow-up: 13.0%, 16.4% and 17.1 % after 1, 2 and 3 years of follow-up, respectively. When patients were classified according BP level at each visit, the development of albuminuria took place at any level of systolic BP maintained, starting in values below 130mmHg and ending above 160mmHg.

	UAE	Baseline	Year 1	Year 2	Year 3
SBP<130	Normo	376 (100)	333 (88.6)	321 (88.2)	322 (85.6)
	Micro	0	43 (11.4)	43 (11.8)	54 (14.4)
SBP 130-139	Normo	338 (100)	298 (88.2)	280 (84.8)	287 (84.9)
	Micro	0	40 (11.8)	50 (15.1)	51 (15.1)
SBP 140-159	Normo	343 (100)	294 (85.7)	268 (80.0)	274 (79.9)
	Micro	0	49 (14.3)	67 (20.0)	69 (20.1)
SBP ≥160	Normo	84 (100)	67 (79.8)	60 (73.2)	63 (75.0)
	Micro	0	17 (20.2)	23 (26.8)	21 (25.0)

n (%)

Conclusions: A long-term office BP control does not exclude the development of de-novo microalbuminuria in patients chronically RAS suppressed. This finding is observed at category of BP, from normal to grade 3 hypertension.

FR-PO411

Long Term Evolution of Blood Pressure Control in Resistant Hypertension Julian Segura, Jose A. Garcia-donaire, Cesar Cerezo, Diego Marquez, Manuel Praga, Luis M. Ruilope. *Hypertension Unit. Department of Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

Background: Therapeutic recommendations for resistant hypertensive patients have been proposed by short-term studies, but information about long term evolution of blood pressure control in these patients is scarce.

Methods: We analyzed the evolution of 106 patients presenting resistant hypertension (RH) after 12 months of follow-up. At baseline, patients were classified as white coat RH (n=50), RH with adequate response to spironolactone (n=43) and RH non responders to spironolactone receiving a combination of aliskiren 300mg+amlodipine 10mg+chlortalidone 25 mg (n=13).

Results: After 12 months of follow-up, those patients presenting RH with adequate response to spironolactone maintain significant reduction both in clinic (mean average reduction -21.7±23.1 and -7.4±10.2 mmHg for systolic [SBP] and diastolic blood pressure [DBP], respectively), ambulatory (-17.7±16.8 and -6.2±8.8 mmHg for 24h SBP and DBP, respectively) and central BP (-17.7±20.1 and -7.4±11.9 mmHg, respectively). These patients showed a significant increase in body weight, body mass index, serum creatinine, potassium and uric acid, and a diminished estimated GFR. Those patients receiving a combination of aliskiren+amlodipine+chlortalidone on top of previous antihypertensive drugs (ACEi, ARB, alpha or betablockers) maintain significant reduction both in clinic (mean average reduction -28.7±23.5 and -13.7±10.9 mmHg for SBP and DBP, respectively), ambulatory (-18.7±20.2 and -8.5±8.5 mmHg for 24h SBP and DBP, respectively) and central BP (-22.9±24.9 and -11.6±12.0 mmHg, respectively). These patients showed non significant changes in body weight, body mass index, serum creatinine, potassium, uric acid and estimated GFR.

Conclusions: In conclusion, after 12 months of follow-up, spironolactone add-on therapy is effective for BP control in true RH, with slight but significant increase in serum creatinine and potassium. In non-responders to spironolactone, the combination of aliskiren+amlodipine+chlortalidone on top of concomitant antihypertensive therapy is effective for BP control, without significant increase in renal parameters.

FR-PO412

Discrepancies between Office and 24-Hour Blood Pressure Control in Hypertensive Patients with Chronic Kidney Disease Julian Segura,[1] Pantelis Sarafidis,[1,2] Manuel Gorostidi,[3] Alejandro De la Sierra,[4] Juan J. De la Cruz,[5] Jose R. Banegas,[5] Luis M. Ruilope.[1] *[1]Hypertension Unit. Department of Nephrology, Hospital 12 de Octubre, Madrid, Spain; [2]Section of Nephrology and Hypertension, 1st Department of Medicine, AHEPA University Hospital, Thessaloniki, Greece; [3]Department of Nephrology, Hospital Central de Asturias, Oviedo, Spain; [4]Department of Internal Medicine, Hospital Mutua Terrasa, Barcelona, Spain; [5]Department of Preventive Medicine and Public Health, Universidad Autonoma, Madrid, Spain.*

Background: Recent evidence suggests major discrepancies between office and 24-hour BP levels in hypertensive populations. We examined control of ambulatory BP in a large cohort of CKD patients.

Methods: A total of 5,624 hypertensive individuals with CKD Stage 1-5 from the Spanish ambulatory BP monitoring (ABPM) Registry were included in this analysis. Office-based BP was calculated as the average of 2 readings, and 24-hour ABPM was performed using a SpaceLabs 90207 device, according to recommendations. Control rates of hypertension were calculated at the <140/90 and <130/80 mmHg office thresholds, and the <130/80 and <120/75 mmHg ABPM thresholds.

Results: Only 21.7% and 7.9% of the total population had office BP <140/90 and <130/80 mmHg, respectively; at both these office thresholds, the rates of control were increasing with advancing CKD stages (*P<0.01*). In contrast, control of ambulatory BP was much higher, with 44.8% of subjects having average 24-hour BP <130/80 and 20.5% 24-hour BP <120/75 mmHg. Furthermore, 52.2% of the population had average daytime BP <135/85 mmHg and 36.6% night-time BP <120/70 mmHg. In multi-variate analysis, age ≥60 years and favourable dipping status were associated with successful 24-hour BP control, whereas male gender, smoking, diabetes, target-organ-damage, and hypertension duration were adversely related to control.

Conclusions: In hypertensive patients with CKD, ambulatory-based control rates were far better than office-based rates. These findings call for wider use of ABPM to evaluate success of hypertension treatment in CKD patients both in clinical practice and randomized trials.

FR-PO413

A Comparative Analysis of Two Methods of Blood Pressure Measurement in an Adult Diabetic Population Pierce Geoghegan, Eadaoin Ni Sheaghdha, Donal John Sexton, Donal N. Reddan, David Lappin. *Medicine, University College Hospital Galway, Galway, Co. Galway, Ireland.*

Background: Blood pressure (BP) control predicts morbidity and mortality in diabetes. However, the optimal approach to monitoring BP in the diabetic population is unclear. Ambulatory blood pressure monitoring reliably predicts prognosis but can be inconvenient. The automated 'BpTRU' device, which provides up to six successive in-office BP measurements, has been shown to correlate more strongly with ambulatory measurements than traditional "once-off" measurements of BP. We investigated whether there was a difference between measurement of BP using traditional methods versus the "BpTRU" device in an outpatient diabetic population.

Methods: Patients were enrolled from an outpatient diabetes day clinic. We recorded the BP of 52 diabetics by traditional measurement and with the BpTRU device. We established whether there was a statistically significant disagreement between the values measured by the two methods and whether these differences had an impact on the assessment of BP control.

Results: Mean systolic BP was significantly lower by BpTRU measurement than by standard assessment, by 7.25 ± 2.94mmHg (95% CI 13.1 to 1.04mmHg, p=0.0154). Significantly more patients achieved their systolic BP target of ≤ 130mmHg by BpTRU measurement than by traditional techniques (69% vs 46%, P=0.0285). No significant disagreement in the measurement of diastolic BP was detected between the two methods and there was no significant difference in the proportion of patients deemed to reach their diastolic BP target when comparing the methods. Overall, significantly more patients simultaneously achieved both their systolic (≤ 130mmHg) and diastolic (≤ 80mmHg) blood pressure targets by BpTRU measurement than by traditional techniques (56% vs 35%, P= 0.0483).

Conclusions: Our study suggests that the BpTRU device can reduce the effect of "white coat" hypertension in a diabetic population. The use of this device in routine practice could improve the overall accuracy of in-office blood pressure assessment in these "hard to treat" patients. This in turn could potentially reduce the cost and morbidity associated with overtreatment of hypertension in diabetes.

FR-PO414

Extreme Blood Pressure Elevations and Risk for Death in CKD Francesco Rainone, James Ritchie, Philip A. Kalra. *Vascular Research Group, Salford Royal Hospital, UK.*

Background: Hypertension exists in over 80% of CKD patients with both cause and effect relationships. In the general population, uncontrolled blood pressure (BP) associates with increased mortality in an exponential fashion. Here we consider the association between extreme BP elevations and risk for death in an all cause CKD-population.

Methods: 1808 patients from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS) were divided by baseline BP: Normal (NBP) - SBP 120-140mmHg;

DBP<80mmHg; Resistant hypertension (RH) - *SBP>160mmHg±DBP>90mmHg; Extreme hypertension (EH) - SBP>190mmHg±DBP>100mmHg; **despite three different classes of antihypertensive medications*. Cox regression (adjusted for age, eGFR, sex, baseline BP, body mass index and number of antihypertensive medications) was used to compare all cause mortality at 1 year and entire follow-up period (censored at death, dialysis, last follow-up or 1/1/2011).

Results: Mean age was 54.5 years, eGFR 36ml/min/1.73m^2, BP 135/75mmHg. Patients with EH (n=65) had significantly increased risk for death at 1 year (HR 5.08, p=0.009) but not over the full follow-up period. For EH, risk for 1 year death was significantly elevated vs NBP (HR 7.8, p=0.008) with a trend to increase vs RH (HR 7.66, p=0.06). Of 52 surviving EH patients with 1 year follow-up data, 10 had persistent EH and 15 NBP. Mean ΔSBP/DBP was -33/-16mmHg. Mean number of antihypertensives increased from 2.6 to 3.0 (no significant difference in types of agents used). Patients with persistent EH, had an increased risk for death vs the remainder of the population (HR 6.97, p=0.008) and vs those who previously had EH (HR 16, p=0.17). The 42 patients with BP no longer defined as EH did not have an ongoing increase in risk for death (HR 1.18, p=0.51).

Conclusions: BP elevations above those defined as RH further increase risk for death in CKD. Most patients with baseline EH can be successfully treated with dose adjustment of medications, reducing risk for death. Patients with persistent EH have significant increases in risk for death and should be considered for intensified therapy including new techniques such as renal sympathetic nerve ablation.

FR-PO415

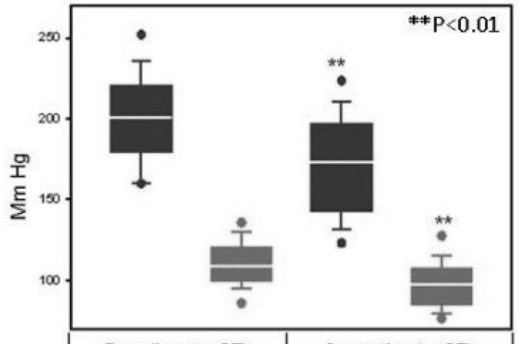
The Standardized Blood Pressure Lowering Effect of Renal Denervation Eva Vink,[1] Willemien Verloop,[2] Evert-jan Vonken,[4] Michiel Voskuil,[2] Wilko Spiering,[3] Peter J. Blankestijn.[1] *[1]Nephrology, UMC Utrecht, Utrecht, Netherlands; [2]Cardiology, UMC Utrecht, Utrecht, Netherlands; [3]Vascular Medicine, UMC Utrecht, Utrecht, Netherlands; [4]Radiology, UMC Utrecht, Utrecht, Netherlands.*

Background: The first studies on renal denervation(RDN) suggest a clear BP-lowering effect. An important limitation of these studies is that BP was measured while patients used antihypertensives which makes it difficult to quantify the net effect of RDN. The aim of our study is to determine the standardized effect of RDN. This abstracts presents an interim analysis of an ongoing study.

Methods: We evaluated the BP-lowering effect of RDN in 2 types of patients who were treated with RDN: patients with resistant hypertension or with intolerance for antihypertensives (office SBP of ≥160mmHg). Secondary causes of hypertension were excluded. At baseline, 6 and 12 months after RDN, office BP is determined. At baseline and 12 months after RDN 24-h ambulatory BP monitoring(ABPM) is done in a standardized fashion: after cessation of all antihypertensives when this is considered to be safe or under the same treatment. For the available within-group paired data, a paired *t* test is used with a 2-sided alpha level of 0.05.

Results: At the moment of submission, 43 patients are treated with RDN. Currently 6 months follow -up data are available of 27 patients, 12 months follow-up data are available of 12 patients. BP decreased significantly during follow-up, but a broad range in effect is observed. The number of antihypertensives did not change significantly during follow-up. Currently 12 months follow-up on 24-h ABPM is available of 8 patients. Mean daytime BP decreased from 161/97mmHg to 147/91mmHg (P=0.103).

Figure 1: Significant decrease in BP after 6 months of follow-up. (N=27)



	Baseline (n=27)	6 months (n=27)
SBP	201 mm Hg	173 mm Hg
DBP	109 mm Hg	97 mm Hg

Conclusions: RDN is a promising treatment for patients with resistant hypertension or intolerance for antihypertensives. It is worth to put more effort in further exploration on this treatment and its options.

FR-PO416

A Clinicopathologic Study of 52 Patients with Essential Malignant Hypertension Confirmed by Renal Biopsy Peng Xia, Xiaoxiao Shi, Lanping Jiang, Yubing Wen, Xuemei Li, Xuewang Li, Limeng Chen. *Nephrology Department, Peking Union Medical College Hospital, Beijing, China.*

Background: This study aimed at evaluating the associations between pathological findings and prognosis in essential malignant hypertension (MHT) patients, with emphasis on the value of peritubular capillary (PTC) loss.

Methods: We retrospectively reviewed the clinical records of 52 patients with essential MHT confirmed by renal biopsy in our hospital from January 2003 to March 2012. The tubular atrophy / interstitial fibrosis (TA/IF), proliferative endarteritis and mucoid changes were evaluated semi-quantitatively. The PTC was evaluated by immunohistochemical staining of CD34, a specific marker of arteriolar endothelium.

Results: The enrolled patients were mostly male (M:F 12:1) and relatively young (age 34.0±8.2y). The Scr was 5.51±4.25mg/dL and the 24h urine protein (24hUpro) was 1.87±1.50g/24h. 21.1% (11/52) of the patients required dialysis. The mean percentage of TA / IF were 56±20%. Lumen occlusion(24.3%), mucoid changes(22.4%) and intimal hyperplasia (22.1%)were commonly seen in 580 observed arterioles. The PTC proportion was 2.27±0.74%, which was significantly less than that of glomerular minimal lesion patients (3.75±0.79%, *P*<0.001). TA/IF correlated positively with Scr, 24Upro and the need for dialysis. PTC proportion correlated negatively with TA/IF, Scr and the need for dialysis. After adequate anti-hypertension therapy, the control rate of BP improved significantly from 5.8% to 76.9% (*P*<0.001), Scr and 24Upro levels also decreased. The mean follow-up time were 29.1±30.1 months and the renal survival rate at 1, 3 and 5 year was 65%,55% and 50%, respectively. The prognostic factors for renal survival identified by COX regression were CKD stage (RR=4.38, 95%CI (1.46, 13.15), P=0.008) and PTC loss (RR=13.21, 95%CI (1.50, 116.69), P=0.020).

Conclusions: Renal involvement in essential MHT patients is severe. PTC loss correlates with renal function well and may predict the long time renal survival.

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FR-PO417

Single Center Experience of Renal Denervation Procedure besides Clinical Studies: Renal Artery Supply Indicates Treatment Success Wolfgang Weiss, Markus Tolle, Markus van der Giet. *Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany.*

Background: Renal Denervation (RDN) gradually moves mainstream as supplementary interventional procedure for treatment of therapy resistant hypertension. 40 patients with therapy resistant hypertension have been identified and treated with RDN procedure. Due to missing predictors and indicators for treatment efficacy we examined which patients benefit most of RDN treatment procedure.

Methods: 40 patients were treated with RDN procedure. Office blood pressure and ambulatory blood pressure monitoring data (ABDM) were collected every third month after intervention for at least 1 year per patient. In practice renal artery anatomy of patients varied over the known limitation of double sided single renal artery supply. Thus only multiple renal artery supply for both kidneys was an exclusion criterion for treatment with RDN procedure. Patients with reduction in systolic pressure ≥ 10 mmHg in office and ABDM measurements were defined as responder.

Results: After RDN treatment the extent of systolic blood pressure reduction within responders did not differ statistically significant between office and ambulatory blood pressure measurements at any time point of observation. 11 of 19 patients (58%) with multiple renal artery supply failed to show a blood pressure reduction and derogated responder rates within treated patients. In contrast effective blood pressure reduction was achieved in 15 of 21 patients (71%) with bilateral single renal artery supply (n=21). One year after RDN responder showed a mean systolic blood pressure reduction of approx. 20 mmHg in office as well as in ABDM measurements.

Conclusions: One year after treatment with RDN procedure a mean systolic blood pressure reduction of approx. 20 mmHg can be estimated. Renal artery supply might be a key point for RDN treatment success and should be judged more critically, although RDN seems to be the last therapeutic option for treatment of therapy resistant hypertension. Hence well-known RDN responder rates of about 70-80% of treated patients could only be achieved if renal artery supply is as highly selected as done for simplicity HTN trial population.

FR-PO418

Renal 123I-MIBG Scintigraphy, a Novel Technique to Assess Efficacy of Renal Sympathetic Denervation: A First Impression Daan W. Eeftinck Schattenkerk,[1] Hein Jan Verberne,[2] Liffert Vogt,[3] Bert-jan Van den Born.[1] *[1]Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands; [2]Nuclear Medicine, Academic Medical Center, Amsterdam, Netherlands; [3]Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

Background: Radiofrequency ablation of the renal sympathetic nerves (RSD) has emerged as a minimally invasive technique to improve blood pressure (BP) control in patients with therapy resistant hypertension. Despite the general effectiveness of RSD, the magnitude of the individual BP lowering response is variable. This variation may partly be due to inconsistency in efficacy of renal ablation, indicated by renal nor-adrenaline spillover studies in human showing an average reduction of 47% (95% CI 28-65%) after RSD. Furthermore, the relative contribution of the kidneys in determining central sympathetic drive may vary and is incompletely understood. Here, in analogy to cardiac

^{123}I-metaiodobenzylguanidine (MIBG) imaging as measure for cardiac sympathetic activity, we introduce renal ^{123}I-MIBG scintigraphy, aiming to give insight into the variable BP effect of RSD.

Methods: Renal, cardiac ^{123}I-MIBG scintigraphy and 24h ambulatory BP monitoring (ABPM) were performed in patients eligible for RSD prior to and 6 weeks after RSD. Subjects received 185 MBq of ^{123}I-MIBG intravenously. At 15 min and 4 hrs post injection scintigrams were made. A low dose CT-scan of the abdomen was made to relate tracer uptake to anatomical structures.

Results: Data of 6 patients (4 male), age 59±11 years, was analyzed. ABPM tended to go down after RSD from 171/106 mmHg (±17/14) to 151/95 mmHg (±29/17) (p=ns). Renal MIBG washout decreased by 21% (from 0.40 to 0.32, p=0.027). Cardiac MIBG washout did not change significantly (from 0.60 to 0.57, p=0.441). However the cardiac / renal MIBG washout ratio decreased by 16% (from 0.67 to 0.56, p=0.026). A correlation between MIBG and ABPM could not be found.

Conclusions: Our preliminary data show that renal ^{123}I-MIBG scintigraphy may prove to be a suitable noninvasive technique to assess the effectiveness of RSD. Furthermore, our data suggest that RSD mainly exerts a renal efferent rather then afferent effect.

FR-PO419

Diastolic Blood Pressure Profile when Targeting Intensive Systolic BP Control B. Peter Sawaya,[1] Hwasoon Kim,[2] Jonathan D. Wilkerson,[1] Leslie Mcclure,[2] Pablo E. Pergola.[3] *[1]Division of Nephrology, Bone and Mineral Metabolism, University of KY, Lexington, KY; [2]Biostatistics, University of Alabama, Birmingham, AL; [3]Renal Associates PA, San Antonio, TX.*

Background: Conflicting reports exist in regard to whether there is a J curve in diastolic blood pressure (DBP) in which cardiovascular events actually increase after lowering DBP below 60-70 mmHg. We hypothesized that DBP is lower in patients undergoing intensive systolic blood pressure (SBP) reduction. We further tested whether factors such as: age, diabetes, isolated systolic hypertension and number of antihypertensive medications contribute to the frequency of observing low DBP.

Methods: The SPS3 study is an international randomized controlled trial that assessed the impact of antiplatelet therapy and optimal level of SBP control in the secondary prevention of subcortical strokes. Patients were assigned to two groups of SBP target (mmHg): Usual Group (UG: SBP 130-149) and Intensive Group (IG: SBP<130), irrespective of DBP.

Results: A total of 3021 patients were enrolled in the study. Mean age (yrs ± SD): UG 63.4 ± 10.8; IG 63.3 ± 10.7. The proportion of visits at which DBP (mmHg) <70 and <60 (measured at 3-month intervals for up to 72 months) were: UG 27.2% and 5.3%; IG 46.9%* and 13.8%*, respectively (* = p <0.0001, UG vs. IG). The mean DBP reached 70 within 12 months in the IG and remained <70 throughout the study period. At no time was the mean DBP <70 in the UG.

DBP Profile

Group	Baseline	12-Month	36-Month	72-Month
UG	80.0 ± 0.3 *(N=1520)*	76.3 ± 0.3 *(N=1356)*	75.6 ± 0.4 *(N=875)*	74.4 ± 0.8 *(N=232)*
IG	78.3 ± 0.3* *(N=1501)*	70.0 ± 0.3* *(N=1354)*	68.1 ± 0.4* *(N=886)*	67.6 ± 0.7* *(N=253)*

**= p <0.0001; Mean ± SD*

Linear regression analysis identified only the following factors as independently associated with increasing percentage of DBP <60 mmHg: targeting SBP <130*, older age* and presence of DM* (* = p <0.0001).

Conclusions: The SPS3 study demonstrates that targeting SBP <130 is more than twice as likely to result in a DBP <60. Older age and DM were the strongest predictors of lower DBP. This study potentially may clarify whether lowering DBP can independently increase cardiovascular events in this high risk population.

Funding: Other NIH Support - NINDS, NCT00059306

FR-PO420

The Association between Annual Change in Systolic Blood Pressure and Mortality in an All-Cause CKD Population James Ritchie,[1] Francesco Rainone,[2] Philip A. Kalra.[1] *[1]Vascular Research Group, Salford Royal Hospital; [2]San Raffaele University, Milan.*

Background: There is a recognized association between increased blood pressure and risk for mortality in both the general and CKD populations. The effects of annual rates of change in blood pressure are less well described.

Methods: 2141 patients from the Chronic Renal Insufficiency Standards Implementation Study (CRISIS) were included. Cox proportional hazards were used to calculate the effect of baseline blood pressure (divided by quartile) on hazard ratio (HR) for all-cause mortality. Time averaged rates of change in systolic blood pressure were calculated and a second Cox model generated to assess differences in HR for death for different rates of blood pressure change. All models were adjusted for age, gender, baseline blood pressure, number of antihypertensive medications and eGFR (CKD-EPI).

Results: Mean baseline age was 54.5 years, eGFR 35.5ml/min/1.73m^2, blood pressure 138/74mmHg on 2.4 different anti-hypertensive medications. No association was observed between quartile of baseline blood pressure and HR for death. However, patients in the highest quartile of time averaged blood pressure change (>4mmHg/year **increase**) had a significantly increased HR for death (HR = 2.04) and patients in the lowest quartile (>4mmHg/year **reduction**) a significantly reduced HR for death (HR=0.33).

Using patients with no annual blood pressure increase as a referent group, significant incremental increases in HR for death were observed for all time-averaged increases in blood pressure over 4mmHg/year. Patients with <4mmHg/year blood pressure increase did not have an increased HR for death.

Baseline blood pressure (mmHg)	HR death	Annual increase in systolic blood pressure (mmHg/year)	HR death
<122	Referent	<1	Referent
122-137	1.08	4	2.04*
137-150	1.05	5	2.17*
>150	1.08	6	2.94*
		7	4.44*
		8	4.90*

* p<0.05

Conclusions: This study demonstrates that in an all-cause CKD population, and independent of baseline blood pressure and eGFR, rate of change in systolic blood pressure may be a predictor of mortality.

FR-PO421

Assessment of Blood Pressure Control and Target Organ Damage in Patients with Chronic Kidney Disease and Hypertension Ran-hui Cha,[1] Hajeong Lee,[2] Yun Jung Oh,[2] Yon Su Kim.[2] *[1]Internal Medicine, National Medical Center, Seoul, Republic of Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: Blood pressure shows diurnal variation, 'dipping'. And the loss of dipping and higher pressure load seems to be related with more target organ damages (TODs).

Methods: APrODiTe is a nationwide multicenter cross sectional study for Korean CKD stage 2, 3, and 4 patients with hypertension. The primary aim is to find out the achievement pattern of BP control and the secondary aim is to identify the relationship between TODs and BP pattern. Clinic BP was measured with OMRON IA-2 automatic BP device and 24 hour ABPM was performed with the A&D Co., Ltd, TM-2430. We used ABP definitions proposed by Fagard et al (daytime BP between 10AM and 8PM and nighttime BP between 12AM and 6AM).

Results: We analyzed data from 1,317 participants. Mean age was 57 year, 62.9% of participants were males and diabetic nephropathy was 24.9%. Mean level of serum creatinine and estimated GFR were 1.64 mg/dl and 48.86 ml/min/1.73m^2, respectively. The proportion of each BP pattern was as below: sustained uncontrolled, 42.3%; masked, 33.9%; white coat, 4.3%; true controlled, 19.4%; Dipper, 33.3%; non-dipper, 34.5%; reverse- dipper, 17.3%; extreme-dipper, 14.9%. Younger age, female gender, less proteinuria, and use of ACEi/ ARB were independently related with true controlled hypertension. Older age, male gender, higher BMI, diabetic nephropathy, and more proteinuria were independently related with sustained uncontrolled hypertension. History of stable angina and heart failure was more prevalent in non-/ reverse-dippers. Diabetic nephropathy was significantly related with reverse-dippers. And higher BP burden (sustained uncontrolled hypertension, non-/ reverse-dippers) was associated with advanced renal dysfunction (CKD stage 3 and 4).

Conclusions: Significant proportion of CKD patients with hypertension were treated inappropriately in Korea. And higher pressure load and loss of dipping were related with more profound TODs. More appropriate BP control in CKD patients is needed and precise BP monitoring has a significant role to improve physicians' practices.

Funding: Pharmaceutical Company Support - sanofi-aventis Korea Co., Ltd.

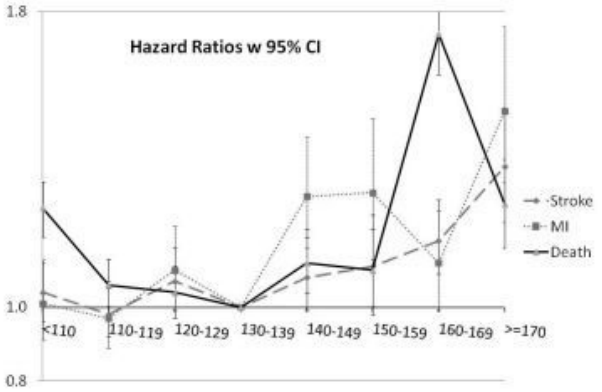
FR-PO422

Exploring a J Curve for Blood Pressure (BP) and Outcomes in a Large Ethnically Diverse Population: Where Is the Ideal BP? John J. Sim,[1] Simran K. Bhandari,[1] Jiaxiao Shi,[1] Joni L. Ricks,[2] Miklos Zsolt Molnar,[2] Kamyar Kalantar-Zadeh.[2] *[1]Nephrology & Hypertension, Kaiser Permanente Los Angeles Medical Center, Los Angeles, CA; [2]Nephrology & Hypertension, Harbor UCLA Medical Center, Torrance, CA.*

Background: Aggressive lowering of BP has not led to superior health outcomes and may even be detrimental based on clinical observations. We sought to determine the relationship of BP with mortality and cardiovascular outcomes in subjects with hypertension (HTN).

Methods: 470,988 HTN subjects from Kaiser Permanente So Cal age ≥ 18 yrs during 1/1/2006 – 12/31/2009 evaluated. Systolic (SBP) and diastolic (DBP), demographics, medications, comorbidities, and outcomes data retrieved from electronic medical records. Cox regressions analyses used to calculate adjusted hazard ratios (HR) comparing different BP's (increments of 10mmHg w SBP 130-139 & DBP 80-89 as reference) for myocardial infarction (MI), stroke, and mortality as independent and competing risk models. Adjustments made for the covariates of age, gender, race, and comorbidities (DM, CKD, cardiovascular disease).

Results: The mean cohort follow up was 3.1 yrs. The lowest crude events rates occurred in SBP 130-139 and DBP 90-99 ranges. Adjusted HR comparing SBP <110, 110-119, 120-129, 140-149, 150-159, 160-169, and >/= 170 vs 130-139 for outcomes were: for MI (1.04, 0.98, 1.07, 1.08,1.11, 1.18, 1.38), stroke (1.01, 0.97, 1.10, 1.30, 1.12, 1.53) and mortality (1.27, 1.06, 1.04, 1.12, 1.10, 1.73, 1.28) respectively. (See figure with 95% CI added). Similar HR was demonstrated for DBP with the nadir however at 90-99 instead of the reference of 80-90.



Conclusions: In a large diverse HTN population, BP relationship to MI and stroke was linear starting at >140 while mortality demonstrated a J shaped relationship across differing BP. Our findings beg the question of where the optimal treatment BP targets should be.

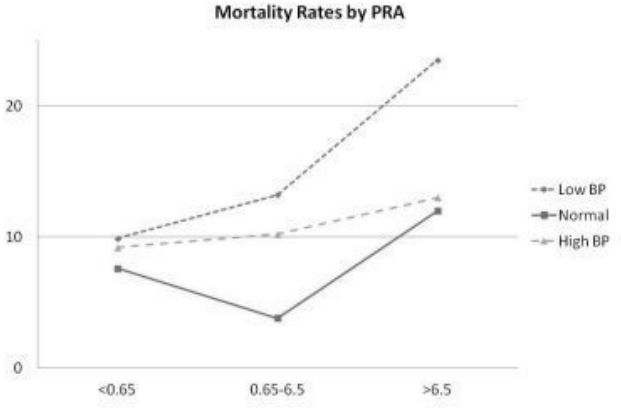
FR-PO423

Plasma Renin Activity (PRA) as a Biomarker for Mortality in Those with Extremes of Blood Pressure (BP) John J. Sim,[1] Jiaxiao Shi,[1] In-lu Amy Liu,[1] Scott A. Rasgon,[1] Kamyar Kalantar-Zadeh.[2] *[1]Nephrology & Hypertension, Kaiser Permanente Los Angeles Med Ctr, Los Angeles, CA; [2]Nephrology & Hypertension, Harbor UCLA Med Ctr, Torrance, CA.*

Background: PRA is reflective of renin angiotensin system activity and works to maintain hemodynamic function. Its values may reflect whether this mechanism is appropriately functioning. We sought to evaluate PRA levels in those with low BP and high BP subjects. We hypothesize that in those with extremes in BP, PRA is a biomarker that can prognosticate poor outcomes as a surrogate for hemodynamic stress and/or a mechanism for it.

Methods: HTN subjects with measured BP and PRA during (1/1/2006 – 12/31/2009). Medication usage, demographics, comorbidities, and outcomes extracted from electronic medical records. Subjects categorized as low (SBP<120), normotensive (120-140), & high (>140). Effective PRA determined based on ACEI/ARB use and classified into <0.65, 0.65-6.5, and >6.5 ng/ml/hr. Crude cardiovascular and mortality event rates and adjusted cox proportional hazards modeling were used to compare outcomes based on the 3 PRA categories.

Results: 1,629 HTN subjects (260 low, 627 normotension, 742 high) were evaluated. Greatest mortality occurred in low (11.9%) and high (9.6%) BP groups compared to normotensives (6.5%). Higher PRA was associated with higher mortality rates within low and high BP groups. Mortality (%) per PRA <0.65, 0.65-6.5, and >6.5 were; 9.9, 13.2, 23.5 in low BP subjects; 9.2, 10.2, 13.0 in high BP subjects whereas it was unrelated in normotensive BP subjects (7.6, 3.8, 12%).



Conclusions: With SBP 120-140 mmHg as the reference range, in subjects with both high (>140) and low BP (<120), higher PRA values>6.5 appear associated with significantly greater mortality risks. Whether PRA serves as a biomarker for worsened outcomes and/or as a mechanism of vascular injury outcomes remains to be determined.

FR-PO424

Progression of Renal Injury after Normotensive Renal Mass Reduction (RMR): Relationship to BP Parameters over 6 Months Karen A. Griffin,[1] Aaron Polichnowski,[1] Hector Licea-vargas,[1] Maria M. Picken,[1**] Rongpei Lan,[3] Christian Rosenberger,[2] Manjeri A. Venkatachalam,[3] Anil K. Bidani.[1] *[1]Medicine (Pathology**), Loyola University Chicago and Hines VA Hospital, Maywood, IL; [2]Medicine, Charite Campus Mitte, Berlin, Germany; [3]Pathology, Univ. of Texas Health Sci Ctr, San Antonio, TX.*

Background: BP is fundamentally labile even in normotensive states and renal autoregulatory mechanisms provide the primary protection against glomerular transmission of BP fluctuations. With impaired autoregulation (RMR), pathogenic glomerular BP transmission may occur despite systemic normotension.

Methods: Male SD rats (Charles River) were prepared for BP radiotelemetry and underwent normotensive 3/4 RMR by surgical excision (SBP during the 2nd wk 128±2 mmHg, n=25). After 6 months, perfusion-fixed kidneys were harvested for correlation of individual BP patterns with a blinded assessment of % glomerulosclerosis (GS) and tubulointerstitial (TI) fibrosis. HIF-1α expression was additionally examined in other rats with 3/4 RMR by either surgical excision (normotensive) or infarction (hypertensive) at 10, 20 and 30 days after RMR (n = ~5/group/time point). Results: Mean ± SE.

Results: Significant heterogeneity was seen for BP courses, with several rats developing progressive BP increases over the last ~ 2 months. Similar variability was observed for proteinuria 151±18 (mg/24h) and % GS (36±5) with a strong correlation ($r^2 = 0.61$). The correlation of GS with BP parameters is shown in the table.

	Average BP (mmHg)		% reading > 150 mmHg		% reading > 175 mmHg	
	Awake	Sleep	Awake	Sleep	Awake	Sleep
BP Parameters	142±2.4	137±2.4*	28.6±4.2	19.5±3.5*	5.9±2.3	4.6±2.1*
Correlation with GS (r^2)	0.52 †	0.54 †	0.57 †	0.67 †	0.45 †	0.49 †

* p < 0.001 vs. respective awake BP parameters; †P < 0.0001

HIF-1α positivity was seen focally, appeared restricted to individual nephrons, was far more abundant in hypertensive vs. normotensive rats, and increased between 10 to 30 days.

Conclusions: These data indicate that in states of impaired autoregulation, glomerular transmission of BP fluctuations, particularly during the sleep periods, remains a major initiating mechanism for CKD progression despite normotension.

Funding: NIDDK Support, Veterans Administration Support

FR-PO425

Normotensive Pheochromocytoma: Clinical, Biochemical, and Radiological Features Anca C. Rafiroiu, Emmanuel L. Bravo. *Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Pheochromocytomas are rare catecholamine-producing tumors with varied clinical manifestations. About 8% are asymptomatic and about 13% are normotensive.

Methods: We report the demographics, clinical and biochemical profile and pathologic findings in seventeen normotensive pheochromocytomas managed in our institution in the last two decades.

Results: Mean age was 48 years (range 32-70 years); 56% were females. None had the cluster of signs and symptoms suggestive of catecholamine (CA) excess. Blood pressure ranged from 95/75 to 130/80 mm Hg. Plasma norepinephrine (NE) was increased in all patients (range 312 to 4548 pg/ml, mean 2237). Plasma epinephrine (E) was increased only in 4 patients (range 52 to 14720 pg/ml, mean 1424), plasma dopamine was slightly increased in 4 patients (range 24 to 173 pg/ml, mean 60). Plasma fractionated metanephrines (n=14) ranged from 0.5 to 32 nmol/L (mean 8) and 24 hr fractionated urinary metanephrines (n=9) ranged form 531 to 31887 ug (mean 6356). At surgery, all tumors had histopathological findings consistent with pheochromocytoma. The tumors ranged in size from 11 to 424 grams. There was no correlation between tumor size and NE levels in the plasma, but there was a strong positive correlation (r = 0.9) between urine NM and tumor size. Postsurgery, CA and MN returned to normal in all.

Conclusions: These studies illustrate the difficulty in detecting pheochromocytomas in some patients. A significant number are normotensive and asymptomatic and are detected only during evaluation for unrelated problems. Since there is no relationship between plasma free CA and the level of blood pressure, it is suggested that NE released from axon terminals of sympathetic postganglionic neurons is biologically more significant than circulating CA. The strong positive correlation between tumor size and urine MN indicate that these tumors metabolize most of the secreted CA resulting in lower circulating levels of plasma free CA and fewer signs and symptoms.

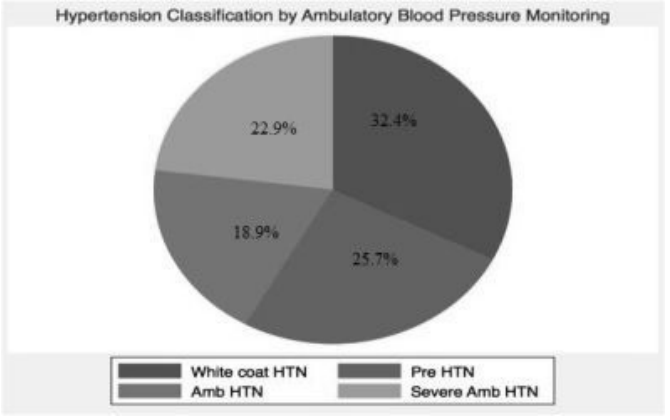
FR-PO426

Association between Kidney Length Adjusted for Body Surface Area and Ambulatory Blood Pressure Parameters Hridya Suman,[1] Oleh M. Akchurin,[1] Frederick J. Kaskel,[1] Robert Woroniecki.[2] *[1]Childrens's Hospital at Montefiore/ Albert Einstein COM, New York, NY; [2]New York-Presbyterian Hospital/Columbia Univ., New York, NY.*

Background: Blood pressure(BP) patterns such as loss of nocturnal dipping,elevated systolic BP (SBP) load & pulse pressure(PP) obtained by 24-hour ambulatory BP(ABP) monitoring can be used to identify children at increased risk of end organ damage.We examined the relationship between kidney size obtained by renal ultrasound & ABP to identify risk groups.

Methods: We conducted a single-center,cross sectional,retrospective analysis of subjects <20 years of age referred for primary hypertension(HTN) from 1/2009 to 12/2011.Exclusion criteria were secondary HTN,hydronephrosis,kidney disease,transplants,congenital heart disease.

Results: 74 subjects with newly diagnosed primary HTN were identified; the distribution is shown in the figure.



Mean age was 16.8(14-17.9) years.15% self-identified as White,44% as Black,39% as mixed race & rest as Asian.There was no significant difference in age,sex distribution,BMI z-score,birthweight,prematurity,eGFR or comorbidities(diabetes, sleep apnea) between subjects based on severity of ABP.Averaged kidney length adjusted for body surface area (K/S) was strongly correlated with mean SBP,PP & SBP variability.Multivariable linear regression of mean SBP showed that K/S was a statistically significant predictor($p=0.006$) after adjusting for age,sex,BMI z-score,family history of HTN & prematurity.This model accounted for 36% of variability in mean SBP.K/S was associated with mean PP by bivariate association($p=0.0001$) & remained an independent predictor of PP on adjustment for age,sex,BMI z-score,family history of HTN & prematurity.

Conclusions: K/S was a statistically significant independent predictor of mean SBP & mean PP.If validated by future studies,K/S may be of use as a non-invasive biomarker to allow identification & management of targeted risk groups.

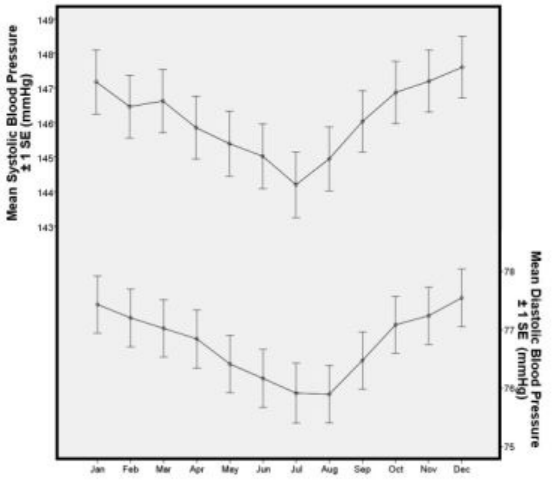
FR-PO427

Seasonal Variation of Blood Pressure and Volume Status in Chronic Hemodialysis Patients: A 10-Year Cohort Study Gretchen Norine De Graav,[1] Charlotte van Noord,[2] Marinus A. Van Den Dorpel.[2] *[1]Internal Medicine, Erasmus MC, Rotterdam, Zuid-Holland, Netherlands; [2]Internal Medicine, Maasstad Hospital, Rotterdam, Zuid-Holland, Netherlands.*

Background: Cardiovascular morbidity and mortality show a seasonal variation in patients on hemodialysis. This variation could be related to higher blood pressure during the winter period. We studied which parameters of volume status and climate have an influence on seasonal variation of blood pressure in patients on maintenance hemodialysis.

Methods: We compared blood pressure and interdialytic body weight gain between summer and winter. Parameters of possible influence on systolic blood pressure were determined with a multivariate regression analysis.

Results: Both systolic and diastolic blood pressure showed a seasonal variation with the lowest average value in summer (144±18 and 76±10 mmHg respectively) and the highest average value in winter (147±18 and 77±10 mmHg). The mean summer-winter difference in SBP and DBP is -2.4 mmHg (95% CI -3.3 to -1.4 mmHg), r=-0.08, and -1.4 mmHg (95% CI -1.8 to -0.9 mmHg), $p<10^{-9}$, r=-0.05, respectively.



Mean Systolic and Diastolic Blood Pressure (plus standard error) of haemodialysis patients from January 2000 to December 2010 (*n*=417)

Volume status had a significant correlation with level of blood pressure, but not with seasonal variation of blood pressure and was not an independent predictor after conducting a multivariate regression analysis. Of all climaticological factors that were studied, only sunshine duration was a significant independent predictor for a lower blood pressure.

Conclusions: In patients on maintenance hemodialysis systolic and diastolic blood pressure are significantly lower in summer. This seasonal variation in blood pressure was not related to differences in interdialytic bodyweight gain between seasons. The underlying mechanism of the relationship between direct sunshine exposure and a lower blood pressure needs further study.

FR-PO428

Plasma Bicarbonate and Risk of Incident Hypertension Ernest I. Mandel,[1,2] John P. Forman,[1,2] Gary C. Curhan,[1,2] Eric N. Taylor.[2,3] *[1]Renal Division, Brigham and Women's Hospital, Boston, MA; [2]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [3]Division of Nephrology and Transplantation, Maine Medical Center, Portland, ME.*

Background: Several biomarkers of metabolic acidosis, including lower plasma bicarbonate, lower urinary citrate excretion, and higher anion gap, have been associated cross-sectionally with prevalent hypertension. However, it is unknown whether lower plasma bicarbonate is an independent risk factor for the development of hypertension among originally non-hypertensive individuals.

Methods: We conducted a prospective, nested case-control study within the Nurses' Health Study II of 695 individuals without hypertension who provided a blood sample between 1997 and 1999 and who subsequently developed hypertension by 2005. Controls were matched according to age, race, time and day of blood draw, and day of menstrual cycle. We used logistic regression to generate odds ratios (OR) for development of hypertension over 6 years of follow-up by quartile of baseline plasma bicarbonate.

Results: Estimated GFR was similar (mean 92 ml/min/1.73m^2; SD 13) among cases and controls. After adjusting for matching factors, BMI, family history of hypertension, plasma creatinine, smoking, physical activity, and dietary intakes of animal protein, potassium, carbohydrate, total fat, calcium, magnesium, dietary fiber, folate, sodium, and caffeine, those in the highest compared with the lowest quartile of plasma bicarbonate had 33% lower odds (OR 0.67; 95% CI 0.48-0.95) of developing hypertension.

Conclusions: Higher plasma bicarbonate was independently associated with a reduced risk of developing hypertension. Further studies are needed to elucidate the mechanism for this relation and to examine the role for alkali therapy in the prevention of hypertension.

Funding: NIDDK Support, Other NIH Support - NCI, Private Foundation Support

FR-PO429

Exercise Training and Post Exercise Hypotension in Kidney Patients Sam A. Headley,[1] Michael J. Germain,[2] Richard J. Wood,[1] Jyovani W. Joubert,[1] Charles M. Milch,[1] Beth Parker,[3] Elizabeth E. Evans,[1] Allen Cornelius,[4] Britton W. Brewer,[1] Linda S. Pescatello.[5] *[1]Springfield College; [2]WNERTA; [3]Springfield College; [4]Hartford Hospital; [5]University of Colorado; [6]Springfield College; [7]Springfield College; [8]University of Connecticut; [9]Springfield College.*

Background: The purpose of the current study was to examine the effect of 16 weeks of aerobic training of moderate intensity on post exercise hypotension (PEH) in stage 3 CKD patients.

Methods: Thirty two CKD patients between the ages of 35-70 attended 4 preliminary sessions before being randomized to either the treatment (TG n=16) or control group (CG n=16). At the first visit, resting blood pressures (BP) were measured using an automated system. At least 3 days later at session 2, a graded exercise test was performed using the Modified Bruce protocol with direct measurement of oxgyen uptake (VO2peak). During sessions 3 & 4, baseline (BL) resting BPs were taken in duplicate after a 5-min rest period. Then subjects either walked for 40 minutes on a treadmill at 50-60% of their VO2peak, or they sat quietly for the same period. Then BPs were measured for 60 min at 10-min intervals as subjects sat in the laboratory. Subjects randomized to the TG received personal training for 16 weeks while those in the CG continued with their usual daily activities. Sessions 2,3,& 4 were repeated after 16 weeks.

Results: There were no differences between the groups for PEH at BL or after the intervention. At BL prior to the 16 weeks of training, SBP (mm Hg) was reduced by 6.8 ± 2.1 while after 16 weeks, this value was 6.3 ± 1.9, p = 0.87. DBP (mm Hg) was reduced at BL by 3.4 ± 1.1 and by 1.7 ± 1.0 after the training program, p = 0.31. The training program did not affect either resting SBP or DBP.

Table 1

Variable	Pre	Post	p =
SBP (mm Hg, TG)	127±17.7	124.6±15.1	p=0.9
SBP(mm Hg, CG)	136.7±19.5	130.9±27.1	
DBP(mm Hg, TG)	78.0±9.8	75.8±9.9	p=0.8
DBP(mm Hg,CG)	79.6±11.3	76.1±15.9	

Conclusions: In conclusion, the magnitude of PEH is not altered by short-term exercise training in stage 3 CKD patients.

Funding: Other NIH Support - NHLBI Grant # 1R15HL096097-01

FR-PO430

Alphacalcidol May Lower Systolic Blood Pressure in Systemic Lupus Erythematosus: 8 Year Follow-Up Study Takeshi Nakatsue,[1] Hiroe Sato,[1] Yoko Wada,[1] Shuichi Murakami,[1] Takeshi Kuroda,[2] Masaaki Nakano,[3] Ichiei Narita.[1] *[1]Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Health Administration Center, Niigata University, Niigata, Japan; [3]School of Health Sciences, Faculty of Medicine, Niigata University, Niigata, Japan.*

Background: Vitamin D3 analogues, such as alphacalcidol (1α-hydoroxycholecalciferol), are often used for glucocorticoid-induced osteoporosis in systemic lupus erythematosus (SLE). It has been reported that 1, 25-dihydoroxycholecalciferol negatively regulate renin-angiotensin system (Li YC et al., J Clin Invest, 2002), and that paricalcitol (1, 25-dihydoroxyergocalciferol) can decrease systolic blood pressure (SBP) and albuminuria in patients with type 2 diabetes mellitus (de Zeeuw D et al., Lancet, 2010). We tested the hypotheses that alphacalcidol reduce proteinuria, maintain estimated glomerular filtration rate (eGFR), lower blood pressure, and thus prevent cardiovascular events in SLE.

Methods: Patients with SLE who were followed-up for 8 years in Niigata University Medical and Dental Hospital were recruited (n=70). They were randomly divided into two groups, alphacalcidol group (n=29) and non-alphacalcidol group (n=41). Their proteinuria, eGFR, SBP, diastolic blood pressure (DBP), and the incidence of cardiovascular events were analyzed. Patients who have received antihypertensive medication after the initiation of alphacalcidol were excluded.

Results: No significant differences were observed in proteinuria, eGFR, the incidence of cardiovascular events in two groups. SBP before the initiation of alphacalcidol were 122±21.5 mmHg in alphacalcidol group and 113±15.5 mmHg in non-alphacalcidol group. SBP after 8 years were 116±11.4 mmHg and 120±14.7 mmHg. The changes of SBP were -6.10±20.7 and 7.00±12.7 mmHg, respectively (p=0.04). DBP before the initiation of alphacalcidol were 72.4±13.4 mmHg in alphacalcidol group and 66.7±11.1 mmHg in non-alphacalcidol group, and at the end of follow-up, 70.2±10.5 mmHg and 72.6±11.1 mmHg, respectively. The changes of DBP were -2.20±12.8 mmHg and 5.90±12.5 mmHg (p=0.11).

Conclusions: Alphacalcidol can lower SBP in SLE.

FR-PO431

Paricalcitol Reduced Left Atrial Volume in Patients with Chronic Kidney Disease Hector Tamez. *Department of Medicine / DIvision of Nephrology, Massachusetts General Hospital. On Behalf of the PRIMO Steering Committee, Boston, MA.*

Background: Left atrial enlargement, a sensitive integrator of left ventricular diastolic dysfunction, is associated with increase risk of cardiovascular related hospitalizations, arrhythmias and mortality. Vitamin D is linked to lower cardiovascular morbidity, possibly modifying cardiac structure and function, however firm evidence is lacking. We assessed the effect of an activated vitamin D analog with changes in left atrial volume index (LAVi) in a post-hoc analysis of the Paricalcitol capsules benefits in Renal failure Induced cardiac MOrbidity randomized trial (PRIMO, clinicaltrials.gov: NCT00497146).

Methods: A total of 196 patients with chronic kidney disease, mild to moderate left ventricular hypertrophy and preserved ejection fraction were randomly assigned to 2 µg of oral paricalcitol or matching placebo for 48 weeks. Two-dimensional echocardiographic measures were obtained at baseline, 24 and 48 weeks after initiation of therapy. We compared change in LAVi (volume changes indexed to body surface area) over the 48 weeks of therapy.

Results: During the study period, there was significant decrease in LAVi of -2.79 mL/m2 (95% confidence interval [CI]: -4.00 to -1.59 mL/m2) in the paricalcitol group compared to the placebo group (-0.70 mL/m2 [95% CI: -1.93 to 0.53 mL/m2]; P=0.002). Paricalcitol attenuated the increase of brain natriuretic peptide (9% in paricalcitol vs. 21% in placebo; P=0.02). Change in brain natriuretic peptide was correlated with change in LAVi (r=0.17; P=0.03). LAVi decreased by 3.04 ml/m2 when comparing the lowest to the highest quartiles of BNP change (P=0.02).

Conclusions: Forty-eight week therapy with paricalcitol significantly reduced LAVi and attenuated the rise of brain natriuretic peptide. This post-hoc result is hypothesis-generating and warrants further confirmation.

Funding: Pharmaceutical Company Support - Abbott Laboratories

FR-PO432

Utility of Spectroscopic Bioimpedance (BIA) in the Management of Refractory Hypertension in Patients with Chronic Kidney Disease (CKD) Ursula Verdalles, Soledad Garcia de Vinuesa, Marian Goicoechea, Borja Quiroga, Javier Reque, David Arroyo, Nayara Panizo, Jose Luno. *Nephrology, HGUGM, Madrid, Spain.*

Background: Expansion of extracellular volume (ECV) is frequent cause of resistant hypertension (RHT) in patients with CKD. Aim of this study was identify patients with RHT, CKD and ECV expansion, trying to control BP by intensification diuretic treatment.

Methods: We included 50 patients with RTH and CKD in whom BIA was conducted. For control of BP, diuretic treatment was increased in patients with ECV expansion and in rest, another antihypertensive drug was added.

Results: Mean age of patients was 68.2±10.4 years, 68% male, 58% diabetic and the mean estimated glomerular filtration rate(eGFR) 50.7±22.4ml/min/1.72m². Baseline SBP 167.2±8.6 mmHg and DBP 84.8±9.5mmHg, and mean number of antihypertensive drugs was 3.7±0.9. ECV expansion was found in 60% of the patients (1.9±0.9L). We could not found significant relation between degree of overhydration and stage of CKD. ECV expansion was found more frequently in diabetics (69%.vs.31%,p<0.01) and in patients with more albuminuria (UACR 745±901 vs 328±450mg/g,p<0.01). At 6 months of follow-up, a decline of 21.4±7.1 mmHg of SBP was observed in group of patients with ECV expansion, versus a decrease of 9.4±3.4 mmHg in normal ECV group(p<0.01). DBP decreased 4.3±3.7 mmHg in both groups. At 6 months of follow-up, 9(30%) of the patients with ECV expansion who increased diuretic therapy reached the target BP,<140/90mmHg, as compared with only 2(10%) of those patients without ECV expansion who added other antihypertensive drug. Total body water decrease 1.9±1.1 L, in group of patients with ECV expansion who intensified diuretic treatment, at expense of a decline of ECV 1.1±1 L. eGFR remained stable in both groups(47,1±21,1 vs 54,1±25,2ml/min/1.73 m²,p=0.37).

Conclusions: ECV expansion is frequent cause of RHT in patients with CKD, independently of stage of CKD. Diabetic and severe proteinuric patients are more exposed to ECV expansion. BIA is a useful method for identifying these patients with CKD, RHT and ECV expansion. Intensification diuretic treatment, in these patients, permits better control BP, without changing eGFR.

FR-PO433

Risk of Coronary Heart Disease in Individuals with History of Kidney Stones: Results from Three Large Prospective Cohort Studies Pietro Manuel Ferraro,[1,2] Eric N. Taylor,[2] Giovanni Gambaro,[1] Eric B. Rimm,[2] Gary C. Curhan.[2] *[1]Division of Nephrology - Renal Program, Department of Internal Medicine and Medical Specialties, Catholic University of the Sacred Heart, Rome, Italy; [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Previous studies analyzed the association between kidney stones (KS) and coronary heart disease (CHD). However, they often failed to control for important risk factors and results were inconsistent. We analyzed the risk of developing CHD for individuals with a history of KS in three large prospective cohort studies.

Methods: We used data from three large ongoing cohort studies: the Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS) I and II. Information on KS and CHD was collected by biennial questionnaires and confirmed through further validated questionnaires (KS) or review of hospital records (fatal and non-fatal myocardial infarction [FMI, NFMI]). The risk of developing CHD (defined as FMI or NFMI or coronary revascularization [CR]) in participants with and without history of KS was assessed with a Cox model adjusted for race, family history of heart disease, smoking status, BMI, physical activity, diabetes, hypertension, gout, elevated cholesterol, use of medications and daily intake of calcium, protein, fat, caffeine and alcohol.

Results: The analysis included 189,339 participants. After up to 22 years of follow-up in men and 16 years in women, 6,937 incident cases of CHD were confirmed. Among women, those with a history of KS compared to those without had an increased risk of CHD in NHS I (HR 1.27, 95% CI 1.03 to 1.56) and NHS II (HR 1.70, 95% CI 1.13 to 2.55); there was no association in the male HPFS cohort (HR 1.01, 95% CI 0.91 to 1.13). Similar results were found when analyzing the individual end-points (FMI, NFMI, CR).

Conclusions: We found a significant independent increased risk of CHD among women with a history of KS but no association was found in men. Mechanisms for such an association remain to be elucidated.

Funding: NIDDK Support, Other NIH Support - NHLBI, NCI

FR-PO434

Soda and Other Beverages and the Risk of Kidney Stones: Results from Three Large Prospective Cohorts Pietro Manuel Ferraro,[1,2] Eric N. Taylor,[2] Giovanni Gambaro,[1] Gary C. Curhan.[2] *[1]Division of Nephrology - Renal Program, Department of Internal Medicine and Medical Specialties, Catholic University of the Sacred Heart, Rome, Italy; [2]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Increasing fluid intake is a well accepted method for reducing the recurrence of kidney stones (KS), but not all types of fluids may be equally beneficial. In particular, it is not clear whether consuming sugar- (SS) and artificially-sweetened (AS) carbonated beverages (CB) increases the risk of developing KS. We analyzed the association between intake of several types of beverages and incidence of KS in three large prospective cohort studies.

Methods: We used data from three large ongoing cohort studies: the Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS) I and II. Information on frequency of consumption of beverages and development of KS was collected by validated questionnaires every four and two years, respectively. The risk of developing KS associated with each beverage was assessed using a Cox model adjusted for covariates including age, race, physical activity, BMI, diabetes, high blood pressure, gout, use of diuretics and intake of calcium, potassium, animal protein, phytate and vitamin C. Pooled results are reported.

Results: During a follow-up of up to 20 years in HPFS, 22 years in NHS I and 16 years in NHS II, 4,462 of the 194,095 participants developed an incident symptomatic KS. We found a significantly higher risk of developing KS across categories of increased consumption of SS cola (p for trend=0.02) and non-cola CB (p=0.003), whereas the trend was border-line for AS cola (p=0.08) and non-cola CB (p=0.05). We found an increased risk of KS for increasing consumption of punch (p=0.04) and a decreased risk for increasing consumption of caffeinated coffee (p<0.001), decaffeinated coffee (p=0.01), tea (p=0.02), wine (p<0.004), beer (p<0.001) and orange juice (p=0.004).

Conclusions: Our analysis confirmed that some beverages (coffee, tea, wine, beer and orange juice) are inversely associated with KS formation while others (SS CBs and punch) are associated with an increased risk.

Funding: NIDDK Support, Other NIH Support - NHLBI, NCI

FR-PO435

Non-Dairy Calcium Intake and Kidney Stone Risk Eric N. Taylor,[1,2] Gary C. Curhan.[2] *[1]Division of Nephrology, Maine Medical Center, Portland, ME; [2]Channing Laboratory, Brigham and Women's Hospital, Boston, MA.*

Background: Higher dietary calcium is associated with lower kidney stone risk. However, high correlations between dietary calcium and dairy intake suggest the possibility that milk, rather than calcium per se, reduces risk of nephrolithiasis.

Methods: We prospectively examined independent associations between non-dairy calcium intake and incident kidney stones in the Health Professionals Follow-up Study (N=30,762 men), the Nurses' Health Study I (N=94,167 older women), and the Nurses' Health Study II (N=101,701 younger women). We excluded men ≥ 60 years old because we previously reported inverse associations between calcium intake and stone risk only in men < 60. Validated food frequency questionnaires were used to assess non-dairy calcium intake every four years. Medical record review confirmed ≥ 95% of self-reported kidney stones in each cohort, and the majority of stones (≥ 77%) were predominantly calcium oxalate. Cox regression was used to adjust for age, BMI, thiazide use, family history of kidney stones, hypertension, diabetes, fluid intake, dairy calcium intake, supplemental calcium, and a wide variety of other dietary and non-dietary factors.

Results: We documented 4,987 incident kidney stones over a combined 50 years of follow-up. Mean energy-adjusted non-supplemental total calcium and non-dairy calcium intakes were 796 and 326 mg/d in men, 720 and 334 mg/d in older women, and 886 and 338 mg/day in younger women. For men, older women, and younger women, the correlation coefficients between energy-adjusted non-supplemental total calcium and dairy calcium intakes were ≥ 0.96. In contrast, the correlation coefficients between energy-adjusted non-supplemental total calcium and non-dairy calcium intakes ranged between 0.13 and 0.22. For participants in the highest compared to lowest quintile of non-dairy calcium, the multivariate relative risks of kidney stones were 0.72 (95% CI 0.56-0.93; P trend 0.01) for men, 0.81 (95% CI 0.67-0.97; P trend 0.11) for older women, and 0.73 (95% CI 0.62-0.87; P trend 0.001).

Conclusions: Both non-dairy and dairy calcium intake are independently associated with a lower risk of kidney stones.

Funding: NIDDK Support

FR-PO436

Association of Prevalent Kidney Stone Disease with All-Cause & Cardiovascular Death among US Adults Jie Tang, Pamela Mettler, M. Chonchol. *Medicine, University of Colorado, Aurora, CO.*

Background: Nephrolithiasis is considered a systemic disorder associated with bone loss, chronic kidney disease and multiple cardiovascular disease (CVD) risk factors. However, its association with all-cause and CVD death is unknown.

Methods: We assessed the relationship between kidney stone disease and risk of mortality among a nationally representative sample of US adults (age ≥18 years), using the Third National Health and Nutrition Examination Survey and its Linked Mortality File (through 2006). Kidney stone disease was defined as self-report of any previous episode of kidney stone. Cox proportional hazard regression analyses were used to estimate the risks of all-cause and CVD death.

Results: Among 15,141 participants, 701 reported a history of kidney stone. Stone formers tended to be male, white and older, had a higher BMI, and more likely to have hypertension, diabetes and CVD. There were a total of 3739 all-cause deaths and 1684 CVD deaths with a median follow up of 15 years. Unadjusted analysis showed that stone formers had a higher risk for death (HR=1.98, 95% CI 1.66-2.36, p<0.0001), and CVD death (HR=2.06, 95% CI 1.61-2.64, p<0.0001) [Table 1]. However, after multivariate adjustment for age, race and sex, stone formers no longer had increased risk for death (HR=1.07, 95% CI 0.92-1.25, p=0.4) or CVD death (HR=1.05, 95% CI 0.83-1.34, p=0.7). After further adjustment for other CVD risk factors, independent associations of prevalent kidney stone disease with all-cause and CVD death remained non-significant.

Table 1. Hazard ratios for all-cause and CVD mortalities in stone formers

	Regression Model	HR (95% CI)	P value
All-cause mortality	Unadjusted	1.98 (1.66-2.36)	<0.0001
	Model 1	1.07 (0.92-1.25)	0.4
	Model 2	0.99 (0.85-1.16)	0.9
	Model 3	0.99 (0.85-1.16)	0.9
CVD mortality	Unadjusted	2.06 (1.61-2.64)	<0.0001
	Model 1	1.05 (0.83-1.34)	0.7
	Model 2	0.94 (0.74-1.20)	0.6
	Model 3	0.94 (0.75-1.19)	0.6

Model 1: adjusted for age, race, sex. Model 2: adjusted for BMI, histories of hypertension, diabetes and CVD, in addition to model 1. Model 3: adjusted for serum CRP, serum 25(OH)D, and MDRD-eGFR, in addition to model 2

Conclusions: Prevalent kidney stone disease is not independently associated with all-cause and CVD death.

Funding: Clinical Revenue Support

FR-PO437

Trend of Incident Kidney Stone Disease in Utah and Idaho Jie Tang,[1] John R. Holmen,[2] Angela Keniston,[1] M. Chonchol.[1] *[1]Nephrology, University of Colorado, Aurora, CO; [2]Intermountain Healthcare, Murray, UT.*

Background: Recent trends of incident kidney stone disease are unclear. Our primary objective was to determine the kidney stone incidence trend through time in an integrated healthcare system serving a defined geographical region.

Methods: Incident kidney stone rates were obtained using Intermountain Healthcare (IHC) Data Warehouse. IHC is a healthcare organization that serves 2.4 million Utah and Idaho residents. Incident stone formers were defined as patients having first ever diagnosis of kidney stone disease and were identified by ICD9 code 592.0 assigned from 1994 to 2010. Negative binomial regression analyses were used to examine the incidence trend.

Results: A total of 176,499 incident stone formers were identified. Adjusted for age, gender and race, kidney stone incidence rates were 0.60% in 1994 and 1.29% in 2010. This represented a significant increase from 1994 to 2010 (p<0.0001 for trend). Age and race adjusted incidence rates for men were 0.81% and 1.51% in 1994 and 2010, respectively. On average, it increased by 2.2% per year during 1994-1999 (p=0.98 for trend) and 4.7% per year during 2000-2010 (p=0.001 for trend). For women, the age and race adjusted rates were 0.44% and 1.12% in 1994 and 2010, respectively. It increased by 2.2% per year during 1994-1999 (p=0.22 for trend) and 5.3% per year during 2000-2010 (p<0.0001 for trend). Age and gender adjusted incidence rates for non-hispanic white (NHW) were 0.59% and 1.26% in 1994 and 2010, respectively. For non-hispanic blacks (NHB), the corresponding rates were 0.55% and 0.61%, respectively, and for Mexican Americans (MA), the corresponding rates were 0.37% and 0.62%, respectively. Rates for NHW increased by about 4.2% per year (p<0.0001 for trend), whereas rates for NHB increased by 0.4% per year (p=0.57 for trend), and the rates for MA increased by 1.6% per year (p<0.0001 for trend). The incidence rate increase in NHW occurred primarily during 2000-2010 (p=0.008 for trend).

Conclusions: Kidney stone incidence rates progressively increased from 1994 to 2010. The increase primarily occurred during 2000-2010 and the causes of these findings remain to be determined.

Funding: Clinical Revenue Support

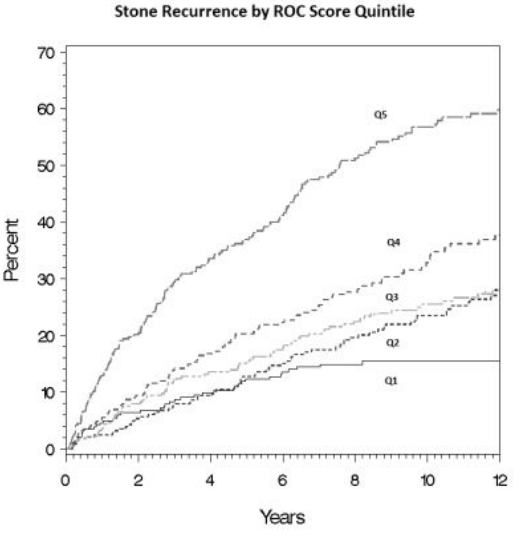
FR-PO438

The Recurrence of Calculus (ROC) Score for Predicting a Second Symptomatic Episode among Stone Formers in the General Population Eric J. Bergstralh, John C. Lieske, Xujian Li, Amy E. Krambeck, Andrew D. Rule. *Mayo Clinic, Rochester, MN.*

Background: A prediction tool for kidney stone recurrence is needed to optimize prevention and treatment strategies. This study was conducted to estimate recurrence rates, evaluate predictors, and develop a risk score for recurrence among incident stone formers.

Methods: We identified first time kidney stone episodes among residents of Olmsted County, MN. Charts were reviewed to validate episodes based on evidence of a passed or obstructing stone with supportive symptoms. Recurrence was defined as having a second validated stone episode >30 days later. Life table methods were used to estimate recurrence rates and predictors of recurrence. Candidate predictors included gender, age, race, family history of stones, body size, signs and symptoms, stone characteristics at the time of the first episode (number, location, size, composition), and prior asymptomatic stones. A recurrence of calculus (ROC) score was developed using the Cox model.

Results: There were 1586 validated stone formers (median age 42 years, 62% male, 87% white) with a median follow-up of 11.2 years. Recurrence rates at 2, 5, and 10 years were 10%, 20%, and 31%, respectively. The full model C-statistic with 18 predictors was 0.657. A more parsimonious multivariable model with 10 predictors had C=0.652. Significant (p<0.05) risk factors in this model included younger age, male gender, family history, prior asymptomatic stone, current pregnancy, multiple stones on imaging, renal pelvic location, and presence of any uric acid in the stone. Ten-year recurrence rates varied greatly (from 16% to 57%) by ROC score quintile.



Conclusions: A scoring system (ROC) was developed that identifies incident symptomatic kidney stone formers at greatest risk for recurrence. Such individuals may benefit from medical intervention and be good candidates for prevention trials.

Funding: NIDDK Support

FR-PO439

Presenting Characteristics of Dent Disease Patients John C. Lieske,[1] Steven J. Scheinman,[2] Lawrence A. Copelovitch,[3] Hae Il Cheong,[4] Eric J. Bergstralh,[1] Ramila A. Mehta,[1] Lada Beara Lasic.[5] *[1]Mayo Clinic, Rochester, MN; [2]SUNY, Syracuse, NY; [3]CHOP, Philadelphia, PA; [4]Seoul National University Children's Hospital, Seoul, Korea; [5]NYU School of Medicine, New York, NY.*

Background: Dent disease is an X-linked disorder characterized by low molecular weight proteinuria, hypercalciuria, kidney stones, and chronic kidney disease. Two causative genes have been identified. The most common presenting phenotype remains poorly defined.

Methods: The Rare Kidney Stone Consortium maintains a voluntary registry for Dent disease. Presenting characteristics of enrolled patients were queried. Data are presented as mean (SD) or % affected.

Results: 71 patients are currently enrolled including 43 from North America, 9 from Europe, 9 from Asia, and 10 unknown/other locations. Disease type was confirmed Dent 1 (*CLCN5* mutation, n=44), Dent 2 (*OCRL1* mutation, n=1), neither Dent 1 nor Dent 2 (n=4), or unknown (n=22). Age at diagnosis was 13.6 (11.7) yrs (range 0-44; 9 patients (13%) diagnosed after age 30). Mean followup was 3.8 yrs. 14 patients had kidney stones at an age of 17.2 (10.2) years, most as the presenting symptom but 3 substantially (6.8 yrs) later. For those with data, low molecular weight proteinuria (95%), hypercalciuria (87%), and hematuria (44%) were common, while kidney stones (27%) and bone disease (11%) were less common. Total protein excretion was 1.8 (1.6) g/24-hr. Random urinary calcium:creatinine ratio was 0.61 (0.60) mg/mg. Among those with normal kidney function and >16 yrs old (n=8), 24-hr urine calcium was 361 (174) mg. First available serum creatinine was 1.1 (1.9) mg/dl at an age of 14.1 (11.6) yrs. 6 patients progressed to ESRD at a mean age of 30.5 (13.2) yrs; 4 currently have functioning renal allografts.

Conclusions: Patients with Dent disease are most often diagnosed in their early teens and only a minority ever experience clinical kidney stones. Moderate proteinuria, hypercalciuria, and hematuria are more commonly present. Clinicians should maintain a high index of suspicion for Dent disease in younger males with unexplained proteinuria and CKD.

Funding: NIDDK Support, Other NIH Support - Office of Rare Diseases Research, Private Foundation Support

FR-PO440

Determinants and Outcome of Renal Calcification in Primary Hyperoxaluria John C. Lieske,[1] Eric J. Bergstralh,[1] Ramila A. Mehta,[1] Craig B. Langman,[2] Dawn S. Milliner.[1] *[1]Mayo Clinic, Rochester, MN; [2]Northwestern University, Chicago, IL.*

Background: Stone formation and nephrocalcinosis are both very common features of Primary Hyperoxaluria (PH), yet the numbers and extent vary markedly between patients. We investigated whether kidney damage from stones, nephrocalcinosis and/or urologic procedures contributed to chronic kidney disease.

Methods: Clinical information collated from 293 of 336 patients enrolled in the Rare Kidney Stone Consortium PH registry with at least one renal image was analyzed including number of stones by imaging; stones passed; urological procedures; urine chemistries; serum creatinine; and measured or estimated GFR. Correlations were assessed by Spearman correlation coefficient.

Results: Mean patient age at last f/u was 25.8yr (25^{th}, 75^{th} = 9.0, 39.8y) and kidney imaging was documented in 293 (87%). Overall, 79% passed a stone, with a mean number of 0.32 passed/yr. One or more urologic procedures were required by 83% of patients ever, with a mean number of 0.16 procedures/yr. Number of stones or procedures was not associated with ESRD risk. Nephrocalcinosis was documented in 36% of all patients. Further, the presence of NC at time of first image or later (time dependent covariate) was associated with a significantly (p=0.0037) increased risk of subsequent ESRD (HR 2.38, 95% CI: 1.30, 4.33). Of urine factors, only low urine citrate was a weak risk factor for stone events. Urinary oxalate, citrate and volume were all associated with risk for nephrocalcinosis, which was more common in PH type 1 compared to other PH types.

Conclusions: Stone events are a hallmark of PH, averaging about 1 every 3 years. Urinary oxalate level does not clearly correlate with stone number, but together with citrate and volume does influence risk of nephrocalcinosis. Stone and nephrocalcinosis appear to be pathophysiologically distinct entities, and the presence of nephrocalcinosis implies a modest risk for ESRD.

Funding: NIDDK Support, Other NIH Support - Office of Rare Diseases Research, Private Foundation Support

FR-PO441

Severity of Renal Tubular Plugging but Not Randall's Plaque Associated with Clinical and Laboratory Characteristics of 78 Stone Formers Undergoing Endoscoping Mapping John C. Lieske, Michael P. Linnes, Eric J. Bergstralh, Xujian Li, Terri J. Vrtiska, Andrew D. Rule, Amy E. Krambeck. *Mayo Clinic, Rochester, MN.*

Background: Two forms of renal papillary micro-calcifications that are thought to be the antecedent of kidney stones have been described, interstitial Randall's plaques and duct of Bellini plugs. We hypothesized that severity of these two forms of micro-calcification reflect stone composition as well as important differences in clinical and laboratory characteristics of stone formers (SF).

Methods: Patients were prospectively enrolled during a percutaneous nephrolithotomy for kidney stone disease. Composition of removed stones was determined by both IR-spectroscopy and micro-CT. Each accessible calyx was endoscopically mapped using a flexible digital nephroscope and representative papillary tip biopsies were acquired. Images of each papillum were digitally analyzed to quantify the area covered with plaque or plug. 24-hr urine samples were analyzed for determinants of supersaturation (SS) and crystal growth inhibition (CGI).

Results: On average 4.5 calyces were mapped in each of 78 patients (39 idiopathic CaOx; 8 CaOx with malabsorption; 12 CaP; 7 struvite; 4 uric acid and 8 others). Randall's plaque was present in 98.7% of kidneys covering an average of 3.0 ±3.3% of the papillary surface, and did not associate with stone type, urinary chemistries, or number of stones. Tubular plugging was less common with moderate amounts (<1%) in 17 SF, and severe amounts (>1%) in 16 others. Severe tubular-plugging associated with higher urinary pH (p=0.03), lower citrate (p=0.02), higher CaP SS (p<0.007), brushite stones, and reduced CGI (27% vs 37%, p=0.003). Those with severe tubular plugging also had more kidney stones (mean 9.2 vs. 2.6, p=0.0002).

Conclusions: Tubular plugs but not Randall's plaques associate with urinary factors and stone severity. The pathogenesis of Randall's plaques may be more complex and involve factors not commonly measured in standard urine panels. Endoscopic papillary mapping documents patterns of microcalcification and improves characterization of stone patients for clinical studies.

Funding: NIDDK Support

FR-PO442

Heritability of Urinary Traits that Contribute to Nephrolithiasis John C. Lieske,[1] Stephen T. Turner,[1] Samuel Edeh,[1] Tracy Fuller,[2] Jennifer Smith,[2] Sharon R. Kardia.[2] *[1]Mayo Clinic, Rochester, MN; [2]University of Michigan, Ann Arbor, MI.*

Background: Kidney stones tend to aggregate in families. Of known risk factors for stones, evidence is strongest for heritability of hypercalciuria.

Methods: Measures of urinary supersaturation were determined from 24 hr urine samples collected from families in the Rochester, MN cohort of the Genetic Epidemiology Network of Arteriopathy. Diet was assessed using the Viocare Food Frequency Questionnaire. Heritability was estimated by using variance components analysis.

Results: Samples were available from 811 individuals (344 men, 467 women, mean (SD) age 66(9) yrs). Age, gender, and weight significantly influenced the vast majority of urinary parameters. Many urine and diet parameters as shown in the Table had strong evidence for heritability (P<0.001), in raw and adjusted models. Overall, 12-36% of the variance in urinary traits could be attributed to heritable factors, while 20-54% of dietary intakes could be.

Heritabilities of 24-Hour Urine and Dietary Measures

Trait	h2 unadjusted	Proportion of variance of measure explained by covariates	h2 adjusted for age, gender, height, weight
Urinary measures			
Calcium	0.41	0.16	0.25
Magnesium	0.34	0.14	0.25
pH	0.35	0.08	0.27
OSM	0.26	0.21	0.20
Citrate	0.39	0.08	0.36
Oxalate	0.11	0.17	0.12
Diet measures			
Animal Protein	0.32	0.12	0.26
Calcium	0.60	0.03	0.54
Fructose	0.25	0.03	0.20
Oxalate	0.28	0.02	0.25
Protein	0.46	0.11	0.38
Sucrose	0.37	0.02	0.37

Conclusions: Evidence from this large cohort suggests a strong genetic component to many nephrolithiasis risk factors, including dietary intake, often considered an environmental influence. Continued efforts to understand genetic influence of kidney stone risk is warranted.

Funding: NIDDK Support, Private Foundation Support

FR-PO443

American and Brazilian Children with Urolithiasis: Similarities and Disparities Maria Goretti Penido,[1] Marcelo S. Tavares,[1] Uri S. Alon.[2] *[1]Pediatric Nephrology Unit, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Pediatric Nephrology, Children's Mercy Hospital, University of Missouri, Kansas City, MO.*

Background: Considering the different racial, socioeconomic backgrounds and geographic locations the aim of this study was to identify the demographic and biochemical differences between American and Brazilian children with urolithiasis.

Methods: We evaluated the data of 222 American and 190 Brazilian children seen between January 1999 and December 2010. Data included age at diagnosis, gender, BMI, imaging technique used and serum chemistries. 24-h urines were analyzed, at least 1 month after stone expulsion while the patients were on their usual diet, for volume, creatinine, calcium, uric acid, citrate, oxalate, cystine. Unpaired T-test was used for statistical analysis.

Results: No abnormalities were noted in serum chemistries in either group. Despite some differences between the populations, like younger age and leaner body mass among Brazilian children, and some differences in the frequencies of various etiologies, the leading

causes of urolithiasis among both groups were "oliguria", hypercalciuria and high Ca/citrate ratio. In neither country was obesity *per se* more frequent in stone patients compared with the general population. US was the most preferred imaging technique excluding more frequent use of CT as the reason for the increased incidence of pediatric urolithiasis.

Clinical, demographic and biochemical data of American and Brazilian children

	American children (n=222)	Brazilian children (n=190)	p
Gender (%)	Male 48%	Male 51%	
Age at diagnosis (years)	11.8±3.8	8.2±3.2	0.001
BMI Z-score	0.36	0.01	0.00001
Overweight (Z-score>2)	15%	2%	
Imaging technique US/CT	73%/27%	98%/2%	
Urine flow <1.0ml/kg/day	63%	49%	
Hypercalciuria (>4.0 mg/kg/day)	47%	69%	
Calcium/citrate ratio (>0.33)	54%	41%	
Hypocitraturia (<180mg/g creatinine)	10%	9.5%	
Hyperuricosuria (factored for GFR)	6.4%	9.5%	
Idiopathic absorptive hyperoxaluria	1.4%	1.6%	
Cystinuria	0.5%	1.0%	
No abnormality	9.0%	13.0%	

Conclusions: Although we observed some differences between the 2 populations, overall etiologies were quite similar justifying combining efforts in addressing the problem.

FR-PO444

A Case-Control Study of Comorbid Disorders Patients in with Kidney Stone Disease Vaka Kristin Sigurjonsdottir,[1,3] Olafur S. Indridason,[2] Runolfur Palsson,[1,2] Vidar O. Edvardsson.[1,3] *[1]Fauculty of Medicine, University of Iceland; [2]Division of Nephrology; [3]Hringurinn, Children´s Hospital, the National University Hospital of Iceland, Reykjavík, Iceland.*

Background: Hypertension and obesity have been associated with an increased risk of nephrolithiasis. The aim of this study was to compare patients with kidney stone disease to control subjects with respect to selected comorbid factors.

Methods: This was a cross-sectional case-control study in which 121 individuals with a history of kidney stone disease were selected from the Icelandic Kidney Stone Registry. Patients with recurrent stone events or a history of a single stone event and elevated serum creatinine (SCr) >100 mmol/l were invited to participate. All study subjects underwent an evaluation of height, weight and blood pressure, and their last SCr value was obtained from medical records. For each patient we randomly selected 2 control subjects without history of kidney stones and matched for age and sex, from participants in a population-based study of bone and mineral health. Wilcoxon-Mann-Whitney and chi-square tests were used to compare the groups.

Results: The 121 subjects with kidney stone disease had a median (range) age of 58 (19-70) years and included 72 (60%) men. Fifty-eight patients had experienced 1-4 stone episodes, 16 had experienced 5-10 episodes and 46 more than 10 stone episodes. The median BMI was 27.9 (19.9-48) kg/m^2 among the cases compared to 26.6 (13.8-46.6) in the controls (p=0.01). The prevalence of hypertension was 55% among the cases and 47% in the controls (P=0.14) and there was no difference between the two groups in measured systolic blood pressure (BP). The diastolic BP was, however, significantly lower among the cases, 78 (38-120) mmHg compared to 81 (54-120), (p=0.01.) Excluding the 6 patients who were selected based on a single stone episode and elevated SCr, the median SCr was 74 (47-363) mmol/l in the patients compared to 66 (27-168), (p<0.001).

Conclusions: Our study suggests an association between obesity and recurrent kidney stone disease but the results do not indicate an association with hypertension. Kidney stone disease appears to have a deleterious effect on kidney function.

Funding: Government Support - Non-U.S.

FR-PO445

A Twin Study of Genetic Influences on Nephrolithiasis in Women and Men David S. Goldfarb,[1] Carolyn Noonan,[3] Jack Goldberg.[2] *[1]Nephrology Division, NY Harbor VA Medical Center, New York, NY; [2]Medicine, University of Washington, Seattle, WA; [3]Epidemiology, University of Washington, Seattle, WA.*

Background: Nephrolithiasis is a complex phenotype influenced by both genetic and environmental factors. Previously we found a genetic component to stone disease using a middle-aged sample of male twin pairs. We now report on the genetic contribution to stones in a younger sample of female and male twin pairs.

Methods: We conducted a classical twin study of kidney stones using the University of Washington Twin Registry. Data were collected by questionnaire to obtain the self-reported history of kidney stones. Proband concordance rates were estimated for kidney stones in monozygotic (MZ) and dizygotic (DZ) pairs; univariate structural equation modeling was used to determine the relative contributions of additive genetics, common environment and unique environment.

Results: There were 4,988 same-sex pairs with kidney stone data. The mean age of the sample was 37 years (range 18-90) and was similar in women and men. The prevalence of kidney stones was 4.7% of women and 4.8% of men. Kidney stone proband concordance rates were higher in MZ than DZ pairs for both women and men.

Kidney Stones in Women and Men in the UW Twin Registry

	MZ Women	DZ Women	MZ Men	DZ Men
Concordant for stones, N pairs	21	5	20	5
Discordant for Stones, N pairs	159	188	75	51
Proband Concordance	0.21	0.10	0.35	0.16

We found significant contributions from genetics and the unique environment ($p < 0.05$ for both) to the liability to stone disease in women and men. There was no significant contribution from the common environment. The heritability was 45% (0.32-0.57) in women and 66% (0.53-0.77) in men; these heritabilities were not significantly different (p=0.27).

Conclusions: Nephrolithiasis in women, as in men, has a substantial heritable component. The size of the genetic contribution in this twin study is similar to our previous study of older male twins. More stone formers are men than women; our data do not support differences in hereditability as the basis for that difference. The genetic basis for stone disease in the general population has not been established.

Funding: Private Foundation Support

FR-PO446

Randomized Controlled Trial (RCT) of Febuxostat (FBX) versus Allopurinol (ALLO) and Placebo (PBO) in Subjects with Hyperuricosuria and Calcium Oxalate (CaOx) Stones David S. Goldfarb,[1] P. MacDonald,[2] L. Gunawardhana,[2] S. Chefo,[2] L. McLean.[2] *[1]NYU Langone Medical Center, New York, NY; [2]Takeda, Deerfield, IL.*

Background: About 1/3 of patients with recurrent CaOx stones have hyperuricosuria as a urinary risk factor. ALLO treatment can reduce the incidence of recurrent CaOx stones in hyperuricosuric stone formers (SF). FBX, a newer xanthine oxidase inhibitor (XOI), may be superior to ALLO in stone prevention. We studied whether FBX would reduce 24h urinary uric acid (uUA) excretion and prevent stone formation and pre-existing stone growth.

Methods: In this 6-mos, double-blind, multicenter RCT, hyperuricosuric (>700 mg/d) patients with a history of CaOx stones and ≥1 3-mm stone seen by multidetector computed tomography (MDCT) were randomized to receive daily FBX 80mg, ALLO 200 or 300mg (based on Ccr), or PBO. Primary end-point was % change from baseline (CFB) to mo 6 in 24h uUA; secondary end-points were % CFB in size of index stone, CFB in no. of stones and in 24h creatinine clearance (Ccr).

Results: 99 patients enrolled, 86 completed the study. Key baseline characteristics were balanced. 86% were men with a mean lifetime history of 10.9 stone episodes, mean largest stone diameter 9.9 mm and a mean of 5.7 stones on MDCT. Mean baseline Ccr was 147mL/min/1.73m^2, serum urate (sUA) 6.3 mg/dL, urine calcium excretion 272.2 mg/d, and uUA 952.7 mg/d. FBX led to significantly greater reduction from baseline in uUA than either PBO or ALLO.

	PBO (n=33)	FBX 80mg (n=33)	ALLO 300mg (n=33)
Baseline uUA (mg/d)	909.4±166.4	1000.6±224.0	948.1±231.2
Treated 6M uUA (mg/d)	783.5±288.0	411.4±288.4	580.0±301.8
CFB in uUA (%)	-12.7±28.8	-58.6±28.6[a,b]	-36.4±37.0
CFB in sUA (%)	-1.04±13.3	-47.3±16.7[b,c]	-26.2±15.0

mean±SD; [a]P=0.003 vs ALLO; [b,c]P<0.001 vs PBO and ALLO, respectively.

Reductions in stone size and number with FBX were not statistically greater than ALLO or PBO. There was no change in serum creatinine.

Conclusions: FBX 80mg lowered 24h uUA significantly more than ALLO 300mg in SF with hyperuricosuria. Neither XOI was associated with reduced stone number compared with PBO in only 6 mos of treatment. Extended duration of FBX treatment leading to greater 24h uUA reductions may demonstrate improved prevention of CaOx stone recurrence.

Funding: Pharmaceutical Company Support - Takeda Pharmaceuticals

FR-PO447

Thiazide Reduces Stone Risk by Increasing Proximal Tubule Calcium Reabsorption and Lowering Urine pH Kristin J. Bergsland, Elaine M. Worcester, Fredric L. Coe. *Department of Medicine, Nephrology Section, University of Chicago, Chicago, IL.*

Background: Calcium (Ca) stone formers with idiopathic hypercalciuria (IH) have decreased reabsorption of Ca in the proximal tubule (rCa_{PT}), as judged by endogenous lithium clearance. Thiazide (TZ) diuretics reduce Ca excretion and prevent stone recurrence. In mice, evidence suggests that TZ acts by increasing fractional rCa_{PT}, thereby reducing urine Ca excretion, but the mechanism in humans is unknown.

Methods: In the General Clinical Research Center, we studied the effect of TZ on renal mineral handling in 4 male IH patients (2 had Ca stones). We collected 15 urines and 20 blood samples over a 15 hour day, both fasting and with 3 meals of known composition. Each subject was studied twice: once before treatment and once after 4-6 months of the TZ chlorthalidone, 25 mg daily.

Results: As expected, urine Ca fell after TZ treatment; the same was true for the fraction of filtered Ca excreted (FECa). Fraction of filtered lithium excreted (FELi) fell sharply with TZ as did calculated distal delivery of Ca out of the PT (DDelCa). This effect was slightly more marked during fasting compared to the fed period. An unexpected effect of TZ was to reduce urine pH; this combined with reduced urine Ca led to a marked fall in CaP SS, but not CaOx SS.

	UCa (mM/hr)	FECa (%)	FELi (%)	DDelCa (mM/hr)	UpH	CaOx SS	CaP SS
PreTx	0.45 ± 0.02	4.7 ± 0.2	28.1 ± 1.2	2.79 ± 0.13	6.43 ± 0.07	7.4 ± 0.5	2.5 ± 0.2
TZ	0.38 ± 0.02*	4.0 ± 0.2*	15.8 ± 1.2#	1.59 ± 0.13#	5.97 ± 0.07#	6.5 ± 0.5	1.2 ± 0.2#

Daily mean ± SEM adjusted for age and weight. *, differs from preTx, p<0.05; #, differs from preTx , p<0.0001.

Conclusions: This may impact stone prevention because CaOx stones begin with an initial CaP overlay on Randall's plaque (RP). In IH, high Ca delivery to the thick ascending limb (TAL) may raise the absolute rate of TAL Ca absorption and increase interstitial Ca concentration. Vasa recta blood, running downward inside the medulla, will be enriched

in Ca, favoring apatite nucleation and RP formation. TZ, by decreasing distal nephron Ca delivery and lowering urine pH, might reduce stone risk by lowering CaP SS as well as slowing progression of RP, providing a new rationale for treatment with TZ.

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FR-PO448

The Contribution of Hydroxyproline Metabolism to Urinary Oxalate and Glycolate Excretion in Normal Subjects Ross P. Holmes,[1] Dean G. Assimos,[1] W. Todd Lowther,[2] John Knight.[1] *[1]Urology, Wake Forest University School of Medicine, Winston-Salem, NC; [2]Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC.*

Background: The metabolism of hydroxyproline (Hyp) produces an equimolar amount of glyoxylate which may be converted to glycolate, glycine or oxalate. Experiments with mouse models of Primary Hyperoxaluria (PH) suggest that this metabolism will make a significant contribution to urinary oxalate excretion in humans with the disease.

Methods: To determine how Hyp is metabolized in humans, 4 normal subjects were infused with 750 nmoles of ($^{13}C_5{}^{15}N$)-Hyp/kg/hr for 6 hrs in the fasted state after a priming dose equivalent to the amount to be infused in 2 hrs that was delivered over a 5 min period. ($^{13}C_5{}^{15}N$)-Hyp was measured in plasma by GC/MS, ^{13}C-glycolate in plasma by IC/MS, and $^{13}C_2$-oxalate and $^{13}C_2$-glycolate in urine by IC/MS.

Results: Enrichment of plasma with ($^{13}C_5{}^{15}N$)-Hyp was constant from 4 – 6 hrs when it reached 19.0 ± 4.3%. Urinary oxalate was enriched by 2.4 ± 1.2% and glycolate by 11.9 ± 1.6%, indicating that the mean contribution of Hyp metabolism to total urinary oxalate excretion was 13.0 ± 5.5% and to urinary glycolate excretion was 63.4 ± 3.7%. There was an 18.5 ± 6.8% increase in the enrichment of urinary glycolate compared to the enrichment in plasma glycolate suggesting that renal metabolism of ^{13}C-Hyp contributed directly to the urinary glycolate pool.

Conclusions: These results suggest that Hyp metabolism is a major source of urinary glycolate and a minor source of urinary oxalate in normal subjects. As much as half of the urinary oxalate may have been obtained from the diet, Hyp metabolism may contribute at least 25% to the urinary oxalate derived from endogenous synthesis. This contribution would increase with the consumption of dietary Hyp. The inability of subjects with PH to efficiently metabolize glyoxylate produced from Hyp breakdown may be a major source of endogenous oxalate production in this disease.

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FR-PO449

Purinergic Signaling Inhibits Oxalate Transport by Human Intestinal Cells Hatim A. Hassan, Sapna Sharma, Sireesha Ratakonda, Ruhul Amin. *Medicine, University of Chicago, Chicago, IL.*

Background: The majority of kidney stones are composed of calcium oxalate, and minor changes in urine oxalate affect stone risk. Intestinal oxalate secretion mediated by anion exchanger SLC26A6 plays a crucial role in limiting net absorption of ingested oxalate, thereby preventing hyperoxaluria and calcium oxalate nephrolithiasis. We previously reported that PKC activation negatively regulates Slc26a6 activity in mouse duodenal tissue. To identify physiologic agonists upstream of PKC, we used the human intestinal Caco2-BBE (C2) cells. We measured ^{14}C-oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity, ≥ 50% of which is mediated by SLC26A6. Purinergic signaling is known to modulate intestinal ion transport through pathways including PKC. We therefore examined whether purinergic stimulation with ATP affects Cl-oxalate exchange activity in C2 cells. We found that ATP significantly inhibited oxalate transport by C2 cells, an effect blocked by the PKC inhibitor Gö6983. Since the response to ATP could be partially mediated by its degradation into adenosine and the activation of the adenosine receptors, we evaluated the effects of UTP and observed significant inhibition of oxalate transport by UTP in C2 cells through a Gö6983-sensitive pathway. We next examined the effect of the selective P2Y2 agonist (2-ThioUTP TSS) since UTP is known to mediate its effects by mainly activating the P2Y2 and/or P2Y4 purinergic receptors. 2-ThioUTP TSS significantly inhibited oxalate transport by C2 cells. Using selective pharmacological inhibitors, we found that ERK1/2, p38, PI3K, and Src kinases are not involved in the observed regulation. Preliminary experiments utilizing immunocytochemistry and surface biotinylation showed that ATP led to reduced surface expression of SLC26A6. These findings are of potential importance because intestinal cells are known to be exposed to extracellular nucleotides (ATP and UTP) under physiological conditions. We conclude that purinergic signaling inhibits oxalate transport by C2 cells, by reducing SLC26A6 surface expression, through signaling pathways that likely include the P2Y2 receptor and PKC.

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FR-PO450

Inhibition of Bone Resorption Suppresses $1,25(OH)_2D_3$-Induced Hypercalciuria in Genetic Hypercalciuric Stone-Forming Rats Kevin K. Frick,[1] John R. Asplin,[2] Ignacio Granja,[2] Nancy Krieger,[1] Christopher D. Culbertson,[1] Kelly Kyker-snowman,[1] David A. Bushinsky.[1] *[1]Univ. of Rochester, Rochester, NY; [2]Litholink Corporation, Chicago, IL.*

Background: Genetic hypercalciuric stone-forming (GHS) rats, inbred from Sprague-Dawley (SD) rats to maximize urine (U) calcium (Ca) excretion, all form kidney stones. GHS rats have increased intestinal Ca absorption, increased bone resorption and reduced renal tubular Ca reabsorption; these sites all have an increased number of vitamin D receptors (VDR). The hypercalciuria in GHS rats is increased further by injection of $1,25(OH)_2D_3$ (1,25D) indicating that VDR are active. The 1,25D-induced increased UCa persists on a low Ca diet (LCD, 0.02% Ca) suggesting enhanced bone resorption. To test this hypothesis, we used the bone resorption blocker alendronate (Aln) in rats fed LCD.

Methods: SD (n=16) and GHS rats (n=16) were fed 13 g/d LCD (2.6 mg/d Ca) and half of each group was injected daily with 1,25D (25ng/100gBW/d). After 8d all rats were also injected daily with Aln (5µg/100gBW/d) until sacrifice at d16.

Results: At 8d, 1,25D increased UCa in SD (from 2.4±0.8 to 13.1±1.0 mg/d, $p<0.01$) and to a greater extent in GHS (from 4.3±0.4 to 23.7±1.7 mg/d, $p<0.01$) indicating that 1,25D induced bone resorption in both groups as UCa exceeded diet Ca. At 16d, Aln eliminated the 1,25D-induced increase in UCa in SD (2.2±0.3 vs 2.1±0.2) but in GHS Aln lessened, but did not eliminate, the 1,25D-induced increase in UCa (2.8±0.3 vs 7.9±1.3, $p<0.01$). Renal mRNA expression of mediators of active transepithelial Ca reabsorption TRPV5, calbindin D28k and PMCA were not altered by 1,25D in either group given Aln, while NCX1 levels were suppressed by 1,25D in both SD and GHS. TRPV6 was stimulated by 1,25D in SD but not GHS. Mediators of paracellular Ca reabsorption CLDN16 and NKCC2 were not altered by 1,25D.

Conclusions: With LCD, the 1,25D-induced hypercalciuria indicates increased bone resorption in SD and GHS. Aln eliminates the increase in UCa in SD; however, in GHS the increased UCa is lessened, but not eliminated, suggesting incomplete inhibition of bone resorption coupled to ongoing decreased renal tubule Ca reabsorption, perhaps due to reduced NCX1 expression.

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FR-PO451

Characteristics of Incident Symptomatic Kidney Stones by Composition in the General Population Andrew D. Rule,[1] Amy E. Krambeck,[1] Eric J. Bergstralh,[1] Xujian Li,[1] John C. Lieske,[1] James Williams.[2] *[1]Mayo Clinic; [2]Indiana University.*

Background: Stone composition analysis is useful for guiding therapy. Little is known about the relationship between stone composition and the clinical and laboratory characteristics of the incident episode.

Methods: Charts were manually reviewed for Olmsted County, Minnesota residents who received their first diagnostic code for kidney stones between 1984 and 2003. Symptomatic stone formers were validated by the presence of symptoms (pain or hematuria) with either a voided stone or an obstructing or infected stone seen on radiographic imaging. Validated stone formers were grouped by their first available stone composition analysis.

Results: There were 4,262 coded stone formers of which 2,059 were validated. Except for 15 with infected stones, all had stone passage or obstruction symptoms. Stone composition analysis was available in 1,281 (62%), but we excluded 7 with drug stones (triamterene, guaifenesin, or ephedrine) and 2 with cystine stones. Stone formers were divided into the following mutually exclusive groups: any brushite (1.1%), any struvite (0.9%), any uric acid (5.3%), and of the remaining with calcium stones, mostly oxalate (76%) or mostly apatite (17%). The table shows presenting characteristics varied by composition.

Characteristics that differ by composition

Characteristic (mean or %)	Missing (n=777)	Oxalate (n=960)	Apatite (n=219)	Brushite (n=14)	Struvite (n=12)	Uric acid (n=67)	p-value
Age, y	43	44	37	46	49	55	<.001
Men, %	59	72	33	50	25	76	<.001
Family history of stones, %	20	30	32	21	25	18	<.001
Body mass index, kg/m2	28	29	27	29	26	32	<.001
Diarrhea, %	9	9	15	14	0	18	.008
Renal colic, %	90	91	86	86	58	90	.004
Gross hematuria, %	20	21	22	21	50	34	.02
Active UTI, %	5	3	11	14	58	6	<.001
Urine pH	6.2	6.2	6.4	6.5	6.8	5.0	.04
Radiographic stone, %	89	78	85	64	83	67	<.001
-Multiple stones, %	28	31	47	58	58	30	<.001
-Stone diameter, mm	2.4	2.1	2.8	6.4	4.6	1.6	<.001
-Staghorn, %	1	1	1	8	42	2	<.001
-UVJ location, %	37	28	32	42	0	19	<.001

Conclusions: Calcium stones are more common among incident stone formers in the general population (94%) than has been reported in referral-based studies (70-80%). Presenting characteristics may help predict stone composition to guide therapy.

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FR-PO452

Endogenous Thiosulfate Excretion Is Related to Protein Intake Andreas Pasch,[1] Felix J. Frey,[1] Harry Van Goor,[3] Brigitte Frey,[1,2] Stefan Farese.[1] *[1]Department of Nephrology & Hypertension, University Hospital Bern, Inselspital, Bern, Switzerland; [2]Department of Clinical Research, University Hospital Bern, Inselspital, Bern, Switzerland; [3]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Background: Thiosulfate (TS, $S_2O_3^{2-}$) is an endogenous intermediate of sulfur metabolism. The sodium salt of TS, sodium thiosulfate (STS, $Na_2S_2O_3$) has been used as a drug for the treatment of cyanide poisoning, cisplatin toxicity, vascular calcifications and nephrolithiasis. Thus, endogenous TS excretion is of potential clinical utility. The determinants of urinary TS excretion are unknown.

Methods: Measurement of 24-hour urinary TS excretion in renal calcium stone formers (n = 40) and age and sex matched healthy controls (n = 40). Determination of volume, creatinine, sodium, calcium, phosphate, urate, oxalate, citrate, sulfate and urea in 24-hour urine. Sequencing of single nucleotide polymorphisms in the genes coding for sulfide:quinone oxidoreductase (SQR, Ile264Thr) and rhodanase (TST, Pro285Ala).

Results: Urinary TS was not different in stone formers and healthy controls (10 ± 8 µmol/day vs. 14 ± 12 µmol/day, p = 0.10) and did not correlate with urine volume or the excretion of creatinine, sodium, calcium, phosphate, urate, oxalate or citrate in 24-hour urine samples from stone formers or healthy controls. TS excretion did also not correlate with the genotype of SQR SNP Ile264Thr or TST SNP Pro285Ala. TS excretion did however correlate with urinary sulfate (p = 0.04) and urea excretion (p = 0.002), two established markers of protein intake. The estimated protein intake, derived from urinary urea excretion, was different in stone formers (1.08 ± 0.31 g/kg bw) and healthy volunteers (1.28 ± 0.33 g/kg bw, p=0.01) in our cohort. The TS-to-sulfate ratios, which might reveal differences in mitochondrial sulfur metabolism showed no differences between stone formers and healthy controls (p = 0.58).

Conclusions: Endogenous TS excretion correlated with markers of protein intake and was not different between stone formers and healthy controls in our cohort. The physiologic role of endogenous TS awaits further clarification.

FR-PO453

Hepatic and Renal sat1 and CFEX Expression in Ethylene Glycol-Induced Oxalate Nephrolithiasis in Rats Birgitta C. Burckhardt,[1] Hrvoje Brzica,[2] Davorka Breljak,[2] Ivana Vrhovac,[2] Vedran Mivek,[2] Mila Lovric,[3] Nina Schnedler,[1] Maja Henjakovic,[1] Waja Wegner,[1] Ivan Sabolic,[2] Gerhard Burckhardt.[1] *[1]Physiology and Pathophysiology, University of Göttingen, Göttingen, Germany; [2]Institute for Medical Research and Occupational Health, Zagreb, Croatia; [3]Laboratory Diagnosis, University Hospital Centre, Zagreb, Croatia.*

Background: The epidemiological incidence of oxalate nephrolithiasis is higher in men than in women. Oxalate is predominantly produced in the liver and then released into the systemic circulation by sat1, a sulfate anion transporter, which is localized in the sinusoidal membrane of hepatocytes, where it mediates exit of oxalate in exchange for sulfate. In renal proximal tubules, sat1 is responsible for basolateral uptake of oxalate into the cell and release of sulfate into the systemic circulation. Finally, oxalate is extruded into the urine by the luminal chloride-formate exchanger, CFEX, which also accepts oxalate as a substrate.

Methods: The model of ethylene glycol (EG)-induced oxalate nephrolithiasis in adult male and female rats was used to monitor changes in sat1, CFEX, and the rate-limiting enzymes of oxalate synthesis, alcohol dehydrogenase (Adh1) and hydroxy acid oxidase (Hao1). Protein and mRNA expression was studied by immunochemistry and real time RT-PCR.

Results: As compared to controls, EG-treated animals exhibited higher concentrations of oxalate in plasma and urine, and a higher abundance of oxalate crystals in urine with male dominance. Sat1 protein in liver and kidneys was male-dominant in controls, increased only in EG-treated females, while sat1 mRNA stayed unchanged. CFEX mRNA in both organs was sex-independent and unaffected by EG treatment. Adh1 and Hao1 mRNA in both organs exhibited distinct sex dependency which remained unchanged upon EG treatment. A general male-dominant Hao1 expression in kidneys of untreated rats was also shown by microarray analysis.

Conclusions: In conclusion, despite hyperoxaluria in EG-treated animals, the expression of sat1 in males and of CFEX in both sexes was sufficient to handle the EG-induced production and secretion of oxalate.

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FR-PO454

Insufficient Adjustment of Water Intake Associated with Western-Rich Diet Increases the Risk of Urine Crystallization Inmaculada Buendia,[1] Oriane Dohein,[1] Marion Vallet,[3] Ivan A. Tack,[3] Michel Daudon.[2] *[1]Danone Research RD128, Palaiseau, France; [2]Dpt of Clinical Physiology, Tenon Hospital, APHP, Paris, France; [3]Dpt of Clinical Physiology, CHU Toulouse, Toulouse, France.*

Background: Calcium oxalate (CaOx) nephrolithiasis is often related to dietary habits and low diuresis. Indeed, dilution of urine is efficient for CaOx kidney stone prevention. High energy intake based on Western diet results in high consumption of salted animal proteins leading to increased protein catabolism responsible for the majority of daily urine waste. Surprisingly, only little attention has been paid to spontaneous adjustment of fluid intake according to the diet.

Methods: We have examined the relationship between Body mass index (BMI), considered to reflect global energy intake, and daily osmotic load, waste excretion (24 hours urine osmolality, oxalate, calcium, citrate, urea and sodium) and the Tiselius crystallization risk index (Tcri) in a population of 312 (F/M: 128/184) recurrent stone formers (RSF). Results were compared to those of 48 healthy control volunteers (F/M: 21/27) from a previous study.

Results: In RSF: 1) BMI positively and significantly correlated with osmotic load, 24h urine urea, sodium, uric acid, oxalate and citrate but not with calcium and magnesium urine excretion; 2) BMI was positively correlated with 24h urine osmolality and Tcri but not with urine volume; as a result, BMI was negatively correlated with free water clearance, a confounding factor of the relationship between BMI and Tcri.

In control subjects: BMI positively and significantly correlated with 24h excretion of most urine waste but also with urine volume. As a consequence BMI was neither correlated with urine osmolality nor with free water clearance or Tcri.

Conclusions: In conclusion, in renal stone formers, by contrast to healthy subjects, high energy and protein intake is not associated with a significantly higher urine volume, leading to more concentrated urine and an increased risk of CaOx crystallization. This observation stresses the interest to screen urine osmolality in RSF exhibiting a western high energy diet and, if necessary, to educate them to adjust water intake in order to prevent CaOx kidney stones.

FR-PO455

Standard Dose Hydrochlorothiazide Is Associated with Less Urinary Calcium Reduction Compared to Chlorthalidone Dawn F. Wolfgram,[1] Brad C. Astor,[1] Amarinder Singh Garcha,[2] Vinod K. Gundu,[2] Stephen J. Knohl,[2] Roy A. Jhagroo.[1] *[1]Medicine, University of Wisconsin, Madison, WI; [2]Medicine, Upstate Medical Center, Syracuse, NY.*

Background: Medical treatment of calcium nephrolithiasis is centered on thiazide diuretics to reduce urinary calcium (UCa) levels; with increased reduction in UCa correlating with decreased risk of stone recurrence. Recent data demonstrate effective BP lowering at low doses of thiazides, prompting use of lower doses in stone prevention, as well. In addition there has been a switch from chlorthalidone (CTL) to less potent hydrochlorothiazide (HCTZ) due to concerns over hypokalemia with CTL. It is unknown whether low doses of HCTZ are effective in lowering UCa levels to target levels. We hypothesize that UCa reduction will be greater with CTL than HCTZ when comparing currently used doses.

Methods: Observational study of stone-forming individuals seen in a metabolic stone clinic over a two year period. Data included patient demographics, co-morbidities, and 24h urine electrolyte composition. Primary outcome was the change in 24 hour UCa comparing HCTZ and CTL.

Results: 322 patients were identified with 112 meeting criteria and used in analysis. The majority were placed on HCTZ (n=42) or CTL (n=47) 25mg QD. Patients on CTL 25mg had a greater reduction in UCa (164 mg; 41%) than those on HCTZ (85mg; 21%), p = 0.006. When comparing a higher dose of HCTZ (50mg) with a lower dose of CTL (25mg) there was no significant differences in 24h UCa reduction at 144mg va 164mg, respectively p = 0.712. Neither CTL nor HCTZ at 12.5mg QD significantly lowered UCa. There was a decrease in serum [K] of 0.5Meq/L (p = 0.001) in patients on CTL 25mg daily, but no serum [K] recorded below 3.3, or cardiac arrhythmia recorded.

Conclusions: In our study there was greater reduction of UCa levels with CTL compared to HCTZ. Although patients on CTL 25mg had a decrease in serum [K] there was no severe hypokalemia or adverse effects documented at that dose. Our data shows that UCa reduction is greater on CTL compared to similarly dosed HCTZ without increased risk of severe hypokalemia.

FR-PO456

Urinary Macromolecular Net Charge: A Promising New Test for Kidney Stone Risk Jeffrey Wesson,[1,2] Ann M. Kolbach,[2] Kevin P. Tucker,[2] Vishal N. Ratkalkar,[2] Jack G. Kleinman.[2] *[1]Medicine, VA Medical Center, Milwaukee, WI; [2]Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Background: There are hundreds of urinary macromolecules (UM) in normal urine, many thought to inhibit stone formation, including strongly anionic proteins like osteopontin and Tamm-Horsfall protein. Though some cationic proteins are found in urine, the overall Net Charge (NC) is anionic. Model polymer studies have shown that polymers or mixtures with very low NC aggregate, which then induces calcium oxalate crystal aggregation. We have tested the hypothesis that stone formers have lower UM NC (weaker inhibitor function) than normals in two independent patient panels, as reported below.

Methods: Freshly voided mid-morning urine samples were obtained from stone formers (SFU) and normals (NU - matched for age, gender, and race) and protease inhibitors were added. UM were isolated immediately by ultradiafiltration through a 10kD cutoff membrane, using a 100mM NaCl buffer for the first panel and 10mM NaCl buffer for the second panel (to prevent loss of UM through precipitation). NC was determined by mixing varied amounts of UM with known quantities of polyarginine (pR) and measuring the absorbance at 400nm. NC was defined as the mass ratio (pR/UM) of the mixture showing maximum absorbance.

Results: The NC results (see Table) show that NC for SFU was about 20% lower than the NC in NU in both panels. The difference in absolute values for NC between the two panels for both SFU and NU was a consequence of differences in pR lots.

Urine Macromolecular Net Charge Results:

+/-,pR/UM	Urine Panel 1		Urine Panel 2	
NU	0.74±0.14	n=22	0.55±0.10	n=13
SFU	0.60±0.09	n=22	0.44±0.14	n=23
p-Values	p=0.0008		p=0.04	

Conclusions: The reduction of NC in SFU is consistent with reduced crystallization inhibition (particularly aggregation) for UM from SFU compared to NU. While the distributions of NC values in the two populations were overlapping, low UM NC appears to be predictive of stone formation and this simple urine test may be useful in identifying patients from a random population at risk for stone disease,and thus allowing for pre-emptive treatment.

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FR-PO457

Can Highly Precise Microanalytical Methods Be Helpful for Understanding the Detail Insight and Growth Behavior of Primary Hyperoxaluria (PH) and Non-PH Stones? Michaela Gessner,[1] Dorrit E. Jacob,[2] Bernd Grohe,[3] Bodo B. Beck,[4] Bernd Hoppe.[1] *[1]Department of Child and Adolescent Medicine, Pediatric Nephrology, University Hospital, Cologne, Germany; [2]Earth System Science Research Centre and Institute for Geosciences, Johannes Gutenberg University, Mainz, Germany; [3]Schulich School of Medicine & Dentistry, Western University, London, ON, Canada; [4]Institute of Human Genetics, University Hospital, Cologne, Germany.*

Background: There are currently 3 types of primary hyperoxaluria (PH) known. All are rare autosomal-recessive inherited disorders of the glyoxylate metabolism. They manifest with varying degrees of nephrolithiasis and/or nephrocalcinosis and also early progression to ESRD in PH1. The diagnosis is frequently delayed and often only made in ESRD. Until now urine excretion parameters and mutation analysis led to the diagnosis. Our study was to determine on whether more specific stone analysis using methods of geosciences allow a faster diagnosis and a deeper insight in stone pathology and growth, so that this data can later be used to develop new therapeutic approaches.

Methods: Kidney stones from PH and non PH-patients were compared. Fragments of stones were embedded in epoxy resin, polished with diamond paste and analyzed by reflected light microscopy and by Raman spectroscopy. Native stone fragments were analyzed by scanning electron microscopy and X-ray microanalysis.

Results: PH1 stones and stones of patients with idiopathic hyperoxaluria consists mainly of calcium-oxalate monohydrate (COM), while the white-yellowish porous PH 3 stones mainly consists of calcium-oxalate dihydrate (COD) and smaller COM inclusions. But PH stones and non-PH stones differ clearly in crystal morphology. The PH stone has a looser and disordered crystal morphology, while the non-PH stone seems to be well ordered. Also, mineral components in the stones such as magnesium, phosphate and sodium were present in different amounts.

Conclusions: The more differentiated breakdown and understanding of the structure and composition of kidney stones can possibly help to understand the differences in clinical expression and may help to open new possibilities for therapeutic approaches.

Funding: Private Foundation Support

FR-PO458

Acid and Protein Enhance Calcium Oxalate Deposition in Experimental Nephrolithiasis Jack G. Kleinman,[1,2] Laura J. Alatalo,[1] Ann M. Kolbach,[1] Jeffrey Wesson.[1,2] *[1]Medicine, Medical College of Wisconsin, Milwaukee, WI; [2]Medicine, Veterans Affairs Medical Center, Milwaukee, WI.*

Background: Acid-ash high protein diets (HP) are recognized as a risk factor for kidney stones. We reported that the mean net charge (NC) of urinary macromolecules (UM) in stone formers is less negative than in non-stone formers. These UM inhibit processes involved in stone formation but are less effective when their NC is less negative. We also reported that enhancing UM NC by infusing a highly anionic macromolecule poly(acrylic acid) inhibits Ca oxalate (CaOx) deposition in ethylene glycol (EG)-fed rats. The studies reported here were designed to ascertain whether HP is associated with a less negative mean NC of UM and also renders rats more susceptible to CaOx deposition when they are fed EG.

Methods: Male SD rats were either fed standard purified diet (NP) with 1% NH_4Cl (acid) or a 40% protein (HP) casein-based diet with acid. 24-h urines were obtained at intervals to ascertain UM NC. Other rats were either fed NP or a 40% HP diet with acid. 24-h urines for chemistries were obtained on the day prior to & on the 8th day of being fed 0.2, 0.4, & 0.8% EG in the drinking water and then kidneys were obtained to determine CaOx deposition.

Results: The NC of UM had declined by 40% at 1 week in the rats on the acid-HP diet & the effect of acid and HP appeared to be additive. Rats on NP did not have significant CaOx deposition until they were fed 0.8% EG; the rats fed the acid-HP had significant CaOx crystal deposition when fed 0.4% EG. The decrease in the threshold for CaOx deposition with acid-HP could not be attributed to a difference urinary oxalate. Calcium excretion & the relative supersaturation (RSS) of the rat urine with respect to CaOx were significantly higher in the acid-HP group; however in the groups that did not receive EG, there were no significant differences in Ca excretion & RSS. This suggests that the RSS change in EG-treated rats was the result rather than the cause of the increased CaOx deposition.

Conclusions: These data are consistent with the hypothesis that a HP diet contributes to the risk of developing Ca nephrolithiasis and that it does so by rendering the NC of UM less negative.

Funding: NIDDK Support

FR-PO459

Effect of pH on Cystine Binding Capacity of Thiol Drugs John R. Asplin, Daniel M. Asplin. *Litholink Corporation, Chicago, IL.*

Background: Pharmacologic therapy for cystinuria consists of alkali salts to raise urine pH and thiol drugs to form cysteine-drug complexes. The large number of pills required for these two therapies affects compliance, leading some clinicians to discontinue alkali when thiol drugs are used. However, the effect of alkalinizing urine on the cystine binding capacity of thiol drugs has not been studied.

Methods: Urine samples were obtained from 4 healthy subjects and 5 aliquots of each urine had pH adjusted from 6.0 to 8.0. Urine samples were incubated with cystine crystals at 37C for 5 minutes and 48 hours. Standard measurements of supersaturation are 48 hours, but shorter incubation times are more germane to in vivo conditions for cystine-thiol drug interaction. Comparisons were made between urine with and without the thiol drugs Tiopronin (TIO) or d-Penicillamine (dPCN), each added at a concentration of 2 mM. The primary endpoint was the amount of cystine that could dissolved in the urine samples, known as cystine capacity (CysCap), measured using a nitroprusside assay (Kidney Int 69:1041, 2006).

Results: In urine without thiol drug, CysCap increased from 230 ± 19 to 286 ±25 mg/l as urine pH was increased from 6.0 to 8.0 at 48 hrs. In the 48 hr incubations CysCap increased dramatically when either of the thiol drugs were added to urine and was not significantly influenced by pH. However, when incubation time was shortened to 5 minutes, representative of the dwell time of urine in the renal pelvis, the effect of thiol drugs to solubilize cystine was greatly dependent on urine pH.

Table 1. Effect of pH and thiols on CysCap (mg/l) at 5 minute incubation

pH	Control	TIO	dPCN
6.0	158 ± 5	207 ± 34	180 ± 26
6.5	163 ± 9	272 ± 58	221 ± 31
7.0	165 ± 14	390 ± 57	298 ± 39
7.5	191 ± 11	460 ± 40	385 ± 35
8.0	226 ± 14	492 ± 33	432 ± 28

Conclusions: Increasing urine pH greatly increased the efficacy of thiol drugs to complex and solubilize cystine. In order to maximize benefit from thiol drugs, alkali therapy should be used in conjunction with thiol drugs with the goal of keeping urine pH at 7.5 or above. Clinical trials are needed to confirm these in vitro findings.

FR-PO460

Heritability of the Coefficient of Variation in a Sample of Stone-Former Probands and First-Degree Relatives Guy M.L. Perry,[1] Steven J. Scheinman,[1] John R. Asplin.[2] *[1]Medicine, SUNY Upstate Medical University, Syracuse, NY; [2]Medicine, SUNY Upstate Medical University, Syracuse, NY; [3]Litholink Inc., Chicago, IL.*

Background: Classical genetics usually consists of the estimation of the heritability of single observations as indicative of genetic means. However, our work indicates genetic variance for *residuals* in renal solutes, including heritability, gender effects and quantitative trait loci (QTL) for the coefficient of variation (*CV*) in urinary calcium in a rodent model of hypercalciuria and kidney stone formation. We hypothesized that urinary solute *CV*s were also heritable in humans.

Methods: We investigated the heritability of the *CV*s in urinary solutes in 949 kidney stone probands from 249 families with relationship structures ranging from proband-sib pairs to full-sib families with multiple offspring measured on two consecutive days.

Results: Narrow-sense (additive) heritability was non-zero for *CV*s for urinary calcium, citrate and sulfate. There was also dominant genetic variance for citrate, sodium, phosphorus and uric acid (Table 1). Sex-by-family interaction effects on *CV* were significant for citrate and uric acid ($P<0.001$).

Table 1. Narrow- (additive, h^2_a) and broad-sense (dominant, h^2_d) estimates of heritability for 2-day coefficients of variation (CV) in urinary calcium (Ca), citrate (Cit), sodium (Na), ammonium (NH_4), phosphorus (P), sulfate (SO_4) and uric acid (UA) in 949 stone-former probands and first-degree relatives.

CV	h^2_a	h^2_d
Ca	0.076 (0.071)	0.041 (0.055)
Cit	0.234 (0.063)	0.079 (0.043)
Na	0.0 (0.051)	0.124 (0.049)
NH_4	0.088 (0.047)	0.0 (0.0)
P	0.0 (0.0)	0.083 (0.035)
SO_4	0.049 (0.048)	0.0 (0.0)
UA	0.0 (0.0)	0.047 (0.030)

Conclusions: Genetic and sex-by-genotype effects on *CV*s in renal solutes corresponds to previous findings for this phenomenon. Genetic variance for urinary solute *CV*s might affect baseline renal phenotyping, produce heritable uncertainty in stone precursors (phosphorus, calcium) or protective solutes such as citrate, or affect the outcome of conventional genetic mapping and/or candidate analysis in renal solute phenotype. Other work of ours suggests that even small (~2%) alterations in residual error are sufficient to affect genetic analysis of solute traits.

Funding: Clinical Revenue Support

FR-PO461

Netrin-1 Regulates Inflammatory Response of Neutrophils by Suppressing COX-2 Mediated PGE2 Production and Suppresses Ischemic Acute Kidney Injury Punithavathi Vilapakkam Ranganathan, Riyaz Mohamed, Calpurnia Jayakumar, Ganesan Ramesh. *Medicine, Vascular Biology Center, Georgia Health Sciences University, Augusta, GA.*

Background: Inflammation is a major causative factor for acute and chronic diseases. The molecule netrin-1 has been shown to regulate inflammation but the mechanism by which this occurs are unknown. We show here the unexpected role of netrin-1 in the regulation of the production of prostanoid metabolite PGE2 from neutrophils in in vitro and in vivo disease models.

Methods: Ischemia reperfusion injury of the kidney was induced by clamping renal pedicle clamping for a period of 26 minutes followed by reperfusion in wild type and RAG-1 knockout mice. Some of these mice received recombinant netrin-1 or vehicle 1hr before renal pedicle clamping. Kidney function, inflammation and immune cell infiltration were quantified by immunostaining, RT-PCR, ELISA, measuring serum creatinine and Western blot analysis.

Results: Ischemia reperfusion in wild-type and RAG-1 knockout mice induced severe kidney injury that was associated with a large increase in neutrophil infiltration and COX-2 expression in the infiltrating leukocytes and proximal tubular epithelial cells. Administration

of netrin-1 suppressed COX-2 expression, PGE2 production, and neutrophil infiltration into the kidney and was associated with reduced apoptosis, inflammatory cytokine and chemokine expression, and improved kidney function. Moreover, administration of PGE2 receptor EP4 agonist enhanced neutrophil infiltration and renal injury which was not inhibited by netrin-1. Consistent with in vivo data, addition of IL-17 increased COX-2 expression and IFNγ production, which was inhibited by addition of netrin-1. Netrin-1-mediated suppression of IFNγ production was abolished by addition of PGE2.

Conclusions: Our results suggest that netrin-1 regulates inflammation and infiltration of neutrophils through COX-2-mediated PGE2 production in neutrophils and renal tubular epithelial cells, and could be a potential drug for treating many inflammatory immune disorders.

Funding: NIDDK Support

FR-PO462

Obesity Magnifies Kidney and Peri-Renal Fat Inflammation and Fibrosis in Experimental Renal Artery Stenosis Xin Zhang,[1] Zilun Li,[1] Alfonso Eirin,[1] Kyra L. Jordan,[1] Amir Lerman,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1,2] *[1]Division of Nephrology and Hypertension, Mayo Clinic; [2]Division of Cardiovascular Diseases, Mayo Clinic, MN.*

Background: Obesity has been increasing in prevalence and is an independent risk factor for chronic kidney disease. Obesity often coexists with ischemic nephropathy due to renal artery stenosis (RAS), yet its impact on the stenotic kidneys (STK) and the underlying mechanisms remain unexplored.

Methods: Obesity-prone Ossabaw pigs were randomized as Lean, Obese, Lean RAS, and Obese RAS (n=6 each group) fed an atherogenic (Obese) or standard (Lean) diet for 16 wks. Unilateral RAS was induced after 12 wks of diet, and 4 wks later single-kidney glomerular filtration rate (GFR) assessed by CT. Oxidative stress was measured in plasma by 8-isoprostane and in the STK by p47phox expression. Inflammation was assessed in both STK and peri-renal fat tissue by the ratio of pro-inflammatory (M1, CD163+/iNOS+) vs. reparative (M2, CD163+/Arginase+) macrophages (MØ), and fibrosis by trichrome staining.

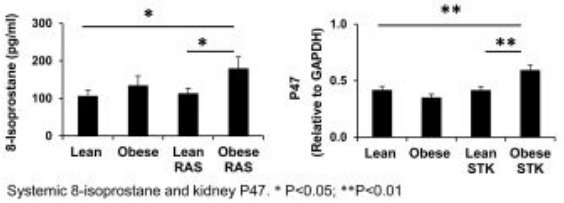
Results: Obese pigs had higher body weight and cholesterol levels than Lean. Both RAS groups achieved comparable stenosis and hypertension. Obesity in RAS increased 8-isoprostane and STK p47 expression (Figure, B), suggesting systemic and local oxidative stress (Figure, B). Obesity increased M1 MØ abundance in both STK and peri-renal fat (Figure, C). Obesity in RAS tended to increase STK fibrosis (P=0.08 vs. Lean STK; P<0.05 vs. Lean) and significantly increased peri-renal fat fibrosis (P<0.05 vs. Lean STK).

A

	Lean	Obese	Lean RAS	Obese RAS
Body weight (kg)	35.5±5.0	48.7±6.2*	34.6±2.1	47.0±1.5*†
Total Cholesterol (mg/dl)	95.5±2.4	431.3±29.5*	90.4±5.2	398.3±38.1*†
MAP (mmHg)	101.5±5.0	110.7±18.7	124.7±4.2*	131.5±6.6*
Single kidney (STK) GFR (ml/min)	53.2±16.8	56.8±10.1	31.8±8.9	35.0±7.4*

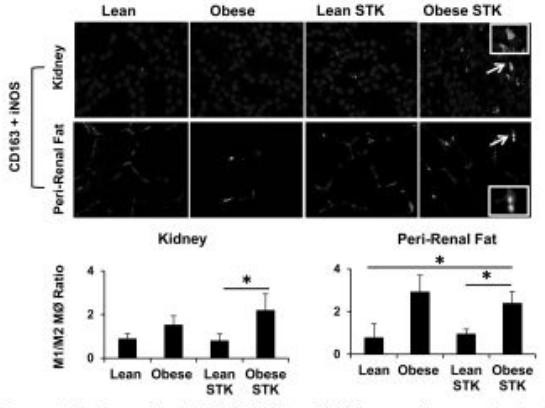
MAP: mean arterial pressure; STK: stenotic kidney; GFR: glomerular filtration rate. * P<0.05 vs. Lean; † P<0.05 vs. Lean RAS.

B



Systemic 8-isoprostane and kidney P47. * P<0.05; **P<0.01

C



Representative images for M1 MØ (CD163+ red /iNOS+ green, 40x, merged yellow) staining in kidney and peri-renal fat, and quantifications for M1/M2 MØ ratio for both. * P<0.05

Conclusions: Obesity aggravates STK oxidative stress, inflammation, and fibrosis. These deleterious effects may be amplified by pathological remodeling of peri-renal fat in RAS, and establish obesity a significant risk factor for kidney disease progression.

Funding: NIDDK Support, Other NIH Support - NHLBI

FR-PO463

Metabolic Syndrome Exacerbates Contralateral Kidney Hyperfiltration and Fat Deposition in Renovascular Hypertension Xin Zhang,[1] Zilun Li,[1] Alfonso Eirin,[1] Aditya S. Pawar,[1] Hui Tang,[1] Kyra L. Jordan,[1] John R. Woollard,[1] Amir Lerman,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1,2] *[1]Division of Nephrology and Hypertension, Mayo Clinic; [2]Division of Cardiovascular Diseases, Mayo Clinic, MN.*

Background: The prevalence of metabolic syndrome (MetS) is increasing and accelerating the progression of many chronic kidney diseases. We hypothesized that MetS aggravates contralateral kidney (CLK) dysfunction in renovascular hypertension (RVH).

Methods: Ossabaw pigs in Lean, MetS, Lean RVH and MetS RVH groups (n=6 each) were fed with atherogenic or standard diet for 16 wks, Unilateral renal artery stenosis was induced after 12 wks of diet, and 4 wks later single-kidney glomerular filtration rate (GFR) assessed by CT. Systemic oxidative stress was assessed by oxidized (ox)-LDL levels and inflammation by TNF-alpha. Kidney fat deposition was assessed by Oil Red O staining and triglyceride (TG) content, inflammation by the ratio of inflammatory (M1, iNOS+) vs. reparative (M2, Arginase+) macrophages (MØ, CD163+), and fibrosis by trichrome staining.

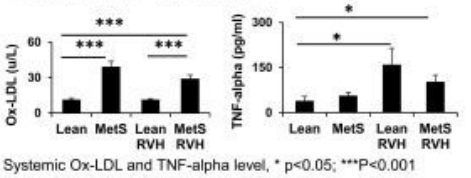
Results: MetS increased insulin resistance, body weight, and cholesterol levels, but did not affect blood pressure in RVH (Figure, A). MetS did not affect stenotic kidney GFR, but increased CLK GFR (P<0.05 vs. Lean), suggesting hyperfiltration. Systemic ox-LDL was increased in both MetS and MetS RVH and TNF-alpha level increased in both RVH groups (Figure, B). MetS increased in the RVH CLK fat deposition, M1/M2 MØ ratio (Figure, C), and fibrosis (P<0.01 vs. Lean; P<0.05 vs. Lean RVH CLK).

A

	Lean	MetS	Lean RVH	MetS RVH
Body weight (kg)	31.4±4.3	45.0±5.7*	35.7±2.1	45.3±1.1*#
HOMA-IR (U/ml×mg/dl)	3.6±0.3	6.7±2.4*	2.6±0.5	10.5±2.9*#
Total Cholesterol (mg/dl)	97.0±2.9	388.0±48.1*	85.4±5.0	346.7±40.6*#
MAP (mmHg)	104.7±6.9	105.1±11.5	122.3±4.4*	130.1±5.9*
Single-kidney (CLK) GFR (ml/min)	50.1±6.9	55.8±2.4	60.8±3.6	75.0±6.8*#

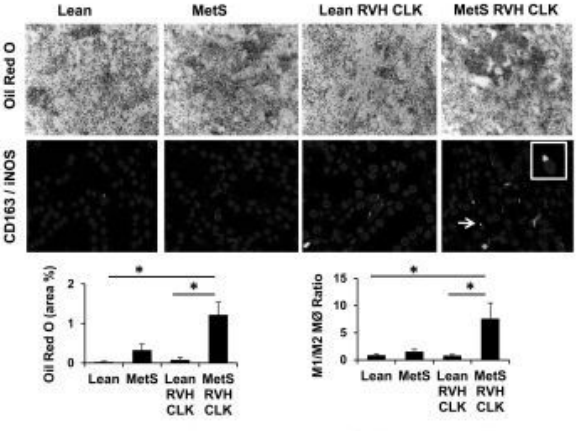
HOMA-IR: homeostasis model assessment insulin resistance; MAP: mean arterial pressure. * P<0.05 vs. Lean; # P<0.05 vs. Lean RVH

B



Systemic Ox-LDL and TNF-alpha level, * p<0.05; ***P<0.001

C



Representative images for Oil red O (10x) and M1 MØ (CD163+ red /iNOS+ green, 40x, merged yellow), and quantifications for oil red o and M1/M2 MØ ratio . * P<0.05

Conclusions: MetS amplifies renal hyperfiltration in the contralateral kidney in RVH, associated with increased fat deposition, recruitment of activated M1 MØ, and fibrosis, indicating adverse tissue remodeling. Thus, MetS is a significant risk factor for kidney injury associated with RVH. Strategies are needed to attenuate its impact on the kidney.

Funding: NIDDK Support

FR-PO464

The Role of Macrophage Stimulating Protein and Its Receptor Recepteur d'Origine Nantais in Renal Ischemia Reperfusion Injury Koeun Lee, Chang Seong Kim, Joon Seok Choi, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: Macrophage stimulating protein (MSP) and its receptor RON, recepteur d'Origine nantais, play an important role in the cell proliferation and migration. We have investigated the role of MSP/RON pathway in tubule regeneration in ischemia-reperfusion (IR) injury.

Methods: We induced bilateral renal IR injury in C57BL/6 mice by clamping both renal pedicles for 30min and sacrificed on 1 or 5 days after IR injury. The expression of MSP, RON, COX-2, Bcl-2, Bax and caspase-3 was determined by immunoblotting. The mRNA level of IL-1β and TNF-α was measured by real-time PCR. Using an in vitro model, human renal proximal tubular (HK-2) cells were incubated with hydrogen peroxide (H_2O_2) for 24h at different concentrations of MSP, and cell viability was measured by MTT assay. The protein expression of mitogen-activated protein kinases (MAPKs), and nuclear factor-kappa B (NF-κB) was detected by immunoblotting. Apoptosis was determined by flow cytometry analysis after HK-2 cells were stained by fluorescein isothiocyanate conjugated annexin V protein and propidium iodine.

Results: Plasma creatinine was markedly increased on day 1 after IR injury compared with controls, which was attenuated on day 5. Accordingly, protein level of Bax/Bcl-2 ratio, COX-2 expression and mRNA level of IL-1β and TNF-α in the kidney were increased on day 1, which were attenuated on day 5. The expression of MSP was upregulated on day 1 and 5, but RON was markedly decreased only on day 5. H_2O_2 treatment decreased cell viability, which was counteracted by MSP. Flow cytometry analysis revealed MSP can prevent H_2O_2-induced apoptosis. Additionally, MSP attenuated phosphorylation of P38 and NF-κB expression.

Conclusions: In conclusion, the protein level of MSP was increased during the regeneration of I/R injured tubular cells, and MSP attenuated H_2O_2-induced apoptosis in HK-2 cells through the modulation of P38 and NF-κB pathways, suggesting a beneficial potential role of MSP/RON in renal tubule cell regenaration.

FR-PO465

Delayed Inhibition of PAR-1 Signaling Ameliorates Renal Ischemia Reperfusion Injury: Potential Role of Sphingosine-1-Phosphate Signaling

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Background: To determine if delayed treatment with a PAR-1 inhibitor SCH79797 could reduce renal injury following renal ischaemia reperfusion injury (IRI). Background: Thrombin may signal via PAR-1 to contribute to renal injury following renal IRI. PAR-1 may signal via Sphingosine -1-phospahe (S1P) generation and receptor signalling. S1P signalling by the S1Pr1 receptor has been suggested to be protective and SIPr3 signalling injurious. Pre-treatment of mice with SCH79797 a PAR-1 inhibitor reduces renal injury following renal IRI. We tested whether delayed treatment with SCH79797 could ameliorate renal injury following reperfusion.

Methods: Using a mouse model of renal IRI we examined the effect of administration of SCH79797 30 min after reperfusion. We also examined the effects of PAR-1 inhibition on SPHK 1 and 2 and SIPr1 and 3 mRNA and protein expression.

Results: SPHK 1 and 2 and S1Pr1 and 3 mRNA and protein expression were induced at 24 h reperfusion in C57BL/6 mice compared to sham operated mice. Mice administered SCH79797 2 h pre ischemia had reduced renal injury as measured by serum creatinine and reduced expression of SPHK 1 and 2 and S1Pr1 and 3 mRNA and protein. To further assess the potential importance and timing of PAR-1 signalling we administered SCH7979 30 min after IRI at time at which time injurious signalling pathways have been shown to be activated.

SCH79797 administered 30 min post reperfusion reduced renal injury as assessed by serum creatinine relative to control mice (133±5 micromol/l vs 163±6 micromol/l p <0.005). Thus suggesting a potential therapeutic role for delayed PAR-1 inhibition.

Conclusions: Taken together our data suggests that PAR-1 inhibition by SCH7979 may be protective when administered both pre and post reperfusion suggesting a potential therapeutic benefit. This protection may at least in partoperate by reducing S1Pr3 signalling and inflammation.

FR-PO466

RGS4 Protects against Vascular Smooth Muscle Cell-Mediated Reperfusion Injury in the Kidney

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Background: Regulator of G protein Signaling 4 (RGS4) is a modulator of smooth muscle vasoconstriction in the kidney while the known systemic anti-inflammatory effects of RGS4 in the same cell type have not been investigated in the microvasculature of the kidney.

Methods: LacZ knock-in RGS4 reporter mouse; transgenic RGS4-eGFP reporter mouse; An $rgs4^{flox/flox}$ mouse was generated and crossed to a smooth muscle myosin heavy chain (SMMHC)-Cre mouse. Animals were used in ischemia-reperfusion injury experiments.

Results: LacZ RGS4 reporter mice demonstrated an increase in RGS4 expression localized to smooth muscle cell-specific myosin-expressing cells in the corticomedullary junction acutely 4 hours after moderate ischemic injury. RGS4-eGFP mice that demonstrate a 5-fold overexpression of RGS4 in the kidney at baseline were found to be resistant to moderate ischemic injury (serum creatinine [Cr], tubular injury score). To isolate the in vivo role of RGS4 in microvascular injury, separate from tubular injury, animals underwent ultra-mild ischemia reperfusion injury (UMIRI), that is, 10 minute unilateral ischemia. Only endothelial injury could be identified 18 hours after injury by morphology, immunostaining, and quantitative analysis. Calponin-1 was not elevated in vasculature of any cohort above baseline indicating a non-vasoconstrcited state. VCAM expression was enriched in congenic controls compared to RGS4-eGFP. To investigate the functional anti-inflammatory role of RGS4 expression in vascular smooth muscle cells of the kidney SMMHC-Cre-$Rgs4^{flox/flox}$ and SMMHC-Cre underwent UMIRI followed by intravenous injection of magnetic iron oxide nanoparticle-47 (MION-47) to detect regional macrophage infiltration. MION-47 sequestration in the corticomedullary junction was elevated in SMMHC-Cre-$Rgs4^{flox/flox}$ compared to SMMHC-Cre as evaluated by high resolution T2-weighted magnetic resonance imaging.

Conclusions: RGS4 expressed in renal vascular smooth muscle cells has an important role in dampening ischemia-induced endothelial dysfunction that results in leukocyte localization and reperfusion injury.

Funding: NIDDK Support

FR-PO467

Adenosine 2A (Adora2a) Agonists Block Pro-Inflammatory Role of Type I IFNs in Kidney Ischemia-Reperfusion Injury (IRI)

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Background: The interplay of innate and adaptive immunity contributes to the pathogenesis of kidney IRI. Type I interferons (IFN-a and b) produced from plasmacytoid dendritic cells (pDC) and known for their role in viral immunity. In this study, we investigated the function of type I IFNs and pDC in kidney ischemia-reperfusion injury (IRI).

Methods: Real-time PCR, FACS and HE staining were used in this study.

Results: IFN-b mRNA increased in kidney as early as 2 hrs during the 24 hr reperfusion period and peaked at 6 hrs (20 fold, P<0.01 compared to sham). Neutralization antibodies for IFN-a or IFN-b (50 μg/mouse) given 18 hrs prior to IRI reduced plasma creatinine compared with isotype control antibody treated mice [37% or 29% % of control](P all <0.05). Gating on the $CD45^{+}7AAD^{-}$ live kidney leukocytes, a significant increase in the number of $CD11c^{+}B220^{+}$ pDC measured by FACS in kidney after IRI compared with sham (P<0.05). To test the role of pDC in kidney IRI inflammation, monoclonal anti-PDCA-1 (Miltenyi Biotec) or anti-pDC/IPC (120G8.04, IMGENEX) antibodies (50 μg) were used to deplete pDC prior to kidney IRI. pDC-depleting antibodies prevented the rise in plasma creatinine following IRI that was observed with isotype control antibody (15% or 27% of control; P all <0.05). To further elucidate the role of type I IFNs in kidney IRI inflammation, $IFNaR^{-/-}$ mice were subjected to kidney IRI. Plasma creatinine in $IFNaR^{-/-}$ mice after IRI was 44% of WT mice (P<0.05) and kidney morphology was preserved. The adenosine A2a receptor (adora2a) agonist, ATL313, administered 18 hrs prior to kidney IRI led to marked kidney protection and reduction of kidney IFN-b mRNA.

Conclusions: We conclude that pDC and/or type I IFN contribute importantly to the pathogenesis of kidney IRI and that targeting this pathway with adora2a agonists may offer a unique and potent strategy to attenuate kidney IRI.

Funding: NIDDK Support, Private Foundation Support

FR-PO468

Identification of a Novel Hypoxia-Inducible Factor 1 (HIF-1) Regulator, CCAAT/Enhancer-Binding Protein Delta (CEBPD) in Kidney Disease

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Background: HIF-1 plays a critical role in tubular cells of both acute and chronic ischemic kidney. Given an important role of HIF in kidney diseases, we aimed to identify a novel gene involved in regulation of HIF-1.

Methods: Microarray analysis of the renal cortex of rat renal artery stenosis (RAS) model (day3 and day7) was used as in vivo screening for potential HIF-1 regulating genes. HeLa cells transfected with shRNA plasmids against continuously regulated genes in RAS kidney compared with sham kidney were evaluated for potency of HIF-1 regulation. Acute and chronic rat ischemic models were analysed for this gene's expression to localize and identify its role in kidney. To confirm its HIF-1 regulation in cultured human renal tubular cells, we performed knockdown and overexpression experiments in HK-2 cells.

Results: 150 genes were extracted from microarray analysis. shRNA library screening revealed CEBPD as the most promising HIF-1 upregulator. CEBPD is a transcription factor involved in regulation of inflammatory responses. In vivo, CEBPD's mRNA was upregulated in the cortex of RAS, 5/6 nephrectomy, ischemia reperfusion injury, and cisplatin nephrotoxicity. Immunohistochemistry clarified its prominent expression in the nuclei of tubular cells of S3 segment, the most hypoxic portion in ischemic renal injury. In HK-2 cells, hypoxia augmented CEBPD protein expression via HIF-1 independent pathway, and siRNA-mediated knockdown of CEBPD resulted in downregulation of HIF-1α protein expression, target genes of HIF-1, and hypoxia- response element (HRE) reporter activity. HIF-1 was regulated at transcription level.

Conclusions: These results altogether demonstrate that CEBPD is a novel HIF-1 upregulator in kidney. We also identified CEBPD's expression profile in various kidney disease models. CEBPD is likely to contribute to protection of tubular cells from its injury through HIF-1, which can be a therapeutic approach for kidney diseases, especially under co-existing condition of ischemia and inflammation.

Funding: Government Support - Non-U.S.

FR-PO469

HIF-1 in Myeloid Cells Is Protective in Acute and Chronic Kidney Injury
Alexander Weidemann,[1] Joanna Kalucka,[1] Gunnar Schley,[1] Bernd Klanke,[1] Jonathan Jantsch,[2] Susanne Olbrich,[1] Jasmin Baumgartl,[1] Kerstin U. Amann,[3] Kai-Uwe Eckardt.[1] *[1]Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Microbiology, University of Erlangen-Nuremberg, Erlangen, Germany; [3]Nephropathology, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Apart from tubular damage, acute (AKI) and chronic kidney injury (CKI) triggers an inflammatory cell response. HIF-1 is central for the adaptation to low oxygen conditions and also critical for inflammatory macrophage responses. Stabilization of HIF-1 is protective in models of AKI and CKI, however the contribution of HIF-1 in inflammatory cells is still unclear. To assess the specific role of myeloid HIF, we used a genetic model of HIF-1 deletion in myeloid cells in models of both, AKI and CKI. Moreover, in a model of CKI, wt and HIF-1ko mice were treated with a PHD-inhibitor to assess whether HIF in inflammatory cells is necessary to confer protection.

Methods: LysM-Cre mice were used to specifically delete HIF-1 in myeloid cells. AKI was induced by bilateral clamping for 30min. CKI was induced by feeding an adenine- rich diet for 3 wks. PHD-inhibitor was administered daily during the adenine diet.

Results: Deletion of HIF-1 in myeloid cells significantly impares recovery after AKI. Acute tubular necrosis is increased, influx of inflammatory cells is accelerated and tubular regeneration reduced. HIF-1 ko mice also exhibit a significantly impared renal function in CKI. Inflammatory and fibrotic genes are upregulated, however with no difference between genotypes. Adenine induced renal failure is ameliorated by concomittant administration of a PHD-inhibitor with significant reduction of creatinine levels. Surprisingly, renal function is not different between PHD-I treated wt and HIF-1ko mice, and inflammation is similarily reduced in both genotypes.

Conclusions: Loss of myeloid HIF-1 is detrimental in AKI and CKI in mice, suggesting a protective role for HIF in these models. Treatment of CKI with HIF-stabilizers is beneficial, however protection is independent of myeloid HIF-1. These data suggest that PHD-inhibition exerts its protective effects in cells other than myeloid cells, or might act HIF-independetly.

FR-PO470

Glomerular Expression of Chemokines and Proinflammatory Cytokines in an Experimental Model of Anti-Neutrophil Cytoplasmic Autoantibody Associated Glomerulonephritis
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Background: Myeloperoxidase (MPO)-anti-neutrophil cytoplasmic autoantibody (ANCA) associated glomerulonephritis (GN) is characterized by necrotizing and crescentic glomerular lesions with leucocyte infiltration. Chemokines and their receptors participate in leukocyte infiltration, which play an important role in the pathogenesis of glomerular injury. However, their expression pattern and function in the early phase of MPO-ANCA necrotizing and crescentic glomerulonephritis remain to be fully elucidated. In the present study, we analyzed gene expression of chemokines and proinflammatory cytokines (namely CCL2, CCL3, CCL20, CXCR1, CXCR2, CXCR8, TNFα, IFNγ, IL-1β, IL-6, IL-17, IL-21, and IL-23) in MPO-ANCA associated GN using an experimental rat model by generation of an immune response to exogenously administered human MPO in adjuvant.

Methods: Necrotizing and crescentic GN was induced in WKY rat by immunization with human MPO (1600 μg/kg or 800μg/kg prime/boost combination). Blood and urine samples were obtained at every week. Quantitative RT-PCR was used to determine gene expression of chemokines and proinflammatory cytokines were analysed in isolated glomeruli in 8 weeks after NCGN induction.

Results: Hematuria (3+) and proteinuria (1000mg/dl) were noted at 4 weeks. hMPO-immunized rats had anti hMPO titers of 1:800 by ELISA. Renal pathology showed GN with elastase+ neutrophil and CD68+ macrophage infiltration in glomeruli. Necrotizing glomerular lesions were mainly constituted with fibrin exudation, infiltration of neutrophils and macrophages, and extracapillary cellular crescents. Several chemokines and proinflammatory cytokines, which are mainly involved in neutrophils and macrophages infiltration, were induced or up-regulated.

Conclusions: This study revealed that expression levels of various chemokines and proinflammatory cytokines were increased in the early phase of MPO-ANCA associated GN. These chemokines provide new insights into the epigenetic factors responsible for crescent formation.

FR-PO471

Genetic Susceptibility in Experimental Autoimmune Vasculitis
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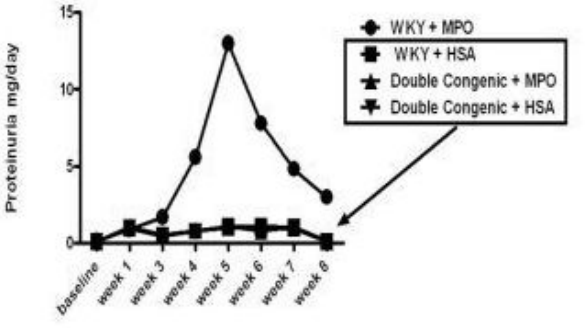
Background: We have developed an experimental model of ANCA associated vasculitis (EAV), in the Wistar Kyoto (WKY) rat that recapitulates human disease. We have previously shown, using a nephrotoxic nephritis model (NTN) in the genetically susceptible WKY rat and compared it with the resistant Lewis strain, that a number of quantitative trait loci (QTL) are important in the pathogenesis of Glomerulonephritis (GN). Congenic strains, using these QTL, were subsequently generated and these animals used to examine their role in EAV. **Aim: To investigate whether EAV susceptibility in WKY rats is determined by QTLs Crgn1/2.**

Methods: Groups of wildtype WKY and double congenic (DC) WKY animals (WKY with QTLs from the Lewis strain) were immunised with myeloperoxidase (MPO) or human serum albumin in Freund's complete adjuvant. Rats were given pertussis toxin on day(s) 0, 2. Weekly urine collections were taken and disease assessed by quantifyng haematuria, proteinuria and by histology.

Results: MPO treated wildtype WKY rats strongly positive (+++) or (++) for haematuria (dipstick) through weeks 3-8. Haematuria was not detectible in either the control group or in any (DC) WKY animals. Proteinuria was also apparent in the MPO treated wildtype WKY group by week 3, this increased to a peak at week 5 before (Fig 1) before decreasing through weeks 6-8. Proteinuria was not detected in control groups or (DC) WKY animals. Abnormal glomeruli were found only in MPO treated WKY animals. Also, all MPO treated groups produced equivalent ANCA autoantibody titres.



Conclusions: These novel findings demonstate that genes located in these QTLs are important in the pathogenesis of EAV.

FR-PO472

Leukocyte and Serum Calprotectin Expression Reflect Disease Activity in ANCA Associated Vasculitis
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Background: Calprotectin (MRP8/14), a TLR4 agonist, expressed in neutrophils, monocytes and infiltrating macrophages, is pro-inflammatory. We have previously shown patients with active renal ANCA associated vasculitis (AAV) have elevated serum levels, and calprotectin deficient (Cal-/-) mice are protected from nephrotoxic nephritis (NTN). We now correlate renal biopsy calprotectin expression with the Berden classification of AAV (therefore outcome), we correlate serum calprotectin expression and relapse in patients with early systemic AAV, and demonstrate abnormalities in neutrophil and monocyte calprotectin surface expression. Finally we explore the interaction of calprotectin with isolated kidney endothelial cells (EC).

Methods: We performed immunohistochemistry on renal biopsies. Serum calprotectin levels were measured by ELISA, and correlated with disease parameters, cell surface expression was assessed by flow cytometry. Accelerated NTN was performed on wild-type (WT) and Cal-/- mice with LPS given during induction. EC were isolated by positive magnetic bead selection from kidneys.

Results: Patients with focal and crescentic glomerular lesions demonstrated the most calprotectin, sclerotic the least ($p<0.05$), linking calprotectin expression with disease activity. In limited systemic disease, calprotectin levels assessed at 1 and 6 months following treatment predict relapse, as shown by ROC (>626 ng/ml at 1 month [sensitivity 78.6%, specificity 92.3%], at 6 months >454 ng/ml [sensitivity 78.6%, specificity 92.3%]. Patients had higher monocyte and neutrophil cell surface calprotectin expression than healthy controls during active and convalescent disease. When given LPS, Cal-/- mice had similar renal disease to WT. Stimulation of primed TLR4-expressing EC with calprotectin increased IL-8 production.

Conclusions: Serum calprotectin is a potential biomarker in patients with AAV predicting future relapse, with glomerular calprotectin representing acute lesions. Calprotectin contributes to inflammation and glomerulonephritis.

FR-PO473

ADAM17 Is Increased in Granulomatosis with Polyangiitis and Contributes to Endothelial Inflammation
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Background: Shedding of membrane proteins like L-selectin (L-Sel), IL-6 receptor or VE-cadherin (VEC) by ADAM proteases is a hallmark of vascular inflammation. ADAM17 is described as the prototype of ADAMs and aggravates vascular inflammation by modulating both innate and acquired immune responses. Granulomatosis with polyangiitis

(GPA) is characterized by increased serum level of ADAM17 substrates like TNF-alpha, sIL-6R or sL-Sel. Therefore, this study aimed to analyze ADAM17 expression in GPA.

Methods: We established an ADAM17-specific ELISA and determined serum ADAM17 levels of active GPA patients and patients with IgA nephropathy or membranous glomerulonephritis (disease controls). ADAM17 whole blood transcript amounts as well as protein distribution in serum samples and kidney biopsy sections were determined by real-time qPCR, immunoblotting and immunohistochemistry. In vitro, endothelial cells (ECs) were exposed to rhADAM17 and alterations in paracellular permeability, junction architecture and release of sVE-Cadherin were analyzed.

Results: Serum ADAM17 levels were highly increased in GPA patients compared to disease controls or healthy individuals. Concordantly, ADAM17 mRNA amounts were significantly upregulated in GPA whole blood samples. Immunoblotting demonstrated that serum-derived ADAM17 exhibited the same molecular size than ADAM17 extracted from tissue lysates. In kidney sections from GPA patients ADAM17 could be localized to matrix deposits whereas in disease controls ADAM17 showed an endothelial staining pattern. In vitro, exposure to rhADAM17 induced enhanced paracellular permeability and shedding of sVE-Cadherin as well as disassembly of intercellular junctions in ECs.

Conclusions: ADAM17 could be detected in serum samples and was highly increased in GPA compared to renal diseases without endothelial involvement. In vitro studies suggest that sADAM17 aggravates the inflammatory response. These data warrant further studies to examine the benefit of ADAM17 inhibition in vascular disorders.

FR-PO474

Subtle Changes in the Glomerular Endothelial Glycocalyx Do Not Lead to Albuminuria but Decrease the Glomerular Leukocyte Influx during Experimental Glomerulonephritis Angelique Rops,[1] Markus Alexander Loeven,[1] Jo H.M. Berden,[1] Ton J. Rabelink,[2] Jeffrey D. Esko,[3] Johan Van der Vlag.[1] *[1]Dept. of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Dept. of Nephrology, Leiden University Medical Centre, Leiden, Netherlands; [3]Dept. of Cellular & Molecular Medicine, University of California, San Diego, CA.*

Background: Proteinuria is manifested in a range of glomerular diseases. Proliferative glomerulonephritis is characterized by glomerular leukocyte influx. The glycocalyx is an endothelial layer consisting of complex polysaccharides, of which HS is the major constituent. HS consists of repeating GlcNAc-GlcA disaccharide units and is extensively modified by a multitude of enzymes of which N-deacetylase-N-sulfotransferase-1 (NDST-1) facilitates the initial modification. The function and structure of the glomerular endothelial glycocalyx is largely unknown. Here, we evaluated the effects of a modified glycocalyx on albuminuria and glomerular leukocyte influx using mice deficient in endothelial and leukocyte NDST-1.

Methods: Albuminuria, plasma creatinine, glomerular leukocyte influx, histology and glomerular HS expression were determined before and after anti-GBM nephritis induction. Leukocyte adhesion was evaluated i*n vitro* using mouse glomerular endothelial cells (mGEnC) with reduced NDST-1 expression and HS-deficient granulocytes.

Results: Glomerular expression of the specific HS domain GlcNS6S-IdoA2S-GlcNS6S was significantly decreased in NDST-1-/- mice corresponding to an altered glomerular mRNA expression of HS. The NDST-1-/- mice showed no albuminuria. However, glomerular leukocyte influx was significantly reduced in NDST-1-/- mice during anti-GBM nephritis, which was accompanied by a decreased glomerular expression of inflammatory HS domains. The NDST-1-/- mice showed lower plasma creatinine levels and less glomerular injury during anti-GBM nephritis. Finally, the *in vitro* leukocyte adhesion assay showed a decreased adhesion of granulocytes upon NDST-1 silencing in mGEnC.

Conclusions: A subtle modulation of the endothelial glycocalyx does not lead to albuminuria but significantly ameliorates anti-GBM nephritis by reduced glomerular leukocyte influx.

Funding: Private Foundation Support

FR-PO475

Effect of Aliskiren on Renal Inflammation and (Pro)renin Receptor in Crescentic Glomerulonephritis Maki Urushihara,[1] Yukiko Kinoshita,[1] Shuji Kondo,[1] Ariunbold Jamba,[1] Takashi Nagai,[1] Toshiaki Tamaki,[2] Shoji Kagami.[1] *[1]Department of Pediatrics, Tokushima University, Tokushima, Japan; [2]Department of Pharmacology, Tokushima University, Tokushima, Japan.*

Background: Crescentic glomerulonephritis (GN) is characterized by severe infiltration of massive inflammatory cells into glomeruli and crescent formation. Although renin angiotensin system (RAS) is the key player in renal injury, the impact of aliskiren on glomerular crescent formation is not elucidated yet.

Methods: To examine whether aliskiren ameliorate renal injury in crescentic GN, we investigated renal injury induced by anti-glomerular basement membrane (GBM) antibodies in Wistar Kyoto rats treated with aliskiren. In addition, using cultured glomerular mesangial cell (MCs) and parietal epithelial cell (PECs), we examined whether recombinant renin could induce monocyte chemoattractant protein-1 (MCP-1) and cell proliferation, respectively.

Results: An anti-GBM nephritis model developed progressive proteinuria and glomerular crescent formation, accompanied by increased MCP-1 and (pro)renin receptor. Interestingly, (pro)renin receptor expressed strongly in the crescent formation area in diseased glomeruli. Proteinuria was significantly reduced by the treatment of aliskiren. Then, aliskiren markedly ameliorated renal injury (% glomerular crescent: 26.0 +/- 1.7 %) compared to vehicle treatment (59.6 +/- 3.6 %). Excretion of urinary protein in a group of rats treated with aliskiren (37.8 +/- 9.5 mg/day) were significantly reduced compared to vehicle-treated rats (128.0 +/- 16.3 mg/day). Aliskiren treatment markedly decreased MCP-1 and (pro)renin receptor mRNA levels in the diseased kidney, measured by quantitative real-time PCR analysis. Next, primary cultured MCs stimulated by recombinant renin showed significant increases of MCP-1 mRNA expression. Furthermore, primary cultured PECs showed an increase in recombinant renin-induced cell proliferation.

Conclusions: These data suggest that therapeutic strategy of aliskiren may prove beneficial for crescentic GN by the suppression of the RAS activation and the decrease of inflammation and cell proliferation in glomerular crescent via (pro)renin receptor.

Funding: Government Support - Non-U.S.

FR-PO476

Toll Like Receptor (TLR) Signaling Is Required for Induction of Experimental Autoimmune Glomerulonephritis (EAG) in CD1 Mice Jitendra K. Gautam, Anjana Gevaria, Kline Bolton. *Department of Medicine, Division of Nephrology and Center for Immunity, Inflammation and Regenerative Medicine, Universtity of Virginia Health System, Charlottesville, VA.*

Results: A single immunization of CD1 mice with recombinant col4α3(IV)NC1 emulsified in complete Freund's adjuvant (CFA-H37Ra) results in the development of EAG. Disease is characterized by heavy albuminuria starting at 4-6 weeks that progresses during the course of the experiment. At sacrifice, mice have abdominal inflammation, fluid accumulation and enlarged spleen and lymph nodes. Most mice show grossly visible kidney damage at 12 weeks. Kidney H&E analysis shows glomeruloneohritis and glomerular sclerosis with infiltration of lymphocytes. By immunofluorescence there is linear IgG deposition on the glomerular basement membrane (GBM). Subclasses IgG1, IgG2a and IgG2b are equally distributed. Kidneys have higher MHC class II expression and infiltration of CD4 T cells, macrophages and dendritic cells.

We hypothesized that EAG in CD1 mice could be induced by synthetic TLR ligands acting as infection mimetics. We tested four TLR agonists with specific signaling pathways: PAM_3CSK_4 (TLR1/2) as a Gram positive infection mimetic, poly IC(TLR3) as a viral infection mimetic, bacterial *lipopolysaccharide* (LPS)(TLR4) as a Gram negative infection mimetic and CpG oligos(TLR9) as unmethylated bacterial DNA. We found that EAG in CD1 mice could be induced with individual TLR agonists. Each TLR agonist's ability to induce EAG differed. PAM_3CSK_4 induced severe EAG followed by poly IC and CpG oligos. EAG in these animals had a comparable course to CFA induced EAG, albeit with a lower overall intensity. LPS did not induce significant EAG but mice still developed linear deposition of IgG on the GBM. Control mice immunized with recombinant col4α3(IV) NC1 in saline did not develop albuminuria or cellular infiltration in the kidney but had linear IgG deposition on the GBM.

Conclusions: These results demonstrate a differential role of TLR stimulation in the development of EAG in CD1 mice. Experiments are underway to understand these differences and what role individual TLR signaling pathways may play in this model.

Funding: NIDDK Support

FR-PO477

Kidney Endothelium-Specific Knockdown of Stanniocalcin-1 Aggravates Anti-GBM Glomerulonephritis Luping Huang,[1] Jie Du,[1] Pumin Zhang,[2] David Sheikh-Hamad,[1] Tatiana Belousova.[1] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX.*

Background: Inflammatory cells (macrophages, T-cells and neutrophils) play critical roles in anti-GBM GN. Stanniocalcin-1 (STC1) inhibits macrophages (leukocyte Biology, 2009); stabilizes endothelial barrier function (ATVB, 2008), and diminishes trans-endothelial migration of leukocytes (AJP-Renal, 2007). Transgenic overexpression of STC1 protects from anti-GBM GN in mice (Am. J. Pathol., 2009). In the following experiments, we sough to determine the contribution of endothelium-mediated effects by STC1 – to protection from anti-GBM GN.

Methods: We employed two powerful and innovative approaches to address the above question: 1) we generated STC1 shRNA Tg mice that express STC1 siRNA conditionally upon removal of floxed reporter stuffer (PGK-driven EGFP), bringing the H1 promoter ahead of the siRNA cassette; 2) using ultrasound microbubble-mediated delivery of Tie2-Cre to the kidney we were able to knockdown the expression of STC1 in kidney endothelial cells in STC1 shRNA Tg mice (within 3-5 days), but not in scrambled shRNA Tg mice.

Results: Applying anti-GBM GN to STC1 shRNA and scrambled shRNA Tg mice 3 days after the delivery of Tie2-Cre to the kidney using ultrasound microbubble-mediated gene delivery confirmed endothelium-specific knockdown of STC1 in STC1 shRNA Tg mice, and revealed worse anti-GBM GN [vacuolization and epithelial cell sloughing, tubular dilatation and cast formation, greater number of crescents, higher creatinine].

Conclusions: These observations suggest that endothelium-mediated effects of STC1 play an important role in preventing anti-GBM disease and suggest that systemic delivery of STC1 may be a viable therapeutic option for kidney inflammation.

Funding: NIDDK Support

FR-PO478

Role for CD4-Lymphocyte Specific NEMO or IKKbeta Deletion in an Experimental Model of Rapid Progressive Glomerulonephritis

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Background: Nephrotoxic serum nephritis (NTN), a T-cell mediated disease induced in mice serves as a model of human rapid progressive glomerulonephritis. Engagement of the T-cell receptor (TCR) by antigen leads to intracellular signaling events causing activation of transcription factors, such as NF-kappa B. We now examined ablation of IKK2 or NEMO specifically in T-cells in NTN.

Methods: T-cell specific ablation of IKK2 or NEMO was achieved through deletion of $IKK2^{fl/fl}$ or $NEMO^{fl/fl}$ alleles by Cre -recombinase expression under control of the $CD4^{cre}$ transgene. NTN-antiserum was injected and functional and histopathologic parameters were determined during a 10 observation period. T cells infiltrating into the kidney were quantitated by immuno-histochemistry and FACS-analysis and kidney chemokine expression was determined by RT-PCR.

Results: Nephritic $CD4^{Cre}IKK2^{fl/fl}$ and $CD4^{Cre}NEMO^{fl/fl}$ mice had an increase in albumin/creatinine-ratio and glomerular crescents ($p<0.01$) at day 10 although the total number of CD4 lymphocytes was reduced in the kidneys. Further analysis showed significantly reduced Tregs in $CD4^{Cre}IKK2^{fl/fl}$ and $CD4^{Cre}NEMO^{fl/fl}$ animals. $CD4^{Cre}IKK2^{fl/fl}$ furthermore showed a higher infiltration of IFN-γ producing Th1-cells when compared with control nephritic mice. Renal expression of cytokines and chemokines was not changed in $CD4^{Cre}IKK2^{fl/fl}$ mice when compared with NTN-controls, however, significantly increased ($p<0.05$) in $CD4^{Cre}NEMO^{fl/fl}$ mice while CCL5 expression was selectively and significantly reduced when compared with NTN-nephritic controls.

Conclusions: Our data demonstrate that ablation of IKK2 or NEMO specifically in $CD4^{+}$T-cells significantly reduced the number of Tregs in kidneys after NTN-induction. Differential regulation of cytokine expression in $CD4^{Cre}IKK2^{fl/fl}$ when compared with $CD4^{Cre}NEMO^{fl/fl}$ mice might reflect further differences in T-cells infiltrating the kidney and point out differential regulation of $CD4^{+}$T-cells via IKK2 and NEMO.

Funding: Government Support - Non-U.S.

FR-PO479

Pirfenidone Inhibits Macrophage Infiltration in 5/6 Nephrectomized Rats

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Background: Macrophage infiltration is involved in the progression of chronic kidney disease(CKD). Pirfenidon as an anti-fibrotic drug is impliacted in the treatment of CKD. This study was to investigate the effects of pirfenidone on M1/M2 Macrophage infiltration in nephrectomized rats.

Methods: Male SD rats underwent a five-sixths nephrectomy. Nephrectomized rats were treated with pirfenidone by gavage for 12 weeks. 24-h urinary protein, NAG activity, Systemic blood pressure(SBP) and CRP were determined. Paraffin-embeded sections were immunohistologically stained for CD68(macrophages)-, CCR7(M1 macrophages)- and CD163(M2 macrophages)-positive cells. MCP-1 and *macrophage inflammatory protein-1α(* MIP-1α) as well as M1 and M2 macrophages secretory proteins were evaluated by Western blotting analysis.

Results: pirfenidone significantly improved the elevated proteinuria and *NAG activity* from 2 to 12th week after surgery(*$p<0.05$).* SNx rats presented with a significant increase of SBP from the 6th week onward, while there was no significant change for SBP in pirfenidone treatment group compared to the control. Pirfenidone attenuated interstitial fibrosis in the cortex and decreased the infiltrating macrophages significantly($p<0.05$). The number of M1 and M2 macrophages were significantly lower after pirfenidone treatment($p<0.05$). the protein expressions of MCP-1 and MIP-1α were increased in rats of control group. Pirfenidone treatment significantly decreased their expressions ($p<0.05$). TNF-α, IL-6 and Nitric oxide synthases 2(iNOS) expressed by M1 macrophages were increased in nephrecomized rats, and Pirfenidone reduced their expressions significantly($p<0.05$). Pirfenidone treatment also significantly decreased Dectin-1 and arginase-1 expressed by M2 macrophages ($p<0.05$).

Conclusions: Pirfenidone attenuates inflammation and inhibits M1 and M2 macrophage infiltration in 5/6 nephrectomized rats, which offers a new insight into the role of pirfenidone in treatment of CKD.

Funding: Government Support - Non-U.S.

FR-PO480

Murine Double Minute (MDM)-2 Mediates Podocyte Loss, Proteinuria, and Subsequent Tubular Injury and Interstitial Fibrosis in Progressive Focal-Segmental Glomerulosclerosis

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Background: Murine double minute (MDM)-2 is a cytoplasmic molecule with p53-dependent mitogenic effects during development and tumor cell growth. We recently described that this mitogenic effect also contributes to tubular repair after acute tubular necrosis. In addition we found that MDM-2 has p53-independent nuclear factor-κB agonistic effect that promotes tubulointerstitial inflammation. Since we noticed prominent MDM-2 expression also in podocytes and parietal epithelial cells, we hypothesized that MDM-2's mitogenic and pro-inflammatory effects also contribute to glomerular pathology, e.g. to adriamycin-induced glomerulosclerosis in Balb/c mice.

Methods: Balb/c mice were procured from Charles River, Germany. All experimental procedures were approved by the local government authorities. Adriamycin (13mg/kg) and Nutlin 3a (20mg/kg) were injected *i.p.* Immunostainings were used for analysing kidney pathology.

Results: After onset of the early glomerular injury phase MDM-2 blockade with nutlin 3a significantly reduced intrarenal cytokine and chemokine expression, glomerular macrophage and T cell recruitment, segmental and global glomerular lesions as well plasma creatinine which correlated with higher podocyte numbers in affected glomeruli. Early MDM-2 blockade had no effect on proliferating parietal epithelial cells. In other mice MDM-2 blockade was initiated only after 2 weeks for 14 days to cover the phase of disease progression, tubular atrophy, interstitial inflammation and fibrosis. MDM-2 blockade significantly improved all these parameters together with a significant reduction in plasma creatinine but without affecting glomerular lesions, proteinuria or tubular cell proliferation.

Conclusions: Together, MDM-2 mediates podocyte loss, proteinuria, and subsequent tubular injury and interstitial fibrosis in progressive glomerulosclerosis, hence, MDM-2 blockade might be suitable therapeutic strategy to prevent the progression of glomerulosclerosis and other disorders that involve nuclear factor-κB-dependent renal inflammation.

FR-PO481

CD36 Knockout Mice Are Protected from Chronic Kidney Disease Progression after 5/6 Nephrectomy with Angiotensin II Infusion

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Background: The class B scavenger receptor CD36 is important in lipid metabolism, inflammation and atherosclerosis. We recently discovered that CD36 knockout (KO) mice are protected from sepsis-induced acute kidney injury and systemic inflammation. Bacteria can also enter cells via CD36. The role of CD36 in chronic kidney disease (CKD) is unknown, but CD36 expression is up-regulated in blood monocytes of dialysis patients, and soluble plasma CD36 is increased in advanced CKD patients. We aimed to study the role of CD36 on CKD progression.

Methods: We compared CKD progression in CD36 KO vs. WT mice (C57BL6 background) using a 5/6 nephrectomy (Nx) model with angiotensin II (AngII, osmotic mini pump 0.75 μg/Kg/min) for 4 weeks. WT 5/6 Nx mice without AngII infusion did not progress to CKD and were used as controls. BUN was measured colorimetrically, Scr by HPLC. Albumin, HMGB1 and IL-6 were measured by ELISA. We used Masson's trichrome and PAS staining to detect interstitial fibrosis and glomerulosclerosis, respectively. Spleen apoptosis was measured by immunohistochemical staining with anti-active caspase-3 antibody. Mean Arterial Pressure (MAP) was measured by radiotelemetry. ANOVA/post hoc was used for statistical analysis.

Results: Kidney function was protected in KO mice (BUN control 95±9, WT 187±36 and KO 119±16 mg/dl; Scr control 0.33±0.32, WT 0.64±0.13 and KO 0.38±0.04 mg/dl, $p<0.05$). KO had less albuminuria than WT mice ($p<0.0001$), glomerulosclerosis score (control 1.8±0.2, WT 3.6±0.3 and KO 2.3±0.2, $p<0.0001$), interstitial fibrosis score (control 1.2±0.2, WT 3.0±0.5, KO 1.8±0.3, $p<0.01$) and weight loss (control -2.9±0.6, WT -8.5±1.8, KO -3.8±0.8 g, $p<0.01$). IL-6 and HMGB1 levels and baseline blood pressure were not different between KO and WT. Spleen apoptosis was not different between the groups.

Conclusions: CKD progression is impaired in CD36 KO mice in a 5/6 nephrectomy + AngII infusion model. The mechanism of protection does not appear to be a global reduction of inflammation as the acute inflammatory cytokines tested were not different in CD36KO.

Funding: NIDDK Support

FR-PO482

Prevention of Chronic Kidney Disease Progression after 5/6 Nephrectomy in TLR4 Mutant Mice C3H/HeJ

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Background: Chronic inflammation is closely linked to several complications of chronic kidney disease (CKD) and predicts CKD progression and mortality. The impact of innate immunity on CKD are largely unknown. Toll-like receptors (TLRs) recognize pathogen associated molecular patterns; TLR4 is the major signaling receptor for endotoxin (lipopolysaccharide) and recognizes host-derived DAMP ligands such as HMGB1.

Methods: We used 5/6 nephrectomy (Nx) to test the role of TLR4 on CKD progression in C3H/HeOuJ (TLR4+) vs C3H/HeJ (TLR4-) mice. As TLR4+ mice did not fully develop CKD by 8 weeks, we infused a low dose (0.0625 μg/Kg/min) of angiotensin II (AngII) by osmotic mini-pump for 4 weeks after 5/6 Nx. BUN was measured colorimetrically; Scr by HPLC; albumin, HMGB1 and IL-6 by ELISA. We stained kidney sections with Masson's trichrome and PAS. Spleen apoptosis was measured by anti-active caspase-3 immunohistochemistry. Student's t-test or ANOVA were used for statistics and log-rank test for survival analysis.

Results: 4 weeks after 5/6 Nx with AngII infusion, TLR4+ mice developed severe kidney dysfunction. Kidney function was protected in TLR4- vs TLR4+ mice (BUN 310.3±34.1 vs 70.1±5.34 mg/dl, $p<0.0001$ and HPLC Scr 0.74±0.12 vs 0.24±0.01 mg/dl, $p<0.001$). TLR4- mice had less albuminuria ($p<0.0001$), glomerulosclerosis score (3.15±0.3 vs 1.65±0.15, $p<0.01$), interstitial fibrosis score (2.29±0.24 vs 1.3±0.09, $p<0.01$), and weight loss (Δ body weight -12.4±1.5 vs +0.09±0.33 g, $p<0.0001$) than TLR4+ mice. Mortality was higher for TLR4+ mice ($p<0.05$) and TLR4+ mice had higher heart weight/100g body

weight ratios than TLR4- (0.61±0.08 vs 0.43±0.02, p<0.05). Serum IL-6 and HMGB1 levels were not significantly different between TLR4+ and TLR4- mice. Spleen apoptosis was higher in TLR4+ mice (18.17±1.53 vs 2.17±0.29 + cells/HPF, p<0.0001).

Conclusions: We found that TLR4 plays a role in AngII-mediated amplification/progression of CKD. AngII is known to up-regulate TLR4 in kidney, but the upstream ligand and the effects on downstream signaling are unkown.

Funding: NIDDK Support

FR-PO483

High suPAR Levels in FSGS Patients Is Associated with Decreased Treg Cells Chang Yien Chan,[1] Wee Song Yeo,[1] Changli Wei,[2] Subhra K. Biswas,[3] Hui Kim Yap.[1] *[1]Pediatrics, National University of Singapore, Singapore; [2]University of Miami; [3]SIgN, A-Star, Singapore.*

Background: Immune-mediated focal segmental glomerulosclerosis (FSGS) is a common cause of end-stage kidney disease in children worldwide. Recent studies have suggested that urokinase receptor (uPAR) signaling is involved in the pathogenesis of FSGS, and that soluble uPAR (suPAR) is increased in sera of FSGS patients. This study aimed to investigate the relationship between elevated suPAR in nephrotic patients and the immune system.

Methods: Plasma suPAR was analyzed in 51 primary nephrotic patients (26 MCNS, 25 FSGS) and 24 healthy controls, using Quantikine Human uPAR Immunoassay (R&D Systems). Patients were stratified into groups I and II, according to whether they had high or low suPAR levels respectively. Peripheral blood mononuclear cells were isolated and MACS-magnetic beads (Miltenyi Biotec) were used to isolate CD4, CD8. CD19 and monocyte fractions. suPAR levels were measured in supernatents following 4-hour cell cultures. Lymphocyte subsets were analyzed by flow cytometry. Results were expressed as mean±SEM. Statistical analysis was done using Mann-Whitney test.

Results: Plasma suPAR levels in patients with FSGS (3216.8±295.2 pg/ml) were significantly higher than controls (2330.8±207.2 pg/ml) and MCNS patients in relapse (2178.9±196.7 pg/ml) and remission (2389.2±225.6 pg/ml)(p<0.02). Following cell culture, suPAR was detected in monocyte supernatants (137.3±11.8 pg/ml) but not in lymphocyte supernatants. Group I patients had significantly higher monocyte supernatant suPAR levels than Group II (166.7±29.4 pg/ml *vs* 78.4±17.4 pg/ml)(p=0.02). Additionally, Group I patients showed a significant decrease in percentage of ICOS on both $CD8^+CD3^+$ (0.61±0.09% *vs* 2.25±0.65%) and $CD4^+CD3^+$ cells (3.95±0.49% *vs* 7.45±1.40%)(p<0.05). The percentage of CD45RA on $CD25^+CD4^+CD3^+$ cells was also significantly lower in Group I patients (4.11±0.63% *vs* 8.13±1.34%)(p<0.001).

Conclusions: Monocytes appear to be the source of suPAR in patients with active FSGS and high suPAR levels was associated with decrease in Treg cells, suggesting a possible regulatory role.

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FR-PO484

Monocyte Cell Death Correlates with Disease Severity in Shiga Toxin Induced Hemolytic Uremic Syndrome in Adult Patients Shuwang Ge, Barbara Hertel, Sang Hi Karen Emden, Jan Beneke, Jan Menne, Hermann G. Haller, Sibylle Von Vietinghoff. *Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: E. coli shiga toxin can induce a hemolytic uremic syndrome (STEC-HUS) that is characterized by hemolysis, intravascular micro- and macrothrombi, renal failure and various degrees of central nervous system involvement. The pathophysiology of STEC-HUS is poorly understood. Data from animal models on the role of leukocytes are controversial. We therefore investigated leukocyte cell death in vivo in adult STEC-HUS patients during acute disease and recovery and studied its association with disease severity.

Methods: Leukocyte cell death in a group of 30 adult patients treated at a tertiary care centre during the STEC-HUS outbreak in Germany in 2011 was assessed by staining for apoptotic and dead cells and multi-color flow cytometry analysis during acute disease and recovery.

Results: A significant leukocytosis was observed in STEC-HUS patients. In addition, concentrations of apoptotic and necrotic monocytes and granulocytes were significantly increased in the circulation of patients compared to healthy controls. This phenotype reverted during recovery. Monocyte apoptosis on admission was significantly higher in patients subsequently assigned to plasma exchange or admitted to the intensive care unit.

Conclusions: In STEC-HUS, elevated numbers of dead leukocytes were detected. Monocyte and granulocyte death are novel markers of acute STEC-HUS that may actively contribute to tissue destruction by liberation of pro-inflammatory enzymes and cytokines.

FR-PO485

Complement Proteins in Neutrophils: A Functional Role in Neutrophil Extracellular Trap Mediated Bacteria Clearance Joshua Yuen,[1,3] Fred G. Pluthero,[1] Walter H. Kahr,[1,2] Nades Palaniyar,[1,3] Christoph Licht.[1,2,3] *[1]Sick Kids Research Institute, The Hospital for Sick Children, Toronto, ON, Canada; [2]Paediatrics, University of Toronto, Toronto, ON, Canada; [3]Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.*

Background: Atypical Hemolytic Uremic Syndrome (aHUS) is a severe renal disorder that is associated with mutations in genes encoding regulatory proteins of the complement alternative pathway (AP). However, clinical insight illustrates that not all carriers precipitate the disease. While cases are often preceded with an infectious episode, the role neutrophils (PMN), key effectors of innate immune and inflammatory responses, play in aHUS is not well established. We set out to characterize the interactions between the complement system and PMN to elucidate their potential pathogenic roles in aHUS.

Methods: PMN purified from human peripheral whole blood by density gradient were subjected to immunoblot analysis and confocal microscopy to identify protein constituents of the complement system. *P. aeruginosa* (mPAO1) was used for bacterial assays.

Results: We identified complement: properdin (CFP), complement factor H (CFH), complement component 3 (C3), and complement factor B (CFB) in resting PMN. Activation of PMN with Phorbal 12-myristate 13-acetate (PMA) induces the formation of neutrophil extracellular traps (NETs), a recently discovered response of PMN to trap bacteria. Activation with PMA leads to a gradual process of intracellular compartmentalized distribution of complement proteins. As the nuclear envelope disintegrates and nuclear chromatin decondenses, C3 mixes with genomic material, while CFP, CFB, and CFH remain at the periphery. When NETs are released, the complement AP proteins are found on their surface and deposited on bacteria.

Conclusions: This suggests complement AP activation on the surface of bacteria ensnared by NETs. We propose that PMN, via this mechanism, create a targeted immune response to microbes through localized activation of AP. Without proper regulation, as found in patients with mutations in the regulatory proteins of AP, this mechanism may precipitate a complement driven disease process.

Funding: Private Foundation Support

FR-PO486

Factor H and Properdin Recognize Different Epitopes on Renal Tubular Epithelial Heparan Sulfate Azadeh Zaferani,[1] Romain Vives,[2] Pieter van der Pol,[3] Gerjan Navis,[1] Mohamed R. Daha,[1,3] Cees van Kooten,[3] Hugues Lortat-jacob,[2] Marc Seelen,[1] Jacob van den Born.[1] *[1]Nephrology, UMCG, Groningen, Netherlands; [2]Institut de Biologie Structurale, Grenoble, France; [3]Nephrology, LUMC, Leiden, Netherlands.*

Background: During proteinuria, renal tubular epithelial cells become exposed to ultrafiltrate-derived serum proteins, including complement factors. Recently, we showed that properdin binds to tubular heparan sulfates (HS). We now document that factor H also binds to tubular HS, although to a different epitope than properdin.

Methods: Binding properties of HS for factor H were evaluated by using competition ELISA and Surface Plasmone resonance experiments. Factor H binding and complement activation on proximal tubular epithelial cells (PTEC) were investigated by FACS analysis.

Results: Factor H was present on the urinary side of renal tubular cells in proteinuric, but not in normal renal tissues and co-localized with properdin in proteinuric kidneys. Factor H dose-dependently bound to PTEC in vitro. Pre-incubation of factor H with exogenous heparin and pretreatment of PTECs with heparitinase abolished the binding to PTECs. Surface Plasmon resonance experiments showed high affinity of factor H for heparin and HS (KD values of 32 and 93 nM respectively). Using a library of HS-like polysaccharides, we showed that chain length and high sulfation density are the most important determinants for glycosaminoglycan-factor H interaction, and clearly differ from properdin-heparinoid interaction. Co-incubation of properdin and factor H did not hamper HS/heparin binding of one another, indicating recognition of different non-overlapping epitopes on HS/heparin by factor H and properdin. Finally we showed that certain low-anticoagulant heparinoids can inhibit properdin binding to tubular HS, with minor effect on factor H binding to tubular HS. As a result, these heparinoids can control the alternative complement pathway.

Conclusions: In conclusion, factor H and properdin interact with different HS epitopes of PTECs. These interactions can be manipulated with some low-anticoagulant heparinoids which can be important for preventing complement-derived tubular injury in proteinuric renal diseases.

FR-PO487

Anti-Aging Gene Klotho Is Essential in the Maintenance of Normal Kidney Structure and Function Zhongjie Sun. *Physiology, University of Oklahoma HSC, Oklahoma City, OK.*

Background: Klotho is a recently discovered anti-aging gene. Genetic mutation of klotho expedites the aging process and shortens the lifespan while overexpression of klotho slows down the aging process and extends the lifespan by 20%. Klotho is predominately expressed in renal distal convoluted tubule cells. The purpose of this experiment is to test our hypothesis that klotho deficiency causes glomerular and tubular damage and impairs kidney function *via* inflammation.

Methods: *In vivo* expression of klotho and IL-10 were achieved by AAV2 delivery of klotho and IL-10 genes.

Results: We found that klotho gene deficiency resulted in leukocyte infiltration in kidneys (T cells, macrophages), glomerular collapse, and tubule atrophy and dilation in KL (+/-) mice. The kidney function was impaired as evidenced by the increased creatinine in the blood. In another experiment, we silenced klotho gene in rats using KL-shRNA. In vivo silencing of klotho gene resulted in macrophage infiltration, upregulation of superoxide production, downregulation of Mn-SOD, and oxidative stress damage in kidneys. Glomerular atrophy and collapse were found in rats treated with KL-shRNA, indicating kidney damage. Interestingly, these devastating effects may be mediated by increased inflammation because they can be abolished by simultaneous over-expression of anti-inflammatory cytokine IL-10 gene. On the other hand, the klotho level was decreased while inflammation was increased in aging-related and hypertension-related kidney damages. Notably, in vivo klotho gene delivery increased the IL-10 level, suppressed superoxide levels, abolished kidney damages (glomerular collapse, tubule atrophy, protein deposition), and improved kidney function in spontaneously hypertensive rats (SHRs).

Conclusions: Therefore, klotho is essential in the maintenance of normal kidney structure and function. In vivo expression of klotho may be a novel approach to the protection of kidneys.

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FR-PO488

Klotho Attenuates Inflammation of Adipose Tissue and Development of Insulin Resistance in High-Fat Induced Obese Mice Minoru Satoh, Kengo Kidokoro, Hajime Nagasu, Chieko Ihoriya, Yuko Nishi, Hiroyuki Kadoya, Tamaki Sasaki, Naoki Kashihara. *Department of Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Chronic inflammation in adipocyte tissues plays a crucial role in the development of obesity-related insulin resistance, which contributes to an increased risk of hypertension, atherosclerosis, and diabetes. The enlargement of adipocytes due to impaired adipocyte differentiation leads to a chronic state of inflammation in the adipocytes. The secretion of proinflammatory cytokines mainly from macrophages enhances local inflammation. In recent years, the Wnt5a/JNK signaling pathway in adipocytes and macrophages has emerged as an important mediator of adipose tissue inflammation that affects systemic metabolism. Secreted klotho protein has been reported to inhibit Wnt signaling. Klotho, an anti-aging gene, is mainly expressed in the kidney tubules, and chronic kidney disease (CKD) patients exhibit low serum levels of klotho. Therefore, we hypothesized that CKD patients show chronic inflammation in adipose tissue due to low levels of klotho. In the present study, we examined whether the klotho protein attenuates high-fat diet–induced chronic inflammation in adipose tissue.

Methods: To determine the in vivo effects of klotho in dietary fat-induced obesity, we investigated klotho-transgenic (KLTG) mice fed with high-fat diet (HFD). Ten-week-old male C57BL/6 wild type (WT) mice and KLTG mice were fed normal fat diet or HFD for 12 weeks.

Results: HFD resulted in insulin resistance, hyperglycemia, and impaired glucose tolerance in WT mice. KLTG mice were resistant to the development of insulin resistance. This lean phenotype was associated with lower leptin and retinol binding protein 4 levels and higher glucose sensitivity than WT mice which consumed similar amounts of HFD. KLTG mice showed reduced macrophage content and an improved inflammatory profile of the adipose tissue.

Conclusions: The klotho protein controls adipose tissue inflammation and systemic insulin resistance in HFD-fed mice. Inhibition of CKD progression may be beneficial to suppress the development of insulin resistance due to obesity.

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FR-PO489

Neuroinflammation Status Plays a Role in Nephrectomy-Induced Cognitive Impairment in Rats Sabrina Degaspari,[1] Carmen B. Tzanno-martins,[2] Clarice K. Fujihara,[3] Roberto Zatz,[3] Tania Araujo Viel,[4] Carolina D. Munhoz,[1] Ana Elisa Bohmer,[1] Larissa De Sá Lima,[1] Cristoforo Scavone,[1] Elisa M. Kawamoto.[1,5] *[1]Institute of Biomedical Science, USP, SP, Brazil; [2]CINE, São Paulo, Brazil; [3]Renal Division-Departament of Clinical Medicine, USP, SP, Brazil; [4]School of Arts, Sciences and Humanities, USP, SP, Brazil; [5]Laboratory of Neurosciences, NIH, Baltimore, MD.*

Background: Renal insufficiency could impair cognitive function; chronic kidney disease (CKD) is associated with increases of the inflammatory process that might play a role in cognitive impairment (CI). The aim of this study is to evaluate if serum and cerebral spinal fluid (CSF) cytokines and corticosterone (CORT) levels correlate with cognitive deficits in 5/6 nephrectomized rats (Nx).

Methods: Wistar rats underwent 5/6 nephrectomy (Sham-operated rats as controls). After 30 days, animals were tested once a month for their cognitive status. Nx with scores 75% less than sham were included in a CI group (Nx-CI, n=12), the remaining rats constituted the Nx group. After 4 months, serum and CSF levels of interleukin-10 (IL-10), tumor necrosis factor (TNF), and CORT were measured.

Results: All Nx showed higher levels of creatinine (1.13±0.05) compared to Sham (0.65±0.05). Nx-CI showed impaired memory retention. Serum TNF (Sham=14.4±3 pg/ml*) and IL-10 (Sham=2904±602 pg/ml*) levels were lower in Nx (TNF=4.0±2 pg/ml; IL-10=321.3±54 pg/ml) and Nx-CI (TNF=4.0±2 pg/ml; IL-10=176.6±657 pg/ml), in the CSF the Nx-CI showed higher levels of TNF (17.4±3 pg/ml*) and lower levels of IL-10 (97.8±7 pg/ml*) compared with Sham (TNF=5.1±0.1 pg/ml; IL-10=888.7±298 pg/ml) and Nx (TNF=3.9±1.4 pg/ml; IL-10=48.1±29). Serum CORT levels (Sham=2680±280 ng/ml) were higher in Nx (8112±106 ng/ml*) and Nx-CI (8130±175 ng/ml*). However, CSF CORT levels were elevated only in the Nx (13052±262 ng/ml*)(*p<0.05).

Conclusions: High serum CORT levels in Nx and Nx-CI rats associated with low levels of proinflammatory cytokines, but CSF measurements indicated a specific central nervous proinflammatory response in Nx-CI rats. These results suggest that neuroinflammation may play a role in the Nx-induced cognitive impairment in CKD rats. **FINANCIAL SUPPORT**: FAPESP; CNPq; USP.

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FR-PO490

Renal Impairment Increases Aortic Antigen Presenting Cell and Lymphocyte Accumulation during Atherosclerosis Development Sibylle Von Vietinghoff,[1,2] Shuwang Ge,[1] Barbara Hertel,[1] Ekaterina Koltsova,[2] Jan T. Kielstein,[1] Klaus Ley,[2] Hermann G. Haller.[1] *[1]Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA.*

Background: Atherosclerosis is a major cause of death in patients with chronic kidney disease. Chronic inflammation of the arterial wall including invasion, proliferation and differentiation of leukocytes is important in atherosclerotic lesion development. While hemodynamics and mineral metabolism have been investigated in detail, how atherosclerotic inflammation is altered in renal impairment is incompletely understood. This study tested whether moderate renal impairment alters leukocyte composition in the aorta of atherosclerotic mice.

Methods: Apolipoprotein E and LDL-receptor deficient mice underwent unilateral nephrectomy or sham surgery and were maintained on high-fat diet for three, six and twelve weeks. Aortic leukocyte infiltration was determined by immunofluorescence staining and confocal microscopy and by flow cytometry of aortic single cell suspensions. Aortic leukocyte interactions were investigated by life cell multiphoton imaging of atherosclerotic aortas.

Results: A 50% reduction in renal function increased atherosclerotic lesion size and aortic leukocyte numbers in both models. The number of aortic leukocytes, among them antigen presenting cells, mostly of myeloid origin, increased significantly. Intravascular interaction of antigen presenting cells with T cells as determined by vascular wall life cell imaging and in vivo aortic T cell proliferation were significantly enhanced in renal impairment.

Conclusions: Mild renal impairment significantly increases aortic inflammatory leukocyte accumulation and antigen presentation in atherosclerosis.

FR-PO491

Kidney-Brain Crosstalk during Sepsis Misako Asada, Motoko Yanagita. *Department of Nephrology, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: Patients with chronic kidney disease (CKD) have a higher prevalence, severity, and mortality of sepsis. However, the mechanism that CKD influences the outcome of sepsis remains unclear. The main cause of death in septic patients is multi-organ failure, and increasing evidences support the presence of crosstalk between kidney and other distant organs via soluble and cellular inflammatory mediators. Here we investigated the influences of CKD on kidney-brain crosstalk in the context of systemic inflammation.

Methods: We divided C57BL/6J male mice (8~9week) into 4 groups: sham-operated mice injected with vehicle (sham/vehicle mice), sham mice injected with lipopolysaccharides (LPS, 2.5mg/kg BW)(sham/LPS mice), mice operated with unilateral ureter obstruction (UUO) and injected with vehicle (UUO/vehicle mice), and mice operated with UUO and injected with LPS (UUO/LPS mice). Mice were sacrificed 5 days after the operation, and organs were subjected to histological analysis and quantitative reverse transcription polymerase chain reaction (qPCR) to evaluate the expression of injury markers and inflammatory mediators.

Results: The expression of IL-6, TNF-a and MCP1 was significantly up-regulated both contralateral and diseased kidneys of UUO/LPS mice compared to the expression of UUO/vehicle mice and sham/LPS mice. Next we evaluated the damages in the brain and demonstrated that the expression of IL-6, IL-6 receptor and glial fibrillary acidic protein (GFAP), a marker for activated glial cells during brain inflammation, was significantly up-regulated in UUO/LPS mice compared to other 3 groups. Induction of GFAP was further confirmed by immunostaining. To analyze the molecular mechanism for kidney-brain crosstalk, we evaluated the expression of neuroprotective factors in the kidneys, and found that the expression of EGF and VEGF was significantly decreased in both contralateral and diseased kidneys in UUO/LPS mice compared to other 3 groups.

Conclusions: Existence of fibrotic kidneys during sepsis aggravates brain injury, possibly due to the reduced expression of neuroprotective factors in the kidneys. Further analysis of the inter-organ crosstalk will provide better insights into the link between sepsis and CKD.

FR-PO492

Stat3 Is Involved in Indoxyl Sulfate-Induced Inflammatory and Fibrotic Gene Expression and Cellular Senescence in Proximal Tubular Cells Toshimitsu Niwa,[1] Fuyuhiko Nishijima,[2] Hidehisa Shimizu.[1] *[1]Department of Advanced Medicine for Uremia, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Biomedical Research Laboratories, Kureha Co., Tokyo, Japan.*

Background: Increased phosphorylation (activation) of signal transducer and activator of transcription 3 (Stat3) on tyrosine 705 leads to renal fibrosis. Indoxyl sulfate, a uremic toxin, induces renal fibrosis through expression of transforming growth factor-β1 (TGF-β1) in proximal tubular cells.The present study aimed to determine whether Stat3 is involved in indoxyl sulfate-induced dysfunction of proximal tubular cells.

Methods: Localization of phosphorylated Stat3 in the kidneys of normal, subtotally nephrectomized, and AST-120-treated subtotally nephrectomized rats was examined by immunohistochemistry. The effect of indoxyl sulfate on phosphorylation of Stat3, and the role of Stat3 on indoxyl sulfate-induced cellular effects were examined using human proximal tubular cells (HK-2 cells).

Results: Subtotally nephrectomized rats showed increased immunostaining of phosphorylated Stat3 in the renal tubules compared with normal rats. Administration of AST-120, which reduces serum level of indoxyl sulfate, to subtotally nephrectomized rats reduced the immunostaining of phosphorylated Stat3 in the renal tubules. Indoxyl sulfate induced phosphorylation of Stat3 in HK-2 cells. Stat3 small interfering RNA (siRNA) suppressed indoxyl sulfate-induced expression of an inflammation maker gene (monocyte chemotactic protein-1), fibrosis maker genes (TGF-β1, α-smooth muscle actin) and a subunit of nuclear factor-κB (p65), and attenuated a cellular senescence maker, senescence-associated β-galactosidase activity.

Conclusions: Stat3 is involved in indoxyl sulfate-induced inflammatory and fibrotic gene expression and cellular senescence in proximal tubular cells.

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FR-PO493

Indoxyl Sulfate, a Uremic Toxin, Downregulates Renal Expression of Nrf2 through Activation of NF-κB Toshimitsu Niwa,[1] Dilinaer Bolati,[1] Maimaiti Yisireyili,[1] Fuyuhiko Nishijima,[2] Hidehisa Shimizu.[1] *[1]Department of Advanced Medicine for Uremia, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Biomedical Research Laboratories, Kureha Co., Tokyo, Japan.*

Background: Indoxyl sulfate is accumulated in the serum of uremic patients, thereby accelerating the progression of kidney failure. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that regulates induction of numerous antioxidant and phase II detoxifying enzymes. In uremic rat kidney, the expression of Nrf2 and its related genes is downregulated. The present study aimed to determine whether indoxyl sulfate affects Nrf2 expression in the kidney.

Methods: AST-120, an oral sorbent which reduces serum level of indoxyl sulfate, was administered to subtotally nephrectomized rats. The effects of indoxyl sulfate on Nrf2 functions in the kidney were determined using following animals: (1) Dahl salt-resistant normotensive rats (DN), (2) Dahl salt-resistant normotensive indoxyl sulfate-administered rats (DN+IS), (3) Dahl salt-sensitive hypertensive rats (DH), and (4) Dahl salt-sensitive hypertensive indoxyl sulfate-administered rats (DH+IS). To determine whether indoxyl sulfate directly downregulates expression of Nrf2, human proximal tubular cells (HK-2 cells) were used.

Results: AST-120 upregulated the expression of Nrf2 and heme oxygenase-1 (HO-1), an antioxidant gene and a target of Nrf2, and suppressed expression of 8-hydroxydeoxyguanosine (8-OHdG), a marker of reactive oxygen species, in the kidney. Expression of Nrf2 and HO-1 was decreased, whereas expression of 8-OHdG was increased in the kidneys of DN+IS, DH, DH+IS compared with DN. Indoxyl sulfate downregulated Nrf2 expression in HK-2 cells. Furthermore, the indoxyl sulfate-induced downregulation of Nrf2 expression was alleviated by pyrrolidine dithiocarbamate, an inhibitor of nuclear factor-κB (NF-κB), and small interfering RNA (siRNA) specific to NF-κB p65.

Conclusions: Indoxyl sulfate downregulates renal expression of Nrf2 through activation of NF-κB. More notably, AST-120 is a novel inducer of renal Nrf2 expression.

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FR-PO494

AST-120 Attenuates Chronic Kidney Disease-Related Monocyte Inflammation through Reduction of Indoxyl Sulfate Shunsuke Ito,[1,2] Sumie Goto,[2] Hideyuki Yamato,[2] Mizuko Osaka,[1] Masayuki Yoshida.[1] *[1]Life Schiences and Bioethics, Tokyo Medical and Dental University, Tokyo, Japan; [2]Biomedical Research Laboratories, Kureha Corporation, Tokyo, Japan.*

Background: Management of cardiovascular disease development is critical in patients with chronic kidney disease (CKD). An oral adsorbent AST-120 retards deterioration in renal function by reducing indoxyl sulfate, a major compartment of uremic toxins, in CKD patients. In the present study, we examined the anti-inflammatory potential of AST-120 in a mouse model of CKD.

Methods: Renal failure was induced by subtotal nephrectomy in ten weeks old male C57BL/6 mice. After four weeks, AST-120 was administered for four weeks. Flow cytometry was performed on the peripheral blood leukocytes stained for monocyte activation marker Mac-1 and dihydroethidium to detect reactive oxygen species (ROS). THP-1 monocytic cells were stimulated with indoxyl sulfate and subjected to flow cytometric analysis and adhesion assay to human umbilical vein endothelial cells (HUVEC) under flow conditions in vitro.

Results: A significant elevation of Mac-1 expression and ROS production were observed in peripheral blood monocyte in nephrectomized mice. These monocytes activation were suppressed by AST-120. Administration with indoxyl sulfate enhanced both Mac-1 expression and ROS production. Treatment with indoxyl sulfate dose-dependently enhanced Mac-1 expression and increased THP-1 adhesion to IL-1β-activated HUVEC. Indoxyl sulfate induced phosphorylation of p38MAPK. SB203580, an inhibitor of p38MAPK, reduced Mac-1 expression and indoxyl sulfate-induced THP-1 adhesion to HUVEC, but not that of JNK and ERK1/2 inhibitors. Indoxyl sulfate enhanced ROS production, and induced membrane translocation of NAD(P)H oxidase subunit p47phox in THP-1 cells. Apocynin, an inhibitor of NAD(P)H oxidase, inhibited Mac-1 expression, ROS production and THP-1 adhesion.

Conclusions: Indoxyl sulfate induced up-regulation of Mac-1 through NAD(P)H oxidase- and p38MAPK-dependent pathways. AST-120 inhibited monocytes activation via reducing indoxyl sulfate in vivo. These observations provide a novel therapeutic approach using AST-120 in CKD-associated cardiovascular disease.

Funding: Government Support - Non-U.S.

FR-PO495

PPARα Activation Improves Glomerular Injury Caused by Anti-Thy1 Nephritis through Suppression of NF-κB Signaling Koji Hashimoto, Makoto Harada, Taro Kanno, Yasufumi Takahashi, Makoto Higuchi, Yuji Kamijo. *Department of Nephrology, Shinshu University, Matsumoto, Japan.*

Background: The vast increase of chronic kidney disease (CKD) has attracted considerable attention worldwide, and the development of a novel therapeutic option against a representative kidney disease that leads to CKD, mesangial proliferative glomerulonephritis (MsPGN), would be significant. Peroxisome proliferator-activated receptor α (PPARα), a member of the steroid/nuclear receptor superfamily, is known to perform various physiological functions. Recently, we reported that PPARα in activated mesangial cells exerted anti-inflammatory effects and that the deficiency of PPARα resulted in high susceptibility to glomerulonephritis. To investigate whether PPARα activation improves the disease activity of MsPGN, we examined the protective effects of a PPARα agonist, clofibrate, in a well-established model of human MsPGN, anti-Thy1 nephritis, for the first time.

Methods: Anti-Thy1 nephritis was induced by a single intravenous injection of a mouse anti-Thy1 monoclonal antibody. The clofibrate-treated rats were fed a 0.02 or 0.1% clofibrate containing diet beginning 5 days before the injection. We examined urine protein excretion and kidney function, and carried out histopathological analysis, transcription factor assay (PPARα and NF-κB), and analysis of mRNA of target molecules of these transcriptional factors.

Results: Pretreatment with clofibrate exerted anti-proteinuric effects and ameliorated the active glomerular pathologic inflammatory changes in rat anti-Thy1 nephritis. The clofibrate treatment continuously activated the glomerular PPARα. The PPARα activation appeared to suppress the NF-κB signaling pathway in glomeruli by the induction of IκBα, resulting in the reduction of proteinuria and the improvement of pathologic glomerular changes.

Conclusions: The current study suggest the anti-nephritic potential of PPARα agonist, clofibrate, against MsPGN. PPARα-related medicines might be useful as a treatment option for CKD.

FR-PO496

Curcumin Ameliorates CKD by Decreasing Paracellular Permeability of LPS Siddhartha S. Ghosh, Richard Krieg, Dominic A. Sica, Todd W. Gehr. *Nephrology, VCU, Richmond, VA.*

Background: Recent studies suggest that the paracellular permeability of uremic toxins such as lipopolysaccharide (LPS) from the intestine plays a proinflammatory role in CKD patients. Our earlier studies show that curcumin is as effective as enalapril in controlling inflammation in our CKD animals. In this study we investigated if paracellular permeability is increased in animal model of CKD and if curcumin can also act locally in the gut to prevent paracellular permeability of LPS and reduce inflammation.

Methods: CKD was induced in CD1 mice by 5/6 nephrectomy (Nx). A group of Nx (n=5) was treated with 100 mg/kg curcumin (CUR) for 12-weeks and results were compared with Nx and sham-operated animals (SH). Proteinuria, BUN and creatinine were used as measures of renal function. To check if curcumin has any effect on paracellular permeability we added LPS to intestinal epithelial cells (Caco2) and monitored permeability of ^{3}H mannitol in presence and absence of curcumin from 0-4 hrs.

Results: Curcumin significantly decreased progressive proteinuria in Nx animals. BUN and creatinine levels of Nx group (62±10 mg/dl and 1.5±0.3) were reduced by 32% ($p<0.05$) and 36% ($p<0.01$) respectively in CUR group. Serum LPS levels of Nx animals vs CUR was 3.2±1.6 and 1.6±0.5 EU/ml respectively, p= 0.0242. LPS is known to stimulate inflammatory biomolecules such as TNFα and IL6 in macrophages. Compared to CUR macrophages from Nx cohort had a 3.6 and 2.8 fold higher TNF ($p<0.01$), and IL-6 ($p<0.05$) mRNA respectively. Intestinal tight junction proteins claudin1 and ZO1 responsible for maintaining the integrity of intestinal smooth muscle measured by immunoblot was significantly lower in Nx vs CUR ($p<0.05$).Paracellular permeability of ^{3}H mannitol in LPS treated Caco2 cells gradually increased with time and was 1.7 ($p<0.01$) and 2.4 fold higher ($p<0.001$) than the CUR+LPS treated cells at 2 and 4 hours respectively.

Conclusions: Our experiments suggest that intestinal permeability is abnormal in animals with renal failure and this leads to leakage of LPS and subsequent inflammation. Curcumin by maintaining the intestinal integrity prevents LPS absorption which ameliorates inflammation and renal failure.

Funding: Private Foundation Support

FR-PO497

Protective Effects of Rosiglitazone on the Rat Kidney under Chronic Ischemia Jinlei Lv,[1] Hong-bo Xiao,[1] Qinkai Chen,[1] Guohua Ding.[2] *[1]Nephrology, The First Affilliated Hospital of NanChang University, NanChang, JInagXi, China; [2]Nephrology, Renmin Hospital of Wuhan University, WuHan, HuBei, China.*

Background: To investigate the expression of PPARγ,$TGFβ_1$ in the rat kidney under chronic ischemia and mechanisms by which rosiglitazone protects the ischemic kidney.

Methods: Sprague-Dawley rats were randomly assigned to four groups:sham-operated (sham), ischemic nephropathy(IN), IN treated early with rosiglitazone(RGE), IN treated late with rosiglitazone(RGL). Biochemical indicators were measured at the end of 12W. Morphological changes of renal tissue were observedby HE,PASM,Masson stain. RealTime-PCR were performed to investigate the mRNA expression of PPARγ,α-SMA,$TGFβ_1$. Immunohistochemistry method was used to observe the protein expression

of PPARγ,α-SMA. The correlations between the mRNA expression of PPARγ,α-SMA, $TGF\beta_1$ and renal pathology counting scores were also conducted.

Results: Compared with sham group, biochemical indicators were significantly increased in IN group($P<0.01$), pathological lesions were observed. Early treated with rosiglitazone significantly decreased biochemical indicators and pathological lesions,The protein expressions of PPARγ,α-SMA were increased significantly in IN group($P<0.01$),meanwhile the protein expressions were decreased in both RGE and RGL group. There were significant increased expressions of PPARγ,αSMA and $TGF\beta_1$ mRNA in IN and RGL group versus sham group;Compared with sham group,the mRNA expressions of αSMA and $TGF\beta_1$ in RGE group increased($P<0.01$).There were positive correlations between mRNA expressions of PPARγ,α-SMA,$TGF\beta_1$ and renal pathology counting scores($P<0.01$).

Conclusions: Rosiglitazone may exert protective effects on chronic ischemic kidney by, at least partially, regulating PPARγ transcription,down-regulating the espression of $TGF\beta_1$, suppressing the activation of renal myofibroblast.

Funding: Government Support - Non-U.S.

FR-PO498

Chronic Renal Failure Increases Monocyte Cell Surface Expression of Galectin-3, a Pro-Inflammatory, Pro-Fibrotic Galectin Andrew Duncan Stewart Findlay, Julius Edward Kieswich, Magdi Yaqoob. *Translational Medicine and Theraputics, William Harvey Research Institute, Queen Mary's School of Medicine and Dentistry, London, United Kingdom.*

Background: Galectin-3 is a pro-inflammatory galactoside binding protein expressed on leucocytes with diverse roles in cardiac fibrosis, inflammation and cell-cell interactions. Whilst both human and animal plasma Galectin-3 is elevated in heart failure and chronic renal failure, the source of the plasma Galectin-3 and implications for elevated Galectin-3 are unknown.

Methods: Murine plasma Galectin -1, -9 and -3 were measured by Elisa over 4 weeks on 0.25% adenine diet (a model of chronic renal failure) vs sham diet. After 2 weeks 0.25% adenine diet vs sham diet Flow Cytometry was performed measuring Galectin-3 cell surface staining of monocytes (Ly6c+ve cells), neutrophils (Ly6g+ve cells) and resting peritoneal macrophages (identified by morphological characteristics).

Results: Plasma Galectin-3 but not 1 and 9 was significantly elevated from 1 week adenine diet and continued to rise over 4 weeks. At 2 weeks diet the chronic renal failure group (0.25% adenine n=8) had a significantly greater proportion of monocytes with cell surface expression of Galectin-3 than sham group (n=8) (Median % of Ly6c +ve cells staining +ve for Galectin-3 = 55.57% vs 30.62% P=0.0079). The median flourescence intensity(MFI) of Galectin-3 positive monocytes was also significantly stronger in the adenine group vs sham ($p<0.05$). There were no significant differences between the 2 groups in neutrophil or peritoneal macrophage expression of Galectin-3 at 2 weeks of diet.

Conclusions: Plasma Galectin-3, derived from leucocytes, is raised in chronic renal failure. Increased cell surface expression of Galectin-3 in the chronic renal failure model was specific to monocytes and not elevated above the sham group in neutrophils or resting peritoneal macrophages. There is an acknowledged correlation between elevated Galectin-3 and heart failure particularly cardiac fibrosis. This is via a proposed monocyte/macrophage derived Galectin-3 activation of cardiac fibroblasts. Galectin-3 may therefore be implicated in uraemic cardiovascular disease.

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FR-PO499

Effect of a Serine Protease Inhibitor on the Progression of Chronic Renal Failure Manabu Hayata,[1] Yutaka Kakizoe,[1] Kohei Uchimura,[1] Jun Morinaga,[1] Rika Yamazoe,[1] Teruhiko Mizumoto,[1] Tomoaki Onoue,[1] Miki Ueda,[1] Sakai Yoshiki,[2] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [2]Research Headquarters, Ono Pharmaceutical Co., Ltd., Osaka, Japan.*

Background: The number of the chronic renal failure (CRF) patients is increasing explosively. Hypertension, proteinuria, inflammation, fibrosis, and oxidative stress are intertwined in a complicated manner that leads to the progression of CRF. However, the therapeutic strategies to delay its progression are limited. Since serine proteases are involved in many aspects of these risk factors, we investigated the effects of a synthetic serine protease inhibitor camostat mesilate (CM) on the progression of CRF in 5/6 nephrectomized (Nx) rats.

Methods: Eighteen male Sprague-Dawley rats were divided into three groups, a sham-operated group (Sham: n=6), a vehicle-treated Nx group (Nx: n=6) and a CM-treated Nx group (Nx+CM: n=6). Following the 9-weeks study period, all rats were sacrificed and blood samples and the remnant kidney were collected for the assessment of the renal injury.

Results: At the end of the experiment, both proteinuria (Sham: 19±1 mg/day, Nx: 158±38 mg/day; $P<0.01$) and serum creatinine levels (Sham: 0.31±0.01 mg/dL, Nx: 0.87±0.07 mg/dL; $P<0.001$) were substantially increased in the vehicle-treated Nx group and treatment with CM significantly reduced proteinuria (Nx+CM: 54±9 mg/day; $P<0.05$ vs. Nx) and serum creatinine levels (Nx+CM: 0.68±0.02 mg/dL; $P<0.05$ vs. Nx). Podocyte-associated proteins such as nephrin and synaptopodin were markedly decreased by 5/6 nephrectomy, and was significantly ameliorated by CM. CM also suppressed the mRNA levels of inflammatory and fibrotic markers including TGFβ1, TNFα, collagen type I, III, and IV, and reduced glomerulosclerosis and interstitial fibrosis in histologic studies. Furthermore, CM decreased the mRNA expression of NADPH oxidase components as well as reactive oxygen species generation and advanced oxidative protein products.

Conclusions: Our current results strongly suggest the possibility that CM might be useful therapeutic strategy against the progression of CRF.

FR-PO500

HIV-Induced Kidney Cell Injury: Role of ROS-Induced Downregulated Vitamin D Receptor Divya Salhan, Nirupama Chandel, Ashaan Subrati, Partab Rai, Tejinder Singh, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Reactive oxygen species (ROS) have been demonstrated to contribute to HIV-induced tubular cell injury. We hypothesized that HIV-induced ROS generation may be causing tubular cell injury through downregulation of vitamin D receptor (VDR) and associated down stream effects.

Methods: Immunoblots of empty vector and HIV (NL4-3) transduced human proximal tubular cells (HPTC) were probed for VDR and actin. To determine the role of ROS, immunoblots of HIV/HPTC-pretreated with catalase or HPTCs directly treated with H_2O_2 were probed for VDR and actin. EV/HPTC and HIV/HPTCs were assayed for the activation of renin-angiotensin system (RAS), ROS generation. EV/PTCs' and HIV/HPTCs' DNA damage and repair in the presence or absence of a VDR agonist (EB1089, 30 nM) and an Ang II blocker (losartan, 10^{-7}M) were assyed by immunofluorescnece studies under confocal microscopy.

Results: HIV not only downregulated tubular cell VDR expression but also inflicted DNA injury. On the other hand, EB1089 inhibited both down regulation of VDR and tubular cell DNA injury in HIV milieu. H_20_2 directlydown regulated tubular cell VDR; whereas, catalase, a free radical scavenger, inhibited HIV-induced down regulation of tubular cell VDR expression HIV also stimulated tubular RAS. Both E1089 and losartan partially inhibited HIV-induced tubular cell ROS generation. HIV/HPTCs exhibited enhanced expression of phospho-p53 and associated downstream signaling. Both EB1089 and losartan not only preserved expression of tubular cell DNA repair proteins but also inhibited induction of double strand breaks. In *in vivo* studies, renal cortical sections of Tg26 mice displayed attenuated expression of VDR both in podocytes and tubular cells. In addition, renal cortical sections of Tg26 mice displayed enhanced oxidative stress-induced kidney cell DNA damage.

Conclusions: These findings indicated that HIV-induced tubular cell downregulation of VDR contributed to the RAS activation and associated tubular cell DNA damage. However, both VDRupregulation and RAS blockade provided protection against these effects of HIV.

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FR-PO501

Loss of Syndecan-1 Induces Endothelial Damage and Albuminuria in Zebrafish and Mice Hermann G. Haller,[1,2] Torsten Kirsch,[1] Joon-Keun Park,[1] Mario Schiffer.[1,2] *[1]Clinic of Hypertension and Nephrology, Hannover Medical School, Hannover, Germany; [2]Mount Desert Biological Laboratory MDIBL, Bar Harbor, ME.*

Background: Syndecans are a family of cell surface heparan sulfate proteoglycans that act as cell surface receptors and are part of the glycocalyx. Recently, syndecan-4 was associated with podocyte function and proteinuria. Since alterations of the enothelial cell glycocalyx seem to be important for the pathogenesis of proteinuria and circulating syndecan-1 is increased in diabetic patients we hypothesized that syndecan-1 plays a role in endothelial cell dysfunction and albuminuria in diabetes.

Methods: Albuminuria was assessed using (1) a novel model albuminuria in embryonic zebrafish and (2) in streptozotocin-induced hyperglycemia in the mouse using immunohistochemistry, immunoblotting real-time PCR and electron microscopy. Syndecan inhibition was achieved by (1) antisense morpholinos and (2) generation of syndecan-1-/- mice. Mice were made hyperglycemic by streptozotocin treatment.

Results: Functional knockdown of syndecan-1 in zebrafish led to endothelial and podocytic cell damage with distorted glomerular capillaries. In addition, vascular development was impaired. Hyperglycemia in a mouse model led to an increased albuminuria after 8 weeks associated with a loss of syndecan-1 and other glycocalyx molecules. The diabetes-induced albuminuria was significantly enhanced in syndecan-/- mice. In addition, the decrease in other gylcocalyx molecules was observed earlier.

Conclusions: Syndecan-1 is important for endothelial function in the glomerulus and rapidly down-regulated by hyperglycemia. Lack of syndecan -1 leads to endothelial dysfunction and albuminuria. Our results support an important role of syndecan-1 in the maintenance of the glomerular barrier.

FR-PO502

Endothelial Cell Responses to Circulating Immune Complexes In Vivo Scott E. Wenderfer, Adisak Suwanichkul. *Pediatrics, Renal Section, Baylor College of Medicine, Houston, TX.*

Background: Antibodies or antibody-antigen immune complexes (IC) are present in the glomeruli in >50% of glomerulopathies. Fixed IC deposits form in distinct regions of the glomeruli of the kidney, and often predominate in the subendothelial space. The response of endothelial cells during deposition and clearance of IC from the subendothelial space is unknown.

Methods: To assess the functional responses of renal endothelial cells to either antibodies or IC in health and in disease, a mouse model of acute circulating IC deposition was adapted for fluorescence microscopic and biological assessment.

Results: The introduction of monoclonal or polyclonal, homologous or heterologous immune complexes resulted in transient glomerular IgG staining, whereas introduction of antibodies alone did not. The accumulation of IC in this model is independent of antigenic specificity. Significant inflammation is not detected in this model, allowing for parenchymal responses to IC accumulation to be studied. The pattern of responses seen in

vivo corresponds to that detected in vitro using cultured renal endothelial cells, and includes induction of pro-inflammatory, pro-angiogenic, and pro-apoptotic factors. In addition, markers of endothelial precursor cell involvement are detected along with wound-healing responses in the resolving phase of the model.

Conclusions: Renal endothelial cells play a primary role in the initial response to circulating IC in the kidney and endothelial precursor cells may play a role in resolving IC kidney diseases. This acute circulating IC model can help to unravel the complexities of kidney disease in autoimmune mouse models and patients with IC diseases, such as lupus nephritis.

Funding: NIDDK Support

FR-PO503

Heme Oxygenase-1 (HO-1) Derived Iron and Ferritin Regulate Macrophage Polarization Subhashini Bolisetty,[1] Abolfazl Zarjou,[1] Reny Joseph,[1] Amie Traylor,[1] Viktória Jeney,[2] James George,[1] Jozsef Balla,[2] Anupam Agarwal.[1] *[1]Medicine/Division of Nephrology, University of Alabama at Birmingham, Birmingham, AL; [2]Medicine, University of Debrecen, Hungary.*

Background: HO-1 is a microsomal enzyme that breaks down heme to generate iron, carbon monoxide and biliverdin. Iron released is sequestered by H-ferritin, which is co-induced with HO-1 as a protective response. The purpose of this study was to determine the role of HO-1 and H-ferritin in macrophage activation and polarization.

Results: HO-1$^{-/-}$ mice display increased renal macrophage infiltration and fibrosis following unilateral ureteral obstruction (UUO) compared to HO-1$^{+/+}$ mice. Proximal tubule (PT)-specific H-ferritin deletion led to a significant increase in macrophage infiltration while PT-specific HO-1 overexpression decreased infiltration. M1 (iNOS, TNF-α) and M2 (Arginase-1, mannose receptor) markers were evaluated in CD11b+ macrophages isolated from UUO kidneys from HO-1$^{-/-}$ and HO-1$^{+/+}$ mice. HO-1 deficiency was associated with marked dysregulation of macrophage polarization with significantly higher expression of M2 markers. In vitro studies demonstrated that HO-1$^{-/-}$ macrophages are more prone to activation and polarization towards both M1 and M2 pathways, a process that was significantly blunted by HO reaction products (biliverdin and carbon monoxide). Iron chelation by deferoxamine and the consequent decrease in H-ferritin provided similar inhibitory effects. On the contrary, addition of iron, apoferritin or recombinant H-ferritin (both devoid of iron) to HO-1$^{+/+}$ cells mimicked the HO-1 deficient state and increased macrophage polarization towards M1 and M2 phenotype. The clinical relevance of these findings was further corroborated in peripheral blood monocytes isolated from patients on chronic hemodialysis with serum ferritin levels above 900ng/ml. Hemodialysis patients (n=9) revealed significantly higher levels of H-ferritin, iNOS and arginase-1 in monocytes compared to healthy volunteers (n=8).

Conclusions: Our findings elucidate the key role of HO-1/ferritin system in macrophage polarization and suggest that modulation of this system in inflammatory disorders may serve as a novel therapeutic strategy.

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FR-PO504

Epigenetic Histone Methylation Primes Uremic Macrophage Inflammatory Responses Neal B. Blatt,[1] Patricia L. Christopherson,[1] Timothy Cornell,[2] Thomas P. Shanley.[2] *[1]Pediatrics-Nephrology, University of Michigan, Ann Arbor, MI; [2]Pediatrics-Critical Care, University of Michigan, Ann Arbor, MI.*

Background: Monocytes from ESRD patients demonstrate increased cellular activation including increased pro-inflammatory cytokine production, and are implicated in the development of an ESRD-mediated chronic inflammatory state leading to excess cardiovascular disease. The mechanism behind these altered cellular responses is currently unknown. We hypothesized that uremia induces changes in histone methylation patterns (epigenetic signatures) that would favor pro-inflammatory gene expression.

Methods: 129SvJ mice underwent subtotal nephrectomy (SNx) or a sham procedure. Bone marrow-derived macrophages (BMDM) were isolated from sham and SNx mice. Chromatin immunoprecipitation (ChIP) was performed using antibodies recognizing trimethylated lysine-4 within histone H3 (H3K4) followed by qPCR for the promoter regions of TNF-α, IL-12, and IL-10.

Results: SNx mice developed increased BUN values (SNx: 38±5 vs Sham: 18±4 mg/dL, P<0.001) indicating the development of uremia. Following stimulation with LPS, BMDM from SNx mice show increased TNF-α production in tissue culture supernatants (SNx: 661±74 vs sham: 394±42 pg/mL, P<0.05) indicating that the BMDM's model human monocyte responses. Analysis of histone methylation patterns showed that SNx BMDM have increased H3K4 methylation at TNF-α and IL-12, but decreased methylation at the IL-10 promoter (see Table).

	TNF-α	IL-12p35	IL-12p40	IL-10 (a)	IL-10 (b)	IL-10 (c)
SNx	2.0 ± 1.2*	1.3 ± 0.4*	2.3 ± 1.7*	0.7 ± 0.3*	0.6 ± 0.4*	0.7 ± 0.4*
Sham	1.0 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

H3K4 methylation at the indicated promoters. Three different regions of the IL10 promoter are indicated by (a-c). For each IP, %input DNA from the qPCR was normalized to sham %input DNA. Data is presented as mean ± SD. * = P<0.05

Conclusions: Using a well-established mouse model of chronic renal failure, we demonstrate that macrophages from uremic mice are primed to respond to TLR ligands. These findings suggest that uremia is able to reprogram the bone marrow to foster the development of a chronic inflammatory state via epigenetic alterations to histone methylation.

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FR-PO505

Statin Directly Inhibit Macrophage Differentiation and Activation, and Provide Anti-Inflammatory Function Shinya Nagasaka, Akira Shimizu, Seiichiro Higo, Go Kanzaki, Takatsugu Iwashita, Toru Iwahori, Yukinari Masuda. *The Department of Analytic Human Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Background: We have reported the anti-inflammatory effects of statin in experimental anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) through modulation of infiltrating macrophages (Mφ). In this study, we assessed the direct effects of statin on Mφdifferentiation and functions, and possibilities of therapeutic approaches with statin-pretreated Mφ on anti-GBM GN.

Methods: We examined the influences of atorvastatin on the differentiation and activation of bone marrow-derived Mφ (BMDMs). BMDMs were differentiated in the presence of several concentrations of atorvastatin. BMDMs were also pretreated with 2.5 μM of atorvastatin for 24 hr followed by stimulation with IFN-γ, and phenotype of Mφ, the expression of Mφ function related molecules including cytokine and chemokine were analyzed. In addition, we administered intravenously statin-pretreated BMDMs (1x10^7 cells) in the anti-GBM GN rats at 3, 4, and 6 days after disease induction, and examined the effects of these cells in anti-GBM GN.

Results: Statin inhibited Mφ development from rat bone marrow progenitor cells with decreased adhesion molecule LFA-1α expression on Mφ. Cox-2 expression and prostaglandin E2 (PGE2) production were significantly increased in Mφ in the presence of statin compared to absence. Statin-pretreated Mφ were enhanced IL-33 expression and down-regulated MCP-1 expression upon IFN-γ stimulation. Furthermore, injection of statin-pretreated Mφ to anti-GBM GN rat resulted in reduced Mφ infiltration and inhibited the formation of crescentic glomerular lesions.

Conclusions: Statin directly inhibits the differentiation and activation of Mφ, and might provide the anti-inflammatory function in Mφ through enhanced PGE2 production by the increased Cox-2 expression. Furthermore, treatment using statin-pretreated Mφ may be usefulfor the inhibition of active and severe GN.

FR-PO506

Active Macrophage Migration Inhibitory Factor (Active MIF) Is a Previously Unrecognized Isoform of MIF and a Potential New Biomarker for Lupus Nephritis Randolf J. Kerschbaumer,[1] Josef H. Kovarik,[2] Dirk Voelkel,[1] Michael Thiele,[1] Patrice Douillard,[1] Frederick W.K. Tam,[3] Mahmoud Loghman-Adham,[4] Hartmut Ehrlich,[1] Hans Peter Schwarz,[1] Friedrich Scheiflinger.[1] *[1]Baxter Innovations GmbH, Vienna, Austria; [2]Department of Nephrology, Wilhelminenspital, Vienna, Austria; [3]Imperial College Kidney and Transplant Institute, London, United Kingdom; [4]Baxter Healthcare Corporation, Westlake Village, CA.*

Background: MIF plays a central role in inflammatory responses and elevated MIF levels have been detected in patients with inflammatory kidney diseases. Some features of MIF are markedly different from those of other pro-inflammatory cytokines. MIF is constitutively expressed, stored in the cytoplasm and constantly present in the circulation of healthy subjects. We have discovered a novel, previously unrecognized conformational isoform of MIF (designated 'active MIF') that is produced in diseases and cannot be detected in healthy subjects. Active MIF is specifically recognized by human monoclonal anti-MIF antibodies showing beneficial effects in animal models of glomerulonephritis.

Methods: New ELISA methods were established that allow for the determination of active MIF as well as non-active MIF. Urine and plasma samples collected from lupus nephritis (LN) patients and healthy subjects were analyzed. MIF concentration in urine was corrected for creatinine (Cr) concentration.

Results: Active MIF could not be detected in the urine and plasma of healthy controls. In LN patients there was a clear correlation between the severity of the disease and the active MIF/Cr ratio in the urine (acute renal flare: median 1780 pg/mg, n=23; healthy controls: median 0 pg/mg, n=38; p<0.01). Concentrations of active MIF in plasma were not increased during acute renal flare, but were elevated during systemic exacerbations of SLE without renal involvement (median 8340 pg/ml, n=9; healthy controls: median 0 pg/ml, n=20; p<0.0001).

Conclusions: Active MIF in urine and plasma of LN patients may be a suitable marker of disease activity or disease progression. Determination of active MIF levels could furthermore be suitable to predict susceptibility for anti-MIF antibody treatment.

FR-PO507

Monodisperse SynBiosys Microspheres for Sustained Intrarenal Drug Delivery Jurjen Zandstra,[1] Christine Hiemstra,[2] Arjen H. Petersen,[1] Johan Zuidema,[2] Audrey Lathuile,[3] Gert Veldhuis,[3] Rob Steendam,[2] Eliane R. Popa.[1] *[1]Medical Biology, University Medical Centre Groningen, Groningen, Netherlands; [2]InnoCore Pharmaceuticals, Groningen, Netherlands; [3]Nanomi B.V., Oldenzaal, Netherlands.*

Background: The alarmingly increasing prevalence and treatment costs of chronic kidney disease call for novel therapeutic strategies that prevent disease progression at an early stage. We propose biodegradable polymeric microspheres (MSP) with physical and chemical properties tailored to sustained intrarenal drug delivery as a tool to modulate processes responsible for chronic kidney disease, such as inflammation and fibrosis. In this study we generated monodisperse microspheres (MonospheresÔ) composed of hydrophilic

phase separated multi-block copolymers (SynBiosys©) and investigated their intrarenal biocompatibility and thus adequacy for future therapeutic application.

Methods: Polymer-only MSP spheres of 30 μm diameter were injected under the capsule of healthy F344 rat kidneys. Kidneys were retrieved 3, 7, 14 and 28 days after MSP implantation. General histology was evaluated by PAS staining. Renal interstitial presence of macrophages (ED-1) and myofibroblasts (αSMA) as a result of MSP implantation was studied by immunohistochemistry.

Results: MSP demonstrated excellent subcapsular injectability. MSP were detected in the subcapsular space at all time points and showed little degradation in time. MSP implantation did not affect tubular integrity at any time point. Low numbers of macrophages were strictly present around MSP. Macrophages and myofibroblasts were virtually absent in the renal interstitium at all time points.

Conclusions: We report for the first time the intrarenal biocompatibility of SynBiosys Monospheres designed for intrarenal drug delivery. Based on their slow degradation rate and the lack of adverse effects of their subcapsular implantation on the renal tissue, we conclude that SynBiosys Monospheres provide a promising tool for sustained intrarenal drug delivery.

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FR-PO508

Renal Afferent Innervation Exhibits an Impaired Excitability in an In Vitro Model of Inflammation Wolfgang Freisinger, Tilmann Ditting, Annalena Karl, Sonja Heinlein, Karl F. Hilgers, Roland E. Schmieder, Roland Veelken. *Department of Nephrology and Hypertension, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.*

Background: Renal denervation may be beneficial in inflammatory renal disease. Recently, we found that afferent renal neurons showed a characteristic excitability, exhibiting predominantly a sustained firing upon current injection. So far, excitability of these neurons under pathological conditions such as renal inflammation is unclear. Hence, in an in vitro model we wanted to test the hypothesis that a proinflammatory mediator like CXCL1 could alter the firing pattern of renal afferent neurons and thus decrease their excitability.

Methods: Dorsal root ganglion (DRG) neurons (Th11-L2) were incubated with the chemokine CXCL1 (1,5nmol/ml) for 12 hours before patch clamp recordings. Labelling (DiI) allowed the identification of renal afferent neurons. Current clamp was used to characterize neurons as "tonic", i.e. sustained action potential (AP) firing or "phasic", i.e. <5 APs according to their firing response to current injections. AP properties were determined in renal and non-renal neurons incubated with CXCL1 and compared to controls.

Results: Renal afferent DRG neurons exhibited in 57% a tonic firing pattern vs.11,7%* in non-renal neurons. However, exposed to the chemokine, renal DRG neurons exhibited significantly less tonic firing (35,6% vs. 57%, *p<0,05) but instead an increased occurrence of phasic firing. Renal DRG neurons with phasic firing pattern showed a significantly lower threshold for AP-firing (600pA [320-1000] vs.1000pA [400-3200]) and a significantly shorter AP-duration at threshold-level (2,095ms [1,75-4,25] vs. 5,15ms [4,3- 8,7]) after exposure to CXCL1.

Conclusions: We could show that after exposure to a proinflammatory mediator like CXCL1, renal afferent DRG neurons exhibited a significantly higher proportion of neurons with a phasic pattern (<5 APs) and decreased excitability as compared to control conditions. Significant changes in action potential properties of phasic neurons point to an altered sodium channel expression likely inducing a faster inactivation of these channels with decreased firing activity.

FR-PO509

Characteristics of T-Cell Migration at the Single Cell Level Using Microfluidics Modeling Namrata G. Jain,[1,2,3] Ian Y. Wong,[2,3] Elisabeth A. Wong,[2,3] Leo Boneschansker,[1,3] A.J. Aranyosi,[2,3] David M. Briscoe,[1,3] Daniel Irimia.[2,3] *[1]Division of Pediatric Nephrology, Children's Hospital Boston, Boston, MA; [2]Massachusetts General Hospital, Boston, MA; [3]Harvard Medical School, Boston, MA.*

Background: Understanding patterns of T cell migration have significant implications for the development of anti-inflammatory therapeutics. However, prior studies evaluating T cell chemotaxis are limited by use of static migration assays (i.e. Boyden and transwell assays). Microfluidics allows for live-time imaging of T cells migrating towards a gradient; characteristics of migration patterns, velocity, stop-start motility events, and footprinting can be evaluated. We developed microfluidics devices to analyze T cell migration at the single cell level.

Methods: Human CD3+ T cells were isolated by negative selection from PBMC, and used either unstimulated or following 24hrs of activation with anti-CD3/anti-CD28 (1mcg/ml). T cells were injected into the main channel of devices, where they have potential to migrate in 15 hrs into a maze of 10um channels towards a chemokine gradient, either 100nM RANTES or 100nM IP-10. The velocity of T cell movement within the maze was evaluated in two dimensions (Vx or Vy), and directional persistence (Px) towards the chemokine gradient was tracked using Image J software.

Results: We found that mitogen-activated T cells displayed greater exploration within the maze (movement in x and y), vs. unstimulated cells ($p<0.001$). However, unstimulated T cells migrated at higher velocities towards IP-10 (Vx=147.2 um/hr, n=96) vs. RANTES (Vx=101.8 um/hr, n=201,$P<0.001$). Mitogen-activated T cells migrated at a greater velocity towards RANTES (Vx=157.4 um/hr, n=227) vs. IP-10 (Vx=119.3 um/hr, n=203, $p<0.001$), and migrated at a slower velocity towards IP-10 vs unstimulated cells ($p<0.001$). Ultimately, both unstimulated and activated T cells persist towards each chemokine, albeit with different migratory patterns.

Conclusions: Our newly developed microfluidic devices allow for the analysis of T cell migration at the single cell level. Understanding the complexity of migratory patterns has potential to support the development of novel anti-rejection therapeutics.

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FR-PO510

Pathogenesis of Hypercholesterolemia in *IL13* Overexpression Rat Model of Minimal Change Nephrotic Syndrome (MCNS) Lauretta Danwei Low,[1] Chang Yien Chan,[1] Tarun K. Maheshwari,[1] Jinmiao Chen,[2] Henry He Yang,[1] Caroline G.L. Lee,[1] Hanry Yu,[1] Hui Kim Yap.[1] *[1]Pediatrics, Biochemistry, Physiology & Cancer Science Institute, National University of Singapore, Singapore; [2]SIgN, A-Star, Singapore.*

Background: Hypercholesterolemia in experimental rat models of NS is thought to be due to abnormalities in hepatic cholesterol metabolism secondary to the marked proteinuria. This study aimed to investigate the pathogenesis of hypercholesterolemia in an *IL13* overexpression rat model of MCNS.

Methods: Plasmid containing rat *IL13* gene was electroporated into rat quadriceps every 10 days. Plasma IL13, cholesterol, albumin and urine albumin levels were assayed weekly. Liver RNA from rats sacrificed at Week 10 was used for microarray analysis. The direct effect of IL13 (25ng/ml) on primary rat hepatocyte and HepG2 cell cultures were studied after 24-hour incubation.

Results: Weekly biochemistry showed significant increase in plasma cholesterol in *IL13* transfected rats (n=58) compared to controls (n=24) from Week 1 (2.08±0.09 vs 1.66±0.05 mmol/l, $p=0.001$), with hypercholesterolemia preceding development of proteinuria by Week 10. Microarray analysis on Week 10 *IL13* transfected rat liver showed >1.9-fold increase in *IL13Ra2* and *CYP7A1*, and 11.3-fold downregulation in *ABCG5*, validated by RT-PCR. Additionally, RT-PCR detected significant increase in *HMGCR* in *IL13* transfected rats (0.056±0.0054 vs 0.039±0.0054, $p=0.034$). Early studies in *IL13* transfected rats with hypercholesterolemia prior to onset of proteinuria at Week 10, showed similar upregulation of *IL13Ra2* (0.15±0.047 vs 0.016±0.0032, $p<0.001$), with downregulation of *ABCG5* (0.019±0.011 vs 0.12±0.016, $p<0.001$) and *LXRa* (0.23±0.021 vs 0.31±0.024, $p=0.043$). IL13 stimulation resulted in upregulation of *IL13Ra2* and downregulation of *LXRa* gene expression in both rat hepatocyte ($p<0.002$) and HepG2 cells ($p<0.05$).

Conclusions: Hypercholesterolemia preceded development of proteinuria in the *IL13* transfected rats. IL13-induced downregulation of LXRa-ABCG5-pathway of hepatic cholesterol elimination through bile appeared to be important early in the genesis of hypercholesterolemia. This was perpetuated by upregulation of *HMGCR* later in the course of the disease.

Funding: Government Support - Non-U.S.

FR-PO511

The SNARE Protein VAMP2 Binds the Carboxy-Terminus of NKCC2 and Mediates cAMP-Stimulated Exocytic Insertion in Thick Ascending Limbs Paulo S. Caceres,[1,2] Pablo A. Ortiz.[1,2] *[1]Int. Med.- Hypert. & Vasc.Res, Henry Ford Hospital; [2]Physiology, Wayne State Univ, Detroit, MI.*

Background: AVP and β-adrenergic receptors enhance NKCC2 activity and NaCl absorption by the Thick Ascending Limb (TAL) *via* cAMP. cAMP increases apical surface NKCC2 by enhancing its exocytic delivery. Inactivation of vesicle associated membrane protein (VAMP) 2 and 3 blocks cAMP-stimulated NKCC2 activity and surface levels. However the molecular mechanism and the specific VAMP that mediates cAMP-stimulated NKCC2 trafficking are not known. We hypothesize that VAMP2 binds NKCC2 and mediates cAMP-stimulated exocytic delivery in TALs.

Methods: We isolated rat TALs and immunoprecipitated (IP) NKCC2 and VAMP isoforms. To map the interacting motif in NKCC2, we generated GST-fusion proteins coding for the amino (N)-terminus of NKCC2 or the carboxy (C)-terminal region containing the apical targeting signal.

Results: We found that NKCC2 co-IP with VAMP2 but not with VAMP7 or VAMP8. VAMP2 bound the C-terminus of NKCC2 but not the N-terminus. We next studied whether VAMP2 co-localized with NKCC2 at the apical surface. To selectively label VAMP2 at the apical surface we expressed VAMP2 tagged with GFP facing the extracellular space in primary cultures of TALs. Endogenous surface NKCC2 was labeled with an antibody against an extracellular epitope. We found that NKCC2 localization at the apical surface was heterogeneous, with most of the NKCC2 present in discrete domains (nanoclusters) with an area of 0.14±0.04 μm^2. After stimulation of cAMP, 45±7% of NKCC2 surface clusters also contained VAMP2. Finally we tested whether VAMP2 mediates cAMP-stimulated exocytic delivery. *In vivo* transduction of the outer medulla with VAMP2-shRNA adenoviruses decreased VAMP2 expression in TALs by 69±7% and completely blocked cAMP-stimulated NKCC2 exocytic delivery without affecting baseline "constitutive" delivery (baseline: 100%, cAMP: 91±12%, n=5). In control TALs (scrambled shRNA), cAMP enhanced NKCC2 exocytic delivery by 88±17% ($p<0.05$).

Conclusions: We conclude that VAMP2 is a novel interacting partner of NKCC2 that mediates cAMP-stimulated NKCC2 exocytic delivery. In addition, NKCC2 and VAMP2 co-localize in clusters at the apical surface of TALs.

Funding: Other NIH Support - NHLBI, Private Foundation Support

FR-PO512

Wnk4 Deficient Mice Manifest Gitelman's Syndrome-Like Phenotype
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Background: By the *in vitro* studies, With-No-Lysine [K] (WNK) kinase 4 exerts dual effects on Na^+-Cl^- cotransporter (NCC) by inhibiting NCC membrane expression directly or activating NCC through WNK4-SPAK/OSR1-NCC phosphorylation signaling. Activation of Spak/Osr1-Ncc phosphorylation signaling had been observed in pseudohypoaldosteronism type II (PHAII)-mutant Wnk4 D561A knock-in mice presenting with typical phenotype of PHAII characterized by salt-sensitive hypertension and hyperkalemic metabolic acidosis.

Methods: The phenotype, mRNA and relevant protein expression and localization in the kidneys were examined.

Results: Unlike the embryonic lethality in global $Wnk1^{-/-}$, global $Wnk4^{-/-}$ mice grew normally and were indistinguishable from wild-type (WT) controls in appearance and behavior. $Wnk4^{-/-}$ mice presented Gitelman-like phenotype with mild hypokalemia and decreased urine calcium excretion. $Wnk4^{-/-}$ mice also revealed a blunted and exaggerated response to thiazide (NCC inhibitor) and furosemide [Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2) inhibitor], respectively, in urine Na^+, K^+ and Cl^- excretion. In kidney tissues of $Wnk4^{-/-}$ mice, decreased phosphor (p-)Spak, total Ncc and p-Ncc as well as increased p-Osr1 and p-Nkcc2 mimicking Spak knockout mice were observed. NHE3 was increased but ROMK1 and ENaC(β) expression were decreased.

Conclusions: These phenomena suggested WNK4 dominantly exerts an activation effect on NCC through SPAK-NCC cascade *in vivo*. Blocking WNK4 activity may be a promising strategy for anti-hypertensive drugs development.

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FR-PO513

Blunted Hypertensive Response to Angiotensin II (AngII) Infusion in CD8 Deficient Mice Is Not Associated with Blunted Stimulation of Na-Cl Cotransporter (NCC)
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Background: Previous work has shown that T cells play a critical role in hypertension. Recently we found that the hypertension caused by AngII infusion (490 ng/kg/min for 14 days) is blunted in mice lacking CD8 cells ($CD8^{-/-}$) but not in mice lacking CD4 cells ($CD4^{-/-}$). AngII increased systolic BP (telemetry) to 169±3, 166±4 and 142± 4 mmHg in the WT, $CD4^{-/-}$ and $CD8^{-/-}$ mice respectively ($p < 0.001$). Moreover, we find that hypertension is associated with a striking polarization of renal $CD8^+$ cells to production of IFN-gand IL-17. We sought to determine the role of T cell subtypes on renal sodium transport.

Methods: The abundance of the NCC and its phosphorylated active form (NCC-p) were measured in whole kidney homogenates by immunoblotting. Density values were normalized to baseline values in sham-infused mice, defined as 1.00; * = $p < 0.05$.

Results: Baseline levels of NCC were significantly lower in both $CD4^{-/-}$ (0.71 ± 0.03*) and $CD8^{-/-}$ mice (0.75 ± 0.02*) vs. WT (= 1). Additionally, baseline NCC phosphorylation in mice lacking either $CD4^+$ or $CD8^+$ cells was only 2-3% of that observed in WT mice. AngII infusion increased NCC total levels in all three groups: 1.31 + 0.13* fold in WT, 1.66 + 0.05* fold in CD4-/- and 1.65 + 0.08* fold in CD8-/-; NCC-p levels were increased in WT (3.56 ± 1.04* fold), and brought back to WT levels in CD4-/- (31.6 ± 2.6* fold) and CD8-/- (60.3 ± 11.6* fold).

Conclusions: 1. At baseline, CD4-/- and CD8-/- mice had very low levels of NCCp (and NKCCp levels, not shown) vs. WT.

2. With AngII infusion, CD4-/- vs CD8-/- mice did not demonstrate blunting of the AngII response to NCC or NCCp.

3. The study demonstrates that T cells of both the $CD4^+$ and $CD8^+$ subtype have a major effect on renal sodium transport, and provide a heretofore unrecognized role of these immune cells in modulation of electrolyte homeostasis.

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FR-PO514

WNK3 Inhibits the Nedd4-2 Mediated Inhibition of the NaCl Cotransporter
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Background: The With No Lysine (K) (WNK) kinases are known regulators of the activity of the renal NaCl cotransporter NCC, which plays a key role in the regulation of blood pressure and the development of arterial hypertension. We have recently shown that NCC, is modulated by the ubiquitin-protein ligase Nedd4-2 and this effect can be prevented by the serum glucocorticoid kinase 1, Sgk1, which interferes with the Nedd4-2/NCC interaction (Arroyo et al. JASN, 2011).

Methods: In the present study we used *Xenopus laevis* oocytes and HEK293 cells.

Results: Searching for novel regulatory proteins, we observed that WNK3, but not WNK4, interacts with Nedd4-2. In HEK293 cells co-transfected with WNK3 and Nedd4-2 cDNA, there is no variation in WNK3 ubiquitylation, suggesting that WNK3 is not a target of Nedd4-2. On the other hand, in *Xenopus* oocytes, WNK3 is capable of recovering the Nedd4-2 mediated inhibition of NCC, without interfering with the Nedd4-2/NCC interaction, suggesting that WNK3 acts differently than Sgk1 on Nedd4-2 action. In support we found that, in contrast to Sgk1, WNK3 does not phosphorylate mNedd4-2 on S222 or S328 and is able to fully prevent the mutant Nedd4-2 S222/328A provoked inhibition of NCC. *In vitro* phosphorylation demonstrates that WNK3 does phosphorylate Nedd4-2 on another yet to be defined serine residue. Co-expression of the WNK3-F242A mutant, which lacks the ability to bind STE20 kinase, SPAK, and thus cannot activate NCC, is able to completely prevent the Nedd4-2 mediated inhibition of NCC, which points to dissimilar mechanisms between NCC activation by WNK3 (SPAK dependent) and WNK3 mediated inhibition of Nedd4-2 (SPAK independent).

Conclusions: WNK3 prevents the Nedd4-2-induced inhibition of NCC, likely by interfering with Nedd4-2 enzymatic activity, representing a novel mechanism for regulation of NCC activity. The effect of WNK3 on Nedd4-2 is different to the Sgk1 because the serine residues 222 and 328 are not implicated, suggesting the possibility for differential regulation of NCC by WNK3-Sgk1.

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FR-PO515

Nitric Oxide-Induced Inhibition of NKCC2 Activity Is Blunted in Thick Ascending Limbs from Angiotensin II-Hypertensive Rats: Role of Phosphodiesterase 5
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Background: Thick ascending limbs (THALs) reabsorb 25% of the filtered NaCl load primarily *via* the Na/K/2Cl cotransporter (NKCC2). NO inhibits NKCC2 activity and NaCl absorption *via* cGMP. In angiotensin (Ang) II-induced hypertension, NaCl reabsorption is increased in THALs; however, whether this is due to impaired NO/cGMP signaling is not known. Intracellular cGMP levels are reduced by cGMP-specific phosphodiesterase 5 (PDE5). Ang II elevates PDE5 and reduces cGMP levels in smooth muscle cells, but whether this occurs in THALs in Ang II-induced hypertension is unknown. We hypothesized that inhibition of NKCC2 by NO is blunted in Ang II-induced hypertension due to increased PDE5 activity.

Methods: Male Sprague Dawley rats were infused with vehicle or 200 ng/kg/min Ang II. On day 5 we measured blood pressure in anesthetized rats and NKCC2 activity in perfused THALs by fluorescence microscopy.

Results: Ang II infusion increased blood pressure from 96 ± 4 to 120 ± 8 mmHg ($p<0.02$). In THALs from vehicle-treated rats, 100 μM of the NO donor spermine NONOate reduced NKCC2 activity by 33% from 1.67 ± 0.31 to 1.11 ± 0.21 AU/s ($p<0.01$) but not in Ang II-hypertensive rats (control: 1.13 ± 0.17 vs NO: 1.32 ± 0.23 AU/s). Addition of 100 μM dibutyryl cGMP reduced NKCC2 activity in control rats from 0.97 ± 0.19 to 0.73 ± 0.15 AU/s ($p<0.01$) but not in THALs from Ang II-hypertensive rats (1.00 ± 0.09 to 1.02 ± 0.30). However, 500 μM dibutyryl cGMP reduced NKCC2 activity in THALs from both controls (1.20 ± 0.32 to 0.76 ± 0.27 AU/s; $p<0.03$) and Ang II-hypertensive rats (1.15 ± 0.07 to 0.68 ± 0.10 AU/s; $p<0.03$). Inhibiting PDE5 with 25 nM vardenafil restored the ability of NO to reduce NKCC2 activity in THALs from Ang II-hypertensive rats (vardenafil: 1.17 ± 0.23 vs vardenafil + NO: 0.44 ± 0.08 AU/s, $p<0.02$).

Conclusions: Inhibition of NKCC2 activity by NO and cGMP is blunted in Ang II-induced hypertension due to elevated PDE5. Increased PDE5 activity in the THAL could contribute to the enhanced THAL NaCl reabsorption seen in this model of hypertension.

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FR-PO516

Dietary Salt Intake and Angiotensin II Regulates WNK-SPAK-NKCC1 Phosphorylation Cascade in Mouse Aorta
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Background: NKCC1 is localized in vascular smooth muscle cells and regulates vascular contractility. Recently, we have reported WNK-OSR1/SPAK-NCC/NKCC1/NKCC2 phosphorylation cascade in the kidney and blood vessels. In this study, we investigated how this cascade in mouse aorta was regulated by dietary salt intake and angiotensin II.

Methods: The phosphorylations of SPAK at Thr383 (a phosphorylation site by WNK kinase) and NKCC1 at Thr206 (a phosphorylation site by SPAK) were examined in the aorta of C57BL/6J mice fed high-salt, normal or low-salt diet for a week. Acute (125ng/kg) and chronic (1.4ug/kg/min for a week) angiotensin II (AngII) infusion was also performed to investigate AngII effect on this signal cascade in aorta.

Results: SPAK and NKCC1 phosphorylations in the mouse aortic tissue was increased by low-salt diet and decreased by high-salt diet, respectively. Both acute and chronic AngII infusion also increased SPAK and NKCC1 phosphorylations. Interestingly, this low-salt diet-induced phosphorylation of SPAK and NKCC1 in aorta was impaired in the WNK3 knockout mice, suggesting that WNK3 may be one of the major WNK kinases in mouse aorta.

Conclusions: In this study, we demonstrated that dietary salt intake and AngII regulated WNK-SPAK-NKCC1 phosphorylation cascade in mouse aorta. This could be an important mechanism of blood pressure regulation by WNK kinases in extrarenal tissues.

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FR-PO517

Nedd4-2 Ablation in Mice Leads to Upregulation of NCC Compensated by Decreased αENaC and Increased ROMK Caroline Ronzaud,[1] Dominique Loffing-Cueni,[2] Sumedha Malsure,[1] Baoli Yang,[3] John B. Stokes,[4] Robert Koesters,[5] Marc P. Maillard,[6] Edith Hummler,[1] Johannes Loffing,[2] Olivier Staub.[1] *[1]Department of Pharmacology and Toxicology, Lausanne University, Lausanne, Switzerland; [2]Institute of Anatomy, Zurich University, Zurich, Switzerland; [3]Obstetrics and Gynecology, Iowa University, Iowa City, IA; [4]Department of Internal Medicine, Iowa University, Iowa City, IA; [5]INSERM UMRS 702, Tenon Hospital, Paris, France; [6]Nephrology, CHUV, Lausanne, Switzerland.*

Background: Control of renal Na^+ and K^+ transport by aldosterone is crucial for maintaining Na^+/K^+ balance and blood pressure (BP). Aldosterone acts in part by preventing ENaC degradation by the ubiquitin ligase Nedd4-2 (N4-2).

Methods: To determine the role of renal N4-2 in mediating salt-sensitive hypertension observed in N4-2 total knockout (KO) mice (Shi *et al*, 2008), inducible renal tubule-specific N4-2 KO mice were generated using the TetOn/CreLoxP systems to delete exons 5-6 of the N4-2 gene. Pax8/LC1 mice, allowing tetracycline-inducible Cre-mediated recombination in renal tubules, were bred with $N4\text{-}2^{fl/fl}$ mice to obtain mutants ($N4\text{-}2^{fl/fl}$/Pax8/LC1) and controls ($N4\text{-}2^{fl/fl}$/Pax8 or $N4\text{-}2^{fl/fl}$/LC1), all treated with doxycyclin.

Results: N4-2 was completely lost in all renal tubular segments in dox-treated mutants. Under both standard and high-Na^+ diets, mutants were able to maintain normal Na^+/K^+ balance, whereas plasma aldosterone and urine volume were increased. Interestingly, mutants displayed hypertension and elevated urine Ca^{2+} excretion under high-Na^+ diet that could be treated with thiazides, suggesting increased NCC activity. Consistently, mutants showed increased NCC protein abundance and phosphorylation. βENaC, γENaC, and ROMK protein expression was increased as well. Unexpectedly, αENaC protein and mRNA were decreased, likely related to the lowered plasma aldosterone levels and compensating the increased NCC activity.

Conclusions: These *in vivo* data show : 1) N4-2 effects on β/γENaC, but not αENaC; 2) importance of N4-2 for controlling BP and Ca^{2+} absorption via regulating NCC; 3) αENaC downregulation and ROMK upregulation that may help preventing hypertension and hyperkalemia, respectively.

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FR-PO518

The Drosophila NKCC Ncc69 Is Required for Normal Renal Tubule Function Aylin R. Rodan, Michel Baum, Chou-Long Huang. *Depts. of Medicine and Pediatrics, UTSW Medical Center, Dallas, TX.*

Background: NKCCs play a key role in renal epithelial ion transport. We are using *Drosophila melanogaster*, which has sophisticated genetics, a rapid life cycle, and easily accessible tubules, to better understand NKCC function and regulation. In the fly tubule, cation flux occurs through the principal cells, which express an apical H^+-ATPase that generates a lumen-positive transepithelial potential difference and drives K^+ secretion by K^+/H^+ exchange. Basolateral K^+ uptake may occur through NKCC, Na^+/K^+-ATPase, and other mechanisms.

Methods: Here, we examine the role of the NKCC Ncc69 in *Drosophila* tubule function by measuring fluid secretion (nl/min/tubule) and K^+ flux (pmol/min/tubule) in isolated tubules from wild-type and *Ncc69* mutant flies.

Results: *Ncc69* mutant tubules have decreased rates of fluid secretion (0.43 +/- 0.03 nl/min vs. 0.55 +/- 0.02 nl/min in wild-type) and K^+ flux (62 +/- 4 pmol/min vs. 93 +/- 4 pmol/min in wild-type), and these phenotypes were rescued by expression of wild-type Ncc69 in the principal cells of the tubule. Ouabain decreased K^+ flux in wild-type tubules, from 92 +/- 6 pmol/min to 64 +/- 6 pmol/min, but had no effect in *Ncc69* mutant tubules, indicating that *Ncc69* is the sole Na^+/K^+-ATPase-dependent transporter in unstimulated tubules. However, in the presence of cAMP, which stimulates diuresis, additional Na^+/K^+-ATPase-dependent K^+ transport pathways are recruited, as ouabain abolished non-Ncc69-mediated, cAMP-stimulated K^+ flux. Na^+ flux was unaltered in *Ncc69* mutants, suggesting Na^+ recycling through the Na^+/K^+-ATPase. This enables the fly, which consumes a K^+-rich, Na^+-poor diet, to secrete K^+ and conserve Na^+. Inhibition of fluid secretion and K^+ flux by the regulatory hormone capa-1, which signals through a NO/cGMPpathway, was abolished in *Ncc69* mutant tubules, indicating that Ncc69 is a target for the inhibitory action of this hormone. This is reminiscent of the inhibition of NKCC2 in thick ascending limb by NO/cGMP signaling.

Conclusions: Thus, the fly renal tubule provides a model in which to study NKCC regulation by pathways conserved in the mammalian tubule. Future studies will examine regulation of Ncc69 by the *Drosophila* homologs of WNK and SPAK/OSR1 kinases.

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FR-PO519

Characterization of the Endocytotic Pathways Involved in Internalization of Renal Na^+-K^+-2Cl^--Cotransporter Anna Daigeler,[1] Christin Dathe,[1] Bridget S. Wilson,[2] Nicholas R. Ferreri,[3] Sebastian C. Bachmann,[1] Kerim Mutig.[1] *[1]Institute of Vegetative Anatomy, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Pathology and Cancer Research and Treatment Center, University of New Mexico, Albuquerque, NM; [3]Department of Pharmacology, New York Medical College, Valhalla, NY.*

Background: The Na-K-2Cl cotransporter (NKCC2) mediates NaCl absorption in the thick ascending limb of Henle's loop (TAL). Vasopressin (AVP) activates NKCC2 by increasing its surface expression to promote urinary concentration. While NKCC2 exocytosis was intensively studied in the last years, little is known about the endocytotic pathways modulating its surface expression. This study was aimed to characterize the pathways involved in the internalization of NKCC2 at steady state and upon AVP.

Methods: To this end we have tested the involvement of NKCC2 in clathrin-, caveolae-, and multiligand type-1receptor sortilin (Sort1)-dependent endocytotic pathways in rat, mice, and cultured TAL and macula densa (MD) cells using immunohistochemistry, electron microscopy, immunoblotting, and co-immunoprecipitation (co-IP). Effects of AVP were evaluated in AVP-deficient Brattleboro rats after short term desmopressin (dDAVP) administration.

Results: In rat and mouse kidneys, NKCC2 was strongly co-localized with clathrin and moderately co-localized with Sort1, whereas no significant expression of caveolins was detected in TAL. These results were confirmed by electron microscopic evaluation of cultured cells and co-IP from rat kidney extracts. Blockade of the clathrin-mediated endocytosis by potassium depletion or chlorpromazine (30 min each) induced significant accumulation of endogenous NKCC2 (+143%) at the apical membrane in cultured cells. By contrast, genetic deletion of Sort1 in mice did not affect the surface expression or phosphorylation of the transporter. Stimulation of TAL in Brattleboro rats by dDAVP markedly increased NKCC2 surface expression, reduced its association with clathrin (-29%), but did not alter its association with Sort1.

Conclusions: We conclude that NKCC2 internalization is chiefly governed by clathrin-mediated endocytosis and can be modulated by AVP.

FR-PO520

WNK4 Modulates WNK3/SPAK-Mediated NCC Phosphorylation and Activation Maria Castañeda-Bueno,[1] Chao-Ling Yang,[2] Lorena Leonor Rojas,[1] Norma Hilda Vázquez,[1] Shaunessy L. Rogers,[2] James A. McCormick,[2] Gerardo Gamba,[1] David H. Ellison.[2] *[1]Molecular Physiology Unit, INNSZ-IIB, UNAM, Mexico City, Mexico; [2]Nephrology & Hypertension, Oregon Health & Science University and VA Medical Center, Portland, OR.*

Background: WNK3 is a positive activator of NCC through a SPAK-dependent phosphorylation of its N-terminal. In contrast, WNK4 exerts a moderate inhibitory effect upon NCC activity. Additionally, wild type WNK4, but not mutant WNK4-PHAII, decreases WNK3-mediated activation of NCC in oocytes. WNK isoforms interact each other and this is prevented by the elimination of two conserved residues (His and Gln) in the C-terminal domain (WNK4/3-HQ).

Methods: Modulation of NCC phosphorylation and activity by wild type and mutant combinations of WNK3, WNK4, and SPAK was assessed in HEK293 cells and in *Xenopus laevis* oocytes.

Results: Cotransfection of WNK3 with NCC in HEK293 cells increased NCC-T53 phosphorylation. This effect was blocked by knocking down endogenous SPAK with siRNA or by co-expressing the kinase-inactive SPAK T243A. Cotransfection of full-length or a C terminal fragment of WNK4 blocked WNK3-mediated NCC phosphorylation. Full length WNK4 also inhibited NCC activation by WNK3 in oocytes. While WNK4-DA (kinase inactive) was also inhibitory, mutants WNK4-PHAII and WNK4-HQ (which does not interact with WNK3) were not. In concordance with this, WNK3 increased the phosphorylation and abundance of glycosylated NCC, and WNK4 or WNK4-DA reduced them; again, WNK4-PHAII and WNK4-HQ were without effect. WNK4 inhibition of WNK3-induced activation of NCC was not associated with changes in WNK3-T-loop phosphorylation. Finally, WNK3-HQ lost the ability to activate NCC, while WNK4-HQ and WNK3-DA-HQ lost the ability to inhibit NCC, suggesting that the basal effects of these kinases depend on interactions between monomers or with endogenous WNKs.

Conclusions: WNK3 promotes NCC phosphorylation in a SPAK dependent manner. WNK4 inhibits WNK3 mediated activation and phosphorylation of NCC. This WNK4 effect is lost when interaction is prevented by the HQ mutations and is also lost with PHAII-WNK4; the results suggest that PHAII mutations may alter WNK interactions.

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FR-PO521

Phosphorylation of Na-Cl Cotransporter by OSR1 and SPAK Kinases Regulates Its Ubiquitination Muhammad Zakir Hossain Khan, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Na-Cl cotransporter (NCC) is phosphorylated in its amino terminus based on salt intake under the regulation of the WNK-OSR1/SPAK kinase cascade. Phosphorylation has been shown to be important for its transport activity and plasma membrane expression. We have observed that NCC protein abundance varies in the kidney based on phosphorylation status.

Methods: To clarify the mechanism, we examined NCC ubiquitination status in mice fed low, normal and high salt diets, as well as in a model mouse of pseudohypoaldosteronism type II (PHAII) where NCC phosphorylation by OSR1/SPAK is constitutively elevated.

Results: Low-salt diet decreased NCC ubiquitination, while high-salt diet increased NCC ubiquitination in the kidney, and this was inversely correlated with total and phosphorylated NCC abundance. In the PHAII model, the ubiquitination of NCC in kidney was also lower when compared to that in wild-type littermates. To evaluate the relationship between phosphorylation and ubiquitination of NCC, we expressed wild-type, phospho-deficient and -mimicking NCC in COS7 cells, and the ubiquitination of immunoprecipitated total and biotinylated surface NCC was evaluated. NCC ubiquitination was increased in the phospho-deficient NCC and decreased in phospho-mimicking NCC in both total and surface NCC.

Conclusions: We demonstrated that NCC phosphorylation by the WNK-SPAK/OSR1 signaling cascade decreased NCC ubiquitination, which may contribute to the increase of NCC abundance in plasma membranes.

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FR-PO522

Generation and Analysis of WT-WNK4 Transgenic Mice Reveal the Physiological Role of WNK4 Takayasu Mori, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Department of Nephrology, Tokyo Medical and Dental Sciences, Bunkyo-ku, Tokyo-to, Japan.*

Background: WNK kinases were identified as the causative genes of pseudohypoaldosteronism type II (PHA II), a hereditary hypertensive disease with hyperkalemia and acidosis. We identified that WNKs form a signal cascade with OSR1/SPAK kinases and SLC12A transporter families. On the other hand, the physiological role of wild-type (WT) WNK4 is still controversial. The purpose of this study is to elucidate this issue by generating and analyzing the transgenic mouse over-expressing WT-WNK4.

Methods: We generated WT-Wnk4 BAC transgenic mice. Purified BAC DNA containing the Wnk4 gene was digested with SwaI, and the desired 36.8-kb fragment was isolated, as reported previously (Lalioti et al.). The purified fragment was injected into one-cell embryos of C57BL/6J mice. We analyzed representative two transgenic lines; one had a low copy number of the transgene (2 copies), and the other had a high copy (30 copies). Blood pressures were measured by telemetry.

Results: WNK4 protein levels in the kidneys of low and high copy (LC and HC) number transgenic mice [Tg($Wnk4^{WT}$)] were increased 1.7±0.1 and 9.1±0.2 fold, respectively, compared with that of wild-type mice. Systolic blood pressure of Tg($Wnk4^{WT}$) mice was increased at night when the WNK4 protein level was increased by the transgene. LC and HC-Tg($Wnk4^{WT}$) mice showed metabolic acidosis, the magnitude of which was larger in HC-Tg than in LC-Tg. The phosphorylations of OSR1, SPAK and NCC in the kidney were clearly increased as the WNN4 protein levels increased in the TG mice.

Conclusions: The increased WT-WNK4 protein level could activate the WNK-OSR1/SPAK-NCC cascade in the in vivo kidney and induce PHAII-like phenotypes.

Funding: Government Support - Non-U.S.

FR-PO523

Sorting Protein-Related Receptor SORLA Modulates Renal Na^+-K^+-2Cl^- Cotransporter through Interaction with the β Isoform of Calcineurin Phosphatase Kerim Mutig,[1] Aljona Borschewski,[1] Christin Dathe,[1] Alexander Paliege,[1] Nicholas R. Ferreri,[2] Sebastian C. Bachmann.[1] *[1]Institute of Anatomy, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Pharmacology, New York Medical College, Valhalla.*

Background: Activity of the Na^+-K^+-2Cl^- cotransporter (NKCC2) of the thick ascending limb (TAL) is dependent on its N-terminal phosphorylation. Sorting protein-related receptor SORLA was shown to facilitate this process, but the underlying mechanisms are not fully understood. We hypothesized that SORLA may interfere with kinases or phosphatases relevant for NKCC2 phosphorylation.

Methods: We have studied the regulation of the proline/alanine-rich kinase (SPAK), oxidative stress responsive kinase 1 (OSR1), and calcineurin phosphatase (CnA) in wildtype (WT) and SORLA-deficient (SORLA-/-) kidneys using immunoblotting and confocal microscopy. Protein-protein interactions were studied by co-immunoprecipitation. Functional analyses included SPAK/OSR1-knockdown in cultured cells and application of the calcineurin inhibitor, cyclosporin A (CsA), in vivo and in cell culture.

Results: SPAK, OSR1 and the calcineurin isoform CnAβ were co-localized with NKCC2 in mouse and rat TAL. Their interactions with the transporter were established by co-IPs from rat kidney tissue. Knockdown of SPAK/OSR1 in cultured rat TAL cells led to a diminished phosphorylation of NKCC2. Application of CsA increased the phosphorylation of the transporter, confirming the functional relevance of these kinases and the phosphatase for NKCC2. SORLA-deficiency was associated with near-absence of NKCC2 phosphorylation (-84%), unchanged abundance and distribution of SPAK and OSR1 kinases, but increased renal abundance of CnAß (+201%) and accumulation of the phosphatase in the apical compartment of TAL. Short term administration of CsA to SORLA-/- mice restored their decreased NKCC2 phosphorylation levels, suggesting that CnAß was responsible for the impaired phosphorylation of the transporter upon SORLA-disruption.

Conclusions: Our results thus suggest that SORLA mediates degradation of CnAß in TAL and thus facilitates NKCC2 phosphorylation.

FR-PO524

A Primary Culture of Distal Convoluted Tubules Expressing Functional Thiazide-Sensitive NaCl Transport René J. Bindels, Nicolas Markadieu, Pedro San-Cristobal, Joost G. Hoenderop. *Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Studying the molecular regulation of the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) is important to understand how the kidney contributes to blood pressure regulation. Until now, a native mammalian cell model to investigate the molecular function of this sodium transporter remained elusive. Our aim was to establish, for the first time, a primary distal convoluted tubule (DCT) cell culture exhibiting transcellular thiazide-sensitive Na^+ transport.

Methods: The Complex Object Parametric Analyzer and Sorter (COPAS) was used to sort fluorescent PV-positive tubules from these kidneys, which were then seeded onto permeable supports. To this end, kidneys from mice expressing enhanced green-fluorescent protein (eGFP) under the PV gene promotor (PV-eGFP-mice) were employed. Parvalbumin (PV) is primarily expressed in the DCT, where it colocalizes with NCC.

Results: Kidneys from PV-eGFP-mice were digested by collagenase and subsequently ~5000 fluorescent tubules per micewere isolated. The purity of the sorted tubules was evaluated by RT-PCR with primers of different tubule-segment specific genes. NCC, TRPM6, ROMK and PV were abundantly detected in the sorted tubules, while NKCC2, TRPV5 and ENaC were less abundant. The isolated DCT fragments were seeded on permeable support and after 6 days of culturing, DCT cell monolayers developed transepithelial resistance values of 630 ± 33 Ω.cm^2. The monolayers also established opposing transcellular concentration gradients of Na^+ and K^+. Radioactive $^{22}Na^+$ flux experiments showed a net apical to basolateral thiazide-sensitive Na^+ transport across the monolayers. Both hypotonic low-chloride medium and 1 mM angiotensinII increased this $^{22}Na^+$ transport significantly by four times, which could be totally blocked by 100 µM hydrochlorothiazide. Angiotensin II-stimulated $^{22}Na^+$ transport was also inhibited by 1 µM losartan. Furthermore, NCC present in the DCT monolayers was detected by immunoblot and immunocytochemistry studies.

Conclusions: A murine primary DCT culture was established which expresses functional thiazide-sensitive Na^+-Cl^- transport.

Funding: Government Support - Non-U.S.

FR-PO525

Phosphorylation of an Alternative Splice Variant of the Thiazide-Sensitive NaCl Cotransporter Markedly Augments Its Transport Activity Pedro San-Cristobal, Henrik Dimke, Jacob (Jaap) Deinum, Jacques W.M. Lenders, Joost G. Hoenderop, René J. Bindels. *Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Despite the importance of the thiazide-sensitive NaCl cotransporter (NCC) in renal electrolyte transport and blood pressure regulation, several aspects related to the control of NCC activity remain unexplained. Recently, a novel splice variant in the carboxyl (C)-terminal domain of NCC (NCC-Sv) was identified, containing a phosphorylation site at residue serine 811. The aim of the present study was to establish the functional role of the newly discovered NCC-Sv.

Methods: NCC-Sv and corresponding phospho mutant's activity were evaluated by $^{22}Na^+$ influx measurements using the *X. laevis* expression system. Moreover, NCC-Sv expression and plasma membrane localization was investigated using fluorescence confocal laser scanning microscopy.

Results: Thiazide-sensitive $^{22}Na^+$ transport rates for NCC-Sv (4250 ± 325 pmol/oocyte/hr) were statistically higher than the wild-type transporter (NCC-wt) (3025 ± 336 pmol/oocyte/hr). Furthermore, the mutant mimicking a constitutively active phosphorylation site (S811D) presents a robust transport (5104 ± 313 pmol/oocyte/hr). In line with this result, elimination of this serine residue in NCC-Sv (S811A) (inactive mutant) prevented the enhanced response (2832 ± 239 pmol/oocyte/hr). All phosphomutants showed similar behavior in transport rates when co-expressed with the With No Lysine "K" Kinases WNK4 (63% inhibition) or WNK3 (250% activation), suggesting that NCC splicing and phosphorylation modulates NCC activity indepedently from the WNK pathway. Confocal microscopy of green fluorescent-tagged protein showed no changes in any of all constructs at plasma membrane abundance. Finally, metolazone affinity appeared unaltered between the different variants.

Conclusions: The present study suggest that phosphorylation of NCC-Sv stimulates the intrinsic activity of the transporter, in addition to the currently described molecular mechanisms. Thus, regulation of the phospho site present in NCC-Sv is a novel pathway whereby NCC activity and hence blood pressure can be regulated.

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FR-PO526

Defective Processing and Trafficking of NKCC2 Proteins in Bartter Syndrome: Role of OS9 Yingying Zhu, Nadia Defontaine, Sylvie Demaretz, Kamel Laghmani. *Universités Paris V, Paris VI, Paris, France.*

Background: Mutations in the renal specific Na-K-2Cl co-transporter, NKCC2, lead to type I Bartter syndrome (BS1). Yet very little is known about the molecular mechanisms underlying the trafficking of NKCC2 mutants in mammalian cells. We have previously shown that wild-type (WT) and NKCC2 mutants are subject to regulation by the endoplasmic reticulum-associated degradation (ERAD). Here, we studied the implication of the ER-resident protein OS9, in the ERAD of BS1-causing mutants.

Methods: The expression of WT and three NKCC2 mutants was monitored in transiently transfected OKP and HEK cells, using immunoblot (IB) and confocal imaging. Protein-protein interactions were investigated by co-immunoprecipitation (Co-IP).

Results: As expected, WT NKCC2 was detected mainly as a complex-glycosylated form, expressed at the cell surface. In contrast, the three BS1-causing mutants exhibited reduced expression levels and were detected mainly intracellularly. Mutant Y477N displayed partial loss of complex glycosylation at the cell surface, and E368G and A628D mutants showed only immature forms and were trapped in the ER. To identify the molecular components of the ERAD of NKCC2 mutants, we focused on OS9, a binding partner of NKCC2 that we identified by the yeast two-hybrid system. Co-IP assay showed that endogenously expressed OS9 interacts with immature forms of WT and NKCC2 mutants. Overexpression of OS9 led to a decrease in the expression and maturation of WT NKCC2, an effect reversed by the proteasome inhibitor MG132. Conversely, knockdown of OS9 by small interfering RNA improved the maturation of WT NKCC2. Importantly, knockdown of OS9 increased the abundance of mutants E368G and Y477N and partially restored the expression of their complex-glycosylated and functional forms. In contrast, knockdown of OS9 had no effect the expression of mutant A628D failing therefore to improve its maturation.

Conclusions: We conclude that OS9 is involved in the ERAD of WT NKCC2 and only some forms of BS1-causing mutants. Moreover, our results suggest that physiological or pathological changes in OS9 expression levels may affect NKCC2 function by modulating its maturation and surface expression.

FR-PO527

Tamm-Horsfall Protein-Deficient Mice Have Increased Sodium Excretion during Acute Sodium Loading Hajamohideen S. Raffi, James M. Bates, Satish Kumar. *Medicine/Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Tamm-Horsfall Protein (THP) is urine's most abundant protein. THP is synthesized in the thick ascending limb of the loop of Henle where it is postulated to play a role in the regulation of sodium transport. Recent studies have shown increased abundance but reduced activity of the Na+,K+,2Cl- cotransporter (NKCC2) in the kidney, in the absence of THP, suggesting that THP increases the activity of NKCC2. In this study, we determined the effect of acute sodium loading in mice in the presence and absence of THP.

Methods: $THP^{-/-}$ mice were created by homologous recombination and backcrossed for eight generations in order to create similar genetic background in $THP^{+/+}$ and $THP^{-/-}$ mice. Six age matched $THP^{+/+}$ and $THP^{-/-}$ mice were selected. Base line urine samples were collected for 4 hours. The mice were gavaged with 1.8 % sodium chloride solution (10 ml/kg body weight). Urine samples were collected for 4 hours. Urine volume and sodium were measured and compared using Student's t test.

Results: At baseline urine volume was higher in $THP^{-/-}$ mice ($THP^{-/-}$, 0.48ml ± 0.21 vs. $THP^{+/+}$, 0.34ml ± 0.09, p = 0.022) but sodium excretion (mEq/4h) was not different between the two groups. After the salt load, urine volume remained higher in $THP^{-/-}$ mice ($THP^{-/-}$, 0.485ml ± 0.04 vs. $THP^{+/+}$, 0.385ml ± 0.04, p = 0.041) and urinary excretion of sodium (mEq/4h) was higher in $THP^{-/-}$ mice compared to $THP^{+/+}$ mice ($THP^{-/-}$, 0.093 ± 0.009vs. $THP^{+/+}$, 0.072 ± 0.007, p = 0.032).

Conclusions: THP-deficient mice have reduced renal sodium absorption and a secondary defect in urinary concentration. THP plays a role in renal handling of sodium.

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WNK4-OSR1/SPAK-NCC Signal Cascade Has Circadian Rhythm Dependent on Aldosterone Koichiro Susa, Eisei Sohara, Kiyoshi Isobe, Motoko Chiga, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Blood pressure and renal salt excretion show circadian rhythms. Recently, it has been clarified that clock genes regulate circadian rhythms of renal transporter expression in the kidney. We found that the activity of WNK-OSR1/SPAK-NaCl cotransporter (NCC) signal cascade is important for regulating salt balance and blood pressure in the body. Although WNK4 and NCC mRNA levels reportedly showed circadian change in the mouse kidney, it remained to be determined whether the activity of this signal cascade also showed circadian change. In this study, we sought to determine whether the protein levels and the phosphorylations of WNK4, OSR1, SPAK, and NCC showed circadian changes in the mouse kidneys.

Methods: Male C57BL/6J mice were sacrificed every 4 h (at 20:00, 0:00, 4:00, 8:00, 12:00, and 16:00), and the expression and phosphorylation of WNK4, OSR1, SPAK, and NCC were determined by immunoblots. (Lights were turned on at 8:00, which was the start of the rest period, and turned off at 20:00, which was the start of the active period, since mice are nocturnal).

Results: Although the protein level of each component of this cascade did not show circadian change, the phosphorylation levels of OSR1, SPAK, and NCC were increased around the start of the active period and decreased around the start of the rest period. Oral administration of eplerenone (10 mg/day) attenuated the overall phosphorylation levels of these proteins and also diminished the circadian change of NCC phosphorylation.

Conclusions: The activity of WNK4-OSR1/SPAK-NCC cascade was shown to have a circadian change in the kidney that may be governed by aldosterone. WNK-OSR1/SPAK-NCC signal cascade may start to be suppressed upon awakening to prepare for adequate sodium excretion during the day. After adequate sodium excretion, this cascade may start to accelerate at bedtime to decrease sodium excretion during the night.

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FR-PO529

Aldosterone Increases Surface NCC Protein Expression via Inhibiting ERK1/2 Phosphorylation-Induced NCC Ubiquitination Xiuyan Feng,[1,2] Matthew Lee,[1] Hui Cai.[1,2] *[1]Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, GA; [2]Renal Section, Atlanta VA Medical Center, Atlanta, GA.*

Background: The thiazide-sensitive Na^+-Cl^- cotransporter (NCC) localizes to the distal convoluted tubule (DCT) and is one of key regulators of sodium balance. The MAPK-ERK1/2 signaling pathway was reported to be mediated in NCC ubiquitination. We have previously shown that knockdown of ERK 1/2 expression increased the surface protein expression of NCC. We also found that aldosteone downregulated the phosphorylation of ERK 1/2. We, therefore, hypothesized that aldosterone up-regulate the NCC surface protein expression via blocking ERK 1/2 phosphorylation-induced NCC ubiquitination.

Methods: We used western blot analysis in mDCT cells and HEK 293 cells.

Results: To further confirm the hypothesis, we treated the mDCT cells with aldosterone 1 μM for 3 hours and found that NCC surface expression was increased whereas ERK 1/2 phosphorylation and NCC ubiquitination were decreased. The NCC ubiquitination was partially increased when we pretreated the mDCT cells with BCI, a specific inhibitor of ERK1/2 phosphatase, suggesting that increase in ERK1/2 phosphorylation enhanced NCC ubiquitination. In addition, when aldosterone treated the HEK 293 cells transiently expressed HA-NCC we found the similar result as that in mDCT cells.

Conclusions: These results suggest aldosterone induced the NCC surface protein expression via inhibiting ERK1/2 phosphorylation-induced NCC ubiquitination.

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FR-PO530

mTORC2 Critically Regulates Distal Tubular Sodium and Potassium Homeostasis Florian Grahammer, Tobias B. Huber. *Renal Division, Department of Medicine, University Hospital Freiburg, Freiburg, Germany.*

Background: The mTOR pathway plays a pivotal role in orchestrating cellular homeostasis. Yet most data relate to the rapamycin sensitive mTOR complex 1 while the organ specific *in vivo* function of mTOR complex 2 has remained more or less elusive.

Methods: Distal tubular cell specific mTORC 2 (*Rictor*) knock-out mice were generated by crossing *Rictor fl/fl* animals with *Ksp*Cre mice. After crossing, these mice were analyzed using functional assays, light and immunofluorescence microscopy, western blotting and primary culture of CCDs.

Results: *Rictor fl/fl*Ksp*Cre mice were viable and did not show any obvious macroscopic, microscopic nor renal functional phenotype under control conditions up to one year of age. Compared to wild-type mice on a low salt diet, *Rictor fl/fl*Ksp*Cre mice were able to adequately reduce Na+ excretion, whereas a high K+ diet led to moderate hyperkalemia. Under a low salt and high K^+ diet *Rictor fl/fl*Ksp*Cre mice rapidly developed weight loss, hyperkalemia and acute renal failure within 4 days. The same phenotype was seen when *Rictor fl/fl*Ksp*Cre mice were treated with the ENaC blocker triamterene indicating a vital salt conserving role of mTORC2 under salt loosing/potassium sparing conditions.

Biochemically we observed a strikingly reduced phosphorylation of sgk1 at Ser422 which seems to be critical to allow K^+ secretion under reduced Na^+ uptake. Experiments with primary cells from *Rictor fl/fl*Ksp*Cre mice could further substantiate the decisive role of mTORC2 for aldosterone mediated distal tubular signalling.

Conclusions: mTORC2 is a vital kinase for the aldosterone mediated renal tubular stress response under clinically relevant conditions and seems decisive for K+ excretion under low salt or salt loosing conditions.

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FR-PO531

High-Throughput NKCC Functional Assay in Adherent Epithelial Cells Monica Carmosino, Federica Rizzo, Giuseppe Procino, Silvia Torretta, Maria Svelto. *Biosciences, Biotechnologies and Pharmaceutical Sciences, University of Bari, Bari, Italy.*

Background: The kidney-specific isoform of the Na-K-2Cl cotransporter NKCC2 is involved in the Na+ reabsorption in the TAL cells and in the regulation of body fluid volume. In contrast, the isoform NKCC1 represents the major pathway for Cl- entry in endothelial cells, playing a crucial role in cell volume regulation and vascular tone. Indeed both NKCC isoforms are involved in the regulation of blood pressure and represent important potential drug targets for hypertension treatment. Accordingly a high-throughput screening for NKCC inhibitors is extremely useful in the development and characterization of new anti-hypertensive drugs. So far the high-throughput screening of NKCC transporters activity has been done by 86Rb+ influx assays.

Methods: We developed a Tallium (Tl+) based fluorescent influx assay that can accurately and rapidly measure NKCC transport activity in adherent epithelial cells in the high-throughput Flex station device (FLEXA). We assessed the feasibility of this assay in the renal epithelial LLC-PK1 cells stably transfected with a previously characterized chimeric NKCC2 construct (c-NKCC2).

Results: In the absence of Cl- in the assay buffer, Tl+ addition did not induce any increase in fluorescence. However a robust Tl+ influx was observed after Cl-addition in c-NKCC2 transfected cells but not in mock-transfected or in parental LLC-PK1 cells suggesting that the Tl+ influx is actually mediated by the c-NKCC2 cotransporter. The c-NKCC2-driven FLEXA signal displays a rapid linear increased phase within the first 20 s after Cl- addition followed by a slower increase and a plateau phase. The initial rate of

Tl+-dependent Cl- influx observed in c-NKCC2 transfected cells is about 3-fold over the background signal in mock-transfected LLC-PK1 cells. The preincubation with furosemide prevented the Cl-dependent Tl+ influx confirming the specificity of the NKCC-mediated Tl+ influx.

Conclusions: We demonstrated that this assay is highly reproducible, offers high temporal resolution of NKCC-mediated ion flux profiles and, importantly, as a continuous assay, it offers improved sensitivity over endpoint NKCC functional assay.

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FR-PO533

The Expression of ClC-Kb, NCC and Calbindin-D_{28k} in Patients with Chronic Hypokalemia Min-hua Tseng,[1,3] Sung-Sen Yang,[2,3] Pei-yi Chu,[3] Shih-Hua P. Lin.[2,3] *[1]Division of Pediatric Nephrology, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan; [2]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan; [3]Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan.*

Background: The actual expression of basolateral membrane chloride channel Kb (ClC-Kb) and apical membrane sodium-chloride cotransporter (NCC) (ClC-Kb) in distal tubules in patients with genetically-confirmed Gitelman's syndrome (GS), classic Bartter's syndrome (cBS), and other causes of chronic hypokalemia has not been well studied.

Methods: Eleven GS patients with different *SLC12A3* mutations (8 missense, 1 nonsense, 1 small deletion, 2 deep intronic), 4 cBS (4 missense, 1 large deletion), and 6 non-GS and non-cBS (3 anorexia nervosa, 2 diuretic abusers, 1 distal renal tubular acidosis) were included. Immunofluoresence (IF) staining for NCC, ClC-Kb and calbindin-D_{28k} of the renal biopsy specimens from these patients and of normal renal tissues obtained from patients receiving total nephrectomy were performed. Urine NCC expression was also measured. All patients underwent renal biopsy have mild proteinuria.

Results: Compared with normal renal tissue, all GS patients showed virtually or markedly attenuated NCC, increased calbidin-D_{28K} but normal ClC-Kb expression. cBS patients had deceased CLC-Kb, but normal NCC and calbidin-D_{28K} expression. Patients with non-GS and non-cBS had normal CLC-Kb, but increased NCC and calbidin-D_{28K} expression. The expression pattern of NCC and CLC-Kb found in the respective GS and cBS was not corrected with the nature and pattern of the corresponding mutations. In contrast to patients with cBS or non-GS and non-cBS, Urinary NCC excretion was markedly decreased in GS patients.

Conclusions: In addition to urinary electrolytes and NCC excretion, the detection of NCC and CLC-kb expression from the renal tissue of patients with chronic hypokalemia may help diagnose the underling causes, especially in patients without *SLC12A3* and *CLCNKB mutations.*

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FR-PO534

Novel Genetic Variant rs623011 Associates with Non-Familial Thyrotoxic and Sporadic Hypokalemic Paralysis Min-hua Tseng,[1,2] Sung-Sen Yang,[3] Pei-yi Chu,[1] Chih-Jen Cheng,[3] Shih-Hua P. Lin.[1,3] *[1]Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan; [2]Department of Pediatric, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan; [3]Division of Nephrology, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan.*

Background: Non-familial hypokalemic periodic paralysis (hypoPP) is mainly composed of thyrotoxic (TPP) and sporadic periodic paralysis (SPP) and features with ictal hypokalemia and muscle paralysis. We have previously suggested that reduced K^+ efflux in skeletal muscle originated from mutations or inhibition of Kir channels may lead to acute hypokalemia and paralysis in these patients (Cheng CJ et al. JBC 2011 and Lin SH et al. JASN 2012). A recent genome-wide association study of Thai TPP patients identified a novel genetic variant rs623011, which may potentially reduce the transcription of Kir2.1 and total Kir current (J Hum Genet 2012). The aim of this study was to evaluate if this genetic variant is present in our patients with TPP and SPP without hyperthyroidism, the second leading cause of non-familial hypoPP in Asia.

Methods: A case-control association study that examines the relationship between rs623011 genetic variant and non-familial hypoPP patients in Taiwanese population sequence rs623011 in 87 patients with TPP, 57 SPP, and 100 healthy subjects. All patients with TPP and SPP did not have mutations in the skeletal muscle voltage-gated Ca^{2+} or Na^+ channels, Kir2.1 and Kir2.6 channels. A chi-square test and SPSS 17.0 software were used to analyze the sequencing results.

Results: Compared with normal control, the frequency of the risk allele A of rs623011 was significantly higher in TPP and SPP patients (73.6% versus 53.2%, p=0.004; 81.5% versus 53.2%, p=2.36E-005, respectively) with the Odds Ratios (95% confidence interval) 2.426 (1.329-4.426) and 4.493(2.194-9.201), respectively. However, the frequency of the A allele of rs623011 was not significantly different between TPP and SPP.

Conclusions: We confirm that rs623011 is the susceptible locus not only in TPP but also in SPP. TPP and SPP may share the pathogenic mechanism of reduced Kir current in skeletal muscle independent of thyroid hormone.

Funding: Government Support - Non-U.S.

FR-PO535

Role of Nedd4-2 and ß$_1$Pix in the Regulation of ENaC by AMPK in Kidney Epithelial Cells Hui Li,[1] Tengis S. Pavlov,[2] Jeffrey Lee,[1] Roland D. Tuerk,[3] Dietbert Neumann,[3] Alexander Staruschenko,[2] Kenneth R. Hallows.[1] *[1]Medicine, Univ. of Pittsburgh, Pittsburgh, PA; [2]Physiology, Medical College of Wisconsin, Milwaukee, WI; [3]Molecular Genetics, Maastricht Univ., Maastricht, Netherlands.*

Background: Renal collecting duct epithelial Na^+ channels (ENaCs) play a key role in total body volume and blood pressure control. The metabolic sensor AMP-activated kinase (AMPK) inhibits ENaC by enhancing ENaC binding to the ubiquitin ligase Nedd4-2, but the mechanisms involved are unclear. The guanine nucleotide exchange factor β_1Pix inhibits ENaC by impairing 14-3-3 protein binding to and sequestration of Nedd4-2, thereby promoting Nedd4-2 targeting to ENaC. We thus hypothesized that β_1Pix participates in ENaC regulation by AMPK.

Methods: Mass spectrometry and in vitro phosphorylation assays were used to detect and define AMPK phosphorylation sites in Nedd4-2 and β_1Pix. Co-immunoprecipitation (co-IP) assays were used to examine modulation of β_1Pix/14-3-3/Nedd4-2 interactions by AMPK in renal epithelial cells. Whole-cell patch-clamp studies were performed in CHO cells co-expressing ENaC and various β_1Pix and AMPK constructs.

Results: AMPK phosphorylates Nedd4-2 at Ser-444, a site previously shown to enhance Nedd4-2 cellular stability. AMPK also directly phosphorylates β_1Pix in vitro. In preliminary co-IP studies the AMPK activator metformin enhanced 14-3-3-β_1Pix binding and inhibited 14-3-3-Nedd4-2 binding in MDCK cells, consistent with our earlier finding of enhanced ENaC-Nedd4-2 association with AMPK activation in cells. Whole-cell ENaC currents were inhibited by the AMPK activator AICAR or by β_1Pix over-expression, but these effects were not additive. Moreover, ENaC current inhibition by AICAR or by over-expression of a constitutively active AMPK mutant (γ1-R70Q) was fully overridden in cells co-expressing a β_1Pix mutant (Δ602-611) that is unable to bind 14-3-3 proteins.

Conclusions: ENaC regulation by AMPK requires both functional Nedd4-2 and β_1Pix. We propose that AMPK inhibits ENaC by phosphorylating Nedd4-2, which enhances Nedd4-2 stability, and by regulating the function of β_1Pix, which then competes with Nedd4-2 for 14-3-3 binding and thereby enhances Nedd4-2 binding to ENaC.

Funding: NIDDK Support

FR-PO536

Scaffold Protein CNK3 Coordinates Assembly of a Multiprotein ENaC-Regulatory Complex Rama Soundararajan,[1] Tim Ziera,[1] Eric Koo,[1] Karen Ling,[1] Jian Wang,[1] Steffen Borden,[2] David Pearce.[1] *[1]Division of Nephrology, Department of Medicine, University of California San Francisco, San Francisco, CA; [2]Strategic Planning, Bayer Healthcare Pharmaceuticals, Berlin, Germany.*

Background: Hormone-regulation of ion transport in the kidney tubules is essential for fluid and electrolyte homeostasis in vertebrates. A large body of evidence has suggested that transporters and channels exist in multiprotein regulatory complexes, however, relatively little is known about the composition of these complexes or their assembly. The epithelial sodium channel (ENaC), in particular, is tightly controlled by the salt-regulatory hormone aldosterone, which acts, at least in part, by increasing expression of the serine-threonine kinase SGK1.

Methods: We used biotinylation assays in transiently transfected HEK293T kidney epithelial cells to study the effect of CNK3 on ENaC surface expression. We also used these cells to test protein-protein interactions in co-immunoprecipitation assays. We used polarized mpkCCD$_{cl4}$ kidney epithelial cells grown on Transwell filters to study effects on ENaC activity. We used GST pull-down assays to analyze direct protein-protein interactions *in vitro*.

Results: We show that aldosterone induces the formation of a native 1.0-1.2 MDa plasma membrane complex, which includes ENaC, SGK1, and the ENaC inhibitor, Nedd4-2, a key target of SGK1. We further show that this complex contains the PDZ domain-containing protein Connector-Enhancer-of-Kinase-Suppressor-of-Ras isoform-3 (CNK3). CNK3 physically interacts with ENaC, Nedd4-2 and SGK1, enhances the interactions among them, and stimulates ENaC surface expression and function in an aldosterone-induced PDZ domain-dependent manner.

Conclusions: These results strongly suggest that CNK3 is a molecular scaffold, which coordinates the assembly of a multiprotein ENaC-regulatory complex at the plasma membrane, and hence plays a central role in Na^+ homeostasis.(NIH Grants DK078679, DK056695 and DK085101).

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FR-PO537

P2X$_4$ Regulates ENaC-Mediated Na Reabsorption and Salt-Sensitivity of BP Eilidh Craigie, David G. Shirley, Scott S.P. Wildman, Robert J. Unwin. *Centre for Nephrology, UCL, London, United Kingdom.*

Background: Nucleotides act via P2 receptors to modify ENaC-mediated Na reabsorption in the ASDN (Bailey & Shirley 2009, Purin Sig). An inhibitory role for P2Y$_2$ receptors upon ENaC activity has been proposed as an intrinsic control system that assists the ASDN to respond appropriately to a high Na load (Toney *et al 2012, Curr Opin Neph Hypert*). An *in vitro* investigation showed that activation of apical P2X$_4$ receptors can potentiate ENaC activity at a normal ASDN Na concentration, highlighting the possibility that P2X$_4$ may also act as a regulator of ENaC (Wildman *et al* 2008, JASN).

Methods: We investigated the role of the $P2X_4$ receptor in the regulation of ENaC activity in $P2X_4$ null mice ($P2X_4^{-/-}$) and littermate controls ($P2X_4^{+/+}$). Mice were maintained on either a standard (SD;0.3%) or a low (LD;0.03%) Na diet for 2 weeks. They were anaesthetised (Inactin, 100mg/kg IP) and surgically prepared for renal clearance studies. After control collections of urine and plasma, mice were given benzamil (1 mg/kg bolus, IV) and further collections were made. Mean arterial (MA) BP was also measured. Data are presented as mean±SEM; statistical comparisons were made using ANOVA and post-hoc Bonferonni.

Results: MABP was significantly elevated in $P2X_4^{-/-}$ compared with $P2X_4^{+/+}$ mice on SD (95±2 vs 85.2±2mmHg; P=<0.01; n=8); LD significantly reduced MABP in $P2X_4^{-/-}$ mice (P=<0.01) while the reduction in dietary Na had no effect in $P2X_4^{+/+}$ mice (87.4±3.2 vs 86.7±2.6mmHg; n=6). On SD ENaC-mediated Na reabsorption (presented as Δ fractional Na excretion [FE_{Na}]) was similar for $P2X_4^{-/-}$ and $P2X_4^{+/+}$ mice (1.9±0.2 vs 1.7±0.2%). On LD $P2X_4^{-/-}$ mice had a significantly lower ΔFE_{Na} than $P2X_4^{+/+}$ mice (1.5±0.2 vs 3.2±0.5%; P<0.01) reflecting an inability to increase ENaC-mediated Na reabsorption on LD.

Conclusions: The BP response to LD in the $P2X_4^{-/-}$ mice suggests that they have a salt-sensitive BP. Moreover, the inability of the $P2X_4^{-/-}$ mice to increase their ENaC-mediated Na reabsorption after LD feeding is compatible with the *in vitro* finding that $P2X_4$ activation potentiates ENaC activity; these observations suggest that $P2X_4$ may act as an intrinsic regulator of ENaC activity in the ASDN in response to normal and/or low Na loads.

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Epigenetic Repression of the Epithelial Sodium Channel α-Subunit Gene Involves Promoter Methylation and a Loss of Sp1 Binding Zhiyuan Yu, Qun Kong, Bruce C. Kone. *Medicine, University of Texas Medical School at Houston, Houston, TX.*

Background: ENaC in the distal nephron constitutes the rate-limiting step for renal sodium and fluid reabsorption. Aldosterone increases tubular sodium absorption in large part by increasing α-ENaC transcription in collecting duct principal cells. We previously demonstrated that epigenetic changes in chromatin repress basal α-ENaC transcription. Here, we investigated the role of promoter methylation in epigenetic control of the gene.

Methods: mIMCD3 collecting duct cells, and stable mIMCD3 cell lines harboring an α-ENaC promoter-reporter construct, were analyzed after vehicle or aldosterone treatment. The effects of DNA methyltransferase (DNMT) inhibitor 5-aza-2'-deoxycytidine (5-Aza-dC) or DNMT isoform gene silencing on α-ENaC mRNA levels and promoter activity were measured by qRT-PCR and luciferase assays, respectively. Bisulfite treatment and sequencing analysis were used to identify methylation of cytosines in the α-ENaC promoter. In vitro methylation and gel shift analysis were used to assay the effects of promoter methylation on α-ENaC promoter activity and Sp1 binding to it.

Results: 5-Aza-dC treatment of IMCD3 cells augmented basal and aldosterone-induced α-ENaC mRNA levels and promoter activity by ~50%. In a corresponding manner, in vitro methylation of the α-ENaC promoter was sufficient to silence its activity in transfected IMCD3 cells. In contrast, siRNA-mediated knockdown of DNMT-3b dramatically increased α-ENaC mRNA levels and promoter activity. Bisulfite treatment and sequencing analysis of the α-ENaC promoter identified methylation of cytosines framing an Sp1 enhancer element. In vitro methylation inhibited binding of Sp1 to this element.

Conclusions: DNMT3b-dependent methylation of α-ENaC promoter sequences framing an Sp1 enhancer element limits α-ENaC transcriptional activity under basal conditions and in response to aldosterone. These results disclose a novel epigenetic mechanism for control of basal and aldosterone-induced α-ENaC transcription.

Funding: NIDDK Support

FR-PO539

Disruption of Bradykinin Signaling Impairs ENaC Adaptation to Dietary Salt Intake Mykola Mamenko, Oleg L. Zaika, Oleh Pochynyuk. *Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX.*

Background: Activation of the renal kinin-kallikrein system promotes natriuresis and diuresis via generation of its principal signaling peptide bradykinin (BK). We have recently identified that BK inhibits activity of the Epithelial Na^+ Channel (ENaC) in the aldosterone sensitive distal nephrons (ASDN) via B2R-PLC pathway.

Methods: The current study combines single channel patch-clamp recordings, fluorescent imaging in split-opened ASDNs of B1R, B2R -/- and wild-type (WT) mice with systemic assessment of urinary Na+ excretion to probe the physiological relevance of BK regulation of ENaC.

Results: We found that in contrast to WT mice BK (500 nM) has no effect on ENaC activity in B1R, B2R knock-outs. ENaC open probability (P_o) is greatly elevated in B1R, B2R -/- mice compared to WT animals during high salt intake (2% Na^+). This difference is less apparent under regular salt intake (0.32% Na^+) and blunted upon salt restriction (<0.01% Na^+). ENaC activity is comparably increased in both mouse strains by saturation of systemic mineralocorticoid status with deoxycorticosterone acetate (DOCA) suggesting that the effect of BK on ENaC is independent of aldosterone. It is accepted that angiotensin converting enzyme (ACE) represents the major pathway of BK degradation. Systemic inhibition of ACE with captopril (30 mg/kgBW for 7 days) significantly decreases ENaC activity and increases urinary Na^+ excretion in WT mice, but this effect is diminished in B1R, B2R -/- mice. At the cellular level, captopril (100 μM) greatly potentiates the inhibitory action of 100 nM BK on ENaC and augments BK-induced elevations of $[Ca^{2+}]_i$ in ASDN cells.

Conclusions: BK reduces ENaC activity especially during elevated sodium intake via a distinct pathway. Moreover, antihypertensive action of ACE inhibitors can be augmented by elevated BK levels, decreasing ENaC-dependent sodium reabsorption in ASDN.

Funding: Private Foundation Support

FR-PO540

PKC and PKA Differentially Regulate TRPV4 Activity and Trafficking in Distal Nephron Mykola Mamenko, Oleg L. Zaika, Oleh Pochynyuk. *Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX.*

Background: Distal nephron (DN) is responsible for final regulation of water and electrolyte balance. The ability of DN cells to reabsorb/secrete numerous ions and water is regulated by mechanical stimuli, such as changes in tubular flow and fluid composition. DN cells respond to increased flow by elevations in $[Ca^{2+}]_i$. It was recently shown that Ca^{2+}-permeable TRPV4 channel is abundantly expressed in DN and functions as a sensor/transducer of flow-induced stimuli. The mechanisms involved in regulation of TRPV4 activity in DN, though potentially important, remain poorly understood.

Methods: The current research combined Fura-2 Ca^{2+} imaging with IHC on split-opened distal nephrons of C57BL/6 mice to study subcellular distribution and functional activity of TRPV4 channels in distal nephron.

Results: We found that activation of PKA pathway with forskolin does not affect TRPV4-mediated Ca^{2+} responses to flow, while markedly shifting subcellular distribution of the channel to the apical membrane. These actions are blocked with a specific PKA inhibitor H-89. On the other hand application of phorbol-12-myristate-13-acetate (PMA), to activate PKC, significantly increases TRPV4-mediated Ca^{2+} responses to flow, without affecting the subcellular distribution of the channel. Inhibition of PKC with BIH-1 diminishes cellular responses to elevated flow.

Conclusions: Functional status of TRPV4 channel in the DN is regulated by two distinct signaling pathways. While PKA cascade is responsible for TRPV4 trafficking and translocation to the apical membrane, PKC-dependent pathway increases the activity of the channel on the membrane.

Funding: Private Foundation Support

FR-PO541

Uncleaved αENaC Is the Predominant form at the Cell Surface of Renal Tubules In Vivo Søren Olesen,[1] Birgitte M. Christensen,[1] Johannes Loffing,[2] Jeppe Praetorius.[1] [1]*Department of Biomedicine, Aarhus University, Health, Aarhus, Denmark;* [2]*Department of Anatomy, University of Zurich, Zurich, Switzerland.*

Background: The principal cells in the renal connecting tubules and collecting ducts reabsorb Na^+ from the preurine by apical entry through the ENaC complex and basolateral extrusion by the Na,K-ATPase. In this process, ENaC is the rate-limiting step. Both α- and γENaC subunits are modulated by proteolytic cleavage. Cleavage of αENaC is believed to occur by furin during transit trough the Golgi apparatus. The cleavage state of ENaC subunits has profound impact on channel activity as well as the resident time in the plasma membrane. The aim of the study was to test the current hypothesis that αENaC subunits are predominantly found in the cleaved form in the cell surface of connecting tubules and collecting ducts.

Methods: Surface-biotinylation of renal tubules was performed after enzymatic treatment of the renal cortex from normal mice.The surface biotinylation of CNT and CD was validated by co-labelling tubules with fluorescent Alexa488-conjugated streptavidin and endogenous green fluorescent tubules in a TRPv5 promoter driven eGFP expressing mouse line. Biotinylated and total protein fractions were immunoblotted with anti-n-terminal αENaC antibodies to determine various processed forms by molecular weight of the protein.

Results: The majority of αENaC in the biotinylated fraction is in full length forms (approximately 100 kDa and 85 kDa). Only a minor fraction (23±4 %) corresponds to the cleaved n-terminal of 30 kDa. In the total homogenate, the 30 kDa band was slightly more pronounced (34±3%) compared to the higher molecular weight forms. Approximately 10% of total αENaC was found in the biotinylated (surface) fraction. These fractions were not altered by aldosterone in preliminary experiments with 6hrs to 7 days aldosterone treatment. Compared to the total homogenate, the biotinylated fraction virtually excluded the intracellular protein α-tubulin.

Conclusions: In conclusion, αENaC at the surface of connecting tubules and collecting ducts exists primarily in a cleavable form accessible to regulation by exo-proteases.

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FR-PO542

Mechanistic Insights into ENaC Na^+ Self-Inhibition Ossama B. Kashlan, Brandon M. Blobner, Thomas R. Kleyman. *Medicine, University of Pittsburgh, Pittsburgh, PA.*

Background: The epithelial Na^+ channel (ENaC) is key to the regulation of extracellular fluid volume and blood pressure through its action in the aldosterone-sensitive nephron. Inhibition of ENaC by extracellular Na^+, referred to as Na^+ self-inhibition, likely occurs through an allosteric mechanism of Na^+ binding to low affinity sites in the large extracellular regions of ENaC subunits. Na^+ self-inhibition reflects Na^+ or Li^+ specific inhibition of ENaC currents. The effector binding sites and transduction pathways remain unidentified. A recent comparative model of the ENaC α subunit revealed an acidic cleft, analogous to the acidic region observed in the resolved structure of acid sensing ion channel 1 (ASIC1).

Methods: We hypothesized that this cleft and analogous clefts in the β and γ subunits host Na^+ effector sites. Mutations of acidic residues in the α subunit acidic cleft led us to identify one site in the peripheral finger domain that has a role in Na^+ self-inhibition. Mutations of analogous sites in the β and γ subunits produced similar results. We examined the effects of mutations of nearby sites on Na^+ self-inhibition, which implicated a loop that connects the central palm and β-ball domains of the α subunit in Na^+ self-inhibition.

To determine whether these sites were involved in effector coordination, we measured the ability of mutants to change the effector specificity of Na^+ self-inhibition, which is $Na^+ > Li^+ >> K^+$ for wild type mouse ENaC.

Results: Several mutations in the palm/β-ball loop of the α subunit induced greater inhibition by Li^+ than by Na^+. Some of these also induced increased K^+ inhibition, compared to wild type. To identify steps in the transduction pathway, we attempted to trap ENaC in a specific conformation by crosslinking sites within the acidic cleft. Introducing Cys to both sites on the finger domain and the nearby loop led to channels that were strongly stimulated by dithiothreitol.

Conclusions: Our results suggest that sites in the acidic cleft influence the effector selectivity of Na^+ self-inhibition, and that conformational changes between nearby structures are associated with channel gating. These data suggest that the acidic cleft may host an inhibitory Na^+ binding site.

Funding: NIDDK Support

FR-PO543

Colon Specific αENaC Gene Inactivation Fully Inhibits Electrogenic Amiloride Sensitive Sodium Transport, Leading to Sodium Loss in Faeces without Compromising Overall Sodium and Potassium Balance

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Background: The epithelial sodium channel (ENaC) composed of three sub-units α, β and γ plays an important role in sodium balance, blood volume and blood pressure. Although the role of ENaC on sodium reabsorption in kidney and lung is well established, less is known in colon. The aim of this project is to investigate the importance of αENaC expression in the distal colon for maintaining sodium and potassium balance.

Methods: It was assessed by generating a double-transgenic mouse in which αENaC expression is deleted in the colonic crypt cells by using the *villin* promoter ("KO" mice). Quantitative RT-PCR and protein expression of αENaC was distal colon specific and near-complete (>98%) in "KO" mice.

Results: Following a standard or sodium-deficient diet, the "KO" mice did not show any difference in body weight compared to WT. By contrast, the "KO" mice exhibited a highly significant decrease in amiloride-sensitive rectal potential difference that was maintained under standard (ko, n=7: -6±1 mV versus wt, n=6: -16 ±5 mV; P< 0.005) and salt-deficient (ko, n=6: -6 ±4 mV versus wt, n=5: -29 ± 3 mV; P< 0.0005) diets indicating that in colon, electrogenic sodium transport is mediated by ENaC. Sodium loss through faeces was significantly increased in "KO" mice, confirming reduced sodium reabsorption via ENaC in colon. Under standard and salt restriction, plasma sodium and potassium electrolytes were normal in "KO" and WT mice. Under standard diet, urine electrolytes were similar in both groups. Under salt restriction, however, sodium retention was significantly increased in KO mice suggesting activation of RAAS.

Conclusions: We conclude that i) upon standard diet, ENaC-mediated sodium reabsorption in colon is not crucial for sodium or potassium balance. ii) under sodium restriction, sodium reabsorption through the colon becomes limiting and it may lead to activation of RAAS to compensate for salt loss through the colon.

Funding: Private Foundation Support

FR-PO544

Neural Precursor Cell Expressed, Developmental Down-Regulated 4 like Protein, Serum- and Glucocorticoid-Induced Protein Kinase 1, and 14-3-3 Proteins Differentially Regulate the Cleaved and Uncleaved Forms of the Epithelial Sodium Channel

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Background: The epithelial sodium channel (ENaC) enhances sodium retention via the principal cells of the distal nephron which is a crucial determinant of hypertension. Nedd4-2 ubiquitin ligase is a major regulator of ENaC activity *in vivo*. ENaC channels require proteolysis of the extracellular domain for full maturation and activation. Aldosterone stimulates ENaC-mediated sodium transport by activating serum and glucocorticoid kinase 1 (SGK1) which phosphorylates Nedd4-2 and promotes its interaction with 14-3-3 proteins, thus avoiding Nedd4-2-mediated inhibition of ENaC. Whether Nedd4-2, SGK1, and 14-3-3 differentially regulate cleaved and uncleaved forms of ENaC is not well known.

Methods: Cell-based assays to analyze exogenously or endogenously expressed proteins by co-immunoprecipitation, biotinylation and subcellular fractionation assays.

Results: Nedd4-2 preferentially binds to cleaved ENaC in transfected HEK293T cells (10.9:1 +/- 0.009, p < 0.05). The majority of Nedd4-2 co-fractionates with cleaved but not uncleaved ENaC in subcellular fractionation assays performed on transfected 293T and mpkCCDc14 cells. SGK1 increases cell surface cleaved and uncleaved ENaC (1.55 +/- 0.17, 2.38+/-1.59, respectively, p<0.05) but increases only cleaved ENaC in the total cell lysate (4.96 +/- 0.22, p<0.05). SGK1 also shifts the preference of Nedd4-2 interaction from cleaved to uncleaved ENaC. R18 is a peptide that interferes with interaction of 14-3-3 and Nedd4-2. R18 reverses the SGK1-mediated increase in cell surface ENaC and total cleaved ENaC.

Conclusions: Aldosterone-induced SGK1 and 14-3-3 proteins coordinately regulate Nedd4-2 and thereby ENaC activity via several mechanisms, and each effect is dependent on the cleavage status of ENaC. The interplay between intracellular regulators of ENaC activity is more complex than currently understood and warrant further investigation.

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FR-PO545

Regulation of Sodium Homeostasis by the Deubiquitylating Enzyme Usp2 In Vivo

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Background: Na^+ homeostasis maintenance is a key process for renal blood pressure regulation in mammals. Na^+ reabsorption occurs all along the renal tubule and about 15% is under the hormonal control of the Renin-Angiotensin-Aldosterone-System in the Aldosterone-sensitive Distal Nephron (ASDN) through the thiazide-sensitive Na^+-Cl^- Cotransporter (NCC) and the amiloride-sensitive Epithelial Na^+ Channel (ENaC). Both have been shown by our lab and collaborators to be regulated by ubiquitin mediated regulation via the SGK1-NEDD4-2 pathway. The deubiquitylating enzyme Usp2-45 was identified as an aldosterone-induced gene and *in vitro* studies showed that it enhances ENaC cell surface expression and activity. Moreover, USP2-45 interacts with the ubiquitin ligase NEDD4-2 and ENaC. Altogether, these data make USP2-45 a putative regulator of ENaC and NCC by counteracting their down-regulation by NEDD4-2.

Methods: We address here the implication of Usp2 in Na^+ homeostasis *in vivo* by taking advantage of a total and constitutive Usp2-knockout mouse model. We subjected these animals to dietary switch from Normal Sodium (0.17% Na^+; NSD) to either Low Sodium (>0.01% Na^+; LSD) or High Sodium (3.2% Na^+; HSD) diets. Their renal phenotype was addressed in metabolic cages, blood pressure measurements by telemetry and Western blot analyses using antibodies against various proteins involved in renal Na^+ reabsorption.

Results: We report here that the Usp2-KO mice have normal Glomerular Filtration Rate and adapt perfectly to Na^+ dietary changes. They display normal plasma Na^+, K^+ and aldosterone levels, comparable expression levels of αENaC, NCC, SGK1 and NEDD4-2 and show no variation in blood pressure under either LSD or HSD.

Conclusions: These unexpected results indicate that Usp2-KO mice are perfectly able to maintain Na^+ and K^+ homeostasis which may suggest that compensatory mechanisms have taken place in these animals.

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FR-PO546

Role of the Renal Mineralocorticoid Receptor for Potassium Homeostasis

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Background: The mineralocorticoid hormone aldosterone (aldo) is released from the adrenal glands upon a dietary K^+ load. Aldo enhances renal K^+ excretion via ROMK channels and stimulates apical epithelial Na^+ channels (ENaC) to provide the electrochemical gradient for K^+ secretion.

Methods: Here we studied knockout mice ($MR^{flox}/AQP2^{cre}$) with targeted inactivation of the mineralocorticoid receptor (MR) in the renal collecting system (CS).

Results: $MR^{flox}/AQP2^{cre}$ mice show mild hyperaldosteronism, but are otherwise healthy. The mice tolerate K^+ loading (5% K^+, 2d) without major illness, but show slightly reduced food intake indicating K^+ avoidance. Immunoblotting (IB) and immunohistochemistry (IHC) reveal a K^+ diet-induced increase in ROMK protein abundance and apical localization that is similar for the CS of control and $MR^{flox}/AQP2^{cre}$ mice. However, $MR^{flox}/AQP2^{cre}$ mice show less K^+ diet-induced α- and γENaC proteolytic activation (assessed by IB) and the apical translocation of ENaC is restricted to the remaining MR-positive late DCT (assessed by IHC). ENaC apical translocation is not seen in the MR-negative CS. Interestingly, $MR^{flox}/AQP2^{cre}$ have a reduced abundance and phosphorylation of the NaCl-cotransporter (NCC) in the DCT. This NCC down-regulation is observed already under basal conditions, but becomes even more pronounced on 5% K^+ diet.

Conclusions: Thus, the K^+ diet-induced activation of ENaC, but not of ROMK, appears to depend on MR. In $MR^{flox}/AQP2^{cre}$ mice, the loss of the MR-dependent activation of ENaC in the CS might be compensated, at least in part, by down-regulation of NCC, which increases Na^+ delivery to cells with remaining ENaC activity, and hence improves the electrochemical gradient for K^+ secretion.

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FR-PO547

The Aldosterone Paradox: How the Kidney Combines Sodium Retention and Potassium Secretion

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Background: The aldosterone paradox refers to the question how aldosterone can promote renal Na^+ retention during hypovolemia and K^+ secretion during hyperkalemia. The main Na^+ transporters in the aldosterone-sensitive distal nephron are the Na^+ Cl^- cotransporter (NCC), which is regulated by WNK and SPAK kinases, and the epithelial Na^+ channel (ENaC), which is regulated by NEDD4-2. Here, we challenged rats not only with low Na^+ (LS) and high K^+ diets (HK) but also with a combined diet (LSHK).

Methods: Normal rats (n=6/group) were fed control diet (0.5% Na^+, 0.8% K^+), LS (0.001% Na^+), HK (5% K^+), or LSHK (0.001% Na^+, 5% K^+) for 8 days. Plasma assays and immunoblotting of kidney homogenates were conducted using standard methods.

Results: Compared to control diet, LS increased plasma renin activity, angiotensin I and II, and aldosterone (429±149 pg/mL), while HK increased aldosterone only (420±183 pg/mL). LSHK caused mild hyperkalemia (5.9±0.2 mmol/L) and increased aldosterone

to a greater extent (1355±228 pg/mL). LSHK increased renin activity similar to LS but failed to increase angiotensin I and II. In the kidney, LS increased NCC 1.4-fold, whereas both HK and LSHK decreased NCC 1.4-fold ($p<0.05$ for all). Both LS and HK increased ENaC (average for all subunits 1.5-fold), while this effect was greater with LSHK (3.0-fold). Only LS increased SPAK (2.4-fold), while both HK and LSHK decreased NEDD4-2 (1.7-fold, $p<0.05$ for all). In a follow-up experiment, LS was still able to increase NCC in the presence of losartan.

Conclusions: LS favors Na^+ reabsorption through NCC, whereas HK favors Na^+ reabsorption through ENaC to indirectly promote K^+ secretion. LS may activate NCC through angiotensin II and SPAK, although this effect was not reversed by losartan. The inhibition of NEDD4-2 by HK and LSHK may contribute to ENaC stimulation. LSHK resulted in maximal aldosterone secretion, but still inhibited NCC, possibly to deliver sufficient Na^+ to ENaC for potassium secretion. The inhibition of NCC during LSHK may be explained by low angiotensin II levels, which, in turn, may be due to an effect of LSHK on the conversion of angiotensinogen to angiotensin I.

FR-PO548

MicroRNA-194 (miR-194) Regulates ROMK Channel Activity by Targeting Intersectin 1(ITSN1) WenHui Wang. *Pharmacology, New York Medical College, Valhalla, NY.*

Background: The aim of the present study is to explore the role of miR-194 in mediating the effect of high K (HK) intake on ROMK channel activities.

Results: Northern blot analysis showed that MiR-194 was expressed in renal cortex and out medulla and that the expression of miR-194 was upregulated by increasing dietary K intake. Moreover, real-time PCR analysis further demonstrated that a HK intake increased the miR-194 expression in the cortical collecting duct. Inserting 3'UTR of ITSN1 which has the seed-sequence for miR-194 into luciferase report gene vector decreased luciferase activity in the cells transfected with human miR-194. Moreover, expression of human miR-194 decreased while application of miR-194 antagomer increased the protein level of endogenous ITSN1 in HEK293T cells. Since a HK intake decreased the expression of ITSN1 which enhanced With-No-Lysine Kinase (WNK)-induced endocytosis of ROMK, we explored whether miR-194 suppressed the expression of ITSN1 thereby increasing ROMK channels in HEK293T cells transfected with GFP-tagged ROMK1. The biotin labeling technique showed that expression of miR-194 increased the ROMK expression in the plasma membrane. The whole-cell recording demonstrated that expression of miR-194 increased ROMK current from 1170pA/25pF to 1750pA/25pF and also abolished the inhibitory effect of WNK4 on ROMK channels. Coexpression of ITSN1 reversed the stimulatory effect of miR-194 on ROMK channels and reduced K currents to 1150pA/25pF, suggesting the role of ITSN1 in mediating the effect of miR-194 on ROMK channels.

Conclusions: We conclude that miR-194 regulates ROMK channel activity by modulating ITSN1 expression and that HK induced increase in miR-194 may be involved in mediating the effect of a HK intake on ROMK channel activity.

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FR-PO549

Src-Family Tyrosine Kinase (SFK) Phosphorylates With-No-Lysine Kinase4 (WNK4) and Modulates the Inhibitory Effect of WNK4 on ROMK Channels WenHui Wang. *Pharmacology, New York Medical College, Valhalla, NY.*

Background: Previous studies have demonstrated that SFK modulates the effect of SGK1-WNK4 interaction on ROMK channels (PNAS, 106:15061-6, 2009). The aim of the present study is to examine whether SFK phosphorylates WNK4 using HEK293T cells transfected with flag-tagged WNK4 and c-Src.

Results: Western blots with anti-phosphotyrosine antibodies demonstrated that c-Src increased WNK4 phosphorylation. The mutation of tyrosine residue 1092 (Tyr^{1092}), Tyr^{1094} and Tyr^{1143} to phenylalanine ($WNK4^{Y1092F}$, $WNK4^{Y1094F}$ and $WNK4^{S1143F}$) decreased the SFK-induced phosphorylation of WNK4, suggesting that they are possibly SFK phosphorylation sites. Mass spectrometry further confirmed that $tyr^{1143}$of WNK4 was phosphorylated by c-Src. Coimmunoprecipitation experiments identified protein tyrosine phosphatase type 1D (PTP1D) in complex with WNK4 and this association was diminished in the cells transfected with $WNK4^{Y1143F}$, suggesting that Tyr^{1143} was a binding site of PTP1D to WNK4. Coexpression of $WNK4^{Y1143F}$ inhibited ROMK channels as potently as those of wt WNK4. However, expression of SGK1 reversed the inhibitory effect of WNK4 on ROMK channels but did not change the inhibitory effect of $WNK4^{Y1143F}$. Moreover, expression of SGK1 failed to stimulate serine phosphorylation of WNK4 at Ser^{1196} in the cells transfected with $WNK4^{Y1143F}$. In contrast, SGK1 increased wt WNK4 phosphorylation at Ser^{1196}. The notion that expression of $WNK4^{Y1143F}$ impairs the association of PTP1D to WNK4 thereby enhancing the effect of SFK was suggested by the finding that SGK1 reversed WNK4's inhibition of ROMK channel in the cells treated with SFK inhibitors.

Conclusions: We conclude that WNK4 is the substrate of SFK and that Tyr^{1143} is one of tyrosine phosphorylation site. The significance of the present study is to illustrate a novel mechanism by which SFK modulates WNK4-dependent regulation of ROMK.

Funding: NIDDK Support

FR-PO550

The Role of Renal SGK1 in the Control of Potassium Homeostasis in Inducible Nephron-Specific Sgk1-KO Mice Lama Al-qusairi,[1] Anne Debonneville,[1] Nourdine Faresse,[1] Johannes Loffing,[2] Olivier Staub.[1] *[1]Dpt. of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland; [2]Institut of Anatomy, University of Zurich, Zurich, Switzerland.*

Background: Dietary K+ load results in an increased kalemia, leading to aldosterone release in order to stimulate K+ secretion in the ASDN. However, the regulatory mechanisms involved in this process remain unclear. Here, we aim to identify new pathways involved in the regulation of K+ secretion. More specifically, we have investigated the role of the aldosterone inducible SGK1 kinase.

Methods: Inducible nephron specific SGK1-KO mice (SGK1fl/fl/Pax8/LC1 model), recently generated in our group (Faresse et al., AJP, 2012), are employed to avoid the compensatory mechanisms which may mask the role of SGK1. Thanks to the previously described TetOn/CreLoxP system, in which rtTA is expressed under the control of the Pax8 promotor (Traykova-Brauch M et al., Nat Med. 2008), inducible inactivation of the floxed SGK1 allele occurs specifically in the renal tubules. Normal and nephron-specific SGK1-KO animals are challenged by different potassium diets. The metabolic parameters are analyzed, together with the protein and the mRNA levels of the key players in K+ regulation.

Results: Our results indicate that K+ secretion is altered in nephron-specific SGK1-KO mice. Indeed, mutant animals exhibit 35% decrease in urine K+ level after 2 days under high K+ diet (5%). Interestingly, urine Na+ level are also decreased in these conditions.

Conclusions: Our results indicate that SGK1 plays an important role in the long-term regulation of K^+ secretion. We are currently investigating accompanied changes in plasma aldosterone and electrolyte levels. Moreover, blood pressure measurements will be undertaken.

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FR-PO551

Role of Big-Conductance K Channels in Principal Cells for K Homeostasis Timo M. Rieg,[1,2] Jessica A. Dominguez Rieg,[2] Volker Vallon.[1,2,3] *[1]Medicine, University of Califonia San Diego, CA; [2]VA San Diego Healthcare System, CA; [3]Pharmacology, University of Califonia San Diego, CA.*

Background: Big-conductance K (BK) channels are expressed in principal cells and intercalated cells of the kidney and contribute to K homeostasis and flow-induced K secretion.

Methods: To define the role of BK channels in principal cells we generated mice with conditionally inactivated BK channels in principal cells of the connecting tubule/collecting duct (AQP2:Cre; $BK^{loxloxCre}$). Cre negative BK^{loxlox} mice served as controls (Con). Mice were placed for 7 days on a low (<0.03%) and subsequently on high (5%) K intake. Spontaneous voided urine was collected daily and K and creatinine determined. Systolic blood pressure (SBP) was measured by tail cuff daily. At the end of each diet regimen blood samples were analyzed for K, pH and aldosterone. ROMK membrane protein expression was determined in renal cortex lysates.

Results: During low K intake (all values are averages over 7 days, Con vs. $BK^{loxloxCre}$, n=12/group), fluid intake (4.4±0.3 vs. 4.7±0.3 ml/day), food intake (3.0±0.2 vs. 2.9±0.1 g/day), urinary K/creatinine (3.7±0.4 vs. 4.2±0.5 mmol/mmol) and SBP (111±5 vs. 112±5 mmHg) were not different between groups. Plasma K (3.9±0.1 vs. 3.9±0.1 mmol/l), aldosterone (445±68 vs. 370±63 pg/ml) and pH (7.38±0.01 vs. 7.4±0.01) were also comparable. After switching to high K diet, fluid intake (9.8±0.8 vs. 9.0±0.4 ml/day) and urinary K/creatinine increased (139±14 vs. 134±17 mmol/mmol), whereas food intake (3.4±0.7 vs. 3.4±0.5 g/day) and SBP were unchanged (110±4 vs. 108±4 mmHg). Increased plasma K (4.8±0.1 vs. 4.6±0.1 mmol/l) triggered higher aldosterone (1589±281 vs. 1262±209 pg/ml) and induced acidosis (7.34±0.02 vs. 7.34±0.01); however, values were not different between groups. After 7 days of high K intake ROMK protein expression in renal cortex was not significantly different between groups (100±8 vs. 104±11%).

Conclusions: Our data indicate that K homeostasis was maintained in mice with conditional inactivation of BK channels in principal cells when mice were challenged with high K intake. ROMK channels in principal cells as well as BK channels in intercalated cells may primarily maintain K balance under these conditions.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO552

Mechanoregulation of BK Channel Activity in the Distal Nephron: Role of the MAP-Kinase (MAPK) Pathway Rajeev Rohatgi,[1,2,3] Cindy Else,[3] Daniel Armando Flores,[2] Beth Zavilowitz,[3] Wen Liu,[3] Lisa M. Satlin.[2,3] *[1]Medicine, James J. Peters VAMC, Bronx, NY; [2]Medicine, Mount Sinai School of Medicine, New York, NY; [3]Pediatrics, Mount Sinai School of Medicine, New York, NY.*

Background: An increase in tubular flow rate, associated with an increase in fluid shear stress (FSS), stimulates BK channel-mediated net K secretion (BK-JK) in the cortical collecting duct (CCD), a segment comprised of intercalated (ICs) and principal cells (PCs). Patch clamp studies in rodent CCD (Li et al, 2006) reveal that inhibitors of ERK and p38 augment BK channel activity in PCs, suggesting that (i) the channel therein is tonically suppressed by MAPK and (ii) MAPK inhibition should stimulate BK-JK in CCDs perfused at a slow flow rate. As FSS stimulates the MAPK pathway in endothelial cells, we hypothesized that FSS regulates MAPK signaling in PCs, which, in turn, modulates BK-JK.

Methods: Immortalized murine CCD cells (mpkCCD) of PC origin were grown to confluence on glass slides and subjected to 0 or 0.4 (physiological) dynes/cm^2 FSS. Western Blot analysis was performed on cell lysates to quantitate phosphorylated p38 and ERK. The effects of MAPK inhibition on net Na (JNa) absorption and K (JK; pmol/min. mm) secretion were measured in microperfused rabbit CCDs in the absence and presence of SB203580, a p38 inhibitor.

Results: mpkCCD cells exposed to FSS for 3, 5, 10, 30 and 60 min expressed greater abundance of phospho-ERK and phospho-p38 than non-sheared cells. SB203580 had no effect on JK (-8.6±1.3 vs. -8.7±2.1) or JNa (14.1±1.5 vs. 14.8±3.7) in CCDs perfused at a slow (1 nl/min.mm) flow rate approximating the "static" conditions used in patch clamp studies.

Conclusions: FSS induces phosphorylation of p38 and ERK in mpkCCD cells (model of PCs), a response expected to inhibit BK channel mediated JK. Our failure to detect an increase in JK in CCDs treated with a MAPK inhibitor, in light of the patch clamp studies by Li et al, lead us to conclude that either 1) MAPK regulates other cellular processes (e.g. the basolateral Na-K-ATPase, NKCC1) which indirectly modulate BK-JK or 2) the BK channel in PCs does not mediate BK-JK.

Funding: NIDDK Support, Veterans Administration Support

FR-PO553

Kv1.1/Kv1.3 Potassium Channel Upregulation by Metabolic Acidosis in the Rat Kidney Rolando Carrisoza-Gaytan, Laura I. Escobar. *Department of Physiology, Facultad de Medicina, Universidad Nacional Autónoma de México, México City, Distrito Federal, Mexico.*

Background: Potassium (K) deficiency is a common electrolyte disorder. Prevalence and severity of different etiologic groups reveals the importance of the mechanisms involved in the maintenance of the external potassium balance. Renal losses represent the most frequent cause of K depletion, which results from a pathologic amplification of several kaliuretic mechanisms, activated simultaneously with additive effects on the kaliuresis. Metabolic acidosis (MA) is one of the conditions where hypokalemia is observed as a secondary abnormality due to an increased renal K secretion. Previously we identified that the voltage gated K channel Kv1.3, in the intercalated cells of the cortical collecting ducts, participates in K secretion in during a high potassium diet (HK) (Carrisoza-Gaytán et al; 2011).

The aim of this wok was to demonstrate the heteromeric expression of Kv1.1/Kv1.3 potassium channel and its regulation by metabolic acidosis.

Methods: From renal cortical and medullary plasma membranes we performed coimmunoprecipitation and immuno blot assays for Kv1.1 and Kv1.3 proteins. Western blot and Immunohistochemistry assays were done for Kv1.1 and Kv1.3 proteins from mebranes of renal cortex and medulla from rats under metabolic acidosis (MA), acute (24h) and chronicle (5 days).

Results: Kv1.1 coimmunoprecipitates with Kv1.3 from the rat kidney plasma membranes. Both, Kv1.1 and Kv1.3 subunits were upregulated to the plasma membranes in MA. Immunohstochemistry assays showed an increased immunoreactivity of the Kv1.1 and Kv1.3 to the apical membranes in collecting ducts in chronic MA.

Conclusions: Upregulation of Kv1.1/Kv1.3 channel under MA suggest its contribution to the increased potassium secretion in collecting ducts. Microperfusion studies of isolated cortical and medullary collecting ducts will allow to support this finding.

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FR-PO554

Protein Carbonylation in Regulation of Renal Proximal Tubular Na/K-ATPase Signaling and Sodium Transport Yanling Yan,[1,3] Anna P. Shapiro,[1] Steven T. Haller,[1] Vinai Kumar Katragadda,[1] Chiamaka Mbaso,[1] Deepak K. Malhotra,[1] Zi-jian Xie,[2,1] Joseph I. Shapiro,[1,2,3] Jiang Liu.[1,3] *[1]Medicine, University of Toledo College of Medicine, Toledo, OH; [2]Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, OH; [3]Pharmacology and Physiology, Marshall University JCE School of Medicine, Huntington, WV.*

Background: We have shown that cardiotonic steroids signaling through the Na/K-ATPase regulate sodium reabsorption in renal proximal tubule (RPT). Here we report that reactive oxygen species (ROS) are critical in Na/K-ATPase signaling and RPT ion transport.

Methods: c-Src phosphorylation, protein carbonylation, sodium transport.

Results: In RPT LLC-PK1 cells, ouabain (100nM) induced ROS production and c-Src activation, enhanced interaction between the α1 subunit and c-Src, stimulated accumulation of Na/K-ATPase α1 and NHE3 in early endosome (EE) fractions, and inhibited transepithelial $^{22}Na^+$ flux. Pretreatment with N-Acetyl-L-Cysteine (NAC) can prevent these effects in a dose-dependent manner. NAC alone had no effect. Both ouabain and glucose oxidase (GO, 1 and 3mU/ml) stimulated protein carbonylation in whole cell lysates and EE fractions. GO alone inhibited the transepithelial $^{22}Na^+$ flux when it was applied on both basolateral and apical aspects. Immunoprecipitation studies indicated that both ouabain and GO caused protein carbonylation of Na/K-ATPase α1 subunit, NHE3 and c-Src. Disruption of the Na/K-ATPase/c-Src signaling complex (α1 subunit knock-down, caveolin-1 knock-out, or Src kinases-null SYF cell) and c-Src inhibitor PP2 abolished ouabain-induced ROS generation and protein carbonylation, but also attenuated GO induced protein carbonylation. Moreover, carbonylation reversed after removal of ouabain from the culture medium, even when evaluated in the presence of protein biosynthesis inhibitor cycloheximide, proteasome inhibitor MG132, and lysosomotropic weak base agent chloroquine.

Conclusions: Protein carbonylation, stimulated by ouabain and glucose oxidase, may regulate Na/K-ATPase/c-Src signaling and related $^{22}Na^+$ flux. The data suggest that the Na/K-ATPase/c-Src signaling complex is a functional receptor of ROS.

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FR-PO555

Regulation of Glomerulotubular Balance III: Implication of Cytosolic Calcium in Flow-Dependent Transport Zhaopeng Du,[1] Laxiang Wan,[1] Sheldon Weinbaum,[2] Alan Mark Weinstein,[3] Tong Wang.[1] *[1]C. & M. Physiology, Yale University, School of Medicine, New Haven, CT; [2]Biomedical Engineering, City College of New York, New York, NY; [3]Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY.*

Background: Underlying glomerulotubular balance (GTB) is the impact of axial flow to stimulate Na^+ and HCO_3^- reabsorption by modulating both NHE3 and H-ATPase activity; the flow effect is proportional to estimated microvillous torque (bending moment). Angiotensin receptor blockade decreases these fluxes, but preserves the relative impact of flow on transport; dopamine leaves baseline fluxes intact, but abrogates the flow-effect. In the present work, we provide evidence implicating cytosolic calcium signals in flow-dependent transport.

Methods: Mouse proximal tubules were microperfused *in vitro* at perfusion rates of 5 and 20 nl/min, and reabsorption of fluid (J_v) and HCO_3^- (J_{HCO3}) were measured. We examined the effect of luminal high Ca^{2+} (5 mM), 0 mM Ca^{2+}, Ca^{2+} chelator BAPTA-AM (10^{-7}M), the IP3 receptor antagonist 2-APB (10^{-7}M), and the CaATPase inhibitor thapsigargin (10^{-5} M).

Results: As previously observed, an increase in perfusion rate from 5 to 20 nl/min increased J_v by 62% and J_{HCO3} by 104% in control tubules. With respect to Na^+ reabsorption, high luminal Ca^{2+} decreased transport at low flow, but preserved the flow-induced increase; low lumen Ca^{2+} had little impact; both BAPTA and 2-APB had no effect on baseline flux, but abrogated the flow effect; thapsigargin decreased baseline flow, leaving the flow effect intact. With respect to HCO_3^- reabsorption, high luminal Ca^{2+} decreased transport at low flow, and mildly diminished the flow-induced increase; low lumen Ca^{2+} had little impact; both BAPTA and 2-APB had no effect on baseline flux, but abrogated the flow effect; thapsigargin decreased baseline flow, and mildly diminished the flow effect.

Conclusions: These data suggest that the IP3 receptor-mediated intracellular Ca^{2+} signaling plays a critical role in transduction of microvillous torque to increases in proximal Na^+ and HCO_3^- transport.

Funding: NIDDK Support

FR-PO556

Sex Differences in Renal Adaptation to High-NaCl Diet Plus Aldosterone Infusion Lijun Li, Lamia Alamri, Carolyn M. Ecelbarger. *Medicine, Georgetown University, Washington, DC.*

Background: Numerous sex and sex hormone differences exist in the regulation of sodium balance; however, the mechanisms are not well defined. High-NaCl diet reduces the abundance of several major sodium transporters. Some, although not all, of these effects can be attributed to the fall in endogenous Aldo. In this study, we test whether sex differences exist in these Aldo-independent responses to changes in dietary NaCl.

Methods: Young male and female mice (n = 12/sex) were placed on a LS diet (0.02% Na+) and implanted with osmotic minipumps to infuse Aldo (1 ug/g body weight/day). Four days later, ½ of the mice were switched to a HS diet (5% NaCl). Urine was collected daily on 1/2 of the mice, and the other 1/2 were used for blood pressure recording by radiotelemetry. Mice were euthanized after 3 more days and kidneys harvested.

Results: Despite clamping Aldo, mice fed HS diet had a 10-fold increase in urine Na+ and Cl- by day 3, with no change in urine K+, as compared to LS-fed littermates. Natriuresis due to HS was slightly diminished in females, but not significantly different from males. BP was not affected by Aldo infusion alone; however addition of HS diet led to a rise in BP in both sexes by day 2: (4.3 and 9.5 mm Hg, for males and females, respectively). HS led to a substantial decrease in cortical levels of the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2), the thiazide-sensitive Na-Cl cotransporter (NCC), and the gamma subunit of the epithelial sodium channle ENaC (70 kDa band), with a rise in the sodium hydrogen exchanger (NHE3), and gamma-ENaC (85 kDa band). The change for NCC and NKCC2 was greater in males. In addition, females had significantly greater levels (over 2-fold) of beta- and gamma-ENaC (both bands) under LS + Aldo, as compared to males.

Conclusions: In summary, young mice of both sexes are able to adapt to HS + Aldo infusion with a robust natriuresis, due to down-regulation several major apical sodium transporter pathways. This likely protects against excessive BP rises. However, the mechanisms whereby this adaptation is achieved may be different, with females showing higher expression of many sodium transporters under LS conditions, and less change overall in response to HS diet.

Funding: NIDDK Support

FR-PO557

Impaired Aldosterone Escape in Transgenic Mice Over-Expressing Human CD39: Role of Altered Sodium Transporter Regulation Yue Zhang,[1] Simon C. Robson,[2] Kaiya L. Morris,[1] Karen M. Dwyer,[3] Bellamkonda K. Kishore,[1] Carolyn M. Ecelbarger.[4] *[1]Medicine, VAMC & Univ of Utah, Salt Lake City, UT; [2]Medicine, BIDMC, Harvard Univ, Boston, MA; [3]Medicine, Univ of Melbourne, Victoria, Australia; [4]Medicine, Georgetown Univ, Washington, DC.*

Background: CD39, an ectonucleotidase (NTPDase1), hydrolyzes extracellular ATP or ADP to AMP. We showed that CD39 is expressed at several sites within the kidney, and thus may impact the availability of ligands for P2 receptors. Recently we reported that transgenic mice (Tg) over expressing hCD39 exhibit defective renal water handling.

Methods: Since P2 receptors regulate both water and sodium transport in the kidney, we examined the effect of low- or high-salt diet (LSD or HSD) feeding for 4 days while clamping the blood aldosterone (Aldo) levels (20 μg/day infusion) in Tg mice vs. wild

type (WT) mice (N = 6). Kidneys were harvested after euthanasia and cortex and medulla were processed separately for Western blotting.

Results: After 2 days of HSD, urine Na started decreasing in Tg mice with a significant difference on day 4 ($P < 0.005$). There were no significant differences in terminal serum osmolality or Na between the genotypes. Two-way ANOVA revealed that mean band density of thiazide-sensitive cotransporter (NCC) was significantly higher in the Tg mice (~14-18%), but was similarly reduced by HSD (15-18%), thus showing no significant interaction. In contrast, the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2) was reduced by HSD in WT mice, but increased in the Tg in both cortex and medulla, resulting in a significant interactive term for both regions. Other proteins, including the subunits of the epithelial sodium channel (ENaC), were also differentially regulated between the genotypes. e.g., major band (85 kDa) associated with γ-ENaC in the cortex significantly increased in HSD fed Tg vs. WT mice.

Conclusions: In summary, hCD39-Tg mice had impaired natriuretic ability in the model of Aldo escape as compared to the WT mice, supporting a role for ATP in facilitating natriuresis. Impaired down-regulation of Na transporters in both medulla and cortex under the conditions of HSD+Aldo may have contributed to this deficiency.

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FR-PO558

Activation of NAD(P)H Oxidase Contributes to Impaired Aldosterone Escape in mPGES-1 Deficient Mice Zhanjun Jia,[1,2] Sunhapas Soodvilai,[1] Ying Sun,[1,2] Tianxin Yang.[1,2] *[1]Nephrology Division, University of Utah; [2]VA Medical Center, Salt Lake City.*

Background: We previously reported mPGES-1 deletion impaired aldosterone (Aldo) escape and potentiated Na retention (Jia et al, AJP-Renal, 2009). The present study examined the role of NADPH oxidase-derived ROS in this phenomenon.

Methods: The Aldo escape was generated by a 2-wk Aldo infusion at 0.35mg/kg/day via mini-pump with a normal Na diet.

Results: After Aldo infusion, urinary TBARS and 8-isoprostane in mPGES-1 KO but not WT mice were increased by 2-fold and 1.9-fold, respectively, contrasting the unaltered levels in WT. By qRT-PCR, Aldo infusion selectively induced a 2-fold increase in renal mRNA expression of $gp91^{phox}$ in KO but not WT mice. The expression of $p47^{phox}$, NOX4, SOD1, SOD2 and SOD3 was unaffected in either genotype. To test the functional role of NADPH oxidase, a specific NADPH oxidase inhibitor apocynin (APC, 10 mg/kg/day by mini-pump), was administered to the KO mice together with Aldo. Interestingly, Aldo alone in KO mice resulted in an Hct reduction by 7.3% on day 4 and 7.8% on day 14, which was completely reversed by APC. Meanwhile, Aldo caused hypernatremia (KO Cont: 141.7±2.8 mmol/L vs. KO Aldo: 155.9±1.2 mmol/L, $P<0.01$) was markedly diminished by APC (145.9±2.5mmol/L vs. KO Cont, $p>0.05$). In parallel, APC entirely blocked Aldo-induced increases in urinary 8-isoprostane, as well as the kidney hypertrophy; the later was confirmed by the kidney to body weight ratio (KO Aldo: 0.96±0.04% vs. KO Aldo+APC: 0.77±0.05%, $p<0.05$) and PAS staining. The impaired Aldo escape in KO mice was associated with upregulations of NCC (+37%) and ENaC-α (+31%) and downregulations of ENaC-γ (-32%) and NKCC2 (-42%), all of which were significantly corrected by APC. Lastly, we examined the effect of APC on Aldo-induced ENaC activity in primary IMCD cells using Ussing chamber. ENaC activity, as reflected by amiloride-sensitive Isc, was increased after 24-h exposure to Aldo (Aldo: 1.57±0.07 vs. Cont: 1.0±0.07, $p<0.01$) and this increase was abolished by APC (Aldo+APC: 1.14±0.08, $p=0.01$ vs. Aldo).

Conclusions: These findings indicated mPGES-1-derived PGE2 attenuates Aldo-induced Na and fluid retention via inhibiting NADPH oxidase.

Funding: NIDDK Support, Veterans Administration Support

FR-PO559

Renal Compensatory Response to TZD-Induced Plasma Volume Expansion: Role of mPGES-1 Zhanjun Jia,[1,2] Ying Sun,[1,2] Tianxin Yang.[1,2] *[1]Nephrology Division, University of Utah; [2]VA Medical Center, Salt Lake City.*

Background: PGE2, a product of prostaglandin E synthase, is considered to participate in renal response to plasma volume expansion owing to its natriuretic/diuretic property. The present study examined the role of mPGES-1 in the renal response to rosiglitazone (Rosi)-induced fluid retention.

Methods: mPGES-1 WT and KO mice were fed with a diet incorporated with or without Rosi (320 mg/kg diet).

Results: A 2-wk Rosi treatment induced a 1.8-fold increase of urinary PGE2 excretion which was completely blocked in KO mice. At day 14, Rosi treated WT mice exhibited increased urinary Na excretion (Rosi:1.23±0.08 mmol/24h vs. Cont: 0.81±0.05 mmol/24h, $p<0.05$) and reduced net Na accumulation (intake-excretion: Rosi 0.25 ± 0.03 mmol/24h vs. Cont 0.47±0.08mmol/24h, $p<0.05$), both of which were completely blocked in KO mice (Na excretion: Rosi: 1.16±0.1 mmol/24h vs. Cont 1.06 ± 0.11 mmol/24h, $p>0.05$; Na^+ intake – excretion: 0.4±0.09mmol/24h vs. 0.44 ± 0.04 mmol/24h, $p>0.05$). The changes in 24-h urine volume and water balance followed the same pattern as Na. Rosi treatment in WT significantly increased plasma volume (PV) as determined by using Evan's blue (Rosi: 29.6 ± 0.56 vs. Cont: 25.6 ± 0.95 ul/gBW, $p<0.05$) and decreased Hct (Rosi: 45.1±0.9% vs. Cont 50.9±0.78%, $p<0.05$) and these changes were greater in KO (PV: 33.7±0.7 ul/g BW, $p<0.05$ vs. WT Rosi; Hct: 41.4±1.0%, $p<0.05$ vs. WT Rosi), indicating accelerated PV expansion. qRT-PCR demonstrated 2-wk but not 2-day Rosi treatment led to widespread downregulation of all Na transporters and aquaporins, including NHE3, NKCC2, NCC, α-Na-K-ATPase, α- and γ-ENaC and AQP2 in WT. In contrast, these compensatory changes did not occur in KO. Rosi treatment in WT mice markedly elevated the urinary output of nitrate/nitrite (Rosi: 338.2 ± 45.2 nmol/24h vs. Cont: 120.0±18.6 nmol/24h, $p<0.01$) and cGMP (Rosi: 11478.8 ± 3496.1 pmol/24h vs. Cont: 2505.8±311.9 nmol/24h, $p<0.05$), both of which were significantly blocked in KO(nitrate/nitrite:197.2 ± 43.1 nmol/24h, $p<0.05$ vs. WT Rosi; cGMP: 3862.6±295.5 pmol/24h, $p<0.05$ vs. WT Rosi).

Conclusions: Renal mPGES-1-derived PGE2 plays an important role in mitigating Rosi-induced plasma volume expansion via NO/cGMP.

Funding: NIDDK Support, Veterans Administration Support

FR-PO560

With-No-Lysine Kinases in Aldosterone Escape Phenomenon Jeonghwan Lee, Jin Suk Han, Kwon Wook Joo. *Internal Medicine, Seoul National University Hospital, Seoul, Korea.*

Background: Escape from aldosterone-induced sodium retention (aldosterone escape) was reported to occur primarily by decreased abundance of the Na-Cl cotransporter (NCC) in distal convoluted tubules. However, underlying mechanisms of decreased expression of NCC is still uncertain. With-no-lysine kinases (WNKs) play an important role in regulating NCC abundances and activity. We investigated the changes of WNKs expression in aldosterone escape rat model.

Methods: Male Sprague-Dawley rats were used. Aldosterone (200 μg/day) was infused for the entire experimental period using osmotic minipump. The control group was kept on a low sodium diet (0.02 mEq/day), and theexperimentalgroup was fed with a higher sodium diet (2.0 mEq/day). Thekidneys were taken on day 4. The protein expression of sodium transporters,WNK1 and WNK4 was determined bysemiquantitative immunoblotting.

Results: Aldosterone escape developed in the experimental group. Aldosterone levels were not different in both groups (11.2 ± 3.2 nM/L in control group and 13.5 ± 3.7 nM/L in experimental group). Sodium excretion rose to the level of the intake in the experimental group (2.0 ± 0.19 mEq/day in experimental group). There was no change in creatinine clearance. The expression of NCC significantly decreased (55 ± 13.4 % of the control group) in the experimental group. The protein abundance of WNK4 was not changed and the expression of WNK1 was decreased (51 ± 11.1 % of the control group) in the experimental group without change of aldosterone level. KS-WNK1 mRNA expression was significantly decreased in the experiment group (58.3 ±24.5 % of the controls).

Conclusions: Decreased expression of NCC in aldosterone escape phenomenon is influenced by WNK1, which is independent of aldosterone level.

FR-PO561

Characterization of NaCl Transport in Mouse Medullary Thick Ascending Limb from P2Y2 Wild-Type and Knock-Out Mice Jens G. Leipziger, Helle A. Praetorius, Rita D. Marques. *Dept. of Biomedicine, Aarhus University, Aarhus, Denmark.*

Background: Local purinergic signals modulate renal tubular transport. Acute activation of renal epithelial P2 receptors causes transport inhibition and thus triggers a diuretic effect. The effects of extracellular nucleotides on ion transport in the thick ascending limb are so far poorly studied. In the medullary thick ascending limb (mTAL) we recently described a basolateral P2X receptor that mediates a marked inhibition (25%) of NaCl absorption. In addition, mTALs also express apical and basolateral P2Y2 receptors. We showed that short term activation of basolateral P2Y2 receptors had no effect on transport in mouse mTAL. Objective: Here we studied, if the absence of the P2Y2 receptor leads to chronic alterations in mTAL NaCl absorption by comparing basal and AVP-stimulated transport rates in tubules isolated from P2Y2 receptor WT and KO mice.

Methods: We used isolated, perfused mouse mTALs to electrically measure NaCl absorption. By electrodes we determined the transepithelial voltage (V_{te}) and the transepithelial resistance (R_{te}) and thus transepithelial NaCl absorption (equivalent short circuit current, I'_{sc}).

Results: No apparent differences of NaCl absorption were observed between WT and KO mTALs (WT: 2064±144 μA/cm², n=53; KO: 1880±174 μA/cm²; n=32). An extended analysis, however, revealed that tubules from male KO mice transported less NaCl as compared to female KO tubules which showed higher rates of NaCl absorption. Both WT and KO tubule showed similar transport inhibition by luminal furosemide and could similarly be activated by basolateral AVP.

Conclusions: Absence of the P2Y2 receptors apparently has no major effects on NaCl absorption in mouse TAL. Intriguingly, however P2Y2 receptor KO mice display a gender-dependent difference in NaCl absorption. These data support a functional role of the P2Y2 receptor in NaCl handling of the thick ascending limb.

Funding: Government Support - Non-U.S.

FR-PO562

Different Classes of Purinoreceptors in the Apical and Basolateral Membranes of Renal Cells Hui-fang Bao, He-ping Ma, Douglas C. Eaton. *Physiology, Emory University School of Medicine, Atlanta, GA.*

Background: Extracellular ATP alters renal sodium transport via P2 purinoceptors, but the receptors in the apical and basolateral membranes are different: the apical membrane contains $P2Y_2$ receptors that inhibit ENaC and the basolateral membrane contains $P2X_4$ receptors that stimulate ENaC.

Methods: Renal cells (A6) were used for single channel patch clamp studies. Cell attached patches on the apical membrane were formed directly. To access the basolateral membrane, a sharp glass needle was used to make a triangular tear in the monolayer and the needle was used to lift up the wedge of cells to provide access to the basolateral surface.

Results: Confocal imaging was used to show that the cellular location of $P2Y_2$ receptors was near or in the apical membrane while $P2X_4$ receptors were near or in the basolateral

membrane. Addition of 100 μM ATP to the apical surface produced a reversible inhibition of ENaC activity (P_0 untreated 0.38±0.16 vs 100 μM ATP 0.06±0.03, n=11, p<0.05). This effect was mimicked by 100 μM UTP, but blocked by 100 μM XMAR ($P2Y_2$ receptor antagonist). Application of either ATP or 2-methylthio-ATP (2-meSATP, a selective P2X receptor agonist) at a final concentration of 50 μM to the basolateral bath significantly elevated ENaC activity. In contrast, no such effect was observed when 2-meSATP was applied to the apical bath. Chelation of extracellular Ca^{2+} with 2 mM EGTA abolished the stimulatory effect of basolateral 2-meSATP, indicating that local Ca^{2+} influx through $P2X_4$ receptors accounts for the stimulation. The stimulation was inhibited when aldosterone was removed from the culture medium for 72 h. Western blots showed that removal of aldosterone strongly reduced $P2X_4$ receptor expression and confocal imaging showed that the $P2X_4$ receptor density in the basolateral region of aldosterone-deprived cells was reduced.

Conclusions: These results suggest a regulatory role for purinoceptors in renal salt and water transport. But the effects of purines on the apical surface produce inhibition while basolateral purines stimulate ENaC. This implies that the same ligand can produce different effects depending upon whether it is present in the tubular fluid or in the blood.

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FR-PO563

A Novel Sodium Pathway in the Proximal Tubule and Its Regulation by Potassium Diets Zinaeli Lopez-Gonzalez, Rolando Carrisoza-Gaytan, Laura I. Escobar. *Fisiología, Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico, D.F., Mexico.*

Background: The Na^+/H^+ (NHE3) and NaPi2 transporters have been recognized as the major pathways for Na^+ reabsorption by the proximal tubule (PT). We identified HCN hyperpolarized-activated sodium channels in the apical membrane of the PT from the rat kidney. Na^+ transport through the renal epithelium is coupled to the distal K^+ secretion. A high K^+ diet inhibits Na+ reabsorption in the PT to increase the flow and loading of Na^+ to the distal nephron and then, K^+ secretion. In contrast, K^+ depletion increases Na^+ reabsorption in the PT and reduces the flow toward the distal nephron, decreasing K^+ secretion. The objective of this work is to determine the regulation of HCN3 channel expression by a high and a deficient K^+ diet in the rat kidney.

Methods: Male Wistar rats of 200 g were fed with a control (1.2%), high (10%) and deficient (0%) K^+ diet for 15 days. Protein expression was quantified in plasma membrane from renal cortex and outer medulla by Western blot assays.

Results: We detected a protein band of ~65 kDa corresponding to the HCN3 channel in the renal tissue. A high K^+ diet caused a decrease of HCN3 of both cortex (44%) and outer medulla (75%). Depletion of K^+ increased the expression of HCN3 only in the outer medulla (60%). These changes were statistically significant ($p < 0.05$) compared to the control diet.

Conclusions: The apical localization of the channel HCN3 and its regulation by high and deficient K^+ diets, support the hypothesis that this channel participates in the reabsorption of Na^+ in the PT. Functional studies in isolated tubules are necessary to confirm this finding.

Funding: Government Support - Non-U.S.

FR-PO564

NO/sGC/cGMP/ERK Pathway Mediates a Unique Dose-Dependent Stimulation of Human Proximal Transport by Angiotensin II Ayumi Shirai,[1] Osamu Yamazaki,[1] Motonobu Nakamura,[1] Hideomi Yamada,[1] Masashi Suzuki,[1] Shoko Horita,[1] Yukio Homma,[2] George Seki.[1] *[1]Internal Medicine, Tokyo University, Tokyo, Japan; [2]Urology, Tokyo University, Tokyo, Japan.*

Background: Although angiotensin II (Ang II) is known to regulate renal proximal tubule (PT) transport in a biphasic manner via AT1 receptor in mouse, rat, and rabbit, little is known about its effect on human PT transport.

Methods: Normal human kidney cortex tissues were obtained during the nephrectomy for renal carcinoma. The Na-HCO_3 cotransporter (NBCe1) activity was determined by cell pH measurement in isolated human PT.

Results: Ang II does-dependently stimulated the NBCe1 activity in human PT (+38%, +60%, and +103% stimulation by 10^{-10} M, 10^{-8} M, and 10^{-6} M Ang II, respectively). Ang II also induced a similar dose-dependent stimulation of the transtubular HCO_3 absorption determined by microfluorometric stop-flow method. AT1 blocker valsartan, MEK inhibitor PD98059, nitric oxide synthetase (NO) inhibitor L-NAME, soluble guanylyl cyclase (sGC) inhibitor ODQ, but not protein kinase G (PKG) inhibitor KT5823 suppressed this stimulation. NO donor sodium nitroprusside (SNP) stimulated NBCe1, which was suppressed by PD98059. 8Br-cGMP also mimicked the stimulation by Ang II. In human renal cortex, Ang II, SNP, and 8Br-cGMP stimulated ERK phosphorylation without enhancing PKG-dependent GSK3b phosphorylation. In mouse kidney, by contrast, 10^{-6} M Ang II, SNP, and 8Br-cGMP inhibited the NBCe1 activity, and stimulated GSK3b phosphorylation without enhancing ERK phosphorylation. Furthermore, L-NAME, ODQ, and KT5823 converted the inhibitory effect of 10^{-6} M Ang II on NBCe1 to stimulation.

Conclusions: These results, for the first time to our knowledge, revealed that Ang II has a dose-dependent stimulatory effect on human PT transport. This stimulation is dependent on the downstream NO/sGC/cGMP pathway, which works inhibitory via PKG in other species but works stimulatory via ERK in human. In view of much higher concentrations of Ang II in kidney than in plasma, the lack of inhibitory effect of high concentration of Ang II on human PT transport may be relevant to the pathogenesis of hypertension.

Funding: Government Support - Non-U.S.

FR-PO565

Regulation of Rat Proximal Tubule Fluid Reabsorption by Nitric Oxide Synthase and Dimethylarginine Dimethylaminohydrolase 1 (DDAH-1): Role of Nitric Oxide and Asymmetric Dimethylarginine (ADMA) Tracy Bell,[1] James Alexander Tomlinson,[2] Christopher S. Wilcox,[1] William J. Welch.[1] *[1]Medicine, Georgetown University, Washington, DC; [2]Medicine, Imperial College London, United Kingdom.*

Background: Nitric oxide (NO) stimulates proximal tubule (PT) Na^+ and fluid reabsorption. ADMA is an endogenous inhibitor of NOS but its effects on tubular function are unknown. ADMA is metabolized by DDAH whose type 1 isoform is predominant in the PT. SNIPs of DDAH-1 predicted the rate of decline of renal function in patients with chronic kidney disease (CKD). Therefore, we tested the hypothesis that DDAH-1 metabolizes ADMA in the proximal tubule and thereby enhances PT fluid reabsorption (J*v*).

Methods: J*v* was measured in anesthetized rats by direct in vivo microperfusion and recollection of artificial tubular fluid (ATF) in S2 segments of the PT.

Results: Addition of a selective inhibitor of DDAH-1, L-257 (10^{-4}M) to ATF did not effect J*v* when compared to addition of vehicle (3.4±0.6 vs 3.1±0.3 nl/min/mm). However, L-257 administered as a bolus i.v. injection (60mg/kg) 2hrs before microperfusion of the PT with L-257 significantly reduced J*v* to 1.8±0.2 nl/min/mm, (P<0.05)and significantly enhanced urine flow rate (2.1±-0.3 vs 9.0±1.8 μl/min; P<0.05). Microperfusion of ADMA (10^{-4} M) or L-NAME (10^{-4} M) into the PT both reduced J*v* significantly to 2.0±0.2 and 1.8±0.2 nl/min/mm, respectively (P<0.05). Rats pretreated with a rapid intravenous injection of siRNA targeted to DDAH-1 that reduced its renal mRNA expression significantly also had reduced J*v* (3.1±0.2 vs 2.2±6.2 nl/min/mm; P<0.05) and had a further reduction in J*v* with 10^{-4}M ADMA added to ATF to 1.4±0.1 nl/min/mm; (P<0.005).

Conclusions: In conclusion, PT fluid reabsorption is regulated by ADMA and its tubular metabolism by DDAH-1. The two hour delay in the effects of a bolus injection of L-257 to diminish J*v* indicated the time required after inhibition of DDAH-1 for accumulation of sufficient tubular ADMA to impair PT reabsorption. Thus, L-257 is a novel regulator of PT function and is a proximal tubule diuretic. PT fluid reabsorption is increased by NOS-dependent NO but decreased by DDAH-1-dependent ADMA.

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FR-PO566

The Alpha-Ketoglutarate Receptor OXGR1 Is Involved in Renal Sodium Handling and Creatinine Excretion Joris Hubertus Robben,[1] Ana Carolina Ariza,[1] Olivier Devuyst,[2,3] Peter M.T. Deen.[1] *[1]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Physiology, University Zurich, Zurich, Switzerland; [3]UCL Medical School, Brussels, Belgium.*

Background: The alpha-ketoglutarate receptor OXGR1 is almost exclusively expressed in the kidney, where its mRNA was found to be expressed in the distal convoluted tubules. Currently its role in renal (patho)physiology is completely unknown. Here, using a knockout mouse strain, we present the first characterization of the function of this receptor in renal physiology.

Methods: Wild-type (WT) and OXGR1-/- mice were housed in metabolic cages to monitor food and water intake and urine output. Subsequently, mice were sacrificed and blood, urine and kidneys were collected for analysis. Urine and plasma were analyzed for levels of individual osmolytes and hormones of the renin-angiotensin system. Kidneys were histologically stained and analyzed for overall morphology. Renal homogenates were prepared for Q-PCR analysis and western blotting.

Results: OXGR1-/- mice showed significant polyuria in comparison to WT littermates (1.74 ± 0.1 vs. 1.27 ± 0.21 mL/24 h, respectively). This may be due to the increased osmolyte secretion observed in the OXGR1-/- animals compared to WT (3700 ± 209 vs 2988 ± 334 mOsm/24 h), including increased sodium excretion (1.82 ± 0.15 vs 1.33 ± 1.33 mmol/24 h). Interestingly, creatinine excretion was significantly increased in the OXGR1-/- mice (56.58 ± 3.89 vs 39.04 ± 3.37 mg/24 h), indicating that OXGR1 may be involved in either creatinine transport, or alternatively in regulating the glomerular filtration rate.

Conclusions: OXGR1 is a novel regulator of renal sodium handling, and potentially regulates the glomerular filtration rate. The sodium transporters involved and whether creatinine secretion or GFR is changed is currently under investigation.

Funding: Private Foundation Support

FR-PO567

Loss of the Succinate Receptor SUCNR1 Yields Increased Glomeruli and Affected Renal Sodium and Potassium Transport Joris Hubertus Robben,[1] Ana Carolina Ariza,[1] Olivier Devuyst,[2,3] Peter M.T. Deen.[1] *[1]Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Physiology, University of Zurich, Zurich, Switzerland; [3]UCL Medical School, Brussels, Belgium.*

Background: The succinate receptor SUCNR1 (also known as GPR91) is expressed in the afferent arteriole and glomerular vasculature, cortical thick ascending limb (including the macular densa) and collecting duct. This receptor recently emerged as a novel regulator of the renin-angiotensin system in diabetes mellitus, while it may also play a role in promoting renal fibrosis in this disorder. However, its role in renal physiology and electrolyte handling remains unknown.

Methods: Wild-type and SUCNR1 knockout mice were housed in metabolic cages to monitor food and water intake and urine output. Subsequently, mice were sacrificed and blood, urine and kidneys were collected for analysis. Urine and plasma were analyzed for levels of osmolytes and hormones of the renin-angiotensin system. Kidneys were histologically stained and analyzed for overall morphology. Renal homogenates were prepared for Q-PCR analysis and western blotting.

Results: The weight of the kidneys of SUCNR1-/- mice was significantly higher than their wild-type littermates, which was accompanied with an increased glomerular size. Serum Na+ levels of SUCNR1-/- mice were slightly but significantly elevated compared to wild-type, while serum potassium, urea, chloride and creatinine values were similar Moreover, SUCNR1-/- mice showed a trend to be polyuric, coinciding with significantly increased osmolyte losses ofmainly sodium, urea and potassium. These osmolyte losses were accompanied by decreased protein levels of the sodium-chloride co-transporter NCC and the epithelial sodium channel ENaC.

Conclusions: SUCNR1 plays a role in the regulation of renal and glomerular morphology, but its mechanism of action in this respect remains to be determined. Moreover, under physiological conditions, SUCNR1 contributes to maintaining a proper sodium and osmolyte balance. Whether SUCNR1 regulates this directly or via the renin-angiotensin system is currently under investigation.

Funding: Private Foundation Support

FR-PO568

Evidence for an Absorptive Paracellular Pathway for Fluid Transport in the Proximal Tubule: Micropuncture Studies in Claudin2-Deficient Mice

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Background: Claudin2 (cldn2), a tight protein expressed in renal proximal tubules, has been shown to create a water conducting pathway in MDCK cell culture and to contribute to net fluid efflux in isolated proximal tubules (Rosenthal R et al J Cell Sci 123, 2010; Muto S et al PNAS 107, 2010). The present studies were performed to confirm these important findings in vivo in a different strain of cldn2-deficient mice (MMRRC, University of Davis, CA).

Methods: Fluid samples obtained by free micropuncture in end-proximal segments of inactin/ketamine anesthetized mice were analyzed for volume, I^{125}-iothalamate (125-io) concentration, and osmolarity.

Results: End-proximal $TF/P_{125\text{-}io}$ ratio used as fluid flux marker showed a marked reduction from 1.95 ± 0.05 (34 tubules/9 mice) in $cldn2^{+/+}$ (WT) to 1.56 ± 0.2 (34 tubules/8 mice) in $cldn2^{y/-}$ (KO) equivalent to a reduction of fractional reabsorption from 47.7 ± 1.4% to 34.6 ± 1.5% ($p < 0.0001$). TF/Posm ratio was slightly below unity in both WT and KO (0.977 and 0.983) demonstrating near isotonicity of claudin2-dependent water absorption. SNGFR was significantly lower in KO than WT (11 ± 0.7 vs. 16.3 ± 1.1 nl/min; $p<0.0001$). Arterial blood pressure during micropuncture was comparable between genotypes (110 ± 2.6 mm Hg in KO, 104 ± 1.9 in WT). Plasma renin concentration and arterial hematocrit were significantly higher in KO than WT. Saline infusion (7% BW/hr) reduced $TF/P_{125\text{-}io}$ to 1.48 ± 0.03 (n=17) in WT, but had no effect in KO (1.5 ± 0.05; n=22).

Conclusions: Our data show that a fraction of 27.5% of proximal fluid reabsorption is claudin2-dependent and is therefore presumably absorbed along the paracellular pathway. Inhibition of fluid reabsorption by ECV expansion is cldn2-dependent suggesting that a reduced paracellular fluid reabsorption is the cause for this inhibition. The force driving paracellular water flux remains to be determined.

Funding: NIDDK Support

FR-PO569

Salt Excretion and Renin Secretion in Mice with a Knock-In Mutation of the AMP-Activated Protein Kinase (AMPK) Site in Acetyl CoA Carboxylase 1

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Background: The master metabolic controller AMPK plays a pivotal role in regulating cellular energy supply and demand. Acetyl CoA carboxylase (ACC) is a major controller of fatty acid metabolism and a well-known substrate for AMPK. We have previously shown that ACC1 was strongly phosphorylated in WT mice in response to salt restriction. In this study, we attempted to determine the role of AMPK and its substrate ACC1 in urinary sodium excretion and renin secretion.

Methods: Mice bearing a knock-in mutation of the AMPK phosphosite in ACC1 ($ACC1^{S79A}$ mice) were placed on a normal salt (0.3%) or salt deficient (0%) diet for 7 days and responses compared with those of wild type (WT) mice.

Results: $ACC1^{S79A}$ mice had no change in urinary sodium excretion when on a normal or salt deficient diet compared with WT mice. There was also no difference in plasma renin concentration (PRC) when receiving a normal salt diet, but $ACC1^{S79A}$ mice receiving a salt deficient diet had a 180% increase in PRC ($p<0.05$), a 170% increase in preprorenin mRNA ($p<0.05$) and a 73% increase in Cox-2 mRNA ($p<0.05$) compared to WT mice. Expression of distal tubule salt transporters was similar in both WT and $ACC1^{S79A}$ mice, apart from a significant increase in expression of NCC in $ACC1^{S79A}$ mice receiving a salt deficient diet and significantly less βENaC in $ACC1^{S79A}$ mice ($p<0.05$) in mice receiving a normal salt diet. Phosphorylation of NCC on T58 and NKCC2 on T100/105 was similar in WT and $ACC1^{S79A}$ mice, with a significant increase in mice receiving a salt deficient diet. Expression of βENaC was significantly reduced in WT mice receiving a salt deficient diet compared with those on a normal salt diet ($p<0.01$), and this also occurred in the $ACC1^{S79A}$ mice ($p<0.01$).

Conclusions: This data suggests that phosphorylation of ACC1 by AMPK within the kidney contributes to control of renin secretion.

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FR-PO570

Involvement of ACCN1 Channel in Sodium Retention in the Nephrotic Syndrome

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Background: Nephrotic syndrome features proteinuria and Na^+retention leading to ascites and edema formation. Previous studies in a corticosteroid-clamped rat model of nephrotic syndrome (CC-PAN) showed that increased Na^+absorption in cortical collecting duct (CCD) is associated with increased Na/K ATPase activity, is independent of aldosterone, does not require activation of ENaC and is sensitive to amiloride.We therefore searched for alternate amiloride-sensitive Na^+channel in CCDs from CC-PAN rats.

Methods: Transcriptomes of CCDs from corticosteroid-clamped (CC) and CC-PAN rats were analyzed. Changes in gene expression were confirmed by RT-qPCR. Protein expression was evaluated by western blot and immunohistochemistry. Sodium transport was evaluated *in vivo* in metabolic cages, and *in vitro* microperfused CCDs.

Results: Metabolic study showed that Na^+excretion and ascites reached a nadir at day 6 after PAN injection and were similar in CC-PAN and PAN rats. Comparison of Na^+absorption in CCDs from CC-PAN and Na^+depleted rats (LN),a model of increased ENaC expression, revealed that Na^+flux was higher in LN than in CC-PAN rats but was fully inhibited by 10µM amiloride in both groups. Na^+flux was inhibited by 0.3M zinc in LN but not CC-PAN rats and it was inhibited by acid pH (pH=6.0) in LN but not CC-PAN rats. These findings confirm that ENaC is not involved in Na^+reabsorption in CC-PAN rats. Transcriptomes and RT- qPCR revealed over-expression of ACCN1 in CC-PAN rats and down-expression of Hsc70, a chaperone that sequesters ACCN in intracellular compartments. Western blot revealed increased expression of ACCN1 in CCDs from CC-PAN rats (arbitrary units±SE; CC-PAN:0.72±0.12; CC:0.31±0.03; $p<0.05$) and immunohistochemistry highlighted an apical labeling in CCDs from CC-PAN but not CC rats.

Conclusions: We found that a channel of the ACCN family (ACCN1) is expressed in CCDs of nephrotic rats where it carry Na^+ reabsorption in low aldosterone nephrotic syndromes. To our knowledge, it is the first demonstration of ACCN channel expression in kidney. Full molecular and functional characterization of this channel will require additional investigations.

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FR-PO571

The Circadian Clock in the Renin-Secreting Granular Cells of the Juxtaglomerular Apparatus Is Involved in the Control of Blood Pressure

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Background: Recent evidence suggest that the circadian timing system controls renal function and blood pressure. Here, we analysed the role of the circadian clock in the renin-secreting granular cells of the juxtaglomerular apparatus.

Methods: The activity of the circadian clock was suppressed by crossing mice expressing Cre recombinase under the control of endogenous $ren1^d$ promoter and mice carrying a floxed allele of Bmal1 gene, an essential component of the molecular clock.

Results: The suppression of the clock activity in the granular cells was validated by qPCR analysis of several clock-dependent genes, including Bmal1, Dbp and Npas2. Urine chemistry parameters were measured in the urine collected hourly from freely moving mice housed individually in the metabolic cages. This analysis has shown that $ren1^d$-Cre/$Bmal1^{lox/lox}$ mice exhibit a significant increase in the urine output and a significant change in the circadian pattern of urinary sodium excretion. Blood chemistry examination revealed that $ren1^d$-Cre/$Bmal1^{lox/lox}$ mice exhibit a significant reduction in plasma aldosterone levels during the light phase of the circadian cycle. In parallel, the expression of aENaC mRNA in the kidney was significantly reduced. Arterial blood pressure, heart rate and general motion activity were measured in conscious unrestrained animals using telemetry. The two latter parameters were not different between wild-type and knockout mice. However, the systolic blood pressure was significantly reduced in $ren1^d$-Cre/$Bmal1^{lox/lox}$ mice during the activity phase.

Conclusions: Collectively, these results indicate that the intrinsic circadian clock in the granular cells is involved in the generation/maintaining circadian rhythms of urinary sodium excretion and blood pressure. This control mechanism potentially involves the circadian control over renin production/secretion in the granular cells.

FR-PO572

Notch Signaling Is Required for the Specification of Mesangial Cells from a Stromal Progenitor during Kidney Development

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Background: Mesangial cells are a specialized type of smooth muscle cell that surround the vascular network within the glomerulous. During development, these cells are derived from mesenchymal stromal progenitors that also give rise to vascular smooth muscle cells (VSMC) and interstitial fibroblasts. VEGF and PDGF govern the migration and assembly

of the mesangial tree during glomerular development, however little is known about how mesangial cells are specified from the stromal progenitor pool.

Methods: To investigate the role of Notch signaling in stromal derived cell types we used Notch activation-dependent fate mapping methods and conditional gene inactivation of Notch pathway components in the stromal progenitor compartment.

Results: Here we show that Notch signaling is active in the stromal mesenchyme and is essential for mesangial cell fate specification. Stromal-specific deletion of RbpJ, the common, obligate DNA binding partner of all active Notch receptors, results in loss of mesangial cells, glomerular hemorrhage and perinatal death due to kidney failure. The defect is evident by the absence of desmin-positive cells within the proximity of the developing glomerulus and in the failure of any stromal-derived cell to invade the vascular cleft of the S-Shaped body. This is in contrast to other mutants lacking a mesangium which specify Desmin-positive mesangial cells that fail to migrate into the cleft. These defects are specific to the mesangial lineage as VSMC and interstitial fibroblasts form normally in the absence of RBPj. Notch1 and Notch2 act redundantly in specifying mesangial cells during development, though Notch2 mutants develop glomerular dysfunction and cysts within the first 4 weeks of life.

Conclusions: These data demonstrate a novel function for Notch signaling during kidney development and provide the first evidence of a pathway required for mesangial cell specification. It also raises questions regarding the proper direction of Notch pathway manipulation in renal diseases.

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FR-PO573

Growth Arrest Specific 1, a Novel and Direct Target of the Wilms Tumor Suppressor, Is Required for Maintenance of Nephrogenesis and Nephron Progenitor Cells

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Background: We recently defined a set of WT1 target genes in the developing kidney, that included Gas1, a GPI-anchored protein expressed in NPCs. In embryonic kidney organ cultures, morpholino knockdown experiments demonstrated that WT1 is required to maintain Gas1 expression, and Gas1 is required to maintain viability of nephron progenitor cells (NPC).

Methods: Luciferase reporter assays in a WT1 expressing immortalized cell line were coupled to siRNA-mediated knock-down of Wt1 expression to delimit the WT1 regulatory region. Mutagenesis and chromatin immunoprecipitation was used to confirm direct interaction of WT1 the Gas1 5' upstream region. Gas1 -/- kidneys were analyzed by immunostaining, and in-situ hybridization.

Results: Luciferase reporter constructs, mutagenesis and siRNA mediated knockdown of Wt1 expression delimited the WT1-dependent regulatory sequence of Gas1 to a 250bp upstream sequence. Kidney development in Gas1-/- kidneys appears to progress normally until E15.5. Thereafter, Gas1 -/- kidneys become hypoplastic, with a 75 percent reduction in nephron mass, and a near complete loss of NPC at birth. At stage E17.5, BrdU incorporation demonstrates decreased proliferation in NPC. Wnt4 expression, marking pretubular aggregates, is lost while NPC marked by Six2 expression are still present, and the inducing signal Wnt9b continues to be expressed in the ureteic bud. A decreased number of S-shaped bodies and renal vesicles supports this finding and accounts for the decreased nephron mass.

Conclusions: Gas1 is a direct target gene of WT1 required to maintain NPC. While Gas1 expression is restricted to NPC, it has a role in the formation of the pretubular aggregate. Unlike other genes that are required to maintain NPC at the earliest stages of kidney development, the Gas1 phenotype demonstrates a novel and distinct requirement for additional signals to maintain NPC during later stages of kidney development.

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FR-PO574

P53 Regulates Progenitor Cell Renewal in the Nephrogenic Niche of the Developing Kidney

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Background: Low nephron number is a predisposing factor for hypertension and associated cardiovascular disease. The self-renewing capability of the Cited1^{+}sub-population of the Six2+ cap mesenchyme (CM) ensures availability of nephron progenitors cells (NPC) for nephrogenesis. p53 plays a key role in cell-fate regulation by transcriptionally regulating genes that control cell cycle arrest, apoptosis and differentiation. Recent studies link p53 with regulating the self-renewal potential of embryonic stem cells. This study examined the role of p53 in NPC renewal.

Methods: Cytometric and molecular marker analyses were performed on embryonic mouse kidneys with conditional deletion of p53 from the Six2+ CM (Six2$^{p53-/-}$).

Results: Six2$^{p53-/-}$ mice have hypoplastic kidneys from E13.5. FACS isolation of genetically labeled GFP^{+}/Six2$^{p53-/-}$ and GFP+/WT CM gave 30% fewer cap cells from mutant than wild-type kidneys (n=6 kidneys, p<0.05). Mutant caps are loosely organized with greatly diminished Cited1 immunoreactivity at E14.5, but maintained Six2 expression. By P1 Cited1 is completely lost and the Six2 domain is reduced. QPCR analysis of CM markers at E15.5 showed significant reduction in expression of Cited1 (80%, p<0.001), Osr1 (40%, p<0.05), Meox1 (45%, p<0.005), tafa5 (75%, p<0.05), Fgf9 (25%, p<0.05) and Pax2 (30%, p<0.005), but Six2 and GDNF expression were maintained. At E13.5 proliferation measured by H3-P-S10 immunostaining showed a 30% decrease in Pax2+ cap cells of mutant kidneys (n=6 kidneys, p<0.05), whereas apoptosis measured by TUNEL was significantly higher (n=6 kidneys, p<0.05). Furthermore, Six2$^{p53-/-}$ kidneys have fewer generations of Lhx1+ nascent nephrons indicative of defective progression of nephrogenesis, supported by decreased Pax8 and Fgf8 expression. Renal vesicles in mutant kidneys have markedly weaker Laminin and Lhx1 immunostaining.

Conclusions: We propose a model wherein p53 promotes the self-renewal of the multipotent NPCs (Cited1^{+}) while promoting the differentiation of the induced Six2^{+} sub-population of NPC to nascent nephrons.

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FR-PO575

Linking Developmental Control Genes to Histone Acetylation

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Background: Specification of cell lineages begins during the time of gastrulation in the mammalian embryo and continues in a sequential fashion as the germ layers become more specialized. Comparmentalization of the mesoderm into paraxial, intermediate and lateral plate is controlled by extrinsic signaling molecules and intrinsic transcription factors. One such factor is the paired domain protein Pax2. Pax2 and the related protein Pax8 are amongst the first markers expressed in the intermediate mesoderm. Mutations in Pax2 result in renal agenesis as intermediate mesoderm cells are unable to generate the epithelial cells of the urogenital system.

Methods: To understand how Pax2 helps specify tissues of the intermediate mesoderm, we have identified and characterized the protein PTIP, which interacts with the Pax2 transactivation domain. PTIP is a ubiquitous protein with six BRCT domains, the most carboxy-terminal of which bind specifically to phosphor-serine peptides. Prior biochemical purification and mass spectrometry revealed multiple histone methyl transferases are part of the PTIP complex.

Results: Here we show that PTIP interacts with proteins that are part of a Histone Acetyltransferase Complex. Immunoprecipitation of PTIP also demonstrates associated histone lysine acetyltransferase activity. At an integrated Pax2 responsive gene as well as an endogenous Pax2 target gene, both histone lysine methylation and histone lysine acetylation are required for gene expression in a PTIP dependent manner.

Conclusions: Thus, PTIP could serve as an adaptor protein that links tissue and cell lineage specific DNA binding proteins to the epigenetic modification complexes that include both histone lysine methylation and histone lysine acetylation. Our data suggests that Pax2 may mark specific regions of chromatin by modification of histones. These epigenetic marks can delineate both active and inactive regions of the genomes in a stable and heritable manner through potential interactions with nucleosome remodeling complexes and other chromatin effectors.

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FR-PO576

Histone Deacetylases 1 and 2 Target p53 and Lhx1 to Regulate Nephron Progenitor Cell Renewal and Differentiation

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Background: Histone deacetylases (HDACs) are a large family of evolutionarily conserved epigenetic regulators, which catalyze the removal of acetyl groups from histones as well as non-histone proteins. We previously demonstrated that HDAC activity mediated by class I HDACs is required for nephrogenesis by regulating a number of key transcriptional regulators, including Pax2, Eya1, WT1, and Lhx1 (JBC, 2011). However, the specific role of individual HDAC members in nephrogenesis remains to be identified. Here, we examined the functions of HDAC1 and HDAC2 in nephron progenitor cell renewal and fate.

Methods: Mice bearing conditional null alleles for HDAC1 and HDAC2 were crossed to Six2-creEGFP mice to delete HDAC1 and HDAC2, singly or in combination, in cap mesenchyme (CM) cells.

Results: Our data show that 1 allele of either HDAC1 or HDAC2 in the CM is sufficient to support normal nephrogenesis, whereas concurrent deletion of all 4 alleles of HDAC1 and HDAC2 causes deficient nephron formation, leading to bilateral renal cystic dysplasia at birth. Immunofluorescence staining of CM$^{HDAC1,2-/-}$ and wild-type kidney sections at E14.5 and P0 revealed that HDAC1/2 are essential for Six2^{+} cell proliferation but have no obvious effect on their survival. CM$^{HDAC1,2-/-}$ cells exhibit hyperacetylated histones and ectopic hyperacetylated p53, but express lower levels of Six2 and Pax2 compared to wild type CM cells. Remarkably, CM$^{HDAC1,2-/-}$ cells can form pre-tubular aggregates and renal vesicles at a slower rate, but fail to progress to comma- and S-shaped bodies. It is known that, in Lhx1$^{-/-}$ mice, tubulogenesis is blocked at the renal vesicle stage. In line with our phenotypic observations, Lhx1 mRNA and protein expression is lacking in renal vesicles of mutant kidneys. By contrast, Wnt4 and Pax8 are still expressed at a lower level in mutant kidneys.

Conclusions: We conclude that HDAC1 and HDAC2 perform redundant yet essential functions during nephrogenesis. HDAC1 and HDAC2 mediate nephron progenitor cell renewal and renal vesicle differentiation via histone and non-histone dependent mechanisms.

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FR-PO577

Defining Nephron Progenitor Gene Regulatory Networks Lori L. O'Brien,[1,3] Joo-seop Park,[2] Andrew P. McMahon.[1,3] *[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; [2]Pediatric Urology and Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [3]Broad CIRM Center, University of Southern California, Los Angeles, CA.*

Background: In the mammalian kidney, nephrons are derived from the cap mesenchyme which surrounds the ureteric bud tips. Maintenance of the cap mesenchyme, the nephron progenitors, is essential to ensure that a sufficient number of nephrons are formed. The progenitors express the transcription factor Six2 which prevents ectopic nephrogenesis and promotes their self-renewal.

Methods: In addition to Six2, the transcriptional regulatory factors Pax2, Hox11, Osr1, Sall1 and Eya1 are expressed within the progenitor population, and they are all required for kidney development. Despite their integral roles, little is known about their transcriptional targets. Identifying regulatory networks will enhance our understanding of nephrogenic programming and facilitate efforts to generate, manipulate and propagate this critical cell population. To this end, we have developed a transgenic strategy to identify targets of any transcriptional regulator-of-interest from nephron progenitors in the developing mouse kidney. The system utilizes a *Six2* enhancer element to drive expression of FLAG-tagged factors specifically within the progenitor population and allows subsequent ChIP-seq to identify targets.

Results: FLAG-Six2, -Hoxd11 and -Osr1 lines have been generated, and their expression recapitulates endogenous Six2 expression. Initial results show significant enrichment of binding for these transcription factors near genes associated with kidney development. Comparison of the Six2 and Hoxd11 datasets reveals that ~ 50% of Hoxd11 bound regions overlap with Six2 bound regions.

Conclusions: Taken together, these data suggest that Six2 and Hoxd11 regulate the expression of progenitor targets through shared networks. Continued investigation and validation of targets and their cis-regulatory input will help shed new light onto the gene regulatory networks operating within the nephron progenitors and lead the way to the discovery of new therapeutic targets and strategies to treat kidney disease.

Funding: NIDDK Support

FR-PO578

Maintenance of Mesenchymal Progenitor Cells in Culture by Aggregation Shunsuke Yuri,[1,2] Naomi Yanagawa,[1] Oak Dong Jo,[1] Masaki Nishikawa,[1,2] Norimoto Yanagawa.[1,2] *[1]Medical and Research Services, GLAVAHS at Sepulveda, North Hills, CA; [2]Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA.*

Background: In the metanephros during kidney development, the metanephric mesenchymal (MM) cells that localize around the ureteric bud (UB) tips, known as cap mesenchyme (CM), are considered to be the progenitor cells capable of self-renewal and differentiation. The potential of this CM for regenerative applications, however, is limited by the lack of culture system whereby its progenitor status can be maintained *in vitro.* In this study, we have tested a wide variety of factors and culture conditions for this purpose.

Methods: UB were separated from E11.5 mouse metanephroi and the remaining cells were dissociated and cultured *in vitro* over 5 days. The ability of various factors and culture conditions to maintain CM was tested by monitoring the expression of marker genes with real time RT-PCR and by the previously described colony-forming assay with Wnt4-3T3 cells (Osafune, Development, 2006).

Results: We found that the use of low calcium KSFM medium (Loo, Method Cell Biol, 2008) in the presence of ROCK inhibitor (Y27632) was effective to sustain the cell proliferation for up to 60 days. While different factors and extra-cellular matrices were effective to prevent the decrease in the expression of marker genes to various extents, they were not able to sustain the colony formation. In contrast, we found that reaggregation with dispersed UB cells enabled colony formation even after 5 days, although at a lower number than the fresh cells. By using metanephroi isolated from Hoxb7-GFP mice, we confirmed the formation of colonies from non-UB cells. This notion was further supported by the expression in the colonies of genes characteristic of podocyte, proximal tubule and loop of Henle. The effect of UB cells in maintaing CM cells appears to require the direct cellular contact because conditioned media derived from UB cells were not effective.

Conclusions: In conclusion, we found that reaggregation with UB cells were effective in maintaining CM cells in culture *in vitro*. On going studies are being conducted to further characterize the underlying mechanism.

Funding: Private Foundation Support

FR-PO579

Impact of Kidney Progenitor Cell Differentiation Status in Wilms Tumorigenesis Le Huang, Sharada Mokkapati, Qianghua Hu, Weihua Tian, Vicki Huff. *Genetics, MD Anderson Cancer Center, Houston, TX.*

Background: Wilms Tumor (WT) is a pediatric cancer of the kidney and is thought to arise from the undifferentiated renal mesenchyme. However, it is unknown from which renal mesenchymal cell types WT can arise. We hypothesize that WT can arise from fetal kidney cells at different differentiation stages and that different progenitor populations at risk will result in tumors with differing histologies and gene expression profiles.

Methods: We used kidney progenitor-specific Cre^{ER} to somatically and mosaically ablate *Wt1* in the context of *Igf2* up-regulation and are determining whether these alterations are tumorigenic. Moreover, we ablated *Wt1* in a high proportion of progenitor cells to study the effects of *Wt1* ablation on kidney development and how *Wt1* ablation predisposes progenitor cell to malignant transformation.

Results: We have generated cohorts of mutant mice and littermate controls and are assessing them for tumor development. Additionally, we found that *Wt1* ablation in stromal progenitors has no obvious effect on nephrogenesis. However, when *Wt1* is ablated in nephrogenic progenitors, nephrogenesis is blocked. The mutant kidneys at E19.5 contain fewer mature nephron structures and an increased number of stromal cells. Furthermore, the nephrogenic zone (NZ), where the progenitor cells reside, is expanded. Cells in the expanded NZ express the progenitor markers and are proliferative. This suggests that *Wt1* ablation in the nephron progenitors prevents the cells from epithelial differentiation and the cells maintain their proliferation and self-renewal property in the NZ.

Conclusions: These results will help us to understand the functions of *Wt1* in the nephron and stroma progenitors and provide significant insights into the mechanisms of Wilms Tumor initiation and progression.

Funding: Other NIH Support - CPRIT

FR-PO580

Distinct Temporally and Spatially-Restricted Roles for Tcf21/Pod1 in Kidney Development Yoshiro Maezawa,[1] Tuncer Onay,[1] Lindsay S. Keir,[2] Henrik Dimke,[1] Chengjin Li,[1] Vera Eremina,[1] Susan E. Quaggin.[1,3] *[1]The Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; [2]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [3]St. Michael's Hospital and University Health Network, Toronto, Canada.*

Background: Tcf21/Pod1 is a basic helix loop helix transcription factor that is required for development of various organs. Conventional Tcf21/Pod1 KO mice die in the perinatal period due to profound cardiac and lung defects. Kidneys of Tcf21 KO mice are hypodysplastic and exhibit defects in branching of the ureteric bud, failure of medullary formation and an arrest in nephrogenesis. Within the metanephros, Tcf21 is expressed in a dynamic pattern in the Six2+cap and Foxd1+stromal mesenchyme, peri-ureteric bud mesenchyme and in developing and mature podocytes, interstitial fibroblasts and pericytes.

Methods: To further dissect the role of Tcf21 in renal development, we generated a conditional allele and bred it to a variety of kidney Cre-driver strains: podocin-Cre; Six2-Cre, Wnt4-Cre and Foxd1-Cre.

Results: Loss of Tcf21 from podocytes results in an FSGS lesion starting at 3 weeks of age, characterized by occasional glomeruli with crescentic transformation and podocyte injury. In situ analysis, immunostaining, microarray analysis and knockdown in podocyte cells in culture identify a variety of downstream targets. Loss of Tcf21 at an earlier stage of nephrogenesis from the cap mesenchyme (Six2-Cre/$Tcf21^{flox/flox}$) leads to glomerular developmental defects characterized by dramatic alterations in glomerular capillary loop formation and abnormal mesangial cell influx. However, in contrast to the conventional KO, ureteric branching, renal medullary formation and renal growth are preserved suggesting distinct roles for Tcf21 at different time-points and in different cell compartments. Analysis of Foxd1-Cre/$Tcf21^{flox/flox}$ and Wnt4-Cre/$Tcf21^{flox/flox}$ mice are currently underway.

Conclusions: Taken together, our results demonstrate multiple roles for Tcf21 during renal development that vary over time and space. Furthermore, these mice provide valuable tool to investigate the transcriptional targets of Tcf21 in cap, stromal mesenchyme and podocyte cell lineage.

Funding: Government Support - Non-U.S.

FR-PO581

Podocyte-Specific Expression of the Podocin Gene Is Combinatorially Regulated by Lmx1b and FoxC Protein Bing He,[1] Lwaki Ebarasi,[2] Sarah De Val,[3] Christer Betsholtz,[2] Karl Tryggvason.[1] *[1]Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; [2]Division of Vascular Biology, Department of Medical Biochemistry, Karolinska Institute, Stockholm, Sweden; [3]Ludwig Institute for Cancer Research, Oxford University, United Kingdom.*

Background: Podocin is a key structural protein of the kidney podocyte slit diaphragm. Mutations in the podocin gene cause familial or sporadic forms of renal diseases owing to disruption of the filtration barrier integrity. The fact that podocin is exclusively expressed in podocytes reflects its unique function, but its regulatory mechanism remains incompletely understood.

Methods: Transgenic zebrafish was used to identify the podocyte-specific enhancer. To characterize the potential DNA binding motif, mutagenesis, EMSA, morpholino-mediated knockdown and in vitro luciferase assay were performed.

Results: Our previous study has localized a potential enhancer in the 2.5-kb zebrafish podocin promoter (JASN 2011;22:1019). Here we define this 5´element and identify a 49-bp enhancer that sufficiently directs expression in zebrafish podocytes. This enhancer contains two adjacent DNA-binding sites (FLAT-E and forkhead) that are synergistically bound by Lmx1b and foxc1a. Importantly, these two transcription factors strongly induce endogenous podocin expression in zebrafish. The cis-acting motif conserved in the human counterpart is bound and activated by LMX1B and FoxC2 and is also present in COL4A4.

Conclusions: We demonstrate a combinatorial control of podocyte expression by the Lmx1b-FoxC enhancer. Our finding provides insights into transcriptional mechanisms required for normal podocyte functions and critical for kidney diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO582

Wnt/β-Catenin Signaling by Exogenous Embryonic CD24(+) Renal Progenitor Cells during Integration into Glycerol-Damaged Renal Tubules

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Background: During nephrogenesis, CD24(+) cells in metanephric mesenchyme are induced to differentiate into renal epithelia and form nephrons by Wnt/βcatenin signals from the ureteric bud (UB). Following acute glycerol-induced proximal tubular damage, exogenous embryonic renal progenitor cells (RPC) are integrated into renal tubules, but the mechanisms involved are poorly understood.

Methods: CD24(+) RPC were isolated by FACS from embryonic day E15 kidneys of mice from heterozygous parents bearing the βCatenin/TCF reporter transgene. Proximal renal tubular injury was induced with i.m. injection of 50% glycerol (8ml/kg) in normal adult mice. Experimental mice were twice infused (via tail vein) with 0.5 million CD24(+) RPC (vs control) three and four days respectively after glycerol injection.

Results: In normal E15-E18 kidney, CD24 (+) cells are seen in the cap mesenchyme, comma- and S-shaped bodies and scattered within the UB trunk of mice bearing a HoxB7/GFP transgene. When isolated by FACS, E15 CD24 (+) RPC express transcripts marking metanephric mesenchyme (Wt1, Osr1, Six2) and UB trunk (Wnt7b), but not the UB tip (Wnt9b, Ret and Wnt11). E15 CD24(+) cells from mice bearing a β-Catenin/TCF reporter (RPC^{TCF}) activate the canonical WNT pathway in response to co-culture with L-cells expressing WNT3a. We infused 0.5 million RPC^{TCF} into adult mice 3 and 4 days after glycerol injection. On day 6, the exogenous RPC^{TCF} were seen widely integrated into the damaged proximal tubules and showed robust re-activation of the canonical WNT signaling pathway. In similar studies, we isolated embryonic CD24(+) cells and stained them with $PKH26^{Red}$ prior to infusion into glycerol mice.The Red-stained RPC were widely integrated into proximal tubules, showing epithelial polarization and staining for LTA, a marker of differentiated proximal tubular cells. The exogenous RPC expressed WNT4 and the proliferation marker, PCNA.

Conclusions: Embryonic CD24(+) renal progenitor cells express WNT4 and re-activate the WNT/β-Catenin signalling pathway during integration into the damaged proximal tubule.

Funding: Government Support - Non-U.S.

FR-PO583

β-Catenin Controls Branching Morphogenesis via the Gdnf-Ret Signaling Axis during Renal Development

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Background: Renal malformation is the major cause of human childhood renal failure. ß-catenin has established roles in the ureteric epithelium by regulating branching and in the mesenchyme by regulating nephrogenesis. We demonstrate that ß-catenin is elevated in human dysplastic renal tissue most notably in the renal mesenchyme. The purpose of this study is to determine the mechanisms by which ß-catenin overexpression in the mesenchyme leads to renal dysplasia.

Methods: We overexpressed or deleted ß-catenin in the metanephric mesenchyme (MM) using Rarß2Cre and floxed ß-catenin mice (*Rarβ2Cre;β-cat$^{GOF\text{-}MM}$* or *Rarβ2Cre;β-cat$^{LOF\text{-}MM}$*). Kidneys were analyzed by histology, immuno-labeling, in situ hybridization, and ChIP analysis.

Results: Rarß2Cre;ß-cat$^{GOF\text{-}MM}$ kidneys were characterized by cystic tubules, dysplastic stroma, and patchy ectopic nephrogenesis. PAX2, CITED1 and SIX2 staining revealed no noteworthy alterations in nephrogenesis. Analysis of branching morphogenesis revealed ectopic ureteric budding off the nephric duct and ureteric bud stalk, and a disorganization of branch patterning. Interestingly, Rarß2Cre;ß-cat$^{GOF\text{-}MM}$ mice exhibited 6 ectopic kidneys consisting of Cytokeratin/PAX2 positive tubules, and WT1 and Nephrin positive glomeruli. Surprisingly, the Cytokeratin/PAX2 positive tubules were not connected to the kidney proper or the nephric duct. In situ hybridization of E11.5 kidneys revealed increased Gdnf, Cret, and Wnt11 expression. Analysis of Rarß2Cre;ß-cat$^{LOF\text{-}MM}$ mice demonstrated renal hypoplasia, reduced ureteric branching and markedly reduced Gdnf expression. No changes in Ret or Wnt11 expression were observed. We further demonstrate that ß-catenin binds to a Tcf consensus site located 4.9kb upstream of the Gdnf transcriptional start site. Molecular cloning of the 4.9kb fragment into pCDNA/LacZ reporter construct was sufficient to regulate LacZ expression.

Conclusions: These data establish that β-catenin is essential in the MM to guide appropriate ureteric budding and branching through the regulation of the Gdnf signaling axis.

Funding: Private Foundation Support

FR-PO584

β-Catenin Expression in the Renal Stroma Is Essential for Branching Morphogenesis and Nephrogenesis

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Background: Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are among the most common of all birth defects, with an incidence rate of 1 in 250 live births. Included in these defects is renal dysplasia, the major cause of childhood renal failure. We recently demonstrated that β-catenin, a protein essential for normal kidney development, is expressed in the renal stroma in the developing and mature kidney. Further we demonstrated that β-catenin is overexpressed in the stromal compartment in dysplastic renal tissue. The functional significance of β-catenin expression and misexpression in renal dysplasia in the renal stroma is not known. *The purpose of this study is to determine the specific functions of β-catenin in the renal stroma during murine kidney development.*

Methods: We generated mice with ß-catenin deficiency or overexpression in the renal stroma using the Foxd1Cre and ß-catenin mice with LoxP sites flanking exon 2-6 (Foxd1Cre;ß-cat$^{LOF\text{-}MM}$) or flanking exon3 (Foxd1Cre;ß-cat$^{GOF\text{-}MM}$) respectively. Embryonic kidneys were analyzed by histology, immunohistochemistry, immunofluoresence, and in situ hybridization.

Results: Analysis of Foxd1-Cre;β-cat$^{LOF\text{-}S}$ kidneys revealed renal hypodysplasia, pancake-like kidneys, an irregular renal capsule, and ill-defined nephrogenic zone. Ureteric branching analysis by cytokeratin immunostaining at E12.5 revealed disorganized and elongated ureteric branches. Remarkably, PAX2, CITED1 and SIX2 immunostaining revealed a marked reduction in condensed mesenchyme around the ureteric tips suggesting impairment in the initial stages of nephrogenesis. Foxd1-Cre;β-cat$^{GOF\text{-}S}$ kidneys demonstrated bilateral renal aplasia or severe hypodysplasia, absence of nephrogenic structures, and increased renal stroma. Analysis of ureteric branching revealed a marked reduction in the number of ureteric branches. Interestingly, PAX2, CITED1, and SIX2 immunostaining revealed a marked increase in condensed mesenchyme around the ureteric tips and essentially no uninduced mesenchyme.

Conclusions: Taken together these studies indicate that β-catenin plays an essential role in the formation of the renal stroma and in regulating ureteric branching and nephrogenesis.

Funding: Private Foundation Support

FR-PO585

A Novel Mechanism Mediating Canonical β-Catenin Signaling during Kidney Development

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Background: Nephrogenesis is driven by signals from the ureteric bud which initiate differentiation of adjacent renal progenitor cells in the metanephric mesenchyme. Our previous studies of mice bearing a β-catenin/TCF reporter demonstrate robust activation of the canonical WNT signaling pathway in the ureteric bud (UB) and the immediately adjacent cap mesenchyme. To explain how the UB signal is so exquisitely targeted to adjacent progenitors, we examined the hypothesis that transfer of membrane-bound proteins from the ureteric bud is required for the progenitor cell response to WNT9b.

Methods: CD24(+) renal progenitor cells(RPC) were isolated by FACS from E15 kidneys of mice bearing a β-catenin/TCF reporter and co-cultured with mouse L-cells transfected with various WNTs and FRZ receptors or with UB cells isolated by FACS from E15 kidneys of mice bearing a HoxB7/GFP(+) transgene. Similar studies were performed by co-culturing WNT9b/L-cells as above with HEK293 cells c0-transfected with TOPFLASH reporter and various FRZs.

Results: E15 CD24/TCF RPC exhibited robust canonical signaling activity when co-cultured with HoxB7 UB cells, but showed no response to co-culture with L-cells expressing WNT9b or WNT11. A screen for expression pattern of various FRZs showed that FRZ4 and FRZ8 are expressed in embryonic ureteric bud but not in cap mesenchyme. Like RPC, HEK293 cells transfected with TOPFLASH were unresponsive to co-cultured L-cells expressing WNT9b, but showed striking responsiveness when they were transfected with FRZ4 or FRZ8. Furthermore, CD24/TCF RPC were responsive to co-cultured L cells co-expressing either WNT9b or WNT11 and FRZ4 or FRZ8. Interestingly, transfected L-cells were shown to shed microvesicles containing FRZ4 or FRZ8 into the culture medium.

Conclusions: These results suggest that renal progenitor cells are unresponsive to WNT9b and WNT11 secreted by neighboring cells. We hypothesize that microvesicle-mediated transfer of FRZ4 or FRZ8 may confer WNT9b- and WNT11 responsiveness to progenitors in the cap mesenchyme.

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FR-PO586

GLI3 Repressor Controls Urinary Tract Development in a Lineage Specific Manner

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Background: The transcription factor GLI3 is proteolytically cleaved to a transcriptional repressor (GLI3R) in states of reduced Hedgehog (HH) signaling. Sonic HH deficiency causes renal agenesis or a single ectopic hypodysplastic kidney. Homozygous loss of both GLI3 and SHH rescues kidney development, implicating GLI3R as a negative regulator of urinary tract (UT) formation. Yet specific events controlled by GLI3R remain undefined.

Methods: GLI3R-dependent control of UT development was analyzed in mouse embryos with constitutive (*Gli3$^{\Delta 699/\Delta 699}$*, Bose et al 2002) or conditional (*Gli3^{TFlag}*, Vokes et al 2008) GLI3R expression.

Results: HH activity in E11.5 WT mice, defined using *Ptc1^{lacZ}*, was strongest in the intermediate mesoderm (IM), common nephric duct (CND) and tail-bud mesenchyme (TM) with moderate activity in the metanephric mesenchyme (MM) but not the Wolffian duct (WD) or ureteric bud (UB). Analysis of *Gli3$^{\Delta 699/\Delta 699}$* mice demonstrated severe renal hypoplasia (100%) and a double collecting system (~47%) at E15.5 (n=12) with hydronephrosis at E18.5 (100%, n=16). Mutants at E11.5 had a marked reduction in HH activity in the MM, CND and TM. UBs were hypoplastic at E10.5 and E11.5 with reduced UB branching at E12.5 (51%, n=16). Induced GLI3R expression at E12.5 in *Esr1-cre;Gli3TFlag* mice caused hypoplasia at E15.5. A double collecting system arose from two primary UBs (67%, n=6) in E11.5 *Hoxb7-cre;Rosa$^{lacZ/+}$;Gli3$^{\Delta 699/\Delta 699}$* mice. The length from cranial WD to bud site(s) indicated normal positioning of one UB with cranial ectopic positioning of the second (p=0.0026). All UBs failed to maintain position caudally with

the CND at E11.5 (p=0.004) and were associated with blind-ended ureters at E16.5 (100%, n=6). UB specific expression of GLI3R in E15.5 *Hoxb7-cre;Gli3^{TFlag}* embryos caused renal hypoplasia (100%, n=14) without a duplex collecting system. IM specific expression of GLI3R using E15.5 *Pax3-cre;Gli3^{TFlag}* embryos caused renal agenesis.

Conclusions: Our data reveal multiple temporal and lineage-specific roles for GLI3R in early UT development by controlling ureteric budding, branching, and ureteric insertion into the bladder.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO587

Deletion of miR-143/145 Leads to Hydronephrosis Silvia Medrano, Maria Luisa S. Sequeira Lopez, Roberto Ariel Gomez. *Pediatrics, University of Virginia.*

Background: Obstructive nephropathy, the leading cause of kidney failure in children, can be anatomical or functional. The underlying causes of functional hydronephrosis are not well understood. microRNAs are small, non-coding RNAs that negatively regulate the expression of target genes at the post-transcriptional level. We recently found that miR-145, a member of the miR143/145 cluster highly expressed in smooth muscle (SM) cells of renal arterioles, is also present in the pelvicalyceal system and the ureter. The present study was conducted to evaluate whether the miR143/145 cluster is involved in urinary tract function.

Methods: Morphological, functional, and gene expression studies were performed in mice carrying a whole body deletion (KO) of miR-143/145.

Results: miR-143/145 KO mice did not exhibit overt morphological changes in the kidney vasculature. However, these mice developed hydronephrosis (100% penetrance), characterized by severe papillary atrophy and dilatation of the pelvicalyceal system without obvious anatomical obstruction.To determine whether ureteral peristalsis is affected in these mice, we recorded the frequency of contractions in exposed ureters of anesthetized animals. The number of contractions was significantly higher in KO mice compared to WT controls. Moreover, in mutant mice, peristaltic waves were replaced by incomplete, short and rapid contractions that failed to propagate in a proximal-distal direction. Microarray analysis of ureters from WT and KO mice showed that of a total of 35,512 genes screened, 109 were upregulated and 216 were downregulated in the KO mice, with at least a 2-fold difference in expression levels, including genes with potential regulatory roles in frequency, intensity and propagation of SM contraction.

Conclusions: Our data suggest that miR-143/145 are important for the normal contractility of the ureter and therefore for the normal transport of urine from the kidney to the bladder. These studies will help understand how ureteric peristalsis is regulated and may provide insights into the etiology and treatment of hydronephrosis in children.

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FR-PO588

DLG1 and CASK Affect Kidney Development through an ERK-Dependent Pathway Sun-Young Ahn,[1] Yeawon Kim,[1] Sung Tae Kim,[2] Jeffrey H. Miner.[2] *[1]Pediatric Nephrology, Washington University in St. Louis, St. Louis, MO; [2]Renal Division, Washington University in St. Louis, St. Louis, MO.*

Background: Dlg1 (discs-large homolog 1) null mice exhibit hydronephrosis, hydroureter, and occasionally hypoplastic kidneys. CASK (calcium/calmodulin-dependent serine protein kinase), which interacts with DLG1 at some membrane-cytoskeleton interfaces, functions as a scaffolding protein that links signaling molecules, receptors and other scaffolding proteins at tight and synaptic junctions. Furthermore, CASK regulates neuronal and epithelial cell polarity. Our aim is to investigate whether DLG1 and CASK cooperate in the developing kidney and to identify the signaling pathways involved.

Methods: We generated mice deficient in both DLG1 and CASK, either globally using null alleles, or specifically in nephron progenitors using Six2Cre/GFP and floxed Dlg1 and Cask alleles. Immunostaining and in situ hybridization were performed on frozen kidney sections. Western blotting was performed using lysates from embryonic kidneys.

Results: Cask-/-;Dlg1-/- (DKO) kidneys were severely hypoplastic and dysplastic and demonstrated a striking premature depletion of nephron progenitors. Importantly, we also observed that Cask-/-;Dlg1+/- (null/het) kidneys were small, though not as small as the DKO kidneys. Nephron-specific null/het mice survived with small kidneys but developed glomerulocystic kidney disease and renal failure. We discovered several cellular and molecular defects in the double mutants. These include reduced proliferation and increased apoptosis of cells in the nephrogenic zone, and a decrease in the number of cells expressing Six2, a transcription factor critical for maintaining the nephron progenitor population. Because CASK binds to syndecan, a co-receptor that modulates FGF receptor (FGFR) activity, we investigated the potential involvement of DLG1 and CASK in the FGF signaling pathway, and found a reduced level of ERK, a mediator of FGF signaling.

Conclusions: Taken together, these results show that DLG1 and CASK play an important role in maintaining the nephron progenitor population, potentially through an ERK-dependent pathway.

FR-PO589

Deletion of Fgfr2 in Tailbud-Derived Stroma Is Associated with Vesicoureteral Reflux, Dysfunctional Voiding and Chronic Kidney Disease Kenneth A. Walker,[1] Irina Zabbarova,[2] Youko Ikeda,[2] Caitlin M. Schaefer,[1] William Chet De Groat,[2,3] Anthony Kanai,[2,3] Carlton M. Bates.[1,4] *[1]Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]Medicine, University of Pittsburgh, Pittsburgh, PA; [3]Pharmacology, University of Pittsburgh, Pittsburgh, PA; [4]Nephrology, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: During development, tailbud-derived stroma (ST) acts on the Wolffian duct to regulate ureteric bud induction and later forms the smooth muscle of the ureter and bladder. Previous data shows deletion of Fgfr2 in ST with Tbx18cre mice (Fgfr2$^{ST-/-}$) results in vesicoureteral reflux (VUR) likely due to induction defects. We aim to use Fgfr2$^{ST-/-}$ mice to investigate the natural history of: 1)VUR 2)the role of Fgfr2 in bladder muscle function, and 3)how bladder defects affect renal morphology and function.

Methods: Bladder function was studied by in vivo cystometry and in vitro bladder sheet assays. Tissue morphology were shown by histology. Renal function was investigated by analyzing urine and serum.

Results: At 1 month, Fgfr2$^{ST-/-}$ mice have high rates of VUR. While bladders from all mice had similar morphology, Fgfr2$^{ST-/-}$ mice had dysfunctional voiding (DV) shown by more frequent and smaller voids. Fgfr2$^{ST-/-}$ mice had higher baseline and threshold bladder pressures and shortened intercontractile intervals than controls. Fgfr2$^{ST-/-}$ mice also have poor compliance, decreased muscle relaxation and decreased muscle contractility. At 6 months, Fgfr2$^{ST-/-}$ mice had high rates of VUR similar to 1 month old mice, often with hydronephrosis. Aged Fgfr2$^{ST-/-}$ mice continued to have DV and develop polyuria. 6 month Fgfr2$^{ST-/-}$ bladders often appear distended with thin, irregular muscle layers. Aged Fgfr2$^{ST-/-}$ mice also have signs of chronic kidney disease (CKD) with fibrosis, elevated blood urea nitrogen and other biomarkers of injury.

Conclusions: Fgfr2$^{ST-/-}$ mice are a novel genetic model linking VUR and DV with impaired bladder function that together pose a significant risk for CKD. This model may ultimately change the way clinicians assess and follow patients with VUR and/or DV and could lead to novel therapeutic approaches to prevent CKD.

Funding: NIDDK Support

FR-PO590

Integrin-Linked Kinase (ILK) Controls Ureteric Bud (UB) Gene Expression via p38MAPK-Dependent and -Independent Mechanisms Joanna Smeeton,[1,2] Norman D. Rosenblum.[1,2,3] *[1]Laboratory Medicine and Pathobiology, U of Toronto, Toronto, ON, Canada; [2]Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada; [3]Division of Nephrology, Hospital for Sick Children, Toronto, ON, Canada.*

Background: In mammals, the renal collecting system is derived via growth, branching and remodeling of the UB, a process termed renal branching morphogenesis (RBM). The intracellular molecular mechanisms that control RBM are largely undefined. Previously, we demonstrated that ILK is required for RBM *in vivo* and controls UB branching via p38MAPK *in vitro* (Smeeton et al, Development, 2010; Leung-Hagestejn et al, Mol Cell Biol, 2005).

Methods: To identify genes that act downstream of ILK during RBM, whole transcriptome microarray analysis was performed using E12.5 kidneys from mutant mice with *Ilk* deficiency targeted to the UB and from controls. p38MAPK signaling was inhibited in kidney explants to investigate whether its activation is required for the expression of candidate genes identified by the microarray analysis.

Results: Microarray analysis identified 227 differentially expressed mRNA transcripts ($p<0.003$). Correlation with the GenitoUrinary Development Molecular Anatomy Project database (gudmap.org) identified 14 UB-enriched genes whose expression was downregulated in mutant kidneys. Quantitative RT-PCR confirmed decreased expression of Ilk, critical gene targets in the Ret/GDNF signaling pathway (Wnt11, Sox8, Myb, CXCR4), and 3 less well-characterized UB-specific genes (SCF, Krt23, Slco4c1). Phospho-p38MAPK protein levels were decreased by 70% in mutant embryonic kidneys ($p=0.004$). The functional requirement for p38MAPK in ILK-mediated gene expression was investigated in kidney explants cultured for 48hr with a p38MAPK inhibitor. While no effect on Sox8, Myb or CXCR4 mRNA expression was observed, p38MAPK inhibition significantly decreased expression of Wnt11 (50%), SCF (45%), Krt23 (85%), and Slco4c1 (58%) ($p<0.05$).

Conclusions: We conclude that (i) ILK regulates downstream gene targets through both p38MAPK-dependent and -independent pathways to control UB branching and (ii) ILK regulates key components of the Ret/GDNF signaling pathway in the developing UB.

FR-PO591

The Transcription Factor Sry-Related HMG Box-4 (Sox4) Is Required for Normal Renal Development *In Vivo* Sunny Hartwig,[1] Junzhuo Huang,[1] Michel G. Arsenault,[1] Martin Kann,[2] Monique C. Saleh,[1] Carlos Lopez,[1] Jonathan Spears,[1] Jacqueline Ho,[2] Glenda M. Wright.[1] *[1]Biomedical Sciences, Atlantic Veterinary College, Charlottetown, PE, Canada; [2]Division of Nephrology, Children's Hospital Boston, Boston, MA; [3]Division of Nephrology, Children's Hospital Pittsburgh of UPMC, Pittsburgh, PA.*

Background: The DNA-binding nuclear transcription factor Wilms' Tumor Suppressor-1 (WT1) plays an essential role in nephron progenitor cell (NPC) differentiation during renal development in humans and mice; however, the gene targets that mediate WT1 function *in vivo* are largely undefined. Genome-wide location analysis using chromatin immunoprecipitation coupled to microarray (ChIP-chip) in embryonic mouse kidney tissues

identified all three members of the *Sry-related HMG box (Sox)-C* subfamily - *Sox4, Sox11* and *Sox12* – as gene targets bound by WT1 *in vivo*. *Sox* genes play master roles in fate determination of specific cell types in a multitude of developmental processes, however, SoxC function during renal development has not been investigated.

Results: We confirmed that Wt1 physically binds the promoter regions of all three *SoxC* genes by direct ChIP. Next, we demonstrate that Wt1 knock-down in embryonic kidney explants results in reduced *SoxC* expression *ex vivo.* Together, these data strongly suggest that *SoxC* genes are transcriptional Wt1 targets *in vivo.* While all three *SoxC* genes are expressed in NPCs, *Sox4* exhibits strongest and most specific expression in NPCs throughout renal development. We therefore investigated the role of *Sox4* in NPC fate by conditionally ablating *Sox4* function in NPCs (*Sox4 NPC*$^{-/-}$ mice). Six2Cre-mediated removal of *Sox4* activity from NPCs leads to reduced nephron number and premature termination of nephrogenesis at birth. Postnatally, *Sox4 NPC*$^{-/-}$ kidneys exhibit multifocal, tubular degeneration and proteinosis, interstititial nephritis and severe segmental to global glomerulopathy by 3 weeks, progressing to renal failure within 5 months.

Conclusions: *SoxC* genes are novel transcriptional Wt1 targets in the developing kidney. Preliminary data demonstrates that at least one SoxC member, Sox4, plays an essential role in controlling renal development *in vivo*.

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FR-PO592

Suppression of Nephrogenesis in Response to Hypoxia Is Mediated via Disruption to Wnt Signaling Melissa H. Little, Calida Neal, Bree Rumballe, Lorine J. Wilkinson. *Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia.*

Background: Kidney development and final nephron number are affected by environmental stressors resulting in long term effects on renal function. We investigated the affect of hypoxia, a common insult to the fetus during pregnancy, on the developing kidney.

Methods: E12.5 kidney explants were cultured in CoCl2, or 1%, 5%, 12% oxygen, and normoxia for 30-40 hours. WISH, RTPCR, electron microscopy (EM), and immunofluorescence were performed to quantitate tip and nephron numbers, and investigate changes in gene expression, and ultrastructure.

Results: The culture of explants in CoCl2 and 1% oxygen resulted in reduced branching and a disproportionate reduction in nephrogenesis. Development was optimal at 5% with increases in nephron number and nephron/tip ratio at this oxygen concentration. Optimal branching was seen at 12% while nephron/tip ratio was equivalent to that of normoxia. Explants cultured in CoCl2 and 1% oxygen retained a progenitor population and nephrogenesis could be rescued by returning the explants to normoxia, suggesting that the progenitors remain competent but do not receive the appropriate signals to undergo MET. Gene expression studies showed that a reduction in the expression of Wnt9b targets occurred in CoCl2 and 1% oxygen cultures.[1] EM revealed accumulation of glycogen granules and a reduction in cell-cell adhesion within the ureteric compartment, supporting the presence of hypoxia and a reduction of b-catenin.[2,3] Indeed, the culture of explants with IWR-1-an inhibitor of canonical Wnt signaling resulted in a similar suppression of differentiation without the loss of cap mesenchyme coupled with altered ureteric morphology. Conversely, suppression of Wnt secretion with the inhibitor IWP-2 resulted in cap mesenchyme loss.

Conclusions: Our data implies an oxygen concentration of 5% is optimal for nephrogenesis but that there is a dissociation between the response of the ureteric epithelium and the cap mesenchyme to oxygen concentration. Our data also highlights an association between hypoxia and suppression of canonical Wnt signaling.

1. Karner et al., 2011, 2. Kaidi et al., 2007, 3.Lyashenko et al., 2011.

Funding: Government Support - Non-U.S.

FR-PO593

Cell Junction Protein TM4SF10 Organizes Actin Filaments at the Adherens Junction Complex in Renal Epithelial Cells Jeffrey S. Simske, Leslie A. Bruggeman. *MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH.*

Background: Regulation of morphogenesis and cell polarity requires the coordinated interaction between the actomyosin contractile apparatus and cellular junctions. We have shown the claudin-like protein TM4SF10 is expressed during glomerular development and is re-expressed in adult podocytes during injury repair following foot process effacement. We propose that TM4SF10 has important regulatory functions in the formation of the podocyte foot process and slit diaphragm.

Methods: We used *C. elegans* and MDCK cells to explore the role of TM4SF10 in epithelial cell junction formation. In vitro, new cell junction formation (newly plated) and re-forming junctions (calcium switch) were used to monitor protein co-localizations. In vivo studies in worms used mutants and re-expression studies with tagged proteins to monitor protein co-localizations and functional rescue.

Results: In MDCK cells, TM4SF10 localized with cadherin at nascent cell junctions and co-immunoprecipitated with adherens junction proteins. In addition, in cells that do not express cadherin, TM4SF10 failed to stably localize to cell-cell contacts. Overexpression of TM4SF10 delayed reformation of cell junctions following calcium switch, reduced apical surface area, and altered cell polarity, with disorganized cell junctions and altered F-actin organization. Knockdown of TM4SF10 resulted in opposite phenotypes. TM4SF10 rescued the phenotype of worms defective in VAB-9, the worm TM4SF10 ortholog. VAB-9, myosin phosphatase, and activated myosin light chain proteins all co-localized at the cell junctions of enclosing worm epidermal cells. In VAB-9 defective worms, myosin phosphatase failed to migrate to cell junctions and had lower levels of activated myosin light chain.

Conclusions: During cell junction formation, TM4SF10 localization at the plasma membrane is dependent on the placement of cadherin. In the absence of TM4SF10, myosin phosphatase is not recruited to the cell junction. Our studies suggest that TM4SF10 plays an important but transient role in stabilizing cell junctions by mediating actomyosin activity through the recruitment of myosin regulatory proteins to points of cell-cell contact.

FR-PO594

FGF9 Supports Survival, Proliferation and GDNF-Receptor Expression of Wolffian Duct Cells Kohei Johkura,[1] Hiroyuki Sakurai,[2] Kevin T. Bush,[3] Sanjay K. Nigam.[3] *[1]Department of Histology and Embryology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan; [2]Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan; [3]Departments of Medicine, Pediatrics, and Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA.*

Background: Fibroblast growth factor (FGF) signaling plays a key role in ureteric bud development in renal organogenesis, while its significance in maintaining Wolffian duct (WD) competence is largely unknown. In isolated WD culture in vitro, GDNF alone did not maintain WD integrity or induce ureteric bud formation. Analogous to isolated ureteric bud culture in vitro, where addition of certain FGFs supported branching morphogenesis, we hypothesized that some FGF(s) may act on WD cells to maintain its integrity and GDNF responsiveness.

Methods: WD and its surrounding mesenchyme were isolated from SD rats at embryonic day 12 and 13. Isolated WDs were cultured in Matrigel in the presence/absence of FGFs. Some cultured WDs were recombined with freshly isolated metanephric mesenchyme (MM) to assess their competence to form ureteric buds. Expression of FGF ligands (all 15 canonical FGFs) and receptors (FGFRs) in mesonephric tissue were examined in rats with real-time PCR and immunohistochemistry.

Results: Several canonical FGFs were expressed in WD and the mesenchyme at meaningful levels, among which only FGF9 successfully supported survival and growth of isolated WD in culture for more than 7 days. Recombination of such WD with MM resulted in ureteric bud formation and nephron tubule induction. FGFRs that can be stimulated by FGF9 were expressed in WD, and the effects of FGF9 on WD were blocked in the presence of FGFR, MAPK, or PI3K inhibitor. The expression levels of GDNF receptors (Ret and Gfrα1) and CyclinD1 were significantly higher in WDs cultured with FGF9 than those of control (10% FBS) while apoptosis marker Bax/Bcl2 ratio was reduced. FGF9 treated WDs formed multiple ureteric bud like structures in response to GDNF.

Conclusions: These results suggest that FGF9 is likely to act on the maintenance of WD cell proliferation, survival and responsiveness to GDNF.

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FR-PO595

MicroRNAs in the Metanephric Mesenchyme Are Critical for Early Kidney Development Jacqueline Ho,[1,2] Sunder Sims-Lucas,[1,2] Jessica Chu,[1,2] Andrew J. Bodnar,[1,2] Jordan A. Kreidberg.[3,4] *[1]Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA; [2]Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Pediatrics, Harvard Medical School, Boston, MA; [4]Medicine, Children's Hospital of Boston, Boston, MA.*

Background: MicroRNAs (miRNAs) are endogenous, small, non-coding RNAs that act as novel regulators of gene expression through the post-transcriptional repression of their target mRNAs. The production of mature miRNAs requires processing by the enzyme, Dicer. Recent studies using a conditionally floxed Dicer allele have demonstrated a critical role for miRNAs in modulating nephron progenitor survival in Six2-expressing cells and their derivatives during kidney development. However, the role of miRNAs in the early metanephric mesenchyme (which gives rise to nephron progenitors and renal stroma) is yet to be defined.

Methods: To elucidate whether miRNAs are necessary in the early metanephric mesenchyme, we utilized the Pax3CreTg allele and the conditionally floxed Dicer allele to generate embryos that lack functional miRNAs in the early metanephric mesenchyme and its derivatives.

Results: The loss of miRNAs in the metanephric mesenchyme results in severe renal dysgenesis. However, the earliest stages of kidney formation is relatively conserved. Ureteric bud outgrowth from the Wolffian duct and the initial specification of the ureteric and metanephric mesenchymal lineages transpires normally, as evidenced by appropriate expression of Six2, Sall1, NCAM, Pax2, and calbindin in the mutant kidneys. Marked apoptosis is observed in the mutant mesenchyme, as measured by TUNEL staining, by E11.5. The ureteric bud fails to branch, and the metanephric mesenchyme involutes, resulting in absence of kidneys by E14.5.

Conclusions: The phenotype observed in this early loss of miRNAs is temporally distinct from that observed when miRNAs are deleted in nephron progenitors, which may reflect differing developmental roles for miRNAs due to the timing and/or cell lineages in which miRNAs are expressed. Taken together, these results demonstrate a previously undetermined requirement for miRNAs during early kidney development.

Funding: Other U.S. Government Support

FR-PO596

Wnt5a and Planar Cell Polarity in Kidney Development and Renal Cyst Formation Liwei Huang,[1] Soo Young Choi,[1] Sarah Mckenna,[1] Xiaofeng Zuo,[1] Yun Kyoung Ryu,[2] Rejji Kuruvilla,[2] Rebecca D. Burdine,[3] Joshua H. Lipschutz.[1] *[1]Division of Nephrology, University of Pennsylvania, Philadelphia, PA; [2]Department of Biology, Johns Hopkins University, Baltimore, MD; [3]Department of Molecular Biology, Princeton University, Princeton, NJ.*

Background: Congenital anomalies of the kidney and urinary tract encompass a large group of disorders that result from abnormal renal developmental processes, and include disorders of terminal differentiation, such as autosomal dominant polycystic kidney disease. Planar cell polarity (PCP) is the coordinated orientation of cells and cellular structures along an axis within the plane of an epithelial surface. Patients with nephronophthisis type II have mutations in the gene INVS/NPHP2, and *Nphp2* knockout mice developed cystic kidney disease via affects on PCP signaling. We previously showed that Wnt5a, a noncanonical Wnt, plays a important role during prostate development, with lumen formation and planar cell polarity being abnormal in the Wnt5a null mouse prostate (Huang et al, *Dev Biol, 2009*). Wnt5a is expressed in metanephroi of mouse embryos. **We hypothesize that Wnt5a acts through planar cell polarity pathway to regulate kidney growth and altered Wnt5a signaling will lead to renal cyst formation.**

Methods: We microinjected zebrafish embryos with antisense *wnt5a* morpholinos at the 1-cell stage and allowed them to grow for 72 hrs to determine the effect of Wnt5a knockdown on pronephric kidney development and PCP pathway. Kidneys/urinary tracts were dissected from wildtype and Wnt5a null mouse before birth.

Results: In zebrafish, Wnt5a morphants had a curly tail down phenotype, which was consistent with a ciliary defect. Wnt5a null mice have either absent or markedly abnormal kidneys. H&E staining showed dilated renal tubules. Since Wnt5a null mice die at birth, kidney cyst formation could occur before or after birth; we plan to generate kidney-specific knockout mice. Floxed Wnt5a mice will be mated with FoxD1-Cre mice.

Conclusions: Knocking out Wnt5a specifically in Wnt5a-expressing kidney cells will directly test our hypothesis that Wnt5a disruption interferes with kidney development, specifically planar cell polarity, and results in developmental defects.

Funding: NIDDK Support

FR-PO597

Differential Requirement of Gfrα1 in Early and Later Kidney Development Thomas K. Davis,[1] Masato Hoshi,[2] Sanjay Jain.[2] *[1]Division of Pediatrics (Nephrology), Washington University School of Medicine, St. Louis, MO; [2]Division of Internal Medicine (Renal), Washington University School of Medicine, St. Louis, MO.*

Background: Glial cell line-derived neurotrophic factor (GDNF) coreceptor α1 (GFRα1) is essential for early ureteric bud induction and kidney development as global Gfrα1-knockout mice die at birth from renal agenesis/aplasia due to absence in Ret tyrosine kinase signaling. A number of studies suggest that GFRα1/GDNF/RET tyrosine kinase signaling remains important after ureteric bud induction however, evidence in vivo for this conclusion is lacking. We have created a mouse model which allows inducible deletion of Gfrα1 in a temporal manner using the tamoxifen inducible Cre-ERT2.

Methods: Animals: Gfrα1 reporter, HoxB7Cre and tamoxifen inducible conditional deletion of Gfrα1 in mice in vivo or in explants cultures using timelapse microscopy.

Immunohistological analysis of tissue sections and wholemounts. Antibodies: E-cadherin, GFP, Gfrα1, phospho-histone H3, phospho-Erk 1/2.

Quantitative real-time polymerase chain reaction (qRT-PCR) using Applied Biosystems 7900 HT Sequence Detection System.

Results: We show Gfrα1 is expressed before UB induction in both epithelial and mesenchymal compartments and remains present during branching. Conditional deletion of Gfrα1 before UB branching in WD epithelia results in renal agenesis. Deletion of Gfra1 after initial UB budding using a tamoxifen inducible Cre strain results in mild renal hypoplasia. The mechanism of hypoplasia is decreased epithelial cell proliferation. Furthermore, this elegant system allows direct visualization and characterization of Gfrα1 negative cells through an EGFP reporter.

Conclusions: Gfrα1 has a broader expression in the urinary tract than either Ret or Gdnf. Gfrα1 is required for metanephric kidney induction but becomes less critical as development progresses. Our results provide further support for redundant pathways other than Gdnf-Gfrα1-Ret signaling to ensure on-going branching morphogenesis.

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FR-PO598

Cytogenomic Aberrations in Children with Isolated Multicystic Dysplastic Kidney (MCDK) Dominique Monlezun, Renfang Song, Tianjian Chen, Graeme James Preston, Adam T. Janssen, Ihor V. Yosypiv. *Pediatrics, Tulane University School of Medicine, New Orleans, LA.*

Background: Cytogenomic microarray has been demonstrated to be a powerful tool to reveal pathogenic mechanisms for multiple congenital anomalies. However, the pathogenic roles of cytogenomic aberrations for isolated MCDK has not been well investigated yet.

Methods: Patients (six females and four males) were diagnosed with MCDK by renal ultrasonography. The mean age of patients was 8.5±1.1 years. Comparative genomic hybridization (CGH) microarray was performed on Agilent 105K array. Data were analyzed using Cytogenomic software package from Agilent.

Results: All patients had normal renal function at the time of collection of the blood specimen. Three pathogenic aberrations were detected in 3 patients. The first aberration was a deletion at 7p14.3 with size of 2.07 Mb. At least 12 genes are located within this deletion region, including *BBS9* and *BMPER*. Mutations in *BBS9* are responsible for Bardet-Biedl syndrome (BBS). Cystic kidney dysplasia is one of the clinical features of BBS. Defects in *BMPER* result in diaphanospondylodysostosis. Nephroblastomatosis with cystic kidneys is the most commonly described extraskeletal finding in patients with diaphanospondylodysostosis. The second aberration was a duplication on 16p13 and 11p12.3 with size of 3.28 Mb. There are more than 20 genes located within this duplicated region. This duplication has not been reported as a benign copy number variant in general population. The third aberration is a monosomy X for female patient. Kidney anomalies are reported in 30% of patients with Turner syndrome. In addition to the 3 aberrations, five of ten patients were detected to carry a duplication at 15q11.2 with size range from 1.2 to 1.9 Mb. This duplication is a common copy number variant and is outside of the epigenetic imprinting region of Prader-Willi/Angelman syndromes.

Conclusions: In summary, our results showed that 30% of patients with MCDK possess cytogenomic aberrations. Exploring the pathogenic changes in MCDK patient genome will reveal new etiology and molecular mechanisms of this disease.

FR-PO599

Sall1 and NuRD Regulate Renal Progenitor Cell Fate Jeannine M. Basta,[1,2] Darcy R. Denner,[1] Lynn R. Robbins,[1] Susan M. Kiefer,[1,2] Michael I. Rauchman.[1,2] *[1]St. Louis University; [2]St. Louis VA Medical Center.*

Background: Renal progenitor cell self-renewal and differentiation must be balanced in order to produce the full complement of nephrons, as reduced self-renewal or accelerated differentiation results in depletion of progenitor cells and renal hypoplasia. The molecular mechanism that regulates this critical cell fate decision in nephrogenesis is not understood.

Methods: We used *Sall1*^{GFP} mice to isolate progenitor cells by FACS from E11.5 and E12.5 kidneys and performed transcriptional profiling. We analyzed informative Sall1 and NuRD mutants.

Results: Genome wide data revealed reduced expression of progenitor genes (*Osr1*, *Cited1*, *Six2*, *Bmp7*, *Eya1*) and ectopic expression of many renal vesicle genes in *Sall1* mutants. There was an increase in the number of Lef1, Lhx1 positive renal vesicles in the mutant, with some in ectopic locations. Cell cycle profiles revealed no difference in proliferation or apoptosis at E12.5 when ectopic vesicles are present indicating that depletion of renal progenitors in *Sall1* deficient kidneys is due to accelerated differentiation. Sall1 associates with the Mi2-NuRD chromatin remodeling complex to regulate gene expression. We thus tested the requirement of the Mi2-NuRD complex and its association with Sall1 in vivo for kidney development. We used *Six2*-Cre to delete NuRD specific components *Mi2* and *Mta2*. Both conditional mutants displayed significant renal hypoplasia with depletion of renal progenitor cells. Moreover, *Sall1* and *Mi2* exhibit a strong genetic interaction in the kidney, supporting a functional link between Sall1 and NuRD. To test if Sall1-NuRD interaction is required in vivo, we prepared mice in which three residues in the N- terminus of Sall1 required for NuRD binding are mutated. These mice die peri-natally due to severe renal hypoplasia and at E13.5 have reduced renal progenitor cells.

Conclusions: We conclude that Sall1 and NuRD coordinately regulate the balance between self-renewal and differentiation of renal progenitor cells. Ongoing studies are aimed at identifying common direct target genes and epigenetic mechanisms of gene regulation by Sall1-NuRD.

Funding: NIDDK Support, Private Foundation Support

FR-PO600

Drosophila as a New Model to Identify and Study Renal Disease Genes Zhe Han. *Internal Medicine, University of Michigan, Ann Arbor, MI.*

Background: Genetic mutations affecting renal function are major cause of renal disease. About 20 genes have been identified to be directly linked to renal disease over the past decade, but the majority of genes required for renal function and involved in renal disease remain unknown. The Drosophila nephrocyte shares striking similarities with mammalian podocytes, making Drosophila a potent model to study genes involved in renal function and renal disease.

Methods: We have developed a novel functional readout for Drosophila nephrocytes. By combining this functional readout and nephrocyte-specific gene knockdown technique, we performed a large-scale genetic screen for genes required for ultra-filtration and protein reabsorption in Drosophila. Our system makes it possible for the first time to examine thousands of genes efficiently for their requirement in renal functions. Using this system, we also developed the Drosophila models for human renal disease caused by specific gene mutations and tested drug treatments.

Results: From our genetic screen, we identified ~150 genes required for ultra-filtration and protein reabsorption in *Drosophila*. Most of these genes are conserved from Drosophila to humans, and encode slit diaphragm components, membrane receptors, glomerular basement membrane components, actin cytoskeletons, TRP channels, vesicle trafficking molecules, myosin and dynein motors, transcription factors and the Coenzyme Q (CoQ) biosynthesis pathways, etc. Many of these genes have been directly linked to renal diseases. Here, we summarize the known renal disease genes that we identified from our screen, and provide a list of genes that are likely to be involved in genetic kidney disease. We also describe the development of the *Drosophila* renal disease models that recapitulate the exact genetic mutations causing renal disease and the use of these fly renal disease models for drug treatment.

Conclusions: Our study demonstrated that the genes involved in ultra-filtration and protein reabsorption are highly conserved from Drosophila to humans, and that Drosophila can be used as a new renal disease model for pathogenic study and drug discovery.

Funding: Other NIH Support - NHLBI

FR-PO601

Identification of Kidney Development Pathways Using Zebrafish Forward Genetics Rebecca A. Wingert, Jennifer Cihlar, Rachel Bounds, Michael Mckernan, Annemarie Fox, Gary F. Gerlach. *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.*

Background: The genetic pathways that pattern nephron segments along the proximo-distal axis remain poorly understood. Zebrafish are a useful genetic model for nephron developmental studies as their nephron segment composition is conserved with humans.

Methods: We have performed a novel haploid screen to identify nephrogenesis genes using zebrafish. Adult wildtype males were mutagenized with ethylnitrosurea and used to make F1 fish, then kidney development was assessed in F1 haploid embryos by whole mount *in situ* hybridization to assay multiple nephron cell types in each sample. We screened 685 females and identified 42 prospective alleles associated with defects in podocytes (17), proximal segments (16), distal segments (7), or axial patterning (2). F1 founders were outcrossed, and lines are now being recovered in the diploid state.

Results: Axial pattern mutant ND154 had reduced proximal and expanded distal segments, hallmarks of a defect in *aldehyde dehydrogenase1a2* (*aldh1a2*), required for retinoic acid (RA) synthesis. We found that ND154 fails to complement $aldh1a2^{m15}$, suggesting it is a new *aldh1a2* allele and providing proof of principle that our strategy will yield nephron patterning mutants. To date we have recovered a total of 10 other lines that represent a unique cohort of kidney mutants. Of these, 5 lines have podocyte formation defects. For example, lines ND172 and ND233 have absent and reduced podocytes, respectively, and display normal development until 3-4 days, when they exhibit renal failure. These data suggest that ND172 and ND233 play specific roles in podocyte formation, while the other podocyte mutants have widespread defects suggesting a disruption in genes with pleiotropic functions. We have also recovered 5 lines with tubule patterning defects that exhibit alterations in proximal or distal segment formation that have not been previously reported.

Conclusions: Taken together, our collection will provide a useful resource to delineate the genes that direct nephrogenesis pathways, and may provide new models to study human congenital kidney defects.

Funding: NIDDK Support, Private Foundation Support

FR-PO602

The Transcription Factor *Evi1* Is Essential for Proximo-Distal Patterning and Differentiation of the Zebrafish Pronephros Rebecca A. Wingert,[1] Valerie Verdun,[1] Alan J. Davidson,[2] Yue Li.[1] *[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN; [2]Department of Molecular Medicine & Pathology, University of Auckland, Auckland, New Zealand.*

Background: Nephron segmentation remains poorly understood. The zebrafish pronephros is a good model to study segmentation, as it is composed of two nephrons that have conserved segments of transporting epithelia: proximal convoluted and straight tubule (PCT, PST), distal early and late (DE, DL) and pronephric duct (PD). Interspersed among the transporting epithelia cells are multiciliated epithelial cells (MCC).

Methods: The transcription factor *MDS1 and EVI1 complex locus* (*evi1*) is broadly expressed in nephron progenitors. To study the role of *evi1*, we performed morpholino knockdowns.

Results: *evi1* morphants had an expanded PST and reduced DL, indicating that *evi1* is necessary for DL formation and may restrict PST fates. In addition, MCC number increased in *evi1* morphants, with a caudally expanded domain. Interestingly, *evi1* morphants had expanded proximal nephron progenitors at early timepoints, suggesting that *evi1* regulates nephron patterning. Retinoic acid (RA) signaling directs proximodistal segmentation early, being essential to form the proximal progenitor domain, while Notch signaling triggers epithelial cell type choice much later. To assess the epistatic relationship between *evi1* and RA, *evi1* morphants were treated with exogenous RA and showed a more severe phenotype than *evi1* knockdown, with a further expanded PST and highly truncated DL. These data indicate that *evi1* acts in parallel to RA or is a downstream RA target. Embryos treated with the Notch inhibitor DAPT exhibited increased MCC numbers in the PST and DE similar to *evi1* morphants, but not the PST domain expansion seen in *evi1* morphants.

Conclusions: Taken together, these studies suggest a model in which *evi1* and RA function early to mediate proximo-distal patterning, and that *evi1* acts later to mediate MCC/transporting-epithelium fate choice, possibly in collaboration with Notch activity. This study has revealed novel developmental roles of *evi1*. Future work will further delineate the relationships between *evi1*, RA, and Notch in nephron formation.

Funding: NIDDK Support

FR-PO603

Formation of a Novel N-Glycosylation Motif in Integrin α3 due to a Rare *ITGA3* Gene Polymorphism Causes Congenital Nephrotic Syndrome and Interstitial Lung Disease Nayia Nicolaou,[1] Coert Margadant,[2] Sietske H. Kevelam,[1] Marc Lilien,[3] Michiel J.S. Oosterveld,[3] Maaike Kreft,[2] Albertien M. Van Eerde,[1] Rolph Pfundt,[4] Paulien A. Terhal,[1] Bert Van der Zwaag,[1] Norman Sachs,[2] Roel Goldschmeding,[5] Nine V. Knoers,[1] Arnoud Sonnenberg,[2] Kirsten Y. Renkema.[1] *[1]Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; [2]Cell Biology, Netherlands Cancer Institute, Amsterdam, Netherlands; [3]Pediatric Nephrology, Wilhelmina Pediatric Hospital, University Medical Center Utrecht, Utrecht, Netherlands; [4]Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [5]Pathology, University Medical Center Utrecht, Utrecht, Netherlands.*

Background: Congenital nephrotic syndrome and interstitial lung disease is a rare disorder, characterized by disrupted basement membrane structures. Causal *ITGA3* gene mutations were recently identified, but the underlying disease mechanism remained poorly understood.

Methods: We describe a patient who presented with glomerulosclerosis, proteinuria and neonatal respiratory distress, who died 7 months after birth due to respiratory insufficiency. A genome-wide screening for structural variations revealed a homozygous region of 19.2 Mb on chromosome 17 that included *ITGA3*. Sequencing of *ITGA3* revealed a homozygous mutation A349S, which was functionally characterized.

Results: Immunostaining in a cell model showed appropriate cell surface localization for the wild type but no surface localization for mutant integrin α3, consistent with the lack of integrin α3 in the kidneys of our patient. The mutation resulted in a *de novo* formation of an N-glycosylation motif. Normally integrin α3 forms heterodimers with integrin β1, though hyperglycosylation disrupted its conformation, preventing it from associating with β1 integrin. Increased ubiquitination indicated that the mutant integrin α3 gets targeted for degradation.

Conclusions: Our findings underscore the role of integrin α3β1 as the main regulator of basement membrane integrity in kidney. We suggest hyperglycosylation of integrin α3 finally resulting in complete lack of expression of α3β1 on the basement membrane, is a new pathogenic mechanism for this severe multiorgan disorder.

Funding: Government Support - Non-U.S.

FR-PO604

Urinary Metabolomic Profiling in Preterm Neonates Mina H. Hanna, Patrick D. Brophy. *Pediatrics, University of Iowa, Iowa City, IA.*

Background: Metabolomics is an emerging field, increasingly being recognized as functionally, the most relevant discipline in personalized health care. Literature regarding the etiology and impact of acute kidney injury (AKI) in the neonatal population is scare. Given the rapid advancement of genomics and proteomics in the identification of renal disease markers, the next logical step is to evaluate the role metabolomics plays as a diagnostic and experimental paradigm in the setting of neonatal AKI. The objective of this metabolomic based strategy pilot project is to define a normative renal metabolomics data set for preterm infants.

Methods: We have prospectively enrolled 3 groups of preterm newborns admitted to the NICU. The 3 groups have been determined by birth weight (BW): 500-1000 grams, 1000-1500 grams, and 1500-2000 grams. Urine samples were collected on days of life 1, 3, 7, 14 and 30. Metabolites present in the urine are evaluated using LC-MS (liquid chromatography mass spectrometry) and GC-MS (gas chromatography mass spectrometry).

Results: 30 preterm infants were enrolled, 10 in each BW group, with mean BWs of: 860 grams, 1275 grams and 1649 grams and mean gestational ages of: 27.0, 29.7 and 31.2 weeks respectively. Infants in the lowest BW group had a significantly longer duration of antibiotics exposure compared to infants in the highest BW group (10.9 ± 7.4 vs 5.2 ± 3.6 days $p<0.05$). Altered excretion of aminoacids suggested an increased aminoaciduria that was more significant in the lower birth weight groups at later time.

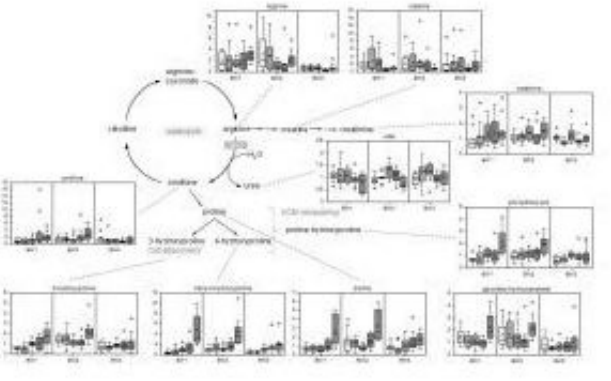


Figure 1: Differences in metabolites associated with Arginine metabolism pathway may indicate decreased renal function in BW1 - and BW2- at the 30 days collection time

Conclusions: Urinary metabolomic profiling provides a robust and readily available approach for defining a normative neonatal data set. We speculate that further metabolite identification and data analysis of our data set is likely to allow delineation of early AKI identification and therefore prompt earlier and possibly preventive intervention.

Funding: Other NIH Support - CTSA Award

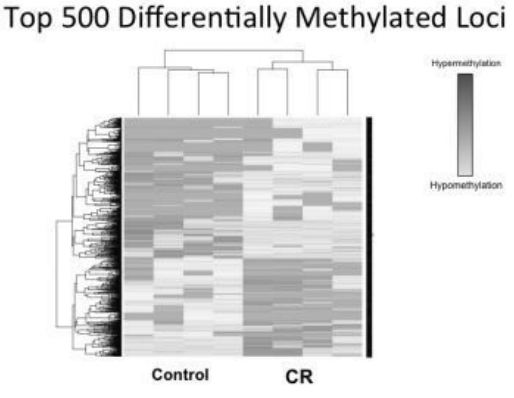
FR-PO605

The Epigenetics of Kidneys Is Altered in Offspring of Maternal Caloric Restriction Howard Slomko,[1] Hye J. Heo,[2] Fabien Delahaye,[2] Yongmei Zhao,[2] Zhongfang Du,[1] Kimberly J. Reidy,[1] Francine Einstein.[2] *[1]Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]OB/GYN, Montefiore Medical Center, Bronx, NY.*

Background: The epigenome serves as the interface between the genome and the environmental exposure. We hypothesize that exposure to a suboptimal intrauterine environment (via maternal calorie restriction) will induce phenotypic and epigenetic renal dysregulation in male offspring that will predispose them to the development of hypertension (HTN) and chronic kidney disease (CKD) later on in life.

Methods: Rat dams were fed 1)standard chow(Con) or 2)50% calorie restricted from gestational day 11 through lactation(CR). Litters were culled(8/lit) and fed standard chow post weaning. Non-invasive tail BP and urine albumin(alb) measured from 2 month(mon) and 6 mon old male pups. Kidneys at 2 mon of age were utilized for glomerular(glom) counts and DNA extraction. Massively parallel sequencing-based HELP assay was used to examine cytosine methylation levels in 2 mon old kidneys.

Results: At 2 mon, while CR and Con have similar bp(145±3/94±3 v 149±2/97±2mmHg,p=NS) and alb/creatinine ratio(0.37±0.07 v 0.34±0.09mg/mg,p=NS), CR already has decreased glom number(20,177±933 v 31,680±1126 /kidney,p<0.01) compared to con. By 6 mon CR developed HTN(161±5/109±4 v 140±5/94±5mmHg,p<0.05) and increased alb/cr ratio(0.69±0.06 v 0.42±0.13mg/mg,p=0.06) compared to con. HELP tagging revealed a trend towards hypomethyation in CR(p<0.0001). A heat map of the top 500 differentially methylated loci in the kidney demonstrate global regional differences.



Conclusions: In utero exposure to CR lead to phenotypic and epigenetic renal dysregulation in young male rats and suggests that the underlying pathophysiology for development of overt CKD and HTN later in life may originate from developmental programming. Validation of loci are ongoing.

Funding: NIDDK Support, Other NIH Support - R01HD063791-03

FR-PO606

Maternal Undernutrition Alters DNA Methylation Profiles in Rat Embryonic Kidney Mariko Hida, Midori Awazu. *Department of Pediatrics, School of Medicine, Keio University, Tokyo, Japan.*

Background: Maternal undernutrition leads to low nephron number. We found that ureteric bud branching, a crucial factor in determining nephron number, is reduced by maternal undernutrition in rats. Reduced nephron number by a nutritional insult is transmitted to the second generation in rats. Furthermore, maternal nutrient restriction alters global methylation in the baboon kidney. We therefore investigated the effect of maternal undernutrition on genome-wide DNA methylation in the rat embryonic kidney.

Methods: The embryonic day 18 kidneys of dams given food ad libitum (CON) and those subjected to 50% food restriction throughout pregnancy (NR) were examined. The DNA methylation landscape around promoter CpG islands was analyzed using methylated DNA immunopreciitation (MeDIP) coupled with microarray (NimbleGen Rat ChIP-chip 385K Promoter array) comparing methylated fractions of CON and NR. MeDIP probe significances were generated using the Kolmogorov–Smirnov test as implemented by NimbleScan. These values were transformed (-log10) to give peak scores, which reflect the probability of methylation at a p-value of less than 0.01. Glomerular number was determined by acid maceration at 3 weeks.

Results: Glomerular number of NR was significantly reduced by 20%. Of 15911 promoter regions included in the array, 7330 regions were hypomethylated and 6310 regions were hypermethylated in NR compared with CON. In NR, nearest genes to methylated regions were categorized as, in descending order of freuency, G-protein coupled receptor signaling pathway, detection of chemical stimulus involved in sensory perception of smell, transcription, transport, apoptosis, development, and others. Hypermethylated genes in NR important in kidney development are, in descending order of peak score, β catenin, heparin-binding EGF-like growth factor,activin A receptor, integrin β4, HGF, BMP4/Smad5, TGF-ß1/Smad4, MMP9, cAMP dependent kinase, integrin linked kinase, PKCδ, and kinases in MAP kinase cascades. Most of these are critical for ureteric branching.

Conclusions: Maternal nutrient restricition changes DNA methylation of genes involved in ureteric branching, which may contribute to reduced nephron number and transgenerational transmission.

Funding: Government Support - Non-U.S.

FR-PO607

A Canadian Study Evaluating Long-Term Impact of a Multifaceted Educational Program on Chronic Kidney Disease Management by Primary Care Physicians Daniel Garceau,[1] Serge Normand,[2] Carl Fournier.[3] *[1]Nephrology, IUCPQ, Quebec, QC, Canada; [2]Office of Evaluation, Faculty of Medicine, University of Montreal, Montreal, QC, Canada; [3]CME Office, Faculty of Medicine, University of Montreal, Montreal, QC, Canada.*

Background: Management of CKD represents a major burden for medical organizations. International societies suggested involvement from PCPs and implementation of large scale teaching programs. However, the long term benefits of such initiatives are unknown. The PREVENIR PROGRAM measured the impact of a large scale educational program intended for 1000 PCPs, from Quebec province, on their clinical behavior, over 33 months.

Methods: Four workshops and a software program on CKD management were developed and updated. From 2009 to 2011, group 1 (500 PCPs) attended 4 workshops and could use the software. Group 2 (500 PCPs) attended 1 to 3 workshops from 2009 to 2010 and did not use the software. Sample of 50 PCPs from both groups and a control group were evaluated before (T0), during (T1) and after (T2) this program using 5 instruments: knowledge questionnaire, interest chart, practice and PCPs profile and structured clinical vignettes. These vignettes measured PCPs skills and reasoning with simulated cases of different complexity. Containing 6-9 items each, they were administered by pairs at T0, T1 and T2.

Results: Group 1 and 2 significant gains in knowledge and clinical skills occurred at T1, and then stabilized without any significant change between T1 and T2. No significant change was observed in CONTROL group.

GLOBAL SCORE FOR KNOWLEDGE AND CLINICAL BEHAVIOR

KNOWLEDGE %				**BEHAVIOR %**		
	EVALUATION PERIOD					
	T0	T1	T2	T0	T1	T2
GROUP	JAN-FEB 2009	APR-SEP 2010	JUL-DEC 2011	JAN-FEB 2009	APR-SEP 2010	JUL-DEC 2011
1	28.4	60.9*	65.2*	34.1	46.1*	47.7*
2	25.6	41.2*	47.7*	32.3	39.8*	42.4*
CONTROL	29.0	32.9	38.0	33.6	33.7	37.9

*p <0.05 vs T0

Conclusions: Our study showed long term benefits on knowledge and clinical behavior of PCPs for CKD management. Of the 8000 PCPs serving a population of 8 million, 1000 were exposed to our program (12.5%). The cost was $250 cad per workshop/physician and $250 cad/physician for the software program. Funding to replicate this program could be provided by governmental or corporate partners.

Funding: Pharmaceutical Company Support - Amgen Canada; BMS Canada; Sanofi-Aventis Canada

FR-PO608

Eleven Key Areas of Renal Nurse Responsibility: The Foundations of Quality Patient Dialysis Outcomes Ali Mohammed Allehbi,[1] Donavilla Paguia,[2] Archie Dumdum Bunani.[3] *[1]Medicine - Nephrology, DaVita Lehbi Care - Saudi Arabia, Riyadh, Central Province, Saudi Arabia; [2]Nursing, DaVita Lehbi Care - Saudi Arabia, Riyadh, Central Province, Saudi Arabia; [3]Clinical Services, DaVita Lehbi Care, Riyadh, Central Province, Saudi Arabia.*

Background: Renal nurses develop their expertise over time and in the exercise of their professional skills deliver the essence of safe, competent, and compassionate care. The knowledge, attitude and skills of a nurse develop progressively where complexities of clinical procedures and experiences are intertwined. This study identifies whether Quality Patient Dialysis Outcomes (QPDO) were directly affected by eleven key areas of nurse responsibility used when evaluating renal staff competency (SC).

Methods: 59 Staff Nurses were appraised evaluating SC while 525 hemodialysis patients were evaluated using the QPDO parameters. Univariate linear regression and Pearson rho moment correlation were used to build relationships.

Results: Data indicated both increase and decrease trends in relation to staff competency. Competencies related to Health Education (↑172.6), Communication (↑147.5), Records Management (↑141.6), Safe and Quality Nursing Care (↑135.0), and Management of Resources (↑133.5) demonstrated increase trends. Competencies related to Research (↑-35.2), Quality Improvement (↑-12.3), and Legal Responsibility (↑-6.68) were relatively decreased as the period of competency evaluation progressed. It was notable that QPDO related to Kt/V, Albumin, Hemoglobin, and Hematocrit Levels were directly proportional to increasing extent of SC ρ=(+0.61) while calcium and phosphorus levels were directly associated to areas where staff were demonstrated an decreasing trend ρ=(+0.66).

Conclusions: The eleven key areas of responsibility used to measure SC in a periodic evaluation demonstrated a strong correlation to the increasing extent of QPDO. Additionally, as the nurses progressed to becoming expert a direct correlation to the QPDO was notable. The study became the foundation for staff training and developing a competency appraisal framework in renal nursing practice thereby promoting quality assurance procedures while attaining QPDO.

Funding: Private Foundation Support

FR-PO609

Variation in the Confidence and Knowledge of General Physicians Managing Acute Kidney Injury: A Need for Better Education Gang Xu,[1] Christopher Thompson,[1] James Trew,[1] Joanne H. Kirtley,[1] Richard J. Baines,[1] Rachel Westacott,[1] Nicholas M. Selby,[2] Sue Carr.[1] *[1]John Walls Renal Unit, Leicester General Hospital, Leicester, Leicestershire, United Kingdom; [2]Renal Medicine, Royal Derby Hospital, Leicester, Leicestershire, United Kingdom.*

Background: Acute Kidney Injury(AKI) presents a global challenge which remains significantly under recognized and yet has a significant effect on mortality and morbidity within the population. In the UK the majority of patients with AKI are looked after by non-nephrologists. Therefore educational initiatives, targeted at general internists are a potential tool to help improve the management and outcomes of patients with AKI. The aim fo the study was to establish the baseline knowledge and confidence of physicians admitting patients with AKI, at University Hospitals of Leicester and Royal Derby Hospital (serving a population of 2 million).

Methods: Physicians and trainees working in the hospitals were surveyed in November 2011. The survey included 15 Multiple Choice Questions designed to test knowledge on AKI.

Results: 342 doctors were surveyed; 39% Interns (pre registration), 42% Residents (post registration), 15% Fellows (Specialised trainee), 4% Consultants. Consultants answered 55% of MCQs correctly, Fellows 53%, Residents 46%, Interns 38%. Fellows felt most confident about diagnosing AKI (92%); however Consultants and Residents scored better on MCQs designed to test knowledge on diagnosis despite being less confident. Only 37% of Interns were confident at starting treatment for AKI despite having knowledge on the subject. 56% had used books/journals to learn about AKI, 56% had attended courses or attended lectures, 69% had received clinical teaching, 37% had used Internet resources, and 18% had used eLearning resources (multiple answers allowed).

Conclusions: Despite being a common presentation many physicians seem to lack both confidence and knowledge on AKI. Perceived confidence levels were higher in all grades of hospital doctor than demonstrated knowledge. This reaffirms the need for educational intervention if patient care and outcomes from AKI are to be improved; electronic learning resources appear underused compared to traditional teaching resources.

Funding: Government Support - Non-U.S.

FR-PO610

Initial Responses to Antihypertensive Therapy in Essential Pediatric Hypertension Stephen D. Cha, Hiren P. Patel, John D. Mahan. *Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH.*

Background: Essential hypertension (HTN) is becoming increasingly prevalent in pediatrics. To develop an educational program for primary care practitioners (PCP) regarding management of pediatric hypertension, we sought to define the pattern of treatments initiated by PCP and the short-term effectiveness of such interventions.

Objective: To assess the choice of initial medication class to treat pediatric HTN and its short term efficacy at 6 months.

Methods: We used our electronic medical records system to look at all referrals to outpatient pediatric nephrology for hypertension/elevated blood pressure (BP) from 1/1/11 through 12/31/11.

Results: Of the 434 patients who were referred for HTN or elevated BP, 35 were started or continued on a hypertensive medication (Rx) by PCP and 82 by pediatric nephrology.

Table 1

	Primary Care Practitioners	Pediatric Nephrology	Total
ACEi/ARB	23	42	65
CCB	4	38	42
Other	8	2	10
Total Started	35	82	117

The majority of patients started on an ACE inhibitor/angiotensin receptor blocker (ACEi/ARB) or diuretics by PCP were continued on it by pediatric nephrology (84%). Of the 117, 98 were followed in nephrology clinic. Only 19 (20%) were at the desired BP goal by 6 months. Of the 65 on an ACEi/ARB, 13 (20%) reached goal BP. Of the 42 on a calcium channel blocker (CCB), 6 (14%) were at goal BP. Ten patients started on other agents (diuretics, beta blockers or clonidine) without improvement. Of the 35 patients with Rx initiated by PCP, none had Rx changes due to side effects.

Conclusions: 1. Only a minority of patients had BP control with an ACEi/ARB or CCB by 6 months. 2. There are opportunities to address gaps in care for these patients by both PCP and pediatric nephrologists reflected by the suboptimal BP control in the short-term period. 3. Effective pediatric HTN education for PCP should address methods to initiate antihypertensive therapy and empower PCP to help such children reach desired BP goals.

FR-PO611

Pediatric Hypertension: Defining the Educational Needs of Primary Care Pediatricians Stephen D. Cha,[1] Deena J. Chisolm,[2] Hiren P. Patel,[1] John D. Mahan.[1] *[1]Pediatric Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Research Institute, Nationwide Children's Hospital, Columbus, OH.*

Background: Essential hypertension (HTN) is becoming increasingly prevalent in pediatrics, affecting 3-5% of the general pediatric population. Suspected pediatric HTN is evaluated with varying degrees of accuracy by primary care pediatricians (PCP). In an effort to improve recognition, evaluation and treatment of HTN among PCP, the comfort level and educational gaps for PCP need to be understood and addressed.

Objective: To identify the educational needs and to develop effective teaching methods to educate and influence practice behaviors of PCP regarding appropriate recognition, diagnostic evaluation, and therapeutic intervention in pediatric essential HTN.

Methods: We conducted 4 separate focus group (FG) discussions with pediatric residents, Adolescent Medicine physicians and 2 outpatient pediatric groups associated with Nationwide Children's Hospital in Columbus, OH. Six to 9 participants in each group discussed approaches to 3 common pediatric HTN scenarios. Sessions were recorded and transcribed for review. Themes were elucidated amongst the focus groups by 4 reviewers.

Results: Five major themes emerged from the focus group sessions (utilization of resources to obtain BP, BP measurement method, co-morbidities, barriers to care, and experience level of training) and 6 minor themes also emerged (differences in BP measurement, accuracy of BP, recognition, practice pattern, education, and differences in level of training). Most participants in each FG wanted further education on pediatric HTN but different groups defined varied needs and identified multiple preferences for how to learn this material.

Conclusions: These results support the need to develop programs to increase PCP knowledge of specific aspects of pediatric HTN. Based on the varied stated preferences of these PCP, education modules and methods will need to employ multiple presentation methods (e-learning, small group sessions, self-study, large group presentations) to be useful and ultimately improve outcomes in pediatric HTN.

FR-PO612

Great Job! Is Not Enough: Faculty Development to Improve Quality of Comments on Evaluations of Fellows Mireille El Ters, Suzanne M. Norby. *Nephrology & Hypertension, Mayo Clinic, Rochester, MN.*

Background: With the upcoming implementation of the ACGME Next Accreditation System, it will be essential for faculty members (FM) to provide meaningful information about trainee performance in order to determine whether milestones are met. Behavior-specific comments (BSC), rather than nonspecific comments (NSC) such as "great job" and "excellent", will be necessary. To improve quality of comments on evaluations of fellows, a live faculty development activity was presented during divisional Grand Rounds, consisting of an interactive didactic presentation along with distribution of copies of FM's own written comments about fellows for critical review during the session.

Methods: To determine whether FM who attended increased % BSC, written summative electronic evaluations of fellows were retrospectively reviewed, and comments were categorized as BSC or NSC. FM not attending the session served as controls. Median % BSC during the 6 months before and 6 months after the presentation were compared using the Wilcoxon rank sums test.

Results: 10 FM attending the session and 9 FM controls completed evaluations of fellows during both pre- and post- time periods. 3 FM (2 attendees, 1 control) with 100% BSC at baseline were excluded, leaving 8 FM in each group. During the pre-activity time period, median % BSC was 0.57 (range 0.10–0.88) for attendees and 0.59 (range 0.07–0.92) for controls (p=0.96). During the post-activity period, median % BSC for attendees was 0.71 (range 0.12–1.0) and for controls was 0.61 (range 0–1.0; p=0.46). Using a paired analysis, attendees demonstrated a 12.1% mean increase in BSC whereas BSC by controls fell by 2.4%. While numerical differences were seen, these changes were not statistically significant.

Conclusions: This faculty development activity was associated with an absolute increase in % BSC written by FM on summative electronic evaluations of nephrology fellows in a single training program. While sample size was small and the improvement did not achieve statistical significance, the findings suggest that a simple targeted faculty development activity may effect behavior change and improve the quality of comments written by FM on summative evaluations.

FR-PO613

Palliative Care Experience of US Adult Nephrology Fellows Hitesh H. Shah, Divya Monga, April Caperna, Lourdes G. Bahamonde, Tanveer Mir, Kenar D. Jhaveri. *Internal Medicine, North Shore LIJ Health System, Hofstra NSLIJ School of Medicine, Great Neck, NY.*

Background: Palliative care (PC) experience and knowledge of current US adult nephrology fellows is not known. It is also unknown if nephrology fellows undergo formal training in PC medicine during fellowship.

Methods: An anonymous online survey was created and subsequently distributed to nephrology fellows via nephrology training program directors.

Results: 65% of the respondents were international medical graduates (IMGs). 74% of fellows had no PC rotation during their medical school. 50% of the respondents had a formal PC elective during residency. Although 91% of the fellows have a PC division in their institution, only 49% had formal didactic PC experience. 85% of the respondent's fellowship program did not offer formal clinical training in PC during their fellowship.

While over 90% of fellows felt most comfortable with either writing dialysis orders or seeing an ICU consult, only 36% felt most comfortable not offering dialysis to a patient in the ICU with multi-organ failure. On a 5-point scale where 1 being least comfortable and 5 being most comfortable, majority of the fellows felt less comfortable in the following areas: not offering renal replacement therapy (RRT), withdrawing RRT, managing pain, treating depression and having end of life discussions. Nearly one out of five fellows surveyed felt obligated to offer RRT to every patient regardless of benefit. Female fellows felt less comfortable not offering or withdrawing RRT as compared to male fellows. US medical graduates felt more comfortable compared to IMGs when not offering or withdrawing RRT and having end of life discussions. 69% felt that a PC rotation during the course of fellowship would be helpful. Majority of the respondents felt that they would benefit from learning "how not to offer aggressive medical management" and "how to discuss withdrawal of life supporting measures".

Conclusions: Education and clinical training in PC medicine is not well experienced by US adult nephrology fellows. To enhance knowledge and clinical experience in PC medicine, a formal rotation during fellowship should be highly considered by nephrology training community.

FR-PO614

Palliative Care Experience of US Internal Medicine Subspecialty Fellows: A Comparative Study Divya Monga, April Caperna, Hitesh H. Shah, Tanveer Mir, Lourdes G. Bahamonde, Kenar D. Jhaveri. *Internal Medicine, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

Background: Palliative care (PC) experience of current US Internal Medicine (IM) subspecialty fellows is unknown. It is unknown if IM subspecialty fellowship programs in US offer formal training in PC medicine during fellowship.

Methods: An anonymous online survey was created and subsequently distributed to IM subspecialty fellows via fellowship training program directors. We compared the PC experience of nephrology fellows to pulmonary/critical care (PCC), hematology/oncology, cardiology and gastroenterology (GI) fellows in training.

Results: Preliminary results showed 26% respondents were from nephrology, 12% from cardiology, 14% from hematology/oncology, 31% from PCC and 17% from GI. 69% of nephrology fellows felt that a formal rotation in PC during fellowship would be very useful compared to 47% of PCC, 32% of cardiology, and 31% of GI fellows. While most IM fellows felt that a PC rotation would help in how not to offer aggressive medical management, majority of PCC fellows felt it would help in learning how to run family meetings. The table below compares the PC experience of IM fellows.

Palliative Care Experience	Nephrology (%)	Cardiology(%)	Hematology/ Oncology (%)	PCC(%)	GI(%)
During medical school	26	19	19	14	25
During medical residency	50	53	56	46	49
Didactics sessions during fellowship	49	32	90	60	20
Formal clinical training during fellowship	14	3	53	24	5
>10 end of life discussions during fellowship	33	32	81	61	46

Comparative Palliative Care Experience of IM fellows

Conclusions: Significant higher percentage of nephrology fellowship programs does not offer a formal clinical training in PC as compared to hematology/oncology and pulmonary/critical fellowships. Majority of the nephrology fellows felt the need of having a formal PC rotation during their fellowship as compared to other IM fellows. More efforts are needed by training community to improve the PC experience of IM fellows.

FR-PO615

Nephrology-Rheumatology Debate Session, an Innovative Educational Experience Kenar D. Jhaveri, Hitesh H. Shah, Divya Monga, Ashish Kataria, Joseph Mattana. *Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

Background: Innovative teaching methods can create an environment that might promote interest and motivation to pursue careers in nephrology. However, such teaching methods might also be useful in maintaining and enhancing both satisfaction and enthusiasm for nephrology amongst fellows during training. Conducting debates created around clinical questions is a learner-centered approach that might be able to accomplish this.

Methods: For the past two years, we have conducted annual combined nephrology-rheumatology debate sessions. Topics were treatment for ANCA vasculitis(AAV) and lupus nephritis. A month prior, questions for the topic to be debated were sent out to all nephrology and rheumatology fellows via e-mail. Fellows were equally divided into two groups mixed from both divisions. On the day of the debate, only fellows were allowed to speak and present their side of the debate (50 minutes). The final 10 minutes was devoted to comments from faculty members in the audience. To assess the value of this tool, an anonymous on-line survey was conducted. Using a 5 point Likert scale, the fellows were asked to assess the impact of these sessions on self study, looking into primary data, usefulness in day to day practice.

Results: 46.2% were first year fellows, 38.5% were second year fellows and 7.7% were third year fellows. The fellows chose on average a score of 4 out of 5 (5 being extremely helpful) when asked about the debate session facilitating their personally looking up primary data on the topic debated, and helping them to better care for their patients with lupus nephritis and AAV. A lower score of 2 out of 5 was reported for the value of having a faculty member assign each member of the team to do a specific topic and allowing for more faculty participation during the debate. Other variables received scores of 3 and above. All fellows reported enjoying the sessions and would want to do it every year.

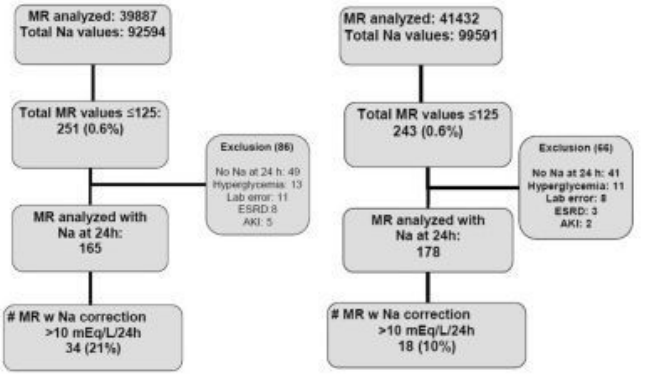
Conclusions: Novel ways of providing nephrology education using methods such as learner-centered debates described above may allow our fellows to learn more effectively and help keep them satisfied and engaged during training.

FR-PO616

Severe Hyponatremia Is Frequently Corrected Too Quickly: A Quality Improvement Project Marie A. Sosa,[1] Jennifer Russo,[2] Francis M. Wanjau,[2] Jeffrey S. Stoff.[1] *[1]Department of Medicine, Division of Nephrology, University of Massachusetts Medical School, Worcester, MA; [2]Department of Medicine, University of Massachusetts Medical School, Worcester, MA.*

Background: Hyponatremia (Na <135 mEq/L) is the most common electrolyte disorder encounter in clinical practice, is considered severe when the Na is below 125 mEq/L. Community-acquired hyponatremia has been described in 38% of hospitalizations and hospital-acquired in 38%. Osmotic demyelination syndrome (ODS) are the neurologic manifestations associated with rapid correction of hyponatremia. ODS can be prevented with Na correction rate of <10mEq/l in 24 h. The incidence of rapid Na correction of hyponatremia and ODS are not known. A quality improvement (QI) project was conducted with the goal of reducing the incidence of rapid Na correction.

Methods: Retrospective review of records of hospitalized patients with Na ≤125 over 4 months in 2011. The rate of correction of serum Na during the first 24h was determined. A second retrospective review of same data was obtained post intervention in 2012.



A root cause analysis identified lack of knowledge of practitioners as one of the causes for rapid correction. QI Intervention: 1)Residents Lecture 2) Review article on hyponatremia 3) Education maintained by small group sessions and the "Na Question of the week". Other data: 47% of the cases were managed by PCP/hospitalist and 24% by ICU staff. Renal was consulted in <1% of the cases. The average time to re-check the initial low Na was 10 h.

Results: In 2011, 21% of the cases of severe hyponatremia were corrected >10mEq/l 24h; the number was decreased to 10% in 2012 after conducting a QI project. Chi-square test 7.33, p value <0.007.

Conclusions: Severe hyponatremia is frequently corrected too quickly. A QI Intervention can be effective in decreasing the number of cases of rapid Na correction.

FR-PO617

Does Lack of Nephrology Fellow Involvement in Patient Education Affect Utilization of Peritoneal Dialysis in the United States? Nand K. Wadhwa,[1] Catherine Messina,[2] Nasser M. Hebah.[3] *[1]Division of Nephrology, Stony Brook School of Medicine, Stony Brook, NY; [2]Preventive Medicine, Stony Brook School of Medicine, Stony Brook, NY; [3]Corporate Home Dialysis, Dialysis Clinics Inc., NY.*

Background: Peritoneal dialysis (PD) use has declined to 7.2% of ESRD patients from a 1984 peak of 15%. The most common barrier to PD is patient preference. However, PD underuse may also reflect a lack of patient education about PD. We examined whether education of nephrology fellows and their involvement in patient education about PD contributed to its' underuse in the US.

Methods: Self-report surveys were administered electronically to directors of US nephrology fellowship programs and medical directors of Dialysis Clinics, Inc (DCI) dialysis units, Oct 2010-Mar 2011. Program directors were identified from the American Society of Nephrology list-serve; medical directors' were identified from the DCI list-serve.

Results: 55% (n=78) of program directors and 21% (n=38) of medical directors responded. Most nephrology programs provide fellow training in PD and HD; PD training was only 20% of total training vs. 80% for HD. Most program and medical directors provided pre-ESRD patient education (80% and 76%) with 98% providing education on treatment options. However, half of medical directors reported that ≤50% of patients attended. 89% of program directors vs. 56% of medical directors reported that ≥65% of patients choose HD (vs. PD) after education on treatment options. Only 33% of program directors reported that nephrology fellows participate in pre-ESRD patient education. 78% of program directors believe that lack of patient education on PD limits utilization, while <50% of medical directors believe this. 88% of program directors believe that limited physician training in PD limits PD use vs. 24% of medical directors.

Conclusions: Inadequate nephrology fellows' PD training and limited involvement in ESRD patient education may contribute to PD underuse in the US. Low patient participation in education programs and differing beliefs among program and medical directors regarding patient and physician education suggest that barriers to PD use may differ by dialysis setting.

FR-PO618

Central Venous Line Placement Competency among Incoming Nephrology Fellows Eric L. Wallace, Kelli R. King-Morris, Jamie P. Dwyer, Julia Lewis. *Nephrology, Vanderbilt University, Nashville, TN.*

Background: In July 2009, the ACGME removed the technical proficiency requirement for Internal Medicine residents to perform central venous line (CVL) placement. Nephrology fellows starting in 2012 are the first who at no time during residency have been required to place CVL to graduate. Little is known about the effect of this policy change on the experience of incoming fellows to place CVL, or how these changes will affect procedural training in Nephrology fellowships.

Methods: Nephrology Fellowship Program Directors and/or Coordinators were sent a survey on CVL placement to distribute to the 2012 Fellows. Fellows answered questions pertaining to their experience with CVL placement, comfort level on the procedure, and ultrasound (US) skills obtained during residency. The survey, data collection, and analysis were performed using REDCap (Research Electronic Data Capture) a secure, web-based application designed to support data capture for research studies.

Results: Of 360 incoming fellows surveyed, 120 (33%) responded. Of respondents, 27% at PGY3 or PGY4 level placed less than 10 CVL. 48% of PGY3 have never placed a large-bore CVL, and 75% of PGY3 have never placed an unsupervised large-bore CVL. 11% of PGY3 have never placed a femoral CVL, and 66% of PGY3 placed <25% of CVL as femoral. 14% of PGY3, 36% of PGY4, and 50% of PGY5 self-identify as novice in US usage. Despite overall low numbers of CVL placed, 90% of respondents reported being somewhat or very comfortable placing CVL.

Conclusions: Faculty supervision of large-bore catheters placement is still required given that 75% of PGY3 fellows have not placed unsupervised large-bore CVL. The femoral CVL may be where most attention is needed as this necessary nephrology procedure is not being done by residents. Decreasing US skill as PGY level increases is likely due to changes in standard of care in placement of CVL. Despite the policy change, residents still place CVL, and perceived comfort level is high, despite low numbers of CVL placed. Perceived comfort level may not correlate with technical proficiency given the low numbers of CVL placed, however; further study is warranted.

FR-PO619

Insertion of Temporary Hemodialysis Catheters by Nephrology Fellows in Canada: A Survey of Training and Current Practice Edward G. Clark,[1] Michael Schachter,[2] Cedric A.W. Edwards.[3] *[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]The British Columbia Renal Agency, Vancouver, BC, Canada; [3]Division of Nephrology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.*

Background: Little is known about the extent to which evidence-based practices regarding central venous catheter insertion have been taught to, and adopted by, nephrology trainees.

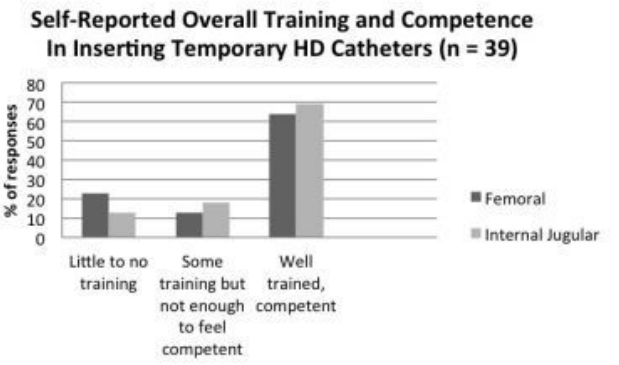
Methods: A web-based survey of fellows enrolled in the 12 English-language adult-nephrology training-programs in Canada assessed the estimated number of temporary hemodialysis (HD) catheters inserted in the prior 6 months of training, adherence to infection control procedures, use of real-time ultrasound, prior training and self-perceived competency in inserting temporary HD catheters.

Results: Completed surveys were received from 68% (39 of 57) of fellowship trainees in the target sample. Eighty-two percent (32 of 39) reported having inserted a temporary HD catheter in the prior 6 months of training.

Self-Reported Adherence To Infection Control Procedures and the Use of Real-Time Ultrasound (n=34)

	RESPONSES, % (n)			
USE OF:	**Always**	**Most of the time**	**Occasionally**	**Never**
Sterile gloves & gown	100 (34)	0	0	0
Facemask	88 (30)	12 (4)	0	0
Cap/hair-covering	62 (21)	18 (6)	21 (7)	0
Head-to-toe sterile drape	38 (13)	27 (9)	15 (5)	21 (7)
Ultrasound at femoral site*	60 (18)	23 (7)	10 (3)	7 (2)
Ultrasound at IJ site§	82 (27)	12 (4)	3 (1)	3 (1)

*n=30, §n=33



Conclusions: Within a representative sample of nephrology fellows in Canada, a majority report having adopted most of the evidence-based practices for temporary HD catheter insertion that were inquired about. Nonetheless, our results highlight many areas for improvement. It is concerning that a significant number of respondents indicated less than adequate training to feel competent in placing temporary HD lines. This finding merits further study.

Funding: Clinical Revenue Support

FR-PO620

Nephrology Journals of the Future: A Quantitative Study Vinay Nair,[1] Shahab Khan,[2] Kenar D. Jhaveri.[3] *[1]Internal Medicine, Mount Sinai Medical Center, New York, NY; [2]American University of Antigua Medical School, Coolidge, Antigua and Barbuda; [3]Nephrology, Hofstra North Shore LIJ School of Medicine, Great Neck, NY.*

Background: Journals are becoming more interactive creating websites that allow public commentary, blogs and sharing features with use of social media. This interaction can encourage all trainees and physicians to discuss important publications in a non-threatening manner.

Methods: We reviewed all journals with an impact factor ≥ 4 using the 2010 Journal Citation Reports Science Edition accessed March 20th 2012. All internal medicine(IM) and subspecialty journals were selected. Each journals website was accessed and reviewed for a blog, Twitter ®, Facebook® or email sharing, and a comment section. If a link to a blog was not found we then performed a google search with the title of the journal and the word blog. Comment section was defined as the ability for readers to publish comments on an article directly on the journals website. We compared the use of blogs, comment section, and social media for sharing between nephrology journals and other internal medicine journals.

Results: 199 journals with an impact factor ≥ 4 were Internal medicine or subspecialty journals. The number of journals in each field of IM with the use of blogs, public commentary and sharing features are seen in table 1.

Journals breakdown by Subspecialty

Specialty	# Journals >=impact factor 4.0	Blogs(%)	Commenting(%)	Sharing(%)
Int Medicine	30	43.3	30	96.7
Hem/Onc	47	2	4.2	91.4
Cards	28	3.5	21.4	96.4
Rheum	26	3.8	7.6	96
Endo	20	0	0	100
ID	18	11.1	11.1	94.4
GI	14	14.2	0	85.7
Pulm	9	0	11	100
Renal	7	14	0	100

No nephrology journal permitted public commentary. One (14%) nephrology journal had a blog, compared to 43.3% of general internal medicine journals. All nephrology journals supported sharing with social media or email.

Conclusions: Adopting social media is a slow process in nephrology academia. Compared to other subspecialties in medicine and general medicine, top nephrology journals had a 100% sharing feature but were slower in adopting blogs and commentary sections. We need our medical journals to be more interactive and allow more features to integrate the current generation of readers.

FR-PO621

Kidney Disease Screening and Awareness Program as a Potential Workforce Model: An Experience from Toronto Cheryl Hao Cui,[1] Li-Li Hsiao.[2] *[1]University of Toronto, Toronto, ON, Canada; [2]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Kidney Disease Screening and Awareness Program (KDSAP) provides free kidney disease screenings and health education for local communities with a specific focus on low-income and ethic minority populations. Such health screenings are run by undergraduate student volunteers, and hence offer students an unparalleled hands-on clinical experience and exposure to nephrology whilecultivating student leadership. With the success of the original KDSAP chapter at Harvard College, we propose that current KDSAP infrastructure can serve as a student organization model and magnify its impact in the community and student education.

Methods: The establishment of the new KDSAP chapters is lead by previous student volunteers who found the KDSAP experience to be rewarding and recognized the impact of the KDSAP in the community. These student leaders took the initiatives to apply for student organization sponsorships and to recruit student volunteers. Because of the well-recognized value of KDSAP in student development, KDSAP received enormous support and initial seeding funds from the school faculty.

Results: We established the first Canadian KDSAP chapter at the University of Toronto, which has demonstrated the scalability of the model. KDSAP Toronto Chapter launched its first community health screening at February 2012. We have successfully recruited 46 student volunteers from various departments. All student volunteers received formal training and participated in the Universal Precaution and Professionalism training session hosted by medical professionals. We have screened 100 participants in one day at the Chinatown Center and received overwhelmingly positive feedback from the participants and volunteers.

Conclusions: Overall, we showed that KDSAP is a reproducible program strategically targeting the undergraduate students and we have successfully validated the scalability of the KDSAP model across the country and internationally. The rapid and organic growth of the KDSAP is driven by the educational benefits it provides for the student volunteers and the remarkable impact it has on the communities.

Funding: Private Foundation Support

FR-PO622

Factors in Career Choice among Contemporary US Nephrologists

Gearoid M. McMahon, Lynette E. Thomas, J. Kevin Tucker, Julie Lin. *Nephrology, Brigham and Women's Hospital, Boston, MA.*

Background: There is a projected future shortage of kidney specialists, and retention of trainees in academic and non-academic nephrology is important. Determining factors that result in choosing a nephrology career could inform future strategies to attract nephrology fellows.

Methods: An anonymous, internet-based survey was sent to members of the American Society of Nephrology (ASN) in June 2009. Respondents answered questions about demographics, training background, and career choices.

Results: Of the 3399 members, 913 (23%) returned the survey. Mean age was 51.1±10.5 years, and 46.1% were academic nephrologists. Interest in nephrology began early in training for most with the intellectual aspects of nephrology, early mentoring and participation in nephrology electives named as the most common reasons in choosing nephrology. Academic nephrologists were more likely to have participated in research in medical school, have a Master's Degree or PhD and successfully obtained research funding during training. Academic debt was higher among non-academic nephrologists. Respondents were generally happy with their career choices. Research opportunities and intellectual stimulation were the main factors for academic nephrologists when choosing their first post-fellowship positions, while geographic location and work-life balance were foremost for non-academic nephrologists. One important limitation is that senior academic nephrologists were disproportionately represented among respondents.

Conclusions: These findings highlight the importance of exposing medical students and residents to nephrology early in their careers through involvement in research, electives, and positive mentoring. Further work is needed to develop and implement effective strategies, including increased early exposure to pre-clinical, clinical, and research medical education, in order to attract future nephrology trainees.

Funding: Private Foundation Support

FR-PO623

Kidney Disease Screening and Awareness Program: Health Education and the Facilitation of Student Career Development

Rena Mei,[1] Li-Li Hsiao.[1,2] *[1]Harvard College, Cambridge, MA; [2]Rena Division, Brigham and Women's Hospital, Harvard Medical School, MA.*

Background: Although Chronic kdiney disease (CKD) is one of the leading causes of death nationwide, there is low patient awareness of CKD along with decreasing interest in the field of nephrology. Initiatives involving renal health education may lead to earlier detection and lower rates of CKD. Health education also exposes students to nephrology and helps raise interest in this field.

Methods: Kidney Disease Screening and Awareness Program (KDSAP) is a kidney health initiative that holds monthly health screenings in underserved communities. KDSAP includes a Health Education component that targets both students and the community. A nephrologist gives a health talk at a partner site prior to a screening; patients then receive individualized advice during the screening. KDSAP also encourages career development of its members, which consists of high school, college and medical students. All new volunteers are trained to act professionally and safely in a clinical setting through the Universal Precaution and Professionalism Session. Blood Pressure Workshops train and certify students to measure blood pressure, a skill that they can then apply at screenings. The "Meet the Patient" and "Meet the Professor" series are campus events that give students insight into the life of a patient and a professional in the medical field specifically in Nephrology practice.

Results: Since its establishment in 2008, KDSAP has successfully trained 109 students for UPP and 99 students for measuring blood pressure. Health talks have been given in 22 communities, educating a total of 5000 patients. Of the 29 KDSAP alumni who were followed, 1 decided to become a nephrologist, 10 enrolled in medical school, 7 joined research groups focused on kidney disease, 4 work at hospitals/medical centers, and 1 works in global health.

Conclusions: KDSAP's Health Education program raises awareness about kidney health in high-risk communities and facilitates student career development by giving students direct exposure to clinical settings and patient interaction. KDSAP hopes to expand this component by training students to lead talks in schools and communities.

FR-PO624

Effectiveness of Multidisciplinary Group Patient Education Programs for CKD Patients: 5 Years Practices and Outcomes

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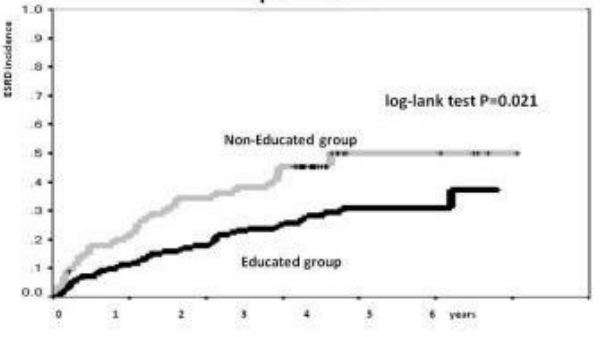
Background: Patients' education is an integral part of managing chronic diseases. However there are a few reports for CKD patients, especially for specific practices and evaluations. We already reported our pilot study on ASN 2007, which our group multidisciplinary education is effective for preserving renal functions in short term. We continued these programs for 5 years, and assessed long term outcomes.

Methods: We organized CKD education class which was designed under specific adult educational methods. This education program is focused on the importance of controlling blood pressure. Classes were held on total 3 hours and instructed by multidisciplinary educators. To evaluate effectiveness of the program we assessed knowledge, behavior and clinical parameters. Incidence of ESRD, which determined by initiation of dialysis, was evaluated for primary end point.

Results: Total 765 individual CKD patients were followed up in our clinic, and 265 patients were (34.6%) participated in this education program. On educated group, mean age 62.4 y.o., 54.5% (N=128) were male, their baseline GFR were 36.2±12.3 mg/dl/min. Their average score of knowledge about CKD or importance of blood pressure was 60% before class, this score significantly improved to 82% after class. After 1 or 5 years later, this score still maintained 75 or 73%. Also, the number of patients who monitored blood pressure themselves, who achieved below 130/80 mmHg were larger than non educated group. On the primary end point, incidence of ESRD patients was significantly lower on educated group than non-educated group. (Figure 1).

Conclusions: Multidisciplinary educational interventions for the CKD patients are effective in long term, and contribute to decrease ESRD patients.

Figure 1. Cumulative incidence rate of ESRD
Kaplan-Meier



FR-PO625

Dietary Sodium Knowledge in Patients with Chronic Kidney Disease

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Background: Adherence to sodium restriction is important for patients with chronic kidney disease (CKD). Little is known about patient knowledge necessary to support adherence.

Methods: One hundred fifty-five adult patients with CKD Stages 1-5, seen in nephrology clinic, were enrolled from April-October 2010. Patients were asked if they received counseling to decrease dietary sodium and if they knew their recommended daily sodium intake limit. They also answered three questions: Which food item has the most sodium? (1 apple, 2 ounces of lunchmeat, 1 banana, 1 baked potato), What is the best way to cut back on sodium? (trim visible fat from meats, eat more fresh vegetables instead of canned, substitute skim milk for whole milk, drink more fresh orange juice instead of canned), and Where does most of the sodium that people eat come from? (salt added at the table, processed/packaged foods, soda pop, plain frozen vegetables). We generated mean (SD) and percentages to describe data. Associations between patient characteristics and sodium knowledge were analyzed using logistic regression.

Results: The mean (SD) age was 56.6 (15.0) years, 46% were female, 77% white, and 79% had stages CKD 3-5. 86% received counseling to limit sodium, and 97% reported making changes as a result. 84% identified the highest sodium food, 83% identified fresh vegetables as a way to cut down on sodium, and 19% believed most sodium comes from table salt. 53% of patients said they did not have a daily sodium intake limit. In analysis adjusted for age, sex, race, health literacy, education, stage of CKD, and kidney education,

women and those of non-white race had a lower odds (OR 0.43 [CI 0.02,0.93; p=0.03] vs. men, and OR 0.35 [0.15,0.81; p=0.01] vs. white, respectively) of answering all sodium knowledge questions correctly.

Conclusions: Opportunity exists to support patient adherence by developing education interventions. Research is needed to determine why sodium knowledge is lower in women and patients of non-white race.

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FR-PO626

Transtheoretical Model of Behavior Change: Application of a Nutrition Education Program to Control Hyperphosphatemia in Hemodialysis Patients Carmen B. Tzanno-martins,[1] Bárbara Margareth Menardi Biavo,[2] Jacqueline Santos,[2] Camila Machado de Barros,[2] Elzo R. Junior,[1] Paul Clesca Troconis.[3] *[1]CINE-HDC-RENALCLASS Group, São Paulo, Brazil; [2]CINE-HDC-RENALCLASS Group, Sao Paulo, Brazil; [3]CINE-HDC-RENALCLASS Group, Sao Paulo, Brazil.*

Background: In patients with chronic kidney disease, it is crucial to begin treatment as early as possible to prevent and decrease the occurrence of complications. Motivating this population of patients to adhere to a treatment plan is a major challenge for health care professionals. In this study, we assessed the impact of a nutritional education program using the transtheoretical model of eating behavior change to control hyperphosphatemia in these patients.

Methods: This prospective observational study performed in satellite dialysis centers included 189 patients out of a total of 378 who exhibited phosphorus concentrations ≥ 5.5 mg/dL. The nutritional intervention consisted of lectures and group dynamics sessions performed during dialysis sessions. The didactic materials used included an illustrated album, photographs of food and brochures. The following data were analyzed pre- and post-intervention: anthropometric, clinical, demographic and laboratory data (serum phosphorus concentration and intact parathyroid hormone (iPTH)) as well as application of the transtheoretical model to eating behavior.

Results: After the nutrition intervention, we found a statistically significant reduction of the serum phosphorus concentration, and most patients exhibited positive changes in their eating behaviors. Reduction of phosphatemia is a measure of compliance with treatment, and it is associated with decreased mortality in patients with chronic kidney disease undergoing hemodialysis.

Conclusions: Thus, we conclude that nutritional intervention through an education program is an effective tool to reduce hyperphosphatemia in this population.

Funding: Private Foundation Support

FR-PO627

An Oral Adsorbent AST-120 Adsorbed the Precursors of Uremic Toxins in the Intestines Ryoko Tateoka, Yoshiharu Itoh, Atsuko Ezawa, Hideyuki Yamato. *Kureha Corporation, Tokyo, Japan.*

Background: An oral sorbent AST-120 is clinically used in Japan for the treatment of chronic kidney disease (CKD) patients to slow the progression of CKD. We found some metabolites such as indoxyl sulfate (IS), p-cresyl sulfate (PCS), phenyl sulfate (PhS), 4-ethylphenyl sulfate (4EtPhS) and hippuric acid (HA) were accumulated in the serum of CKD rats, and their serum levels were reduced by administration of AST-120. In this research, we investigated the toxic effect of these metabolites on proliferation of a pig kidney-derived cultured renal epithelial cell line (LLC-PK_1). In addition, we analyzed their precursors which were adsorbed on AST-120 recovered from the feces of CKD rats to elucidate the role of AST-120 in the intestines.

Methods: Cell proliferation assay: The LLC-PK_1 cells were incubated with metabolites for 4 days. Then, the cell number in each well was estimated using a cell counting kit. Analysis of adsorbates on AST-120 recovered from the feces of CKD rats: AST-120 was administered to CKD rats by oral gavage at a dose of 1.0 g/kg, 4.0 g/kg. Twenty four-hour feces were collected and separated into AST-120 and residue, and then extracts were obtained from each by liquid extraction. By using gas chromatography/mass spectrometry (GC/MS), we analyzed indole (ID), p-cresol (PC), phenol (Ph), 4-ethylphenol (4EtPh) and phenylpropionic acid (PPA) in the extracts from AST-120 which are considered precursors of IS, PCS, PhS, 4EtPhS and HA, respectively.

Results: Cell proliferation assay: IS, PCS, PhS, 4EtPhS and HA inhibited significantly the cell proliferation relative to control medium, and their inhibitions were dose-dependent. Analysis of adsorbates on AST-120 recovered from the feces of CKD rats: ID, PC, Ph, 4EtPh and PPA were detected in the extracts from AST-120. The amounts of them were dose-dependently increased by administration of AST-120.

Conclusions: We showed that IS, PCS, PhS, 4EtPhS and HA induced an inhibition of renal epithelial cell proliferation in a dose-dependent manner. We detected ID, PC, Ph, 4EtPh and PPA as adsorbates on AST-120. These results indicate that AST-120 reduce the serum level of uremic toxins by adsorbing their precursors in the intestines.

FR-PO628

Central Role of Urea in Disruption of Intestinal Tight Junction and Barrier Dysfunction in CKD Nosratola D. Vaziri, Gaytri Gandotra, Hamid Moradi, Jun Yuan. *Medicine, Division of Nephrology and Hypertension, University of California, Irvine, CA.*

Background: CKD causes intestinal barrier dysfunction which contributes to systemic inflammation. Recently we found depletion of key trans-cellular [claudin-1 and occludin) and intracellular [ZO1] protein constituents of epithelial tight junction (TJ) in the colon of CKD animals [Vaziri et al NDT, 2012]. These findings elucidated the mechanism by which uremia impairs intestinal barrier function. Present study explored the effect of simulated uremia on intestinal epithelial barrier function/structure and possible causal role of urea whose influx into the gut and conversion to ammonia by bacterial urease is well known.

Methods: TJ-forming T84 cells were seeded on the apical compartment of Transwell plates to achieve polarization and utilized when trans-epithelial electrical resistance (TER) approached 1,000 $\Omega.cm^2$ to ensure full polarization and TJ formation. The cells were then incubated for 24 hr in media containing 10% plasma from ESRD or healthy individuals or in media containing zero, 70, or 120 uM urea with or without urease to recapitulate the effect of microbial flora.TER was then measured (Millicell ERS-2 meter) and cells harvested for Western blot and immunohistology.

Results: TER fell by 48% in presence of uremic plasma and by 28-30% in presence of clinically-relevant urea levels reflecting barrier dysfunction. Incubation in media containing ESRD patients' plasma and urea respectively reduced cluadin-1 (by 85% & 90%), occludin (15% & 80%), and ZO1 (by 70% & 75%). The effect of urea was dramatically amplified by urease.

Conclusions: Exposure to uremic milieu impairs intestinal barrier function and structure which by enabling influx of endotoxin and other noxious products can contribute to inflammation and accumulation of gut-derived uremic toxins. This is partly mediated by urea which is generally known as a nontoxic metabolite.

FR-PO629

Myocardial Infarction Accelerates Renal and Cardiac Impairment Post Subtotal Nephrectomy: Implications for Cardiorenal Syndrome Shan Liu,[1] Andrew Kompa,[1] Darren J. Kelly,[2] Henry Krum,[1] Bing Hui Wang.[1] *[1]Department of Epidemiology & Preventive Medicine, Monash University, Melbourne, Australia; [2]Department of Medicine, University of Melbourne, St. Vincent's Hospital, Melbourne, Australia.*

Background: Renal failure with co-morbid cardiac dysfunction is associated with significant worsening of mortality requiring further investigation into the pathophysiology and related mechanisms. We investigated the renal and cardiac pathophysiological changes that occur when myocardial infarction (MI) follows chronic kidney injury (induced by 5/6 nephrectomy – STNx).

Methods: Male Sprague Dawley rats (n=36) were randomized into four groups: Sham-operated STNx + Sham-operated MI (Sham+Sham), Sham-operated STNx + MI (Sham+MI), STNx+Sham-operated MI (STNx+Sham) and STNx+MI. STNx/Sham surgery was first induced followed by MI/Sham surgery 4 weeks later. Renal function was assessed prior to the 2nd surgery and 8 weeks later. Thereafter, tissues were collected for analysis.

Results: Survival rate was 100%, 77.8%, 60.9% and 39.8% in Sham+Sham, Sham+MI, STNx+Sham and STNx+MI rats, respectively. All rats that underwent STNx had significantly reduced glomerular filtration rate (Sham+Sham: 8.6±0.5, Sham+MI: 8.1±0.5, STNx+Sham: 0.7±0.4, STNx+MI: 0.2±0.4 ml/min/kg) and creatinine clearance (Sham+Sham: 242.6±26.6, Sham+MI: 255.5±39.7, STNx+Sham: 32.2±8.7, STNx+MI: 33.9±12.4 ml/min), and significantly increased urine total protein (Sham+Sham: 23.1±2.0, Sham+MI: 16.2±1.6, STNx+Sham: 344.4±48.9, STNx+MI: 380.0±89.1 mg/day) compared to rats that did not undergo STNx. Increases in renal interstitial fibrosis ($p<0.05$) and kidney injury molecule-1 ($p<0.001$) in the non-infarct zone were observed in STNx+MI compared to STNx+Sham rats, despite no difference in blood pressure (BP) ($p=0.27$). Heart weight ($p<0.05$), cardiomyocyte cross-sectional area ($p<0.01$) and cardiac interstitial fibrosis ($p<0.05$) was greater in STNx+MI compared to STNx+Sham rats.

Conclusions: Combined STNx and MI accelerated renal fibrosis, and cardiac hypertrophy and fibrosis; all of these alterations were BP independent. This study demonstrates the potential of this model in assessing the pathophysiology and mechanisms of cardiorenal syndrome.

FR-PO630

Immunomediated Mechanism of Organs Damage in Cardiorenal Syndrome Type1 Grazia Maria Virzì,[1,2] Massimo de Cal,[1,2] Dinna N. Cruz,[1,2] Elisa Scalzotto,[1,2] Claudio Ronco.[1,2] *[1]Nephrology Dep, St Bortolo Hosp, Vicenza; [2]International Renal Research Institute Vicenza, IRRIV, Italy.*

Background: Cardiorenal syndrome Type1 (CRS1) is characterized by acute cardiac events leading to acute kidney injury (AKI). CRS1 pathophysiology is very complex. Given circulating nature of many inflammatory mediators, it is tempting to examine the immunomediated mechanism as mediator of organs crosstalk in CRS1. The main objective was to examine *in vitro* that CRS1 plasma was able to trigger a response in monocytes and renal tubular cells (RTC), resulting in apoptosis and in cytokine-release.

Methods: We enrolled 12 patients with Heart Failure (HF)(age 73.5±12.9yrs, sCr 0.87mg/dl (IQR 0.82-0.96), 7 patients with CRS1 (75.8±10.8yrs, sCr 0.93mg/dl (IQR 0.89-0.99) and 5 healthy controls (CTR)(63±8yrs). Plasma from different groups were

incubated with monocytes and RTCs for 24h and, subsequently, cell apoptosis was evaluated by different methods and quantitative levels of TNF-α and IL-6 in supernatants was performed by ELISA.

Results: Both cell lines treated with CRS1 plasma showed higher DNA ladder formations, suggesting presence of apoptotic events. In fact, a quantitative analysis of apoptosis showed significantly higher apoptosis rates in CRS1(30.5%, IQR 24-39.9) compare to HF (8%, IQR 6.7-9.5) and CTR (5%, IQR 4.5-7.5)(p<.005). Moreover, Caspase-3 levels in cells incubaed with the plasma from CRS1 patients demonstrated a significantly higher concentration (1.2ng/ml, IQR 1.1-2 vs HF 0.78, IQR 0.6-0.9 and CTR 0.1, IQR 0.1-0.2).When compared with CTR (2.4, IQR:1.5-4.1), TNF-α levels in supernatant were significantly elevated both in HF (28.2pg/ml, IQR:20.7-32.8) and in CRS1 group (28.45, IQR 22.4-29.6)(both p<.005). Furthermore, in CRS1 patients IL-6 was significantly higher compared with HF patients and CTR (46.1pg/ml, IQR 24.4-70 vs HF 21.25, IQR 18.4-36.5 and CTR 2.4, IQR 1.8-3.4)(p<.005).

Conclusions: This pilot study suggests the presence of defective regulation of apoptosis in monocytes and RTCs in CRS1, and the presence of an immunomediated mechanism. Furthermore, these preliminary results imply that inflammatory pathways may have a central role in pathogenesis of CRS1 and may be fundamental to damage distant organs.

FR-PO631

Nicotine and the Progression of Chronic Kidney Disease: Role of COX-2 Derived Prostaglandins Gabriel Rezonzew,[1] Phillip H. Chumley,[1] Gene P. Siegal,[2] Wenguang Feng,[1] Ping Hua,[1] Edgar A. Jaimes.[1,3] *[1]Medicine/Nephrology, University of Alabama at Birmingham; [2]Pathology, University of Alabama at Birmingham; [3]Nephrology, VA Medical Center, Birmingham, AL.*

Background: Cigarette smoking is a risk factor in the progression of CKD. Nicotine (NIC), a major component of tobacco, worsens renal injury in 5/6 nephrectomy rats (Nx), a well validated model of CKD (AJP'12). COX-2 derived prostaglandins (PGs) have been postulated to participate in the pathogenesis of renal injury. Herein we determined the role of COX-2 on the adverse effects of NIC in the severity of injury in Nx rats.

Methods: Male SD rats were divided in five groups: Sham, Nx, Nx+NIC (0.1 gm/L, DW), Nx+NIC+COX-2 innhibitor (NS, 1.5 mg/Kg/day, SQ), Nx+NS. Urine was collected for proteinuria and PGs (PGI_2 and PGE_2) and blood pressure (BP) measured by tail cuff. Rats were euthanized after 12 weeks and kidneys saved for western blot (WB) and histology.

Results: Nx did not affect COX-2 expression (WB) and had a small effect on the urinary excretion of PGI_2 and no effect on PGE_2 (Table). NIC did not modify COX-2 expression in Nx rats but increased the urinary excretion of PGI_2. NS increased COX-2 (WB) and reduced urinary excretion of PGI_2 but not of PGE_2 (Table). COX-2 inhibition significantly reduced BP in Nx and Nx+NIC rats and improved glomerular injury score (GIS), proteinuria and fibronectin in Nx+NIC rats but not in Nx rats.

	Sham (n=8)	5/6Nx (n=9)	5/6Nx+NIC (n=7)	5/6Nx+NIC+NS (n=8)	5/6Nx+NS (n=8)
COX-2 (A.U.)	1.1±0.01	1.02±0.11	1.04±0.03	1.30±0.1*,#,**	1.47±0.1*,#,**
PGI2 (ng/mg creat)	7.7±0.4	13.3±6.7	37.4±7.2 *,#	3.2±0.9#,**	2.4±0.5#,**
PGE2 (ng/mg creat)	2.6±0.3	1.6±0.4	2.6±1.2	2.8±1.0	2.5±0.5
SBP(mmHg)	128±5.1	162±8.4*	159±7.8*	135±7.7**	140±6.6#
GIS	0.27±0.03	0.85±0.2*	1.6±0.26 *,#	0.26±0.05 #,**	0.86±0.28*
Proteinuria(mg/mg creat)	1.97±0.45	8.4±1.3 *	13.24±1.7 *,#	4.35±0.72**	20.1±3.8 *,#
Fibronectin(fold increase)	1±0.09	1.64±0.34*	2.56±0.28 *,#	1.78±0.06**	1.7±0.03 *

* p<0.05 vs Sham, # p<0.05 vs 5/6Nx, ** p <0.05 vs 5/6Nx+NIC

Conclusions: We have demonstrated that COX-2 plays an important role as mediator of the deleterious effects of nicotine in CKD making it a potential target for the prevention of accelerated progression of CKD in smokers.

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FR-PO632

Nicotine Receptors Are Widely Expressed in the Human, Rat and Mouse Kidney: A Potential Role in Tobacco Induced Renal Injury? Phillip H. Chumley,[1] Gabriel Rezonzew,[1] Ping Hua,[1] Wenguang Feng,[1] Edgar A. Jaimes.[1,2] *[1]Medicine/Nephrology, University of Alabama at Birmingham; [2]Nephrology, VA Medical Center, Birmingham, AL.*

Background: Nicotine acetylcholine receptors (nAChRs) are formed by the combination of five subunits to form pentameric receptors that function as agonist-regulated Ca^+ channels. Their activation by nicotine in the central nervous system is responsible for the addictive properties of tobacco smoking. Recently several nAChR subunits have been identified in other tissues including the systemic vasculature, lung, and skin. Nicotine contributes to the progression of chronic kidney disease (CKD) via the promotion of mesangial cell proliferation, extracellular matrix production and reactive oxygen species generation. We previously showed the presence of nAChR subunits in human mesangial cells (AJP '07) and recently demonstrated that blockade of the α7-nAChR subunit reduces renal injury associated with the administration of nicotine in 5/6 nephrectomy rats, a validated model of CKD (AJP '12). To shed further insight into the importance of the different nAChR subunits it is critical to characterize their presence and primary distribution in the renal parenchyma.

Methods: Western blot analysis and immunofluorescent methods were used to determine the expression and localization of several subunits (α1-7, ß1-4) in the normal kidney from human, rat (Sprague-Dawley) and mouse (C57BL/6) origin.

Results: As shown in table 1 we observed the presence of several nAChR subunits in the normal kidney of three different species.

Table 1

subunit	Human IF	Mouse IF	Rat IF	Mouse western	Rat western	Primary location
α1	++	-	-	-	-	Human - glomeruli and tubules
α2	-	-	-	-	+	n/a
α3	+	-	-	-	-	Human - tubules
α4	+	++	++	++	++	glomeruli and tubules
α5	+	++	++	++	+++	tubules
α6	++	++	++	++	+++	glomeruli, tubules, interstitial and vascular
α7	+++	+++	+++	++	+++	tubules, glomeruli in human
β1	-	-	-	-	-	n/a
β2	+	+	+	++	-	tubules
β3	++	++	-	+++	-	glomeruli and tubules
β4	+++	+++	+++	+++	+++	glomeruli and tubules

Conclusions: We conclude that several nAChR subunits are present in the normal kidney, that when activated by nicotine may play a role in the pathogenesis of accelerated CKD in smokers.

Funding: Other NIH Support - NIEHS, Private Foundation Support

FR-PO633

Induced Overexpression of VEGF Ameliorates Glomerulosclerosis Progression in Mice Anne P. Wilson,[1] Haichun Yang,[1] Ji Ma,[2] Agnes B. Fogo.[1] *[1]Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN; [2]Pediatric Medicine, Vanderbilt University, Nashville, TN.*

Background: Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor produced by the podocyte in glomeruli. In healthy mice, overexpression of VEGF leads to proteinuria. However, in the setting of chronic renal disease, podocyte loss reduces VEGF in glomeruli, which may contribute to glomerulosclerosis progression. We investigated whether induced overexpression of VEGF after glomerulosclerosis could decrease progression.

Methods: VEGF loxp (V) mice were mated with TetO-podocin-Cre (R) mice to create double transgenic mice (RV) that permit inducible overexpression of VEGF, or single transgenic mice (R), which will not induce VEGF. All mice underwent 5/6 nephrectomy (Nx). At 8 weeks after 5/6 Nx, overexpression of VEGF was induced by administration of 2 mg/mL doxycycline in the drinking water. Mice were sacrificed at week 12, or earlier in severely ill mice. Systolic blood pressure and proteinuria were measured at week 0, week 8, and the time of sacrifice, and glomerulosclerosis assessed.

Results: RV mice did not have proteinuria at baseline (ACR 72.2 ± 8.2 mg/mg). At week 8, both RV and R mice had developed proteinuria (*RV:* 8835.6 ± 2996 mg/mg; *R:* 7760.6 ± 1791 mg/mg). Systolic BP increased significantly in both groups from week 0 to week 8 (*RV:* 116.4 ± 4.96 week 0 to 140.9 ± 9.1 mmHg at week 8; *R:* 117.7 ± 2.4 week 0 to 139 ± 5.7 mmHg at week 8, p<0.05). After administering doxycycline at week 8, RV mice survived longer than R mice (17.3 days vs. 15.2 days). Systolic BP did not change significantly from week 8 to sacrifice in RV or R mice.In R mice, there was a 3.5 fold increase in proteinuria (measured by ACR) on average from week 8 to sacrifice, while in RV mice, ACR remained steady over this time period. However, despite longer survival times, there was less glomerulosclerosis in RV mice (sclerosis index 1.05 ± 0.18, 0-4 scale) than in R mice (1.31 ± 0.16).

Conclusions: Our data indicate induced overexpression of VEGF slows the progression of existing glomerulosclerosis and prevents increased proteinuria by local mechanisms, not through systemic blood pressure effects.

Funding: NIDDK Support

FR-PO634

Establishing a Biomarker for the Vasculopathy of Fabry Disease Liming Shu, Anuradha Vivekanandan-Giri, Subramaniam Pennathur, James A. Shayman. *Internal Medicine, University of Michigan, Ann Arbor, MI.*

Background: Vascular endothelial dysfunction of Fabry disease, resulting from a deficiency of α-galactosidase A (α-Gal A), is a consequence of the accumulation of globotriaosylceramide (Gb3). We have previously established models of vasculopathy in the α-Gal A null mouse including oxidant induced thrombosis, accelerated atherogenesis, and impaired arterial reactivity to better delineate the mechanistic basis for the vasculopathy. These studies have demonstrated that excess Gb3 deposition in both lysosomes and caveolae of plasma membranes cause decreased nitric oxide bioavailability as a result of both reduced eNOS activity and uncoupling.

Methods: To better understand the pathogenesis of Fabry disease in humans, we generated a human cell model of Fabry disease by using RNA interference. EA.hy926 cells, a hybridized human endothelial cell line, were transiently transfected with either control or siRNA specifically directed against α-Gal A.

Results: α-Gal A knockdown was greater than 85% and was confirmed by immunoblotting and Gb3 accumulation. Enzyme expression and Gb3 levels were proportionate to the level of transfection. eNOS activity was correspondingly decreased and the reduction was greater than 60%. The oxidized amino acids were then quantified by tandem mass spectrometry. 3-Nitrotyrosine, a specific marker for reactive nitrogen species (RNS) was markedly increased (40 to 120 fold) without corresponding changes in other oxidized tyrosines, including chlorotyrosine, dityrosine, and orthotyrosine, consistent with eNOS as the source of the RNS.

Conclusions: These data confirm the previously observed association between Gb3 accumulation, decreased NO bioavailability, and RNS resulting from eNOS uncoupling. These findings raise the possibility that 3-nitrotyrosine may serve as a biomarker for the vascular involvement in Fabry disease.

Funding: NIDDK Support

FR-PO635

Role of SDF-1/CXCR4/eNOS Signaling in Maintaining Endothelial Integrity and Renal Function in Chronic Kidney Disease Li-Hao Chen,[1] Suzanne Advani,[1] Darren A. Yuen,[1] Kim Connelly,[1] Ian W. Gibson,[2] Manish M. Sood,[2] Philip A. Marsden,[1] Richard E. Gilbert,[1] Andrew Advani.[1] *[1]Dept. of Medicine, St. Michael's Hospital, Toronto, ON, Canada; [2]Health Sciences Centre, University of Manitoba, Winnipeg, MB, Canada.*

Background: Therapeutic strategies antagonizing the angiogenic chemokine stromal cell-derived factor-1 (SDF-1), or its cognate receptor CXCR4, are being keenly investigated for the treatment of malignancies and other chronic diseases. In the present study, we explored the role of the SDF-1/CXCR4 system in chronic kidney disease (CKD), where decreased angiogenic factor activity and capillary loss are pathophysiologically linked to renal decline.

Methods: Gene expression was determined in both rat and human kidney tissue. CXCR4 antagonism was achieved by treating subtotally nephrectomized (SNx) rats with the bicyclam, AMD3100. SDF-1 signaling was induced by infusing recombinant SDF-1 into the kidneys of normal rats prior to glomerular isolation. In vitro studies were performed in wildtype and eNOS$^{-/-}$ glomerular endothelial cells (GECs).

Results: CXCR4 protein was noted in endothelial cells of both glomerular and peritubular capillaries in normal adult human kidney tissue, while renal expression of the receptor was increased in the kidneys of SNx rats and in biopsies of patients with secondary focal segmental glomerulosclerosis. In contrast, while SDF-1 mRNA was reduced in SNx kidneys, it was increased following treatment with the ACE inhibitor, perindopril. Chronic CXCR4 antagonism accelerated renal decline and capillary loss in SNx rats, while acute infusion of SDF-1 induced CXCR4-dependent glomerular endothelial signaling through endothelial nitric oxide synthase (eNOS) activation. Exposure of cultured GECs to recombinant SDF-1 resulted in tube formation, proliferation and migration, each of which was impaired in eNOS deficient GECs.

Conclusions: These observations indicate that local SDF-1/CXCR4/eNOS signaling functions to preserve endothelial integrity in CKD. While caution should be applied with the chronic systemic use of anti-SDF-1/CXCR4 agents in the renally impaired population, augmentation of this pathway, by conventional or novel agents, may attenuate declining function in CKD.

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FR-PO636

The Sphingosine-1-Phosphate Receptor Agonist FTY720 Improved Expression of eNOS in Kidneys from Subtotal Nephrectomized Rats Haifeng Ni, Junfeng Chen, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.*

Background: Previous study suggested that decreased bioavailability of endothelial nitric oxide (NO) produced from endothelial NO synthase (eNOS) plays a crucial role in the progression of kidney damage. Sphingosine 1-phosphate (S1P) has been highlighted as an endothelial barrier-stabilizing mediator. FTY720 is a S1P analog originally developed as a novel immunosuppressant. In this study, we investigated the effects of FTY720 on the levels of nitric oxide (NO) and the expression of eNOS in the renal tissue from subtotal nephrectomized rats.

Methods: Seven days after surgery, SD rats were allocated to the following groups: Sham, subtotal nephrectomy (SNX), and SNX +FTY720. Rats were killed on week 12 after surgery and blood, urine and kidneys were collected for analyses. The renal histological changes were investigated by light and transmission electron microscopy. The expression of eNOS were detected by immunohistochemical staining, RT-PCR and Western blot.

Results: FTY720 attenuated the increase of BP, proteinuria, SCr and NAG in SNX($P<0.01$). FTY720 treatment prevented the downregulation of nitric oxide(NO) in kidney in SNX. SNX rats had severer glomerular sclerosis and vascular lesions than sham rats ($P<0.01$). FTY720 treatment ameliorated these effects ($P<0.01$). There was endotheliosis in glomerulus and widening of subendothelial spaces in SNX by Electron microscopy, these abnormalities were attenuated by FTY720. Immunohistochemistry showed the tissue localization of eNOS protein in the sham rat kidneys was in endothelial cells in glomeruli and vasculature. Semiquantitative analyses showed that eNOS expression in the glomeruli and vessles was lower in SNX rats compared with the sham ($P<0.01$), whereas FTY720 abrogated this change($P<0.01$). FTY720 treatment improved eNOS mRNA expression ($P<0.01$) and protein expression ($P<0.01$) compared with the untreated SNX.

Conclusions: FTY720 ameliorates endothelial injury in glomeruli and vasculature in kidneys from SNX. Alterations of eNOS may be an explanation for the underlying mechanism of FTY720 renoprotection.

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FR-PO637

Sirtuin 1 (SIRT1) Deficiency in Endothelial Cells Leads to Microvascular Rarefaction, Premature Senescence, and Cardiomyopathy Julien Maizel,[1,2] Sandhya Xavier,[2] Jun Chen,[2] Radovan Vasko,[2] Michael S. Goligorsky.[2] *[1]INSERM 1088, Jules Verne University of Picardie and University Medical Center, Amiens, France; [2]New York Medical College, Valhalla, NY.*

Background: Cardiovascular diseases are the leading cause of death in CKD. Recently several studies demonstrated the role of impaired angiogenesis in the development of cardiomyopathies. SIRT1 regulates angiogenesis. Therefore we analyzed the cardiac functions of mice deficient in SIRT1 in endothelial cells.

Methods: The left ventricular diastolic function of Sirtuin1$^{f/f}$;Tie2-Cre (KO), Sirtuin1$^{f/+}$Tie2-Cre (Het), Tie2-Cre, Sirtuin1$^{f/f}$ (all controls) mice was examined echocardiographically: isovolumic relaxation time (IVRT) and myocardial performance index (Tei index). Hearts were examined by Masson's trichrome staining, CD31 immunostaining and analyzed for RNA abundance for Collagen 1 and 3, short and long endoglin, MMP14, TIMP2 and VEGF.

Results: The KO mice compared to control groups of animals exhibited diastolic dysfunction with increased IVRT and Teï index (Table 1). The percentage of fibrosis in the left ventricle of KO mice was increased. The number of CD31+ structures in the left ventricle was significantly lower in the KO compared to controls animals. The RNA expression of collagen 1 and collagen 3 and the ratio of short-to-long endoglin, a marker of senescence were all higher in the KO group. The RNA expression of VEGF, MMP14 and TIMP2 were all lower in the KO group.

Table 1. Echocardiographic parameters of heart function and markers of remodeling

	Het Cont	Het	KO Cont	KO
IVRT, ms	18.5±3.9	18±4.3	19.2±1.1	23.2±2.4
Tei index	0.28±0.06	0.29±0.08	0.29±0.1	0.37±0.04
Trichrome, %	0.2±0.1	0.4±0.2	0.3±0.1	0.6±0.4
CD31 +	60±17	66±14	78±2	29±13
VEGF-A	0.58±0.2	0.47±0.44	1.12±0.7	0.18±0.15
Collagen 1	1.1±0.5	1.3±1.5	0.3±0.3	5±0.02
Collagen 3	2.1±2.8	3.1±4	0.5±0.6	2.7±1.9
MMP14	1.2±0.7	0.6±0.4	1.1±0.5	0.5±0.8
TIMP2	1.1±0.5	0.9±0.7	1.4±1	0.3±0.4
Endo S/ Endo L	1±0.3	1.1±0.1	1±0.2	4.3±0.4

Conclusions: the inactivation of SIRT1 in endothelial cells is associated with a) the left ventricular diastolic dysfunction, b) premature senescence, c) microvascular rarefaction and reduced expression of VEGF, and d) cardiac fibrosis due to MMP14 deficiency.

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FR-PO638

Sirt3 Is a Target for Acetyl-L-Carnitine Protection against Angiotensin II-Induced Insulin Resistance Daniela Macconi,[1] Luca Perico,[1] Simona Buelli,[1] Marina Morigi,[1] Paola Cassis,[1] Lorena Longaretti,[1] Federica Casiraghi,[1] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [2]Unit of Nephrology and Dialysis, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy.*

Background: Sirt3 deficiency is causally linked to IR and metabolic syndrome and data are available that angiotensin II (Ang II) down-regulates Sirt3 gene expression in cultured cells. Since acetyl-L-carnitine (ALCAR) has been found to ameliorate hypertension and insulin resistance (IR) in subjects at increased cardiovascular risk, here, we explored whether ALCAR ameliorates Ang II-induced IR through Sirt3 modulation.

Methods: Rat skeletal muscle cells, L6 myotubes, were incubated in the absence or presence of 100 nM Ang II for 24 h before and during 30-min stimulation with 100 nM insulin. IR was evaluated as inhibition of insulin-induced glucose uptake and GLUT4 translocation to the plasma membrane. Surface GLUT4 was assessed in L6 GLUT4-myc. Mitochondrial ROS were measured by MitoSOX. Sirt3, phospho/total AMPK, acetylated proteins were analyzed by western blot. NAMPT mRNA was measured by real time PCR. Silencing of Sirt3 was induced by siRNA Sirt3 transfection in L6 GLUT4-myc myotubes.

Results: ALCAR prevented Ang II-induced IR by improving insulin-stimulated GLUT4 transport and glucose uptake in L6 myotubes. These effects were associated with reduced mitochondrial oxidative stress and upregulation of manganese superoxide dismutase (MnSOD). Similarly to ALCAR, MnTBAP, a mitochondria SOD mimetic, enhanced cell surface GLUT4-myc in Ang II-treated cells suggesting that control of mitochondrial superoxide is a crucial step for improving insulin sensitivity. ALCAR reversed the inhibitory effect of Ang II on Sirt3 activity/expression. Reduced mitochondrial protein acetylation by ALCAR was associated with activation of AMPK and up-regulation of NAMPT. Silencing of Sirt3 in L6 GLUT4-myc myotubes induced IR in unstimulated cells mimicking the effect of Ang II and completely abrogated the beneficial effect of ALCAR on Ang II-induced IR.

Conclusions: Sirt3 mediates the protective effect of ALCAR in Ang II-induced IR.

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FR-PO639

Epigenetic Alterations at Laminin Genes in Aging Kidneys Karol Bomsztyk, Daniel S. Mar, Oleg N. Denisenko. *UW Medicine SLU, University of Washington, Seattle, WA.*

Background: Aging is associated with a gradual decline of kidney function. Glomerular filtration rates decrease due to changes in structure and composition of basement membrane. We hypothesized that in a rat model aging induces changes in renal expression of laminin genes that encode key components of basement membrane.

Methods: To control for the effect of genetic background we used two rat lines, i) Fisher 344 (F344), and ii) F344xBN hybrid (FBN-F1) with median life spans 24 and 33 months respectively RT-PCR and multiplex Matrix chromatin immunoprecipitations were used to assess levels of mRNA and transcription/chromatin changes in kidneys from young (4 mo) and old animals (F344 - 24/28 mo, FBN-F1 - 32 mo).

Results: In both animal lines, aging was associated with increased transcript levels of laminin chains a3, b3 and c2, with little or no changes in other laminin mRNAs. These changes were more pronounced in old F344 animals that have shorter life span. Aging-induced laminin genes showed increased density of RNA polymerase II at these loci, suggesting altered transcription. There was a correlation between increases in laminin transcript levels and loss of the repressive epigenetic mark H3K27m3 at these genes during aging. These observations suggested a causal link between the loss of this silencing mark and

upregulation of laminin genes. *In vitro* experiments supported this suggestion, as inhibition of activity of cognate methyltransferase, Ezh2, in kidney cell line HEK293 decreased H3K27m3 levels and upregulated Lama3/b3/c2 expression. However, Ezh2 levels were not reduced in old kidneys, indicating that a decrease in the histone methyltransferase cannot account for the loss of H3K27m3 density and aberrant laminin gene expression during aging. Instead we found that levels of H3K27m3 demethylase KDM6A were increased along Lamc2 gene in old compared to young kidneys.

Conclusions: These data suggest that upregulation of Lama3/b3/c2 expression in aging kidneys is caused by increased activity of KDM6A histone demethylase at these genes. Thus, deregulation of epigenetic enzymes could be a major contributor to altered composition of basement membranes and the decline in kidney function associated with aging.

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FR-PO640

FAT10-Knockout Mice Are Protected from HIV-Associated Nephropathy

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Background: HIV-associated nephropathy (HIVAN) is a rapidly progressive form of focal segmental glomerulosclerosis that is accompanied by tubular dilation and tubulointerstitial inflammation and fibrosis. We have previously shown that the ubiquitin-like protein FAT10 is upregulated in renal tubular cells of HIVAN and that FAT10 mediates NF-kB activation and HIV-induced apoptosis in renal tubular cells in vitro.

Methods: To determine whether FAT10 expression is necessary for HIVAN pathogenesis in vivo, we studied whether FAT10 $^{-/-}$ HIV transgenic mice are protected the HIVAN phenotype that occurs in the Tg26 HIV-transgenic model of HIVAN. After fully backcrossing FAT10$^{-/-}$ mice onto the FVB/N genetic background (same as Tg26), we bred them with Tg26 mice and compared the renal phenotype of FAT10 $^{-/-}$ HIV mice with FAT10 $^{+/+}$ HIV mice (Tg26) at 3 months of age.

Results: We found that 76% (19 of 25 mice) developed typical HIVAN pathologic abnormalities including glomerulosclerosis, microcystic tubular dilatation and inflammatory cells infiltration while these abnormalities occurred in only 24% (8 of 33 mice) of FAT10 $^{-/-}$ HIV mice. FAT10 $^{-/-}$ HIV mice developed less severe proteinuria (albumin/creatinine ratio 1.29±0.31 vs 2.19 ± 0.31, $p<0.05$), but there was no significant difference in serum levels of BUN, creatinine, or TNFα. FAT10 $^{-/-}$ HIV mice also had markedly lower expression of renal chemokines and cytokines (*CCL2, CCL20, CXCL1, CXCL2, CXCL5, IL-6* and *VCAM*) as compared to Tg26 mice however, there was no difference in renal expression of HIV RNA (*tat-rev, nef, vpr*).

Conclusions: Together, the results suggest that FAT10 has an important role in the pathogenesis of HIVAN and that FAT10 may mediate these effects via enhanced expression of NF-kB dependent pro-inflammatory mediators.

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FR-PO641

Increased Mortality in the Tg26 Mouse Model of Human Immunodeficiency Virus Associated Nephropathy when Podocan Expression Is Decreased

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Background: Podocan was originally identified as an upregulated gene in mouse podocytes expressing HIV-1 viral genes. Tg26 mice, a murine model of HIVAN, also have an increased expression of podocan in sclerotic glomeruli. To test the hypothesis that decrease in podocan expression would improve renal function in Tg26 mice, we crossed Tg26 mice with podocan deficient mice.

Methods: Body weight, blood urea nitrogen (BUN), and proteinuria from at least 10 mice per group of wild type, podocan deficient, Tg 26, and Tg 26/podocan deficient mice (Tg 26/podn$^{+/-}$) were monitored from 3 to 24 weeks of age. Kidneys from above groups at 12 weeks of age were analyzed by PAS staining.

Results: Podocan deficient mice showed normal development, life span, and renal function. Similar to Tg26 mice, Tg 26/podn$^{+/-}$ mice had onset of proteinuria at three weeks postnatally, and the severity of proteinuria was comparable between these two groups ($p>0.05$). BUN levels of Tg 26/podn$^{+/-}$ mice were normal. Although both Tg 26 and Tg 26/ podn$^{+/-}$ mice had histopathological changes characteristic of HIVAN including diffuse collapsing glomerulosclerosis, microcystic tubular changes, and proteinaceous casts, these changes were less severe in Tg 26/podn$^{+/-}$ mice. Notably, in the kidneys of Tg 26/podn $^{+/-}$ mice, there was increased monocytic interstitial infiltration. However, there was increased mortality of Tg 26/podn $^{+/-}$ mice (n=15) compared to age-matched Tg 26 mice (n=25) ($p<0.0001$): more than half of Tg 26/podn $^{+/-}$ mice were dead by 12 weeks of age and by 20 weeks of age only 13% of Tg 26/podn $^{+/-}$ mice were still alive, while the survival rates of Tg26 mice at the age of 12 and 20 weeks were 84% and 68%, respectively.

Conclusions: Our results indicate that podocan deficiency alleviates clinical and histological manifestations in experimental HIVAN, suggesting that increased expression of podocan contributes to HIVAN pathogenesis. Our results also suggest that podocan has certain functions that are essential for the survival of these HIV transgenic animals, and these functions are currently under investigation.

Funding: NIDDK Support

FR-PO642

Kidney Proximal Tubular Epithelial Cell-Specific Overexpression of Netrin-1 Suppresses Inflammation and Albuminuria through Suppression of COX-2-Mediated PGE2 Production in Streptozotocin-Induced Diabetic Mice

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Background: Inflammation plays a key role in development and progression of diabetic kidney disease. However the role of the anti-inflammatory molecule netrin-1 in diabetic kidney disease is unknown. Here we examined the role of netrin-1 in diabetes-induced kidney inflammation and injury using tubule-specific netrin-1 transgenic mice.

Methods: Diabetes was induced using streptozotocin in wild type and netrin-1 transgenic animals. Kidney function, fibrosis, albuminuria and inflammation were determined by measuring serum creatinine, Western blot analysis, immunostaining, ELISA and RT-PCR. The mechanism of netrin-1-induced suppression of inflammation was studied in vitro using a proximal tubular epithelial cell line.

Results: Diabetes was associated with increased infiltration of neutrophil and macrophage, chemokine expression, tubular epithelial cell apoptosis and interstitial fibrosis in the kidneys. These changes were minimal in the kidney of netrin-1 transgenic mice. In addition, diabetes induced a large increase in the excretion of prostaglandin E2 (PGE2) in urine, which was suppressed in netrin-1 transgenic mice. Netrin-1 induced suppression of PGE2 production was mediated through suppression of NFkB mediated cyclooxygenase-2 (COX-2) in renal tubular epithelial cells. In addition, netrin-1 also increased albumin uptake by proximal tubular epithelial cells through the PI3K and ERK pathways but does not alter glucose excretion and uptake by tubular epithelial cells.

Conclusions: Our results suggest that netrin-1 is a major regulator of inflammation and apoptosis in diabetic nephropathy and may be a useful therapeutic molecule for treating chronic kidney diseases such as diabetic nephropathy.

Funding: NIDDK Support

FR-PO643

Manipulation of Hif2a to Treat Anemia of CKD: A Viable Option?

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Background: Anemia management is a major component of caring for CKD patients. EPO and ESAs are a mainstay of therapy. However, there is interest to develop new agents that stimulate endogenous EPO production in a more physiologic manner. Prolyl hydroxylase inhibitors stabilize HIF2a, thus increasing EPO production and are currently under investigation for the treatment of anemia. Here we sought to understand how HIF2a stabilization affects erythropoiesis *in vivo*.

Methods: We generated an inducible gain-of-function tetO-HIF2a transgenic mouse and bred it to Pax8-rtTA and Rosa-rtTA driver strains. We also generated a stress model of anemia (30% reduction in hematocrit following blood-letting) in wildtype mice.

Results: Pax8 is expressed in renal tubular epithelial cells and some hepatocytes; induction results in Hif2a mRNA upregulation in these cells (4,500-fold and 7,800-fold, respectively). Within 2 weeks, hematocrits doubled, resulting in thrombosis, cardiovascular events and death. Upon removal of induction (- dox), hematocrits promptly returned to baseline. In Pax8-rtTA-tetO-HIF2a mice, most Epo is produced by hepatocytes (550-fold increase), not renal cells. In contrast, stress anemia (decrease in Hct 30% after blood-letting) in wildtype mice results in increased Epo production from renal interstitial cells (2,500-fold) but not liver. Using a Rosa-rtTA-driver strain bred to tetO-Hif2a mice, results in more modest Hif2a upregulation (600~2,400-fold; all cells), resulting in massive expansion of erythroblasts in bone marrow, liver and spleen. Interestingly, these mice are anemic due to failure of RBC maturation. Switching off HIf2a (-dox) permits rapid differentiation of erythroblasts. Epo levels were modestly increased (70~100-fold; kidney and liver).

Conclusions: Our data demonstrate multiple roles for Hif2a in control of erythropoiesis through Epo production and effects on RBC differentiation. Pharmacologic manipulation of Hif2a (e.g. prolyl hydroxylase inhibitors) will require careful dosing and monitoring to avoid serious complications.

FR-PO644

Assessment of Erythropoietin Effects in the Progression of Experimental Chronic Kidney Disease

Fernando Felippe Carvalho, Vicente de Paulo Castro Teixeira, Waldemar S. Almeida, Nestor Schor. *Nephrology Division, UNIFESP, Sao Paulo, Brazil.*

Background: Erythropoietin (EPO) has been used primarily to treat anemia caused by chronic kidney disease. Recent studies have shown a renoprotective EPO effect in ischemic kidney diseases. The mechanisms of renoprotection include antiapoptotic effects and stimulation of endothelial progenitor cells. Thus, the aim of this study is to evaluate the influence of EPO on progression of kidney disease in experimental chronic kidney disease.

Methods: Male Wistar rats weighing 280-300g underwent 5/6 nephrectomy and were divided into two groups: (NX) only nephrectomized(n=6) and (NX-EPO) nephrectomized(n=6) and treated with a weekly dose of erythropoietin (250UI/kg/ip). All animals were sacrificed 8 weeks after surgery. Hematocrit, serum creatinine, proteinuria, indirect blood pressure measurement, immunohistochemical analysis, glomerular score and tubular lesion were assessed.

Results: The NX-EPO group showed significant improvement in serum creatinine (NX 1.6 ± 0.4 versus NX-EPO 0.8 ± 0.1, P ≤ 0.001) and protein/urine creatinine ratio (NX 11.2 ± 6.0 versus NX-EPO 4.1 ± 2.2, P = 0.021). Histopathological results demonstrate a lower rate of glomerular sclerosis (NX =33% versus NX-EPO=17%) and tubulointerstitial fibrosis (grade III NX versus grade I NX-EPO) according to Banff classification. Preliminary results of immunohistochemistry reveal an increased expression of desmin in NX group.There were no significant differences in hematocrit and blood pressure between the two groups.

Conclusions: Our study suggests a beneficial effect of EPO in the model of progression of experimental chronic renal disease reflected by improvement of serum creatinine, proteinuria and attenuation of glomerular lesion score and a lower expression of desmin in podocytes, regardless of its effect on hematocrit and blood pressure.

FR-PO645

Effects of H.P. Acthar Gel® in the NZB/W Mouse Model of Spontaneous Lupus Nephritis Shaun Jordan, Dima Decker, David Young. *Research & Development, Questcor Pharmaceuticals Inc., Ellicott City, MD.*

Background: H.P. Acthar Gel® (Acthar) is a long-acting porcine-derived highly purified preparation of ACTH1-39 that may include other proopiomelanocortin (POMC) peptides and is FDA approved "to induce a diuresis or a remission of proteinuria in the nephrotic syndrome without uremia due to lupus erythematosus." We investigated whether Acthar can reduce proteinuria and other renal measures of disease severity and progression in the spontaneous lupus nephritis NZB/W mouse model.

Methods: Eligible female NZB/W F1 mice (spot urine protein test strip score < 3 between 20 to 26 weeks of age) were randomly assigned into 3 treatment groups at 28 weeks. Treatments were administered by s.c. injection once every other day (160 IU/kg Acthar or 0.4 ml/kg Acthar Placebo Gel, APG) or once daily (5 g/kg prednisolone, PRED) for 18 weeks. Animals were terminated if they developed either severe proteinuria, lost ≥ 20% body weight, or exhibited prostration. Proteinuria, survival, and body weight were monitored weekly, and serum creatinine and renal histopathology and IgG deposition were conducted on terminal serum and kidney samples.

Results: Proteinuria and weight loss occurred after two weeks of APG and PRED treatment, albeit to a lesser extent in PRED treated mice. In contrast, proteinuria was not observed in any Acthar treated mice, and this effect persisted beyond 10 weeks of treatment. Furthermore, body weight remained stable, and in some cases slightly elevated, in Acthar treated mice. These effects of Acthar were consistent with improvements in serum and renal measures of disease severity and progression.

Conclusions: Acthar produced beneficial effects on renal measures of disease severity and progression, as well as survival, in a mouse model of spontaneous lupus nephritis, which were superior to those produced by PRED, a standard of care that extends survival in NZB/W mice. These are the first preclinical evidence supporting the on-label indication of Acthar as a treatment for lupus nephritis. Preclinical research is ongoing to determine whether these effects represent potential immunomodulatory and anti-inflammatory effects of Acthar mediated through melanocortin receptors.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals Inc.

FR-PO646

Active Involvements of Glucagon-Like Peptide-1 (GLP-1)/Dipeptidyl Peptidase IV (DPP4) System in the Development of Renal Injury in a Rat Model of Chronic Kidney Disease (CKD) Ryotaro Ando,[1] Seiji Ueda,[1] Nana Obara,[1] Yusuke Kaida,[1] Kei Fukami,[1] Sho-ichi Yamagishi,[2] Seiya Okuda.[1] *[1]Division of Nephrology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan; [2]Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Fukuoka, Japan.*

Background: GLP-1 not only increases the postprandial insulin secretion, but also has various extra-pancreatic actions. GLP-1 receptor is presented in the kidney and DPP4 is abundantly expressed in renal tubular cells. However, the precise roles of GLP-1/ DPP4 in the pathogenesis of CKD remain unclear. Here we examined whether manipulation of GLP-1 or DPP4 could affect the development of renal injury in a rat model of CKD.

Methods: Sprague-Dawley (SD) rats and F344/DuCrlCrlj (F344) rats, genetically DPP4-deficit rats, were divided into sham-operated rats (sham) and 5/6 nephrectomized rats (5/6Nx), respectively (SD-sham; n=10, SD-5/6Nx; n=69, F344-sham; n=12, F344-5/6Nx; n=15). SD-5/6Nx rats were subdivided into 1) hydralazine, 2) exendin-4, an agonist of GLP-1 receptor, and 3) exendin(9-39), an antagonist of GLP-1 receptor -treated groups. Four weeks after the operation, the rats were killed to evaluate their urinary protein excretion, metabolic data, histology, and morphometry.

Results: In SD-5/6Nx rats, progressive increases in blood pressure, proteinuria and BUN levels were observed, all of which were significantly attenuated in F344-5/6Nx rats. Similarly, administration of exendin-4 into SD-5/6Nx rats decreased these deteriorated effects, whereas these were aggravated by exendin(9-39). Further, immunohistochemistry revealed that progressive capillary and podocyte loss observed in the SD-5/6Nx rats were also attenuated in both genetically and pharmacologically GLP-1-activated models.

Conclusions: These results indicate that GLP-1 may exert renoprotective effects, at least in part by preventing endothelial and podocyte injury in 5/6Nx rats and therefore, could be a novel therapeutic tool for CKD.

FR-PO647

Development and Treatment of HUS in Nonhuman Primates Induced by Toxins from Enterohemorrhagic *E.coli* Deborah Stearns-Kurosawa, Shinichiro Kurosawa. *Pathology, Boston University School of Medicine, Boston, MA.*

Background: Hemolytic uremic syndrome (HUS) is a potentially lethal complication of infection with enterohemorrhagic *E.coli* O157:H7 bacteria (EHEC), a food-borne pathogen. Bacterial Shiga-like toxins (Stx1,Stx2) induce HUS, characterized by thrombocytopenia, hemolytic anemia and thrombotic microangiopathy, with acute renal injury. Nonhuman primates (Papio baboons, 4-6kg) were evaluated for response to toxins, development of HUS, response to therapeutics and discovery of damage associated molecular pattern (DAMPs) biomarkers reporting cell injury.

Methods: Animals were challenged i.v. with Stx1 (10-100ng/kg), Stx2 (10-50ng/kg), or Stx1+Stx2 (25ng/kg each). Physiology was monitored; blood and urine were collected over 7-28 days for analysis of: kidney & liver chemistries (IDEXX labs), cytokines by multiplex immunoassays (Luminex, Millipore), HMGB1(ELISA), plasma mitochondrial DNA (qPCR). Therapeutic interventions were Rescue Protocols beginning up to 24 hours after toxin with synthetic anti-toxin peptide (TVP) or activated protein C anticoagulant (APC; drotrecogin alfa, activated; Eli Lilly). Primary outcomes were survival and measures of kidney function.

Results: Animals challenged with Stx1 or Stx2 developed HUS and dose-dependent acute kidney injury. Mixed Stx1+2 toxin was synergistic, with reduced survival ($p<0.001$), reduced platelets ($p<0.05$), and more rapid loss of kidney function relative to similar single toxin load. Stx1 elicited a more pro-inflammatory cytokine profile and high DAMPs (HMGB1, mtDNA). Treatment with cell permeable TVP peptide or APC resulted in surviving otherwise lethal Stx2.

Conclusions: Up to 25% of EHEC patients who develop HUS have life-long kidney problems. Outbreaks are sporadic, so animal models are vital. This project describes the only animal model that develops HUS in response to the EHEC toxins. We demonstrate disease similar to patients, and reduced disease severity with a synthetic peptide that regulates toxin cytotoxicity and a natural enzyme that reduces coagulopathy to protect organ function. Availability of this animal model provides the opportunity to evaluate novel therapeutics and to identify biomarkers that report HUS risk.

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FR-PO648

Nlrp3 Is a Key Modulator of Metabolic Syndrome-Induced Nephropathy Pieter J. Bakker,[1] Loes Butter,[1] Lotte Kors,[1] Gwendoline J.D. Teske,[1] Fayyaz Sutterwala,[2] Sandrine Florquin,[1] Jaklien Leemans.[1] *[1]Pathology, Academic Medical Center, Amsterdam, Netherlands; [2]Inflammation Program, University of Iowa, Iowa City, IA.*

Background: Metabolic syndrome (MetSyn) is defined as a cluster of five risk factors such as obesity and insulin resistance. MetSyn-driven nephropathy is characterized by microalbuminuria, fibrosis and inflammation which ultimately leads to end-stage renal disease. The innate immune receptor Nlrp3 has been implicated in obesity and type 2 diabetes. We hypothesized that the MetSyn-driven nephropathy is mediated by Nlrp3.

Methods: Nlrp3 -/- (Nlrp3KO) and wild-type C57BL/6J mice (n=8 per group) were fed a control diet (CD) or Western diet (WD) for 16 weeks. Blood was collected and kidneys were harvested, formalin-fixed or snap frozen. Snap frozen kidneys were used to determine cytokine, lipid and mRNA levels by respectively ELISA, enzymatic reaction and quantitative PCR. Paraffin-embedded kidneys were used for immunohistochemistry. Statistics were done by Mann-Whitney U test.

Results: A Western diet induced renal Nlrp3 expression. Nlrp3 deficiency protected against kidney function decline in WD-fed mice as seen by increased plasma urea and micro-albuminuria. Renal pathology in WD-fed wild-type mice was characterized by lipid accumulation, fibrosis and inflammation. Nlrp3 deficiency prevented WD-induced renal cholesterol accumulation. Furthermore, proximal tubules showed extensive Nlrp3-dependent vacuolization in WD-fed mice. Vacuoles contained phospholipids as indicated by Nile Red staining. Moreover, WD-induced fibrosis consisted of an increase in Collagen1$^+$ glomeruli in wild-type mice. Fibrosis was absent in Nlrp3KO mice. Renal inflammation in WD-fed wild-type mice was enhanced as seen by a profound accumulation of F4/80$^+$ macrophages and an increase in MCP-1. No macrophage influx was observed in Nlrp3ko mice despite increased MCP-1 levels. We established that Nlrp3 mediates WD-induced renal ICAM-1 suggesting a role for Nlrp3 in regulating macrophage influx through ICAM-1.

Conclusions: Here we demonstrate that Nlrp3 is a key modulator of renal lipid accumulation, inflammation and fibrosis leading to decreased renal function in a model for MetSyn-induced nephropathy.

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FR-PO649

Serum miR-148b as a Novel Non-Invasive Biomarker of IgA Nephropathy (IgAN) Grazia Serino,[1,2] Fabio Sallustio,[1,2] Francesco Pesce,[1] Sharon N. Cox,[1] Giuseppe De Palma,[2] Francesco Paolo Schena.[1,2] *[1]Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy.*

Background: IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide characterized by aberrant O-glycosylation in the hinge region of IgA1. Our recent work demonstrates that miR-148b regulates the enzyme core 1, β1,3-galactosyltransferase 1 (C1GALT1) explaining the abnormal glycosylation process in

IgAN (JASN, 23:814-824; 2012). miR-148b was reported to be overexpressed in PBMCs of IgAN patients, but its levels in serum have never been investigated. The aim of our study was to evaluate if serum levels of miR-148b may be considered as a novel non-invasive biomarker for the diagnosis of IgAN.

Methods: Serum miRNA was extracted from patients and controls using QIAzol Lysis Reagent and miRNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Using quantitative real-time PCR, we detected the expression of circulating miR-148b in 60 patients with IgAN and 60 healthy blood donors (HBD) with negative urine analysis. IgA1 O-glycosylation serum levels using helix aspersa agglutinin (HAA) lectin binding assay were also measured in each sample.

Results: We found that miR-148b serum levels were higher in IgAN patients compared to HBD (IgAN 0.49±0.03; HBD 0.39±0.01; p<0.01). Similarly, deglycosylated IgA1 serum levels were significantly higher in IgAN patients (p<0.01). Logistic regression analysis showed that both miR-148b (p=0.01) and deglycosylated IgA1 (p=0.02) levels were significantly able to predict the IgAN affection status. Moreover, subjects with higher miR-148b serum levels (≥75th percentile) were at high risk for IgAN (OR 2.9, 95% CI: 1.3-6.9).

Conclusions: These results suggest that miR-148b may be a novel non-invasive biomarker for the diagnosis of IgAN which, also combined with deglycosylated IgA1 serum levels, could better define the disease state.

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FR-PO650

IgA Nephropathy: A Murine Model that Displays Typical IgAN Pathology after Passive Administration of Immune Complexes Zina Moldoveanu,[1] Hitoshi Suzuki,[2] Kenji Satake,[2] Yusuke Suzuki,[2] Lea Novak,[1] Zhi Qiang Huang,[1] Colleen J. Winstead,[1] Darrell B. O'Quinn,[1] Bruce A. Julian,[1] Casey T. Weaver,[1] Jiri F. Mestecky,[1] Yasuhiko Tomino,[2] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University, Faculty of Medicine, Tokyo, Japan.*

Background: IgA nephropathy (IgAN) is an autoimmune glomerulonephritis wherein immune complexes (IC) composed of galactose-deficient IgA1 (Gd-IgA1; autoantigen) and anti-glycan IgG autoantibodies deposit in the glomeruli. Here we developed an animal model by using *in vitro*-formed IC from human Gd-IgA1 and anti-glycan IgG for induction of IgAN-like disease in mice.

Methods: Gd-IgA1 and anti-glycan IgG, either recombinant or isolated from sera of IgAN patients, were used to form IC to be injected *i.v.* into SCID or nude mice. Samples of blood and urine were collected to determine serum IgA1 and IgG, urinary protein and creatinine and hematuria. The excised kidneys were evaluated by light-, immunofluorescence- and electron-microscopy (EM).

Results: Gd-IgA1 and anti-glycan IgG formed IC that deposited in the mesangium with murine C3, and induced hematuria and proteinuria. Albuminuria increased by ~50% at 24 h after injection of IC. EM confirmed electron-dense mesangial deposits and showed podocyte injury and erythrocytes in Bowman's space. In control mice injected with only Gd-IgA1, IgA1 deposited only transiently and did not cause renal injury. Morphometric analysis of glomeruli in PAS-stained renal tissue sections showed mesangial hypercellularity and matrix expansion. Mean number of nuclei per unit area was the same in naïve mice and mice injected with Gd-IgA1 (38.5 ± 4.8 *vs.* 39.4 ± 6.5), but was higher in mice injected with IC (51.6 ± 8.0). Matrix expansion and enhanced mesangial expression of alpha-smooth muscle actin were observed in the mesangium of mice injected with IC.

Conclusions: We confirmed the role of Gd-IgA1-IgG IC in IgAN. This new animal model provides a useful tool for elucidation of pathogenesis of IgAN and for testing and development of future therapeutic strategies.

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FR-PO651

Role of *N*-Acetylgalactosaminyl Transferases in the Synthesis of Aberrant IgA1 *O*-Glycans in IgA Nephropathy Milan Raska,[1,2] Koshi Yamada,[2] Tyler J. Stewart,[2] Milada Stuchlova-Horynova,[1,2] Zhi Qiang Huang,[2] Hitoshi Suzuki,[3] Zina Moldoveanu,[2] Bruce A. Julian,[2] Robert J. Wyatt,[4] Jiri F. Mestecky,[2] Matthew B. Renfrow,[2] Ali G. Gharavi,[5] Jan Novak.[2] *[1]Palacky University, Olomouc, Czech Republic; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Juntendo University, Tokyo, Japan; [4]University of Tennessee, Memphis, TN; [5]Columbia University, New York, NY.*

Background: IgA nephropathy (IgAN) is associated with the presence of IgA1 with galactose (Gal)-deficient *O*-glycans in the hinge region (HR) which are recognized as an autoantigen by specific antibodies. The mechanisms leading to the aberrant glycosylation are related to changes in the expression, activity, and/or localization of specific glycosyltransferases. The initiation step in *O*-glycan formation is attachment of *N*-acetylgalactosamine (GalNAc) to the IgA1 HR, catalyzed by GalNAc-transferases (GalNAc-Ts). Among the 17 known human GalNAc-Ts, only GalNAc-T14 is differentially expressed in IgAN patients and healthy controls. Here, we determined the effect of down-regulation of GalNAc-T14 expression on IgA1 glycosylation in IgA1-producing cells.

Methods: EBV-immortalized IgA1-secreting cellsderived from circulatory B cells of IgAN patients (n=3)and healthy controls (HC, n=3) were subjected to GalNAc-T14 siRNA knock-down. RealTime PCR was used for determination of GalNAc-T14 expression and lectin ELISA for determination of Gal deficiency of HR *O*-glycans.

Results: We confirmed that all tested IgAN cell lines overexpressed GalNAc-T14 in comparison to HC cell lines. The efficacy of GalNAc-T14 siRNA knock-down was >80% for all tested cell lines. Lectin ELISA confirmed that decrease in GalNAc-T14 expression leads to less Gal deficiency, especially in IgAN cell lines. This observation indicates that overexpression of GalNAc-T14 observed in IgAN cell lines could contribute to Gal deficiency of IgA1 molecules in IgAN.

Conclusions: GalNAc-T14 is the only overexpressed GalNAc-T in IgAN cell lines. As knock-down of its expression resulted in decrease of Gal deficiency on IgA1, we conclude that disregulation of GalNAc-T14 expression represents a new mechanism contributing to etiology of IgAN.

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FR-PO652

Enalapril Treatment in Early Life Causes Lifelong Progressive Renal Injury in the Kidneys of Adult Male Rats Hyung Eun Yim, Kee Hwan Yoo, In Sun Bae, Young Sook Hong. *Department of Pediatrics, Korea University Medical Center, Seoul, Republic of Korea.*

Background: Interruption of the renin angiotensin system (RAS) during the period of ongoing nephrogenesis produces renal histological abnormalities and functional defect. We have previously shown that the RAS block even after the completion of nephrogenesis induces renal injury in young male rats. However, at present there is a lack of studies as to long-term renal effects exposed to blockade of the RAS after the achievement of nephrogenesis. The aim of the present study was therefore to determine the longstanding renal consequences of early postnatal RAS inhibition after the completion of nephrogenesis in adult male rats.

Methods: Newborn Sprague-Dawley male pups were given enalapril (30 mg/kg/day) or vehicle by orogastric tube between the ages of 2 and 4 weeks postnatally. Body weight, blood pressure (BP) and renal alterations were determined at 6 and 12 months, respectively.

Results: Pups in the neonatally enalapril-treated rats weighed less than rats in the control group between 16 days and 5 weeks of age and more than those between 14 weeks and 12 months ($P < 0.05$). Mean BP levels in the enalapril-treated rats were not different from the controls at 6 months; however, they were higher than the LC group at 12 months ($P < 0.05$). At 12 months, apoptotic renal cortical cells were increased in the enalapril-treated rats, compared to the controls ($P < 0.05$). The enalapril-treated group showed increased glomerulosclerosis and tubulointerstitial fibrosis at 6 and 12 months ($P < 0.05$). In the immunoblotting and immunohistochemistry, neonatally enalapril-treated ratsshowed increased intra-renal expression of matrix metalloproteinase (MMP)-9 and decreased angiotensin II receptor type (AT) 1 at 6 months ($P < 0.05$). At 12 months, the expressions of tissue inhibitor of MMP-1 and plasminogen activator inhibitor-1 were increased and AT2 expression was decreased in the kidneys of neonatally enalapril-treated rats ($P < 0.05$).

Conclusions: Our findings suggest that angiotensin II inhibition even after the completion of nephrogenesis can induce deferred systemic hypertension and progressive renal damage in adult male rats.

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FR-PO653

Early Treatment with Enalapril Is Not Renoprotective in 'Programmed' Obese 3 Month-Old Rats Hyung Eun Yim, Kee Hwan Yoo, In Sun Bae, Young Sook Hong. *Department of Pediatrics, Korea University Medical Center, Seoul, Republic of Korea.*

Background: Countering the renin angiotensin system (RAS) has been reported to be beneficial in obese patients with chronic kidney disease.We have shown that early postnatal overnutrition leads to the development of renal injury in adult rats. This study was aimed to investigate that the RAS block in early life can ameliorate renal injury induced by early postnatal overnutrition.

Methods: Three or 10 male pups per mother were assigned to either the small litter (Obese group) or normal litter (Lean group) rats during the first 21 days of life. With this, all pups were randomized into 4 groups, and treated with enalapril (Obese enalapril, OE; Lean enalapril, LE) or vehicle (Obese control, OC; Lean control, LC) between the ages of 2 and 4 weeks postnatally. Body weight, blood pressure (BP) and renal alterations were determined at 3 months.

Results: Pups in the OC group weighed more than rats in the LC group between 7 days and 3 months of age ($P < 0.05$). Enalapril decreased body weights in the Lean group at weaning (22 days) and at 3 months ($P < 0.05$); however, body weights in the OE group were not different from the OC group at 3 months. Mean BP levels in the LE, OC and OE groups were higher than the LC group at 3 months ($P < 0.05$) while there was no difference between the OC and OE groups. At 3 months, the LE group showed increased renal cell apoptosis, glomerulosclerosis, and tubulointerstitial fibrosis and decreased renal cell proliferation, compared to the LC group ($P < 0.05$). The index scores of glomerulosclerosis and tubulointerstitial fibrosis were higher in the OE group than the OC group ($P < 0.05$). In immunoblotting and immunohistochemistry, the LE group showed increased intra-renal angiotensin II receptor type (AT) 2 and matrix metalloproteinase (MMP)-9 and decreased renin and tissue inhibitor of MMP (TIMP)-1 expression, compared to the LC group ($P < 0.05$). The OE group also demonstrated increased intra-renal AT2 and decreased AT1 and TIMP-1 expression, compared to the OC group (< 0.05).

Conclusions: The RAS block in early life can induce the detrimental renal impact and may be not renoprotective in 'programmed' obese adult rats.

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FR-PO654

Lack of Renoprotective Effect of Angiotensin 1-7 and Angiotensin 2-10 in a Rat Model of Hypertension and Focal Segmental Glomerulosclerosis

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Background: Angiotensin (Ang) II mediates progressive glomerulosclerosis (GS). We previously showed that Ang I is largely converted to Ang 1-7 and Ang 2-10 by glomeruli, and observed that Ang 2-10 binds to the AT_2 receptor (R) with more affinity than to the AT_1 R. Ang 1-7 may be antifibrotic. We hypothesized that Ang 1-7 and Ang 2-10 could be renoprotective in a model of focal segmental GS, the Fawn-Hooded Hypertensive (FHH) rats.

Methods: Rats (n=9/group) underwent uninephrectomy at week (wk) 6 to accelerate GS and implantation of osmotic minipumps at wk 18 for intravenous (IV) delivery of saline (S), Ang 1-7 or Ang 2-10 (100 or 400 ng/kg/min) until wk 30. Two other groups received oral captopril (CAP, 100 mg/kg/d) or losartan (LOS, 20 mg/kg/d).

Results: S-treated rats were hypertensive at wks 24 (179 ± 2 mmHg) and 30 (185 ± 3 mmHg). CAP and LOS decreased systolic blood pressure (SBP) (156 ± 7 and 155 ± 5 mmHg at wk 24, 145 ± 5 and 146 ± 5 mmHg at wk 30; for CAP and LOS, respectively; p<0.0001 vs. S). In contrast, low-dose Ang 1-7 had no effect on SBP, low-dose Ang 2-10 raised it by wk 30 (194 ± 3 mmHg; p<0.001 vs. S), and high-dose Ang 1-7 and Ang 2-10 raised SBP (188 ± 3 and 187 ± 3 mmHg at wk 24, 197 ± 3 and 196 ± 4 mmHg at wk 30; for high-dose Ang 1-7 and Ang 2-10, respectively; p<0.001 vs. S). Proteinuria in S-treated rats was 2 (1–5), 537 (184–862), 978 (433–2839) and 738 (622–2032) mg/d, at wk 6, 18, 24 and 30, respectively. The course of proteinuria between wks 18 and 24 was significantly regressed by CAP (+ 342 ± 289 vs. - 225 ± 453 mg/d, S vs. CAP, respectively; p<0.01), but was otherwise not modified by any of the other treatments. CAP significantly reduced global GS (34 ± 9 vs. 9 ± 4%, p=0.03) and tubular injury score (2.6 ± 0.4 vs. 1.3 ± 0.4, p=0.04) compared to S, whereas structural damage was not halted by Ang 1-7 or Ang 2-10 treatment.

Conclusions: In summary, chronic IV administration of Ang 1-7 and Ang 2-10 failed to ameliorate kidney damage in uninephrectomized FHH rats.

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FR-PO655

Inhibition of Renin Activity Slows down the Progression of HIV-Associated Nephropathy (HIVAN)

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Background: Renin-angiotensin system has been reported to play an important role in the development of HIVAN. In the present study, we evaluated the effect of aliskiren, an inhibitor of renin activity (aliskiren) on the progression of renal lesions in two different mouse models (Vpr and Tg26) of HIV-associated nephropathy (HIVAN).

Methods: In protocol A, Vpr mice were fed either water (C-VprA) or doxycycline (D-VprA) in their drinking water for six weeks. In protocols B and C, Vpr mice received either saline (C-VprB/C), Doxy (D-VprB/C) + normal saline, or Doxy + aliskiren (AD-VprB/C) for 6 weeks (protocol B) or 12 weeks (protocol C). In protocols D and E, Vpr mice were fed Doxy for 6 weeks followed by kidney biopsy. Subsequently, half of the mice were administered either saline (D-VprD/D) or aliskiren e(AD-VprD/D) for 4 weeks (protocol D) or 8 (protocol E) weeks. In protocol F, Tg26 mice were administered vehicle, aliskiren, or hydralazine for 4 weeks. Subsequently, renal biomarkers were determined and severity of renal lesions were scored.

Results: All D-VprA showed renal lesions in the form of focal segmental glomerular sclerosis and dilatation of tubules. In protocol B and C, aliskiren diminished both progression of renal lesions and proteinuria. In protocol C, aliskiren also diminished (P<0.01) rise in BUN. In all groups, Doxy-treated mice displayed increased serum Ang I levels (the product of plasma renin activity, PRA); on the other hand, all aliskiren-treated mice displayed diminished serum Ang I levels. Renal tissues of D-VprC displayed increased Ang II content; however, aliskiren attenuated renal tissue Ang II production in AD-VprC. In protocol D, AD-VprD showed 24% increase in number of sclerosed glomeruli when compared to 139% increase in sclerosed glomeruli in D-VprD (P<0.01) from their baseline. The attenuating effect of aliskiren on the progression of renal lesions continued in AD-VprE. Aliskiren also diminished blood pressure, proteinuria and progression of renal lesions in Tg26 mice.

Conclusions: These findings indicate that renin activity inhibition retards the progression of HIVAN.

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FR-PO656

Functional Role of Aquaporin-1 in Renin Angiotensin System-Induced Hypertension and Kidney Injury

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Background: Aquaporin-1 (AQP1), a major water channel in proximal tubules is responsible for reabsorbing 80% of glomerular filtrate fluid. Angiotensin II (Ang II) increases AQP1 gene expression in cultured rat immortalized renal proximal tubular cells (IRPTCs) and in rat kidneys *in vivo*. Little is known about AQP1 regulation in animal models of renin angiotensin system (RAS) -induced hypertension and kidney injury. We reported that RAS blockade prevents intrarenal RAS activation, hypertension, and nephropathy progression in angiotensinogen (Agt)-transgenic (Tg) mice specifically overexpressing Agt in their RPTCs. The present studies explored the role of AQP1 in RAS-induced hypertension and kidney injury, focusing on underlying molecular mechanisms.

Methods: Male Agt-Tg mice (and non-Tg littermates served as control) ± RAS blockade (losartan and perindopril treatment from 13 to 20 weeks). Systolic blood pressure (SBP), renal reactive oxygen species (ROS) generation, glomerular filtration rate (GFR), urinary albumin/creatinine ratio and Ang II levels, as well as renal morphology were measured. Renal proximal tubular Agt, AQP1 and HO-1 (heme oxygenase-1) gene expression was assessed by real time-qPCR, western blot and immunohistochemistry.

Results: Untreated, Agt-Tg mice developed hypertension, renal hypertrophy and tubulointerstitial fibrosis with upregulation of AQP1 and downregulation of HO-1expression in their RPTCs as compared with non-Tg littermates. RAS blockade prevented these abnormalities. Furthermore, *in vitro* studies showed that AQP1 gene expression is significantly upregulated in an Agt-RPTCs stable clone (stably transfected with rat Agt cDNA), and this augmented AQP1 gene expression was normalized by losartan via the activation of HO-1 gene.

Conclusions: Our data suggest that the counter balance of AQP1 and HO-1 gene expression plays a key role in RAS -induced hypertension and kidney injury, both *in vivo* and *in vitro.*

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FR-PO657

Angiotensin II Blockade and Calorie Restriction Prevent Progression by Different but Complementary Mechanisms

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Background: Podocyte depletion drives progression in glomerular diseases. Angiotensin II drives progression through promoting podocyte loss from destabilized glomeruli (Fukuda et al, Kidney Int 2011). Progressive podocyte loss leading to progression and ESKD can also be triggered by growth-dependent glomerular enlargement, as demonstrated using the podocin promoter-driven AA4EBP1 transgenic rat model. In this model proteinuria, glomerulosclerosis and progression to ESKD is triggered by body growth alone and can be completely prevented by calorie restriction to prevent weight gain and glomerular enlargement (Fukuda, JASN, 2012, in press).

Methods: To compare mechanisms by which calorie restriction and angiotensin II blockade prevent progression we used the uninephrectomized podocin-AA4EBP1 transgenic rat model on an ad lib or 40% calorie restricted diet, with and without enalapril/losartan delivered in the drinking water. Morphometry was used to measure glomerular tuft volume, podocyte number per tuft, podocyte density and glomerulosclerosis.

Results: Both angiotensin II blockade on an ad lib diet and calorie restriction prevented progression (prevented proteinuria, reduction in podocyte density and glomerulosclerosis), but by different mechanisms. Calorie restriction maintained normal podocyte density by preventing glomerular tuft enlargement. In contrast angiotensin II blockade on an ad lib diet did not prevent glomerular tuft enlargement, but reduced the rate of podocyte loss so that podocyte number per tuft increased to maintain normal podocyte density.

Conclusions: Reduced podocyte density as the trigger and driver of podocyte depletion-dependent progression can be prevented by two different mechanisms (calorie restriction and angiotensin II blockade), which should be complementary in the clinic.

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FR-PO658

Aldosterone Induces Proteasome Aggregation in Distal Renal Tubules

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Background: Aldosterone is the main mineralocorticoid and thereby responsible for body Na^+ conservation. Prolonged plasma aldosterone elevation induces gene transcription and protein synthesis. We hypothesized that in renal tubules, the aldosterone induced increase in protein synthesis and turnover would challenge the cellular protein degradation by proteasomes.

Methods: Male Wistar Rats (Taconic) were given 50µg/kg body wt· day^{-1} aldosterone or vehicle for 7 days through subcutaneous osmotic minipumps. Kidney samples were prepared for immunoblotting and immunohistochemistry. Electron microscopy was also used to resolve the structure of protein aggregates. The kidney homogenate was labelled with aggresome dye and sorted by using Fluorescence activated particle sorting. The resulting samples were vaccum dried and processed for mass spectrometry.

Results: The cellular accumulation of specific proteins and the development of aggresomes were compared in kidney samples from rats receiving 50 µg aldosterone/ kg body weight/ 24 hrs and vehicle in osmotic minipumps for 7 days by immunohistochemistry and electron microscopy. Aldosterone induced a 1.5-fold increase in proteasome 20s abundance in distal renal tubules with a concomitant increase in aggresome-like structures. An alternative antibody revealed a much stronger induction of the aggresome-like punctate labeling, with 80% co-localization with proteasome 20s labeling, but negligible

co-localization with endosomal and lysosomal markers. Fluorescence activated particle sorting and subsequent mass spectrometry identified several proteins in the aggresomes including rat keratins specific to simple epithelia. The accumulation of aggresomes was specific to the aldosterone sensitive renal tubules, especially the distal convoluted tubules.

Conclusions: We conclude that high physiological concentrations of a key hormone in the regulation of renal function challenges the protein degradation system to such a degree that certain proteins accumulate in aggresomes.

FR-PO659

Inhibition of Angiotensin II (AngII) Induced Increases in Glomerular Permeability by Scavengers of Reactive Oxygen Species (ROS), Inhibitors of Small GTPases and by Calcineurin Blockade Josefin Axelsson, Anna Rippe, Kristinn Sverrisson, Bengt Rippe. *Department of Nephrology, Lund University, Lund, Sweden.*

Background: AngII can induce acute increases in the permeability of the glomerular filtration barrier (GFB). After binding of AngII to its receptor (AT1R) there is Ca^{2+} influx into the cell, which activates signaling cascades, involving e.g. calcineurin, kinases, and, further downstream, small GTPases, such as Rac1 and RhoA. In the present study we sought to interact with elements of this signaling cascade in order to test new antiproteinuric agents during AngII activation.

Methods: In anaesthetized Wistar rats the left urether was cannulated for urine collection and blood access was achieved. Rats were infused with AngII (16 ng/kg/min) alone, or together with the ROS scavengers, 4-Hydroxy-TEMPO (TEMPOL) or dimethylthiourea (DMTU), or the RhoA-kinase inhibitor, Y-27632, the Rac-1 inhibitor, NSC23766, or the calcineurin inhibitor, tacrolimus (Prograf®). Polydisperse FITC-Ficoll-70/400 (mol.radius 13-80Å) and ^{51}Cr-EDTA were infused throughout the experiment. Plasma and urine samples were taken during baseline and at 5 and 15 min after the start of the infusions and analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients for $Ficoll_{13\text{-}80Å}$. GFR was analyzed from the plasma to urine clearance of ^{51}Cr-EDTA.

Results: AngII infusion into rats caused rapid, partly reversible increases in glomerular permeability to large Ficoll molecules ($Ficoll_{50\text{-}80Å}$), peaking at 5-15 min. The increases in glomerular permeability were totally abrogated by TEMPOL and partly by DMTU. RhoA and Rac-1 inhibition were also effective in reducing the permeability actions of AngII, as was tacrolimus (max effect at 15 min).

Conclusions: AngII markedly increased the permeability of the GFB. This effect cannot not only be blocked by AngII receptor inhibition, but also by interactions with several elements of the AT1R downstream signaling cascade. Scavengers of ROS are also able to reduce the permeability response to AngII, pointing to new avenues for the treatment of proteinuria in states of AngII activation.

Funding: Government Support - Non-U.S.

FR-PO660

Epigenetic Role of WHSC1L1 in Nephrin Gene Expression Yugo Ito, Zentaro Kiuchi, Yukino Nishibori, Kunimasa Yan. *Pediatrics, Kyorin University, Tokyo, Japan.*

Background: The epigenetic regulation of nephrin gene expression is poorly understood. Unbiased transcriptome profiling identified WHSC1L1 (Wolf-Hirschhorn syndrome candidate 1-like 1) to be one of the highly glomerulus-specific transcripts (EMBO J, 25:1160, 2006). WHSC1L1 is a histone methyltransferase known to be involved in epigenetic control in cancers. Thus, the present study aimed to investigate whether WHSC1L1 plays a regulatory role in nephrin gene expression.

Methods: HEK-293 cell line stably expressing human full-length WHSC1L1 (WHSC1L1-293) and anti-human WHSC1L1 antibody were generated. The localization and the expression pattern of protein and mRNA of WHSC1L1 in the adult and fetal mouse kidney were determined by immunohistochemistry, western blot study and RT-PCR. Identification of methylated lysine on histone H3 interacting with WHSC1L1 was studied by siRNA experiment with WHSC1L1 using wild-293 cells. The interaction of WHSC1L1 with nephrin promoter was studied by the chromatin immunoprecipitation assay using anti-WHSC1L1 antibody in WHSC1L1-293 cells. Finally, the expression of nephrin and WT1 mRNA in the samples from wild-293 and WHSC1L1-293 cells was compared by RT-PCR.

Results: WHSC1L1 of the kidney consisted of 2 isoforms: long-form 200-kDa and short-form 100-kDa proteins. Glomerulus expressed both forms of WHSC1L1 whereas tubulus expressed only short-form. The glomerular WHSC1L1 in the adult kidney predominantly located at the podocyte nucleus, which was also apparent at the pre-capillary stage. Inhibition of WHSC1L1 gene expression in wild-293 cells revealed the exclusive reduction of methylated histone H3K36. Nephrin promoter was precipitated by chromatin-WHSC1L1 complex. Finally the expression of nephrin mRNA was accelerated by the WHSC1L1 induction.

Conclusions: WHSC1L1 is a novel podocyte molecule that up-regulates nephrin by epigenetic regulation.

FR-PO661

The Lipid-Modifying Enzyme PON2 as a New Slit Diaphragm Protein Henning Hagmann,[1] Dontscho Kerjaschki,[2] Bernhard Schermer,[1] Thomas Benzing,[1] Paul T. Brinkkoetter.[1] *[1]Nephrology, University Hospital Cologne, Cologne, Germany; [2]Clinical Pathology, University of Vienna, Vienna, Austria.*

Background: Signalling at the mammalian slit diaphragm (SD) via the non-selective cation channel TRPC6 plays an important role in podocyte disease. It has been shown that TRPC6 protein expression as well as TRPC6 channel activity are increased in glomerular diseases such as focal segmental glomerulosclerosis, minimal change disease and membranous nephropathy.

Previous studies suggest that the activity of the TRPC6 channel depends largely on the lipid environment directly surrounding the channel protein. The mammalian Podocin-TRPC6 complex shows remarkable homology to the neuronal touch sensor complex of C.elegans where the Podocin homologue MEC-2 orchestrates the lipid environment necessary for proper touch sensation via the degenerin cation channel MEC-4/-10. In addition the lipid modifying enzyme MEC-6 interacts with and enhances the channel activity of MEC-4/-10.

Results: Here, we identify the mammalian homologue of MEC-6, the paraoxonase family protein PON2, as a novel slit diaphragm protein. PON2 not only associates with cholesterol-rich detergent resistant membrane domains but also co-precipitates directly with SD-proteins in pull-down experiments. In addition, we provide first-time evidence that PON2 is an integral transmembrane protein that localizes to the plasma membrane with its enzymatically active domain facing extracellular.

To understand the implication of PON2 on cellular lipid metabolism we generated a stable PON2-deficient mouse podocyte cell line by lentiviral gene transfer. Functional studies on cellular lipid-profiles revealed that total cholesterol levels are increased whereas DAG levels appeared to be decreased in PON2-deficient cells.

Conclusions: In conclusion we identified PON2 as a novel transmembrane protein at the slit-diaphragm. It not only interacts directly with known SD proteins but also modifies the lipid surrounding at the plasma membrane. It could be easily envisioned that PON2 represents an important regulator of TRPC6 channel activity similar to its homologue MEC-6 in the nematode C.elegans.

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FR-PO662

Shp2 a Non-Receptor Tyrosine Phosphatase Regulates Nephrin Phosphorylation and Podocyte Mobility Rakesh Verma, Madhusudan M. Venkatareddy, Puneet Garg. *Internal Medicine/ Nephrology, University of Michigan, Ann Arbor, MI.*

Background: Recent studies suggest that podocyte injury or foot process effacement is a form of podocyte mobility, a result of increased lamellipodia formation. Nephrin's ability to regulate podocyte actin dynamics, lamellipodia formation and focal adhesion dynamics in a phosphorylation dependent manner suggests its role in foot process mobility or effacement. Furthermore, increase in Nephrin tyrosine phosphorylation has been observed following podocyte injury and during podocyte development. Src kinase Fyn has been shown to be responsible for Nephrin phosphorylation. Though the consequences of Nephrin phosphorylation are better understood, the upstream molecular events responsible for Src kinase activation and Nephrin phosphorylation are not known.

Methods: We used biochemical and cell biology studies to demonstrate association between Nephrin and Shp2. Mice with podocyte specific deletion of shp2 were generated by breeding Shp2 floxed mice with Podocin-Cre mice.

Results: In a biased screen we found association between purified recombinant Nephrin and non-receptor phosphatase Shp2. Shp2 has been shown to dephosphorylate a regulatory tyrosine (Y416) on Src kinases, resulting in increased kinase activity. Using the CD16 clustering approach we observed recruitment of shp2 to CD16-Nephrin clusters at the membrane. We observed increase in Nephrin phosphorylation in the presence of shp2 in transfected cells. As anticipated, immunostaining of newborn mouse kidneys as well as kidneys perfused with protamine sulfate showed increased Shp2 activity. Furthermore, mouse kidneys perfused with Shp2 small molecule inhibitor prior to protamine sulfate abrogated both Nephrin phosphorylation and foot process spreading. Similarly, mice with podocyte specific deletion of Shp2 are resistant to foot process spreading following injury.

Conclusions: Our results suggest that, Nephrin phosphorylation is necessary for foot process spreading observed in glomerular diseases that present with proteinuria. Furthermore, inhibition of signaling events that increase podocyte mobility or lamellipodia formation prevent podocyte foot process spreading following injury.

Funding: NIDDK Support

FR-PO663

The Novel Nephrotic Syndrome-Causing Gene Products, ARHGDIA and KANK2 Interact with and Regulate Rho Family Small GTPases in Podocytes Heon Yung Gee,[1] Shazia Ashraf,[1] Pawaree Saisawat,[1] Toby W. Hurd,[1] Virginia Vega-Warner,[1] Humphrey Fang,[1] Lutz T. Weber,[2] Julia Hoefele,[3] Bodo B. Beck,[4] Corinne Antignac,[5] Friedhelm Hildebrandt.[1,6] *[1]Departments of Pediatrics and of Communicable Diseases, University of Michigan, Ann Arbor, MI; [2]Pediatric Nephrology, Ludwig-Maximilian's University, Munich, Germany; [3]Center for Human Genetics and Laboratory Medicine Dr. Klein and Dr. Rost, Martinsried, Germany; [4]Institute of Human Genetics, Institute of Human Genetics, Cologne, Germany; [5]Inserm U983, Necker Hospital, Paris, France; [6]Howard Hughes Medical Institute.*

Background: Nephrotic syndrome (NS) is a heterogeneous group of disorders characterized by gross proteinuria with hypoalbuminemia and edema. We performed homozygosity mapping (HM) and whole exome resequencing (WER) in 63 sibling cases of childhood onset NS from 28 different families. This resulted in the identification of ARHGDIA and KANK2 as novel recessive single-gene causes of NS.

Methods: The function of ARHGDIA and KANK2, and their relation to Rho family GTPases, were examined in podocytes and rat kidney tissues using cell biological and molecular approaches.

Results: We found two ARHGDIA mutations in 2 patients with steroid-resistant NS, a nonsense (p.R120X) and a missense (p.G173V) mutation. We demonstrated by co-immunoprecipitation that ARHGDIA, KANK2, and Rho family GTPases physically interacted in cultured human podocytes and rat glomeruli, which was also confirmed by GST pulldown assay. Interestingly, both mutations in ARHGDIA abrogated interaction with RHOA, RAC1 and CDC42. In addition, the mutations increased the active GTP-bound RAC1 and CDC42 which result in a more migratory phenotype of podocytes. We found a homozygous missense mutation in KANK2 (p.S181G) in two siblings with steroid-sensitive NS. This missense mutation enhances its interaction with ARHGDIA and Rho GTPases and decreased the active states of RHOA, RAC1 and CDC42.

Conclusions: By our combined gene strategy we found two novel NS-causing genes ARHGDIA and KANK2, which interact within a protein complex of glomerular podocytes. Our data suggest that imbalances between RHOA and RAC1/CDC42 signaling in podocytes can cause NS.

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FR-PO664

Like Crk1/2, Podocyte-Specific CrkL Deletion Prevents Foot Process Spreading Following Mouse Podocyte Injury Britta George,[1,2] Abdul A. Soofi,[3] Jidong Zhang,[1] Rakesh Verma,[3] Lawrence B. Holzman.[1] *[1]Renal-Electrolyte and Hypertension, University of Pennsylvania, Philadelphia, PA; [2]Renal, University of Münster, Münster, Germany; [3]Renal, University of Michigan, Ann Arbor, MI.*

Background: Activation of Nephrin induces actin cytoskeletal remodeling resulting in lamellipodia formation in podocyte culture in a PI-3 kinase, FAK, p130Cas, and Crk1/2-dependent fashion that is distinct from the previously described nephrin-Nck1/2 pathway necessary for assembly and polymerization of actin filaments (J Clin Invest. 2012;122(2):674-92). In mice, podocyte-specific deletion of Crk1/2 prevented foot process effacement in one model of podocyte injury and attenuated foot process effacement and associated proteinuria in a second model. These observations suggested that cellular mechanisms governing lamellipodial protrusion in culture are similar to those used in vivo during foot process effacement. Because Crk1/2 null mice were normal at birth and survived to 20 months without developing glomerulopathy, we hypothesized that the Crk1/2 paralog, CrkL, functionally complements Crk1/2 in a podocyte-specific context.

Methods: Podocyte-specific CrkL null mice or podocyte-specific Crk1/2;CrkL double knockout mice were derived and characterized by light microscopy, scanning and transmission electron microscopy, and by examining urine albumin/creatinine ratio over time.

Results: Podocyte-specific CrkL null mice had a phenotype identical to that observed in Crk1/2 null mice: CrkL null mice developed and aged normally but were protected from protamine sulfate-induced foot process effacement. Further, simultaneous podocyte-specific deletion of Crk1/2 and CrkL resulted in proteinuria that was first detected by six weeks post-gestation and that was associated with unusual foot process elongation detected by scanning electron microscopy.

Conclusions: These results are consistent with the conclusions that Crk1/2 and CrkL functionally complement each other in the podocyte, and that Crk1/2 and CrkL are required for developing typical foot process architecture. These results also emphasize that Crk1/2 or CrkL or their associated protein complex might serve as therapeutic targets for glomerular disease.

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FR-PO665

The Calcium Channel TRPC6, Known to Cause FSGS when Chronically Hyperactive, Protects Podocytes from Acute Complement-Mediated Injury Andreas D. Kistler,[1] Jeffrey W. Pippin,[2] Geetika Singh,[3] Katherina Walz,[1] Amit K. Dinda,[3] Christian Faul,[1] Stuart J. Shankland,[2] Jochen Reiser.[1] *[1]University of Miami; [2]University of Washington, Seattle, WA; [3]All India Institute of Medicine, New Delhi, India.*

Background: Gain-of-function mutations in the calcium channel TRPC6 lead to autosomal dominant FSGS and podocyte expression of normal TRPC6 is increased in acquired human glomerular diseases, particularly in membranous nephropathy (MN). We therefore speculated that overexpression of TRPC6 in cultured podocytes may lead to loss of actin stress fibers through calcineurin-mediated dephosphorylation of synaptopodin and subsequent cleavage by cathepsin L.

Methods: We used standard methods, including podocyte culture, lentiviral gene transfer, Ca-imaging and cell surface biotinylation.

Results: Overexpression of TRPC6 in differentiated podocytes did not affect synaptopodin levels or actin stress fibers despite correct membrane localization and activity of the channel. Unexpectedly, overexpression of TRPC6 protected podocytes from complement-induced injury, an in vitro model of MN. In contrast, overexpression of dominant-negative TRPC6, knock down of TRPC6 and the administration of a TRPC antagonist increased podocyte sensitivity to complement. This effect was mediated by CaMKII: complement attack activated CaMKII in podocytes and the degree of activation correlated with TRPC6 levels. Pretreatment of podocytes with a CaMKII inhibitor phenocopied the effect of TRPC6 inhibition. Human MN biopsy samples, where induced TRPC6 expression has been previously shown, displayed increased activity of CaMKII. In the nephrotoxic serum (NTS) nephritis model, where complement contributes to glomerular injury, podocyte-specific TRPC6 transgenic mice showed stronger CaMKII activation, reduced podocyte FP effacement and reduced levels of proteinuria. In contrast, TRPC6 knock out mice exhibited reduced CaMKII activation and higher levels of proteinuria after NTS injection compared to WT littermates.

Conclusions: These data suggest a dual role of TRPC6 in podocytes: whereas chronic hyperactivity leads to FSGS, acute activation protects from complement-mediated damage in the short term.

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FR-PO666

Matrix Metalloproteinase-7 Promotes Podocyte Injury and Proteinuria by Proteolytic Shedding of Nephrin Ying Li, Dong Zhou, Youhua Liu. *Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Recent studies implicate Wnt/β-catenin signaling in the pathogenesis of podocyte dysfunction and proteinuria. However, the underlying mechanism remains poorly understood. As a transcriptional target of the Wnt/β-catenin signaling, matrix metalloproteinase-7 (MMP-7) is demonstrated as a surrogate biomarker for Wnt/β-catenin signaling in the kidney. Here, we tested the hypothesis that MMP-7 may mediate the pathogenic action of Wnt/β-catenin in the development and progression of podocyte injury and proteinuria.

Methods: We assessed the potential role of MMP-7 in the proteolytic shedding of slit diaphragm proteins. Using MMP-7 null mice, we investigated its effect on podocyte dysfunction and proteinuria after injury in vivo.

Results: In podocytes, MMP-7 expression was transcriptionally controlled by Wnt/β-catenin signaling. In ex vivo glomerular culture system, MMP-7 induced nephrin degradation in a time- and dose-dependent manner. Furthermore, this MMP-7–mediated nephrin cleavage was prevented by MMP inhibitor II. MMP-7 treatment leaded to flattening and fusion of the podocyte foot processes and increased glomerular permeability to albumin in ex vivo glomerular culture. The proteolytic shedding of nephrin mediated by MMP-7 was also confirmed in vitro using recombinant nephrin protein. Interestingly, onlyMMP-7, but not MMP-2 or MMP-9, mediated cleavage of nephrin. To delineate the role of MMP-7 in vivo in causing proteinuria, we utilized genetic MMP-7 knockout mice. Comparing with wild-type controls, MMP-7 null mice were protected from developing albuminuria after adriamycin injection. Conversely, intravenous injection of recombinant MMP-7 protein or ectopic expression exogenous MMP-7 plasmid in vivo caused podocyte dysfunction and de novo albuminuria in normal healthy mice. Inhibition of Wnt/β-catenin by Paricalcitol, a synthetic vitamin D analogue, repressed MMP-7 expression and prevented proteinuria after adriamycin injection.

Conclusions: These results identify podocyte slit diaphragm protein nephrin as a novel substrate of MMP-7. Our data also suggest that MMP-7 plays a critical role in mediating Wnt/β-catenin-induced podocyte injury and proteinuria.

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FR-PO667

Endothelium-Derived tPA Promotes Podocyte Injury and Proteinuria by Activating LRP-1/β1 Integrin Signaling Ying Li, Youhua Liu. *Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Background: Endothelium and podocytes are essential, integral constituents of the glomerular filtration barrier. However, whether or how endothelial dysfunction/activation induces podocyte injury and proteinuria is elusive.

Methods: We studied this issue by investigating the role of endothelium-derived tissue-type plasminogen activator (tPA) in mediating podocyte injury and proteinuria.

Results: We showed that tPA was specifically upregulated in the glomerular endothelium in human biopsies of patients with proteinuric kidney diseases such as focal segmental glomerulosclerosis (FSGS) and diabetic nephropathy. tPA was also rapidly induced in mouse glomeruli in adriamycin (ADR) nephropathy, prior to the onset of proteinuria in this model. To delineate the role of tPA in proteinuria, we utilized genetic tPA knockout mice. Comparing with wild-type controls, tPA null mice were almost completely protected from developing albuminuria after ADR injection. Similarly, tPA ablation also preserved podocyte integrity, nephrin, podocin and WT1 expression in ADR nephropathy. Interestingly, LDL receptor-related protein 1 (LRP1), known as tPA functional receptor, was activated in glomerular podocytes in proteinuric nephropathies. In vitro, tPA induced tyrosine phosphorylation of LRP1 in cultured mouse podocytes, which triggered focal adhesion kinase (FAK) phosphorylation, ERK1/2 activation, p90RSK and GSK-3β phosphorylation, leading to Snail1 induction. In ex vivo glomerular culture system,tPA suppressed nephrin expression and increased glomerular permeability to albumin. Intravenous infusion of recombinant tPA protein caused podocyte dysfunction and de novo albuminuria in normal healthy mice. Conversely, podocyte-specific ablation of LRP1 in conditional knockout mice ameliorated ADR-mediated proteinuria and podocyte injury.

Conclusions: These results suggest that endothelium-derived tPA promotes podocyte injury and proteinuria by activating a novel signaling cascade of LRP-1/β1 integrin/GSK-3β/Snail1.

Funding: NIDDK Support

FR-PO668

NF-kB-Dependent Chemokine Production in Podocytes Contributes to Glomerular Damage and Prolonged Proteinuria

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Background: Inflammation is a major mechanism in the development of glomerular injury and is an important cause of progressive kidney disease. NF-kB controls the expression of pro-inflammatory signaling molecules such as chemokines. The role of this pathway in podocytes is poorly understood.

Results: Here, we disrupted NF-kB signaling in podocytes by specific knockout of the NF-kB essential modulator (NEMO). Podocyte-specific NEMO-deficient mice ($NEMO^{pko}$) were healthy and did not show proteinuria or morphological kidney changes. After induction of glomerulonephritis both $NEMO^{pko}$ and control mice developed severe proteinuria. However, $NEMO^{pko}$ mice showed a rapid recovery, with remission of proteinuria and restoration of podocyte morphology. Moreover, we observed a strongly activated endothelial cell layer at day 7 following NTN-injection in control mice which was absent in the NEMO-deficient mice. At day 7 of disease, expression of proinflammatory chemokines including MCP1, MCP3, RANTES was strongly reduced in glomeruli of $NEMO^{pko}$ mice. However, the altered chemokine expression did not result in differences in the amount of infiltrating macrophages, T-lymphocytes and granulocytes. In line with these findings, cell culture experiments in NEMO-knockdown mouse podocytes showed a reduced secretion of chemokines after stimulation with IL-1 and TNFa.

Conclusions: Collectively, we hypothesize that endothelial or mesangial cells are one of the primary targets of the podocyte-dependent pro-inflammatory chemokine secretion and that the proinflammatory activity of NF-kB in podocytes aggravates proteinuria in experimental glomerulonephritis in mice. Based on these data it can be speculated that immunosuppressive drugs may not only target professional immune cells but also podocytes directly to convey their beneficial effects in various types of glomerulonephritis.

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FR-PO669

Complete Regression of Existing Podocyte Injury by Mineralocorticoid Receptor Blockade in Podocyte-Specific Arhgdia Knockout Mice

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Background: We previously reported that systemic depletion of Arhgdia (also designated as RhoGDIα) causes podocyte damage, possibly via 'Arhgdia-Rac1-mineralocorticoid receptor (MR)' cascade. In the present study, we established podocyte-specific Arhgdia knockout mice ($Arhgdia^{pod-/-}$), and analyzed morphological and functional alterations of podocytes and their underlying mechanisms.

Methods: $Arhgdia^{pod-/-}$ were established by mating $Arhgdia^{flox/flox}$ with Nephrin-Cre mice. Primary culture of podocytes was performed by cultivation of glomeruli isolated using Dynabeads method.

Results: Arhgdia was predominantly expressed in glomerular podocytes and tubular cells in control mice. In $Arhgdia^{pod-/-}$ mice, Cre recombinase was highly and exclusively expressed in WT1-positive podocytes. Podocyte-specific deletion of Arhgdia was confirmed by immunostaining of Arhgdia in the kidney and reduced expression in primary cultured podocytes from $Arhgdia^{pod-/-}$ mice. $Arhgdia^{pod-/-}$ mice spontaneously developed massive albuminuria, podocyte injury, and FSGS. At 5 weeks of age, $Arhgdia^{pod-/-}$ mice already exhibited podocyte damage (albuminuria 6730 ± 1065 μg/d vs 26± 4 μg/d in control mice), when administration of MR blocker eplerenone was initiated. Albuminuria was gradually reduced, and after 8 weeks of treatment, podocyte injury was totally regressed with disappearance of albuminuria (30 ± 8 μg/d) and normalization of nephrin staining. In cultured podocytes, Arhgdia knockdown resulted in increased active Rac1. In addition, MR expression was enhanced in the glomeruli of $Arhgdia^{pod-/-}$ mice.

Conclusions: Our results suggest a role for the 'Arhgdia-Rac1-MR' cascade within podocytes in the pathogenesis of podocyte damage in $Arhgdia^{pod-/-}$ mice. Recently, Arhgdia and Rac1 regulator Arhgap24 were identified as causative genes for some patients with nephrotic syndrome and FSGS. Our experimental data, together with these clinical findings, would provide mechanistic insights into podocyte pathobiology.

Funding: Government Support - Non-U.S.

FR-PO670

Activation of Podocyte NMDA Receptors Leads to Generation of Reactive Oxygen Species, Mobilization of TRPC6 Channels, NFAT Activation, and Apoptotis

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Background: NMDA receptors mediate several forms of synaptic plasticity. However, excessive activation of neuronal NMDA receptors evokes neurodegeneration, owing to calcium overload and oxidative stress. Podocytes express an atypical population of NMDA receptors that are preferentially activated by metabolites such as L-homocysteic acid. Here we analyze if similar effects occur in podocytes.

Methods: Experiments were performed on the MPC-5 mouse podocyte cell line, and in 21-week *db/db* mice and lean litter-mate controls. ROS generation was assayed using fluorometric assays. TRPC6 mobilization was assayed by cell-surface biotinylation and whole-cell recordings. Localization of NFAT was analyzed by confocal microscopy and cell fractionation/immunoblot. Expression of podocyte markers and NMDA receptors was by confocal microscopy. Apoptosis was assessed by TUNEL and caspases assays.

Results: A 24-hr application of NMDA to cultured podocytes increased production of ROS. NMDA-evoked ROS generation drove an increase in surface expression of TRPC6 channels, which was blocked by the NMDA antagonist MK-801 and by MnTBAP, a scavenger of ROS. Increases in TRPC6-like cationic currents were also observed in NMDA-treated podocytes. NMDA increased nuclear localization of NFAT, which was blocked by MK-801, MnTBAP, cyclosporine, and by the TRPC inhibitor SKF-96365. NMDA treatment for 24 hr reduced expression of nephrin and podocin but there was no loss of cells. With 72 hr NMDA exposure we observed cell death, increased expression of caspases and nuclear fragmentation. In obese *db/db* mice, we observed markedly increased glomerular expression of NMDA receptor NR1 subunits compared to lean litter-mate controls.

Conclusions: Excessive activation of NMDA receptors may contribute to podocyte dysfunction and degeneration owing to oxidative stress and calcium overload, mediated in part through mobilization of TRPC6 channels and their associated pathways. Sustained increases in NMDA receptor activation could occur as a result of hyperhomocysteinemia, or in conditions such as type II diabetes in which expression of these receptors appears to be increased.

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FR-PO671

Synergistic Effect of Mesangial Cells Induced CXCL1 and TGF-β1 in Promoting Podocyte Loss in IgA Nephropathy

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Background: Podocyte loss was reported to reflect disease severity and progression in IgA nephropathy (IgAN). But the mechanisms of podocyte loss are still unclear. Recent evidence showed that IgA1 complexes from IgAN patients could directly activate mesangial cells, and further induce podocyte injury through indirect mesangial cell-podocyte cross-talk. In the present study, we explored the mechanism of mesangial cells induced podocyte apoptosis and adhesion dysfunction in IgAN.

Methods: IgA1 complexes (cIgA1) were purified from serum of 28 IgAN patients and 20 health controls (HC). Cultured human mesangial cells were then treated with 100μg/ml cIgA1. Multiple cytokines produced by mesangial cells were detected using Human Cytokine Array and confirmed by ELISA. Then the diluted mesangial conditional medium or recombinant human (rh) cytokines up-regulated in mesangial cells (2ng/ml CXCL1 and/or 2ng/ml TGF-β1) were used to treat cultured human podocyte. Podocyte apoptosis was detected by TUNEL and adhesion capability was detected by counting the adhesive cells.

Results: cIgA1 from IgAN significantly up-regulated the expression of CXCL1 and TGF-β1 in mesangial cells compared with HC (CXCL1: 718.4±532.1pg/ml vs. 311.6±191.8pg/ml, P=0.002; TGF-β1: 1535.5±255.5pg/ml vs. 1304.9±363.8pg/ml, P=0.031). The podocyte over-expression of CXCR2 (the receptor of CXCL1) was induced by mesangial cell conditional medium from IgAN (IgAN-MsCM) and rhTGF-β1, but not rhCXCL1. Increased podocyte apoptosis (19.48%±1.9% vs 4.5%±1.7%, P<0.05) and reduced podocyte adhesion (5790±377 vs 9272±736, P=0.002) were induced by IgAN-MsCM, as well as rhCXCL1 together with rhTGF-β1. Moreover, both the effect of increased podocyte apoptosis and reduced podocyte adhesion recovered partially by the blocking antibody of CXCR2.

Conclusions: Our present study implied that the cIgA1 from IgAN patients could up-regulate the secretion of CXCL1 and TGF-β1 in mesangial cells; and then the synergistic effect of CXCL1 and TGF-β1 further induced the apoptosis and adhesion dysfunction in podocyte via CXCR2. It might be a potential mechanism of podocyte loss in IgA nephropathy.

FR-PO672

Protein 4.1O Is a Novel Linker between Nephrin and the Actin-Cytoskeleton and Protects from High Glucose Mediated Nephrin Endocytosis
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Background: Microalbuminuria is an early marker for diabetic nephropathy. A GWAS for diabetic nephropathy revealed FRMD3 as a candidate gene in type 1 diabetics. FRMD3 encodes for 4.1O which belongs to the family of band 4.1 proteins. These proteins are adaptors of plasma membrane receptors to the actin cytoskeleton. In zebrafish, moe, the 4.1 orthologue is expressed in podocytes and is required for slit diaphragm formation. The function of 4.1O in human podocytes is unkown so far. Interaction of nephrin with β-arrestin2 preceeds nephrin endocytosis which is perceived to promote proteinuria.

Methods: RNA was isolated from human podocytes and subjected to rt-PCR for 4.1 family members. Cells expressed 4.1O, nephrin, podocin, Glepp1, Neph1, FAK and IQGAP1. After cell lysis coimmunoprecipitation was performed.

Results: Protein 4.1 family members 4.1O, 4.1G, 4.1B and 4.1N are expressed in human podocytes. 4.1O interacts with nephrin, podocin, Glepp1 but not with Neph1. 4.1O binding to nephrin maps to nephrin 1158-1190, whereas nephrin binds to the c-terminal domain of 4.1O. GLEPP1 interacts with 4.1O within its phosphatase domain. 4.1O binds to GLEPP1 by its FERM domain. 4.1O shows a strong interaction with FAK and IQGAP1. The FERM-domain of 4.1O is required for IQGAP1 binding. Nephrin endocytosis is ameliorated in the presence of 4.1O. 4.1O reduces the interaction of nephrin with β-arrestin2 in high glucose media while 4.1O does not influence the nephrin-β-arrestin2 interaction under low glucose condition.

Conclusions: Protein 4.1O is a candidate gene for diabetic nephropathy in type 1 diabetics. The deletion of its orthologue in zebrafish causes proteinuria. Due to the interaction with nephrin and actin 4.1O is a promising adaptor for slit diaphragm proteins connecting to the actin cytoskeleton. Its interaction with IQGAP1 and FAK may influences the migration of podocytes. We therefore postulate that 4.1O plays an important role in the development and progression of proteinuric kidney diseases.

FR-PO673

A Molecular Mechanism for Angiotensin II Induced Proteinuria
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Background: Microalbuminuria is as an early marker for glomerular permeability in hypertensive patients. Inhibitors of the RAAS but not calcium channel blockers reduce albuminuria in these patients. Albuminuria results from a defect in the glomerular filter that is composed of endothelium, basal membrane and podocytes with slit diaphragms. Nephrin is a major component of the glomerular slit diaphragm which is endocytosed upon binding to the adaptor protein β-arrestin2.

Methods: Cells expressing the AT1-receptor (AT1R) or its mutant D125AR126L, nephrin and β-arrestin2 were stimulated with Angiotensin II (Ang2). Co-immunoprecipitation with subsequent westernblot analysis was performed. Cells were pretreated with the inhibitor before stimulation with Ang2. For siRNA experiments cells were transfected with Gaq siRNA. The effect of Ang2 on the β-arrestin2 binding was studied with two nephrin mutants. For the endocytosis assay, cells were stimulated with Ang2. Glomeruli were isolated from mice, lysed and β-arrestin2 was precipitated.

Results: Ang2 stimulation increases the interaction between nephrin and β-arrestin2. This Ang2 effect is dependent on the AT1R and is inhibited by AT1R blockers. The G-protein signalling is essential for the Ang2 effect, as the AT1R mutant D125AR126L abolishes all G-protein signalling and inhibits the Ang2 mediated increase of the nephrin β-arrestin2 interaction. Gaq siRNA as well as a phospholipase C (PLC) inhibitor block the Ang2 effect. The phosphorylation status of nephrin Y1217 is crucial for the Ang2 effect. Stimulation with Ang2 increases endocytosis of nephrin, which can be inhibited by AT1R- and PLC-blockers. In podocytes, nephrin endocytosis is enhanced after Ang2 treatment. The Ang2 effect on nephrin-β-arrestin2 binding can be reproduced in isolated glomeruli.

Conclusions: Ang2 weakens the integrity of the slit diaphragm thru increased nephrin endocytosis and is perceived to promote proteinuria. This previously unknown molecular effect of Ang2 could help to understand the molecular mechanisms of Ang2 induced proteinuria beyond hemodynamic effects.

FR-PO674

Targeted Deletion, but Not Systemic Inhibition of NF-kappa-B Essential Modulator Preserves Podocyte Morphology and Function *In Vivo* and *In Vitro*
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Background: NF-kappa-B (NFkB) essential modulator (NEMO) is necessary for the activation of the NFkB signaling cascade, which plays a pivotal role in proteinuric glomerular disease. As systemic, unspecific inhibition of NFkB impairs both protective and damaging mechanisms we investigated NEMO-inhibition by selectively targeting podocytes *in vivo* and *in vitro*.

Methods: First, consequences of systemic inhibition of NFkB with pyrrolidine dithiocarbamate (PDTC) were studied in mice with nephrotoxic nephritis (NTN). Secondly, by crossbreeding mice expressing Cre-recombinase behind the podocin-promoter with floxed NEMO-mice, we generated podocyte specific NEMO -/- mice. Course and renal outcome of NTN at days 7 and 14 following disease induction were studied in these mice (n=14) and compared to wildtype littermates (n=16). Third, the consequences of NEMO-inhibition were studied in podocytes transfected with NEMO-siRNA.

Results: Systemic inhibition of the NFkB pathway with PDTC in NTN improved neither proteinuria, renal function nor podocyte morphology and function. In contrast, podocyte specific NEMO deletion significantly ameliorated proteinuria, albuminuria and creatinine clearance on d7, which was mirrored by improved glomerular morphology (reduced GBM thickness and podocyte effacement) on d14. Immunohistochemical analysis on d14 showed a significant preservation of glomerular podocin expression in NEMO -/- mice compared to controls. *In vitro*, podocytic NEMO-silencing increased the gene and protein expression of podocin and improved F-actin organisation in comparison to control.

Conclusions: In conclusion, targeted deletion of podocytic NEMO, but not systemic NF-kB inhibition, ameliorated podocyte damage and preserved expression of podocin, a key component of the slit diaphragm, and improved cell morphology and function *in vivo* and *in vitro*. Thereby NEMO represents a new modulator of glomerular damage.

FR-PO675

Podocyte-Specific Deletion of Prohibitin 2: A Novel Protein at the Slit Diaphragm: Leads to Progressive Proteinuria and Glomerular Sclerosis
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Background: Prohibitins as well as Podocin belong to the PHB domain protein family. Loss or SNPs of Podocin are known to cause steroid-resistant forms of nephrotic syndrome. So far, very little is known about the function of other PHB-domain proteins in podocytes. Prohibitin 1 and Prohibitin 2 (Phb1/Phb2) are two closely related proteins that reside within the inner mitochondrial membrane where they are essential for membrane stability and cristae structure without affecting mitochondrial membrane potentials or activity of the respiratory chain.

Results: Here, we report on a podocyte-specific Phb2 knockout mouse. At postnatal day 14 Phb2 knockout mice showed no signs of glomerular dysfunction. However, starting postnatal day 18 the absence of Phb2 led to significant proteinuria and massive loss of body weight resulting in premature death. Kidney histology revealed proliferation of parietal epithelial cells with partial vacuolization and collapsing glomerular capillaries as well as highly disorganized mitochondrial cristae. Floxed Phb2;Cre negative mice served as control mice and showed normal kidney morphology and function. As a Phb2-knockout does not affect mitochondrial function we further addressed its subcellular localization in podocytes. Immunogold labeling localized Phb2 not only to mitochondria but also to the slit diaphragm. Coimmunoprecipitation experiments identified Phb2 as a novel interactor of Podocin. On a molecular level, the formation of multimeric Podocin complexes was found to be reduced in Phb2 knockdown cells as analyzed by sucrose gradient ultracentrifugation.

Conclusions: In conclusion, loss of Phb2 leads to massively impaired glomerular filtration in mice. Not only the localization of Phb2 to the slit diaphragm but also the interaction with Podocin point to an important extra-mitochondrial role of Phb2. As loss of Phb2 affects the formation of multimeric Podocin complexes we hypothesize a role of Phb2 as a stabilisator of the podocyte slit diaphragm complex. Phb2 – a novel player at the slit diaphragm?

FR-PO676

Podocyte-Derived VEGF Regulates Glomerular Tuft Development through the Suppression of PDGF Receptor β Phosphorylation
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Background: Glomerular podocyte-derived VEGF is indispensable for migration and proliferation of endothelial cells, as evidenced by the findings from podocyte-specific *Vegf* knockouts. In contrast, whereas podocyte-specific *Vegf* overexpression, that can mimic diabetic nephropathy or POEMS syndrome, led to the collapse of glomerular tufts and thickening of GBM, the details of phenotype have not been reported earlier. The aim of this study is to clarify further the function of VEGF on the glomerular cells.

Methods: Since the podocyte-specific *Vegf* transgenic mice die within a few weeks after birth, we have established dual transgenic mice (Tg), which can express *Vegf* only in podocytes under the control of "Tet-On system" (*Pod-rtTA; TetO-Vegf*). *Vegf* was induced only by administration of Doxycycline, as confirmed by the positivity of podocyte-*lacZ* driven by bi-directional *TetO* promoter.

Results: Macroscopic and microscopic inspections found that prominent cortical hemorrhages were present, and Bowman's capsules and tubules were occupied with red blood cells in Tg. In addition, endothelial cells increased in number with subendothelial spaces enlarged. Endothelial fenestration was impaired and PV-1 remained to be expressed in mature glomeruli. In contrast, mesangial cells lacked or decreased in number, resulting in the defect of intussusceptive splitting of the glomerular tufts. We analyzed the effect of VEGF on the PDGF-PDGF receptor β axis. In Tg, the expression of PDGF per se in endothelial cells were lower, when compared to the wild type. Moreover, in cultured mesangial cells, VEGF inhibits ligand-induced PDGF receptor β phosphorylation.

Conclusions: Taken together, podocyte-derived VEGF positively and negatively acts on endothelial and mesangial cells, respectively, thereby regulating the development of glomerular capillary tufts.

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FR-PO677

A Novel Mouse Model of Podocyte Depletion Liming Wang,[1] Phillip Ruiz,[2] Robert F. Spurney.[1] *[1]Medicine, Duke University Medical Center, Durham, NC; [2]Pathology, University of Miami, Miami, FL.*

Background: A decrease in the number of glomerular podocytes is observed in both animal and human kidney diseases. Because podocytes are terminally differentiated cells with a limited capacity for replication, it has been suggested that podocytes which are lost may not be effectively replaced, causing instability of the glomerular tuft and glomerulosclerosis. As a result, some investigators contend that a reduction in glomerular podocytes may be a final common pathway causing progressive renal injury in glomerular diseases. The goal of these studies was to examine the capacity for glomerular repair after a podocyte depleting injury.

Methods: We created transgenic (TG) mice expressing the yeast enzyme cytosine deaminase specifically in glomerular podocytes. In these TG animals, the prodrug 5-flucytosine (5-FC) is converted to 5-fluorouracil (5-FU) and promotes podocyte death.

Results: Treatment with increasing dosages of 5-FC caused graded increases in proteinuria 1-2 weeks after treatment, which returned to control levels by the 10-week time point. Light microscopic examination revealed minimal pathology at the 2-week time point, but electron microscopy revealed found foot process effacement as well as focal areas of glomerular basement membrane duplication, and immunohistochemical studies detected podocyte apoptosis and a decrease in the number of Wilms tumor protein 1 (WT-1) positive cells. By the 10-week time point, however, the number of WT-1 positive cells was similar to controls and a few mice had developed focal areas of glomerulosclerosis. Consistent with the effects of 5-FC on podocyte number, expression of the podocyte proteins nephrin, podocin, synaptopodin and podocalyxin were altered in a similar temporal fashion.

Conclusions: The glomerulus has a significant capacity for repair after a podocyte depleting injury.

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FR-PO678

Differential Role of Notch1 and Noth2 in Podocytes Ae Seo Deok Park, Bhaskar Vadla, Katalin Susztak. *Renal Electrolyte and Hypertension Division, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA.*

Background: Our lab has recently demonstrated that ectopic activation of Notch signaling in podocytes resulted in proteinuria and glomerulosclerosis. There are four Notch isoforms: Notch1-4, however, the relative contribution of each of these isoforms and the cognate ligand are largely unknown. The aim of this study is to understand the mechanism of activation and the role of Notch1 and Notch2 in podocytes and their contribution to diabetic kidney disease development.

Methods: Animals with podocyte specific deletion of Notch1 or Notch2 were generated by crossing podocin-cre ($NPHS2^{Cre}$) driver mice with Notch1 or Notch2 flox/flox mice. Mice were injected streptozotocin intraperitoneally and were sacrificed at 20 weeks of age. The urinary albumin/creatinine ratio measurements were performed and histology was analyzed by PAS staining. For *in vitro* studies we used conditionally immortalized mouse podocyte cell lines.

Results: Mice with podocyte-specific deletion of Notch1 or Notch2 had no baseline phenotype, consistent with the findings that Notch1 and Notch2 are silenced in differentiated podocytes. Mice with podocyte specific deletion of Notch1 but not Notch2, had significantly reduced albuminuria, when we made them diabetic. We also observed improved renal histology in diabetic Notch1 null mice. We found significant decrease in podocyte number in wild-type diabetic animals, while the podocyte loss was much less in diabetic Notch1 knock-out animals. These data indicate that Notch1, but not Notch2, is critical for diabetes-induced podocyte damage. *In vitro* results indicate that upon TGF-beta treatment, there is an increase in endogenous Jagged1 expression in the podocytes, suggesting a cell-autonomous activation of Notch1 pathway (via Jagged1). The activation of Notch pathway is reflected in the expression of Notch downstream genes and an increase in Notch1 protein activity. Notch2 activity is relatively unchanged under these circumstances.

Conclusions: Thus we conclude that the Notch isoforms are differentially activated by the different Notch ligands. The Jagged1 induced Notch1 activation seems to play a critical role in podocyte damage.

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FR-PO679

mTORC1 Signaling in Focal Segmental Glomerulosclerosis Stefan Zschiedrich, Tobias B. Huber. *Renal Division - Department of Medicine, University Hospital Freiburg, Freiburg, Germany.*

Background: Mammalian target of rapamycin (mTOR) represents an evolutionary conserved protein kinase regulating a variety of essential cellular processes including growth, proliferation and survival. mTOR forms two distinct functional complexes, termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), whereby mTORC1 is sensitive to rapamycin and consists of multiple components including Raptor. We could recently decipher the role of mTOR complexes in podocyte homeostasis and diabetic nephropathy. However, the role of mTORC1 in the course of focal-segmental glomerulosclerosis (FSGS) remained elusive.

Methods: For a detailed analysis of mTOR function in the course of FSGS we combined gene expression analysis of human biopsies, transgenic mice targeting mTORC1 as well as pharmacological mTOR inhibitor studies of the adriamycin FSGS mouse model.

Results: Both mTORC1 target genes and mTOR mRNA itself were induced in human FSGS and the FSGS mouse model, indicating that induction of mTORC1 activity is a general characteristic of FSGS. Strikingly, curtailing mTORC1 signaling by genetically reducing mTORC1 copy number in podocytes prevented glomerulosclerosis and significantly ameliorated the progression of glomerular disease in FSGS, evidencing that mTORC1 is centrally involved in the progression of FSGS. However, podocyte-specific abrogating of mTORC1 activity resulted in massive proteinuria, stressing the importance of an initial mTORC1 response to glomerular disease. In addition, pharmacological inhibition of mTOR by rapamycin could ameliorate proteinuria and podocyte loss in the FSGS mouse model. Interestingly, these rapamycin effects were dose dependent with lower doses of rapamycin being most efficient in preventing glomerular disease progression.

Conclusions: Genetical or pharmacological reduction - but not abolition - of mTORC1 activity can prevent the consequences of mTOR hyper-activation in podocytes in FSGS mouse model, suggesting that a carefully titrated inhibition of mTORC1 may prevent podocyte injury and ameliorate the progression of human FSGS.

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FR-PO680

PodNet: A Protein-Protein Interaction Network of the Podocyte Gregor Warsow,[1,2,3] Nicole Endlich,[1] Eric Schordan,[1] Sandra Schordan,[1] Ravi Kumar Chilukoti,[4] Georg Homuth,[4] Marcus J. Moeller,[5] Georg Fuellen,[2] Karlhans Endlich.[1] *[1]Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany; [2]Biostatistics and Informatics in Medicine and Ageing Research, University of Rostock, Rostock, Germany; [3]Mathematics and Informatics, University of Greifswald, Greifswald, Germany; [4]Functional Genomics, University of Greifswald, Greifswald, Germany; [5]Division of Nephrology and Immunology, University Hospital of the Aachen University of Technology (RWTH), Aachen, Germany.*

Background: Interactions between proteins crucially determine cellular structure and function. Thus, differential analysis of the interactome in protein-protein interaction (PPI) networks may considerably help to elucidate cellular mechanisms and transitions. Though podocytes play a central role in kidney function and pathology, podocyte specific PPI networks do not exist.

Methods: We screened the literature for podocyte genes and PPIs, and built PodNet, a mouse podocyte PPI network in Cytoscape format. Using database PPIs, we expanded PodNet to XPodNet. Interactome analysis was done with the Cytoscape plugin ExprEssence, based on the law of mass action.

Results: Mapping podocyte transcriptomes on XPodNet, the most abundant PPIs were observed between slit diaphragm proteins, actin-associated proteins and the apical membrane/adapter proteins podocalyxin/Nherf2 (Podxl/Slc9a3r2) and claudin 5/ZO-1 (Cldn5/Tjp1). To demonstrate the performance of XPodNet in differential interactome analysis, we examined developmental differentiation and the effect of cell culture, using transcriptomes of podocytes in 10 different states. PPIs between slit diaphragm proteins are most significantly upregulated during podocyte development and most significantly downregulated in culture. On the other hand, our analysis revealed that PPIs, which are lost during podocyte differentiation, are not regained in culture, suggesting rather a loss than a reversal of differentiation for podocytes in culture.

Conclusions: In summary, we have developed PodNet as a valuable tool for differential interactome analysis in podocytes, identifying potential key regulated interactions in developing and cultured podocytes.

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FR-PO681

A Combined Proteomic and Transcriptomic Ex Vivo Fingerprint of the Mouse Podocyte Florian Grahammer, Tobias B. Huber. *Renal Division Department of Medicine, University Hospital Freiburg, Freiburg, Germany.*

Background: Application of high-throughput methods to the endogenous podocyte have been hampered by low yields of current isolation procedures preventing reliable and reproducible determination of its transcriptome and proteome.

Methods: A *hNPHS2* Promoter driven Cre recombinase in a dual color membrane tagged GFP/Tomato reporter mouse was used. First, a modified and optimized bead perfusion protocol was applied to establish a highly enriched glomerular fraction of kidney lysates. Further single cell dissociation based on collagenase digest and vigorous mechanic disruption of the glomerular architecture led to a more than 100 fold increase in podocyte yield as compared to previous reports, which greatly facilitates downstream applications such as gene arrays and proteomics.

Results: On average 500.000 cells could be obtained per mouse. Gene ontology analysis of gene arrays comparing podocytes with non-podocyte glomerular cells revealed an enrichment of processes related to cytoskeleton, cell differentiation, receptor and synapse activity as well as endosomal transport. qPCR confirmed selected targets from differentially expressed sets of genes. Both label-free and SILAC proteomics with spiked "heavy" glomerula were performed. A total of 1448 proteins could be detected by MS with 161 being differentially expressed. Again, proteins from cytoskeleton, plasma membrane, cell junction, synapse and membrane rafts were found to be enriched in podocytes. Several differentially expressed proteins were confirmed by Western Blot.

Conclusions: We have established a method for large-scale isolation of podocytes allowing efficient use in downstream screening applications. For the first time we were able to analyze a proteome of the podocyte and hence correlate mRNA and protein expression. Analysis of splice variants, miRNA and transcription factor networks yielded interesting and novel insights into podocyte biology.

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FR-PO682

The Slit Revisited: Analyzing the Role of Nephrin and the Whole Neph Family Using an In Vivo Comparative Approach Christoph Schell, Florian Grahammer, Tobias B. Huber. *Renal Division, Department of Medicine, University Hospital Freiburg, Freiburg, Germany.*

Background: The slit diaphragm constitutes the final component of the glomerular filtration barrier. NEPHRIN and NEPH1 are essential components of the SD, while the role of other members of the NEPH family (NEPH2 and 3) is unclear. By using conditional alleles we try to decipher the precise function of these genes.

Methods: We crossed conditional Nephrin and Neph1 alleles to the *hNPHS2-cre* line. Furthermore constitutive knockout mice were generated. Generation of Neph2 and Neph3 mice was performed in a similar way. By employing *Nphs2rtTA*TetOCre* models timed deletion in adulthood could be induced. Detailed analysis was performed with functional, phenotypic assays, light microscopy, IF, WB, SEM and TEM.

Results: As expected podocyte specific *Nephrin* knock-out mice show congenital proteinuria, growth retardation and die within ten days. Interestingly, Neph2 and Neph3, constitutive KO mice did not show any obvious phenotype over a 12 week period. Surprisingly the analysis of *Neph1* conditional and constitutive knock-out mice displayed totally differing results. While constitutive *Neph1* mice present with almost 100% lethality during 24h after birth, conditional *Neph1* mice show a proteinuric, non lethal phenotype resembling a FSGS like glomerulopathy. NEPH1 plays a fundamental role in the enteric nervous system being responsible for the early lethality of constitutive NEPH1 KO mice. Ultrastructural analysis revealed that both, NEPH1 and Nephrin molecules, are required for a fine tuned targeting of podocyte foot processes. Interestingly, the slit diaphragm can still be formed in the absence of NEPH1, but not in the absence of Nephrin.

Conclusions: This unique comparative approach revealed for the first time distinct functions for the slit diaphragm molecules NEPHRIN, NEPH1, NEPH2 and NEPH3 *in vivo*. While NEPHRIN seems to be essential for the formation of the slit diaphragm, NEPH1 rather appears to have a guidance function for podocyte foot processes; on the contrary NEPH2 and NEPH3 appear to be dispensable In addition, our data point to an unexpected fundamental role of NEPH1 for the innervation of the intestine.

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FR-PO683

Role of Microtubule Associated Protein 1b (Map1b) in Glomerular Podocytes Stefan Kohl,[1,4] Markus Gödel,[1] Friedrich Propst,[2] Beat M. Riederer,[3] Tobias B. Huber.[1] *[1]Renal Division, University Hospital Freiburg, Freiburg, Germany; [2]Max F. Perutz Laboratories, Department of Biochemistry and Cell Biology, University of Vienna, Vienna, Austria; [3]Center for Psychiatric Neurosciences, Proteomics Unit, Psychiatric Hospital, CHUV, Prilly-Lausanne, Switzerland; [4]Department of Pediatrics, University of Michigan, Ann Arbor, MI.*

Background: Glomerular podocytes are process-bearing, octopus-like specialized epithelial cells. The cytoskeleton of podocyte main processes and processes of neurons have a similar basic architecture: Both are supported by prominent bundles of microtubules (MT). Furthermore, they share a common set of proteins (e.g. Map Tau) which are needed to assemble, maintain, and utilize their complex MT cytoskeleton but are absent in simple shaped cells.

Methods: We used immunofluorescence microscopy of human and rat kidney sections, as well as human cell culture podocytes. Common molecular cloning methods were employed to generate lentiviral constructs of GFP/mCherry labeled Map1b. A newly created transgenic podocyte cell line was used for live cell imaging (Nikon Biostation). Kidneys of total Map1b KO mice were examined by light and electron microscopy.

Results: We studied the microtubule cytoskeleton of podocytes and identified the neuronal microtubule associated protein 1b (Map1b) as a new protein to be highly enriched in podocyte main processes. Expression of Map1b in differentiating glomerular podocytes of newborn rats was detected in very early stages of glomerulogenesis (comma shaped bodies). Live cell imaging of GFP/mCherry-tagged Map1b in cell culture podocytes revealed the highly dynamic organization of the microtubule cytoskeleton and its close association with Map1b. Surprisingly, mice being constitutively depleted of Map1b did not show structural podocyte defects.

Conclusions: We identified the "neuronal" Map1b as a new "podocytic" protein. It is associated with a highly dynamic MT skeleton of podocyte main processes. However, Map1b is not essential for glomerulogenesis and podocyte differentiation in mice.

FR-PO684

Dynamin Ring Stabilizer Protect the Actin Cytoskeleton in Proteinuric Kidney Disease Changkyu Gu, Sanja Sever. *Nephrology, Massachusetts General Hospital, Charlestown, MA.*

Background: Dynamin oligomerization into rings has been linked to stimulation of F-actin elongation and protection of damaged kidney podocytes from dynamin proteolysis. Therefore, development of dynamin ring stabilizer compounds as novel therapeutics is important for the treatment of actin-based diseases such as proteinuric kidney disease.

Methods: Dynamin ring stabilizer compound was characterized and tested for the effects on dynamin activity using the various approaches including GTPase assay, electron microscopy, endocytosis assay and protein binding assay. Cell staining assay and PAN or LPS-induced transient proteinuric animal models were used to reveal the effects of dynamin ring stabilizer compound on actin cytoskeleton in podocytes and proteinuria respectively.

Results: 1. Dynamin ring stabilizer compound induces formation of long-lived dynamin rings.

2. Dynamin ring Stabilizer compound regulates formation of focal adhesions and stress fibers.

3. Dynamin ring Stabilizer compound does not inhibit clathrin-mediated endocytosis, unless it is actin-dependent clathrin-mediated endocytosis.

4. Effects dynamin ring Stabilizer compound on actin dynamics are independent of RhoA signaling.

5. Dyamin ring Stabilizer compound reduces proteinuria.

Conclusions: Dynamin oligomerization regulates actin dynamics independent of the endocytosis and the well-known actin cytoskeleton regulator, Rho GTPase family. Our data showed that dynamin ring stabilizer compound promotes formation of stress fibers and focal adhesion in podocytes. Furthermore, its actin stabilizing action could be harnessed to reduce the symptoms of an actin-based disorder in two rodent models of proteinuric kidney disease. The dynamin ring stabilizer compound has therefore provided key new insights into dynamin oligomerization, actin dynamics and introduces a possible new candidate therapeutic.

FR-PO685

A VEGFR2-Gαi3-GIV Molecular Complex Regulates Actin Remodeling, Cell Survival and Cell Migration in Podocytes Honghui Wang,[1] Taro Misaki,[1] Akiko Eguchi,[2] Pradipta Ghosh,[2] Marilyn G. Farquhar.[1] *[1]Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA; [2]Department of Medicine, University of California San Diego, La Jolla, CA.*

Background: We previously demonstrated that after acute injury in Puromycin Aminonucleoside Nephrotic (PAN) rats expression of GIV increases as the nephrin level decreases. GIV is phosphorylated and phospho-GIV binds VEGFR2 and Gαi3, and Akt signaling is increased. These results suggest that GIV takes over the task of enhancing Akt survival signaling in response to VEGF. The objective here was to investigate the mechanistic role of GIV in actin remodeling, cell survival and cell migration of cultured podocytes.

Methods: To mimic early PAN injury, in vitro differentiated mouse podocytes were treated with PA (30 μg/mL) for 24-72 h. Expression and phosphorylation of GIV and VEGFR2 and Akt were assessed by quantitative immunoblotting, endogenous interaction between VEGFR2 and GIV in podocytes by co-immunoprecipitation, and in vitro interaction between GIV and Gαi3 by GST-pulldown assay. To study the function of GIV, GIV was depleted by siRNA, actin remodeling was assessed by rhodamine-phalloidin staining, cell migration by wound healing assay, and apoptosis by caspase 3 cleavage and Annexin V assays.

Results: In PA treated podocytes, phosphorylation of GIV is increased, phosphorylation of VEGFR2 is elevated, and the interaction between GIV, VEGFR2 and Gαi3 is enhanced. In addition, interaction between inactive but not active GST-Gαi3 and GIV is increased in PA treated podocytes. These results are consistent with previous findings in PAN rats and validate that PA injury of podocytes is a suitable in vitro model for investigating early podocyte injury. In GIV depleted podocytes, Akt activation is attenuated, the actin cytoskeleton reorganizes, and cell migration is impaired. Upon PA injury, Akt activation in response to VEGF stimulation is abolished, and cell apoptosis is dramatically increased.

Conclusions: We conclude that upon PAN injury GIV interacts with VEGFR2, assembles a VEGFR2-Gαi3-GIV signaling complex that stimulates Akt activation, actin remodeling and cell survival in podocytes.

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FR-PO686

INF2 Preserves Lamellipodial Actin Dynamics and Slit-Diaphragm Trafficking by Modulating Rho/mDia Signaling Hua Sun, Johannes S. Schlondorff, Martin R. Pollak. *Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: Mutations in INF2 are a common cause of familial focal segmental glomerulosclerosis (FSGS). We have previously established that INF2 antagonize Rho-mediated activation of diaphanous related formins (mDia). Here we investigated the role of INF2 in regulating actin dynamics and trafficking of slit diaphragm (SD) proteins in podocytes.

Methods: Cultured podocytes were treated with INF2-targeting siRNA. The effect of INF2 knockdown on actin dynamics, actin-dependent lamellipodial morphology and trafficking of SD proteins were examined by immunofluorescence microscopy. Wild-type and mutant INF2 were transfected to ascertain their ability to rescue the observed phenotypes.

Results: Knockdown of INF2 in podocytes led to a loss of lamellipodia and the associated actin meshwork, accompanied by disruption of nephrin and podocin trafficking. Both the changed phenotypes were reversed by overexpression of dominant negative Rho or mDia. Overexpression of wild-type INF2 restored lamellipodia and SD trafficking disrupted by active Rho or mDia, while overexpression of INF2 mutants that are unable to bind and inhibit mDia, led to SD protein mislocalization. The ability of INF2 to direct SD trafficking is mediated by its interaction with podocin and caveolin-1.

Conclusions: INF2 plays an essential role in preserving lamellipodial actin dynamics by antagonizing the Rho/mDia pathway. INF2 directs lipid-raft mediated trafficking of SD proteins to lamellipodia via an interaction with podocin. FSGS-causing mutations disrupt the ability of INF2 to inhibit mDia, thereby impairing actin dynamics critical for podocyte morphology and trafficking of SD proteins. These results suggest that excess mDia activation play a central role in the development of INF2-related FSGS.

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FR-PO687

Loss of Apoptosis Antagonizing Transcription Factor (AATF) in Podocytes Leads to FSGS Katja Hoepker,[1] Safiya Khurshid,[1] Heike Goebel,[2] Linus A. Völker,[1] Heiko Schweizer,[3] Bernhard Schermer,[1] Thomas Benzing.[1] *[1]Internal Medicine II and Center of Molecular Medicine Cologne, University of Cologne, Cologne, Germany; [2]Institute of Pathology, University of Cologne, Cologne, Germany; [3]Internal Medicine IV, University of Freiburg, Freiburg, Germany.*

Results: AATF is expressed in developing podocytes. Because AATF´s conventional knockout is early embryonically lethal we generated a mouse containing a floxed AATF allele and crossed it with the Podocin-Cre line. This created an embryonic podocyte knockout (pod ko) at approx. E12.5. Surprisingly the pod ko shows a considerably late phenotype. The mice are born in a normal mendelian ratio and are not to be distuingished from their littermates until the age of 12 weeks when they become proteinuric. At varying age, between 4-7 months the pod ko animals loose weight and show ESRD. Histologically, their kidneys present with the classical criteria of focal segmental glomerulosclerosis (FSGS). The genomic integrity of podocytes is being challenged by ROS, ionizing irradiation or chemotherapeutic agents. Their specific features of the DNA damage response (DDR) as postmitotical cells are largely being uninvestigated. We and other groups have identified AATF as a key protein involved in DDR pathways. The DDR signaling cascade ultimately activates p53, which then eventually determines the fate of the cell: DNA repair or elimination by apoptosis. We show that in resting cells AATF resides in the cytoplasm where it interacts with MRLC3. On encountering DNA damage MAPKAP Kinase 2 gets activated and migrates out of the nucleus into the cytoplasm where it phosphorylates AATF, abrogating its interaction with MRLC3 and thus allowing AATF to translocate into the nucleus. In the nucleus AATF localizes on to the promoters of the p53 dependent pro-apoptotic target genes like PUMA, Bak and BAX and hence negatively regulates p53 dependent apoptosis.

Conclusions: We therefore bring forth a DNA damage player, which on a systems level, acts in a switch-like manner to control the quality of the p53 response to DNA damage and plays a central role in podocyte maintanance.

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FR-PO688

PTEN Deletion in Podocytes: A Potential General Mechanism Causing Foot Processes Effacement and Proteinuria Jamie Lin,[1] Jing Xu,[2] William E. Mitch,[1] Zhaoyong Hu.[1] *[1]Internal Medicine, Nephrology Division, Baylor College of Medicine, Houston, TX; [2]Internal Medicine, Changhai Hospital, Shanghai, China.*

Background: Proteinuria is a serious complication of chronic kidney disease (CKD), including diabetic nephropathy (DN). Podoycytes play a prominent role in maintaining the integrity of the glomerular filtration barrier but they can undergo cytoskeletal remodeling resulting in foot processes effacement and increasing albuminuria. We studied PTEN (phosphatase and tensin homolog) because it is known to be down-regulated in glomeruli of type 1 (T1DM) or type 2 (db/db) mice and PTEN regulates cytoskeletal remodeling(e.g., fibroblast).

Methods: In kidneys of DN patients or db/db mice, we assessed PTEN expression in podocytes. In cultured podocytes, we examined whether down-regulation of PTEN stimulates Rac1 to disrupt the cytoskeleton. We also created mice with podocyte-specific PTEN KO (PPKO) to examine structural changes in podocytes and albuminuria.

Results: In glomeruli of DN patients or db/db mice, PTEN protein levels were decreased as was the level of Nephrin. In cultured podocytes, we inhibited PTEN with BpV(Hopic), a specific PTEN inhibitor, and found disorganization of F-actin in the cytoskeleton plus an increase in albumin permeability. In contrast, forced expression of PTEN in cultured podocytes blocked RAC1/Cdc42 activation and the disorganization of the cytoskeleton stimulated by PDGF. We hypothesize that reduced PTEN in podocytes is responsible for a cytoskeletal disorganization leading to effacement and proteinuria. To test our hypothesis, we examined PPKO mice and found about a 2-fold increase in albuminuria over control, lox/lox mice. PAS staining of kidney sections from PPKO mice revealed early glomerulosclerosis. Nephrin immunostaining exhibited interrupted, granularity vs smooth pattern in podocytes of normal lox/lox mice. By electron microscopy, there was extensive foot process effacement in podocytes of PPKO mice.

Conclusions: PTEN down-regulation in podocytes causes effacement and albuminuria. Because PTEN is reduced in kidneys of DN patients, targeting glomerular PTEN could become a therapeutic strategy for suppressing proteinuria.

Funding: NIDDK Support

FR-PO689

Vitamin D Reduces the Enhanced TRPC6 Expression in Injured Podocytes and Glomerular Disease Tom Nijenhuis,[1] Ramon Sonneveld,[1] Silvia Ferrè,[2] Joost G. Hoenderop,[2] Henry Dijkman,[3] Jo H.M. Berden,[1] René J. Bindels,[2] Jack F. Wetzels,[1] Johan Van der Vlag.[1] *[1]Nephrology, Radboud University Nijmegen Medical Centre, Netherlands; [2]Physiology, Radboud University Nijmegen Medical Centre, Netherlands; [3]Pathology, Radboud University Nijmegen Medical Centre, Netherlands.*

Background: Transient Receptor Potential channel C6 (TRPC6) is a slit diaphragm protein expressed by the podocyte. TRPC6 gain-of-function mutations cause hereditary focal segmental glomerulosclerosis (FSGS). Glomerular TRPC6 expression is increased in acquired proteinuric disease. We demonstrated that acquired increased TRPC6 expression is ameliorated by anti-proteinuric angiotensin receptor blockers and angiotensin converting enzyme inhibitors. Because vitamin D also has an anti-proteinuric effect, we hypothesized that it may affect podocyte TRPC6 expression.

Methods: Podocyte injury was induced by adriamycin exposure in cultured mouse podocytes. TRPC6 promoter activity was studied in a TRPC6 promoter-luciferase assay. Adriamycin-induced Nephropathy (AN) in rats was used as a model for FSGS and 1α-hydroxylase (1α-OHase) knockout mice as model for 1,25-D_3 deficiency.

Results: Adriamycin-induced podocyte injury increased TRPC6 expression, which was dose-dependently reduced by 1,25-D_3 treatment. Accordingly, 1,25-D_3 reduced TRPC6 promoter activity. AN rats showed increased TRPC6 mRNA and glomerular TRPC6 protein expression and proteinuria. 1,25-D_3 treatment normalized TRPC6 expression and reduced proteinuria. In 1,25-D_3-deficient 1α-OHase KO mice, TRPC6 mRNA and glomerular TRPC6 protein expression were increased, accompanied by podocyte foot process effacement and proteinuria. 1,25-D_3 supplementation normalized TRPC6 expression, podocyte morphology and proteinuria in these mice.

Conclusions: Our results demonstrate that 1,25-D_3 reduces TRPC6 expression in injured podocytes, possibly by inhibiting TRPC6 promoter activity. *In vivo*, 1,25-D_3 reduced glomerular TRPC6 expression in FSGS, whereas 1,25-D_3 deficiency was associated with enhanced TRPC6 expression, which correlated with signs of podocyte injury and proteinuria. Thus, this TRPC6 expressional regulation could be relevant in the anti-proteinuric effect of vitamin D.

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FR-PO690

Motor Protein Myo1c Is Critical in Organizing the Slit Diaphragm Protein Neph1 at the Podocyte Cell Membrane Ehtesham Arif,[1] Leena Mallik,[2] Yogendra Singh Rathore,[2] Babita Kumari,[1] Lawrence B. Holzman,[1] E. Michael Ostap,[3] Fnu Ashish,[2] Deepak Nihalani.[1] *[1]Department of Medicine, Renal Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA; [2]Protein Engineering, CSIR Institute of Microbial Technology, Chandigarh, Punjab, India; [3]Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA.*

Background: Podocytes are the key components of the glomerular filtration system and their dysfunction is often associated with massive proteinuria and loss of kidney function. Our previous studies have demonstrated that the slit diaphragm components Nephrin and Neph1 fail to localize to the podocyte cell membrane in the absence of motor protein Myo1c that is critical for podocyte functioning.

Methods: We performed Immuno-precipitaiton, peptide walking and small/wide angle X-ray scattering (SWAXS) approaches to characterize the interaction between Myo1c and Neph1.

Results: In this study, we provide molecular details of this interaction and identify critical residues in Myo1c and Neph1 that mediate this binding. We identified mutations in the C-terminal domain of Myo1c and the cytoplasmic domain of Neph1 that nearly abolished this interaction. Further, the Neph1 binding mutants demonstrated their inability to localize at the membrane suggesting an active role for Myo1c in Neph1 localization. Similar results were obtained when Myo1c mutants including the dominant negative and the Neph1 binding mutant, were substituted in this assay. To further understand the structural details of this interaction, we determined the solution structures of full length Myo1c and its truncated mutants individually and in complex with the cytoplasmic domain of Neph1 using SWAXS.

Conclusions: The preliminary analysis of this data shows that this association is mediated via multiple binding regions in Neph1 and Myo1c and the binding of Neph1 induces a shape change in the C-terminus of Myo1c. Collectively, these results highlight the mechanism through which Myo1c serves as a major transporter of Neph1 to its physiological location in podocytes and therefore, contributes to the maintenance of the glomerular filtration assembly.

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FR-PO691

Def-6 Expression and Localization in Atypical PKCλ/ι Deficient Mice and Podocytes Kirstin Worthmann,[1] Michael Leitges,[2] Beina Teng,[1] Irini Schaefer,[1] Hermann G. Haller,[1] Tobias B. Huber,[3] Mario Schiffer.[1] *[1]Division of Nephrology, Medical School Hannover, Hannover, Germany; [2]Biotechnology Centre of Oslo, University of Oslo, Oslo, Norway; [3]Renal Division, University Hospital Freiburg, Freiburg, Germany.*

Background: Podocyte specific deficiency of the atypical PKC isoform λ/ι in mice leads to a severe glomerular phenotype. In vitro studies of PKCλ/ι knockout podocytes revealed the guanine nucleotide exchange factor (GEF) Def-6 as a possible target gene of PKCλ/ι.

Methods: Monoclonal cell lines were isolated from control and deficient mice and their phenotype observed under the microscope. Cells were stained with phalloidin to visualize the actin cytoskeleton and vinculin to mark the focal adhesions. Furthermore, the cells were tested for the activity of small GTPases. Microarrays of the PKCλ/ι deficient and wildtype cells revealed several genes that are differentially regulated. For rescue experiments in deficient podocytes we generated adenoviral constructs of PKCλ/ι-GFP. Moreover, we analyzed the Def-6 expression and localization in PKCλ/ι deficient and wildtype kidney sections.

Results: When we compared PKCλ/ι deficient and wildtype cells we observed strong differences regarding cell shape and cell size. Furthermore, we observed increased cytoskeletal rearrangements in the deficient cells and less focal adhesions. Small GTPases assays revealed an increased activation of Rac1 and decreased amounts of total Cdc42. In

microarrays we detected Def-6 as one of the strongest upregulated genes in the deficient cells. Stainings of fixed podocytes and fractionizations revealed a strong membranous Def-6 localization in vitro. The actin cytoskeletal phenotype and the mRNA expression of Def-6 in the deficient cells could be rescued in overexpression experiments. Furthermore, we detected a strong glomerular expression of Def-6 in PKCλ/ι deficient and wildtype mice while the expression pattern of Def-6 changes obviously in glomeruli of deficient mice.

Conclusions: In summary, we can show that the actin cytoskeleton of podocytes is negatively influenced by increased Def-6 levels and that the expression and localization of this regulator is highly affected in PKCλ/ι deficient podocytes and glomeruli.

Funding: Government Support - Non-U.S.

FR-PO692

Protective Effects of Everolimus on Puromycin-Induced Cytoskeletal Alterations in Human Podocytes Are Mediated by RhoA Signaling Stefanie Weber,[1] Stefanie Jeruschke,[1] Anja K. Büscher,[1] Jun Oh,[2] Perihan Nalbant.[3] *[1]Pediatrics II, University Duisburg-Essen, Essen, Germany; [2]University Children's Hospital Hamburg, Hamburg, Germany; [3]Center for Medical Biotechnology, Molecular Cell Biology, University Duisburg-Essen, Essen, Germany.*

Background: Podocytes are highly differentiated cells playing an important role in maintaining the glomerular filtration barrier. Damage of the podocyte actin cytoskeleton is a major cause of proteinuria associated with foot process effacement and loss of slit diaphragms. Recently, antiproteinuric actions of immunosuppressants have been attributed to the recovery of the actin cytoskeleton in podocytes.

Methods: In this study, actin-related effects of treatment with the mTOR inhibitor everolimus (EV) were investigated in a puromycin aminonucleoside (PAN) in vitro model of proteinuric disease.

Results: EV substantially recovered PAN-induced defects in human cultured podocytes as increased apoptosis, decreased adhesion and enhanced migration. These protective effects were associated with restored cell morphology and recovery of actin stress fibers. Biochemical studies revealed that activities of the Rho GTPase RhoA and myosin light chain (MLC), both regulators of actin stress fiber formation, were substantially increased by EV. Y-27632, an inhibitor of the RhoA effector ROCK *(Rho*-associated protein kinase), abolished phosphorylation of MLC and stress fiber recurrence, substantiating that the RhoA-ROCK-MLC pathway is the mediator of EV induced stress fiber reorganization. In addition, live-cell imaging of RhoA activity in untreated podocytes using a FRET-based biosensor revealed elevated activation in dynamic protrusions and contractile regions indicating tight spatial and temporal regulation of the GTPase.

Conclusions: Together, our results indicate stabilizing effects of EV on podocyte viability and structure, in particular regarding the actin cytoskeleton involving the RhoA-ROCK-MLC pathway in an experimental setting of proteinuria. These observations might prompt further studies focusing on mTOR inhibitors in proteinuric disease.

Funding: Pharmaceutical Company Support - Novartis Pharmaceutical Cooperations

FR-PO693

Na^+/H^+ Exchanger-1 Reduces Injury of Podocytes due to Endoplasmic Reticulum Stress via Activation of Autophagy Zhe Feng, Shaoyuan Cui, Quan Hong, Guangyan Cai, Xiang-Mei Chen. *Department of Nephrology, Nephrology Development of the State Key Laboratory, Chinese PLA General Hospital, Beijing, China.*

Background: Injury of podocytes plays a critical role in glomerular proteinuria. Induction of endoplasmic reticulum (ER) stress in podocytes is thought to lead to loss of the cytoskeleton and subsequent injury of podocytes, but the clearance mechanism of unfolding or misfolding of protein due to ER stress is not well understood.

Methods: A passive Heymann nephritis model of membranous nephropathy and conditionally immortalized mouse podocyte cells (MPCs) were used with these experiments. pcDNA-NHE-1 plasmid was constructed and siRNA-NHE-1 was designed.Activation of autophagy was evaluated by GFP-LC3 detection, measurement of autophagic vacuoles by AO and LC3 conversion (LC3-I to LC3-II).

Results: We found that induction of ER stress and activation of autophagy in podocytes were related to cytoskeleton injury and an increase in proteinuria in a passive Heymann nephritis model of membranous nephropathy. In vitro experiments showed that ER stress induced the loss and recombination of the cytoskeleton in MPCs. The efficiency of autophagy could be a pivotal degradation system for unfolding or misfolding of protein due to ER stress in podocytes. Disturbance of autophagy resulted in cells being vulnerable under ER stress, and it aggravated the loss of the cytoskeleton. Furthermore, Na^+/H^+ exchanger-1 (NHE-1) may exert a protective effect by reducing the loss of synaptopodin in MPCs exposed to ER stress, which were recognized using overexpression of a full NHE-1 clone plasmid and NHE-1 siRNA deficiency methods.

Conclusions: This protective mechanism is due to NHE-1 activated autophagy of podocytes via PI3K/Akt phosphorylation, which reduces ER stress, and this may provide a pathway to prevent podocyte injury and decrease proteinuriaduring the course of glomerular disease.

Funding: Government Support - Non-U.S.

FR-PO694

Increased Mesangial Cell Number and Podocyte Effacement in Calponin h-2 Knockout Mice Shane Joychan,[1] Haiping Chen,[1] Jian-ping Jin,[1] Moazzem Hossain,[1] Noreen F. Rossi.[1,2] *[1]Physiology and Internal Medicine, Wayne State University School of Medicine, Detroit, MI; [2]John D. Dingell VA Medical Center, Detroit, MI.*

Background: Calponin, an actin filament-associated protein, is found in smooth muscle and non-smooth muscle cells. H-2 calponin is highly expressed in mature kidney and has been implicated in regulating the actin cytoskeleton in other tissues especially during mechanical stress. We hypothesized that h-2 calponin knockout mice would display changes in mesangial cell number and abnormal podocyte morphology compared with wild type mice.

Methods: Expression of calponin h-2 in knockout mice, heterozygous and wild type C57B/L6 mice was performed by western blot using anti-h-2 calponin polyclonal antibodies RAH1 and mAB CP21. Kidneys from each group were perfusion fixed with glutaraldehyde, then evaluated by an investigator blinded to the genetic background of the mice. Mesangial cell number was quantitated on 60 independent sections per kidney (~ 4 glomeruli per section) on periodic acid-schiff stained sections. The extent of podocyte effacement was assessed on electron microscopic images by counting the number of slit diaphragms per millimeter of glomerular basement membrane (GBM) length.

Results: Calponin h-2 knockout mice exhibited significant mesangial proliferation compared with wild type mice ($P < 0.01$). The number slit diaphragms per length were significantly fewer 863 ± 52 per mm GBM in the h-2 calponin knockout mice compared with 1033 ± 24 per mm GBM in the wild type mice. Segments of podocyte effacement were evident along the GBM in the h-2 calponin knockout mice but none in the wild type mice. Heterozygotes more closely resembled the calponin h-2 knockout mice.

Conclusions: These findings suggest that calponin h-2 influences mesangial cell number and is involved in podocyte morphology in basal conditions. Calponin h-2 may be involved in the cytoskeletal changes observed during mechanical stress that occurs in glomeruli under pathological conditions.

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FR-PO695

Sphingomyelin Phoshodiesterase-Like 3b (SMPDL3b) Affects β3 Integrin Signaling in Podocytes Tae-hyun Yoo,[1,3] Changli Wei,[1] Rodrigo Villarreal,[1] Christopher E. Pedigo,[1] Johanna Guzman,[1] Christian Faul,[1] Jochen Reiser,[1] George William Burke,[2] Sandra M. Merscher-Gomez,[1] Alessia Fornoni.[1] *[1]Department of Medicine, University of Miami; [2]Department of Surgery, University of Miami, Miami; [3]Yonsei University College of Medicine, Seoul.*

Background: Deficiency of SMPDL3b renders podocytes susceptible to injury in focal segmental glomerulosclerosis (FSGS), and down-regulation of SMPDL3b in FSGS can be prevented by rituximab. However, little is known about the mechanisms by which SMPDL3b deficiency may cause podocyte injury. The aim of this study is to investigate if and how SMPDL3b and rituximab affect β3 integrin activation and signaling induced by either urokinase-type plasminogen activator receptor (uPAR) or by its soluble form (suPAR).

Methods: Co-immunoprecipitations (Co-IP) and competitive Co-IPs were performed to detect interactions between SMPDL3b, uPAR or suPAR and β3 integrin. Wildtype (WT), SMPDL3b knockdown (KD), and overexpressing (OE) human podocytes were cultured with or without lipopolysaccharide (LPS) or recombinant human uPAR (rhuPAR). in the presence or absence of rituximab. Total (AP3) and activated (AP5) β3 integrin, as well as Src and focal adhesion kinase (FAK), were analyzed by Western blot (WB) as downstream effectors of uPAR signaling. Activation of β3 integrin was also analyzed by immunofluorescence (IF) staining for AP3 and AP5.

Results: Co-IPs showed interactions between SMPDL3b and uPAR/suPAR. Competitive Co-IPs indicated that SMPDL3b competes with the ability of uPAR/suPAR to immunoprecipitate β3 integrin. The degree of β3 integrin activation, phospho-Src and -FAK protein expression was increased in LPS- and rhuPAR- stimulated cells, and was prevented in OE and rituximab pretreated podocytes. KO podocytes were characterized by constitutive activation of β3 integrin, Src and FAK and increased susceptibility to LPS- and rhuPAR. IF staining confirmed the findings obtained by WB.

Conclusions: In summary, we demonstrated that down-regulation of SMPDL3b renders podocytes susceptible to LPS and uPAR induced activation of β3 integrin in podocytes. These findings, suggests a possible mechanism by which SMPDL3b affects podocyte function in FSGS.

Funding: NIDDK Support

FR-PO696

GIV Binds VEGFR and Assembles a GIV-Gαi3 Signaling Complex during Puromycin Aminonucleoside Nephrosis (PAN) Taro Misaki,[1,3] Honghui Wang,[1] Hirotaka Fukasawa,[1] Vanessa Taupin,[1] Pradipta Ghosh,[2] Marilyn G. Farquhar.[1] *[1]Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA; [2]Department of Medicine, University of California San Diego, La Jolla, CA; [3]Division of Nephrology, Seirei Hamamatsu General Hospital, Hamamatsu, Shizuoka, Japan.*

Background: Podocyte injury is a common determining factor in glomerular disease progression. Nephrin dependent signaling has been proposed to maintain podocyte survival via Akt activation. However, in PAN at early time points (7d) Akt phosphorylation is increased when nephrin expression is decreased and no apoptosis is detected, suggesting that

there is an alternative survival signaling pathway. GIV is a novel GEF that binds receptor tyrosine kinases and activates Gαi3 which leads to Akt activation, rearrangement of the actin cytoskeleton and cell migration. Here we investigated the role of GIV in PAN rats.

Methods: Glomeruli were isolated from normal and 7d PAN rats. Expression and phosphorylation of GIV, VEGFR2 and Akt, and expression of VEGF were assessed by quantitative immunoblotting; localization of GIV was determined by immunofluorescence; interaction between endogenous VEGFR2 and GIV by co-immunoprecipitation; and in vitro interaction between GIV and Gαi3 by GST-pulldown assay.

Results: Both the expression and phosphorylation of GIV are significantly increased in PAN glomeruli, and the increased GIV is localized in podocytes. Expression of both VEGF and VEGFR2 is elevated and activation (phosphorylation) of VEGFR2 and Akt are greatly enhanced in PAN. Moreover, VEGFR2 binds phospho-GIV from PAN glomeruli but not GIV from normal glomeruli. Finally, GIV from PAN glomeruli binds inactive but not active GST-Gαi3 whereas GIV from normal glomeruli does not bind either active or inactive GST-Gαi3, indicating that GIV binds and presumably activates Gαi3 and Akt only in PAN rats.

Conclusions: Our results show that GIV is induced after PAN injury, and enhanced GIV expression coincides with decreased nephrin expression and activation of VEGFR2 and Akt. Thus GIV interacts with VEGFR2 and activates Gαi3 in PAN which most likely enhances podocyte survival via activation of Akt.

Funding: NIDDK Support

FR-PO697

Percutaneous Renal Biopsy of Native Kidneys: A Single Center Experience of over 1000 Biopsies Stephen M. Korbet, William Whittier. *Nephrology, Rush University Medical Center, Chicago, IL.*

Background: Percutaneous renal biopsy (PRB) of native kidneys is an invaluable tool in the diagnosis and management of renal disease. We report a single academic center's experience.

Methods: PRB of native kidneys was performed in 1055 stable adult patients by an attending nephrologist (15%) or supervised fellow (85%) from 6/1983 to 3/2012 using real-time ultrasound guidance and 14-gauge biopsy needles. All patients were observed for 23-24 hours post-biopsy. From 1991 forward data was prospectively obtained and an automated needle was used.

Results: The primary indications for PRB were proteinuira (49%), SLE/vasculitis (26%), ARF (12%) and hematuiria (8%). Pts were 46±17 yo with 23% ≥60 yo, 38% were male, 40% white, 43% AA, 12% Hispanic and 4% Asian/other. The pre-PRB BT was 7±2 min with only 6% >9 min and the PTT was normal in 95% of pts. The SCr was 2.3±2.3 with 47% >1.5 mg/dl and the pre-Hgb was 12±2 g/dl with 21% ≤10 g/dl. The post-Hgb was 10.8±2 with a 1.0±0.9 delta Hgb (P <0.0001) overall. By light microscopy glomeruli were obtained in 99.6% of cases with 90% having ≥10 glomeruli and the average number of glomeruli per biopsy was 23±12. Glomeruli for immunofluorescence and EM were present in 98% of cases. Biopsies done with the automated 14-gauge needle obtained more glomeruli than with the manual needle (21±13 vs. 23±12, P 0.0001). Adequate tissue for diagnosis was obtained in 99% of biopsies and 88% of lesions were glomerular (SLE/Vasc 28%, FSGS 14%, IgAN/TBM 10%, MGN 8%, MCD 8% and MPGN 8%), 7% had tubulointerstitial lesions and 4% had nondiagnostic changes. There was no difference over time in the proportion of pts with FSGS, MGN or IgAN. Minor complications (no intervention) occurred in 8% of biopsies (mainly gross hematuria 4.5%). Major complications (required intervention) occurred in 6.6% of biopsies and resulted in death in 2 (0.18%: 1 due to bleeding and 1 sudden and unexplained), cystoscopy in 2 (0.18%), embolization in 9 (0.85%), transfusion in 48 (4.6%) and other/readmission 9 (0.8%). There was no difference in major complication rates between fellows or attendings or over the period of study.

Conclusions: PRB of native kidneys remains a highly successful and safe procedure.

FR-PO698

Safety and Outcomes According to Practitioners and Techniques in Percutaneous Native Renal Biopsy Eun Sil Koh, Sungjin Chung, Hyun Chul Whang, Yu Ah Hong, Seok Joon Shin, Cheol Whee Park, Yong-Soo Kim, Yoon-Sik Chang. *Department of Internal Medicine, The Catholic University of Korea College of Medicine, Seoul, Korea.*

Background: The purpose of this study is to compare the safety and the adequacy of tissue yield in percutaneous renal biopsy according to practitioners and techniques based on ultrasound.

Methods: This study included 658 native renal biopsies performed from 2005 to 2010 at a single center. The biopsies were performed by the nephrologists or the ultrasound (US) expert radiologists, and performed by the US-marked blind or the real-time US-guided technique.

Results: During the study period, 271 US-marked blind biopsies were performed by nephrologists, 170 real-time US-guided biopsies by nephrologists, and 217 real-time US-guided biopsies by US expert radiologists. There were no differences in post-biopsy complications such as hematoma, need for intervention, gross hematuria, pain or infection among groups. In the results of the adequacy of tissue yield, there was no significant difference in glomerular yield according to renal biopsy technique based on US. However, glomerular yield from renal biopsy performed by nephrologist was superior to that performed by US expert radiologist ($p<0.001$).

Conclusions: Percutaneous renal biopsy performed by nephrologist is equivalent to biopsy performed by US expert radiologist in post biopsy complications, and is even superior in glomerular yield.

FR-PO699

Timing of Complications of the Percutaneous Native Kidney Biopsy Kaelin C. Volpini, Stephen M. Korbet, William Whittier. *Nephrology, Rush University Medical Center, Chicago, IL.*

Background: Percutaneous kidney biopsy (PKB) of native kidneys is a safe procedure; however, in the modern era, the timing or rate of complications may have changed.

Methods: PKB of native kidneys was performed in 1055 adult pts by a nephrologist or supervised fellow from 6/1983-3/2012 using real-time US guidance and 14-gauge biopsy needles. Baseline demographic and laboratory data at the time of PKB was recorded. All pts were observed for at least 23-24 hours (h) post-PKB for the presence, severity, and timing of complications. From 1991 forward data prospective and an automated needle was used. Data was divided into Era 1 (1983-1990), Era 2 (1991-2000) and Era 3 (2001-2012) and complications were divided into major (requiring intervention) and minor (no intervention required).

Results: On avg at the time of PKB pts were 46 ± 17 years old, 38% male and had a systolic BP (SBP) of 133±18 mmHg. The avg baseline bleeding time (BT) was 7±2 min, Hgb was 12±2 g/dl, and SCr was 2.3±2.3 mg/dl. Overall, the avg post-PKB Hgb was 10.8±2 with a change of 1.0±0.9 g/dl (P < 0.0001). PKB related complications occurred in 14.6% of pts; minor complications in 8% of pts and major ones in 6.6% of pts. Two pts died (0.18%: 1 PKB related). Baseline characteristics with increased risk for complication included female gender (69% vs. 61%, p= 0.0493), elevated SCr (2.8±2.8 vs. 2.2±2.2 mg/dl, p=0.0019), increased BT (7.2±2.0 vs. 6.7±1.9 min, p= 0.0026), anemia (Hgb 11.4±2.2 vs. 11.9±2.0 g/dl, p=0.0101) and HTN (SBP 137±20 vs. 133±18 mmHg, p=0.0145) compared to none. There was no change in complication rate over time (overall for Era 1, 13.4%; Era 2, 13.6%; Era 3, 16.5%, P=NS). Complications post-PKB were discovered in 58% of pts at ≤ 4h, 75% ≤ 8h, 88% ≤ 12h, 91% ≤ 24h. Major complications were noted in 56% of pts at ≤ 4h, 74% ≤ 8h, 89% ≤ 12h, 91% ≤ 24h. Complications post-PKB were discovered sooner in Era 3 than in Era 2 (overall complications ≤ 8h in Era 3 87% vs. Era 2 56%, p=0.0022; major complications ≤ 8h in Era 3 83% vs. Era 2 56%, p=0.033).

Conclusions: PKB of native kidneys remains a safe procedure. The rate of complications has not changed over time, but they are discovered sooner post-PKB in the modern era.

FR-PO700

Validation of the Oxford Classification in IgA Nephropathy, a Systematic Review and Meta-Analysis Sufang Shi,[1] Jicheng Lv,[1] Damin Xu,[1] Hong Zhang,[1] Stephan Troyanov,[2] Daniel C. Cattran,[3] Haiyan Wang.[1] *[1]Renal Division, Department of Medicine, Peking University First Hospital; Peking University Institute of Nephrology, Beijing, China; [2]Department of Nephrology, Ho´pital du Sacre´-Coeur de Montre´al, University of Montreal, QC, Canada; [3]Division of Nephrology, Toronto General Hospital, University of Toronto, Otaria, Canada.*

Background: Oxford Classification of IgA nephropathy, recently developed in 2009, has been validated in different populations. However the results remained inconsistent. We undertook a systematic review and meta-analysis to synthesize all available data and evaluate Oxford Classification utilization in IgA nephropathy.

Methods: We systematically searched Medline, Embase, and the Cochrane Library for trials published between 2009 and December, 2011. Studies assessing Oxford Classification in IgA nephropathy were included. Summary estimates of relative risk reductions were calculated with a random effects model. Renal outcome was defined as the composite of doubling of serum creatinine concentration or 50% decline of eGFR or end-stage kidney disease.

Results: Twelve identified studies provided data for 3449 patients among whom 501 kidney failure events were recorded. Overall, Oxford Classification including mesangial hypercellularity (M), segmental glomerulosclerosis (S), and tubular atrophy /interstitial fibrosis (T), showed a good correlation with kidney progression. In the multivariate model, the hazard ratio (HR) was 0.65 (95% CI 0.53 to 0.81, p<0.001), 1.63(95% CI 1.24 to 2.13, p<0.001) and 4.78(95%CI 2.37, 9.66, p<0.001) in M0,S1 and T1-2 lesions respectively without evidence of heterogeneity. Although the pooled results showed endocapillary hypercellularity (E) lesions were associated with kidney failure (HR 1.60, 95%CI 1.05 to 2.46, p=0.030) with evidence of heterogeneity (I^2=60.5%, p=0.007), subgroup analysis showed that E lesions were only reported risk factor in small studies with few end points (p=0.023) while not in large studies (p= 0.46).

Conclusions: This study validated Oxford Classification MST but not E lesions in IgA nephropathy.

FR-PO701

Is the Oxford System Associated with Changing Therapy in Canadian Pediatric IgAN/HSPN? Maury N. Pinsk,[1] Chantal Bernard,[2] Ian W. Gibson,[3] Aicha Merouani,[4] Christoph Licht,[5] Aviva M. Goldberg,[3] Pavel Geier,[6] Keith K. Lau,[7] Andrew W. Wade,[8] Tom D. Blydt-Hansen.[3] *[1]U Alberta, Canada; [2]McGill U, Canada; [3]U Manitoba, Canada; [4]U Montreal, Canada; [5]U Toronto, Canada; [6]U Ottawa, Canada; [7]McMaster U, Canada; [8]U Calgary, Canada.*

Background: IgA and HSP nephropathies are common glomerulonephritites affecting children. We examined the degree to which the Oxford Scoring System was associated with a change in therapy from pre- to post- biopsy in a cohort of 46 biopsy- proven IgAN and HSPN Canadian children.

Methods: Canadian children undergoing kidney biopsy for initial diagnosis of IgAN or HSPN were consented and enrolled in the REDDCAPP registry at four centres across Canada

once the histological diagnosis was confirmed. Clinicians independently managed the care of these patients with the available local interpretation of the histopathology. Pre-biopsy and post-biopsy therapies were recorded. Tissue histology was scored centrally according to the Oxford Criteria using the M (0,1), E(0,1), S(0,1) and T(0,1,2) scoring rubric by a single pathologist. Statistical methods used the $\chi 2$ analysis of differences in categorical variables.

Results: Statistical analysis indicated that a biopsy alone was significantly associated with change of therapy ($\chi^2 = 24.2$, $p<0.05$). Specifically, performing a biopsy was most associated with a change in therapy with respect to ACEi ($\chi^2 = 25.1$, $p<0.05$), daily prednisone use ($\chi^2 = 15.5$, $p<0.05$), and azathioprine use ($\chi^2 = 19.6$, $p<0.05$), but not ARB, alternate day prednisone, mycophenolate, cyclosporine, tacrolimus or fish oil use. Assessment of the components of the Oxford Scoring System showed that no individual component of the scoring rubric was associated with change of therapy with ACEi, daily prednisone or azathioprine use.

Conclusions: Performing a biopsy is strongly associated with changing therapy for pediatric IgAN/HSP, but there is no component of the Oxford Scoring System that showed an association with changing therapy. This suggests that in clinical practice the Oxford criteria do not reflect subjective clinical or histological characteristics of disease severity that clinicians identify as triggers to alter management.

Funding: Clinical Revenue Support

FR-PO702

Validity of Oxford Classification of IgA Nephropathy in Arabs

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Background: Several classification systems has been developed trying to predict renal outcome. The aim of this study is to assess the validity of this classification in a cohort of adult Saudi patients with biopsy proven IgAN.

Methods: A retrospective review of clinical and histological data of patients with biopsy proven IgA nephropathy seen from May 1998 to May 2011 was undertaken. The study was conducted at the King Khalid University Hospital and at the King AbdulAziz Medical City, Riyadh, Saudi Arabia. A total of 70 patients with primary IgA nephropathy were included in the analysis. The primary endpoint was a worse renal outcome which was defined as decrease of estimated glomerular filtration rate by 25% of their baseline value at last follow up.

Results: The study included 70 patients with the mean age of 32.2 ± 12.9 years and a median of 3.5 years of follow up. Higher stages of mesangial score and segmental glomerulosclerosis were associated with a trend for higher degree of proteinuria and lower estimated GFR at presentation and higher rate of worsening of renal function, but did not reach statistical significance. In multivariate logistic regression worsening of renal function was not predicted by any histologic class (Table 1).

Table 1. The odd ratios for worsening of renal function and Oxford Histological classifications of kidney biopsies.

Oxford Class	25% decrease in eGFR
M1 (M0 Control)	0.36 (0.07-1.75)
S1 (S0 Control)	1.35 (0.26-6.97)
E1 (E0 Control)	1.85 (0.35-9.83)
T1 (T0 Control)	1.55 (0.23-10.49)
T2 (T0 Control)	1.67 (0.18-15.31)

Mesangial hypercellularity in > or <50% of glomeruli (M0/1); Segmental sclerosis/adhesions–(S0/1); Endocapillary hypercellularity–(E0/1); Tubular atrophy/interstitial fibrosis–0-25%, 26-50%, >50% (T0/1/2).

Conclusions: The Oxford classification system is a useful tool that reflects the severity of the initial clinical presentation in Arabs with IgA nephropathy. However, it did not predict long term renal outcomes.

FR-PO703

Characteristics of Periglomerular Angiogenic Small Vessels in IgA Nephropathy

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Background: In diabetic nephropathy, several angiogenic small vessels are frequently observed as polar vasculosis at the glomerular vascular pole. In IgA nephropathy (IgA GN), angiogenic small vessels at the periglomerular areas are also detected sometimes.

Methods: Periglomerular angiogenic small vessels are defined as small vessels with pericytes or monolayered smooth muscle cells around Bowman's capsule, except for afferent and efferent arterioles. We examined 114 cases of IgA GN. Clinical and histopathological characteristics of IgA GN with angiogenic small vessels were clarified.

Results: In the 114 cases of IgA GN, 46 cases (40.4%) had the glomeruli with periglomerular angiogenic small vessels, and showed higher incidence of glomerular (global and segmental) sclerosis, crescentic lesions than the rest ($P<0.001$). Interestingly, no periglomerular angiogenic small vessels could be detected in the cases with only active glomerular lesions. Angiogenic small vessels were present at glomerular hilus (42 glomeruli in 28 cases), glomerular adherence lesions (47 glomeruli in 28 cases), and the other regions (24 glomeruli in 19 cases). Serial sections revealed that periglomerular angiogenic small vessels were connected between glomerular capillaries and peritubular capillaries, sometimes with red blood cells, indicating functional vessels. We also found angiogenic small vessels, located in neither glomerular hilus nor glomerular adherence lesions, would be glomerular hilus or adherence lesions in consecutive slices. In clinical findings, IgA GN patients who had glomeruli with angiogenic small vessels showed past-history of macrophematuria, however, renal function and daily proteinuria at the biopsy were not significantly differences between the cases with and without periglomerular angiogenic small vessels ($P=0.02$).

Conclusions: Periglomerular angiogenic small vessels might result from the historical active glomerular lesions, but would demonstrate resolution of these activity and development of glomerular repair.

FR-PO704

Endothelial Cell Injury in Acute Active and Chronic Glomerular Lesions in IgA Nephropathy

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Background: Glomerular capillary injuries may contribute to the progression of various glomerular diseases. We examined glomerular capillary injuries in active and chronic glomerular lesions in IgA nephropathy. We selected renal biopsy samples of IgA nephropathy (n = 149).

Methods: Glomerular endothelial cell injuries were assessed by immunohistochemistry for CD34 and electron microscopy, and the correlation between these lesions and renal functions was examined. The characterization of endothelial cells injury were examined in various glomerular lesions that were difined by Oxford classification. Acute active and chronic glomerular lesions were defined using ISN/RPS 2003 classification of lupus nephritis.

Results: Injured glomerular capillaries in active glomerular lesions (endocapillary hypercellularity, fibrinoid necrosis, karyorrhexis, rupture of glomerular basement membrane (GBM), and cellular and fibrocellular crescents) were characterized by separation of endothelial cells from GBM, and loss of glomerular capillaries, together with inflammatory cell infiltration, fibrin exudation, rupture of GBM, and/or cellular and fibrocellular crescent formation. Injured capillaries in chronic glomerular lesions were characterized by loss of capillaries and segmental or global glomerular sclerosis with or without fibrous crescents.

In acute active glomerular lesions, necrosis and crescents correlated significantly with hematuria (necrotic lesion, $p<0.05$; crescents, $p<0.01$) and daily proteinuria (necrotic lesion, $p< 0.01$; crescents, $p<0.01$). In chronic glomerular lesions, segmental or global sclerosis correlated significantly with daily proteinuria (segmental sclerosis, $p< 0.01$; global sclerosis, $p<0.01$) and renal dysfunction (segmental sclerosis, $p< 0.01$; global sclerosis, $p<0.01$).

Conclusions: In conclusion, glomerular endothelial cell injuries involved in the formation of severe and progressive glomerular injuries, and contributed to disease activity and the progression of chronic lesions in IgA nephropathy.

FR-PO705

The Correlation between Histological Parameters and Renal Prognosis with or without Steroid Therapy in IgA Nephropathy

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Background: The Japanese guideline for IgA nephropathy by Ministry of Health, Labour and Welfare is widely used in Japan but the prognostic factor is different from oxford classification. These guidelines were produced using relationship between renal prognosis and pathology. The renal prognosis may have been affected by various treatments including steroid therapy.

Methods: We retrospectively analyzed 56 patients who could be pursued over five years after renal biopsy out of 220 patients diagnosed with IgA nephropathy between 1991 and 2000. Renal prognosis was evaluated using alteration rate of estimated glomerular filtration rate (eGFR). Correlation between histological parameters and eGFR decline rate was evaluated in all patients (ALL), moreover evaluated separating two groups; steroid using (S) group (N=18) and steroid non-using (N) group (N=38).

Results: On the histological analysis using each parameter, the % of total crescent, adhesion, collapsing, segmental sclerosis (SS), global sclerosis (GS), tubular atrophy/interstitial fibrosis (TI) had significant positive correlation with eGFR decline rate in ALL($P<0.001$on TI, others $P<0.01$). Those parameters didn't show correlation with eGFR decline rate in group S, but showed significant positive correlation with eGFR decline rate in group N ($P<0.001$on TI, others $P<0.01$).

Using the Japanese guideline for IgA nephropathy, the % of chronic lesion (SS, GS, and fibrous crescent) didn't correlate with eGFR decline rate in group S, but showed significant positive correlation with eGFR decline rate in ALL and group N ($P=0.002$, 0.012). Meanwhile the % of active lesion (cellular crescent and fibrocellular crescent) didn't correlate with eGFR decline rate in any group.

Conclusions: The correlation between histological parameters and renal prognosis in IgA nephropathy was different whether steroid therapy was given or not. Steroid therapy might influence histological parametersconcerning renal prognosis. Further analysis of the retrospective study is needed to clarify the relationship not using steroid therapy.

FR-PO706

Membranoproliferative-Like Henoch-Schönlein Purpura Nephritis in Children: Clinico-Pathological Features and Outcome Yanjie Huang,[1] Xiaoqing Yang,[1] Tatsuo Yamamoto.[2] *[1]Pediatrics, The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, China; [2]Second Department of Medicine, Numazu City Hospital, Japan.*

Background: The prognostic value of renal clinical symptoms and histological lesion in children with membranoproliferative-like Henoch-Schönlein purpura nephritis (HSPN), which belongs to International Study of Kidney Disease in Children (ISKDC) grade VI, remains to be clarified.

Methods: We analyzed retrospectively the clinico-pathological features and outcome of 9 cases of membranoproliferative-like HSPN, which were all hospitalized patients in our hospital between 2008 and 2010. The morbidity of The ISKDC grade VI was 3.67% in 245 cases of HSPN with renal biopsy at the same period. Among the 9 patients, 7 (77.78%) cases presented with hematuria and nephrotic syndrome, and received steroids (oral prednisone or venous methylprednisolone pulse therapy) and immunosuppressive drugs (oral tripterygium glycosides or venous cyclophosphamidepulse therapy); 1 (11.11%) with hematuria and heavy proteinuria (>50mg/kg/24h) received oral prednisone and tripterygium glycosides; 1 (11.11%) with hematuria and moderate proteinuria (25~50mg/kg/24h) were only treated with oral tripterygium glycosides.

Results: Light microscopic examination showed that the diffuse membranoproliferative-like lesions, including glomerular mesangial and endothelial cell proliferation, mesangial interposition, and double contours formation in all cases. The predominant deposition of IgA and fibrinogen in mesangium and segmentally in capillary walls were observed in all cases. Nine patients were followed up for 1 to 3 years, and all had recovered.

Conclusions: In this study, the clinical manifestation of Membranoproliferative-like HSPN in children was serious, but the outcome was well. It may reason from all specimens with very mild glomerulosclerosis and tubulointerstitial fibrosis, and without severe destruction of the glomerular basement membrane.

FR-PO707

Low Proximal Tubular Expression of Megalin and Cubilin in Highly Albuminuric Patients with IgA Nephropathy Jia Sun,[1] Kjell R. Hultenby,[2] Annika Wernerson,[1] Jonas Axelsson,[3] Karin Elisabet Lindström.[1] *[1]CLINTEC, Karolinska Institutet, Stockholm, Sweden; [2]Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.*

Background: Reabsorption of polypeptides from the glomerular filtrate occurs via receptor-mediated endocytosis in the proximal tubule (PXT), primarily mediated by the receptors, megalin and cubilin. While previous studies in dogs have indicated altered PXT lysosome formation in proteinuria, megalin and cubilin expression was not investigated and there are few human data. The aim is to investigate human PXT expression of megalin and cubilin in nephritis with high and low levels of albuminuria.

Methods: Using immunogold electron microscopy (iEM), we studied renal biopsy samples from subjects with clinical and immunohistochemically verified IgA nephropathy. From each biopsy, 10 PXT were randomly selected, and from each three images of the epithelium were obtained using a random procedure. PXT epithelial and intracellular expression of megalin, cubilin and albumin were tested using polyclonal antibodies.

Results: We assayed 6 patients with low (LA; n=2, GFR: 98-114ml/min, U-alb: 58-150mg/24h), medium (MA; n=2, GFR: 65-68ml/min, U-alb: 1499-1925mg/24h) and high (HA; GFR: 61-132ml/min, U-alb: 6938-9767mg/24h). Compared to LA, both MA and HA patients had significantly higher intra cellular but not membrane-bound albumin. Meanwhile, expression of cubilin and megalin in both locations decreased as albuminuria increased (Fig 1).

Conclusions: Highly albuminuric IgA nephropathy is characterized by decreased expression of megalin and cubilin, while intracellular albumin is increased.

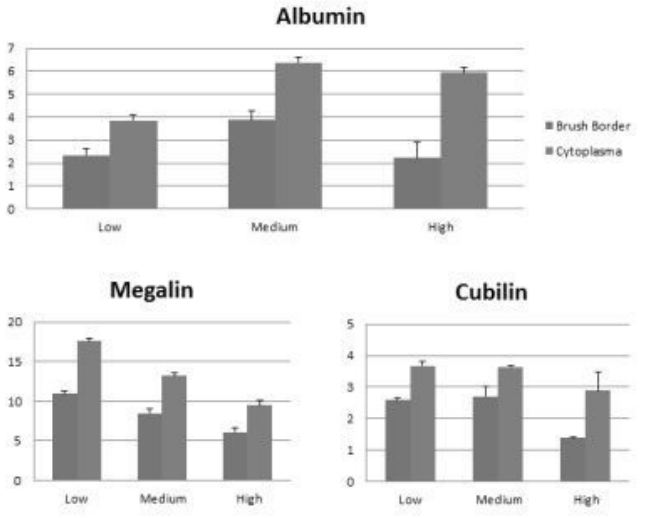

Figure 1. Expression of megalin, cubilin and albumin in LA, MA and HA patients.

Funding: Government Support - Non-U.S.

FR-PO708

Thrombotic Microangiopathy in IgA Nephropathy Is Strongly Associated with Hypertension and Chronic Lesions Jamie S. Chua,[1] Johan W. De Fijter,[2] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden University Medical Center, Leiden, Netherlands.*

Background: In a recent publication from Karoui et al, 53% of 128 cases with IgA nephropathy (IgAN) had lesions consistent with thrombotic microangiopathy (TMA). The aim of our study was to validate these findings.

Methods: In this study, 69 consecutive biopsies from the period 2005-2012 were incorporated, of which 56 were previously diagnosed with IgAN and 13 with Henoch-Schonlein nephritis (HSN). Biopsies were re-evaluated according to the Oxford Classification. TMA, arterial intimal sclerosis and arteriolar hyalinosis were scored according to the guidelines of Cattran et al. Clinical data comprising age, sex and blood pressure were collected.

Results: TMA occurred in 20.3% (n=14) of all biopsies and in 23.2% (n=13) of all IgAN patients. Only 1 patient with HSN had TMA. TMA lesions were chronic in 78.6% (n=11) and acute in 21.4% (n=3). Mesangial hypercellularity, IFTA and arterial intimal fibrosis were found more frequently in patients with TMA than in those without TMA (X-square test, respective p-values 0.02; <0.001 and <0.001). Also hypertension was present more frequently in patients with TMA than in those without TMA (82% versus 41%, p=0.02). All reported associations remained statistically significant when omitting patients with HSN.

Conclusions: It was hypothesized by Karoui et al. that in their study, the high frequency of TMA in IgAN could be explained by the selection of patients from a hypertension-clinic. Our results confirm this hypothesis. Moreover, we also find TMA in IgAN to be associated with chronic damage. Although in our study, TMA was found less frequently in patients with IgAN than recently reported, it seems clear that IgAN-associated TMA is an underappreciated phenomenon.

FR-PO709

An Overview of Idiopathic Renal Thrombotic Microangiopathy Saeed Iftikhar Khilji, Catherine M. Brown, Anthony Dorman, Patrick O'Kelly, John J. Walshe. *Nephrology Department, Beaumont Hospital, Dublin, Ireland.*

Background: RenalThrombotic Microangiopathy is a well-recognized complication of numerous established medical conditions. We have observed a number of cases where no known cause was found; Idiopathic Renal Thrombotic Microangiopathy (IRTMA). However, reports on IRTMA are limited. The aim of this study was to give an overview and outcomes of IRTMA in our centre.

Methods: We conducted a retrospective review of all renal biopsies performed in our institution from 1996 to 2009 inclusive. The pts presenting with a known cause for TMA were excluded. Indications for renal biopsy, clinical course and outcomes were studied.

Results: A total of 5338 renal histopathology reports were reviewed and 436 pts were identified with a diagnosis of TMA. Follow up in our centre was available for 282 patients, with 80 patients confirmed as cases of IRTMA. Characteristics of IRTMA included a higher male to female ratio (n=46/34) and average age at the time of biopsy was 47 years (SD 13.52) ranging from 25 to 81 years. Indications for biopsy in patients with IRTMA were impaired renal function and hypertension(gp A),ARF(gp B),isolated proteinuria(gp C) and hypertension and proteinuria(gp D).Outcomes for the 4 groups are shown in Table 1.

Table 1: Indications and outcomes for patients with IRTMA

Outcomes	Group A- Impaired renal function and HTN 41%(n=33)	Group B- ARF 25%(n=20)	Group C- isolated proteinuria 23%(n=18)	Group D- HTN and proteinuria 11%(n=9)
Normal renal function	0	3	13	9
Impaired renal function	22	3	1	0
Haemodialysis or peritoneal dialysis	8	6	4	0
Renal Transplantation	3	8	0	0

Overall patient survival was 90%. Age at the time of biopsy and female sex were associated with better renal function and survival but were not statistically significant. A multivariate analysis revealed no independent risk factor for recovery to normal renal function or survival.

Conclusions: We believe that IRTMA is an under reported condition which requires further study. It presents most commonly with impaired renal function and hypertension followed by ARF. Our data suggests that the outcome is variable but patients with isolated proteinuria have the best clinical course.

FR-PO710

The New Histopathologic Classification for ANCA-Associated Glomerulonephritis Predicts Renal Relapse Arda Goceroglu,[1] Chinar Rahmattulla,[1] Robert A. De Lind van Wijngaarden,[2] Annelies Evaline Berden,[1] Kerstin W. Westman,[3] Oliver Flossmann,[4] Herbert Hauer,[5] David R.W. Jayne,[6] Niels Rasmussen,[7] Laure-Helene Noel,[8] Franco Ferrario,[9] Ruediger Waldherr,[10] Charles D. Pusey,[11] Ron Wolterbeek,[1] Ernst C. Hagen,[12] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Leiden University Medical Center; [2]Erasmus Medical Center Rotterdam; [3]Skane University Hospital Malmö; [4]Royal Berkshire Hospital; [5]Medical Center Leeuwarden; [6]Addenbrooke's Hospital, Cambridge; [7]Statens Seruminstitut; [8]Necker Hospital; [9]San Gerardo Hospital; [10]University of Heidelberg; [11]Imperial College London; [12]Meander Medical Center.*

Background: This study investigates whether the new histopathologic classification of ANCA-associated glomerulonephritis (AAGN) [Berden, JASN 2010] predicts renal relapse during long term follow-up.

Methods: Diagnostic renal biopsies with minimally 10 glomeruli and clinical data of 109 patients with mild to severe AAGN from 2 multi-center European randomized clinical trials were available. Cox regression analysis was performed with the histopathologic classification, age and baseline eGFR (eGFR0) as candidate predictors. Diagnosis of renal relapse was based on clinical manifestations attributable to active AAGN. Data were censored for end-stage renal failure and last visit.

Results: The median follow-up was 91 months (range 0.2–136 months). During follow-up 16 (15%) patients had a renal relapse. The AAGN classification was, corrected for age and eGFR0, independently related to renal relapse (p=0.027). Patients with a focal class (HR=0.095, 95% CI 0.013-0.671, p=0.018) or crescentic class (HR=0.191, 95% CI 0.054-0.675, p=0.010) had a lower risk for renal relapse than patients with a sclerotic class biopsy. Patients with a mixed class did not show a lower risk than patients with a sclerotic class (HR=0.230, 95% CI 0.042-1.263, p=0.091).

Conclusions: In this long term follow-up study, the new histopathologic classification for AAGN proved to be an important predictor for renal relapse. Regarding the focal, crescentic and sclerotic class, the risk for renal relapse increased with ascending class.

FR-PO711

Dutch TRAnsplantation in VAScultis (DUTRAVAS)-Study: A Multi-Center Study on Renal Transplantation in ANCA-Associated Vasculitis (AAV) Arda Goceroglu,[1] Chinar Rahmattulla,[1] Annelies Evaline Berden,[1] Marlies E.J. Reinders,[1] Marcory van Dijk,[2] Carine Peutz-Kootstra,[3] Maarten H.L. Christiaans,[3] Iris Noorlander,[4] Roel Goldschmeding,[5] Arjan D. Van Zuilen,[5] Eric Steenbergen,[6] Luuk Hilbrands,[6] Lorraine Harper,[7] Mark A. Little,[8] Ernst C. Hagen,[9] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Leiden University Medical Center; [2]University Medical Center Groningen; [3]Maastricht University Medical Center; [4]Erasmus Medical Center Rotterdam; [5]University Medical Center Utrecht; [6]Radboud University Nijmegen Medical Center; [7]University of Birmingham; [8]Trinity College Dublin; [9]Meander Medical Center.*

Background: It is practically unknown how a disease recurrence of AAV in a renal allograft defines graft outcome. We investigated the outcome of renal transplantation in Dutch patients with AAV with special focus on disease recurrence and graft survival within 5 years after transplantation.

Methods: Patients with AAV and a renal transplant were retrospectively recruited through the Dutch national pathology database PALGA. Transplant biopsies and clinical data were available for an analysis on 83 patients. Biopsies were scored according to Banff '09. Renal disease recurrence was scored according to the new histopathologic classification of ANCA-associated glomerulonephritis.

Results: Five years after transplantation, 16 grafts were lost: 4 due to recurrence (1 focal, 1 crescentic, 2 mixed class), 4 due to infarction, 3 due to acute rejection, 3 due to interstitial fibrosis and tubular atrophy, 2 due to sepsis and 1 due to PTLD. Ten patients had a relapse: 6 intra- and extrarenal, 3 intrarenal (3 focal, 1 crescentic, 4 mixed class and 1 unknown) and 1 extrarenal. There were 19 histologically proven acute rejections in 17 patients.

Conclusions: This is one of the largest studies to date investigating long-term outcome of renal allografts in AAV patients. The relapse rate in this cohort was 3.3% per patient year within 5 years follow-up. Five years graft-survival was 78.3%. In a substantial proportion of patients with disease recurrence in the kidney (4 out of 9) the recurrence led to graft loss within 5 years after transplantation.

FR-PO712

CCL-18 Is a Potential Target in Renal Tissues of Patients with ANCA Associated Rapidly Progressive Glomerulonephritis Gesa Stege,[1] Silke R. Brix,[1] Thorsten Wiech,[2] Elion Hoxha,[1] Benjamin Otto,[3] Kristin Klaetschke,[3] Ulf Panzer,[1] Gunter B. Wolf,[4] Wolfram J. Jabs,[4] Fedai Özcan,[4] Frieder Keller,[4] Dirk Bokemeyer,[4] Peter J. Heering,[4] Karl Wagner,[4] Saskia Schröder,[1] Ursula Kneissler,[2] Udo Helmchen,[2] Rolf A. Stahl.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Germany; [2]Nierenregister Hamburg, Universitätsklinikum Hamburg-Eppendorf, Germany; [3]Klinische Chemie, Universitätsklinikum Hamburg-Eppendorf, Germany; [4]RPGN Study Group, Germany.*

Background: In order to characterize potential new molecular targets in the pathogenesis of ANCA associated rapidly progressive glomerulonephritis (RPGN) we prospectively analyzed 62 patients with necrotizing glomerulonephritis.

Methods: RNA from 27 renal biopsies of these patients and from 17 control kidneys was used for microarray analysis. CCL-18 mRNA levels referred to 18S RNA were determined by real-time RT-PCR (TaqMan). CCL-18 protein was detected in serum by ELISA (R&D Systems) and by staining of renal biopsies with anti-CCL-18-antibody (PeproTech).

Results: Among 1,300 differentially regulated transcripts 935 were up-regulated. One of the most up-regulated transcripts in patients with ANCA associated RPGN was the chemokine C-C-type motif ligand 18 (CCL-18).

CCL-18 is a chemokine which plays a role in macrophage maturation and is a chemoattractant of T-cells. Compared with controls CCL-18 expression was 94 times higher in renal biopsies from patients with RPGN when measured by RT-PCR. Immunohistologic analysis of renal biopsies from patients with RPGN showed expression of CCL-18 protein in a subpopulation of tubulointerstitial cells, which belong to the monocytic or dendritic cell lineage. CCL-18 serum levels were significantly higher in patients with ANCA associated RPGN (AR) compared to those with non-ANCA associated RPGN (NR) (32 AR: median 95.1 ng/mL; IQR: 62 - 163.7 ng/mL vs 12 NR: median 37.1 ng/mL; IQR:20.3 - 67.4 ng/mL; p=0.0013).

Conclusions: Our findings suggest, that CCL-18 may play a pathophysiologic role in ANCA associated RPGN but needs further prospective clinical evaluations.

FR-PO713

Need of Interstitial Fibrosis Parameter on the Newly Proposed Simplified Glomerular Histological Classification to Predict the Longterm Outcome in Japanese Cohort of MPO-ANCA Associated RPGN Eri Muso,[1] Tomomi Endo,[1] Wako Yumura,[2,3] Kensuke Joh.[4] *[1]Division of Nephrology and Dialysis, Kitano Hospital the Tazuke Kofukai Medical Research Institute, Osaka, Japan; [2]4th Dept Internal Medicine, Tokyo Women Medical College, Tokyo, Japan; [3]Department of Nephrology, Jichi University School of Medicine, Jichi University School of Medicine, Tochigi, Japan; [4]Sendai Shaho Hospital, Sendai, Japan.*

Background: The newly proposed classification categorized into focal(F), crescentic(C), mixed(M), and sclerotic(S) showed prognostic value for 1- and 5-year renal outcomes (Berden et al :JASN 21: 2010). The predictive potency of this newly proposed categorization was performed in Japanese cohort with dominant MPO-ANCA positive MPA patients.

Methods: 87 patients,median age 63 years, male: female:37:50 Renal biopsy was performed in all before treatment. At least 5 year prognosis was observed and eGFR and renal survival at onset and 6months, one year and 5 years after renal biopsy period were analyzed. The histological categories based on glomerular lesion was performed as Berden et al (JASN 2010) In addition, the severity of interstitial fibrosis(IF) were categorized into three grade from .<50%, 50-75%, >75% and eGFR change of various classes adding these parameters on the 4 glomerular classes were compared.

Results: In 87 Japan cases, all were MPA patients. 41were F, 6:C, 26:M and 14:S, respectively. Higher frequency of F and rarer in C were noted. Median number of glomeruli per biopsy, renal survival data available for 5-years and life survival in one year were higher in Japan than JASN cases (25.9vs 14.8, 60/87 vs 47/100 and 76/87 vs 75/100, respectively) Survival rate of S was significantly poor. EGFR outcomes at 5years independently favorable in F and poor in S were consistent with JASN cases, however, no significant difference between C and M in Japan. After adding interstitial parameter, M with >75% IF showed significantly lower eGFR than F, C with same IF and M with less IF and higher than S with same IF.

Conclusions: In Japan, only in S group, the prediction was potent by glomerular classification. For other glomerular classes, the evaluation adding interstitial fibrosis parameter was necessary.

Funding: Private Foundation Support

FR-PO714

Necrotizing Vasculitis of Small Renal Arteries in Patients with Anti-Neutrophil Cytoplasmic Antibody Associated Nephritis Akiko Endo,[1,2] Yoshifumi Ubara,[1] Keiichi Sumida,[1] Noriko Hayami,[1] Tatsuya Suwabe,[1] Junichi Hoshino,[1] Kenmei Takaichi,[1] Yoshihiro Arimura,[2] Akira Yamada.[2] *[1]First Department of Internal Medicine, Kyorin University School of Medicine, Tokyo, Japan; [2]Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: Anti-neutrophil cytoplasmic antibody (ANCA)-associated nephritis is considered to be a vasculitis of small vessels, including the glomerular capillaries, arterioles, and interlobular to arcuate arteries. Although crescent formation affecting glomerular capillaries had been reported as representative of this disease, the significance of coexisting vasculitis of small arteries (such as the arterioles, interlobular arteries, and arcuate arteries) has not been investigated.

Methods: The subjects were 50 patients with rapidly progressive nephritis and ANCA positivity whose renal biopsy specimens contained arterioles and interlobular arteries. They were retrospectively evaluated. Cellular crescents and/or necrotizing glomerulonephritis were noted in all 50 patients. Patients were classified as having necrotizing vasculitis with inflammatory cells and fibrinoid necrosis affecting their arterioles and interlobular arteries (Group A, n=10) or as being without necrotizing vasculitis (Group B, n=40), and were compared clinically.

Results: Granulomatosis with polyangiitis (GPA) was diagnosed in 40% of Group A versus only 2.5% of Group B ($p<0.05$). C-reactive protein (CRP) was significantly higher in Group A compared with Group B (11.58±6.19 versus 2.7±3.55 mg/dl, $p<0.05$). Pulmonary involvement was significantly more frequent in Group A compared with Group B (80% versus 37.5%, $p<0.05$), and the relapse rate was significantly higher in Group A than Group B ($p<0.05$).

Conclusions: Necrotizing vasculitis of small renal arteries may be closely related to elevation of CRP and pulmonary involvement.

FR-PO715

Intrarenal Plasma Cells in ANCA Associated Rapidly Progressive Glomerulonephritis Might Be a Marker of Disease Activity and Predict Clinical Outcome Silke R. Brix,[1] Thorsten Wiech,[2] Gesa Stege,[1] Ulf Panzer,[1] Gunter B. Wolf,[3] Wolfram J. Jabs,[3] Fedai Özcan,[3] Frieder Keller,[3] Dirk Bokemeyer,[3] Peter J. Heering,[3] Karl Wagner,[3] Udo Helmchen,[2] Rolf A. Stahl.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Nierenregister, Institut für Pathologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [3]RPGN Study Group, Germany.*

Background: The potential pathogenic role of B cells in the development of ANCA associated rapidly progressive glomerulonephritis (RPGN) becomes increasingly evident and is supported by the successful treatment of these patients with Rituximab. The significance of antibody producing plasma cells in these inflamed kidneys is, however, unclear.

Methods: We therefore studied the appearance and localisation of plasma cells in 10 renal biopsies of patients with ANCA associated RPGN (MPO-ANCA, n=5; PR3-ANCA, n=5). Glomeruli were categorised for the presence of necrosis, crescents, sclerosis and capsular rupture. Plasma cells of 94 periglomerular and 50 interstitial fields were counted using immunohistochemical stains (Immunoglobulins A, G and M).

Results: Plasma cells were seen in all stages even in the early stages of glomerular damage. The number of periglomerular plasma cells increased significantly when glomeruli had a capsular rupture (64 vs. 30; $p=0.04$). According to the classification of Berden et al., these 10 biopsies contained 1 focal case, 3 crescentic and 5 mixed cases as well as 1 sclerotic case. Initiation of immunosuppressive treatment before biopsy did not alter the density of plasma cells. In our cohort a high number of plasma cells correlated with a poor renal function at the time of biopsy ($p=0.03$). Patients with plasma cell rich biopsies showed greater improvement in renal function after six months compared to those with few plasma cell infiltrates ($p=0.03$).

Conclusions: A high number of intrarenal plasma cells might be a marker of disease activity. Furthermore the presence of plasma cells may predict a better response to treatment and could influence the decision towards a therapy of B cell depletion.

FR-PO716

Clinico-Pathological Characteristics of Rapidly Progressive Glomerulonephritis (RPGN) of Post-Infectious Glomerulonephritis (PIGN), Compared with RPGN of Anti-Neutrophil Antibody-Associated Glomerulonephritis (ANCA-GN) Yu Tateishi,[1] Toshiyuki Komiya,[2] Tatsuo Tsukamoto,[2] Eiji Ishimura,[1] Masaaki Inaba,[1] Eri Muso.[2] *[1]Osaka City University Graduate School of Medicine, Japan; [2]Kitano Hospital, Japan.*

Background: PIGN, a relatively rare type of RPGN, has been reported to recently increase in the elderly. The aim of the present study is to reveal the clinico-pathological characteristics of RPGN of PIGN to make a rapid differential diagnosis from RPGN of ANCA-GN.

Methods: Fifty five cases of RPGN, which consisted of 8 PIGN (7 males and 1 female, 69.3±8.4 years) and 47 ANCA-GN (29 males and 18 females, 69.2±12.1 years) who were admitted from 2001 to 2010, were retrospectively examined. Clinical data at renal biopsy were compared between the two types of RPGN. Pathological findings of renal biopsy, evaluated according to the pathological classification of ANCA-GN (Clin Exp Nephrol 2008, 12: 277), were compared between the two.

Results: All 8 PRGN of PIGN had bacterial infection with negative titer of ANCA, and was treated by antibiotics after the diagnosis. All 47 RPGN of ANCA-GN were treated by corticosteroid after the diagnosis. Urinary protein levels of PIGN group were significantly higher than those of ANCA-GN group (8.9±5.7 v.s. 1.1±0.3 g/day, $p<0.05$). Serum IgA and compliment C3 levels of PIGN were significantly higher than those of ANCA-GN group (631±256 v.s. 332±41 mg/dL, $p<0.05$; 86.1±15.5 v.s. 110.1±8.3 mg/dL, $p<0.05$; respectively). Regarding pathological findings, mesangial cell proliferation (46.9±14.0% v.s. 22.9±21.2% of all glomeruli, $p<0.01$), and endocapillary proliferation (24.9±15.1% v.s.10.3±15.9%, $p<0.05$) were significantly more frequently observed in PIGN group than in ANCA-GN group, but cellular and fibrocellular crescents were significantly less in the former ($p<0.05$). Immunopathologically, positive staining of IgA was significant in PIGN, while negative staining in all ANCA-GN.

Conclusions: RPGN of PIGN was characterized by nephrotic range of proteinuria, higher serum IgA and complement 3 levels, and pathological findings of mesangial and endocapillary proliferation. These clinico-pathological features are helpful to differentiate RPGN of PIGN from RPGN of ANCA-GN.

FR-PO717

Laser Microdissection and Proteomic Analysis of Amyloidosis, Fibrillary and Immunotactoid Glomerulonephritis Sanjeev Sethi,[1] Julie A. Vrana,[1] Jason David Theis,[1] Anjali Sethi,[3] Samih H. Nasr,[1] Lynn D. Cornell,[1] Nelson Leung,[2] Fernando C. Fervenza.[2] *[1]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2]Internal Medicine, Mayo Clinic, Rochester, MN; [3]Drexel University College of Medicine, Philadelphia, PA.*

Background: Organized deposits are present in amyloidosis, fibrillary and immunotactoid glomerulopathy. The deposits are fibrillar in amyloidosis and fibrillary glomerulonephritis and microtubular in immunotactoid glomerulopathy. We performed these studies to determine the composition of the deposits to better characterize these conditions.

Methods: We performed laser microdissection of glomeruli in 5 cases of amyloidosis and 3 cases each of fibrillary and immunotactoid glomerulopathy. This was followed by tandem mass spectrometry based proteomics analysis.

Results: We divide the results into 3 major groups: structural proteins, complement proteins, and immunoglobulin/others.

Structural Proteins: Large spectra of vimentin, collagen alpha-3, basement membrane heparin sulfate, vinculin and fibrillin are noted in immunotactoid glomerulopathy compared to fibrillary glomerulonephritis. On the other hand, large spectra of apolipoprotein E, vitronectin, and clusterin are noted in fibrillary glomerulonephritis compared to immunotactoid glomerulopathy. Other than apolipoprotein E, vitronectin and serum amyloid P component, amyloid deposits do not contain large spectra of any other structural proteins compared to fibrillary and immunotactoid glomerulopathy. Surprisingly, small spectra of SAP are also noted in fibrillary and immunotactoid glomerulopathy.

Complement Proteins: Fibrillary and immunotactoid glomerulopathy contain large spectra of complement factors of the classical pathway compared to amyloidosis.

Immunoglobulins and other: The cases tested for fibrillary and immunotactoid glomerulopathy contain spectra for Ig gamma-1C region while amyloidosis contain spectra for light chains, serum amyloid A protein or leukocyte derived chemotaxin-2 (depending on the type).

Conclusions: There is a distinct pattern of deposition of structural, complement and fibrillar/amyloidogenic proteins in amyloidosis, fibrillary and immunotactoid glomerulopathy.

FR-PO718

Distinguishing Features of κ Light Chain AL Amyloidosis in Clinical Manifestation and Renal Biopsy Ying Yao, Youkang Zhang, Su-xia Wang. *Renal Division, Department of Medicion, Peking University First Hospital, Beijing, China.*

Background: The aim of this study was to compare the clinical and pathological features among patients with renal AL amyloidosis derived from κ light chain (AL-κ) and λ light chain (AL-λ).

Methods: We collected 190 patients with biopsy-proven renal AL amyloidosis in our institute between 1990 and 2011. AL was diagnosed by the demonstration of selective λ or κ staining of the amyloid by immunohistochemistry. Among them, 25 cases were confirmed as AL-κ, and 165 cases were AL-λ. The clinical and laboratory data at renal biopsy was recorded and used for analysis. We reassessed the kidney biopsies of all patients, and the extent of amyloid deposition in glomeruli (GA), blood vessels (VA) and interstitium (IA) were evaluated semiquantitatively. The renal amyloid load (TA) was defined by the sum of GA, VA, and IA.

Results: Monoclonal proteins were detected by serum immunofixation electrophoresis(IFE) in 39.1% of AL-κ and 59.7% of AL-λ patients ($P=0.06$). The most common monoclonal immunoglobulin type was IgM (21.7%) in AL-κ, whereas IgG (27.1%) in AL-λ, $P<0.001$. AL-κ patients presented with higher incidence of extrarenal organ involvement than AL-λ (hepatic involvement 75% versus 24.4%, $P<0.001$; cardiac involvement 72.7% versus 34.2%, $P=0.020$). Renal function and proteinuria were similar between the two groups. In renal biopsy, the extent of vascular deposition and the renal amyloid load were more severe in AL-κ than AL-λ.

Comparison of main clinical and pathological characteristics between patients with AL-κ and AL-λ

	AL-κ	AL-λ	P value
Male, n (%)	13(52)	107(64.8)	0.215
Age, years	53.9±11.2	57.6±10.6	0.115
Positive in serum IFE, n (%)	9(39.1)	80(59.7)	0.06
Positive in urine IFE, n(%)	7(53.8)	56(75.7)	0.104
Proteinuria, g/day	6.38±4.1	6.39±3.9	0.989
Nephrotic syndrome, n(%)	19(76.0)	115(76.2)	0.986
Renal insufficiency, n(%)	4(17.4)	27(18.2)	1
Heart involvement, n(%)	8(72.7)	32(34.2)	0.020
Liver involvement, n (%)	12(75)	21(24.4)	<0.001
VA, median(IQR)	3.5(3-4)	2(1-3)	0.002
TA, median(IQR)	8(5.75-9.25)	6(4-7)	0.004

IQR, interquartile range

Conclusions: Patients with AL-κ were more likely to presenting with hepatic and cardiac involvement and had severe vascular amyloid deposition in renal biopsy.

FR-PO719

The Modern Spectrum of Renal Biopsy Findings in Diabetics Shree G. Sharma,[1] Andrew S. Bomback,[2] Jai Radhakrishnan,[2] Leal C. Herlitz,[1] Michael B. Stokes,[1] Glen S. Markowitz,[1] Vivette D. D'Agati.[1] *[1]Pathology, Columbia University Medical Center (CUMC); [2]Nephrology, Columbia University Medical Center (CUMC); [3]Nephrology, CUMC; [4]Pathology, CUMC; [5]Pathology, CUMC; [6]Nephrology, CUMC; [7]Pathology, CUMC.*

Background: With the epidemic of diabetes mellitus (DM), renal biopsies performed in diabetics are increasing in number and complexity.

Methods: To assess modern trends, we performed a single-center study of clinical-pathologic findings in all diabetics biopsied over the past year. Among 2642 native kidney biopsies, 620 (23.5%) were from diabetics.

Results: The cohort included 376 (60.7%) males with mean age 60.1 ± 12.3 y and mean duration of DM 11.4 ± 8.8 y. Mean creatinine was 3.39 mg/dL, including 22% with stage 1 or 2, 27% stage 3, 25% stage 4 and 27% stage 5 CKD. On renal biopsy, 36% had nondiabetic renal disease (NDRD) alone; 37% had diabetic nephropathy (DN) alone; and 26% had DN plus NDRD as shown.

Table 1

	DN	DN + NDRD	NDRD	p-value*
Number (%)	227 (37)	164 (26)	220 (36)	
Age (y)	57	62.7	61.4	<0.001
Duration of DM (y)	13.8	13.7	7.2	<0.001
Creatinine (mg/dL)	2.9	3.9	3.4	0.004
eGFR	42.2	33.0	45.8	0.003
Nephrotic proteinuria	123 (54.2%)	77 (47%)	69 (31.4%)	<0.001
Active urine sediment	63 (27.8%)	62 (37.8%)	74 (33.6%)	0.3
AKI (no baseline CKD)	101 (44.5%)	85 (51.8%)	110 (50.0%)	0.4
AKI on CKD	37 (16.3%)	43 (26.2%)	37 (16.8%)	0.04
All AKI	138 (60.8%)	128 (78.1%)	147 (66.8%)	0.001
Low C3/C4	2 (0.9%)	16 (9.8%)	12 (5.5%)	<0.001
M-spike	16 (7.1%)	13 (7.9%)	30 (13.6%)	0.04

* One way ANOVA

In NDRD alone, FSGS (27%), ATN (17%), hypertensive nephrosclerosis (12%), IgA nephropathy (10%), MGN (8%) and pauci-immune GN (7%) comprised 80% of diagnoses, compared to ATN (43%), hypertensive nephrosclerosis (24%), FSGS (7%) and IgA nephropathy (7%) for NDRD plus DN. Among serologies, ANCA, M spike and low C3/C4 were more likely than HCV to identify a related NDRD. In multivariate analyses, nephrotic range proteinuria (p=0.02) and longer duration of DM (p<0.001) were associated with increased odds of any DN. DM duration ≥12 y was the best predictor (57% sensitivity, 73% specificity) of DN alone.

Conclusions: Judicious use of renal biopsy has uncovered NDRD alone or superimposed on DN in the majority (62%) of biopsies.

FR-PO720

Collapsing Glomerulopathy in Advanced Diabetic Nephropathy Steven P. Salvatore,[1] Chandra B. Chandran,[2] Chike N. Okechukwu,[3] James M. Chevalier,[1] Surya V. Seshan.[1] *[1]Weill Cornell Med Coll, NY, NY; [2]St. Joseph's Med Center, Paterson, NJ; [3]Crozer-Chester Med Center, Chester, PA.*

Background: Collapsing glomerulopathy (CG) is a pattern of podocyte injury presenting with massive proteinuria, rapid progression, and relative resistance to therapy. Etiologies include viral infections, drugs, and autoimmune conditions in native kidneys, and obliterative arteriopathy in transplants, previously proposed due to ischemic podocyte injury. Glomerular ischemia also occurs in diabetic nephropathy (DN) with severe vascular hyalinosis.

Methods: DN was seen in 12.5% of native kidney biopsies (2003-2011) of which 4.8% had at least 1 glomerulus with CG. Immunostaining for vascular endothelial growth factor (VEGF), cytokeratin (CK) and podocyte markers WT-1, synaptopodin, podocin, and beta-dystroglycan were analyzed. A DN control group without CG was used for comparison.

Results: The 26 patients were 26-80 years old and mostly type 2 diabetics (78%) with long-standing disease (mean (M) 14 years), insulin dependence (67%), and hypertension (83%). Serum creatinine (Cr) levels were elevated, M 3.8 mg/dL (1.1-10.4). Most had nephrotic range proteinuria, M 9.8 g/d (2-29). No patients had HIV. Renal biopsy showed segmental or global glomerulosclerosis (GS) in 2% (0-23) and 33% (0-80), respectively. DN classification was Class IV (12), III (8), IIb (4), and IIa (2). CG was present in 2-30% (M 16%) of glomeruli. Vascular disease was prominent, moderate in 44% and severe in 56%. Extensive arteriolar hyalinosis with >50% luminal stenosis was seen in 85.2% of cases. Markers of podocyte differentiation were lost in the glomeruli with CG for 100% synaptopodin and podocin, 75% beta-dystroglycan, and 70% WT-1. CK was positive in 70% and VEGF overexpressed in 43%. Follow-up on 17 patients: 13 developed chronic renal failure M 7 months from the biopsy (0-36), significantly more than controls (P=.005). The 4 remaining, 5-24 months follow-up, had increasing Cr with stable proteinuria.

Conclusions: CG contributes to an increased level or new onset of proteinuria in DN. Identification of CG in advanced DN with significant vascular hyalinosis is presumably due to podocyte ischemia and is of prognostic significance.

FR-PO721

IgG Subclass Distribution in Different Stages of Membranous Glomerulonephritis Tibor Nadasdy,[1] Sergey V. Brodsky,[1] Anjali A. Satoskar,[1] Gyongyi Nadasdy,[1] Brad H. Rovin,[2] Lee A. Hebert.[2] *[1]Pathology, The Ohio State University, Columbus, OH; [2]Medicine, The Ohio State University, Columbus, OH.*

Background: Recent breakthrough findings revealed that most patients with idiopathic (primary) membranous glomerulonephritis (MGN) have IgG4 antibodies to the phospholipase A2 receptor (PLA2R). These IgG4 antibodies can be detected in the glomerular immune complexes and they colocalize with PLA2R. In secondary forms of MGN, such IgG4 antibodies are absent or less prevalent. There are no studies addressing the IgG subclass distribution across different stages of MGN.

Methods: During a 25 month period, we identified 157 consecutive biopsies with MGN with adequate tissue for light, immunofluorescence and electron microscopy. Of the 157 MGN cases, 114 were primary MGN; 43 were secondary MGN. The glomerular stage distribution was the following: stage 1 n=22; stage 2 n=50; stage 3 n=25, Stages 3 to 4 (overlap between stages 3 and 4) n=17. We compared the Intensity of IgG subclass staining (on a semiquantitative scale of 0 to 3+) and the IgG subclass dominance between primary and secondary MGN and between the different stages of MGN.

Results: In primary MGN most (76% of cases) were IgG4 dominant. In contrast, in secondary MGN IgG1 was dominant in 60% of biopsies (p=0.0018). Interestingly, in early stage (stage 1) primary MGN, IgG1 was the dominant IgG subclass (64% of cases); in all later stages IgG4 dominated (82%, 80% and 76% in stages 2, 3 and 3 to 4, respectively) (p=0.0493). The staining intensity for IgG1 in stage 1 MGN was 2.1 +/- 0.8 versus a 1.6 +/- 1.3 intensity for IgG4. In all later stages, IgG4 showed the strongest staining. In secondary forms of MGN, we did not find such correlations (heterogeneous group with low case numbers).

Conclusions: In early stage MGN, antibody response is different from later stages, with IgG1 dominant deposits. It is possible that early on, antigens other than PLA2R play an important role, Alternately, there may be a subclass switch in the antibody response with IgG4 taking over later as the dominant immunoglobulin.

Funding: Clinical Revenue Support

FR-PO722

Nonmuscle Myosin Heavy Chain IIA (NMMHC-IIA) as a Dominant Target Antigen in Human Idiopathic Membranous Nephropathy Chang Ying Xing,[1] Suyan Duan,[1] Peter W. Mathieson,[2] Moin Saleem,[2] Gang Liu,[3] Haiyan Wang.[3] *[1]Dept. of Nephrology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China; [2]Academic Renal Unit and Children's Renal Unit, University of Bristol, Bristol, United Kingdom; [3]Renal Division, Peking University First Hospital, Beijing, China.*

Background: Idiopathic membranous nephropathy (IMN) is the main pathological type in adult nephrotic syndrome. Although the NEP and PLA2R, etc. as target antigens have been identified in the pathophysiology of IMN, there are still lots of unanswered questions to be resolved. Our preliminary study generated a new rat model induced by rabbit anti-human podocyte protein antibody, whose clinical and pathological changes resembled the human MN, and the target antigen of this model was sequentially identified to be NMMHC-IIA expressed on podocytes.

Methods: Cell lysates were prepared from human podocytes. Western blotting(WB) and co-immunoprecipitation(Co-IP) were performed with the total cell lysates using serum from patients with IMN or other glomerular diseases or normal individuals. Mass spectrometry was used to analyze the reactive protein bands and confirm the identity of the target antigen with specific antibodies. ELISA was used to compare the antibody concentration in different patients.

Results: In Co-IP, serum from 29 of 35 patients (82.9%) with biopsy-proven IMN specifically identified a 227-kD protein, which was sequentially identified to be the NMMHC-IIA confirmed by mass spectrometry. By WB under reducing and nonreducing conditions respectively, 14 of other 15 IMN(93.3%) had circulating antibodies reacting to the NMMHC-IIA protein, while 13 of them(80.0%) had detectable anti-PLA2R antibodies. There were 11(73.3%) patients had both antibodies. Furthermore, ELISA showed much higher concentrations of the antibodies in IMN than other proteinuric diseases and normal controls(p<0.05). The follow-up results in 10 patients indicated that the concentration of autoantibodies against NMMHC-IIA was significantly positively correlated with 24-hour urinary protein.

Conclusions: Antibody against NMMHC-IIA, a podocyte protein, can be detected in a majority of patients with INM, indicating that is a major autoantigen in this disease.

FR-PO723

Autoantibody Epitope Mapping of the Phospholipase A2 Receptor as Autoantigen in Membranous Nephropathy Astrid Behnert,[1] Beina Teng,[1] Meifeng Zhang,[2] Andrej Skoberne,[1] Marvin J. Fritzler,[2] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Hannover Medical School, Hannover, Germany; [2]University of Calgary, Calgary, AB, Canada.*

Background: In idiopathic membranous nephropathy (IMN) PLA_2R was discovered as autoantigen and thus far, the published evidence suggested a conformation dependent antigen. An indirect immunofluorescence assay on cells overexpressing the receptor is the standard to detect and quantify PLA_2R autoantibodies. We examined antibody-antigen interactions in order to identify specific epitopes and reactive domains of PLA_2R that could be used to improve testing by using small peptides in various diagnostic platforms. We also tested recombinant overexpressed receptor as target antigen on ELISA and Luminex beads.

Methods: Epitope mapping was done using SPOT technology. Immunoblotting was then performed with patient serum as primary antibody (ab) and anti-human IgG_4 as a secondary ab. ELISA plates were coated with PLA_2R peptides and analyzed with patient sample as primary ab and HRP conjugated anti-human IgG_4 as detection ab. A capture immunoassay was performed by coupling anti-GFP ab to Luminex beads followed by capture of GFP-tagged PLA_2R from transfected HEK293 cell lysates. The beads were incubated with patient sample and diluted PE conjugated anti-human IgG and reactivity determined on a Luminex 100.

Results: Seven reactive antigen determinants were identified using epitope mapping and when these putative epitope regions were analyzed by testing synthetic peptides in ELISA, the absorbance of positive samples was higher than of negative and control samples but the difference was not significant ($p>0.05$). However, in the Luminex based capture assay, the median fluorescence units of positive samples was significantly higher than that of negative and healthy control samples.

Conclusions: In summary, we developed a novel observer independent assay for detecting PLA_2R autoantibodies. We could confirm that antibody binding to PLA_2R seems to depend to a large extent, on a conformational epitope. However, in our SPOT technology assay we identified multiple different epitopes that PLA_2R antibodies of patients with IMN were reacting to, confirming that epitope spreading occurs in IMN.

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FR-PO724

Rebiopsy in Idiopathic Membranous Nephropathy (IMN) on Long Term Tacrolimus Marie Condon, H. Terence Cook, Megan Griffith. *Imperial College Kidney and Transplant Centre, London, United Kingdom.*

Background: Remission of nephrotic syndrome due to IMN can be achieved with tacrolimus (Tac). Relapse rates are high on withdrawal of therapy and many pts require long term treatment. Although stable renal function is maintained in most pts, little is known about the underlying histology. This study reports histology in repeat biopsies (RBs).

Methods: 23 pts on Tac for > 12 mths have had RBs at a median time of 3.39 yrs (range 1.69 – 8.79). 11/23 in complete remission (CR) for consideration of Tac withdrawal (WD); 3/23 for rising creat in partial remission (RCPR); 9/23 for nephrotic relapse (NPR) (6/9 on Tac; 3/9 during/after withdrawal).

Results:

	Biopsy 1 - Pre Tac			Biopsy 2 > 12months on Tac			
n=23	M(R) Creat umol/L	M(R) % Segmental Sclerosis (SS)	M(R) % Tubular Atrophy (TA)	M(R) Creat umol/L	M(R) % SS	M(R) % TA	No. (%) with Arteriolar Hyaline
WD=11	97(83-203)	0(0-67)	10(0-40)	132(54-188)	8(0-20)	20(5-50)	4(36)
RCPR=3	68(61-71)	0(0-9)	5(5-10)	130(123-265)	11(10-50)	30(20-30)	2(67)
NPR=9	117(74-172)	0(0-29)	10(0-15)	122(71-239)	9(0-55)	30(10-50)	2(22)
All	96.5(61-203)	0(0-67)	10(0-40)	131(54-265)	9(0-55)	20(5-50)	8(35)

M(R) = Median(Range). Change in % SS: WD (p=0.31), RCPR (p=0.14), NPR (P=0.08). Change in % TA: WD (p=0.18), RCPR (p=0.02), NPR (p=0.001).

2/11 WD pts had stage I&II deposits on RB, despite being in CR for > 12 mths. Tac withdrawal was commenced on the other 9; 3/9 relapsed at 5, 5, & 7 mths after Tac was stopped (On RB at withdrawal 1/3 had no deposits; 2/3 had stage IV deposits). 2/3 biopsied for RCPR had a TIN (1/2 with stage I&II deposits); 1/3 had stage I&II in addition to III&IV deposits, arteriolar hyaline was also present.

Conclusions: Pts who do not achieve/sustain a CR, appear to have more progression of scarring on RB. However, even in pts with stable GFR and in CR (the WD group), an increase in SS and TA may occur. It is not clear whether these histological changes are part of the natural history of IMN or as a result of Tac; it will be important to follow the GFR in these pts. In our pre withdrawal biopsies, the absence of new immune deposits did not predict sustained remission and no specific histological features predicted relapse. More work is required to identify pts at risk of relapse.

FR-PO725

Increased Risk of Treatment Failure and End-Stage Renal Disease in Familial FSGS Xu Hao, Jingyuan Xie, Jun Ma, Qiongxiu Zhou, Wen Xue, Hong Ren, Nan Chen. *Nephrology, Nephrology Department, Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: Few studies comparedsporadicand familial focal segmental glomerular sclerosis (FSGS). The aim of this study is to study the clinical features and prognosis of FSGS patients with or without family history.

Methods: We enrolled 124 family FSGS patients from 83 families (FFSGS group) and 124 age and sex matched sporadic FSGS patients (SFSGS) in this study. FSGS patients were proven by renal biopsy. The mean follow up time was 26.5±19.5 month in SFSGS group and 28.3±12.5 month in FFSGS group ($p>0.05$). Baseline clinical characteristics of allparticipants were recorded. Primary outcomes of this study were ESRD and remission of proteinuria (defined as 50% reduction from baseline proteinuria).

Results: There were no difference in age and gender between the two groups. Patients in SFSGS group had higher urine protein secretion (2.0±1.8 vs 1.4±1.4g/24h) and lower serum albumin (3.0±1.1 vs 3.6±6.2g/dL) than FFSGS group ($p<0.01$). Accordingly, more patients had NS in SFSGS group than FFSGS group (22.6% vs 13.3%, $p=0.003$). The baseline serum creatinine and eGFR between the two groups were similar. In addition, 35.16% of sporadic and 43.75% of familial patients had high blood pressure when biopsy was performed ($p=0.08$). During follow-up period, remission of proteinuria was observed in 48.39% sporadic patients while only in 23.08% familial patients ($p=0.006$).The median ESRD-free survival times for SFSGS and FFSGS group were 96 and 72 months respectively (Log rank $p=0.04$).

Conclusions: Familial FSGS patients had increased risk to proteinuriatreatment failure and progression to ESRD compare to sporadic FSGS patients.

FR-PO726

Low Glomerular Density with Glomerulomegaly in Primary Focal Segmental Glomerulosclerosis Kentaro Koike, Nobuo Tsuboi, Yasunori Utsunomiya, Tetsuya Kawamura, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

Background: Studies have shown that glomerular size in patients with focal segmental glomerular sclerosis (FSGS) is large. Also, it is known that FSGS lesions are more frequently observed in juxtamedullary cortex in which the hemodynamic stress is more prominent. Observations of FSGS in very low birth weight infants suggest a link between FSGS and low nephron number. These findings indicate that increased glomerular capillary pressure associated with low number of glomeruli may be one of the important mechanisms implicated in the development of FSGS. We have recently reported that a low glomerular density (GD, number of glomeruli per renal cortical area) in the renal biopsy is associated with an enlarged glomerular size as well as a lesser response to corticosteroid therapy in patients with minimal change disease (MCD) (AJN, 2011). The present study aimed to prove our hypothesis that GD represents the disease status of primary FSGS.

Methods: The GD and the glomerular volume (GV) of the primary FSGS patients (n=23) were measured using a computed imaging analyzer. Biopsies of kidney transplant donor (KTD) (n=20) and those of MCD (n=50) were used as comparison.

Results: As compared to KTD and MCD, the GD of the FSGS patients was low (3.1±1.0 vs. 3.6±1.1 vs. 2.1±1.0/mm^2, $p<0.001$). In contrast, the GV in the FSGS patients was significantly larger than those of the KTD and MCD (2.4±0.6 vs. 2.5±0.8 vs. 5.0±0.3×$10^6$$\mu m^3$, $p<0.001$). Interestingly, the GD showed significant inverse correlations to the rate of FSGS lesions ($r=-0.427$) as well as the GV values ($r=-0.420$). A comparison of FSGS patients with nephrotic syndrome (NS) (n=10) and those without NS (n=13) showed different patterns of GD/GV distribution. In the FSGS patients with NS, the GD showed a relatively extensive distribution like those observed in the KTD and the MCD patients. In the FSGS patients without NS, the GD was extremely low and the GV tended to show further enlargement.

Conclusions: These results suggest that a low GD, together with glomerulomegaly, is a histological characteristic in renal biopsies of primary FSGS patients.

FR-PO727

Upper Limit of Normal for Number of Globally Sclerotic Glomeruli on a Sectioned Core-Needle Biopsy of the Renal Cortex Walter K. Kremers,[1] Hisham Elsherbiny,[1] Mariam P. Alexander,[1] Emilio D. Poggio,[2] Hatem Amer,[1] John C. Lieske,[1] Andrew D. Rule.[1] *[1]Mayo Clinic, Rochester, MN; [2]Nephrology, Cleveland Clinic, Cleveland, OH.*

Background: Global glomerulosclerosis is characteristic of chronic kidney disease but also occurs with normal aging. The objective of this study was to determine the upper limit of normal (ULN) for number of globally sclerotic glomeruli.

Methods: Living kidney donors had a core-needle biopsy of the donated kidney cortex during the transplant surgery at the Mayo Clinic from 1999 to 2009. One paraffin-embedded section (3 um thickness) stained with periodic acid-Schiff was scanned into a high resolution image file and the number of non-sclerosed glomeruli (NSG) and globally sclerosed glomeruli (GSG) were counted. Linear regression was used to predict mean number of GSG based on demographics and biopsy characteristics and quantile regression estimated the 95^{th}%ile for number of GSG.

Results: After excluding 4 donors with >25% interstitial fibrosis on renal biopsy, there were 952 donors (mean age 44 years, 43% were male, 6.3% had hypertension). Predictors of the number of GSG on renal biopsy were age, number of NSG, area of cortex, presence

of corticomedullary junction, presence of capsule, and hypertension (p<.05 for all). In the multivariable model only age, cortical area and hypertension were independent predictors (p<.05 for all). For ease of use in clinical practice, the quantile regression model was fit with age and number of NSG as predictors with only a slight decrease in the model goodness-of-fit. The 95th percentile for number of GSG is shown in the Table.

Upper limit of normal for the number of GSG on a renal biopsy section

Age	0-4	5-8	9-16	17-32	33-64
18-30	0	0	0	1	1
31-35	0	1	1	1	2
36-40	1	1	1	1	2
41-45	1	1	1	2	3
46-50	1	1	2	3	4
51-55	2	2	2	3	5
56-60	2	2	3	4	6
61-65	2	3	3	5	7
66-70	3	3	4	5	8
71-75	3	4	4	6	9

Conclusions: The ULN for number of GSG varies by age and the number of NSG on the renal biopsy. These thresholds may help identify chronic pathological injury in patients who do not have significant interstitial fibrosis.

Funding: NIDDK Support

FR-PO728

Distribution of Glomerular Density in Different Cortical Zones of the Human Kidney Go Kanzaki,[1] Nobuo Tsuboi,[1] Yasunori Utsunomiya,[1] Akira Shimizu,[2] Tetsuya Kawamura,[1] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan; [2]Department of Analytic Human Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Background: Recent studies have suggested that low nephron number associated with low birth weight is a risk factor for progression to ESRD and cardiovascular events in later life. We have recently reported that glomerular number per renal cortical area in biopsies (glomerular density: GD) differs significantly between individuals, and that a low GD predicts a worse renal outcome in patients with IgA nephropathy and idiopathic membranous nephropathy (CJASN2010, NDT2011). These results suggest that GD represents each nephron number and is useful in the prediction of CKD progression. However, variations of GD in anatomically different cortical zones remain undetermined in human kidney.

Methods: A total of 100 autopsies without CKD were included in this study. The GD and the glomerular volume (GV) in both of the superficial and juxtamedullary cortexes were measured using a computed imaging analyzer. The measured values were analyzed in relation to the clinicopathological features of these autopsies.

Results: As a whole, the GD and the GV had approximately 3.5-fold and 4.9-fold differences between the individuals, respectively. In addition, a close inverse correlation was observed between the GD and the GV (r=-0.494, p<0.001). The GD in juxtamedullary cortex was lower than that in the superficial cortex (2.2±0.6 vs. 3.0±0.7/mm^2, p<0.001). In contrast, the GV in juxtamedullary cortex was higher than that in the superficial cortex (3.1±0.8 vs. 2.7±1.0 x10^{6}μm^3, p<0.001). The GD of the juxtamedullary cortex was associated with the kidney weight. On the other hand, the GD of the superficial cortex was associated with the rate of global glomerulosclerosis and the presence of atherosclerosis lesions. Age, BMI, kidney function, heart weight and history of hypertension did not show significant relationships with the GD levels of each cortex.

Conclusions: Independent of potential variations observed between individuals, there are significant zonal differences in the distribution of GD in human kidney.

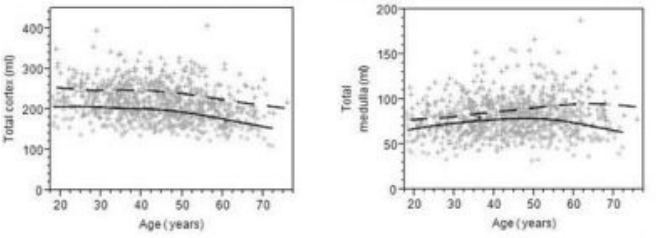
FR-PO729

Kidney Cortical Volume, but Not Medullary Volume, Decreases with Age and Partially Accounts for the Age-Related Decline in Glomerular Filtration Rate Xiangling Wang, Ramesh Avula, Hatem Amer, Leah Walters, Terri J. Vrtiska, Lilach O. Lerman, Andrew D. Rule. *Mayo Clinic.*

Background: Age-related changes in kidney morphology are poorly understood, particularly in relationship to kidney function. We investigated age trends in kidney cortical and medullary volume and assessed the relationship between the decline in GFR with normal aging and age-related changes in kidney volume.

Methods: Kidney cortical and medullary volume was measured on contrast-enhanced abdominal CT images of healthy subjects (n=878) evaluated as potential kidney donors between 2001 and 2008 at the Mayo Clinic. ITK-Snap Version 2.2.0 was used to segment kidneys and calculate three-dimensional (3D) cortical and medullary volume. Total kidney volume was sum of cortical and medullary volume; volumes of both kidneys were compiled. GFR was measured by iothalamate clearance. Kidney volumes were regressed on age and compared between genders. Multivariable regression was used to determine whether the decline in GFR with age occurred independent of age differences in kidney volumes.

Results: Among the 878 donors (59% female, ages range 18 to 75 years), kidney cortical volume decreased with age (p<.001 for both genders), and medullary volume increased with age in men (p<.001), while no linear change was evident in women (p=0.77) (Figure).



GFR declined (all per age-decade) by 7.1 (95% CI 6.1 to 8.0) ml/min/1.73 m^2 unadjusted, by 5.4 (95% CI 4.5 to 6.4) ml/min/1.73 m^2 after adjusting for cortical volume, by 7.7 (95% CI 6.7 to 8.7) ml/min/1.73 m^2 after adjusting for medullary volume, and by 6.0 (95% CI 5.0 to 6.8) ml/min/1.73 m^2 after adjusting for total kidney volume.

Conclusions: Kidney cortical (but not medullary) volume decreased with age in healthy adults, but only partially accounted for age related decline in GFR. Further work needed to identify the primary mechanism of GFR decline with aging.

Funding: NIDDK Support

FR-PO730

Glomerular Number and Individual Glomerular Volume in Female Americans Victor G. Puelles,[1] Rebecca N. Douglas-Denton,[1] Wendy E. Hoy,[2] John F. Bertram.[1] *[1]Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria, Australia; [2]Center of Chronic Disease, University of Queensland, Brisbane, Queensland, Australia.*

Background: The study of individual glomerular volume (IGV) in male Americans has provided new insights into the relationship between glomerular size and glomerular number (N_{glom}). IGV mean and heterogeneity per subject are significantly higher in Caucasian American men with low N_{glom}, compared to those with high N_{glom}. The aim of this study is to determine for the first time if this trend is also present in female Americans.

Methods: We included renal autopsy tissue from 24 females, 12 African (AA) and 12 Caucasian (CA) Americans (sampled from 129 available adult females - 77 AA and 52 CA), based on extremes of N_{glom} (low or high) for a total of 6 age-matched pairs between N_{glom} categories per race. Age and body surface area (BSA) were obtained from the autopsy report and medical records. N_{glom} was estimated by the disector/fractionator principles and IGV by the Cavalieri estimator (a total of 30 glomeruli per subject; 10 superficial, 10 middle and 10 juxtamedullary glomeruli). Analysis was performed using non-parametric tests within N_{glom} categories.

Results: In AA with high N_{glom}, N_{glom} was >1.06 million (mean: 1.2 million) and in those with low N_{glom}, N_{glom} was <0.65 million, (mean: 0.58 million). AA with high N_{glom} had a 3-fold range in IGV, with a median of 3.9; while those with low N_{glom} had a 7.6-fold range of IGV, median 4.7 (P=0.24 for mean, P=0.26 for variance). In CA with high N_{glom}, N_{glom} was >0.99 million glomeruli (mean: 1.1 million) and in those with low N_{glom}, N_{glom} was <0.65 million glomeruli, (mean: 0.45 million). CA with high N_{glom} had a 4.2-fold range in IGV, with a median of 3.5; while those with low N_{glom} had a 4.8-fold range of IGV, median 3.9 (P=0.59 for mean, P=0.13 for variance). It is noteworthy that BSA was greater in AA compared to CA females (P=0.01).

Conclusions: AA females with lower N_{glom} tended towards a higher mean IGV and IGV variability than those with high N_{glom}. Such a trend was not apparent in CA. The higher BSA in the AA females might be exacerbating the glomerular hypertrophy in the setting of modestly lower N_{glom}.

Funding: NIDDK Support

FR-PO731

Calcineurin Inhibitors (CNI) Cause Glomerulosclerosis in Children in the Absence of Arteriolar Hyalinosis: An In Vivo Model for CNI Nephrotoxicity in Non-Renal Conditions Matthew Tabinor,[1] Martin T. Christian,[1] Thomas A. Mcculloch.[2] *[1]Children's Renal and Urology Unit, QMC Campus, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [2]Department of Histopathology, City Campus, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom.*

Background: The calcineurin inhibitors (CNI) ciclosporin (CsA) and tacrolimus (Tac) cause chronic nephrotoxicity which is demonstrated on renal biopsy by arteriolar hyalinosis (AH). We have observed glomerulosclerosis (GS) in the absence of AH during chronic CNI therapy. We hypothesise GS can occur without AH, making GS an independent marker of CNI nephrotoxicity.

Methods: Retrospective analysis of biopsies from a histopathology database between 1/1/1997 and 1/3/2012 in patients ≤ 18y with nephrotic syndrome caused by minimal change nephropathy (MCN). Other histological lesions, which could confound for GS, were excluded.

Results: 109 biopsies were grouped into preCNI therapy (n=83), postCNI therapy without AH (n=22) and postCNI with AH (n=4). The difference in mean %GS between preCNI (1.1%) and postCNI without AH (10.6%) was significant (p=0.001). %GS was significantly associated with duration of CNI therapy (n=26, R=0.28, p<0.001) but not disease duration (n=60, R=0.009, p=0.81). Analysis of serial biopsies revealed a significant change in the mean %GS between the 1st (preCNI therapy) and 2nd biopsy (n=13; 9 treated with CsA; 4 treated with Tac; Δ%GS=+11.9%; p=0.006), representing significant

progression of GS on CNI therapy. There was a positive association between average CNI plasma trough level and %GS, but this was non-significant for both CsA (n=14, R=0.37, p=0.29) and Tac (n=5, R=0.13, p=0.55).

Conclusions: GS occurs without AH in children with MCN treated with CNI and correlates with the duration of CNI therapy. Progressive GS does not occur in MCN, indicating the development of GS during CNI therapy represents an *in vivo* model of nephrotoxicity caused by CNI when used for non-renal indications. Our data suggest a need for periodic assessment of nephrotoxicity in these children.

FR-PO732

Development of Steroid-Resistant Pediatric Nephrotic Syndrome Following Prolonged Steroid Therapy Is Associated with Alternative Macrophage Activation Yohei Ikezumi,[1] Toshiaki Suzuki,[1] Takeshi Yamada,[1] Hiroya Hasegawa,[1] David J. Nikolic-Paterson,[2] Akihiko Saitoh.[1] *[1]Department of Pediatrics, Niigata University Medical and Dental Hospital, Niigata, Japan; [2]Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

Background: Most cases of pediatric nephrotic syndrome (NS) are steroid-sensitive; however, some patients become steroid-resistant during treatment. We have previously shown that chronic lesions, including matrix expansion and fibrosis, seen during steroid therapy are associated with alternatively activated (M2) macrophages (MΦ). This study examined M2-type MΦin pediatric steroid resistant NS (SRNS).

Methods: Renal biopsies from a group of 12 children with NS were examined. Of these 9/12 were steroid sensitive (SSNS), but 5/9 of those initially responding became steroid resistant (SRNS) with worsening proteinuria and were re-biopsied.CD68+ MΦand M2 markers (CD36, CD163, CD204) were assessed by immunofluorescence. Dexamethasone (Dex) and oxidized LDL (oxLDL) stimulation of cultured human monocyte-derived MΦwas also analysed.

Results: Analysis of all 17 biopsies showed a 3-fold increase in glomerular CD68+ MΦ in SRNS vs SSNS ($p<0.001$), with increased numbers of glomerular $CD36^+$, $CD163^+$ and $CD204^+$ cells ($P<0.05$ vs SSNS). Analysis of the 5 patients who developed steroid resistance showed significantly increased glomerular infiltrates of $CD68^+$, $CD36^+$, $CD163^+$ and $CD204^+$ cells in the second biopsy compared to the first (all $P<0.05$). Double immunostaining showed that close to 100% of glomerular $CD68^+$ MΦexpressed the oxLDL scavenger receptor, CD36. In vitro studies showed up-regulation of CD36 by human MΦ in response to M2 (Dex, oxLDL, IL-4, IL-13) but not M1 (IFNγ, LPS) stimuli. Dex augmented oxLDL induced up-regulation of CD36 expression. Also, Dex plus oxLDL had an additive effect on up-regulating expression of TGFβ, ACE, and LR11; a smooth muscle cell migration factor.

Conclusions: A substantial increase in glomerular M2-type MΦwas seen in pediatric SRNS. In vitro studies support a role for glucocorticoids and oxLDL in promoting this M2-type response. Further studies are warranted to determine whether M2-type MΦare involved in the pathogenesis of SRNS.

Funding: Government Support - Non-U.S.

FR-PO733

Significance of Foot Process Effacement in Minimal Change Disease with Acute Kidney Injury in Humans and Animal Models Akihiro Tojo, Kensuke Asaba. *Division of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan.*

Background: Some patients with minimal change disease develop acute kidney injury (AKI) associated with severe proteinuria. Recently, we have reported that albumin was selectively transported across the podocyte cell body in minimal change nephrotic syndrome (KI 2011; 80: 1328), in which effaced foot processes with tight-junction like connections cover the glomerular capillary and may reduce the glomerular filtration rate. We examined the mechanism of AKI related to foot process effacement in minimal change disease.

Methods: We evaluated retrospectively the clinical and pathological characteristics of 38 patients with biopsy-proven minimal change disease, and also investigated the pathogenesis of AKI compared to the puromycin aminonucleoside nephrotic rat model with a single injection (10mg/100g BW), or two injections with a 2 week interval.

Results: Of 38 patients with minimal change disease, 13 patients (34%) developed AKI. Patients with AKI were older (41±6 years vs. 31±3 years, $P<0.05$) and had lower levels of serum albumin (1.8±0.1 vs. 2.2±0.2 g/dL, $P<0.05$) compared to patients without AKI. Although baseline serum creatinine (0.86±0.05 vs. 0.79±0.04 mg/dL) was not significantly different between these two groups, serum creatinine levels were elevated in patients with AKI (1.74±0.37 mg/dL). There were no differences in renal histological lesions including glomerulosclerosis, arteriolosclerosis and interstitial fibrosis. The extent of foot process effacement and urinary protein were correlated significantly ($R=0.48$, $P<0.001$). In puromycin nephrotic rats, the extent of foot process effacement became severer in rats with two injections of puromycin than in the single injection model (90.4±2.0 vs. 69.6±4.9% of capillary wall length, $p<0.001$), urinary protein was increased (79.1±3.4 vs 27.2±1.2 mg/mgCr), and serum creatinine increased in the twice injection model suggesting the onset of AKI (sCr 1.22 vs.0.49 mg/dL).

Conclusions: In minimal change disease, the extent of foot process effacement is correlated with the amount of urinary protein, whereas severe foot process effacement may impede water and creatinine filtration, resulting in AKI.

Funding: Government Support - Non-U.S.

FR-PO734

Leukocyte Infiltration and Glomerular Capillary Injury in Endocapillary Proliferative Lesions in Various Glomerulonephritis Akiko Mii, Yukinari Masuda, Akira Shimizu. *Nippon Medical School, Tokyo, Japan.*

Background: Leukocyte infiltration and glomerular capillary injury may involve in the progression of various glomerular diseases. In this study, we assessed the association between infiltrating cell phenotype and glomerular capillary injury in post-streptococcal acute GN (PSAGN), lupus nephritis (LN), and Henoch-Schonlein purpura nephritis (HSPN).

Methods: The renal biopsy samples presenting endocapillary proliferative lesions of PSAGN (n=18), LN (n=23) and HSPN (n=23) were selected. To assess the character of infiltrating cells, we performed immunostaining against esterase, MPO, CD3, CD68, CD163 (a marker of M2 Mφ), CD169 (a marker of M1 Mφ), MPO+CD68 Mφ. To identify the alteration of glomerular capillaries, we examined electron microscopic findings, and performed immunostaining against CD34.

Results: In mild endocapillary lesions of PSAGN, inflammatory cells existed in glomerular capillaries, and CD34+ glomerular capillary lumina were retained in periphery of glomeruli. In the severe lesions, leukocytes migrated into subendothelial space, and CD34+ glomerular capillaries separated from glomerular basement membrane (GBM), however, many capillaries remained in glomerular lobes. In contrast, in LN and HSPN, endocapillary proliferative lesions were characterized by the loss of CD34+ endothelial cells with leukocyte infiltration, necrotizing lesion, and GBM rupture. Glomerular infiltrating cell phenotype was distinct between PSAGN and LN/HSPN. In PSAGN, esterase+ neutrophils were remarkable, and MPO+ cells showed similar distribution. Many CD68+ Mφ and a few CD3+ T cells were noted, and most of CD68+ Mφ were CD163+ M2 Mφ. However, in LN and HSPN, some MPO+ cells were MPO+/CD68+ Mφ. A large number of CD169+ M1 Mφ and a small number of CD163+ M2 Mφ were evident.

Conclusions: Glomerular capillary injury develops in endocapillary proliferative lesions. Glomerular capillary network is remained in PSAGN. However, in LN and HSPN, capillary destruction and endothelial injury is prominent and contributes to the formation of active necrotizing lesions. M1 Mφ, MPO+ Mφ, and a small number of M2 Mφ may mediate active inflammation as well as glomerular capillary injury in LN and HSPN.

FR-PO735

Changing Trends of Primary GN in Chulalongkorn University Talerngsak Kanjanabuch, Kearkiat Praditpornsilpa, Somchai Eiam-Ong, Kriang Tungsanga. *Medicine, Chulalongkorn University, Bangkok, Thailand.*

Background: The nationwide renal registry has not yet been organized in Thailand, thus the aim of the present study was to examine the prevalence and changing trends of primary glomerular diseases in the King Chulalongkorn Memorial Hospital (KCMH) in the last 3 decades (1980-2010).

Methods: the patient profiles and renal biopsy specimens of KCMH were reviewed. Final diagnosis was made for each patient based on clinicopathologic correlations. The comparison among individual decade and with literature report from Asian countries according to the demographics and patient outcome were studied.

Results: A total of 1,493 primary glomerular diseases in 2,099 native renal biopsies were retrieved. 69.8% were female and 30.2% were male. Their age average was 37±14 (13-80) years. The most common indication for renal biopsy was nephrotic syndrome (NS: 36.5 %). IgAN (19.5%) and FSGS (19%) are the most 2 common causes, followed by minimal change disease (16.9%) and membranous nephropathy (MN: 13.3%). In recent decades, the prevalence of IgMN has tended to decline, but high rates persist in IgAN and increasing rates are found in FSGS and MN, although the indication of renal biopsy is not change from the last 2 decades. Compared to other reports from Asian population, our IgAN population had worse prognostic factors and higher Oxford score. 30% had hypertension (HT), 34% had Scr ≥ 1.5 mg/dL and urine protein values of > 3 gm/dL, and one fourth had moderate-severe tubulo-interstitial fibrotic score. However, the annual change in eGFR was -0.82 mL/min/m^2. FSGS, the second contributor, most of them presented with NS with HT. Average Scr and serum albumin were 1.5 (0.4-8.2) mg/dL and 2.8 (0.9-4.5) g/dL, respectively. NOS and tip were the leading pathologic variants of the FSGS (84%). Majority of them were steroid responder (70%), only 1 case had spontaneous remission. This responsiveness to steroid was not changed during the 3 decades.

Conclusions: IgMN trended to decline their prevalence during the last 3 decades. On the contrary, the prevalence of IgA nephropathy remained unchanged while the rate of FSGS was increase.

Funding: Government Support - Non-U.S.

FR-PO736

Histiocytic Glomerulopathy Associated with Macrophage Activating Syndrome Alfonso Eirin, Maria V. Irazabal, Fernando C. Fervenza, Sanjeev Sethi. *Mayo Clinic, Rochester, MN.*

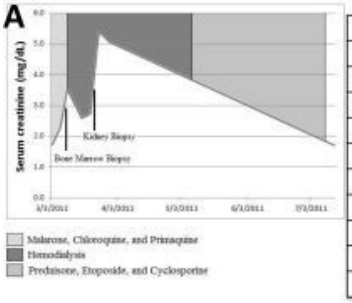
Background: Macrophage-activating syndrome (MAS) is a severe condition due to a hyperinflammatory response resulting from exaggerated activation and proliferation of non-malignant macrophages. MAS falls under the umbrella group of diseases known as of hemophagocytic lymphohistiocytosis (HLH) or hemophagocytic syndrome (HPS). Acute tubular necrosis is the most common renal manifestation in HLH/MAS, but little has been reported about glomerular involvement in acute renal failure.

Methods: A 37-year old man presented with acute renal failure following a febrile illness. His serum creatinine was 1.69 mg/dL, with an estimated glomerular filtration rate of 57.9 mL/min/1.73m^2. Initially, malarial infection was suspected and the patient was treated with malarone, chloroquine, and primaquine for 7 days without any improvement.

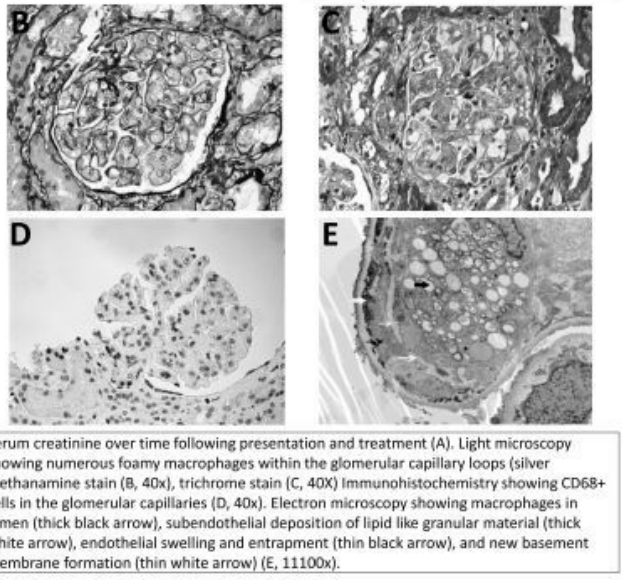
Laboratory results showed features of macrophage activating syndrome with anemia, thrombocytopenia, hypofibrinogenemia, and elevated ferritin levels (Table). Bone marrow aspirate showed granulocytic and megakaryocytic hyperplasia, slightly left-shifted erythropoiesis and polyclonal plasmacytosis. At that time, the patient developed a progressive renal dysfunction requiring dialysis.

Results: Renal biopsy was then done to determine the cause of renal failure and showed unique glomerular findings with massive histiocytic infiltration ("Histiocytic Glomerulopathy") with numerous endocapillary macrophages and features of endothelial injury (Figure). The patient was referred to our institution to initiate therapies to target activated macrophages/histiocytes (prednisone 60mg/day, etoposide, and cyclosporine 75mg/day) and responded well.

Conclusions: Recognizing that the histiocytic infiltrate and endothelial injury is a part of the macrophage activating syndrome is important since early recognition and treatment is of utmost importance since the disease can be fatal.



Serum creatinine (mg/dL)	1.72	0.8-1.3
BUN (mg/dL)	15	8-24
eGFR (mL/min/1.73m²)	57.9	>60
Hemoglobin (g/L)	7.3	13.5-17.5
Hematocrit (%)	20.5	38.8-50.0
White blood cells (x 10³/μL)	22.3	4.5-11.0
Platelet count (x 10³/μL)	342	130-400
Fibrinogen (g/L)	6.76	1.5-2.77
Ferritin (μg/L)	1727	24-336
CRP (mg/L)	296	<6
Urinalysis	unremarkable	



Serum creatinine over time following presentation and treatment (A). Light microscopy showing numerous foamy macrophages within the glomerular capillary loops (silver methanamine stain (B, 40x), trichrome stain (C, 40X) Immunohistochemistry showing CD68+ cells in the glomerular capillaries (D, 40x). Electron microscopy showing macrophages in lumen (thick black arrow), subendothelial deposition of lipid like granular material (thick white arrow), endothelial swelling and entrapment (thin black arrow), and new basement membrane formation (thin white arrow) (E, 11100x).

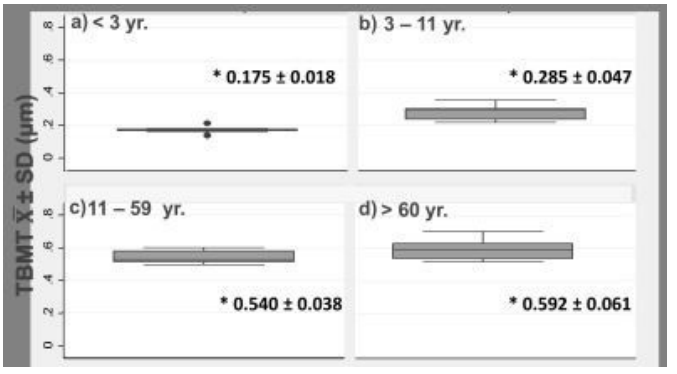
FR-PO737

Tubular Basement Membrane Thickness Increases over the Life Span in an Age Specific Manner Randa Razzouk,[1] Ellen Brooks,[1] Anthony Chang,[2] Michael Z. David,[3] Craig B. Langman.[1] *[1]Kidney Diseases, Children's Memorial Hospital, Chicago, IL; [2]Pathology, University of Chicago, Chicago, IL; [3]Medicine, University of Chicago, Chicago, IL.*

Background: Kidney basement membranes of glomerular (G) and tubular (T) origin serve critical functions including permselectivity, filtration, transport, & cell anchoring. Measurement of G basement membrane thickness (GBMT) in human biopsies is a practical method to diagnose several kidney diseases, including thin GBM & diabetic nephropathies (DN). GBMT can be divided into 4 sub-groups based on developmental stage: infancy(<3yrs);childhood(3-11yrs);adulthood(11-59 yrs) & older age (>60 yrs) while no gender effect is seen. T basement membrane (TBM) ultrastructure demonstrates two distinct layers, the lamina densa & the lamina lucida. Evaluation of TBM thickness (TBMT) has been rarely studied & only in adults with DN where TBMT is thicker, compared to controls.Thus, TBMT measurement may provide relevant information in the development of renal pathology.

Methods: We measured TBMT from proximal tubules (PT) of archived kidney biopsies obtained over the last decade: across all ages, both genders, & excluding cases of DN,IgA Nephropathy, Membranous Nephropathy, Thin GBM & moderate to severe tubular atrophy. Using electron microscopy, we examined N =40 biopsies (10 per age category: <3 yrs, 3-11 yrs, 11-59 yrs & >60 yrs). We performed 39± 4(x ¯± SD) measurements/patient/PT at 5800x. For each age stratum, we determined the x ¯± S.D.TBMT. Results were compared by ANOVA & Spearman's Rank test.

Results: Figure a-d. Mean TBMT Differ by Age,*$p<10^{-3}$. There was a strong positive relationship between Proximal TBMT across age ($r=0.93$, $p<10^{-3}$).



Conclusions: We demonstrated an age-related increase in TBMT from <3yrs to >60 yrs. We are now expanding our research to compare GBM & TBM thicknesses in thin GBM disease.

FR-PO738

Immunopathology of Omeprazole-Induced Acute Interstitial Nephritis Linda Berney-meyer,[1] Noelyn Hung,[1] Tania L. Slatter,[1] A. Richard Kitching,[2] Robert J. Walker.[1] *[1]Medicine and Pathology, University of Otago, Dunedin, New Zealand; [2]Nephrology, Monash Medical Centre, Melbourne, Australia.*

Background: Omeprazole is now a common cause of drug-induced acute interstitial nephritis (AIN). How omeprazole-induced AIN is mediated is unknown. It is not the classical allergic AIN, rather the histological appearances are more characteristic of an acute tubulitis as seen in vasculitis or cellular rejection.

Methods: Data from 25 biopsy-proven cases of omeprazole-induced AIN were reviewed. The H&E stained biopsies were scored using the Banff criteria for tubulitis. The interstitial infiltrate was then analysed using immunohistochemical and fluroscopic chromogenic insitu hybridization techniques to identify and semi-quantitate the cellular infitrate, using CD20 for B-cells, CD4, CD8, IL17A, IL17F and Foxp3 staining to identify and characterise T-cells.

Results: All patients presented with evidence of AKI. Urinalysis demonstrated a sterile pyuria with varying amounts of proteinuria. There were no urinary eosinophils. There were no systemic symptoms to suggest a vasculitis and ANCA (PR3 & MPO titres) were negative in all cases. Histologically, all cases had evidence of inflammatory cells crossing the tubular basement membrane and attacking the tubular epithelial cells. 56% had evidence of mild tubulitis (t1), 24% moderate tubulitis (t2) and 20% severe tubulitis, and 78% of all cases were a mononuclear cell infiltrate,. There was no glomerular involvement. There was no significant eosinophilic infiltrate seen. Immunohistochemical staining confirmed the predominant inflammatory cells to be CD4 positive cells, present in clusters, in 77% of cases and combined CD4 IL17A/F staining in 44 - 48% of cases, suggesting a predominantly Th1 - Th17 mediated inflammatory process.

Conclusions: This is the largest reported biopsy series of omeprazole-induced AIN. The tubulitis evident with the intense interstitial infiltrate appears to be mediated by a Th1 - TH17 process analagous to vasculitis. How omeprazole induced this Th1 - Th17 inflammatory response is unclear. Once better defined, more appropriate therapy can be introduced.

Funding: Government Support - Non-U.S.

FR-PO739

Frequency and Associations of C4d Deposits in Explanted Kidney Allografts Jolanta Kowalewska,[1] Beata Naumnik,[2] Gabriela Korakiewicz,[1] Jerzy Glowinski,[3] Lech Chyczewski.[1] *[1]Pathology, Medical University of Bialystok, Bialystok, Poland; [2]Nephrology and Transplantation with Dialysis Unit, Medical University of Bialystok, Bialystok, Poland; [3]Vascular Surgery and Transplantology, Medical University of Bialystok, Bialystok, Poland.*

Background: The presence of C4d deposits in peritubular capillaries (PTC) in kidney allograft is one of the histological markers of antibody-mediated rejection (AMR). There is emerging evidence that AMR may contribute to late kidney allograft function deterioration. It has been shown that significant proportion (in some studies 39%) of kidney allografts have significant PTC C4d deposits and this finding is associated with an increased risk of subsequent graft failure.

Methods: We searched the Department of Pathology database for kidney allograft nephrectomy specimens. The time after the transplantation (tx) and the cause of graft loss were recorded. All identified cases were reviewed for histological findings and retrospectively stained for C4d using immunohistochemical method.

Results: We identified 31 kidney allograft nephrectomies. Among the recipients were 17 (55%) females and 14 (45%) males, ages 16 to 70 (average 41 years old at the time of tx). The average time post tx was 8 years (range 1 month to 19 years). The causes of allograft failure were: chronic allograft nephropathy (24 cases), infection (2 cases), CNI toxicity (2 cases), acute rejection (2 cases), and renal artery stenosis (1 case). Twenty two (22/31, 71%) grafts showed strong diffuse staining for C4d in PTC. Additionally, in 7 cases there was focal weak staining for C4d. Two cases were negative. Strong C4d deposition was associated with transplant glomerulopathy (TG; 20/22, 90%) and chronic active transplant arteriopathy (19/22, 86%). In the groups of focal or negative C4d, TG and arteriopathy were seen in two cases.

Conclusions: The majority of removed kidney allografts show strong PTC C4d staining (in our study 71% grafts), pointing to humoral rejection process as a significant contributor to the allograft failure.

C4d deposits in explanted grafts are associated with transplant glomerulopathy and chronic active transplant arteriopathy.

FR-PO740

IgG4-Positive Plasma Cells in Renal Allograft Biopsies Thomas Paulraj Thamboo,[1] Vathsala Anantharaman,[2] Ming Teh.[1] *[1]Pathology, National University Health System, Singapore, Singapore; [2]Medicine, National University of Singapore, Singapore, Singapore.*

Background: IgG4 is thought to play an anti-inflammatory role or be a response to inflammation, with attention focussed recently on its role in the newly-recognised IgG4-related disease. This retrospective study investigated the presence of IgG4-positive plasma cells (IgG4PC) in renal allograft biopsies.

Methods: Archival formalin-fixed and paraffin-embedded tissue from consecutive renal allograft biopsies over a 3 year period was studied. Immunostaining for CD138 and IgG4 was performed and the number of IgG4PC and total plasma cells present in each biopsy was assessed.

Results: 146 cases that met the entry criteria had residual tissue for study. Overall, IgG4PC were identified in 65 cases (44.5%). In cases with acute T-cell mediated rejection, the mean number of IgG4PC per biopsy was significantly higher than in cases with no rejection (65.46 versus 5.47; p=0.049) (t-test; 1 tail) as was the mean number of IgG4PC per HPF (5.18 versus 0.79; p=0.046). The mean total number of all plasma cells per biopsy was also significantly higher in the former group (765.08 versus 80.53; p=0.019). When cases of acute antibody-mediated rejection were compared to cases with no rejection, the mean total number of all plasma cells per biopsy was significantly higher (p=0.011). However, there was no significant difference in the mean number of IgG4PC per biopsy and the mean number of IgG4PC per HPF between the two groups. In cases with "Borderline rejection" (Banff Category 3), the mean number of IgG4PC per biopsy, the mean number of IgG4PC per HPF and the total number of all plasma cells per biopsy were less than those of cases with acute rejection and more than those of cases with no rejection, but these differences were not statistically significant. When all cases were considered, there was no significant difference in the number of IgG4PC or in the number of all plasma cells between cases with mild and severe interstitial fibrosis and tubular atrophy.

Conclusions: IgG4PC are present in a significant number of renal allograft biopsies. Significantly more IgG4PC are present in cases with acute T-cell-mediated rejection compared to cases with no rejection.

FR-PO741

Distributions of eGFR in Patients Examined by Contrast-Enhanced Computed Tomography before and after eGFR Self-Report Yoshinari Yasuda,[1] Kanako Shibata,[1,2] Sawako Kato,[1] Motomitsu Goto,[1] Mutsuharu Hayashi,[1] Shoichi Maruyama,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]CKD Initiatives/Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Pharmacy, Kinjyo Gakuin University School of Pharmacy, Nagoya, Japan.*

Background: Renal insufficiency is the most important risk factor for contrast induced nephropathy (CIN), however it is difficult to assess renal function only by serum creatinine (sCr). Estimated glomerular filtration rate (eGFR) is globally recommended for evaluation of renal function, and eGFR self-report may affect clinical decision making to prevent CIN. Since Nagoya University Hospital (NUH) started eGFR self-report in December 2009, we analyzed distributions of eGFR in patients who were examined by contrast-enhanced computed tomography (CE) in NUH before and after eGFR self-report.

Methods: Study subjects were 1,123 and 967 patients who were examined by CE in NUH in April 2008 and 2011. Patients under 18 years of age were excluded. Age, gender and sCr before CE (until 4 months before) were collected from medical record and eGFR was calculated by Japanese eGFR equation. Distributions of age, gender and eGFR categories (G1: 90 and above, G2: 60-89, G3a: 45-59,G3b: 30-44 ,G4: 15-29 and G5: less than 15 mL/min/1.73m^2) were compared before and after eGFR self-report.

Results: Age and gender distributions were not different in 2008 and in 2011. Although most of hospitalized patients were examined sCr before CE, 20% of outpatients were not examined sCr in NUH before CE both in 2008 and in 2011. In remaining patients, proportions of eGFR category G1, G2, G3a, G3b, G4 and G5 were 26.2%, 55.5%, 12.7%, 5.3%, 0.3% and 0.1% in 2008, and 21.8%, 56.8%, 16.9%, 3.2%, 0.5% and 0.9% in 20011, respectively. Proportion of G3b was significantly decreased, while that of G4 and G5 was increased in 2011. In most of G4 and G5 patients in 2011, referral to nephrologists had been completed previous to CE.

Conclusions: Distributions of eGFR categories among patients examined by CE were different before and after eGFR self-report in NUH, suggesting its preferable effect to prevent CIN.

Funding: Government Support - Non-U.S.

FR-PO742

Renal Safety Evaluation after Gd-DOTA-Enhanced-MRI Compared with Non-Enhanced-MRI in Patients at High Risk of Developing Contrast Medium Induced Nephropathy Jean Marie Gustave Billiouw,[1] Luis Martibonmati,[3] Olivier Rouviere,[4] Lorenzo Bacigalupo,[10] Bart Dirk Maes,[5] Thierry P. Hannedouche,[6] Francois Vrtovsnik,[7] Claire Rigothier,[8] Paolo Campioni,[9] Gilbert Deray.[2] *[1]Nephrology, Ziekenhuis, Aalst, Belgium; [2]Nephrology, Salpétrière Hospital, Paris, France; [3]Radiology, Valencia Hospital, Valencia, Spain; [4]Department of Urinary and Vascular Imaging, Hospices Civils de Lyon, Department of Urinary and Vascular Imaging, Hôpital E. Herriot, Lyon, France; [5]Nephrology, Heilig Hartziekenhuis, Roeselare, Belgium; [6]Nephrology, Strasbourg Hospital, Strasbourg, France; [7]Nephrology, Bichat Hospital, Paris, France; [8]Nephrology, Pellegrin Hospital, Bordeaux, France; [9]Radiology, Sant'Anna Hospital, Ferrara, Italy; [10]Radiology, Ospedali Galliera, Genova, Italy.*

Background: To assess the safety profile of Gd-DOTA in patients with chronic renal failure.

Methods: Phase IV, non-randomized, comparative, multinational trial (RESCUE study) including 135 adult patients with a known stable stage III/IV renal insufficiency (15<estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m²) scheduled to undergo a MRI with Gd-DOTA (Dotarem®) or unenhanced-MRI. The primary endpoint was the percentage of patients with a nephrotoxicity, defined as a serum creatinine level increase at 72±24h of at least 25% or 0.5mg/dl compared to baseline, using a non-inferiority analysis. Main secondary criteria were serum creatinine and eGFR variations, clinical safety.

Results: The difference (unenhanced-MRI–Gd-DOTA-MRI) in nephrotoxicity incidence was -1.4% and significantly (p=0.001) superior to the clinical non-inferiority limit, demonstrating the non-inferiority. The serum creatinine variation from baseline was -1.4±10.4% for Gd-DOTA-MRI and -3.5±9.9% for unenhanced-MRI (p=0.291). No relevant differences for the other secondary endpoints were noted. The good general safety profile of Gd-DOTA was also confirmed.

Conclusions: The non-inferiority of Gd-DOTA-MRI over unenhanced-MRI in terms of nephrotoxicity was demonstrated. Among the few contrast medium-induced-nephropathy studies with gadolinium products, this prospective study included a control group emphasizing the very good renal tolerance of Gd-DOTA in at-risk patients.

FR-PO743

Incidence of Infectious Complications after Rituximab in Glomerular Disease Claire Trivin,[1] Alexandre Karras,[1] Gabriel Choukroun,[2] Cecile M. Vigneau,[3] Gatault Philippe,[4] Cécile Courivaud,[5] Jean Francois Augusto,[6] Maxence Ficheux,[7] Bruno Moulin,[8] Eric Thervet.[1] *[1]Hôpital Européen Georges Pompidou, Paris; [2]CHU Amiens; [3]Hôpital Pontchaillou, Rennes; [4]Hôpital Bretonneau, Tours; [5]Hôpital St Jacques, Besançon, France; [6]CHU Angers; [7]CHU Caen; [8]Hopitaux Universitaires de Strasbourg, France.*

Background: Rituximab (RTX) is largely used in many renal diseases but the related risk is still debated. The aims of this study were to describe infectious complications after RTX treatment, to consider potential risk factors and the outcome of these patients.

Methods: In 7 French renal units, we retrospectively analyzed RTX treated patients for renal disease, excluding renal transplant recipients and patients with hematological indications. We retrospectively determined the incidence and the characteristics of infectious complications and retrieved clinical and biological data.

Results: Between 2005 and 2011, we individualized 92 patients who had received RTX for glomerular disease. The mean duration of follow-up was 25.9±19.9 months. Patient survival was 94.5%. Overall, a total of 38 infectious episodes were observed in 28 (30.4%) patients. Pneumonia (n=11), Septicemia (n=7) and cellulitis (n=6) were the most frequent infections. Compared to patients without infection, patients who presented any infectious complication were significantly older (55.1±18.9 vs 45.7±19.2; p=0.03), exhibited more frequent diabetes mellitus and had cryoglobulinemia as initial nephropathy (p=0.008 and p=0.001 respectively). The total RTX dose was positively correlated with the risk of infection. Serum creatinine before RTX was significantly higher, and gammaglobulin concentration during the follow-up was significantly lower in patients with infection (p=0.01 and 0.04 respectively).

Conclusions: Infectious risk after RTX therapy in nephrology is high, especially in elderly patients and when higher dose of treatment are used. Cryoglobulinemia, renal failure and diabetes mellitus are independent risk factors. Patient should be monitored for gammaglobulin levels to evaluate the infectious risk leading to the question of systematic intravenous gammaglobulin treatment in this population.

Funding: Clinical Revenue Support

FR-PO744

Potential Medication Risk for Restless Legs Syndrome in Dialysis Patients: Part D Data in the United States Renal Data System Donald L. Bliwise, Rebecca H. Zhang, Nancy G. Kutner. *USRDS Rehab/QOL SSC, Emory University, Atlanta, GA.*

Background: ESRD is a significant risk factor for restless legs syndrome (RLS). The presence of RLS in dialysis patients is associated with poorer quality of life, and in several studies, higher rates of mortality. Because RLS is commonly associated in the general population with medications with central nervous system effects, we examined USRDS data to investigate whether use of these drugs might be associated with RLS Dx.

Methods: Cases were identified by the unique ICD-9 code for RLS (333.94) that became effective October 1, 2006. The RLS code was present for 1,508 patients who initiated regular dialysis therapy October 1, 2006 through December 31, 2008. Four controls who started dialysis in the same time period, matched by age, race, and sex, were selected for each case (n=6,032). Part D medication data were searched for October 1, 2006 through December 31, 2008. Prevalence of prescribed drugs was investigated in four classes: (I) tricyclic antidepressants, selective serotonin uptake inhibitors, serotonin–norepinephrine reuptake inhibitors, and buspirone; (II) first generation anti-histamines; (III) anti-psychotics with partial dopamine blockade; and (IV) metoclopramide. The study hypothesis was that usage prevalence for these classes would be higher in patients with RLS than in those without an RLS Dx.

Results: Even after matching for age, race and sex (all known risk factors for RLS), usage of each of the 4 medication classes was strongly associated with RLS Dx: Class I (OR = 2.18; 95% CI 1.92-2.48); Class II (OR = 1.75; 95% CI 1.23-2.48); Class III (OR = 1.19; 95% CI 1.10-1.30); Class IV (OR = 2.07; 95% CI 1.77-2.43).

Conclusions: These data raise the possibility that some medications that see widespread usage in dialysis patients may actually increase risk for a comorbidity that commonly distresses many patients. Clearly, for any individual patient, the risk/benefit ratio for these medication classes may warrant such usage. On the other hand, these data also imply that a careful review of concurrent medications should be undertaken when a dialysis patient expresses troubling RLS symptoms.

Funding: NIDDK Support

FR-PO745

The Osmolality of Nonionic, Iodinated Contrast Agents as an Important Factor for Renal Safety Diana C. Lenhard,[1] Hubertus Pietsch,[1] Philipp D. Lengsfeld,[2] Gregor Jost.[1] *[1]CT and MR Contrast Media Research, Bayer Pharma AG, Berlin, Germany; [2]Radiology & Interventional Global Medical Affairs, Bayer Pharma AG, Berlin, Germany.*

Background: Non-ionic contrast agents (CA) can be classified into low-osmolar and iso-osmolar CA. Targeting the differences in the CA's osmolalities, we analyzed and compared renal iodine retention and potentially occurring kidney injury.

Methods: Rats were injected with low-osmolar iopromide 300, iso-osmolar iodixanol 320 and with an iodixanol/mannitol mixture containing an osmolality of 610 mOsm/kg H2O, equal to iopromide 300. Iodine retention was determined 2h post injection (p.i.) on the basis of CT measurements. Potential injury of kidneys was analysed by qRT-PCR of acute kidney injury (AKI) markers.

Results: CT analysis exhibited twice as much residual iodine in the kidneys 2h after iodixanol administration compared to iopromide injection. Interestingly, iodine retention after iodixanol/mannitol administration was reduced down to iopromide level. 2h p.i. Iodixanol caused a significantly upregulated expression of the AKI marker PAI-1 occurring to a much lesser degree in iopromide treated rats. Additionally, 2h and 24h after Iodixanol administration KIM-1 and NGAL transcripts were significantly increased compared to iopromide and NaCl application. However, the iodixanol enhanced AKI marker induction was reversed by iodixanol/mannitol down to almost Iopromide transcript levels in all cases.

Conclusions: Increased renal iodine concentrations and elevated AKI marker expression seem to be mainly caused by the explicitly higher viscosity of Iodixanol and appear to be reversed by raising Iodixanol's osmolality, demonstrating a positive osmodiuretic effect and a higher renal tolerance of low-osmolar CAs.

Funding: Pharmaceutical Company Support - Bayer Pharma AG

FR-PO746

The Activity Report of Dialysis Service Continuity and the Staging Base Function for Wide Area Medical Transportation on the Great East Japan Earthquake Mariko Miyazaki,[1,2] Yaeko Murata,[1] Tae Yamamoto,[1] Toshinobu Sato,[3] Hiroshi Sekino,[4] Sadayoshi Ito.[2] *[1]Blood Purification, Tohoku University Hospital, Sendai, Japan; [2]Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [3]Nephrology, Sendai Shakaihoken Hospital, Sendai, Japan; [4]Urology, Kojinkai Hospital, Sendai, Japan.*

Background: Twenty thousands of people fell victim to the Great East Japan Earthquake. The half of them had lived at the coast of Miyagi prefecture. In this area, electrical power service and water supply were interrupted widely after earthquake. It was the great difficulty for the people who need renal replacement therapy.

Methods: We examined the activity record of disaster response in Tohoku University hospital.

Results: Over 2,000 patients needed the dialysis in Sendai city area. They were treated in their area by full working of Sendai Shakaihoken Hospital and Kojinkai Hospital and one after the other recovered facilities supplied by water truck. After 3.11, Kesennuma city hospital worked using emergency generators and water tank, however, couldn't recover the electrical power supply after more than 72 hours. Furthermore, the prolonged harsh conditions threatened the patients staying at devastated area. We coordinated the wide-area medical transportation for 80 patients using Japanese Self Defense Force Aircraft to Hokkaido, 400 miles north of Miyagi. The function of staging base by Tohoku University Hospital enabled the long-distance transportation safely in medical check and stabilizing the condition for the suffered dialysis patients. That was the first systematical and largest operation planned by Japanese government associating prefectural governments, Japanese Associations of Dialysis Physicians, and many dialysis facilities containing outside of Miyagi.

Conclusions: The scheme for large number of dialysis patients' staging base was different from injured victims' transportation. Network centric information management enabled the co-operation of many organization.

Acknowledgment: Dr. Seiji Ueno, Dr. Kazuhiko Orikasa, and Dr. Hiroshi Ohtomo in Dialysis center of Kesennuma city hospital.

FR-PO747

A Novel Assessment of Fall Risk in Hemodialyis Patients: Preliminary Findings Emaad M. Abdel-Rahman,[1] Rahul Soangra,[3] Thurmon E. Lockhart,[3] John Lach,[2] Rasheed A. Balogun.[1] *[1]Nephrology, UVA, Charlottesville, VA; [2]Engineering, UVA, Charlottesville, VA; [3]Engineering, Virginia Polytechnic University, Blacksburg, VA.*

Background: Falls among the hemodialysis (HD) patients are more common after HD than before (Cook et al, 2006) with resulting high morbidity and mortality. The functional and mobility mechanisms that contribute to falls need to be studied. The timed up and go (TUG) test is a reliable and valid clinical test for quantifying functional mobility and fall risk (Mathias et al, 1986). Sit-to-walk (STW) component of TUG is a complex sequential postural locomotor task (Magnan, 1996). Impaired timing of STW events and sequencing may place patients at higher risk of falling. Aim: Assess the effects of hemodialysis therapy on fall risk in ESRD patients using a validated novel instrument.

Methods: Measurement of locomotor performance using time to complete the phases of STW tasks as a marker of dynamic stability (fall risk) immediately before and after 22 HD sessions in 6 ESRD patients (54±4 years; 4 females and 2 males). Each HD session lasted four hours with no intradialytic complications. Instrumentation: A novel inertial measurement unit (IMU) - TEMPO (Technology-Enabled Medical Precision Observation (Barth et al, 2009).

Results: After HD, significant increases in time to complete all phases of STW tasks were found.

Time interval from initiation to reach peak flexion (PF) and extension (PE) events and the time taken to complete all phases of STW (C) task.

Time between events	Pre HD(seconds)	Post HD (seconds)	P
STW PF	0.471±0.037	0.799±0.073	0.030*
STW PE	1.533±0.196	1.907±0.205	0.026*
STW C	2.462±0.566	2.900±0.893	0.041*

Conclusions: There is a demonstrable decrease in locomotor performance and dynamic stability immediately after HD. This increases fall risk. This study has high potential to shift current clinical practice paradigms with an immediate impact because this type of assessments can be easily implemented in most clinical settings. Furthermore, this study has the potential to elucidate the mechanism underlying increased fall risk in this population outlining the specific deficiencies potentially leading to novel approaches to intervention in the future.

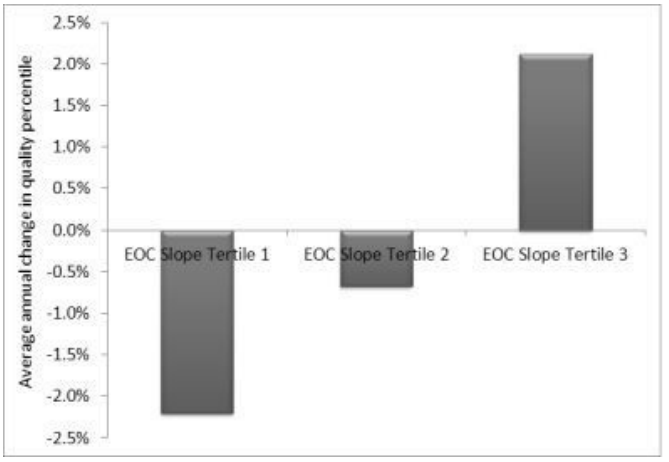
FR-PO748

Improvements in Clinical Quality Are Associated with "Environment of Care" Rounds Penny Faith Sheppard, Seth Johnson, Robert Levin, Nancy Ginsberg, Len A. Usvyat, Mary T. Sullivan, Paul Balter, Peter Kotanko, Paul M. Zabetakis. *Renal Research Institute, NY, NY.*

Background: "Environment of Care" (EOC) rounds are a monthly process in the RRI clinics. It was introduced in 2008 and involves a detailed inspection of each clinic based on 28 operational and clinical criteria that involve Infection Control/Patient Care/Housekeeping & Medication preparation, handling, storage and administration. Scores on a scale from 0% to 100% (best) are assigned to each clinic. We aimed to understand whether these scores are aligned with quality outcomes in the clinics.

Methods: All RRI clinics are ranked monthly based upon their quality outcomes. These rankings cover patient parameters, such as anemia, phosphorus control, nutrition, vascular access, dialysis adequacy, as well as mortality and hospitalization rates. The best clinics are assigned the highest percentile (on a scale from 1 to 100%). We studied all clinics bn Jan 2009 and April 2012.

Results: We studied 19 RRI clinics; mean EOC score was 89.2% (SD 3.4%). We found a positive and significant correlation between EOC scores and clinical quality percentile ($r=0.36$, $p<0.05$). We also found that clinics with improvements in EOC scores experienced an improvement in the clinical quality ranking during the study period ($r=0.22$, $p<0.05$). Dividing clinics into tertiles of EOC slopes [tertile1: below -0.9%/year; tertile 2: -0.9 to +0.5%/year; tertile 3: greater +2.5%/year] over the 40 month study period, we found a gradient relationship with clinics with highest increases in EOC scores having the highest ranking improvements.



Conclusions: Environment of care rounds aimed at monthly checks of 28 clinic operation issues covered in CMS's Conditions of Coverage are associated with not only improved operational procedures in the dialysis clinics but are also with improved clinical quality and patient outcomes.

FR-PO749

User Profiles of a Smartphone Application to Support Drug Adherence: Experiences from the iNephro Project Stefan Becker,[1] Anna Mitchell,[1] Andreas Kribben.[2] *[1]Medizinische Klinik I, Marienhospital Herne, Klinikum der Ruhr-Universität Bochum, Herne, Germany; [2]Klinik für Nephrologie, Universitätsklinikum Essen, Essen, Germany.*

Background: One of the key problems in the drug therapy of patients with chronic renal failure is drug adherence. In 2010 the initiative iNephro was launched (www.inephro.de). A "context sensitive" software to support regular and correct drug intake was developed for a smartphone platform (iOS). The present study investigates, whether and how such an application is deployed by smartphone users.

Methods: Together with cooperating partners the mobile application "medication plan" was developed. Users are able to keep and alter a list of their regular medication. A memory function supports regular intake. The application can be downloaded free of charge from the iTunes App Store. After individual consent of users from 21/10/2010 to 04/02/2012 2,042,338 actions were recorded and analyzed from the downloaded applications. In 2,279 cases, demographic data were collected by a questionnaire.

Results: Overall the application was used by 11,688 smartphone users, in 3,406/11,688 (29.1%) cases at least once a week for at least 28 days. 3,209/11,688 (29.5%) used the application at least 84 days. 1,554/2,279 (68.2%) of users surveyed were male, the stated age of all users was between 6-87 years (mean 44). 1,697/2,279 individuals (74.5%) declared to be suffering from cardio-vascular disease, 292/2,279 (12.8%) had a previous history of transplantation and 161/2279 (7.1%) suffered from diabetes mellitus. 1,568/2,279 (68.8%) of users were on <6 different medications, 201/2,279 (8.8%) 6 – 10 and 26/2,279 (1.1%) on more than 10.

Conclusions: A smartphone application that supports drug adherence is already regularly used by chronically ill users with a wide range of diseases over a longer period of time.

Funding: Pharmaceutical Company Support - Roche Pharma AG, Grenzach-Wyhlen

FR-PO750

Is Frequency of Renal Tumors Increased in Lithium-Treated Patients? Mohamad Zaidan,[1] Fabien Stucker,[2] Benedicte Stengel,[3] Aurelie Hummel,[1] Paul Landais,[1] Viorel Vasiliu,[1] Jean-Jacques Boffa,[1] Pierre M. Ronco,[2] Jean-pierre Grünfeld,[1] Aude Servais.[1] *[1]Necker Hospital, Paris, France; [2]Tenon Hospital, Paris, France; [3]Inserm U1018, Villejuif, France.*

Background: Prevalence of renal cancers (RC) is increased in some toxic interstitial nephropathies. Our aim was to determine whether the frequency of renal tumors in lithium-treated (Li2+) patients was increased.

Methods: 170 Li2+-patients with renal imaging were included. The diagnosis of renal tumor was confirmed by pathological analysis. Two sex-, age- and eGFR-matched CKD patients with renal imaging but no Li2+ exposure were randomly selected for each Li2+-patient.

Results: 108 females and 62 males were included (mean age 65±12 years) with a mean follow-up of 6±6 years and eGFR of 40±17 ml/min/1.73m². 14 patients (8F/6M, mean age 64±9 years) had renal tumor including 7(4%) cancers and 7(4%) benign tumors. Pathological analysis was performed for all cases but 2 with typical angiomyolipomas. RC included 3 clear cell renal cell carcinoma (RCC), 2 papillary RCC, 1 hybrid tumor characterized by the association of chromophobe RCC and oncocytoma, and 1 clear cell carcinoma with leiomyomatous stroma. Benign tumors included 4 oncocytomas, one of which was associated with numerous papillary adenomas, 1 mixed epithelial and stromal tumor and 2 angiomyolipomas. The mean duration of Li2+ exposure at diagnosis was 23±10 years.

Standardized Mortality Ratio of RC in Li2+-patients compared to the French population were: 7.5 (95%CI [1.51-21.95]) and 13.7 (95%CI [3.68-35.06]) in men and women, respectively, suggesting the incidence of RC was significantly increased. The prevalence of RC cancer was also increased in Li2+-patients compared to 340 sex-, age- and eGFR-matched CKD patients without Li2+ exposure (4% *vs* 0%, p <0.001), indicating that the higher frequency in Li2+-patients does not result from detection bias.

Conclusions: Our study demonstrates that the frequency of renal tumors, particularly RC, is increased in Li2+-patients and underscores the importance of a screening by renal imaging. The high frequency of other benign tumors, including oncocytomas, also suggests a potential role of Li2+ exposure in the development of these tumors.

FR-PO751

Risk Factors and Timing of Native Kidney Biopsy Complications Marie-christine Simard-meilleur,[1] Louise Roy,[1] Stephan Troyanov,[2] Soumeya Brachemi.[1] *[1]Nephrology, CHUM Saint-Luc, Montreal, QC, Canada; [2]Nephrology, Hopital Sacre-Coeur, Montreal, QC, Canada.*

Background: The optimal observation period after percutaneous renal biopsy is uncertain. Identifying risk factors and timing of complications is essential to patient care.

Methods: We retrospectively studied all native kidney biopsies performed between January 2007 and July 2011.

Results: The cohort consisted of 287 patients and a total of 313 biopsies, 147 of which were done as an outpatient procedure; 98 patients were kept under observation for 8 hours (h) and 49 for 24h. Fourteen had an abnormal bleeding time, 3 had an INR ≥ 1.5, 33% received dDAVP, 3% platelets and 4% FFP. Outpatients had fewer comorbidities. Eighteen percent of inpatients developed minor complications, a symptomatic hematoma or hematuria not requiring intervention, (12% in outpatients); 17% had major complications, bleeding requiring transfusion or angiographic intervention, (2% in outpatients). Fifteen percent experienced a symptomatic hematoma (12% in outpatients), 8% had macroscopic hematuria (4% in outpatients), 17% received a red blood cell transfusion (2% in outpatients) and 3 inpatients required angiographic intervention. Overall, 79% of complications manifested within the first 8h, 83% at 12h and 91% at 24h. In outpatients, >95% of complications manifested within the first 8h. The risk of symptomatic hematoma correlated with the level of platelets. It increased from 10, 15, 29 to 40% in individuals with >200, 140-200, 100-140 and <100 x 10^9/L platelets who did not receive transfusions, respectively (p =0.03). By contrast, in those with <100 x 10^9/L platelets who did receive a platelet transfusion, a hematoma was observed in only 17%. A needle gauge ≤ 15 tended to predispose to hematomas. Associations between comorbidities, hemostasis (INR, bleeding time), preventive therapy (dDAVP, FFP) and complications were not statistically significant.

Conclusions: Outpatients presented with fewer comorbidities and experienced fewer complications than inpatients. Complications also manifested earlier in outpatients with >95% observable at ≤8 hours. The platelet count was the most predictive risk factor of bleeding. Same-day discharge in selected patients is safe.

FR-PO752

Safety of Native and Transplant Kidney Biopsy Jason M. Kidd, Eddie R. Fuller, Vimal K. Derebail. *Division of Nephrology and Hypertension, University of North Carolina Kidney Center, Chapel Hill, NC.*

Background: Renal biopsy is an important tool for the diagnosis and treatment of patients with kidney disease. The benefits of biopsy must be weighed against potential risks. The purpose of this study was to identify predictors and frequency of complications in native and transplant kidney biopsies.

Methods: We retrospectively examined 301 consecutive percutaneous renal biopsies performed from December 2010 through December 2011. Biopsies were performed with a 16-gauge spring-loaded biopsy needle under real-time ultrasound guidance. The primary outcome measured was any bleeding associated with biopsy. Major complications were defined as significant bleeding requiring blood transfusion, interventional radiology procedure or hospital admission. Minor complications were defined as active bleeding seen radiologically without resultant hematoma formation, arteriovenous fistula (AVF) formation or hematoma without need for intervention.

Results: 301 total biopsies were performed (129 native and 172 transplant). The frequency of complications varied significantly between native and transplant biopsies (Table 1). The risk of significant bleeding requiring transfusion was 5.4% in native kidneys and 0.6% in transplant kidney biopsies. No significant risk factors were identified between patients with and without complications. 37 patients had multiple transplant biopsies performed in the period studied with no major complications.

Type of Complication by Kidney Biopsy

	Native (N=129)	Transplant (N=172)	
	N (%)	N (%)	p-value
Bleeding (incl hematoma/ retroperitoneal bleed)	68 (52.7)	44 (25.6)	<0.001
Major Complication	12 (9.3)	1 (0.6)	<0.001
Transfused blood	7 (5.4)	1 (0.6)	0.02
Bleeding seen radiologically without hematoma formation	6 (4.7)	25 (15)	0.02
AVF	4 (3.1)	16 (9.3)	0.04
Hematoma	61 (47.3)	19 (11.1)	<0.001

Conclusions: Transplant kidney biopsies have a significantly lower risk of major complications compared with native kidney biopsies despite an increased risk of AVF formation. Native kidney biopsies had more bleeding complications. Multiple biopsies of renal allografts did not increase the risk of major complications.

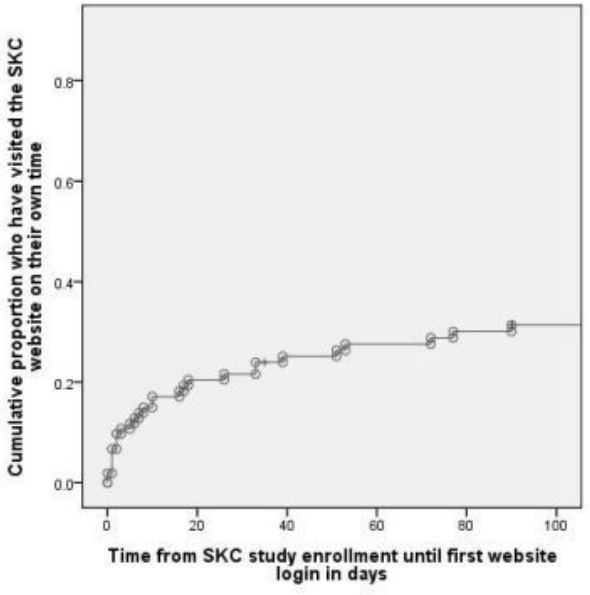
FR-PO753

If You Build It, Will They Come? Use of an Educational Patient Safety Web Site by Patients with Chronic Kidney Disease (CKD): Results from Safe Kidney Care Clarissa Jonas Diamantidis,[1] Marni Zuckerman,[1] Wanda Fink,[1] Peter F. Hu,[2] Shiming Yang,[3] Jeffrey C. Fink.[1] *[1]Medicine, University of Maryland School of Medicine, Baltimore, MD; [2]Anesthesiology, University of Maryland R. Adams Cowley Shock Trauma Center, Baltimore, MD; [3]Computer Science, University of Maryland Baltimore County, Baltimore, MD.*

Background: Use of health information technology (health IT) has become increasingly common in the delivery of care to patients with chronic diseases. We evaluated how frequently patients with pre-dialysis CKD visited an informational Web site aimed at providing disease-specific safety information.

Methods: As part of the Safe Kidney Care (SKC) study, an educational Web site was designed to provide information on specific safety concerns for patients with CKD. Participants in Phase 1 of the study were provided a medical alert bracelet with a unique ID which can be entered into the SKC Web site to track participant entry into the Web site by participants after an in-person tutorial. Participants' visits and dwell times on specific safety modules were tracked.

Results: Of 108 participants, 27% (n = 30) visited the Web site a median of 1 time (range 1-7) from study enrollment after 3 month of follow-up.



Median access time was 7 minutes (range <1-44 minutes). The three most frequently visited pages were "Renal function calculator," "Pills to avoid," and "Foods to avoid." In multivariate adjustment, more than high school education and annual household income >$50,000 were associated with Web site entry. Pertinent factors not found to be predictive were age, race, gender, or GFR.

Conclusions: Preliminary results show general interest in a Web-based platform designed to improve patient safety in CKD.

Funding: NIDDK Support, Pharmaceutical Company Support - American Medical ID

FR-PO754

Outcomes of Renal Dosing of Tenofovir in HIV Infected Individuals Alfredo B. Tiu, William Christopher Mathews. *Medicine, Owen Clinic UCSD and Veteran's Admnistration Oceanside Clinic, Oceanside, CA.*

Background: Tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor, has been reported to cause proximal renal tubular dysfunction and acute kidney injury. This study evaluates safety and efficacy of extended-interval TDF dose.

Methods: Retrospective, case-control, study of HIV-positive patients prescribed extended-interval dose of TDF between October 2001 to March 2010. Data were collected from Owen Clinic at University of California, San Diego. The primary endpoints were viral load (VL) rebound and CD4 count. Secondary endpoints were serum creatinines (SCr) and estimated glomerular filtration rates (eGFR), proteinuria and glycosuria on random urinalysis, serum phosphate and bicarbonate levels. eGFR was calculated based on MDRD and CKD-EPI equations.

Results: 65 patients met inclusion and exclusion criteria. Average VL prior at start of daily TDF was 89045 copies/mL. 63 patients acheved VL suppression in 6 months. CD4 counts of the 65 patients increased from average of 343 to 406 cells/µl. After dose adjustment of TDF, CD4 counts were stable. 11 patients had TDF discontinued. 13 patients had TDF dose adjusted back to daily dose. 41 patients remained on extended-interval dosing of TDF. 6 on extended-interval dose of TDF had viral rebound. The average SCr was 1 mg/dL, corresponded to an eGFR of 87 mL/min by MDRD at the start of TDF. The average SCr and eGFR when TDF dose was changed to extended-interval were 1.5 mg/dL and 55 mL/min. eGFR by both MDRD and CKD-EPI were stable or improved when TDF dose was adjusted. Proteinuria and glycosuria were higher on extended-interval TDF dose. Hypophosphatemia and metabolic acidosis were not significant.

Conclusions: In HIV positive adult patients on dose-adjusted TDF for renal impairment, viral rebound appears unlikely and CD4 counts are likely to remain stable. Renal function stabilize or slighly improve after dose adjustment. Proteinuria and glycosuria were better markers for renal injury compared to low serum phosphate or bicarbonate. This study provides insight and clinical confidence in continuation of TDF at renally adjusted dose, which allows preserving alternative HAART regimens for the future.

FR-PO755

Cross Reacting Antibodies to Fab 2 Fragments Causing Liver Toxicity after Rituximab and Adalimumab Therapy Joerg Latus,[1] Peter Fritz,[2] Martin Kimmel,[1] Mark Dominik Alscher,[1] Niko Braun.[1] *[1]Nephrology, Robert- Bosch Hospital, Stuttgart, Germany; [2]Division of Pathology, Diagnostic Medicine, Stuttgart, Germany.*

Background: We present a case ofacute liver toxicity in a patient with refractory ANCA-associated GPA, first after treatment with Rituximab and secondly after Adalimumab therapy. We hypothesized the presence of cross reactive antibodies to the two different therapeutic monoclonal antibodies (Tmab) and performed a detailed scientific work-up.

Methods: Three sera from the patient were analyzed for antibodies to rituximab and adalimumab by ELISA. As controls, the microtiter plates were coated with the Fab fragment of an unrelated humanized monoclonal antibody, with human Fc proteins as well as a mouse IgG globulin. Additionally, lymphocyte transformation test (LTT) using rituximab and adalimumab as antigens was performed.

Results: Viral serology for hepatitis A, B, C and autoantibodies specific for autoimmune liver disorders were negative. Transjugular liver bopsy showed histopathological findings compatible with drug-induced liver toxicity. In all three sera from the patient antibodies to rituximab could be detected by ELISA. Two samples had also antibodies to adalimumab, although the patient had not received adalimumab at that time, indicating cross reactivity between both substances. Testing against an unrelated human Fab fragment also revealed positive results, indicating that the patient had developed antibodies against human Fab fragments in general. The Fc proteins were negative, and patients' sera did also not react with mouse IgG globulins. LTT revealed negative results.

Conclusions: This is the first study demonstrating liver toxicity induced by two different Tmabs. Cross reacting antibodies to Fab2 fragments are probably involved. Whether drug toxicity or treatment failure of Tmab therapy are associated with the presence of antibodies to Fab2 fragments has to be evaluated in further studies.

FR-PO756

Disparity in the Treatment of Pediatric Nephrotic Syndrome with Rituximab: Survey of Pediatric Nephrology Centers in the United States Teri L. Crumb, Alejandro Quiroga. *Pediatric Nephrology, Helen DeVos Children's Hospital, Grand Rapids, MI.*

Background: Off-label use of rituximab has been increasingly reported in recent literature for treatment of refractory nephrotic syndrome (NS). An assessment of center practices would ascertain areas of variability and establish the foundation for future research that promotes the safe utilization of this therapy.

Methods: A survey was designed to assess pediatric nephrology centers use of rituximab across the U.S. We assessed for standard practices and experiences by examining: indications, dosing, treatment intervals, clinical side effects, clinical markers used, concomitant therapies and weaning parameters.

Results: 46 pediatric centers were contacted and 25 responses were received with a rate of response of 54%. 4 centers (16%) reported no use of rituximab for NS and 2 (8%) had not used it in the last year. 19 (76%) reported treatment utilization and those results follow. Indication for treatment identified 84% use rituximab as a last resort option and 90% reported dosing at 375mg/m^2. Markers commonly followed were CD 19 and urine Pr/Cr ratio (both 84%). Concomitant medications used were steroids (84%), calcineurin inhibitors (47%) and mycophenolate mofetil (42%). Centers identified the 1st therapy to be weaned post infusion was steroids (74%). The areas of great variability were intervals of dosing, indications for weaning concomitant therapy and the weaning period. The following intervals were reported: single dose (21%), 4 doses/1wk interval (26%), 2 doses/2wk interval (32%), initial/1yr (11%) and variable (26%). When to start weaning therapies was reported as time interval (11%), clinical exam (11%), markers (37%) and variable (47%). Weaning periods were 2 wk (5%), 4 wk (11%), 8 wk (26%), >12wk (11%) and variable (42%).

Conclusions: Rituximab is widely used in the U.S. for NS (76% of survey centers). However, the lack of current guidelines and standardization given the widespread use of rituximab could affect patient safety. We have identified variability in three major areas. This great disparity among centers in the dosing intervals and weaning parameters should prompt us towards the establishment of consensus group guidelines.

FR-PO757

Managing Peritoneal Dialysis-Related Peritonitis Using a Cloud-Based Web App Dimas Yusuf, Daniel Schwartz. *Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada.*

Background: Peritonitis is a common and serious complication of peritoneal dialysis. Most cases of uncomplicated peritonitis may be safely treated on an outpatient basis, however this requires timely patient care and rigorous follow-up involving a multidisciplinary team approach.

Methods: We sought to improve our management of peritonitis with the goal of improving care and reducing the frequency of subsequent infections. We created a secure web-based app to optimize day-to-day management and ensure adherence to our evidence-based care pathway. This app allows us to monitor our cases and ensure that we: (1) obtain samples for culture and sensitivity; (2) start empiric antimicrobial therapy; (3) adjust the treatment based on culture results; (4) obtain a follow-up culture to ensure resolution of infection; and (5) complete a home visit to provide additional patient support. Any divergence or delay from this protocol is detected by the app, which generates a visual alert that prompts corrective action. The app also assists with drug dosing and provides reminders for fluconazole prophylaxis. Lastly, it provides a platform for data collection and analysis to aid in CQI activities.

Results: Since our app was launched in November of 2010, our peritonitis rate decreased from 1 in 24.4 months in the 6 months prior to launch, to 1 in 29.2 in the 6 months prior to June 2012 (NS). Pre-and-post launch staff surveys revealed increased confidence in quality of care—the proportion of staff who "strongly agree" that all cases of peritonitis are thoroughly managed and followed-up increased from 6.3% to 45.8% ($p < 0.001$).

Conclusions: A web-based app to manage and track infectious peritonitis can be used to encourage adherence to a clinical care path. While we demonstrated improvement in team confidence in quality of care, further study is required to determine if patient outcomes can be improved.

FR-PO758

Peritoneal Dialysis Patients in Thailand's Worst Flood Piyatida Chuengsaman. *CAPD Service and Training Center, Banphaeo Hospital, Bangkok, Thailand.*

Background: Last year, from late September till early December, Thailand's worst flood occurred in the central region of Thailand. This affected everyone included PD patients. We provide special services to make sure the patient's safety.

Methods: There are three options which we provide for our patients.

First: move to other provinces.

Our staff will inform the patient destination's PD center. In case of emergency, or shortage of any supplies, our patients can contact them. Our patients should bring all the supplies and the medicines they needed along with them. They will check for the quality of water supply and area for exchange procedure. If they would like to have any suggestions, they can phone or MMS to our staff.

Second: move to our own shelter "Banphaeo shelter for PD patients".

We set Banphaeo shelter in Samutsakorn province near Bangkok. This shelter contains all the basic need for normal daily living. And the most important things are to provide PD solution, water supply, space to make the exchange procedure and medical care.

Third: decide to"not move".

We provide a specific guideline for our patients. The guideline contains suggestion for hand hygiene in case of shortage of water supply or water treatment in case of suspect the quality of tap water. Additionally, it contains how to prepare the space for exchange procedure in case of any limitation. We also keep contact with them to provide any help.

Results: We analyze for the patient's safety in this time period compare to the three months before the disaster. The number of peritonitis episodes from June till December 2011 are as followed; 29, 23, 23, 24, 19, 18 and 22 respectively. In the first group, which is about 50 patients, one patient expired due to acute myocardial infarction, one got peritonitis and one got exit site infection. In the second group, which are about 40 patients, one patient expired due to sudden death and one got peritonitis. In the third group, which are about 550 patients, who decide to "not move", the number of dead cases, peritonitis did not change compare to normal situation.

Conclusions: Peritoneal dialysis patients are at risk when disaster occurred. But if PD center helps our patients to manage, we can ensure our patient safety in this special situation.

FR-PO759

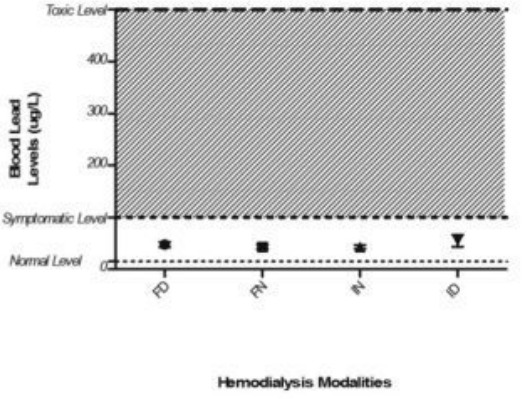
Elevated Blood Lead Levels in Home Hemodialysis Patients: Are They Toxic or Acceptable? Shih-Han S. Huang,[1] Guido Filler,[2] Benjamin Ka Thomson,[1] Peter G. Blake,[1] Robert M. Lindsay.[1] *[1]Medicine, Division of Nephrology, London Health Sciences Centre, London, ON, Canada; [2]Paediatric, London Health Sciences Centre, London, ON, Canada.*

Background: Hemodialysis patients with minimal or no residual renal function may be liable to trace metals (example: lead) exposure (dialysate and phosphate binders) and accumulation. In this study, we reported the blood lead levels in our home hemodialysis patients and assessed variables that may influence the levels.

Methods: Blood lead levels were taken from 46 home hemodialysis patients during their clinic visits. These were compared with those found in the general population and with known toxic levels. The blood lead concentrations of patients on different treatment modalities (conventional vs. quotidian) were compared. The relationship between the blood lead levels and other continuous variables was examined.

Results: The median (Shapiro-Wilk test indicated non-normal distribution) blood lead level was 44.4 ug/L (inter quartile range: 32.2, 55.9), which was significantly different (Wilcoxon Signed Rank test, $p<0.001$) than normal (15 ug/L) and toxic (>100-500 ug/L) levels.

Figure 1. The blood lead levels (ug/L) of patients on different treatment modalities: frequent daily (FD), frequent nocturnal (FN), intermittent nocturnal (IN) and intermittent daily/conventional (ID) hemodialysis.



There were no significant differences in median blood lead levels between patients who were on different hemodialysis modalities (Kruskal-Wallis test, P=0.81). There were no significant correlations between blood lead levels and any of the variables we assessed (Spearman's test, P=0.09-0.66).

Conclusions: The blood lead levels were elevated but not related to age, dialysis vintage or modalities, residual renal function nor parathyroid hormone levels. Thus potentially iatrogenic contribution of dialysis to lead accumulation was excluded. These elevated blood lead levels may represent the "acceptable" levels in this population. Further prospective studies are indicated to establish the acceptable levels for other trace metals with various dialysis modalities.

FR-PO760

Epoetin Zeta for Treatment of Anemia in Chronic Kidney Disease: 4-Year Post-Marketing Surveillance Helen Phillips, Andrew Buckley. *Hospira UK Ltd, Royal Leamington Spa, United Kingdom.*

Background: Epoetin zeta (Retacrit®, Hospira UK) is a biosimilar erythropoiesis-stimulating agent (ESA) indicated for treatment of anemia associated with chronic renal failure in adult and pediatric patients on hemodialysis, adult patients on peritoneal dialysis, and for treatment of severe anemia of renal origin accompanied by clinical symptoms in adult patients with renal insufficiency not yet undergoing dialysis. Approval of biosimilar ESAs in the EU requires extensive scientific evaluation and stringent regulatory procedures, ranging from preclinical/clinical studies to tightly controlled manufacturing processes. Post-marketing pharmacovigilance is an integral part of regulatory requirements. This post-marketing surveillance study was undertaken as part of periodic safety update reports submitted to the regulatory authorities. **Aim:** To assess the continuing safety of epoetin zeta in treating renal anemia.

Methods: Data were obtained from 18 December 2007 – 31 December 2011. Adult patients with symptomatic anemia received subcutaneous epoetin zeta as per the EU summary of product characteristics (50 IU/kg 3 times/week for 4 weeks). Depending on response, subsequent dosage varied from 75–300 IU/kg/week. A 3-month dosing period was used as average exposure time and total cumulative post-marketing exposure estimate was based on sales of finished product.

Results: Patients received approximately 45,000 IU during the initial course of treatment and 112,500 IU during maintenance therapy. The average total dose per patient was 157,500 IU. During the surveillance period, 8,522,817,000 IU or 54,113 patient-courses were used according to the epoetin zeta nephrology indication. The total cumulative post-marketing and clinical exposure estimate (oncology and nephrology data) was approximately 25,770,847,000 IU or 86,159 patient-courses. No reports of neutralizing antibodies or cases of pure red cell aplasia have been received to date.

Conclusions: The total epoetin zeta patient exposure now exceeds 86,000 patient-courses, of which over 54,000 patient-courses were in the nephrology indication. In these patients, no new safety issues have been identified which impact on the established safety profile of the product.

Funding: Pharmaceutical Company Support - Hospira UK Ltd

FR-PO761

Antibiotic Spacers in the Treatment of Infected Prostheses: A Systematic Review and Risk-Benefit Clinical Decision Analysis Andrew Luu, Madhumathi Rao. *Nephrology, Tufts Medical Center, Boston, MA.*

Background: Two-stage arthroplasty is currently the treatment of choice for infected joint prostheses. There is limited data regarding safety or risk benefit ratio. In a systematic review of the literature we examined the rate of acute kidney injury (AKI) and infection recurrence as a measure of efficacy, and assessed the risk-benefit ratio of antibiotic spacer use to aid clinical decision making.

Methods: We applied screening filters to searches from Ovid. Using data inputs from systematic review, primary analyses and expert opinion we computed probabilities for AKI and infection recurrence for different therapeutic approaches - medical therapy, two stage arthroplasty without antibiotic spacers, low dose or high dose aminoglycoside spacers or other antibiotic spacers. Risk benefit analysis was run with TreeAge Pro 2012 modeled for age and baseline CKD to find the best utility and quality-adjusted life expectancy by the DEALE method.

Results: We found 10 studies addressing AKI and 31 studies addressing infection recurrence in patients undergoing 2-stage arthroplasty. The incidence of AKI in 544 patients ranged from 1.8 to 17% depending on definition of AKI. Reported risk factors were aminoglycoside dose, age, baseline chronic kidney disease (CKD), vascular comorbidity and hypotension. Infection recurrence occurred in 6.6% of 1019 patients and was associated with knee rather than hip prostheses (56% vs 44%), but not with type of antibiotic in the spacer or organism including MRSA. Limitations of the literature included inadequate and heterogenous data precluding metaanalysis.

The best utility and quality-adjusted life expectancy calculated by the DEALE method was seen in the younger patient without comorbidity.

Conclusions: Antibiotic spacers are associated with significant nephrotoxicity and systemic complications. Judicious patient selection with treatment tailored to patient comorbidities guided by robust data and application of decision analyses models would support the best outcomes.

Funding: Clinical Revenue Support

FR-PO762

Renal and Cardiovascular Outcomes in Patients with Fabry Disease under Long Term Enzyme Replacement Therapy Ricardo M. Heguilen,[1] Amelia Rita Bernasconi,[1] Juan Politei,[1] Gustavo H. Cabrera,[1] Roland C. Blantz.[2] *[1]Medicine & Neprhology, Hospital Fernandez, Universidad de Bs As., Bs As, Caba, Argentina; [2]Medicine, University of California San Diego and VASDHS, San Diego, CA.*

Background: Variable degree of proteinuria and progressive renal failure, along with CV and CNS involvement are major features in adult patients with Fabry disease (FD). Enzyme replacement therapy (ERT) must be initiated as early as possible to prevent terminal organ damage; ERT has been proved to reverse substrate storage in lysosomes. We report the renal and CV outcomes of a cohort of FD pts, most of them on ERT.

Methods: 29 pts (18 F) aged 17-56 years (35.2 ± 2.1y) with a history of FD based upon typical manifestations and a reduced activity of leukocyte a-gal were prospectively studied for up to 9.5 y (median 5y). 22 pts were on ERT with IV agalsidase α or agalsidase β; administered according to manufacturer´s directions. Glomerular and tubular, as well as cardiac functions were evaluated clinically and through standard laboratory methods and echocardiographically.

Results: 25 individuals displayed renal involvement (mild proteinuria and/or excretory dysfunction). Baseline Cr clearance was 89.0 ± 5.3 mL/min and 8 individuals exhibited baseline urine protein excretion > 0.5 g/d. Hypercalciuria, was present in 8 patients. Proteinuria as well as excretory function remained. The average CrC at the end of follow up was 80.0 ± 6.0 mL/min (p: NS vs. baseline). $TRPO_4$ decreased slightly although significantly from 86.2 ± 1.1 to 80.3 ± 2.0% ($p<0.05$). Similarly, $TmPO_4$/GFR decreased from 3.3 ± 0.2 to 2.7 ± 0.1 mg/dL ($p< 0.05$). There was a trend for this reduction be more pronounced in males than in females. 7 patients displayed non progressive concentric myocardial hypertrophy with prolonged isovolumetric ventricular relaxation time (IVRT).

Conclusions: Renal and cardiac involvement are frequent in individuals suffering from FD but may be stabilized by ERT. The long term subtle reduction in $TRPO_4$ may be an early marker of proximal tubular dysfunction due to the disease or as a consequence of drug - induced toxicity.Enzyme replacement therapy is safe and effective in preserving renal and CV function in patients with Fabry.

FR-PO763

Static and Dynamic Balance Function in Dialysis Patients Yafei Yang,[1] Hsiu-chen Lin,[2] Shuya Chen,[2] Chiu-Ching Huang.[1] *[1]Nephrology, China Medical University Hospital, Taichung, Taiwan; [2]Physical Therapy, China Medical University, Taichung, Taiwan.*

Background: Falls in dialysis patients are common and cause significant morbidity and mortality. Multiple risk factors of falls have been identified, such as age, falling history, lower extremities weakness, balance problems, arthritis, orthostatic hypotension, and anemia. Computerized dynamic posturography (CDP) system, which can perform sensory organization test (SOT), limits of stability (LOS) and rhythmic weight shifting (RWS) tests, is widely used for evaluating static and dynamic balance problems in the elderly and various patient groups. However, limited literature addressed the issue of balance function in dialysis patients. The aim of this study was to fill this gap.

Methods: Twenty-six patients (19 peritoneal dialysis, 7 hemodialysis) receiving dialysis more than 3 months were enrolled. Twenty-eight age-matched healthy volunteers were also recruited as control group. Each participant received anthropometric measurements, physical fitness (5-repetition sit-to-stand (STS), grip strength test, sit-bend test and 6-minute walk test) and static and dynamic balance function tests on the CDP system.

Results: There were no differences in height, weight, body fat and body mass index between groups. All the physical fitness parameters, except for sit-bend test, were significantly worse in the dialysis group. They also demonstrated worse performance in the balance tests, i.e. lower composite equilibrium score in SOT, lower composite score of reaction time, movement velocity, endpoint excursions in LOS test, and directional control in LOS and RWS tests. The composite equilibrium score, the average score of four SOT conditions, was moderate correlated with 5-rep STS time (-0.41, *P=0.04)*, grip strength (0.43, *P=0.03)*, 6-min walk distance (0.55, *P=0.004)*, and sit bend length (0.50, *P=0.01)*.

Conclusions: Balance functions of dialysis patients are significantly impaired compared to age-matched controls. The composite equilibrium score was the best balance variable that related to all the physical fitness variables.

Funding: Government Support - Non-U.S.

FR-PO764

Percutaneous Insertion of Peritoneal Dialysis Catheters by Nephrologists: The Learning Curve Matthew R. Todd, Gerry Endall, Mark Dominic Uniacke, Paul Gibbs. *Wessex Renal & Transplant Service, Queen Alexandra Hospital, Portsmouth, Hampshire, United Kingdom.*

Background: The Wessex Renal & Transplant Service has been using a combination of percutaneous and open surgical techniques for peritoneal dialysis catheter insertion since 2006. Around 1/3 of catheters are inserted percutaneously under conscious sedation by nephrologists. We have recently instituted a formalized training program for nephrologists-in-training learning catheter insertion.

Methods: We retrospectively analyzed catheter survival (censored for non-patency-related change of modality and death) and compliance with ISPD audit standards for technical success (<1% bowel perforation/hemorrhage, <5% exit site infection/peritonitis within 2 weeks of insertion, <20% need for manipulation/replacement or technique failure). Catheters were grouped by the experience of the operator at the time of insertion (number of catheters previously inserted). Catheter survival was plotted using the Kaplan-Meier method.

Results: 133 catheters were inserted percutaneously by nephrologists over the 6-year period by 15 different operators (range 1-31 catheters per operator). Overall all ISPD audit standards were met. The technical failure rate (catheter not usable at day 14 post-insertion) was higher with inexperienced operators: 1st catheters 13% (2/15), 2nd-10th catheters 10% (7/67), >10th catheters 5% (2/44). There were no technical failures in 28 catheters inserted by operators with experience of 13 or more prior catheter insertions.

Conclusions: Our data show a distinct learning curve for percutaneous peritoneal dialysis catheter placement by nephrologists in training. This has implications for training programs for interventional nephrologists. In the UK, the Renal Association recommend that all nephrology trainees have the opportunity to learn peritoneal dialysis catheter placement; however, the needs of trainees needs to be balanced with the outcomes for patients. Our data would suggest that, with our current rate of around 25 percutaneous insertions per year, it may not be possible to give all trainees adequate experience in peritoneal dialysis catheter insertion whilst giving patients the highest likelihood of technical success.

FR-PO765

Safety and Long Term Outcomes of Percutaneous Peritoneal Dialysis Catheter Insertion under Local Anaesthesia Jonathan Dick, Kate Bramham, Elaine Bowes, Ravi Kumar, Sean Main, Elaine Ruth Sylvester, Claire C. Sharpe, Hugh Cairns, Satish Jayawardene. *Renal Unit, King's College Hospital, London, United Kingdom.*

Background: Percutaneous peritoneal dialysis (PD) catheter placement under local anaesthesia (LA) allows PD to be offered as the initial mode of renal replacement therapy. However, theoretical concerns over safety and outcomes may influence the choice of this access approach amongst clinicians.

Methods: Records of 543 catheter insertions performed from 2002-2011 were retrospectively reviewed. LA catheters were inserted using the Seldinger technique by nephrologists or a specialist nurse. Surgical insertion took place under general anaesthetic (GA). Procedure failure was defined as failure to insert a catheter or it was not functioning at day 0 or 1. Failures were not included in analysis of other complications. Patients who died, received a renal transplant, or transferred to another unit during the period assessed were excluded from analysis.

Results: Patient baseline characteristics were not significantly different between access approaches.

Outcomes of LA/GA Catheter insertions

	LA	GA	P Value
Total Catheter Insertions (No. Patients)	324 (254)	219 (112)	-
1st Catheter Insertion	251 (78%)	99 (45%)	<0.0001
Procedure Failure	32 (10%)	9 (4%)	0.013
Received Haemodialysis before 1st Catheter Insertion	49 (19%)	37 (38%)	<0.0001
Catheter Functioning at 6 Months / 1 Year	138 (51%) /102(36%)	109 (57%)/76 (41%)	NS
Exit Site Infection <30 Days	32 (11%)	28 (13%)	NS
Peritonitis <30 Days	16 (5%)	15 (7%)	NS
Inpatient Stay Days Median (IQR)	0 (0-5)	4 (3-6)	<0.0001

Conclusions: To our knowledge this is the largest series of LA PD catheter insertions. Patients who have LA PD catheter insertion have significantly shorter inpatient stays and are less likely to require haemodialysis prior to the procedure. LA PD catheter insertion is associated with a higher immediate procedure failure, however rates of infective complications and catheter patency at one year are similar to insertions under GA.

FR-PO766

The Development and Success of an Outpatient Peritoneal Dialysis Catheter Placement Program Randall L. Rasmussen,[1] Rajeev Narayan,[2] Aris Q. Urbanes,[1] Gerald A. Beathard,[1] Terry Litchfield.[1] *[1]Lifeline Vascular Access, Vernon Hills, IL; [2]San Antonio Kidney Disease Access Center, San Antonio, TX.*

Background: Peritoneal dialysis (PD) success depends upon timely and skilled placement of a PD catheter (PDC). Most PDCs are placed by surgeons, but advances in percutaneous or laparoscopic placement have resulted in non-surgeons placing more PD catheters. In this study, a large outpatient vascular access system developed a national program to provide this service to patients.

Methods: Ten (10) centers were trained in percutaneous and peritoneoscopic placement of PD catheters by an expert physician. Training including a didactic training; followed by simulation training and performance of procedures with the training physician present. During the training period, the local dialysis provider and PD nurses were engaged in the program. Data was collected in an electronic health record and analyzed using SPSS software.

Results: One hundred and forty seven (147) PD catheters were placed during the one year period following training and program launch. The program has been a clinical success with a 95.9% success rate and a 1.4% complication rate including a .7% major complication rate. The mean age of the patients were 55 years old; 54% diabetic. More than 70% of the patients have come from one large dialysis organization (LDO), reflecting their active engagement in patient PD starts.The most common reasons for PD catheters were to either start dialysis (43.5%) or modality change (44.2%). Average case times were 74.39 minutes. The early failures are small (n=3) and 2 of the 3 successfully got another PD catheter.

Conclusions: A dedicated outpatient program for the placement of PD catheters can be very successful when training is formal along with focus on quality outcomes. In addition, the support of LDOs is critical to program development and success.

FR-PO767

Emergent and Urgent Peritoneal Dialysis Instead of Hemodialysis with a Central Venous Catheter Mukesh Sharma, Kenneth D. Abreo, Bharat Sachdeva. *Nephrology, Louisiana State University Health Sciences Center, Shreveport, LA.*

Background: In the US, 60-80% of ESRD patients start HD with a central venous catheter (CVC), resulting in an unacceptably high morbidity and mortality from infections. Selecting PD as the initial dialysis modality is an excellent CVC avoidance strategy.

Methods: Emergent PD (EPD) is defined as starting PD immediately (24-48 h) after PD catheter (PDC) placement whereas urgent PD (UPD) is defined as starting PD within 2 weeks of PDC placement. 5 new ESRD patients were educated by their nephrologist on the merits of each dialysis modality and chose PD over HD. Of these 5 patients only 1 had a vascular access in place. 3 patients did EPD and 2 UPD. Indications for EPD and UPD were uremia, volume overload, and hyperkalemia. Two EPD and 1 UPD patients received 1-3 HD treatments through a CVC and AVF respectively prior to PDC placement. Interventional nephrologists placed PDCs with fluoroscopy guidance under moderate sedation and local anesthesia. A purse string suture was intentionally placed in the anterior rectus sheath encircling the catheter to prevent PD fluid leak. Low volume (1 liter) exchanges were used with the patient in the supine position.

Results: There were no PDC infections, leaks, malfunction, or any procedure related complications in any of the patients immediately after placement. Demographics and laboratory results for EPD and UPD were as follows:

#	PD Type	Gender/Age/ Race	Indication	eGFR	Pre-PD Crt	Post PD Crt	Pre-PD BUN	Post PD BUN	Pre-PD K	Post PD K	Pre-PD HCO3	Post PD HCO3
1	EPD	M/30/AA	Uremia	4	19.5	16.5	139	92	5.1	3.8	18	27
2	EPD	M/62/AA*	Hyperkalemia	11	8.6	6.8	93	51	6.3	4.9	25	25
3	EPD	F/57/AA*	Hyperkalemia	8	4.7	5.2	63	60	5.5	4.8	17	23
4	UPD	F/21/AA	Uremia	10	8.7	5.7	123	64	4.4	4.3	25	25
5	UPD	F/62/W†	Volume	11	3.5	3.2	38	44	5.8	5.4	33	24
			Mean	8.8	8.2	7.5	87.2	62.2	5.0	4.6	23.6	24.8
			Std. Dev.	2.9	6.5	5.2	40.5	18.4	1.3	0.6	6.3	1.5

AA=African-American, W=White, Pre-PD=before start of PD, Post PD for EPD=hospital discharge day, Post PD for UPD=after ~ 7 d of PD
*Patient had one HD treatment for Hyperkalemia, †Patient had HD for 3 days for volume overload using AVF

Conclusions: ESRD patients are often amenable to PDC placement, especially if the physician can provide modality options and the pros and cons of PDC versus CVC. Insertion of PDC at short notice is a sine qua non for starting EPD or UPD. PD can be started immediately with no complications and continued in the outpatient setting thus avoiding the perils of a CVC in patients without a vascular access.

FR-PO768

A Comparison of Swan-Neck and Conventional Straight Catheters with Upper Abdominal Exit-Site Locations Masahiro Eriguchi,[1] Hisako Yoshida,[2] Kazuhiko Tsuruya,[2] Takanari Kitazono.[1] *[1]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Catheter related infections are crucial problem among peritoneal dialysis patients. Compared to a conventional method with a lower abdominal exit-site location, clinical studies of extended catheters with an upper abdominal exit-site (UAE) location tended toward lower incidences of exit-site infection (ESI) and catheter infection-related peritonitis. Also, several studies have been reported with the use of swan neck (SN) catheters compared with conventional straight (CS) catheters. But a beneficial effect on clinical outcomes by a SN catheter with an UAE location is not critically investigated.

Methods: This is a non-randomized comparison of 56 consecutive patients who were applied CS catheter with an UAE location (CS group) and 21 consecutive patients who were applied SN catheter with an UAE location (SN group). Prospective data collection included patient demographics, infectious complications and catheter survival. To evaluate infectious complications, we use Kaplan-Meier survival curves for first episode of infectious complications and recurrence data analyses for recurrent episodes of infections.

Results: Less exit-site infections (1/15.6 vs 1/10.3 patient-month) and peritonitis (1/41.6 vs 1/28.9 patient-month) were observed in the SN group than in the CS group. Survival rate for first episode of ESI of the SN group tended better than that of the SN group (p=0.062). But survival rate for first episode of peritonitis and catheter survival were not different. By recurrence data analyses, mean cumulative episodes of ESI tended less in the SN group than the CS group, but this was not statistically different.

Conclusions: For an upper abdominal exit-site location, the extended swan-neck shaped catheter may contribute to reduce exit-site infections.

Funding: Pharmaceutical Company Support - Baxter (20th Baxter PD Fund)

FR-PO769

Acute Peritoneal Dialysis in the Pediatric Population of New Zealand: A Review of the Introduction of a New Catheter Insertion Technique Maria P. Stack, Hemal Kodikara, Tonya Kara. *Pediatric Nephrology, Starship Children's Hospital, Auckland, New Zealand.*

Background: Starship Hospital is the only national referral centre providing acute dialysis for pediatric patients in New Zealand. The advantages of using peritoneal dialysis (PD) in these children include the ability to perform continuous renal replacement therapy, with optimized nutrition, avoiding central venous access. The disadvantages include the potential for early leaks, delayed use and restricted volumes with an open insertion. To allow early use the surgeons at this centre have been using a laparoscopic technique since 2005. We specifically examined the introduction of laparoscopic assisted PD catheter insertion compared with open insertion technique.

Methods: We performed a 10 year review of acute peritoneal dialysis in Starship Hospital from 2001 to 2011. We excluded peritoneal dialysis carried out post cardiac surgery and catheters placed for chronic use. We analyzed data based on insertion technique, either open or laparoscopic assisted.

Results: Data on 99 children who had acute kidney injury (AKI) and received PD was analyzed. The mean age of the group was 3.9 years (SD 3.3years). Predominant diagnosis was HUS, accounting for 69/99 cases, followed by sepsis related AKI and acute glomerulonephritis. There were no cases of peritonitis in the 18 children who had laparoscopic approach, and 5/81 (6%) cases in the standard open approach. In the lapraoscopic group 2/18 (11%) required further manipulation of catheter after initial insertion, compared to 14/81 (17%) in the open group. Conversion to HD due to catheter related complications was seen in 2/18 (11%) laparoscopic cases and 9/81 (11%) of open cases.

Conclusions: Overall we have had success with acute peritoneal dialysis in New Zealand and the introduction of the new technique has not resulted in an increased rate of catheter related complications when compared to standard approach. The infection rates are lower with the laparoscopic approach, acknowledging the low number of cases in our group.

FR-PO770

Peritoneal Dialysis Solutions Containing Low Polydispersity Glucose Polymers as Osmotic Agents: Theoretical Predictions from the Three-Pore Model J. Ken Leypoldt, Alp Akonur, Clifford J. Holmes. *Medical Products R&D (Renal), Baxter Healthcare Corporation, Deerfield, IL.*

Background: Icodextrin is an osmotic agent for peritoneal dialysis (PD) solutions containing glucose polymers with a weight-average molecular weight (Mw) between 12 and 20 kilodaltons (kD) and a number-average molecular weight (Mn) between 5 and 6.5 kD; the polydispersity (Mw/Mn) for icodextrin has been reported to be 2.6 (Vonesh et al, Perit Dial Int 2006). We used mathematical modeling based on the three-pore model to determine combinations of Mw (1-24 kD), polydispersity (1.6-2.4) and glucose polymer concentration (4.0-15.0%) that achieved higher ultrafiltration (UF) without an increase in carbohydrate absorption compared with icodextrin.

Methods: Mathematical modeling of UF as a function of time during a long dwell of 12 hours was performed based on PD Adequest 2.0 for high, high-average and low-average transport patients, according to their UF profile. All glucose polymer, including icodextrin, distributions were assumed to be log-normal, and carbohydrate absorption was calculated as the mass of glucose polymer infused minus that remaining after 12 hours. Glucose polymers were defined as having improved UF performance if they achieved higher UF after 12 hours than that using icodextrin at any concentration between 7.5 and 15% without additional carbohydrate absorption for all patient transport types (with a tolerance of 3 g).

Results: Computed results demonstrated that 1) no glucose polymer with Mw between 1 and 7 kD had improved UF performance at any polydispersity or concentration; 2) glucose polymers with Mw between 8 and 14 kD at a concentration of 7.5% achieved modestly improved UF performance (up to 20% higher UF, depending on polydispersity); 3) glucose polymers with Mw between 8 and 24 kD at concentrations greater than 7.5% achieved more substantial improvements in UF performance at specific low polydispersity-concentration combinations.

Conclusions: As osmotic agents for PD solutions, glucose polymers can achieve higher UF than icodextrin without additional carbohydrate absorption by decreasing their polydispersity and increasing the glucose polymer concentration.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO771

Peritoneal Dialysis Solutions Containing Low Polydispersity Glucose Polymers as Osmotic Agents Achieve Higher Ultrafiltration than 7.5% Icodextrin without an Increase in Carbohydrate Absorption: Validation in a Rabbit Model J. Ken Leypoldt, Catherine M. Hoff, Clifford J. Holmes. *Medical Products R&D (Renal), Baxter Healthcare Corporation, Deerfield, IL.*

Background: Peritoneal dialysis (PD) solutions containing icodextrin as the osmotic agent have advantages during long dwells. The glucose polymers comprising icodextrin have been reported to have a polydispersity (ratio of weight-average to number-average molecular weight or Mw/Mn) of 2.6 (Vonesh et al, Perit Dial Int 2006). We hypothesized that a PD solution containing glucose polymers with low polydispersity at high concentration could achieve higher ultrafiltration (UF) without an increase in carbohydrate absorption (CA) compared with 7.5% icodextrin solutions.

Methods: Experimental studies were performed during 8-hour dwells in New Zealand White rabbits (2.35-2.90 kg body weight). Study 1 compared solutions containing 7.5% icodextrin (N = 9) with 11% 19K glucose polymer (Mw = 18.8 kilodalton & Mw/Mn = 2.06, N = 9). Study 2 compared solutions containing 7.5% icodextrin (N = 12) with 11% 18K glucose polymer (Mw = 18.0 kilodalton & Mw/Mn = 1.98, N = 12). A control study compared solutions containing 11% icodextrin (N = 9) with 11% 19K glucose polymer (N = 9). Net UF was measured by complete fluid collection, and CA was measured by subtracting the total carbohydrate in the collected fluid from that initially infused.

Results: In Study 1, UF for the 11% 19K glucose polymer was higher than that for 7.5% icodextrin (89±31 ml [mean±SD] vs. 49±15 ml, P = 0.004) without an increase in CA (5.2±0.9 g vs. 5.0±0.9 g, P = 0.7). In Study 2, UF for the 11% 18K glucose polymer was higher than that for 7.5% icodextrin (96±18 ml vs. 66±17 ml, P < 0.001) without an increase in CA (4.8±0.7 g vs. 5.2±0.6 g, P = 0.2). In the control study, UF for 11% 19K glucose polymer and 11% icodextrin were similar (68±20 ml vs. 57±15 ml, P = 0.2), but CA was lower for the 19K glucose polymer solution (5.3±0.8 g vs. 6.4±1.1 g, P = 0.03).

Conclusions: These findings suggest that long dwell PD solutions containing 11% 18-19K glucose polymers with low polydispersity can provide higher UF than 7.5% icodextrin solutions without an increase in CA.

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FR-PO772

4-Hour Dwell Period Ultrafiltration Volume with Icodextrin Solution Is Better than 4.25% Glucose Solution to Predict Peritoneal Equilibrium Test (PET) Results Sara Mohrbacher, Erica A. Guimaraes, Rosilene M. Elias, Benedito J. Pereira, Manuel C. Castro, Hugo Abensur. *Division of Nephrology, University of São Paulo School of Medicine, Sao Paulo, Brazil.*

Background: The PET is a well-defined, yet cumbersome, method to classify patients on peritoneal dialysis (PD) by transport pattern. The ultrafiltration (UF) volume for a 4-hour dwell period with 4.25% glucose is then employed to estimate peritoneal membrane capacity. With icodextrin (ICO), UF is mainly through the small pores of the peritoneal membrane. ICO was not yet tested to evaluate membrane capacity. Here, we compared UF with 4-hour dwell period with ICO and 4.25% glucose solutions, correlating to PET.

Methods: We included 11 patients on chronic PD (mean age, 44 ± 19 years; male, 50%). The median time on PD was 14.5 months. Each patient underwent three assessments: standard PET, UF with 4-hour dwell period with 4.25% glucose and UF with 4-hour dwell period with ICO.

Results: Table 1 shows the UF volume, as well as the PET classification by glucose at 4h/0h (G4/G0) and by dialysate/plasma creatinine (D/P Cr). Patients were divided into two groups: low (L) + low average (LA) or high (H) + high average (HA). The UF with ICO correlated with G4/G0 (r=-0.758, p=0.007) and with D/P Cr (r=0.674, p=0.023). ROC determined that the best cut-off point to predict transport pattern with ICO was 181 ml based on PET classification by G4/G0 (area under the receiver operating curve, 1.0; p=0.006) and 152 ml for PET classification by D/P Cr (area under curve 1.0, p=0.008). The sensitivity and specificity were 100% for both cut-off points.

	L-LA	HA-H	L-LA	HA-H
	G4/G0 (n=5)	G4/G0 (n=6)	D/P Cr (n=4)	D/P Cr (n=7)
Dialysate	UF volume, ml			
4.25% glucose	749±179	588±157	719±166	645±207
ICO	48±103	344±131*	17±88	319±136*

*p<0.05 vs. L-LA

Conclusions: UF volume with 4-hour dwell period with ICO can predict peritoneal membrane transport pattern better than 4-hour dwell period with 4.25% glucose. High transporters have more small pores than low transporters. ICO acts through small pores, and is less absorbed than is glucose, which explains the high UF volume in HA-H patients. Further studies are needed to confirm these results in a larger sample.

FR-PO773

Fluorescent Albumin to Assess Water Transport in a Mouse Model of Peritoneal Dialysis Johann Morelle,[1] Sara Terryn,[1] Yvette Cnops,[1] Anna Rippe,[2] Eric Goffin,[1] Bengt Rippe,[2] Olivier Devuyst.[1,3] *[1]Nephrology, Saint-Luc UCL, Brussels, Belgium; [2]Nephrology, University Hospital, Lund, Sweden; [3]Institute of Physiology, University of Zurich, Zurich, Switzerland.*

Background: Intraperitoneal volume (IPV) tracers are warranted to accurately assess fluid transport during peritoneal dialysis (PD), but their current use is limited by stringent biosafety regulations and tracer kinetics requiring correcting equations. Here we demonstrate that albumin labelled with Alexafluor, a particularly bright, photostable and pH-insensitive dye, is an easy-to-use and highly reliable IPV tracer for investigation of water transport in mouse models of PD.

Methods: Transport across the mouse peritoneum was investigated during a 2-h exchange in 41 SV129 mice, with a PD solution in which 100 µg of fluorescent albumin was added. Dialysate microsamples were taken at 0-10-30-60-90-120min to measure fluorescence, assess tracer kinetics and determine IPV changes; volumetric measure of IPV was considered as the gold standard. The technique was further applied to test the influence of various concentrations of glucose (G) and to assess the role of the ultrasmall pore aquaporin-1 (AQP1) in *Aqp1* mice.

Results: The intra-peritoneal mass of fluorescent albumin remained constant during the whole duration of the dwell performed with G3.86%, indicating no disappearance of the tracer (relative albumin mass at 120min 98±2%, p>0.05). Calculated IPV closely correlated with the volumetrically assessed IPV at each time point (p=0.31 between IPV curves) and calculated and measured net ultrafiltration (UF) were not different (41.0±2.4 vs 39.6±2.4 µl/g, p=0.34). Fluorescent albumin had no effect on albumin concentration in the dialysate, dialysate osmolality, or net UF. G1.36% reduced by 68% and G7% increased by 56% the net UF, as compared to G3.86% (p<0.001 between IPV curves). Aqp1 wildtype and knockout mice had an initial UF rate of 25.1±1.4 µl/min and 7.2±1.7 with G3.86%, respectively, while the net UF in the latter was decreased by 40% (p<0.001).

Conclusions: Alexafluor labelled-albumin is an accurate IPV tracer and a promising tool to unravel the molecular mechanisms and test new strategies to improve water removal during PD.

Funding: Pharmaceutical Company Support - Baxter Extramural Grant, Private Foundation Support

FR-PO774

Aquaporin-1 Mice Provide Novel Insights into the Physiology of Crystalloid and Colloid Osmosis across the Peritoneal Membrane Johann Morelle,[1] Sara Terryn,[1] Yvette Cnops,[1] Eric Goffin,[1] Olivier Devuyst.[1,2] *[1]Nephrology, Saint-Luc UCL Academic Hospital, Brussels, Belgium; [2]Institute of Physiology, University of Zurich, Zurich, Switzerland.*

Background: The principle of osmosis has now been applied for more than 50 years to generate ultrafiltration (UF) across the peritoneum of peritoneal dialysis (PD) patients. The use of transgenic mouse models of PD has contributed to the understanding of crystalloid osmosis, by demonstrating that the water channel aquaporin-1 (AQP1) is the molecular counterpart of the ultrasmall pore. We tested the hypothesis that colloid osmotic agents induce UF independently of AQP1 and that combinations of crystalloid and colloid osmotic agents have a synergistic effect on water and sodium removal through the so-called small pore system.

Methods: Peritoneal transport was investigated during 2-h exchanges in transgenic *Aqp1* mice (knockout, in which water transport occurs exclusively through the small pores, vs. wild-type littermates) with PD solutions including glucose (G) 1.36% and 3.86%, icodextrin (I) 7.5% and combinations of G/I (CIG 1.36% and CIG 3.86%).

Results: Water removal was not affected by the absence of AQP1 when using the colloid agent icodextrin, as indicated by the same net UF in *Aqp1* knockout and wild-type mice. CIG 3.86% and CIG 1.36% resulted in a 9.4 and 5.4-fold increase in net UF, and in a 4.8 and 2.8-fold increase in sodium removal, respectively, as compared to icodextrin 7.5% alone. Net UF and sodium removal obtained with glucose and icodextrin were superior to the sum of the results generated by each osmotic agents used separately. The estimated amount of water generated through small pores increased from 11.6±1.2 µl/g to 31.1±1.4 µl/g (p<0.001) when icodextrin 7.5% was added to glucose 3.86%, while the contribution of AQP1 remained unchanged.

Conclusions: Using various osmotic agents alone or in combination in the *Aqp1* mice unequivocally demonstrates that (1) colloid osmosis occurs independently of the ultrasmall pore AQP1; (2) bimodal solutions synergistically enhance UF and sodium removal; (3) these synergistic effects result from an increased contribution of small pores to water removal.

Funding: Pharmaceutical Company Support - Baxter (Extramural Grant), Private Foundation Support

FR-PO775

Peritoneal Fluid Flow Can Be Detected and Quantified with Dynamic Electrical Impedance Tomography Using an In Vivo Peritoneal Dialysis Model Edward A. Ross,[1] Aaron S. Tucker,[2] Jennifer Paugh-Miller,[1] Rosalind J. Sadleir.[2] *[1]Division of Nephrology, Univ of Florida; [2]Dep't of Biomedical Engineering, Univ of Florida, Gainesville, FL.*

Background: Bioimpedance technology has many applications including body-content analyses during ultrafiltration. We previously showed how noninvasive multi-sensor arrays and reconstruction algorithms could monitor abdominal fluid flow and accumulation using Electrical Impedance Tomography (EIT), and reported proof of principle during PD exchanges. We now studied refined algorithms and an 8-electrode sensor array to image and quantify PD fluid flow.

Methods: PD subjects underwent a manual exchange during continuous EIT monitoring: 8, 5x10 cm electrodes attached to the anterior abdomen near the umbilicus level. Our EIT device (EPack 2 system) measured impedances between pairs of electrodes in multiple configurations during 2000 ml fluid infusion. Data were reconstructed into color-coded images, and then post-processed to calculate a measure (Quantity Index or QI) of the total impedance variation. QI data were used to calculate fluid accumulation rate and total volume, which was compared to that measured by weighing the PD bag during instillation.

Results: We studied 9 PD exchanges in 3 subjects. We found it possible to detect fluid accumulations of >60 g with 98% sensitivity and 95% specificity. The color-coded tomography-like reconstructed images visually demonstrated the pattern of fluid flowing

into the abdomen. Since this technology may be extended to emergent detection of intra-abdominal bleeding, we calculated that our frame acquisition rate of 1 Hz could show 60 ml of hemorrhage within 60 seconds, which is less than half the imaging time required using rapid ultrasound methodologies.

Conclusions: This study clearly demonstrates the feasibility of EIT to monitor fluid flow and accumulation in the abdomen. This dynamic technology has merit for noninvasively studying PD fluid flow patterns and may extend to the rapid and potentially automated detection of other abdominal fluid collections, such as that in traumatic hemorrhage. Thus optimizing the imaging algorithms, as well as the number and placement of electrodes, warrants further investigation.

FR-PO776

Long Term Follow Up of Volume Status in Peritoneal Dialysis Patients
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Background: Volume overload is prevalent in ESRD patients and is an independent risk factor of morbidity and mortality in dialysis patients. We evaluated the long term effects of volume status in peritoneal dialysis patients.

Methods: 98 patients were enrolled in a single center and were followed up for 36 months. Body composition was measured using body composition monitor (BCM, FMC): overhydration(OH), lean tissue mass index(LTI), fat tissue mass index(ATI). Overhydration was defined as OH value over 1.1L. Geriatric nutritional risk index (GNRI=[14.89x albumin(g/dl)]+[41.7x body weight/ideal body weight]) were used as a nutritional marker. Aortic calcification score (0-24) was assessed using lumbar spine x-ray.

Results: Total seventy eight patients completed the study with mean follow up period of 30months. Patients with volume overload increased up to 82.1% from 74.5% of total study patients during follow up without any intervention. Those who were overhydrated remained constantly overhydrated after 36months. The patients with excess fluid showed poorer nutritional parameters (lower GNRI, lower LTI, lower lipid profile) and the results remained similar after the completion of the study. Aortic calcification score was significantly increased overall after 2.5year(3.2±4.8 to 5.3±5.6, p=0.001). However, the score was not significantly different from patients with or without overhydration. During mean follow-up of 30months, 12(15%) died. All-cause mortality was increased in fluid overload state (unadjusted HR 1.26(95% CI 1.009-1.563), adjusted HR 1.31(95% CI 1.01-1.713). Those with higher protein, albumin and LDL cholesterol level showed decreased risk of all cause mortality.

Conclusions: Volume overload is associated with malnutrition and seems to be an independent predictor of mortality in PD patients. Further study should evaluate the effects of intervention of volume control in PD patients.

	Harzard Ratio	95% HR Confidential Interval		p value
Age	1.066	1	1.136	0.049
The absence of cardiovascular event	0.232	0.067	0.0806	0.021
Overhydration(Δ9651;OH)	1.316	1.01	1.71	0.041

FR-PO777

Should PD Patients Be Kept 'Wet' to Preserve Residual Renal Function?
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Background: Preservation of residual renal function (RRF) is a cornerstone in the management of PD patients, with loss of RRF associated with technique failure, LVH and death. Objective: to clarify whether long-term alterations in volume status measured using bioimpedance are associated with preservation of RRF.

Methods: Patients undergoing peritoneal dialysis between March 2003 and January 2011 at 2 tertiary university hospitals, who had a set of paired bioimpedance measurements and dialysis adequacy measurements 12 months apart were considered (n=427). RRF as measured by urine Kt/V (uKt/V), was correlated with baseline demographic, biochemical and physiological parameters. Additionally to investigate whether a relative change in hydration status affected RRF, the cohort was divided into tertiles both by baseline ECW/TBW and 12-month follow up ECW/TBW. We examined whether a change from baseline hydration tertile to follow up hydration status tertile was associated with alterations in loss of RRF over the 12 month follow-up.

Results: loss in RRF as measured by change in uKt/V at 1 year compared to baseline was not correlated with dialysis vintage, PD modality (CAPD v APD), ethnicity, sex, BMI, presence of diabetes, HbA1c, PTH, albumin, CRP, number of antihypertensive medications, hemoglobin, serum sodium or change in hydration status as measured by change in ECW/TBW between baseline and year 1. However there was a correlation between loss of RRF and baseline mean arterial pressure (r=0.13, p<0.02), a younger age(r=0.15, p<0.0005), greater daily UF volume (r=0.12, p=0.03) and a higher baseline RRF as measured by daily UO (r=0.4, p<0.0001). In addition changes in volume status (overhydrated v neutral v underhydrated) over a 12-month follow up did not alter loss in RRF (1-way ANOVA p=0.21: Dunn's multiple comparison test no demonstrated significant difference between any group).

Conclusions: Both relative and absolute changes in volume status over a 12-month period do not appear to significantly alter loss in RRF. Clinicians should not 'chase' hydration status to ensure preservation of RRF.

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FR-PO778

Comparative Study of Enalapril versus Losartan on Residual Renal Function Preservation in Automated Peritoneal Dialysis: A Randomized Controlled Study
Arturo R. Marin. *Nephrology, Hospital ISSEMYM, Satelite, Estado de Mexico, Mexico.*

Background: Residual renal function (RRF) is an important determinant of mortality and morbidity in patients receiving peritoneal dialysis (PD). Recent studies have shown a positive effect of angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) on RRF in PD patients**.** Treatment with ACEi and ARBs has been shown to be associated with decreased risk of residual renal function (RRF) decline in PD patients and was independently associated with a 32% decrease in the development of anuria in patients with chronic kidney disease (CKD) irrespective of the dialysis modality. A 70% decrease in mortality of PD patients treated with either ACEi or ARBs therapy has been shown. The objective was comparing enalapril and losartan for RRF preservation in automated peritoneal dialysis (APD) patients.

Methods: An open label randomized controlled trial (RCT) with a 12 month follow-up period was conducted to compare the effect of enalapril versus losartan on RRF preservation in 60 APD patients. Measurements were done at the start of the study (baseline), 6 and 12 months. The primary outcome measures were the longitudinal change in RRF and the time to anuria. Secondary outcome measures included peritonitis, duration of hospitalization for any cause, drug effects, cardiovascular events and cerebrovascular events with permanent neurologic deficit.

Results: RRF was similar at baseline in both groups, 3.65±1.6 in the enalapril group and 4.1±2.01 ml/min/1.73 m^2 in the losartan group (ns). At 6 months, RRF was 3.0±0.5 in the enalapril group and 3.14±1.6 ml/min/1.73 m^2 in the losartan group (ns) and at 12 months RRF was 2.36±0.38 in the enalapril group and 2.54±0.47 ml/min/1.73 m^2 in the losartan group (ns). RRF declined by 1.29±1.21 in the enalapril group compared with 1.56±1.54 ml/min/1.73 m^2 in the losartan group (ns). The average decline in RRF in patients receiving losartan was 0.27 ml/min/1.73 m^2 less than that in enalapril group (ns).

Conclusions: There was not significant difference on RRF preservation between enalapril and losartan groups. The treatment with any of the drugs is useful in preserving RRF in APD patients.

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FR-PO779

Incremental Peritoneal Dialysis Prescriptions Including Icodextrin for Patients Having Residual Kidney Function
Alp Akonur, Steven Guest. *Renal, Baxter Healthcare Corporation, Deerfield, IL.*

Background: Incremental peritoneal dialysis (PD) has been proposed as a gradual way to introduce dialysis and is based on the concept that patients may have significant residual kidney function (RKF) at the initiation of dialysis and therefore do not require full-dose therapy at initiation. We have performed kinetic modeling of glucose and icodextrin-based regimens to better understand the RKF requirements that would allow incremental PD to be a viable option.

Methods: Prescriptions of 1-3 exchanges per day were modeled using a modified 3-pore model. Average patient characteristics (e.g. BSA=1.86 m^2, 4-hr D/P creatinine=0.65), 2.27% glucose (G) during 5-hour, and icodextrin (I) during 8-hour exchanges with 2L fill volume were considered. The minimum glomerular filtration rate (GFR) and residual urine volume required to achieve a total weekly urea Kt/V of 1.7 and total daily fluid removal of 1L were calculated.

Results: Three levels of incremental prescriptions consisting of only glucose, only icodextrin or combinations of the two resulted in three levels of minimum required residual GFR and urine volume as shown in the Figure. At each level, prescriptions including icodextrin provided improved ultrafiltration requiring approximately 150 mL less residual urine volume compared with 2.27% glucose.

Conclusions: Incremental PD allows for gradual introduction of dialysis in certain patients. We demonstrated that incremental prescriptions including icodextrin meet current adequacy targets in patients with residual GFR and residual urine volume as low as 3.7 mL/min/1.73 m^2 and 215 mL. By providing improved UF, icodextrin may especially benefit patients with lower residual urine volume and help avoid excess glucose exposure.

		Model Predictions		Required RKF	
		UF (mL/day)	Kt/V (per week)	Urine Volume (mL/day)	GFR (mL/min/1.73 m^2)
Level I	Glu	209	0.35	791	>7.7
	Ico	356	0.39	644	
Level II	2xGlu	429	0.70	571	5.7–7.7
	Glu+Ico	565	0.74	435	
Level III	3xGlu	648	1.05	352	3.7–5.7
	(2xGlu)+Ico	785	1.09	215	

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO780

Periostin and Peritoneal Inflammation Aditi Nayak, Ying Wang, Tiane Dai, Cynthia C. Nast, Lan Quang, Janine A. La Page, Ali Andalibi, Sharon G. Adler. *Nephrology, LA Bio Medical Research, Torrance, CA.*

Background: Periostin(Postn) is implicated in inflammation, scarring, and angiogenesis, characterizing peritoneal membrane(PMemb) failure. Postn's promoter has binding sites for STATs. We did studies to show associations between inflammation, JAK/STAT activation and Postn.

Methods: Met5A mesothelial cells were incubated in 1) filter-sterilized peritoneal dialysate (1.5% dextrose; fPDF); 2) fPDF at pH7.3 (7.3mPDF); 3) heat-sterilized PDF (hPDF); 4) low concentration glucose degradation products (lGDP) in fPDF; and 5) high concentration GDP in fPDF (hGDP). Media Postn was measured by immunoblotting. PD effluent (PDE) was collected from patients on PD ≤ 2wks (New (N), n=8) and ≥6 mos (Long-term (LT), n=8), centrifuged, and Postn measured. CA125 and JAK/STAT activators were measured in PDE in N and LT patients by electrochemiluminescence (Meso Scale Discovery, Gaithersburg, MD). Results are expressed as [analyte], [analyte]/[CA125] ratio, and analyte appearance rate (AR) ([analyte]xPDE volume/dwell time,mins). PMembs were stained for Postn in pts with encapsulating peritoneal sclerosis (EPS) and in PD (13mos).

Results: GDPs, and less so PDF glucose, induced Postn secretion by Met5A($p<0.01$). Postn in PDE was significantly higher in LT PD pts vs N. Table shows values for CA125 and JAK/STAT activating cytokines.

	[Analyte]		[Analyte]/[CA125]		AR	
Marker,pg/ml	N,(n=8);LT,(n=7)	p	N,(n=8);LT,(n=7)	p	N,(n=5);LT,(n=3)	p
IL 4	0.43;0.14	**0.03**	10.96;4.77	0.08	5.14;0.07	**0.03**
IL 13	0.89;0.17	**0.04**	20.37;6.80	0.12	8.36;2.43	0.18
IL 6	32.54;41.44	0.49	649.19;10968.04	**0.04**	147.18;179.67	0.18
GM-CSF	0.40;0.59	0.2	10.31;46.87	**0.03**	4.04;3.59	0.88
IFNγ	0.50;0.81	0.11	14.71;64.91	**0.05**	5.92;7.19	0.46
CA 125	46.55;20.33	**0.04**			527.80,183.16	**0.03**

Postn in PMemb was in peritoneal vascular smooth muscle cells, pericytes, fibroblasts, macrophages and basement membrane; in EPS also co-localizing with collagen in matrix.

Conclusions: Postn is induced in GDP/glucose containing media and LT PDE; and in LT and EPS PMemb. IL6, GM-CSF and IFNγ are candidate stimulators of JAK/STAT-mediated Postn transcription. Decrements in IL 4 and 13 may represent attenuation of macrophage repair in LT pts. These identify potential therapeutic targets.

Funding: Pharmaceutical Company Support - Meso Scale Discovery, Private Foundation Support

FR-PO781

Clinical Investigation of Relationship between Peritoneal Monocyte/Macrophage and Peritoneal Solute Transport Rate Xiao-jun Chen,[1] Xing Chen,[1] Yan Ge,[1] You-ming Peng,[1] Lin Sun,[2] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University, Changsha, Hunan Province, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: Recent studies have shown that macrophage may involved in the angiogenesis on peritoneum which results in increased effective surface area, and thus in a decrease in the glucose-driven osmotic pressure of the PDE leading to UF loss.

Methods: We investigated Overnight (8-10-hour) PDE (with glucose 2.5%) collected from 40 incident PD patients (duration<3 months)and 23 long-term PD patients (duration>12 months). The population of peritoneal cells was studied by flow cytometry analysis, especially the percentage of monocyte/macrophages. Overnight dialysate level of VEGF, TNF-α, IL-10 were measued by ELISA. Baseline dialysate-to-plasma ratio for creatinine (D/P Cr) was determined within 3 months of PD induction.

Results: Baseline D/P Cr was correlated with percentage of monocyte/ macrophages ($R=0.683$, $P<0.001$), dialysate appearance rate of VEGF ($R=0.561$, $P<0.001$), and low serum albumin($R=0.440$, $P=0.005$). However, baseline peritoneal permeability was not associated with serum C-reactive protein, TNF-α or other clinical factors. The ratio of monocyte/macrophages in incident patient was correlated with dialysate appearance rate of VEGF ($R=0.586$, $P<0.001$). On multiple linear regression analysis, the ratio of monocyte/macrophages in overnight dialysate was an independent factor influencing baseline peritoneal permeability ($P<0.001$). D/P Cr of Long-term patients was correlated with dialysate appearance rate of VEGF ($P < 0.05$), but correlation with other clinical or laboratory parameters were not found.

Conclusions: These data suggested that the number of peritoneal monocyte/macrophage in peritoneal dialysate is correlated with peritoneal transport characteristic and is a possible determinant of baseline peritoneal transport rate. Macrophage infiltration probablely changes peritoneal solute transport rate via expressing VEGF.

Funding: Clinical Revenue Support

FR-PO782

Effect of Wnt/β-Catenin Signaling on Human Peritoneal Mesothelial Cells Epithelial-Mesenchymal Transition Yuanyuan Guo,[1] Li Xiao,[1] Xun Zhou,[1] You-ming Peng,[1] Yashpal S. Kanwar,[2] Lin Sun,[1,2] Fu-You Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University; [2]Departments of Pathology and Medicine, Northwestern University.*

Background: Recently, the role of Wnt/β-catenin signaling in regulating epithelial-mesenchymal transition (EMT) during organ fibrosis has been estabilised. In the present study, we determine the hypothesis that Wnt/β-catenin signaling may involve in the process of peritoneal dialysis induced peritoneal mesothlial cells EMT.

Methods: Here, we analyzed the expression of Wnts, β-catenin, E-cadherin and α-SMA in peritoneal mesothelial cells isolated from peritoneal dialysis effluents of patients (Real time PCR and/or Western Blot).

Results: Compared with new patients, the levels of Wnt1, Wnt5a, Wnt7b, Wnt8a and β-catenin in patients undergoing peritoneal dialysis over one year were higher, along with higher α-SMA and lower E-cadherin, indicating reactivation of the Wnt/β-catenin signaling. In vitro, human peritoneal mesothelial cell line (HMrSV5) was exposed to different concentrations of high glucose (30, 60, 90mM D-glucose) for 24 hours, and the expressions of Wnt1, Wnt5a, β-catenin, E-cadherin and α-SMA were examined (Western Blot, Real time PCR and Immunofluorescence). HMrSV5 cells treated with 90mM mannitol served as control. Results showed that high glucose upregulated the expression of Wnt1, Wnt5a and β-catenin in concentration-dependent manner, along with increased α-SMA and decreased E-cadherin. Futhermore, delivery of the Wnt antagonist Dickkopf-1 (DKK1) gene significantly reduced the levels of β-catenin, along with the upregulation of E-cadherin and downregulation of α-SMA, compared to high glucose group. Additionally, TGF-β1 could also elevated the levels of Wnt1, Wnt5a and β-catenin in HMrSV5 cells.

Conclusions: Taken together, these results establish a role for Wnt/β-catenin signaling in the process of peritoneal mesothelial cell EMT and identify this pathway as a potential therapeutic target.

FR-PO783

The Size and Viability of Mesothelial Cells Affect Peritoneal Membrane Function Masanobu Akazawa,[1] Eiichiro Kanda,[1] Tomomi Uno,[2] Yoshitaka Maeda,[2] Sei Sasaki.[3] *[1]Tokyo Kyosai Hospital, Japan; [2]JA Toride Medical Center; [3]Tokyo Medical and Dental University.*

Background: Various tests including the peritoneal equilibrium test (PET) and measurement of the surface area of peritoneal mesothelial cells are used for the evaluation of the deterioration of peritoneal membrane in peritoneal dialysis (PD) patients. By flow cytometry (FCM), we can evaluate all cells including mesothelial cells in dialysate. We aimed to evaluate the relationship between peritoneal membrane function and the characteristics of mesothelial cells in dialysate.

Methods: A cross-sectional analysis of 40 patients (29 males and 11 females) on PD was performed. Overnight dialysates were collected for PET. By FCM, we analyzed the cell populations in the overnight dialysate to evaluate the characteristics of mesothelial cells: size and viability. Cell size was measured by forward scatter levels. By using 7-Amino-Actinomycin D, the viability of mesothelial cells was measured. A multivariable regression analysis was adjusted for age, gender, diabetic nephropathy and peritonitis.

Results: The average age was 64.6 years and the average PD duration was 42.0 months. D/P creatinine correlated with the size of peritoneal mesothelial cells (Spearman's rank correlation coefficient $\rho=0.545$, $p=0.0003$) and the viability of peritoneal mesothelial cells ($\rho=0.4494$, $p=0.0036$). A positive correlation between the size of peritoneal mesothelial cells and the viability of mesothelial cells was observed ($\rho=0.6050$, $p<0.0001$). An adjusted multivariable regression analysis showed that D/P creatinine was significantly associated with the size of peritoneal mesothelial cells ($p=0.0034$). Another adjusted multivariable analysis showed that the size of peritoneal mesothelial cells associated with the viability of peritoneal mesothelial cells ($p<0.0001$).

Conclusions: Our findings showed that the size of peritoneal mesothelial cells in dialysate was associated with D/P creatinine and the viability of peritoneal mesothelial cells. It was suggested that enlarged mesothelial cells may become easily detached from peritoneal membrane in viable condition and lead to increase the membrane permeability.

FR-PO784

Inadequate Cellular Stress Response in Peritoneal Dialysis: A Novel Pathomechanism and Its Therapy Klaus Kratochwill,[1,2] Rebecca Herzog,[1] Anton Lichtenauer,[1,2] Lilian Kuster,[1,2] Andreas Vychytil,[3] Christoph Aufricht.[1,2] *[1]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria; [2]Zytoprotec GmbH, Austria; [3]Universitätsklinik of Medicine III, Medical University of Vienna, Austria.*

Background: PD-fluid (PDF) has cytotoxic effects on mesothelial cells (MC), which form an essential part of the peritoneal dialysis membrane. Recent results indicated an inadequate cellular stress response (ICSR) upon PDF exposure as a novel mode of cytotoxic action, leading to further increased vulnerability of cells. Our aim was to elucidate molecular mechanisms involved in ICSR and to test novel cytoprotective strategies.

Methods: In experimental PD and samples from patients of the PD-protec phase I trial (ClinicalTrials.gov; NCT01353638), gel-based fluorescent detection of protein abundance and MALDI-MS together with systems biology was applied to yield protein interaction networks of enriched biological processes following PD with and without therapeutic cytoprotective interventions.

Results: Combined proteomics and bioinformatics identified an ICSR associated with sterile inflammation via interleukin-1 receptor (IL-1R) associated pathways. Addition of an IL-1R antagonist led to significant cytoprotection of MC, blockade of cytokine levels and altered expression of the stress proteome. Addition of alanyl-glutamine (Ala-Gln) ameliorated "dampening" of the MC stress proteome, associated with increased survival of MC. Exposure to PDF with and without Ala-Gln also revealed different patterns of post-translational modification of proteins with N-acetyl-glucosamine (O-GlcNAc) potentially explaining the cytoprotective effects of Ala-Gln during in-vitro PD. Early data from the PD-protec trial also suggest cytoprotective cellular responses induced by Ala-Gln in clinical PD.

Conclusions: This work describes the ICSR of MC following PDF exposure as a novel pathomechanism. Depressed cytoprotective responses increased the susceptibility of MC during experimental PD. Supplementation of PDF restored the cytoprotective stress proteome, resulting in improved cellular resistance. The implication of Ala-Gln addition to PDF on outcome during clinical PD is currently under investigation.

Funding: Pharmaceutical Company Support - Zytoprotec GmbH

FR-PO785

Transcription Factor SRF Enhances Fibrosis of Human Peritoneal Mesothelial Cells via Modulating miRNA-199a/214 Cluster Dependent Epithelial-Mesenchymal Transition

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Background: High glucose (HG) induced epithelial-mesenchymal transition (EMT) of primary human peritoneal mesothelial cells (HPMCs) is characterized by markers, as E-cadherin and claudins. Our previous study showed that Serum response factor (SRF), a transcription factor that binds to CArG boxes, is a major regulator of HPMCs EMT and fibrosis. However, the target and pathway of SRF in PD process hasn't to be elucidated.

Methods: All these immortal HPMCs were characterized by phenotype markers and tested the expression of SRF and miRNA-199a/214 cluster by real time PCR and Western blot.

Results: Our study showed that a series of miRNAs, such as miR-214/miR-199a-5p cluster from selected primary HPMCs miRNA array and have CArG element in the promotor, were found to be regulated by SRF. ChIP and luciferase reporter assays revealed that nuclear translocation of SRF directly promotes the transcription of miR-214 and miR-199a-5p, which are examined to be highly expressed in HG-induced HPMCs. Depletion of miR-214/miR-199a cluster in HPMCs reduces EMT marking by upregulating E-cadherin, claudins and downregulating α-SMA in vitro. Mechanistically, miR-199a-5p and miR-214 were characterized to target the E-cadherin and claudin-2 messenger RNA CDH1 and CLDN2 to contribute to the epithelial-mesenchymal transition (EMT).

Conclusions: Together, this study reveals a novel function of SRF in HPMCs EMT and fibrosis and identified a new SRF-miR-199a/miR-214 cluster-CDH1/CLDN2 EMT axis which highlights the potential association between TFs and miRNA-mediated EMT and fibrosis in PD.

FR-PO786

Peritoneal Membrane Vessels in CKD Stage 5 Patients Starting PD and Its Relationship with Cardiovascular Status, Dialysis Adequacy and Peritoneal Membrane Characteristics after 12months of Dialysis Treatment

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Background: Peritoneal dialysis (PD) causes alterations in peritoneal membrane (PM)-vasculopathy with vessels obliteration, also reported before PD initiation. Cardiovascular system (CV) damage, changes in dialysis adequacy and PM transport are reported during PD. We wanted to evaluate PM vessels before starting PD and to establish their relationship with CV, lab, adequacy and PM transport data during 12months of PD.

Methods: 23 pts,CKD aetiology were: diabetic nephropathy–10pts, non-diabetic nephropathies-13pts. PM specimen was taken during PD catheter insertion. Histological analysis-using-NIS ELEMENTS AR. Peritoneal vessels thickness, ratio of luminal diameter to vessel diameter (L/V) were calculated. CV evaluation was: EF, LVMI, IMT performed before PD start. PET test and kt/v calculation - after 12months of PD. Albumin, CRP, Hb, cholesterol, PTH at the onset and at 6 months intervals.

Results: CV assessment, PM vessels data are shown in table 1. There were no statistically significant correlation between peritoneal vessels indices, CV data, lab data, clinical variables and kt/v. There were no differences between HA and LA transporters.

Results expressed as mean + SD in diabetic and non-diabetic pts.

Variables	non-diabetic	diabetic	P
Age	48,4±20,4	55,0±11,47	0,37
EF (%)	64,05±5,67	60,73±9,27	0,30
LVMI (g/m2)	119,51±38,25	118,58±21,19	0,94
PWV(m/s	10,12±2,94	14,83±3,57	0,002946
IMT (mm)	0,65±0,16	0,78±0,21	0,11
PV Wall Thickness (μm	9,89±3,34	10,01±2,00	0,92
L/V ratio	0,60±0,92	0,60±0,95	0,94
kt/v (after 12months)	2,43	2,19	0,0401

Conclusions: Initial peritoneal vessels status and CV system status in diabetic and non-diabetic pats who enter PD therapy has no influence on dialysis adequacy and PM transport after one year of PD.

FR-PO787

The Effect of Celecoxib on Peritoneal Lymphangiogenesis in Uremic Peritoneal Dialysis Rats

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Background: To investigate the effect of COX-2 inhibitor(Celecoxib)on the expression of (lymphatic vessel endothelial hyaluronan receptor-1,LYVE-1) in uremic peritoneal dialysis rats.

Methods: 48 male SD rats (180-200g) were randomly divided into 5 groups: normal control (A,n=6), rats with sham operation (B,n=6), uremic rats without PD (C,n=6), uremic rats dialyzed with 4.25% PD solution (D,n=24), uremic rats dialyzed with 4.25% PD solution and treated with celecoxib (20 mg/kg BW) via oral gavage(E ,n=6).The rats from D groups were given regular peritoneal dialysis (3ml/100g BW) and divided into 4 groups(0w/2w/3w/4w,each group=6). The rats from E groups were treated by celecoxib (20 mg/kg BW) via oral gavage. A 2-hour peritoneal equilibration test (PET) was performed before rats were killed. Immunohistochemistry or RT-PCR was applied to detect the expression of LYVE-1,VEGF-C,COX-2 in rats'peritoneal tissues,and record the net ultrafiltration quantity of group D and E. Statistical analysis was performed.Significance was defined as a=0.05.

Results: There was no significant difference of LYVE-1 VEGF-C,COX-2 expression between group A and group B. And compared with group A or B, the expression of these proteins were significantly increased in group C, D or E($P<0.05$). There was a positive correlation between COX-2 and VEGF-C, VEGF-C and LYVE-1. Compared with group D(0w), the peritoneal thickness was significantly increased in the submesthelial compact zone and the net ultrafiltration quantity was significantly decreased in group D(2W/3W/4W, $P<0.05$). Compared with roup D, the peritoneum thickness was significantly decreased, the net ultrafiltration quantity was significantly decreased in group E.

Conclusions: The expression of LYVE-1 might be up-regulated by peritoneal dialysis. Celecoxib may decrease lymphangiogenesis by inhibiting VEGF-C pathway and increase the net ultrafiltration quantity.

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FR-PO788

Daikenchuto, a Herbal Medicine, Suppresses the Progression of Peritoneal Fibrosis in Mice

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Background: Long-term peritoneal dialysis (PD) leads to histological changes in the peritoneal membrane and results in peritoneal fibrosis. One of the mechanisms of these structural changes is considered to depend on inflammation. Daikenchuto (DKT) is a herbal medicine, and widely used to treat postoperative ileus in Japan. Although DKT has been reported to ameliorate intestinal inflammation, its effect on peritoneal fibrosis remains unknown. Here we investigated the effect of DKT on peritoneal fibrosis in animal model.

Methods: Male ICR mice were divided into three groups, the chlorhexidine gluconate (CG) group, the CG + DKT group, and the control group. 15% ethanol in saline with or without CG was injected intraperitoneally three times per week and DKT was dissolved drinking water and administrered for 3 weeks, approximate dose 3000 mg/kg/day. The CG group and the control group were receiving drinking water. For histological examination, 4-μm-thick paraffin-embedded tissues were stained using the Masson-trichrome method. The expression of collagen type III, heat shock protein (HSP)-47, α-smooth muscle actin (α-SMA), transforming growth factor (TGF)-β, monocyte chemotactic protein-1 (MCP-1), and F4/80 were examined by immunohistochemistry.

Results: In the CG group, peritoneal tissues showed marked thickening of the submesothelial zone, and the numbers of HSP-47, α-SMA, TGF-β, MCP-1 positive cells and F4/80 positive macrophages were significantly increased compared to those in the control group. On the contrary, these changes were significantly suppressed in the CG + DKT group.

Conclusions: These results suggested that DKT could prevent peritoneal fibrosis through the suppression of inflammation. We conclude that DKT might be one of the candidates for a novel therapeutic agent in preventing peritoneal fibrosis.

FR-PO789

Espironolactone to Prevent Peritoneal Fibrosis in Peritoneal Dialysis Patients: A Randomized Controlled Trial

Armando Vazquez-Rangel, Virgilia Soto, Rafael G. Toledo, Edgar A. Castillo, Nasser Abdel Polanco Flores, Ilse Falcon-Chavez, Magdalena Madero. *INCICH.*

Background: Progressive loss of peritoneal function is prevalent in peritoneal dialysis (PD) patients and in part related to infections and hypertonic solutions. These latter factors are responsible for the inflammatory cascade which stimulate peritoneal fibrosis (PF). In animal studies the renin angiotensin system (RAA) is implicated in PF. There are no human data regarding the impact of RAA blockade on PF. The objective of this study was to evaluate the role of espironolactone on PF.

Methods: This was a randomized double blind controlled trial that included PD patients recruited from 2008 to 2011 at our institution. Patients were elegible if they were 18 years

or older, were not assigned to RAA blockade meds and had signed informed consent. Exclusion criteria included pregnancy or hiperkalemia (>5.5 meq/l). The primary endpoint was PF and thickness in peritoneal biopsies. Patients were randomly assigned to receive either spironolactone 25 mg or placebo for 6 months. Peritoneal biopsies were performed at the time the PD catheter was placed and at the end of follow up.Clinical and laboratory data were assesed monthly by the nephrologist. Peritoneal biopsies were evaluated for peritoneal fibrosis, thickness and inflammation.

Results: A total of 20 patients were included in the study. Median age was 41 (26-59) years, 60% were male and 35% were diabetic.There was no difference between groups in the percentage of diabetics, glucose exposure in PD bags or peritoneal membrane transport chacarteristics. Compared to baseline, at the end of follow up there was a significant increase in peritoneal fibrosis, thickness and inflammation (median % increase 19, 75 and 39, respectively) ($p<0.01$ for all). Spironolactone did not prevent fibrosis, peritoneal thickness or inflammation. There was a trend towards higher peritonitis rates($p=0.09$) and hiperkalemia (K>5.5 meq/l) ($p=0.07$) in the espironolactone group.

Conclusions: We found a significant increase in PF, peritoneal thickness and inflammation at the end of follow up, effects which were not attenuated by use of spironolactone.

Funding: Government Support - Non-U.S.

FR-PO790

PDF-Induces Peritoneal Membrane Damages via Hypoxic Pathway Talerngsak Kanjanabuch,[1,2] Wasin Manuprasert,[1,2] Kearkiat Praditpornsilpa,[1] Somchai Eiam-Ong,[1] Kriang Tungsanga.[1] *[1]Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Kidney and Metabolic Disorders Research Center, Chulalongkorn Univeristy, Bangkok, Thailand.*

Background: To investigated the role of hypoxia as a pathogenic factor in peritoneal membrane damages after exposed to peritoneal dialysis fluids (PDFs).

Methods: Twenty Sprague-Dawley rats were subjected to intraperitoneal injection with normal saline and 3.86%G PDFs as well as hypoxic conditions. After 4 weeks, the peritoneum hypoxia was assessed by hypoxyprobe (pimonidazol) and HIF-1α expression. The hypoxia-responsive protein and mRNA levels were quantitated by western blot and RT-PCR. Human peritoneal mesothelial cells (HPMCs) were incubated with 0.1%FCS, various concentrations of PDF, and 100μM cobalt chloride (chemical-induced hypoxic milieu) in normoxia (95%room air, 5% CO2) and hypoxic (94%N2, 5%CO2, 1%O2) conditions. Cell injuries were determined by LDH assay and number of apoptotic HPMC was counted by TUNEL-labeling flow cytometry. The intra-cellular hypoxia and its response to hypoxia were demonstrated similarly to the *in vivo*.

Results: Pimonidazol (an exogenous hypoxic marker) and the expression of HIF-1α (an endogenous hypoxic marker) were significant detected both in animal treated with PDF and hypoxic conditions. The hypoxia-responsive proteins and genes, including TGF-β and VEGF, were up-regulated both in the PDF and hypoxia-treated groups. All findings above were correlated well with histo-morphologic changes of peritoneal membrane (submesothelial thickening and neoangiogenesis). Of interest, up-taken of pimonidazol as well as expressions of HIF-1α and hypoxia-responsive genes correlated well with the degree of HPMC injuries and the number of cellular apoptosis and the concentration of glucose in the PDF. The exposures of the cells to hypoxic conditions by both chemical and physical inducers caused cell injury and death at similar degree of severity to the PDF exposure.

Conclusions: The hypoxic milieu in peritoneum was induced by exposure to the PDF and may play a pathogenic role in the peritoneal membrane changes.

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FR-PO791

Overexpression of the Klotho Protein Attenuates Peritoneal Fibrosis by Suppressing Wnt-Signaling Yuko Nishi, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Japan.*

Background: For peritoneal dialysis (PD) patients, one of the obstacles of successful long-term PD is peritoneal fibrosis in the peritoneal membrane after exposure to nonphysiological dialysis solutions. Several studies have demonstrated that peritoneal mesothelial cells undergo epithelial-to-mesenchymal transition (EMT) after expose to injury. Furthermore, the induction of EMT is associated with activation of Wnt-dependent beta-catenin signaling.Klotho, secreted from the kidneys and characterized as an anti-aging gene, has been shown to function as a Wnt antagonist. Therefore, we explored the possibility that the klotho protein could reduce peritoneal fibrosis by inhibiting Wnt signaling.

Methods: Transgenic mice that overexpress the alpha-klotho gene under the control of the human elongation factor 1 alpha promoter (KLTG; C57BL/6 background) and C57BL/6 (wild type) mice underwent scratched peritoneal injury and were examined after 7 and 14 days. The activation of beta-catenin signaling was examined by crossing the transgenic mice with BAT-LacZ mice that overexpress the beta-galactosidase gene under the control of 7 repeats of the T cell factor/lymphoid enhancer-binding factor 1 promoter.

Results: At 7 days after peritoneal injury, Wnt/beta-catenin signaling was activated in the regenerated endothelium in wild type mice and to a lesser extent in KLTG mice. The expression of α-smooth muscle actin, as a marker of EMT, also increased during peritoneal mesothelial regeneration in wild type mice and to a lesser extent in KLTG mice. At 14 days after peritoneal injury, wild type mice showed progression of peritoneal fibrosis and adhesion of the peritoneum was demonstrated by Masson's trichrome stain. In addition, enhanced mRNA expression of connective tissue growth factor and fibronectin in peritoneal tissue was also observed. On the other hand, KLTG mice showed attenuated progression of peritoneal fibrosis associated with a reduction of EMT.

Conclusions: Overexpression of the klotho protein protects the peritoneal membrane by attenuating Wnt signaling. Present results give us deeper insight into clinical benefit of preserving residual renal function in PD patients.

FR-PO792

Analyses of microRNAs Targeting CTGF in Peritoneal Fibrosis Mouse Model Kenichi Koga, Masashi Mukoyama, Hideki Yokoi, Kiyoshi Mori, Masato Kasahara, Takashige Kuwabara, Hirotaka Imamaki, Tomoko Kawanishi, Akira Ishii, Keita P. Mori, Yukiko Kato, Shoko Ohno, Naohiro Toda, Akira Sugawara, Kazuwa Nakao. *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by blocking protein translation. Peritoneal fibrosis is a major complication upon continuous ambulatory peritoneal dialysis (CAPD), leading to a loss of peritoneal function. Some cytokines or growth factors are reported to be involved in the pathogenesis of peritoneal fibrosis. Connective tissue growth factor (CTGF) is one of the growth factors that can potentially induce peritoneal fibrosis; PDGF is also reported to be associated with peritoneal induction of angiogenesis, fibrosis and mesenchymal transition, but little is known about miRNAs that regulate gene expressions including CTGF gene in the course of peritoneal fibrosis.

Methods: We searched miRNAs that target the CTGF gene by database. We analyzed miRNA expressions in a human mesothelial cell line, MeT-5A cells, under the treatment with PDGF-BB, AngII or TGF-β. Total RNA was extracted at 24 hours after the stimulation and miRNA expressions were assayed by TaqMan PCR. We also assessed the miRNA expressions in peritoneal membrane of chlorhexidine gluconate (CG)-treated mice at days 7, 14, 21 and 28.

Results: We identified miR-26a and miR-30c that were expressed in Met-5A cells, targeting the CTGF gene. miR-30c expression in MeT-5A cells was not changed with PDGF-BB, AngII or TGF-β1 stimulation. In contrast, PDGF-BB tended to increase miR-26a expression by 1.5-fold in Met-5A cells. In the peritoneal tissue of CG-treated mice, miR-26a expression was gradually increased after CG treatment, and showed significant increase at 14 days after the treatment by 2.3-fold compared with control mice.

Conclusions: These results indicate that miR-26a is upregulated in the mouse peritoneum during a course of peritoneal fibrosis, suggesting that miR-26a may regulate CTGF expression in PDGF-BB-induced peritoneal injury.

Funding: Pharmaceutical Company Support - Japan Baxter PD Fund

FR-PO793

Decorin Regulates Cell Activation and Fibrotic Processes in Human Peritoneal Mesothelial Cells through Inhibition of ERK Phosphorylation Susan Yung, Mel Chau, Qing Zhang, Andy Yim, Jiang Na, Daniel Tak Mao Chan. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: Mesothelial cell dysfunction contributes to peritoneal fibrosis during long-term peritoneal dialysis (PD). We previously demonstrated that mesothelial cells synthesize abundant decorin, a dermatan sulfate proteoglycan with anti-fibrotic properties. In this study, we investigated the mechanisms through which decorin regulates fibrogenesis and epithelial-to-mesenchymal transition (EMT) in human peritoneal mesothelial cells (HPMC) in the setting of PD.

Methods: Growth arrested HPMC were stimulated with spent non-infected or infected PD fluid in the presence or absence of exogenous decorin (0-1000 ng/ml) for 24h. Cell morphology, expression of fibronectin and fibroblast specific protein-1, and ERK activation were assessed. The effect of decorin gene silencing on cell activation and fibrotic processes in HPMC was also investigated.

Results: Non-infected PDF induced EMT and cell detachment in HPMC. These changes were more marked in cells exposed to infected PD fluid. Pre-incubation of HPMC with decorin preserved HPMC morphology and inhibited PD fluid-induced EMT, accompanied by decreased fibronectin synthesis and increased E-cadherin expression. The effect of decorin was mediated through the suppression of ERK phosphorylation. Knockdown of decorin expression in HPMC resulted in increased expression of fibroblast specific protein-1, fibronectin and TGF-β1 in both control and PD fluid stimulated HPMC ($P<0.01$, for all), accompanied by decreased E-cadherin expression ($P<0.01$, for all).

Conclusions: The data demonstrate that decorin regulates cell activation and fibrotic processes in HPMC during PD through modulation of the ERK signaling pathway.

Funding: Government Support - Non-U.S.

FR-PO794

Therapeutic Use of Murine Peritoneal Macrophages Changqi Wang,[1] Qi Cao,[1] Ya Wang,[1] Guoping Zheng,[1] Ye Zhao,[1] Hong Zhao,[2] Thian Kui Tan,[1] Yiping Wang,[1] David C. Harris.[1] *[1]Centre for Transplant and Renal Research, Westmead Millennium Institute, Westmead, NSW, Australia; [2]Biochemistry Faculty, Shanxi Medical University, Taiyuan, Shanxi, China.*

Background: Each day patients treated with chronic peritoneal dialysis discard dialysate which contains a large number of mononuclear phagocytes (MP, predominantly comprising macrophages, MΦ and dendritic cells, DCs). As a rich source of MP, peritoneal dialysate may be useful to better understand the biology of these cells and to explore their therapeutic potential.

Methods: MP were isolated from peritoneal dialysate and differentiated in vitro into MΦ with M-CSF or into DCs with IL-4 and GM-CSF. The MΦ and DCs were modulated

into effector and regulatory phenotypes. Murine peritoneal MΦ were modulated into M2 MΦ with IL-4/IL-13 and transfused into mice with adrimycin nephropathy (AN).

Results: Dialysate of PD patients contains a large number of MP (4.4 ± 2.8 x10^6 Mφ and 0.93 ± 0.55 x 10^6 DCs per 2 L). Peritoneal Mφ; expressed a higher percentage of CD163, a marker for alternatively activated macrophages, than did those from blood (26.6±3.8% vs 8.3±1.6%). These MP could be differentiated *in vitro* into Mφ or DCs with high purity (Mφ 90% and DCs 85%).Human MP were modulated into M1 or M2 Mφ and immunogenic or tolerogenic DCs, that showed a similar cytokine expression pattern to those of mice.Adaptive transfer of M2 Mφ derived from mouse peritoneum provided a level of protection against AN injury that was similar to those derived from spleen, whereas M2 derived from bone marrow were not protective.

Conclusions: MP from dialysate could be differentiated into Mφ and DCs, and further modulated to an effector or regulatory phenotype. M2 derived from mouse peritoneum reduced renal injury. These data indicate the possibility of using human peritoneal MP to treat kidney disease.

Funding: Government Support - Non-U.S.

FR-PO795

Effect of Thalidomide in Experimental Peritoneal Fibrosis Developed in Uremic Rats Dayana G. Viloslada, Filipe M. Silva, Irene L. Noronha. *Nephrology, University of Sao Paulo, SP, Brazil.*

Background: Peritoneal fibrosis (PF) is considered the most serious complication of long-term peritoneal dialysis, responsible for ultrafiltration failure. The use of drugs with anti-inflammatory and anti-fibrotic properties, such as thalidomide, represent an alternative strategy of treatment. The aim of this study was to analyze the effect of thalidomide in experimental model of PF developed in uremic rats.

Methods: Chronic kidney disease (CKD) was induced in male Wistar rats by adenine in the diet (0.75%), for 30 days. At day 15, rats with CKD, characterized by hypertension (170±8 mm/Hg) and high serum levels of BUN (78±8 mg/dL) and creatinine (0.7±0.4 mg/dL), were subjected to intraperitoneal injections of 0.1% chlorhexidine gluconate (CG) daily for 15 days, to induce PF. Animals (n=30) were divided into 3 groups: **CKD,** CKD rats receiving only vehicle; **CKD+PF**, PF induced in CKD rats; **CKD+PF+Thalid**, CKD rats+PF treated with thalidomide (100mg/Kg/d daily by gavage) for 15 days. At day 30, animals were sacrificed and the following parameters were analyzed: thickness of peritoneal membrane (masson thrichrome staining), number of macrophages (MØ), T-cells and α-smooth muscle actin (α-SMA) (immunohistochemistry), and collagen III, TGF-β, TNF-α and IL-1β expression (real time PCR).

Results: At day 30, CKD with uremia was confirmed by increased serum BUN and creatinine levels (134 ±17 and 1.81±0.21 mg/dL, respectively). PF was also successfully induced. Thalidomide significantly reduced the peritoneal membrane thickness, α-SMA and the number of MØ and T-cells. Reduction of TGF-β, collagen III, TNF-α and IL-1β mRNA levels was also observed.

Table 1

	CKD	CKD+PF	CKD+PF+Thalid
Peritoneal Membrane thickness (μm)	30±10	123±32*	42±9#
MØ (cells/mm²)	379±120	958±204*	233±108#
T-cells (cells/mm²)	208±161	806±217*	101±83#
α-SMA (%)	2.1±1.4	6.4±2*	0.5±0.2#
Collagen III (mRNA level)	1.0±0.8	18.9±2.4*	10.8±0.7#
TNFα (mRNA level)	1.0±0.8	8.0±0.3*	0.3±0.3#
IL-1β (mRNA level)	1.0±1.0	7.1±1.8*	0.5±0.8#
TGFβ (mRNA level)	1.0±0.4	2.1±0.1*	1.0±0.2#

*p<0.05 vs CKD, #p<0.05 vs CKD+PF

Conclusions: Thalidomide was effective in reducing the thickness of peritoneal membrane in this experimental model, possibly due to its anti-inflammatory and anti-fibrotic properties.

Funding: Government Support - Non-U.S.

FR-PO796

Not Only the Selected Peritoneal Cell Therapy but Also the Secreted Factor from the Implanted Cells Is Important to Ameliorate Peritoneal Fibrosis Shinji Kitamura, Kenji Tsuji, Hitoshi Sugiyama, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Long-term peritoneal dialysis (PD) causes chronic peritoneal damage. Peritoneal mesothelial cells (PMCs) play an important role to peritoneal function. We investigated the possibility of cell therapy using the PMCs to prevent peritoneal damage in PD patients.

Methods: We harvested human PMCs from the peritoneal dialysis effluent of PD patients. The PMCs were separated based on morphological characteristics into epithelial-like (Epi) cells and fibroblast-like (Fib) cells by the limiting dilution method. We transplanted these cells into nude mice whose parietal and visceral peritoneum were scratched by mechanical scraping. After 14days transplantation, we sacrificed the mice and evaluated peritoneal thicknesses, intra-abdominal adhesion number.

Results: The transplanted cells were detected at the parietal and visceral peritoneum. Compared with the positive control, the Epi cell therapy group showed very few adhesions and exhibited no thickening of the parietal and visceral peritoneum (parietal peritoneal thicknesses: positive control group 46.5±8.1μm vs. Epi cell therapy group 18.2±6.8μm, P<0.05). However, the group with Fib cell therapy could not inhibit peritoneal adhesion and thickening (parietal peritoneal thickness: Fib cell therapy group: 59.1±13.1μm). In addition, HGF was expressed by the grafted Epi cells but not Fib cells. Fib cells expressed VEGF stronger than Epi cells. These findings suggest that selected PMCs cell therapy contribute to the regeneration of damaged peritoneum by both direct cellular interactions and cytokine secretion from the grafted cells. Moreover, when we injected the culture medium that included the secreted factor from Epi cells into the peritoneal injured mice, the peritoneal adhesion and thickening in the mice were inhibited as same as the Epi cell therapy.

Conclusions: We suggested that not only the effect of selected transplanted Epi cell attachment but also the secreted factor from implanted Epi cells contribute the regeneration process for peritoneum.

FR-PO797

Stimulation of Cyclooxygenase 2 Protein Expression in Cultured Peritoneal Mesothelial Cells Michael E. Ullian, Linda Walker, Megan Hicks, Thomas Morinelli. *Medicine/Nephrology, Medical University of South Carolina, Charleston, SC.*

Background: Peritoneal fibrosis decreases peritoneal dialysis (PD) efficacy. Angiotensin II (AngII) and glucose (PD fluid osmotic agent) each causes inflammation/fibrosis. We investigated whether AngII or glucose activates cyclooxygenase 2 (Cox2, profibrotic) in cultured peritoneal mesothelial cells (MC).

Methods: From rat peritoneum we harvested MC, which were cobblestone in appearance in culture passages 0-1, for experiments [N 4-9 (N of 1=cells from 1 rat)]. Techniques included: equilibrium receptor binding (125I-AngII, 90 min, 4°C); intracellular free calcium (Calcium-3 probe, fluorescent plate reader); immunoblotting of Cox2 protein normalized for ß-actin protein, expressed as fold-increase over control; inhibitor concentrations 10 μM.

Results: AngII binding was 95% inhibited by unlabeled AngII or losartan (AT1 receptor antagonist) and 36% inhibited by PD123319 (AT2 receptor antagonist), suggesting that most receptors are AT1. AngII stimulated concentration-dependent, losartan-inhibitable increases in intracellular free calcium, confirming surface receptor coupling to intracellular signals. Increase in Cox2 expression by 100 nM AngII and 4.25% (240 mM) glucose was maximal at 6 and 24 hr, respectively. AngII (1–100 nM) and glucose (1.5%-4.25%) elicited concentration-dependent increases in Cox2 expression at optimal exposure times; maximal responses were 6-fold with 100 nM AngII and 30-fold with 4.25% glucose. Mannitol (4.25%, non-metabolizable osmotic control) stimulated Cox2 expression at 24 hr but only by 6-fold. Maximal AngII stimulation of Cox2 expression was completely inhibited by losartan and only 35% inhibited by PD123319. Maximal glucose stimulation of Cox2 expression was not significantly inhibited by losartan, 45% inhibited by resveratrol (antioxidant), and 95% inhibited by RO1069920 (NFκB inhibitor).

Conclusions: In primary rat peritoneal MC, glucose activates Cox2 through oxidants and NFκB but not AT1 receptors. Glucose is more potent than mannitol, suggesting that glucose stimulates Cox2 via both osmotic and metabolic mechanisms. Inhibitors (oxidants, Cox2, NFκB) may slow peritoneal membrane fibrosis in PD patients.

Funding: Pharmaceutical Company Support - Dialysis Clinic Incorporated

FR-PO798

Effect of miRNA-302c on Epithelial Mesenchymal Transition of Peritoneal Mesothelial Cells and the Mechanism Xiejia Li,[1] Li Xiao,[1] Xun Zhou,[1] Lin Sun,[1,2] Fuyou Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University, Changsha, Hunan Province, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: Epithelial mesenchymal transition (EMT) is the initiate and reversible process of TGF-β-induced peritoneal fibrosis. Connective tissue growth factor (CTGF) acts as an effector in the downstream cascade of TGF-β-induced fibrosis, and it also can facilitate EMT in the process of fibrosis. MicroRNAs are important regulators in the process of TGF-β induced EMT such as microRNA-200 family and microRNA-205. In our pilot study, we found that the expression of miRNA-302c was downregulated in TGF-β1-treated human peritoneal mesothelial cells (HPMCs) and we also found that CTGF is a target gene of miRNA-302c. So our object is to investigate whether miRNA-302c plays a role in TGF-β1-induced EMT by regulating the expression of CTGF.

Methods: We constructed a lentiviral vector to stably express has-miRNA-302c. Then we treated HPMCs with 5ng/ml TGF-β1 for 48 hours in the presence or absence of LV-hsa-miR-302c. The expression of miRNA-302c was analysed using TaqMan quantitative fluorescence probe real time PCR, the expression of E-cadherin,α-SMA,collagenIand CTGF were analysed using real time PCR and western blot.

Results: Compared with control group, the expression of miRNA-302c was downregulated with the stimulation of TGF-β1, but the expression of miRNA-302c was rised in the presence of LV-hsa-miR-302c. The presence of LV-hsa-miR-302c can ameliorate the morphology change induced by TGF-β1. The expression of E-cadherin in TGF-β1-treated HPMCs was decreasing and the expression of α-SMA and collagenIwas increasing, but the presence of LV-hsa-miR-302c can ameliorate the changes. Meanwhile, compared with the control group, the expression of CTGF was rising with the stimulation of TGF-β1, but the presence of LV-hsa-miR-302c can ameliorate the increase of CTGF.

Conclusions: The infection of LV-hsa-miR-302c can upregulate the expression of miR-302c in HPMCs; miR-302c can ameliorate the TGF-β1-induced EMT through suppressing the expression of CTGF.

Funding: Government Support - Non-U.S.

FR-PO799

Downregulation of miRNA-29c Inhibits High Glucose Induced Epithelial-Mesenchymal Transition in Human Peritoneal Mesothelial Cells Yan Ge,[1] Li Xiao,[1] Xiao-jun Chen,[1] Yuanyuan Guo,[1] Xiejia Li,[1] Xun Zhou,[1] Lin Sun,[1,2] Fuyou Liu.[1] *[1]Department of Nephrology, Second Xiangya Hospital, Center of Kidney Disease and Dialysis in Hunan Province, Central South University, Changsha, Hunan Province, China; [2]Departments of Pathology and Medicine, Northwestern University, Chicago, IL.*

Background: It is believed that epithelial-mesenchymal transition (EMT) of peritoneal mesothelial cells plays a pivotal role in the pathogenesis of peritoneal fibrosis in peritoneal dialysis patients. Recent studies demonstrated that miRNA could regulate EMT in different kind of cells and inhibit organ fibrosis. However, the role of miRNA in EMT of peritoneal mesothelial cells and peritoneal fibrosis is largely unknown.

Methods: We identified that miR-29c upregulated in peritoneal mesothelial cells derived form dialysis effluent in long term PD patients compared to newly starters using miRNA array analysis and we validated that the expression of miR-29c also increased both in derived peritoneal mesothelial cells from 24 PD patients and human peritoneal mesothelial cell line (HMrSV5) treated with high glucose in vitro by real-time PCR.

Results: And the upregution of miR-29c was correlated with the expression of EMT markers (E-cadherin, α-SMA, Collagen-Iand finbronectin) both ex vivo and in vitro examined by RT-PCR, western-blot and immunofluorescence cell staining. We hypotheses that miR-29c may regulate the EMT of peritoneal mesothelial cells, thus the miR-29c inhibitor was transferred into peritoneal mesothelial cells treated with high glucose and the expression of Sprouty homolog 1 (Spry1), a confirmed target of miR-29c was also measured. Knockdown of miR-29c unregulated the expression of E-cadherin, but downregulated the expression of α-SMA, Col-Iand FN in peritoneal mesothelial cell induced by high glucose. And the expression of Spry1 in peritoneal meosthelial cell was decreased while inhibition of miR-29c upregulate the expression of spry1 in vitro.

Conclusions: These findings demonstrated that miR-29c could regulate EMT of peritoneal mesothelial cell and the effect of miR-29c on the regulation of EMT in peritoneal mesothelial cells may be mediated by Spry1.

Funding: Government Support - Non-U.S.

FR-PO800

Long-Term Effect of Low Glucose Degradation Product Dialysis Solution on Ex Vivo Phenotype of Human Peritoneal Mesothelial Cells in Continuous Ambulatory Peritoneal Dialysis Patients Kyu-hyang Cho,[1] Jun-Young Do,[1] Seokhui Kang,[1] Jong-Won Park,[1] Kyung Woo Yoon,[1] Sun Young Jung.[2] *[1]Department of Internal Medicine, Yeungnam University Hospital, Daegu, Republic of Korea; [2]Department of Internal Medicine, Soonchunhyang University Gumi Hospital, Gumi, Republic of Korea.*

Background: The purpose of this study was to analyze the long-term effect of low GDP dialysis solution on the changes of ex vivo phenotype of human peritoneal mesothelial cells (HPMCs) and epithelial to mesenchymal transition (EMT) of HPMCs in CAPD patients.

Methods: Among new CAPD patients from May 2001 to January 2009 in our hospital, 74 patients (DM: 27, mean age: 48 years) finished a 60 month protocol.Patients were assigned to one of four groups, group D (n=21, high GDP, Dianeal®), group P (n=24, low GDP, Physioneal®), group S (n =10, high GDP, Stay-safe®), group B (n=19, low GDP, Balance®).HPMCs were also cultured from overnight dwell effluent at months 1, 6, 12, 24, 36, 48 and 60. We scored HPMCs (1: cobble stone appearance mesothelial cell, 2: mixed, 3: fibroblast) as morphologic characteristics by the same researcher and measured CA125 levels.

Results: There were significant decreases in CA125 level and increases in cell score in effluent over time on peritoneal dialysis. There were no significant differences in cell score in effluent between the high GDP group and the low GDP group during the first 5 years. However, the group B showed lower cell score than the group S during the first 5 years as a result of subgroup analysis. The group B showed significant higher CA125 level in effluent than the group S at months 1, 6 and 12. There were no differences in cell score between the group D and the group P during the first 5 years. Factors associated with higher cell score was old age and the high GDP solution.

Conclusions: There were significant decreases in CA125 level and increases in cell score of HPMCs in effluent over time on peritoneal dialysis. The group B showed significant lower cell score of HPMCs in effluent than the group S during the first 5 years in incident CAPD patients. Further study is needed to explain the differences in long-term effects of two kinds of low GDP solutions on EMT of HPMCs.

FR-PO801

The Effect of Icodextrin on Long-Term Patient Survival in Continuous Ambulatory Peritoneal Dialysis Patients Kyu-hyang Cho,[1] Jun-Young Do,[1] Seokhui Kang,[1] Jong-Won Park,[1] Kyung Woo Yoon,[1] Sun Young Jung.[2] *[1]Department of Internal Medicine, Yeungnam University Hospital, Daegu, Republic of Korea; [2]Department of Internal Medicine, Soonchunhyang University Gumi Hospital, Gumi, Republic of Korea.*

Background: We conducted this study to analyze the effect of icodextrin dialysis solution on long-term patient survival and the factors associated with patient survival in CAPD patients.

Methods: 183 incident patients were enrolled from May 2001 to October 2004 in our hospital and 75 (DM:28, icodextrin:36, follow-up range:1-132 months) patients finished a complete a 36 month protocol among 183 patients. We defined icodextrin group as once daily use of icodextrin more than 6 months.Serum leptin and adiponectin were measured at months 1 (baseline), 6, 12, 24, and 36. Degree of comorbidity was assessed on the basis of the Davies comorbidity score. For the analysis of patient survival, we included all 183 (DM:92, icodextrin:57, follow-up range:38-132 months) patients who entered into the study (intention-to-treat analysis). We also conducted analysis of patient survival, by including only 75 patients who fulfilled a 36 month protocol (per-protocol analysis).

Results: Icodextrin group showed higher serum adiponectin than non-icodextrin group. Icodextrin group showed lower serum leptin/adiponectin ratio than non-icodextrin group. As a result of intention-to–treat analysis, icodextrin group had a better patient survival rate compared to non-icodextrin group (89.5% vs. 59.1% in 3 years and 82.3% vs. 45.2% in 5 years). As a result of per-protocol analysis, icodextrin group also showed a better patient survival rate compared with non-icodextrin group (97.1% vs. 78.8% in 5 years and 75.0% vs. 53.2% in 10 years). The factors associated with patient survival were young age, non-DM, low Davies comorbidity score, use of icodextrin.

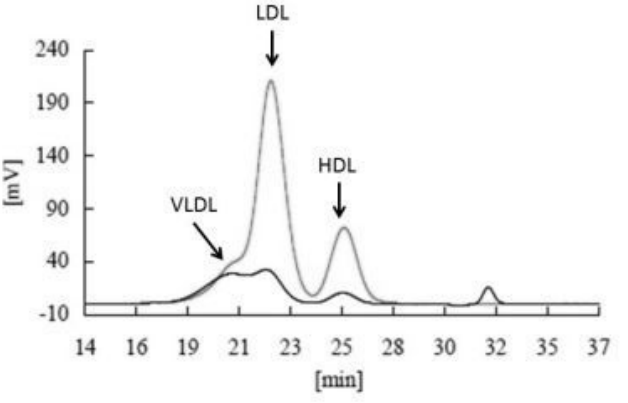
Conclusions: This study suggests that the application of icodextrin solution may be a better option to improve leptin/adiponectin ratio in CAPD patients. The icodextrin group showed a better long-term patient survival in CAPD patients compared to the non-icodextrin group. We need further studies to clarify the factors associated with patient survival.

FR-PO802

A New High-Performance Liquid Chromatography Showed that Icodextrin Dialysate Favorably Improved Lipid Profiles in Peritoneal Dialysis Patients Eiichiro Kanda,[1] Masumi Ai,[2] Yoshitaka Maeda,[3] Mitsuyo Okazaki,[2] Sei Sasaki,[2] Masayuki Yoshida.[2] *[1]Tokyo Kyosai Hospital; [2]Tokyo Medical and dental University; [3]Toride Medical Center.*

Background: Peritoneal dialysis (PD) patients show an atherogenic lipid profile. Lipoprotein profile consists of a continuous spectrum of particles of different sizes and densities. We investigated lipoprotein subclasses in PD patient, and evaluated the effects of icodextrin on lipid metabolism.

Methods: 49 patients were enrolled in this cross-sectional study in Japan. Cholesterol levels of 20 lipoprotein subclasses were measured using an improved method of high performance liquid chromatography: CM, fraction (F) 1-2; Large VLDL, F3-5; Medium VLDL, F6; Small VLDL, F7; Large LDL, F8; Medium LDL, F9; Small LDL, F10; Very small LDL, F11-13; Very large HDL, F14-15; Large HDL, F16; Medium HDL, F17; Small HDL, F18; Very small HDL, F19-20.



Lipoprotein Profile of A Diabetic

Results: 26 patients used icodextrin: average age, 64.1 years; male, 77.6%; diabetics, 42.8%; duration of PD, 39.9 months; statin use, 32.6%; total cholesterol level, 183.1 mg/dl; total triglyceride level, 139.3 mg/dl. Although no significant difference was observed in age, gender, diabetics, or statin use between patients using icodextrin (icodextrin group) and those not using (control group), the icodextrin group showed significantly lower cholesterol levels of F10 (t-test p=0.05) and F11 (p=0.019), and significantly higher cholesterol levels of F15 than the control group (p=0.037). Adjusted multivariate regression analysis showed that icodextrin use was associated negatively with cholesterol levels of F10 (p=0.031) and F11 (p=0.023), and positively with those of F15 (p=0.037).

Conclusions: Icodextrin dialysate improved lipid profiles with regard to the prevention of atherosclerosis. It can be recommended to PD patients to reduce the risks of atherosclerosis.

FR-PO803

Low GDP Peritoneal Dalysis Regimen Lowers Plasma Levels of Proinflammatory Ligands of Receptor for Advanced Glycation End Products Sylvie Opatrna,[1] Anna Popperlova,[1] Marta Kalousova,[2] Zima Tomás.[2] *[1]Medicine I, Charles University Medical School and Teaching Hospital, Pilsen, Czech Republic; [2]Institute of Medical Biochemistry and Laboratory Medicine, 1st Faculty of Medicine, Charles University and General Hospital, Prague, Czech Republic.*

Background: Intraperitoneal glucose degradation products (GDP) load from instilled peritoneal dialysis (PD) solutions has recently been shown to directly influence systemic advanced glycation end products (AGEs) levels. AGEs exert part of their effects after engagement with the receptor for AGEs (RAGE). We studied the effect of three PD regimen that differ in GDP load on plasma and effluent levels of s-RAGE and its proinflammatory ligands: extracellular newly identified RAGE (EN-RAGE) and high mobility group box-1 protein (HMGB-1).

Methods: PD regimen with high GDP load (glucose-lactate PD fluid, D; n=8) was compared with a low GDP load (glucose-bicarbonate/lactate with icodextrin exchange for overnight dwell, E; n=9) and a very low GDP load (glucose-bicarbonate/lactate, P; n=16).

Results: D group demonstrated higher plasma EN-RAGE levels, 77.8 ng/mL, vs. both E, 11.2, p<0.001 and P, 27.0, p<0.001 as well as higher plasma HMGB1 levels, 2.2 ng/mLvs. both E, 1.1, p<0.01 and P, 1.5, p<0.01. Plasma s-RAGE did not differ between the three PD regimen used. Peritoneal clearance of s-RAGE and EN-RAGE was higher with E compared to both D and P (p<0.001 resp. p<0.01). In the whole PD patients´ group, those with dialysate-to-plasma creatinine ratio (D/Pcr) > 0,65 tended to have higher s-RAGE plasma levels (p= 0.056); and those with CRP level above median demonstrated higher HMGB-1 and EN-RAGE (p<0.05 for both).

Conclusions: Lower intraperitoneal GDP load is associated with decreased plasma levels of EN-RAGE and HMGB-1 thus possibly reflecting reduced systemic AGEs generation. Peritoneal transport characteristics, microinflammation as well as the capability of icodextrin to increase removal of middle molecular weight substances might also exert an effect on plasma RAGE ligands levels.

Funding: Government Support - Non-U.S.

FR-PO804

The Effect of Biocompatibility of PD Solutions on Pro-Inflammatory Cytokine Secretion by Peripheral Blood Mononulclear Cells Gyu Tae Shin, Inwhee Park, Heungsoo Kim. *Department of Nephrology, Ajou University School of Medicine, Suwon, Kyunggi, Republic of Korea.*

Background: Previous studies showed that in vitro exposure to biocompatible amino acid solution or bicarbonate-buffered solutions resulted in more pronounced secretion of pro-inflammatory cytokines by PBMC compared to conventional glucose based lactate-buffered solutions, and this observation was interpreted as improved host defense status by biocompatible solutions and conversely immune suppressive effect of conventional solutions. In this study, we investigated whether such in vitro results are reproduced in PD patients.

Methods: We enrolled 32 patients on PD as well as 10 healthy controls. Sixteen patients (P-E-N group) were using amino acid solutions (Nutrineal), bicarbonate/lactate-buffered glucose solutions (Physioneal) and icodextrin solutions (Extraneal), and the other 16 (D-E group) were on lactate-buffered glucose solutions (Dianeal) and icodextrin solutions. PD patients' PBMC were incubated with lipopolysaccharide (LPS). Additionally, control PBMC were exposed to dialysis solutions and then incubated with LPS.

Results: Serum CRP, serum TNF-alpha and IL-6 levels as well as unstimulated TNF-alpha and IL-6 production by PBMC were comparable between the the P-E-N group and the D-E group. LPS stimulated TNF-alpha production was significantly higher in the D-E group (2969.07±1835.08pg/ml) compared to the P-E-N group (1743.43±828.79pg/ml, p=0.021) and healthy controls (1609.02±822.48pg/ml, p=0.038). Similarly, LPS stimulated IL-6 production was higher in the D-E group (65807.27±30090.67pg/ml) compared to the P-E-N group (48385.19±18111.04pg/ml, p=0.056) and healthy controls (37588.63±10814.56pg/ml, p=0.009). In vitro exposure of PBMC to PD solutions showed that TNF-alpha levels were comparable between Dianeal1.36% and Physioneal1.36%, whereas Nutrineal showed higher TNF-alpha levels compared to both solutions. IL-6 levels were highest in Nutrineal followed by Dianeal1.36% and then Physioneal1.36%.

Conclusions: PD patients on biocompatible solutions showed normal ex vivo pro-inflammatory cytokine secretion by PBMC, whereas patients on lactate-buffered solutions showed hyper-responsive secretion of pro-inflammatory cytokines.

FR-PO805

The Effect of HMG-CoA Reductase Inhibitor on Insulin Resistance in Patients Undergoing Peritoneal Dialysis Seung Hyeok Han,[1] Dae-Suk Han,[1] Mi Jung Lee,[1] Shin-Wook Kang,[1,2] Hye-young Kang,[2] Seong Hun Kim,[2] Dong Ho Shin.[1] *[1]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea; [2]Severance Biomedical Science Institute, Brain Korea 21, Yonsei University, Seoul, Republic of Korea.*

Background: Insulin resistance is associated with the progression of atherosclerosis and is reported to predict cardiovascular mortality in patients with end-stage renal disease (ESRD). Although statin exerts pleiotropic effects, it is uncertain whether statin therapy may improve insulin resistance in these patients.

Methods: We conducted a prospective open randomized trial to investigate the effects of statin on insulin resistance in 70 patients undergoing peritoneal dialysis (PD). After a 4-week run-in period, patients were randomized into a statin group (n=35) or a control group (n=35). The statin group received 10 mg/day of rosuvastatin for 6 months. Insulin resistance was determined using homeostatic model assessment-IR (HOMA-IR). We also measured serum concentrations of adipokines and inflammatory markers.

Results: Compared to baseline values, statin treatment significantly decreased HOMA-IR index (2.37±1.08 to 2.05±0.82, P=0.014). In addition, there was a concordant decrease in high sensitivity C-reactive protein (hsCRP) levels in the statin group (2.05±1.57 to 1.21±0.84 mg/L, P<0.001). Such improvements were not observed in the control group. When between-group differences in the changes of these parameters were compared, hsCRP levels were more decreased in the statin group than in the control group (P=0.021 for between-group difference), but the changes in HOMA-IR index were comparable between the two groups (P=0.189 for between-group difference). During this period, altered adipokine profiles did not improve in both groups.

Conclusions: Statin failed to improve insulin resistance in PD patients despite a significant decrease in hsCRP levels by statin treatment, suggesting that reducing inflammation by statin is of limited help to fully attenuate insulin resistance in these patients.

FR-PO806

Oxidative Stress Assessed by Modified Assay of Advanced Oxidation Protein Products (AOPP) in Peritoneal Dialysis Patients Hong Xu, Abdul Rashid Tony Qureshi, Olof Heimburger, Peter F. Barany, Björn Anderstam, Ann-christin Bragfors Helin, Bengt Lindholm, Peter Stenvinkel. *Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: End-stage renal disease (ESRD) patients (pts) have increased circulating levels of markers of inflammation and oxidative stress (OS). The relations between these markers, how they should be assessed, and if they are linked to antioxidants such as vitamin E, α-tocopherol (α-T) are unclear in peritoneal dialysis (PD) pts.

Methods: In 83 prevalent PD pts (57 men, median age 64 years) we analyzed circulating markers of inflammation (interleukin-6 (IL-6), tumor necrosis factor (TNF), and high-sensitivity C-reactive protein (hs-CRP)) and two markers of OS: 8-hydroxy 2'-deoxy-guanosine (8-OH-dG) which reflects oxidative damage to DNA, and a modified assay of AOPP (mAOPP), which takes into account the impact of raised levels of s-triglycerides on AOPP. We also analyzed serum lipids, and other biochemical parameters including α-T at baseline, and after 1, 2, and 3 months. The variation of the studied variables was calculated as the intra-class correlation (ICC) from estimates of between-pts and within-pts variance (Mixed model).A high ICC indicates that most variability is between pts and less within pts.

Results: mAOPP levels correlated with 8-OH-dG (rho=0.29, p<0.01) and TNF (rho=0.27; p<0.05), while correlations with IL-6, hs-CRP, α-T and cholesterol were not significant. 8-OH-dG correlated with IL-6 (rho=0.35; p<0.001) and hs-CRP (rho=0.32; p<0.003). In women, median mAOPP was higher in diabetic than in non-diabetic pts (134 (106-168) vs 116 (97-161) umol/L; p=0.06). In men, median mAOPP was higher in malnourished than in well-nourished pts (150 (118-224) vs 135 (104-172) umol/L; p=0.05). ICC (95% CI) for mAOPP was 0.80 (0.73-0.86), TNF 0.72 (0.64-0.80), IL-6 0.33 (0.22-0.46) and α-T 0.70 (0.60-0.79), respectively. Within-pts variation of mAOPP was lower than that of IL-6, TNFand α-T.

Conclusions: In PD, pts, AOPP is associated to inflammation, but less so than 8-OH-dG. AOPP levels vary less than inflammation markers. AOPP should be considered when designing studies targeting increased OS and inflammation in ESRD pts.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

FR-PO807

Serum 8-Hydroxy 2'-Deoxy-Guanosine (8-OH-dG) as a Marker of Oxidative Stress in Peritoneal Dialysis (PD) and Hemodialysis (HD) Patients Hong Xu, Abdul Rashid Tony Qureshi, Olof Heimburger, Peter F. Barany, Björn Anderstam, Monica Irene Eriksson, Bengt Lindholm, Peter Stenvinkel. *Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: 8-OH-dG reflects oxidative damage of DNA and is considered to be a suitable marker of oxidative stress. Here we analyzed 8-OH-dG and its relationship with inflammatory biomarkers in a cohort of prevalent PD patients (pts) and compared the results to those obtained in a group of prevalent hemodialysis (HD) pts .

Methods: In a cross-sectional study, serum 8-OH-dG and interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) were measured in 83 prevalent PD (27 men, median age 64 years) and in 224 prevalent HD (100 men, median age 63 years) pts along withbiochemical markers, assessments of Davies comorbidity score and presence of wasting (subjective global assessment, SGA). 8-OH-dG was analyzed by ELISA (Japan Institute for Control of Aging, Shizuoka, Japan). Overall mortality was assessed after 31 months (1-52) of follow up.

Results: The median levels of 8-OH-dG level (1.34 vs 0.51 ng/ml; P<0.001), serum IL-6 (8.6 vs 6.5 pg/ml; P<0.001), hs-CRP (6.5 vs 4.4 mg/L; P=0.031), and the mean albumin level (34.6±4.5 vs 31.3±4.6 g/L; P<0.001) were greater in HD than in PD pts while age, gender, Davies comorbidity score, and SGA score did not differ between PD and HD pts. 8-OH-dG and IL-6 (rho=0.347; p<0.001) and hs-CRP (rho=0.322; p<0.003) were correlated in PD pts but did not attain statistical significance in HD pts. During a mean follow up of 34 months (3-52) in HD pts, 102 (46%) died, higher 8-OH-dG level was associated with a greater hazard ratio for death, 1.72; 95% confidence interval 1.15-2.56). During a mean follow up of 22 months (1-48) in PD pts, 18 (22%) died, 8-OH-dG level was not significantly associated with mortality.

Conclusions: Serum 8-OH-dG levels differed between HD and PD pts, along with differences in inflammatory biomarkers, suggesting that factors related to dialysis modality may modify oxidative stress in pts with end-stage renal disease. Oxidative stress assessed by using 8-OH-dG level is a strong predictor of mortality in HD pts.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

FR-PO808

Serum Interleukin-18 Levels Predict 3-Year Mortality in Peritoneal Dialysis Patients Yao-Lung Liu,[1,2] Jiung-hsiun Liu,[1,2] Che-yi Chou,[1,2] Huey-Liang Kuo,[1,2] Chiu-Ching Huang.[1,2] *[1]Division of Nephrology and Kidney Institute, China Medical University, Taichung, Taiwan; [2]School of Medicine, China Medical University, Taichung, Taiwan.*

Background: Interleukin-18 (IL-18), a pro-inflammation cytokine, is up-regulated and associated with high hospitalization in maintenance dialysis patients. However, it has not been determined whether serum IL-18 levels may predict mortality in dialysis patients. The aim of this study was to evaluate the association between serum IL-18 levels and all-cause mortality in peritoneal dialysis patients.

Methods: A total of 63 patients (27 male and 36 female) with median age of 53 (45-65) yr with end-stage renal disease underwent incident peritoneal dialysis were included in this prospective cohort study. Serum IL-18 levels were measured at one month after the start of peritoneal dialysis therapy. The follow-up period was 36 months.

Results: Ten patients (15.9%) expired, three patients (4.7%) received kidney transplantation and two patients (3.2%) transferred to hemodialysis therapy during the 36-month follow-up period. The death group showed significantly higher serum IL-18 levels than the survival group [903.8 (843.0-1173.1) pg/ml vs. 784.6 (633.1-959.9) pg/ml, p =0.022]. On the basis of the receiver-operator curve, the most appropriate cut-off point for serum IL-18 levels to predict all-cause mortality was 857.2 pg/ml (sensitivity 80.0%; specificity 64.2%; area under the curve = 0.730). The Kaplan-Meier survival analysis showed significantly higher mortality rate in the high serum IL-18 group (IL-18>857.2 pg/ml) compared with the low group (IL-18<857.2 pg/ml) (p=0.049).

Conclusions: High serum IL-18 levels predicted a high all-cause mortality rate in incident peritoneal dialysis patients.

FR-PO809

Impact of Prior Hemodialysis on First Year Peritoneal Dialysis Outcomes Eduardo K. Lacson, Nien-chen Li, Franklin W. Maddux, Joseph P. Pulliam. *Fresenius Medical Care, North America, Waltham, MA.*

Background: Patients initiating maintenance peritoneal dialysis (PD) are known to have good outcomes in their 1st year. We investigated whether prior history of HD influences 1st year PD outcomes.

Methods: Incident patients who started PD in Fresenius Medical Care North America facilities within their first year of dialysis were stratified into 3 groups: (A) direct to PD, (B) ≤90 days of HD prior to PD, or (C) >90 days of HD prior to PD. Mortality, hospitalization, and peritonitis incidence in the first PD year was recorded. Kaplan-Meier survival curves were created and log-rank tests were performed. Cox's proportional hazards models with stepwise selection were constructed with initial covariates: age, vintage, BMI, gender, race, comorbidities (diabetes, amputation, CHF, and PVD and baseline albumin, hemoglobin, and phosphorus levels. For stepwise selection, alpha for model entry and deletion were both set at 0.05.

Results: Group A (N=1,309) patients were older (58 vs. 55 vs. 56 years) compared to B (N=492) and C (N=403), respectively. They also had higher weight (85 vs. 83 vs. 82 kg.) but with similar BMI at 32 kg/m^2. Residual CrCl was 10 vs. 9.0 vs. 8.6 ml/min with A>B>C. Other trends showed fewer comorbidities, lower phosphorus and lower creatinine at baseline for Group A, with Groups B and C being similar to each other. Crude mortality rates were 9% vs. 11 % vs. 14%, respectively, with A<B<C (p=0.009). Hospitalization rates were also A<B<C at 56% vs. 63% vs. 65% (p<0.0001). Peritonitis rates were not different at 29% vs. 32% vs. 27% (p=0.26). Adjusted hazard ratio (HR) of death for B vs. A was 1.64 (1.17, 2.31); and that of C vs. A was 1.92 (1.36, 2.69); HR of hospitalization for B vs. A was 1.27 (1.11, 1.45); and that of C vs. A was 1.35 (1.17, 1.56); and HR of peritonitis for B vs. A was 1.36 (1.09, 1.70); and that of C vs. A was 1.67 (1.03, 2.69).

Conclusions: Among incident patients in this national cohort, prior HD before starting PD was associated with higher risk for mortality, hospitalization and peritonitis within the first year. Predialysis care, early modality selection, and/or self-care ability may explain these findings.

FR-PO810

Vascular Access at Transfer from Peritoneal Dialysis to Hemodialysis Predicts Survival Patrick Lan,[1] Philip A. Clayton,[1,2] John Saunders,[1] Kevan Polkinghorne,[3] Paul Snelling.[1] *[1]Royal Prince Alfred Hospital, Sydney, Australia; [2]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, Australia; [3]Monash Medical Centre, Melbourne, Australia.*

Background: Peritoneal dialysis (PD) patients commonly transfer to hemodialysis (HD), however the literature describing the outcomes of such transfers is limited. The aim of our study was to describe the predictors and outcomes of these transfers.

Methods: Data were obtained from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry on adult patients commencing PD as their initial renal replacement therapy in Australia or New Zealand between 2004-2010. Follow-up was until 31 December 2010. Logistic regression models were constructed to determine predictors of transfer within 6 and 12 months of PD commencement. Cox analysis and competing risks regression were used to determine the predictors of survival and transplantation post-transfer.

Results: The analysis included 4781 incident PD patients, of whom 1699 transferred to HD during the study period. The main reasons for transfer were: peritonitis (42%); inadequate dialysis (15%); dialysate leak (7%); or an inability to manage self-care (7%). 51% of transfers occurred within 12 months of commencing PD. Logistic models did not identify any clinically useful predictors of transfer within 6 or 12 months (c-statistics 0.54 and 0.55 respectively). 67% of patients commenced HD with a central venous catheter (CVC). CVC use at transfer was associated with increased mortality (hazard ratio 1.38, 95%CI 1.12-1.70, p=0.002) and a reduced incidence of transplantation (subhazard ratio 0.76, 95% CI 0.58-1.00, p=0.05). A sensitivity analysis restricted to patients who remained on HD for 30 or more days, confirmed that transfer with a CVC was associated with increased mortality.

Conclusions: It is difficult to predict when incident PD patients will require transfer to HD. Post-transfer survival and access to transplantation are lower in those who commence HD via a CVC. PD patients require adequate vascular access at the time of transfer, but the optimal time of vascular access creation is not known and requires further research.

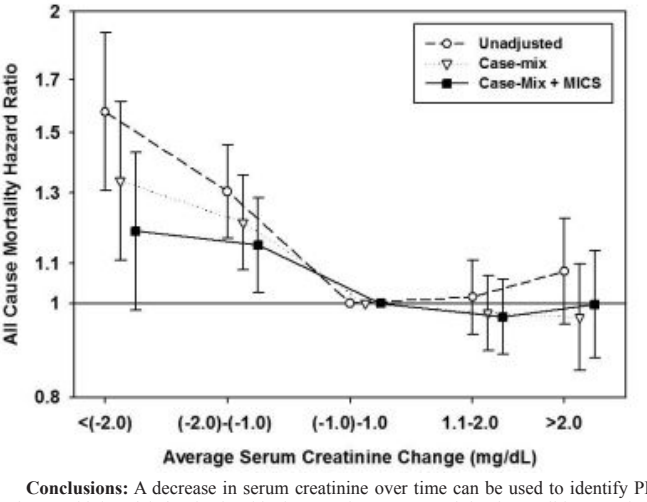
FR-PO811

Decrement of Serum Creatinine Level over Time and Increased Death Risk in Peritoneal Dialysis Patients Lilia R. Lukowsky,[1,2] Rajnish Mehrotra,[3] Jongha Park,[1,4] Miklos Zsolt Molnar,[1] Sapna Singh Patel,[3] Joel D. Kopple,[2,3] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,2,3] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]David Geffen School of Medicine and Fielding School of Public Health at UCLA, Los Angeles, CA; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]Ulsan University Hospital, Ulsan, Republic of Korea; [5]University of Tennessee, Memphis, TN.*

Background: Protein-energy wasting includes sarcopenia and is associated with increased mortality in end-stage renal disease. There are little data concerning whether muscle wasting is associated with mortality in peritoneal dialysis (PD) patients.

Methods: Change in serum creatinine (Cr) levels over 3 months, as a dynamic surrogate of muscle mass change, was evaluated for its association with all-cause mortality in 8,981 PD patients treated in 580 US dialysis units from July 2001 to June 2006. Survival models were adjusted by baseline serum Cr, case-mix and malnutrition-inflammation cachexia syndrome.

Results: The patients were 55±16 (mean±SD) years old and included 47% women, 25% blacks and 48% diabetics. Compared to patients for whom serum Cr level did not change (-1.0 to 1.0 mg/dl), patients with decrements of >2.0 and 2.0-1.1 mg/dl had a 19% (HR 1.19, 95%CI: 0.98-1.43) and 15% (HR: 1.15, 95%CI: 1.03-1.28) higher risk of death, respectively; however, there was no association of increments of +1.1-2.0 and >2.0 mg/dl with survival (adjusted HR: 0.97, 95%CI: 0.89-1.06 and HR: 1.00, 95%CI: 0.88-1.13).



Conclusions: A decrease in serum creatinine over time can be used to identify PD patients at higher risk for death. This decrease in serum creatinine is possibly a result of muscle wasting, although it may also reflect anorexia with reduced meat intake. The risk may, in some cases, be modifiable with nutritional intervention.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK077341

FR-PO812

Suberoylanilide Hydroxamic Acid Attenuates Peritoneal Fibrosis Induced by Chlorhexidine Gluconate in Mice Kumiko Io,[1] Tomoya Nishino,[1] Mineaki Kitamura,[1] Yoko Obata,[1] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan; [2]Department of Histology and Cell Biology, Nagasaki University School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan.*

Background: Long-term peritoneal dialysis causes peritoneal fibrosis in submesothelial areas. However, the mechanism of peritoneal fibrosis is unclear. Epigenetics is defined as a heritable change that occurs outside the modification of DNA coding sequence. Among epigenetic modifications, histone acetylation leads to the transcriptional activation of genes. Recent studies indicate that histone acetylation involves the progression of fibrosis. Therefore, we examined the effect of suberoylanilide hydroxamic acid (SAHA), one of HDAC inhibitors, on the progression of peritoneal fibrosis in mice.

Methods: 10 week-old male ICR mice were divided into three groups, chlorhexidine gluconate (CG), CG + SAHA, and control group. Peritoneal fibrosis was induced by the injection of CG into peritoneal cavity in mice every other day for 3 weeks. SAHA or vehicle was administered subcutaneously every day from the start of CG injection for 3 weeks. The mice were sacrificed 3 weeks after the first CG injection and peritoneal tissues were dissected out. Morphologic peritoneal changes were assessed by Masson's Trichrome staining and fibrosis-associated factors were assessed immunohistochemically.

Results: In CG-injected mice, the marked thickening of the submesothelial compact zone was shown. In contrast, the administration of SAHA suppressed the progression of submesothelial thickening and typeIII collagen accumulation in CG-injected mice. The numbers of fibroblast-specific protein-1-positive cells and phosphorylated-Smad 2/3-positive cells were decreased to a significantly greater extent in CG + SAHA group than CG group. Histone acetylation was reduced in the peritoneum of CG group, whereas it was increased in CG + SAHA group.

Conclusions: Our results indicate that SAHA can suppress peritoneal thickening and fibrosis in mice. These results suggest that SAHA may have therapeutic potential for peritoneal fibrosis.

FR-PO813

Ambulatory Arterial Stifness Index as a Determinant of Mortality in Continuous Ambulatory Peritoneal Dialysis Patients Ayse Mukadder Bilgic,[1] Ali Akcay,[1] Siren Sezer.[2] *[1]Nephrology, Fatih University Medical School, Ankara, Turkey; [2]Nephrology, Baskent University Medical School, Ankara, Turkey.*

Background: Cardiovascular disease is the most important cause of mortality and morbidity in patients with end stage renal disease(ESRD). "Ambulatory Arterial Stiffness Index(AASI)" has been proposed recently as an indicator of arterial stiffness and shown to provide prognostic information on cardiovascular mortality. We aimed to determine the relation between AASI and clinical-laboratory parameters and survival in peritoneal dialysis(PD) patients.

Methods: Fifty patients (M/F:17/33; mean age, 41.9±11.8 years, mean PD duration, 60.0±27.6 months) receiving PD at least 6 months were enrolled. Demographic, clinical and laboratory data and left ventricle mass index(LVMI) were recorded. AASI were obtained from 24-hour ambulatory blood pressure monitoring records. The patients have been followed for 18 months.

Results: Mean AASI values were 0.41±0.17 and positively correlated with duration of ESRD, comorbidity index, serum calcium, calciumxphosphorus value, parathyroid hormone, LVMI, and dipper/nondipper status. Calciumxphosphorus value (b=0.277, p=0.03), parathyroid hormone (b=0.290, p=.02), dipper/nondipper status (b=-0.388, p=.009), and daytime systolic blood pressure load (b=0.870, p=.009) were independent predictors of AASI. During 18 months following period, four patients disappeared. The shows Kaplan-Meier survival curves for all-cause mortality according to AASI above and below the mean (0.41). All-cause mortality was significantly elevated in PD patients with AASI > 0.41.

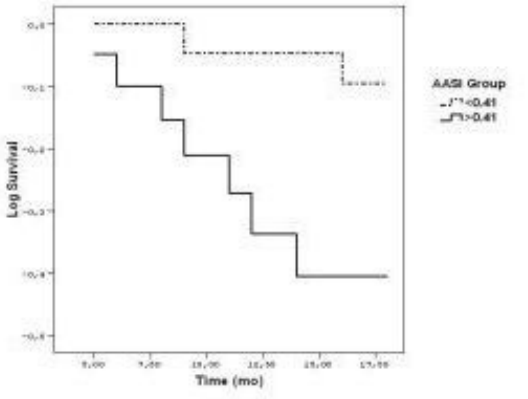


Figure 1: Kaplan-Meier survival curves for all-cause mortality according to AASI above and below the mean (0.41) in Peritoneal dialysis patients.

Conclusions: This is the first study which evaluated AASI in patients with ESRD. We obtained the results similar to previous studies about arterial stiffness measures. Also, we showed that AASI has significant value as a marker of all-cause-mortality in PD patients.

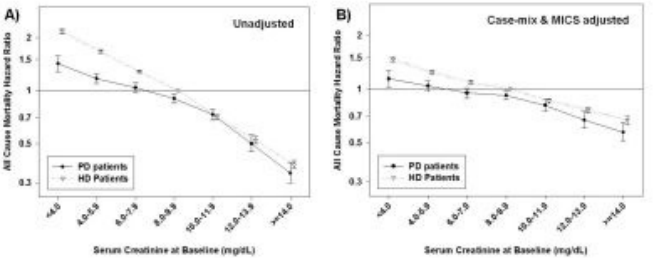
FR-PO814

Comparing Association of Serum Creatinine and Mortality between Peritoneal Dialysis and Hemodialysis Patients Jongha Park,[1,2] Rajnish Mehrotra,[3] Miklos Zsolt Molnar,[1] Lilia R. Lukowsky,[1,4] Sapna Singh Patel,[3] Joel D. Kopple,[3,4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3,4] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Ulsan University Hospital, Ulsan, Republic of Korea; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]David Geffen School of Medicine and Fielding School of Public Health at UCLA, Los Angeles, CA; [5]University of Tennessee, Memphis, TN.*

Background: Peritoneal dialysis (PD) and hemodialysis (HD) patients appear to have similar mortality patterns despite protein losses via peritoneal dialysate and lower serum albumin levels in the former. We hypothesized that the muscle-death association is similar between HD and PD patients.

Methods: Association of baseline serum creatinine (Cr) level, which is affected by muscle mass, with all-cause mortality was compared between 10,922 PD and 113,617 HD patients treated in 580 outpatient dialysis clinics in the US from July 2001 to June 2006. Stabilized serum Cr in PD was compared to pre-dialysis serum Cr in HD patients.

Results: PD patients were 55±15 yrs old and included 52% women, 24% blacks and 48% diabetics; while HD patients 59±14 yrs, 41%, 38% and 52%. With HD patients with serum Cr 8.0-9.9 mg/dl as reference group for estimating HR, PD patients showed lower adjusted HRs for death than HD patients with serum Cr ≤9.9 mg/dl; mortality risk with <4.0, 4.0-5.9, 6.0-7.9, and 8.0-9.9 mg/dl were lower with PD vs. HD by 33%, 20%, 14%, and 8%, respectively. However, there were no significant differences with PD vs. HD for higher serum Cr categories ≥10.0 mg/dl.



Conclusions: At lower ranges of serum Cr levels, reflecting lower muscle mass, PD patients had lower risk of death than HD patients, but this observation should be qualified for potential role of residual renal function and meat intake in PD and HD patients.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK077341

FR-PO815

Automated Peritoneal Dialysis in Hong Kong: There Are Two Distinct Groups of Patients Cheuk-Chun Szeto, Bonnie Kwan, Kai Ming Chow, Chi-bon Leung, Philip K.T. Li. *Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong.*

Background: Although 80% of the dialysis patients in Hong Kong are treated with peritoneal dialysis (PD), most received continuous ambulatory PD (CAPD), while automated PD (APD) accounts for 5% of the patients. Recent evidence suggests that clinical outcomes are different for CAPD and APD in specific subgroups of patients.

Methods: We reviewed the clinical outcome of 90 consecutive incident APD patients and 180 CAPD patients (control group) in our center.

Results: The average follow up was 56.6 ± 47.5 months. The APD group was younger and had a lower Charlson's score than the control group. Furthermore, the APD group had a highly skewed, bimodal distribution of the Charlson's score, indicating the presence of two different groups of patients. Multivariate analysis showed that in addition to the treatment mode (APD versus control) and Charlson's score, there was a significant interaction between the two ($p = 0.002$) on patient survival. For patients with Charlson's score ≤6, the APD group had a significantly better patient survival than the control group (85.4% vs 66.6% at 5-year, $p = 0.0002$), while for patients with Charlson's score ≥7, the patient survival was similar. Similarly, Charlson's score and its interaction with treatment mode, but not the treatment group *per se*, were independent predictors of technique survival ($p = 0.013$). For patients with Charlson's score ≥7, the APD group had a significantly lower technique survival than the control group (8.8% vs 34.3%, $p = 0.001$), while for patients with Charlson's score ≤6, the technique survival was similar (44.4% vs 42.5%, $p = 0.15$). Peritonitis-free survival was 35.2% and 32.2% for APD and control groups, respectively ($p = 0.021$), and the difference was not affected by Charlson's score.

Conclusions: Comorbid diseases had a significant interaction with the mode of PD on patient and technique survival of incident PD patients. Our result suggests that APD is associated with a survival advantage in, and only in, young patients with minimal comorbid diseases.

Funding: Clinical Revenue Support

FR-PO816

Peritoneal Dialysis in the Elderly: Comparison of Outcomes Jeffrey Laut, Ann Starinovich, Helen Brickel. *Nephrology/Home Dialysis, Fresenius Medical Care, East Hartford, CT.*

Background: The dialysis population is aging. Finding age appropriate dialysis modalities for the elderly continues to be a challenge to the dialysis community. Peritoneal dialysis (PD) appears to be a gentler, less traumatic modality for this aging population, allowing for an easier transition, and ultimately a more normal lifestyle once on dialysis. Existing literature supports the view that functional status and quality of life are better on PD then HD. However, literature is lacking in regards to measurable outcomes of elderly patients on PD when compared to their younger cohorts.

Methods: We reviewed our single center experience with an elderly (>75 years) population of PD patients over 5 years (2006 to 2011) and compared outcomes to that of a younger (<75 years) PD population.

Results: During the study period 152 patients participated in our PD program, 115 patients in the younger group (group A), mean age 54.1 yrs. (range 20-74), and 37 patients in the elderly group (group B), mean age 81.2 yrs. (range 75-89). The mean duration time on PD was not different between the groups; group A, 18.7 months v group B, 17.1 months. 35 patients (30.4%) from group A are still active in the program compared with 11 patients (29.7%) from group B ($p=0.94$). As expected the mortality rate over the observation period was higher (35%) in the elderly (group B) as compared to 22% in group A, though the difference was not statistically significant ($p=0.11$). A similar pattern was seen in regards to withdrawl from dialysis, with 16% (n=6) of group B and only 3% (n=4) of group A withdrawing entirely from dialysis; this did reach statistical significance ($p=< 0.01$). Of interest, only 10.8% (n=4) of the elderly failed PD due to peritonitis, as compared with 20% (n=23) of the younger group ($p=0.2$).

Conclusions: Our data demonstrate that PD as a dialysis modality for the elderly, has acceptable outcomes when compared to their younger cohorts, in regards to 1) longevity of PD, 2) expected mortality, and 3) failure of PD due to peritonitis. Currently an equal percentage of patients from both groups are still active in our program. PD should be considered a viable option for the elderly.

FR-PO817

The Association between Body Mass Index and Mortality in Patients with Peritoneal Dialysis Yong Kyun Kim,[1] Su Hyun Kim,[2] Young Ok Kim,[1] Ho Cheol Song,[1] Euy Jin Choi,[1] Yong-Lim Kim,[3] Yon Su Kim,[4] Shin-Wook Kang,[5] Nam Ho Kim,[6] Chul Woo Yang.[1] *[1]Department of Internal Medicine, College of Medicine the Catholic University of Korea; [2]Department of Internal Medicine, College of Medicine Chung-Ang University; [3]Department of Internal Medicine, School of Medicine Kyungpook National University; [4]Department of Internal Medicine, College of Medicine Seoul National University; [5]Department of Internal Medicine, College of Medicine Yonsei University; [6]Department of Internal Medicine, Chonnam National University Medical School.*

Background: Previous studies have demonstrated that increasing body mass index is associated with decreased mortality in hemodialysis (HD) patients. In patients with peritoneal dialysis (PD), the association between body mass index (BMI) and survival has not been well established. The aim of study was to determine the association between BMI and mortality in the PD population in the Clinical Research Center (CRC) for End Stage Renal Disease (ESRD) cohort in Korea.

Methods: Prevalent patients with PD were selected from CRC for ESRD, a prospective cohort study in dialysis patients in Korea. Patients were categorized into four groups by quartile of BMI. Cox regression analysis was used to calculate adjusted hazard ratio (HR) of mortality with a BMI of quartile 2 (21.35 - 23.48 kg/m^2) as the reference.

Results: In total, 900 patients with PD were included. The median follow-up period was 24 months.The multivariate Cox proportional hazard models showed that the lowest quartile of BMI was associated with increased mortality (HR 3.14, 95% CI, 1.39-7.08, $p=0.006$). But highest quartile of BMI was not associated with improved survival (HR 1.55, 95% CI, 0.65-3.67, $p=0.320$).

Conclusions: In PD patients, lower BMI was a significant risk factor for death. But increasing BMI was not associated with decreased mortality in PD patients, which are inconsistent with the results from studies in HD patients.

FR-PO818

Socioeconomic Status & Barriers to Peritoneal Dialysis: A Mixed Methods Study Suma Prakash,[1] Adam T. Perzynski,[1] Peter Austin,[3] Fangyun Wu,[2] Mary Ellen Lawless,[1] Michael Paterson,[2] Robert R. Quinn,[4] Ashwini R. Sehgal,[1] Matthew J. Oliver.[3] *[1]Case Western Reserve University; [2]Institute for Clinical Evaluative Sciences; [3]University of Toronto; [4]University of Calgary.*

Background: Lower socioeconomic status (SES) has been associated with lower use of peritoneal dialysis. This study aimed to determine 1) the effects of SES (income & education) on eligibility for peritoneal dialysis (PD) and starting dialysis on PD and 2) the effects of SES on barriers to PD eligibility and initiation.

Methods: This was a mixed-methods study of incident end stage renal disease (ESRD) patients of age ≥ 18 years from eight dialysis programs in Ontario, Canada. Patients were assessed for PD candidacy, educated and made a modality choice. Income and education were estimated as mean values for the dissemination area (DA) of residence (400-700 people per DA) using Canadian census data. PD initiation was analyzed in patients eligible for both PD and hemodialysis (HD). Multivariable logistic regression models estimated using generalized estimating equations were used to describe the relationship between SES and the likelihood of PD eligibility and of PD initiation. Frequencies of reasons for PD non-eligibility & not initiating PD were coded using a thematic, constant comparative approach and compared across SES categories.

Results: There were 1314 and 857 patients in the eligibility and initiation analyses respectively. SES was not a significant independent predictor for PD eligibility or initiation. Reasons for PD non-eligibility were consistent across SES categories. However, SES did influence barriers to initiating PD. In lower income DAs, space issues ($p=0.02$) and social support ($p=0.03$) were more frequently cited as barriers to PD. Space issues ($p=0.049$) and support issues ($p=0.0003$) were more common in lower education DAs while schedule barriers were more common in higher education DAs ($p=0.049$).

Conclusions: In a universal health care system, SES did not affect PD eligibility or initiation. However, specific barriers to starting PD differed significantly by SES. Further examining ways to incorporate information on various barriers during education may impact patient receptiveness to PD.

FR-PO819

Mannose-Binding Lectin Gene Polymorphism May Predict Response to Leflunomide in Patients with Progressive IgA Nephropathy Beili Shi, Zhaohui Ni, Liou Cao, Shan Mou, Min Zhang, Qin Wang, Renhua Lu, Minjie Zhou, Jia Qi Qian. *Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.*

Background: The optimal treatment or progressive IgA nephropathy remains undefined. The aim of this study was to investigate risk factors which may predict response to Leflunomide (LEF) in a prospective, randomized, controlled trial.

Methods: Patients (renal biopsy proven, Lee SMK grade II-IV, with urinary protein > 1.0g/d, and/or baseline eGFR 29-60ml/min/1.73m^2) were randomly enrolled. LEF group was given oral LEF (40mg/d for 3 days followed by 20mg/d) plus oral prednisone (0.8mg/kg/d tapered to10mg/d), and steroid group prednisone alone (1mg/kg/d tapered to 10mg/d), both for 12 months. The efficacy and safety was evaluated after 6, 12 and 24 months. Integrated capillary electrophoresis was used to detect MBL gene polymorphism in peripheral blood DNA. The clinical and pathological factors which could predict patients' response to LEF was investigated by Logistic regression analysis.

Results: 85 patients (38 in LEF group and 47 in steroid group) were enrolled. There was no difference in baseline data. All patients received an ACEI or ARB. At 6, 12, 24 months of treatment, decrease of proteinuria ($p<0.05$), improvement of serum albumin ($p<0.05$) were observed in both groups and renal function maintained stable. Total effective rate in LEF group were 68.42% and 68.09% in steroid group, respectively ($p=0.26$). The adverse events prevalence was low and slight in both groups. Compared with those who response to LEF therapy, NR (no response) patients had higher baseline serum creatinine ($P=0.035$), uric acid level ($P=0.027$), severer histological tubular-interstitial damage ($P=0.028$), accompanied with a higher mutation prevalence in MBL gene exon 1 (+54) ($P<0.001$). Logistic regression analysis found that MBL gene exon 1 (+54) polymorphism may predict renal response to leflunomide ($\beta=-0.469$, $P=0.04$) in IgAN patients.

Conclusions: LEF combination with low dose of predinisone appears to be a safe and effective treatment in progressive IgAN, while those with MBL gene variation may predict lower response probability.

Funding: Government Support - Non-U.S.

FR-PO820

Transcriptome Sequencing Identifies Potential Steroid Resistance Biomarkers in Childhood Nephrotic Syndrome William E. Smoyer,[1,2] Audrey Carol Papp,[3] Amy Webb,[3] Milan Popovic,[3] Rainer Benndorf,[1,2] Shipra Agrawal,[1] Richard F. Ransom.[1,2] *[1]Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH; [2]Program in Pharmacogenomics, Department of Pharmacology, The Ohio State University, Columbus, OH.*

Background: Glucocorticoids (GC) induce remission of nephrotic syndrome (NS) in most children, though ~20% present with or develop GC resistance. The molecular basis for differences in GC efficacy between steroid-sensitive (SSNS) and steroid resistant (SRNS) children remain largely unknown. This study seeks to determine the mechanisms responsible for differential responses to GC in children with SSNS and SRNS.

Methods: Total mRNA from leukocytes, collected at presentation (S1) and after the first course of GC therapy (S2) from children with SSNS and SRNS, was used to produce cDNA libraries for deep sequencing using the SOLiD™ Series 4 sequencer. Each sample produced ~100 million sequence reads of ~50 bases/read, sufficient to obtain whole transcriptome sequences over a wide range of transcript expression.

Results: Transcriptome-wide deep sequencing identified numerous GC-regulated genes and splice variants, as well as structural and regulatory SNPs. As expected, expression of genes known to be induced by GC in many cell types, including leukocytes (*TSC22D3, DUSP1, RNF130*), was greater in S2 than S1 in both SRNS and SSNS patients. In contrast, the most striking difference in gene expression between SRNS and SSNS patients was the inability of GC to down-regulate expression of several genes in SRNS (but not SSNS) patients, including those encoding specific cytokines (*IL8, TNF, TNFSF10*), transcription factors (IRF1), and enzymes (PTGS2) with known or suspected relevance to NS.

Conclusions: In this preliminary, small-scale analysis from our large available patient cohort, we can already conclude that transcriptome-wide deep sequencing of leukocyte mRNA from children with SSNS and SRNS holds the potential to identify novel molecular mechanisms underlying both the development of, and effective therapy for, NS in children, and may also improve our understanding of the many other diseases treated with GC.

Funding: Other NIH Support - National Institute of General Medical Sciences

FR-PO821

Novel Sets of Genes Correlated with Progressive Tubular Damage and Tubulointerstitial Fibrosis in Patients with Chronic Kidney Disease Shunsaku Nakagawa,[1] Haruka Shinke,[1] Kumiko Nishihara,[1] Tomoko Sato,[1] Hitomi Miyata,[2] Takeshi Matsubara,[2] Noriyuki Iehara,[2] Yoshinobu Igarashi,[3] Hiroshi Yamada,[3] Atsushi Fukatsu,[2] Motoko Yanagita,[2] Kazuo Matsubara,[1] Satohiro Masuda.[1] *[1]Department of Pharmacy, Kyoto University Hospital, Kyoto, Japan; [2]Department of Nephrology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Toxicogenomics Project, National Institute of Biomedical Innovation, Osaka, Japan.*

Background: In chronic kidney disease (CKD), the irreversible loss of nephron causes not only glomerular sclerosis but also progressive tubular atrophy and tubulointerstitial fibrosis. In this study, we aimed to identify the molecular characteristics that reflect the histopathological changes seen in the kidneys of CKD patients.

Methods: A total of 47 patients with histopathologically confirmed renal disease were enrolled in the study after obtaining written informed consent. The gene expression profiles of renal biopsy specimens of CKD patients were determined using microarray analysis (Whole Human Genome Microarrays, Agilent Technologies) and assessed with respect to the severity of pathological lesions related to tubular cell damage and tubulointerstitial fibrosis.

Results: Analysis involving 43,376 probes indicated that 23 and 105 genes showed significantly increased expression with the progression of tubular cell injury and tubulointerstitial fibrosis, respectively. Eight genes, including neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), were common between these 2 sets. We determined the tubular damage score on the basis of the expression levels of these 8 genes in the biopsy specimens. Composite area under the receiver operating characteristics curve (AUC-ROC) analysis showed that the score had high sensitivity for diagnosing severe tubular cell damage (AUC = 0.952, $P < 0.01$) and tubulointerstitial fibrosis (AUC = 0.894, $P < 0.05$). In addition, we found that the tubular damage score was inversely correlated with the estimated glomerular filtration rate ($r = -0.594$, $P < 0.01$).

Conclusions: Novel sets of genes correlated with progressive tubular damage and tubulointerstitial fibrosis were identified in patients with CKD.

Funding: Government Support - Non-U.S.

FR-PO822

Metabolomic Approach for Clarifying the Effect of AST-120 in 5/6 Nephrectomized Rats by Capillary Electrophoresis Mass Spectrometry Yasutoshi Akiyama,[1,2] Yoichi Takeuchi,[2,5] Eikan Mishima,[2] Takehiro Suzuki,[2] Sadayoshi Ito,[2] Tomoyoshi Soga,[3] Takaaki Abe.[2,4,5] *[1]Department of Community Health Promotion, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Hospital, Sendai, Japan; [3]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan; [4]Division of Medical Science, Ohoku University Graduate School of Biomedical Engineering, Sendai, Japan; [5]Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan.*

Background: An oral adsorbent AST-120 consists of spherical carbon particles and prevents the progression of chronic kidney disease (CKD) by adsorbing uremic toxins or their precursors in the intestine. The best-known uremic toxin whose plasma level is decreased by AST-120 treatment is indoxyl sulfate. Other than indoxyl sulfate, some compounds have been reported whose plasma levels are decreased by AST-120 treatment. However, the target compounds of AST-120 have not been fully elucidated.

Methods: Plasma concentrations of 147 compounds (62 anions and 85 cations) in 5/6 nephrectomized rats with or without 4 weeks of AST-120 treatment were comprehensively evaluated by capillary electrophoresis mass spectrometry (CE-MS).

Results: We identified 7 anions and 17 cations that were significantly decreased by AST-120 treatment. In contrast, we also identified 2 cations that were significantly increased by AST-120. Among them, 5 anions and 19 cations except for 3-indoxyl sulfate and hippurate were newly identified in this study. Plasma levels of 10 compounds (N-acetyl-neuraminate, 4-pyridoxate, 4-oxopentanoate, glycine, γ-guanidinobutyrate, N-γ-ethylglutamine, allantoin, cytosine, 5-methylcytosine and imidazole-4-acetate) were significantly increased by 5/6 nephrectomy and significantly decreased by AST-120 treatment.

Conclusions: These 10 compounds newly identified in this study could be added as the uremic compounds that indicate the effect of AST-120 treatment in CKD rats. This study provides the useful information not only for identifying the indicators of AST-120, but also for clarifying the change of metabolic profile by AST-120 treatment.

FR-PO823

Efficacy of a Human Anti-Macrophage Migration Inhibitory Factor Antibody in a Rat Model of Proliferative Nephritis Bernhard Baumgartner,[1] Jeff McKee,[1] Randolf J. Kerschbaumer,[2] Frances A. Clemo,[1] Audrey M. Hutchcraft,[1] Martin Wolfsegger,[2] Alfred Weber,[2] Gerald Hoebarth,[2] Frederick W.K. Tam,[3] Friedrich Scheiflinger,[2] Hans Peter Schwarz,[2] Eva-Maria Muchitsch,[2] Werner Hoellriegl.[2] *[1]Baxter Healthcare Corporation, Round Lake; [2]Baxter BioScience, Vienna, Austria; [3]Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom.*

Background: Baxter developed a fully human monoclonal antibody (BAX B01) against the macrophage migration inhibitory factor (MIF). BAX B01 exerts anti-inflammatory properties by neutralizing MIF and is currently in a phase I clinical trial for the treatment of lupus nephritis. The pharmacodynamic evaluation of BAX B01 in a model for proliferative nephritis was required for filing IND.

Methods: Proliferative glomerulonephritis in Wistar Kyoto rats was induced by intravenous (i.v.) injection of nephrotoxic serum (NTS), an anti-rat glomerular basement membrane serum raised in rabbits. BAX B01 was injected i.v. at 5 different dose levels (1, 5, 10, 20, and 40 mg/kg) 4 and 11 days after NTS administration. Urine total protein, albumin, and TNF-α as well as histological kidney changes (glomeruli crescent formation and ED-1 positive cells) were measured and compared to 40 mg/kg isotype control antibody and buffer solution-treated animals.

Results: The lowest BAX B01 dose showing a therapeutic effect was a single 10 mg/kg application that significantly reduced urine total protein compared to the isotype control antibody- and buffer-treated animals (25% and 33% respectively). Administration of 20 or 40 mg/kg BAX B01 (either single dose on day 4 or two doses on days 4 and 11) led to further significant reduction in glomerulonephritis severity (up to 63% reduction in proteinuria, 61% reduction in albuminuria, and 38% reduction in crescent formation). Evaluation of pharmacokinetic parameters of i.v. administered BAX B01 in healthy Wistar rats gave a terminal half-life of approximately 12 days. No BAX B01 related signs of toxicity were observed during the efficacy and PK studies.

Conclusions: The results of our studies show that BAX B01 is efficacious in a rat model of proliferative nephritis, even after a single administration.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation

FR-PO824

Impact of Cyclin-Dependent Kinase Inhibition with Roscovitine on Actin Cytoskeleton and Intracellular Calcium Signaling in Renal Cells: Dual Effect of the S and R Enantiomer Grazia Tamma, Marianna Ranieri, Annarita Di Mise, Alessia Spirlì, Maria Svelto, Giovanna Valenti. *Dept. Biosciences Biotechnologies and Pharmacological Sciences, University of Bari, Bari, Italy.*

Background: Cyclin-dependent kinases (CDK) inhibitors represent interesting therapeutic candidates due to their ability to target cell cycle proteins. Of these, roscovitine is currently entering phase II clinical trials against cancers and phase I clinical tests against glomerulonephritis because reduces the abnormal cell proliferation. The two roscovitine

enantiomers (R and S) are both very promising therapeutic tools due to their ability to regulate cell proliferation, however they might exert distinct actions at tissue levels. Here we evaluated roscovitine effect on actin cytoskeleton and intracellular calcium signaling in MDCK cells.

Methods: Intracellular calcium was evaluated by Fura-2AM microfluorimetry. Actin filaments were visualized by phalloidin-TRITC.

Results: The two enantiomers had opposite effects on actin organization as R-roscovitine caused actin depolymerization whereas S-roscovitine stabilized actin filaments. *Long term* R-roscovitine treatment significantly reduced basal cytosolic calcium compared to control cells. In contrast, S-roscovitine treated cells showed a significant increase in basal intracellular calcium. S*hort term* exposure to S-roscovitine induced a cytosolic calcium peak, which was abolished after store depletion with cyclopiazonic acid (CPA). Instead R-roscovitine caused cytosolic calcium oscillations followed by a small calcium plateau. Calcium oscillations were prevented after store depletion with CPA or treatment with the PLC inhibitor U73122. Bafilomycin, a selective vacuolar H+-ATPase inhibitor abolished the small calcium plateau.

Conclusions: To our knowledge this is the first study revealing the differential effect of S- and R-roscovitine on cytoskeleton and intracellular calcium signaling in renal cells. Since calcium and CDKs are pleiotropic cellular regulators and both exert powerful effects on cell proliferation and regulation of membrane transporter trafficking through actin dynamics, the use of S- and R-roscovitine as therapeutic tools has to be carefully evaluated.

Funding: Government Support - Non-U.S.

FR-PO825

Renal Damage Induced by Iron Sucrose Similars in a Rat Model

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Background: IV iron preparations are non-biological complex drugs (NBCD) based on a polynuclear iron(III)-oxyhydroxide core stabilized by various carbohydrates (sucrose, dextran, gluconate or carboxymaltose). This study assessed kidney function after IV administration of either the original iron sucrose (IS, Venofer) or iron sucrose similar (ISS) preparations.

Methods: SD rats received IV iron preparations (40mg iron/kg bw weekly for 4 weeks, blinded samples) with: J1 (Ferri, India); K1 (Encifer, India); L1 (Ijzehydroxide Saccharose Complex, Netherlands);M1 (Likefer, Russia);N1 (Venofer, Switzerland); controls with normal saline. Proteinuria, cr.cl, NGAL in urine and kidney tissues, oxidative/nitrosative stress and iron parameters were comparatively evaluated. Iron disposition, hepcidin and apoptosis were also assessed.

Results: Differences in iron disposition in the kidney were observed between IS and ISS groups in tubular and glomerular cells. Markers for functional kidney parameters from animals treated with N1 (originator) and J1-M1 (ISS preparations) differed significantly ($p<0.01$).

Mean ± SD	J1	K1	L1	M1	N1 (originator)	Control
Proteinuria (mg/day)1	79.1± 9.0	83.2± 10.3	39.9 ± 4.4†	28.9 ± 4.0†	7.5 ± 3.4**	4.1 ± 2.3*
Urine NGAL (ng/ml)1	1871.0±247.1	2662.2±398.7*	1428.1±263.2†	974.8±118.5†	441.5±47.9**	366.5 ± 63.0**
Iron (Prussian Blue positive staining /area)	6.6 ± 0.4	6.5 ± 0.5	4.4 ± 0.6†	4.5 ± 0.7†	1.5 ± 0.3**	0.4 ± 0.2 *
L Ferritin (positive staining /area)	2.6 ± 0.4	2.7 ± 0.3	3.9 ± 0.4†	4.2 ± 0.3†	9.1 ± 0.3**	0.8 ± 0.3*
Nitrotyrosine (positive staining /area)	14.8 ± 1.2	14.5 ± 1.3	9.7 ± 1.0†	9.5 ± 0.9†	3.4 ± 0.8**	3.0 ± 0.5**
Caspase 3 (number of positive cells /area)	8.1 ± 1.5	8.7 ± 1.2	7.5± 1.6	7.2 ± 1.4	1.0 ± 0.5**	0.2 ± 0.2*
Hepcidin (positive staining /area)	12.5 ±2,4	11.1±1,8	8,5±1,5†	7,8±1,6†	3.3±0,8**	2.1± 0.9*

1 Values a day 29.* $p<0.01$ vs. all. † $p<0.01$ vs. J1 and K1. ** $p<0.01$ vs. J1, K1, L1, and M1 $p<0.01$

Conclusions: ISS preparations showed a different pattern of iron disposition in the kidneys, a higher level of oxidative/nitrosative stress and kidney injury versus the original IS. These results may be due to subtle and not always predictable structural differences of these non-biological complex drugs.

Funding: Pharmaceutical Company Support - Vifor Pharma

FR-PO826

Citrate Pharmacokinetics in Severe Acute Kidney Injury Patients

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Background: Regional citrate anticoagulation (RCA) has been increasingly used in severe acute kidney injury (AKI) patients at high risk for bleeding. However, citrate metabolism has never been evaluated in this patients group. This study systematically evaluated the pharmacokinetics of citrate in severe AKI patients and applied the kinetic parameters to the prediction of systemic citrate concentrations during continuous veno-venous hemofiltration (CVVH) using citrate anticoagulation.

Methods: Critically ill patients with AKI (n=12) and healthy volunteers (n=10) were infused 4% sodium citrate and substituted calcium for 2 hours. Serial blood samples were taken before, during and up to 120 min after citrate infusion. Systemic citrate concentrations were measured. Subgroup analyses of severe AKI patients according to RIFLE class, SOFA scores and the necessity for vasopressors were performed. The kinetic parameters were applied to a citrate kinetic equation to predict the systemic citrate concentrations during 10 patients' CVVH using citrate anticoagulation.

Results: Total body clearance of citrate was similar in patients with AKI and healthy volunteers (648.04 ± 347.00 L/min versus 686.64 ± 353.60 L/min ; P = 0.624). There were no significant differences in citrate basal and peak concentrations between groups (p=0.423 and 0.247, respectively). Subgroup analyses showed there were tendencies of increasing AUC and reducing clearance in patients with higher SOFA scores or using vasopressor agents, though no statistical differences were found. Measured citrate concentrations fitted well with the predicted citrate curve. No patient developed citrate accumulation and citrate-related metabolic complications during CVVH using citrate anticoagulation.

Conclusions: Citrate clearance in critically ill patients with AKI is not impaired. Patients with higher SOFA scores or using vasopressor agents are suggested to be under careful monitoring during long-term CRRT procedures. With the help of citrate kinetic equation, citrate pharmacokinetic data could provide a basis to predict the risk of citrate accumulation.

Funding: Government Support - Non-U.S.

FR-PO827

Dialyzability and Pharmacokinetics of Sitafloxacin Following Multiple Oral Dosing in Infected Hemodialysis Patients: Comparison with Levofloxacin

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Background: The pharmacokinetics and dialyzability of oral sitafloxacin, a newly available quinolone, in hemodialysis patients have not been reported previously.

Methods: Fourteen maintenance hemodialysis patients with acute pyelonephritis were randomly assigned either oral sitafloxacin (50 mg daily for three days, N=7) or levofloxacin (500 mg on the first day and 250mg on the third day, N=7) after obtaining their informed consent. On the 3rd day, pharmacokinetic parameters and dialyzability of the drugs were evaluated in these patients by arterio-venous difference method.

Results: All patients exhibited improved symptoms without major problems. Drug concentrations in all arterial samples were compatible with that of healthy subjects orally receiving 100 mg daily and above their MIC of targeted bacteria. Dialyzer clearance of sitafloxacin was slightly but significantly higher than that of levofloxacn (57.8 ± 3.9 and 49.9 ± 0.9 ml/min/m^2, sitafloxacin and levofloxacin, respectively). Apparent half-lives of both drugs during dialysis session were almost same and they were significantly increased after the dialysis; however the increment after the session was significantly prominent in sitafloxacin (4.0 ± 0.4 and 46.5±3.8 hours for sitafloxacin, 4.6±0.5 and 27 .1 ±3.6 hours for levofloxacin, during and after the session, respectively). Dialyzer clearances were positively correlated with urea reduction ratio and negatively correlated with serum albumin concentration in both drugs. Rebound of the drug concentrations after the dialysis were not seen.

Conclusions: Oral dosing of sitafloxacin at 50 mg daily in maintenance hemodialysis patients provides a safe drug concentration compatible with that of healthy subjects orally receiving 100 mg daily. Because significant amount of both drugs were removed, administration might be undertaken after the dialysis session. Comparing with levofloxacin, higher dialyzer clearance and reduction of half-life during the HD session of sitafloxacin may be due to smaller protein-binding rate of the drug.

Funding: Government Support - Non-U.S.

FR-PO828

Validation Pharmacokinetic Study for Minimum Dose Oseltamivir Using LC/MS/MS in Peritoneally Dialyzed Patients

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Background: The present study was undertaken for developing and validating a pharmacokinetic(PK) study using minimum dose oseltamivir for patients receiving peritoneal dialysis (PD) using high-performance LC-MS/MS.

Methods: The study was performed in three steps. First, we developed a highly sensitive and specific assay for oseltamivir and its active metabolite, oseltamivir carboxylate. Second, dose-finding pilot PK study was performed for PD patients (n=4) in order to verify the linearity and concordance of PK profiles among 2.5mg, 5mg and 10mg oseltamivir, for which the regular dose is 75mg b.i.d. Third, two separate PK studies in CAPD patients (n=10) with a minimum and regular therapeutic doses of oseltamivir were conducted and the two PK parameters were compared.

Results: Validation for the determination of oseltamivir in plasma and dialysate by LC-MS/MS was carried out with LLOQ of 0.5 ng/mL and linear range between 0.5 and 500 ng/mL. In the dose-finding study, the three oseltamivir doses (2.5mg, 5mg and 10mg) exhibited linear pharmacokinetics. Therefore, PK study with 2.5mg oseltamivir was performed in CAPD patients (n=10) in order to validate the accuracy of minimum dose, compared with regular therapeutic dose (35mg). There were high concordance rates between 2.5mg and 35mg PK studies both for oseltamivir and oseltamivir carboxylate - i.e., AUC_{last} / Dose, CL/F, and drug excreted by dialysis (%). No significant differences between the two doses were observed in the dose adjusted parameters such as C_{max} / Dose (0.64+/-0.24 vs 0.65+/-0.27 ng/mL/mg, p:NS), and AUC_{last} / Dose (1.15+/-0.76 vs 1.48+/-0.68 ng·h/mL/mg, p:NS) for oseltamivir. No differences were observed in the C_{max} / Dose, and AUC_{last} / Dose for oseltamivir carboxylate, either.

Conclusions: The present study could be a model of minimum dose PK study predicting the PK parameters at a therapeutic dose and can be extended to other drugs in such vulnerable patients with end stage renal disease.

FR-PO829

Population Pharmacokinetics and Dose Adjustment Strategy of Cyclosporine in Steroid-Resistant Nephrotic Patients Satoru Ogahara, Takao Saito, Yasuhiro Abe, Yoshie Sasatomi, Katsuhisa Miyake, Hitoshi Nakashima. *Nephrology and Rheumatology, Internal Medicine, Fukuoka University Hospital, Fukuoka, Japan.*

Background: Cyclosporine A (CsA) is widely used in the treatment for steroid-resistant nephrotic patients. Therapeutic drug monitoring is essential to avoid adverse effects while maximizing immunosuppressive efficacy. The aims of this study were 1) to determine the population pharmacokinetic parameters of CsA in these populations; and 2) to propose a simplified strategy to predict AUC_{0-4}, a parameter that has recently been recommended as an index for the dose adjustment of CsA.

Methods: A total of 300 CsA concentration data were collected from 22 patients, who had received CsA once daily in the morning, either before or after breakfast. Population pharmacokinetic analysis was performed using the NONMEM program. A one-compartment pharmacokinetic model with first-order absorption and elimination were used to fit the data. The CsA concentrations predicted using the individually adjusted pharmacokinetic parameters by Bayesian method were compared with the observed CsA concentrations. Then, the AUC_{0-4} predicted by the 1-point sampling strategy using C_1 were compared with the AUC_{0-4}.

Results: The estimated mean population pharmacokinetic parameters (interindividual variability) for oral clearance (CL/F) and apparent volume of distribution for the central compartment (Vd/F) were 19.6 L/hr (34.5%) and 40.5 L (39.5%), respectively. The CsA concentrations predicted using the individually adjusted pharmacokinetic parameters shown an excellent correlation with observed CsA concentrations. The AUC_{0-4} predicted by the 1-point sampling strategy using C_1 exhibited an excellent correlation with the AUC_{0-4} calculated by the trapezoidal method ($R^2 = 0.668$). The final regression model was: $AUC_{0-4} = 1.5(C_1) + 633$(dosing time) + 8.27(dose) where dose timing = 0 for before breakfast, dose timing = 1 for after breakfast.

Conclusions: The present findings suggest that 1-point sampling strategy using C_1 based on population pharmacokinetics can accurately predict AUC_{0-4}, providing an excellent method for the daily dose adjustment of CsA.

FR-PO830

Novel Model for Prediction of Pharmacokinetics at a Therapeutic Dose with a Low-Dose Pharmacokinetics in End-Stage Renal Disease on Hemodialysis: Low-Dose Oseltamivir Pharmacokinetics Yun Jung Oh,[1] Dong Ki Kim,[1] Kwang-hee Shin,[2] Jeonghwan Lee,[1] Kook-Hwan Oh,[1] Kyung-sang Yu,[2] Yon Su Kim,[1] Kwon Wook Joo.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: Adverse drug reaction due to drug overdosing may give rise to safety issues in pharmacokinetic (PK) study in patients with end-stage renal disease (ESRD). To reduce the risk, we aimed to develop a model for prediction of PK at a therapeutic dose (TD) with a low-dose (LD) PK study in ESRD patients using oseltamivir as a model drug.

Methods: Two-step PK studies using LC-MS/MS were performed in 14 anuric ESRD patients undergoing hemodialysis. First, the dose-finding pilot step (n=4) was designed as dose-escalation study by dose-doubling from 2.5mg of single oral oseltamivir to find the LD that shows linear PK with two higher doses. Once the LD was determined, PK predictability assessment study (n=10) was performed using the LD defined in the pilot study and TD (35mg) with a 3-week washout period. The PK profiles of oseltamivir and its active metabolite oseltamivir carboxylate (OC) at a TD simulated from the LD data were compared with observed PK.

Results: In the dose-finding pilot study, a 2.5mg of oseltamivir was defined as the LD that shows dose-proportionality with two higher doses (5 and 10mg). Therefore, PK study with 2.5mg oseltamivir was performed to validate its predictability of the PK profiles at a TD. Despite the 14-fold difference in dose, the plots of the plasma oseltamivir and OC concentration-time profiles for the LD and TD have a similar shape. The population PK analysis demonstrated that the simulated PK parameters of oseltamivir and OC were well correlated with observed values, and that the simulated OC concentration-time profiles were well corresponded to the measured values.

Conclusions: The close agreement between the observed and the model-simulated concentrations indicated the rational to use the LD PK study for predicting the PK profiles of drugs at a TD in ESRD patients undergoing hemodialysis.

Funding: Government Support - Non-U.S.

FR-PO831

Safety and Pharmacokinetics of Single and Multiple Doses of a First in Class Dual NADPH Oxidase 1 and 4 Inhibitor Administered Orally in Healthy Subjects Philippe Wiesel,[1] Lionel Hovsepian,[2] Peter J. Mutch,[3] Jacques Herve,[1] Freddy Heitz,[1] Patrick Page.[1] *[1]Genkyotex S.A., Plan-les-Ouates, Switzerland; [2]SGS-Aster, Paris, France; [3]Aptiv Solutions, Stevenage, United Kingdom.*

Background: NADPH oxidases (NOX) are important sources of reactive oxygen species (ROS), and excessive NOX-mediated ROS production has been implicated in a broad range of human diseases, including respiratory, cardiovascular, renal, and metabolic disorders and other conditions where oxidative stress, inflammation and fibrosis play a role. The NOX1 and NOX4 isoforms seem to be centrally implicated in the development of diabetic complications. We report the single and multiple dose safety and pharmacokinetics (PK) of the first in class dual NOX1/4 inhibitor, GKT137831.

Methods: A first, open label single ascending dose study assessed increasing doses of oral GKT137831 (10, 30, 100, 300, 900, 1500, and 1800mg) in 36 healthy adult male subjects. A second, randomized, double-blind, placebo controlled, multiple ascending dose study assessed 10 consecutive (daily or twice daily) doses of oral GKT137831 in 4 sequential cohorts of 12 subjects each (9 active and 3 placebo). GKT137831 was administered at doses of 100mg OD, 300mg OD, 400mg BID, and 900mg OD.

Results: Single-dose and multiple-dose GKT137831 was safe and well tolerated, and dose limiting toxicities were not identified at the doses tested. The incidence of adverse events (AEs) was low and comparable across the placebo and all presumed biologically active doses, and there were no serious AEs. Following a single oral dose, GKT137831 was rapidly absorbed ($T_{max} \sim 1$ hour) and had dose-proportional PK (AUC_{0-24} and C_{max}) up to 900mg. The terminal half-life was approximately 12 hours at the anticipated therapeutic dose. There was no change in PK characteristics following 10 consecutive daily or twice daily administrations, and no accumulation occurred.

Conclusions: The excellent safety and pharmacokinetic profiles of single-dose and multiple-dose GKT137831 in healthy subjects warrant further investigations in patients. Phase 2 clinical studies in patients with diabetic nephropathy and other disorders are planned.

FR-PO832

Pharmacokinetics (PK) of Single Dose Oral Ranolazine in Subjects Receiving Maintenance Hemodialysis (HD) Bruce A. Mueller,[1] Bridget Scoville,[1] Rachel F. Eyler,[3] Michael Heung,[2] Jonathan H. Segal.[2] *[1]College of Pharmacy, U Michigan; [2]Division of Nephrology, U Michigan School of Medicine; [3]College of Pharmacy, U Connecticut.*

Background: Ranolazine is an anti-anginal medication that requires dose adjustment for renal insufficiency but does not have manufacturer's dosing recommendations for patients receiving HD in the product label. The objective of this study was to characterize the PK of ranolazine in subjects with CKD-5 receiving HD.

Methods: This adaptive design study was a prospective, open-label, single dose PK study of ranolazine in non-fasting subjects with CKD-5 receiving HD. A single Ranexa ER 500mg tablet was administered. Plasma samples were collected at t=1, 2, 4, 8, 12, 15, 18, 20, 22, 23, 30 and 65 hours post dose. A 4 hour HD session began 18 hours post-dose. Pre/post dialyzer plasma samples were obtained at 20, 22 hours post dose. Samples were analyzed by LC-API/MS/MS with a lower limit of detection of 50 ng/mL.

Results: Three subjects (mean±SD age 38±12 years, weight 56.6±10.2 kg) completed the study. The Cmax ranged from 372 to 906 ng/mL and the Tmax ranged from 4 to 12 hours. Ranolazine AUC0-24 was 8281.0 ng*hr/mL, 9550.0 ng*hr/mL, and 2818.9 ng*hr/mL for subjects 1, 2, and 3, respectively. Subject 3 had undetectable concentrations starting at 18 hours post dose. The t1/2 of 3.6±1.7 hours was calculated from samples t=23 and 30 for subject 1 and 2 and t=12 and 15 for subject 3. Post dialyzer plasma ranolazine concentrations were 10.2%±7.6% lower than pre dialyzer plasma ranolazine concentrations. Post-HD rebound 1 hr after HD ranged from 3.6%-7.6%.

Conclusions: The Tmax varied widely in subjects receiving HD and was longer than the 2-5 hours reported in subjects with normal renal function. The t½ of 3.6 hours was shorter than the 7 hour t½ reported in subjects with normal renal function. The ~10% difference between pre and post dialyzer samples, ranolazine's large Vd, and significant plasma protein binding suggests that ranolazine is not very dialyzable. Our ranolazine PK findings are in-line with published data from subjects with renal insufficiency. Further evaluation in this population is necessary for proper PK characterization.

Funding: Other NIH Support - UL1RR024986, Pharmaceutical Company Support - Gilead

FR-PO833

Continuous versus Intermittent Infusion of Loop Diuretics in Hospitalized Patients: A Meta-Analysis of Randomized Controlled Trials Fahad S. Alqahtani,[1] Ioannis Koulouridis,[1,2] Khagendra Dahal,[1] Bertrand L. Jaber.[1,2] *[1]Department of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Department of Medicine, Tufts University, Boston, MA.*

Background: Several studies have examined the potential benefits of continuous vs. intermittent intravenous loop diuretic administration in hospitalized patients with conflicting results. We conducted a meta-analysis to compare the efficacy of continuous vs. intermittent infusion of loop diuretics in hospitalized patients with fluid overload of various causes.

Methods: We searched MEDLINE (through December 2011) and prior meta-analyses for randomized controlled trials comparing the efficacy of continuous vs. intermittent infusion of loop diuretics. We extracted data on dosing algorithm, mean diuretic dose, daily urine output, and weight change. Random-effects model meta-analyses were used to examine net change in daily urine output and body weight.

Results: We identified 7 cross-over and 11 parallel-arm randomized controlled trials (936 patients) of adults and children. Continuous infusion of loop diuretics resulted in a non-significant net increase in daily urine output of 334 mL (95% confidence interval [CI] CI -74, 742; P = 0.109; I^2 = 84%), but a significant net increase in urine output (normalized to furosemide-equivalent dose) of 147 ml/100mg (95% CI 8, 285; P = 0.038; I^2 = 83%). Studies that used a loading dose prior to the continuous diuretic infusion had a significant net increase in daily urine output of 294 mL (95% CI 31, 557; P = 0.028) compared to studies that did not use a loading dose (340 ml; 95% CI -417, 1096; P = 0.379). Body weight loss was significantly greater in the continuous vs. intermittent infusion group (-0.8 kg; 95% CI -1.5, -0.1; P = 0.041; I^2 = 0%). There was significant heterogeneity in effect sizes among individual studies based on an I^2 index value of greater than 50%.

Conclusions: Continuous infusion of loop diuretics preceded by a loading dose results in greater diuresis in hospitalized patients with fluid overload compared to intermittent dosing regimens. Studies are required to examine whether these benefits translate into improved clinical outcomes.

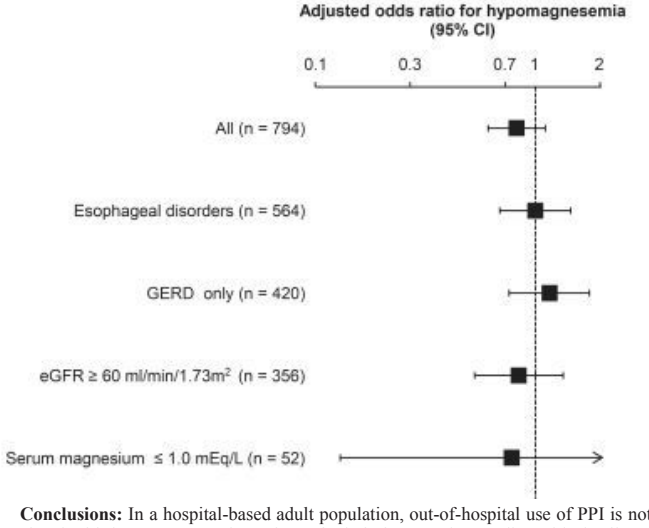
FR-PO834

Out-of-Hospital Use of Proton Pump Inhibitors and Hypomagnesemia at Hospital Admission: A Nested Case-Control Study Ioannis Koulouridis,[1,2] Mansour Alfayez,[1] Hocine Tighiouart,[3] Nicolaos E. Madias,[1,2] David M. Kent,[2,3] Jessica K. Paulus,[2,4] Bertrand L. Jaber.[1,2] *[1]Department of Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Department of Medicine, Tufts University School of Medicine, Boston, MA; [3]Biostatistics Center, Tufts University Clinical Research, Boston, MA; [4]Epidemiology, Harvard School of Public Health, Boston, MA.*

Background: Case series suggest that chronic use of proton pump inhibitors (PPIs) is associated with hypomagnesemia. Current literature lacks systematically collected data. We examined whether the presence of hypomagnesemia at time of hospital admission is associated with PPI use.

Methods: Nested, age- and sex-matched case-control study of 402 adult cases of hypomagnesemia (<1.4 mEq/L) and 402 controls (1.4-2.0 mEq/L). All magnesium levels were measured at time of hospital admission. Out-of-hospiral PPI use was ascertained from medical records. We included the first hospitalization documenting an ICD-9-CM diagnosis code for disorders of the esophagus, stomach or duodenum. Omeprazole equivalent daily dose was calculated whenever possible. Conditional logistic regression was performed to examine the association of PPI use with hypomagnesemia. Adjustment variables included the Charlson-Deyo comorbidity index, diabetes, diuretic use, eGFR, and gastro-esophageal reflux disease (GERD).

Results: PPI use was not associated with hypomagnesemia (adjusted odds ratio [OR] 0.82; 95% CI 0.61, 1.11). Neither PPI type nor omeprazole equivalent daily dose was associated with hypomagnesemia (data not shown). Several sensitivity analyses were unrevealing (Figure).



Conclusions: In a hospital-based adult population, out-of-hospital use of PPI is not associated with hypomagnesemia at time of admission. Further studies with details on exposure and duration of PPI use are needed to address this potential medication-related issue.

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FR-PO835

Influence of Kidney Disease on Drug Pharmacokinetics: An Assessment of Industry Studies Submitted to Food Drug Administration for New Molecular Entities 1999-2010 Gary R. Matzke,[1] Samantha A. Marks,[1] Thomas C. Dowling,[2] John E. Murphy,[3] Gilbert J. Burckart.[4] *[1]School of Pharmacy, Virginia Commonwealth University, Richmond, VA; [2]School of Pharmacy, University of Maryland, Baltimore, MD; [3]Colleges of Pharmacy and Medicine, University of Arizona, Tucson, AZ; [4]Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD.*

Methods: The quality of renal studies reported in the Food and Drug Administration approved prescribing information (FDA-API) and the clinical pharmacology section of the FDA review (FDA-CPR) of each new molecular entity (NME) approved from 1999 to 2010 were reviewed. The relationship between NME characteristics and the type of renal study and labeling language as established in the 1998 FDA Guidance for Industry were evaluated.

Results: 194 small molecules intended for oral or parenteral use were approved by FDA during 1999-2010. Renal studies were conducted for 89.6% of the 48 NMEs that had > 30% of the drug eliminated renally unchanged (fe); in contrast 65.8% of the 146 NMEs with fe < 30% had a renal study conducted ($p<0.01$) Hemodialysis studies were conducted in just 39.5% and 17.1% of the high and low fe NMEs ($p<0.01$) and CRRT studies were done for less than 1% of NMEs. The study design evaluation revealed that: single dose were more common than multiple dose studies; studies in 3 or more renal function groups were more common than studies conducted in only 2 groups for high (65% vs 18.6%) and low (43.8% vs 35.4%) fe NMEs. Dosage regimen reduction was recommended for 75% ± 9.7 of high fe NMEs and 21.2% ± 5.4 of low fe NMEs ($p<0.0001$). The specificity of the drug dosage adjustment recommendations was better for high (85% ± 13.3) than low fe NMEs (73.4% ± 4.8).($p< 0.0001$) FDA-API language improved over time for high but not low fe NMEs.

Conclusions: Renal function study methodologies, especially dialysis studies, are rarely well described in FDA-API. Dosing recommendations often lack specificity sufficient for individualizing patients' drug therapy. Modifications of FDA guidance to improve the quality of renal studies and FDA-API content appear warranted.

FR-PO836

Pharmacokinetics, Efficacy and Safety of Lesinurad, a Novel URAT1 Inhibitor, in Individuals with Mild to Moderate Renal Impairment Jeffrey N. Miner, Bradley Kerr, Zancong Shen, Li-tain Yeh, David T. Hagerty, Vijay Hingorani, Matthew Cravets, Kimberly J. Manhard, Barry D. Quart. *Ardea Biosciences, San Diego, CA.*

Background: Lesinurad is novel URAT1 inhibitor that blocks reabsorption of uric acid in the proximal tubule of the kidney. Serum urate (sUA) lowering effect, pharmacokinetics (PK), safety, and tolerability of lesinurad were examined in subjects with normal and impaired renal function in 3 clinical trials.

Methods: In 2 double-blind, placebo-controlled, Phase 2b studies, 331 gout patients were randomized to receive a 28-day once-daily course of placebo or lesinurad from 200 to 600 mg as monotherapy or in combination with allopurinol (ALLO). In a phase 1 study, 24 subjects with varying degrees of renal function were given a single oral dose of lesinurad 200 mg. Full PK profiles were obtained in the phase 1 study and in a sub-study of the Phase 2b ALLO-combination study and predose concentrations were measured weekly in both phase 2b studies. Urinary lesinurad concentrations and sUA levels were also evaluated.

Results: Lesinurad C_{max} increased ≤30% in the various renal impairment categories compared to subjects with normal renal function. Lesinurad AUC increased ~33% in subjects with mild renal impairment and approximately doubled in subjects with moderate renal impairment. Renal clearance of lesinurad was similar between subjects with normal renal function and with mild renal impairment, but appeared to decrease as the creatinine clearance declined below around 40 ml/min. Median sUA reduction with the 400 mg dose in patients with normal, and mild to moderate renal impairment was 32%and 28% for monotherapy and 21%and 25% for combination with ALLO, respectively. Across the 3 studies, the tolerability and safety of lesinurad was similar in patients with normal and impaired renal function.

Conclusions: In Phase 2B clinical trials, there were no meaningful differences in lesinurad exposure in patients with mild-to-moderate renal impairment. Similar serum urate reductions were observed in these patients compared to patients with normal renal function. The efficacy and safety profile of lesinurad was similar in patients with normal and impaired renal function.

Funding: Pharmaceutical Company Support - Ardea Biosciences

FR-PO837

Pharmacokinetics (PK): Pharmacodynamics (PD) of Peginesatide in Patients with Chronic Kidney Disease on Dialysis Richard Czerniak, Michael J. Kukulka, Himanshu Naik, Ping Qiu, Max Tsai, Eric Schmidt, Majid Vakilynejad. *Takeda Global Research and Development Center, Inc., Deerfield, IL.*

Background: Peginesatide (OMONTYS®) is an erythropoiesis-stimulating agent indicated for treatment of anemia due to chronic kidney disease (CKD) in adult patients on dialysis. Peginesatide cyclically increased reticulocyte count and maintained hemoglobin (Hb) levels within ±1 g/dL of baseline following intravenous (IV) or subcutaneous (SC) doses in dialysis patients switched from epoetin treatment. A phase 3 randomized, open-label, active-controlled study in dialysis patients showed that similar Hb levels were achieved with similar doses by IV or SC routes.

Methods: A population PK-PD model was developed using NONMEM VI and data from 4 phase 2 studies and 1 phase 3 study in CKD patients on and not on dialysis. Non-compartmental methods used WinNonlin v5.2.

Results: Volume of distribution (Vd) after IV dose was 35 mL/kg, terminal elimination half-life ($t_{1/2}$)was 48 hr, and plasma clearance (CL) was 0.5 mL/h•kg. After SC dose, time to C_{max} was 2 days. Absolute bioavailability (BA) was ~ 40%. The Vd/F after SC dose was 74 mL/kg, $t_{1/2}$ ranged from 33 to 77 hours, and CL/F was 1.5 mL/h•kg. No accumulation was observed following IV or SC doses every 4 weeks (Q4W).

A 2-compartment model with first order absorption and Michaelis-Menton elimination well described the PK. The relationship between peginesatide plasma concentrations and Hb levels in dialysis patients was well characterized by a modified precursor-dependent indirect PD response model. The exposure and BA estimated were similar to those obtained following noncompartmental analysis. The estimates for EC_{50} and E_{max} were 0.4 µg/mL and 0.54, respectively. None of the identified covariates including concomitant medications were considered clinically relevant, based on their impact on simulated peginesatide exposure (<±30%) and Hb levels (<±0.2 g/dL). Based on simulations, following 10 mg IV and SC injection, plasma concentrations were sustained above the estimated EC_{50} for similar durations with 4QW dosing interval.

Conclusions: Peginesatide produced similar PD response with IV or SC administration, despite the differences in PK.

Funding: Pharmaceutical Company Support - Takeda Global Research and Development Center, Inc.

FR-PO838

PK/PD-Guided Development of a Novel Hepcidin Antagonist for the Treatment of Functional Iron Deficiency in CKD Andreas M. Hohlbaum,[1] Andrea Allersdorfer,[1] Nicole Andersen,[1] Jürgen Christian,[1] Hendrik Gille,[1] Martin Huelsmeyer,[1] Galina Katzmann,[1] Max Mayr,[1] Bernd Meibohm,[2] Stefan Trentmann,[1] Laurent P. Audoly.[1] *[1]R&D, Pieris AG, Freising, Germany; [2]Pharmaceutical Sciences, Univ. of Tennessee, Memphis, TN.*

Background: Many CKD hemodialysis patients exhibit functional iron deficiency and will become anemic despite treatment with IV iron and ESA's. Blockade of hepcidin offers a novel pharmacological point of intervention for addressing functional iron deficiency in CKD by modulating iron availability and/or the response to ESA's. We recently presented the discovery of a highly specific and potent hepcidin-specific Anticalin (PRS-080) that can effectively increase serum iron levels in a non-human primate model (ASH#687). We are now trying to identify an appropriate half-life for our drug candidate to match hepcidin production rates and consequently optimize pharmacodynamic responses.

Methods: Different versions of PRS-080 were rationally engineered using PEGylation to modify the half-life of the drug. PK properties of PRS-080 bearing either a PEG 12, 20, 30, or 40kDa moiety were measured in mice, rats and cyno's.

Results: Human volumes of distribution and clearance were extrapolated by allometric scaling and half-lives were then calculated to be 6, 17, 50 and 298 hours depending on the PEG moiety. The different versions of PRS-080 also caused a significant increase in total plasma iron in cyno's. A PK/PD model for the interaction between PRS-080 and hepcidin was developed to predict format, dose and time-dependent suppression of hepcidin in a chronic setting. The model accurately described the observed concentration-time profiles of total and hepcidin-free Anticalin in cyno's after single IV administration while the predicted profiles of free hepcidin correlated well with observed iron responses. The model also revealed that therapeutics with antibody like half-lives result in the accumulation of hepcidin-drug complexes, but do not prolong hepcidin suppression.

Conclusions: These results have provided a rationale for identifying the most appropriate serum half-life for our drug candidate while maximizing efficacy. Clinical development of PRS-080 in CKD is planned.

Funding: Government Support - Non-U.S.

FR-PO839

Comparison of PR3-ANCA and MPO-ANCA Epitope Specificity upon Disease Relapse Aleeza J. Roth,[1] Witold M. Winnik,[2] JulieAnne G. McGregor,[1] Caroline Jennette Poulton,[1] Elisabeth Berg,[1] Susan L. Hogan,[1] Yichun Hu,[1] Gloria A. Preston,[1] J. Charles Jennette,[1] Ronald J. Falk.[1] *[1]Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC.*

Background: Relapse is a major clinical problem in ANCA vasculitis that causes increased morbidity and mortality. Compared to MPO-ANCA patients, patients with PR3-ANCA run a significantly increased risk of experiencing relapses. We hypothesized that a relapsing patient is producing autoantibodies with epitope specificities differing from those at disease onset or previous active disease.

Methods: Epitope mapping entailed immobilizing ANCA to native PR3 or MPO, which protects epitopes from enzymatic digestion. Epitopes were eluted and identified by matrix-assisted laser desorption mass spectrometry (MALDI-MS). ^{16}O-to-^{18}O exchange was used to detect peptide targets of low level autoantibodies from healthy individuals. Autoantibody specificity was determined for 25 patients (active vs. relapse) and 15 healthy individuals.

Results: A total of 14 PR3 epitopes or 25 MPO epitopes were detected from patients with active disease. Upon reactivation of disease PR3-ANCA patients developed additional unique anti-PR3 autoantibodies whereas MPO-ANCA patients did not. MPO-ANCA target the same MPO epitopes upon reactivation of disease. In contrast, PR3-ANCA patients develop anti-PR3 that do not target the same epitopes. Natural PR3-ANCA (3/14) and MPO-ANCA (12/25) from patients in remission and healthy controls reacted at very low levels with unique epitopes.

Conclusions: ANCA titers in clinical assays reflect a combination of both pathogenic and nonpathogenic ANCA. Relapse of disease in patients with PR3-ANCA, un-like MPO-ANCA, develop a different repertoire of B cell clones which target previously unrecognized epitopes. This abstract does not necessarily reflect EPA policy.

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FR-PO840

Regulatory B Cells in Patients with ANCA Vasculitis Lydia Aybar,[1] Susan L. Hogan,[2] Yichun Hu,[2] Elisabeth Berg,[2] Caroline Jennette Poulton,[2] Ronald J. Falk,[2] Donna O. Bunch.[2] *[1]Microbiology and Immunology, UNC, Chapel Hill, NC; [2]UNC Kidney Center, UNC, Chapel Hill, NC.*

Background: ANCA disease is a relapsing and remitting autoimmune disease where pathogenesis is B cell dependent; yet, how particular B cell subsets contribute to immunopathogenesis remains unknown. Regulatory B cells (Bregs) are phenotypically characterized as CD19^{+}CD24^{hi}CD38^{hi} and play a role in immunological tolerance by suppression of TH1 cells' inflammatory cytokine production via IL-10 secretion. We hypothesize that a decrease in functional, IL-10 producing B cells is a component of B cell dysregulation that accompanies active disease.

Methods: We examined 140 samples from 94 ANCA patients and 19 healthy individuals by flow cytometry. To identify Bregs, PBMCs were stained with antibodies against CD19, CD24, CD38, and CD5. A smaller cohort of 15 ANCA patients and 3 healthy controls was examined for IL-10 producing B cells. To determine the competency of B cell subsets to produce IL-10 in patients with ANCA disease during active and remittent disease states, PBMCs were stimulated with CD40 ligand and CpG DNA, a TLR9 agonist, for 72-96h and processed for intracellular staining of IL-10.

Results: ANCA patients, regardless of disease activity, and healthy individuals had similar percentages of B cells with a regulatory phenotype: 14±2% vs 10±1% respectively (p=0.2). Patients in remission are similar to healthy individuals with regard to IL-10 producing B cells (mean=29±3%) and 29±3% (p=0.9) respectively. In contrast, B cells from patients with active disease produced significantly less IL-10 (13+4%) than ANCA patients in remission (p=0.003).

Conclusions: ANCA patients and healthy individuals had similar percentages of Bregs; yet, active ANCA patient B cells produced significantly less IL-10. These data suggest that Bregs are present in ANCA patients but functionally impaired. These findings emphasize the importance of functional IL-10 producing Bregs in maintaining disease remission and support the hypothesis that a loss of functional Bregs may permit the immunopathogenesis of active ANCA disease. Assessing Breg ability to secrete IL-10 may be an important biomarker of ANCA remission.

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FR-PO841

Abstract Withdrawn

FR-PO842

AIRE Is Necessary for Myeloperoxidase Tolerance in Autoimmune Anti-Myeloperoxidase Glomerulonephritis Poh-Yi Gan, Diana S. Tan, Shaun A. Summers, A. Richard Kitching, Stephen R. Holdsworth. *Medicine, Monash Medical Centre, Monash University, Melbourne, Victoria, Australia.*

Background: The autoimmune regulator (AIRE) transcription factor is important in the maintenance of central tolerance. AIRE has been found to regulate the promiscuous presentation and expression of tissue-restricted antigens in medullary thymic epithelial cells. The role of central tolerance mechanisms in the maintenance of tolerance to the potential auto-antigen, MPO is unknown. This study aims to define the role of AIRE in nephritogenic autoimmunity to myeloperoxidase (MPO).

Methods: We compared AIRE and MPO mRNA expression in isolated thymic cell subpopulations in AIRE-/- and AIRE+/+ littermate control C57Bl/6 mice. We then assessed the development of induced MPO autoimmunity and renal injury among AIRE-/- and AIRE+/+ mice in a model of autoimmune anti-MPO GN.

Results: AIRE and MPO mRNA were predominately expressed in thymic medullary epithelial cells. MPO mRNA was not detectable in AIRE-/- thymii. Compared with AIRE+/+ littermates, AIRE-/- mice immunized with MPO and developed significantly greater MPO autoimmunity with increased ANCA titres (0.59±0.05 vs 0.37±0.04 OD450nm), greater frequency of IFNγ-producing CD4+ cells (234±33 vs 130±16 ELISPOT) and delayed type hypersensitivity responses to MPO (0.1±0.01 vs 0.03±0.001 mm). Associated with increased autoimmunity, GN in AIRE-/- mice was significantly enhanced (glomerular CD4+ cells: 0.6±0.1 vs 0.3±0.1 cells/glomerular cross section [c/gcs]; macrophages: 1.1±0.2 vs 0.5±0.04 c/gcs) and proteinuria (1.6±0.3 vs 0.2±0.1 relative score), all $p<0.05$.

Conclusions: This study highlights the importance of AIRE in controlling the maintenance of central tolerance to MPO and deficiency in AIRE resulting in enhanced autoimmunity to MPO and increased severity of GN in a model of autoimmune anti-MPO focal necrotizing GN.

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FR-PO843

Defective Treg Function Exacerbated by Expansion of a Suppression-Resistant Effector Population in Human ANCA Disease Meghan E. Free,[1] Donna O. Bunch,[2] Elisabeth Berg,[2] Madelyn Burkart,[2] Susan L. Hogan,[2] Yichun Hu,[2] Gloria A. Preston,[2] J. Charles Jennette,[1,2] Ronald J. Falk,[2] Maureen Su.[3] *[1]Pathology and Laboratory Medicine, UNC Chapel Hill, Chapel Hill, NC; [2]UNC Kidney Center, UNC Chapel Hill, Chapel Hill, NC; [3]Pediatrics, UNC Chapel Hill, Chapel Hill, NC.*

Background: The development of pathogenic anti-neutrophil cytoplasmic autoantibodies (ANCAs) can result in systemic small vessel vasculitis. However, the breakdown in immune tolerance that results in the induction and persistence of ANCAs is not well-understood. We hypothesized that abnormal T cell regulation is central to disease pathogenesis and demonstrate here two separate abnormalities in T cell regulation in patients.

Methods: Analyses were performed using flow cytometry on lymphocytes stained with appropriate antibodies. Functional studies were completed using a standard suppression assay.

Results: First, we show that the Treg frequency in the peripheral blood of active disease patients is increased, but have decreased suppressive function. Tregs from active disease patients disproportionately utilize a FOXP3 isoform lacking exon 2, which may alter Treg function. Second, we identify a markedly expanded CD4+ T cell population that is resistant to Treg suppression, produces pro-inflammatory cytokines, and is antigen-experienced.

Conclusions: Thus, not only is the suppressive network of Tregs disrupted, but a distinct pro-inflammatory effector T cell subset comprises the majority of peripheral CD4+ T cells in ANCA disease patients.

Funding: NIDDK Support

FR-PO844

Detection of Newly Synthesized MPO and PR3 Protein in Peripheral Neutrophils from Patients with ANCA Vasculitis Elizabeth A. Alderman, Anshul K. Badhwar, Jia Jin Yang, Olivier Lardinois, J. Charles Jennette, Ronald J. Falk, Gloria A. Preston. *UNC Kidney Center, UNC-CH.*

Background: Generally, mature neutrophils are considered pre-apoptotic cells minimally synthesizing RNA and protein. Studies of ANCA vasculitis revealed neutrophils are environmentally-responsive cells whose normal biology can be disrupted in disease. Peripheral neutrophils from patients in active disease were transcribing genes normally silenced during granulopoesis including autoantigens PR3 and MPO. We determine whether disease-associated transcripts of PR3 and MPO are translated.

Methods: Neutrophils were separated from blood of patients with ANCA disease (BVAS range 2-22), and control subjects. Cells were incubated (20hrs) in methionine free medium supplemented with methionine analogue, L-azidohomoalanine with or without anisomycin. PR3/MPO were immunopurified and the proteins that incorporated AHA were biotinylated using a Click chemistry approach. Biotin-labeled PR3/MPO was purified using an anti-biotin Ab and detected by western blot probed with streptavidin conjugated to alkaline phosphatase and validated using anti-PR3 and anti-MPO Abs. Gel purified product was analyzed by mass spectrometry. Transcript levels were monitored by QPCR.

Results: Neutrophils produce a plethora of newly translated proteins, absent when cultured with the protein synthesis inhibitor anisomycin. Seven patients were analyzed for translation of PR3 and MPO: PR3 ANCA (n=3) and MPO ANCA (n=4). QPCR values for PR3 ranged from 0.76 to 86.73 and MPO ranged from 46.37 to 384.99 fold above normal. Seven patients were positive for biotinylated PR3,and five positive for biotinylated MPO. Remission samples were negative. Mass spetrometry analysis confirmed de novo synthesize of PR3 and MPO. A patient with chronic neutropenia injected daily with GM-CSF served as a control**.** Although the patient had ample protein synthesis in cultured neutrophils, the patient was negative for PR3 and MPO message by TaqMan and negative for PR3 and MPO by western blot.

Conclusions: Neutrophils from patients are actively synthesizing proteins, including PR3 and MPO. These newly synthesized proteins may play a role in disease by increasing antigen availability.

FR-PO845

Toll Like Receptor 9 Enhances Glomerular Injury in Anti-Neutrophil Cytoplasmic Antibody Vasculitis Shaun A. Summers, Sharon Lee Ford, Poh-Yi Gan, Stephen R. Holdsworth, A. Richard Kitching. *Department of Medicine and Nephrology, Monash Medical Centre and Monash University, Melbourne, Victoria, Australia.*

Background: Neutrophils are early effector cells in ANCA vasculitis. Clinical studies suggest infections promote vasculitis, with Toll Like Receptors (TLRs) a link between infection and glomerulonephritis. We sought to define the role of TLR9 in ANCA vasculitis.

Methods: We used myeloperoxidase (MPO) deficient mice to generate MPO-ANCA. TLR9 was assessed in human and murine MPO-ANCA glomerulonephritis by immunofluorescence. C57BL/6 wild type (WT) mice were injected with: 1) TLR9 ligand (CpG-ODN) alone, 2) MPO-ANCA alone or 3) CpG-ODN+MPO-ANCA. Neutrophil recruitment was assessed after 5 hours. TLR9$^{-/-}$ mice were injected with CpG-ODN+MPO-ANCA. WT and TLR9$^{-/-}$ mice were irradiated and reconstituted with WT or TLR9$^{-/-}$ bone marrow cells (BM) to generate bone marrow chimeras. WT BM→ WT mice (BM+ Tissue Cell[TC]+), TLR9$^{-/-}$BM→WT mice (BM-TC+) and WT BM→TLR9$^{-/-}$mice (BM+TC-) all received CpG-ODN+MPO-ANCA.

Results: TLR9 immunostaining is increased in murine and human biopsies with active MPO-ANCA glomerulonephritis. TLR9 ligation enhances MPO-ANCA induced glomerular neutrophil recruitment. Compared to WT mice receiving CpG-ODN or MPO-ANCA alone, neutrophil recruitment synergistically increased after CpG-ODN+MPO-ANCA (CpG-ODN alone 0.3±0.1, MPO-ANCA alone 0.9±0.1, CpG-ODN+MPO-ANCA 1.5±0.1 neutrophils/ glomerular cross section (n/gcs), P<0.01). Enhanced neutrophil recruitment corresponded with increased kidney mRNA CXCL1, CXCL2 and TNF expression. Control oligonucleotides (GpC-DNA) did not affect recruitment. In TLR9$^{-/-}$ mice receiving CpG-ODN+MPO-ANCA, neutrophil recruitment (WT 1.4±0.1 vs. TLR9$^{-/-}$ 0.6±0.1n/gcs, P<0.001) and kidney mRNA expression (CXCL1, CXCL2, TNF) decreased. In chimeric mice, neutrophil recruitment was similarly decreased in BM-TC+ and BM+TC- mice (BM+TC+1.7±0.2, BM-TC+0.8±0.1, BM+TC-1.0±0.1n/gcs, P<0.001). In further experiments functional and histological renal injury was increased in WT mice 6 days after receiving CpG-ODN+MPO-ANCA, compared to mice receiving either CpG-ODN or MPO-ANCA alone.

Conclusions: TLR9 ligation enhances glomerulonephritis in ANCA vasculitis.

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FR-PO846

Histone H3K9 Methylation Contributes to the Epigenetic Silencing of Proteinase 3 (PR3) and Myeloperoxidase (MPO) Genes in Patients with ANCA Disease Jia Jin Yang,[1] Kerry R. Colby,[1] Chao Guo,[1] Caroline Jennette Poulton,[1] Elisabeth Berg,[1] Madelyn Burkart,[1] J. Charles Jennette,[2,1] Gloria A. Preston,[1,2] Ronald J. Falk,[1,2] Dominic J. Ciavatta.[3,1] *[1]Medicine, UNC-CH, Chapel Hill, NC; [2]Pathology, UNC-CH, Chapel Hill, NC; [3]Genetics, UNC-CH, Chapel Hill, NC.*

Background: We described a role for histone H3K27 methylation in the epigenetic silencing of PR3 and MPO autoantigen genes, and a defect in this mechanism in patients with ANCA disease (J Clin Invest 120: 3209). In this study, we investigated whether histone H3K9 methylation, a repressive histone modification, might also be disrupted in ANCA patients.

Methods: Expression levels of H3K9 methyltransferase genes, EHMT1 and EHMT2 (G9a), were determined by Affymetrix microarray (Array) and quantitative PCR (Q-PCR), and H3K9 trimethylation (H3K9me3) level was measured by chromatin immunoprecipitation (ChIP).

Results: Array analysis revealed that expression of EHMT1 (ANCA: 51±32 vs HC: 72±24, p=0.03) and EHMT2 (ANCA: 42±27 vs HC:60±11, p=0.005) were significantly depleted in leukocytes from ANCA patients (n=25) compared to healthy controls (n=16). The expression levels of EHMT1 and EHMT2 negatively correlated with PR3 and MPO mRNA level, which were markedly elevated in ANCA patients. Expression of EHMT1 was significantly lower in MPO-ANCA (n=12) than PR3-ANCA patients (n=13). Q-PCR confirmed the reduced expression of EHMT1 and EHMT2 in ANCA patients (n=80) compared to controls (n=20), and the negative correlations for EHMT1 and EHMT2 compared to PR3 and MPO genes. EHMT1 and EHMT2 mRNA levels were significantly lower in patients with active disease (n=40) than patients in remission (n=40). ChIP showed that levels of H3K9me3 were statistically depleted at the PR3 and MPO promoter regions in MPO-ANCA patients (n=7, PR3: 3.0±4.1% of input, p=0.01; MPO: 3.0±5.1, p=0.01) compared to controls (n=23, PR3: 21.2±28.6; MPO: 20.0±26.0), but not in PR3-ANCA patients (n=8, PR3: 20.7±28.2; MPO: 18.7±23.9).

Conclusions: These results further implicate epigenetic mechanisms in the regulation of ANCA autoantigen genes, and suggest that transcriptional control of autoantigen genes may differ in MPO-ANCA versus PR3-ANCA patients.

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FR-PO847

Combination Treatment of Beraprost and Telmisartan Remarkably Ameliorates Renal Dysfunction in Anti-Glomerular Basement Membrane Nephritis Rats Mitsuko Miyamoto, Fuko Matsuda, Shigeo Fujii, Hidenori Mochizuki, Hajimu Kurumatani. *Pharmaceutical Research Laboratories, Toray Industries, Inc, Kanagawa, Japan.*

Background: A prostacyclin analogue beraprost sodium or an angiotensin II type I receptor antagonist telmisartan has separately been reported to have renoprotective effects in rats. We examined the effect of the combination treatment of beraprost and telmisartan on chronic renal failure in progressed glomerulonephritis (GN) rats by concomitantly administering both drugs at the doses not showing clear effects.

Methods: GN was induced to rats by injecting anti-glomerular basement membrane antibody. Fourteen days after the induction, serum creatinine levels of GN rats were significantly elevated compared with those of normal rats. Then, we divided GN rats into four groups and started the administration of vehicle, beraprost (0.1 mg/kg, twice a day), telmisartan (3 mg/kg, once a day), or their combination.

Results: At day 35 after GN induction, systolic blood pressure was significantly reduced in the beraprost/telmisartan combination group compared with that of control group. At day 42 after GN induction, serum creatinine level significantly decreased in the beraprost/telmisartan group compared with that of control group, while beraprost or telmisaltan alone tended to improve serum creatinine levels, but it is not statistically-significant. In addition, the oxidative stress-related factors in the renal cortex, Nox2 and p47-phox subunits of NADPH oxidase, were decreased significantly in the beraprost/telmisartan group; inflammation-related factors monocytes/macrophages infiltration in the kidney and plasma level of MCP-1 were also decreased in the beraprost/telmisartan group. Furthermore, endothelial cell damage rather than podocyte damage in the kidney was found to be markedly ameliorated in the combination treatment of beraprost and telmisartan.

Conclusions: The combination treatment of beraprost and telmisartan is likely to ameliorate chronic renal failure in progressed GN more effectively than mono-treatment of each drug through both the endothelial protection and glomerular podocyte protection which have been reported in beraprost and telmisartan, respectively.

FR-PO848

The Immunodominant T Cell Goodpasture Epitope Induces Anti-Glomerular Basement Membrane Glomerulonephritis in Susceptible HLA-DR Transgenic Mice A. Richard Kitching,[1] Joshua D. Ooi,[1] Janet Chang,[1] Vadim Pedchenko,[2] Billy G. Hudson,[2] Stephen R. Holdsworth.[1] *[1]Medicine, Monash University, Clayton, VIC, Australia; [2]Medicine, Vanderbilt University Medical Center, Nashville, TN.*

Background: Anti-glomerular basement membrane (GBM) glomerulonephritis (GN) is strongly HLA-DRB1*15:01 associated. The autoantigen is the non-collagenous domain of the α3 chain of Type IV collagen (α3(IV)NC1), but the pathogenic T cell epitope in humans is not known.

Methods: These studies aimed to define the immunodominant and pathogenic T cell Goodpasture epitope using humanized mice deficient in all elements of mouse MHCII but transgenic for susceptible (HLA-DRB1*15:01; 1501tg) or non-susceptible (HLA-DRB1*01:01; 0101tg) human MHCII.

Results: Immunizing 1501tg or 0101tg mice with overlapping α3(IV)NC1 20-mers defined an HLA-DRB1*15:01 restricted T cell epitope ($\alpha3_{133-152}$). This epitope was immunoreactive in *ex vivo* recall responses by T cell proliferation and IFN-γ/IL-17A ELISPOT, but was non-reactive in 0101tg or C57BL/6 mice. Mouse $\alpha3_{133-152}$ and human $\alpha3_{132-151}$ were cross-reactive. Further studies refined the epitope to an 11-mer ($\alpha3_{136-146}$), including 4 critical residues. 1501tg mice immunized with human α3(IV)NC1 or C6 (an α1/α3 chimeric molecule with $\alpha3_{127-141}$ in the non-immunogenic α1 backbone) responded to $\alpha3_{136-146}$ but not $\alpha3_{9-28}$ (containing another putative epitope). However, α1(IV)NC1 or C2 (containing $\alpha3_{17-31}$)-immunized mice responded to neither peptide. Transferring $1x10^7$ $\alpha3_{136-146}$-specific CD4+ Th1 clones generated from 1501tg mice into naive 1501tg mice with LPS (0.5μg/g) induced crescentic GN (day 35: glomerular necrosis 59±6%, crescents 33±5%; control cells 0%), albuminuria (control cells 0.01±0.01, $\alpha3_{136-146}$ clones 0.44±0.03 mg/μmol creatinine, P<0.001) and glomerular CD4+ T cell and macrophage infiltrates. Active immunization with $\alpha3_{136-146}$ (100μgx3) induced GN in 1501tg.FcγRIIB-/- mice (Fcγ receptors are implicated in disease susceptibility) but not in 0101tg.FcγRIIB-/- mice (day 42). Similarly, α3(IV)NC1 immunization induced anti-GBM GN only in 1501tg. FcγRIIB-/- mice.

Conclusions: T cell autoimmunity and injury in anti-GBM GN are defined by the HLA-DRB1*15:01 restricted epitope $\alpha3_{136-146}$.

Funding: Government Support - Non-U.S.

FR-PO849

B7x Deficiency Exacerbates Renal Injury in Antibody Mediated Nephritis in Mice

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Background: B7x (B7-H4, B7S1) is an important member of the B7/CD28 superfamily of T cell costimulatory proteins. B7x is a negative regulator of $CD4^+$ and $CD8^+$ T cell activation and proliferation, and is primarily expressed in peripheral, non-lymphoid tissues.

Methods: We hypothesized that B7x may modulate the pathogenesis of antibody mediated nephritis through its effects on T cells. We induced nephritis in B7x-deficient (B7x-/-) (n=10) and B7x-WT B6 mice (n=10) by intravenous injection of nephrotoxic sera (rabbit anti-mouse glomerular antibodies).

Results: Following nephritis induction, we observed a significant increase in the levels of serum IgG (p=0.023), particularly of the IgG2b and IgG1 isotype, in B7x-/- mice as compared to WT mice. At the mRNA level, IL-23 (p=0.046), CCL2 (p=0.061) and CCR5 (p=0.045) was found to be upregulated in B7x-/- kidney tissue. Immunohistochemistry revealed increased glomerular infiltration of $CD3^+$ T lymphocytes (p=0.034) and $CD68^+$ macrophages (p=0.003) in B7x-/- mice kidneys. Furthermore, kidney histopathology was more severe in B7x-/- mice, in terms of infiltrating polymorphonuclear cells in glomeruli (p<0.0001) and interstitium (p=0.004), crescent formation, and tubular atrophy/dilatation. Finally, serum creatinine and BUN were elevated in the B7x-/- group receiving nephrotoxic sera, as compared to WT mice.

Conclusions: In conclusion, B7x plays a protective role in the pathogenesis of antibody mediated nephritis, likely through exerting an inhibitory effect on T cell activation. Our results provide the first demonstration of a potential role for modulation of the B7x signaling pathway as a new approach for the treatment of immune mediated nephritis.

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FR-PO850

Lipocalin-2 Is an Endogenous Inhibitor of Inflammation in Murine Nephrotoxic Serum Nephritis

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Background: Lipocalin-2 (Lcn-2) is involved in divergent processes such as acute kidney injury or bacterial host defence. Our study was designed to evaluate the functional role of Lcn-2 in nephrotoxic serum nephritis (NTS).

Methods: NTS was induced in wild-type (WT) and Lcn-2 knock-out mice as well as Lcn-2 chimeras.

Results: Mice lacking Lcn-2 exhibited more glomerular damage with increased proteinuria and interstitial leukocyte accumulation compared to WT mice. Since Lcn-2 is expressed in tubular epithelial cells as well as cells of innate immunity such as macrophages and polymorphonuclear neutrophils (PMN), we induced NTS in Lcn-2 chimeras. Chimeras with Lcn-2 expressed in macrophages and PMN were found to develop NTS comparable to wild-type controls. In contrast, chimeras with Lcn-2 expressed in tubular epithelial cells developed increased NTS due to decreased concerted apoptosis but increased necrosis and formation of damage-associated molecular patterns (DAMPs) such as high-mobility group box 1 (HMGB-1) in the kidney. *In vivo* blockade of HMGB-1, a toll-like receptor (TLR)-2 agonist, significantly reduced inflammation and NTS in Lcn-2 knock-out mice. *In vitro*, we found IL-6 to be significantly upregulated in immortalized tubular epithelial cells and macrophages by TLR-2 stimulation. In parallel, Lcn-2 was found to be increasingly transcribed by TLR-2 signalling *in vitro*.

Conclusions: Taken together, Lcn-2 expressed in innate immune cells is protective in NTS by inducing concerted apoptosis and inhibiting the formation of HMGB-1 thereby limiting cytokine production via TLR-2 signalling. In parallel, TLR-2 dependent transcription of Lcn-2 is an endogenous inhibitor of inflammation in NTS.

Funding: Government Support - Non-U.S.

FR-PO851

IL-6 Mediates Crescentic Glomerulonephritis via Differential Effects on Th17 Responses and Macrophage Activation

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Background: IL-6 is central for generation of pro inflammatory Th17 responses but also mediates anti inflammatory effects by dampening macrophage activation. Aim of the current study is to clarify the controversial role of IL-6 in crescentic glomerulonephritis, especially in the light of newly developed IL-6 directed therapies.

Methods: The Th17 and macrophage dependent model of nephrotoxic nephritis (NTN) was induced in wildtype and IL-6-/- mice. Antibodies for differential blockade of IL-6 signalling pathways were used.

Results: NTN induction in IL-6-/- mice resulted in abscence of nephritogenic Th17 responses and amelioration of disease. To dissect opposing effects of IL-6 on Th17 responses and macrophage activation, neutralization studies were performed. An IL-6 antibody was applied at a dose insufficient to block Th17 responses which in contrast to the results in IL6-/- mice aggravated NTN. Aggravation was most likely due to increased macrophage activation with augmented renal infiltration and TNFa production. To verify this hypothesis, aIL-6 treatment was started late in the macrophage dependent effector phase of NTN after Th17 responses had already developed. Again, IL-6 blockade aggravated nephritis and increased macrophage infiltration. Worsening of nephritis was not observed after treatment with sgp130Fc fusion protein which specifically blocks alternative but not classical IL-6 signalling.

Conclusions: In summary our data show that partial IL-6 blockade is not sufficient to suppress nephritogenic Th17 responses and aggravates nephritis via increased macrophage activation. This pro inflammatory effect is mediated by classical but not alternative IL-6 signalling. Complete lack of IL-6, however, prevents development of Th17 responses which overrules macrophage activation and ameliorates disease. Our studies identify IL-6 as differential mediator of crescentic GN and underline the central role of Th17 cells for disease pathogenesis.

Funding: Government Support - Non-U.S.

FR-PO852

Regulatory T Cell-Derived Interleukin-10 Protects Mice against Crescent Glomerulonephritis

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Background: Regulatory T cells (Tregs) exert their immunosuppressive activity through different immune-regulatory mechanisms including the production of anti-inflammatory cytokines such as IL-10. The suppressive mechanisms of Tregs in renal autoimmune disease, however, remains to be elucidated. In the present study we investigate the role of Treg cell-derived IL-10 in the T cell-dependent murine glomerulonephritis (GN) model of nephrotoxic nephritis (NTN).

Methods: NTN was induced by intraperitoneal injection of nephrotoxic sheep serum in mice. To identify renal IL-10-producing $Foxp3^+$ Tregs we used transgenic reporter (FIR x *tiger*) mice, which enable simultaneous detection of IL-10 and Foxp3 via flow cytometry. To analyze the role of $IL\text{-}10^+$ Tregs we first adoptively transferred $CD4^+CD25^+$ Tregs from either naïve wt C57BL/6 mice or $IL\text{-}10^{-/-}$ mice in nephritic mice. Furthermore, we performed analyses in nephritic $Foxp3^{Cre}$ x $Il10^{flox/flox}$ mice carrying a specific deletion of *Il10* in $Foxp3^+$ Tregs.

Results: By using the FIR x *tiger* mice we identified a significant frequency of IL-10-producing Tregs in the kidney, which was increased upon NTN induction. Adoptive cell transfer experiments revealed a protective effect by wt but not $IL\text{-}10^{-/-}$ Tregs in NTN mice since wt Tregs dampened glomerular crescent formation and albumin/creatinine ratio, whereas $IL\text{-}10^{-/-}$ Tregs did not. Moreover, nephritic mice deficient of endogenous $IL\text{-}10^+$ Tregs exhibit an increased renal and systemic Th1 and most notably Th17 immune response, as determined by flow cytometry, qRT-PCR and ELISA. This was associated with an aggravated course of glomerulonephritis as measured by glomerular crescent formation and albumin/creatinine ratio.

Conclusions: In conclusion, our results contribute to the understanding of the suppressive mechanisms of Tregs in crescentic glomerulonephritis by highlighting the indispensability of Treg-derived IL-10 in reduction of disease severity and regulation of the Th17 immune response. Hence, the immunosuppressive cytokine IL-10 has to be kept in mind with respect to therapeutic approaches in GN.

FR-PO853

Serum Starved Adipose-Derived Stromal Cells Ameliorate Rat Crescentic Glomerulonephritis by Promoting the Generation of M2 Immunoregulatory Macrophages Kazuhiro Furuhashi, Naotake Tsuboi, Hangsoo Kim, Takayuki Katsuno, Waichi Sato, Enyu Imai, Seiichi Matsuo, Shoichi Maruyama. *Nephrology, Nagoya University, Nagoya, Aichi, Japan.*

Background: We have reported that adipose tissue-derived stem cells (ASC) promoted regeneration in a rat model of acute kidney injury and more recently that ASC more strongly modulate T-cell immune reaction than bone marrow derived mesenchymal stem cells (BM-MSC). In the present study, we examined the renal protective effects of ASC focusing on their immunomodulatory properties in anti-GBM GN.

Methods: Necrotizing crescentic glomerulonephritis was induced in WKY rats by intraperitoneal injection of anti -GBM mAb. Renal function and histology were assessed in animals treated with ASC or BM-MSC.To evaluate ASC-driven functional M2 polarization in macrophage(MΦ), we cultured peritoneal MΦ with ASCs or BM-MSC.

Results: Intravenous injection of ASC significantly prevented renal dysfunction and proteinuria in diseased animals. Crescent formation was significantly decreased in ASC group compared to control group. Interestingly, infiltration of M2 MΦ in glomeruli was increased only in ASC group despite comparable number of infiltrated MΦ to control group. In vitro co-culture system clearly demonstrated that ASC, but not BM-MSC, directly turned MΦ into M2 phenotype. Moreover, these effects of ASC were more prominent in low serum cultured ASC (LASC) than high serum cultured ASC (HASC). The number of ASC in glomeruli was 1.5/glomerulus/cross section without any difference between HASC and LASC, but this would be efficient since individual LASC could polarize around 200 MΦ to M2 phenotype in vitro. These results collectively suggest that LASC recruited into diseased glomerulus make stronger effect on MΦ for protection of glomerular injury than HASC. Moreover we found PGE2 derived from LASC induced MΦ into M2 phenotype.

Conclusions: ASC exerted profound immunoregulatory properties especially on MΦ by PGE2 and ameliorated glomerular injury in rat anti-GBM GN model. In human immunosuppressive therapy is often restricted for their adverse effect. Therefore, LASC administration would be desirable therapeutic approach to improve prognosis of anti-GBM GN patients.

FR-PO854

Th17/IL-17 Immune Response Drives CXCL5 Dependent Neutrophil Infiltration and Renal Damage during Crescentic Glomerulonephritis Erik M. Disteldorf,[1] Geraldine Nouailles,[3] Hans-Joachim Paust,[1] Anett Peters,[1] Sabrina Bianca Bennstein,[1] Hans-willi Mittrücker,[2] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]Universitätsklinikum Hamburg Eppendorf, Nephrology, Hamburg, Germany; [2]Universitätsklinikum Hamburg Eppendorf, Immunology, Hamburg, Germany; [3]Max-Planck-Institut, Department for Infectious Biology, Berlin, Germany.*

Background: The Th17/IL-17 immune response plays a pivotal role in the pathogenesis of autoimmune diseases including human and experimental crescentic glomerulonephritis. The mechanism of Th17/IL-17-induced tissue injury, however, remains to be fully elucidated.

Methods: We used a murine model of crescentic glomerulonephritis (nephrotoxic nephritis).

Results: Here we show that the Th17/IL-17 immune response is paralleled by the expression of the chemokine CXCL5 in tubular cells and consecutive tubulointerstial infiltration of pathogenic $CXCR2^+$ neutrophils. In line with this, IL-17A strongly induced CXCL5 expression in tubular cells, and renal CXCL5 levels and neutrophil recruitment were markedly reduced in nephritic $IL\text{-}17^{-/-}$ mice and in $RAG1^{-/-}$ mice transferred with $IL\text{-}17^{-/-}$ lymphocytes. To test whether CXCL5 plays a functional role in renal inflammation, we generated CXCL5-deficient mice and induced nephritis in $CXCL5^{-/-}$ and wild-type mice. CXCL5-deficient mice developed less severe nephritis in terms of reduced renal neutrophil infiltration, less renal tissue injury and better preserved renal function. Finally we show that CXCL5 expression is highly up-regulated in the kidneys of patients with crescentic glomerulonephritis.

Conclusions: Our data therefore identify a unique role of CXCL5 in Th17-mediated glomerulonephritis and suggest that CXCL5 may be an intriguing therapeutic target.

FR-PO855

Deficiency of Limp-2, an Integral Lysosomal Membrane Protein, Attenuates Renal Injury in Experimental Crescentic Glomerulonephritis Darren H.K. Lee,[1,2] Poh-Yi Gan,[3] Marina Katerelos,[4] Scott Andrew Fraser,[4] Kurt Gleich,[4] Stephen R. Holdsworth,[3] David A. Power.[1,2,4] *[1]Department of Nephrology, Austin Health, Melbourne, Victoria, Australia; [2]Department of Medicine, Austin Health, University of Melbourne, Melbourne, Victoria, Australia; [3]Centre of Inflammatory Diseases, Department of Medicine, Monash University, Monash Medical Centre, Melbourne, Victoria, Australia; [4]The Institute of Breathing and Sleep, Austin Health, Melbourne, Victoria, Australia.*

Background: Deficiency of lysosomal membrane protein SCARB2 (Limp-2 as murine homologue) causes FSGS in humans, and tubular proteinuria in humans and mice. Limp-2 deficiency in mice, however, leads to failure of fusion of phagosomes with lysosomes, defective macrophage activation and innate immunity in response to *Listeria infection.*

Methods: To define the role of Limp-2 in experimental crescentic glomerulonephritis (GN), wild-type (WT) and $Limp\text{-}2^{-/-}$ littermates received i.p. injections of 2 mg of nephrotoxic sheep serum per gram of body weight. Renal injury and immune response were assessed at day 14.

Results: Compared with WT, $Limp\text{-}2^{-/-}$ mice had significantly reduced crescent formation (15.6±10.2% vs 40.2±22.5%, $P<0.005$), interstitial inflammation ($P<0.05$) and a trend for reduced tubulointerstitial injury. On day 1, urinary albumin/creatinine ratio was significantly increased in WT (4921±5180 vs 18±11 mg/mmol, $P<0.05$) but not $Limp\text{-}2^{-/-}$ mice (1655±1842 vs 315±173 mg/mmol) compared to baseline. At day 14, albuminuria and renal function were similar. There was, however, a significant reduction in the influx of glomerular macrophages (0.6±0.2 vs 1.2±0.4 cells per glomerular cross section (c/gcs), $P<0.05$) and $CD4^+$ T cells (0.2±0.1 vs 0.6±0.2 c/gcs, $P<0.05$) in $Limp\text{-}2^{-/-}$ vs WT mice. Renal MCP-1 mRNA expression was also reduced ($P<0.05$). Systemic humoral immune response, determined by glomerular mouse IgG deposition and mouse anti-sheep IgG subclass production, was similar in both groups.

Conclusions: The data suggest that Limp-2 is essential in mediating the local immune response in experimental crescentic GN. The likely reason is a failure of macrophage activation and cytokine release. This study identifies a novel role for a lysosomal protein in autoimmunity.

FR-PO856

Lipoxin A4 and Resolvin E1 Reduce the CD16+ Pro-Inflammatory Subsets of Monocytes Eileen Nolan,[1,2] Debra F. Higgins,[1] Yvonne M. O'Meara,[2,3] Catherine Godson.[1,2] *[1]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4, Ireland; [2]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland; [3]Mater Misericordiae University Hospital, Dublin 7, Ireland.*

Background: Monocytes are a structurally and functionally heterogeneous cell population, with three subsets defined on the basis of their surface expression of CD14 and CD16. The classical subset is CD16- and produces anti-inflammatory IL-10, whereas the intermediate and non-classical subsets are CD16+ and produce the inflammatory cytokines TNF-α and IL-6. Expansion of CD16+ monocyte populations occurs in CKD and is associated with chronic inflammation and increased cardiovascular mortality. Lipoxins and resolvins are endogenously-produced eicosanoids with established anti-inflammatory and pro-resolution properties. The purpose of this study was to determine whether lipoxin A_4 (LXA_4) and resolvin E1 (RvE1) would prove effective in modulating monocyte responses to inflammatory stimuli.

Methods: The human cell line THP-1 was used as a model of monocytes. Cells were treated with LXA_4 (10 nM), RvE1 (1 pM), or vehicle (0.1% EtOH) for 30 minutes, before exposure to pro-inflammatory stimuli (IFN-γ 200 ng/ml and LPS 0.1 ng/ml). Cells were stained with monoclonal antibody to CD16 and analysed by flow cytometry. Cytokine content of cell supernatants was assayed by ELISA.

Results: Stimulation of THP-1 monocytes with IFN-γ and LPS resulted in increased expression of CD16 and increased production of both TNF-α and IL-6. A reduction in CD16 expression was observed following exposure to LXA_4 ($p<0.01$) or RvE1, suggesting that these pro-resolution mediators reduce inflammatory monocyte subsets. Treatment of monocytes with RvE1 or LXA_4 also resulted in reduced production of TNF-α ($p<0.01$) and IL-6 ($p<0.05$), as compared with IFN-γ and LPS-stimulated cells, suggesting that these agents attenuate pro-inflammatory monocyte behaviour.

Conclusions: Our results suggest that both lipoxin A_4 and resolvin E1 may have therapeutic potential in reprogramming pro-inflammatory monocytes, thereby reducing their detrimental influence in kidney disease.

Funding: Government Support - Non-U.S.

FR-PO857

Characterization of the Renal CD4+ T-Cell Response in Experimental Autoimmune Glomerulonephritis Hans-willi Mittrücker,[1] Stefanie Hünemörder,[1] Julia Holzer,[1] Hans-Joachim Paust,[2] Ulf Panzer,[2] Helmut Hopfer.[3] *[1]Institute for Immunology, University Medical Center Hamburg-Eppendorf, Germany; [2]Department of Internal Medicine III, University Medical Center Hamburg Eppendorf, Germany; [3]Institute for Pathology, University Hospital Basel, Switzerland.*

Background: Anti-glomerular basement membrane (GBM) glomerulonephritis results from an autoimmune reaction against the Goodpasture antigen, the non-collagenous domain of the alpha3 chain of type 4 collagen (alpha3IV-NC1). The production of autoantibodies and their deposition along the basement membrane of glomeruli and pulmonary alveoli are central to pathogenesis of disease. However, there is also increasing evidence from clinical and experimental data for a contribution of T cells to disease development.

Methods: We use the mouse model of experimental autoimmune glomerulonephritis (EAG) to analyze the role of T cells in renal destruction.

Results: Following immunization with recombinant alpha3IV-NC1, DBA/1 mice display a pathogenesis that reflects all hallmarks of human anti-GBM glomerulonephritis. We observe an initial alpha3IV-NC1-specific IgG response with deposition of autoantibodies along the GBM and progressive proteinuria. In this stage, the kidney shows only marginal signs of inflammation. After 9-13 weeks, mice develop severe signs of glomerulonephritis including crescent formation as well as massive tubulointerstitial damage with accumulation of T cells and macrophages. Renal inflammation eventually results in a severe loss of kidney function accompanied by massive edema formation. CD4+ T lymphocytes isolated from the kidneys of end stage EAG mice display a highly activated phenotype and produce

TNFalpha, IFNgamma and IL-17A upon polyclonal in vitro stimulation. Using highly sensitive FACS-based techniques, we can also detect renal CD4+ T cells that produced cytokines in response to stimulation with alpha3IVNC1.

Conclusions: From our data, we conclude that renal accumulation of autoreactive T cells might play a role in the progression from mild renal inflammation to severe glomerulonephritis with cell-mediated destruction of glomerular structures and severe tubulointerstitial inflammation.

Funding: Government Support - Non-U.S.

FR-PO858

Experimental Study of Application of Anti-Glomerular Basement Membrane Antibodies Neutralizing Monoclonal Antibody on Anti-Glomerular Basement Membrane Nephritis Rats

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Background: The development of anti-GBM disease is often sudden onset,extremely dangerous,and the prognosis is poor. On the anti-GBM nephritis rats, we try to use the neutralizing monoclonal antibodies to the anti-GBM antibody to improve the progression of the disease,aim to observe the effect of the neutralizing monoclonal antibodies to the anti-GBM antibody on anti-GBM nephritis rats.

Methods: Wistar rats were randomly divided into five groups:(1)Anti-GBM nephritis group was injected with human anti-GBM antibody via the caudal vein, meanwhile the same dose of Freund's complete adjuvant(FCA) was injected.(2)Control group I was injected with healthy human IgG via the caudal vein.(3)Control group II was injected with neutralizing monoclonal antibodies to anti-GBM antibody via the caudal vein, also the same dose of FCA.(4)Intervention group I was injected with human anti-GBM antibody, the same dose of FCA via the caudal vein and then neutralizing monoclonal antibodies to anti-GBM antibody at day 7.(5)Intervention group II was injected with human anti-GBM antibody, the same dose of FCA via the caudal vein and then neutralizing monoclonal antibodies to anti-GBM antibody at day 14.At day 7, 14 and 21, serum ,urine and renal specimen were collected for analysis.

Results: At day 21, there were significant decreases in intervention group I compared with anti-GBM nephritis group in 24-hours proteinuria[(16.62±5.53)g], BUN[(11.53±2.26) mmol/L] and Scr[(102.46±16.86)umol/L](P<0.05), and also in intervention group II as compared to anti-GBM nephritis group,but no significant difference was found(P>0.05). There was obvious decrease of renal cell proliferation,crescent formation and deposition of immune complexes in intervention group I and intervention group II compared with anti-GBM nephritis group, while such improvement in intervention group I was more significant. There was no significant change in control group I and control group II.

Conclusions: Early application of neutralizing monoclonal antibodies to anti-GBM antibodies can effectively improve the kidney lesion of anti-GBM nephritis rats.

Funding: Government Support - Non-U.S.

FR-PO859

Beraprost Sodium Improves Survival Rates in Anti-Glomerular Basement Membrane Glomerulonephritis and 5/6 Nephrectomized CKD Rats

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Background: In order to clarify whether beraprost sodium inhibits the progress to end-stage renal disease, the effects of beraprost sodium on survival rates in two rat chronic kidney disease (CKD) models were evaluated.

Methods: (A) 5/6 nephrectomized CKD rats

Beraprost sodium was administrated at a dose of 0.6 mg/kg/day from Day 29 after 5/6 nephrectomized (Nx) operation. Death or serum creatinine doubling time of each rat was evaluated, and the slope of plots of reciprocal serum creatinine was also calculated as the renal function.

(B) Glomerulonephritis (GN) rats induced by anti-glomerular basement membrane serum

Beraprost sodium was administrated at a dose of 0.2 or 0.6 mg/kg/day from Day 5 after GN induction when serum creatinine increased significantly.

Results: In 5/6 Nx CKD rats, 190 days after operation, survival rates of vehicle and beraprost sodium groups were 4 and 32%, respectively. In GN rats, 70 days after nephritis induction, survival rates of vehicle, beraprost sodium 0.2 and 0.6 mg/kg/day groups were 17, 50 and 83%, respectively. In both models, beraprost sodium improved survival rates statistically significantly. In 5/6 Nx rats, the slopes of reciprocal of serum creatinine of beraprost sodium groups were sweeper than those of vehicle group, suggesting that beraprost sodium suppressed the reduction of renal function.

Conclusions: In this study it was confirmed that beraprost sodium improved the survival rates in both anti-GBM GN and 5/6 Nx rats; therefore, beraprost sodium is likely to suppress the progression of CKD to ESRD.

FR-PO860

The mTOR-Inhibitor Rapamycin Mediates Proteinuria in Nephrotoxic Serum Nephritis by Activating the Innate Immune Response

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Background: The mTOR inhibitor Rapamycin (Rapa) is currently used in the prevention of allograft rejection in renal transplantation. Proteinuria is a limiting factor in the clinical use of Rapa. We have previously shown that Rapa deteriorates murine nephrotoxic serum nephritis (NTS), when application is started 14 days after disease induction. This study was designed to study the glomerular effects of Rapa in NTS in order to improve the understanding of Rapa-induced proteinuria.

Methods: Glomeruli of nephritic Rapa- or vehicle-treated mice were laser-microdissected and qRT-PCR was performed to study the expression of glomerular cell markers, inflammatory chemokines and cytokines. Immunohistochemistry was performed to assess glomerular infiltration by inflammatory cells and to assess viability of podocytes and glomerular endothelial cells.

Results: The increase in urinary albumin/creatinine ratio in Rapa-treated compared to control animals went along with a more prominent glomerular infiltration by $CD4^+$ T cells and macrophages. Glomeruli from animals treated with Rapa showed increased mRNA levels of the proinflammatory cytokines IL-6 and TNFα, as well as the chemokines MCP-1 and MIP-1β and their cognate receptors CCR2 and CCR5, both of which are expressed on monocytes. Also, we detected a higher transcription level of the regulatory T cell transcription factor Foxp3 in the Rapa-group, while the expression levels of the Th1 marker t-bet and the Th17 marker RORγt were unchanged.

To assess the effect on resident glomerular cell types, we studied the expression of the podocyte marker nephrin, the endothelial-cell marker CD31 as well as the growth factor VEGF-A and did not find any differences in their glomerular expression on mRNA or protein level.

Conclusions: In conclusion, these data suggest that the Rapa-mediated increase in proteinuria in the setting of murine NTS is a result of the activation of the innate immune system rather than a direct cytotoxicity to prodocytes or glomerular endothelial cells.

Funding: Pharmaceutical Company Support - Pfizer

FR-PO861

Leukocyte-Derived Matrix Metalloproteinase 9 Is Crucial for the Recruitment of Monocytes/Macrophages in Experimental Glomerulonephritis

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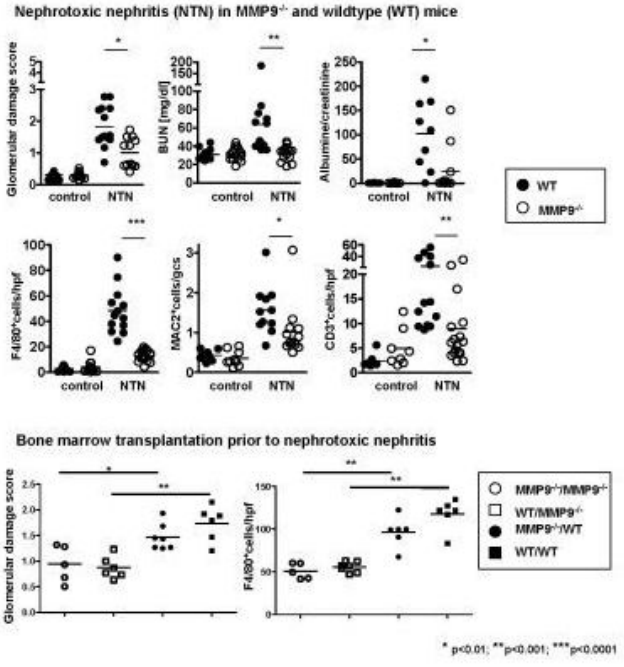
Background: Matrix Metalloproteinase 9 (MMP9) is a conditionally expressed collagenase and upregulated in glomerulonephritides. Its ultimate function in these diseases is, however, still unclear. In this study, we investigated the leukocyte-specific role of MMP9 in crescentic nephrotoxic nephritis (NTN) in mice.

Methods: Nephrotoxic nephritis (NTN) was induced in 10 wk-old male $MMP9^{-/-}$ and $MMP9^{+/+}$ mice by injection of nephrotoxic sheep serum (C57BL/6 background, microsatellite analysis). Albuminuria and BUN were determined at day 10. Kidneys were harvested for immunohistochemistry, RT-PCR or flow cytometry. Migratory capacity of peritonitis-elicited macrophages was assessed in chemotactic-assays. The specific function of leukocyte-derived MMP9 was investigated performing bone marrow transplantation prior to the induction of NTN.

Results: NTN in wildtype mice resulted in upregulation of MMP9 followed by profound leukocyte infiltration, albuminuria, glomerular crescents and renal failure. MMP9-deficiency ameliorated the course of NTN and significantly reduced histological injury. The absence of MMP9 attenuated the infiltration of $F4/80^+CD11b^+$ macrophages as determined by flow cytometry and immunohistochemical analyzes. Migration of MMP9-deficient macrophages was impaired in chemotactic assays. Bone marrow transplantation was able to restore renal tissue injury and macrophage recruitment when wildtype-derived donor cells were transplanted onto MMP9-deficient mice prior to the induction of NTN.



Conclusions: MMP9-deficiency ameliorates renal impairment during nephrotoxic nephritis in C57BL/6 mice by attenuating migration and renal infiltration of monocytes/macrophages. As bone marrow transplantation studies show, this effect is mediated by leukocyte-derived MMP9 and not by resident kidney cells.

FR-PO862

Comprehensive Re-Classification of Resident Renal Mononuclear Phagocytes Identifies 5 Subpopulations, One of Which Is an IL-10 Producing Reno-Protective Subpopulation Takahisa Kawakami, Prasanthi Karna, Peter J. Nelson, Jeremy Stuart Duffield. *Division of Nephrology, Institute for Stem Cell & Regenerative Medicine, University of Washington, Seattle, WA.*

Background: Monocytes, Macrophages and Dendritic Cells (DCs), although lineally linked, have been thought to be separate cell types. However, there are no definitive discriminatory markers, & their functions & origins are increasingly recognized to be overlapping. Recently, we proposed that they be unified & re-classified as Renal Mononuclear Phagocytes (MPCs) (Nelson et al JASN 2012 23:194). The function, ontogeny & fate of resident MPCs is poorly understood but they play critical roles not only in inflammatory diseases but also in nephrogenesis, angiogenesis, & the maintenance of homeostasis and tolerance in tissues, including kidney.

Methods: We developed a 7-color flow cytometry assay to comprehensively analyze MPCs in kidney using an unbiased approach, in which granulocytes, B cells, T cells, and NK cells are excluded.

Results: Analysis of MPCs simultaneously by CD11b and CD11c expression identified 5 subpopulations: CD11b^{hi} CD11c^{hi} (H-H), CD11b^{hi} CD11c^{lo} (H-L), CD11b^{int} CD11c^{int} (I-I), CD11b^{int} CD11c^{hi} (I-H), CD11b^{lo} CD11c^{int}, and a small CD11b- CD11c- population of undefined cells. Each subpopulation demonstrated marked differences in other surface markers: e.g. I-I MPCs are F4/80^{hi} while H-H and H-L are F4/80^{int}. H-H, I-I, and I-H are MHC-II^{high}. Further, I-H cells exclusively express CD103, an integrin associated with cells thought to function as DCs in non-lymphoid tissues. Transcriptional analysis of sorted populations identified other marked differences: e.g. H-H cells expressed high levels of CCR7 (p=0.017), found on cells that migrate to lymph nodes. Using a novel mouse model that reports IL-10 producing cells, in normal kidney, IL-10 expressing cells reside almost exclusively in the I-I population. For each of these populations we will additionally report ontogeny, relative capacity to induce tolerance or activate T cell responses, phagocytose, & follow their cell fate in response to injury.

Conclusions: The renal MPCs comprise 5 phenotypically and functionally distinct subpopulations which share macrophage and DC properties.

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FR-PO863

Glomerular IgM Exacerbates Glomerular Disease Progression Sarah E. Panzer,[1] Matthew C. Pickering,[2] Joshua M. Thurman.[1] *[1]Renal Diseases and Hypertension, University of Colorado Denver, Denver, CO; [2]Imperial College, London, United Kingdom.*

Background: Glomerular deposits of IgM and the complement protein C3 are observed in a variety of proteinuric renal diseases. The clinical significance of IgM in the glomerulus is a source of debate. We hypothesized IgM binds to neoepitopes in the diseased glomerulus and tested whether IgM plays a role in the progression of glomerular disease.

Methods: Mice with a targeted genetic deletion of factor H (fH$^{-/-}$) were used as a model of non-immune complex mediated glomerular disease. We crossed these mice onto μMT mice, which lack B cells and therefore have no ability to produce IgM, generating fH/μMT double knockout mice. Urine, blood, and renal tissue were harvested at 9 months of age. Urine albumin was determined by ELISA and BUN and Cr were measured. Glomerular IgM and C3 were assessed by immunofluorescence on frozen kidney sections. In addition, a murine mesangial cell line was used to test whether IgM in normal mouse serum binds to epitopes on mesangial cells by flow cytometry.

Results: Glomerular IgM deposition progressively accumulated in fH$^{-/-}$ mice, expanding throughout the glomerular tufts as the mice aged. Albuminuria in fH/μMT mice was attenuated at 9 months compared to fH$^{-/-}$ mice (1596 ng/mg Cr, N=10 vs 2847 ng/mg Cr, N=13, P = 0.065). Glomerular C3 deposits were similar among fH$^{-/-}$ and fH/μMT mice (mean fluorescence intensity 55.3 vs 56.5, P=NS). Also, flow cytometry of cultured murine mesangial cells exposed to serum demonstrated binding of IgM to the cells, but no binding of IgG.

Conclusions: In the fH$^{-/-}$ model we demonstrate IgM binds within the injured mesangium as disease progresses. Mice deficient in B cells develop milder disease. We also demonstrate that, *in vitro*, IgM binds cultured murine mesangial cells. Our data suggest glomerular IgM deposition occurs after injury, which may expose mesangial epitopes. IgM specific for these epitopes appears to contribute to disease progression. Glomerular IgM may provide an important new therapeutic target for slowing progression of glomerular disease.

Funding: NIDDK Support

FR-PO864

Immune Tolerance toward NC1 Hexamers of α3α4α5(IV) Collagen Is Selectively Breached by Proteolysis: A Mechanism Eliciting Non-Nephritogenic Anti-GBM Autoantibodies Florina Olaru,[1] Xu-Ping Wang,[1] Wentian Luo,[1] Linna Ge,[1] Jeffrey H. Miner,[3] Andrew Wasiluk,[2] Xochiquetzal J. Geiger,[2] Dorin-Bogdan Borza.[1] *[1]Vanderbilt University School of Medicine, Nashville, TN; [2]Mayo Clinic Hospital, Jacksonville, FL; [3]Washington University, St. Louis, MO.*

Background: Goodpasture/anti-GBM disease usually presents as rapidly progressive glomerulonephritis (GN) associated with autoAbs against the NC1 domain of α3(IV) collagen. Rare atypical presentations can provide new insights into the disease pathomechanisms.

Methods: We characterized novel anti-GBM autoAbs associated with atypically mild, non-progressive GN. The mechanisms breaching self-tolerance to α3α4α5(IV) collagen and eliciting non-nephritogenic anti-GBM autoAbs were determined using mouse models.

Results: We identified a novel type of anti-GBM autoAbs that specifically bound to α3α4α5NC1 hexamers but not monomeric NC1 subunits, were restricted to human IgG4 subclass, and did not mediate rapid progressive GN. In both wild type and FcγRIIB-/- mice, non-nephritogenic anti-GBM autoAbs selectively targeting α3α4α5NC1 hexamers and restricted to mouse IgG1 subclass were induced by immunization with NC1 hexamers solubilized from murine GBM. Although mIgG1 autoAbs bound to the GBM of immunized mice, glomerular inflammation and C3 deposition were absent, while kidney function and histology remained normal. Unlike its NC1 hexamer fragment, intact α3α4α5(IV) collagen from murine GBM was not immunogenic in wild type mice, yet elicited robust IgG antibody responses in Alport mice lacking α3α4α5(IV) collagen.

Conclusions: These results reveal that self-tolerance toward NC1 hexamers of α3α4α5(IV) collagen is established in wild type but not Alport mice. Self-tolerance is selectively breached by limited proteolytic cleavage of the autoantigen, eliciting atypical anti-GBM autoAbs against NC1 hexamer epitopes accessible in the native GBM. This autoimmune response is associated with an extreme polarization of the anti-GBM autoAbs toward Th2-associated IgG subclasses (hIgG4, mIgG1), which likely accounts for their lack of nephritogenicity. Hence, complement-fixing subclasses of anti-GBM autoAbs are likely required for the development of rapid progressive GN.

Funding: NIDDK Support, Private Foundation Support

FR-PO865

Development of Novel In Vivo Model for Rapid Characterization of Allelic Variants in Systemic Lupus Erythematosus William Franklin Pendergraft,[1-3] Amit Prasad,[3] Terry K. Means,[1,3] Nir Hacohen.[1,3] *[1]Broad Institute, Cambridge, MA; [2]Joint Fellowship Program in Nephrology, Brigham and Women's Hospital and Massachusetts General Hospital (MGH), Boston, MA; [3]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Department of Medicine, MGH, Charlestown, MA.*

Background: With the advent of genome-wide association studies (GWAS) came tantalizing allelic variants in patients with systemic lupus erythematosus (SLE); however, a critical barrier to understanding their role, especially pertaining to lupus nephritis, is that their characterization has been limited in vivo. We address this critical barrier by creation of a temporally-controlled in vivo system of lupus nephritis-prone mice harboring an immune system reconstituted with hematopoietic bone marrow progenitor cells (HPCs) that either overexpress allelic variants or their respective short hairpin RNA (shRNA).

Methods: To validate this system, HPCs from mice constitutively overexpressing actin were infected with IPTG-inducible actin short hairpin RNA (shRNA) and injected into irradiated wild-type B6 mice after which reconstitution occurred followed by dietary IPTG administration. An identical method was then applied to HPCs from lupus-prone NZB/W F1 female mice using either myd88 shRNA or the doxycycline-inducible human allelic variant recently implicated in SLE, TREX1^{R114H} followed by disease activity monitoring.

Results: GFP expression was abolished after IPTG administration in wild-type B6 mice harboring an immune system reconstituted with hematopoietic cells overexpressing GFP and GFP shRNA. Anti-nuclear and anti-nucleosome autoantibodies did not develop in NZB/W F1 mice exposed to IPTG whose hematopoietic cells overexpressed myd88 shRNA, and autoantibody development occurred faster in those mice exposed to doxycycline whose hematopoietic cells overexpressed TREX1.

Conclusions: This model provides a new mechanism to characterize the role of proteins implicated in the pathogenesis of not only SLE, but also immune-mediated diseases in general and has broad applicability to other fields of science.

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FR-PO866

Spontaneous Development of Lupus in Mice Deficient in SCARF1 William Franklin Pendergraft,[1,2,3] Zaida G. Ramirez-ortiz,[3] Amit Prasad,[3] Nir Hacohen,[1,3] Terry K. Means.[1,3] *[1]Broad Institute, Cambridge, MA; [2]Joint Fellowship Program in Nephrology, Brigham and Women's Hospital and Massachusetts General Hospital (MGH), Boston, MA; [3]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Department of Medicine, MGH, Charlestown, MA.*

Background: Defects in mechanisms governing apoptotic cell detection and clearance in vivo can lead to accumulation of apoptotic cells, which has been associated with systemic lupus erythematosus (SLE); however, the phagocyte-specific receptor responsible for apoptotic cell clearance has yet to be identified. Interestingly, ced-1 is required for apoptotic cell engulfment in the worm, and it encodes a transmembrane protein homologous to the human scavenger receptor expressed by endothelial cells-1 (SREC1 also known as SCARF1).

Methods: In order to analyze the role of the scavenger receptor SCARF1 in apoptotic cell clearance and autoimmunity, we generated Scarf1-deficient mice and analyzed their ability to capture and clear apoptotic cells as well as their propensity for developing autoimmunity.

Results: Scarf1$^{-/-}$ mice, as compared to their wild-type counterparts, have a roughly 3-fold increase in the percentage of endogenous circulating apoptotic cells and CD11c^{+}CD8^{+} dendritic cells (DCs) isolated from Scarf1$^{-/-}$ mice capture ~50% less apoptotic cells when compared to wild-type DCs in vitro. Using an in vivo phagocytic assay in which apoptotic B cells were labeled with CFSE and injected intravenously, we found that splenic DCs from Scarf1$^{-/-}$ mice captured significantly less apoptotic cells than wild-type DCs. In addition, six-month old Scarf1$^{-/-}$ mice spontaneously develop autoantibodies to nuclear antigens and concomitant dipstick hematuria and proteinuria, all of which appear to be more severe in females.

Conclusions: The scavenger receptor, SCARF1, functions in the recognition and phagocytosis of apoptotic cells to maintain tissue homeostasis and prevent lupus-like autoimmune disease. This also represents a new spontaneous model of SLE, which yearns to be explored further.

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FR-PO867

Anti-Annexin II Level in Serum Correlates with Disease Manifestations in Lupus Nephritis Daniel Tak Mao Chan, Kwok Fan Cheung, Andy Yim, Desmond Y.H. Yap, Susan Yung. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: We have reported that anti-dsDNA antibodies from patients with lupus nephritis could bind to annexin II on the surface of human mesangial cells and mediate downstream inflammatory processes. In this study we investigated the relationship between serum anti-annexin II activity, measured with ELISA, and clinical parameters.

Methods: Serial serum samples from 23 patients with biopsy-proven severe proliferative lupus nephritis were included. Sera from patients with non-lupus renal diseases and healthy subjects were used as controls.

Results: A total of 487 serum samples were from patients with lupus nephritis, including 97 during active disease and 390 samples during remission. IgG anti-annexin II activity was significantly higher in patients with lupus nephritis compared with controls (P<0.001). Sero-positivity rate for anti-annexin II IgG was 20.3%, 5.3%, and 3.1% in patients with lupus nephritis, non-lupus glomerular diseases, and healthy controls respectively (P<0.001 for both). Anti-annexin II titre (IgG) correlated with the level of anti-dsDNA antibodies (r=0.26, P<0.001), total IgG (r=0.45, P<0.0001), and serum creatinine (r=0.45, P<0.001), while there was an inverse correlation with C3 level (r=-0.31, P<0.001). In 15 of 23 patients investigated, the levels of anti-annexin II and anti-dsDNA were both higher during active disease than during remission.

Conclusions: The data show that IgG anti-annexin II titre correlates with serological and clinical markers indicating disease activity in lupus nephritis.

Funding: Government Support - Non-U.S.

FR-PO868

The Role of IL-17-Producing Invariant NKT Cells in the Autoimmune Lupus Nephritis Seung Hee Yang,[1] Su Mi Lee,[2] Yun Jung Oh,[2] Jung Pyo Lee,[3] Yon Su Kim.[2] *[1]Internal Medical Research Center, Seoul National University, Seoul, Republic of Korea; [2]Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; [3]Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Republic of Korea.*

Background: CD1d-restrictive invariant NKT cells modulate various autoimmune diseases through secreting multiple cytokines, but the major determinant cytokines in these autoimmune diseases and the functional relationship between these cytokines are still poorly understood. The purpose of this study was to examine the role of the NKT cells as IL-17 producer in the development of glomerulonephritis (GN) using a murine autoimmune lupus nephritis (ALN) model.

Methods: ALN was induced by administering pristane either to C57BL/6 (B6), NKT cell-deficient B6 (B6.CD1d$^{-/-}$), Balb/C, or IL-17A deficient Balb/C (Balb/C.IL-17A$^{-/-}$) mice.

Results: Compared with wild-type mice, B6.CD1d$^{-/-}$ andBalb/C.IL-17A$^{-/-}$ mice showed attenuation of GN in terms of mesangial proliferation and the deterioration of renal function. With the induction of ALN, renalNKT cell infiltration increased, which suggests that NKT cells might actively participate in the renal injury produced by ALN. Expressions of STAT3, IFN-γ, TGFβ, CXCL16, IL-23 and IL-17 mRNA were up-regulated in the ALN mice kidneys, whereas the levels of those mRNA were suppressed by the deletion of NKT cells. In *in-vitro* mesangial cell-T cell interaction system, we found that IL-17 producing NK1.1^{-}NKT cells enhanced the secretion of pro-inflammatory cytokines. αGalCer-primed NK1.1^{-}NKT cells produced IL-17 and the activation of IL-17 by NK1.1^{-}NKT cells increased proliferation of mesangial cells. In addition, treatment of anti-CXCL16 antibody reduced the IL-17 mediated proinflammatory reaction in this system.

Conclusions: These results suggest that NKT cells play an important pro-inflammatory role in pristane-induced lupus nephritis by promoting the secretion of IL-17 and proinflammatory cytokines, and lead to renal injury in this model.

FR-PO869

Lack of Basigin Exacerbates Lupus Nephritis in Experimental Lupus Model Kayaho Maeda, Tomoki Kosugi, Waichi Sato, Hiroshi Kojima, Yuka Sato, Hiroshi Nagaya, Mayuko Maeda, Tomohiro Masuda, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Basigin (Bsg/CD147), a glycosylated transmembrane protein, plays important roles of cell survival, invasion and metastasis. Recently, we demonstrated deleterious effects of Bsg in renal inflammation caused by ischemia and renal fibrosis. As Bsg identifies activated regulatory T cell (Treg), the attention has become extended to the autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus (SLE). Particularly, IL-17/IL-23 axis and Treg also serve important roles in the pathogenesis of SLE. However, the molecular mechanism involving Bsg remains unknown yet. We therefore investigated the role of Bsg in lupus nephritis.

Methods: Lupus nephritis was induced in Bsg deficient mice (Bsg$^{-/-}$) or wild-type mice (Bsg$^{+/+}$) with an intraperitoneal injection of pristane (0.5ml/each mice). They were sacrificed at 6 months after an injection for histological and immunohistochemical analyses. Kidney, spleen and thymus were analyzed.

Results: There was no difference between Bsg$^{+/+}$ and Bsg$^{-/-}$ in serum anti-nuclear antibody and anti-dsDNA antibody during the experimental period, whereas serum C3 decreased in Bsg$^{-/-}$. Albuminuria in Bsg$^{-/-}$ was more than Bsg$^{+/+}$. Mesangial and endothelial cells proliferations, macrophage and T lymphocyte infiltration, and wire loop lesion were prominent in Bsg$^{-/-}$ mice. Consistent with these data, IgG, C3, and C1q depositions in Bsg$^{-/-}$glomeruli were predominantly observed. By flow cytometry analysis, IL-17A producing CD3^{+} and CD4^{+} T cells were higher in Bsg$^{-/-}$ spleen than Bsg$^{+/+}$. Interestingly, no obvious difference in the number of Treg is found in both genotypes, whereas IL-10 production and CTLA4 expression strikingly decreased in Bsg$^{-/-}$ Treg than Bsg$^{+/+}$ Treg. Suppression assay also demonstrated the effect of Bsg$^{+/+}$ Treg in the proliferation of CD4^{+}CD25^{-}T cell compared with Bsg$^{-/-}$Treg. These data suggest that Bsg deficiency causes Treg dysfunction, eventually leading to the development of lupus nephritis.

Conclusions: This study identifies Basigin/CD147 as a key molecule in lupus nephritis.

FR-PO870

Switch from Proteasome to Immunoproteasome in Children with Idiopathic Nephrotic Syndrome Roberta Camilla, Elisa Loiacono, Maria Elena Donadio, Licia Peruzzi, Rachele Gallo, Laura Morando, Alessandro Amore, Rosanna Coppo. *Nephrology, Dialysis, Transplantation, R. Margherita Hospital, Turin, Italy.*

Background: The etiology and pathogenesis of idiopathic nephrotic syndrome (NS) in children is still unknown, but signs of involvement of innate immunity through viral infections and activation of the interferon pathway have been reported. The proteasome (PS) is converted into immunoproteasome (iPS) by interferons (INF γ and α) by replacing β1, β2 and β5 PS subunits with LMP2, MECL-1 and LMP7 iPS subunits. The switch to iPS improves the catalytic proteasomal activities and leads to production of optimal MHC-I ligands, shaping T cell response. We investigated in peripheral mononuclear cells (PBMC) of children with NS the innate immunity activation focusing on Toll-like receptor (TLR) expression and the switch from PS to iPS.

Methods: PBMC from 28 children (2-18 y.o.) with NS and 30 healthy controls were tested with real time PRC (Taqman) to measure mRNA expression of TLR3, TLR4, TLR9 and of PS (ß1, ß2, ß5) and iPS (LMP2, MECL-1, LMP7) subunits. The iPS/PS switch was expressed as ratio between iPS and corresponding PS mRNA fold changes.

Results: iPS/PS mRNA subunits ratio of MECL-1/β2 was significantly increased in PBMC from NS patients in comparison to healthy controls (1.26 ± 0.61 versus 1.02 ± 29, P= 0.04) while LMP2/β1 and LMP7/β5 in NS patients were not different from controls (respectively 1.00±0.51 vs 0.91±0.42 and 0.91±0.5 vs 1.07±0.33; p ns). The switch MECL-1/β2 was significantly correlated with TLR3 mRNA (P= 0.02). This switch remained unchanged in different phases of clinical activity, however, in 3 patients treated with a protease inhibitor provided with anti-PS activity, saquinavir, a reversal to normal values of the MECL-1/β2 mRNA ratio was observed (2.02 before treatment vs 0.86 after treatment). No significant changes were detected in mean levels of TLR3-4-9 between patients and healthy controls.

Conclusions: We reportfor the first time an abnormal switch from proteasome to immunoproteasome in children with NS which was found to be correlated with TLR3 activation. These results suggest an involvement of the interferon pathway of innate immunity in this disease.

FR-PO871

Efficacy of CD40 DNA Vaccine in Preventing Heymann Nephritis Is Enhanced by Dendritic Cell Targeting Yuan Min Wang,[1] Ya Wang,[2] Yiping Wang,[2] Guoping Zheng,[2] Geoff Yu Zhang,[1] Jimmy Jianheng Zhou,[1] Thian Kui Tan,[2] Qi Cao,[2] Min Hu,[2] Debbie Watson,[1,3] Huiling Wu,[4] Dong Zheng,[2] David C. Harris,[2] Stephen Alexander.[1] *[1]Centre for Kidney Research, Children's Hospital at Westmead, Westmead, NSW, Australia; [2]Centre for Transplant and Renal Research, Westmead Millennium Institute, Westmead, NSW, Australia; [3]Centre for Medical Bioscience, University of Wollongong, Wollongong, NSW, Australia; [4]Collaborative Transplant Research Group, Royal Prince Alfred Hospital, Camperdown, NSW, Australia.*

Background: Heymann Nephritis (HN) is an experimental rat model of human membranous nephropathy (MN), a common cause of chronic kidney disease (CKD) in adults. CD40 and its ligand CD154 are critical costimulatory molecules required for T cell activation and B cell differentiation, which make them important therapeutic targets for treating CKD. Studies have shown the benefits of targeting antigen to DC using single-chain Fv antibody directed at DEC205. Here we utilized this approach in generating the DNA vaccine targeting CD40 to DC and compared the efficacy of CD40 DNA vaccine enhanced by DC targeting (DEC-CD40) to that without DC targeting (con-CD40).

Methods: Lewis rats were immunized twice with either DEC-CD40 or con-CD40 (3 weeks apart with 300mg/rat/injection) via intramuscular injection with electroporation two weeks before the induction of HN via immunization of crude renal tubular antigen (Fx1A). Twelve weeks after HN induction, renal function and histology were assessed.

Results: DEC-CD40 induced a stronger immune response with higher levels of serum anti-CD40 autoantibody as compared to con-CD40. DEC-CD40 completely prevented proteinuria throughout the 12 week period. con-CD40 delayed the onset of proteinuria, demonstrating an effect though less potent than DEC-CD40. Renal structural injury was ameliorated by CD40 DNA vaccines with DEC-CD40-HN showing much less injury than con-CD40-HN. Immune cell infiltrations ($CD4^+$, $CD8^+$ and macrophage) and glomerular IgG deposition were reduced by DEC-CD40. The protective effects of CD40 vaccine were not associated with B cell depletion.

Conclusions: By targeting antigens to DCs, the efficacy of DNA vaccines can be greatly improved.

FR-PO872

EGFR and Src Are Essential for TLR3-Mediated Podocyte Injury Michifumi Yamashita, Saurabh Chattopadhyay, Volker Fensterl, Ganes C. Sen. *Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.*

Background: Podocyte injury is a leading factor of many glomerular diseases, and leading to renal failure. We reported viral innate immunity, especially Toll-like receptor 3 (TLR3), disturbs podocyte cell function. TLR3 is a sensor for double-stranded (ds) RNA, a common byproduct of viral replication. Recently, we reported that TLR3 has five tyrosine (Tyr) residues in the cytoplasmic domain, and that Tyr759 and Tyr858, and those phosphorylations are essential for the signaling (Nat Struct Mol Biol. 2004). However, the detailed mechanisms: who phosphorylates Tyr residues and how it does, are not understood at all.

Methods: To identify the candidate protein kinases for TLR3 Tyr residues, various Tyr kinase inhibotors, shRNA knock-down cells, and knock-out cells were used. Co-immunoprecipitation was used to test the interactions between EGFR, Src, TRIF, and wild type- or various mutant TLR3. To test TLR3 phosphorylation, *in vitro* kinase assay and immunoprecipitation-based phospholylation assay were employed.

Results: Using various Tyr kinase inhibitors, knock-down cells, and knock-out cells, we identify Epidermal Growth Factor Receptor (EGFR/ErbB1) and Src as candidate kinases for TLR3 Tyr residues. EGFR and Src were physically interacted with TLR3 upon dsRNA stimulation. This interaction was independent of TLR3 Tyr residues, suggesting this event precedes TLR3 phosphorylation. Interestingly, TLR3-EGFR interaction did not require Src, while TLR3-Src interaction needed EGFR. Finally EGFR directly phosphorylated Tyr858, whereas Src phosphorylated Tyr759 in *in vitro* kinase assay. Furthermore, the phosphorylation of the two Tyr residues were essential for the recruitment of TRIF, the obligatory adaptor molecule, leading to the downstream signalings.

Conclusions: EGFR/ErbB1 and Src, bind sequentially to dsRNA-activated TLR3 and phosphorylated the two Tyr residues: EGFR phosphorylates Tyr858, Src phosphorylates Tyr759, a step essential for the recruitment of the obligatory adaptor protein, TRIF. EGFR and Src are essential for TLR3-mediated podocyte injury.

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FR-PO873

The Calcineurin-NFAT Pathway Allows for Urokinase Receptor Mediated beta3 Integrin Signaling to Cause Podocyte Injury Wei Shi, Juan Ma, Bin Zhang. *Department of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Clinically, calcineurin inhibitors (e.g., cyclosporine A, CsA) have been used to reduce proteinuria in focal segmental glomerulosclerosis (FSGS) or other proteinuric kidney diseases. A novel role for CsA has been reported in inhibiting the dephosphorylation of podocyte synaptopodin by calcineurin. It is possible, however, that the suppression of calcineurin-NFAT signaling in podocytes also plays a role in the antiproteinuric effect of CsA. *In vivo* conditional NFATc1 activation in podocytes can lead to podocyte injury and proteinuria in mice. Podocyte urokinase receptor (uPAR)-β3 integrin signaling axis is involved in podocyte injury, proteinuria and FSGS.

Methods: we used the 5/6 nephrectomy and LPS animal models in vivo and cultured podocytes in vitro.

Results: Here we show that calcineurin inhibition by CsA reduced podocyte uPAR expression and with that it suppressed β3 integrin activation in podocytes. This finding raises the possibility of a potential molecular link between calcineurin and uPAR-β3 integrin signaling in podocytes. Our results further show that, in LPS- or inomycin-induced NFAT activation podocyte model, podocyte uPAR expression and activated β3 integrin increased, but β3 integrin expression remained unchanged. In contrast, NFAT-siRNA reduced uPAR expression and inhibited β3 integrin activation but not its expression. Meanwhile, we show that CsA inhibited podocyte motility in a NFATc1-dependent manner. We also showed that NFAT inhibition using a cell-permeable NFAT inhibitor (11R-VIVIT) can reduce proteinuria and suppress podocyte uPAR expression and β3 integrin activation in LPS-induced proteinuric SCID mice that are devoid of T and B cells.

Conclusions: These findings suggest the involvement of uPAR-β3 integrin signaling in calcineurin/NFAT activation-induced podocyte injury.

Funding: Government Support - Non-U.S.

FR-PO874

The Generation of Inducible Podocyte Specific Human Phospholipase A_2-Receptor-1 Transgenic Mice Gunther Zahner,[1] Elion Hoxha,[1] Udo Helmchen,[2] Rolf A. Stahl.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Nierenregister, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: The Phospholipase A_2 Receptor-1 (PLA_2R), which is in human kidneys exclusively expressed on glomerular podocytes, is the major antigen in 70% of patients with primary membranous nephropathy. In order to get more insight in the pathogenetic role of the PLA_2R and to be able to create a murine model of membranous nephropathy (MN), we generated transgenic mice which exclusively express the human PLA_2R on glomerular podocytes.

Methods: To achieve this goal the full length coding region of the human PLA_2 R was cloned into an expression vector carrying a tetracycline responsible promotor (pWTT1). After successfull expression in HEK 293 cells cotransfected with a Tet-Off vector (Clontech), mice expressing the human PLA_2R were generated by microinjection of the PLA_2R construct in pronuclei of single cell mouse embryos (PLA_2Rtg mice). These mice were cross bred with mice which express a tetracycline activator under the control of the podocin promotor (Podo-TA mice). The litters, PodoTA/PLA_2Rtg mice, were used for further studies.

Results: Feeding these mice with 2 mg/ml doxycycline in the drinking water for 14 days markedly induced the mRNA expression of the human PLA_2R in isolated glomeruli. When PLA_2R positive antisera from patients with primary MN were used as developing antibodies PLA_2R protein was detected by Western blotting only in isolated glomeruli from PodoTA/PLA_2Rtg mice following doxycycline.

Conclusions: Thus, doxycycline-inducible PodoTA/PLA_2Rtg mice may serve as a murine model of human membranous nephropathy and will eventually allow to get a better understanding of the role of PLA_2R in membranous nephropathy.

Funding: Clinical Revenue Support

FR-PO875

Membranous Nephropathy and Anti-PLA2R Antbody Induced by an Epitope in Nonmuscular Myosin Heavy Chain IIA (NMMHC-IIA) in Rabbits Suyan Duan,[1] Chang Ying Xing,[1] Peter W. Mathieson,[2] Moin Saleem.[2] *[1]Dept. of Nephrology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China; [2]Academic Renal Unit and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: Our previous study found the antibody against NMMHC-IIA, a podocyte protein, could be detected in a majority of patients with INM, indicating that is a major autoantigen in this disease. To study the immune activity of a synthetic epitope-peptide in NMMHC-IIA and its pathogenicity in rabbits.

Methods: This epitope-peptide were synthesized and coupled with keyhole limpet hemocyanin(KLH), which were used to immune male New Zealand rabbits to prepare epitope-specific antibody. Western blotting(WB) and co-immunoprecipitation(Co-IP) were performed with the total cell lysates and anti-peptide rabbit serum. The kidneys of rabbits were performed renal pathological examination by LM, IF and EM.

Results: Compared with the normal control group (saline-injected group), the epitope-peptide immunized rabbits could produce specific anti-peptide antibodies. The serum from the epitope-peptide immunized rabbits had antibody combined with a 227-kD protein by Co-IP, which was sequentially identified to be NMMHC-IIA by mass spectrometry. The same distribution of special protein in cultured human podocyte was visualized by rabbit serum and commercial anti-NMMHC-IIA antibody. The epitope-specific antibodies were reactive to the commercial NMMHC-IIA protein (Tagged-His Tag) by WB. At 8 week, the rabbit produced another antibody against PLA2R by WB under nonreducing condition. Furthermore, the immunostaining study of rabbit kidney tissues showed that the rabbit IgG was granular deposits along the GBM, while the renal pathological changes under the electron microscope were foot process fusion, irregular thickness of GBM and mesangial widening. The rabbits had proteinuria either.

Conclusions: The synthesized epitope-peptides in NMMHC-IIA as an antigen can induce rabbit to produce epitope-specific antibody against NMMHC-IIA, and another antibody against PLA2R, both podocyte proteins, and it can lead rabbit to resemble pathological change of human MN. It may be a good animal model of MN.

FR-PO876

Retinoic Acid-Inducible Gene-I and Melanoma Differentiation-Associated Protein 5 Are Induced by Polyinosinic-Polycytidylic Acid in Human Podocytes in Culture Michiko Shimada,[1] Yoshiko Shutto,[1] Ikuyo Narita,[1] Tadaatsu Imaizumi,[1] Moin Saleem,[2] Peter W. Mathieson,[2] Richard J. Johnson,[3] Hideaki Yamabe,[1] Ken Okumura.[1] *[1]Nephrology, Hirosaki University, Hirosaki, Japan; [2]University of Bristol; [3]University of Colorado.*

Background: Immunologic factors are implicated as a cause of proteinuira, since it sometimes occurs after or is exacerbated by virus or bacterial infection. In innate immunity, pathogens are recognized by various pattern recognition receptors (PRR). Among them, several toll-like receptors are proved to be expressed in podocytes and their activation induces phenotype changes of podocytes causing proteinuria. However, expression of other PRR such as retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) have not been proved in podocytes. In this study, we examined the expressions of RIG-I and MDA5 in human podocytes stimulated with double stranded RNA, polyinosinic-polycytidylic acid (Poly-IC), mimicking viral infection.

Methods: Conditionally immortalized human podocytes were grown at 33°C then converted to differentiated cells by incubating at 37°C for 10 days. The differentiated cells were stimulated with poly-IC (2-500 ng/ml) for 6-30 hours. Total RNA was harvested and mRNA expression of RIG-I and MDA-5 were analyzed by quantitative RT-PCR. The cell lysates were analyzed by Western blotting.

Results: In the differentiated human podocytes, poly-IC dose-dependently increased the relative mRNA expressions of RIG-I and MDA5. 6-hour incubation of Poly-IC (500ng/ml) increased mRNA expressions of RIG-I (7.3±0.7 fold: $p<0.0001$) and MDA5 (16.5±0.7 fold: $p<0.0001$). Besides, mRNA expressions of RIG-I and MDA5 increased over time: 30 hours incubation with Poly-IC (500ng/ml) increased mRNA expression of RIG-I (13.1±1.5 fold, $p<0.001$) and MDA5 (20.3±1.4 fold, $p<0.001$). Similarly, Western blotting showed that expression of both RIG-I and MDA-5 were dramatically increased by poly-IC in a dose- and time-dependent fashion.

Conclusions: RIG-I and MDA5 are expressed in human podocytes and their expression was increased by poly-IC. Induction of these viral receptors may also contribute to podocyte changes in viral infections.

Funding: Government Support - Non-U.S.

FR-PO877

Calcium-Oxalate Crystals Induce Renal Inflammation and Acute Kidney Injury by Activating NLRP3 Inflammasome-Mediated Interleukin-1β Secretion in Renal Dendritic Cells Shrikant R. Mulay,[1] Onkar Kulkarni,[1] Khader Valli Rupanagudi,[1] A. Migliorini,[1] Murthy Darisipudi,[1] Helen Liapis,[2] Hans J. Anders.[1] *[1]Ludwig Maximillians University, Munich, Germany; [2]Washington University School of Medicine, MO.*

Background: Calcium oxalate (CaOx) crystal nephropathy cause intrarenal inflammation & tissue damage but the molecular mechanisms remain elusive. The aim of the study was to investigate whether CaOx crystals have the potential to activate IL-1β-dependent innate immunity via the NLRP3-ASC-caspase-1 axis in kidney, and how?

Methods: All mice were procured from Jackson Laboratories (Bar Habour, MA). All experimental procedures were approved by the local government authorities. Immunostaining and electron microscopy were used for analysing kidney pathology. Bone marrow derived dendritic cells (DC) & renal DC was generated by established protocols.

Results: We show that CaOx crystals activate renal DC to secrete IL-1β via NLRP3-ASC-caspase-1 axis. CaOx crystal phagocytosis and potassium efflux were essential for this. CaOx crystals killed tubular cells (TEC) in culture to release a NLRP3 agonist ATP; however ATP-degradation with apyrase did not abrogate IL-1β release by DC upon exposure to supernatants of TEC killed by CaOx crystals. In experimental oxalate nephropathy, CaOx localized to tubular lumen as well as inside tubular and interstitial cells as detected by Pizzolato staining, TEM, SEM, FFEM. Intrarenal crystal desposition induced severe tubular damage, cytokine expression, neutrophil recruitment, and renal dysfunction. These effects were abrogated upon DC-depletion or in mice deficient in MyD88, IL1-r1, NLRP3, ASC or caspase-1 despite a similar extent of CaOx crystal deposition. In addition, ATP depletion or therapeutic IL-1 antagonism had same effect.

Conclusions: CaOx crystals directly damage tubular epithelial cells. Inside renal DC they activate the NLRP3-ASC-caspase-1 axis to trigger IL-1β release. Subsequent IL-1R signaling sets off MyD88-dependent renal inflammation, neutrophil recruitment and accelerates tubular damage & renal dysfunction. Therapeutic blockade of IL-1 is protective in murine model and might potentially be protective in human crystal nephropathies.

FR-PO878

Indoleamine 2,3-Dioxygenase Inhibition and Kidney Gene Expression Following Ischemia-Reperfusion Injury Todd D. Merchen,[1] Erika I. Boesen,[1] Eiko Kitamura,[1] Rachel Harbarger,[1] Andrew L. Mellor,[1] James J. Wynn,[1] Robert Podolsky,[1] N. Stanley Nahman,[1,2] David M. Pollock.[1] *[1]Georgia Health Sciences University, Augusta, GA; [2]Charlie Norwood VAMC, Augusta, GA.*

Background: In kidney transplantation (KTx) ischemia reperfusion injury (IRI) is associated with delayed function and chronic injury. In rats with IRI, recovery was improved by inhibiting indoleamine 1, 2-dioxygenase (IDO) (JASN 22:817A). IDO also reduces rejection in rat renal allografts (J Gene Med 13:373). We have hypothesized that IDO-induction of pro-tolerant pathways in KTx may carry the paradoxical risk of slowing recovery from IRI. To define changes in genes modulated by IDO in IRI, we inhibited IDO in the face of IRI and assessed renal gene expression.

Methods: Male SD rats underwent IRI (30 mins of bilateral renal pedicle clamping, followed by 1 hr of recovery (N=4)) or sham surgery (N=4). IDO was blocked with 140 mg/kg of oral 1-methyl-D-tryptophan (1MT). 1MT treated were subjected to IRI (N=4) or sham surgery (N=4). Following IRI recovery, the kidneys were taken, the cortex isolated, and total RNA hybridized to the GeneChip®Rat Gene 1.0 ST Array (Affymetrix).

Results: IRI vs sham: significant changes in 102 transcripts (2.00 – 45.51 (range of fold change)), dominated by genes coding for cell death and proliferation. 1MT without IRI vs sham: significant changes ($p<0.002$) in 32 transcripts (1.04 – 1.4) with immune cell trafficking molecules predominating. Pretreating with 1MT followed by IRI: significant change ($p<0.001$) in 54 genes (1.07 – 2.63), with cell death and proliferation predominating, without evident change in immune cell trafficking molecules. These data indicate substantial alterations in gene expression following IRI or 1MT therapy. IDO inhibition with 1MT prior to IRI induced changes in cell death and proliferation genes.

Conclusions: Inhibition of IDO with 1MT alters immune cell trafficking molecules in the absence of IRI. In the face of IRI, IDO inhibition results in predominant changes in cell death, growth and proliferation transcripts. We would theorize that IDO inhibition with 1MT improves renal recovery from IRI by altering expression of genes associated with death and/or cellular proliferation.

Funding: Clinical Revenue Support

FR-PO879

Renal Tubular Cells (RTCs) from Hibernating Squirrels Are Protected against Apoptosis Swati Jain, Charles L. Edelstein, Alkesh Jani. *Univ of CO Denver, CO.*

Background: Donor kidney cold (4°C) ischemia (CI) of >24 hrs is an important cause of DGF. The 13-lined ground squirrel (GS) is a hibernating mammal that undergoes winter hibernation, when its core body temperature falls to 4°C for 6-18 days. Since hibernation is a normal part of the GS life-cycle, we hypothesized that RTCs from hibernating GS are protected from apoptosis due to CI.

Methods: Kidneys of C57BL6 mice and hibernating GSs were exposed to CI in UW solution for 72 hrs. Apoptotic RTCs were scored by a pathologist. Immunoblots were performed for caspase-3, XIAP (an inhibitor of caspase-3 and apoptosis) and phosphoAKT (pAKT) which converts BAD to pro-survival factor phosphoBAD (pBAD).

Results: RTC apoptosis and caspase-3 activity were significantly increased in mouse vs. GS kidneys. XIAP, pAKT and pBAD were significantly increased in hibernating GS kidneys, but were undetectable in mouse kidneys.

Table 1

Stage	Caspase-3 activity (nmol/min/mg)	Apoptosis (cells/hpf)	pAkt protein	pBAD protein	XIAP protein
GS (0 hr CI)	6.5	0.1	+++	+++	+++
GS (72 hr CI)	13.6	0	+++	+++	+++
Mouse (0 hr CI)	0	0	ND	ND	ND
Mouse (72 hr CI)	800*	1*	ND	ND	ND

n = 3; * $p<0.05$ vs mouse and 0 hr ; ND = not detected

To determine the mechanism of resistance of GS RTC to apoptosis, GS and mouse RTCs were treated with cisplatin (50uM), an agent known to cause apoptosis. Cisplatin treated GS RTC had significantly less apoptosis, no active caspase-3, increased XIAP, pAkt and pBAD vs. mouse RTC. (Table 2)

	Mouse RTE	Mouse RTE + 50uM cisplatin	Squirrel RTE	Squirrel RTE + 50uM cisplatin
Apoptosis (TUNEL + cells/hpf)	3.4%±0.88	24.6%±2.5*	0.5%±0.28	1.32 %±0.32**
Caspase-3 protein	+	+++	ND	ND
XIAP protein	+++	+	++	++++
pAkt protein	+++	+	++	+++
pBAD protein	++	+	++	++++

n = 3; * p <0.05 vs mouse RTE. ** p < 0.0001 vs mouse RTE + 50uM cisplatin; ND = not detected

Wortmannin inhibition of pAKT reduced pBAD, and increased caspase-3 in GS RTC demonstrating the importance of Akt signaling in GS RTC survival.

Conclusions: We have shown for the first time that GS RTCs are protected against apoptosis induced by CI and cisplatin, associated with upregulation of pro-survival factors XIAP, pAKT and pBAD. Inhibition of pAKT in GS RTC results in reduced anti-apoptotic pBAD and increased pro-apoptotic caspase-3 expression.

Funding: NIDDK Support

FR-PO880

Increased Autophagic Flux, Caspase-3 and Apoptosis in Renal Tubular Epithelial Cells Undergoing Warm Reperfusion (WR) after Cold Ischemia (CI)

Swati Jain, Charles L. Edelstein, Alkesh Jani. *Univ of CO Denver, CO.*

Background: Delayed graft function (DGF) is primarily caused by cold ischemia (CI) and warm reperfusion (WR). The mechanism by which CI/WR cause cell renal tubular epithelial (RTE) cell death is not known. Autophagy is a cell-survival strategy employed by cells during stresses such as ischemia and reperfusion. The relationship between apoptosis and autophagy during CI/WR of donor kidneys is not known. We hypothesized of increased autophagic flux and apoptosis during CI/WR of RTE cells.

Methods: Renal tubular epithelial cells (LLC-PK1 cells) were subjected to CI in University of Wisconsin (UW) solution at 4°C for 24h. To simulate rewarming UW solution was replaced with DMEM containing bovine serum at 37°C for 24h. LLCPK cells incubated at 37°C served as controls. Immunoblot and densitometry were used to assess active caspase-3 (17kDa), caspase-1 (45kDa) and LC3–II (14kDa) (autopahgic flux). Autophagic flux is measured by comparing the amount of LC3-II with and without Bafilomycin, a lysosomal inhibitor. Cells were treated with Bafilomycin (150nM) one hour before CI.

Results: During CI, Caspase-1 is not increased but apoptosis and active caspase 3 were significantly increased vs. controls.

table1

	Control	CI	CI + Baf	WR	WR + Baf
TUNEL	2.4%±0.83	24.6%±4.7*	36%±2.1	7.4%±1.4	42.9%±5.4**
Caspase-3	++	+++	++++	+	+++++
Caspase-1	+	+	+	++++	++++
LC3-II	+	+	+	+++	++++

n = 3; * p <0.05 vs control, **p<0.001vs control and WR

Treatment with Bafilomycin did not change LC-3 II protein during CI vs. controls, indicating that CI did not increase autophagic flux. In contrast, during WR, apoptosis and caspase-3 did not increase whereas caspase-1 was increased vs. controls. Treatment with Bafilomycin resulted in significantly more apoptosis and LC-3II expression indicating an increase in autophagic flux during WR.

Conclusions: Caspase-3 and apoptosis are increased during CI. Autophagic flux is increased during WR. Caspase-1 (a proinflammatory marker) is increased in WR vs. CI. Lysosomal inhibition with Bafilomycin, increases caspase-3 and apoptosis during both CI and WR. Further study of relationship between apoptosis and autophagy during CI and WR is merited.

Funding: NIDDK Support

FR-PO881

Mitochondria-Targeting Peptide (SS-31, Bendavia®) Prevents Microvascular Rarefaction, Inflammation, and Fibrosis Caused by Ischemia-Reperfusion Injury

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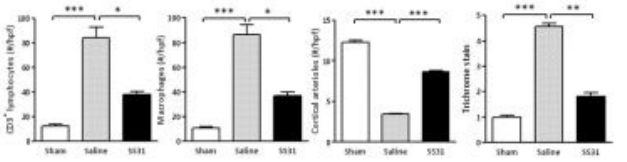
Background: Interstitial fibrosis and tubular atrophy is the primary cause of chronic graft dysfunction resulting in end-stage renal disease. Ischemia-reperfusion (IR) injury during renal transplantation leads to acute kidney injury, oxidative stress, inflammation, microvascular rarefaction, and interstitial fibrosis. SS-31 is a mitochondria-targeting tetrapeptide that has been shown to accelerate ATP recovery, reduce oxidative stress, and minimize ischemic kidney injury (Szeto et al., JASN 22:1041-1052, 2011). Here we examined the effects of SS-31 on microvascular rarefaction, inflammation, and fibrosis after acute IR injury.

Methods: Sprague-Dawley ratswere assigned to sham, IR+saline and IR+SS-31 (n=4-6). Rats were subjected to bilateral renal ischemia for 45 min followed by 4 weeks reperfusion. SS-31 (2.0 mg/kg, sc) was administered 30 min before ischemia and immediately before reperfusion. Renal function was determined on day 28, and kidneys were harvested for histopathology. Results are expressed as mean ± SEM.

Results: 45 min ischemia resulted in apoptosis and necrosis of proximal tubular epithelial cells, and damage to the microvasculature. This was followed by significant interstitial fibrosis (trichrome, aSMA, FSP), inflammation (CD3, CD68), and loss of microvasculature (aSMA, CD31) observed after 28 days reperfusion. Treatment with SS-31 significantly reduced inflammation, microvascular rarefaction, and interstitial fibrosis.



Conclusions: These results indicate that protection of mitochondrial function during IR injury prevents long-term changes in renal microvasculature and prevents inflammation and fibrosis. SS-31 (Bendavia®) is a promising drug candidate for renal transplantation.

Funding: Pharmaceutical Company Support - Stealth Peptides Inc.

FR-PO882

Angiopoietin-1 Administration in a Brain Death Rat Model

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Background: Kidneys derived from deceased brain dead (DBD) donors have inferior outcomes after transplantation compared to kidneys from living donors. Also, DBD donors suffer from bacterial translocation and endotoxemia. A link between Angiopoietin 1 (Ang1), Angiopoietin 2 (Ang2) and endotoxemia has been established. Ang1 and Ang2 are antagonictic ligands that bind to the Tie2 receptor. We aimed to modify the Ang1 levels in our brain death rat model to clarify whether exogenous administration of Ang1 has a protective role and may be of therapeutical value to improve outcome after DBD transplantation.

Methods: We administrated 0,1µg/kg Ang1 or 0,9% saline 30 min before brain death (BD) induction. BD was induced by inflating a subdurally placed balloon catheter in rats. Two groups of sham operated animals were injected with either Ang1 or 0,9% saline (n = 7 for all groups). The animals were monitored for 4 hrs. Just before sacrificing the animals blood was collected and kidneys were harvested for histology and PCR.

Results: Plasma levels of ALT, AST, creatinine, LDH and urea increased in the BD groups compared to sham operated groups ($p<0.05$). The 2fold induction of E-selectin, P-selectin, VCAM-1, ICAM-1, IL-6, KIM-1 and HO-1 in the kidney is elevated in the BD groups compared to the sham operated groups ($p<0.05$). The renal TNF-α fold induction of the BD+Ang1 group is increased compared to both sham groups ($p<0.05$). Tie2 fold induction in the kidney is not influenced by Ang1 administration and decreased significantly in the BD groups (mean fold induction 0.45 BD+saline and 0.38 BD+Ang1) compared to the sham groups (mean fold induction 2.5 sham+saline and 2.6 sham+Ang1).

Conclusions: Functional and inflammatory markers were increased in the BD groups and not affected by this dosage of Ang1. These results show a remarkable effect of brain death on Tie2 fold induction. This reduction suggests a functional role for Tie2 in BD which could not be compensated by administrating Ang1.

Funding: Government Support - Non-U.S.

FR-PO883

Identification of MicroRNAs Associated with Delayed Graft Function(DGF) in Deceased Donor Kidney Allografts

Mita M. Shah,[1] Kristin Mekeel,[1] James B. Tee.[2] *[1]UC San Diego; [2]University of Calgary.*

Background: DGF following kidney transplantation impacts immediate and long-term graft function. The molecular mechanisms that contribute to DGF remain poorly uncerstood. MicroRNAs (miRNAs) act to reduce the expression of their target genes, and their expression has been implicated in a variety of processes that affect allograft function. The role of miRNAs in controlling the genetic processes that predispose to DGF in kidney allografts is unknown.

Methods: Kidney tissue from 10 individual allografts were obtained prior to implantation. Total RNA from each specimen was concurrently evaluated by microarray for whole-transcript mRNA and miRNA expression. Samples from transplants were compared based on the presence (n=5) or absence of DGF (n=5) post-transplant. Differentially expressed genes and miRNAs were narrowed down to those satisfying 1-way ANOVA and a >=2-fold (for mRNA) or >= 1.5-fold (for miRNA) change in expression. Hierarchical clustering of both significantly expressed genes and miRNAs was observed for DGF and matching donors. Predicted target mRNAs were generated for each miRNA using two algorithms (MicroCosm and TargetScan) and correlated with this subset of differentially expressed genes.

Results: Nine upregulated miRNAs paired with 8 target genes and 12 downregulated miRNAs matching 29 target genes were identified in the presence of DGF.

Differentially regulated miRNA

miRNA	Target Gene	r value*
Upregulated miRNA		
miR-885-3p	PPP1R16B	-0.89
miR-663b	RHOBTB1	-0.74
miR-885-5p	RHOBTB1	-0.74
miR-498	COL1A1	-0.59
miR-122	SLC25A34	-0.46
Downregulated miRNA		
miR-509-3-5p	DCLK1	-0.77
miR-146b-5p	SLC2A14	-0.73
miR-510	IRF1	-0.71
miR-509-3p	EGR1	-0.71
miR-506	BTG2	-0.51

*pearson correlation coefficient

Many of the top correlated miRNA-target gene pairings are implicated in cell-cycle regulation, an important process during recovery from ischemia-reperfusion injury.

Conclusions: This report identifies a number of dynamically regulated miRNAs in kidney allografts that suffer DGF. Further investigation will help to elucidate the molecular mechanisms that underlie DGF as well as potentially identify novel biomarkers to predict allografts at risk for DGF and therapeutic targets to ameliorate the risk of DGF.

Funding: Other NIH Support - O'Brien Center

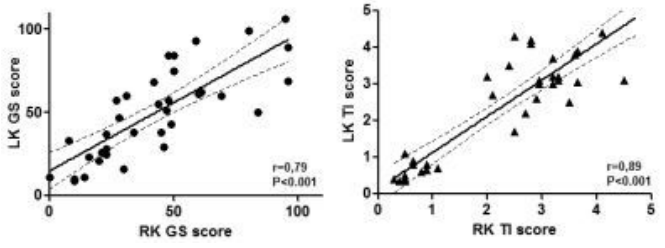
FR-PO884

Systemic Predictors of Single Kidney Function and Injury in a Bilateral Ablation Model of CKD in the Rat Diana A. Papazova, Marianne Christina Verhaar, Arianne van Koppen, Maarten P. Koeners, Jaap A. Joles. *Nephrology & Hypertension, UMC Utrecht, Utrecht, Netherlands.*

Background: Renal transplantation from expanded–criteria donors (ECD) increases mortality and graft failure [Molnar. AJKD 2012]. Organ shortage demands fundamental research on ECD including accurate predictors of function and injury prior to experimental transplantation. However, traditional models of chronic kidney disease always include uninephrectomy. Avoiding uninephrectomy in ablation models would allow prediction of function and injury at the time-point of experimental transplantation. To establish an ECD model we examined systemic predictors of single kidney function and injury as well as symmetry of injury in a novel bilateral ablation (BA) model in the rat.

Methods: Male Lewis rats underwent 2/3 ablation of each kidney in a one-step procedure (BA, n=20), controls underwent bilateral sham surgery (CON, n=16) and systolic BP, urea and proteinuria were measured regularly. A terminal measurement, performed when proteinuria exceeded 100mg/24h in BA rats, included split-urine collection for single kidney function (GFR: inulin and ERPF: PAH) in left and right kidney (LK and RK). Glomerulosclerosis (GS) and tubulo-interstitial injury (TI) were scored. CON rats were age-matched.

Results: Proteinuria, in comparison to BP and urea, was the best predictor for GFR, RPF, TI and GS(r=-0.72; r=-0.63, r=0.81 and r=0.82 respectively, all P<0.001). Symmetry: LK-GS and LK-TI correlated strongly with RK-GS and RK-TI (r=0.79, P<0.001, r=0.89, P<0.001) respectively.



Conclusions: In this model of BA, the best systemic predictor for function and injury was proteinuria. Injury (GS and TI) was symmetrical. These findings firmly position this new bilateral ablation model in the field of ECD transplantation.

Funding: Government Support - Non-U.S.

FR-PO885

Epithelial (EMT) and Endothelial (EndMT) to Mesenchymal Transition in Renal Transplant Recipients with Antibody-Mediated Allograft Rejection (R) G. Castellano,[1] Antonia Loverre,[1] C. Divella,[1] Claudia Curci,[1] Alessandra Stasi,[1] Anna Zito,[1] Loreto Gesualdo,[1] Giuseppe Grandaliano.[2] *[1]Nephrology Unit, DETO, University of Bari, Italy; [2]Nephrology Unit, Dept of Medical and Surgical Sciences, University of Foggia, Italy.*

Background: Antibody-mediated graft R is one of the main causes leading to chronic kidney graft damage and loss. EMT and EndMT processes contribute to the progression of chronic allograft damage.The aim of our study was to investigate the role of IL-17 and Complement (C) in the induction of EMT and EndMT in acute (AHR) and chronic (CHR) humoral R.

Methods: IL-17, E-cadherin, CD31 and FSP1 protein expression were investigated by confocal microscopy in graft biopsies with acute T-cell-mediated rejection (ATMR, n=10), chronic T-cell-mediated rejection (CTMR, n=10), AHR (n=10), CHR (n=10) and in cultured tubular and endothelial cells.

Results: Both CHR and AHR were characterized by a significant increase in tubular IL-17 protein expression (CHR .12±.04, AHR 11.6±4.9 IL-17$^+$pixel/total area) compared to CTMR (.03±.008; p=.004 vs CHR) and ATMR (1.0±.3; p<.0001 vs AHR). Interestingly, CHR and AHR were characterized by a significant increase of tubular E-cadherin$^+$/FSP1$^+$ cells (CHR 15.3±1.5, ABMR 5.9±2.1) compared to CTMR (5.2±1.2, p=.001 vs CHR) and ATMR (2.0±.4, p=.02 vs AHR), suggesting the presence of EMT. Moreover, CHR and AHR showed a significant increase of CD31$^+$/FSP1$^+$cells (CHR 53.4±7.5, AHR 77.6±16.2) compared to CTMR (26.8±6, p=.004 vs CHR) and ATMR (14.7±5.2, p=.004 vs AHR) in peritubular capillaries, suggesting the occurrence of EndMT. Interestingly, the number of FSP1$^+$cells correlated with IL17 expression in both AHR and CHR (R^2 =.41, p<.05).

Finally, we observed a significant increase of FSP1$^+$ cells both in EpC (basal 2.34±.6; IL-17+C3a 7.5±2.4, p<.04) and in EndC (basal:3.06±.2; C3a:3.50±.23; IL-17 4.45±1.15; IL-17+C3a: 6.03±2.5)(p<.05 IL-17+C3a vs basal)stimulated for 24 hours.

Conclusions: In conclusion, our data suggest a synergistic role of IL-17 and Complement in the induction of EMT and EndMT in CHR and AHR. The ttherapeutic inhibition of these systems may be essential to prevent the progression of renal fibrosis in transplanted kidney.

FR-PO886

Endothelial Cell Proliferation Augments Endothelial-to-Mesenchymal Transition in the Presence of Inflammation and Hypoxia Craig Bryan Woda,[1,2] Sarah Bruneau,[1,2] David M. Briscoe.[1,2] *[1]Division of Nephrology, Children's Hospital Boston, Boston, MA; [2]Department of Pediatrics, Harvard Medical School, Boston, MA.*

Background: Cellular and humoral allograft rejection is associated with microvascular endothelial cell (EC) proliferation (identified by Ki67 staining) within renal biopsies. EC proliferation has also been reported to precede chronic allograft rejection. We hypothesize that proliferating EC de-differentiate into fibroblasts in the presence of local cytokines and/or hypoxia, and thus endothelial-to-mesenchymal transition (EndMT) drives chronic allograft fibrosis.

Methods: We treated human umbilical vein endothelial cells (HUVEC), that had undergone <20, 40-60, 80-100 or >120 cellular divisions, with TGFβ1 (10 ng/ml) or TGFβ2 (2.5 ng/ml) for 7-10 days. EndMT was evaluated by FACS (CD31 and N-cadherin co-expression) and confirmed by qPCR (N-cadherin, αSMA, and Snail).

Results: Contrary to publications using commercial EC lines, neither TGFβ1 nor TGFβ2 alone induced significant EndMT in primary cultures of HUVEC, and overall, we found EndMT to be a rare event (<5% cells, P=NS vs. untreated cells, n=3-6 per group). However, EndMT by both FACS and qPCR increased significantly in EC that had undergone >80 cellular divisions when treated with TNFα (100U/ml), in combination with TGFβ1 or TGFβ2. Interestingly, 70% of EC that underwent >120 divisions developed EndMT following treatment with TGFβ2 + TNFα (P<0.01, n=4) as compared to ~25% of EC treated with TGFβ1 + TNFα (p<0.05, n=5). Finally, to evaluate the role of hypoxia on inflammatory EndMT, EC were exposed to 10% pO2 for 7-10 days. We found that hypoxia alone induced EndMT in only 10% of cells as compared to ~3% of EC in normoxia (p<0.03, n=6), but again EndMT was significantly induced in proliferating EC with >120 doublings (~35%, P<0.02, n=6) following activation with TGFβ1 + TNFα.

Conclusions: Collectively, these findings indicate that intragraft inflammatory conditions known to be associated with rejection induce EndMT selectively in proliferating EC. Monitoring EC proliferation/doubling within grafts may identify risk for the development of chronic rejection.

Funding: NIDDK Support

FR-PO887

FGF-23: A Novel Role in Transplant Vasculopathy and Chronic Antibody Mediated Rejection Eileen W. Tsai,[1] Yiping Jin,[2] Isidro B. Salusky,[1] Katherine Wesseling-Perry,[1] Robert B. Ettenger,[1] Elaine F. Reed.[2] *[1]Pediatric Nephrology, UCLA, Los Angeles, CA; [2]Pathology and Laboratory Medicine, UCLA, Los Angeles, CA.*

Background: Fibroblast growth factor 23 (FGF-23) has been associated with allograft rejection, CKD progression, and poor graft survival in pediatric and adult renal transplant recipients. FGF-23 binds to the FGF receptor, which is also up-regulated by HLA class I antibodies (Abs) in the endothelium. Therefore, we aimed to determine whether FGF-23 induced endothelial cell (EC) signal transduction and proliferation, contributing to transplant vasculopathy.

Methods: Primary human aortic EC, passages 2-8, were treated with varying concentrations of FGF-23 (1-100 ng/ml) and basic fibroblast growth factor (bFGF, 1-10ng/ml). Phosphorylation of mTOR Ser2448, S6 kinase Thr389, S6 Ribosomal Protein (S6RP) Ser235/236, Akt Ser473, 4E-BP1 Thr37/46 and ERK Thr202/Tyr204 was measured by Western blot. EC were stimulated with either FGF-23 or bFGF alone or in combination with the murine anti-Class I HLA mAb W6/32 (1ug/ml) directed against a monomorphic epitope on all HLA class I molecules. EC proliferation was assessed by flow cytometry using CSFE labeling.

Results: FGF-23, similar to bFGF, stimulated a 60% increase in mTOR phosphorylation when compared to untreated EC (p<0.05). FGF-23 induced a 70% and 20% increase in S6 kinase and S6RP phosphorylation, which are downstream targets of mTOR responsible for EC proliferation (p<0.05). FGF-23 stimulated a 60% increase in Akt Ser473 phosphorylation, an essential regulator of survival proteins Bcl-2 and Bcl-xL (p<0.05) and a 35% increase in ERK phosphorylation, a known mediator of cell proliferation (p<0.05). Additionally FGF-23 induced a 30% increase in EC proliferation compared to untreated EC (p<0.05) which was significantly augmented to 80% by the addition of HLA class I Ab (p<0.05).

Conclusions: FGF-23 induction of PI3K/Akt, mTOR and MAPK/ERK pathways may play an important and novel role in EC proliferation, which is augmented by HLA class I Ab. Thus, together they can be synergistic in promoting antibody-mediated rejection and development of transplant vasculopathy, leading to poor transplant outcomes.

FR-PO888

Non-Anticoagulant Heparinoid Reduces Inflammation in Experimental Renal Transplant Dysfunction Saritha Adepu,[1] Kirankumar Katta,[1] Ditmer Talsma,[1] Pramod Kumar Agarwal,[1] Saleh Yazdani,[1] Annamaria Naggi,[3] Giangiacomo Torri,[3] Jan-luuk Hillebrands,[2] Jacob van den Born.[1] *[1]Nephrology, UMCG, University of Groningen, Netherlands; [2]Pathology & Med. Biology, UMCG; [3]G. Ronzoni" Institute for Chemical and Biochemical Research.*

Background: Renal chronic transplant dysfunction (CTD) is a major cause of long-term renal allograft failure. The pathogenesis of CTD is multifactorial but essentially involves tissue remodeling partly related to inflammation. To date, immunosuppressive therapies are ineffective in attenuating renal CTD. Endogenous heparan sulfate proteoglycans maintain tissue homeostasis by modulation of inflammation by their heparan sulfate polysaccharide side chains. We hypothesized that intervention with heparan sulfate-like glycomimetics limits CTD, knowing their involvement in inflammation.

Methods: To test this hypothesis a rat model of renal CTD was used. Rats were treated subcutaneously daily with 2 mg/kg of unfractionated heparin (n=4) or with the non-anticoagulant heparinoids N-acetyl heparin (n=5) or periodate-oxidized, borohydrate-reduced heparin (RO-heparin; n=7) for 9 weeks. Saline-treated rats served as control (vehicle group; n=7). Immunostaining was performed to quantify inflammation. Neointima formation was quantified on Verhoeff stained sections. Blood pressure was measured and biochemical analyses were done in 24h-urine and in plasma.

Results: Compared to the vehicle group, RO-heparin-treated rats had >2-fold lower cortical tubulo-interstitial accumulation of CD45+ leukocytes ($p<0.02$) and >2-fold reduced proteinuria 9 weeks after transplantation (NS). Regular heparin increased neointima formation in arteries <200 µm (week 9: heparin vs vehicle group; $p=0.006$), whereas both non-anticoagulant heparinoids did not enhance neointima formation. Heparin(oid) treatment did not affect body weight, graft survival, blood pressure, and renal function, neither reduced glomerular and interstitial tissue remodeling.

Conclusions: These data demonstrate beneficial effects of non-anticoagulant heparinoids in experimental CTD. We suggest to investigate selected non-anticoagulant glycomimetics as potential adjunct therapy to attenuate CTD.

FR-PO889

Systematic Histological Analysis of Thrombotic Microangiopathy in a Porcine Xeno-Kidney Transplant-Model Jan U. Becker,[1] Wolf Ramackers,[2] Juliane Wittig,[1] Putri Andina Agustian,[1,3] Maximilian Ernst Daemmrich,[1] Clemens L. Bockmeyer.[1] *[1]Institute of Pathology, Hannover Medical School, Hannover, Germany; [2]Clinic for General, Visceral- and Transplant Surgery, Hannover Medical School, Hannover, Germany; [3]Clinic for Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: Despite a variety of pharmacologic and transgenic interventions thrombotic microangiopathy (TMA) is still a big challenge in porcine to human xenotransplantion. A detailed molecular analysis of histological patterns in xenokidneytransplant-models is necessary for a targeted anticoagulant therapy.

Methods: Porcine kidneys were perfused with human and porcine (control) whole blood (each n=4). The histological alterations as well as the function of these kidneys were analysed in different intervention groups (each n=4). Microthrombi positive for fibrin (Fib+MT) and platelets (CD61+MT) were defined as lumen occlusion of more than 50% with fibrin and platelets. 100 glomeruli per sample were analyzed for thrombi count as well as PAI-1, tPA, KLF4 mRNA and miRNA-143 expression.

Results: In total CD61+MT were more frequently observed than Fib+MT. Glomerular CD61+MT were increased in xenotransplants with and without intervention compared to controls. Only aPC was able to significantly diminish glomerular CD61+MT in xenotransplants. There was a significant positive correlation of glomerular and preglomerular CD61+MT as well as of glomerular PAI-1 and tPA mRNA expression. There were no differences in glomerular PAI-1, tPA, KLF4 mRNA expression between xenotransplants and controls and no correlation of these mRNA transcripts with thrombus deposition. However there was an elevated expression of miRNA-143 in xenotransplants with and without intervention.

Conclusions: Microthrombi in xenotransplants seem to be composed predominantly of platelets. aPC seems to be the most effective antithrombotic intervention. There seems to be a sufficient mRNA expression of the protective KLF4 and fibrinolytic tPA in contrast to our recent human data. miRNA-143 seems to have no effect on mRNA expression of KLF4 in this model. Therefore this xenotransplant model seems to be different from human acute humoral rejection associated TMA.

FR-PO890

Glomerular microRNA Expression Profiles in Transplant Associated Thrombotic Microangiopathy Jan U. Becker,[1] Juliane Wittig,[1] Maximilian Ernst Daemmrich,[1] Putri Andina Agustian,[1,2] Clemens L. Bockmeyer.[1] *[1]Institute of Pathology, Hannover Medical School, Hannover, Germany; [2]Clinic for Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: Thrombotic microangiopathy (TMA) in renal transplants occurs in CNI-toxicity (CNI-TMA) and acute humoral rejection (AHR-TMA). microRNAs regulate the expression and transcription of mRNA transcripts. So far nothing is known about the compartment-specific microRNA dysregulation in the glomerular capillary bed in CNI-TMA and AHR-TMA. We hypothesized (i) that transplant biopsies with CNI-TMA, AHR-TMA and without TMA (each n=5) display different microRNA profiles and (ii) that there is an association of differentially expressed microRNAs with pro- and antithrombotic mRNA transcripts that could explain microthrombosis.

Methods: The expression of 675 microRNAs and selected pro- and antithrombotic mRNA transcripts was assessed in microdissected glomeruli from transplant biopsies by real time PCR.

Results: From 88 microRNAs expressed in all samples only miR-195, miR-222 and miR-532-3p were differentially expressed. miR-195 was significantly up-regulated in CNI-TMA compared to AHR-TMA. miR-222 and miR-532-3p were up-regulated in all TMA samples as well as in CNI-TMA compared to controls. miR-532-3p was also up-regulated in AHR-TMA compared to controls. miR-532-3p was correlated positively with CD59, CD73 and PAI-1 and negatively with fibrinogen, tPA and ADAMTS13. miR-222 correlated positively with PAI-1 and negatively with KLF-2.

Conclusions: microRNAs may play a role in the regulation of the altered expression levels of pro- and antithrombotic mRNA transcripts in CNI-TMA and AHR-TMA. In particular miR-222 and miR-532-3p could lead to impaired fibrinolytic activity in the glomerular capillary bed, directly via up-regulation of PAI-1 and/or down-regulation of tPA as well as indirectly via the transcription factor KLF2. This potentially novel pathomechanism should be studied in vitro and in vivo.

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FR-PO891

The Metabolic Reprogramming in a Kidney as a Consequence of CypD Ablation Jelena Klawitter, Alexander T. Pennington, Jacek Klepacki, Jost Klawitter, Joshua M. Thurman, Uwe Christians. *University of Colorado Denver, Aurora, CO.*

Background: Cyclophilin D (CypD, encoded by *Ppif*) modulates the opening of the MPTP. The immunosuppressant CsA inhibits CypD, leading to desensitization of MPTP opening, Ca^{2+} overload and ROS stimulation. In this study we aimed to investigate (A) the role CypD ablation has on the kidney metabolism and (B) the role CypD plays in CsA-induced nephrotoxicity.

Methods: *Ppif-/-* and *Ppif* +/+ wild type (WT) male mice were treated with either CsA (25 mg/kg/day) or vehicle (skim milk) from 8-12 weeks of age. Following perchloric acid extraction, water-soluble kidney tissue fractions (n=5-7) were used for mass spectrometry-based measurement of mitochondrial nucleotide loads and Krebs cycle intermediates.

Results: (A) The concentrations of Krebs cycle intermediates citrate and succinate were significantly higher in kidneys and urine of *Ppif-/-* as compared to WT mice. The consequence of this metabolic reprogramming towards mitochondrial energy production was a significantly higher energy charge in *Ppif-/-* as compared to WT mice kidneys.

(B) After treatment with CsA, no significant changes in the Krebs cycle metabolites within the kidney of either knockout or WT mice were observed. Their urinary concentration was however significantly reduced in *Ppif-/-* mice. This suggested that *Ppif-/-* mice try to retain the Krebs cycle intermediates inside the kidney in order to cope with the declining ATP production. And while the kidney energy charge significantly decreased and plasma creatinine significantly increased in *Ppif-/-* mice, both remained stable in WT mice. It is interesting to note that the described metabolic changes occurred before any histological changes in the kidney became detectable.

Conclusions: Our data suggest that CypD knockout and dysfunction in the MPTP opening, as a coping mechanism, increases the energy demand of the kidney. This in turn activates kidney's mitochondrial energy production pathways. The observation that this metabolic reprogramming makes the kidney more vulnerable to the negative effects of CsA suggests that there is an alternative pathway, independent of CypD, by which CsA causes mitochondrial dysfunction.

FR-PO892

An Investigation of Calcineurin Inhibitor Toxicity and Cadherin Expression in Human Kidney Nileshkumar Shah,[1] Seema Jain,[1] Roel Goldschmeding,[2] Iain Macphee,[3] Mysore Keshavmurthy Phanish,[1] Mark Edward Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]UMC, Utrecht, Netherlands; [3]St George's, University of London, United Kingdom.*

Background: Calcineurin inhibitors (CNI) are the mainstay of transplant immunosuppressive regimes although long term graft life remains limited due to their nephrotoxicity, the mechanism of which remains unclear. We investigated the effects of CNIs on human primary proximal tubule cells (PTEC) with respect to their effects on native cadherins, K & N. We also investigated the role of Cytochrome P450 (CYP) enzyme activity on calcineurin mediated nephrotoxicity.

Methods: Biopsy samples demonstrating Chronic Allograft Nephropathy (CAN) and controls, were probed for K & N cadherins. In experiments carried out on human PTEC,

cells were treated with vehicle, Cyclosporine, CsA 1μg/ml,Tacrolimus, FK 0.1μg/ml, TGFβ1 5ng/ml for 72hr. An ALK5 inhibitor was used to assess the role of TGFβ1 in CNI mediated effects. Secreted TGFβ1 was measured by BioPlex. Cells were treated with CYP enzyme inhibitor, Ketoconazole 10μM with or without CNI. Expression of cadherins and profibrotic proteins were measured.

Results: Biopsy tissue from patients with CAN revealed loss of K & N cadherin compared to healthy controls. Treatment with FK caused loss of K cadherin in human PTEC similar to treatment with TGFβ1. CsA and FK induced significant 4 and 3 fold increases in TGFβ1 secretion. CNI caused activation of Smads and P38 at 48 h. CNI caused a significant rise in pro-fibrotic proteins (Fn, MMP2 & CTGF). These effects were abrogated by inhibition of Alk5. There was no increase in N cadherin expression with CNI treatment. CYP3A expression and inhibition of its activity by Ketoconazole was observed in PTEC. Combination of Ketoconazole and CNI augmented loss of K cadherin. Ketoconazole alone caused loss of K cadherin without significant cell death.

Conclusions: Our results suggest that CNI induced nephrotoxicity is mediated by increased TGFβ1 production rather than activation of the latent form; lack of N cadherin rise suggests differential activation of TGFβ-mediated outcomes. In addition, ketoconazole augmented the effects of CNI but also directly damaged human PTEC.

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FR-PO893

The Effect of Calcineurin-Inhibition on the Renal Renin-Angiotensin System: New Place for Renin Excretion Agnes Prokai,[1] Nora Himer,[1] Katalin Kis-petik,[1] Janos Peti-Peterdi,[2] Attila Szabo.[1] *[1]Semmelweis University and Research Laboratory of Hungarian Academy of Sciences, Hungary; [2]University of Southern California.*

Background: Tacrolimus (Tac) and Cyclosporin A (CyA) are two potent immunosuppressants being essential therapeutic solutions for the prevention of allograft rejection, at the same time possessing nephrotoxic side effects. However, the underlying patomechanism how these drugs act as nephrotoxic agents are still not fully understood. In this study we investigated *in vivo* the effect of calcineurin-inhibitors (CNI) on the renal renin-angiotensin system (RAS).

Methods: Three week old, male C57B6 mice (n=15) were divided into three groups: controls (C) and mice treated with 0.075 mg/kg/day of Tac twice a day (Tac) or 2mg/kg/day of CyA (CyA). Following three weeks of administration serum creatinine was measured. The renin content in the collecting duct (CD) and juxtaglomerular apparatus (JGA) were evaluated applying FACS and multi-photon microscopy. The extent of vessel contraction was assessed and the consequent fibrosis was determined by Masson staining.

Results: The trough level of CNI was comparable to that used in clinical practice. Serum creatinine was significantly elevated in both Tac and CyA groups. Applying FACS analysis we demonstrated that both the JGA and CD renin content increased 4-fold following the administration of CNI. These data were further supported by *in vivo* multi-photon microscopy; renin granulation increased remarkably in both locations. As a result of local RAS activation vasoconstriction was present in both treated groups and as early as the third week of immunosuppression fibrotic islands developed.

Conclusions: In summary, our studies visually revealed that CNI possess nephrotoxic effect on the kidney parenchyma due to enhanced renin activity not only in the JGA but in the CD segment as well. Therefore, RAS inhibition could be beneficial by preventing the nephrotoxic effect of the CNI. However, further studies are needed to reveal what kind of inhibitors provide the most efficient treatment.

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FR-PO894

The Effect of Korean Red Ginseng on Chronic Cyclosporine-Induced Renal and Pancreatic Injury Kyoung Chan Doh,[1,2] Sun Woo Lim,[1,2] Shang Guo Piao,[1,2] Ohk Bun Jung,[4] Soo Kyung Bae,[4] Gyu Hyun Hwang,[5] Gyeong Il Min,[5] Byung Ha Chung,[1,2,3] Chul Woo Yang.[1,2,3] *[1]Convergent Research Consortium for Immunologic Disease, Catholic University of Korea, Seoul, Republic of Korea; [2]Transplant Research Center, The Catholic University of Korea, Seoul, Republic of Korea; [3]Division of Nephrology, Catholic University of Korea, Seoul, Republic of Korea; [4]College of Pharmacy, The Catholic University of Korea, Bucheon-si, Republic of Korea; [5]School of Medicine, Catholic University of Korea, Seoul, Republic of Korea.*

Background: The study was performed to investigate whether the Korean red ginseng (KRG) has protective effect in an experimental model of chronic CsA toxicity.

Methods: Mice were treated with CsA (30 mg/kg/day, s.c.) and KRG (0.5g or 1 g/kg/day, P.O.) under 0.01% salt diet for 4 weeks. Body weight, urine volume, and blood glucose level were measured before sacrifice. Blood level of CsA was evaluated using LC/MS/MS. Induction of renal fibrosis and islet mass were analyzed in tissue sections. Immunohistochemistry using F4/80 antibody was performed to detect macrophage infiltration. Oxidative stress was measured with urinary excretion and serum level of 8-hydroxy-2'-deoxyguanosine (8-OHdG).

Results: After 4 weeks, there were no differences in Δ body weight and urine volume. Blood glucose level of CsA treated mice was normalized concurrent with KRG 0.5 but not in KRG 1. Blood level of CsA does not differ with concurrent with KRG 0.5 but was significantly increased compared with CsA+KRG 1 group. KRG 0.5 treatment significantly decreased both of CsA-induced renal fibrosis and islet mass but not in KRG 1. Consistently, increased macrophage infiltration in kidney and islet in CsA group was markedly decreased in only CsA+KRG 0.5. 8-OHdG level detecting oxidative stress in urine and serum was also significantly decreased concurrent with KRG 0.5+CsA group compared with CsA, however, there was no difference in CsA+KRG 1.

Conclusions: From our results, we suggest that KRG have protective effect against CsA-induced renal and pancreatic injury through decreasing oxidative stress. However, high dose of KRG on CsA toxicity may aggravate CsA level in blood, and there is possibility synergistic organ toxicity.

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FR-PO895

Gene Therapy with Indoleamine 2,3-Dioxygenase Inhibits Development of Chronic Transplant Dysfunction in Rat Diana Vavrincova-Yaghi,[1] Leo E. Deelman,[1] Marc Seelen,[2] Harry Van Goor,[3] Robert H. Henning,[1] Maria Sandovici.[1] *[1]Department of Clinical Pharmacology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands; [2]Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, Netherlands; [3]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.*

Background: Chronic transplant dysfunction (CTD) is the primary reason for late allograft loss in kidney transplantation. Indoleamine 2, 3-dioxygenase (IDO) is involved in foeto-maternal tolerance, and prevents allograft rejection. In our previous experiment, we showed gene therapy with IDO inhibits acute rejection of renal allograft. The aim of current study is to show whether IDO is also able to improve CTD.

Methods: Transplantation was performed in Dark-Agouti to Wistar-Furth CTD model. Adenovirus carrying IDO gene (AdTIDO, n=7) and gene for GFP (AdTL, n=5) and saline were injected into renal artery of donor kidney. Recipients were immunosuppressed with cyclosporine for 10 days. After, the contra lateral kidney was removed. Body weight, serum creatinine and blood pressure (BP) were measured and 24 hour urine was collected every two weeks. Rats were sacrificed after 12 weeks.

Results: IDO treatment significantly improved body weight during whole experiment compared to AdTL and saline groups. Although there was no significant change in plasma creatinine or creatinine clearance, treatment with IDO decreased significantly elevated proteinuria (20.3±5 mg/24 h, vs 56.8±19 mg/24 h and±32.8 mg/24 h). Additionally, IDO therapy decreased BP significantly (137±2 mmHg vs 154±10 mmHg and 163±8 mmHg). Moreover, it decreased the incidence of focal glomerulosclerosis (2.57±0.74 %) compared to AdTL and saline (7.92±4.19 %; 9.60±5.47 %). Furthermore, IDO gene therapy decreased significantly the number of graft-infiltrating macrophages and expression of α-smooth muscle actin. The level of foxp3 mRNA, the marker of tolerogenic T cells, was elevated in the IDO treated group.

Conclusions: Here we show for first time the beneficial effect of local IDO gene therapy in a model of CTD.

FR-PO896

Antithymocytes Globulines and Immune Senescence in Renal Transplant Patients Jamal Bamoulid,[1,2,3] Caroline Roubiou,[1,2,3] Thomas Crépin,[1,2,3] Béatrice Gaugler,[2,3] Jean Marc Chalopin,[1,2,3] Philippe Saas,[2,3] Didier Ducloux.[1,2,3] *[1]Nephrology, CHU Besançon, Besançon, France; [2]INSERM 1098, IFR 133, Besançon, France; [3]Faculté de Médecine et de Pharmacie, Université de Franche-Comté, Besançon, France.*

Background: CD4 T cell reconstitution after antithymocyte globulins (ATG) is dependent on pre-transplant thymic function and persistent ATG-induced CD4 T cell lymphopenia is associated with abnormalities close to those observed in immune senescence. We hypothesized that ATG could be responsible of accelerated immune senescence in renal transplant recipients (RTR).

Methods: We analyzed a prospective cohort of 66 incident RTR. Thymic output of recent thymic emigrants (RTE), regulatory T cells (Treg), $CD8^+$ T cell subsets were studied by flow cytometry at transplant and one year after transplantation. Twenty out of 66 patients were also studied for T lymphocyte relative telomere length and telomerase activity at both times. Age, gender, induction therapy (ATG or anti-CD25 monoclonal antibody [mAb]), immunosuppressive regimen, and CMV status were analyzed as potential confounding factors.

Results: 46 patients received ATG whereas 20 received monoclonal anti-CD25 mAb. Pre-transplant RTE cell count predicted $CD4^+$ T cell count 1 year after transplantation. RTE cell count significantly decreased and were lower in ATG than in anti-CD25 mAb-treated recipients. Proportion of $CD8^+CD28^-$T cells increased after transplant in ATG patients. This increase was more pronounced in RTR with an inverted CD4/CD8 T-cell ratio and in CMV-positive RTR. Treg were more frequent after transplant in ATG than in anti-CD25 mAb recipients. In ATG recipients, Treg peripheral expansion was related to poor thymic function one year post-transplant. The relative telomere length of T lymphocyte increased in anti-CD25 mAb but not in ATG recipients after transplantation. No statistical differences were observed in relative telomerase activity after transplantation.

Conclusions: ATG is associated with reduced thymic output of naive T cells, increased Treg, lymphocyte phenotype and relative telomere length evocative of immune senescence. Mechanisms underlying this thymic alteration and clinical consequences remain to be studied.

FR-PO897

Treatment with a Spleen Tyrosine Kinase (SYK) Inhibitor Reduced the Severity of Experimental Renal Allograft Rejection Jennifer Smith,[1] Gurjeet Bhangal,[1] Asim Syed,[1] Esteban S. Masuda,[2] H. Terence Cook,[1] Nadey S. Hakim,[1] Charles D. Pusey,[1] Frederick W.K. Tam.[1] *[1]Imperial College Kidney and Transplant Institute, Hammersmith Hospital, Imperial College London, London, United Kingdom; [2]Rigel Pharmaceuticals Inc, South San Francisco, CA.*

Background: Spleen tyrosine kinase (SYK) is important in downstream signalling of immune receptors, including B cell receptors and Fc receptors in lymphocytes, monocytes/ macrophages and intrinsic renal cells. Fostamatinib, a SYK inhibitor, was effective in prevention and treatment of antibody mediated experimental glomerulonephritis (Smith J et al. J Am Soc Nephrol. 21:231-6, 2010). However, it is not known whether inhibition of SYK will be effective in prevention of renal allograft rejection.

Methods: We investigated the effect of fostamatinib (provided by Rigel Pharmaceuticals and AstraZeneca) in a rat model of severe rapidly progressive renal allograft rejection. In each operation, the left kidney of a Lewis rat was removed and replaced by a left kidney from a Brown Norway rat. The native right kidneys of the Lewis rats were kept in situ. Fostamatinib treatment or vehicle was given by oral gavage twice daily. The extent of renal allograft rejection was analysed in renal tissue collected 7 days after transplantation. The severity of cortical infarction was assessed with a scale from 0 to 5.

Results: In the vehicle treated rats (n=12), there was extensive infarction of the renal allograft, grades 5 (4-5), median (quartiles). In the fostamatinib treated rats, there was complete prevention of allograft infarction 0 (0-0) and 0 (0-0) in rats treated with 20 mg/kg (n=7) and 30 mg/kg (n=7) respectively twice daily ($p<0.001$). There was dose dependent reduction of the interstitial infiltrates in the fostamatinib treated rats. Interstitial macrophages were reduced by 93 %, $p<0.01$; CD8+ cells reduced by 96 %, $p<0.05$ and endothelial expression of C4d reduced by 78% ($p<0.05$) in the rats treated with 30 mg/kg fostamatinib in comparison to the vehicle treated rats.

Conclusions: To our knowledge, this is the first report showing that treatment with a SYK inhibitor is effective in reducing the severity of experimental renal allograft rejection.

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FR-PO898

Thrombin Modulate T Cells Response Induced by Dendritic Cells (DC) in Kidney Transplant Recipients with Delayed Graft Function (DGF) Paola Pontrelli,[1] M. Cariello,[1] Raffaella Verrienti,[1] T. Tataranni,[1] Giovanni Stallone,[2] Francesco Paolo Schena,[1] Loreto Gesualdo,[1] Giuseppe Grandaliano.[2] *[1]DETO-Nephrology Unit, Univ. of Bari, Italy; [2]Dept. of Medical and Surgical Sciences, Univ. of Foggia, Italy.*

Background: Coagulation and complement (C) activation represent key pathogenic events in ischemia-reperfusion-induced renal injury leading to DGF. Aim of our study was to evaluate if thrombin (thr) may induce renal C production and influence the ability of DCs to modulate Th1/Th2 bias.

Methods: PAR-1, BDCA1 (myeloid DC), BDCA4 (plasmacytoid DC), fibrin and C3c protein expression were evaluated by confocal microscopy in pre-transplant and DGF graft biopsies (n=10). Cultured DCs were obtained incubating monocytes with IL-4 and GM-CSF. DC maturation was achieved using a cytokine cocktail. PAR1 protein expression on cultured DC was evaluated by flow cytometry. C receptors, C3, IL12p40 and IL10 gene expression were evaluated by real time PCR.T cell phenotype was analyzed by ELISPOT, after a 2 weeks-exposure to thr-treated DC.

Results: PAR1, the main thr receptor, was expressed by myeloid, but not plasmacytoid, infiltrating DCs in DGF patients. BDCA1+ DCs localized in interstitial areas characterized by fibrin and C3c deposits and expressed C3. To investigate the link between thr and C activation, we evaluated the ability of thr to modulate C3 expression in cultured DC. PAR-1 protein abundance increased in cultured immature and mature DC compared to Ms (73.8±14.4; 73.5±10.4; 32.1±12.4 MFI, respectively p=.03). Thr stimulation induced an increase in C receptors 1, 2 and 3 expression, with a peak at 6 hours ($p<.05$). PAR-1 activation caused a time-dependent increase in C3 (6 fold at 6 hours) and IL-12 p40 gene expression (2.7 fold at 6 hours), while strikingly reducing IL-10 mRNA abundance (2.4 fold at 6 hours). ELISPOT confirmed the ability of thr-treated DC to increase IFNg production by T cells (1.6 fold), suggesting the activation of the Th1 bias.

Conclusions: Our data suggest that thr-activated PAR-1 may induce renal C production and modulate Th-1 response by DC. This observation may suggest a potential pathogenic link between DGF and acquired alloresponse leading to progressive graft damage.

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FR-PO899

Macrophage Transcripts ADAMDEC1, CXCL13 and CCL18 Are a Hallmark of T Cell-Mediated Rejection in Humans Dina F. Badr,[1] Luis G. Hidalgo,[2] Konrad S. Famulski,[2] Philip F. Halloran.[1] *[1]Medicine, University of Alberta, Edmonton, AB, Canada; [2]Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada.*

Background: The two types of rejection recognized in the Banff classification are T cell-mediated rejection(TCMR) and antibody-mediated rejection(ABMR). Macrophages and T cells are the dominant cells infiltrating acutely rejecting renal allografts.

Methods: Four hundred and three kidney transplant biopsies for cause (BFC) were classified using a modified Banff classification. Using microarray analysis, we compared the gene expression in biopsies with TCMR (n=35) vs ABMR (n=75) and defined transcripts that are preferentially expressed in TCMR. We studied their relationship to Banff lesions and macrophage/T cell burden.

Results: CXCL13, ADAMDEC1 and CCL18 were the top three transcripts with higher expression in TCMR over ABMR (FDR<0.0001). All 3 transcripts were significantly higher in TCMR than in BFC with no major abnormalities, acute kidney injury,borderline rejection and interstitial fibrosis and tubular atrophy. All 3 transcripts correlated particularly well with Banff interstitial inflammation (i-score), tubulitis (t-score) and intimal arteritis (v-score) in all 403 BFC (table 1). Higher expression of ADAMDEC1, CXCL13 and CCL18 was associated with increased T cell/macrophage burden in all 403 BFC and in TCMR biopsies. In a primary human cell panel, only macrophages expressed higher levels of all 3 transcripts compared to nephrectomies (figure 1).

Conclusions: Macrophage transcripts ADAMDEC1, CXCL13 and CCL18 expression distinguish TCMR from other Banff categories. Their correlations to Banff lesions for TCMR and to T cell/macrophage burden suggest a role for macrophages in alloimmune mechanisms implicated in TCMR. Better understanding of the role of macrophages in rejecting kidneys may offer helpful diagnostic tools as well as possible future therapeutic targets.

Table 1. Correlations of ADAMDEC1, CXCL13 and CCL18 to Banff scores and burdens of macrophages and T cells in 403 BFC†

Probe set ID	Gene	Banff lesion score			T cell burden	Macrophage burden
		i-score	t-score	v-score		
206134_at	ADAMDEC1	0.44	0.40	0.27	0.72	0.78
205242_at	CXCL13	0.37	0.39	0.21	0.74	0.53
32128_at	CCL18	0.30	0.28	0.22	0.48	0.58

† Spearman correlation coefficient, p values <0.0001

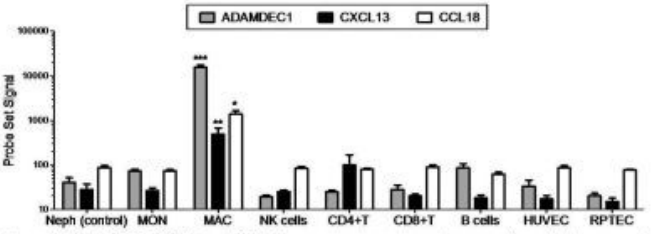

Figure 1. ADAMDEC1, CXCL13 and CCL18 expression in a primary human cell panel (microarrays). RPTEC; renal proximal tubule epithelial cells, HUVEC; human umbilical vein endothelial cells; CD8+T: CD8+T cells, CD4+T: CD4+T cells, NK cells: natural killer cells, MAC; macrophages and MON: monocytes. Probe set signal (mean ± SE of ≥3 donors) in each cell type was compared to control nephrectomies (Neph) using One-way ANOVA with Dunnett's Multiple Comparison Test, *p<0.05 , **p<0.01, ***p<0.001

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FR-PO900

The Role of TIM-4 on Antigen Presenting Cell Function in Alloimmunity Martina M. McGrath, Melissa Y. Yeung, Nader Najafian. *Brigham and Women's Hospital, Harvard Medical School.*

Background: TIM4, expressed on DCs and macrophages, functions both as a costimulatory molecule and a phosphotidylserine receptor, mediating phagocytosis of apoptotic cells. Costimulatory blockade of TIM4 in vivo is associated with increased allograft survival. However, this is associated with splenic accumulation of apoptotic cells. The impact of this effect on allograft outcome is unclear. We have sought to specifically examine the effect of TIM4 blockade on phagocytosis.

Methods: Apoptotic cells were generated by incubating CFSE-labeled Balb/c thymocytes for 6 hours in 10mM Dexamethasone, where >75% of cells become apoptotic. In vitro phagocytosis assays were carried out using apoptotic cells incubated with B6 splenocytes for 2 hours. Phagocytosis was assessed using flow cytometry, analysing cells positive for CFSE and either CD11c or F4-80. For in vivo phagocytosis assays, $20x10^6$ apoptotic cells were injected IV into B6 recipients. At 2 hours, spleens were harvested for flow analysis as outlined.

Results: TIM4+ and TIM4neg DCs have a similar capacity for in vitro phagocytosis (67.7% vs 68.2% p=NS). TIM4+ macrophages show greater phagocytosis than TIM4neg (32.9%vs 15.9% $p<0.01$). In vitro treatment with RMT 4-53 has no effect on uptake of apoptotic cells by DCs but leads to marked decrease in phagocytosis by peritoneal macrophages (PMs) (48.78% to 27.68% $p<0.01$).

DC phagocytosis in vivo is increased after treatment with RMT4-53 (18% to 27.5% p=0.049). This appears to be due to a shift in uptake to DCs, as phagocytosis by F4-80+ splenic macrophages is not blocked. After exposure to apoptotic cells in vivo, DCs downregulate MHC Class II expression (from 94% to 80% by 6hrs).

Conclusions: TIM-4 plays a non-crucial role in phagocytosis of apoptotic cells by splenic DCs. It is highly expressed on PMs, and blockade impairs their ability to phagocytose. Despite widespread expression on macrophages, it remains unclear which subset is most affected in vivo, leading to observed splenic accumulation of apoptotic cells. We hypothesise that the shift in phagocytosis of apoptotic cells to DCs after blockade of TIM4 may promote a more tolerogenic DC phenotype and may contribute to allograft survival.

FR-PO901

Role of T Regulatory Cells (CD4+CD25+FOXPS3+) in Renal Transplant Patients with Chronic Allograft Dysfunction Raj K. Sharma, Sharad K. Mittal, Amit Gupta, Sita Naik. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Scineces, Lucknow, UP, India.*

Background: We studied the role of CD4+CD25+FOXP3 T regulatory cells (Tregs) in renal transplant patients with chronic allograft dysfunction.

Methods: Patients with chronic allograft dysfunction (n=20, Serum Creatinine; range 2.0 to 4.23), patients with normal graft function (n=20, Serum Creatinine; 0.8 to 1.5) surviving for >2.5 years, and healthy volunteers (n=10) were enrolled. Of these 20 patients with chronic allograft dysfunction (CAD), 10 patients had history of acute rejection in past and or evidence of chronic immune mediated rejection on graft biopsy and 10 patients had non immunological graft dysfunction but had interstitial fibrosis and tubular atrophy. CD4+CD25+FOXP3+ Tregs were enumerated by flow cytometry. CD4+CD25+ T cells (Tregs) and CD4+CD25- T effector cells (Teff) from PBMC of renal patients were isolated. Teff cells were stimulated with anti CD3 for 3 days and cocultured in the presense and absence of Tregs at ratios (Tregs:Teff) of 1:1 and 2:1. Proliferation was measured using the thymidine (H3) uptake.

Results: The median suppressive capacity of CD4+CD25+ Tregs of patients with chronic allograft dysfunction (n=20) was 20.4% (range 0 to 51.29) at 1:1 ratio and 8.95% (0 to 28.5) at 2:1 ratio. This was significantly less than the patients with normal graft function (median 66.38%; range 30.1 to 95.35; $p<0.05$ at 1:1 ratio and 23.3%; 0 to 59; $p<0.05$ at 2:1 ratio) and healthy control (72.56; 19.8 to 92.5 at 1:1 ratio and 30.65; 7.22 to 46.05 at 2:1 ratio) at both ratios. Frequency of Tregs in patients with chronic allograft dysfunction (median 7.54%; range 2.61 to 15.45) and patients with normal graft function (8.21%; 3.25 to 19.1) was higher than healthy control (5.53%; 3.79 to 10.79; $P<0.05$). However, it was comparable between the patients with normal graft function and with chronic allograft dysfunction.

Conclusions: We conclude that Tregs in patients with chronic allograft dysfunction though expanded were less suppressive as compared to patients with normal graft dysfunction. Suppressive functionality of expanded Tregs may be important for the maintenance of good graft function in transplant recipients.

FR-PO902

Immune Response Indictors in Kidney Transplant Recipients within the First Year after Transplantation Magdalena Krajewska,[1] Katarzyna Koscielska-Kasprzak,[1] Marcelina Zabinska,[1] Katarzyna Madziarska,[1] Waclaw Weyde,[1] Dariusz Janczak,[3] Agnieszka Gomulkiewicz,[2] Piotr Dziegiel,[2] Marian Klinger.[1] *[1]Dept. of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Dolnoslaskie, Poland; [2]Dept. of Histology and Embryology, Wroclaw Medical University, Wroclaw, Dolnoslaskie, Poland; [3]Dept. of Vascular and General Surgery, Wroclaw Military Hospital, Wroclaw, Dolnoslaskie, Poland.*

Background: In previously examined 170 pts (7y after KTx) we observed expansion of $CD8^+CD28^-$ cells.

The goal of this research was to investigate regulatory and cytotoxic gene expression in a group of Ktx pts during the 1 year post-KTx. The phenotype analysis of T cell subpopulations was also performed.

Methods: 36 KTx pts and 20 volunteers have been included. The research involved PBMC gene expression analysis on Taqman LDAs of FOXp3, ILR2A, GZMB, PRF1, GAPDH genes. The cells were flow cytometry phenotyped and the $CD3^+CD8^+CD28^-$, $CD3^+CD4^+CD25^+CD127^{low}$ populations were assessed. GZMB expression by $CD3^+CD4^+$ and $CD3^+CD8^+$ cells has been determined.

Results: The number of $CD3^+CD8^+CD28^-$ cells increases after KTx and is correlated with GZMB and PRF1 gene expression. However, this subpopulation does not predict the transplant outcome.

The level of nTregs observed 1 m after KTx is similar to controls and is decreasing over time reaching significance 6 m after KTx p=0.011. The nTreg number observed 3 m after KTx is a predictor of 1 y transplant outcome rs=-0.43, p=0.015. The nTreg population is correlated with FOXp3 and ILR2A expression in PBMC.

The FOXp3 and ILR2A expression in KTx pts is lower than in controls and remains unchanged over the first y post-transplant. The higher regulatory gene expression is related to better transplant outcome FOXp3 expression 3 m post-KTx and 1 y sCr correlation rs=-0.66, $p<0.0001$. The GZMB expression is also developed post-KTx in $CD3^+CD4^+$ cells and is related to CD28 negativity. Additionally initial study shows that $CD3^+CD4^+CD28^-$ population may be negatively correlated with graft function >1 year post-KTx, SCr, rs=0.44, p=0.039.

Conclusions: The immune state in KtX is characterized by nTreg fall and $CD8^+CD28^-$ T cells induction. The latter seems to have cytotoxic properties.

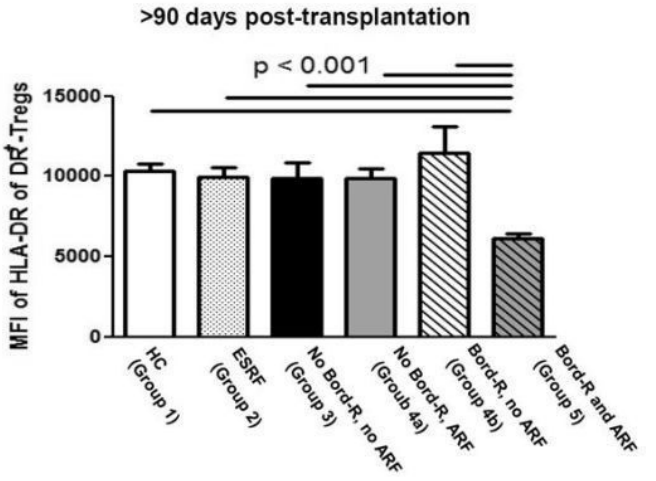
FR-PO903

The Extent of HLA-DR Expression of DR^+-Tregs Is Significantly Reduced in Transplanted Patients with Clinically Relevant Borderline Rejection Matthias Schaier,[1] Nicole Seissler,[1] Luis Eduardo Becker,[1] Claudia Sommerer,[1] Sebastian Schäfer,[1] Ruediger Waldherr,[3] Andrea Steinborn,[2] Martin G. Zeier.[1] *[1]Department of Nephrology, Univeristy of Heidelberg, Heidelberg, Germany; [2]Department of Gynecology, Univeristy of Heidelberg, Heidelberg, Germany; [3]Department of Pathology, Univeristy of Heidelberg, Heidelberg, Germany.*

Background: Regulatory T cells (Tregs) were shown to be involved into the pathogenesis of acute rejection after transplantation. The suppressive activity of the total regulatory T cell pool depends on its percentage of highly suppressive $HLA\text{-}DR^+$-Treg cells.

Methods: Therefore, both the suppressive activity of the total Treg pool and the extent of HLA-DR expression of DR^+-Tregs (MFI HLA-DR) were estimated in non transplanted volunteers, patients with end-stage renal failure (ESRF), healthy renal transplant patients with suspicion on rejection, due to sole histologic Bord-R or sole acute renal failure (ARF), and patients with clinically relevant borderline rejection (Bord-R and ARF).

Results: Transplanted patients with clinically relevant borderline rejection show significantly reduced suppressive activity of their $CD4^+CD127^{low+/-}CD25^+$-Tregs. Our data propose that the HLA-DR MFI strongly depends on its composition with DR^{high+} and DR^{low+} Tregs. Patients with clinically relevant borderline rejection have significant lower HLA-DR MFI´s and significantly reduced proportions of DR^{high+}-Tregs, which have the highest suppressive capacity within the total Treg pool.



Conclusions: Our findings clearly demonstrate that the determination of the HLA-DR MFI of the $HLA\text{-}DR^+$-Treg subset allows a highly sensitive, specific and non-invasive discrimination between patients with clinically relevant Bord-R (Bord and ARF) and patients with subclinical rejection or other causes of transplant failure.

FR-PO904

The Pathological Characteristics of Kidney in Acute Graft-Versus-Host Disease after DA-to-Lewis Rat Bone Marrow Transplantation Seiichiro Higo,[1,2] Akira Shimizu,[1] Shinya Nagasaka,[1] Go Kanzaki,[1] Yukinari Masuda,[1] Akiko Mii.[2] *[1]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Department of Internal Medicine, Divisions of Neurology, Nephrology and Rheumatorogy, Nippon Medical School, Tokyo, Japan.*

Background: It is known that allogeneic bone marrow transplantation (allo-BMT) causes acute or chronic graft-versus-host-disease (GVHD). Liver, gut, and skin are known to primary target sites of acute and chronic GVHD. However, kidney is still uncertain to be influenced by GVHD. In the present study, we examined the pathology of kidney in acute GVHD in DA-to-Lweis rat allo-BMT.

Methods: Acute GVHD was induced in Lewis rats (RT1l) by transplantation of DA rat (RT1a) bone marrow cells ($6.0x10^7$ cells) after lethal irradiation (10Gy). We examined the clinical and pathological characteristics of acute GVHD in various organs, after BMT without immunosuppression.

Results: Almost all white blood cells in peripheral blood were constituted with DA phenotype. Between 21 and 40 days after allo-BMT, acute GVHD was developed with decreased body weight (>20%), skin rush with alopecia, and diarrhea. Liver and kidney dysfunction was detected: asparate aminotransferase (AST:143±103U/L), alanine aminotransferase (ALT:105.5±6.5U/L), and blood urea nitrogen (BUN:30.6±7.0mg/dL). The pathology of liver, skin, and gut showed acute GVHD that was characterized by very similar findings of severe acute T-cell mediated rejection in transplanted liver, skin, and small intestine grafts. In kidney, many $CD3^+$ T cells and $CD68^+$ macrophages infiltrated that mediated the interstitial inflammation around small arteries that expanded into peritubular interstitium with severe peritubular capillaritis. Acute glomerulitis and endarteritis in small arterioles were evident. Acute tubulitis with $CD3^+$ T cells was also noted. No obvious IgG, IgM, and C3 deposition was detected in the kidney.

Conclusions: Kidney was one of the target organs of acute GVHD. The pathology of acute GVHD in the kidney was characterized by the T cell-mediated injury for the microvascular endothelium and renal tubules, and these findings were very similar of acute T-cell mediated rejection in transplanted kidney grafts.

FR-PO905

Impact of Immunosuppressive Treatment on Digestive Tract Colonization of Pyelonephritis Strain Escherichia Coli 536 Jerome Tourret,[1] Ben P. Willing,[2] Gilbert Deray,[1] Corinne Isnard-Bagnis,[1] Brett B. Finlay.[2] *[1]Dpt of Urology, Nephrology and Transplantation, AP-HP, Pierre and Marie Curie University, Paris, France; [2]Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.*

Background: Uropathogenic *E. coli* (UPEC) strains cause urinary tract infections (UTIs) which are a major concern in kidney transplant recipients (KTRs). Acute graft pyelonephritis is responsible for long term kidney graft function deterioration. The determinants of *E. coli*'s establishment in the host's digestive tract before it reaches the urinary tract and the impact of immunosuppressive (IS) drugs on intestinal colonization have not been studied.

Methods: C3H mice were orally challenged with UPEC strain 536. Digestive tract colonization was assessed by plating of fecal samples. Mice were sacrificed at various time points and cytokine expression was measured in different segments of the digestive tract. Strain-specific anti-*E. coli* antibodies were measured in blood. The experiment was repeated after 2 weeks of treatment with prednisolone, mycophenolate mofetyl and tacrolimus.

Results: Pyelonephritis *E. coli* strain 536 efficiently colonized the mouse digestive tract, with fecal counts as high as 10^8 CFU/g after a single oral challenge. Intestinal colonization remained stable for at least 2 weeks. The incoming *E. coli* strain was specifically detected in the distal intestine through a peak of the inflammatory cytokines IL6 and TNF 48 hrs after bacterial oral challenge. Yet, no inflammatory infiltrate was observed in intestinal sections. Even though specific anti-*E. coli* IgG could be detected in blood 2 weeks after intestinal colonization, an inflammatory response was observed 48 hrs after a second oral challenge in pre-colonized mice. When mice were treated for 2 weeks with IS drugs before oral challenge, host's digestive inflammatory response was abrogated and *E. coli*'s digestive tract colonization increased 1000 fold.

Conclusions: Innate and adaptive immunity cooperate to control UPEC intestinal colonization. IS drugs might favor UTIs in kidney transplant recipients through enhanced digestive colonization. Trying to block digestive colonization by UPEC could be a new strategy to prevent UTIs in KTRs.

Funding: Pharmaceutical Company Support - Novartis, Astellas, Private Foundation Support

FR-PO906

United Kingdom Study of Living-Kidney Donor Relationships: Gender and Ethnic Variations Rishi Pruthi,[1] Rommel Ravanan,[2] Anna Casula,[1] Paul J. Roderick.[3] *[1]UK Renal Registry, Bristol, England, United Kingdom; [2]Renal Unit, Southmead Hospital, Bristol, England, United Kingdom; [3]Public Health, University of Southampton, Southampton, England, United Kingdom.*

Background: Ethnic minorities constitute a disproportionate number on the waiting list, and are subject to longer waiting times. By analysing the relationships between live donors and their recipients, we aim to gain a better understanding of how different communities/ populations view living donation.

Methods: Using data from NHS Blood & Transplant we reviewed the demographic characteristics of all living donor renal transplant recipients and their respective donors between 2001-2010 in the UK. The relationship between recipients and their donors was analysed together with their ethnicity and gender.

Results: 6580 patients and their respective donors were analysed. Women contributed 54.2% to the donor group, and 40.1% to the recipient group. Ethnic minorities received fewer transplants; Asian 7.2% (n=471) and Black 3.7% (n=244) than their proportional representation on the waiting list 26.2% (n=1760). Spousal donation was similar in both white 23.8% (n=1360) and Asian 21.7% (n=102) populations, but significantly reduced in the Black population 13.5% (n=33) p=0.0001 which had significantly higher sibling donation (43.6%) p<0.0001. Whilst spousal gender disparity (female preponderance) was universal in all groups this was significantly greater in the Asian population where women donated 79.4% compared to black 60.6% and white 61.4% p=0.003. Gender disparity was not seen amongst offspring donating. The proportion of parental donations has reduced over time, with a rise in kidney donation from offspring and from 'other' relationships p<0.001.

Conclusions: Living kidney donation is subject to significant unexplained relationship differences amongst ethnic minorities. Spousal donation is significantly lower in the Black population which has higher sibling donation. Gender disparity is greatest in the Asian spousal population, and donation from 'other' relationships has risen over time. There is a need to understand these differences, and develop a strategy to increase donation rates in ethnic minorities especially from male spouses.

FR-PO907

Quality of Life after Living Renal Donation Claudia Sommerer,[1] Ralf A. Dikow,[2] Matthias Schaier,[1] Christian Morath,[1] Vedat Schwenger,[1] Martin G. Zeier.[1] *[1]Nephrology, University Hospital Heidelberg, Heidelberg, Germany; [2]Dialysis Center, Bruchsal, Germany.*

Background: Living renal transplantation represents a favourable alternative to deceased renal transplantation with excellent recipient and allograft survival results. Until now, only limited data about short- and longterm quality of life and psycho-social consequences for the living donor are available.

Methods: In an open, prospective observational study renal allograft donors were evaluated concerning quality of life and psycho-social results of living donation. Standardized questionnaires as well as additional questions related to living donation were used (Zerssen Symptome Score, SF-12).

Results: Altogether, 125 renal allograft donors were evaluated (43 male, median age 52.7±11.4, mean time after transplantation 3.7±3.8). None of the donors had any serious post-transplant complications and the renal function was stable. Most of the donors were satisfied with their organ donation (n=119); 3 patients denied and 3 patients were uncertain about the question if they would be willing to donate again. In addition, 18 donors were unsatisfied with their present living situation, 6 donors complaint about negative familial consequences. In 17 donors quality of life was worse after donation and 13 patients stated that their discomforts were caused by renal donation. Mean Zerssen Symptom score of the donors was 10.0±4.9 (min 0, max 56) compared to 14.3±10.8 in a healthy population. In 8 patients (6.4%) the Zerssen score was above the cut-off of 27 (5 female), and in another 6 patients the Zerssen Score was between 22 and 27. Mean age of the eight patients with a Zerrsen Score above 27 was 49.2±9.1 years, mean time after donation was 3.5±2.1.years, 6/8 patients were married, 80% were employed, and 3/8 donors reported a migration background. Medical problems of the recipients were indicated by 3 of these 8 donors. Most complaints were about back pains and sleeplessness.

Conclusions: Concerning no less than 10% of living donors complaining impaired QoL directly related to living renal donation there is a need to improve pre and post-transplant psycho-social donor supervision.

FR-PO908

Blood Pressure Characteristics Measured by Three Different Modalities in Living Kidney Donors Yasushi Ohashi, George Thomas, Martin J. Schreiber, Emilio D. Poggio. *Nephrology and Hypertension, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH.*

Background: Most kidney transplant centers exclude prospective living kidney donors with hypertension from donation because of concerns that nephrectomy may increase medical risks associated with hypertension after donation. It is unclear, however, how various approaches to measure blood pressure (BP) correlate with each other to provide accurate measurements. Our objective was to assess BP in donors using 3 different modalities.

Methods: 93 living kidney donors seen between 2009 to 2011 had BP measured by the following modalities before donation: in-office BP (single BP using aneroid sphygmomanometer in clinic), BpTRU BP (average of 5 automated readings in clinic using BpTRU device), and 24 hour ambulatory blood pressure monitoring.

Results: Hypertension (defined as average 24-h BP ≥ 130/80 mmHg) was present in 20 donors. Masked hypertension (normal BP in clinic but elevated 24-h BP) was noted in 13 donors when BP was measured in-office, and in 14 donors with BpTRU. White coat hypertension (BP ≥ 140/90 mmHg in clinic but normal 24-h BP) was seen in 2 donors when BP was measured in-office.

	In-office BP, mmHg			BpTRU BP, mmHg		
BP Classification	<120/80	120–139/80–89	≥140/90	<120/80	120–139/80–89	≥140/90
Normal 24-h BP, n (%)	41 (44.1)	30 (32.3)	2 (2.2)	52 (55.9)	21 (22.6)	0 (0.0)
24-h BP ≥ 130/80 mmHg, n (%)	2 (2.2)	11 (11.8)	7 (7.5)	9 (9.7)	5 (5.4)	6 (6.5)

A higher 24-h systolic BP (SBP) was significantly associated with higher serum creatinine, lower HDL, higher LDL, higher hemoglobin, higher kidney volume, pre-diabetes, and smoking. Smoking was significantly associated with nocturnal non-dipping. BP was significantly lower on BpTRU than on in-office measurement ($p<0.05$). Sensitivity and specificity for elevated 24-h BP are 77.8% and 84.5% by in-office BP, and 100% and 83.9% by BpTRU BP.

Conclusions: While BpTRU BP has higher sensitivity for elevated 24-h BP than in-office BP, average 24-h BP has the highest correlation with risk factors among the 3 modalities and is also useful to detect masked hypertension.

FR-PO909

Changes in Serum Biomarkers 6 Months after Living Kidney Donation Shiv Kapoor,[1] Megan E. Donato,[1] Stephanie DeLoach,[3] Raymond R. Townsend,[1] Peter P. Reese,[1] Kevin E.C. Meyers.[2] *[1]Univeristy of Pennsylvania, Philadelphia, PA; [2]Pediatrics, CHOP, Philadelphia, PA; [3]Thomas Jefferson University, Philadelphia, PA.*

Background: Living kidney donation is an important means to improve health in patients with advanced chronic kidney disease or who are receiving dialysis therapy. Kidney donation is considered to be of low risk when donors are carefully selected, but some concern exists especially in young donors who are anticipated to have only one kidney for the rest of their life. Although we showed recently that blood pressures by ABPM do not change substantially 6 months after donation, we were concerned that more subtle changes, such as in the levels of important serum biomarkers, would change adversely after living kidney donation. Thus, we studied markers of endothelial function (ARG, homoARG, ADMA, SDMA), bone metabolism (FGF23) & inflammation (TNF,hsCRP,IL-6) pre-, and 6 months post-nephrectomy in living kidney donors.

Methods: We obtained blood samples on 14 subjects (6 men/8 women; mean(S.D.) age of 41(10) years) who donated a kidney at the University of Pennsylvania or the Children's Hospital of Philadelphia. Their eGFR by CKD-EPI decreased, from 86(11) to 60(12) mL/min/1.73m2. *We measured FGF-23, TNF, and IL-6 by ELISA, ARG, H_ARG, ADMA, SDMA by HPLC and hsCRP by the Roche Analyzer.*

Results: These data indicate a small but definite adverse change in several biomarkers that are important surrogates for inflammation (TNF), endothelial function (ADMA) and bone metabolism (FGF23) and suggest potential targets for monitoring and potentially intervention in living kidney donors.

Marker	ARG	ADMA	SDMA	FGF-23	TNF1-α	IL-6	hsCRP
Units	μM	μM	μM	RU/ml	pg/ml	pg/ml	mg/l
Pre	66.5+26.2	0.40+.05	0.56+.07	57.8+40.7	1.62+.37	1.33+.82	3.89+6.38
Post	82.0+22.6	0.44+.05	0.77+.09	74.1+48.1	2.07+.55	1.61+1.04	2.05+2.52
p-value	0.06	0.006	<0.001	<0.001	0.006	0.44	0.34

Conclusions: Current follow-up of living kidney donors is often minimal, perhaps related to the perception of low CV risk as reflected in our subject's young age and normal BP, which may overlook modest but important changes in metabolism occurring after nephrectomy that could influence future health outcomes.

Funding: Private Foundation Support

FR-PO910

Racial and Ethnic Differences in Determinants of Live Donor Kidney Transplantation in the United States Tanjala S. Purnell,[1] Ping Xu,[2] Yoshio N. Hall.[2] *[1]Johns Hopkins University; [2]University of Washington.*

Background: Few studies have comprehensively examined multi-level determinants of live-donor kidney transplantation (LDKT) across all major US racial/ethnic groups.

Methods: We performed a retrospective cohort study to examine racial/ethnic differences in determinants of LDKT among 162,308 non-elderly patients who initiated dialysis in 2005-2008. We linked patient-level data from the USRDS and UNOS registries and area-level socioeconomic data from the 2000 US Census. The primary outcome was time from dialysis initiation to receipt of LDKT through Sept. 30, 2008. We analyzed associations of race/ethnicity and time to LDKT using Cox proportional hazards models, and we estimated racial/ethnic differences in the degree of delayed LDKT attributable to demographic, socioeconomic and clinical factors in bootstrap analyses.

Results: Overall, 6126 subjects received a first LDKT during 221,185 person-years. Mean crude rates of LDKT were lowest among blacks (1.05 per 100 person-years [95% CI: 0.1-1.1]), American Indians/Alaska Natives-AIANs (1.22 [0.9-1.7]) and Pacific Islanders (1.47 [1.1-2.0]), intermediate among Hispanics (2.23 [2.1-2.4]) and Asians (3.20 [2.8-3.6]), and highest among whites (4.77 [4.6-4.9]). Disparities in LDKT attenuated but remained clinically and statistically significant after adjustment for demographic, socioeconomic and clinical factors. The largest fraction (34%, 21% and 33%, respectively) of the disparity among blacks, Hispanics and AIANs compared with whites was attributed to adjustment for health insurance and zip code poverty. Measures of pre-dialysis care accounted for 6%, 7%, 4% and 3% of the disparity among blacks, Hispanics, AIANs and Pacific Islanders but none of the disparity among Asians. Among Asians, Hispanics and Pacific Islanders, notable fractions were attributable to household linguistic isolation (4%, 5% and 5%, respectively).

Conclusions: In the US, rates of LDKT remain significantly lower among nonwhites relative to whites, but determinants of these disparities appear to vary according to race/ethnicity. Targeted efforts to address race/ethnicity-specific determinants of delays in LDKT may help mitigate these disparities.

Funding: NIDDK Support, Private Foundation Support

FR-PO911

Subclinical Abnormalities in Post-Perfusion Biopsies of Living Donor Kidneys Elizabeth A. Kendrick,[1] Kelly D. Smith,[2] Connie L. Davis.[1] *[1]Division of Nephrology, University of Washington, Seattle, WA; [2]Department of Pathology, University of Washington, Seattle, WA.*

Background: To identify preexisting abnormal findings in the donor kidneys, we performed post-perfusion (T0) intraoperative biopsies(bx) of kidney transplants performed at our center.

Methods: We retrospectively reviewed results of T0 bx in live donor kidneys performed from 10/1/2008 to 4/1/2012. 116 LD txs were performed of which 73 T0 bxs were available for review. Bx reports were reviewed for glomerulosclerosis (GS), interstitial fibrosis and tubular atrophy (IFTA), acute tubular injury(ATN), vascular abnormalities(VAB), and positive immunoflorescence (IFAB). None of the LDs had any urinalysis abnormalities, microalbuminuria, hypertension, or abnormal glucose tolerance.

Results: ATN was seen in 44 T0 biopsies(60.3%) consistent with reperfusion injury; 5 also had focal glomerular capillary thromboses. GS was seen in 35 (47.9%) of biopsies; <5% in 19(26.0% of all bxs); 5-10% in 9 (12.3%); >10-15% in 7 (9.6%). Three of 7 bx with >10% GS were kidneys from donors younger than 50 years, all male. Focal IFTA was seen in 17 (23.3%); more mild diffuse IFTA in 6(8.2%). Focal or greater VAB(arteriosclerosis or hyalinosis) were seen in 29(39.7%); moderate VAB in 5(6.8%). IFAB was seen in 12(16.4%); 6 had weak staining in various patterns, felt due to non-specific binding of doubtful clinical significance. Six biopsies showed more significant IFAB, most often mesangial IgA deposition. Electron microscopy (EM) demonstrated mesangial immune deposits in 4 of 6 cases, and one case also had subepithelial deposits. One biopsy showed IFAB in areas of GS but EM was negative for deposits. IFAB was generally seen in the absence of any significant glomerular abnormalities on light microscopy, and not generally seen with GS, IFTA or VAB.

Conclusions: Bx abnormalities are frequent in kidneys from healthy living donors. If unknown they can potentially confound interpretation of post tx kidney bxs. Importantly, these abnormalities could have implications for future health risk of LDs and counseling them.

FR-PO912

Pregnancies amongst Australasian Living Kidney Donors Andrew J. Mallett,[1] Nicole M. Isbel,[1] George T. John,[2] Helen G. Healy,[2] Philip A. Clayton.[3] *[1]Department of Nephrology, PAH, Brisbane, Queensland, Australia; [2]Department of Renal Medicine, RBWH, Brisbane, Queensland, Australia; [3]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, RAH, Adelaide, South Australia, Australia.*

Background: Living kidney donors consistently account for >33% of Australasian renal transplant procedures. Females represent >50% of this population; their obstetric outcomes are unreported. This study aims to describe pregnancies amongst living kidney donors reported to the ANZDATA living kidney donor registry (LKDR).

Methods: The ANZDATA LKDR was interrogated for reported pregnancies from its commencement (January 2004) to 31 December 2010. The size of the potentially fertile population (female living kidney donors ≤50years) was also assessed. This registry currently has a ~60% loss to follow up rate by 1year post donation.

Results: The population of potentially fertile living kidney donors was 1197, representing 57.5% of all living kidney donors ≤50years. 12 pregnancies amongst 12 donors were identified, resulting in 5 livebirths, 3 spontaneous abortions (<20weeks), 1 surgical termination, and no stillbirths (>20weeks). The outcome of 3 pregnancies is unknown. No information is known about other obstetric complications or outcomes. Mean donor age at time of kidney donation was 32.67yrs (24-46yrs) and mean time to first report of pregnancy after donation was 18.17months (2-50months). Mean blood pressure at donation was 120.96/74 mmHg (106-134/60-89mmHg), with a mean GFR of 123.89ml/min/1.73m² (108-138ml/min/1.73m²). 83.3% were related to their recipient. None have been reported to be deceased, receive dialysis or undergo transplantation, with a mean follow up time of 33.42months (12-57months).

Conclusions: Whilst little meaningful information is known about Australasian living kidney donor obstetric outcomes, this report of 12 pregnancies amongst 12 donors in a potentially fertile population of 1197 donors may be interpreted with optimism. Vigilance to maximise and improve living kidney donor follow up to similar levels as organ recipients is required. An appreciation of obstetric outcomes in Australasian living kidney donors may help inform the choices of potential female living kidney donors.

FR-PO913

Living Kidney Donation Appears Safe in African-American Donors Based on Short-Term Follow-Up of Renal Function at 6 Months Post Nephrectomy Lavanya Kodali, Jyothishree R. Pinnaka, Sanjaya Satapathy, Oleksandra Dryn, Melissa Moore, James D. Eason, Luis Campos, Kasturi Vinay Ranga. *Methodist Hospital Transplant Institute, University of Tennessee Health Sciences Center, Memphis, TN.*

Background: Living donation has been the most promising solution to the shortage in organ availability for renal transplantation worldwide. While short-and long-term safety have been reported, studies have been largely limited to Caucasian donors.Given the mix of both African-Americans (AA) and non-African-Americans (Non AA) in our donor population, we aimed to compare short term outcomes of renal function post-donation.

Methods: Retrospective, cohort study of all living kidney donors at a single center from 2007 to 2011. Statistical calculations were performed using SPSS software.

Results: There were a total of 100 donors, M: F= 31:69, in this time period. Of them,only 67 donors had follow-up labs at 6 months (group 1 non- AA, n =45 ; group 2-AA, n = 22). Mean age (yrs) was 41.76 vs. 37.0 (NS),mean BMI in kg/m2, 24.98 vs 29.08 (S). baseline MDRD GFR (ml/min) was significantly higher in group 2 compared to group 1 at baseline (96.49 vs 108.89) and at 6 months post-donation (61.5 vs 73.0),

Factors significantly predicting a higher 6 month GFR on univariate analysis were younger age, race, and BMI. Upon multivariate analysis, only baseline MDRD GFR achieved statistical significance.

As noted above, 33 / 100 donors did not return for their 6 month follow-up, which is a cause for worry, reflecting a national trend.

Conclusions: We conclude that living kidney donation is safe, at least in the short-term post donation, whether African-American or not, in a well selected population based

on age and BMI. Larger multicenter studies and the donor registry should validate our findings.These observations are encouraging in a population consisting of large numbers of African –Americans as a majority of patients on the waiting list and have historically had low rates of living transplantation. Given the higher prevalence of hypertension and kidney disease in the African -American population, careful screening prior to donation is of utmost importance.

FR-PO914

Impact of Simultaneous Liver Kidney Transplant Policy on Patients with End-Stage Renal Disease Anton I. Skaro,[1] Yaojen Chang,[2] Daniela Ladner,[1] John J. Friedewald,[1] Lorenzo G. Gallon.[1] *[1]Comprehensive Transplant Center, Northwestern University; [2]Oncology, Georgetown University.*

Background: Simultaneous liver kidney (SLK) transplant provides better survival to end-stage liver disease (ESLD) patients with renal impairment than liver transplant alone (LTA). During the era of the Model for End-Stage Liver Disease (MELD) allocation system, the number of SLK transplants has increased. However, this could prolong the waiting period for kidney transplants for ESRD. Consequently, the most equitable and efficacious use of deceased donor kidneys remains controversial.

Methods: A discrete time state transition Markov model was constructed to simulate ESLD patients following the proposed UNOS SLK guidelines to receive SLK transplant or LTA. The simulation included the transition between MELD quintiles, change in dialysis status, re-transplantation for chronic/acute graft failure, and the probability of native renal recovery based on the dialysis duration and transplant strategy. The model output, measured in life years, was estimated from the results of one million micro-simulations for each scenario. Validity of the model output was examined by comparing projected survival rates of each transplant strategy in the model with data from UNOS.

Results: In the base case model the UNOS SLK guidelines led to 273 kidneys released at the expense of 777 life years lost from ESLD patients. When the 10-year difference in life expectancy between KTA and dialysis patients was 2.48 years, the 677 life years gained from 273 KTA is less than the 777 life years lost from ESLD patients. According to the 2009 Annual Report of the USRDS, KTA recipients survived longer than dialysis patients by 3.5 years over a 10-year period. Under these circumstances, the 956 life years gained from 273 kidneys exceeded 777 life years lost from ESLD patients.

Conclusions: An unintended consequence of MELD-based liver allocation has been an increase in SLK transplant in the US. The development of SLK transplant listing criteria would restore access to kidney transplant for patients with ESRD. The SLK guidelines underscore the dichotomy of life years lost for ESLD patients and life years gained for ESRD patients on the kidney waitlist.

Funding: NIDDK Support

FR-PO915

Re-Examining the Impact of Pre-Donation Renal Function Criteria for Living Kidney Donor Selection on Long-Term Graft Survival Ann Young,[1] Joseph Kim,[1,2] Amit X. Garg,[3] Charmaine E. Lok.[1,2] *[1]Department of Medicine, University of Toronto, Toronto, ON, Canada; [2]Division of Nephrology, University Health Network, Toronto, ON, Canada; [3]Division of Nephrology, Western University, London, ON, Canada.*

Background: Individuals with reduced kidney function are being accepted as living kidney donors. The impact of liberalizing this criterion in the most recent era of transplantation on long-term outcomes needs to be revisited.

Methods: This retrospective, population-based cohort study followed kidney allograft recipients from Ontario, Canada from July/1992 to March/2010. Recipients and donors were identified through the provincial transplant database, and linked to large electronic healthcare databases. Baseline donor GFR was estimated using the CKD-EPI equation. Outcomes were defined using reliable codes compared to chart review. Multivariable survival analysis was performed with the primary outcome of graft loss with death as a competing event.

Results: In total, 2,087 living donor kidney transplants were studied. Baseline eGFR of donors (in mL/min) were: ≥ 110 (n = 508, 24%), 100-109 (n = 480, 23%), 90-99 (n= 471, 23%), 80-89 (n = 337, 16%), and < 80 (n = 291, 13%; reference group). The majority of transplants (74%) occurred after the year 2000. Recipients of the lowest eGFR kidneys were older than those receiving highest eGFR kidneys (46 vs. 42 years). Mean follow-up time for the cohort was 7.6 years after transplant. There was no significant difference in the HR for graft loss when comparing recipients in each eGFR category to the reference group (adjusted HRs (95%CI) from highest (≥ 110 mL/min) to lowest (80- 89 mL/min): 0.79 (0.52-1.19), 0.97 (0.66-1.42), 0.97 (0.67-1.41), and 1.13 (0.77-1.65), respectively).

Conclusions: The risk of graft loss with death as a competing event was not significantly higher for recipients of living donor kidneys with eGFR < 80 mL/min. Transplant centres utilizing explicit eGFR cut-off criteria for living kidney donor selection may consider cautious liberalization, especially for potential donors who are otherwise deemed suitable.

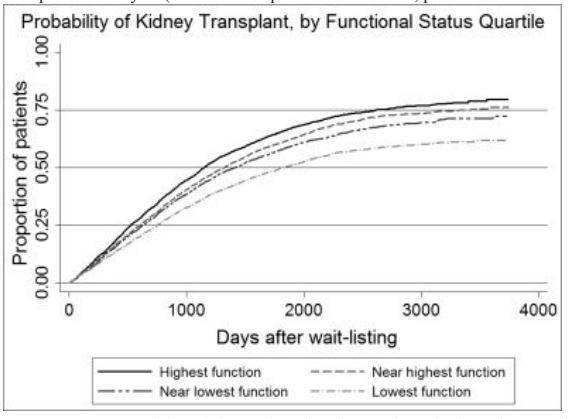
FR-PO916

The Influence of Functional Status on Outcomes for Patients Awaiting Kidney Transplant Peter P. Reese,[1] Roy D. Bloom,[1] Justine Shults,[1] Adam S. Mussell,[1] Sylvia E. Rosas,[1] Kirsten L. Johansen,[2] Peter Abt,[1] Matthew H. Levine,[1] Arthur Caplan,[3] Jason Karlawish,[1] Harold I. Feldman.[1] *[1]University of Pennsylvania; [2]UCSF; [3]NYU.*

Background: Functional status is a summary measure of global health that might help nephrologists counsel patients about the value of kidney transplant. We hypothesized that patients with the lowest functional status would (1) be less likely to receive a transplant, and (2) not derive a survival benefit from kidney transplant.

Methods: Using Organ Procurement and Transplantation Network data linked to data on functional status (physical function [PF] scale of the Medical Outcomes Study Short Form-36) assessed by a dialysis provider, we performed a cohort study of 19,640 adults who were wait-listed from 6/1/2000 – 5/31/2006. First, we fit a MV Cox regression model to examine the association of PF with the receipt of a kidney transplant. Second, we used Cox regression, with transplant status as a time-dependent covariate, to evaluate the survival benefit associated with transplant vs dialysis, stratified by quartile of PF score.

Results: Median age 50 years; 36% were black and 60% male. As in the figure, better PF had a "dose-dependent" association with the probability of receiving a kidney transplant (adjusted hazard ratio [aHR] 1.43 for highest vs lowest PF quartile, p<0.001). Within 9 months post-transplant, patients in every PF quartile derived a survival benefit from transplant vs dialysis (For each PF quartile: aHR <0.60, p<0.001 at 9 months).



Conclusions: Although lower functional status is an independent predictor of lower likelihood of kidney transplant, patients at every level of function can derive a survival benefit from kidney transplant by 9 months beyond transplant. Future studies should examine whether low functional status patients should accept extended criteria donor kidneys in order to be transplanted sooner.

Funding: NIDDK Support, Private Foundation Support

FR-PO917

Evaluation for Kidney Transplantation in the Elderly Nicole Beauvais,[1] Ashley E. Davis,[2] Anton I. Skaro,[2] Bing Ho,[2] Daniela Ladner.[2] *[1]Northwestern Memorial Hospital; [2]Comprehensive Transplant Center, Northwestern University.*

Background: Elderly patients (pts) >65 years comprise 44.6% of the ESRD population but make up only 19.6% of the kidney transplant (KT) waitlist. Little is known about how transplant centers evaluate elderly pts. Recipient age is a significant negative risk factor for post transplant outcomes, and KT recipient selection varies widely across the U.S., especially with regard to age. We describe how listing and transplanting the elderly at a large transplant center compares to the non-elderly and examine reasons for rule-out. A comparative analysis using national data are presented.

Methods: A single-center (NMH) retrospective chart review of all pts evaluated for KT between 2/2007-5/2012 was performed. Demographics and characteristics were obtained from the EMR. Statistical analyses using Fisher and chi-square tests were performed.

Results: 3,993 pts were evaluated for KT. 645 (16.2%) were elderly. The rate of KT eval for the elderly increased by 176% from 2007 to 2011. 57% of the pts <65 yrs were listed compared to 38% of elderly. 83% of all elderly at NMH who had a KT received a living donor (LD) which is statistically significantly higher than nationally (p<0.001).

	≥65 yr (n=645)	<65 yr (n=3348)
Currently listed	144	1096
Transplanted (p=0.06 Fisher's)	101	783
Living donor	84	582
Deceased donor	17	201
Ruled out (p<0.0001 Fisher's)	279	905
Medical	63	181
Financial	3	53
Non-adherence	28	78
Did not complete testing	50	187
Pt choice	38	190
Pre-emptive	7	7
Other/Unknown	90	209
Expired in evaluation or listed	46	149
In evaluation	58	222
Tx elsewhere	17	124

χ^2 $p < 0.001$ for table

Mortality on KT waitlist

	NMH	National	p-value
>65 yr (%)	21	44	<0.001
<65 yr (%)	12	28	<0.001

Conclusions: As ESRD in elderly is rising, pts >65 yrs are increasingly being evaluated for KT. Interestingly, medical rule out is only slightly higher in elderly (23%) than in younger pts (19%). At NMH, an elderly pt is as likely to receive a LD KT as is a younger pt. Compared to national rates, waitlist mortality in the elderly is two times lower at NMH and LD KT in both the elderly and younger pts is significantly higher. Identifying barriers that the elderly face in completing KT testing and determining why they remove themselves from evaluation may offer opportunities to increase LD KT.

FR-PO918

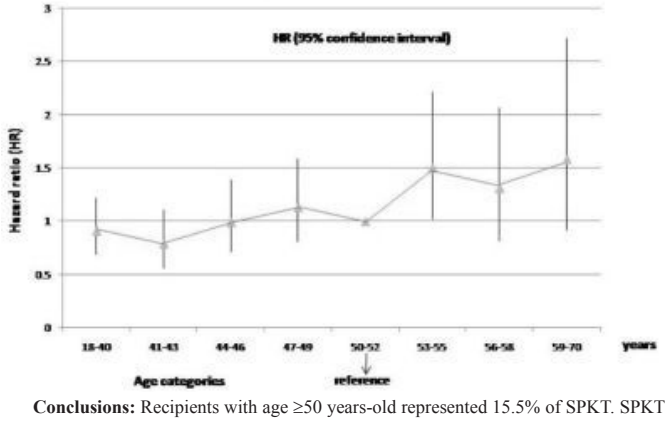
Simultaneous Pancreas-Kidney Transplantation Outcomes in Recipients Older than 50 Years Naowanit Nata, Mandana Kamgar, Edmund Huang, Marcelo Santos Sampaio, Suphamai Bunnapradist. *UCLA, Los Angeles, CA.*

Background: Simultaneous pancreas kidney transplantation (SPKT) is the treatment of choice for type 1 diabetics (T1D) with end-stage renal disease. Many institutions do not accept older patients as SPKT candidates, and therefore SPKT outcome data on recipients older than 50 year-old is limited.

Methods: Using UNOS/OPTN database as of March 2011, we selected all T1DM adult recipients who received a SPKT between 2000 and 2009, and categorized by age at the time of transplant as ≥50 and <50 years old. Five-year kidney, pancreas and patient survival were examined. Multivariate analysis was used to define hazard ratios (HR) of graft loss and death in ≥50 years, with <50 as reference.

Results: We found 6387 and 1172 recipients with <50 and ≥50 year-old, respectively. The mean age of SPKT≥50 was 53.7±3.4 and SPKT<50 was 38.2±6.4 years. Five-year patient survival was 85 and 88% (p<0.001); death-censored kidney (DCK) survival was 87 and 86% (p-value=0.32) and death-censored pancreas (DCP) survival was 83 and 78% (p=0.001) for ≥50 and <50, respectively. Risk of death (HR=1.32; 95% CI 1.10-1.59) and kidney loss (HR=1.19; 95% CI 1.02-1.38) were increased in SPKT≥50. Hazard-ratios of pancreas failure, DCK failure and DCP failure in SPKT ≥50 recipients were 0.95 (95% CI 0.83-1.10), 0.97 (95% CI 0.79-1.20) and 0.75 (95% CI 0.64-0.90), respectively. Adjusted risk of death for age categories of 18-40, 41-43, 44-46, 47-49, 53-55, 56-58 and ≥59 compared with 50-52 year-old is showed in figure1.

Figure 1. Risk of death



Conclusions: Recipients with age ≥50 years-old represented 15.5% of SPKT. SPKT recipients ≥50 year-old had an increased risk of death, decreased risk of death-censored pancreas failure, but a similar risk of death-censored kidney failure when compared to SPKT recipients<50 year of age.

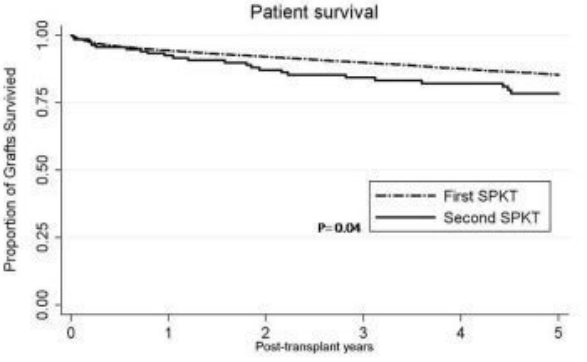
FR-PO919

Outcomes of Simultaneous Pancreas-Kidney Re-Transplantation after a First Simultaneous Pancreas-Kidney Transplantation Naowanit Nata, Edmund Huang, Mandana Kamgar, Marcelo Santos Sampaio, Suphamai Bunnapradist. *UCLA, Los Angeles, CA.*

Background: Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for end stage renal disease diabetic patients. Data on outcome of a SPK re-transplant after a first SPKT is limited.

Methods: Using OPTN/UNOS database as of March-2011, we found 16788 adult first SPKT (f-SPKT) between years 1987 and 2011. Of those, 5440 (32.4%) failed both grafts, and only 140 (2.6%) were subsequently re-transplanted with a SPKT (SPK-rT). Kidney graft, pancreas graft, and patient survival were compared between f-SPKT and SPK-rT. Adjusted hazard ratios (HR) of kidney and pancreas failure, and death were examined in SPK-rT recipients, with f-SPKT as the reference group.

Results: Mean age was 39.5±8.2 and 39.7±8.2 years; median (25^{th}-75^{th} p) PRA was 0 (0-5.0) and 29 (2.0-79.5); and median follow-up were 1949 (771-3370) and 1457 (432-2449) days, for f-SPKT and SPK-rT, respectively. Five-year patient survival was 78 and 85% (p=0.04) (Figure 1); death-censored kidney (DCK) survival were 74 and 84% (p=0.01) and death-censored pancreas (DCP) survival were 73 and 77% (p=0.32) for SPK-rT and f-SPKT, respectively.



First SPKT	16788	14452	12911	11597	10192	8869
Second SPKT	140	115	96	84	70	55

Risk of kidney failure (HR=1.39; 95% CI 1.01-1.91) and DCK failure (HR=1.52; 95%CI 1.02-2.25) were increased in SPK-rT recipients. Risk of death (HR=1.25, 95% CI 0.83-1.89), pancreas failure (HR=1.14; 95% CI 0.84-1.56), and DCP failure (HR=1.13; 95%CI 0.79-1.62) were not significant increased in SPK-rT recipients.

Conclusions: Only 2.6% of a first failed SPKT received a second SPKT. A second SPKT after a first failed SPKT was associated with inferior overall and death censored kidney survival when compared to a first SPKT.

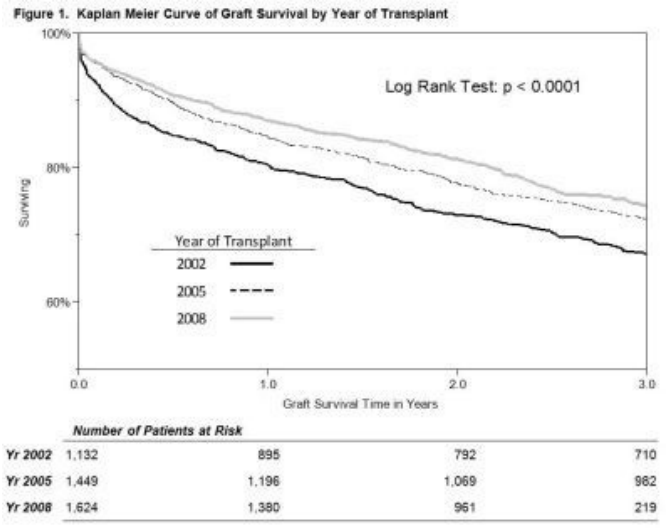
FR-PO920

Trend in Graft Survival for Expanded Criteria Donor Kidney Transplants in the United States: Is There a Learning Curve? Anthony W. Castleberry,[1] Kadiyala V. Ravindra,[1] Deepak Vikraman,[1] Mathias Worni,[1] Andrew S. Barbas,[1] Matthew Jay Ellis,[1] William Irish,[2] Debra Sudan.[1] *[1]Duke University Medical Center; [2]Clinical Trial and Consulting Services.*

Background: The trend in graft survival nationwide in United States (US) expanded criteria donor (ECD) kidney transplants remains poorly understood. Our objective was to perform a trend analysis of transplant characteristics and graft survival for all US ECD kidney transplants performed over the past decade.

Methods: We performed a longitudinal cohort study of all US ECD transplants performed between 01/2002 and 12/2010 recorded by the United Network for Organ Sharing. Exclusion criteria included pediatric recipients, living donors, non heart beating donors, repeat or multiorgan transplants, and pre-dialysis recipients. Kaplan-Meier method was used to assess graft survival while trends in transplant characteristics and survival were analyzed by ordinary least squares regression and the Cochran–Armitage trend test.

Results: 12,731 ECD transplants were available for analysis (mean age 60 years, 32% Black and 64% male). Use of ECD kidneys increased from 16.9% in 2002 to 20.9% in 2010 (p<0.001) during which time graft survival significantly improved (Figure 1). The rate of delayed graft function declined from 35.2% in 2002 to 27.8% in 2010 (p<0.001), the mean donor kidney risk index increased from 1.88 to 1.97 (p<0.001), the percent of ECD kidneys pumped increased from 26.5% to 62.7% (p<0.001) as did the percent of pre-transplant kidney biopsies (79.9% in 2002 versus 94.4% in 2010; p<0.001).



Number of Patients at Risk				
Yr 2002	1,132	895	792	710
Yr 2005	1,449	1,196	1,069	982
Yr 2008	1,624	1,380	961	219

Conclusions: Despite an increasing mean donor kidney risk index over time among ECD kidneys, graft survival has improved, suggesting a learning curve. Contributory factors may include an increased use of pumping and biopsies to improve selection of ECD kidneys.

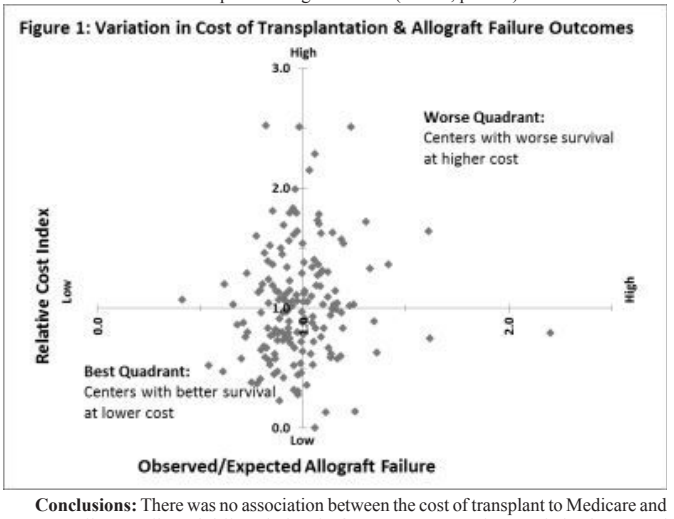
FR-PO921

Is Medicare Getting What It Pays for in Kidney Transplant Outcomes? Suying Li,[1] Nicholas Salkowski,[2] Craig Solid,[1] Mark Schnitzler,[3] Jon J. Snyder,[1] Joseph Kim,[4] Bertram L. Kasiske,[1,2] Ajay K. Israni.[2] *[1]USRDS Coordinating Center, MMRF, MN; [2]Hennepin County Medical Center, University of Minnesota - Medicine, MN; [3]University of St. Louis, MO; [4]University of Toronto, School of Medicine, Canada.*

Background: The relationship between cost and kidney allograft failure has not been investigated in the US.

Methods: Using Medicare claims from the United States Renal Data System, we determined Part A and B costs for all adult Medicare kidney recipients transplanted between 1/1/2007 and 6/30/2009. We compared the relative cost (observed/expected cost) for the first year posttransplant for all transplant centers, adjusting for recipient, donor and transplant characteristics, region, and the local wage index. Using program-specific reports (PSRs) created by the Scientific Registry of Transplant Recipients, we correlated relative cost with observed/expected allograft failure between centers. We excluded small transplant centers with less than 3.69 expected allograft failures.

Results: Among 20,757 transplants at 165 centers, the mean observed cost was $65,366 (IQR=55,094-71,624) and the relative cost was 0.99 (IQR=0.88-1.08). The mean observed/expected allograft failure was 1.03 (IQR=0.61-1.37). There was no correlation between relative cost and observed/expected allograft failure (r=0.10, p=0.20).



Conclusions: There was no association between the cost of transplant to Medicare and a center's all cause allograft failure during the first year posttransplant. Variation in cost and outcomes suggests a need to determine the most cost- effective practices to reduce costs.

Funding: NIDDK Support

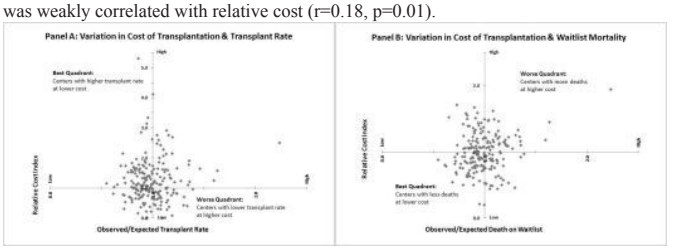
FR-PO922

Does an "Aggressive" Transplant Center Lead to Higher Medicare Costs? Suying Li,[1] Nicholas Salkowski,[2] Craig Solid,[1] Mark Schnitzler,[3] Jon J. Snyder,[1] Joseph Kim,[4] Bertram L. Kasiske,[1,2] Ajay K. Israni.[2] *[1]USRDS Coordinating Center, MMRF, MN; [2]Hennepin County Medical Center, University of Minnesota - Medicine, MN; [3]University of St. Louis, MO; [4]University of Toronto School of Medicine, MN, Canada.*

Background: Higher wait-list mortality for a center could suggest that the center accepts high-risk candidates for transplant. A higher transplant rate suggests that the center accepts higher-risk donors. We hypothesized that cost of kidney transplant at such centers may be higher.

Methods: Using Medicare claims data, we determined Part A and B costs for all adult Medicare kidney recipients transplanted between 1/1/2007-6/30/2009. We then developed a relative cost (observed/expected cost) for the first year posttransplant for all transplant centers, adjusting for transplant characteristics, geographic region, and local wage index. Using program-specific reports (PSRs) created by the Scientific Registry of Transplant Recipients, we correlated relative cost with adjusted wait-list mortality and adjusted transplant rate between centers. We excluded small centers with expected wait-list mortality or expected transplant rate less than 3.69 in 2009.

Results: The study included 20,726 transplants at 203 centers for transplant rate. The mean observed cost was $64,903 (IQR=54,741-71,624) and the relative cost was 0.99 (IQR=0.88-1.08). The mean observed/expected wait-list mortality of 0.99 (IQR=0.78-1.18) was weakly correlated with relative cost (r=0.18, p=0.01).



The mean observed/expected transplant rate on the waiting list of 1.24 (IQR=0.82-1.59) was not correlated with the relative cost (r=0.08, p=0.26).

Conclusions: There was no association between being an "aggressive center" and the cost of transplant to Medicare.

Funding: NIDDK Support

FR-PO923

Development and Validation of a New Statistical Model for Prognosis of Long-Term Graft Function after Pediatric Kidney Transplantation Lars Pape,[1] Thurid Ahlenstiel,[1] Antonia Zapf.[2] *[1]Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Biostatistics, Hannover Medical School, Hannover, Germany.*

Background: No adequate statistical model has been established to estimate future glomerular filtration rate (GFR) in children after kidney transplantation (KTX). In adults, equations based on simple linear regression analysis are used, but it is questionable if this approach is also suitable for children.

Methods: To address this issue, an optimal prognostic model of GFR was generated for 63 children at 3-7 years after KTX. The main regression model for prediction of the log-transformed GFR (logGFR) included the mean monthly change of GFR in the period 3-24 months after KTX (ΔGFR), the baseline GFR at 3 months after KTX (bGFR), and an intercept. Additionally it was investigated if the inclusion of cofactors leads to more precise predictions. The model was validated by leave-one-out-cross-validation for years 3-7 after KTX. Prognostic quality was determined with the mean squared error (MSE) and mean absolute error (MAE). Results were compared with the simple linear regression model used in adults.

Results: Compared to the simple linear model for adults, lower MSE and MAE values, this means more precise predictions, were observed with the new pediatric model. The benefit of inclusion of cofactors was not relevant.

Conclusions: To our knowledge, this is the first statistical model to predict long-term graft function in children with a very high precision. The next step would be to validate the results in an independent cohort.

FR-PO924

Predictors of Early and Late Graft Loss in Recipients with Kidneys from the Same Donor: Analysis of the UNOS Database Joyce P. Samuel, Cynthia S. Bell, John S. Bynon, Joshua A. Samuels, Rita Swinford. *UT-Houston Medical School, Houston, TX.*

Background: When a deceased donor provides kidneys to 2 different recipients, a unique opportunity to study recipient-specific risk factors for graft loss is created. The UNOS database from 1987-2010 was queried to identify matched pairs of transplant recipients who had received their kidney allograft from the same donor. We sought to identify recipient factors that predicted early vs late allograft loss independent of donor factors.

Methods: This analysis included only donor-matched recipient pairs with divergent graft survival determined if one of the allograft kidneys failed within 2 ys, and the other

survived at least 7.5 ys. These time points were chosen as they represented the 25th and 75th percentile for kidney allograft loss among donor-matched pairs in the database.

Results: We identified 2315 donors with 4630 recipients who had divergent graft survival. Median graft survival time was 5 mo in the early graft loss (EGL) group vs 122 mo in the late graft loss group. Those with early graft loss were older (median age at transplantation of 47 yr [interquartile range 35,57] vs. 45 [IQR 35, 54]), and had a higher proportion of Blacks (33% vs. 22%). Multivariate mixed effects logistic regression analysis for early vs late graft loss showed a 68% increase in odds of EGL if the recipient was Black (odds ratio 1.68, 95%CI 1.47, 1.92), had a higher BMI (OR 1.01, 95%CI 1.002, 1.02), or was older (1.01, 95%CI 1.004, 1.012). Other significant covariates were whether the recipient had been transplanted previously (OR 1.63, 95%CI 1.26, 2.11), increasing cold ischemia time (OR 1.63, 95%CI 1.25, 2.11), and high peak PRA (greater than 80%) (OR 1.60, 95%CI 1.24, 2.06). Recipient gender, dialysis prior to transplant, and time on transplant waiting list were not associated with an increased risk for EGL in this analysis.

Conclusions: By reporting the outcomes of both kidneys from 2315 individual donors, we confirmed previously identified risk factors for EGL while controlling for donor factors. Black race, higher BMI, older age, previous transplant, increasing cold ischemia time, and higher antibody sensitization predicted EGL in this cohort.

FR-PO925

Long-Term Results of Renal Transplantation from Donors with Acute Renal Failure Jacqueline Apaza, Esther Gonzalez Monte, Natalia I. Polanco Fernandez, Laura Garcia-puente Suarez, Ignacio Bengoa, Enrique Morales, Amado Andres, J. Morales. *Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain.*

Background: In order to reduce the waiting time for renal transplantation the selection criteria for kidney grafts have been extended in recent years. The aim of our study was to evaluate the initial and long-term evolution of patients transplanted from a donor with acute renal failure (ARF).

Methods: Between 1990 and 2009 were analyzed 40 patients who received a kidney transplant from a donor with Serum Creatinine ≥ 2 mg / dl at the time of extraction, and compared the evolution with their respective transplant patient with donor serum creatinine < 2 mg/dl, transplanted in the same period of time and similar ages and sex.

Results: The table 1 analyzes the characteristics and evolution of the group of patients with ARF and controls. In the donor group with SCr ≥ 2 mg / dl there was a higher proportion of males (p = 0.01). The mean SCr at the time of removal from donors with ARF was 2.8mg/dl compared to 0.9mg/dl in the control group (p <0.001). Cold ischemia time was higher in donors with ARF (21.8 min vs 17.8 min, P <0.01). There was no difference in the rate of primary graft non-function, in the mean time to recover renal function or the rate of acute rejection. SCr at 3, 6 and 12 months of evolution was similar in both groups. In the analysis of survival at 5 years there were no differences when comparing both groups (90% in the group with ARF and 95% in controls).

Characteristics of transplant patients from donors with ARF compared with controls

Donors	SCr≥ 2 mg/dl	SCr <2mg/dl	P
Age (years)	42,2±17	39,9±16	NS
Gender (M/F) %	80/20	60/20	0,02
SCr (mg/dl)	2,8±0,7	0,9±0,28	<0,001
Recipients			
Age (years)	49±12	45,2±11	NS
Gender (M/F) %	60/40	57,5/42,5	NS
PRA>50%	5 %	12,5 %	NS
Transplantation			
Cold ischaemia time (min)	21,8±4	17,8±6,3	0,01
acute tubular necrosis(days)	14±12	10±9	NS
Primary non-functioning grafts (%)	2,5	0	NS
Acute rejections (%)	15	15	NS
SCr 3 months	1,4±0,46	1,6±1,1	NS
SCr 6 months	1,4±0,7	1,4±0,38	NS
SCr 12 months	1,3±0,46	1,4±0,7	NS

Conclusions: Donors with ARF secondary to acute tubular necrosis may be good donors with excellent graft outcome and long survival.

FR-PO926

KDPI Scores: Observed Outcomes Exceed Expectations at Three Years Cynthia Leaphart, Martin L. Mai, Sherry Sonnenwald, Stephanie Koonce, Mary B. Prendergast, Hani Wadei, Thomas A. Gonwa, Burcin Taner. *Department of Transplant, Mayo Clinic Florida, Jacksonville, FL.*

Background: The Kidney Donor Profile Index(KDPI) proposes allocation of donor kidneys to decrease waitlist times. To understand the KDPI system and outcomes, we performed a retrospective analysis to determine a) KDPI scores of organs transplanted at our institution and b) correlation of actual recipient outcomes with KDPI scores.

Methods: Retrospective chart review of transplanted kidneys(1/1/2000-12/31/2008) extracted donor characteristics(age, height, weight, race, history of hypertension or diabetes, cause of death, serum creatinine(highest,lowest,&final), Hepatitis C Viral status, donation after cardiac death(DCD) status) to calculate KDPI. KDPI highest(**KH**), lowest(**KL**), and final(**KF**) percentages were calculated from donors' highest, lowest, and final creatinine. Recipient demographics including age at transplant, graft and recipient survival, & length of follow up were collected. Recipients of dual transplant, living kidney, & multi-organ transplants were excluded from analysis. Donor and recipient matching was categorized by ascending KDPI.

Results: Of 330 complete records, mean±s.d., median, and range of KDPI(%) are:**KH:**41.37±24.2,40,(1-93), **KL:**33.4±24.6,30,(1-88), **KF:**37.3±24.6,37,(1-93), suggesting that acceptable donors span the extent of KDPI scores. Recipient graft survival at 1-,2-, and 3-years analyzed by **KH** are shown in Table 1. Strikingly, allograft survival at 3-years post-transplant exceeded predicted outcomes from the KDPI allocation model, excluding 3-year survival for KDPI=80-89%.

Actual Graft Survival

KDPI(%)	1-Yr(%)	2-Yr(%)	3-Yr(%)	3-Yr Predicted
1-4	100	100	93.3	87.8
5-9	100	100	100	86.8
10-19	97	93.6	85.1	85.9
20-29	95.7	91.5	91.5	84.5
30-39	95.2	92.9	88.1	83.1
40-49	97.5	95	90	81.5
50-59	97.3	94.6	86.5	79.7
60-69	83.9	77.4	74.2	77.9
70-79	91.9	83.8	83.8	75.7
80-89	100	86.7	66.7	73.1
90-94	80	80	80	69.2

Conclusions: Previously accepted kidneys used for transplant ranged broadly across KDPI percentage. Graft survival exceeded predictions of the KDPI range for this single transplant center. Matching strategies using the KDPI may improve outcomes and shorten waitlist times.

FR-PO927

Dual Kidney Transplant Outcomes in the US: The UNOS Database Analysis Sowmini Medavaram, Zouhair M. Kabbara, Sumit Mohan, Mark A. Hardy, Lloyd Ratner, David J. Cohen, Bekir Tanriover. *Columbia University Medical Center.*

Background: Dual kidney transplant (DKT) is not a consideration in the current allocation algorithm in the US for waitlisted transplant candidates. Many kidneys used for DKT would otherwise have been discarded because of their high-risk characteristics (donor age>60yrs, terminal creatinine>2.5mg/dL, eGFR<65mL/min, donor diabetes and/or hypertension, and moderate glomerulosclerosis 15-50%). We report the rate of complication and outcomes of DKT compared to expanded criteria kidney (ECD) transplants to assess their potential utilization as a form ECD allocation.

Methods: From 1994 to 2001, a total of 1,601 DKT and 23,457 ECD transplants from donors aged ≥50years are identified in the UNOS STAR file. Donor quality (Kidney Donor Risk Index, KDRI), allograft survival, renal function at last follow-up and rate of surgical complications are primary outcomes.

Results: DKT comprised 3.7% of kidney transplants form donors aged ≥50years. The comparative results are summarized on below table:

		Dual donor age>50	ECD	p value
N		1,601	23,457	
±Donor age (years)		64.1±7.5	59.8±6.1	< 0.001
Donor gender (female %)		55.7	55.1	<0.001
Terminal Creatinine (mg/dL)		1.3±1.3	1.2±1.4	0.202
Diabetic donors (%)		16.8	11.7	<0.001
KDRI - Rao		1.8±0.5	1.8±0.4	0.884
Recipient age (yrs)		59±10.8	56.4±12.2	<0.001
Recipient gender (female %)		37.9	37.2	0.612
Cold ischemia time (hrs)		23.9±12.6	19.7±9.3	<0.001
Delayed allograft fucntion (%)		27.8	33.6	<0.001
Follow up years		3.8±3.2	3.9±3.4	0.0334
eGFR (MDRD) at last follow up (mL/min/1.73m2)		47.7±22.9	41.2±18.4	<0.001
Cause of kidney failure				0.861
	Acute rejection(%)	14.8	13.4	
	Chronic rejection (%)	35	38.4	
	Primary nonfunction(%)	12.2	11.3	
	Graft thrombosis (%)	6.5	5.1	
	Urological complication (%)	0.7	0.6	
	Other (%)	20.7	22	
Death functioning kidney (%)		6.7	4.3	0.077
Posttransplant 90 day mortality (%)		3.9	3.1	0.057
5 yr death censored graft survival(%)		51.1	51	0.22

Conclusions: Outcomes with DKTs are comparable to those with ECD transplants but are performed relatively infrequently. DKT utilization needs to be optimized by offering them as a part of ECD algorithm to and should be considered prior to discard of available kidneys.

FR-PO928

Renal Transplantation in the over 70s Is Safe and Successful Miriam R. Berry,[1] Anil Chalisey,[2] Andrew K. Coutinho,[3] Kate S. Wiles,[4] Sourjya Kar,[5] Nicholas Torpey.[1] *[1]Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Norfolk and Norwich University Hospital; [3]Broomfield Hospital, Chelmsford; [4]Ipswich Hospital; [5]Lister Hospital, Stevenage.*

Background: Improving survival of patients with end stage renal failure in the context of an aging population demands that the safety and efficacy of renal transplantation in the elderly is evaluated.

Methods: We performed a retrospective analysis of every renal transplant performed in a patient in their 70s at our institution since January 1st 2000. A control group was identified comprising the recipient of the paired kidney where possible (n=10), or the subsequent cadaveric transplant recipient at our institution.

Results: Since 2000, 26 patients have received a renal transplant after their 70th birthday; 14 from cadaveric non heart-beating donors, 9 cadaveric heart-beating donors and 3 living donors. Demographic and outcome data were collected for the 23 recipients of cadaveric grafts and compared to a control group comprising 21 patients. The median age was 72 (range 70-74) vs 57 (range 41-68) yrs ($p<0.001$). There were no significant differences in duration of renal replacement therapy nor time spent on the transplant waiting list. There was a trend towards older patients receiving kidneys from older donors: 63 (57-71.5) vs 58 (52-63) yrs ($p=0.051$). Neither graft nor patient survival were significantly different between study and control groups: 1 year graft and patient survival 84 vs 95% ($p=0.60$) and 100 vs 95% ($p=1.00$) respectively, and to date: 87 vs 90% ($p=1.00$) and 96 vs 90% ($p=0.60$) with a median follow up period of 34 (range 5-117) months. Current level of graft function was similar: median creatinine 151 vs 170 µmol/l ($p=0.42$). Surprisingly, delayed graft function was significantly more common in the control group: 90 vs 48% ($p=0.003$) despite comparable cold ischemic times. Neither length of stay (10 vs 10 days, $p=0.13$) nor re-admission rate at 3 months (22 vs 14, $p=0.20$) were different. There was no difference in the frequency of acute and late complications between groups.

Conclusions: Renal transplantation in patients in their 70s is safe and successful. Longer term follow up will show if this is an effective use of donor organs.

FR-PO929

Long-Term Outcome of En Bloc Paediatric-Kidney Transplantation in Adult Recipients Hildegard Hafner-Giessauf,[1] Astrid Mauric,[1] Helmut Mueller,[2] Philipp Eller,[3] Florian Iberer,[2] Alexander R. Rosenkranz,[1] Kathrin Eller.[1] *[1]Clinical Division of Nephrology, Medical University Graz, Austria; [2]Clinical Division of Transplant Surgery, Medical University Graz, Austria; [3]Clinical Division of Angiology, Medical University Graz, Austria.*

Background: Renal transplantation has been shown to be the best therapeutic option in end stage renal disease (ESRD) patients. Nowadays, non-heartbeating as well as old and very young donor kidneys are accepted for transplantation. En bloc transplantation of paediatric-kidneys into adult recipients (EBKT) is one strategy to increase the donor pool.

Methods: We here report on 10 to 22 years of follow-up of patients receiving EBKT in a single centre retrospective cohort study approach.

Results: The mean donor age and donor body weight was 14±12 months and 8±3 kilograms, respectively. Thirteen recipients (6 females, 7 males) were followed for 10 to 22 years. The mean recipient age was 44±13 years at the time of transplantation. Two of 13 patients lost their grafts in the first week after EBKT because of haemorrhagic infarction of the kidney transplants or sepsis. The latter patient died due to septic shock. Only one patient had an acute cellular rejection that was successfully treated with steroids and anti-CD3-antibody. Eleven out of 13 patients after EBKT survived and have a functioning graft 10 to 22 years after successful EBKT. The serum creatinine was 1.34±0.6 mg/dl 5 years (n=11), 1.37±0.7 mg/dl 10 years (n=11), 1.40±0.6 mg/dl 15 years (n=4), 1.08 mg/dl 20 years after EBKT (n=2). The eGFR evaluated by using MDRD-2 was 66.5±22 ml/min/m^2 5 years (n=11), 62±28 ml/min/m^2 10 years (n=11), 56±23 ml/min/m^2 15 years (n=4), 61 ml/min/m^2 20 years after EBKT (n=2).

Conclusions: In summary, EBKT in our hands has a brilliant long-term graft and patient survival when the acute post-operative phase is uncomplicated.

FR-PO930

Year of the Transplantation and/or Early Renal Graft Function: Influence on Long-Term Outcomes in Renal Trasplantation Maria Luisa Agüera, Alberto Rodriguez-benot, Maria Dolores Navarro, Raquel Ojeda, Sagrario Soriano, Alejandro Martin-Malo, Pedro Aljama-Garcia. *Renal Unit, H.U.Reina Sofia, Cordoba, Spain.*

Background: There is not any consensus if the year of the transplantation and/or the early renal graft function has any influence on long-term outcomes of kidney transplantation recipients.

Methods: We included all transplant recipients of a renal graft alone in our centre from 1979 to 2005. Demographic, clinical, analytical and therapeutic variables have been recorded for each patient pre, peri and post-transplantation. Time of transplantation was defined by quartiles. Early renal graft function was defined by glomerular filtration rate (GFR) estimated from different equations (Cockroft-Gault, MDRD, Nankivell) and by stratification on End Stage Renal Disease Staging. It was performed survival test of cumulative survival (uni and multivariate), actuarial survival and projected half mean live calculated using the most adjusted mathematics' equation to 156 or 130 observed real survival data (weekly data).

Results: 778 kidney graft's recipients were included and divided in four quartiles depending on the moment of the transplantation. The most adjusted mathematics equation was potential equation for Q1-Q4 and second grade polynomial equation for Q2-Q3 at the 156-observed-data analysis. Using these equations, the kidney graft survival initially improved (Q1-Q2) and lastly was stabilized (Q3-Q4). Classical methods of survival and real half mean life confirmed these results. GFR at 12 months post-transplant could have influenced on renal graft survival censored by death of the patient after adjusted by the rest of the covariables.

Conclusions: Patient and kidney graft's survival improved until the introduction of the calcineurin inhibitor and after that it has been stabilized, regardless of the survival test used. Projected half mean life based on a high number of observed data after the six months post-transplantation is an adequate method to compeer renal graft outcomes of recent times. Early renal graft function, defined on GFR estimated at 12 months post-transplantation, has some influence in long-term renal graft outcomes (censored by death of the patient).

FR-PO931

Five Year Graft Survival and Delayed Graft Function Predicted by Pre-Existent Renal Damage Welmoet H. Westendorp,[1] Hilde Tent,[3] Hendrik Sijbrand Hofker,[1] Rutger J. Ploeg,[4] Henri G.D. Leuvenink,[1] Marcory van Dijk,[2] Harry Van Goor.[2] *[1]Surgery, UMCG, Groningen, Netherlands; [2]Pathology, UMCG, Groningen, Netherlands; [3]Nephrology, UMCG, Groningen, Netherlands; [4]Nuffield Dept. of Surgical Sciences, University of Oxford, Oxford, United Kingdom.*

Background: Organs derived from deceased brain dead (DBD) donors show worse function and more acute rejection episodes than those from living donors (LD). Brain death induces a progressive inflammatory response in potential donor organs. Minor renal damage is present in healthy donors. We hypothesize that pre-existent renal damage is more prominent in kidneys from DBD donors compared to LD and might predict graft failure after transplantation.

Methods: We used renal biopsies of 125 living and 73 DBD donors. Associations with donor characteristics and recipient outcome were determined. The degree of FGS, interstitial fibrosis (IF), intima thickness and vascular hyalinosis were scored. Sections were scored for macrophages, granulocytes and pre-fibrosis (α-SMA).

Results: Vascular hyalinosis, FGS, macrophages and granulocytes are markedly increased in DBD compared to LD ($p<0.05$). Intima thickness and IF are not significantly different between the donor types. The degree of FGS and IF are associated with donor age ($\rho = 0.265$, $\rho = 0.315$ resp; both $p<0.01$). IF correlates with diastolic blood pressure and effective renal plasma flow prior to donation ($\rho = 0.144$, $\rho = -0.197$ resp; both $p<0.05$). Macrophages and intima thickness correlated univariate with delayed graft function (DGF) (Exp(B)=261.1, Exp(B)=1.021 resp; both $p<0.05$). In multivariate analysis, 5 year graft survival was best predicted by granulocytes ($p<0.05$). Separately analyzed, 1 year graft survival in DBD donor is best predicted by IF ($p<0.05$) and in LD by the degree of intima thickness. Five year graft survival in DBD donor is best predicted by IF and in LD by the degree of vascular hyalinosis.

Conclusions: Pre-existent renal damage is more prominent in kidneys of DBD donors than in kidneys from LD. Therefore, therapeutic interventions in the DBD donor could be a tool to reduce inflammatory damage of the graft-to-be and thereby improve organ quality and graft survival.

Funding: Government Support - Non-U.S.

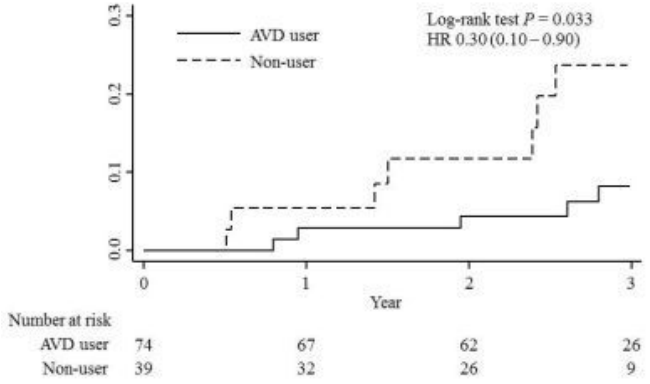
FR-PO932

Oral Active Vitamin D Therapy for Potential Chemoprevention of Post-Transplant Malignancy Yoshitsugu Obi,[1] Naotsugu Ichimaru,[2] Takayuki Hamano,[3] Isao Matsui,[1] Masayoshi Okumi,[4] Jun-Ya Kaimori,[5] Koji Yazawa,[4] Yoshiharu Tsubakihara,[3] Hiromi Rakugi,[1] Shiro Takahara,[5] Yoshitaka Isaka.[1] *[1]Geriatric Medicine & Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Urology, Osaka Central Hospital, Osaka, Japan; [3]Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [4]Specific Organ Regulation (Urology), Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [5]Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Post-transplant malignancy (PTM) is a limiting factor for survival in kidney transplant recipients (KTRs). We hypothesized that active vitamin D compounds (AVDs) could reduce PTM development in KTRs.

Methods: Ambulatory KTRs were prospectively followed from 2007 to 2010. A propensity score (PS) of having received AVDs was estimated using 26 clinically relevant factors. We used Cox models to estimate the effect of AVDs on newly diagnosed PTM.

Results: Among 218 participants, median age was 50 (IQR, 40–59) years, 63.3% were male, median time since transplantation was 11.2 (IQR, 5.2–17.1) years, and mean estimated GFR was 41.3 (SD, 15.6) ml/min/1.73 m^2. At baseline, 42.2% had been treated with AVDs mainly for glucocorticoid-induced osteoporosis. AVDs used were calcitriol (58.7%) and alfacalcidol (41.3%). During follow-up, PTM developed in 5.4% of 92 AVD users and 8.7% of 126 non-users. Poor vitamin D status was common in the participants, but serum 25-hydroxyvitamin D levels did not predict PTM. After stratifying patients by PS tertiles, we found that AVDs were significantly associated with a lower risk of PTM (HR 0.25 [0.07–0.82]). Sensitivity analyses using PS matching yielded similar results.



Also, HR was 0.30 (0.10-0.90) by IPW method.

Conclusions: AVDs are potential chemopreventive agents against PTM in KTRs.

FR-PO933

A Single Daily Dose Enhances the Adherence to Immunosuppressive Treatment in Kidney Transplant Recipients Yoshitsugu Obi,[1] Naotsugu Ichimaru,[2] Taigo Kato,[3] Jun-Ya Kaimori,[4] Masayoshi Okumi,[3] Koji Yazawa,[3] Hiromi Rakugi,[1] Norio Nonomura,[3] Yoshitaka Isaka,[1] Shiro Takahara.[4] *[1]Department of Geriatric Medicine & Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Urology, Osaka Central Hospital, Osaka, Japan; [3]Department of Specific Organ Regulation (Urology), Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [4]Department of Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Nonadherence (NA) to immunosuppressive agents is a major risk factor for allograft failure in kidney transplant recipients (KTRs). The aim of this study was to estimate the relative effect of daily dosing on treatment adherence, not to identify how patients are non-adherent, in long-term KTRs.

Methods: In January 2009, a cross-sectional, anonymous, and voluntary questionnaire survey was given to ambulatory KTRs. A self-reporting questionnaire underestimates NA, but we reasoned that the effect of the dosing regimen should be estimated with relative accuracy by using the generalized ordered logit/partial proportional hazard odds model given that the distribution patterns in the degree of NA have been shown to be similar with other measures.

Results: Of 336 eligible patients, 312 (92.9%) participated in this study. Two hundred seventy-four patients (87.8%) were more than 3 years post-transplant. Univariate analysis revealed that a single daily dose was significantly associated with better adherence. After controlling for age, sex, time since transplantation, and the number of prescribed drugs, the effect of single daily dose still remained significant (odds ratio, 0.40 [95% confidence interval, 0.20–0.83]; p = 0.013). Several sensitivity analyses yielded similar results.

Adjusted Odds Ratios for Single-daily Compared with Twice-daily Dosing

Adjusted covariates	OR	95% CI	p
age and sex	0.46	(0.23-0.91)	0.027
age, sex, and the number of prescribed drugs	0.47	(0.23-0.94)	0.033
age, sex, the number of prescribed drugs, and time since transplantation	0.40	(0.19-0.81)	0.011

Conclusions: A single daily regimen—one of few modifiable factors—might improve treatment adherence and allograft survival in long-term KTRs.

FR-PO934

Graft Loss among Kidney Diseases with a High Risk of Post-Transplant Disease Recurrence: The Size of the Problem in Pediatric Transplantation, Results on Behalf of the ESPN/ERA-EDTA Registry Karlijn J. Van Stralen,[1] Enrico Verrina,[2] Franz S. Schaefer,[3] Kitty J. Jager.[1] *[1]ESPN/ERA-EDTA Registry, Medical Informatics, Netherlands; [2]G Gaslini Hospital, Italy; [3]University of Heidelberg Center for Pediatrics and Adolescent Medicine, Germany.*

Background: Some kidney diseases tend to recur in the renal allograft after transplantation. We studied the risk of graft-loss among primary renal disease (PRDs) known for their high risk of recurrence and compared it to that of patients with hypoplasia or dysplasia.

Methods: Within the ESPN/ERA-EDTA registry, we studied children from 33 countries who received a kidney transplant before the age of 20 between 1990 and 2009. Patients were censored after 5 years of follow-up and cumulative incidence competing risk analysis was used to calculate survival curves.

Results: Patients with focal and segmental glomerulosclerosis (FSGS), haemolytic uremic syndrome (HUS), membranoproliferative glomerulonephritis type I or II (MPGN), primary Hyperoxaluria (oxalosis), IgA nephropathy or Henoch Schönlein (IgA/HS), or Systemic Lupus Erythomatosus (SLE) received significantly less often a pre-emptive transplantation as compared with patients with hypo/dysplasia. The rate of living donation was lower among patients with oxalosis, FSGS and SLE than in patients with hypo/dysplasia. Patients with FSGS had a 5-year risk of graft loss of 25.7%, while it was only 14.4% among hypo/dysplasia patients. The risk of graft loss was more strongly increased among FSGS children who started RRT aged 6-12 (27.7%), or >12 (32.4%) as compared to younger children (17.1%). Patients with MPGN (28.1%, type I; 22.5%, type II; 54.8%), and oxalosis (20.2%) also had significantly increased rates of graft loss, which remained after adjustment for age at start of RRT, time on dialysis, gender, and period of transplantation. No significantly different risks were found for patients with HUS (18.9%), IgA/HS (16.3%) or SLE (20.3%).

Conclusions: The risk of graft loss is increased among PRDs with a high risk of post-transplant recurrence. Physicians seem to anticipate on this higher risk of graft loss by performing less pre-emptive transplantation and providing fewer grafts from living related donors.

Funding: Clinical Revenue Support

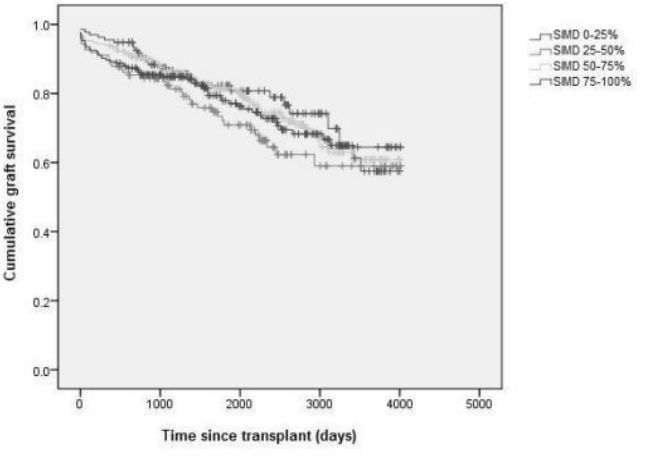
FR-PO935

Social Deprivation Does Not Affect Outcome Following Renal Transplantation Emma L. Aitken, Marc J. Clancy, David Kingsmore. *Department of Renal Surgery, Western Infirmary, Glasgow, United Kingdom.*

Background: Social deprivation is associated with increased mortality for patients on RRT. Similarly patients from lower socioeconomic categories have poorer access to transplantation. However the impact of social deprivation on outcomes following renal transplantation is unknown.

Methods: We undertook a retrospective analysis of all patients undergoing renal transplantation at a single centre serving the West of Scotland over a ten year period 2000-2010 (n=705). Postcode data permitted calculation of a Scottish Index of Multiple Deprivation (SIMD) score which was analysed in quartiles from 0-25% (least deprived) to 75-100% (most deprived). Outcome measures were graft loss, mortality, creatinine at 1 year, DGF and BPAR. Kaplan Meier survival analysis was undertaken ($p<0.05$ is significant). Results are presented as percentages of the total population in SIMD quartiles 0-25%, 25-50%, 50-70% and 75-100% respectively.

Results: Mean follow-up was 5.86+/-0.11 years. There was no difference in overall survival following transplantation depending on SIMD (89.6%, 87%, 88.4%, 90.2%; p=0.928). There was a trend towards improved graft survival in the least socioeconomically deprived however this was not significant (80%, 69.9%, 74.1%, 73.8%; p=0.34) (Figure 1). Similarly, there was a non-significant trend towards lower creatinine at 1 year in the least deprived patients (163.5+/-12.8, 211.7+/-19.5, 170.2+/-9.8, 197.1+/-6.9; p=0.07). There was no difference in rates of DGF (p=0.47) or BPAR (p=0.97) depending on socioeconomic status. The proportion of patients undergoing live donor transplantation was similar across the range of SIMD (23.9%, 27.6%, 25.5%, 21.8%; P=0.76).



Conclusions: Social deprivation does not affect either graft or patient survival after renal transplantation. Additionally it did not influence uptake of live donor transplantation in our patient population.

Funding: Government Support - Non-U.S.

FR-PO936

Comparison between CKD-EPI and MDRD Equation in the Estimation of Renal Function before and after Kidney Donation in Kidney Donors Jeong Gwan Kim, Hyun Chul Whang, Myung Hyun Lee, Yul Hee Cho, Gun Hee An, Yu Ah Hong, Byung Ha Chung, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim, Bumsoon Choi. *Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea.*

Background: The aim of this study is to investigate the usefulness of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula to predict renal function in subject in kidney donor before and after kidney transplantation.

Methods: We analyzed the CKD-EPI formula in comparison to Modification of Diet in Renal Disease (MDRD) equation in 207 pontential kidney donors before KT and 71 donors after kidney donation. Correlation, bias, precision and accuracy within 30 % of measured GFR (mGFR) were determined. mGFR was measured by technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) clearance.

Results: Before kidney donation, both of the results of MDRD and CKD-EPI equations correlated well with GFR (0.49; 0.52, respectively). eGFR calculated by MDRD underestimated mGFR significantly (100.7 ± 20.4 vs. 110.3 ± 20.7 mL/min/1.73 m^2,P<0.01). In contrast, eGFR calculated by EPI (108.7 ± 18.0 mL/min/ 1.73 m^2) did not showed significant differences to mGFR (P=0.23). Accuracy within 30 % of mGFR was higher for CKD-EPI (91.8 %) compared to MDRD (84.1 %)(P<0.01). After kidney donation, remained kidney showed significantly higher GFR compared to before kidney donation. (58.1 ± 10.3 vs. 78.8 ± 15.7 mL/min/1.73m^2, P<0.01). Both of the results of MDRD and CKD-EPI correlated well with mGFR, the correlation coefficient was lower in EPI (0.48; 0.27, respectively). MDRD equation underestimate mGFR (71.9 ± 14.vs. 78.8 ± 15.7 5 mL/min/1.73 m^2, P<0.01), but eGFR calculated by CKD-EPI (76.9 ± 21.2 mL/min/1.73 m^2, P=0.951 vs. mGFR) did not show significant differences compared to mGFR. However, the accuracy of the CKD-EPI (67.6%) within 30% of mGFR was lower than the accuracy of the MDRD equation (83.3%)(P<0.01).

Conclusions: In potential kidney donor, the healthy population, CKD-EPI equation showed better performance compared to MDRD equation to predict mGFR. However, after kidney donation, in uninephrectomy state, it was inferior to MDRD equation to predict mGFR.

FR-PO937

Effect of Utility-Based Deceased Donor Kidney Allocation on Living Donor Transplantation Rates Philip A. Clayton,[1] Blair S. Grace,[1] Keith McCullough,[2] Robert Merion,[2] Alan B. Leichtman,[2] Scott Campbell,[3] Jenni Wright,[4] Jeremy Chapman,[4] Stephen P. McDonald,[1] Steven J. Chadban.[1] *[1]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, SA, Australia; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]Princess Alexandra Hospital, Brisbane, QLD, Australia; [4]National Organ Matching System, Sydney, NSW, Australia.*

Background: Allocation of deceased donor (DD) kidneys in order to maximise utility would preferentially allocate DD kidneys to younger, healthier recipients. An unintended consequence of such an allocation system may be to reduce the number of living donor (LD) kidney transplants performed.

Methods: We adapted the US Scientific Registry of Transplant Recipients Kidney-Pancreas Simulated Allocation Model software to simulate allocation of Australian DD kidneys. We constructed two simulations – one replicating the current Australian allocation rules, and a simple utility model that allocated kidneys nationally to the patient with the highest predicted benefit based on donor and recipient age, HLA match and peak PRA. We made a simplifying assumption that wait-listing practice and LD transplant planning were independent of allocation rules.

Results: The simulation included 4146 wait-listed patients and 1882 kidneys from 1043 donors over 06/28/06-12/31/10. Compared with the current rules the utility model allocated kidneys to younger patients (mean 39.7 vs 51.9 years, P<0.001) with fewer co-morbidities and more HLA mismatches. Of the wait-listed patients not allocated a DD kidney, the number of LD grafts fell from 454 to 359 and the LD graft recipients were older (56.5 vs 48.1 years, P<0.001) with more co-morbidities. The total projected life-years following DD transplant increased from 38,130 to 44,687, and projected life-years following LD transplant fell from 10,603 to 7,386. Expected survival of younger patients increased substantially at the expense of smaller reductions in the expected survival of older patients.

Conclusions: The simulated utility model for DD kidney allocation prioritised transplantation of younger patients with fewer co-morbidities, resulting in a fall in LD and thus total transplants in those wait-listed for a DD.

Funding: Government Support - Non-U.S.

FR-PO938

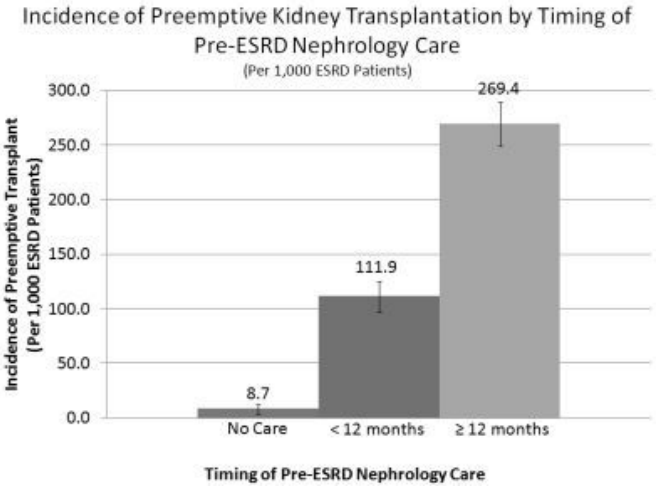
Access to Nephrology Care Prior to End Stage Renal Disease Is Associated with a Higher Incidence of Preemptive Kidney Transplantation among Pediatric Patients Rachel E. Patzer,[1,2] Nancy G. Kutner,[3] William M. McClellan,[2,4] Sandra Amaral.[5] *[1]Emory Transplant Center, Atlanta, GA; [2]Department of Epidemiology, Rollins School of Public Health, Atlanta, GA; [3]USRDS Rehabilitation/QoL Special Studies Center, Emory University, Atlanta, GA; [4]Division of Nephrology, Emory University School of Medicine, Atlanta, GA; [5]Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: In 2005, the United States Renal Data System (USRDS) began collecting information about whether ESRD patients received care from a nephrologist prior to ESRD. It is unknown how pre-ESRD nephrology care impacts access to preemptive kidney transplant (PKT) in children with ESRD.

Methods: We examined all incident pediatric (< 21 yrs) ESRD patients 2005-2009 in USRDS. Multivariable logistic regression was used to calculate odds ratios (OR) for PKT by timing of pre-ESRD nephrology care.

Results: Among 5,776 pediatric ESRD patients, 741 patients (12.8%) received a preemptive kidney transplant. The incidence of PKT was significantly higher among patients who had more than 12 months of pre-ESRD nephrology care (269.4 transplants per 1,000 ESRD patients; 95% CI: 249.5 - 290.1) compared to those with < 12 months (111.9 per 1,000 ESRD patients; 95% CI: 98.2-126.8) and no (8.7 per 1,000 ESRD patients; 95% CI: 5.1 - 13.9) pre-ESRD nephrology care (p<0.0001). Pediatric ESRD patients with nephrologist care within 12 months prior to ESRD had an adjusted odds of PKT 17 times higher than patients without access to pre-ESRD nephrology care (OR=17.6; 95% CI: 10.3-29.8). Patients with more than 12 months of nephrology care prior to ESRD had an adjusted odds of PKT 24 times that of patients with no access to pre-ESRD nephrology care (OR=23.9; 95% CI: 13.9-41.7).

Conclusions: In the US, access to pre-ESRD nephrology care significantly influences the likelihood of receiving a PKT among pediatric ESRD patients.



FR-PO939

Medication Adherence, Treatment Understanding, and Health Literacy in Kidney Transplant Recipients Rachel E. Patzer,[1] Marina Serper,[2,3] Jennifer King,[3] Kamila Przytula,[3] Titilayo O. Ilori,[1] Audra R. Williams,[1] Daniela Ladner,[2] Michael S. Wolf.[3] *[1]Emory Transplant Center, Emory University School of Medicine, Atlanta, GA; [2]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL; [3]Health Literacy and Learning Program, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Background: Kidney transplant (KT) recipients must take complex drug regimens and lifelong immunosuppression to maintain graft function. Little is known about health literacy, medication adherence, and treatment understanding in KT recipients.

Methods: Structured interviews were conducted with 28 KT recipients at two transplant centers in Chicago, IL and Atlanta, GA from January to May 2012. Basic demographics, health literacy (as measured by the Rapid Estimate of Adult Literacy in Medicine), cognitive function, medication adherence, treatment understanding, and drug regimen consolidation were assessed.

Results: Table 1 shows demographic and baseline characteristics of the study sample.

Demographics and Baseline Characteristics (N=28)	N (%)
Male	16 (57.1%)
Age, mean (SD)	51.3 (±13.7)
Race/Ethnicity	
White	15 (53.6%)
Black	9 (32.1%)
Hispanic	4 (14.3%)
Income < $50,000	19 (67.9%)
Months from KT, median (IQR)	38 (17.5, 60.5)
Number of daily medications, median (IQR)	9 (7,12)
Inadequate Health Literacy	9 (33.3%)

Patients were taking a median of 9 medications and 19.6% had a medication change within the previous month. A total of 28.6% of patients were non-adherent with at least one medication as measured by self-report. 33.3% of the study sample had limited health literacy. One-fourth of patients demonstrated inefficient dosing and spacing of at least one medication and 21.4% were unable to identify the indication for specific medications. Factors associated with non-adherence included lower health literacy, minority race, female gender, and lower income(p=< 0.05).

Conclusions: This is the first study to deconstruct the knowledge and skills of KT recipients for managing complex medication regimens. Limited health literacy was prevalent and associated with self-reported nonadherence. This is an actively ongoing study with a target enrollment of 100 participants planned by Fall 2012.

Funding: NIDDK Support

FR-PO940

Trends in Kidney Donor and Recipient Risk in the Era Surrounding Initiation of Medicare Conditions of Participation Sarah L. White,[1,2] Dawn Zinsser,[1] Matthew Paul,[1] Gregory Levine,[1] Tempie H. Shearon,[1] Valarie B. Ashby,[1] John C. Magee,[1,3] Alan B. Leichtman.[1,2] *[1]Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI; [2]Department of Medicine, Division of Nephrology, University of Michigan, Ann Arbor, MI; [3]Department of Surgery, Section of Transplantation, University of Michigan, Ann Arbor, MI.*

Background: In 2007, the US Centers for Medicare and Medicaid Services implemented Conditions of Participation (CoPs) for organ transplant programs, under which programs not attaining specified one-year survival rates are flagged for review. Case-mix is accounted for in the review process, yet anecdotal reports suggest that increased oversight may have prompted some programs to modify their organ and candidate selection criteria.

Methods: Data for the 221 adult kidney transplant programs active in 2007 were collated from CMS ESRD and Medicare claims and the SRTR database. Programs were classified according to whether they were flagged in any of the program-specific reports (PSR) released after the CoPs went into effect. A Cox proportional hazards model of one-year graft survival was constructed based on the July 2007 PSR cohort, and this model used to derive expected hazards for all adult deceased-donor kidney transplants between 2001 and 2010.

Results: Relative to the 2007 case-mix, from 2001 to 2010 the program average risk of one-year graft loss increased 6% (P<0.0001) due to donor characteristics, and 7% (p<0.0001) due to recipient characteristics. No inflection in donor or recipient risk was observed coincident with the introduction of the CoPs.

Conclusions: Trends in average program risk of one-year kidney graft loss do not support the assertion that the introduction of CoPs has reduced overall opportunities for marginal candidates or that there has been a systematic shift away from utilization of higher-risk deceased donor kidneys.

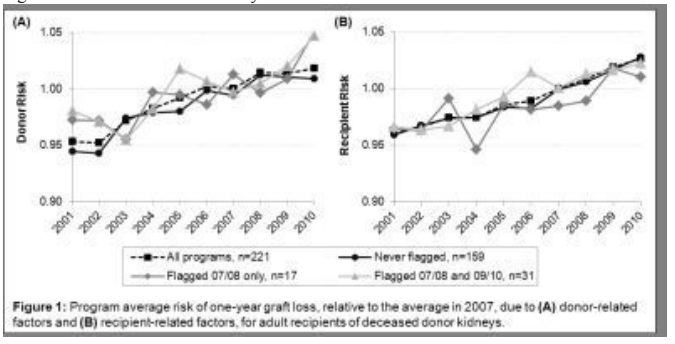


Figure 1: Program average risk of one-year graft loss, relative to the average in 2007, due to **(A)** donor-related factors and **(B)** recipient-related factors, for adult recipients of deceased donor kidneys.

Funding: Other U.S. Government Support

FR-PO941

Pre-Transplant Virtual PRA and Long-Term Outcomes of Kidney Transplant Recipients Lu Y. Huber,[1] Nils Lachmann,[2] Matthias Niemann,[2] Marcel Naik,[1] Danilo Schmidt,[1] Lutz Liefeldt,[1] Hans-Hellmut Neumayer,[1] Constanze Schönemann,[2] Klemens Budde.[1] *[1]Medizinische Klinik m. S. Nephrologie, Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]HLA-Labor, Institut für Transfusionsmedizin, Charité - Universitätsmedizin Berlin, Berlin, Germany.*

Background: Virtual panel reactive antibody(vPRA) is being implemented worldwide to gauge sensitization against HLA antigens. Its impact on longterm outcomes is however largely unknown.

Methods: Data from all 18-65 years(y) kidney-only transplantation(Tx) performed 1.1.1996-31.7.2011 in our centre were collected. vPRA was calculated based on antibody specification by solid phase techniques. Patients(pts) were divided into nonsensitized(NS, <5%), low(LS, 5-50%) or high sensitization(HS, >50%) group based on pre-Tx vPRA.

Results: Total of 896 Tx were performed during this period, 726 cases had vPRA available. There were 617(85%), 44(6%) and 65(9%) cases in NS, LS and HS group, respectively. More females(P=0.002) were in LS(45.5%) and HS(56.9%) than in NS group(34.6%), and more had previous Tx (69.2% in HS, 54.5% in LS, 7.3% in NS, P<0.001). The HS group had longer waiting time(4.6±3 vs. 2.9±3(LS) and 2±2.6y(NS), P< 0.001) and less living donation(9.2% vs. 27.3%(LS), 38.1%(NS), P< 0.001). Sensitized pts had more delayed graft function(38.3% (HS), 31.8%(LS), 17.5%(NS), P<0.001), biopsy proven rejection(12.3%(HS), 18.2%(LS), 7.1%(NS), P=0.017) and shorter followup(4±3.3(HS), 4.2±3.1(LS) and 5.8±3.9y(NS), P<0.001). Post-Tx donor specific antibody was more frequent in HS(35.4% vs. 18.2%(LS), 17.2%(NS), P<0.001). Sensitized group had lower GFR than NS in the first 3y. HS and LS had significantly lower death-censored and non-death censored graft survival(death-censored, 8y: 79%(NS), 57.1%(LS), 62.8%(HS), P< 0.001; non-death-censored: 8y, 78%(NS), 49.6%(LS), 45.9%(HS), P< 0.001), but patient survival rates were not different(8y: 77.0-88.9%, P=0.243), all were significantly better than N=704 waitlisted pts (69%, P< 0.001).

Conclusions: Sensitized pts defined by vPRA show higher graft losses but similar pts survival compared with non-sensitized pts. Level of vPRA does not proportionally correlate to the outcome.

FR-PO942

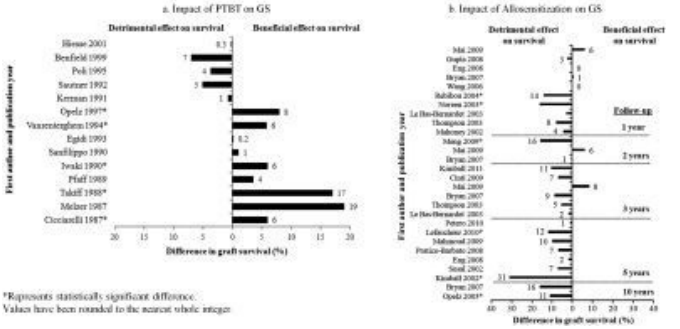
A Systematic Review on the Impact of Pre-Transplant Transfusions and Allosensitization on Allograft Survival Jeffrey Petersen,[1] Juan Scornik,[2] Jonathan Bromberg,[3] Douglas J. Norman,[4] Mayank Bhanderi,[5] Matthew Gitlin.[1] *[1]Amgen Inc.; [2]University of Florida; [3]University of Maryland; [4]Oregon Health & Science University; [5]HERON Health.*

Background: A recent review conducted by the Agency for Healthcare Research and Quality suggested that pre-transplant blood transfusion (PTBT) had a neutral to beneficial effect on graft survival (GS) compared with no transfusion. This review does not address the potential causative pathways of PTBT on outcomes, selection bias, or improvement in graft survival with newer immunosuppressive agents.

Methods: We performed a systematic review to examine the impact of: 1) PTBT on GS; 2) PTBT on allosensitization (the presumed causative pathway) and 3) resulting allosensitization on GS. A search conducted using MEDLINE®, Embase®, and the Cochrane Library for English-language publications identified 7,494 records from which 180 studies met eligibility criteria. Data were summarized descriptively and interpreted clinically.

Results: While many older studies demonstrated a beneficial effect of PTBT on GS, the magnitude of benefit has diminished over time, with most recent evidence suggesting a trend towards a neutral to detrimental effect of PTBT on GS (Figure 1a). PTBT increases the risk of allosensitization (data not shown). Recent data for a 10-year period indicate that allosensitization leads to graft rejection (data not shown) and poor GS (Figure 1b).

Figure 1. Impact of PTBT and Allosensitization on GS



Conclusions: Based on more recent literature, PTBT may be neutral to detrimental when current immunosuppressive regimes are taken into account, thus avoiding PTBT when possible is a sound management option that prevents a number of detrimental effects in patients awaiting a kidney transplant. The design and evaluation of systematic reviews can benefit from clinical judgment.

Funding: Pharmaceutical Company Support - Amgen Inc.

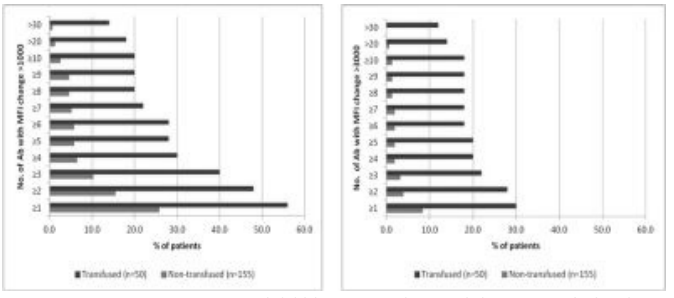
FR-PO943

RBC Transfusions Are Associated with Allosensitization in Patients Awaiting Kidney Transplantation Julie M. Yabu,[1] Matthew W. Anderson,[2] Deborah Kim,[3] Brian D. Bradbury,[3] Calvin D. Lou,[2] Jeffrey Petersen,[3] Jerome A. Rossert,[3] Glenn M. Chertow,[1] Dolly B. Tyan.[2] *[1]Medicine, Stanford Univ, Palo Alto, CA; [2]Pathology, Stanford Univ, Palo Alto, CA; [3]Amgen, Thousand Oaks, CA.*

Background: Human leukocyte antigen (HLA) sensitization is a major barrier to successful kidney transplantation resulting in prolonged wait times and decreased graft survival. This study determined the association of transfusions with the breadth, magnitude and specificity of HLA antibody (Ab) formation using highly sensitive and specific methods.

Methods: We linked demographic, comorbidity and transfusion data from United States Renal Data System (USRDS) with HLA Ab data obtained from Luminex single antigen beads for dialysis patients (pts) awaiting primary kidney transplant. We required pts to have ≥2 HLA Ab measurements. We matched pts who had a transfusion event between 2 Ab measurements (transfused group) with up to 4 non-transfused pts (non-transfused group) by age, sex, race, transfusion history and time on dialysis. We compared changes in mean fluorescence intensity (MFI) and specificities in Ab measurements for transfused and non-transfused pts.

Results: Of 654 pts with ≥2 HLA Ab measurements, there were 50 transfused pts and 155 non-transfused pts. The proportion of pts with change in MFI>1000 and >3000 for ≥1 HLA Ab was significantly higher for transfused compared to non-transfused pts (Fig 1): 20% of transfused vs 3% of non-transfused pts had ≥10 HLA Ab with MFI increase >1000 (p<0.0001); 18% of transfused vs 1% of non-transfused pts had ≥10 HLA Ab with MFI increase >3000 (p<0.0001). The association of transfusion with HLA Ab remained after controlling for other potential sensitizing events.



Conclusions: Among potential kidney transplant recipients, transfusion is an independent risk factor and results in significant increase in HLA Ab strength and breadth.

Funding: Pharmaceutical Company Support - Amgen, Inc.

FR-PO944

Antibody-Mediated Rejection in Pediatric Kidney Allograft Recipients: Risk Factor Analysis Jiwon L. Lee,[1] Yo Han Ahn,[2] Su Jung Park,[1] Hye Jin Chang,[1] Sang Taek Lee,[1] Hye Won Park,[3] IL-Soo Ha,[1] Hae Il Cheong,[1] Hee Gyung Kang.[1] *[1]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea; [2]Center for Pediatric Oncology, National Cancer Center, Goyang-si, Gyeonggi-do, Korea; [3]Health Promotion Center, Seoul National University Bundang Hospital, Bundang, Gyeonggi-do, Korea.*

Background: Antibody-mediated rejection(AMR) is an important cause of graft dysfunction. Non-adherence to drugs and disturbed quality of life are reported to be related to rejection in childhood. The present study compared two groups of kidney rejection, a group with AMR and the other with acute cell mediated rejection(ACR) to investigate risk factors of AMR.

Methods: Retrospective review of allograft recipients(n=138) of the last decade in our hospital was done. Acute rejection was in 49. Excluding protocol biopsies(n=22), there were 7 of AMR, 3 of ACR and 17 of borderline T-cell mediated rejection. Factors including age at transplantation, gender, donor types, HLA mismatches, puberty, socioeconomic status(SES) and compliance were analyzed to compare the AMR group and ACR/borderline group.

Results: While the mean age at rejection were comparable(15.8 years(yrs) vs. 15.5 yrs), AMR group was younger at transplantation(mean age 9.5 vs. 12.6 yrs). One of AMR was diagnosed in ten years after transplant and the rest in 5.6 yrs, after some period of poor compliance, coinciding with family crisis such as divorce or economic collapse. Six (85.7%) AMR patients showed poor compliance while 2(12.5%) of ACR/borderline had the problem. The donor type or HLA mismatch did not show significance. Rejection was treated with methylprednisolone and additional intravenous immunoglobulin, plasmapheresis, and rituximab were given for AMR. At follow-up, 3 of AMRs (43%) were losing their graft (eGFR<15 mL/min/1.73m^2) in 3.5 months after rejection, while 1 ACR lost his graft in 12 months.

Conclusions: Here we found that AMR in pediatric patients developed in those with poor compliance with low SES. While further study is necessary, we suggest that better surveillance and multi-disciplinary support is required to help our patients to keep adherence to their medication, thus may reduce AMR from low compliance.

FR-PO945

Removing HLA Antibodies: What Is the Best Technique? Nicolas Maillard,[1] Manolie Mehdi,[1] Ingrid Masson,[1] Lena Absi,[2] Christopher R. Mariat.[1] *[1]Nephrology, Dialysis, Transplantation, CHU Saint Etienne, Saint Etienne, France; [2]Laboratoire d'Histocompatibilité, Etablissement Français du Sang Auvergne Loire, Saint Etienne, France.*

Background: Antibody mediated rejection represents one of the main cause of graft loss. Several techniques to clear HLA antibodies are available such as plasma exchange methods (PE) and protein A based immunoadsorption (IA). The aim of our study was to compare the performances of PE and IA to lower HLA antibodies concentration.

Methods: Pre-transplant candidates or renal transplant patients presenting an indication of HLA desensitization were non randomly allocated to IA (one single session of 100 mL/kg) or PE (3 consecutive daily PE sessions of 45 mL/kg/session). Single antigen Luminex assays were performed on sera obtained before and immediately after the PE or IA treatment. Concentration of each anti-HLA antibody was estimated by measuring the Mean Intensity of Fluorescence (MFI).

Results: Thirteen patients were allocated to the PE group allowing the analysis of 253 HLA antibodies. In the IA group, 2 patients underwent 9 non-consecutive sessions allowing the analysis of 157 HLA antibodies. MFI were significantly reduced after both types of treatment: from 3518 to 1638 and 5475 to 2330 for the IA group and the PE group, respectively ($p<0.001$) with a similar relative reduction for both treatment (-65,4% and -65,0%, ns). However while class I antibodies were more efficiently removed in the IA group (-72% vs. -69%, p=0.01), the relative MFI reduction for class II antibodies was more pronounced in the PE group (-32% vs -50%, p=0.006). Surprisingly, some of anti- class II antibodies had a higher MFI immediately after treatment. This phenomenon occurred significantly more frequently after IA (20% vs 10% of HLA antibodies, p=0.02) and was abolished by pre-treating sera with DDT.

Conclusions: This study is the first one that compares the efficiency of IA against PE to lower MFI levels of HLA antibodies, using a single antigen Luminex assay. The global efficacy is equivalent between one single high volume IA session and 3 daily consecutive PE sessions. The apparent lack of efficacy of IA on class II antibodies was due to a C1q prozone effect.

Funding: Clinical Revenue Support

FR-PO946

Low Long-Term Antibody Formation and Preserved Kidney Allograft Function with an "Aggressive" Chronic Immunosuppression Protocol Mita M. Shah, Jennifer C. Joliat, Janice Kerr, Robert W. Steiner. *UC San Diego.*

Background: Recent studies suggest that de novo anti-donor antibody (DSA) is a major threat to kidney transplant (KT) survival, and on balance higher CNI exposure may improve outcomes. This study evaluated the rate of DSA formation in a KT recipient (KTR) population that has been maintained on an aggressive immunosuppression (IS) protocol.

Methods: Our long term KT clinic is strictly protocolized, with target trough TAC levels 8-10 ng/ml or CSA 2 hour levels 800-1000 ng/ml, prednisone at 0.1 mg/kg, and MMF 500-750 BID for all patients. IS is not tapered for KTRs who are "doing well". Importantly, empty-stomach CNI dosing is reinforced with all KTRs, improving CNI AUC by 30- 40%, with minimal effect on trough levels. We collected DSA in 61 KTRs perceived to be at higher risk for chronic rejection while maintained on CNIs (11 AA, 27 Hispanic).

Results: At the time of DSA determination, TAC trough levels were 8.3 +/- 2.0 ng/ml and CSA 2 hour levels 811 +/- 217 ng/ml. In the DSA- group who were 3.4 +/- 4.2 yrs post-transplant, serum Cr was 1.5 +/- 0.5 mg/dL with urine protein/creatinine ratio (UP/C) of 0.2 +/- 1.1. Of 61 KTRs, only 4 (7%) had detectable DSA at a mean of 5.6 +/- 2.75 post-transplant yrs, with serum Cr 1.5 +/- 0.3 mg/dL and UP/C of 0.14 +/- 3.7. Three DSA+ KTRs with an acute rejection due to noncompliance were excluded. In the total clinic population of ~400 KTRs, occasional BK viremia occurs with standard IS and is treated with tailored IS reduction. In this population, we documented 2 uneventful cases of CMV viremia and 1 severe CMV infection in a KTR who had TAC exposure above that dictated by protocol. Among all biopsies performed for cause, only 1 suggested CNI toxicity.

Conclusions: The DSA+ rate in KTRs maintained on our "aggressive" IS protocol was 7%; this compares well to the 10-30% prevalence of published DSA positivity in unselected KTRs during long-term follow-up. DSAs were more often surveyed in KTRs felt to be at high risk; thus this rate may represent a worst case outcome. We continue to develop and analyze our database, but such studies of DSA formation under a protocolized IS regimen may help determine optimally effective chronic regimens.

FR-PO947

Does Virtual Cross Matching Affect Outcomes in Renal Transplantation? A 2 Year Experience in Glasgow, UK Matthew Wickham, Emma L. Aitken, Marc J. Clancy. *Renal Transplant Unit, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom.*

Background: Cold Ischaemic Time (CIT) is well established as a key risk factor for Delayed Graft Function (DGF), which is an important predictor of both short and long-term graft outcomes[1]. This study aims to assess the impact of the introduction of virtual cross matching (vXM) in our renal transplant unit.

Methods: vXM was introduced in May 2010. A prospective comparison of all subsequent deceased donor transplants is presented. All live donor transplants were excluded.

Outcome measures were CIT, DGF and creatinine at 6 months. DGF was defined as either dialysis required beyond day 1 or failure of creatinine to fall by 50% in the first week post transplant.

Results are presented as mean +/- SEM. Student's t-test was used to compare continuous variables and x^2 test to compare categorical data ($p<0.05$ is significant).

Results: A total of 140 deceased donor transplants were performed. 74 (52.9%) had a vXM. 6-month follow up data was available for 100 transplants, of which 52 had a vXM. There was no significant difference between the numbers of DCD/DBD transplants with a vXM or full cross matching.

Demographic parameters were otherwise equivalent. CIT was significantly shorter in patients who had a vXM (9.94+/-0.43hr vs. 15.25+/-2.74; p=0.04). DGF was less likely in patients transplanted with a vXM compared to full XM (36.7% vs. 47%; p=0.007).

However, no difference was seen between vXM and full XM in 6-month creatinine (153.73+/-17.2µmol/l vs. 140.52+/-20.2 ; p=0.75) or all cause mortality (0% vs. 2.1%; p=0.48). There was a trend towards improved 6 month graft survival in patients with vXM however this was not statistically significant (98.1% vs 89.6%; p=0.1).

Conclusions: Virtual cross matching significantly reduces the CIT and DGF in deceased donor transplantation, however this does not have an identifiable impact upon graft function at 6 months.

Increased numbers and longer follow up will be required to confirm any beneficial effect on long term graft/patient survival.

[1]Taylor CJ et al. *Ten-year experience of selective omission of the pretransplant crossmatch test in deceased donor kidney transplantation.Transplantation. 2010 Jan 27;89(2):185-93.*

Funding: Government Support - Non-U.S.

FR-PO948

List Paired Exchange Reduces Waiting Times for Kidney Transplantation
Muhammad Saim, Dhiren Kumar, Martha Behnke, Anne L. King. *Department of Transplant Nephrology, Virginia Commonwealth University, Richmond, VA.*

Background: Waiting times on the list have continued to increase over the past decade. Living donor (LD) transplantation can address this long waiting time issue but donor-recipient incompatibility is a major obstacle to (LD) kidney transplantation. In list paired exchange, an incompatible LD provides a kidney to a candidate on the deceased donor (DD) waiting list in return for which the living exchange recipient receives priority on the DD waiting list.

Methods: We retrospectively compared the variance living kidney recipients (VLD) to direct living kidney (LD) recipients and the variance deceased donor (VDD) recipients to the standard criteria deceased donor (SCDD)recipients from October 2004 until August 2011 during which time 19 list paired exchanges were performed at our center.

Results:

Table 1

	VLD	LD	VDD	SCDD
N	19	341	13	389
Race AA	10 (52.6%)	153 (44.9%)	6 (31.6%)	287 (73.8%) *
Age	43.8 +/- 10.1	46.1 +/- 13.4	50.9 +/-12.7	48.6 +/- 12.9
Retransplant	2 (10.5%)	27 (8%)	1 (5.3%)	52 (13.4%)
PRA	13 +/- 18.8	17.3 +/- 26.0	15.9 +/- 24.0	41.1 +/- 38.7 *
Donor Age	42.9 +/- 14.1	40.1 +/- 11.2	25.8 +/- 10.2	34.0 +/- 14.9 *
Wait List (days)	755 +/- 322	305 +/- 368 *	313+/- 252	624 +/- 491 *
CIT	23.3 +/- 6.1	28.3 +/- 23.4	856 +/- 471	1093 +/- 453 *
DGF	2 (10.5%)	15 (4.4%)	3 (15.8%)	174 (44.7%) *
Patient Survival				
1 yr	94.3 +/- 5.6	93.4 +/- 0.4	100	98.4 +/- 0.7
5 yr	94.3 +/- 5.6	89.3 +/- 2.1	100	90.0 +/- 2.1
Death censored graft survival				
1 yr	100	98.8 +/- 0.6	100	95.1 +/- 1.1
5 yr	82.8 +/- 11.1	84.7 +/- 2.4	92.9 +/- 6.9	77.9 +/- 2.9

*p value < 0.05

There was no difference between the VLD and LD groups in any of the parameters. The VDD recipients had a significantly shorter waiting time and lower DGF rate but equivalent graft and patient survivals when compared to the SCDD recipients. The mean time to transplant for the VDD after VLD was 37.5 days.

Conclusions: Variance donation was beneficial to both the VLD and the VDD recipients and produced equivalent patient and graft survivals. This strategy resulted in the availability of high quality living donor kidneys with excellent outcomes for all groups and can be considered as another strategy to address the organ donor shortage.

FR-PO949

Preemptive Plasmapheresis (PP) and CMVIg Improves Survival in Highly Sensitized Renal Transplant Recipients
Anne L. King,[1] Amit Sharma,[2] Qing Ren,[1] Martha Behnke,[2] Pamela Kimball,[2] Todd W. Gehr.[1] *[1]Nephrology, VCU, Richmond, VA; [2]Surgery, VCU, Richmond, VA.*

Background: Kidney transplantation in the highly sensitized recipient carries with it an increased risk of rejection and poor graft survival. We developed a unique scoring system to standardize our approach to the use of preemptive adjuvant PP/CMVIg following deceased donor kidney transplantation in highly sensitized (PRA>50, FCXM>100 channel shifts) but lymphocytotoxic crossmatch negative recipients.

Methods: We retrospectively analyzed all highly sensitized adult deceased donor kidney transplant (KT) recipents at our center from 2006-2009 who were enrolled in this preemptive algorithm and compared them to a historical cohort (2000-2006) who did not receive preemptive adjuvant PP/CMVIg. Standard immunosuppression consisted of 4 doses of rabbit antithymocyte globulin with tacrolimus, mycophenolate mofetil, and steroids. Pateints were scored on a number of parameters at the time of KT including: PRA>50(1point), FCXM>100(1 poimt), FCXM>200(2 points), DGF(1 point). Patients with scores >3 points received preemptive PP/CMVIg(150 mg/kg) thrice weekly for 2 weeks beginning on the first preoperative day.

Results:

Pre- versus Post Protocol PP-CMVIg

	PreProtocol(n=54)	Protocol(n=40)	p-value
Length of Stay(d)	11.1(9.1)	8.2(4.2)	0.06
Delayed Graft Function	36(66.7%)	28(70%)	0.82
rejection 1st year	33(61.1%)	8(20%)	<0.0001
SCr 1 year	2.2(1.9)	1.5(0.5)	0.03
SCr 3 year	1.8(1.0)	1.5(0.6)	0.09
Patient survival 1 yr	92.3(3.6)	97.5(2.5)	<0.005
Patient survival 3 yr	83.3(5.1)	91.7(4.6)	<0.005
Graft survival 1 yr	83.3(5.1)	95.0(3.5)	<0.005
Graft survival 3 yr	68.5(6.3)	89.9(4.8)	<0.005

Mean(SD)

Conclusions: The addition of preemptive PP/CMVIg to a standard immunosuppressive regimen conferred a survival advantage for highly sensitized patients compared to a historical cohort in this single center study. This strategy should be more extensively evaluated in a multicenter trial. This algorithm can be easily applied at the time of kidney transplantation with excellent outcomes in this very high risk patient population.

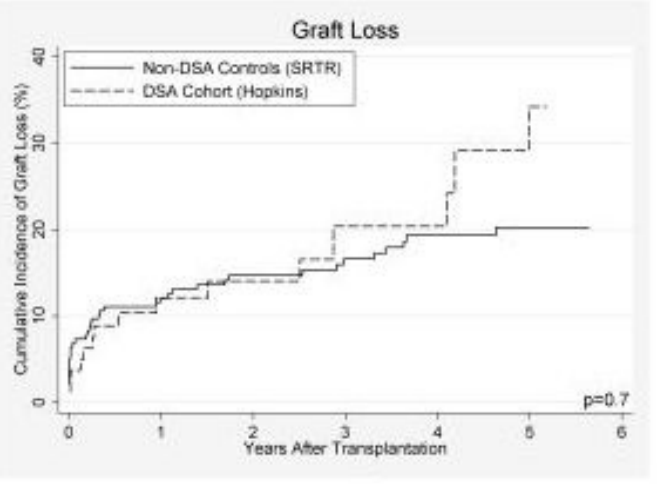
FR-PO950

The Outcome of Deceased-Donor Kidney Transplantation with Preformed Donor Specific Antibodies
John R. Montgomery, Andrew Lawrence Singer, Dorry L. Segev, Niraj Desai, Edward S. Kraus, Gaurav Gupta, Nabil N. Dagher, Robert Avery Montgomery, Hamid Rabb, Nada Alachkar. *Johns Hopkins University.*

Background: Successful live donor kidney transplantation with positive crossmatch has been achieved by utilizing desensitization therapy. Data is limited, however, on the treatment and outcome of deceased donor kidney transplant (DDKT) with preformed donor specific antibody (DSA). We report the outcome of these patients at our institution.

Methods: We identified 80 patients from 2000-2012 who underwent negative cytotoxic-crossmatch DDKT, but found to have DSA (Hopkins-DSA) at time of transplant. These patients were treated preemptively with plasmaphersis and low-dose IVIG ± rituximab immediately after transplantation to prevent DSA escalation and antibody mediated rejection (AMR). We compared this group with matched DDKT controls from the Scientific Registry of Transplant Recipients (SRTR-non-DSA). Subgroup analyses among Hopkins patients, Hopkins-DSA vs. Hopkins-non-DSA (n=1084), were performed to compare allograft function and rejection rate.

Results: When compared with SRTR-non-DSA controls, long-term patient survival of Hopkins-DSA (P=0.2) and graft survival (P=0.7) were comparable.



Comparing with Hopkins-non-DSA, there was a higher incidence of biopsy-proven AMR (31.2 vs. 5.1%, P<0.001) and cellular rejection (50.6 vs. 29.6%, P<0.001) in Hopkins-DSA. Additionally, there was increased chronic glomerular injury manifested on biopsies as chronic transplant glomerulopathy (33.3% vs. 20.0%, P=0.006). Allograft function, was similar after one year in the two groups.

Conclusions: Despite higher rates of acute and chronic rejection in positive DSA group, our data demonstrates equivalent long-term graft and patient survival to DDKT recipients without DSA. This can be achieved by preemptive plasmapheresis and low-dose IVIG ± rituximab along with close DSA monitoring.

FR-PO951

The Clinical Outcome of ESRD Patients Who Return to Peritoneal Dialyssi after Renal Allograft Failure
Keunsuk Yang, Hae Min Lee, Jeong Gwan Kim, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, Seoul, Korea.*

Background: With the increase of the accumulating numbers of kidney transplantation, the number of patients who return to dialysis after graft loss (DAGL) has increased The aim of this study is to investigate the safety and efficacy of peridoneal dialysis (PD) after graft loss compared to transplant naive PD patients.

Methods: This study is conducted on 714 patients who started PD in Seoul St. Mary's between 1988 and 2009. Out of them 47 patients of them belong to DAGL group while remained 667 patients were transplant-naive PD (TN-PD) group. We compared the overall patient survival rate and the clinical outcome of PD.

Results: The mean age was 40.8±10.7 in DAGL group and it was51.03±14.20 in TN-PD group (P<0.01). In comparison of primary renal disease, the distribution of it showed significantly different pattern (P>0.05). The most common cause of ESRD in DAGL was primanry glomerulonephritis (76.6 %) but it was diabetes mellitus (38.9 %) in TN-PD group. Patient survival rate was not significantly different between two groups. The 1, 5, 10 year patient survival rate was 100%, 86% and 57 % in DAGL group, respectively and they were 92%, 71% and 62% in TN-PD group, respectively. PD survival rate did not show significant differences between two groups as well. The 1, 5 and 10 year PD survival rate were 98%, 95% and 88 % in DAGL group respectively, and they were 95%, 80% and 66 % in TN-PD group, respectively. The most common cause of death in both groups were infection (DAGL :26.7 % TN-PD : 25.8 %) and cardiovascular disease (DAGL : 18.7 %, TN-PD :18.7%), and the distribution of the cause of death did not differ significantly. (P>0.05) The most common cause of PD failure was peritonitis and tunnel infection in both group, and the distribution did not differ between two groups as well. (P>0.05).

Conclusions: In conclusion, the clinical outcome of PD in DAGL group is comparable with that of TN-PD patients. Therefore, PD could be considered for dialysis modality in patients who experienced allograft failure.

FR-PO952

Impact of Nephrologists' Training and Education on Referral for Transplantation Nasrollah Ghahramani. *Medicine (Nephrology), Penn State College of Medicine, Hershey, PA.*

Background: Transplantation (Tx) is the treatment of choice for the majority of patients with ESRD. There is wide variation in the rate of referral for kidney Tx. A key component of the Tx process is the referral by the primary nephrologist. The objective of this study is to evaluate the impact of nephrologists' training and continuing medical education (CME) on referral of patients for Tx.

Methods: Invitations to participate were mailed to 2100 nephrologists practicing in the Mid-Atlantic and Southeastern States. Of these, 822 expressed interest. Two-hundred-fifty nephrologists were randomly selected and invited to complete a survey (online or paper) consisting of questions probing demographics, practice characteristics and perception of suitability for Tx. A total of 206 nephrologists completed the survey. Excluding responses from 19 Tx nephrologists yielded 187 responses. Chi-square and stepwise logistic regression were performed. Variables in the model included: age, race, gender, location, Tx training during fellowship, attendance at major national nephrology meetings and at Tx-related CME sessions.

Results: attendance at > 3 of the national nephrology meetings over the previous 5 yearswas associated with higher likelihood of referral for Tx at an earlier stage (OR:1.56; 95% CI: 1.07 to 2.28; p=0.02). Having received > 4 months of Tx training during fellowship was also associated with earlier referral (OR:2.94; 95% CI: 1.09 to 7.95; p=0.03) and higher likelihood of withholding referral due to the concern about patient's lack of understanding (OR:1.29; 95% CI: 1.05 to 1.59; p=0.01). Nephrologists who regularly attended Tx-related CME activities were more likely to have an established protocol and at least one designated nurse coordinator for workup and referral of the Tx candidate (OR:1.47; 95% CI:1.02 to 2.10; p=0.03).

Conclusions: The training and continuing education that nephrologists receive play a significant role in establishing an infrastructure for patient referral for Tx. Training is associated with earlier and more selective Tx referral with an emphasis on the patient's understanding of the process.

Funding: NIDDK Support

FR-PO953

The Clinical and Molecular Phenotype of Non-Adherence in Kidney Transplant Patients Gunilla Einecke,[1] Jeff Reeve,[2] Joana Sellares,[3] Philip F. Halloran.[2] *[1]Nephrology, Medical School Hannover, Hannover, Germany; [2]Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, Canada; [3]Nephrology, Hospital Vall d'Hebron, Barcelona, Spain.*

Background: We assessed the clinical presentation of non-adherence (NA) and the corresponding histologic and molecular features in 403 allograft biopsies (Bx) for clinical indication in 315 kidney transplant patients (pts) 6 days to 32 years post Tx.

Methods: Concerns about NA at or before Bx were recorded by the attending clinicians in 22/280 pts whose charts were available for review.

Results: 82/280 kidneys (29%) progressed to failure. Despite similar GFR at Bx, pts with NA had lower GFR 6 months after Bx (46±33 vs 55±23) and higher rates of graft loss (73% vs 19%). NA was associated with younger age (p<0.001), African American ethnicity (31% vs 8%, p=0.006), and rapid deterioration (45% vs 22%) but not stable impaired function (3% vs 19%). 17/22 Bx (77%) in pts with NA were diagnosed as borderline or rejection, 64% had antibody-mediated rejection (20% in pts without NA). Bx of pts with NA had more interstitial inflammation (1.6±0.9 vs 1.1±0.9, p=0.024), tubulitis (1.4±1.1 vs 0.7±0.9, p=0.001), peritubular capillaritis (1.5±1.2 vs 0.4±0.9, p=0.001), glomerulitis (0.7±0.9 vs 0.3±0.6, p=0.03), and fibrosis (1.7±0.8 vs 1.2±0.9, p=0.008), higher expression of inflammatory and injury gene sets and higher molecular risk scores predictive of graft loss. Based on the molecular changes in the Bx we built a classifier to detect NA and assigned a molecular NA-score to each Bx (Fig 1). 52 Bx had NA-scores > 0.5 (14 from pts with NA, 32 from pts without known NA; sensitivity 64%, specificity 91%, PPV = 0.30, NPV = 0.97). Bx with false-positive NA-scores were diagnosed as TCMR, borderline, or atrophy/fibrosis.

Conclusions: Whether this reflects non-specificity of the molecular classifier or undetected NA cannot be concluded from this data.

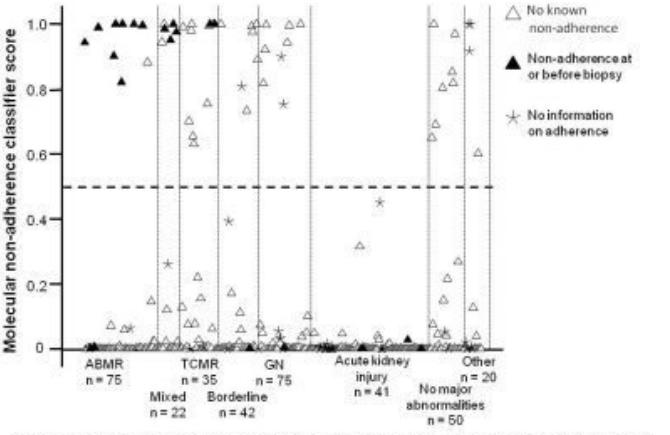


Figure 1. We developed a molecular classifier for non-adherence based on gene expression in the biopsy and calculated a molecular non-adherence score for each biopsy. Biopsies are grouped by their histologic diagnosis. Patients were classified as non-adherent based on physicians notes in their charts.

Funding: Pharmaceutical Company Support - Roche Molecular Systems, Hoffmann-La Roche Canada Ltd, Astellas Canada, Private Foundation Support, Government Support - Non-U.S.

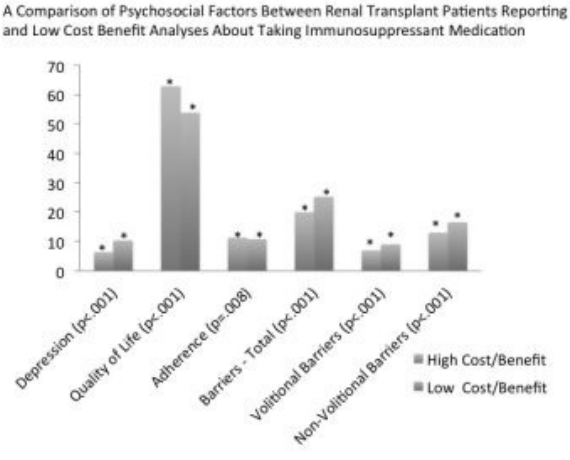
FR-PO954

Kidney Transplant Patient's Beliefs about Immunosuppressant Medication Utility Predict Adherence in a Multi-Ethnic Sample Daniel Cukor,[1] Melissa Constantiner.[2] *[1]Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY; [2]Clinical Health Psychology, Ferkauf Graduate School, Bronx, NY.*

Background: Adherence to immunosuppressant medication is essential for the maintenance and functioning of organ grafts, yet is often below clinical recommendations.

Methods: Subjects completed demographic, medical and psychological questionnaires.

Results: In 312 renal transplant patients from three New York City sites, the average age was 49.63 ± 12.65 years. The sample was 46.9% male, had 13.34 ± 3.68 years of education, and 46.6% were Black, 26.9% Hispanic, and 22.2% White. The Beliefs About Medication-Specific subscale was completed and a single score reflecting the individual cost-benefit analysis for immunosuppressant medications was created. The sample was divided by median split. No significant differences were found when comparing the two groups with regards to gender, age, time since transplantation, income, education, ethnicity, or employment (p> .05). Those endorsing higher cost-benefit ratios endorsed higher immunosuppressant adherence and fewer overall barriers to medication adherence (p< .05) when compared to subjects with a lower cost/benefit ratio.



Conclusions: Subjects who believed more strongly in the utility of their medication had better rates of adherence, a higher quality of life, lower rates of depression, and fewer barriers to adherence, but no significant demographic differences. Even adherence behaviors that are thought of as "non-volitional," such as forgetting a dose or being short of money, were less of a problem for subjects who more strongly believed in the utility of their immunosuppressant medications. Implications for intervention are strong in that increasing transplant patients' beliefs about the utility of taking their medication may have significant positive consequences on adherence behaviors and other measures of psychological health.

FR-PO955

The Effect of a Transition Program on Adherence in Adolescent Kidney Transplant Recipients Moving from a Pediatric to an Adult Care Setting
Rory F. McQuillan,[1] Alene Toulany,[2] Jeffrey Schiff.[1] *[1]Division of Nephrology, University Health Network, Toronto, ON, Canada; [2]Division of Adolescent Medicine, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: Medication non-adherence is prevalent in adolescent kidney transplant recipients and may account for poor long term graft survival. The process of transition from paediatric to adult care is a potentially modifiable factor in influencing adherence. The hypothesis for this study is that adherence can be improved by adult nephrology services engaging with patients in a paediatric setting prior to transfer in a bi-annual transition clinic and by a single nephrologist assuming responsibility for patients upon transfer.

Methods: Thirty-two consecutive patients, transferring from The Hospital for Sick Children to Toronto General Hospital were included. The first 16 transferred prior to the transition clinic; the subsequent 16 attended the clinic. Baseline data included date of end stage kidney disease; date, type and number of transplants; previous rejection; non-adherence and graft function at transfer. After transfer, measures of adherence including attendance at clinic and blood test appointment, drug levels and self reported non-adherence were identified. A patient was considered non-adherent if they directly admitted missing medication doses or exhibited at least 2 of the following 3 characteristics: missing appointments, missing blood tests or undetectable drug levels. The primary outcome was non-adherence during the first year post transfer; the secondary outcome was change in graft function after one year.

Results: There were no significant differences in baseline characteristics. Ten of 16 patients were non-adherent in the group transferred before the transition clinic, compared to 3 of 16 in the group who attended the clinic (p=0.03). The median change in serum creatinine was + 11.5μmol/L (0-16μmol/L) in the pre-transition clinic group compared to - 3.5 μmol/L (-9 - 11μmol/L) in the group who attended the clinic (p=0.03).

Conclusions: This study demonstrates change in the transition process improves adherence in adolescent kidney transplant patients and may lead to better graft function.

FR-PO956

Polycystin-1 Modulates Polycystin-2 Expression
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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a genetic disorder characterized by formation of fluid filled cysts that displace renal parenchyma and lead to end stage renal disease in ~50% of the patients. Mutations in PKD1 or PKD2 genes lead to ADPKD. Polycystin-1 (PC1) and polycystin-2 (PC2) are gene expression products of PKD1 and PKD2 respectively. Knockout of Pkd1 or Pkd2 genes leads to polycystic kidney disease in mice. On the other hand, overexpression of PC1 in Pkd1 transgenic mice or PC2 in TAZ knockout mice also leads to polycystic kidney disease. These data imply that up-regulation or down-regulation of PC1 and/or PC2 protein may play a role in pathogenesis of ADPKD. Here we investigated interrelation between PC1 and PC2.

Methods: MDCK cells were stably transfected with an expression system where expression of full length PC1 or mutant PC1-R4227X can be induced by tetracycline. Next MDCK cells were grown on transwell plates and PC1 expression was induced with tetracycline in the presence or absence of proteasome inhibitors (MG132). Finaly, we studied expression of PC2 in the presence or absence of PC1 and the interaction between PC1 and PC2 by co-immunoprecipitation.

Results: We found that induction of full length PC1 in MDCK cells down-regulates PC2 expression whereas induction of PC1-R4227X has no effect on PC2 protein level. Proteasome inhibitors stabilize PC1 expression leading to up-regulation of PC1 and further down-regulation of PC2 in PC1 induced or un-induced MDCK cells. Up-regulation of PC1 expression in the presence of proteasome inhibitors leads to increased interaction between PC1 and PC2 while total level of PC2 is decreased.

Conclusions: Our results suggest that PC1 may regulate the steady state level of PC2 protein and the extent of their interaction in vivo. C-terminal interaction between PC1 and PC2 may play a role in modulation of PC2 protein expression. Further studies will be conducted to elucidate the mechanism of PC2 regulation by PC1.

Funding: NIDDK Support

FR-PO957

The C-Terminus but Not the Channel Function of PC2 Is Required for Maintaining Normal Expression Levels of PC1 C-Terminal Fragment
Yiqiang Cai,[1] Sorin V. Fedeles,[1] Ke Dong,[1] Hongzhi Quan,[1] Yuehong Wang,[1] Seung H. Lee,[1] Xin Tian,[1] Ming Ma,[1] Ann-hwee Lee,[2] Stefan Somlo.[1,3] *[1]Internal Medicine, Yale University; [2]Immunology and Infectious Diseases, Harvard University, Boston, MA; [3]Genetics, Yale University, New Haven, CT.*

Background: PC1 is a polytopic receptor-like protein that undergoes cleavage at the GPS site to yield the extracellular N-terminal fragment (NTF) and the intra-membranous C-terminal fragment (CTF) that remain non-covalently associated with each other. PC2 is a cation channel in the TRP family. PC1 and PC2 interact via their respective C-termini and it has been proposed that PC1 and PC2 form a gated signaling complex. We have shown that mutations in genes for polycystic liver disease resulted in cyst formation by indirectly affecting PC1 protein levels. Here we sought to define the mechanism of cyst formation following mutation in PC2.

Methods: We generated conditionally immortalized mTAL-derived cell lines from $Pkd2^{fl/fl}$;$Pkd1^{F/H}$-BAC transgenic mice carrying the ImmortoMouse transgene. These cells were made null for Pkd2 ($Pkd2^{-/-}$;$Pkd1^{F/H}$-BAC) by adenoviral-mediated Cre infection. FLAG and HA tagged PC1 expression was examined in $Pkd2^{fl/fl}$ and $Pkd2^{-/-}$ cells and in $Pkd2^{-/-}$ cells in which either myc-tagged PC2, truncated PC2 (L703X) or "channel dead" full-length PC2 (D511V) had been re-expressed.

Results: The steady-state levels of PC1-CTF was dramatically decreased in the absence of PC2; PC1-NTF expression is not altered showing that GPS cleavage was unaffected. Re-expression of wild-type PC2 rescued the expression of PC1-CTF whereas re-expression of L703X, which does not interact with PC1, did not. Re-expression of D511V, which interacts with PC1 but lacks channel activity, also rescued PC1-CTF expression levels.

Conclusions: Interaction with PC2 is required to maintain the stability of PC1–CTF but not for GPS cleavage. Considering that most PKD2 mutations are truncating, the findings define a novel role for PC2 in the PC1-PC2 complex and post the hypothesis that defects in the maintenance of normal levels of PC1-CTF may underlie cyst formation in many cases of ADPKD due to mutations in PKD2.

Funding: NIDDK Support

FR-PO958

Filamin-A Stabilizes Polycystin-2 Expression by Preventing Its Degradation
Qian Wang, Zuocheng Wang, Jungwoo Yang, Xing-Zhen Chen. *Physiology, University of Alberta, Edmonton, AB, Canada.*

Background: Polycystin-2 (PC2), encoded by the PKD2 gene, is a Ca-permeable cation channel mainly located on the ER membrane. Mutations in PKD2 account for 10-15% of the autosomal dominant polycystic kidney disease. Filamins are actin-binding protein implicated in scaffolding, membrane stabilization and signal transduction, through interaction with ion channels, receptors and signaling proteins. We recently found that filamin reduces PC2 channel activity through direct binding. Here we studied the effect of filamin on PC2 degradation.

Methods: Cycloheximide treatment, western blotting, shRNA knockdown, ^{35}S pulse labeling, RT-PCR and real-time RT-PCR.

Results: Using synthesis inhibitor cycloheximide we showed that the half life of PC2 protein in filamin-A deficient human melanoma M2 cells is much shorter than that in filamin-A-replete A7 cells. Knockdown of filamin-A by shRNA in A7, Hela and HEK cells significantly shortened the PC2 half life. Furthermore, transient expression of human filamin-A C terminus (aa 2150~2647) prolonged the PC2 half life in M2 cells. Taken together, these data indicate that filamin slows down PC2 degradation, which is consistent with our observation that the steady state level of over-expressed PC2 in A7 is higher than in M2 cells. On the other hand, by use of ^{35}S pulse labeling and synthesis inhibitor cycloheximide we showed that filamin has no effect on the PC2 synthesis rate after normalization by the PC2 mRNA level. We are currently examining whether the effect of filamin on PC2 degradation is through their direct binding, by the use of blocking peptides that disrupt or weaken the PC2-filamin interaction.

Conclusions: Filamin is an important regulator of stabilization and cellular handling of PC2, presumably through direct binding and anchoring to the actin cytoskeleton.

Funding: Government Support - Non-U.S.

FR-PO959

Characterization of Post-Translational Modifications of Native Polycystin-2 (TRPP2)
Alexis Hofherr, Claudius Wagner, Michael Kottgen. *Division of Nephrology and General Medicine, University Medical Centre Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany.*

Background: Autosomal dominant polycystic kidney disease is the most common lethal monogenetic disorder in humans. Mutations in the PKD2 gene account for approximately 30 % of cases. PKD2 encodes the transient receptor channel polycystin-2 (TRPP2), a non-selective Ca^{2+}-permeable cation channel. The physiological function of ion channels is affected by chemical modification after translation. Nascent peptides are subjected to a battery of specific enzyme-catalyzed modifications on their amino acid side chains. These structural changes and the attachment of biochemical functional groups extend the range of protein functions. So far, several studies have shed light on specific post-translational modifications of TRPP2. However, a comprehensive analysis of posttranslational modifications of TRPP2 is lacking.

Methods: Here we characterize the post-translational modifications of TRPP2 using liquid chromatography tandem mass spectrometry. Endogenous TRPP2 was isolated from canine and mouse cell lines as well as mouse kidneys by immuno-precipitation.

Results: We identified five N-linked glycosylation sites in TRPP2. Mutation of these sites abolished N-linked glycosylation completely and significantly changed the biochemical properties of TRPP2. Furthermore, we mapped known and novel phosphorylation sites in TRPP2. The physiological relevance of these phosphorylation sites is currently under investigation.

Conclusions: These results may be a starting point for a more detailed analysis of the functional implications of post-translational TRPP2 modifications.

Funding: Government Support - Non-U.S.

FR-PO960

Impaired Trafficking of Polycystin-1 May Be a Key Mechanism of Cyst Formation in the Aquaporin-11 Knockout Mouse Yuichi Inoue,[1] Eisei Sohara,[1] Katsuki Kobayashi,[2] Tatemitsu Rai,[1] Kenichi Ishibashi,[3] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Department of Nephrology, Tokyo Medical and Dental Sciences, Bunkyoku, Tokyo, Japan; [2]Molecular Genetics, Chiba-East National Hospital, Chiba, Japan; [3]Pharmaceutical Department, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan.*

Background: We previously reported that the disruption of aquaporin-11 (AQP11) gene in mice resulted in cyst formation in the kidney. However, the mechanism of cyst formation in the AQP11-/- mouse is still unknown.

Methods: To investigate the mechanism, we analyzed the AQP11-/- mouse and AQP11 BAC transgenic (Tg^{AQP11}) mouse that expresses 3xHA–tagged AQP11, by focusing on the polycystic kidney disease-related gene products such as polycystins.

Results: Cyst formation in the AQP11-/- kidney was rescued by mating AQP11-/- mice with Tg^{AQP11} mice, confirming that 3xHA-tagged AQP11 expressed in the AQP-/- Tg^{AQP11} mouse behaved as a native AQP11 in vivo. Immunofluorescence of the kidney from Tg^{AQP11} mice revealed that 3xHA-AQP11 was localized in the cytoplasm of proximal tubules. Double immunofluorescence with organelle markers revealed that 3xHA-AQP11 was partially co-localized with KDEL, an endoplasmic reticulum (ER) marker. Immunoblots of isolated ER fraction of the kidney of Tg^{AQP11} mice also confirmed the ER localization of AQP11.Since ER is essential for folding, quality control, and translocation of newly synthesized proteins, we hypothesized that the absence of AQP11 in ER might result in defective sorting of the proteins known to be involved in polycystic kidney diseases. Therefore, we performed the immunoblots of those proteins in ER and plasma membrane fractions obtained by a density gradient centrifugation of the AQP11-/- kidney. As a result, polycystin-1 protein expression in the plasma membrane and ER fractions was decreased and increased, respectively, in the AQP11-/- kidney compared to that in wild-type kidney.

Conclusions: In this study, we could clearly demonstrate that the major site of AQP11 expression within the kidney cells was ER. The absence of AQP11 might affect the proper polycystin-1 trafficking from ER to the plasma membrane, thereby causing the cyst formation in the AQP11-/- kidney.

Funding: Government Support - Non-U.S.

FR-PO961

Polycystin-1 Cleavage at GPS Is Essential for Liver Homeostasis Marie Trudel,[1] Almira Kurbegovic,[1] Feng Qian.[2] *[1]Institut de Recherches Cliniques de Montréal; [2]Johns Hopkins University School of Medicine.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease that displays renal and hepatic cysts as well as non-cystic anomalies. The PKD1 gene responsible for most cases of ADPKD encodes polycystin-1 (Pc1). Pc1 is partially regulated by post-transcriptional modifications via cleavage at the GPS domain that generates N- and C-terminal fragments. Functional germline inactivation of Pkd1 in mice results in renal and pancreatic cysts and lethality by birth. Significantly, $Pkd1^{V/V}$ knockin mice without GPS cleavage of Pc1 escape embryonic/neonatal lethality of null mice but developed cystic kidneys and mild liver anomalies postnatally, and die by ~1month of age. Paradoxically, high levels of Pkd1 expression are also pathogenic in mice. Transgenic mice generated with a Pkd1-BAC driven from its native or renal specific promoter developed severe renal cysts leading to renal insufficiency at 4-6 months of age.

Methods: To investigate the role of the GPS cleavage of Pc1 in extra-renal tissues, we questioned whether Pkd1-BAC transgenesis could rescue the renal phenotype of $Pkd1^{V/V}$ mice and examined the hepatic phenotypes.

Results: The Pkd1 kidney or systemic expressors on the $Pkd1^{V/V}$ background rescue at P10 substantially or totally the renal cystic phenotype respectively, as evaluated by kidney to body weight ratio and histomorphometric analysis of cystic area. Lifespan of these $Pkd1^{V/V}$ mice with kidney-specific Pkd1 transgene was extended till ~3-4 months of age. By this age, these binary mice displayed severe liver phenotype with cystic clusters that affected virtually all biliary ducts and network. These results revealed a critical role for the Pc1 cleavage in liver homeostasis. In contrast, mice with the systemic Pkd1 transgene on $Pkd1^{V/V}$ background are healthy and have a lifespan expectancy >1year of age. In these older mice, the livers from each mouse (n=5) developed focal cystic anomalies in periphery of hepatic lobes that were significantly milder than with the kidney Pkd1 transgene expressors.

Conclusions: Together these results support an essential role for Pc1 GPS cleavage in ADPKD kidney but also liver pathogenetic mechanism.

Funding: Government Support - Non-U.S.

FR-PO962

Physiological Regulation of Polycystin-1 Cleavage and the Creation of the P100 Product Owen M. Woodward, William B. Guggino. *Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Mutations in PKD1 are responsible for 85% of polycystic kidney disease, but the physiological function of its protein product, PC1 remains unclear. Germ line knockout of PC1 in mice causes severely cystic kidneys before birth, where as the conditional knock out of PC1 later in development results in a much slower development of kidney cysts. This developmental dependent role is reflected in the protein expression of PC1: expression is high in the embryonic kidney but drops to undetectable levels after birth. However, although the full length protein disappears at birth, two c-terminal cleavage products persist after birth, the CTF and P100 cleavage products.

Results: We explored the mechanism regulating this dynamic cleavage of PC1 into the P100 product. Previously, we showed that the P100 product inhibits store operated calcium entry, and does so by inhibiting the translocation of the ER Ca2+ sensor protein STIM1 upon ER store depletion. Here, we demonstrate that extracellular Ca2+, activating a G-protein coupled Ca2+ receptor (CaSR), appears to regulate the creation of the P100 product in our MDCK cell line model system, and does so through a STIM1 dependent manner. And finally we probe the physiological consequence of STIM1 inhibition in kidney development with a kidney specific STIM1 knockout mouse.

Conclusions: Extracellular Ca2+, activating a G-protein coupled Ca2+ receptor (CaSR), appears to regulate the cleavage pf PC-1 and the creation of the P100 product in our MDCK cell line model system, and does so through a STIM1 dependent manner.

Funding: NIDDK Support, Other NIH Support - Johns Hopkins PKD Core Center, Private Foundation Support

FR-PO963

Renal Epithelial Disruption of TGFβ/Alk5 Signaling Does Not Affect Cystogenesis and Fibrosis in Pkd1-Mutant Mice: Novel Evidence for a Role of the Activin-A/Alk4 Signaling Pathway in ADPKD Wouter N. Leonhard,[1] Martijn H. Breuning,[1] Peter Dijke,[2] Emile De Heer,[3] Dorien J.M. Peters.[1] *[1]Human Genetics; [2]Molecular Cell Biology; [3]Pathology, Leiden University Medical Center, Netherlands.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD), caused by mutations in *PKD1 / 2*, is characterized by progressive cyst formation and renal fibrosis. Smad2/3 target-genes are highly upregulated at advanced PKD. However, we previously showed that epithelial deletion of *Alk5* (type-I receptor for TGFβ) neither affected fibrosis, nor cyst formation in adult tamoxifen-inducible kidney-specific *Pkd1*-deletion mice. Since the Hippo pathway has previously been described to be activated in PKD, and the YAP/TAZ target activin-A is known to stimulate Smad2/3-phosphorylation by binding to both the activin type-II receptor and its type-I receptor *Alk4*, we investigated whether this pathway may be involved.

Methods: Double inducible *Pkd1;Alk5*-deletion mice were treated with tamoxifen at post-natal day (PN)11 or PN40 in order to disrupt *Pkd1* and *Alk5*. We also generated various primary and immortalized cell-lines to study the effect of stimulation by Activin-A. Immuno-histochemistry, Western blotting and RT-MLPA were used to analyze the activin-A/*Alk4* pathway and Smad2/3 dependent signaling.

Results: *Alk5* disruption had no effect on nuclear translocation of phosphorylated Smad2, cyst formation, or renal survival in mice with fast (PN11) or slowly (PN40) progressing PKD. Activin-A was highly upregulated concordant with increased Hippo signaling and both activin type-I and type-II receptors were found to be highly expressed *in vivo*. In addition, activin-A indeed stimulated Smad2 phosphorylation *in-vitro*.

Conclusions: In two models of PKD, we confirmed that *Alk5* disruption in renal epithelium neither affects cystogenesis, nor fibrosis. However, activin-A was highly upregulated and its receptors abundantly expressed. These data suggest that the activin-A/*Alk4* cascade is likely to contribute to the pathogenesis of ADPKD. We are currently targeting the *Alk4*-gene to further demonstrate its involvement and potential new therapeutic interventions for ADPKD.

FR-PO964

Bioactive Sphingolipid Signaling in PKD: New Mechanistic Insights into the Role of Sphingosine Kinase 1 Herve Husson,[1] Thomas A. Natoli,[1] Kelly A. Rogers,[1] Bing H. Wang,[2] Yeva Budman,[2] Katherine W. Klinger,[3] Steven R. Ledbetter,[1] Oxana Beskrovnaya.[1] *[1]Tissue Protection and Repair, Genzyme Corporation a Sanofi Company, Framingham, MA; [2]Analytical Research and Development, Genzyme Corporation a Sanofi Company, Framingham, MA; [3]Genetics and Genomics, Genzyme Corporation a Sanofi Company, Framingham, MA.*

Background: Cyst formation in polycystic kidney disease (PKD) is associated with abnormal rates of epithelial cell proliferation, apoptosis, and mitogenic signaling. Accumulating evidence shows that sphingolipids (SL) and glycosphingolipids (GSL) are important regulator of these cellular processes. Recent data demonstrate that elevated levels of structural GSL promote cystogenesis; however, it is not known whether bioactive SL play a pathogenic role in PKD. To investigate the role of bioactive lipids in PKD, we compared SL levels in normal kidneys to cystic kidneys of jck mice. We found elevated levels of sphingosine-1-phosphate (S1P) in cystic kidneys. Analysis of S1P synthetic enzymes showed that sphingosine kinase 1 (Sphk1) mRNA, but not Sphk2, was highly overexpressed in cystic kidneys compared to normal kidneys. In addition, Serial Analysis of Gene Expression (SAGE) showed upregulation of Sphk1 but not Sphk2 mRNA in human ADPKD kidney epithelial cells relative to normal cells. We set out to test whether genetic loss of the Sphk1 gene could inhibit cystogenesis in jck mice. Surprisingly, loss of the Sphk1 gene aggravated PKD with increased activation of mitogenic signaling, proliferation and apoptosis. These data suggest that S1P produced by Sphk1 is protective. Therefore, we tested this hypothesis by pharmacological inhibition of S1P lyase activity to increase S1P levels. Although S1P lyase inhibition significantly increased kidney S1P levels, there was no effect on cystogenesis or GSL levels. Our data provide new insights into imbalances of sphingolipid homeostasis associated with PKD and help define viable therapeutic targets within this pathway.

Funding: Pharmaceutical Company Support - Genzyme Corporation

FR-PO965

Progressive Glomerulotubular Injury and Formation of Atubular Glomeruli in Murine and Human Polycystic Kidney Disease: A Role for Tubular Obstruction Carolina I. Galarreta,[1] Jared J. Grantham,[2] Michael S. Forbes,[1] Robin L. Maser,[2] Darren P. Wallace,[2] Robert L. Chevalier.[1] *[1]Dept of Pediatrics, University of Virginia, Charlottesville, VA; [2]Kidney Institute, University of Kansas, Kansas City, KS.*

Background: Ureteral obstruction in the mouse causes proximal tubular degeneration and formation of atubular glomeruli (ATG) (Forbes MS. AJP-Renal 2011, 2012). We hypothesized that tubular obstruction by cysts leads to progressive nephron damage in polycystic kidney disease (PKD).

Methods: Formation of ATG was examined in kidneys from mice with early (cpk) or late (pcy) development of PKD, and in nephrectomy tissue from an adult autosomal-dominant PKD patient. Cysts form in medullary collecting ducts in each of these examples. Kidneys from cpk mice were studied at 10 and 19 days of age (early maturation), while kidneys from pcy mice were studied at 5 and 9 weeks of age (adulthood). Sections were also stained with *Lotus tetragonolobus* lectin, which binds to functional proximal tubular cells and mature columnar cells of Bowman's capsule in mice with intact glomerulotubular junctions (GTJ).

Results: Serial sections revealed normal GTJ in 100% of 21 glomeruli from 10 day-old cpk mice, whereas in 19 day-old mice, only 74% of 27 glomeruli were normal, with atrophic GTJ in 22% and ATG in 4%. In 9 week-old pcy mice, 74% of 73 GTJ were intact, 8% had atrophic GTJ, and 18% were ATG. In human advanced PKD, 75% of 20 GTJ were atrophic, and the remainder (25%) were ATG. There were fewer *Lotus*-positive glomerular capsules in 10 day-old cpk mice, but some proximal tubules were preserved even at 19 days. *Lotus*-positive proximal tubules were preserved in 5 week-old pcy mice, but there were patches of *Lotus*-negative tubules by 9 weeks: these areas contained ATG. In human PKD, there were few scattered *Lotus*-positive tubules.

Conclusions: In summary, there was progressive injury to the GTJ with the formation of ATG in murine PKD; and similar formation of ATG in advanced human PKD. These findings support the possibility that obstruction of medullary tubules by enlarging cysts contributes to the loss of proximal tubule mass, the formation of ATG, and the decline in GFR characteristic of PKD.

Funding: NIDDK Support

FR-PO966

Role of Ras GTPase Isoforms in Renal Cystic Disease Progression in a *PKD1* Mutant Mouse Model Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Dorien J.M. Peters,[3] Claire C. Sharpe,[1] Bruce M. Hendry.[1] *[1]King's College London; [2]Cambridge University; [3]Leiden University Medical Center.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by abnormal cell proliferative responses that lead to cystogenesis and progressive fibrosis, resulting in loss of renal function. Ras-GTPase signalling has been implicated in these processes. We aim to examine the role of Ras isoforms and their interacting pathways in renal cystic disease progression in an orthologous ADPKD model.

Methods: *PKD1*$^{nl/nl}$hypomorphic mice were bred on a B6xCD1 genetic background. Immunohistochemistry (IHC), PMT stain, qPCR, immunoblotting and serum biochemistry were used to study renal phenotypic and molecular changes at sequential time points.

Results: On this genetic background, renal cystic disease progresses over 7-8 weeks. An initial rise in kidney volume peaking at P35, together with an early phase of increased tubular and cystic epithelial cell proliferation occurs. This is followed by a later stage of volume loss associated with a dramatic decline in epithelial cell proliferation at P35-49, progressive fibrosis and uraemia. Sustained activation of phospho-ERK up to P49 occurs, localizing predominantly to cystic epithelia in 26-37% of cells compared to 5-10% in tubular epithelia on IHC. In parallel, significant upregulation of Kirsten (Ki)-Ras mRNA is seen, with a peak rise at P35 by 3-4 fold compared to wt ($p=<0.001$), and ~2 fold increases at P21 and P49 ($p=<0.05$). Neural (N)-Ras expression is elevated by 2-3 fold through P21-49 ($p=<0.01$). Harvey (Ha)-Ras was not increased. Investigation of Ras-interacting pathways found a significant rise in Raf1 expression between P21 to P49, and late increase in pyk2 at P49. The pattern of Src expression followed that of N-Ras.

Conclusions: A sustained upregulation of Ki- and N-Ras expression together with activation of ERK occurs through both phases of renal disease progression in this model. This suggests Ki- and N-Ras signaling may have dual roles in the early proliferative growth of cysts and the later fibrotic stage associated with renal failure. This is under our further investigation.

FR-PO967

Cyst Fluid from Polycystic Kidney Disease Patients Activates Multiple Chloride Channels in Renal Principal Cells Bonnie L. Blazer-Yost,[1,2] Stephanie Flaig,[1] Vincent H. Gattone,[1,2] Robert L. Bacallao.[3] *[1]Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN; [2]Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN; [3]Nephrology, Indiana University School of Medicine, Indianapolis, IN.*

Background: In autosomal dominant polycystic kidney disease (ADPKD) renal cysts enlarge slowly during the patient's lifetime. Despite the growing cysts, renal function is not typically compromised until midlife but then the decline is precipitous and progresses to renal failure within a few years. Renal injury, whether traumatic, chemical or hypoxic, exacerbates renal decline. It is our hypothesis that renal injury or natural aging of cysts in ADPKD patients causes cyst rupture thereby releasing factors that accelerate expansion of remaining cysts thus promoting rapid disease progression. The objective of the studies was to characterize the factors and channels involved in cyst growth during late stage ADPKD.

Methods: Electrophysiological techniques were used to characterize the ion transport response of normal renal principal cells (mouse principal cells of the kidney cortical collecting duct; mpkCCD) after exposure to cyst fluid from human ADPKD patients.

Results: We have previously shown that addition of cyst fluid to the basolateral side of principal cells stimulated a Cl^- secretory response that, in vivo, would result in cyst expansion. The active component of the cyst fluid is lysophosphatidic acid (LPA) and cyst fluid contains sufficient LPA to maximally stimulate secretory Cl^- transport. The Cl^- secretory flux involved ion movement through the cystic fibrosis transmembrane conductance regulator (CFTR) and through an alternative channel with an inhibitory profile consistent with TMEM16a. Thus, the secretory activity is mediated by both CFTR and a Ca^{2+}-activated Cl^- channel.

Conclusions: This is the first demonstration of the involvement of Ca^{2+}-activated Cl^- channels in the cellular response to ADPKD cyst fluid. Consideration of alternative chloride channels is important when designing interventions to inhibit cyst expansion.

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FR-PO968

Polycystic Kidney Disease 1 Gene in Fibroblast and Cyctic Disease Progression Yunxia Tao, Zonghan Dai, Zijuan Liu, Hongxing Tang. *Internal Medicine, Texas Tech University Health Sciences Center, Amarillo, TX.*

Background: Cyst expansion and loss of renal function in ADPKD kidneys are associated with progressive renal fibrosis, the mechanism of which is not fully understood. Renal interstitial myofibroblast, the active form of fibroblast, are the principal source for the synthesis of ECM components as well as the enzymes that regulate ECM turnover. It is established that the mutation of Pkd1 in renal tubular epithelia cells leads to increased proliferation and cyst development. However, little is known about the function of the Pkd1 in renal interstitial fibroblasts.

Methods: In this study, we show that Pkd1 is expressed in fibroblast cell lines and primary fibroblasts isolated from mouse kidneys. We also show that Pkd1 is expressed in bone marrow and circulating fibroblast precursor cells (FSP-1-positive cells). In order to study the function of Pkd1 in fibroblasts, we knocked down the expression of Pkd1 in NIH 3T3 fibroblasts by shRNA-mediated gene silencing. We tested a panel of lentiviruses expressing various Pkd1 shRNAs and were able to knock down the Pkd1 mRNA expression by up to 80% in fibroblasts.Using BrdU incorporation assay, we found that the knockdown of Pkd1 expression significantly increased fibroblast proliferation.

Results: A significant increase in the aSMA expression, a marker of myofibroblast, was observed in the Pkd1 knockdown fibroblasts compared with parental fiboroblasts, suggesting that the Pkd1 knockdown induces fibroblast activation to myofibroblast. We have previously shown that co-culture of fibroblasts with cystic epithelial cells induces cyst formation and enlargement. Here, we provide evidence that the knockdown of Pkd1 expression in fibroblasts significantly increased their ability to stimulate cyst formation and expansion.

Conclusions: Taken together, our results suggest that Pkd1 plays an important role in fibroblast growth and activation and that Pkd1 mutation in fibroblast in ADPKD kidneys may play a key role in renal fibrosis and cystic disease progression.

Funding: NIDDK Support

FR-PO969

Transforming Growth Factor Beta: A Molecular Target of Polycystin-1 and Nuclear Factor of Activated T-Cell Signaling Tarundeep Kaur,[1] Nidhi Mahajan,[1] Veena Puri,[2] Vivekanand Jha,[3] Surinder Kumar Singla,[1] James P. Calvet,[4] Sanjeev Puri.[5] *[1]Dept of Biochemistry, Panjab Univ, Chandigarh, India; [2]Centre for Systems Biol & Bioinformatics, Panjab Univ, Chandigarh, India; [3]Dept of Nephrology, PGIMER, Chandigarh, India; [4]Kidney Institute, Univ of Kansas Med Ctr, Kansas City, KS; [5]Centre for Stem Cell & Tissue Engineering, and Biotechnology Branch, UIET, Panjab Univ, Chandigarh, India.*

Background: Autosomal dominant polycystic kidney disease is a common hereditary disorder characterized by development of multiple fluid filled cysts in both kidneys. Loss-of-function mutations in the PKD1 gene are the major cause of this slowly progressive, fatal disease. Diminished levels of the PKD1 gene product polycystin-1 (PC1) are thought to result in abnormal cellular signaling. Previously we had shown that PC1 upregulates the NFAT transcription factor (Puri et al J Biol Chem 2004 279:55455). We now demonstrate that TGFβ is a target of this signaling.

Methods: Transient transfection of HEK293T cells using PC1 C-tail constructs with or without augmentation with Gα12; TGFβ RT-PCR expression analysis; NFAT promoter-reporter analysis and NFAT-GFP nuclear localization; antisense and chemical inhibitors of NFAT signaling; cell proliferation by MTT and FACS analysis.

Results: Transient transfection analysis with constructs expressing the PC1 C-tail showed that PC1 upregulates endogenous TGFβ expression in comparison to that seen with a control construct sIg0 that does not contain the PC1 C-tail. PC1-mediated NFAT activation and nuclear localization were augmented by coexpression of the Gα12 subunit of heterotrimeric G-proteins, and this concomitantly augmented expression of TGFβ. This increased TGFβ expression was completely abolished by cyclosporine, an inhibitor of calcineurin/NFAT signaling, and by antisense inhibition of Gα12 and PLCδ. The functional outcome of this signaling crosstalk was a considerable reduction ($p<0.05$) in cell proliferation.

Conclusions: These results demonstrate the importance of PC1/NFAT signaling in understanding PKD1 gene function and suggest a potential pathway through TGFβ crosstalk for the maintenance of normal renal epithelial organization.

Funding: NIDDK Support, Government Support - Non-U.S.

FR-PO970

Heat-Shock Protein 90 Regulates Cystic Epithelial Cell Proliferation through Histone Methyltransferases Mediated Downstream Pathways

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Background: The heat-shock protein 90 (HSP90) is essential for the creation, maintenance, and destruction of proteins. Its normal function is critical for maintaining the health of cells, whereas its dysregulation may contribute to not only carcinogenesis but also cystogenesis in polycystic kidney disease (PKD).HSP90 has been reported to regulate cell cycle progression through p53 and retinoblastoma (RB) mediated signaling pathways. Based on the fact that HSP90 was upregulated in *Pkd1* mutant renal epithelial cells, we hypothesized that HSP90 mediated pathways involved in regulating cyst formation in autosomal dominant PKD (ADPKD).

Methods: We investigated the expression of HSP90 and its relationship with histone methyltransferases (HMTs) in the renal cystic epithelial cells treated with or without HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17-AAG). We also tested the effect of 17-AAG on cyst development in *Pkd1* knockout mouse embryonic kidneys.

Results: We found that in *Pkd1* mutant cystic epithelial cells: 1) in addition to HSP90, HMTs were also upregulated; 2) HSP90 formed a complex with HMTs; 3) inhibition of HSP90 resulted in the downregulation of HMTs; 4) downregulation of HMTs with siRNAs or HSP90 inhibitor resulted in the upregulation of p21, which might be mediated through the methylation of p53 by HMTs; 5) inhibition of HSP90 decreased the phosphorylation of Rb and the expression of cyclin-dependent kinase 2 (Cdk2) and 4 (Cdk4), which might be through increasing the formation of Rb-E2F1 complex to decrease the cell proliferation. We further found that treatment with 17-AAG delayed cyst formation in *Pkd1* knockout mouse embryonic kidneys.

Conclusions: HSP90 regulated cystic epithelial cell proliferation through histone methyltransferases mediated methylation of p53 and Rb and inhibition of HSP90 delayed cyst formation in *Pkd1* knockout mouse embryonic kidneys. Understanding the role of HSP90 signaling in cystic epithelial cells allows us to develop new therapeutic strategies for ADPKD.

Funding: NIDDK Support

FR-PO971

Aberrant VEGF and sFlt-1 Expression in ADPKD Patients

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Background: Emerging evidence indicates that angiogenesis occurs in human renal cysts in autosomal dominant polycystic kidney disease (ADPKD). We have shown that the serum level of vascular endothelial growth factor (VEGF) correlates with total renal volume in children and young adults with ADPKD. This suggests that VEGF may play a key role in early cystogenesis. VEGF activity is mediated by signaling through its receptors including VEGF receptor -1 (VEGFR-1/Flt-1). Soluble Flt-1 (sFlt-1), a splice variant of the VEGF receptor without transmembrane and cytoplasmic domains, inhibits VEGF signaling and has been shown to contribute to endothelial dysfunction in CKD. We hypothesized that sFlt-1 level may contribute to aberrant VEGF mediated angiogenesis in ADPKD.

Methods: Adult ADPKD patients were divided into two groups for analysis : group A: eGFR > 90ml/min/1.73m^2 and group B: eGFR <40ml/min/1.73m^2. Serum VEGF and sFlt-1 levels were measured by ELISA. Renal VEGF mRNA levels were measured by real-time RT-PCR and expressed as ratio of VEGF/GAPDH.

Results: Serum sFlt-1 levels were significantly lower in group A than in group B (40.9±46.4pg/ml, n=19 vs 71.7±32.3pg/ml, n=18, p<0.05). Group A sFlt-1 levels were similar to that reported for healthy individuals. Serum VEGF level did not differ between the two groups (593.8±500.6 pg/ml, group A, n=19 and 490.6±271.1 pg/ml, group B, n=18, p=NS) but were higher than reported levels in healthy individuals. The ratio of VEGF/sFlt-1 was higher in group A than in group B (16.8±18.1, n=19 vs 8.9±6.3, n=18, p=0.08). VEGF gene expression was significantly lower in ADPKD kidneys compared to normal kidneys (0.11±0.05, n=6 vs 0.25±0.14, n=5, p<0.05) but was higher that levels in kidneys from patients with other renal diseases.

Conclusions: Abnormalities in serum sFlt-1 and VEGF levels, including the increased VEGF/sFlt-1 ratio observed early in ADPKD are indicative of increased bioavailability of VEGF and support a role for angiogenesis in cyst growth. Decreased renal VEGF expression in advanced disease may exacerbate vascular damage in the kidney. Further studies are needed to verify the causal relationship between VEGF/sFlt-1 and cystogenesis in ADPKD.

Funding: NIDDK Support, Private Foundation Support

FR-PO972

Over-Expression of the Polycystin-1 (PC1) C-Tail Enhances the Sensitivity of M-1 Cells to the Effects of Ouabain

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Background: We had previously shown that physiological levels of the hormone ouabain enhance proliferation of epithelial cells derived from renal cysts of patients with autosomal dominant polycystic kidney disease (ADPKD cells). This effect is mediated via the Na,K-ATPase signaling complex and activation of the epidermal growth factor receptor (EGFR), Src kinase and the extracellular regulated kinase (ERK) pathway. Here, we have determined whether ouabain had an effect on mouse cortical collecting duct cells stably expressing the C-terminal cytosolic portion of polycystin-1 (M-1 C20), which had been previously shown to cause a PKD phenotype, and compared it with a control cell line which does not express the PC1 C-tail (M-1 C17).

Methods: CellTiter 96 MTT Assays, immunobloting and Na,K-ATPase activity assays were used.

Results: Our results show that while ouabain stimulated proliferation of M-1 C20 cells, it did not modify the growth rate of M-1 C17 cells. Ouabain-enhanced cell proliferation was dose dependent and increased with ouabain amounts between 3 nM to 100 nM, which correspond to the circulating levels of ouabain commonly detected in plasma. Dose response curves for the interaction of ouabain with Na,K-ATPase activity showed that, in contrast to M1-17 cells, in M1-20 cells, approximately 20% of the Na,K-ATPase had higher affinity for ouabain. In addition, M-1 C20 cells, but not M-1 C17 cells, responded to ouabain with an increase in phosphorylation of ERK, which was abrogated by AG1478 and PP2, inhibitors of EGFR and Src, respectively. Altogether, these results show that expression of the C-tail of PC1 makes M1 cells sensitive to physiologic levels of ouabain, stimulating the EGFR-Src-ERK pathway and the proliferation of the cells. This increased response to ouabain resembles that previously found in ADPKD cells.

Conclusions: Over-expression of the C-terminal domain of PC1 transforms M1 cells to the abnormal ouabain sensitive phenotype of ADPKD cells.

Funding: NIDDK Support

FR-PO973

The PKD1 Gene Expresses a Second Transcript Using Intron 41 as Its Start Site

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Background: As part of a genome-wide cDNA library construction using a 5' cap approach to identify and capture mRNA, Kimura et al, identified three alternative transcripts in the PKD1 locus (Kimura et al., Genome Research, 16, 55-65, 2006).

Results: We have sequenced one transcript and found that its start site is in intron 40. The cDNA is 1925 bp long and it has a unique splice junction between the 3' end of exon 41 and the 5' end of exon 43 in the PKD1 gene. Northern blot analysis confirms expression of a 1900 bp message found in kidney and placenta. Based on sequence analysis no canonical start site could be found. To evaluate the start site of the cDNA we cloned the gene into pCTAP vectors which permit expression all three reading frames. We left the 5' end of the cDNA intact to provide an unbiased expression profile. Based on the cloning strategy we predicted that one reading frame would result in a full-length protein containing strepavidin and calmodulin binding sites that are encoded by the CTAP vector and can be assayed by immune blot. A unique 42 kDa band was identified in one reading frame by immune blot. The open reading frame (ORF) uses a CAG codon as its putative start site. The ORF predicts that this transcript encodes a 48 kDa protein with a 46 amino acid cleavable signal sequence suggesting that the protein is secreted. Strikingly when this cDNA is transfected into COS-1 cells mesenchymal markers are such as snail and slug are decreased. Furthermore transfected cells increase expression of E-cadherin and occludin.

Conclusions: A second transcript is encoded by the HmPKD1 locus with its start site in intron 40. This transcript codes for a 48 kDa protein which dramatically induces expression of epithelial markers in a mesenchymal cell line.

Funding: Private Foundation Support

FR-PO974

Inhibition of Ca^{2+}/Calmodulin Activates RAS and Enhances cAMP-Dependent ERK Activation in Human ADPKD Cells

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Background: cAMP plays a central role in the pathogenesis of ADPKD by stimulating cyst epithelial cell proliferation through activation of B-Raf and MEK/ERK signaling. By contrast, in normal renal cells (NHK), cAMP inhibits Raf-1, ERK and cell proliferation. The molecular mechanism for this phenotypic difference in the mitogenic response to cAMP remains unclear. Activated RAS (GTP bound) recruits Raf to the plasma membrane and mediates Raf activation. Ca^{2+} /calmodulin (CaM) can stimulate or inhibit RAS, depending on cell type. Previously, we showed that CaM inhibition with W-7, alone or in the presence of AVP, significantly increased ERK phosphorylation (P-ERK) in ADPKD cells, while having no effect in NHK cells. Here, we determined if Ca^{2+}/CaM differentially regulates RAS activity to account for differences in cAMP-dependent ERK activation between ADPKD and NHK cells.

Methods: ADPKD and NHK cells were treated with W-7 ± AVP and RAS activity was determined by RAS-GTP affinity pull-down assay. NHK cells were treated with a Ca^{2+} chelator BAPTA to restrict intracellular Ca^{2+}, mimicking ADPKD cells.

Results: cAMP stimulated RAS to similar levels in ADPKD and NHK cells; however, it only increased P-ERK and proliferation of ADPKD cells. In ADPKD cells, CaM inhibition with W-7 alone increased RAS activity and P-ERK. cAMP caused a further increase in P-ERK, while having only a modest effect on RAS activation, suggesting cAMP had an effect downstream of RAS. In NHK cells, W-7 increased RAS activity, but did not stimulate ERK. In the presence of W-7, cAMP had no additional effect on RAS and failed to stimulate ERK. Restriction of intracellular Ca^{2+} with BAPTA increased RAS activity 3-fold and stimulated ERK in NHK cells. cAMP had no further effect of RAS but stimulated ERK and cell proliferation, suggesting that the Ca^{2+}-induced phenotypic switch in the cAMP mitogenic response was below the level of RAS.

Conclusions: These data support the hypothesis that the differential effect of cAMP on the ERK signaling pathway between NHK and ADPKD cells is due to regulation of components downstream of RAS, notably the Raf kinases.

Funding: NIDDK Support, Private Foundation Support

FR-PO975

N-myc Downstream Regulated Gene 1 Is Engaged in the Regulation of Cyst Growth in PKD2 Transgenic Mice Jong Hoon Park, Bo Hye Kim, Eun Young Park. *Biological Science, Sookmyung Women's University, Seoul, Korea.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is an inheritable and progressive kidney disease featured by formation of fluid-filled cysts. N-myc downstream regulated gene 1 (NDRG1) is involved in cellular proliferation and differentiation. Our research group focused on this gene as a candidate regulator in cyst growth.

Methods: To check expression level of genes, real time RT-PCR and western blot were performed. Protein localization on the specimen was detected by immunohistochemistry. Transient transfection or siRNA treatment was utilized for regulating gene expression level. Also, three dimensional culture (3D culture) of MDCK cells was used for observing *in vitro* cystogenesis.

Results: NDRG1 expression level was higher in the kidney of PKD2 TG mice. Furthermore, NDRG1 protein was highly expressed in the cyst lining epithelial cells. We found that PKD2 gene regulated NDRG1 expression level *in vitro*. Also, NDRG1 knockdown attenuated cyst growth in 3D culture of Madin-Darby canine kidney cells (MDCK). Now, we try to find how PKD2 gene regulates NDRG1 expression level. We investigate transcription factors which bind to NDRG1 promoter as mediators between PKD2 and NDRG1.

Conclusions: In conclusion, overexpression of PKD2 gene upregulates NDRG1 expression level. This could aggravate cyst growth.

Funding: Government Support - Non-U.S.

FR-PO976

Inactivation of Mxi1 Induces Cilia Disassembly through IFT20 Reduction in Polycystic Kidney Jong Hoon Park, Je Yeong Ko, Kyung Hyun Ryu, Eunji Lee. *Department of Biological Science, Sookmyung Women's University, Seoul, Korea.*

Background: Primary cilia are antenna-like projections from the basal body bounded by the cell surface and act as sensory organelles that detect mechanical and chemical signals from extracellular environment. They consist of 9 doublets microtubules but no central microtubules termed 9+0. Intraflagellar transport (IFT) is involved in ciliogenesis so mutation of these genes induces ciliopathies. It is critical that the size control system of cilia be elucidated to enable understanding of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. In previous study, multiple tubular cysts, inflammation and fibrosis were observed in kidneys of Mxi1 KO mice aged six months or more.

Methods: To observe primary cilia phenotype *in vitro* and *in vivo*, SEM and ICC were used. To identify the relationship between Mxi1 and cilia desassembly, Q-PCR, WB and promoter assay are used in this study.

Results: Here, we identify the relationship between inactivation of Mxi1 and cilia disassembly. The length of primary cilia is decreased and p-ERK level induced by cilia defect is increased in kidneys of Mxi1 KO mice. Ciliogenesis of Mxi1 KO MEFs is decreased and this abnormality is restored by Mxi1 transfection to Mxi1 KO MEFs. To elucidate the cilia regulatory mechanism related to Mxi1, IFT genes are validated >*in vitro* and *in vivo* using Q-PCR and IFT20 is selected for candidate gene in this study. Ciliogenesis defects and down-regulation of IFT20 observed in Mxi1-siRNA treated mIMCD-3 compared to controls. Also we identified that Mxi1 regulates IFT20 promoter activity via Ets1 binding to IFT20 promoter.

Conclusions: In conclusion, these results suggest that Mxi1 has an effect on regulation of ciliogenesis through IFT20 expression in polycystic kidneys.

Funding: Government Support - Non-U.S.

FR-PO977

Development of Renal Cystic Cell Lines of Autosomal Dominant Polycystic Kidney Disease and Characterization through Microarray Ah-Young Kang,[1] Hayne C. Park,[2] Young-Hwan Hwang,[3] Insuk So,[4] Chang-seok Ki,[5] Curie Ahn.[1,2] *[1]Department of Immunology, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [3]Department of Internal Medicine, Eulji General Hospital, Seoul, Korea; [4]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea; [5]Department of Laboratory Medicine & Genetics, Samsung Medical Center, Seoul, Korea.*

Background: Research on the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD) has been hampered by the lack of well-characterized cyst-derived cell lines. Because each cyst from one patients is thought to have a different somatic mutation, tissue in whole is not an adequate tool to study the effect of various second hits. Therefore, developing various ADPKD cell lines with a different cystic origin is necessary to understand heterogenous nature of the disease.

Methods: We established 6 renal cyst epithelial cell lines from 5 ADPKD patients (one cell line each for 4 patients and 2 cell lines from different cyst origin in one patient) using SV40 viral DNA. *PKD 1* and *PKD2* sequencing was conducted in each cell line with its corresponding blood samples, and the cyst origin was confirmed by immunostaining with tubule markers of DBA and LTA. The cDNA microarray was performed to elucidate genetic expression profiles in ADPKD. Further, basal intracellular calcium levels were also measured.

Results: All of these cell lines were found to have *PKD1* gene defects with different germline and somatic mutations. Each cell line was originated from either proximal or distal tubule. The expression of genes associated in calcium-related pathways or calcium entry were altered in ADPKD. Both intracellular and ER storage calcium levels varied among different cell lines.

Conclusions: Our ADPKD cell lines developed from different cyst origins may facilitate the research in unraveling the pathogenesis of ADPKD. Further comprehensive analysis of microarray may provide a potential therapeutic target for ADPKD.

FR-PO978

Hepatocyte Nuclear Factor-1β Regulates Transcription of MicroRNA miR-200 Sachin S. Hajarnis, Massimo Attanasio, Karam S. Aboudehen, Vishal Patel, Peter Igarashi. *Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX.*

Background: The transcription factor hepatocyte nuclear factor-1β (HNF-1β) regulates tissue-specific gene expression in the kidney and other epithelial organs. HNF-1β is essential for normal kidney development, and mutations of HNF-1β produce kidney cysts by inhibiting the expression of cystic disease genes. We hypothesized that HNF-1β regulates the expression of miRNAs that are important in kidney development and PKD.

Methods: MicroRNA microarray analysis was performed on renal epithelial cells expressing dominant-negative mutant HNF-1β (DN-HNF-1β). Chromatin immunoprecipitation and DNA sequencing (ChIP-seq) were performed on mIMCD3 cells that express wild-type HNF-1β. Combining the results identified miRNAs that are directly regulated by HNF-1β. miRNAs were validated by ChIP, expression analysis, and functional studies. Target Scan and miRanda were used to identify candidate miRNA targets, which were validated by qRT-PCR and luciferase reporter assays.

Results: Microarray analysis and ChIP-seq identified members of the miR-200 family as candidate HNF-1β targets. miR-200 is enriched in the kidney and is down-regulated in cells expressing DN-HNF-1β. HNF-1β and active RNA polII bind to sites located 28 kb upstream to the miR-200 locus and stimulate transcription. mIMCD3 cells express miR-200 and form tubules when grown in collagen gels. Treatment of mIMCD3 cells with antagomirs that specifically target miR-200 disrupts tubulogenesis. Bioinformatic analysis identified two conserved miR-200 binding sites in the 3'UTR of *Pkd1*. Levels of *Pkd1* mRNA are increased in miR-200 antagomir-treated cells. miR-200a/b inhibit the expression of a luciferase reporter gene linked to the 3'UTR of *Pkd1*.

Conclusions: HNF-1β directly regulates the transcription of miR-200, a kidney-enriched miRNA family. miR-200 is required for renal tubulogenesis and post-transcriptionally regulates *Pkd1*. Since deficiency or overexpression of *Pkd1* causes kidney cysts, regulation by miR-200 may be important for maintaining renal tubular structure. These studies reveal a novel pathway by which HNF-1β controls the levels of mRNAs involved in cystogenesis.

Funding: NIDDK Support

FR-PO979

ErbB4 Deletion Accelerates Polycystic Kidney Disease Progression in Cys1^{cpk} Mice Fenghua Zeng, Tomoki Miyazawa, Raymond C. Harris. *Medicine, Vanderbilt University, Nashville, TN.*

Background: ErbB4 is a type I transmembrane protein that belongs to the EGF receptor family. There are four isoforms of ErbB4, of which JM-a/CYT-1 and JM-a/CYT-2 are expressed in kidney. In other systems JM-a/CYT-1 has been shown to promote cell differentiation while JM-a/CYT-2 increase cell proliferation. JM-a/CYT-1 is mainly expressed in the renal cortex, while JM-a/CYT-2, the predominant isoform of ErbB4 in kidney, is localized to renal medulla. Our previous studies have shown that JM-a/CYT-2 promotes renal epithelial cell tubulogenesis in vitro, but its function during renal disease is unknown.

Methods: We generated Cys1^{cpk} mice carrying a heart-rescued ErbB4 deletion (ErbB4^{heart}/Cys1^{cpk}) by crossing Cys1^{cpk}, an ARPKD mouse model, with ErbB4^{heart} mice.

Results: ErbB4 is highly expressed in the cyst lining cells of the kidneys of Cys1^{cpk} mice. In ErbB4^{heart}/Cys1^{cpk} mice, accelerated cyst progression and renal function decline were noted as early as 10 days postnatally, as indicated by larger cystic kidney size, higher kidney weight to body weight ratio and elevated BUN level compared to Cys1^{cpk} mice. At this age, no notable renal abnormality can be detected in ErbB4^{heart} mice, although dilated tubes can be seen later in adult ErbB4^{heart} mice. Cell proliferation, assessed by Ki67 immunostaining, showed that Ki67 immunoreactivity was predominately seen in the cortex of the cystic kidneys in both ErbB4^{heart}/Cys1^{cpk} and Cys1^{cpk} mice, with the ratio of Ki67 positive cells to total cyst-lining epithelial cell (TCEC) significantly higher in ErbB4^{heart}/Cys1^{cpk} mice compared to Cys1^{cpk} mice (18.56 ± 2.03 vs. 7.35 ± 0.82, $p < 0.01$). On the other hand, TUNEL staining localized apoptotic cells mainly to the renal medulla. TUNEL positive cell to TCEC ratio was again higher in ErbB4^{heart}/Cys1^{cpk} mice compared to Cys1^{cpk} mice (37.2 ± 4.57 vs. 5.01 ± 1.28, $p < 0.001$).

Conclusions: Our results indicate that ErbB4 deletion in Cys1^{cpk} mice can induce abnormal cell proliferation and increase cell apoptosis, resulting in accelerated cyst formation and earlier renal function deterioration and suggest that ErbB4 may exert cytoprotective and antiproliferative effects in renal epithelia.

Funding: NIDDK Support

FR-PO980

Characterisation of a New Mouse Model of Polycystic Kidney Disease Brigitte Lelongt,[1] Zeineb Bakey,[1] Laure Delestre,[2] Marie-thérèse Bihoreau,[3] Pierre M. Ronco,[1] Dominique Gauguier.[2] *[1]INSERM UMR_S 702, Paris, France; [2]INSERM UMR_S 872, Paris, France; [3]Centre National de Génotypage, Evry, France.*

Background: We have demonstrated that a missense mutation in the SAM domain of Anks6 induces renal cysts in the cy/+ rat. Homozygous rats died at 4 weeks. Heterozygous rats displayed a slow progression of the disease leading to death after 1 year. Approximately 75% of the cysts derived from the proximal tubule. Cysts in the liver and pancreas were also observed in about half of old-affected females.

The objective of this study is to further investigate the role of Anks6 in the pathogenesis of PKD by using a mouse model.

Methods: We identified in a library of ENU treated mice a mouse carrying a mutation in the SAM domain of Anks6, six amino acid away from the PKD-causative mutation in the cy/+ rat strain. We rederived the mouse, transferred the mutation on a C3H background by 7 successive backcross breedings and analysed the mutant mouse phenotype.

Results: Anks6 was detected in cilia of normal and cystic tubules. A very slow progression of the disease is observed in homozygous mutant mice which die after 16 months. Mice heterozygous for the mutation do not display any cysts. Cysts are detected in glomeruli and also in different nephron segments in cortex, medullary and papilla. Immunohistochemical markers showed that cysts derive from collecting ducts and thick ascending limb of Henle's loop, whereas only few cysts were observed in proximal tubules. We could not detect cysts in other organs, such as liver and pancreas. No differences in cyst origin, cyst size and cyst number were noticed between males and females.

Conclusions: This new mouse model provides unambiguous evidence of the role of Anks6 mutations in PKD and opportunities to investigate the *in vivo* effects of Anks6 mutation in combination with other PKD-causative mutations in mice. Discrepancies in phenotypes and inheritance mode between the cy/+ rat and the ENU mouse strongly suggest that the mutations in the SAM domain of Anks6, although very close, disrupt sites of interactions to different Anks6 protein partners in the two species and that these different partners are involved in cyst formation.

Funding: Government Support - Non-U.S.

FR-PO981

Roles of N-Terminal Cysteine 38 in the Dimerization and Channel Function of PKD2L1 Wang Zheng, Jungwoo Yang, Xing-Zhen Chen. *Physiology, University of Alberta, Edmonton, AB, Canada.*

Background: Polycystic kidney disease 2-like 1 (PKD2L1) is a homologue of PKD2 but is not related to ADPKD. It is a Ca-activated non-selective cation channels and is present in multiple tissues including retina, tongue, brain and kidney. It acts as part of an acid sensor in the tongue. It was reported that the N-terminal domain D21-S42 is important for the channel activity and that the C-terminus trimerizes in vitro. Here we studied residues that are important for PKD2L1 oligomerization and channel function.

Methods: Mutant and WT PKD2L1 channel function was measured with two-electrode voltage clamp to *Xenopus oocytes*. PKD2L1 Oligomerization was examined with non-reducing SDS-PAGE, and co-IP between GFP- and Flag-tagged PKD2L1.

Results: We examined truncation mutants Δ1-36 (lacking aa M1-R36) and Δ1-38 by electrophysiology in oocytes and found that Δ1-36 exhibits similar function to WT PKD2L1 while Δ1-38 loses channel function. We examined mutants V37A, C38A and T39A, and found that while V37A and T39A have similar function to WT, C38A exhibits very low activity, suggesting the importance of C38 for channel activity. We then examined whether C38 affects PKD2L1 oligomerization. Using PKD2L1 over-expression in oocytes and HeLa cells, and native mouse tissues under non-reducing SDS-PAGE conditions, we observed oligomer bands. Mutation C38A or addition of 2ME (or DTT) substantially reduced oligomerization, suggesting that C38 is part of a disulfide bond critical for PKD2L1 dimerization. Using co-IP between GFP- and Flag-tagged PKD2L1 we found that the WT-C38A interaction is much weaker than the WT-WT interaction. Mutations to the other cysteine residues outside transmembrane spans do not affect channel function. Of note, C38-mediated dimerization is insufficient to account for tetramerization. Interestingly, the N-terminus truncation mutant Δ1-95 did not oligomerize. We are identifying N-terminal residues involved in dimerization via peptide-peptide interaction.

Conclusions: Two PKD2L1 proteins dimerize through a disulfide bond formed by their C38, which is necessary for its channel function. There may exist N-terminal residues involved in dimerization via peptide-peptide interaction.

Funding: Government Support - Non-U.S.

FR-PO982

Modelling Genetic and Environmental Synergy in Metanephric Cystogenesis Corina Anders,[1] Nick Ashton,[2] Mark R. Dilworth,[1] Adrian S. Woolf.[1] *[1]School of Biomedicine, University of Manchester, Manchester, United Kingdom; [2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.*

Background: Cyclic adenosine monophosphate (cAMP) drives cyst growth in polycystic kidney diseases (PKD) and, in autosomal dominant (AD) and recessive PKD, kidney cystogenesis initiates antenatally. In some PKD families, differences in kidney disease severity exist between affected individuals, and genomic and/or environmental modifying factors have been evoked to explain such observations. We hypothesized that PKD cystogenesis is accentuated by an aberrant fetal milieu, specifically by exposure to glucocorticoids, molecules mediating developmental programming of postnatal diseases.

Methods: Cyst formation was assessed in explanted wild-type mouse embryonic day 13 metanephroi, using 8-Br-cAMP as a chemical model for genetic cystogenesis, and the glucocorticoid dexamethasone as an environmental modulator. On days 3 and 6, cyst areas and numbers were quantified by an observer blinded to culture conditions. Tubules were phenotyped with megalin, aquaporin-1, uromodulin and calbindin-28 antibodies and *Dolichhos biflorus* agglutinin. Glomeruli were counted by stereology.

Results: Dexmethasone, over a physiological to pathological glucocorticoid concentration range, significantly synergized with cAMP increasing cytogenesis. We defined concentrations of each molecule which, on their own, were not cystogenic; when applied together at these same concentrations, however, cysts began to form in embryonic kidneys. Notably, cAMP or dexamethasone alone generated cysts arising in both differentiating proximal tubules and descending limbs of loops of Henle. When these cystogens were applied together, however, a striking glomerulocystic phenotype occurred and we note that glomerular cysts have been reported in histology from fetuses and young children with ADPKD. Glomerular numbers were similar in all culture conditions.

Conclusions: These results provide evidence for concerted cystogenic actions of genetic (i.e. cAMP activity in PKD) and environmental (i.e. glucocorticoid activity in developmental programming) factors. The data support the idea that an adverse antenatal environment exacerbates genetic cystic disease.

FR-PO983

Constitutive Activation of NF-κB Causes Tubule Enlargement and Cyst Formation in Cultured Mammalian Collecting Duct Cells Liping Sun,[1] Xinzhou Zhang,[1] Shixuan Wang,[2] Wanfan Zhang.[1] *[1]Division of Nephrology, Shenzhen People's Hospital, Shenzhen, Guangdong, China; [2]Renal Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Background: Polycystic kidney disease (PKD) is characterized by progressive enlargement of renal cysts. We have previously demonstrated an increase in NF-κB in fibrocystin-decreased kidney cells.The aim of the present study was to determine the effect of NF-κB signaling on cyst formation, tubular cell apoptosis and proliferation in the mCCD cells.

Methods: We designed an *in vitro* model of tubulogenesis in mCCD cells.To assess whether the activation of the NF-κB signaling pathway can induce the enlargement of established tubular structures, mCCD cells were treatment with TNF-α. NF-κB activation was determined by measuring IκB degradation and phosphorylation, NF-κB p65 nuclear levels and EMSA.Subsequent addition of caspase-3 inhibitor to test that TNF-α-induced tubule enlargement was mediated through the activation of caspase-3.Lumen diameter was measured using an inverted photomicroscope. PCNAstaining was performedfor proliferation, TUNEL methodand flow cytometry was used to detectapoptotic cells.

Results: Selective activation of NF-κB pathway using TNF-α resulted in progressive dilatation of existing tubules, leading to the formation of cyst-like structures. The caspase-3 inhibitor reduced tubular apoptosis and proliferation and prevented tubule enlargement and cyst formation.

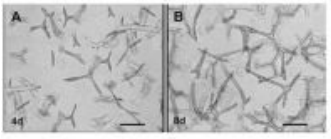


figure1. mCCD cells form well-organized tubular structures

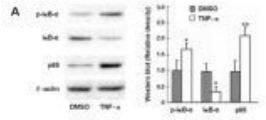


figure2. analysis of NF-κB activity after TNF-α treated

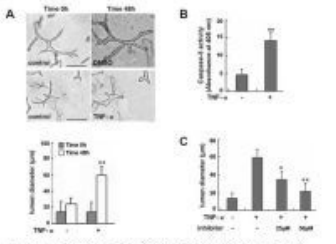


figure3. activation of the NF-κB pathway induces cystic dilatation of existing tubules

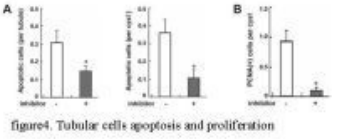


figure4. Tubular cells apoptosis and proliferation

Conclusions: We conclude that NF-κB signaling plays a key role in renal cyst formation, at least in part by inhibiting caspase-3 activity. These observations provide a potential platform for the future treatment of the renal manifestations of PKD.

Funding: Government Support - Non-U.S.

FR-PO984

TRPV4 Participates in Primary Cilia-Mediated Osmosensation of Renal Epithelial Cells Bradley P. Dixon, Brian J. Siroky, John J. Bissler. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Primary cilia detect extracellular mechanical and chemical stimuli and are located on the surface of nearly all cells. Defects in primary cilia are associated with cystic kidney diseases and some forms of cancer. Signals transduced by the primary cilium affect cell proliferation, differentiation, migration and apoptosis. The mechanism by which cilia sense changes in osmolality is not well understood. The transient receptor potential vanilloid channel TRPV4 localizes to the primary cilium in hepatic duct cells, and appears to sense changes in osmolality. It is unknown whether TRPV4 participates in the osmosensation of the primary cilium of renal epithelial cells. We assessed the expression and function of TRPV4 in renal epithelial cells. Specifically, we assayed for a role for TRPV4 in sensing osmolality.

Methods: 176-5 renal epithelial cells that contain a floxed *Kif3a* (a kinesin motor required for ciliogenesis) and a tamoxifen-inducible Cre recombinase were cultured with tamoxifen to develop conditionally deleted *Kif3a* (176-5Δ cells). TRPV4 expression was assessed by RT-PCR and immunofluorescence. Both the 176-5 and 176-5Δ cell lines were gradually adapted to 600mOsm/kg with NaCl or urea, or maintained under control conditions, either in the presence or absence of a TRPV4 antagonist, HC-067047 100nM. Cell cycle distribution was assessed by flow cytometry. Cell proliferation was measured by crystal violet viability assay.

Results: 176-5Δ cells continued to proliferate in spite of hyperosmolal adaptation, whereas 176-5 cells arrested cell proliferation upon adaptation to hyperosmolal NaCl or urea. However, blockade of TRPV4 activity with HC-06747 caused an increase in S phase of 176-5 cells in hyperosmolal NaCl, but had no effect on cell cycle distribution in cilia-deficient 176-5Δ cells.

Conclusions: These results indicate that TRPV4 is expressed in renal epithelial cells, and may participate in osmosensation directed by cilia. Further studies are needed to determine the cellular localization of TRPV4 and the mechanism by which TRPV4 influences the response to hyperosmolality.

Funding: NIDDK Support

FR-PO985

Renal Chymase, but Not Angiotensin-Converting Enzyme, Is Upregulated in Polycystic Kidney Disease Mice Michifumi Yamashita,[1,2] Saurabh Chattopadhyay,[2] Ganes C. Sen.[2] *[1]Pathology, University Hospitals Case Medical Center, Cleveland, OH; [2]Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.*

Background: Polycystic kidney disease (PKD) is a common genetic disorder, which affects around 6 million worldwide. Renin Angiotensin System (RAS) has been implicated in cyst growth and hypertension in PKD patients. Now a large-scale clinical trial (HALT-PKD) is ongoing to test the effects of RAS blockades on the cyst growth of ADPKD. However, the precise mechanism has not been studied in detail. We have used Juvenile Cystic Kidney (*jck*) mice, a murine model of PKD, to analyze RAS in PKD.

Methods: *Jck* mice (female, 100 days old) and age-matched wild type (WT) mice were used to test blood pressure by non-invasive tail cuff method. Critical components of RAS, Angiotensin Converting Enzyme (ACE), ACE2 and chymase levels were analyzed in the sera and the kidney sections. Serum ACE activity and Angiotensin II (Ang II) level in plasma and kidney were measured by colorimetric assay based on Hip-His-Leu cleavage and radio-immune assay (RIA), respectively. Urine ACE was measured by murine ACE ELISA, and normalized by urinary creatinine level.

Results: *Jck* mice exhibited higher blood pressure as compared to its WT control mice. However, *jck* mice showed reduced serum ACE activity and plasma Ang II level. On the other hand, kidney Ang II was significantly higher in *jck* mice than WT mice. Surprisingly, renal ACE and ACE2 levels as well as urinary ACE in *jck* mice were significantly reduced than WT mice. Furthermore, renal chymase level was significantly increased in the interstitial area and the cystic wall in *jck* mcie than WT mice.

Conclusions: Our study clearly showed that in *jck* mice, systemic RAS is suppressed, and renal RAS is upregulated by chymase, with significant reductions of renal ACE and ACE2. In view of the fact that ARB and/or ACE inhibitor have clinical roles in PKD patients, it will be interesting to test the chymase inhibitors in future clinical studies for controlling the renal RAS activity.

Funding: Other NIH Support - HL048258

FR-PO986

Kidney Injury Molecule-1 (Kim-1) Is Specifically Expressed in Cystically Transformed Proximal Tubules of the PKD/Mhm (cy/+) Rat Model for Polycystic Kidney Disease Nicholas Obermueller,[1] Juergen Engel,[1] Stephanie Schlitt,[1] Sigrid C. Hoffmann,[2] Bettina Kraenzlin,[2] Norbert Gretz,[2] Helmut Geiger,[1] Stefan Gauer.[1] *[1]Division of Nephrology, III. Medical Clinic, University Hospital, University of Frankfurt, Frankfurt, Germany; [2]Medical Research Center, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.*

Background: Kidney injury molecule-1 (Kim-1) is rapidly expressed after acute tubular injury and is considered as a biomarker for kidney damage. We examined the localization of renal Kim-1 in the PKD/Mhm(cy/+) rat, an established model for autosomal dominant polycystic kidney disease.

Methods: Male adult heterozygous (cy/+) rats as well as (+/+) littermates were perfusion-fixed with PFA, after the left kidney had been removed and snap frozen. The right kidney was paraffin embedded and Kim-1 expression was determined by immunohistochemistry (IHC) using a polyclonal antibody, and by western blot analysis.

Results: By IHC Kim-1 was not detectable in wildtyp (+/+) kidneys, whereas a robust de novo expression could be observed solely in cystically transformed proximal tubules of varying size. In detail, Kim-1 was distributed mostly apically in dedifferentiated cystic epithelia, e.g. with an already reduced or even a completely lost brush border. Distal tubules and collecting ducts, as double-labeled with calbindin did not show any expression of Kim-1. In contrast to vimentin and osteopontin (other markers of tubular damage and dedifferentiation) Kim-1 was strictly confined to structures of proximal tubular origin; in cysts, the staining of the three markers did only partially overlap as shown in serial sections. De novo protein expression of Kim-1 in (cy/+) kidneys was corroborated by western blotting.

Conclusions: Our data show upregulation of Kim-1 in the PKD/Mhm(cy/+) model, specifically in the diseased proximal tubular segment, contrasting to other molecules indicating renal injury (e.g. osteopontin), which are also expressed in distal nephron segments in this model. This may indicate, that Kim-1 represents not only a pure marker of tubular injury, but may be causally involved in the events during de-differentiation, cyst formation, and fibrotic remodeling.

Funding: Clinical Revenue Support

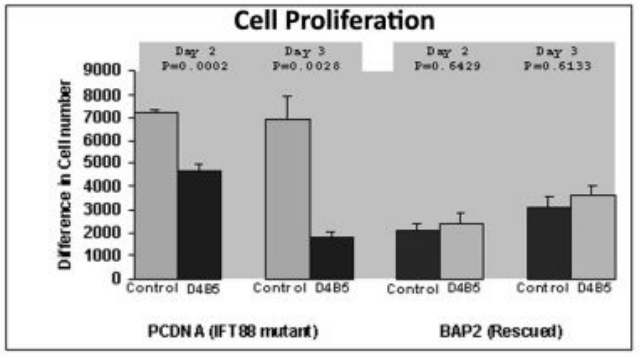
FR-PO987

Aberrant Expression of Laminin-332 in ARPKD Contributes to Cystogenesis by Altering Principal Cell Proliferation Soundarapandian Vijayakumar,[1] Tricia M. Nienaber,[1] Matt Peter Marinkovich,[4] Bradley K. Yoder,[2] Vicente E. Torres.[3] *[1]Pediatrics, University of Rochester, Rochester, NY; [2]University of Alabama, Birmingham, AL; [3]Mayo Clinic, Rochester, MN; [4]Stanford University, Stanford, CA.*

Background: A hypomorphic mutation in the mouse laminin α5 gene causes polycystic kidney disease associated with aberrant accumulation of laminin-332 (α3β3γ2) (JASN17:1913, 2006). Laminin-332 promotes proliferation of ADPKD cells and cystogenesis in 3D culture (JBC 281:29181, 2006). Here we examine whether aberrant ECM assembly plays a role in ARPKD cystogenesis (PCK rat and orpk cell lines).

Methods: Real-time PCR using Taqman chemistry was performed on cDNA from Postnatal (PN) day 2, 7, 10, 21, 30 and 40 and 3 month PCK and SD rat kidneys with specific primers to laminin-α3, β3, γ2, integrin β4, collagen XVII and SRY genes. Frozen sections from PCK kidneys were stained with a rabbit polyclonal to laminin-332. Celltiter 96 Aqueous One Solution reagent was used to perform cell proliferation assays for up to three days.

Results: qPCR show significant upregulation of laminin-332 chains and integrin β4 (10 fold increase in laminin-γ2, 8 fold increase in integrin β4) in 3 month PCK compared to SD kidneys. A 3.4 fold increase in laminin-γ2 was observed as early as PN40. Immunohistochemistry shows the presence of ECM laminin-332 not only in the cysts but also in precystic collecting ducts of PCK but not SD rats. A four fold increase in laminin-β3 and a 3-fold increase in integrin β4 were observed in orpk mutant cell lines compared to rescued cell lines. Mutant cells showed increased proliferation (7000 cells vs 3000 cells) which was inhibited by laminin-332 function blocking antibody D4B5. D4B5 had no effect on the proliferation of rescued cells.



Conclusions: Aberrant laminin-332 expression in ARPKD contributes to cystogenesis by inducing abnormal proliferation of collecting duct principal cells.

FR-PO988

Possible Contribution of Fibrocytes to Renal Fibrosis in Cpk Mouse, a Model of ARPKD Taketsugu Hama,[1] Koichi Nakanishi,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Yuko Shima,[1] Masayasu Miyajima,[2] Hisahide Takahashi,[3] Shizuko Nagao,[3] Kazumoto Iijima,[4] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical University, Wakayama, Japan; [2]Laboratory Animal Center, Wakayama Medical University, Wakayama, Japan; [3]Education and Research Center of Animal Model for Human Disease, Fujita Health University, Toyoake, Aichi, Japan; [4]Pediatrics, Kobe University, Kobe, Hyogo, Japan.*

Background: The pathophysiology of cystic epithelia in PKD is characterized by altered proliferative activity, a secretory rather than absorptive function, and an abnormal matrix microenvironment. However, the aspect of extracellular matrix abnormality, especially fibrosis, has not been fully investigated in PKD. Previously, we demonstrated epithelial-to-mesenchymal transition (EMT)-like phenotype in *cpk* mouse, a rapid progressive ARPKD model (JASN 21:520A,2010, 22:576A,2011). Although EMT is thought to be one of key features in PKD fibrosis, it seems to have only a partial role for fibrosis. Recently, circulating fibrocytes expressing both leukocyte (CD45) and mesenchymal (collagen type I) antigens have been clarified to have a key role of progressing fibrosis in any organ and they have the possibility of a new target for therapy. Therefore, we investigate fibrocytes contribution in *cpk* mouse to explore another candidate of fibrosis promoter.

Methods: Kidneys from 5 male *cpk* and control mice (each on day 0, 7, 14, 21) were harvested, and stained with anti-collagen type I antibody and Masson's trichrome stain to evaluate fibrosis. We also performed real-time PCR for collagen type I. We conducted a double-color immunofluorescence analysis using both anti-CD45 and anti-procollagen type I antibodies to identify fibrocytes in the kidney of *cpk* and control mice.

Results: Collagen type I expression area and gene level increased significantly according to age in *cpk* mice (1.4 ± 0.7, 1.8 ± 0.6, 2.9 ± 0.8, 4.3 ± 1.4 [mean ± SD]; relative to each control on day 0, 7, 14, 21), and dual-positive cells were detected predominantly in *cpk* mice.

Conclusions: These findings suggested that fibrocytes were recruited in *cpk* and participated in the progression of fibrosis. Therefore, they may be new targets for a disease-specific intervention.

Funding: Government Support - Non-U.S.

FR-PO989

RNA SEQ Validates the Transcriptional Complexity of *Pkhd1* Ravindra Boddu,[1] Gregory G. Germino,[2] Luiz F. Onuchic,[3] Lisa M. Guay-Woodford.[4] *[1]UAB, Birmingham, AL; [2]NIDDK/NIH, Bethesda, MD; [3]Univ Sao Paulo, Brazil; [4]CNMC, Washington, DC.*

Background: We previously analyzed a kidney-specific plasmid library generated from *Pkhd1* exon 1 and 67-containing amplicons and demonstrated that this mouse ARPKD gene orthologue is transcriptionally complex. Bioinformatic analyses and minigene experiments indicated that exon-splice enhancer (ESE) motifs modulate alternative exon usage. In the current study, we sought to use RNA-Seq strategies to validate the predicted splice variants and evaluate whether human missense variants that disrupt ESE motifs impact *PKHD1* splicing.

Methods: We initially applied standard RNA-Seq to WT kidney total RNA and detected all canonical *Pkhd1* exon junctions, as well as several novel junctions, though the read number for the latter was below the significance threshold. We then developed a novel, targeted strategy to specifically enrich IMCD cell-derived RNA for *Pkhd1* transcripts encoded by approximately 500 kb genomic sequence.

Results: The targeted RNA Seq strategy identified all canonical *Pkhd1* exon junctions, as well as the novel junctions captured in our amplicon catalogue. In addition, this unbiased approach identified novel exon junctions not represented in our catalogue. All novel junctions were expressed at levels at least 100-fold lower than the canonical junctions. These data are consistent with a recent report demonstrating low expression of alternative *Pkhd1* isoforms versus the full-length RNA (Ward *et al*, 2011). Given our evidence for alternative *Pkhd1* splicing, we evaluated 313 publically available missense mutations (http://www.humgen.rwth-aachen.de), as well as those reported to the UAB Hepatorenal Fibrocystic Disease Core Center. Many variants were predicted to disrupt one or more ESE motifs and minigene assays demonstrated that the R760H variant altered *PKHD1* splicing.

Conclusions: We conclude that *Pkhd1/PKHD1* is transcriptionally complex; transcriptional processing is modulated in part by ESEs; and alternative isoforms are expressed at significantly lower levels than the full-length RNA. We propose that mutational disruption of *PKHD1* ESEs may represent an under-appreciated pathogenic mechanism in ARPKD.

Funding: NIDDK Support

FR-PO990

A Singe Mutated Inv Gene Can Modify Murine pcy Nephronophthisis but Not cpk ARPKD Vincent H. Gattone,[1] Alexander J. Carr,[1] Robert L. Bacallao.[2] *[1]Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN; [2]Nephrology Division, Dept of Medicine, Indiana University School of Medicine, Indianapolis, IN.*

Background: Modifier gene loci for PKD were found at chromosomal locations known to harbor other cystic disease genes. In *cpk, jck, bpk* and *pcy* models, a modifier loci was found on chromosome 4 in the general area known to harbor the *inv* and *mks3* genes. We hypothesized that a single mutant *inv* gene (nphp2) would influence the severity of the cystic pathology caused by the *pcy/nphp3* gene. There are known interactions in cultured cells between nephronophthisis protein products, *inv* with *nphp3* and *nphp9* (Cytoskel. 67:112-119,2010). The *inv* locus is thought to play an important role in ciliogenesis, a central defect in PKD. We tested whether such an interaction between *inv* and *nphp3* would cause an alteration in severity of the cystic phenotype *in vivo*.

Methods: FVB-*inv* mutant mice (*NPHP2*) were crossed with CD1-*pcy* mice (*NPHP3*) and the renal cystic pathology of resulting *pcy/pcy* mice that were *inv/+ or +/+* were compared. Similarly, *inv* mice were crossed with Balb/c-*cpk* mice with ARPKD and renal / biliary pathology assessed. *pcy/pcy* mice were evaluated at 4 weeks of age while *cpk/cpk* mice studied at 10 and 17 days of age.

Results: Non-pcy-cystic *inv/+* and *+/+* were not different suggesting minimal strain background influence on results. However, a single mutant *inv* gene made *pcy/pcy* renal cystic pathology worse at 4 weeks of age. The modifier loci for *cpk* severity on chromosome 4 was described as *kif12* (JASN 16:905-916, 2005). Unlike the *pcy* results, *inv* did not influence the severity of *cpk* ARPKD at either 10 or 17 days of age.

	Body Weight	Kidney Weight	KW%BW	Heart Wgt	Heart % BW
inv/+-pcy/pcy (30)	14.91± 0.42	0.673± 0.046	4.48 ± 0.26	0.098 ± 0.004	0.662 ± 0.026
+/+-pcy/pcy (7)	15.12 ± 0.91	0.460 ± 0.019	2.65 ± 0.11	0.089 ± 0.004	0.511% ± 0.051
	Body Weight	Kidney Weight	KW%BW	Bile Duct Diam.	Bile Duct/BW
inv/+-cpk/ cpk (8)	5.01 ± 0.33	0.333 ± 0.030	6.86 ± 0.70	2.46 ± 0.41	0.51 ± 0.12
+/+-cpk/cpk (3)	5.69 ± 0.47	0.420 ± 0.076	7.39 ± 1.28	2.90 ± 0.42	0.53 ± 0.10

Conclusions: A single mutant nphp gene can act as a modifier gene and alter disease severity, but this may not always be the case.

Funding: Clinical Revenue Support

FR-PO991

SDCCAG8 Interacts with RABEP2 and Modulates Sonic Hedgehog Signaling Rannar Airik,[1] Jens S. Andersen,[2] Friedhelm Hildebrandt.[1,3] *[1]Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; [3]Departments of Pediatrics and of Human Genetics, Howard Hughes Medical Institute, Ann Arbor, MI.*

Background: *SDCCAG8/NPHP10* is a novel ciliopathy gene, which if mutated, causes nephronophthisis-related ciliopathy (NPHP-RC), characterized by cystic kidneys, retinal degeneration and mild mental retardation in humans (1). We have recently generated a knock-out mouse model of *Sdccag8,* that recapitulates the human ciliopathy phenotypes.

Methods: In order to study the molecular defects in *Sdccag8*-deficient cells we isolated mouse embryonic fibroblasts (MEFs) from wild type and *Sdccag8*$^{-/-}$ embryos. Confluent MEFs were serum starved to induce ciliogenesis and treated with Sonic hedgehog (Shh) pathway agonist SAG to activate the pathway. qRT-PCR was used to analyze the changes in SHH target genes. To identify novel SDCCAG8 protein interaction partners SILAC-assay was performed in collaboration with J.A.

Results: While 80% of wild type cells were ciliated upon serum starvation, only 10% of *Sdccag8*$^{-/-}$ MEFs grew cilia. Moreover, *Sdccag8*$^{-/-}$ MEFs demonstrated very low mRNA levels of Patched1, Gli1 and Gli3 indicating defects in Shh pathway activation. This finding is corroborated by the presence of polydactyly phenotype in 80% *Sdccag8*$^{-/-}$ mice – a common phenotype of dysregulated Shh signalling in the developing limb bud. SILAC-assay identified RABEP2 as a novel interaction partner of SDCCAG8. RABEP2 has been previously implicated in promoting ciliogenesis (2). The protein interaction of SDCCAG8 and RABEP2 was confirmed by immunoprecipitation experiments.

Conclusions: Sdccag8 is required for ciliogenesis and Shh pathway activation. Our data indicate that defects in ciliogenesis and Shh pathway activation may explain some of the ciliopathy phenotypes in *Sdccag8*$^{-/-}$ mice. SDCCAG8 interaction with RABEP2 implicates SDCCAG8 in endosomal trafficking. Further understanding the role of *Sdccag8* in causing NPHP-RC will provide valuable insight into the pathogenetic mechanisms of ciliopathies.

(1) Otto et al., Nat Genet 42:840-850, 2010 (2) Kim et al., Nature 1048–1051, 2010.

Funding: NIDDK Support

FR-PO992

Loss of Function of Ruvbl1, a Regulator of DNA Damage Response, Causes Pronephric Cysts in Zebrafish Embryos Weibin Zhou,[1] Friedhelm Hildebrandt.[1,2,3] *[1]Pediatrics, University of Michigan, Ann Arbor, MI; [2]Human Genetics, University of Michigan, Ann Arbor, MI; [3]Howard Hughes Medical Institute.*

Background: Genotoxicity and DNA Damage Response (DDR) have been implicated in a variety of human diseases. Recent identification of mutations in proteins (CEP164 and FAN1) involved in DDR as the causes of nephronophthisis (1) and karyomegalic nephritis (2), suggests that defects in DDR may be a cause for renal cystic kidney diseases. ruvbl1 is a protein essential for DNA mismatch repair. It regulates DNA damage- induced histone 2X phosphorylation. We and others (3) found that zebrafish *ruvbl1* mutants displayed pronephric cysts and other ciliopathy-like phenotypes (such as hydrocephalus and retinal degeneration).

Methods: In order to understand the pathogenic mechanism of DDR defects in kidney diseases and test therapeutical compounds, we characterized a zebrafish cystic mutant model for *ruvbl1*.

Results: However,*ruvbl1* mutants had normal cilia in pronephros while the cysts developed. These fish mutant also showed an elevated gH2X and massive neuronal degeneration, indicating DDR defects. Interestingly, rapamycin treatment, which has been shown to protect cells from irradiation-induced DDR, rescued pronephric cysts in ruvbl1 mutants.

Conclusions: Our results confirm that DDR defects are linked to ciliopathy-like phenotypes and suggests that modulators of DDR may also be used to reduce renal cysts. Further study of the connection of DDR and cilium function is warranted to uncover the disease mechanism of "ciliopathy".

Funding: NIDDK Support, Other NIH Support - Howard Hughes Medical Institute

FR-PO993

Inactivation of the Epigenetic Factor Reptin Causes Kidney Cyst in Zebrafish Lu Zhao,[1] Shiaulou Yuan,[1] Ying Cao,[2] Zhaoxia Sun.[1] *[1]Yale University, New Haven, CT; [2]Tongji University, Shanghai, China.*

Background: The critical role of the cilium, a cell surface sensory organelle, in polycystic kidney diseases has been well appreciated in recent years. However, how ciliary defects lead to cyst formation remains unclear. In this study, we present evidence that inactivation of the AAA+ ATPase Reptin (also known as RuvbL2/TIP48), a conserved protein component in multiple chromatin-remodeling complexes, causes kidney cysts in zebrafish.

Methods: We show that zebrafish embryos with a proviral insertion in *reptin* (*reptin*^{hi2394} mutants) develop typical cilia associated phenotypes, including kidney cysts and ventrally body curvature. These phenotypes are reproduced in wildtype embryos injected with a morpholino against *reptin*. In addition, both mutants and morphants can be significantly rescued by expressing *reptin* mRNA.We further investigated potential functional interactions between Reptin and cilia. Genetic interaction studies suggest that *reptin* functions in the same genetic pathway as *ift172*, a gene essential for cilia biogenesis. Interestingly, unlike IFT mutants, *reptin*^{hi2394} mutant has no observable defect in cilia generation, maintenance, or size.

Results: Taken together, these results suggest that inactivation of Reptin can lead to kidney cyst formation. However, although *reptin* functions in the same genetic pathway with *ift172*, *reptin*^{hi2394} mutant has no observable ciliary morphologic defects.

Conclusions: In summary, our data suggest that loss of Reptin induces kidney cysts formation through a cilia related mechanism, although not through cilia biogenesis. Since Reptin has a well-defined role in epigenetic control through its interaction with various transcription factors, it will be interesting to investigate how Reptin inactivation leads to kidney cysts formation, and whether epigenetic regulation is involved in the cilia-kidney cysts model.

FR-PO994

The Novel Joubert Gene KIF7 Regulates Microtubular Dynamics and Cellular Polarity Max C. Liebau,[1,2] Claudia Dafinger,[2,3] Thomas Benzing,[2] Hanno Jörn Bolz,[3,4] Bernhard Schermer.[2] *[1]Department of Pediatrics, University Hospital of Cologne, Cologne, Germany; [2]Department of Internal Medicine II, Renal Division, University Hospital of Cologne, Cologne, Germany; [3]Institue of Human Genetics, University Hospital of Cologne, Cologne, Germany; [4]Bioscientia Center for Human Genetics, Ingelheim, Germany.*

Background: Joubert syndrome (JBTS) is characterized by a specific brain malformation with various possible additional pathologies including a nephronophthisis-like renal phenotype. It results from mutations in various different genes. JBTS has been linked to dysfunction of primary cilia since the gene products localize to this ancient organelle.

Methods: We recently identified a novel disease locus, *JBTS12*, with mutations in the *KIF7* gene in a consanguineous JBTS family by linkage analysis. Cellular KIF7 function was now assessed by multiple approaches.

Results: KIF7 regulates the structure of cilia, centrosomes and the Golgi network most likely by control of cytoplasmic tubulin acetylation and microtubular dynamics. Novel studies point to a role of KIF7 in the regulation of cellular polarity and cellular migration. KIF7 can act as a kinesin in intracellular trafficking and interacts with end-binding protein 1 indicating a potential role of KIF7 at microtubular plus ends. Imaging studies are compatible with these findings and confirmative for a function of KIF7 at the cilium and at the ciliary base.

Conclusions: The data suggest a regulation of cytoplasmic cellular microtubules through a mostly ciliary protein.

FR-PO995

RuvBl1 Is a Novel Nephrocystin-1 Interactor and Is Required for Tubular Cell Survival Max C. Liebau,[1,2] Claudia Dafinger,[2] Sandra Habbig,[1,2] Oliver Rinner,[3] Ruedi Aebersold,[3] Thomas Benzing,[2] Bernhard Schermer.[2] *[1]Department of Pediatrics, University Hospital of Cologne, Cologne, Germany; [2]Department of Internal Medicine II, Renal Division, University Hospital of Cologne, Cologne, Germany; [3]Institute of Molecular Systems Biology, Swiss Federal Institute of Technology, Zurich, Switzerland.*

Background: Nephronophthisis is the most common genetic cause of end stage renal failure during childhood and adolescence. Mutations in *NPHP1* encoding the ciliary protein nephrocystin-1 (NPHP1) are a frequent cause of nephronophthisis. However, the cellular NPHP1 protein function is poorly understood.

Methods: In a recently described immunoprecipitation-based gel-free mass spectrometry approach candidates for NPHP1-interacting proteins were identified. Interactions were confirmed with independent methods. For various interactors cellular function was studied in vitro and *in vivo*.

Results: We identified novel candidate proteins and known nephrocystins. One of the confirmed novel interactors was RuvBl1, an AAA ATPase with known roles in the regulation of WNT signalling and DNA damage response signalling. Knockdown of the RuvBl1 homolog in zebrafish leads to cystic kidneys. To further study renal RuvBl1 function *in vivo*, we generated a tubule-specific *Ruvbl1*-knockout mouse. Tubule-specific *Ruvbl1*-deficiency led to neonatal death and tubular cell degradation.

Conclusions: The data support the recently established link between ciliary proteins and members of the DNA-damage response signalling pathway.

Funding: Government Support - Non-U.S.

FR-PO996

Wtip and Vangl2 Are Required for Mitotic Spindle Orientation and Cloaca Morphogenesis Tomoko Obara. *Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK.*

Background: Defects in cilia and basal bodies function are linked to ciliopathies, which result in kidney cyst formation. Recently, cell division defects have been observed in cystic kidneys, but the underlying mechanisms of such defects remain unclear. Wtip is an LIM domain protein of the Ajuba/Zyxin family, but its role in ciliogenesis during embryonic development has not been previously described.

Methods: Cloning full length of zebrafish *wtip* and *wtip* gene expression by RT-PCR, *in situ* hybridization, whole-mount immunochemistry, morpholino and mRNA injections, histological analysis, electron microscopy, high-speed video microscopy.

Results: We report Wtip is enriched in the basal body and knockdown of *wtip* leads to pronephric cyst formation, cloaca malformation, hydrocephalus, body curvature, and pericardial edema. We additionally show that *wtip* knockdown embryos display segment-specific defects in the pronephros: mitotic spindle orientation defects are observed only in the anterior and middle pronephros; cloaca malformation is accompanied by a reduced number of ciliated cells; and ciliated cells lack the striated rootlet that originates from basal bodies, which results in a lack of cilia motility. Our data suggest that loss of Wtip function phenocopies Vangl2 loss of function, a core planar cell polarity (PCP) protein located in the basal body protein. Furthermore, we demonstrate that *wtip* and *vangl2* interact genetically.

Conclusions: Taken together, our results indicate that in zebrafish, Wtip is required for mitotic spindle orientation in the anterior and middle of the pronephros, cloaca morphogenesis, and PCP, which may underlie the molecular etiology of ciliopathies.

Funding: NIDDK Support

FR-PO997

Knockdown of *BBS10* in Renal Cells Affects Apical Targeting of AQP2: A Possible Explanation for the Polyuria Associated with Bardet-Biedl Syndrome Giuseppe Procino,[1] Miriam Zacchia,[2] Claudia Barbieri,[1] Monica Carmosino,[1] Giovambattista Capasso,[2] Maria Svelto.[1] *[1]Department of Biosciences, Biotechnologies and Pharmacological Sciences, University of Bari Aldo Moro, Bari, Italy; [2]Department of Internal Medicine, 2nd University of Naples, Naples, Italy.*

Background: Bardet-Biedl syndrome (BBS) is a autosomal-recessive ciliopathy characterized by defects in multiple organ systems causing retinal degeneration, obesity, hypogonadism, polydactyly, mental retardation, and renal dysfunction. In particular, polyuria and polydipsia, with impairment of renal concentration ability, are the earliest signs of renal dysfunction.

Among the 14 identified genes *(BBS1-14),* mutated in BBS patients, *BBS10* and BBS1 contribute approximately 40% of all known mutations. BBS1 partecipates to the formation of the BBSome, a BBS proteins complex having importance in ciliary biogenesis and elongation. BBS10 is not associated with the BBSome and may also be involved in non-ciliary-related microtubule-based transport. Interestingly, patients with BBS10 mutations show a more severe renal phenotype.

Methods: In order to investigate whether the polyuria associated with BBS might be related to a defect in the shuttling of the water channel AQP2 in the kidney collecting duct, we studied the effect of *BBS1* or *BBS10* knockdown in AQP2-expressing renal cells.

Results: Interestingly, apical surface biotinylation indicated that siRNA-mediated silencing of BBS10 but not BBS1 dramatically and specifically prevented the forskolin-induced exocytosis of AQP2 at the apical membrane.

In the same experimental condition, immunofluorescence, followed by confocal analysis, showed that silencing of *BBS10* but not *BBS1* strongly affected the organization of the microtubules cytoskeleton within the cell. As a consequence, we observed that, upon FK treatment, AQP2 mostly redistributed to the basolateral membrane with negligible increase at the apical membrane.

Conclusions: Taken together, these results suggest that lost of proper microtubule-based polarized transport in the collecting duct cells might cause basolateral misrouting of AQP2 and might explain the urine concentration defect of BBS patients carrying mutations of BBS10.

FR-PO998

Role of the Small GTPase Cdc42 in Renal Ciliogenesis Soo Young Choi,[1] Liwei Huang,[1] Xiaofeng Zuo,[1] Sarah Mckenna,[1] Rebecca D. Burdine,[2] Joshua H. Lipschutz.[1] *[1]Renal Electrolyte and Hypertension Division, University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Molecular Biology, Princeton University, Princeton, NJ.*

Background: Primary cilia have been strongly implicated in ADPKD pathogenesis, and disruption results in cysts that destroy the kidney. In renal tubule cells, we showed that the highly conserved eight-protein exocyst complex, which is involved in targeting and docking vesicles carrying membrane proteins, was necessary for ciliogenesis; that *sec10* knockdown phenocopied *pkd2* knockdown in zebrafish; and that the small GTPase Cdc42 interacted with exocyst Sec10 to regulate ciliogenesis in MDCK cells.

Methods: Given the importance of Cdc42 for cell function, to study how *cdc42* affects ciliogenesis and cystogenesis *in vivo*, we used two different animal models and techniques: antisense morpholinos (MOs) to knockdown *cdc42* in zebrafish, and the Cre-Lox system to knockout Cdc42 in a kidney-specific manner in mice. Zebrafish embryos were injected with *cdc42* start-site MOs. Cdc42 renal tubule specific knockout mice were generated by breeding Cdc42 floxed and Ksp-cadherin Cre mice.

Results: *cdc42* morphants developed pericardial edema, short tail, small eyes, abnormal cilia, left-right patterning defects, and glomerular expansion that phenocopied *sec10* and *pkd2* morphants. Co-injection of small amounts of *cdc42* and *sec10* MOs, which individually had no effect, together resulted in an abnormal phenotype, which suggests that *cdc42* and *sec10* act in the same pathway. For Cdc42 kidney-specific knockout, 7 female $Cre^{ksp/+}$-$Cdc42^{fl/+}$ mice were backcrossed against 2 male $Cdc42^{fl/fl}$ mice, and 38 pups evaluated. Of 38 pups, 9 $Cdc42^{fl/+}$, 14 $Cdc42^{fl/fl}$, and 14 $Cre^{ksp/+}$-$Cdc42^{fl/+}$ living mice, and only 1 dead $Cre^{ksp/+}$-$Cdc42^{fl/fl}$ knockout mouse, were identified. The non-Mendelian ratio (1/38) suggests that kidney-specific knockout of Cdc42 leads to early postnatal lethality.

Conclusions: In zebrafish, *cdc42* knockdown results in a ciliary phenotype, and *cdc42* acts synergistically with exocyst *sec10*. Cdc42 kidney-specific knockout in mice leads to an early postnatal death. Taken together, these data suggest that Cdc42 is essential for normal cilia function and nephrogenesis.

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FR-PO999

Septin 7b Participates in Ciliogenesis and Regulates Kidney Function in Zebrafish Surjya Narayan Dash,[1] Eero Lehtonen,[1] Pertti Panula,[2] Sanna H. Lehtonen.[1] *[1]Haartman Institute, University of Helsinki, Finland; [2]Neuroscience Center and Institute of Biomedicine/Anatomy, University of Helsinki, Finland.*

Background: The small filament-forming, GTP-binding protein septin 7 is expressed in glomerular epithelial cells but its function in the kidney is unknown.

Methods: To study the role of septin 7 in the kidney, we used morpholino antisense oligonucleotides to knock down septin 7b in zebrafish and analysed the kidney function by using a labelled tracer.

Results: qRT-PCR showed septin 7b mRNA expression already at 2 hours post fertilization and by whole mount in situ hybridization, we found that septin 7b is enriched in the brain, pronephros and cloaca in 1-4 days post fertilization (dpf) larva. Immunostaining of 4 dpf larva revealed that septin 7 concentrates in the apical region of the pronephric tubules and ducts. Suppression of septin 7b with morpholinos caused pericardial and yolk sac edema, pronephric cysts, dorsal body axis curvature and hydrocephalus in 3.5 dpf larva indicating defects in kidney and cilia function. The phenotype of the morphants was rescued by co-injecting in vitro transcribed capped zebrafish septin 7b mRNA along with the morpholinos. Light microscopy indicated that the morphant embryos had distended glomeruli, pronephric tubules and ducts. Electron microscopy revealed podocyte foot process effacement and irregular microvillar-type cell processes extending from the apical surface of podocytes. Pronephric tubules appeared dilated with sparse microvilli that appeared irregular and atrophic. Further, injection of labelled dextran indicated defects in fluid flow in septin 7b morphant zebrafish suggesting dysfunction of pronephric cilia. Staining for acetylated tubulin revealed disorganization and shortening of the cilia in morphants suggesting that septin 7b participates in ciliogenesis. We also found that septin 7 is present at the base of the cilia in cultured mIMCD-3 (mouse inner medullary collecting duct) cells further suggesting that septin 7 may play a role in ciliogenesis.

Conclusions: Ciliary dysfunction and cyst formation in the kidney due to suppression of septin 7b provides new insights into the role of septin 7 in kidney diseases and ciliopathies.

FR-PO1000

The Nephrocystins NPHP9 and NPHP4 Corporately Activate the Hippo Downstream Effector TAZ Sandra Habbig,[1,2] Malte P. Bartram,[2] Roman-ulrich Mueller,[2] Mareike Franke,[2] Thomas Benzing,[2] Bernhard Schermer.[2] *[1]Pediatric Nephrology, University Childrens Hospital, Cologne, Germany; [2]Department II of Internal Medicine, University Hospital, Cologne, Germany.*

Background: Mutations in NPHP genes cause Nephronophthisis (NPH), an inherited cystic kidney disease leading to end stage renal failure in children and adolescents. To date, 12 NPHP genes have been identified. Interestingly, all of them encode for proteins that assemble functional complexes at centrosomes and cilia, linking NPH to a group of oligogenetic disorders called ciliopathies. Despite numerous studies the function of the NPH protein complex in cellular signaling is still not well understood.

Recently, we identified NPHP4 to be an inhibitor of the Hippo signalling pathway implicating that the pathogenesis of NPH and related ciliopathies is linked to dysregulation of the Hippo signaling cascade. De-repressed Hippo signalling and the resulting decrease in the proliferative potential due to the loss of NPHP4 function presents a potential disease mechanism that could explain the rather small, degenerative kidneys, the phenotypic hallmarks in NPH. However this finding had not been extended to other NPH proteins.

Results: Here, we demonstrate that the kinase Nek-8, which is a part of the NPH protein complex and encoded by the NPHP9 gene, also represses hippo signalling and enhances the activity of TAZ/TEAD transcription. Strikingly, all three previously described patient mutations did not affect TAZ/TEAD transcriptional activity. Biochemical analysis revealed that Nek-8 interacts with the hippo effector protein TAZ thereby stabilizing TAZ in its active form in the nucleus. Interestingly, this effect is enhanced in the presence of NPHP4 which promotes the nuclear accumulation of NPHP9.

Conclusions: Our data suggest that NPHP4 acts upstream of NPHP9/ Nek8 and provides further evidence for a role of dysregulated hippo signalling in the pathogenesis of NPH. Both, NPHP4 and NPHP9 antagonize hippo signalling and consecutively stimulate cell proliferation.

FR-PO1001

The Intracelluar Signaling Pathway in Renal Cyst Development of *inv* Mutant Mice Noriyuki Sugiyama, Takahiko Yokoyama. *Anatomy and Developmental Biology, Kyoto Prefecture University of Medicine, Graduate School of Medical Science, Kyoto, Japan.*

Background: Recent studies have identified several genes whose defects cause hereditary renal cystic diseases. Most of these gene products are located in the primary cilia. It has been proposed that primary cilia are involved in signaling pathways. Defects in these signaling pathways are considered to result in abnormal cell proliferation and random oriented cell division in the kidney, which lead to renal cyst formation. The *inv* mutant mouse, a model for human nephronophthisis (NPHP) type 2, develops multiple renal cysts. However, it was not clarified that the intracellular signaling pathway caused renal cyst development in *inv* mutant mice. Then, we searched for the intracellular signaling pathway changed during renal cyst development in *inv* mutant mice.

Methods: We analyzed some intracellular signaling pathways, canonical Wnt/b-catenin, MAPK (ERK, P38 MAPK, and JNK/c-Jun), AKT, STAT, hedgehog intracellular signaling pathway. In *inv* mouse kidneys. Further, we examined the effects of ERK and p38 MAPK inhibition on renal cyst development in *inv* mutant mice.

Results: ERK and p38 MAPK signaling were continued to activate in an early phase in renal cyst development. Activations of STAT3 and JNK/c-Jun signaling were detected in a late phase. Activation of other signals was not detected in *inv* kidneys. Inhibition of ERK signaling reduced renal cyst expansion, abnormal tubular cell proliferation, and fibrosis without affecting p38 MAPK activation. In contrast, inhibition of p38 MAPK decreased the degree of fibrosis. However, the FR167653 did not prevent cyst expansion, abnormal cell proliferation.

Conclusions: These results suggest that the ERK signaling pathway were important in all phenomena of renal cyst development in *inv* mutant mice and the p38 MAPK signaling pathway was related in renal fibrosis but not cyst development. Further, our results suggest that p38 MAPK and ERK signaling pathways independently affect renal fibrosis in *inv* mutant mice.

Funding: Government Support - Non-U.S.

FR-PO1002

Defining Uraemic Arterial Functional Abnormalities in HD Patients: Combination of In Vivo and Ex Vivo Arterial Assessment Adil Mohamed Abushufa,[1] Mohamed Tarek Eldehni,[1,2] Aghogho Odudu,[1,2] Philip D. Evans,[1,2] Saoirse O'Sullivan,[1] Chris W. McIntyre.[1,2] *[1]Graduate Entry Medical School, University of Nottingham, Derby, United Kingdom; [2]Department of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom.*

Background: Haemodialysis (HD) patients exhibit significant abnormalities in vascular structure and function, which impact on cardiovascular mortality. The mechanisms underlying these changes are still unclear. We aimed to examine vascular function in isolated subcutaneous arteries of HD patients using myography having first characterised the patients in vivo.

Methods: Abdominal subcutaneous fat biopsies were obtained from 11 HD patients and 26 normal controls. Arteries were dissected, mounted and conducted on a wire myography. Cumulative concentration-response curves were constructed to the following; noradrenalin (NA), endothelin-1 (ET-1), thromboxane A2 (U46619), angiotensin II (AngII), vasopressin,

bradykinin (BK), acetylcholine (Ach) and sodium nitroprusside (SNP). Pulse Wave Velocity (PWV) was measured in HD patients in addition to blood pressure.

Results: Greater contractile responses to U46619, AngII, and vasopressin were observed in all HD arteries compared to controls. The maximum vasorelaxant response to Ach and BK (endothelium-dependent vasodilators) was significantly lower in HD patients than controls; while a similar response to SNP (endothelium-independent response) was obtained in both groups (table1). PWV correlated with the maximum contractile to Vasopressin in large arteries (r=0.829, P=0.042).

Conclusions: Larger vessel responses were correlated to in vivo assessment of arterial function. HD patients are primed to hypertension and end organ demand ischaemia by a markedly elevated pressor response to a wide range of stimuli. The failure of arterial relaxation is mediated by endothelial dysfunction. **Table 1 Maximum responses (R max) to different vasoactive agents in small arteries.**

		HD	Control	P
R Max	NA	5.73 ± 0.16	5.61 ± 0.19	0.6872
	ET-1	11.17 ± 0.27	8.97 ± 0.60	0.0155
	U46619	14.03 ± 0.35	9.31 ± 0.36	< 0.0001
	AngII	7.39 ± 0.22	4.80 ± 0.25	< 0.0001
	Vasopressin	15.13 ± 0.39	7.011 ± 0.42	< 0.0001
% Relaxation	BK	35.67 ± 0.78	40.08 ± 0.62	0.0008
	Ach	36.22 ± 1.29	53.83 ± 1.32	< 0.0001
	SNP	59.40 ± 1.25	63.40 ± 1.33	0.156

Funding: Government Support - Non-U.S.

FR-PO1003

Gene Expression Analysis of Neointimal Hyperplasia in Arteriovenous Grafts Sun Hyung Kwon,[1] Li Li,[2] Christi M. Terry,[2] Yan-Ting E. Shiu,[2] Huan Li,[2] Ilya S. Zhuplatov,[2] Donald Blumenthal,[1] Alfred K. Cheung.[2,3] *[1]Pharmacology and Toxicology, Univ. of UT, Salt Lake City, UT; [2]Medicine, Univ. of UT, Salt Lake City, UT; [3]Medicine, VASLCHCS, Salt Lake City, UT.*

Background: Failure of arteriovenous grafts (AVG) occurs frequently and predominantly as a consequence of neointimal hyperplasia (NH) formation at the venous anastomosis. Development of effective therapies for NH is impeded by our limited understanding of the molecular genesis of NH.

Methods: A porcine model of AVG stenosis was employed and global gene expression microarray analysis was used to determine the genomic responses of the vein at early time points following AVG placement. We explored genes that were differentially expressed in the NH-prone (venous anastomosis) and NH-resistant (downstream venous segment) regions, compared to the un-operated vein at 5 days and at 14 days post-AVG placement (ANOVA $p<0.05$ with at least 4-fold changes).

Results: Statistical analysis revealed that, compared to the un-operated control vein, the number of significantly altered genes was higher in the NH-prone region than in the NH-resistant region (262 vs. 150 and 224 vs. 108 at 5 days and 14 days, respectively). A substantial number (65%) of such spatially and/or temporally regulated genes in the NH-prone region are associated with gene ontology terms that are consistent with processes occurring during NH development, such as cell proliferation, adhesion and migration, vasculogenesis, extracellular matrix (ECM) organization, and immune response. Notably, genes related to ECM organization and cell motility (e.g., IBSP, SPP1, ADAM7, DCHS2, PHACTR3 and COL11A1) were among the most highly up-regulated in the NH-prone region. The up-regulation of these genes may contribute to the early pathogenesis of NH.

Conclusions: Global gene expression profiling of NH-prone regions at the AVG anastomosis provides clues to the molecular basis underlying the pathophysiology of NH, and may suggest strategies for the development of targeted anti-NH therapies.

FR-PO1004

ANCA-Associated Systemic Vasculitis Is Associated with Impaired Dilatory Capacity of the Conduit Brachial Artery: A Link to Increased Cardiovascular Risk? Peter Clausen, Wladimir M. Szpirt, Bo Feldt-Rasmussen. *Department of Nephrology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.*

Background: ANCA-associated systemic vasculitis (AAV) affects small vessels but has also been shown to be associated with subsequent increased macrovascular cardiovascular risk. This study tested the dilatory capacity of the conduit brachial artery and the plasma concentration of the potential vascular marker von Willebrand Factor (vWF) at diagnosis and the effect of immunosuppressive treatment on these parameters in order to examine potential pathophysiological mechanisms behind the association.

Methods: The dilatory responses of the brachial artery (percentage of baseline) to post-ischemic increased blood flow (endothelium-dependent flow-associated dilatation (FAD) and to sublingually administered nitroglycerin (endothelium-independent nitroglycerin-induced dilatation (NID)) were measured by ultrasound in 24 patients with newly diagnosed AAV (female/male: 9/15, age: 55±12 years) and compared to measurements in 24 healthy controls (female/male: 9/15, age: 59±7 years). Plasma concentration of vWF was measured by ELISA. Patients were reexamined after 6 and 12 months of immunosuppression (plasmapheresis, corticosteroids and cyclophosphamide followed by azathioprine). All patients reached remission during follow-up.

Results: NID was impaired in patients with AAV as compared to controls (109±6 vs. 123±5%, $p<10^{-8}$) whereas a tendency to impaired FAD did not reach statistical significance (102±3 vs. 104±4%, p=0.17). Plasma vWF concentration was increased in patients (2.0 (1.0-3.8) kIU/L vs. 1.1 (0.4-1.8), $p<10^{-6}$). One year of immunosuppressive treatment did not affect neither NID, FAD nor vWF in patients.

Conclusions: The dilatory capacity of the brachial artery is impaired and the plasma concentration of von Willebrand Factor, a potential marker of vascular function, is increased in ANCA-associated systemic vasculitis. These disturbances are not reversed by 1 year of immunosuppression. This may indicate a sustained damage also to major vessels which could be a potential mechanism underlying the demonstrated association between ANCA-associated systemic vasculitis and increased cardiovascular risk.

FR-PO1005

Sildenafil Reduces Arterial Stiffness in ESRD Patients Treated with Hemodialysis William D. Paulson,[1,2] Allison Dubner,[2] John White,[2] David M. Pollock,[2] Jennifer S. Pollock,[2] Gaston K. Kapuku.[2] *[1]Charlie Norwood VAMC, Augusta, GA; [2]Georgia Health Sciences University, Augusta, GA.*

Background: ESRD is characterized by increased arterial stiffness, which contributes to high cardiovascular morbidity and mortality. Measures that reduce arterial stiffness may potentially improve cardiovascular outcomes, but there are currently no established treatments for this problem. In this study, we tested the hypothesis that sildenafil, which enhances and prolongs the effects of nitric oxide, decreases pulse wave velocity (PWV) and increases small artery elasticity index (SAE) in hemodialysis patients.

Methods: Chronic hemodialysis patients (mean age = 47 years, range 19-78) were randomly assigned to ingest a placebo (N = 20) or 50 mg sildenafil capsule (N = 20). Just before, and 60 and 120 min post ingestion, patients underwent measurement of arterial blood pressure, carotid-femoral PWV (SphygmoCor System), and SAE (HDI/PulseWave System).

Results: Mean values in the 40 patients were consistent with increased arterial stiffness: high systolic (144.0 ± 3.5 mmHg [±SE]) and pulse pressures (61.9 ± 1.9 mmHg), high PWV (9.36 ± 0.47 m/sec), and low SAE (5.37 ± 0.40 ml/mmHg·100). After ingesting the capsule, PWV in the control group increased by 0.56 ± 0.29 m/sec and decreased in the sildenafil group by 0.39 ± 0.32 m/sec (P = 0.037) during the next 120 min. Similarly, SAE decreased in the control group by 0.65 ± 0.52 ml/mmHg·100 and increased in the sildenafil group by 0.80 ± 0.63 ml/mmHg·100 (P = 0.08). Patients with the highest baseline PWV had the largest decrease in PWV at 120 min after sildenafil ($R^2 = 0.247$, P = 0.05), and patients with the lowest baseline SAE had the largest increase in SAE ($R^2 = 0.205$, P = 0.045).

Conclusions: ESRD patients treated with hemodialysis have increased arterial stiffness, manifested by high PWV and low SAE. In this randomized study, a single 50 mg dose of sildenafil significantly improved PWV and SAE when compared with a control group. These results suggest that treatments that enhance and prolong the effects of nitric oxide could potentially improve cardiovascular outcomes in ESRD.

Funding: Clinical Revenue Support

FR-PO1006

Matrix Metalloproteinase 10 and Coronary Artery Calcification in Chronic Kidney Disease Patients without Cardiovascular Disease Joaquin Manrique,[1] Alvarez Virginia,[3] Carolina Purroy,[1] Fernanda Slon,[1] Josune Orbe,[2] José A. Paramo,[2] Jesus Arteaga,[1] Jose A. Rodriguez.[2] *[1]Nephrology, Complejo Hospital Navarra, Pamplona, Spain; [2]Atherosclerosis Research Lab, Division of Cardiovascular Sciences, CIMA, Universidad de Navarra, Pamplona, Spain; [3]Cardiology, Complejo Hospital Navarra, Pamplona, Spain.*

Background: Cardiovascular disease is the main cause of mortality among chronic kidney disease (CKD) patients and atherosclerosis is the common pathophysiological substrate for ischemic vascular diseases and their thrombotic complications. Accelerated vascular dysfunction and calcification is also highly prevalent in patients with CKD. The imbalance between matrix metalloproteinases (MMP) and their inhibitors has been proposed to play a main role in atherosclerosis progresion and plaque rupture. We hypothesized that MMP-10 can be associated with atherosclerosis burden in CKD patients, measured by coronary artery calcification.

Methods: a cross-sectional, observational study including 75 CKD patients stages III–V, without previous cardiovascular disease (stroke, cardiac or peripheral), and 30 healthy control subjects. Serum MMP10 levels were measured by enzyme-linked immunosorbent assay and coronary artery calcification (CAC) was determined by multi-detector computed tomography (Agatston score).

Results: CKD patients exhibited significantly augmented serum MMP-10 levels as compared with control subjects ($p<0.001$), and also higher incidence of coronary calcification ($p<0.01$). Patients on dialysis showed increased MMP-10 and vascular calcification, in comparison with earlier stages of CKD ($p<0.05$ both). However, no relationship could be found between MMP-10 and vascular calcification in CKD patients, that was mainly associated with age. MMP10 was associated with PTH levels ($p<0.05$) and, among patients with stages III&IV, correlated inversely with GFR ($p<0.01$).

Conclusions: increased serum MMP10 levels in CKD are closely related with renal function but not with coronary calcification in CKD patients without previous cardiovascular disease.

FR-PO1007

The Protein Acyl Transferase ZDHHC21 Is a Novel Regulator of α1 Adrenergic Receptor Signaling, Vascular Tone, and Blood Pressure
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Background: Multiple proteins involved in α1 adrenergic signal transduction require palmitoylation, the reversible post-translational attachment of a lipid (typically palmitate) to specific cysteine residues. Protein palmitoylation is generally catalyzed by members of the newly-described ZDHHC family of protein acyl transferases, The role of ZDHHC enzymes in vascular function is unknown.

Methods: We have characterized cardiovascular function in a mutant mouse strain that expresses a nonfunctional mutant of ZDHHC21, an isoform widely expressed in vascular and other tissues. Experiments have included (1) ex vivo myograph studies of isolated aortic rings and mesenteric arteries, (2) in vivo studies using ambulatory blood pressure measurements and echocardiography, and (3) in vitro studies of signaling in cultured cells derived from ZDHHC21 mutant mice.

Results: Aortic rings and mesenteric arteries isolated from ZDHHC21 mutant mice both displayed specific defects in response to phenylephrine, an α1 adrenergic receptor agonist. In vivo, the mutant mice displayed significant tachycardia and nocturnal hypotension. Stroke volume and fractional shortening were normal. There was also significant blunting of circadian cycles of heart rate and blood pressure. Cultured vascular smooth muscle cells from mutant mice mobilized calcium normally in response to phenylephrine.

Conclusions: Together the data support the presence of hypotension and tachycardia due to diminished peripheral vascular resistance in ZDHHC21 mutant mice. The underlying mechanism likely involves reduced vascular responsiveness to α1 adrenergic agonists. The molecular defect in adrenergic signaling may lie in pathways that modulate the sensitivity of smooth muscle contraction to calcium concentration rather than calcium mobilization itself. In sum, these data identify ZDHHC21 as a novel regulator of vascular α1 adrenergic receptor signaling and may reveal new targets to modulate vascular tone and blood pressure.

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FR-PO1008

Glyoxalase I Ameliorates Age-Related Endothelial Dysfunction
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Background: Endothelial dysfunction is a major culprit of chronic kidney disease (CKD) and cardiovascular diseases (CVD), especially in elder people. Extracellular advanced glycation end-products (AGEs) are associated with age-related endothelial dysfunction, however, the role of intracellular glycation affecting endothelial function is not elucidated. We investigated effect of glycation in thoracic aorta of rats systemically overexpressing glyoxalase I (GLO-1), which detoxifies methylglyoxal (MG), a typical reactive intermediate carbonyl formed in early glycation process.

Methods: Four groups of rats: young (13-week)/ old (48-week) and wild type (WT)/ glyoxalase I-overexpressing (Tg) Wistar rats, were examined. GLO-1 activity was measured for all groups. Glycated protein level and senescence markers of thoracic aorta were evaluated by immunohistochemistry. Finally, we examined endothelium -dependent and -independent vasorelaxation by tension studies of aortic ring treated with acetylcholine or sodium nitroprusside.

Results: There was no significant difference in blood pressure and glucose tolerance among 4 groups. Plasma lipid levels of old WT and Tg were comparable. GLO-1 activity was twice as high in young and old Tg as WT rats of the same age. MG-modified protein (argpyrimidine) was increased in aortic intima of old WT rats compared with young, while age-related accumulation of argpyrimidine was significantly reduced in Tg rats. Expression of senescence markers p53, p21, and p16 was decreased in aortic intima of old Tg compared with WT of the same age. Tension studies showed attenuation of age-related endothelial dysfunction in Tg compared with WT, while there was no difference in endothelium-independent vasorelaxation between WT and Tg rats.

Conclusions: The present study revealed that glycation was accelerated in intima of thoracic aorta with aging, and that age-related endothelial dysfunction was attenuated by GLO-1 overexpression. GLO-1 appears to be a potent target for prevention and treatment of CKD and age-related CVD.

FR-PO1009

Defective EphrinB2 Reverse Signaling Promotes Capillary Rarefaction and Fibrosis after Kidney Injury
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Background: Microvascular disease is a characteristic of acute and chronic kidney diseases, which leads to rarefaction of peritubular capillaries (PTCs) and promotes secondary injury due to ischemia, a process that may be central to kidney disease progression. We recently showed that pericytes detach themselves from PTCs in response to injury and this detachment is an important step both for PTC rarefaction and fibrosis, but our understanding of the molecular signals in this process is limited. Bidirectional signaling by EphB4 receptor and ephrinB2 ligand has been identified as a critical angiogenesis cue during embryogenesis and fetal development. Therefore, we investigated the role of ephrinB2 reverse signaling in injury-induced angiogenesis, and subsequent PTC rarefaction and fibrosis in the kidney.

Results: EphrinB2 reverse signaling was activated in the kidney only after injury induced by unilateral ureteral obstruction (UUO). Mice lacking the PDZ intracellular signaling domain of ephrinB2 (ephrinB2 dV) experienced impaired angiogenesis followed by increased PTC rarefaction and increased fibrosis after kidney injury. EphrinB2 dV primary kidney pericytes were more migratory than wild type (WT) pericytes, had impaired capacity to stabilize capillaries generated in 3D culture, and impaired capacity to stimulate capillary basement membrane synthesis. EphrinB2 dV primary kidney microvascular endothelial cells (MVECs) migrated and proliferated less than WT MVECs in response to vascular endothelial growth factor A (VEGFA) and showed impaired internalization and activation of VEGF receptor-2 (VEGFR2).

Conclusions: Defective PDZ binding motif dependent ephrinB2 reverse signaling promotes PTC rarefaction via impaired angiogenesis and impaired vascular stability during kidney injury. Additionally, defective PDZ binding motif dependent ephrinB2 reverse signaling in kidney pericytes promotes pericyte to myofibroblast transition and myofibroblast activation, leading to enhanced fibrosis.

FR-PO1010

Cyclosporine Attenuates Arginine Transport, in Human Endothelial Cells, through Modulation of Cationic Amino Acid Transporter-1
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Background: The spectrum of cardiovascular toxicity by cyclosporine (CSA) includes hypertension, accelerated atherosclerosis, and thrombotic microangiopathy, all of which are the result of endothelial cell dysfunction (ECD). ECD is characterized by decreased endothelial nitric oxide synthase (eNOS) activity. Cationic amino acid transporter-1 (CAT-1) is the specific arginine transporter for eNOS. Cyclosporine has been shown to attenuate nitric oxide generation. However, the mechanism remains elusive.

Methods: We studied the effect of cyclosporine on arginine uptake by human umbilical vein endothelial cell cultures (HUVEC) in the absence and presence of L-arginine.

Results: Cyclosporine (0.1-0.5 mmole/L) significantly attenuated arginine transport in a dose and time dependent manner, a phenomenon which was prevented by co-incubation with L-arginine (1 mM). The aforementioned findings were accompanied by increased protein nitration, a measure of peroxynitrite accumulation. Protein abundance of CAT-1, PKCα, and phsphorylated PKCα (CAT-1 inhibitors) were not affected by cyclosporine. Co-incubation of cells exposed to CSA with a JNK inhibitor abolished CSA effect on arginine transport.

Conclusions: Cyclosporine inhibits arginine transport in HUVEC through post translational modulation of CAT-1, possibly through activation of the MAP kinase JNK pathway.

FR-PO1011

Loss of the Endothelial Glucocorticoid Receptor Prevents Rescue by Dexamethasone in a Mouse Model of Sepsis
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Background: The glucocorticoid receptor (GR) is ubiquitously expressed in most cell types and previous studies suggest that elimination of GR in specific tissues may have profound effects. The synthetic steroid dexamethasone (DEX), which acts through GR, is often given systemically in sepsis, sometimes with questionable benefit.

Methods: In vivo, mice with a tissue specific deletion of endothelial GR (eGR) and their littermate controls were pre-treated with DEX 2 mg/kg and then sepsis was induced by injecting LPS 12.5 mg/kg. Survival was monitored over a period of 4 days. Corticosterone, IL-6 and nitric oxide levels were measured at baseline and 8 hours after LPS injection. Continuous blood pressure and heart rate monitoring were also performed in anesthetized mice at baseline and after DEX pre-treatment and acute LPS injection.

Results: Survival in control animals after DEX+LPS treatment was 100% (n=8 animals) while survival in similarly treated eGR knockout mice was only 66% after 1 day and 22% after 2 days (n=9 animals, p=0.0031). eGR knockout animals demonstrated statistically significant increases in IL-6 (116.4 ± 37.1 vs. 5.4 ± 1.4 ng/ml, p=0.04) and nitric oxide (43.5 ± 9.3 μM vs. 12.0 ± 0.9 μM, p=0.01) after DEX+LPS treatment compared to similarly treated controls. Corticosterone levels were similar between the two groups. Mean BP in control animals that had been pretreated with DEX was 71.7 ± 2.1 mm Hg (n=5) 4 hours after LPS while BP was only 42.9 ± 6.2 mm Hg in similar treated eGR knockout animals (n=5, p <0.01).

Conclusions: In conclusion these data suggest that:

(1) eGR knockout mice have more hemodynamic instability and inflammation than control mice after DEX+LPS treatment

(2) DEX confers no protective benefit in eGR knockout mice and actually appears to worsen outcomes

(3) the presence of eGR is necessary for the beneficial actions of DEX in this model.

Funding: NIDDK Support

FR-PO1012

Arginase Inhibition Mediates Renal Tissue Protection in Diabetic Nephropathy via a Nitric Oxide Synthase 3-Dependent Mechanism

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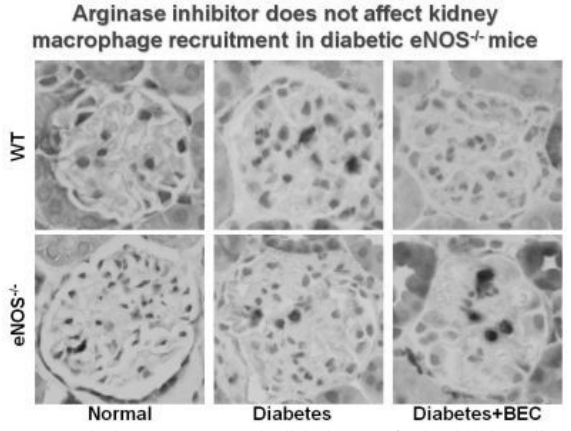
Background: Our recent publication (Diabetes 60:3015-22; 2011) shows increased kidney arginase activity in diabetic mice that was associated with a reduction in renal medullary blood flow. Furthermore, pharmacological blockade or genetic deficiency of arginase-2 confers kidney protection in diabetic mouse models. However, the mechanism by which arginase inhibition mediates renal tissue protection is not clear. Here we hypothesize that the protective effect of arginase inhibition is nitric oxide synthase 3 (eNOS) dependent in diabetic nephropathy.

Methods: Experiments were conducted in eNOS knockout ($eNOS^{-/-}$) and their wild type littermate ($eNOS^{+/+}$) mice using multiple low doses of vehicle or streptozotocin (STZ; 50 mg/kg ip for 5 days) treated with continuous subcutaneous infusion of vehicle or the arginase inhibitor S-(2-Boronoethyl)-L-cysteine (BEC; 2.3 mg/kg/day) via osmotic pump for 6 weeks.

Results: Using immunohistochemistry, we confirmed the localization of arginase-2 but not arginase-1 in human and murine kidneys, mainly in the endothelial cells. Blocking arginases using BEC for 6 weeks in diabetic $eNOS^{+/+}$ mice significantly attenuated albuminuria ($p<0.05$), the increase in plasma creatinine ($p<0.01$), histopathological changes and kidney macrophage recruitment compared to vehicle-treated diabetic $eNOS^{+/+}$ mice.



Interestingly, BEC treatment in diabetic $eNOS^{-/-}$ mice failed to affect any of these parameters and was not significantly different from vehicle-treated diabetic $eNOS^{-/-}$ mice.

Conclusions: These findings indicate that arginase inhibition mediates renal tissue protection in diabetic nephropathy via eNOS-dependent mechanism.

Funding: NIDDK Support

FR-PO1013

Regulation of Renal NADPH Oxidase Activity and Vascular Function by Gut Microbiota

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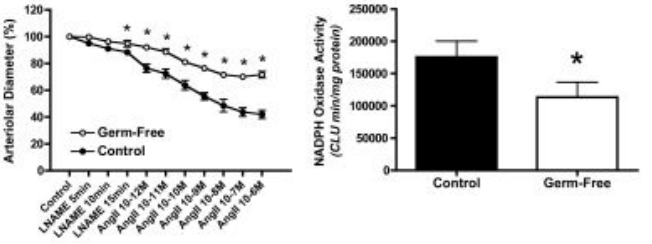
Background: Angiotensin II mediated contraction of the vasculature involves activation of NADPH oxidases. Angiotensin II induced oxidative stress is associated with vascular remodeling and increased preglomerular resistance that are both implicated in the pathogenesis of renal and cardiovascular disease. Recent studies have demonstrated that changes in gut microbiota may influence obesity and diabetes, conditions associated with oxidative stress and inflammation. We used germ-free mice to investigate the hypothesis that host microorganisms regulate NADPH oxidase activity and vascular responses to Angiotensin II in the kidney.

Methods: Isolated and perfused afferent arterioles were used to investigate the arteriolar responses to Angiotensin II (10^{-12} to 10^{-6} mol/L, each dose 2 min), after inhibition of nitric oxide synthase with L-NAME (10^{-4} mol/L). NADPH oxidase activity was measured as Lucigenin-dependent chemiluminescence of superoxide.

Results: Arterioles from conventional mice displayed greater L-NAME induced contraction (-12±2%) and maximal Angiotensin II responses (58±3%) compared with that of germ-free mice (-5±3% and -30±6%, respectively). Basal NADPH oxidase activity in the kidney, measured as Lucigenin-dependent chemiluminescence of superoxide, was markedly reduced in germ-free mice (113±21x10^3 CLU min/mg protein) compared with conventional mice (176±25x10^3 CLU min/mg protein).



Conclusions: The gut microbiota modulate renal NADPH oxidase activity and Angiotensin II mediated contraction of preglomerular vessels. The mechanisms are currently being investigated.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1014

Impact of Renal Denervation on Inflammation

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Background: Renal Denervation (RDN) is a new tool in resistant hypertension. First clinical trials (HTN1/HTN2-Trial) could demonstrate a significant reduction in blood pressure in these cardiovascular high risk patients. The kidney with its sensory nerves plays an important role in blood pressure regulation. Animal data and observations on patients after nephrectomy support this hypothesis. However, the underlying mechanisms on blood pressure reduction are not fully understood. In addition, it is unclear, why it is taking weeks to months before the effect can be observed. There is new evidence that inflammatory cells are regulated by the autonomic nervous system. Furthermore, direct correlation of C-reactive-Protein (CRP) and cardiovascular risk has already been shown.

The aim of this prospective analysis was to find the influence of RND on the inflammation markers CRP and TNFalpha.

Methods: Patients with refractory hypertension were prospectively evaluated for blood pressure and high-sensitive CRP and TNFalpha at baseline and after 6 months. In n=55 patients RDN and in n=38 optimal medical therapy (OMT) was applied.

Results: Systolic blood pressure dropped significantly in both groups to -23mmHg (RDN) and -7mmHg (OMT), respectively. Only in the RDN group CRP was reduced significantly (4265ng/ml to 2900ng/ml). No significant reduction was observed in OMT group (3184ng/ml to 3020ng/ml). TNFalpha declined from 2,97pg/ml to 2,45pg/ml in RDN patients.

Conclusions: For the first time it could be shown, that RND has an impact on systemic inflammation. The reduction of inflammation might be one explanation for the time-shifted response in blood pressure. Ongoing analyses on isolated leucocytes will elucidate underlying mechanisms.

FR-PO1015

Nitric Oxide Inhibition Enhances the Susceptibility to Glomerular Injury as Compared to Angiotensin II Infusion Despite Similar Levels of Blood Pressure in Rats

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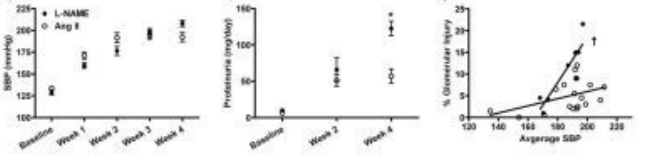
Background: Experimental models of hypertension induced by elevated Ang II or reduced nitric oxide (NO) are commonly used to investigate the pathogenesis of renal injury. Recent studies have suggested that Ang II attenuates, while reduced NO potentiates, RBF autoregulation, predicting differences in the relationship between BP and glomerular injury. However, the quantitative relationships between BP and renal injury have not been examined in these models of renal vasoconstriction-associated hypertension.

Methods: Male SD rats (Harlan) were prepared for BP radiotelemetry and 1 wk later administered either Ang II (n=18, 300-500 ng/kg/min SQ via Alzet minipump) or the NO synthase inhibitor, L-NAME (n=9, 50 mg/kg/day via drinking water), for 4 wks. BP was measured every 10 minutes/24 hr. day, 24-hr. proteinuria was assessed at 2 and 4 wks, and the kidneys were perfused fixed at 4 wks.

Results: Ang II and L-NAME led to significant and similar patterns of hypertension (Fig. 1a). Proteinuira was greater in L-NAME vs. Ang II rats at 4 wks (* $p<0.05$ vs. Ang II, Fig. 1b). Glomerular injury was greater in L-NAME (10.8 ± 2.2%) vs. Ang II (4.9 ± 0.8%) rats ($p<0.05$). Furthermore, the slope of the relationship between BP and glomerular injury was > 5-fold higher in L-NAME vs. Ang II rats († $p<0.05$ slope vs. Ang II, Fig. 1c). Results are mean±SE.



Conclusions: These data suggest that BP transmission to the glomerular capillaries is attenuated in Ang II- vs. L-NAME-induced hypertension. Thus, the consequences of impaired RBF autoregulation on glomerular injury are different in experimental models of hypertension associated with renal vasoconstriction (Ang II) vs. vasodilation (renal mass reduction).

Funding: NIDDK Support, Veterans Administration Support

FR-PO1016

Role of ADMA in Endothelial Dysfunction in Adenine CKD Mice

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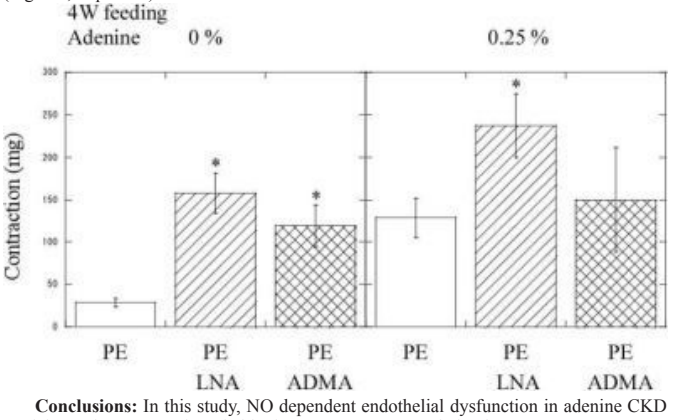
Background: Chronic kidney disease (CKD) is a serious risk factor for cardiovascular diseases (CVD). Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase (NOS) inhibitor which plays a key role both in renal deterioration and arteriosclerosis in CKD through endothelial dysfunction, however its pathophysiological mechanisms in CKD have not been fully clarified. In this study, we analyzed vascular function in adenine CKD mice in association with ADMA.

Methods: C57BL/6 mice (10 weeks old) were randomized to chow with or without 0.25% adenine. Thoracic aortas were analyzed ex-vivo by wire myography at 4 weeks.

Results: Plasma creatinine levels were elevated in adenine treated group (A) compared to control (C) (A: 0.21+-0.09, C: 0.013 +- 0.006 mg/dL). After phenylephrine (PE) pretreatment, concentration-dependent curves for acetylcholine -induced relaxation was decreased in A compared to C, and sodium nitroprusside, NO donor, induced relaxation was not different between A and C, suggesting NO dependent endothelial dysfunction. PE induced contraction after NOS inhibitor treatment was significantly increased by N-nitro-L-arginine (NLA) both in A and C, however it was only increased in C by ADMA (Figure1, *: p<0.05).



Conclusions: In this study, NO dependent endothelial dysfunction in adenine CKD model was demonstrated, probably due to ADMA accumulation in CKD.

Funding: Government Support - Non-U.S.

FR-PO1017

Acute Antihypertensive and Renal Hemodynamic Effects of Tempol and PEG-Catalase in CKD

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Background: Excess reactive oxygen species (ROS) increase blood pressure (BP) and renal vascular resistance (RVR). Administration of superoxide dismutase mimetic Tempol normalizes BP and RVR in various models of hypertension. There are fewer reports on H_2O_2 reduction by catalase. No such data are available in chronic kidney disease (CKD). We assessed acute antihypertensive and renal vasodilatory effects of Tempol or polyethylene glycol (PEG)-catalase in CKD and control (CON) rats.

Methods: In male Lewis rats 2/3 of each kidney was ablated (CKD). CON were sham-operated. 24h proteinuria, lipid peroxide (TBARS), 8-isoprostane and H_2O_2 urine excretions were determined. Hemodynamics were studied when proteinuria exceeded 100mg/24h. Under isoflurane we measured mean arterial pressure (MAP) and renal blood flow (RBF, Transonic flow probe on left renal artery), allowing calculation of RVR (MAP/RBF) during continuous infusion of Tempol (CKD n=6, CON n=5), PEG-catalase (CKD n=8, CON n=5) or vehicle (CKD n=4, CON n=4). GFR (inulin), glomerulosclerosis (GS) and tubulo-interstitial injury (TI) were measured.

Results: CKD rats had low GFR (P<0.0001) and high GS and TI (both P<0.05) compared to CON. 24h TBARS (P<0.05), 8-isoprostane (P<0.001) and H_2O_2 excretions (P<0.1) were elevated. Baseline MAP (123±13 vs. 99±8 mmHg, P<0.0001) and RVR (21±11 vs. 7±1 units, P<0.0001) were consistently increased in all CKD groups. Versus baseline Tempol caused marked reduction in MAP in CON (81±10 vs. 99±11 mmHg, P<0.05) but not in CKD (127±16 vs. 130±13 mmHg), without much change in RVR in CON or CKD. PEG-catalase had effect on MAP in both CON (94±8 vs. 102±4 mmHg, P<0.05) and CKD (110±12 vs. 118±12 mmHg, P<0.05), but the difference in MAP between CON and CKD was unchanged. PEG-catalase slightly decreased RVR in CON and CKD (-0.8±0.3 and -2.7±2.0 units, both P<0.05). Vehicle did not change MAP or RVR.

Conclusions: In CKD ROS were increased but, contrary to our hypothesis, BP and RVR did not depend more on ROS than in CON. Limited reduction of RVR by Tempol and PEG-catalase in both CON and CKD suggests that in general ROS-mediated vasoconstriction is mainly extrarenal.

Funding: Government Support - Non-U.S.

FR-PO1018

The Endothelial Progenitor Cell (EPC) Migration Inhibitor FTY720 Augments Malignant Nephrosclerosis in Deoxycorticosterone Acetate (DOCA)/Salt Hypertensive Rats

Yoshitaka Iwazu, Shigeaki Muto, Yuko Watanabe, Kana Iwazu, Takashi Ioka, Eiji Kusano. *Division of Nephrology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

Background: The novel immunosuppressant FTY720 induces lymphocyte migration and homing in secondary lymphatic organs by regulating egress from lymph nodes. FTY720 also supports bone marrow homing of circulating EPCs, which are incorporated into foci of neovascularization. We explored how FTY720 affects the renal vascular damage in DOCA/salt hypertensive rats, a model of human malignant nephrosclerosis.

Methods: Uninephrectomized rats were divided into control, control plus FTY720, DOCA alone, and DOCA plus FTY720 groups, which were given vehicle, vehicle plus FTY720 (0.5 mg/kg/day), DOCA alone, and DOCA plus FTY720 (0.5 mg/kg/day) for 4 wks, respectively. All rats were given 0.9% NaCl/0.3% KCl to drink. We compared systolic blood pressure (SBP), the number of circulating EPCs, and renal tissues stained with picro-Sirius red among the groups of rats.

Results: SBP was higher in rats given DOCA plus FTY720 than in rats given DOCA alone, although it was higher in the two groups than in controls. In rats given DOCA plus FTY720 and rats given DOCA alone, the onion-skin lesion was prominently observed in their renal arterioles, with the number being greater in rats given DOCA plus FTY720. The number of circulating EPCs was less in rats given DOCA plus FTY720 than in controls and in rats given DOCA alone. Control rats given FTY720 exhibited less circulating EPCs than control rats given vehicle alone, but had no onion-skin lesions in their renal arterioles.

Conclusions: We conclude that FTY720 administration augments malignant nephrosclerosis in the DOCA/salt hypertensive rats. These findings suggest that in the DOCA/salt hypertensive rats, FTY720 may reduce circulating EPCs from the blood, inhibit their infiltration into the diseased kidney, and thus suppress repair processes of the renal arteriole damage.

FR-PO1019

Association of Uric Acid with Vascular Stiffness in the Framingham Heart Study

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Background: Uric is associated with increased risk of cardiovascular disease as well as with arterial stiffness in patients with hypertension or stroke. It remains unknown if uric acid is associated with arterial stiffness in the general population.

Methods: Cross sectional study evaluating the relationship between serum uric acid levels and carotid femoral pulse wave velocity (CF- PWV) using multivariable linear regression in 3914 participants from the Generation 3 Framingham cohort.

Results: Mean (SD) age, MDRD estimated GFR (eGFR) and uric acid were 40.0 (8.8) years, 101.4 (22.1) mL/min/1.73 m^2 and 5.3 (1.5) mg/dL, respectively. Mean (SD) CF- PWV was 7.0 (1.4) m/s. Individuals in the highest quartile of uric acid (>6.3 mg/dl) were more likely to be male, have a higher prevalence of hypertension, higher BMI, fasting glucose and insulin, and lower estimated GFR. Multivariate adjusted means of CF-PWV by uric acid quartiles are show in Table 1. In unadjusted analysis each 1 mg/dL higher serum uric acid was associated with a 0.27 ± 0.01 higher CF-PWV (R^2= 0.09, p < 0.0001). This was attenuated but remained significant after adjusting for age, sex, smoking, hypertension, BMI, fasting glucose, insulin, and eGFR (β= 0.07 ± 0.02, Model R^2= 0.45, p < 0.0001).

Conclusions: Serum uric acid levels are significantly associated with CF-PWV in a younger Caucasian population. It remains to be determined however whether this is of clinical importance as the variation in CF-PWV explained by uric acid is relatively small.

Table 1: Association between uric acid quartiles and carotid-femoral pulse wave velocity:

Uric acid quartile (mg/dL)	Carotid-Femoral PWV LS MEAN* ± SE	P -value
< 4.2	6.90 ± 0.04	0.002
4.2-5.1	6.94 ± 0.04	0.005
5.2-6.3	7.06 ± 0.04	0.339
> 6.3	7.15 ± 0.04	--

*: Adjusted for age, sex, estimated GFR, smoking, hypertension, fasting glucose, insulin, and body mass index. P-value for the comparison with the 4th quartile

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FR-PO1020

Calcimimetic R-568 Impacts Arterial Structure and Function but Does Not Lower Blood Pressure via the Renin Angiotensin System Navid Shobeiri, Kimberly J. Laverty, Michael A. Adams, Rachel M. Holden. *Queen's University.*

Background: Calcimimetics modulate calcium sensing receptors (CaRs) of the parathyroid gland, thus decreasing parathyroid hormone release. Lowering of PTH levels with calcimimetics (R-568) has been linked to a decrease in vascular calcification and a short-term lowering of blood pressure in animal models. CaRs have also been identified in the renin-angiotensin system (RAS) as well as within the arterial wall, however the direct effect of R-568 on these receptors has not been studied. We investigated the impact of R-568 in RAS mediated blood pressure control as well as its direct impact on vascular calcification and function.

Methods: Spontaneously hypertensive rats (SHR) were given a low salt diet to enhance the RAS and were put on two doses (20 and 50 mg/kg/day, gavage) of the calcimimetic, R-568 for 5 days. Furthermore, rats were also treated with enalapril at a later time point to assess the integrity of their RAS. Blood pressure was recorded every 4 minutes by radio-telemetry for 60 days. Calcification was induced by incubating aortic rings in 3.8mM phosphate in DMEM for four days. Contractility of aortic rings was assessed by wire myography.

Results: Both doses of R-568 significantly reduced serum PTH (52±17% and 65±17% reduction) and serum calcium (34±8% and 34±5% reduction) four hours after dosing. There was no impact of either R-568 dose on blood pressure either short-term (minutes to hours) or long-term (days); however enalapril lowered blood pressure in the same SHR rats on low salt diet (150.8±15.4 to 93.7±14.0 mmHg, $p<0.0001$). R-568 dilated phenylephrine pre-contracted vessels in myographs. Incubation of aortic ring in 3.8mM phosphate in DMEM for four days increased calcification (180±87 nmol/mg); however, addition of R-568 (30μM) decreased calcification (79.9±54.3nmol/mg, $p<0.005$).

Conclusions: R-568 appears to directly affect vascular function and vascular calcification independent of its effect on PTH. The blood pressure lowering effect of R-568 is most likely not RAS mediated.

Funding: Pharmaceutical Company Support - Amgen, Government Support - Non-U.S.

FR-PO1021

AMPK Plays a Significant Role in Modulation of Myogenic Constriction of the Renal Artery Mahdi Hamidi Shishavan, Sjoerd W. Landheer, Hendrik Buikema, Leo E. Deelman, Robert H. Henning. *Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Background: Adenosine Monophosphate Protein Kinase (AMPK) is involved in cellular energy homeostasis. In diabetes, activation of AMPK improves vascular function, resulting in attenuation of diabetic vascular complications. Myogenic tone, the contractile response of vascular smooth muscle cells to increased intralumal pressure, is important in the autoregulation of renal blood flow and pressure.

In the present study we investigated the effects of AMPK activation on myogenic constriction of the renal artery of lean and Zucker Diabetic Fatty rats (ZDF).

Methods: ZDF (n=8) and Lean (n=8) rats, age 17 weeks, rats were sacrificed and intrarenal vessels were mounted in a vascular perfusion setup. Vessels were denuded by phenylephrine (1mM). After equilibration of arteries for 30 min, the first myogenic pressure response curve was assessed by stepwise increasing the pressure in the range of 20-140mmHg. Subsequently, vessels were incubated with AICAR(0.2mM) for 30 min before assessment of a second myogenic pressure response curve. Time controls were included and passive properties of vessels were obtained in Krebs without calcium.

Results: Myogenic responses did not differ between Lean (2427±889) and ZDF (2496±656) but incubation with AICAR resulted in a reduction of myogenic responses in both groups (1511±541, 1236±621, respectively). Although the reduction was most pronounced in ZDF, it did not reach significance($p=0.4$) compared to Lean rats. Time controls did not show a significant reduction in myogenic response. The inhibitory effect of AICAR was significantly correlated with the primary myogenic response in the Lean group ($p=0.03$) but not in ZDF ($p=0.6$).

Conclusions: Findings indicate that AMPK activation inhibits renal artery myogenic constriction. The effect of AICAR was similar in Lean and ZDF, indicating that the effects of AICAR were independent of metabolic status. Results suggest that AMPK activation could contribute to impaired renal autoregulation resulting in increased glomerular pressure. Future studies on the effects of AMPK activation on myogenic response in-vivo are therefore warranted.

Funding: Government Support - Non-U.S.

FR-PO1022

Higher Potassium Intake Is Associated with Reduced Large Elastic Artery Stiffness, Oxidative Stress, and Inflammation in Healthy Middle-Aged and Older Adults Kristen L. Jablonski,[1] M. Chonchol,[1] Matthew Racine,[2] Candace Geolfos,[2] Bradley S. Fleenor,[2] Douglas R. Seals.[2] *[1]Internal Medicine/Renal, University of Colorado Denver, Aurora, CO; [2]Integrative Physiology, University of Colorado Boulder, Boulder, CO.*

Background: Large elastic artery stiffness increases with age and is a strong independent predictor of incident cardiovascular diseases (CVD) in healthy middle-aged and older (MA/O) adults. Dietary potassium may attenuate this age-associated stiffening, but the relation and associated physiological mechanisms are unclear. We hypothesized that greater potassium intake (measured by 24-hr urinary excretion) would be associated with reduced large elastic artery stiffness (as indicated by aortic pulse-wave velocity [aPWV]) and reduced circulating and/or endothelial cell markers of oxidative stress and inflammation.

Methods: Potassium excretion, aPWV and markers of oxidative stress/inflammation were measured at 2 time-points in 14 MA/O adults (62±2 years, mean±S.E.) with moderately elevated systolic blood pressure [SBP] (138±2 mmHg).

Results: Linear regression analysis of these pooled timepoints demonstrated that potassium excretion (72±4 mmol/d) was inversely related to aPWV (817±33 cm/sec; $r=-0.38$, $p<0.05$). This relation persisted when models were adjusted for age, SBP, serum creatinine, sodium excretion, body-mass index and cholesterol ($\beta= -0.38\pm0.15$, $p<0.05$). Potassium excretion was then divided into tertitles. Compared to the lowest tertile (n=10; 29-65 mmol/d), the highest tertile (n=9; 83-106 mmol/d) demonstrated increased antioxidant activity (glutathione peroxidase, 8570±919 vs. 6297±438 U/L; total antioxidant status, 1.48±0.02 vs. 1.40±0.02 mmol/L; endothelial cell manganese superoxide dismutase, 0.61±0.03 vs. 0.43±0.04 A.U.), reduced inflammation (log C-reactive protein, -0.22±0.18 vs. 0.33±0.13 mg/L), as well as increased endothelial cell nitric oxide synthase (eNOS) protein expression (0.42±0.03 vs. 0.24±0.03 A.U.; all $p<0.05$).

Conclusions: These results support the hypothesis that greater dietary potassium intake is associated with reduced large elastic artery stiffness, oxidative stress and inflammation in MA/O with moderately elevated SBP.

Funding: Other NIH Support - AG013038, AG006537, AG03114, AG033994, RR025780

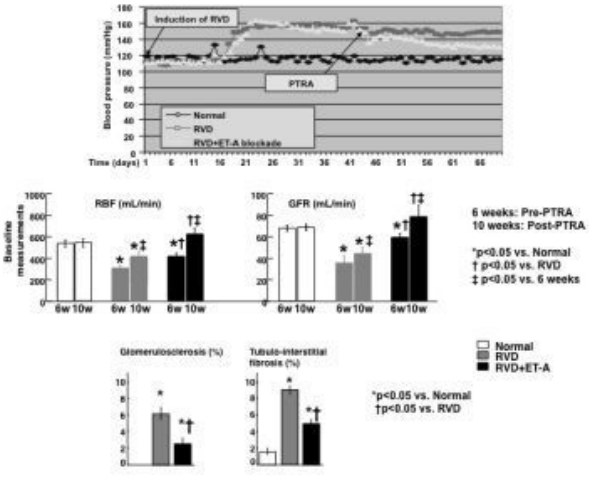
FR-PO1023

Chronic Endothelin-A Receptor Blockade Improved the Responses to Renal Revascularization in Experimental Renovascular Disease Alejandro Chade. *Physiology and Biophysics, and Medicine, University of Mississippi, Jackson, MS.*

Background: Renal angioplasty (PTRA) is the most frequent therapeutic approach to treat renal artery stenosis. However, PTRA improves renal function and/or resolves hypertension in only 1/3 of the patients due to still unknown causes. We have shown that chronic blockade of the ET-A receptor attenuates functional and structural damage in the stenotic kidney during progression of renovascular disease (RVD). However, whether ET-A receptor blockade could improve renal function and hypertension in response to PTRA in RVD is unknown.

Methods: Unilateral RVD was induced in 8 pigs and randomized into untreated (RVD) or chronically treated with ET-A blocker (RVD+ET-A, 0.75 mg/kg/day, oral, n=4 each). After 6 weeks, single-kidney blood flow (RBF) and glomerular filtration rate (GFR) was quantified in the stenotic kidney using multi-detector CT (MDCT). Then, PTRA was performed in all RVD and RVD+ET-A pigs and observed for 4 additional weeks. At 10 weeks, in vivo MDCT studies were repeated. Pigs were then euthanized, stenotic kidneys were removed, and ex vivo studies performed.

Results: Renal stenosis and hypertension were similar in RVD and RVD+ET-A. On the other hand, the blunted RBF and GFR in RVD pigs were largely preserved by ET-A blockade. PTRA resolved RVD in all pigs. However, improvements in blood pressure and renal function were greater in RVD+ET-A+PTRA than in RVD+PTRA, accompanied by a substantial decrease in renal fibrosis.



Conclusions: Chronic ET-A blockade slowed the progression of renal functional and structural injury in RVD and significantly improved the responses to PTRA. These data suggests ET-A blockade as a potential therapeutic co-adjuvant intervention to improve the outcomes of renal revascularization in RVD.

Funding: Other NIH Support - NIH-NHLBI

FR-PO1024

Sustained Renal Vein NGAL Elevation in Human Atherosclerotic Renal Artery Stenosis Despite Reversal of Tissue Hypoxia and Improved Blood Flow after Renal Artery Stenting Ahmed Saad,[1] Sandra Herrmann,[1] Behzad Ebrahimi,[1] John A. Crane,[1] James Glockner,[2] Michael A. Mckusick,[2] Sanjay Misra,[2] Lilach O. Lerman,[1] Stephen C. Textor.[1] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Radiology, Mayo Clinic, Rochester, MN.*

Background: Atherosclerotic renal artery stenosis (ARAS) is known to reduce renal blood flow (RBF) and amplify kidney hypoxia, The relationship between post-stenotic kidney tubulo-interstitial injury and these factors is poorly understood. We hypothesized that renal injury reflected by renal vein levels of acute phase reactant, neutrophil gelatinase associated lipocalin (NGAL), would correlate with RBF, tissue perfusion and hypoxia in subjects with ARAS.

Methods: Inpatient studies performed in ARAS patients (n=18 kidneys, 71±5.5% occlusion) before and 3 months after stent revascularization, or with essential hypertension (EH) (n=42 kidneys), during fixed Na+ intake and ACE/ARB Rx. Single-kidney (SK) cortical,medullary perfusion and RBF measured using multidetector CT, and glomerular filtration rate (GFR) by iothalamate clearance. Tissue deoxyhemoglobin levels (R2*) measured by Blood Oxygen Level Dependent (BOLD) MRI at 3T, as was fractional kidney hypoxia (% of axial area with R2*>30/s).

Results: SK-RBF, perfusion, and GFR were reduced in the post-stenotic kidney. Renal vein NGAL and fractional hypoxia were higher in untreated ARAS than EH. NGAL levels and fractional hypoxia at baseline correlated inversely with RBF and tissue perfusion (r= -0.6, p<.03).GFR correlated inversely with NGAL (r=-0.5, p<.003). After stenting, fractional hypoxia fell with modest increase in RBF, while GFR and NGAL remained unchanged.

Single-kidney	RAS N=18 Before stenting	RAS N=18 After stenting	EH N=42
NGAL (ng/ml)	131±54	123±34	65.8 ± 36**
RBF(ml/min)	269±127	305.6±179*	377±164**
Fractional hypoxia(R2*>30/s)	19.4±14	10.7±9.2*	12.9±9.3**
GFR(ml/min)	27±14	31±17	43.2±13**

Mean ± SD, *P<.05 VS RAS Before. ** P<.02 VS RAS

Conclusions: These data demonstrate that despite reversal of hypoxia and partial restoration of RBF after revascularization, renal vein NGAL remains elevated and GFR fails to recover in ARAS, consistent with ongoing tubulo-interstitial injury in post-stenotic kidneys.

Funding: Other NIH Support - NHLBI

FR-PO1025

Endothelin-Converting Enzyme-1 Is Regulated by Calcitriol in Human Endothelial Cells Patricia Martinez-miguel,[2] Diana Medrano,[2] Vanesa Lopes-martin,[2] Alicia Luengo,[1] Sergio De Frutos Garcia,[1] Ignacio Arribas,[2] Diego Rodriguez-Puyol,[2] Susana Lopez-ongil.[2] *[1]Department of Physiology, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain; [2]Research Unit, Hospital Universitario Principe de Asturias, Alcalá de Henares, Madrid, Spain.*

Background: Secondary hyperparathyroidism is a cardiovascular risk factor. Active analogues of vitamin D such as 1a-25 di-hidroxi-vitamin D3 (Calcitriol) are successfully used in its treatment but with several disadvantages, as its possible effects on the endothelium. Since chronic kidney disease patients present endothelial dysfunction, the present study was designed to assess the intrinsic effects of Calcitriol over endothelial cells, which express vitamin D receptors, where we evaluated endothelin (ET1)-converting enzyme 1 (ECE-1) regulation.

Methods: Human endothelial cells (EA) were incubated at different times and doses of Calcitriol, to evaluate ECE-1 regulation. Expression levels of mRNA and proteins were analysed by Western and Northern blot and its promoter activity by using luciferase reporter plasmids. The ET-1 levels and ECE-1 activity was determined by ELISA.

Results: Calcitriol induced in a dose and time-dependent manner the increase in the ECE-1 transcriptional activity and protein expression, with a slight increase on ET-1 production. The use of ECE-1 promoter serial deletions reversed the transcriptional activity when AP-1 binding sites where depleted. AP-1 activity was confirmed by EMSA analysis and the use of the specific Erk1/2 inhibitor PD98059 or the JNK inhibitor SP-600125: both AP-1 activity inhibitors were able to block Calcitriol-dependent ECE-1 activation, suggesting this effect as AP-1-dependent.

Conclusions: Calcitriol increased ECE-1 expression through activation of AP-1 transcription factor. These results suggest Calcitriol as a potential endothelial function regulator, at least in our in vitro model. However, animal studies will be necessary to go into its physiological consequences.

FR-PO1026

Blood Pressure Independent Effects of ACE Inhibition on Myogenic Tone in Chronic Kidney Disease Sjoerd W. Landheer, Peter Vavrinec, Hendrik Buikema, Leo E. Deelman, Robert H. Henning. *Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands.*

Background: In patients with chronic kidney disease (CKD), angiotensin converting enzyme (ACE) inhibition results in better renal protection than treatment with other antihypertensive drugs. It is suggested that this is caused by additional intrarenal effects of ACE inhibition, beside its systemic antihypertensive effects. Myogenic tone (MT): the ability of arteries to constrict in response to increased intraluminal pressure, protects the kidney from fluctuations in systemic blood pressure. We hypothesized that the additional protective effects of ACE inhibitors include improvement of intrarenal MT.

Methods: We compared the effect of ACE inhibition by lisinopril with an antihypertensive triple therapy that only effects blood pressure on MT in 5/6 nephrectomized rats. Male wistar rats (n=21) underwent a 5/6 nephrectomy or were sham operated (n=7). Six weeks after 5/6 nephrectomy, rats were stratified based on proteinuria into 3 groups and treated for 6 weeks with: vehicle (control, n=7), Lisinopril (n=7) and a triple therapy of reserpine, hydrochlorothiazide and hydralazine (n=7). After sacrification, mesenteric and intralobular arteries with a diameter of 100-200µm were mounted in a vessel perfusion setup for assessment of MT.

Results: After 5/6 nephrectomy, rats became hypertensive and progressively proteinuric. Both lisinopril and triple therapy reduced blood pressure to the level of sham rats, while proteinuria was only reduced by lisinopril. 5/6 nephrectomized rats showed impaired myogenic constriction in both systemic mesenteric and local intralobular arteries. Lisinopril and triple therapy both improved myogenic tone in mesenteric arteries, while myogenic tone in intralobular arteries was only improved by lisinopril.

Conclusions: The protective effect of ACE inhibition on MT in systemic mesenteric arteries is due to its antihypertensive properties, while in intrarenal arteries MT was improved by its additional protective effects. Improved intrarenal MT could be a possible explanation for the finding that ACE inhibitors outperform other antihypertensive drugs in renoprotection in CKD.

Funding: Government Support - Non-U.S.

FR-PO1027

Augmentation of GFR in Critically Ill Traumatic Brain Injured (TBI) Patients Is Dependent on Acuity of Critical Illness and Severity of Trauma Krunal M. Patel, George N. Coritsidis, Parth Rali, Win Naing, Marie France R. DeLeon, Nechama Diamond. *Elmhurst Hospital Center, New York, NY.*

Background: Recent studies have shed light on the augmentation of GFR in the critically ill and primarily in young patients. This may play an important role in pharmacodynamics and especially proper antibiotic dosage.

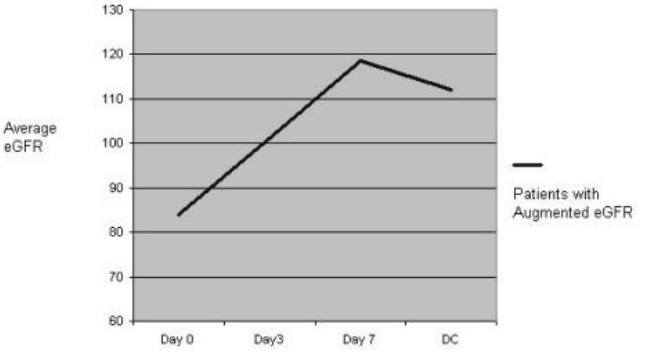
Methods: 135 TBI patient's record were reviewed who admitted to the surgical/trauma ICU between 2008 and 2011 at Elmhurst Hospital, a level 1 trauma center in New York. Patients with CKD-EPI GFR (eGFR) <60cc/min on admission and those that developed AKI were excluded leaving 110 patients. APACHE II, injury severity scores (ISS), eGFR,hospital length of stay (hLOS) were calculated. Patients with increased eGFR by >25% (Augmented GFR) were compare to those that failed to reach >25%(Not Augmented GFR), and first week vancomycin levels were also recorded (n=27 a vs n=24, respectively). Data presented as average ± SEM.

Results: Age, and hLOS of the total 110 patients were 48±1.8 years and 30±2 days respectively, eGFR increased by 18% from admission baseline (98.6± to 116.3±, p<.0001) and declined after 7 days. eGFR increased by 41% in augmented GFR group compare to 8% in non augmented. There were no differences in age, degree of proteinuria, incidence of sepsis,vasopressor use, hLOS between groups. ISS and APACHE were greater in augmented GFR group.

Table 1

Group (N=110)	Augmented(n=43)	Not Augmented (n=67)	p
Δ eGFR	34.5 (41%)	8.9(8%)	NA
Age (years)	47±3	48±2	NS
APACHE II	11.6± 0.9	9.5± 0.7	0.0648
ISS	21.9±2.6	15.8±1.01	0.0161
hLOS (days)	32±3	28±6	NS
Vanco Level(mcg/ml)	8.27	9.05	NS

hLOS= hospital length of stay



Conclusions: In our neuro critically ill patients, eGFR increased significantly by 18%. This rise persisted for at least a week before returning to baseline. When correcting for age, patients achieving higher eGFR had increased trauma and acuity scores.

Funding: Clinical Revenue Support

FR-PO1028

Mitochondrial Protection with Pre- and Postconditioning during Renal Ischemia-Reperfusion Sandrine Lemoine,[1,2] Nicolas Rognant,[1,2] Fitsum Guebre-egziabher,[1,2] Lionel Augeul,[2] Maurice Laville,[1,2] Michel Ovize,[2,3] Laurent Juillard.[1,2] *[1]Néphrologie, Hospices Civils de Lyon, France; [2]CARMEN 1060, Université Lyon 1, France; [3]Cardiologie, Hospices Civils de Lyon, France.*

Background: Ischemia-reperfusion (IR) injuries often cause kidney dysfunction after renal transplantation or aortic clamping. A study on cardiac IR injuries showed that the mitochondrial permeability transition pore (mPTP) plays a key role in activating cell death. The aim of our study is to see whether PreCsA and PostCI help prevent early mPTP opening and electron transport chain dysfunction in kidney mitochondria.

Methods: We conducted a right unilateral nephrectomy with 30-minute contralateral clamping of the left renal artery on C57BL6 mice. The animals were divided into 4 groups: a control group (C), an ischemic group (IR), a preconditioning group with 3mg/kg cyclosporine IV injections 10 minutes before clamping (preCsA), and an ischemic postconditioning group with 3 cycles of 5 minutes each (postCI). We measured calcium retention capacity (CRC) to study mPTP opening, and mitochondrial respiration (expressed in RCR ratio: stage 3/stage 4) 24 hours after reperfusion. Means were compared using Fisher's exact test, with a significant difference $p<0.05$.

Results: In the C group, 404 ± 56 nmol ca^{2+}/mg-proteins were needed for opening the mPTP, while 97±54 nmol ca^{2+}/mg in the IR group, 214±62 nmol ca^{2+}/mg in the preCsA group and 246±68 nmol ca^{2+}/mg in the postCI group. CRC was increased either by preCsA and postCI vs IR ($p=0.006$ and $p=0.002$, respectively); RCR (stage 3/stage 4) for complex I in the electron transport chain was at 5.4 ± 0.49 for group C, 3.5 ± 0.98 for group IR, 4.9 ± 1.2 for group preCsA and 5.4 ± 0.67 for group postCI. Mitochondrial respiration was also improved significantly between preCsA group ($p=0.02$) and postCI group ($p=0.009$) vs IR.

Conclusions: Both preCsA and postCI help prevent IR by delaying the early opening of mPTP that leads to cell death, and by protecting mitochondria from sustained dysfunction of electron transport in the mitochondrial chain.

Funding: Pharmaceutical Company Support - Amgen and Roche

FR-PO1029

Humans with IgA Nephropathy Have Increased Systemic RAS Activity but Decreased Arterial Sensitivity to Angiotensin II Compared to Healthy Controls Ahmed Abdi Ali, Michelle C. Mann, Brenda Hemmelgarn, Jennifer M. MacRae, Tanvir Chowdhury Turin, Darlene Y. Sola, Sofia B. Ahmed. *Deparment of Medicine, University of Calgary, Calgary, AB, Canada.*

Background: IgA nephropathy (IgAN) is associated with increased cardiovascular (CV) risk. Arterial response to angiotensin II (AngII), a measure of endothelial health and local intrinsic RAS tone, is an intermediate measure of CV risk. We sought to compare arterial stiffness, both at baseline and in response to angiotensin (Ang) II challenge, in humans with mild IgAN (eGFR>60, proteinuria < 1g/d) to that of healthy individuals.

Methods: Eight (age 45±5, 88% men) normotensive, non-diabetic, non-obese subjects with IgAN were compared to 37 healthy controls (age 37±2, 35% men). Subjects were studied in high salt balance and women were studied in the same phase of their menstrual cycle. Brachial blood pressure and arterial stiffness, expressed as pulse wave velocity (PWV) and aortic augmentation index (AIx) were measured manually and by tonometry respectively, at baseline and in response to AngII infusion (3ng/kg/min x 30 min then 6ng/kg/min x 30 min). The primary outcome was the impact of mild IgAN on arterial response to AngII challenge at 60min. Baseline variables were adjusted as appropriate for gender, age and BMI using ANCOVA.

Results: At baseline, IgAN subjects demonstrated greater MAP (94±3 vs. 84±2 mmHg, $p=0.009$), PWV (9.2±0.8 vs. 7.6±0.2 m/s, $p=0.07$) and circulating RAS components (plasma renin activity: 0.56±0.2 vs. 0.22±0.03 ng/l/hr, $p=0.006$; AngII: 26±6 vs. 18±1 ng/l, $p=0.03$; aldosterone: 164±34 vs. 131±11 pmol/l, $p=0.3$). The baseline AIx values were similar between the groups(13±4 vs. 12±2.7 %, $p=0.6$). IgAN was associated with a blunted BP response (ΔMAP: 19±3 vs. 30±1 mmHg, $p=0.04$) and arterial stiffness response (ΔPWV: 0.5±0.8 vs. 1.7±0.2 m/s, $p=0.01$; ΔAIx: 12±2 vs. 13±2%, $p=0.9$) to AngII challenge.

Conclusions: IgAN, even in the setting of normal kidney function, is associated with increased blood pressure, arterial stiffness and systemic RAS activity but decreased arterial sensitivity to AngII compared to healthy controls. Increased CVD risk in the setting of IgAN may be mediated, in part, by changes in vascular RAS activity.

FR-PO1030

Increased 25- and 1,25-Hydroxy Vitamin D Levels Are Associated with Enhanced Blood Pressure Responsiveness to Angiotensin II in Healthy Humans David Donald McTavish Nicholl, Ahmed Abdi Ali, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary, Calgary, AB, Canada.*

Background: Reduced 25- and 1,25 Hydroxy Vitamin D (25VD and 1,25VD) levels are risk factors for kidney and cardiovascular disease. Limited studies suggest a role for VD in the modulation of the renin angiotensin system (RAS), though in humans the mechanism remains unclear. We sought to determine the relationship between 25VD and 1,25VD levels and the blood pressure response to Angiotensin II (AngII) challenge, a well-accepted measure of RAS activity, in healthy humans.

Methods: Thirty-five normotensive, non-obese, healthy subjects (11 men, 24 women; 35±2y; 25VD, 68±4 nmol/L; 1,25VD, 100±5 pmol/L) were studied fasted in high salt balance, a state of maximal RAS suppression. Female subjects were studied in the follicular phase of their menstrual cycle. 25VD and 1,25VD were measured at baseline. Blood pressure (BP) was measured every 15 min at baseline, in response to a graded AngII infusion (3ng/kg/min x 30min followed by 6ng/kg/min x 30min), and after a 30 min recovery period. The primary outcome was the relationship between 25VD and 1,25VD levels and greatest BP response to AngII.

Results: 25VD levels were associated with an enhanced mean arterial pressure (MAP), systolic BP (SBP), and diastolic BP (DBP) responses to AngII infusion at 45 min (MAP: $R=0.503$, $p=0.002$; SBP: $R=0.382$, $p=0.02$; DBP: $R=0.482$, $p=0.003$). These relationships remained even after adjustment for covariates (MAP, $\beta=0.16$, $p=0.001$; SBP, $\beta=0.17$, $p=0.007$; DBP, $\beta=0.15$, $p=0.003$). 1,25VD levels were associated with an enhanced SBP response to AngII infusion at 45 min ($R=0.4$, $p=0.02$) and non-significant trends with the MAP ($R=0.339$, $p=0.054$) and DBP ($R=0.2$, $p=0.17$) responses. However, these relationships remained after adjustment for covariates (MAP, $\beta=0.1$, $p=0.023$; SBP, $\beta=0.13$, $p=0.017$; DBP, $\beta=0.08$, $p=0.061$).

Conclusions: Higher levels of both 25VD and 1,25VD are associated with an enhanced hemodynamic response to AngII challenge in healthy humans. Larger, prospective studies are needed to further examine the relationship between VD and the RAS in humans.

Funding: Government Support - Non-U.S.

FR-PO1031

Obstructive Sleep Apnea Treatment Improves Arterial Stiffness and Alters Vascular Sensitivity to Angiotensin II in Humans David Donald McTavish Nicholl, Patrick Hanly, Jennifer M. MacRae, George Handley, Brenda Hemmelgarn, Marc Poulin, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary, Calgary, AB, Canada.*

Background: Obstructive sleep apnea (OSA) is a recognized risk factor for the development of vascular disease, particularly hypertension. Limited studies suggest a prominent role for the renin angiotensin system (RAS), activation of which is deleterious to kidney and cardiovascular function. We sought to determine the effect of continuous positive airway pressure (CPAP) therapy on arterial stiffness and the RAS at baseline and in response to Angiotensin II (AngII), in humans with OSA.

Methods: Ten newly diagnosed (8 men, 2 post-menopausal women; 50±4y) OSA subjects (respiratory disturbance index [RDI]>15) with nocturnal hypoxia [oxyhemoglobin saturation {SaO_2} <90% for >12% of night]) who were otherwise healthy were studied pre- and post-CPAP therapy (1 month of adequate therapy [>4h/night]). Subjects were studied in high salt balance, a state of maximal RAS suppression. Arterial stiffness (aortic augmentation index [Aix] and carotid femoral pulse wave velocity [PWV]) was measured by applanation tonometry at baseline and in response to a graded AngII infusion (3ng/kg/minx30min followed by 6ng/kg/minx30min, and a 30min recovery). The primary outcome was the effect of CPAP treatment on the Aix and PWV responses to AngII at 60 min and the recovery period.

Results: CPAP corrected OSA (RDI: 52±6 vs 5±1hr^{-1}, $p=0.005$; duration SaO_2<90%: 33±5 vs 5±3% of night, $p=0.005$) and reduced baseline AIx (23.2±3.6 vs 17.2±4.7%, $p=0.025$), but did not affect baseline PWV (7.93±0.33 vs 8.15±0.31m/s, $p=0.8$). There was a non-significant increase in AIx (9.2±1.9 vs 12.3±2.9%, $p=0.2$) and a non-significant decrease in PWV (1.41±0.43 vs 0.99±0.66m/s, $p=0.4$) sensitivity to AngII (all values pre- vs post-CPAP). There was no change in how quickly AIx returned to baseline after AngII challenge (3.1±1.8 vs 1.7±3.1%, $p=0.8$), but a more rapid recovery was observed with PWV post-AngII challenge (1.69±0.42 vs 0.43±0.26m/s, $p=0.017$).

Conclusions: Our preliminary observations suggest OSA treatment with CPAP may improve arterial stiffness through changes in vascular sensitivity to AngII.

Funding: Government Support - Non-U.S.

FR-PO1032

Obstructive Sleep Apnea Treatment Improves Renin Angiotensin System Activity in Humans David Donald McTavish Nicholl, Patrick Hanly, George Handley, Brenda Hemmelgarn, Marc Poulin, Darlene Y. Sola, Sofia B. Ahmed. *University of Calgary, Calgary, AB, Canada.*

Background: Obstructive sleep apnea (OSA) is strongly associated with kidney and cardiovascular disease. Limited studies suggest a prominent role for the renin angiotensin system (RAS), activation of which is deleterious to cardiorenal function. We sought to determine the effect of continuous positive airway pressure (CPAP) therapy in patients with OSA on mean arterial pressure (MAP) and RAS components at baseline and in response to Angiotensin II (AngII).

Methods: Ten newly diagnosed (8 men, 2 post-menopausal women; 50±4y) OSA subjects (respiratory disturbance index [RDI]>15) with nocturnal hypoxia [oxyhemoglobin saturation {SaO_2} <90% for >12% of night]) who were otherwise healthy were studied pre- and post-CPAP therapy (1 month of adequate therapy [>4h/night]). Subjects were studied in high salt balance, a state of maximal RAS suppression. MAP, plasma renin activity (PRA), and aldosterone were measured during each study period at baseline and in response to a graded AngII infusion (3ng/kg/minx30min, 6ng/kg/minx30min, 30min recovery). MAP was measured every 15min. The primary outcome was the effect of CPAP therapy on MAP and RAS components at baseline and in response to AngII.

Results: CPAP corrected OSA (RDI: 52±6 vs 5±1hr^{-1}, $p=0.005$; duration SaO_2<90%: 33±5 vs 5±3% of night, $p=0.005$) and reduced baseline MAP (97±1 vs 89±2mmHg, $p=0.007$), PRA (0.27±0.02 vs 0.19±0.03ng/L/s, $p=0.02$), and aldosterone (195±30 vs 122±16pmol/L, $p=0.009$), but did not affect AngII (20±4 vs 20±4ng/L, $p=0.7$). CPAP increased MAP sensitivity to 3ng/kg/min AngII (15min: 10±2 vs 16±2mmHg, $p=0.01$; 30min: 12±3 vs 15±3mmHg, $p=0.1$), but did not affect the MAP response to 6ng/kg/min AngII or recovery. There was a blunted PRA response post-CPAP (3ng/kg/min: -0.10±0.02 vs -0.07±0.02ng/L/s, $p=0.097$; 6ng/kg/min: -0.17±0.02 vs -0.12±0.03ng/L/s, $p=0.05$), but improved recovery (-0.14±0.02 vs -0.09±0.03ng/L/s, $p=0.038$).There were no changes in the aldosterone response to AngII.

Conclusions: CPAP treatment for OSA results in decreased RAS activity and increased hemodynamic sensitivity to AngII. Our results support a role for the RAS in mediating OSA-induced hypertension.

Funding: Government Support - Non-U.S.

FR-PO1033

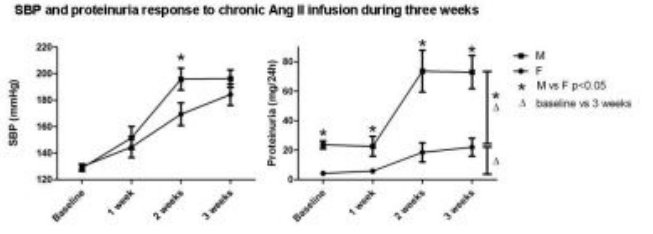
Gender Differences in Vascular and Renal Response to Chronic Angiotensin II in Rats Tsjitske Toering,[1] Anne Marijn van der Graaf,[2] Mienke van der Wiel,[3] Robert H. Henning,[4] Hendrik Buikema,[4] Marijke M. Faas,[2] Gerjan Navis,[1] Titia Lely.[3] *[1]Nephrology; [2]Medical Biology; [3]Obstetrics&Gynaecology; [4]Clinical Pharmacology UMCG, Groningen, Netherlands.*

Background: Epidemiological data show gender differences in susceptibility to cardiovascular and renal disease, possibly due to differences in AngII sensitivity. We studied gender differences in vascular and renal response to chronic infusion of AngII in rats.

Methods: Nineteen weeks old female (F) and male (M) Wistar rats were treated with AngII for three weeks (200ng/kg/min, i.p; F/M:n=11/10) or sham treated (F/M:n=13/10). Systolic blood pressure (SBP) and proteinuria were measured at baseline and weekly. Aortic rings of sham treated rats were mounted for isotonic measurement of vasotonus. AngII sensitivity was studied using response curves in the presence of vehicle, AT2-R blocker PD123319 or AT1-R blocker losartan. Endothelium mediated relaxation was studied after vehicle, nitric oxide (NO) synthase inhibitor L-NMMA or cyclooxygenase (COX) inhibitor indomethacin incubation.

Results: M show a more rapid increase in SBP and proteinuria reaching a plateau after two weeks of AngII (figure). No M-F differences were seen in in-vitro AT1-R-mediated contraction. AT2-R-mediated relaxation was found in F, but not in M ($p=0.03$). Endothelium mediated relaxation was more pronounced in F compared to M ($p<0.01$). L-NMMA reduced relaxation more in M (78±19%) than in F (50±22%;$p<0.01$), while indomethacin increased relaxation in M ($p<0.01$), but not in F. Relaxation in M depends to a larger extent on NO, and is counteracted by constrictive COX products.

Conclusions: These data show gender differences in vascular and renal response to chronic AngII. Differences in AT2-R function and mediators of endothelial relaxation might be involved. Gender should be taken into account in regimens for cardiovascular and renal protection.



FR-PO1034

Long-Term Hemodynamic and Molecular Effects after Renin Angiotensin System Blockade in Humans with Type 1 Diabetes Mellitus David Cherney,[1] Bernard Zinman,[2] Rahim Moineddin,[3] Vesta S. Lai,[1] Judith A. Miller,[1] James W. Scholey,[1] Heather N. Reich.[1] *[1]Division of Nephrology, Toronto General Hospital, University of Toronto; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Department of Medicine, University of Toronto; [3]Department of Family and Community Medicine, University of Toronto.*

Background: Early intensive glycemic control is associated with long term clinical benefits in type 1 diabetes mellitus [T1DM], an effect referred to as "metabolic memory". Animal studies have similarly suggested that blockade of the renin angiotensin system (RAS) can lead to a "memory" effect on vascular tissue and long term benefit. In this context we evaluated the effect of prior RAS blockade on subsequent renal and systemic vascular function and inflammatory cytokine excretion in patients with T1DM.

Methods: We recruited 27 subjects who had completed the 5-year RAS Study [Renin Angiotensin System Study] in Toronto (9 placebo-treated, 18 RAS blockade-treated [enalapril or losartan]). Four years after completing the RASS and off study medication for the same period, we measured renal hemodynamic function, arterial stiffness, flow-mediated vasodilatation (FMD/flow), nitroglycerin responsiveness and urine cytokines/chemokines during clamped euglycemia and hyperglycemia.

Results: Placebo and RAS-blockade treated subjects had similar baseline characteristics. The renal hemodynamic response to clamped hyperglycemia was greater in the RAS blockade group (106±15 to 120±16 ml/min/1.73 m^2) compared with the placebo group (109±11 to 110±16 ml/min/1.73 m^2, repeated measures ANOVA $p=0.025$). In the systemic circulation, FMD/flow during clamped euglycemia was higher in RAS-treated vs. placebo-treated subjects (repeated measures ANOVA $p=0.021$), while arterial stiffness and nitroglycerin responses were the same. Urinary cytokine/chemokine excretion in response to clamped hyperglycemia was exaggerated in placebo vs. RAS-blockade subjects.

Conclusions: A 5 year period of RAS blockade confers long-term protective renal and systemic vascular changes and alters the response to hyperglycemia. Our data suggest RAS-related memory effects on vascular function and inflammatory responses can occur in humans.

Funding: Private Foundation Support

FR-PO1035

Filtered Angiotensinogen, Not Renal Renin, Determines the Renal Angiotensin II Synthesis when Podocytes Lose Their Molecular Barrier Function Iekuni Ichikawa,[1,3] Fumio Niimura,[1] Akira Nishiyama,[2] Taiji Matsusaka.[1] *[1]Tokai University, Japan; [2]Kagawa University, Japan; [3]Vanderbilt University.*

Background: Urinary tract obstruction activates the renal renin-angiotensin (A) system (RAS) through enhancement of renin release. In view of the well-established detrimental effect of AII on the glomerular structure, ureteral obstruction in glomerular disease is expected to exacerbate destruction of glomerular architecture. It was, therefore, truly surprising to find in our recent study with the NEP25 transgenic model of inducible podocyte-selective injury that unilateral ureteral obstruction (UUO) near completely prevented progression of podocyte injury and subsequent glomerulosclerosis in obstructed kidneys (Am J Physiol 2011;300:F792). The results led us to test the possibility that podocyte injury causes derangement in renal RAS regulation.

Methods: Thus, the above UUO protocol was duplicated in NEP25 mice (n=7) by inducing unilateral ureteral obstruction (UUO) for 7 days following induction of podocyte injury.

Results: The level of renal renin activity measured at the completion of study was comparable in obstructed kidneys and kidneys of control sham-operated mice (6.1±0.7 vs. 6.4±0.7 µg AI/H/mg protein). In contrast, the renal tissue AII content was significantly downregulated in the obstructed kidneys when compared to contralateral kidneys (121±31 fmol/g tissue vs. 210±42, $P<0.05$) along with marked suppression of renal angiotensinogen (Agt) protein. These were not observed in sham operated NEP25 mice (n=7) (AII, 308±74 and 316±66).

Conclusions: In conjunction with our recent findings from tissue-specific Agt knockouts that renal AII is derived primarily from the Agt of liver origin (JASN, In press), the results indicate that the protective effect of ureteral obstruction on the glomerulus is, at least in part, attributed to diminution of filtered Agt. Moreover, the study demonstrated that podocyte injury causes shift of the primary regulatory step for the renal RAS from renin activity to filtration of circulating angiotensinogen.

FR-PO1036

Inorganic Nitrite Attenuates Angiotensin II Mediated Contraction of the Renal Microcirculation by Reducing NADPH Oxidase and Increasing Nitric Oxide Xiang Gao,[1] Ting Yang,[2] Jon Lundberg,[2] Erik G. Persson,[1] Mattias Carlstrom.[2] *[1]Medical Cell Biology, Uppsala University, Sweden; [2]Physiology & Pharmacology, Karolinska Institutet, Sweden.*

Background: Oxidative stress and nitric oxide (NO) deficiency are associated with renal and cardiovascular disease. Stimulation of a nitrate-nitrite-NO pathway has shown therapeutic effects, however, the mechanisms are not clear. We investigated the hypothesis that nitrite may moderate renal oxidative stress and influence microvascular function.

Methods: Mouse afferent arterioles were used to investigate effects of nitrite on luminal diameter and NO production (DAF-FM). Contractions to angiotensin II (AngII; 10^{-12} to 10^{-6}M) and L-NAME (10^{-4}M) were studied with nitrite (10^{-5}M) acutely and after nitrate supplementation (10^{-2}M, 7 days). NADPH oxidase activity was measured as Lucigenin-chemiluminescence of superoxide.

Results: Nitrite increased arteriolar diameter and enhanced NO bioavailability (7±1%). Contraction to AngII alone (31±3%), and during NOS inhibition (56±4%), was attenuated with simultaneous nitrite treatment (17±4% & 25±2%, respectively). Dietary nitrate increased circulating nitrite and attenuated the L-NAME+AngII response (32±2%).

Attenuation of the AngII+L-NAME response with nitrite was abolished with NO scavenger (cPTIO; 66±2%) and guanylyl cyclase inhibitor (ODQ; 58±4%). NADPH oxidase activity in isolated/cultured preglomerular VSMC (50±8 x10^3 CLU/min/cells) was reduced with nitrite (33±5 x10^3 CLU/min/cells). In whole kidney, nitrite reduced NADPH oxidase activity and abolished AngII induced stimulation of the NADPH oxidase.

Effects with nitrite were investigated in a mouse model with oxidative stress (SOD1 knockouts). SOD1-deficient arterioles had abnormally strong AngII contraction (89±5%), which was normalized by simultaneous nitrite (48±6%).

Conclusions: Stimulation of a novel nitrate-nitrite-NO pathway attenuates AngII-mediated contraction in the renal microcirculation. Mechanistically, this is associated with reduced NADPH oxidase activity and increased NO. Dietary approaches with nitrate/nitrite may have therapeutic implications in renal and cardiovascular disease.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1037

Angiotensin Converting Enzyme and Glomerular Arteriole Reactivity in Normal and Diabetic Mice Nadine Bouby,[1] Catherine Chollet,[1] Nathalie Caron,[2] Annette Hus-citharel,[3] Lise Bankir.[1] *[1]INSERM U872, Paris, France; [2]FUNDP, Namur, Belgium; [3]INSERM U1050, Paris, France.*

Background: Angiotensin 1 converting enzyme (ACE=kininaseII) insertion/deletion polymorphism is associated with renal prognosis. We previously demonstrated in mice a causal link between the constitutive level of ACE and the renal complications of diabetes mellitus (DM). The present work examined whether this susceptibility to DM nephropathy is related to changes in renal hemodynamic and vascular reactivity induced by ACE level.

Methods: Mice with 2 or 3 copies of the ACE gene (ACE2+, ACE3+, respectively) exhibiting normal or high concentration of plasma and tissue ACE were studied 3 weeks after type 1 DM induction by low doses of streptozotocin. Control ACE2+ and ACE3+ mice received vehicle. Contractile response to AngiotensinII (AngII) or bradykinin (BK) of

microdissected juxtamedullary afferent (AA), and muscular (mEA) or thin efferent (tEA) arterioles was evaluated by intracellular calcium $[Ca^{2+}]_i$ mobilization. Endothelial nitric oxide synthase (eNOS) expression was quantified by RT-qPCR.

Results: Genotype or DM did not alter the dose-response curves to AngII in AA and tEA. In contrast, the maximal response of mEA to 10^{-8} mol/L AngII was decreased by DM ($p<0.01$) in both genotypes. Application of 10^{-6} mol/L BK during the plateau phase of the response to AngII induced a $[Ca^{2+}]_i$ decrease by 10% in tEA and 25% in mEA and AA, with no effect of genotype or DM. eNOS mRNA expression was found only in mEA of ACE3+ mice, and inhibition of NOS with L-Name (2.10^{-5} mol/L) blunted the vasodilatory response to BK or acetylcholine only in mEA of ACE3+ mice, in both control or DM conditions. Prostanoid inhibition with indomethacin (2.10^{-5} mol/L) had no effect on BK response.

Conclusions: An increase in constitutive ACE level induced specific change in signalling pathway of BK in mEA in normal or diabetic conditions. The emergence of the NO pathway might prevent marked vasoconstriction in mEA, that supply medullary blood flow, in situation of high Ang II level and might partially blunt alterations in glomerular filtration rate and medullary blood flow during the early phase of DM.

Funding: Government Support - Non-U.S.

FR-PO1038

Acetazolamide Reduces Glomerular Hyperfiltration and Renal Plasma Flow in Obese Subjects: A Randomized Controlled Trial Boris Zingerman,[1] Arie Erman,[1,2] Sarit Bar Sheshet Itach,[1] Yaacov Ori,[1,2] Michal Herman-Edelstein,[1,2] Uzi Gafter,[1,2] Avry Chagnac.[1,2] *[1]Nephrology, Rabin Medical Center, Israel; [2]Sackler School of Medicine, Tel Aviv University, Israel.*

Background: Glomerular hyperfiltration is one of the factors involved in the pathogenesis of obesity-associated glomerulopathy. Treatment with acetazolamide (Aceta), a diuretic acting on the proximal tubule, increases delivery of sodium chloride to the macula densa and thus has the potential of decreasing hyperfiltration by blunting tubulo-glomerular feedback. The aim of the present randomized controlled study with cross-over design is to examine whether administration of Aceta may reduce obesity-associated increase in glomerular filtration rate (GFR) and renal plasma flow (RPF). Furosemide (Fur) was used as control using an equipotent natriuretic dose.

Methods: Eight male obese subjects (BMI 36.5 [34-53]), with no diabetes mellitus and no hypertension, aged 40±8 yrs participated in the study. Serum creatinine was 0.79±0.08 mg/dl. In Phase 1, following baseline measurement of GFR (inulin clearance) and RPF (aminohippurate clearance), they received by random assignment either intravenous Aceta (5 mg/kg BW) or Fur (2 mg). GFR and RPF were measured during one hour after administration of Aceta or Fur (two 30 min periods). Phase 2 was performed 2 weeks later using the same protocol, where the diuretic not injected at Phase 1 was injected. Volemia was maintained constant throughout the 2 phases.

Results: Natriuresis increased from 1.7± 0.6 to 4.0± 1.1 ($P<0.0005$) and from 1.9± 0.7 to 4.5± 1.5 ($P<0.0005$) mEq/min following Aceta and Fur administration respectively.

		Baseline	Change (%)	
			Post 0-30 min vs Baseline	Post 31-60 min vs Baseline
Aceta	GFR (ml/min)	154±25	-21% ($P<0.0005$)	-24% ($P<0.0002$)
Aceta	RPF (ml/min)	675±64	NS	-17% ($P<0.01$)
Fur	GFR (ml/min)	155±17	NS	NS
Fur	RPF (ml/min)	771±168	NS	NS

vs: versus

Conclusions: Acute treatment of obese subjects with Aceta, a proximally acting diuretic, results in a decrease in glomerular hyperfiltration. This effect may have therapeutic implications for alleviation of obesity-associated hyperfiltration.

Trial registration: NCT01146288.

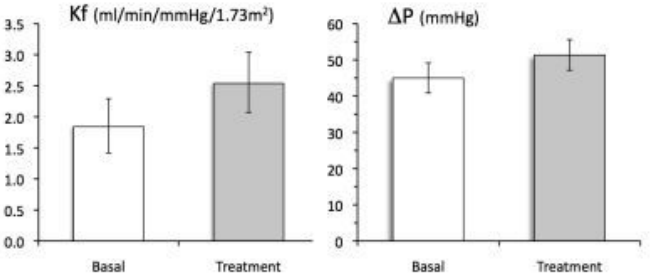
FR-PO1039

Effect of Bardoxolone Methyl on the Determinants of Glomerular Ultrafiltration Andrea Remuzzi,[1,2] Fabio Sangalli,[2] Giuseppe Remuzzi.[2,3] *[1]University of Bergamo, Italy; [2]Mario Negri Institute, Bergamo, Italy; [3]Ospedali Riuniti, Bergamo, Italy.*

Background: It has been recently reported (Pergola et al. NEJM 2011) that a 24 week treatment with bardoxolone methyl was associated with improvement in estimated GFR in patients with advanced CKD and type 2 diabetes, suggesting that this treatment may have promise for amelioration of CKD. We used theoretical analysis of the determinants of GFR to estimate potential changes associated with bardoxolone methyl treatment.

Methods: We used the theoretical model of glomerular ultrafiltration of WM Deen (AJP 1972) to calculate expected values of whole kidney glomerular ultrafiltration coefficient (Kf) before and after 24 week treatment with bardoxolone (150 mg). We initially assumed constant glomerular transmembrane pressure difference (ΔP) to calculate changes in Kf associated with treatment. Subsequently, we assumed constant values of Kf and calculated expected changes in ΔP. Assumed values of eGFR were 32.3 and 42.7 ml/min/1.73m^2 for basal and after treatment evaluation, respectively. We assumed that drug treatment did not affect afferent arteriolar plasma flow and protein concentration.

Results: As shown in the Figure, assuming a constant value of ΔP=45 mmHg, we estimated that the basal value of Kf = 1.84 must increase to 2.54 ml/min/mmHg/1.73m^2 to justify the increase in eGFR associated with treatment, with an average increase of 37.4%. Assuming that Kf remained constant with treatment, we calculated a corresponding increase in ΔP from 45 to 51.3 mmHg, with an average increase of 14.1%.



Conclusions: The results of our theoretical analysis show that the elevation in eGFR observed in type 2 diabetic patients treated with Bardoxolone is more likely the result of an elevation in glomerular capillary pressure (of 6.3 mmHg in average) rather than of a change in Kf that should imply an unattainable increase in glomerular filtering surface area.

FR-PO1040

Renal TGF-β in Transjugular Biopsies Is Associated with Reduced Blood Flow Rather than Hypoxia in Human Atherosclerotic Renal Artery Stenosis (ARAS) Sandra Herrmann,[1] Monika L. Gloviczki,[1] Ahmed Saad,[1] Alfonso Eirin,[1] Michael A. Mckusick,[2] Lilach O. Lerman,[1] Stephen C. Textor.[1] *[1]Nephrology, Mayo Clinic; [2]Radiology, Mayo Clinic, Rochester, MN.*

Background: ARAS reduces renal blood flow (RBF) and amplifies regional kidney hypoxia, leading to inflammatory injury. Transforming growth factor-beta (TGF-β) is an inflammatory cytokine and our previous studies demonstrate higher tissue expression of TGF-β in ARAS as compared to normal kidney donors. The relationships of TGF-β to RBF and tissue oxygenation in ARAS are unknown.

Methods: Single kidney measurements were obtained in the stenotic kidney (STK,n=23) before and 3 months after stent revascularization and compared to non-stenotic kidneys (NSTK,n=11) from the same subjects. Single Kidney GFR and RBF were measured using multi-detector CT. Tissue deoxyhemoglobin levels (R2*) were determined by Blood Oxygen Level Dependent MRI at 3T in the cortical region as was the fractional tissue hypoxia (% of entire axial kidney area with R2*>30s^{-1}). Kidney biopsies obtained from a subset of patients (n=10) before revascularization, stained for TGF-β, were graded on a scale 0=0%, 1<25%, 2=25-50%, 3>50%.

Results: Single kidney RBF and GFR were reduced in the STK. TGF-β tissue score correlated inversely with RBF ($r=-0.67$; $p=0.03$) but not with renal levels of deoxyhemoglobin. Fractional hypoxia was elevated in the STK compared to NSTK and fell to similar levels after revascularization.

Single Kidney Parameter	STK N=23 Before Revascularization	STK After Revascularization	NSTK N=11 Baseline
% R2*>30s^{-1}	20.1±13.4	9.7±8.2†	11.5±5.7*
RBF(mL/mn)	216.2±106.7	272.4±156.9	257.6±164*
GFR(mL/mn)	28.1±13.5	28.9±14.4	50.5±17.4*

†STK before vs STK after Revasc $\rho<0.05$;*NSTK vs STK before Revasc $\rho<0.05$

Conclusions: These data demonstrate reduced RBF in human ARAS is associated with progressively higher tissue TGF-β expression. Our results suggest pre-stent TGF-β expression is not dependent on renal hypoxia. Persistently reduced GFR despite increased RBF and reduction in hypoxic signal to NSTK levels suggest sustained tissue injury. Revascularization may require additional measures, such as those targeted to counter TGF-β, for effective repair.

Funding: Other NIH Support - NIHBL

FR-PO1041

Evaluation of Renal Blood Flow in High-Fat Diet-Induced Kidney Disease Using Contrast Ultrasound Imaging Anne-Emilie Decleves,[1] Joshua J. Rychak,[2] Dan J. Smith,[2] Kumar Sharma.[1] *[1]University of California, San Diego; [2]Targeson, Inc.*

Background: Obesity is a major contributor of progressive renal disease which occurs as a result of complex interactions between metabolic and hemodynamic, and is characterized by alterations in renal blood flow (RBF). However, the changes of RBF are difficult to assess, emostly in small animal models of disease. In this study, we tested the use of microbubble contrast agents for ultrasound (US) to quantitate changes in the RBF.

Methods: C57BL/6J mice were randomized to a standard diet (STD) or a high-fat diet (HFD). To better evaluate the changes in RBF, at 30 week on diet, mice were treated either with Losartan (an Angiotensin II receptor 1 antagonist) or the placebo (normal saline) for 6 weeks. A perfusion contrast agent was administered i.v. to anesthetized mice, and real-time imaging data was acquired at 14 MHz using a clinical ultrasound scanner. The kidney was imaged in the long axis, and cortical blood flow was assessed at low mechanical index (non-destructive imaging). Renal functional and structural studies were also performed.

Results: Ultrasound perfusion imaging provided an excellent method of analyzing blood flow in the mouse kidney. A cortico-medullary flow gradient was readily visualized in all mice. We observed that the time required for the contrast agent to perfuse the cortex, quantified by time-to-peak analysis, was significantly longer in HFD mice relative to STD mice (10.49±1.64 vs 2.71±0.63 sec, $P<0.05$). The treatment with losartan alleviated this change (5.89±0.84 sec, $P<0.05$). These data were concurrent with an increased glomerular filtration rate in HFD mice compared to STD (270.48±30.41 vs 178.5±10.6μl/min,

P<0.05) or HFD+losartan-treated mice (270.48±30.41 vs 183.4±21.51µl/min, P<0.05). Immunostaining of CD31, a marker of endothelial cells, significantly decreased in the HFD suggesting a rarefaction of peritubular capillaries in the tissues.

Conclusions: These data showed for the first time that ultrasound contrast imaging represents a non-invasive method for the evaluation of changes in RBF in a mouse model of obesity. This technique may be useful both in a research setting and in the clinic.

Funding: Other NIH Support - SBIR 10

FR-PO1042

A Novel Vascular Morphology in Perfusion Fixed Human Glomeruli
Chris R. Neal. *Microvascular Research Labs, Physiology and Pharmacology, University of Bristol, Bristol, United Kingdom.*

Background: Human glomeruli have perfused volumes which are large compared to their input arterioles compared to mice or rats. In this study, the human glomerular vascular tree has been reconstructed from perfusion fixed human kidneys, to search for structural changes that may compensate for these large perfusion volumes.

Methods: Unusable human transplant kidneys (4) were perfused with Marshall's solution and transported on ice. Renal arteries were cannulated and the vasculature flushed at 100mmHg hydrostatic pressure with mammalian HEPES Ringer containing Ficoll 400 (25mmHg colloid osmotic pressure). This was followed by a glutaraldehyde fixative solution at the same colloid osmotic and hydrostatic pressures. Tissues were dehydrated, embedded in resin and serial section reconstructions made of glomeruli. Vascular diameters (d) and branching intervals were measured using Image J software from reconstructed images of vascular poles.

Results: In human glomeruli (n=12) both afferent arterioles (d=21±2µm) and efferent arterioles (d=15±2µm) lead into ellipsoidal vascular chambers (VCs). Afferent VCs (mean d=29x50x42 µm) are twice the volume of efferent VCs (mean d=24x36x41 µm) and lead into an average of 6 high capacity unbranching conduits (Capcon vessel; d=14±1µm) that convey blood to peripheral glomerular sectors. The smaller efferent VCs have on average 11 narrower highly branched vessels (d=9±0.5µm) with an occasional wider efferent vessel draining into them.

Conclusions: VCs and Capcon vessels are not apparent in the collapsed vasculature of human renal biopsy. Perfusion fixation at the correct pressures reinflates the glomerular vascular tree to *in vivo* diameters, allowing these vessels to be observed and measured in an *in vivo* state for the first time. Human glomerular VCs and Capcon vessels are unique structures that may effectively distribute pressure and flow among a large population of filtration capillaries in human glomeruli. There are huge implications in glomerular disease where complications can originate at the vascular poles and glomerular periphery at the location of VCs and peripheral Capcon vessels.

FR-PO1043

Vascular Endothelial Growth Factor A and C Modify Glycosaminoglycans in Human Glomerular Endothelial Cells
Rebecca R. Foster,[1] Sian Louise Baker,[1] Robert H. Jenkins,[2] Robert Steadman,[2] Gavin Iain Welsh,[1] Peter W. Mathieson,[1] Simon C. Satchell,[1] Raina D. Ramnath.[1] *[1]School of Clinical Sciences, University of Bristol, Bristol, United Kingdom; [2]Institute of Nephrology, Cardiff University, Cardiff, United Kingdom.*

Background: Glomerular endothelial cells (GEnC) in culture express a surface glycocalyx, consisting of proteoglycan core proteins and glycosaminoglycan side chains (GAG), which contribute to the permeability barrier to macromolecules [1, 2]. Given that vascular endothelial growth factor (VEGF)A and C are both produced by podocytes and affect a wide range of endothelial behaviour including permeability, our aim was to investigate whether they could modify components of the glycoclayx.

Methods: GEnC were treated with vehicle, 1nM VEGFA or 10nM VEGFC in serum containing media in the presence of 20µci/ml [6-^{3}H] glucosamine for 48h to label newly synthesised GAG. Cell media and cell lysate (ammonium hydroxide) were separated and injected onto a DEAE ion exchange column. A salt gradient was used to elute GAG depending on charge (sulphation). Fractions were read on a scintillation counter, then ethanol precipitated, digested with hyaluronidase or left undigested and injected onto a size exclusion column and fractions were read again. Cells were also treated as above in serum free media without radiolabel for 48h and charged GAG were quantified in the media using a colourimetric Alcian blue assay.

Results: Both VEGFA (p<0.05) and VEGFC (p<0.01) increased the synthesis of non-sulphated GAG (0.3M NaCl elution) in the media of cells, confirmed to be hyaluronic acid using size exclusion analysis. VEGFC was shown to increase the degree of charge overall (>0.6M NaCl elution), mostly in the cell media. In contrast, under conditions of stress, VEGFA induced a significant increase in charged GAG release (p<0.05).

Conclusions: In conclusion, we demonstrate for the first time that VEGFs differentially regulate GAG in GEnC in terms of synthesis, charge and release, which may have implications in glomerular filtration barrier regulation.

1. Singh, A., et al. J Am Soc Nephrol, 2007. **18**(11): p. 2885-93.
2. Foster, R.R., et al. *Renal Association*2011: Birmingham.

Funding: Private Foundation Support

FR-PO1044

Renal Tubular Creatinine Secretion Changes in Thyroid Disease
Jubi Den Haan, Marinus A. Van Den Dorpel, Arie Berghout. *Internal Medicine, Maasstadziekenhuis, Rotterdam, Netherlands.*

Background: Serum creatinine levels are decreased in hyperthyroidism and increased in hypothyroidism. Usually creatinine levels return to baseline after treatment, resulting from alterations in renal hemodynamics or an direct effect of thyroid hormone on tubular transporters as studied in animals. We hypothesize that thyroid hormone enhances renal tubular creatinine secretion (RTCS).

Methods: Patients with hyperthyroidism (TSH<0.05mU/L) or hypothyroidism (TSH>12mU/L) were subjected to clearance studies before and 3 months after treatment. Clearance of inuline (Cin) and creatinine (Ccr) were determined. Cin was determined using the continuous infusion method(Inutest®, Fresenius Kabi, Austria) and concomitant Ccr using timed urine collection. RTCS was denominated as the ratio between Ccr and Cin (Ccr/Cin).

Results: Seven patients with hyperthyroidism due to Graves' disease and three patients with autoimmune hypothyroidism were included; two hyperthyroidism patients withdrew and were excluded. In the remaining five hyperthyroid subjects TSH (0.02±0.01 and 1.15±1.00mU/l), FT4 (57.0±25 and 12.2±2.4pmol/l), total T3 (6.8±3.0 and 1.7±0.37pmol/l) were changed (all p<0.05) before and after treatment respectively. Serum creatinine (37±6 and 53±8umol/l) increased and GFR (169±21 and 147±17ml/min/1.73m2) decreased (NS). In the hypothyroid patients TSH (51±17 and 3.63±3.41mU/l), FT4 (5.9±3.5 and 14.3±3.6pmol/l), total T3(1.5±0.26 and 1.7±0.2pmol/l) changed before and after treatment. Serum creatinine (85 ±28 and 75±20umol/l) decreased and GFR (155±24 and 186±39ml/min/1.73m2) increased(NS). The fractional excretion of sodium, chloride, phosphate, calcium and uric acid remainde stable. After treatment of hyperthyroidism Ccr/Cin ratio fell from 2.1±1.80 to 0.91±0.27 (p=0.06), but after correction of hypothyroidism Ccr/Cin remained stable (0.88±0.16 vs 0.92±0.31. Pooled analysis of the change in Ccr/Cin ratio in both groups was near significant (1.65±1.5 vs 0.88±0.28;p=0.07) compared to euthyroidism.

Conclusions: We demonstrated a near significant change in RTCS in a small group of hyperthyroid and hypothyroid patients, supporting our rationale that renal tubular function might be influenced by thyroid hormone.

Funding: Private Foundation Support

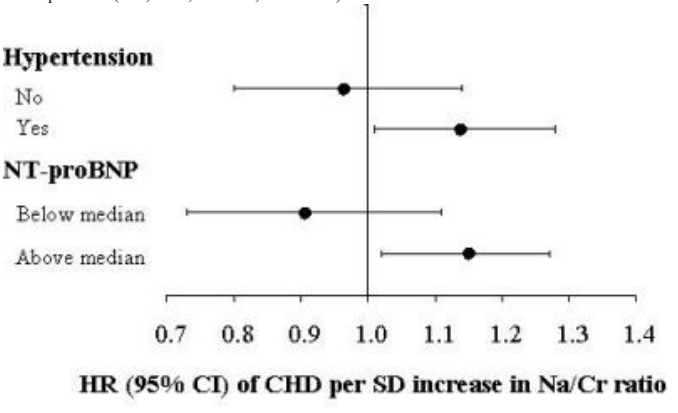
FR-PO1047

Sodium Intake and Risk of Coronary Heart Disease in the Overall Population and Specific Sub-Populations
Michel M. Joosten,[1,2,3] Ron T. Gansevoort,[2] Kenneth J. Mukamal,[3] Hiddo Jan Lambers Heerspink,[2] Johanna M. Geleijnse,[1,4] Gerjan Navis,[2] Stephan J.L. Bakker.[1,2] *[1]TI Food & Nutrition; [2]University Medical Center Groningen; [3]BIDMC; [4]Wageningen University.*

Background: Whether sodium intake is a risk factor for coronary heart disease (CHD) has recently been challenged, despite effects on blood pressure and extracellular volume. We hypothesized that the association between sodium intake and CHD risk would be more pronounced in subjects potentially more susceptible to the adverse effects of high sodium intake.

Methods: We followed 7779 adults without prior CHD at baseline (1997-8) from the observational, community-based PREVEND study. Sodium intake was measured in two 24h urine collections and normalized to creatinine excretion. Hypertension (n=2453 of which 42% used antihypertensive drugs) and N-Terminal pro-B-type Natriuretic Peptide (NTproBNP) levels > the sex-specific median (n=3796) were defined as predisposing factors.

Results: Mean (SD) sodium intake was 144 mmol/24h (51 mmol/24h). During a median follow-up of 10.5 y we documented 464 events. Sodium intake was not clearly associated with risk of CHD in the entire cohort after adjustment for age, traditional CHD risk factors and potassium/creatinine ratio [multivariable hazard ratio (HR), 1.06; 95% confidence interval (CI), 0.97-1.17 per SD increase], but was associated with higher risk in subjects with hypertension (HR, 1.14; 95% CI, 1.01-1.29) and also in subjects with higher levels of NTproBNP (HR, 1.14; 95% CI, 1.02-1.28).



Conclusions: Sodium intake was associated with increased risk of CHD in subjects with hypertension and in subjects with relatively high levels of NTproBNP. These findings warrant emphasis on sodium reduction, particularly in these risk groups. Differences between cohorts in vulnerability to the adverse effects of sodium may partially explain the inconsistencies on this topic.

Funding: Private Foundation Support, Government Support - Non-U.S.

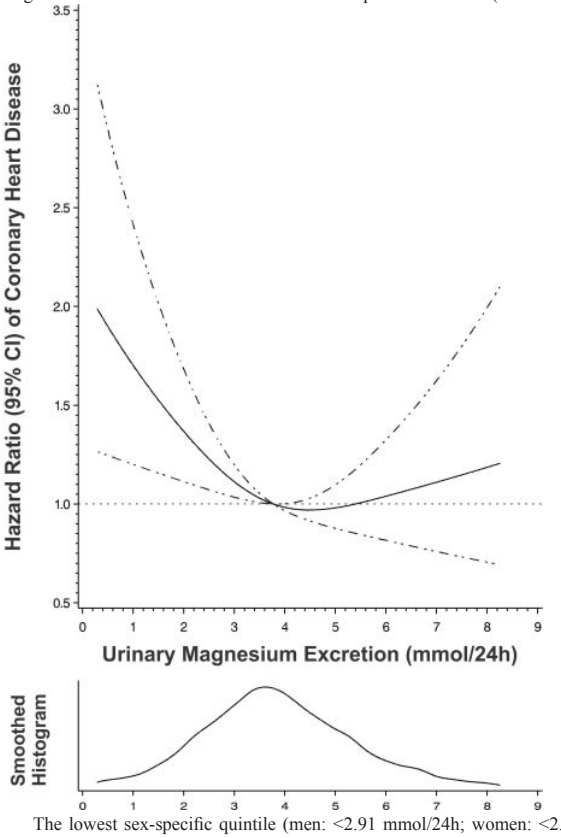
FR-PO1048

Urinary Magnesium Excretion and Risk of Coronary Heart Disease Michel M. Joosten,[1,2,3] Ron T. Gansevoort,[2] Kenneth J. Mukamal,[3] Gerjan Navis,[2] Stephan J.L. Bakker.[1,2] *[1]Top Institute Food and Nutrition, Wageningen, Netherlands; [2]University Medical Center Groningen, Groningen, Netherlands; [3]Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Previous studies on dietary magnesium and risk of coronary heart disease (CHD) have yielded inconsistent results, possibly due to the use of dietary questionnaires, which reflect food magnesium content, but not intestinal magnesium uptake. We investigated whether urinary magnesium excretion, a direct reflection of dietary magnesium uptake, is associated with CHD risk.

Methods: We prospectively followed 7780 adults free of diagnosed CHD at baseline (1997-98) from the community-based, observational PREVEND Study. Urinary magnesium excretion was measured in two 24-h urine collections at baseline.

Results: During a median follow-up of 10.5 year, 465 CHD events occurred. After adjustment for age, traditional CHD risk factors, excretion of creatinine and other cations, magnesium excretion had a threshold relationship with CHD risk (P for curvature=0.02).



The lowest sex-specific quintile (men: <2.91 mmol/24h; women: <2.47mmol/24h) had an increased risk of fatal and non-fatal CHD [multivariable hazard ratio (HR), 1.66; 95% confidence interval (CI), 1.33-2.07) compared with the upper four quintiles. A similar increase in risk for the lowest quintile was observed for mortality related to CHD (HR, 1.57; 95% CI, 1.01-2.46), but not for mortality related to cancer (HR, 0.92; 95% CI, 0.65-1.29) or all-cause mortality (HR, 1.10; 95% CI, 0.88-1.38).

Conclusions: Low urinary magnesium excretion was associated with a higher risk of CHD incidence and mortality. These results suggest that increasing dietary magnesium intake or intestinal uptake, particularly among those with the lowest urinary magnesium excretion, could reduce the risk of CHD. These findings also emphasize the value of 24h-urine collections to assess dietary exposure.

Funding: Government Support - Non-U.S.

FR-PO1049

Identification of Proteins that Interact with NFAT5, Using Peptide Affinity Chromatography and Mass Spectrometry Yuichiro Izumi, Jinxi Li, Maurice B. Burg, Joan D. Ferraris. *NHLBI/SBC, National Institutes of Health, Bethesda, MD.*

Background: Nuclear Factor of Activated T-Cells 5 (NFAT5) induces transcription of osmoprotective genes in response to hypertonicity. NFAT5 is associated with many other proteins, only a few of which have been identified. In order to identify additional proteins that regulate NFAT5 by interacting with it, we performed peptide affinity chromatography. Interactions with transcription factors often involve intrinsically disordered regions, so we concentrated on regions of NFAT5 predicted to be intrinsically disordered.

Methods: Biotin-tagged peptides were synthesized, each containing 20 amino acids from a region in the N-terminus of NFAT5 predicted to be intrinsically disordered (UniProtKB/Swiss-Prot: O94916.1). HEK293 cells were grown in light, medium, or heavy SILAC medium for several passages, then incubated at 200, 300, or 500 mOsm (NaCl varied), respectively, for 30 min. Cytoplasmic or nuclear extracts from the different osmolalities were combined and incubated with peptides and streptavidin-coated magnetic beads to isolate proteins associated with the peptides. Proteins eluted from the beads were digested with trypsin and the resultant peptides analyzed on an Eksigent nanoLC Ultra coupled with a Thermo LTQ-Orbitrap Velos mass spectrometer. LC-MS/MS files were searched via Proteome Discoverer (PD) against Sprot human database for peptide and protein identification.

Results: Coomassie staining of SDS gels showed protein enrichment associated with peptides corresponding to NFAT5 amino acids 191 – 210 and 206 – 225 in cytoplasmic fractions and 161 – 180, 191 – 210, and 206 – 225 in nuclear fractions. Numerous proteins were reproducibly identified in biological replicates associated with peptide baits 161 – 180, 191 – 210 and 206 – 225 in nuclear and/or cytoplasmic fractions. PSPC1, SFPQ and NONO are notable among the proteins identified. We are currently investigating their importance for regulation of NFAT5 by hypertonicity.

Conclusions: Peptide-based affinity chromatography revealed proteins that are associated with NFAT5 and that presumably regulate it in response to hypertonicity.

Funding: Other NIH Support - The Intramural Research Program of NHLBI, NIH

FR-PO1050

Focal Adhesion Kinase Regulates NFAT5 Activity Wolfgang Neuhofer,[1,2] Christoph Küper,[2] Julia Lichtnekert,[1] Maria-luisa Fraek,[2] Franz Beck.[2] *[1]Dept. of Nephrology, Medical Clinic and Policlinc IV, University of Munich, Munich, Germany; [2]Dept. of Cellular Physiology, University of Munich, Munich, Germany.*

Background: NFAT5 is a major regulator of the urinary concentrating process and is essential for the osmoadaptation of renal medullary cells. Focal adhesion kinase (FAK) is a mechanosensitive non-receptor tyrosine kinase that is expressed abundantly in medullary nephron segments. The present study thus addressed the question of whether FAK is involved in NFAT5 activation by hypertonicity.

Methods: FAK activation was monitored by phospho-specific antibodies. FAK inhibition was performed by PF-228 and siRNA-mediated knockdown of FAK. Expression of NFAT5 and appropriate target genes was assessed by qRT-PCR and Western blot analysis. Transcription of the NFAT5 gene was determined using a reporter construct containg 1.0 kb of the human NFAT5 promoter. Transcriptional activity of NFAT5 was monitored by reporter constructs under control of NFAT5, and NFAT5 transactivating activity was analyzed using the binary GAL4 assay. The contribution of the NFAT5 mRNA 3'-UTR on mRNA stability, a luciferase construct containing the full-length NFAT5 3'-UTR was used.

Results: Osmotic stress induced time-dependent activation of FAK as evidenced by phosphorylation at Tyr-397. Both pharmacological inhibition of FAK with PF-228 and siRNA-mediated knockdown of FAK drastically reduced NFAT5 transcriptional activity and target gene expression in HEK293 cells. This effect was not mediated by impaired nuclear translocation or by reduced transactivating activity of NFAT5. However, NFAT5 abundance under isotonic and hypertonic conditions was diminished by 50% by FAK inhibition or siRNA knockdown of FAK. This effect was not caused by reduced transcription of the NFAT5 gene as assessed in reporter gene assays using a 1.0-kb fragment from the human NFAT5 promoter. Rather, NFAT5 mRNA stability was diminished significantly by FAK inhibition, which correlated with reduced activity in a reporter construct containing the NFAT5 mRNA 3'-UTR.

Conclusions: In conclusion, FAK is a major regulator of NFAT5 activity by increasing its abundance via stabilization of the mRNA. This in turn, depends on the presence of the NFAT5 3'-UTR.

Funding: Government Support - Non-U.S.

FR-PO1051

Hypertonicity Increased Intracellular Glutathione (GSH) Content with Reduced GSH Efflux from Cells Masaru Horio. *Functional Diagnostic Science, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Oxidative stress in renal tubular cell is a possible factor inducing cell damage. Hypertonicity is one of the main causes of the oxidative stress. Glutathione (GSH) is an important antioxidant. It is synthesized in the cell and exported to the extracellular space. Intracellular GSH content depends on the rates of the synthesis and the efflux. In the present study, intracellular GSH content and GSH efflux from cells were evaluated in hypertonic MDCK cells.

Methods: Hypertonicity was made by addition of NaCl. GSH efflux was evaluated by GSH content in culture medium.Multiple drug resistance protein (MRP) has been implicated in the GSH efflux. Transport activity of MRP was measured by efflux of carboxyfluorescein, a substrate of MRP. Effects of MK571, a specific inhibitor of MRP, on efflux of GSH and carboxyfluorescein were studied.

Results: Hypertonicity of 400 and 450 mOsm significantly increased intracellular GSH content up to 1.5 fold the value of isotonic cells after 8h. Content of GSH in culture medium was significantly lower in 450mOsm cells compared with 300mOsm cells after 8h (8.8±2.0 and 25±2.2 nmoles/mg protein, respectively). Hypertonicity made by addition of mannitol showed similar results. MRP transport activity measured by efflux of carboxyfluorescein was significantly increased after hypertonic exposure. The efflux of carboxyfluorescein was partially inhibited by 2.5μM MK-571 in both isotonic and hypertonic conditions. On the contrary, the efflux of GSH was stimulated by 2.5μM MK-571 in both isotonic and hypertonic conditions. Addition of MK571 significantly decreased intracellular content of GSH in 450mOsm cell.

Conclusions: Hypertonicity decreased GSH efflux and increase intracellular content of GSH. Low GSH efflux in hypertonic condition might be one of the mechanisms of increasing intracellular GFR content. Effect of MK571 on GSH efflux suggested the participation of MRP in GSH transport, although the effect of MK571 was opposite direction compared with the effect on carboxyfluorescein efflux. The mechanism of GSH transport has remained elusive. Further study is needed.

Funding: Government Support - Non-U.S.

FR-PO1052

Cell Cycle Checkpoint Activation and DNA Damage Response under Hyperosmolal Conditions Are Tissue-Specific Bradley P. Dixon, Brian J. Siroky, John J. Bissler. *Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Children with complex urogenital anomalies frequently require bladder augmentation, and the gastrointestinal tissues utilized for these surgeries are at increased risk for malignancy. We previously demonstrated aberrant cell cycle distribution in colon epithelial cells under hyperosmolal conditions, whereas bladder cells have normal cell cycle distribution under the same conditions. We sought to characterize the cell cycle checkpoint activation and DNA damage response of gastrointestinal and bladder epithelial cells adapted to a hyperosmolal microenvironment.

Methods: Conditionally immortalized colon (YAMC), small intestine (MSIE), gastric (ImSt), and bladder (ULTI) epithelial cell lines were gradually adapted to hyperosmolal conditions with either NaCl or urea, or maintained under isoosmolal conditions, and exposed to etoposide. Cell cycle analysis was performed by propidium iodide staining and flow cytometry. Activation of the DNA damage response was assessed in cell lysates by western blot analysis.

Results: Bladder epithelial cells were able to activate a G1/S phase cell cycle checkpoint under both isoosmolal and hyperosmolal conditions. In contrast, the gastric and small intestine epithelial cells were only able to activate this checkpoint under isoosmolal conditions. Colon, small intestine, and gastric epithelial cells demonstrated an attenuated DNA damage response under hyperosmolal conditions, whereas bladder epithelial cells had a more robust activation of the DNA damage response under such conditions.

Conclusions: The failure of gastrointestinal cells to activate cell cycle checkpoints and the DNA damage response following induction of DNA damage under hyperosmolal conditions may explain the susceptibility to carcinogenesis experienced by gastrointestinal tissues in the augmented bladder.

Funding: NIDDK Support

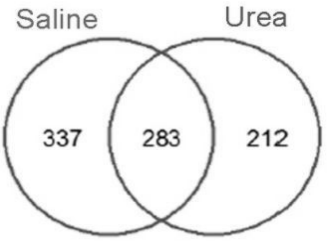
FR-PO1053

Correction of Hyponatremia with Saline versus Urea Induces Deferential Gene Expression Changes in the Brain Fabrice Gankam Kengne, Bruno Couturier, Alain Georges Soupart, Guy Decaux. *Medicine, ULB - Hopital Erasme, Brussels, Belgium.*

Background: Osmotic demyelination syndrome (ODS) occurs after rapid correction of chronic hyponatremia (HypoNa) which can be prevented with urea. The gene expression profile of the brain during ODS and after urea treatment could provide some insight into the pathophysiology and treatment of ODS.

Methods: We performed gene expression analysis in 3 groups of rats: Hyponatremic rats(I), and after its correction with hypertonic saline (II) or urea (III). hypoNa was induced by DDAVP and liquid diet for 4 days. 24 hrs after the correction, the brain RNA was collected and microarray analysis performed (Illimuna platform) and results analysed with genomestudio software. Significant gene changes (fold change >1.5) were uploaded into Metacore software to identify the major differentially affected pathways in the two treatments.

Results: All animals (urea = 5, hypertonic saline =5 and chronic hypoNa =4) had severe hypoNa (Na<120mEq/L) and its correction in group II and III induced an significant increase in serum Na at 24 hrs (30mEq/L). 620 genes were differentially regulated in group II vs 495 in III and only 283 genes were common to both treatments.



Pathway analysis revealed that apoptosis/survival trough endoplasmic stress response and protein folding were the highest differentially affected networks between group II and III.

Top Deferentially affected networks after correction of hypoNa with urea vs saline

Pathways	pValue
apoptosis-ER stress	8.7e-6
Protein folding ER and cytoplasm	1e-4
Reproduction-Gonadotropin regulation	3.9e4
Cytoskeleton Actin filament	4e-4
DNA dammage checkpoint	9e-4

Conclusions: There is a different response of the brain to correction of hyponatremia with urea and hypertonic saline including gene affecting the ER stress response-protein folding. These results could illuminate the pathophysiologic mechanisms involved in ODS.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1054

Analysis of Gene Expression Changes in Rat Brain after Acute and Chronic Hyponatremia Fabrice Gankam Kengne, Bruno Couturier, Alain Georges Soupart, Guy Decaux. *Internal Medicine, Universite Libre de Bruxelles, Brussels, Belgium.*

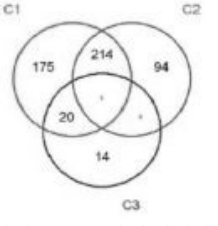
Background: Acute hyponatremia (hypoNa) in contrast to chronic hypoNa produces several CNS symptoms including potentially fatal brain edema. Little is known about the genetic changes underlying that differential effect. Analysis of the gene expression profile in acute vs chronic hypoNa could provide a basis for the understanding of the pathophysiology of the brain adaptation to hypoNa and its treatment.

Methods: We performed gene expression analysis in 3 groups of rats: Normonatremic rats(I), acutely hyponatremic rats (12 hrs of hypoNa) and chronic hyponatremic rats (4 days). hypoNa was induced by DDAVP and liquid diet. We collected brain mRNA and performed miccroarray analysis (Illimuna platform and genome studio software) to identify differentially affected genes in the 2 conditions.

Results: Acute (group I n =5) and chronic hyponatremic rats (II, n=4) had severe hypoNa (Na<120mEq/L) vs normonatremic rats (III, n=5)(Na =140mEq/L). Compared to controls, acute hypoNa induced significant changes (fold change >1.5) in 417 genes and chronic hypoNa induced changes in expression of only 51 genes. When comparing group I and II, we found that 326 genes were differentially regulated.

Comparison	UP-regulated	DN-regulated
c1 – Acute vs. Cont.	225	192
c2 – Acute vs. Chronic	192	133
c3 – Chronic vs. Cont.	32	19

(cutoff: FDR < 5%, FC > 1.5)



Top regulated genes in acute hyponatremia vs chronic hyponatremia included transcription factor arc and hmgn1 which is a downstream target of the osmotic regulator p38MAPK.

Top 5 of upregulated genes in acute vs chfonic hyponatremia

Gene Name	Fold change Acute vs Chronic
Hmgn1	7.39
Gatad2b	6.78
Arc	5.88
Abcg2	4.56
osteoprotegerin	4.09

Conclusions: Chronic hyponatremia induces fewer gene changes in the brain when compared to acute hyponatremia. This could explain the paucity of symptoms seen in chronic hyponatremia. Careful analysis of the differentially affected genes could provide better understanding of the brain adapatation to various forms of anisosmolarity.

FR-PO1055

Functional Expression of P0 (Adenine) Receptor in the Collecting Duct Intercalated Cells in Rat and Mouse Janos Peti-Peterdi,[1] Yue Zhang,[2] Anush Gevorgyan,[1] Donald E. Kohan,[2] Christa E. Müller,[3] Bellamkonda K. Kishore.[2] *[1]Physiology, Univ of Southern California, Los Angeles, CA; [2]Medicine, VAMC & Univ of Utah, Salt Lake City, UT; [3]Pharmaceutical Chemistry, Univ of Bonn, Bonn, Germany.*

Background: The P0 is a Gi-coupled receptor (R), which binds adenine with high affinity as compared to adenosine, AMP/ADP/ATP, and reduces cellular cAMP levels. Blood levels of adenine are markedly increased in chronic kidney disease (CKD) and positively correlate with the duration or severity of CKD. We previously reported that rat kidneys express P0-R mRNA and protein. Hence, intrarenal expression and distribution of P0-R may have a potential role in the pathogenesis of CKD.

Methods: We generated and characterized a peptide-derived polyclonal antibody specific for an 18-amino acid C-terminal sequence of rat P0-R. This antibody detects both rat and mouse P0-R. Using this antibody in confocal immunofluorescence (IF) microscopy we localized P0-R protein in the medulla of rat and mouse and compared to the distribution of AQP2 protein. The inhibitory effect of adenine (10 μM) on the dDAVP (desmopressin; 10 nM)-stimulated cAMP production was determined in freshly isolated inner medullary collecting duct (IMCD) fractions.

Results: In both rat and mouse medulla IF showed P0-R labeling on the apical aspect of the collecting duct. The P0-R protein was predominantly localized to non-AQP2 expressing collecting duct cells, although a few AQP2-positive cells also had sparse PO-R labeling. In IMCD fractions from the rats, adenine significantly reduced dDAVP-stimulated cAMP production (by 43%). Surprisingly, adenine did not affect dDAVP-stimulated cAMP production in mouse IMCD.

Conclusions: P0-R is predominantly expressed in the intercalated cells of the rodent kidney, with minor expression in principal cells. The functional data suggest that significant levels of P0-R may be present in the principal cells of rat, but not in the mouse, to reduce dDAVP-stimulated cAMP production. Conversely, a signal transduction from intercalated cells to principal cells may also be possible. Thus, the markedly increased blood adenine levels in CKD may perturb urinary concentration/medullary function in the kidney.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO1056

Aldosterone Reduces Uptake of Interstitial Albumin into Renal Type A Intercalated Cells Thomas Buus Jensen, M. Umar Cheema, Jeppe Praetorius. *Department of Biomedicine, Aarhus University, Health, Aarhus, Denmark.*

Background: Albumin has been identified in preparations of distal renal tubules or collecting ducts by mass spectrometry. This study aimed to establish whether albumin was a contaminant in those studies or actually present in the tubular cells, and if so, determine the origin of the albumin.

Methods: Immunohistochemistry and immuno-gold electron microscopy were applied to localize albumin in distal tubules. Albumin mRNA expression was assessed by RT-PCR and cellular albumin-fluorescein uptake into MDCK cells on permeable support was determined by fluorescence microscopy. The effect of 24-hour aldosterone administrationon distal tubular albumin uptake was studied in c57/bl6 mice.

Results: Albumin was localized to mouse renal intercalated cells by three different anti-albumin antibodies. In addition to the expected proximal tubular albumin immunoreactivity, double fluorescence labeling with various tubule and cell markers identified type A intercalated cells as the site of albumin in the distal renal tubules and collecting ducts. Albumin did not colocalize with markers for early endosomes (EEA1), late endosomes/lysosomes (Cathepsin D) or recycling endosomes (Rab11). Ultrastructural analysis confirmed the presence of albumin-containing large membrane associated bodies in the basal parts of the intercalated cells. Albumin mRNA was detected in mouse renal cortex but was absent from isolated connecting tubules and cortical collecting ducts. This suggests that type A cells are taking up albumin rather than producing it. Wild type type I MDCK cells showed robust uptake of fluorescein-albumin from the basolateral side but not from the apical side when grown on permeable support. Albumin-aldosterone conjugates were internalized from the basolateral side as well by MDCK cells. Aldosterone administration for 24 and 48 hours decreased albumin abundance in connecting tubules and cortical collecting ducts from mouse kidneys.

Conclusions: We conclude that albumin is taken up from the basolateral side by type A intercalated cells and speculate that the protein might acts as a carrier of less water-soluble substances from the blood to the tubular cells.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1057

Effect of Isotonic and Hypertonic Sodium Chloride and Glucose on Excretion of Urinary Aquaporin2 in Healthy Subjects Janni Majgaard Jensen, Frank H. Mose, Jesper N. Bech, Erling B. Pedersen. *Department of Medical Research, Holstebro Hospital and Aarhus University, Holstebro, Denmark.*

Background: Urinary excretion of aquaporin2 (u-AQP2) is currently used to evaluate the water transport in AQP2 water channels in the principal cells in the nephron. The effect of different types of volume expansion on u-AQP2 has never been studied in a randomized controlled trial in healthy humans. The aim of this study was to measure u-AQP2 after isotonic saline, hypertonic saline and glucose infusion in healthy subjects.

Methods: We studied the effect of isotonic saline 0.9% (23 ml/kg), hypertonic saline 3.0 % (7 ml/ kg) and isotonic glucose 5 % (23 ml /kg) at the end of three periods each of 5 days duration with wash out periods of two weeks between interventions, in a randomized, placebo-controlled crossover study. The study comprised of 23 healthy subjects, who consumed a standardized diet regarding calories, sodium and fluid for 4 days before each examination day. GFR was measured as ^{51}Cr-EDTA renal clearance using continuous infusion technique. We measured blood pressure (BP), free water clearance (CH2O), fractional excretion of sodium (FE_{Na}), plasma concentrations of renin (PRC) and aldosterone (p-Aldo) and urinary concentrations of AQP2 at baseline and after infusion.

Results: After isotonic saline infusion, u-AQP2 and CH2O were unchanged, whereas FE_{Na} increased (167%). After hypertonic saline infusion there was an increase in u-AQP2 (344%) and FE_{Na} (144%), whereas CH2O decreased (-186%). After isotonic glucose infusion there was a decrease in u-AQP2 (-64%) and FE_{Na} (-31%) whereas CH2O increased (230%). Systolic BP, pulse rate and GFR increased slightly and to the same extend during all three infusions. PRC and p-Aldo decreased after isotonic and hypertonic saline infusion, but not after glucose infusion.

Conclusions: The study documented that u-AQP2 reflects changes in water channel activity in the kidney tubule during isotonic and hypertonic saline and isotonic glucose infusion in healthy subjects. The study underlines the value of u-AQP2 to estimate water transport in the distal tubule during changes in extracellular volume.

Funding: Private Foundation Support

FR-PO1058

Effects of Oral Tolvaptan in Patients with Heart Failure, Chronic Kidney Disease and Hyponatremia Mari Katsumata,[1] Nobuhito Hirawa,[1] Keisuke Yatsu,[1] Sanae Saka,[1] Gen Yasuda,[1] Satoshi Umemura.[2] *[1]Nephrology, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan; [2]Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Kanagawa, Japan.*

Background: Tolvaptan, a vasopressin V2 receptor blocker, has a diuretic effect for patients with heart failure. Tolvaptan has been applied to the excessive body fluid state, regardless of using the clinical doses of diuretics. However, there were few data concerning the effects of tolvaptan in patients with chronic kidney disease (CKD).

Methods: We compared the urinary volume and laboratory findings of 10 patients with CKD stage 3-5. Tolvaptan (15mg/day) was administered every day with other diuretics in use. In order to clarify the factors, which relate to the response to tolvaptan, we compared the difference between the responder and the non-responder. Furthermore, we examined the correlations between baseline data and the alterations of urinary volume.

Results: The urinary volume increased from 798 ml/day to 951 ml/day by use of tolvaptan. Concurrently, creatinine increased slightly (from 3.64 mg/dl to 4.16 mg/dl, p=0.02) and urine osmolarities decreased significantly (from 256.0 to 220.7 mOsm/kg, p=0.01). When the patients were divided to the responder (n=5) group and the non-responder group, the urinary volume was significantly higher in the responder group (from 675 ml/day to 1058 ml/day, p=0.017). The responder exhibited a higher serum creatinine levels than that of the non-responder group (4.25 mg/dl vs 2.12 mg/dl, p=0.05). However, the reason was not clear. Hyponatremia was improved dramatically to the normal value, and the augmentations of the sodium concentration were negatively associated with the basal sodium levels (r=-0.91,p<0.001).

Conclusions: Add on treatment with tolvaptan is partly useful to increase diuresis, even in patients with CKD stage 3-5. Furthermore, tolvaptan improve the hyponatremia seen in CKD patients. Thus, tolvaptan might be a good alternative drug for patients with chronic heart failure, renal failure and hyponatremia.

FR-PO1059

Renal Escape from Antidiuresis: Involvement of Key Intrarenal Factors Saad Hussain, Joseph G. Verbalis. *Endocrinology & Metabolism, Georgetown University, Washington, DC.*

Background: Downregulation of renal arginine vasopressin (AVP) V2 receptors (V2R) and subsequent removal of aquaporin-2 (AQP2) water channels play a critical role in escape from AVP-induced antidiuresis. Previous studies have implicated endothelial and neuronal nitric oxide synthetase (eNOS & nNOS) and prostaglandins (PG) in the decrease in renal AQP2 levels that leads to escape, while angiotensin II (Ang II) signaling appears to antagonize escape.

Methods: We studied the role of intrarenal pathways by creating a mouse model of escape and comparing single gene knockout (KO) mice to C57BL/6 wild type (WT) mice. For each experiment, 15 mice were divided into three groups of n=5: 1) KO mice (eNOS, nNOS, or Ang II receptor 1a [$AT_{1a}R$]) fed a gel diet and infused with desmopressin (dDAVP); 2) WT mice fed a gel diet and infused with dDAVP; 3) WT mice fed a solid diet and infused with dDAVP. All experiments were repeated to yield a final n=10 for each group. Urine volume and osmolality, plasma [Na^+] and [K^+], ad libitum water and food intake, and body weight were measured daily. Escape from dDAVP-induced antidiuresis was observed over the course of 5 days.

Results: eNOS KO mice escaped to the same level as gel diet controls without delay in escape (WT Uosm=626 & KO Uosm=633 mOsm/kg H_2O on day 5), but produced significantly lower urine volumes on days 3 & 4. nNOS KO mice showed delayed escape, but eventually reached the same level of diuresis by day 5 (WT Uosm=696 & KO Uosm=639 mOsm/kg H_2O). Escape in $AT_{1a}R$ KO mice was also significantly delayed, but these mice similarly escaped to the same level by day 5 (WT Uosm=637 & KO Uosm=603 mOsm/kg H_2O).

Conclusions: Our data demonstrate that each of these intrarenal signaling pathways likely plays a role in escape from antidiuresis, because no KO strain exhibited the same pattern of escape as the WT mice. However, each of the KO strains did eventually escape from dDAVP-induced antidiuresis. Thus, our data suggest that renal escape in chronically hyponatremic patients is due to a complex signaling system that involves intrarenal eNOS, nNOS, and Ang II, but is not solely attributable to changes in any one of these signaling pathways alone.

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FR-PO1060

A Vasopressin-Induced Change in Prostaglandin Receptor Subtype Expression Provides an Explanation for the Paradoxical Effect of Prostaglandin E_2 on Urine Concentration Marleen L.A. Kortenoeven,[1,3] Michelle Boone,[1] Horst Schweer,[2] Robert A. Fenton,[3] Jack F. Wetzels,[4] Peter M.T. Deen.[1] *[1]Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Department of Pediatrics, Philipps-University Marburg, Marburg, Germany; [3]Department of Biomedicine, Aarhus University, Aarhus, Denmark; [4]Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Urine concentration involves vasopressin (AVP), which via a Gs-cAMP cascade increases transcription of aquaporin-2 (AQP2) water channels and AQP2 redistribution to the apical membrane in renal collecting duct principal cells. Besides AVP, prostaglandins are also involved in water balance regulation. Intriguingly, without AVP, prostaglandin E_2 (PGE_2) increases water reabsorption in the collecting duct, while in the presence of AVP, PGE_2 and $F_{2\alpha}$ ($PGF_{2\alpha}$) decrease water reabsorption.

Methods: To delineate how prostaglandins can exert their diverse effects on water reabsorption, we utilized mouse cortical collecting duct ($mpkCCD_{cl4}$) cells, which endogenously express AQP2 in response to AVP.

Results: In the absence of dDAVP, AQP2 abundance was increased by PGE_2. In the presence of dDAVP, blocking prostaglandin production by indomethacin application increased AQP2 levels, while both PGE_2 and $PGF_{2\alpha}$ application reduced AQP2. dDAVP significantly increased PGD_2 and PGE_2 production, while $PGF_{2\alpha}$ was decreased. RT-PCR showed that, without dDAVP, the prostaglandin receptors EP1, EP4 and FP were expressed. dDAVP application increased the expression of the Gi/q-coupled EP1 and FP receptors, while expression of the Gs-coupled EP4 receptor was decreased.

Conclusions: In agreement with in vivo effects on water permeability, our study showed that PGE_2 increased AQP2 abundance without dDAVP, but PGE_2, as well as $PGF_{2\alpha}$, decreased it in the presence of dDAVP. dDAVP increased $PGD_2$and PGE_2 release, and reduced that of $PGF_{2\alpha}$. As AQP2 abundance is cAMP/Gs-dependent, which is counteracted by activity of Gi/Gq-coupled receptors, the dDAVP-induced increase in Gq-coupled EP1 expression and the decrease in Gs-coupled EP4 expression may explain the paradoxical PGE_2-mediated effects on water permeability.

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FR-PO1061

Impaired Vasopressin Escape in Transgenic Mice Over-Expressing Human CD39: Role of Prostaglandins Bellamkonda K. Kishore,[1] Yue Zhang,[1] Kaiya L. Morris,[1] Karen M. Dwyer,[2] Simon C. Robson.[3] *[1]Medicine, VAMC & Univ of Utah, Salt Lake City, UT; [2]Medicine, Univ of Melbourne, Victoria, Australia; [3]Medicine, BIDMC & Haravard Univ, Boston, MA.*

Background: CD39, an ectonucleotidase (NTPDase1) that hydrolyzes ATP/ADP to AMP, is expressed at several sites within the kidney, and thus may impact the availability of ligands for P2 receptors. Recently we reported that transgenic mice (Tg) over expressing hCD39 have impaired ability to concentrate urine under basal conditions. Hence, we hypothesized that the Tg mice may have enhanced ability to escape from vasopressin-induced anti-diuresis.

Methods: WT and Tg mice (N = 5/genotype) were infused with dDAVP (desmopressin; 2 ng/h) via subcutaneous osmotic mini pumps, with free access to chow and drinking water. After 4 days of dDAVP infusion, the mice were switched to a high water-containing gelled diet as the sole ration (water-loading). Urine output, osmolality and PGE2 excretion were monitored. After 11 days of infusion, the mice were euthanized and renal medullary AQP2 protein abundance was determined by immunoblotting.

Results: Both WT and Tg mice responded equally to dDAVP infusion with no significant difference in urine output and osmolality by day 4. However, water loading caused the onset of AVP escape in WT mice as assessed by increase urine output, and prostaglandin excretion, associated with decreased urine osmolality, which were clear by days 10 and 11. These responses in Tg mice on day 10 and 11 were significantly different ($P < 0.05$) from the WT mice showing a lack of AVP-response. No significant differences in AQP2 protein in WT and Tg mice kidney medullas were found.

Conclusions: The above observations in Tg mice are in line with the previous report of synergistic effects of nitric oxide and prostaglandins in renal escape from AVP-induced antidiuresis (Am J Physiol 284:R354, 2003) and our observations that P2Y2 receptor knockout mice have delayed onset of AVP-escape (JASN 18:114A, 2007). Thus, increased scavenging of extracellular nucleotides in Tg mice might have alter the ability of the kidney to recover from AVP-escape by impaired production of PGE2 and/or impaired P2 receptor signaling.

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FR-PO1062

Forskolin Stimulation Induces Cell Membrane UT-A1 Mono-Ubiquitination and Lysosomal Degradation Hua Su, Conner Carter, Jeff M. Sands, Guangping Chen. *Renal Division, Department of Medicine, Emory University, Atlanta, GA.*

Background: Urea transporter UT-A1 plays an important role in the urinary concentrating mechanism. We previously reported that forskolin stimulation promotes UT-A1 ubiquitination, endocytosis and degradation.

Methods: The cell plasma membrane was isolated by sucrose gradient ultracentrifugation. The type of ubiquitination was judged by immunoblotting with specific ubiquitin antibodies to different linkages of ubiquitin chains. We used ubiquitin activating E1 enzyme inhibitor PYR-41 and deubiquitylase inhibitor PR-619 to block ubiquitination and deubiquitination. The endocytosis and recycling of UT-A1 were measured by cell surface biotinylation and MesNa treatment.

Results: UT-A1-MDCK cells were treated with forskolin for 1 h and the cell plasma membrane was isolated. Forskolin stimulation mainly induced UT-A1 ubiquitination on the plasma membrane. Interestingly, the forskolin-induced UT-A1 ubiquitination is mono-ubiquitination. Inhibition of ubiquitination by ubiquitin activating E1 enzyme inhibitor PYR-41 significantly reduced forskolin-induced UT-A1 endocytosis and degradation. We further found that forskolin stimulated UT-A1 degradation is blocked by lysosome inhibitors chloroquine and NH4Cl. Deubiquitylase inhibitor PR-619 promotes cell surface UT-A1 degradation by reducing protein recycling back to the membrane.

Conclusions: Our study reveals that forskolin stimulation induces UT-A1 mono-ubiquitination on the cell plasma membrane and subsequent protein degradation in the lysosome.

Funding: NIDDK Support

FR-PO1063

Altered Renal Expression of AQP2 and UT-B in α-Galactosidase A Deficient Mice Ji-eun Kim,[1] Su-Youn Lee,[1] Sae Jin Lee,[1] Sung-chul Jung,[2] Ki-Hwan Han.[1] *[1]Department of Anatomy, Ewha Womans University School of Medicine, Seoul, Republic of Korea; [2]Department of Biochemistry, Ewha Womans University School of Medicine, Seoul, Republic of Korea.*

Background: Fabry disease is an inherited disorder caused by deficiency of α-galactosidase A (α-Gal A) resulting in lysosomal accumulation of globotriaosylceramide (Gb3). The purpose of this study was to investigate the mechanism of the decreased urinary concentration, one of the earliest renal manifestation of Fabry disease, in α-Gal A deficient mice.

Methods: Kidney tissues were processed for α-Gal A enzyme activity assay, Gb3 level quantification, immunocytochemistry, and immunoblot analysis.

Results: α-Gal A deficiency caused typical histopathology and significant polyuria that was associated with increased renal Gb3 level. Fabry kidneys showed a significantly increased expression of ER stress proteins, Bip and CHOP. Immunocytochemistry revealed that the expression of CHOP was induced mainly in glomeruli, outer medullary vascular bundles, and medullary collecting duct. CHOP immunoreactivity was also detected in the descending thin limb (DTL) of the loop of Henle. UT-B, AQP2, and AQP1 are key transport proteins involved in urine concentration in vascular bundles, collecting duct, and DTL, respectively. Expression of UT-B and AQP2 proteins significantly decreased in Fabry kidneys, but the abundance of AQP1 protein remained unchanged. Confocal microscopy demonstrated that AQP2 was abnormally localized in the cytoplasm in CHOP-expressing medullary collecting ducts.

Conclusions: These findings suggest that altered expression of AQP2 and UT-B may play an important role in the urinary concentration defect in Fabry disease.

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FR-PO1064

Resveratrol Increases NO Bioavailability in the Rat Thick Ascending Limb via a NOS-Dependent Mechanism Agustin Gonzalez-Vicente, Jeffrey L. Garvin. *Hypertension and Vascular Research Division, Henry Ford Hospital, Detroit, MI.*

Background: About 30% of the NaCl filtered through the glomerulus is reabsorbed in the thick ascending limb (TAL). Nitric oxide (NO) produced by nitric oxide synthase 3 (NOS3) inhibits salt absorption in this segment. The phytoalexin resveratrol has been reported to increase NOS3 expression and activity in vascular endothelium cells. It also inhibits NADPH oxidase, a source of superoxide anion (O_2^-) which can scavenge NO. We hypothesized that in the TAL resveratrol increases NO bioavailability via NOS activation.

Methods: To test this TAL suspensions were prepared from collagenase-perfused rat kidneys, and NO bioavailability measure using 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate.

Results: In the presence of 100μM L-arginine, resveratrol acutely increased NO bioavailability in a dose dependent manner, reaching a maximum at 100μM (2.5 ± 0.3 *vs* 1.6 ± 0.2 AFU/mg/min in vehicle, $p<0.05$, $n=4$). This rise in NO bioavailability was blunted by 80% in the presence of 5mM N_ω-Nitro-L-arginine methyl ester (L-NAME), a NOS competitor inhibitor (1.0 ± 0.3 *vs* 0.2 ± 0.2 AFU/mg/min, $p<0.05$, $n=4$). When 100μM TEMPOL, a O_2^- scavenger, was added to the media, resveratrol was able to significantly increase NO bioavailability to the same extent as in its absence (1.6 ± 0.4 *vs* 1.1 ± 0.3 AFU/mg/min in vehicle, $n=6$).

Conclusions: We conclude that: 1) resveratrol increases NO bioavailability in the TAL; and 2) this is due to activation of NOS rather than a reduction in O_2^-.

Funding: Other NIH Support - NHLBI

FR-PO1065

Individual Roles of the Homologous STE20-Like Kinases SPAK and OSR1 in Vasopressin Signaling along the Distal Nephron Sebastian C. Bachmann,[1] Turgay Saritas,[1] Eric J. Delpire,[2] James A. McCormick,[3] David H. Ellison,[3] Kerim Mutig.[1] *[1]Department of Anatomy, Charité Universitätsmedizin, Berlin, Germany; [2]Department of Anesthesiology, Vanderbilt University, Nashville, TN; [3]Division of Nephrology and Hypertension, Oregon Health & Science University, Portland, OR.*

Background: The Na^+-K^+-2Cl^--cotransporter (NKCC2) of the thick ascending limb (TAL) and the Na^+-Cl^--cotransporter (NCC) of the distal convoluted tubule (DCT) are critical for renal salt handling. Activation of these transporters by vasopressin (AVP) includes their phosphorylation at defined, conserved N-terminal threonine and serine residues. Little is currently known, however, about the kinase pathways mediating this action of AVP. Two homologous Ste20-like kinases, SPAK and OSR1, have been recognized as key enzymes within a signaling cascade providing phosphorylation of the cotransporters. Interactions between the catalytically active, full-length variant (FL-SPAK), the dominant-inhibitory, short variant (KS-SPAK) and OSR1 determine this process. Our study aimed at dissecting the individual functions of SPAK and OSR1 in substrate activation along the nephron at baseline and after AVP stimulation.

Methods: To this end, acute and chronic effects of V2 receptor-specific desmopressin (dDAVP) on the kinases and transporters were evaluated in SPAK-deficient mice and AVP-deficient Brattleboro rats.

Results: SPAK variants displayed prominent regulatory changes along TAL and DCT along with an activation of the cotransporters, whereas OSR1 was less involved. The KS- and FL-SPAK variants were modulated by AVP for their selective interaction with NKCC2 in control of its activation, whereas the phosphorylation of NCC was essentially governed by FL-SPAK alone.

Conclusions: In sum, our data specify how SPAK may serve as a hallmark kinase in modulating Na+ reabsorption along the distal nephron under the endocrine control of AVP.

FR-PO1066

Heat Shock Protein 70-kDa Plays a Role in AQP2 Trafficking in Kidney Collecting Duct Cells Euijung Park, Jung-suk Lim, Hyun Jun Jung, Tae-Hwan Kwon. *Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, Korea.*

Background: The AQP2 is a vasopressin-regulated water channel protein in the kidney collecting duct. Several proteins (Heat shock protein 70-kDa (Hsp70 and Hsc70), annexin II, tropomyosin 5b and heat shock protein 70-5 (BiP)) were identified as interating proteins with C-terminus of AQP2, where phosphorylation of AQP2 (Ser256) occurs. However, it remains unknown whether these proteins play a role in AQP2 trafficking.

Methods: The changes in protein expression were examined by semiquantitative immunoblotting. The effects of siRNA-mediated knockdown of Hsp70 on the AQP2 phosphorylation (Ser^{256}) and trafficking were examined by semiquantitative immunoblotting and cell surface biotinylation; and the effects of dDAVP on the regulation of Hsp70 transcription were examined by in silico analysis and luciferase reporter assay.

Results: Renal abundance of AQP2, Hsp70 and annexin II was significantly decreased in rats with hypokalemia-induced NDI. Immunohistochemistry demonstrated decreased immunolabeling intensity of AQP2, Hsp70, and annexin II and apical targeting of AQP2 in the inner medulla (IM). In contrast, AQP2 and Hsp70 were significantly increased in the IM of dDAVP-infused rats for 5 days. In silico analysis of 5'-flanking regions of AQP2, Hsp70-1 and Hsp70-2 genes revealed that transcriptional regulator binding elements associated with cAMP response were identified at both the Hsp70-1 and Hsp70-2 promoter regions, in addition to AQP2. Luciferase reporter assay demonstrated the increase of luminescence after dDAVP stimulation (10^{-8}M, 6 h) in LLC-PK1 cells transfected with 1 kb of 5'-flanking region in Hsp70-2 construct, but unchanged luminescence in cells transfected with Hsp70-1 construct. Cell surface biotinylation analysis demonstrated that forskolin (10^{-5}M, 15 min)-induced AQP2 trafficking to the apical plasma membrane was significantly attenuated in the mpkCCDc14 cells with siRNA-mediated knockdown of Hsp70-2, which was associated with markedly decreased phosphorylation of AQP2 (Ser256).

Conclusions: Taken together, Hsp70-2 is likely to play a role in AQP2 trafficking, possibly by affecting phosphorylation of AQP2 at Ser 256.

Funding: Government Support - Non-U.S.

FR-PO1067

Dynamics of Aquaporin 2 Phosphorylation at Serine 256 and Serine 261 upon Co-Expression of Constitutively Active Variants of the Calcium-Sensing Receptor (CaSR) in Renal Cells Marianna Ranieri,[1] Grazia Tamma,[1] Annarita Di Mise,[1] Peter M.T. Deen,[2] Maria Svelto,[1] Giovanna Valenti.[1] *[1]Dept of Biosciences, Biotechnologies and Pharmacological Sciences, University of Bari, Bari, Italy; [2]Dept of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: We have recently shown that in MCD4 renal cells, cell surface AQP2 expression in cells exposed to CaSR agonists was higher than in control cells and did not increase significantly in response to short term exposure to forskolin. Those findings were in line with data obtained in hypercalciuric subjects displaying at baseline significantly higher AQP2 excretion and no significant increase in AQP2 excretion and urinary osmolarity after acute DDAVP administration compared to normocalciurics (Procino et al Plos One 2012). This indicates that CaSR-AQP2 interplay represents an internal renal defense to mitigate the effects of rising of calcium during antidiuresis on the risk of calcium precipitation.

Methods: Human wild-type CaSR (hCaSR-wt) and its constitutively active variants (hCaSR-R990G; hCaSR-N124K) were functionally expressed in renal HEK cells stably expressing hAQP2. The N124K mutation is one of eight naturally occurring activating mutations in subjects with autosomal dominant hypocalcemia, whereas R990G is a gain-of-function of the CASR gene polymorphism. Western blotting analysis of a crude membrane fraction was performed using phospho-specific antibodies.

Results: Compared to mock cells, pS256-AQP2 abundance was significantly increased in cells expressing either the wt-CaSR or its activating variants. Of note, we also found a significant increase in pS261-AQP2 in hCaSR-wt expressing cells compared to mock. Interestingly, the expression of pS261-AQP2 was significantly higher in cells expressing the constitutively active CaSR variants with respect to wt-CaSR expressing cells. No change in the pS269 was observed.

Conclusions: Since previous data demonstrated that the amount of pS261 significantly decreases in response to short-term vasopressin exposure, it can be speculated that the increase in pS261 observed in cells expressing constitutively active CaSR variants might counteract the vasopressin response.

Funding: Government Support - Non-U.S.

FR-PO1068

Sildenafil (Sil) Treatment Increases AQP2 Production and Insertion in Lithium (Li)-Induced Nephrogenic Diabetes Insipidus via cGMP-Mediated PKA Activation Talita R. Sanches, Leticia U. De Castro, Antonio C. Seguro, Lucia Andrade. *Nephrology, University of Sao Paulo School of Medicine, SP, Sao Paulo, Brazil.*

Background: Plasma membrane accumulation of AQP2 results from arginine vasopressin type 2 receptor stimulation of adenylyl cyclase, cAMP-mediated activation of PKA, and AQP2 phosphorylation, as well as PKA-induced phosphorylation of the cAMP responsive element binding protein (CREB), which stimulates transcription of the AQP2 promoter. Sil, a type-5 cGMP phosphodiesterase inhibitor, elevates intracellular cGMP and might induce AQP2 membrane insertion, independently of vasopressin receptor activation, by activating a parallel, cGMP-mediated signal transduction pathway. In experimental Li-induced NDI, Sil is known to decrease urinary volume by upregulating renal AQP2.

Methods: Wistar rats received 4 weeks of Li (40 mmol/kg food) or not (control), some also receiving Sil (200 mg/kg food) in weeks 2-4, with or without Li (Li+Sil or Sil). We immunoblotted renal tissue to evaluate expression of AQP2, GSK3β, CREB and phosphorylated CREB (pCREB). In suspensions of medullary collecting duct cells from the papillae of Li-group kidneys—Li+susp (n = 6); Li+Sil+iPKA+susp (n=6, incubated with 5μM Sil and 5 nM PKA inhibitor); and Li+Sil+iPKG+susp (n=6, incubated with 5μM Sil and 100μM Protein Kinase G Inhibitor)—we immunoblotted for AQP2 expression. Data are mean±SEM.

Results: In renal tissue, AQP2 expression in Li+Sil rats was higher than in Li rats but lower than in controls and Sil rats. GSK3β expression was slightly lower in the Li group than in the other groups. Although CREB abundance was similar across groups, pCREB was higher in the Li+Sil group than in the control, Li and Sil groups (355±60.1 vs. 100±0.7, 162±13.4 and169±9.4; $p<0.01$). AQP2 expression was higher in Li+Sil+iPKG+susp than in Li+susp and Li+Sil+iPKA+susp (57.37±4.33 vs. 37.7±5.31 and 36.13±1.73; $p<0.01$).

Conclusions: cGMP mediates activation of PKA-induced AQP2 phosphorylation, which is necessary for membrane accumulation of AQP2. PKA also induced pCREB phosphorylation, which stimulates transcription of the AQP2 promoter. Sil might provide significant clinical benefits to patients suffering from Li-induced NDI.

FAPESP, CNPq.

Funding: Government Support - Non-U.S.

FR-PO1069

Chlorpromazine Inhibits Clathrin-Mediated Endocytosis of AQP2 from the Basolateral Membrane by Selective Basolateral F-Actin Depolymerization Naofumi Yui,[1,2] Hua Ann Jenny Lu,[1] Ying Chen,[1] Richard Bouley,[1] Dennis Brown.[1] *[1]Center for Systems Biology, Program in Membrane Biology, Nephrology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA; [2]Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.*

Background: Recently, we found that AQP2 is continuously targeting to the basolateral plasma membrane in polarized MDCK cells, from which it is rapidly retrieved by clathrin-mediated endocytosis. It then undertakes microtubule-dependent transcytosis. We also found that treatment with chlorpromazine (an inhibitor of clathrin-mediated endocytosis) results in AQP2 accumulation in the basolateral, but not the apical, membrane of epithelial cells.

Results: In this study, we investigated how the chlorpromazine effect on AQP2 might occur. In MDCK cells, both AQP2 and clathrin were concentrated in the basolateral membrane after chlorpromazine treatment (100 μM, 15 min). Interestingly, we found that basolateral, but not apical, cortical F-actin was selectively depolymerized by chlorpromazine. Next, we applied chlorpromazine to rat kidney slices in vitro. After incubation with chlorpromazine, basolateral accumulation of AQP2 and clathrin were significantly increased in collecting duct principal cells, which showed a significant decrease of their basolateral F-actin. As for the MDCK cells, the drug-induced reduction in F-actin was also restricted to the basolateral domain of rat kidney collecting duct cells.

Conclusions: These results further support our new trafficking model of AQP2, which demonstrates continuous basolateral targeting and subsequent apical transcytosis. We show that chlorpromazine selectively depolymerizes basolateral F-actin, and this results in an inhibition of clathrin-mediated endocytosis of AQP2 from the basolateral membrane.

Importantly, the regulation of cortical F-actin organization seems to be different between apical and basolateral domains. We previously showed that vasopressin mediated F-actin depolymerization requires the presence of AQP2. Whether this "catalytic" effect of AQP2 on actin depolymerization is enhanced by chlorpromazine, even in the absence of vasopressin, will be examined in future studies.

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FR-PO1070

Cationic Uremic Toxins Interact with the Organic Cation Transporter, OCT2, in Human Kidney Proximal Tubule Cells Carolien M.S. Schophuizen,[1] Martijn J. Wilmer,[2] Jitske Jansen,[3] Joost G. Hoenderop,[3] Lambertus V. Heuvel,[1] Rosalinde Masereeuw.[2] *[1]Department of Pediatric Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Department of Pharmacology & Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Several organic cations, such as guanidines and polyamines, have been found to accumulate in the plasma of uremic patients due to inadequate renal clearance. Here, we studied the interaction of these cationic uremic toxins with the organic cation transporter, OCT2, in a conditionally immortalized human proximal tubule epithelial cell line (ciPTEC).

Methods: Transporter activity was measured in cell suspensions by studying the uptake of the fluorescent substrate, 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP^+). IC_{50} values were determined for cationic uremic toxins, cadaverine, putrescine, spermine and spermidine (polyamines), acrolein (polyamine breakdown product) and guanidine and methylguanidine (guanidines).

Results: Concentration-dependent inhibition of ASP^+ uptake by TPA, cimetidine, quinidine and metformin confirmed functional, endogenous OCT2 expression in ciPTEC. All cationic uremic toxins tested inhibited ASP^+ uptake by OCT2, of which acrolein was the most potent (IC_{50} = 116 ± 30 μM). A Dixon plot was constructed for acrolein using three independent inhibition curves (10, 20 or 30μM ASP^+), demonstrating a competitive type of interaction (Ki = 93 ±16 μM). This suggests that acrolein is a substrate for the OCT2 transporter. Exposing the cells to a uremic toxin mixture resulted in a more potent and biphasic inhibitory response curve, indicating complex interaction between the toxins and OCT2. Plotting the inhibitory potencies against logP values revealed a correlation of -0.67 ($P < 0.05$), illustrating that hydrophobicity plays a significant role in OCT2 interaction.

Conclusions: OCT2 interactions can be elucidated using ciPTEC. Here, the interaction of cationic uremic toxins, and especially acrolein, was demonstrated, indicating their involvement in the accumulation of uremic toxins in renal disease.

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FR-PO1071

ER-Stress and Endosomal Abnormalities in the Kidney of AQP11 Null Mice Shintaro Kondo,[1] Tadashi Yamamoto,[2] Yasuko Tanaka,[1] Kenichi Ishibashi.[1] *[1]Meiji Pharmceutical University, Japan; [2]Niigata University School of Medicine, Japan.*

Background: AQP11 null mice suffer from fatal kidney failure a month or so after birth due to polycystic kidneys following the huge intracellular vacuole formation in the proximal tubule formed a week or so after birth.

Methods: To obtain the insights into the mechanisms for the vacuole and cyst formations, we compared the gene and the protein expression profiles between the kidney of AQP11 null and wild mice.

Results: At the very beginning of the vacuole formation (postnatal day 3: p3), nine genes were expressed less than half and five genes were expressed more than twice than wild mice including proapoptotic Chac1 and Trib3. As our previous microarray analysis at p7 with full vacuoles documented up-regulation of ER-stress genes, Der13 was compared by real-time PCR to reveal the fifteen fold increase even at p3. At the polycystic stage (p30), 821 genes were expressed less than half and 1172 genes were expressed more than twice including genes related inflammation (Lcn2, Serpina3n, Lyz2, C3, Egr2, Havcr1) and apoptosis (Casp12). As active LC3-II protein was not induced at p3, the vacuole seemed not to be due to autophagy but more likely due to endosomal abnormality as the staining of both EEA1 (early endosome antigen 1) and M6PR, a late endosome marker, were decreased at p1 and the staining of M6PR recovered at p3 with still decreased EEA1 staining. Two-dimensional gel electrophoresis was employed to compare the protein profile of the kidney at the beginning of the cyst formation (p17). Three increased spots were found in AQP11 null mice and the two of them were identified by LC-MS/MS as ER-stress related 78kDa glucose regulated protein (Hspa5) and Major urinary protein 3 (MUP3). Interestingly, MUP3 has also been reported to be increased in the cysts of another PKD model mouse (jck).

Conclusions: In the kidney of AQP11 null mice endosomal abnormalities appeared at the early vacuole formation, when ER-stress was initiated to continue to the stage of the polycysts with epithelial vacuoles.

Funding: Government Support - Non-U.S.

FR-PO1072

Impaired Aquaporin2 Trafficking in Medullary Collecting Ducts of Sodium Chloride Co-Transporter (NCC)/Pendrin Double Knockout Mice Kamyar A. Zahedi,[1] Hassane Amlal,[1] Jie Xu,[1] Sharon Barone,[1] Manoocher Soleimani.[1,2] *[1]Medicine, University of Cincinnati, Cincinnati, OH; [2]Veterans Administration Research Services, Veterans Administration Hospital, Cincinnati, OH.*

Background: Mice with combined deficiency of the sodium chloride co-transporter and Cl^-/HCO_3^- exchanger pendrin (NCC/pendrin-dko) are polyuric and have increased levels of salt excretion compared to wild type (WT) animals. As a result these animals are severely volume depleted and develop pre-renal kidney failure. The adverse hemodynamic effects of combined NCC/pendrin deficiency can be corrected by salt replacement. Furthermore, despite appropriately elevated vaspressin levels, the volume depleted NCC/pendrin-dko mice have impaired urine concentrating ability.

Methods: Western blotting and immunofluorescent studies were performed using aquaporin2 (AQP2) antibodies.

Results: The effect of NCC/pendrin deficiency on the expression and distribution of AQP2 was examined in order to determine the cause of urine concentrating defect and polyuria. Immunofluorescent studies indicated that AQP2 is diffusely distributed in the apical, sub-apical and cytoplasmic regions of principal cells in kidneys of NCC/pendrin-dKO mice. Thus, we hypothesized that the trafficking of AQP2 is altered in NCC/pendrin-dko mice. Phosphorylation of AQP2 on serine residues 256, 264 and 269 is important in its cell surface expression while its phosphorylation on serine 261 is important in its internalization and recycling. In order to test our hypothesis we compared the phosphorylation of AQP2 on serine 261 in wt and NCC/pendrin double ko mice by western blot analysis. Our results indicate that the abundance of serine 261-phosphorylated AQP2 is elevated in the protein extracts from NCC/pendrin-dko mice compared to their WT counter parts. Immunofluorescent examination of kidneys from both genotypes using anti-phosphoserine 261AQP2 confirmed its increased subapical and intracellular localization in NCC/pendrin-dko compared to WT mice.

Conclusions: These studies suggest that altered cellular trafficking and localization of AQP2 is in part responsible for the impaired urine-concentrating ability and polyuria in NCC/pendrin-dko animals.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

FR-PO1073

The Activation of Renal Unfolded Protein Response Reduces Urinary Exosomal Aquaporin-1 Excretion Kanako Shigemura, Hiroko Sonoda, Saki Takahashi, Yoshiki Higashijima, Masahiro Ikeda. *Veterinary Pharmacology, University of Miyazaki, Miyazaki, Japan.*

Background: The unfolded protein response (UPR) is an intracellular signaling pathway in response to the accumulation of misfolded proteins in the endoplasmic reticulum. Renal ischemia-reperfusion (I/R) injury is an important cause of acute kidney injury and several studies have indicated that the UPR is activated after renal I/R mainly in the outer medulla. Also, it has been reported that the UPR activation has a protective role against I/R-induced cell injury. Recently, our group found that urinary exosomal aquaporin-1 (AQP1) excretion was reduced in response to renal I/R, leading to discovery of a novel biomarker. However, the mechanism of the reduction is largely unknown.

Methods: In this study, we asked whether the induction of the UPR affects the urinary exosomal excretion of AQP1, using chemical UPR inducers, including tunicamycin and thapsigargin. Tunicamycin (0.3 mg/kg) was administered to rats by sub renal capsule injection to minimize any indirect action under in vivo conditions. Urine samples were collected at 18-24 h after the administration. Urinary exosomes were isolated from the urine samples by differential ultracentrifugation.

Results: Real-time PCR analyses showed that the treatment with tunicamycin dramatically increased GRP78 and CHOP mRNAs (known to be markers for activation of the UPR), and decreased AQP1 mRNA in the outer medulla. These responses were accompanied with the decreased abundance of urinary exosomal AQP1 protein, but not with that of AQP2 protein. When tunicamycin (3 mg/ml) or thapsigargin (1 mM) was added to cultured proximal tubule cells (NRK-52E cells), up-regulation of GRP78 and CHOP mRNAs, and down-regulation of AQP1 mRNA were also observed.

Conclusions: These findings suggest a possible mechanism by which the UPR pathway mediates the reduction of urinary exosomal AQP1 protein excretion following renal I/R.

FR-PO1074

Compartmental Discrepancies in Uremic Toxin Composition and Implications on Solute Removal by Dialysis Leonard Ebah, Milind Nikam, Anuradha Jayanti, Angela M. Summers, Paul E. Brenchley, Sandip Mitra. *Manchester Institute of Nephrology and Transplantation, Manchester Royal Infirmary, United Kingdom.*

Background: Uremic toxin (UT) removal is a key objective of dialysis, its efficacy often measured by plasma solute clearance. Little is known about extraplasmatic UT composition and the effect of dialysis on extravascular compartments such as interstitial fluid (IF). We compared IF and plasma UT composition.

Methods: Microdialysis was used to sample subcutaneous IF and measure its composition in a range of UTs (urea-MW 60, creatinine-MW 113, phosphate-MW 95, urate, MW 168) both off and during haemodialysis(HD). A novel minimally invasive

microneedle array was developed and used to directly sample subcutaneous IF. GCMS and LCMS-MS were applied to paired plasma and IF samples of CKD patients to compare their metabolomic and proteomic profiles.

Results: Off dialysis, IF small toxin levels correlated closely to that of plasma (r=0.98, 0.94, 0.74 and 0.82 for plasma vs. IF urea, creatinine, phosphate and urate respectively). However, paired analysis of the whole group revealed subtle but significant differences between plasma and IF urea (15.4±7 vs. 14.4±7mmol/L; p=0.01), creatinine (397.4 ±286 vs. 344.7±255 μmol/L; p=0.02) and urate (0.4±0.2 vs. 0.3±0.2 mmol/L; p=0.03). During HD, a significant lag of IF toxin decay curves occurred in ~30% of cases. Metabolomics revealed over 5000 metabolites of <1000Da within IF; 40 of these corresponded to known small and protein-bound UTs. Thousands of other metabolite peaks were detected (some possibly yet unidentified UTs), with marked discrepancies between plasma and IF levels, indicating that the two fluid types exhibit different metabolite profiles in CKD. Proteomics of IF and plasma revealed markedly different profiles, with middle molecules such as beta-2 microglobulin, cystatin C, IgG kappa light chain, IgG lambda light chain and complement factor D consistently found in significantly higher quantities in IF than in plasma.

Conclusions: IF is a toxin-rich environment and certain toxins may be unevenly distributed within extracellular fluid with a possible sequestration in IF, potentially rendering them harder to clear by dialysis.

FR-PO1075

Copeptin as a Novel Serum Marker of Upper and Lower Urinary Tract Infections Anna Masajtis-zagajewska, Ilona Kurnatowska, Malgorzata Jadwiga Wajdlich, Michal P. Nowicki. *Department of Nephrology, Hypertension and Kidney Transplantation, Medical University, Lodz, Poland.*

Background: A differentiation between lower and upper urinary tract infection (UTI) depends mainly on clinical presentation with atypical and variable symptoms posing a diagnostic challenge.

Vasopressin has recently attracted scientific interest as a diagnostic and prognostic biomarker, e.g. in sepsis and pneumonia. Copeptin is a stable C-terminal pro-vasopressin Thus, it could become an important biomarker in infections, including UTI. In this pilot study we compared the discriminatory usefulness of serum copeptin with conventional inflammation markers such as C-reactive protein (CRP), procalcitonin (PCT) and IL-6 in patients with upper and lower UTI.

Methods: The study comprised 24 patients; 11 with lower (9F,2M) and 13 with upper UTI (10F,3M). Serum markers and urine cultures were assessed immediately before commencing an antibiotic treatment and 24, 48 hours and 7 days thereafter.

Results: Before an antibiotic therapy serum levels of all biomarkers and copeptin were increased in both groups. In upper UTI group only serum CRP (93.1±71.2 mg/dl) and PCT (1.6±3.1 ng/ml) were significantly higher than in patients with lower UTI (14.5±38 mg/dl and 0.05±0.001 ng/ml, respectively). On day 7 of the antibiotic treatment all inflammatory markers and copeptin levels decreased significantly Serum copeptin levels were decreased to the similar extend in both groups of patients. In contrast, a decrease of serum CRP and IL-6 levels was significantly greater in patients with upper than lower UTI. A decrease of PCT level was much lower in both groups.

Conclusions: Serum Copeptin seems to be a useful inflammation marker in UTI. However, serum levels of CRP and IL-6 may be more suitable for the discrimination between lower and upper UTI and for the assessment of the effect of antibiotic therapy.

Funding: NIDDK Support

FR-PO1076

Drinking Effect on Aquaporins 1 and 2 and eNOS Expression, in Renal Medulla of Acute Sodium Overload Rats Silvana L. Della Penna,[1] Maria Ines Rosón,[1] Gabriel Cao,[2] Elsa Zotta,[3] Marcela Pandolfo,[1] Andrea Fellet,[1] Ana Balaszczuk,[1] Lorena Sarati,[1] Jorge E. Toblli,[2] Belisario Fernandez.[1] *[1]Pathophysiology Department, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Buenos Aires, Argentina; [2]Experimental Medicine Laboratory, Hospital Aleman, Buenos Aires, Argentina; [3]Physiology Department, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.*

Background: The objective was to investigate the effects of water drinking on aquaporin 1 and 2 expressions (AQP1 and AQP2 respectively) in renal medulla of rats subjected to an acute sodium overload and to explore its association with eNOS expression.

Methods: Male Sprague-Dawley rats were intraperitoneally injected with 2ml/100g of body weight with the following solutions: Controls: C group (saline: 0.15 mol/L NaCl) and experimental Na group (hyperosmolar: 0.80 mol/L NaCl). Then the animals rested in the animal care room for 2 h with free (+W) or restricted (-W) access to drinking water. Then, plasma levels of Na^+, Cl^-, K^+ and proteins were measured and eNOS, AQP1 and AQP2 expressions were detected in renal medulla by Western-blot and immunohystochemical stain.

Results: Plasma Na^+ and proteins were significantly higher in Na–W group than in C-W group ($P<0.05$). AQP2 expression increased in Na+W group compared with C+W group: AU (arbitrary units); C+W: 1.20 ±0.07, Na+W: 1.84±0.08, $P<0.05$. AQP2, AQP1 and eNOS expression decreased in Na-W group compared with Na+W group (AU, AQP2: Na-W: 1.47±0.10, Na+W: 1.84±0.08, AQP-1, Na-W: 0.77±0.11, Na+W: 1.27±0.19, $P<0.05$; eNOS, Na-W: 0.77±0.03, Na+W: 1.49±0.09, $P<0.05$).

Conclusions: Urinary sodium concentration may be favored in animals subjected to an acute saline overload, by increased AQP2 levels in renal medulla, when they have free access to drinking water. However, drinking restriction to these rats could facilitate urinary sodium excretion, by decreasing AQP1, AQP2 and eNOS expression in renal medulla.

Funding: Government Support - Non-U.S.

FR-PO1077

Aqp5 Is a New Dot1a Transcriptional Target and Links Aldosterone to Polyuria Wenzheng Zhang,[1] Hongyu Wu,[1] Lihe Chen,[1] Qiaoling Zhou,[2] Oleh Pochynyuk,[1] Xi Zhang.[1] *[1]UTHSC, Houston, TX; [2]Central South Univ, Houston, TX.*

Background: Histone methyltransferase Dot1a represses ENaC in aldosterone (aldo) signaling network. High [aldo] induces polyuria. We developed $Dot1l^{AC}$ mice that lack Dot1l and thus H3 dimethylation (H3m2K79) in $Aqp2^+$ cells. $Dot1l^{AC}$ vs. $Dot1l^{f/f}$ mice increased intercalated cells (IC) by 20% in the expense of principal cells (PC) (Wu et al, 2011 ASN abstract # 21278).

Methods: Mice on the normal Na+ pellet were used for urine analysis, patch clamping, RT-qPCR, IB, IF and ChIP. Urine analysis was also done with mice on a normal Na+ gel diet, after 24-h H2O deprivation or aldo infusion. RNAi knockdown and luciferase assays were done in IMCD3 and MLE15 cells.

Results: $Dot1l^{AC}$ vs. $Dot1l^{f/f}$had normal ENaC mRNA levels, but significantly reduced ENaC Po and effective ENaC activity; 2) $Dot1l^{AC}$ vs. $Dot1l^{f/f}$ elevated the urine volume by 83 ± 29% and decreased urine osmolarity by 34 ± 9.1% with normal Aqp2 mRNA and protein expression. $Dot1l^{f/f}$and $Dot1l^{+/+}$ Aqp2Cre had similar urine volume and osmolarity. $Dot1l^{AC}$ also had polyuria on the gel diet or after water deprivation; 3) Aqp5 mRNA is >100-fold and 12-fold higher in $Dot1l^{AC}$ and $Dot1l^{+/+}$ Aqp2Cre than in $Dot1l^{f/f}$,respectively. Aqp5 immunostaining was robustly detected in most of PC in $Dot1l^{AC}$, but barely detectable or very faintly in controls; 4) ChIP showed strong binding of Dot1l and H3m2K79 in 4 out of 12 regions covering the 6.5 kb between Aqp2 and Aqp5 in $Dot1l^{f/f}$. The binding was significantly impaired in each of these 4 regions in $Dot1l^{AC}$; 5) Dot1a depletion significantly increased Aqp5 mRNA by >10-fold in IMCD3 cells; 6) Dot1a overexpression significantly decreased expression of Aqp5 and Aqp5-promoter luciferase reporters in MLE15 cells. 7) Aldo-infused mice significantly increased Aqp5 mRNA and urine volume.

Conclusions: ENaC mRNA level in $Dot1l^{AC}$ may be counterbalanced by loss of Dot1a-mediated repression and a decreased PC population. The PC towards IC shifting may lead to the impaired ENaC function. Aqp5 is likely a new aldo and thus Dot1a transcriptional target. Dot1a and Aqp5 seem to be the missing components linking aldo to polyuria.

Funding: NIDDK Support

FR-PO1078

Aqp5 Interacts with Aqp2 and Impairs Its Membrane Localization Wenzheng Zhang,[1] Lihe Chen,[1] Hongyu Wu,[1] Qiaoling Zhou,[2] Xi Zhang.[1] *[1]UTHSC, Houston, TX; [2]Central South Univ, Changsha, China.*

Background: While Aqp5 is the closest homolog of Aqp2 and shares 66% sequence identity with it, Aqp5 expression is undetectable in normal mouse kidney. $Dot1l^{AC}$ mice lack H3 K79 dimethylation (H3m2K79) in $Aqp2^+$ cells, express substantial Aqp5 mRNA and protein, and display polyuria (Wu et al, 2012 ASN abstract # 1439). We hypothesize that Aqp5 contributes to polyuria, partially by impairing Aqp2 membrane expression.

Methods: IF, Co-IP, and confocal microscopy were used to examine if AQP2 and AQP5 interact in vivo in mouse kidney and in kidney biopsies from patients with minimal change disease (MCD) and patients with diabetic nephropathy (DN), and in vitro in transfected IMCD3 cells. Cell surface biotinylation was done to determine the effect of AQP5 overexpression on AQP2 membrane localization in IMCD3 cells.

Results: Aqp5 and Aqp2 colocalized in most of Aqp2+ cells in $Dot1l^{AC}$, with barely detectable Aqp5 in $Dot1l^{f/f}$. $Dot1l^{AC}$ vs. $Dot1l^{f/f}$ had apparently reduced apical distribution of Aqp2, with diffuse Aqp2 at both apical and basolateral sides; 2) All of the 15 MCDs had undetectable AQP5, with AQP2 primarily seen at the apical side. All 17 DN samples contained $AQP2^+AQP5^-$, $AQP2^+AQP5^+$, $AQP2^-AQP5^+$ and $AQP2^-AQP5^-$ tubules. In the $AQP2^+AQP5^+$ tubules, these two proteins were typically colocalized as large discrete foci near the perinuclear region. 3) All MCDs had strong H3m2K79 expression. Significant reduction of H3m2K79 was seen in all of the DN samples. Such reduction occurred throughout the whole biopsies in some cases or focally in others, to various degrees. Most of AQP5+ cells had weak or no H3m2K79 at all in the nuclei; 4) RFP-AQP5 and GFP-AQP2 colocalized as large discrete foci in the perinuclear region in IMCD3 cells; 5) GFP-AQP2 was specifically coimmunoprecipitated with FLAG-AQP5 in IMCD3 cells; 6) Myc-AQP5 significantly decreased plasma membrane-associated FLAG-AQP2 to about 21% of Myc vec in IMCD3 cells.

Conclusions: Aqp5 interacts with Aqp2 and apparently impaired Aqp2 membrane localization in vivo in kidneys of Dot1lAC mice and in kidney biopsies of patients with DN, and in vitro in transfected IMCD3 cells.

Funding: NIDDK Support

FR-PO1079

Remission of Crescentic Glomerulonephritis due to Henoch Schonlein Purpura in an Elderly Adult Achieved with Rituximab and Glucocorticoid Therapy Panupong Lisawat, Varun Agrawal, Pamela C. Gibson. *Fletcher Allen Healthcare.*

Background: Henoch-Schonlein Purpura (HSP) is an uncommon systemic vasculitic disease in adults due to immunoglobulin A (IgA) deposits predominantly in the skin, joints and glomeruli. Eleven percent of the patients with HSP are reported to progress to end stage kidney disease. We present an interesting case of crescentic HSP nephritis in an elderly adult that was successfully treated with rituximab and glucocorticoids.

Methods: A 78-year-old woman was hospitalized for lower extremity palpable rash for 3 weeks. She had acute kidney injury (serum creatinine [Cr] 1.5 mg/dl, with baseline Cr 0.9 mg/dl six months ago). She complained of swelling involving her face and both feet.

She had hypertension and temporal arteritis that was treated with prednisone 15 months ago. Biopsy of skin rash revealed leukocytoclastic vasculitis that stained with anti-IgA conjugate. Urine studies were notable for 3+ blood, protein: creatinine ratio of 20 g/g and dysmorphic RBCs. Her Cr continued to increase to 1.8 mg/dl. Kidney biopsy was significant for diffuse necrotizing glomerulonephritis with cellular crescents (involving 53% and 9% glomeruli respectively). Promient subendothelial, mesangial and paramesangial staining with IgA (3+) was seen. Crescentic HSP was diagnosed. Cyclophosphamide therapy was considered, but was not favored for concerns of toxicity in the elderly. We initiated induction therapy with rituximab and pulse methylprednisolone, followed by maintenance therapy with prednisone (1mg/kg/day) tapered over 6 months. At 6 months, her Cr was 1.6 mg/dl with hematuria but no proteinuria (urine protein: creatinine ratio of 0.1g/g).

Conclusions: Crescentic glomerulonephritis in HSP is an uncommon cause of RPGN with a paucity of randomized clinical trials to guide therapy. We speculate that Rituximab, by its anti CD-20 activity, may have altered IgA production by B lymphocytes resulting in remission in our patient. Our case highlights rituximab with glucocorticoids as a therapeutic option for treating crescentic GN associated with HSP in an elderly adult while avoiding cyclophosphamide toxicity.

FR-PO1080

Successful Stenting of Acute Renal Artery Dissection in a Patient with Vascular Ehlers Danlos Syndrome Panupong Lisawat, Richard J. Solomon. *Division of Nephrology, University of Vermont, Burlington, VT.*

Background: Endovascular procedures have increased in treating many vascular complications in Vascular Ehlers Danlos Syndrome (VEDS). However, the experience in treating renal artery complications is limited. We describe a case of VEDS that had bilateral renal artery dissections and renal infarction that was successfully treated with endovascular stenting.

Methods: A 41-year-old female with a history of Vascular Ehlers-Danlos syndrome (VEDS) presented with right-sided back pain that resembled the pain that led to left nephrectomy 14 years previously. On examination, she was not in distress but her blood pressure was >200 mmHg systolic during episodes of uncontrolled pain. She had mild right upper abdominal tenderness with right costovertebral angle tenderness. Her serum creatinine was 1.2 (baseline of 0.8.). She underwent renal angiography which revealed a dissection with decreased flow in the posterior division of right renal artery and focal dissection with focal stenosis of the anterior division right renal artery. Another emergent interventional angiogram was performed a few day later as she had abrupt back pain associated with an increase in creatinine. She was found to have a new occlusion of posterior division of right renal artery. Stenting was successfully performed. Her serum creatinine peaked at 2.46 on the day of procedure and then began to trend downward. Two weeks after stenting, her creatinine was 1.4.

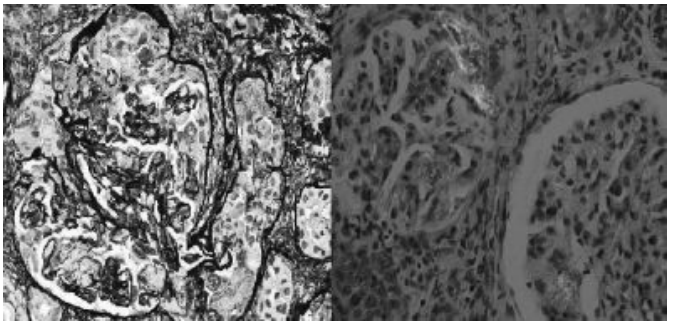
Conclusions: There have been successful endovascular procedures attempted to control the control vascular complications in VEDS patients (Lum et al). However, due to the collagen defect and tissue fragility, invasive procedure mortality can exceed 40% (Pepin et al). No renal stenting procedures have been reported for management of dissection in these patients. Our patient is the first case of VEDS that has a second symptomatic renal artery dissection after prior contralateral nephrectomy for renal artery dissection. It is also the first case in a VEDS patient with renal artery dissection successfully treated with an interventional stent placement.

FR-PO1081

Crescentic Glomerulonephritis with AA Amyloidosis in a Patient with Sarcoidosis Katherine Duello,[1] Xochiquetzal J. Geiger,[2] Walter Hellinger,[1] Nabeel Aslam.[1] *[1]Medicine, Mayo Clinic Florida, Jacksonville, FL; [2]Pathology, Mayo Clinic Florida, Jacksonville, FL.*

Background: Sarcoidosis is a granulomatous disease which rarely causes Crescentic Necrotizing Glomerulonephritis (CNG). AA amyloidosis (AA) has been reported with sarcoidosis. However, co-existent CNG with AA in sarcoidosis has not been previously described. Here we report a case of CNG with AA amyloidosis in a pt with sarcoidosis.

Methods: A 66 yr old female presented with wt loss, anorexia, and liver lesions of 8 months duration. Prior workup showed splenic lesions concerning for abscesses prompting splenectomy, pathology showed granulomatous inflammation without infection. Liver biopsy showed non-necrotizing epitheloid granulomas with negative stains for AFB, fungi and negative cultures. Chest CT showed lymphadenopathy and pleural effusion. ACE level was elevated. Based upon these findings, sarcoidosis was diagnosed. She developed hematuria, rise in creatinine (Scr) to 3.2 mg/dL and nephrotic range proteinuria. Lupus and vasculitis serology were negative. Renal biopsy showed CNG, Congo red was positive for amyloid in the glomeruli and vessels.



EM confirmed amyloid fibrils and typing via liquid chromatography mass spectrometry showed amyloid AA. She was treated with steroids with improvement in her renal function (Scr 1.3) and reduction in proteinuria.

Conclusions: AA amyloidosis has been associated with chronic inflammatory states, but rarely with sarcoidosis. However, previously reported cases did not show CNG. In our case, renal biopsy showed CNG with AA amyloidosis. We speculate that AA amyloid deposits lead to glomerular basement membrane injury leading to crescentic necrotizing lesions in our patient similar to cases reported with with rheumatoid arthritis. This correlation should be considered in patients with sarcoidosis who develop RPGN or nephrotic range proteinuria.

FR-PO1082

Dilated Cardiomyopathy: An Unusual Presentation of Cryoglobulinemic Glomerulonephritis Successfully Treated with Rituximab Allyson Hart, Marc L. Weber, Milind Y. Junghare. *Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN.*

Background: Hepatitis C virus (HCV) infection is the main cause of mixed cryoglobulinemia vasculitis and may affect any organ system. We describe a patient with HCV-associated cryoglobulinemia with new-onset dilated cardiomyopathy and glomerulonephritis (GN) who dramatically improved with plasmapheresis and rituximab.

Methods: A 49 woman with a history of HCV presented with new onset non-ischemic cardiomyopathy, rash on her lower extremities and acute kidney injury (AKI). She was found to have an ejection fraction (EF) of 35% and no significant valvular disease. She had microscopic hematuria, a rise in HCV RNA, hypocomplementemia and type II cryoglobulnemia. Kidney biopsy showed cryoglobulin-associated membranoproliferative GN.She was treated with plasmapheresis and rituximab with subsequent resolution of cryoglobulinemia, rash, and AKI. Repeat echocardiogram showed normalization of her left ventricular EF.

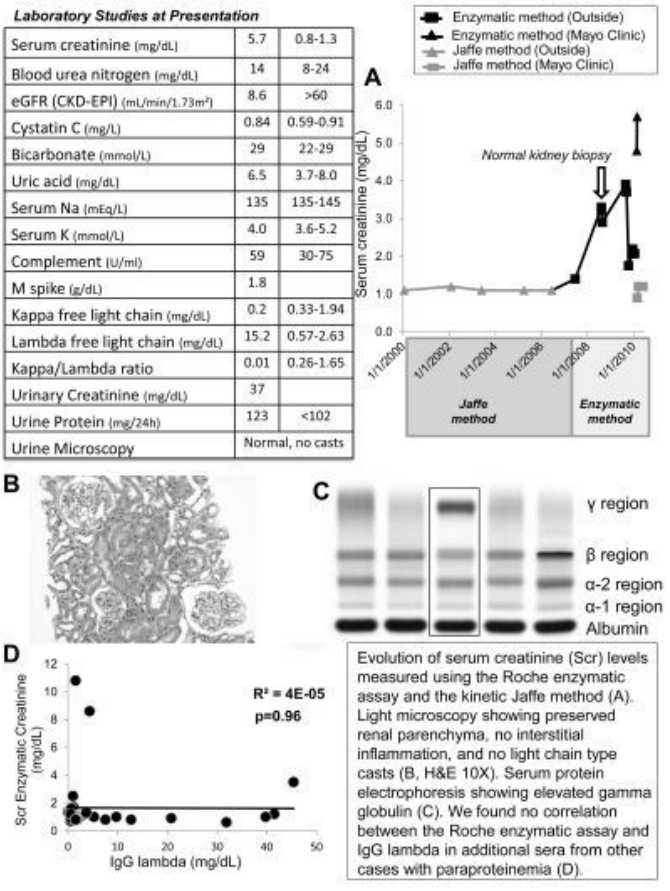
Conclusions: Heart involvement in HCV-associated cryoglobulinemic GN is rarely reported in the literature, while up to up to 21% of deaths in this disease have been attributed to acute myocardial infarction or heart failure. Maestroni and Gorevic et al. reported small and medium sized coronary vasculitis in 22% and 55% of patients with cryoglobulinemia and heart disease at autopsy, respectively, suggesting that alterations in immune function amendable to therapy may frequently go unrecognized. Antiviral and immunosuppressive therapies remain the mainstay of treatment for HCV-associated cryoglobulinemic GN; however, reduced kidney function often precludes ribavirin therapy. Rituximab has been reported to be effective in treating HCV-associated cryoglobulinemic GN in conjunction with plasmapheresis, thus allowing subsequent treatment of underlying HCV. This case demonstrates that rituximab is well tolerated, does not necessarily lead to increased HCV viral levels and may be effective in treating HCV-associated cyroglobulinemic GN. Furthermore, rituximab proved successful in treating "idiopathic" dilated cardiomyopathy in the setting of HCV and cryoglobulinemia.

FR-PO1083

Falsely Elevated Serum Creatinine in a Patient with a Monoclonal Gammopathy Alfonso Eirin,[1] John C. Lieske,[1] Francis Buadi,[2] Timothy S. Larson,[1] Marie C. Hogan.[1] *[1]Div of Nephrology; [2]Hematology, Mayo Clinic, MN.*

Background: Estimation of glomerular filtration rate (eGFR) using serum creatinine (Scr) is widely employed to assess renal function. In recent years, Scr assays have been standardized. Many labs have changed from the kinetic Jaffe to enzymatic methods, in part due to concerns regarding potential interfering substances such as ketoacids.

Methods: An 80yo man was evaluated for a 10 year history of "progressive chronic renal insufficiency", during which time Scr had progressively increased from 1.1 to 3.3mg/dL (fig 1A). At presentation, Scr was 5.7mg/dL, measured on a Roche C-501 Modular analyzer using a Roche enzymatic assay (IDMS-traceable). BUN, potassium, and cystatin C levels were normal (Table). Further evaluation identified elevated lambda immunoglobulin free light chain and a corresponding IgG lambda paraprotein on serum protein electrophoresis(fig 1C). A renal biopsy revealed normal cortex (fig 1B) with no evidence of light chain deposition, amyloid, or cast nephropathy. Subsequently, a Scr measured using a Roche kinetic Jaffe assay was normal at 0.9mg/dL. Analysis of 34 additional sera from other cases with paraproteinemia did not interfere with the Roche enzymatic assay (Fig 1D).



Evolution of serum creatinine (Scr) levels measured using the Roche enzymatic assay and the kinetic Jaffe method (A). Light microscopy showing preserved renal parenchyma, no interstitial inflammation, and no light chain type casts (B, H&E 10X). Serum protein electrophoresis showing elevated gamma globulin (C). We found no correlation between the Roche enzymatic assay and IgG lambda in additional sera from other cases with paraproteinemia (D).

Conclusions: Although the enzymatic Scr assay is thought to have less interference than the Jaffe assay, this study suggests that they can still occur. Previous studies have identified dopamine and/or related metabolites as potential substances that interfere with the enzymatic Scr assay, and this case suggests that paraproteins can also rarely interfere with the test. Thus, regardless of assay, nephrologists should suspect interfering analytes when discrepancies are found between the Scr levels, clinical presentation, and other measures of renal function (e.g., cystatin C). Most labs can measure SCr by an alternative assay when an interfering substance is suspected.

FR-PO1084

Reversible Renal Failure and Minimal Change Disease (MCD) Induced by Vascular Endothelial Growth Factor (VEGF) Blockade with Sunitinib
Tarun Chugh, Savneek S. Chugh, Rajan Kapoor, Sreedhar R. Adapa, Dominick F. Reda, Praveen N. Chander. *New York Medical College, Valhalla, NY.*

Background: VEGF produced by podocytes helps maintain the integrity of glomerular endothelium and podocytes. Blockade of VEGF by specific antibodies such as Bevacizumab can cause acute kidney injury (AKI) and proteinuria mostly with thrombotic microangiopathy (TMA) i.e. endothelial injury. Recently, nephrotic syndrome and/or irreversible AKI have been reported with VEGF blockade by tyrosine kinase receptor inhibitors (TKI) like Sunitinib. The pathology, however, is not well characterized. We report the first case of Sunitinib induced reversible AKI with acute interstitial nephritis (AIN) and marked proteinuria (38g/day) with MCD.

Methods: An 82 year old male with history of renal cancer, on Sunitinib for 2 years, was admitted with 4 weeks of lower extremity edema and progressive renal failure, requiring dialysis. He was on Insulin, Furosemide, Simvastatin and denied taking herbal or OTC products. Physical examination was remarkable for pedal edema; BP 138/78 mmHg. Relevant labs: BUN 70mg/dl, creatinine (Cr) 5.3mg/dl, total protein 5.2g/dl, S.albumin 1.8g/dl. Urinalysis 3+ protein, 3+ blood, urine protein 38g/day. Immunofixation and serological work-up was negative. Renal imaging was normal. Renal biopsy showed primarily MCD and severe AIN. Sunitinib was discontinued and he was started on tapering doses of Prednisone followed by marked improvement. Dialysis was discontinued in a week; Cr stabilized at 1.8mg/dl and proteinuria decreased to <1g/day.

Conclusions: VEGF blockade is known to cause renal disease with variegated pathology. Most cases are reported with Bevacizumab and show TMA. Rare case reports of TMA, FSGS with TMA and acute tubular necrosis (ATN) alone or with MCD (one case) have been reported with TKIs, the latter suggesting podocyte injury. Only three cases of AIN have been described so far; none were associated with MCD. In conclusion, our case shows: 1) TKIs can result in a constellation of AKI with AIN and MCD, 2) TKIs can result in podocytic damage in addition to endothelial injury, and 3) the pathology can be reversed with appropriate therapy.

FR-PO1085

Lupus Nephritis with Severe Renal Failure: Successful Induction with Mycophenolate Mofetil
Tarun Chugh, Rajan Kapoor, Savneek S. Chugh, Sreedhar R. Adapa, Karim B. Solangi. *Department of Nephrology, New York Medical College, Valhalla, NY.*

Background: Mycophenolate mofetil (MMF) has been shown to be noninferior to cyclophosphamide (CYC) as an inducing agent in patients with active lupus nephritis (LN) class III, IV or V. The role of MMF in severe renal failure (CrCl < 30ml/min) has not yet been defined. We present a case of LN (class IV/ V) in severe renal failure (CrCl 8ml/min) who had successful induction with MMF.

Methods: A 25 year old African American (AA) male with history of SLE for past 4 years, was admitted with progressive bilateral lower extremity swelling of 6 weeks duration. He denied similar complaints in the past and was currently not on any medications. His vitals were stable with BP 146/92 mmHg. Physical exam was unremarkable except for anasarca. Relevant labs: Hemoglobin 10.1 g/dl, serum K^+ 5.8 meq/L, BUN 60 mg/dl, creatinine 5.9 mg/dl which trended up to 6.8mg/dl on day 5, albumin 1.7g/dl. Urinalysis 3+ protein, 3+ blood. Urine protein 4.5gm/day. Renal US/Doppler: normal. C3, C4 were extremely low and ANA, anti dsDNA titers were high. Rest of the serological work up including HIV was negative. Renal biopsy showed Class IV-G and V active LN. He was then treated with pulse steroids and MMF. About 16 weeks later his creatinine decreased to 1.1mg/dl, proteinuria decreased and complements normalized. He never required dialysis.

Conclusions: Majority of the trials comparing MMF vs CYC as inducing agent excluded patients with CrCl < 30ml/min and/or creatinine > 1.5 mg/dl. However, in ALMS trial, 11% of the patients in MMF arm had CrCl < 30ml/min and mean creatinine was 1.2 ± 1.1 mg/dl, in contrast to our patient with creatinine of 6.8mg/dl. Post-hoc analysis of this trial also suggested better outcomes with MMF in AA population. After discussion regarding treatment options (CYC vs MMF) and the side effects of each therapy, MMF was chosen per patient preference. Successful outcome with MMF as inducing agent in a LN patient with such severity of renal involvement is encouraging. In view of this, MMF may be used as an alternate inducing agent in LN even with severe renal failure, especially in young AA patients where CYC toxicity is of concern.

FR-PO1086

Renal-Limited Thrombotic Microangiopathy Associated with Clopidogrel
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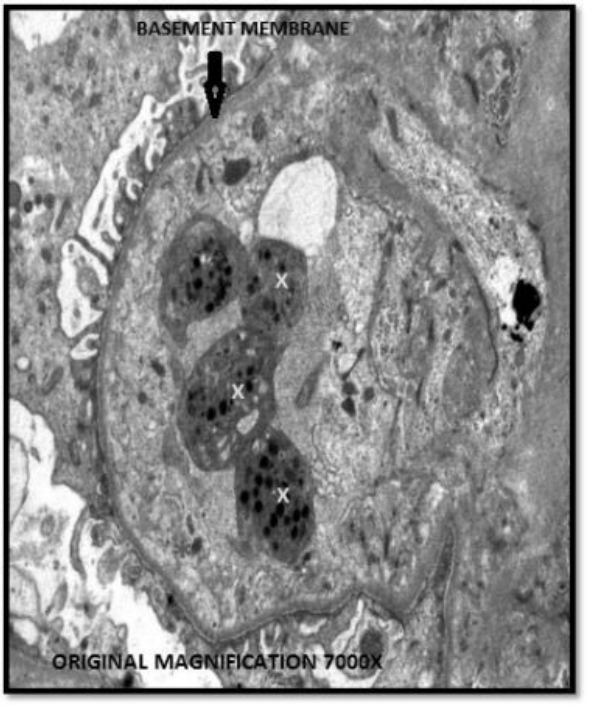
Background: Thrombotic microangiopathy (TMA) in the form of TTP-HUS with renal manifestations has been well described. TMA limited to the kidneys is rare. We describe the first case of clopidogrel-associated renal-limited TMA without thrombocytopenia.

Methods: An 82 year old Caucasian female was referred to the Renal clinic for sub-acute kidney injury. Her serum creatinine (SCr) had increased from 1.03 to 2.23 mg/dl over 22 months. Her medical history was significant for hypertension, hyperlipidemia and peripheral vascular disease. She had been on clopidogrel for 5 years. Labs showed hemoglobin 10.7 gm/dl with platelet count 237,000/ul. Urine microscopy, serological evaluation and Renal ultrasound were unremarkable. A renal biopsy was performed. Biopsy findings were notable for electron microscopy that showed platelet clumps(X) within capillaries, endothelial cell swelling(*) and subendothelial space expansion.



These were consistent with acute TMA. Clopidogrel was stopped. Serologic testing for ADAMTS13 was within normal limits. The patient's SCr had acutely increased to 2.95mg/dl and was admitted to the hospital. Admission labs showed normal platelet count and Serum LDH level. Schistocytes were absent on peripheral blood smear. She received daily plasma exchange(PE) for 10 days along with dexamethasone. SCr was 1.87 on day 10, 1.40 in one month and was 1.83mg/dl six months later.

Conclusions: Our patient presented with sub-acute kidney Injury and a *normal* platelet count while on clopidogrel. Renal biopsy revealed findings consistent with TMA. Patient had no systemic symptoms. With drug withdrawal, PE, and glucocorticoid therapy, her renal function improved to baseline. We report the first case of renal-limited TMA associated with clopidogrel.

FR-PO1088

Complication of Nephrotic Syndrome: It Can Be a Headache

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Background: Venous thromboembolism is a recognized complication of patients with nephrotic syndrome. The nephrotic syndrome, defined by proteinuria (>3.5grams/24 hours), edema, hyperlipidemia, hypoalbuminemia and lipiduria, has a significant impact on the regulation of clotting factors. This increases patients' risk for life threatening thrombotic complications. In this case report we present the rare complication of a cerebral venous sinus thrombosis in a young woman with nephrotic syndrome.

Methods: A 20 year old woman with a relapse of minimal change disease presented to the hospital with a two day history of frontal headaches. Her headache was accompanied by photophobia, nausea, decreased appetite and emesis. Physical exam was notable for pretibial and periorbital edema, photophobia and an otherwise normal neurologic exam. Laboratory values were notable for: WBC 22,000, Hb 18.2g/dL, Platelets 286,000, Creatinine 0.7mg/dL, INR 1.0, Albumin 1.4g/dL and a Protein/Creatinine 19.7. A lumbar puncture was without evidence of infection or intracranial bleed. An MRI venogram was completed and revealed a cerebral venous sinus thrombosis. The patient was initiated on anticoagulation using unfractionated heparin and her steroid dose changed to intravenous methylprednisolone. The patient's neurological symptoms completely resolved.

Conclusions: Nephrotic syndrome is associated with a 7-8 fold increase in the rate of venous thromboembolism compared to the general population. This is secondary to both urinary loss of anticoagulants such as Antithrombin and Protein S as well as hepatic upregulation of procoagulants. Patients at greatest risk for venous thromboembolism are those with a serum albumin less than 2.9g/dl and those within the first six months of their diagnosis [Barbour,S. KI, 81, 190-195]. The duration of treatment as well as primary prophylaxis remains uncertain for this patient population. Given the significant morbidity and mortality associated with cerebral venous sinus thrombosis early recognition and initiation of anticoagulation is necessary for optimal clinical outcomes. At this time, thrombolysis is considered only in patients who are not responsive to anticoagulation with heparin.

FR-PO1089

Belimumab for ANCA-Associated Vasculitis

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Background: A 55-year-old woman with a 28-year history of refractory antinuetrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is effectively treated with belimumab, a B cell modulator that does not deplete B regulatory cells.

Methods: This patient presented in 1984 with subglottic stenosis. Laryngeal biopsy revealed acute and chronic inflammation. Immunfluorescence for myeloperoxidase (MPO) ANCA was positive. Diagnosis was organ limited AAV. Despite treatment with oral methotrexate, azathioprine, corticosteroids, inhaled and intralesional steroids, and topical mytomycin C, repeat biopsies were unchanged. In 2005, she presented with fever, malaise, weight loss, episcleritis, otitis, cough, arthralgias, myalgias, and rash. With negative infectious workup but newly positive enzyme-linked immunosorbent assay for MPO ANCA, symptoms were attributed to systemic AAV. She was treated with mycophenolate and subcutaneous methotrexate but continued to have smoldering disease and recurrent infections. Kidney biopsy in 2006, prompted by active urine sediment, noted pauci-immune glomerular sclerosis and fibrocellular crescents. She was prescribed 6 courses of rituximab over 5 years, and methotrexate and mycophenolate for maintenance. She continued to have frequent disease flares and recurrent infections. In December 2011, belimumab was prescribed every 2 weeks for 3 infusions, then monthly at a dose of 10mg/kg. The patient had rapid improvement of subglottic stenosis, glomerulonephritis, and fatigue, and resolution of episcleritis.

Conclusions: Belimumab, a monoclonal antibody approved for treatment of systemic lupus erythematosus in 2011, prevents binding of soluble human B lymphocyte stimulator protein (BLyS) to B lymphocyte receptors, thereby suppressing B-cell mediated immunity. This is a case of a patient with AAV effectively treated with belimumab. AAV is known to be mediated by B-cell activity, but not all B cells are equal. Research now shows B regulatory cells may be important in preventing relapse in AAV. Belimumab may be an option for refractory AAV by modifying B cell response without obliterating B regulatory cells.

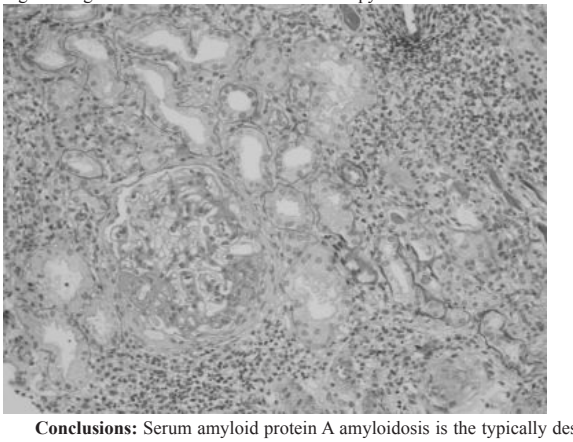
FR-PO1090

Proteinuria and Hematuria in a Patient with Familial Mediterranean Fever

Shuchi Anand, Neeraja Kambham, Neiha Arora. *Stanford University, Palo Alto, CA.*

Background: Familial Mediterranean fever (FMF) is a rare serositis, so named due to its classic presentation of fever and autosomal recessive inheritance among Mediterranean individuals. Patients present with periodic fever, abdominal pain, pleurisy, joint pain, or rash. Since the cloning of the FMF gene (MEFV), mutations in pyrin, leading to an inability to downregulate neutrophil activation, are considered causative. Colchicine is the mainstay of therapy.

Results: Our patient is a 22 year-old Indian female who was referred for microhematuria and proteinuria. She had been diagnosed with FMF after presenting at age 21 with episodic fevers. Lab work up revealed elevated inflammatory markers, with unremarkable serologies. Gene testing for FMF was heterozygous for a known mutation. Colchicine therapy was initiated 10 mo prior to renal evaluation, with resolution of fevers. A urinalysis at that time showed microhematuria and proteinuria, with subsequent urine protein/creatinine ratios of 1.5-2.8g/g. Serum creatinine rose from 0.9 to 1.2 mg/dL over the next 9 months. On exam, she was normotensive without edema. A renal biopsy was performed, demonstrating patchy chronic active interstitial nephritis, segmental membranous nephropathy and secondary segmental glomerulosclerosis. Prednisone therapy was recommended.



Conclusions: Serum amyloid protein A amyloidosis is the typically described renal manifestation of FMF, thought to occur in patients with prolonged untreated disease. Our patient received prompt treatment, making AA amyloidosis less likely. Whether the observed pathology in our case is idiopathic vs. secondary to FMF is unclear, given that her systemic disease was quiescent at the time of renal biopsy. Management will focus on treatment of interstitial nephritis, as we feel that her impaired kidney function and sclerotic changes were out of proportion to mild membranous changes seen.

Funding: NIDDK Support

FR-PO1091

An Unusual Histologic Presentation of Lambda Light-Chain Deposition Disease Concomitant with De Novo Thrombotic Microangiopathy in a Renal Allograft Recipient

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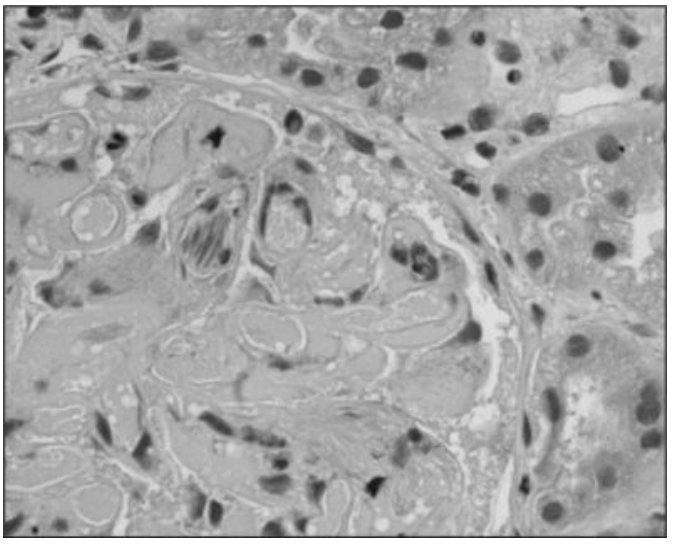
Background: Light chain deposit disease (LCDD) is an uncommon monoclonal gammopathy characterized by deposition of monoclonal kappa or lambda-immunoglobulin (Ig) light chains in various organs. Invariably, LCDD recurs after renal transplantation leading to early graft loss.

Methods: We report a patient with no known history of plasma cell dyscrasia, who presented with renal allograft dysfunction shortly after transplantation. A 44-year-old female presented with acute kidney injury (AKI) 4 weeks following living related donor kidney transplantation. Plasmapheresis (PP) was initiated with presumption of acute humoral rejection. Renal biopsy demonstrated intracapillary fibrin thrombi with focal collections of eosinophilic rhomboid-shaped crystalline material in glomeruli without evidence of rejection.



Immunofluorescence stained positive for lambda light chains in the glomeruli. Subsequent workup for plasma cell dyscrasia demonstrated IgG lambda in serum and bone marrow biopsy contained 10% plasma cells. Pretransplant banked sera revealed a monoclonal IgG lambda spike. After treatment with 3 cycles of bortezomib, the patient again developed AKI. Renal biopsy revealed changes identical to the initial biopsy with new thrombotic microangiopathic (TMA) changes. PP was reinstituted with improvement in renal function. A diagnosis of atypical hemolytic uremic syndrome was entertained and eculizumab was initiated with discontinuation of PP and stabilization of renal function.

Conclusions: We hypothesize that the unusual histologic glomerular changes with crystalline deposition represent precipitation of light chains which in turn may have triggered the development of TMA in this patient.

FR-PO1092

Rapid Renal Recovery in a Case of Crescentic Glomerulonephritis Secondary to Infective Endocarditis Kunal Malhotra, Vibhu Dhawan, Venkatesh Kumar Ariyamuthu, Preethi Yerram. *Department of Nephrology, University of Missouri, Columbia, MO.*

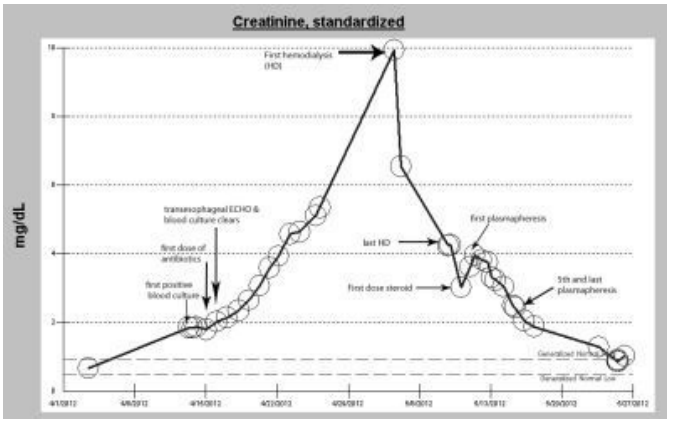
Background: Post infectious immune-complex related glomerulonephritis (GN) usually resolves with treatment of IE. We present a patient who became dialysis dependent despite being on appropriate antibiotic therapy and resolution of bacteremia.

Methods: 59-year-old female with history of congenital Ventricular Septal Defect and baseline creatinine of 0.7 mg/dl presented with rash on her legs, night sweats, and weight loss for 3 weeks. Cardiac exam revealed a new diastolic murmur. Mobile vegetations on aortic and mitral valves were seen on Trans-esophageal echocardiogram. Her blood cultures grew gamma-hemolytic streptococcus, which subsequently cleared on appropriate antibiotic therapy.

Relevant labs

WBC /mcL	6000
Hb g/dL	9.3
Platelets	105000
BUN mg/dL	12-38
Creatinine mg/dL	0.7- 9.9
CRP mg/dl	7.6
C3 mg/dl	28
C4 mg/dl	<9
ANA	1:320
ANCA	negative
UA	>30 RBC
Hepatitis B & C	negative

Her creatinine progressively worsened with a peak value of 10 mg/dl, and required hemodialysis. Kidney biopsy showed immune–complex mediated necrotizing and crescentic GN with disruption of Bowman's capsule and minimal interstitial fibrosis. She was started on plasmapheresis (PE) and high dose steroids with rapid taper, with subsequent improvement in her creatinine to 0.8 mg/dl. She subsequently had aortic valve replacement and VSD closure.



Conclusions: Our patient with IE-related GN did not improve as expected with antibiotic therapy, but turned around dramatically with steroids and PE. In literature, there is limited evidence supporting this therapy, while antibiotics alone have been successful in many cases. Our case supports the possible beneficial role of PE and steroids in IE-related crescentic GN worsening despite appropriate antibiotic therapy, although the risk of immunosuppression and aggravating endocarditis needs to be considered.

FR-PO1093

Factitious Antistreptolysin-O Activity in a Case of Multiple Myeloma with Combined Light Chain Cast Nephropathy and Light Chain Deposition Disease Ekamol Tantisattamo, Chuong Dinh. *Medicine, University of Hawaii, Honolulu, HI.*

Background: Antistreptolysin-O (ASO) is well-known to be elevated in poststreptococcal glomerulonephritis (PSGN). However, falsely elevated ASO can occur in paraproteinemia like multiple myeloma (MM) and leads to delay in diagnosis and treatment. We report a case of a woman presented with acute kidney injury (AKI) secondary to combined light chain cast nephropathy (LCCN) and light chain deposition disease (LCDD) from MM with high ASO level.

Methods: A 42-year-old Caucasian woman presented with nausea and vomiting for 2 months. She had low-grade fever but no sore throat. Three days prior to admission, her serum creatinine was elevated to 5.4 mg/dl from normal baseline. Repeated creatinine elevated up to 11.2 mg/dl. Workup for AKI revealed markedly elevated ASO of 3,036 IU/ml and streptozyme of > 800 STZ Units. Complements and anti-DNase-B Ab were normal. Urine microscopy did not reveal dysmorphic RBC or red cell casts. ANA, ANCA, and anti-GBM were negative. Renal ultrasound was normal. Initially, she was supportively treated as PSGN; however, serum creatinine was still high. Kidney biopsy revealed κ type LCCN and κ LCDD. Given high UPCR of 5 mg/g of creatinine, workup for paraproteinemia was performed. She had unexplained anemia with Hb of 8.9 g/dl. Serum calcium was 9.4 mg/dl. SPEP showed 2 monoclonal proteins. Serum IFE revealed 2 free κ light chain monoclonal proteins. There were high free κ chain and normal free λ chains. β-2 microglobulin was elevated to 26.3 mg/l. Bone survey was negative. Bone marrow biopsy revealed plasma cells > 90% of all cells consistent with MM. Intermittent hemodialysis and plasmapheresis were initiated with bortezomib-based chemotherapy.

Conclusions: Many chemical laboratory measurements can interfere with paraproteins. Falsely elevated ASO is one of those conditions due to coating of the ASO antigen-laden latex particles by the paraprotein. If we relied only on the serologic diagnosis of PSGN, then the diagnosis of myeloma kidney would have been missed. Our patient presented with AKI with highly elevated ASO. Therefore, paraproteinemia should be considered in a patient with a high ASO but with atypical features of PSGN.

FR-PO1094

Type 1 Cryoglubulinemia Presenting as Acute Kidney Injury (AKI) from Thrombotic Microangiopathy Gaurav Agarwal,[1] Olatokunbo O. Shobande,[1] Maria Alejandra Alfonso-Jaume,[1] Micah R. Chan,[1] Jose R. Torrealba,[2] Weixiong Zhong.[2] *[1]Department of Medicine and Division of Nephrology, University of Wisconsin School of Medicine and Public Health, Madison, WI; [2]Department of Pathology, University of Wisconsin School of Medicine and Public Health, Madison, WI.*

Background: The main renal presentation of cryoglobulinemia is Membrano-proliferative glomerulonephritis (MPGN) type 1 from immune complex mediated injury. Cryoglobulinemia causing TMA is not described in literature. Here we describe a rare presentation of type 1 cryoglobulinemia as TMA causing AKI.

Methods: A 53-year old male presented with fever and generalized rash, digital gangrene. His baseline creatinine was 1.1mg/dl. The next day his creatinine increased to 4mg/dl and he became oliguric. On urinalysis there was 2+ proteinuria and 2+ hematuria and no active sediments. He was also noted to have a low platelet count of 20K/uL. Serology including complement, ANCA, anti-GBM, Hep panel, ASO, ANA, and HIV were negative.
Intervention: We treated the patient as an unknown RPGN with pulsed high dose steroids and plasmapharesis.

Results: Renal biopsy showed severe thrombotic microangiopathy and 70% acute cortical necrosis. A skin biopsy showed thombotic occlusion of small size arteries with cryoglobulins without any vasculitis. Days later, labs confirmed type 1 cryoglobulinemia with monoclonal band. Bone marrow examination was also done which confirmed B cell lympho- proliferative disorder. He was then started on Rituximab. He remained dependent upon dialysis.

Conclusions: In conclusion, this patient had B cell lymphoproliferative disorder causing type 1 cryoglobulinemia. The cryoglobulin burden caused acute TMA and presented as AKI. **Review of literature:** Type 2 and type 3 cryoglobulinemia (mixed cryoglobulinemia) usually present as type 1 MPGN and mechanism are immune complex mediated injury causing vasculitis. Type 1 cryoglobulinemia usually presents as thrombotic occlusion of small size vessels without any vasculitis. Renal biopsy should be promptly done in type 1 cryoglobulinemia to look for TMA and early institution of plasmapharesis can potentially make a difference in outcome.

FR-PO1095

Beware Maslow's Hammer: A Case of Sirolimus Induced Pneumonitis

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Background: A 54 year old male received a second cadaveric renal transplant from a 40 year old diabetic. Both donor and recipient were cytomegalovirus (CMV) IgG positive. Maintenance immunosupression included mycophenolate mofetil (MMF), tacrolimus and prednisone. His post-operative period was complicated by delayed graft function and one month later by biopsy proven CMV esophagitis. Valganciclovir was started; tacrolimus levels were maintained around 10ng/ml, while MMF dosage was reduced. A kidney biopsy was performed two months later because of acute renal failure. Tacrolimus was switched to sirolimus after the biopsy revealed isometric tubular vacuolization with acute thrombotic microangiopathy. His creatinine stabilized between 2.5-3 mg/dl after the switch. Sirolimus levels were maintained at 10 ng/ml. Two weeks later, he presented with fever, generalized malaise and dry cough. His oxygen saturation on room air was 89%. His laboratory data included platelet count 62 x10^3 cells/ul, WBC 5.2 x 10^3 cells/ul (86% neutrophils and 13% bands), BUN 56 mg/dl and creatinine 3.4 mg/dl. The chest X-Ray (CXR) showed diffuse bilateral infiltrates. MMF was discontinued and empiric antibiotics started. Blood cultures were negative for bacterial and fungal etiologies. Serologies remained negative for atypical organisms and CMV. 2-D echocardiogram showed preserved systolic function. While his CXR worsened inspite of antibiotics, he was peculiarly not dyspneic and his chest was normal to auscultation. Sirolimus induced pneumonitis (SIP) was suspected. Sirolimus was discontinued and 60mg IV solumedrol initiated. Within the next few days there was complete resolution of fever, CXR findings, and thrombocytopenia. Pt was eventually discharged on everolimus. SIP is an idiosyncratic reaction and is a diagnosis of exclusion. It commonly occurs in patients with limited kidney function, especially those who are switched to sirolimus later because of CNI toxicity. Respiratory distress is very rare. Pulmonary infiltrates on CXR maybe the lone finding. Treatment involves either dose reduction or a switch to everolimus and high dose steroids. This case highlights the diagnostic dilemma of SIP.

FR-PO1096

Acute Kidney Injury of a Transplanted Kidney Secondary to Donor Rhabdomyolysis

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Background: Donor rhabdomyolysis after live donor laparoscopic nephrectomy has been reported in the past but in general the recipients were reported to have uneventful courses after transplantation. We report the first case of acute kidney injury requiring dialysis treatment secondary to donor rhabdomyolysis.

Methods: A 64-yo-male, with medical history of End Stage Kidney Disease on hemodialysis received a living related kidney transplant. He developed decreased urine output after surgery and his serum creatinine and BUN started rising. Kidney biopsy on post transplant day 8 showed "degenerative and regenerative changes in tubules compatible with acute tubular necrosis (ATN)". Patient was on hemodialysis for a week (totally four treatments), then his urine output started to pickup and his creatinine and BUN started to come down. His serum creatinine was 0.96 mg/dl six months after transplantation. The donor was a 34 years old healthy male with a BMI of 32. Although he tolerated the open nephrectomy procedure well and had good urine output post operatively, his serum creatinine was elevated at 2.64 mg/dl with a CPK of 14214 Units/L on postop day three, consistent with rhabdomyolysis. Given the donor was diagnosed with rhabdomyolysis after the nephrectomy, an immunohistochemical staining for myoglobin was performed on the transplanted kidney, which showed focal staining in the apical cytoplasm of the renal tubular cells, as well as in the interluminal granular cast material. The recipient had ATN and slow graft function due to his donor's rhabdomyolysis.

Conclusions: Acute kidney injury in a recipient due to rhabdomyolysis/myoglobinuria after living donor nephrectomy is quite rare. Prolonged surgery as well as long lateral decubitus positioning, increased body mass index of the donor, and volume depletion may be predisposing factors. Since rhabdomyolysis after kidney transplantation can be associated with allograft loss and death, increased surveillance for rhabdomyolysis is warranted in donors as well as in recipients who are at increased risk.

FR-PO1097

Acremonium Skin and Soft Tissue Infection in a Kidney Transplant Recipient

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Background: Acremonium species are ubiquitous fungal organisms found in soil and air that can lead to superficial infections after traumatic inoculation. Not much is reported regarding this organism in patients with kidney transplantation.

Methods: A 66 year old male with LURT (2 years prior) and DM type 2 presented with a soft mass that had been growing over his left lateral malleolus for two months. The patient was a 5/6 HLA mismatch, induced with thymoglobulin and corticosteroids. Donor and recipient were CMV positive and the patient had received a CMV prophylaxis post-transplant. The patient had an episode of acute cellular rejection secondary to medication noncompliance for which he was treated with pulse corticosteroids 2 months prior to presentation. The patient's immunosuppression included MMF, tacrolimus, and prednisone. The physical exam was remarkable at the left ankle, there was a 2X4 cm soft mass palpable superior to the lateral malleolus. The lesion was tender to palpation with mild erythema. His laboratory results were unrevealing. He had a normal baseline creatinine of 1.2mg/dl. Initially, the patient was managed with intravenous antibacterials including vancomycin for a presumed bacterial abscess. After one week of therapy and no improvement, the mass was biopsied and found on culture to grow an Acremonium species susceptible to posaconazole. The patient improved while on oral posacanozole but there was recurrence once the medication was discontinued. Subsequently he underwent surgical debridement and treatment with voriconazole. He has had resolution to date.

Conclusions: To the best of our knowledge, there are only five reported cases of Acremonium species documented in kidney transplant recipients and only one previously documented case that resulted in a subcutaneous abscess. There are no standard therapy guidelines for the treatment but in several case reports excision and long-term therapy with a susceptible antifungal. This is the second reported case of successfully treated subcutaneous Acremonium infection in a kidney transplant patient.

FR-PO1098

Third Time's a Charm: A Case of Recurrent Transplant FSGS

Nashila AbdulRahim, Angelina Edwards, Horacio E. Adrogue, Aleksandra De Golovine. *Renal Diseases and Hypertension, University of Texas at Houston, Houston, TX.*

Background: Circulating permeability factors have been implicated in recurrence of focal segmental glomerulosclerosis (FSGS) in as many as 30% of renal transplant patients. Therapies are targeted towards decreasing levels and blocking their effects. We describe here a case of recurrent transplant FSGS treated with a combination of therapies thereby leading to graft salvage.

Methods: A 39 year old Vietnamese male with end-stage renal disease; living-unrelated kidney transplant in 2002, complicated by recurrent FSGS leading to transplant nephrectomy in 2007; received a cadaveric kidney transplant on February 16, 2012. The donor kidney belonged to a 41 year old Caucasian woman who died of cerebral brain death; prior to procurement, her serum creatinine (sCr) was 0.4 mg/dl. Our patient had an uncomplicated intraoperative course. Thymoglobulin and methylprednisolone were used as induction agents. He was started on tacrolimus, mycophenolate mofetil (MMF) and prednisone as maintenance agents. The patient's sCr remained elevated at 8.3 mg/dl; hemodialysis was initiated on post-operative day 3 secondary to a rise in blood urea nitrogen and fall of serum bicarbonate. A transplant kidney biopsy was done on February 24, which showed diffuse podocyte foot process effacement, suspicious for early recurrent FSGS, with no evidence of acute rejection. His immunosuppression was changed from tacrolimus to cyclosporine (CsA), and MMF and high dose prednisone were continued. The patient continued to receive hemodialysis until April 4 as well as consecutive plasmapheresis, followed by a dose of rituximab. He was also started on galactose 12 grams twice daily. Galactose has been shown in vitro to block the activity of a permeability factor implicated in FSGS. A repeat transplant kidney biopsy on April 5 showed no evidence of acute rejection; the diffuse pattern of foot process effacement was no longer observed. His sCr improved to 1.7 mg/dl on May 24. The circulating factor was sent and results are pending at this time.

Conclusions: Our case illustrates a successful therapeutic approach in a patient at a 50-80% risk for a second graft loss due to recurrent FSGS.

FR-PO1099

Acute Necrotizing Gingivitis Following Kidney Transplantation

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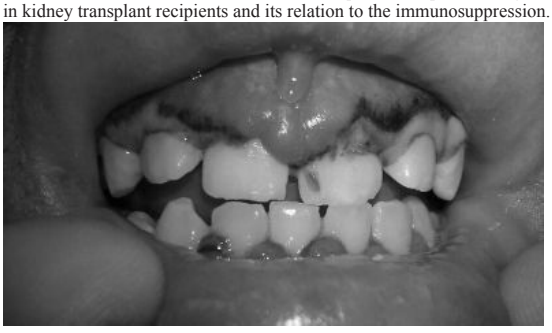
Background: Acute necrotizing ulcerative gingivitis (ANUG) is a rare complication after transplantation. It is important to recognize this condition as it can be confused with other gingival lesions, e.g. herpetic infections, drug-induced gingival hyperplasia from cyclosporine or calcium channel blockers and PTLD.

Methods: An 18 year old male with ESRD secondary to FSGS in a solitary kidney received a deceased donor renal transplant from an EBV and CMV positive donor. Patient's serology was EBV and CMV negative. Induction immunosuppression consisted of basiliximab, tacrolimus and glucocorticoids, maintenance with tacrolimus, MMF and prednisone. No episodes of acute rejection. The hypertension was treated with amlodipine. Four months post transplant, patient developed gingival swelling, and amlodipine was discontinued. He then presented with oral pain, fever, severe gingival hyperplasia with

areas of necrosis, and cervical adenopathy. No systemic lymphadenopathy or visceromegaly was present. Laboratory results: WBC 3,600 cells/mcL with 0% granulocytes, 23% lymphocytes, and 76% monocytes. EBV PCR, CMV PCR and HSV culture were negative. Gram stain of a gingival lesion showed 1-2+ polys, bacterial culture revealed normal oral flora, and blood cultures were negative. Based on the clinical presentation and negative laboratory findings, the patient was diagnosed with ANUG due to poor dental hygiene. After debridement, the patient was treated with piperacillin/tazobactam and MMF was held until the infection resolved. Patient was advised to use a soft bristle toothbrush. Two weeks later, the patient fully recovered.

Conclusions: Further observations are required to explore the significance of ANUG in kidney transplant recipients and its relation to the immunosuppression.



FR-PO1100

A Case of Squamous Cell Carcinoma Arising from Long-Term Indwelling Urinary Catheter in Patient with Chronic Kidney Disease Eleni Chelioti,[1] Evdokia Efthimiou,[1] Maria Sotiraki,[1] Kassiani Manoloudaki,[2] Maria Tsilivigou,[1] Gabriel Papadakis.[1] *[1]Dept of Nephrology, General Hospital of Piraeus, Athens, Greece; [2]Dept of Pathology, General Hospital of Piraeus, Athens, Greece.*

Background: Patients with chronic indwelling urinary catheter are known to have an increased risk of urinary malignancy. This condition has been attributed to chronic inflammation and mechanical stimulus from the catheter. However, squamous cell carcinoma (SCC) around the inguinal area is relative rare. We report a case of lower abdominal SCC arising from the long-term indwelling urinary catheter.

Methods: A 72 years-old male with chronic kidney disease due to chronic obstructive uropathy presented in a uremic state that necessitated the urgent initiation of dialysis treatment. After a 5 sessions of hemodialysis his renal function was improve. The patients also had a history of long – term indwelling urinary catheter-less than 5 years- due to prostatic hypertrophy and chronic urinary infections. Physical examination revealed an abdominal mass around the left inguinal area. Abdominal enhanced computed tomography (CT) showed a 6-cm mass at the left inguinal area, an osteolitic lesion at the left pubosciatic adhesion and a small hepatic mass. Histopathology examination of percutaneous biopsy specimens revealed SCC with moderate differentiation. The cystoscopy suggested ulcerative infiltration of the left ureterobladder conjuction and the lateral bladder wall. Histology at biopsy specimens was consistent with SCC grade II. CT of lungs was negative. Four months after his admission he died with multiple visceral metastization.

Conclusions: In the western world SCC represent <5% of all bladder tumors and 10% in patients with a catheter indwelling for ≥10years. Predisposing factors: patients with spinal cord injury with prolonged indwelling catheter and chronic irritation to bladder wall either by chronic urinary infections, calculi, long-term indwelling catheter or bladder diverticula. Although distant metastasis is infrequent (8-10%) the prognosis is poor and most patients die. Our patients had many predisposing factors for the development of SCC and in the literature there have been only four reported cases.

Funding: Other U.S. Government Support

FR-PO1101

Tumor Induced Osteomalacia (TIO): Associated with Elevated Circulating Plasma Fibroblast Growth Factor (FGF)-7 along with FGF-23 Khaled Khazim,[1] Christine L. Gear,[1] Rajeev Suri,[2] Paolo Fanti,[1] Shweta Bansal.[1] *[1]Division of Nephrology, University of Texas HSC at San Antonio, TX; [2]Dept of Radiology, UTHSC at San Antonio, TX.*

Background: FGF-23 is regarded as the prominent phosphatonin causing TIO. Other phosphaturic hormones including FGF-7, secreted frizzled related protein-4, and matrix extracellular phosphoglycoprotein have been identified by in vitro studies of tumors resected from patients with TIO, but limited information is available about their circulating levels. In many cases identification of tumor is a challenge in itself.

Methods: 64 year male was seen for fatigue, weakness and hypophosphatemia for 10 years. During 4-year follow-up, serum phosphorus was as low as <1.0mg/dL, calcium 9.3mg/dL, 25(OH)VitD 22ng/mL, 1,25$(OH)_2$VitD 37pg/ml, iPTH 63pg/mL, urine phosphorus 3.6gm/24hr with fractional excretion of 90%, and CrCl 95ml/min. Plasma FGF-23 mildly elevated at presentation increased gradually over next 3 years. The presence of hypophosphatemia, renal phosphate wasting, and osteomalacia, and absence of primary hyperparathyroidism, Fanconi's syndrome or familial rickets, raised the suspicion of TIO. However, a tumor could not be identified using CT scans, MRI scan, bone scan, octreotide scintigraphy and FDG-PET/CT. Simultaneous regional blood sampling revealed high levels of both FGF-23 and FGF-7 and lateralization in the left femoral as compared to the contralateral vessels, with more pronounced imbalance for FGF-7 than FGF-23; e.g., FGF-7 concentration was 15.77 vs. 1.66 pg/ml in the left vs. right femoral veins, while FGF-23 concentration was 170 vs. 152 RU/ml, respectively, confirming initial suspicion of likely source in left lower extremity. The focused MRI showed only degenerative joint disease. In absence of identifiable tumor patients was started on cinacalcet with significant improvement in serum phosphorus and decrease in fractional excretion of phosphorus to 29% over next 3 months.

Conclusions: We are first to demonstrate that FGF-7 can be analyzed in the circulation and assist in the diagnosis and localization of TIO-inducing tumors. Clinicians should be aware of association between TIO and phosphatonins other than FGF-23.

Funding: Veterans Administration Support

FR-PO1102

Acute Kidney Injury in a Patient with Metastatic Urothelial Cancer Silvi Shah, Neha Nainani, Pradeep Arora. *Department of Nephrology, University at Buffalo, Buffalo, NY.*

Background: Acute kidney injury (AKI) occurring secondary to renal metastasis from solid tumors is rare. We report a case of 71-year-old white male with history of urothelial cancer that presented to the hospital with acute kidney injury.

Methods: A 71-year-old male was admitted to the hospital with one-month history of nausea and was found to have elevated serum creatinine of 2.2 mg/dl (estimated glomerular filtration rate [eGFR] 30 ml/min/1.73m^2). Baseline serum creatinine was 1.1 mg/dl (eGFR 72 ml/min/1.73m^2). Past medical history was significant for stage II transitional cell carcinoma of bladder diagnosed 5 years ago and was in remission following treatment. The physical examination revealed blood pressure of 160/77 mmHg and mild lower extremity edema. He was non-oliguric; and was not dehydrated or volume overloaded. Urine analysis showed 0-1 RBC/hpf, 30mg/dl proteins and was negative for leukocyte esterase and nitrite. No casts were seen and 24-hour urinary protein excretion was 0.7 grams. Renal ultrasound revealed bilaterally enlarged unobstructed kidneys. His renal function continued to deteriorate and he became lethargic and confused. Hemodialysis was initiated. CT scan showed bilateral kidney enlargement, pulmonary consolidation, multifocal lytic lesions and retroperitoneal nodules. Renal biopsy was consistent with diffuse metastatic infiltration by poorly differentiated urothelial cancer cells. Due to poor prognosis, he was not considered a candidate for chemotherapy and died.

Conclusions: AKI due to renal parenchymal invasion from solid tumors is distinctly unusual. This case illustrates the rare potential of solid tumors to metastasize to kidneys and cause irreversible acute kidney injury. Early recognition with renal biopsy and treatment of solid tumor with chemotherapy or radiation may prevent progression to end stage renal disease.

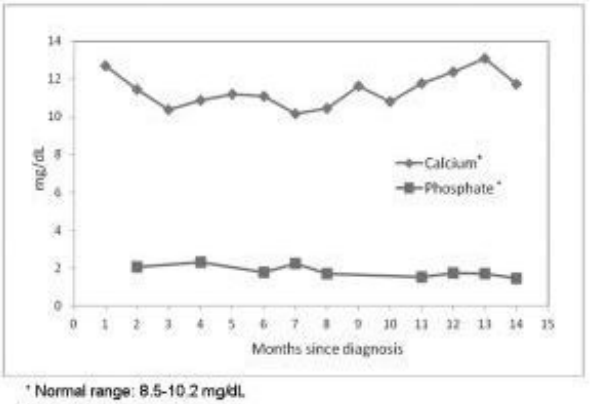
FR-PO1103

Non PTH-rP Related Hypercalcemia in a Patient with Neuroendocrine Tumor of the Pancreas: First Case Report Ioan-andrei Iliuta, Felix Couture, Fabrice Mac-Way. *Medicine, L'Hotel-Dieu de Quebec, Quebec, QC, Canada.*

Background: Neuro-endocrine tumours (NET) of the pancreas complicated by hypercalcemia are rare entities that have rarely been described in the literature. All case-reports have shown that hypercalcemia was related to the production of PTHrP by the tumour. We describe for the first time the case of a non PTH-rP related hypercalcemia in a patient with pancreas NET.

Methods: We report the case of a 48 year-old man diagnosed with a NET of the pancreas associated with liver metastasis. Octreotide was rapidly started but he was seen in nephrology for multiple episodes of severe hypercalcemia that was treated with i.v Bisphosphonates and hydration. Evolution of blood calcium and phosphorus are shown in Figure 1.

Figure 1. Evolution of serum calcium and phosphate since diagnosis



* Normal range: 8.5-10.2 mg/dL
* Normal range: 2.4-4.1 mg/dL

Biochemistry anomalies included hypophosphatemia, slightly decreased blood 25(OH) vitamin D level, high 1.25 (OH)vitamin D, low PTH and increased urinary phosphate secretion.

Table 1. Bone and mineral parameters at first visit

	First visit	+ 3 months
PTH (pg/mL)	0	0
PTHrP (pg/mL)*	5.5	6.0
25(OH) vit D (ng/mL)+	-	20.8
1,25 (OH) vit D (pg/mL)**	-	102.7
24 hour urinary calcium (2.5-7.5 mmol)	7.06	-
24 hour urinary phosphate (10-32 mmol)	35.3	-
Creatinine (mg/dL)	0.73	0.84
TSH (mU/L)	normal	-

*Normal < 15; + Normal range 32-100 ng/mL ** Normal range 15-51 pg/mL

Surprisingly, blood PTHrP level measured twice was also in the lower range. As serum calcium was consistently elevated, monthly i.v Bisphosphonate was recently introduced. Despite Octreotide treatment, liver metastasis progressed and blood calcium remained elevated.

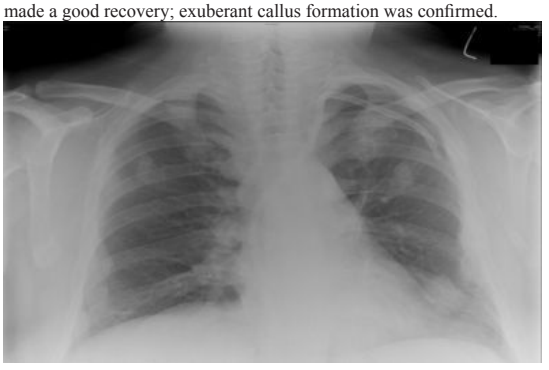
Conclusions: This is the first case describing hypercalcemia in a patient with a NET of the pancreas without elevation of PTHrP. We hypothesized that hypercalcemia and hypophosphatemia were respectively caused by 1.25 (OH)vitamin D and FGF-23 tumour production. The production of a PTH-like fragment molecule that has yet to be identified is also possible.

FR-PO1104

Skin and Bone: Skeletal Demineralisation Following Treatment of Calciphylaxis Miriam R. Berry, Calum Neil Ross. *Department of Renal Medicine, Norfolk and Norwich University Hospital, Norwich, Norfolk, United Kingdom.*

Background: Calcific uraemic arteriolopathy (CUA) - calciphylaxis - is a feared complication of end stage renal failure (ESRF) characterised by medial calcification of subcutaneous arterioles and tissue necrosis. Management should optimise components of the calcium-phosphate-parathyroid axis. Sodium thiosulphate (STS) is an emerging adjuvant therapy which enhances solubility and subsequent excretion of chelated extraskeletal calcium. We report a unique complication of this life-saving therapy.

Methods: A 35-year-old female haemodialysis patient, with ESRF due to ANCA-positive vasculitis, developed severe proximal CUA on her trunk, breasts and limbs three months after total parathyroidectomy; she was not expected to survive. Treatment was escalated to include STS three times weekly, increased dialysis dose with low calcium dialysate, bisphosphonate therapy and correction of hyperphosphataemia. The extensive skin lesions resolved by secondary intention over 18 months. Six months after discontinuing STS therapy, our patient complained of skeletal pain; imaging confirmed insufficiency fractures of multiple ribs and pubic rami. Bisphosphonate therapy was discontinued and our patient made a good recovery; exuberant callus formation was confirmed.



Conclusions: Our patient demonstrates a hitherto unreported complication of CUA treatment. Skeletal demineralisation may be a predictable complication of STS therapy which reduces total body calcium. The high mortality of CUA means that most patients will not survive to develop this iatrogenic complication. The enhanced bone mineralisation mediated by bisphosphonates is outweighed by the decalcifying action of STS leading to multiple insufficiency fractures. Withdrawal of bisphosphonate therapy may reverse the potential for adynamic bone disease and permit osteoblastic activity to resume.

FR-PO1105

Sildenafil as Adjunct to Therapy in Lithium Induced Nephrogenic Diabetes Insipidus Humberto C. Sasieta-Tello,[1,2] Thomas P. Monson,[1,2] Robert L. Safirstein.[1,2] *[1]Department of Medicine, Central Arkansas Veterans Healthcare System, Little Rock, AR; [2]Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: The pathogenesis of lithium-induced diabetes insipidus (LiDI) is not fully elucidated. It is uncommon, chronic and difficult to treat. Its treatment is based on four principles: Low-sodium diet, hydrochlorothiazide, amiloride and NSAIDs. This agents decrease polyurea, allowing the patient to maintain euvolemia and normonatremia relying on oral intake. Hypovolemic hypernatremia is a common complication.

Methods: 61 year old male with proven LiDI was admitted with the following diagnosis: LiDI, Acute Renal Failure, Hypernatremia and Hyperkalemia. His prior medical history included Bipolar Disorder, managed with Lithium Carbonate. LiDi was managed as outpatient with HCTZ-Amiloride and NSAID. After initial evaluation and stabilization, amiloride and NSAIDs were suspended to prevent further nephrotoxicity and he received intravenous fluids trying to maintain water balance and achieve normonatremia. HCTZ was maintained throughout all hospital stay. Normonatremia and return to baseline creatinine was achieved after 4 days, with a urine output of 10 liters. On his 6th hospital day he was started on Sildenafil 20mg PO TID, due to prior AKI and hyperkalemia. Within 48 hrs, his urine output decreased to 5.5 liters, he was able to match his free water requirements by the oral route and was safely discharged. After 90 days, he remains on sildenafil and HCTZ, and maintains normonatremia.

Conclusions: The decrease in the AQP2 density in the luminal aspect of the collecting duct is the end-step in the development of LiDI. cAMP is the mediator of the main pathway of AQP2 luminal positioning. Recently, cGMP was found to be a mediator in a minor luminal expression pathway. Inhibition of cGMPase leading to increased expression of AQPs has been found, in both a molecular and animal model setting. Sildenafil has been extensively in this patient's demographic group; side effects and pharmacological interactions are known and predictable. Sildenafil could be an effective drug to treat LiDI. Further testing is required.

Funding: Veterans Administration Support

FR-PO1106

Recurrent Uric Acid Renal Stones: Novel Phenotype for a Known Mutation Badreldin M. Bedri, Davoud Mohtat, Alda Tufro. *Pediatric Nephrology, Yale University, New Haven, CT.*

Background: The incidence of renal stones has increased significantly in United States and industrialized countries in the past decades. Uric acid renal stones constitute 5-10% of renal stones in children in United States. Hyperuricemia is observed in most children with uric acid renal stones and is mostly due to a genetic cause. The most common genetic mutations that lead to hyperuricemia are in hypoxanthine phosphoribosyltransferase 1(HPRT1) gene with a wide spectrum of clinical phenotypes. The most severe mutations in HPRT1 gene are associated with Lysch-Nyhan syndrome, characterized by hyperuricemia, hyperuricosuria, severe developmental delay with self-mutilation behavior and severe neurological abnormalities.

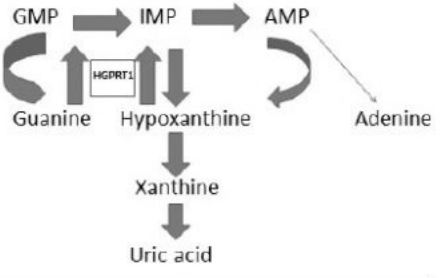


Fig 1: Purine metabolism. AMP: adenosine monophosphate, GMP: guanine monophosphate, HGPR1: hypoxanthine guanine phosphoribosyltransferase, IMP: inosine monophosphate.

Methods: 11 years old boy who had mild developmental delay, recurrent uric acid stones, hyperuricemia and hyperuricosuria. He initially presented with gross hematuria and then developed left renal Staghorn stone associated with left hydronephrosis and underwent laparoscopic percutaneous nephrolithotomy. Stone analysis revealed a composition of 100% calcium, sodium and magnesium urate. His work up showed normal renal function and excluded secondary causes of hyperuricemia. Genetic testing identified a hemizygous single nucleotide substitution, c.527C>T(p.P176L), in exon 7 of the HPRT1gene which is associated with an incomplete Lesch-Nyhan syndrome.

Conclusions: More than 400 mutations in HPRT1 gene have been reported, with a wide spectrum clinical phenotype. The identified mutation in our patient has been reported in the literature as associated with partial HPRT1 deficiency and incomplete lysch-Nyhan syndrome but no clinical phenotype information was reported. To our knowledge, our patient is the first in the literature to have this mutation along with a clinical phenotype.

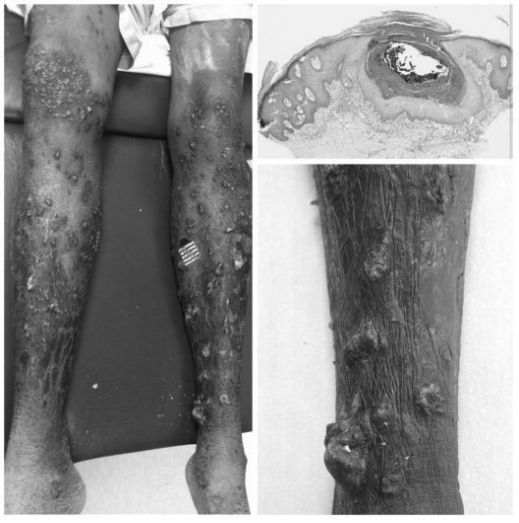
FR-PO1107

Severe and Advanced Form of Acquired Perforating Dermatosis in a Patient with End Stage Renal Disease Sasan Raeissi,[1] Antoine L. Samaha,[2] Maryam Pourpaki.[1] *[1]Internal Medicine, Good Samaritan Hospital, Cincinnati, OH; [2]Nephrology, Good Samaritan Hospital, Cincinnati, OH.*

Background: Acquired Perforating Dermatosis (APD) is usually seen in adults with end stage renal disease (ESRD) and/or diabetes mellitus (insulin dependent > non-insulin dependent). The lesions of APD are usually dome-shaped papules or nodules, 1 to 10 mm in diameter, with a central crust-filled crater. They occur predominantly on the trunk and extensor limb surfaces and are severely pruritic.

Methods: A 34-year-old African American Female with a 23 years history of uncontrolled diabetes mellitus type 1 and diabetic nephropathy that progressed to ESRD at age 28 years. She was initially on automated peritoneal dialysis and later switched to hemodialysis at age 31 years due to non adherence to therapy. She had multiple hospitalizations for diabetic ketoacidosis and uremic symptoms. She also had uncontrolled

bone and mineral disease. She developed pruritic and papular skin lesions (initially <5mm in diameter) over lower extremities within few months of initiating dialysis and was referred to a dermatologist who made the diagnosis APD. This was confirmed with a skin biopsy showing epidermal invagination with a keratotic plug containing basophilic cellular debris. She had erratic visits with dermatology and non adherence with therapy (retinoids, phototherapy, etc.). Her APD progressed to diffuse 4 to 32mm hyperpigmented papulo-nodular skin lesions, covered with keratotic film. Some of the lesions were umbilicated and some coalesced and formed large vegetating lesions.



Conclusions: This case represents APD in extremis and at late stage due to non adherence to therapy. The precise pathogenesis of APD is unknown. Topical retinoids, topical and intradermal steroid and phototherapy have variable effects. In some instances, APD has remitted completely after renal transplantation.

FR-PO1108

Cognitive Function at a Creatinine of 28.8 mg/dL Sabrina Schneider,[1] Anne Kathrin Malecki,[2] Olaf Boenisch,[1] Hermann G. Haller,[1] Jan T. Kielstein.[1] *[1]Department of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany; [2]Institute of Psychology, Martin-Luther University, Halle-Wittenberg, Germany.*

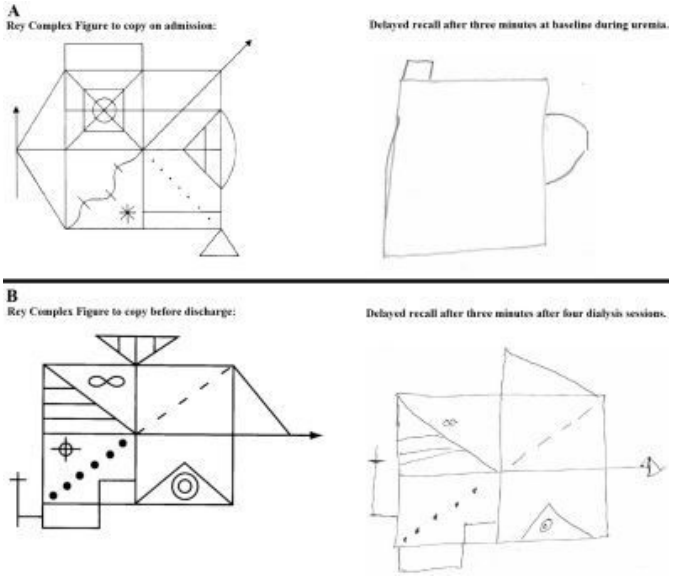
Background: We report one of the rare cases of severe uremia and describe the day to day improvement in neuro-cognitive function by dialysis starting at a serum creatinine of 27.8 mg/dl.

Methods: A 27-year-old male with s/p kidney transplantation presented with graft failure due to non-adherence to immunosuppressive medication. Performance in memory, attention and executive function was regularly assessed by: (digit span, Rivermead Behavioral Memory Test, Trail Making Test (TMT) A and B, test battery for attentional performance, word fluency test and Rey Complex Fgures).

Results: In parallel to the correction of uremia we saw a general improvement of cognitive function. While memory and attention improved over time yielding average performance, executive function (assessed by TMT), despite improvement remained below average.

Raw scores and percentile of neuropsychological test battery

				RAW SCORES (PERCENTILE)			
Hospital day #		0	2	3	5	7	8
TESTS							
Story RBMT (words)							
	recall	8	5.5	10	11	11.5	13
Wechsler Digit Span							
	forward	6	6	7	7	7	9
Trail Making Test (seconds)							
	A	55 (<10)	45 (<10)	34(10-20)	35 (10.20)	31 (20-30)	24 (40-50)
	B	129 (<10)	109 (<10)	101 (<10)	74 (<10)	73 (<10)	64 (20)
TAP (milliseconds)							
	Alterness						
	with acoustic signal	260±57 (24)	226±47 (38)	216±30 (38)	273±40 (12-14)	208±27 (50)	200±31 (73)
	without acoustic signal	279±49 (10)	241±34 (31)	233±29 (38)	257±39 (27)	225±38 (58)	207±23 (73)
LABORATORY PARAMETERS							
	Serum Creatinine (µmol/l)	27.8	18.1	12.4		10.7	7.1
	Serum Urea (mmol/l)	67.6	41.6	32.7		29.9	17.8
	Phosphorus (µmol/l)	2.69	2.2	2.1			
	Hemoglobin (g/dl)	6.3	8.8	7.6			8.7



Conclusions: Severe uremia impairs memory, attention and executive function. Dialysis improves this impairment acutely and remarkably. The reversible (functional) component of cognitive impairment in CKD remains to be elucidated.

Funding: Clinical Revenue Support

FR-PO1109

Anaphylaxis due to Ethylene Oxide Used to Sterilize the Dialysis Needle Tubing Set Iqbal Masood, Paul Robbins. *Nephrology, Lankenau Medical Center & Lankenau Institute for Medical Research, Wynnewood, PA.*

Background: Dialyzer reactions both type A and type B have declined with less use of ethylene oxide for dialyzer sterilization and an increase in the use of bio-compatible membranes .Ethylene oxide is still used for sterilizing the needle set and blood tubing of the dialysis circuit. We are presenting a case of anaphylaxis due to the use of ethylene oxide for sterilization in dialysis needle tubing set, noted when a reaction occured even before connecting to the dialysis circuit.

Methods: 62 year black female with a history of failed kidney transplant necessitating reinitiation of hemodialysis .After starting hemodialysis with Polysulfone dialyzer she was noted to have intermittent pruritis, treated with benadryl.Patient had an episode of facial swelling, prompting change of the dialyzer to cellulosynthetic membrane(ASAHI 100) but 10 minutes into the first session ,she was noted to have stridor, chest pain and required intubation, inspite of pre-treatment rinsing of the dialysis circuit. The dialyzer was again changed to cellulose tri-acetate(Exeltra 150) but yet nother episode of facial swelling occurred within a minute of cannulation and flushing of the needle set tubing prior to connecting to the dialysis circuit, indicating allergy to ethylene oxide used to sterilize the needle tubing set.We found that at our facility needle set tubing and dialysis circuit blood tubing are sterilized with ethylene oxide .Patient did well with pre-treatment rinsing of the entire circuit before cannulation, in additon to dexamethasone,famotidine and benadryl and has not had any more episodes, since she is receiving the same pre-treatment for almost one year before every dialysis treatment.

Conclusions: Dialyzer reactions with anaphylaxis (type A)are mostly due to allergy to ethylene oxide, dialyzer membrane, heparin or bacterial contamination.Ethylene oxide is still used to sterilize the tubing of needle set and dialysis circuit and patients may still face the same reaction. This case illustrates that in addition to the dialyzer, sensitivity to ethylene oxide used in tubings of dialysis circuit and needle set can cause life threatening anaphylaxis.

FR-PO1110

Acute Kidney Injury due to Alvimopan (Entereg) Iqbal Masood, Alejandro Diez. *Nephrology, Lankenau Medical Center & Lankenau Institute for Medical Research, Wynnewood, PA.*

Background: Medications can frequently cause acute kidney injury (AKI). We encountered a case of post renal AKI due to Alvimopan (Entereg). A review of the literature failed to show other documented cases.

Methods: 51 year old black female with normal renal function (serum creatinine: 0.9 mg/dl) underwent an uneventful reversal of a Hartman's procedure. On post operative day 2 her indwelling urinary catheter was removed. On post operative day 4 she was noted to have an elevated creatinine at 3.8mg/dl. Bladder revealed approximately 900mL of urine prompting re-insertion of an indwelling urinary catheter. Serum creatinine improved to 1.0 mg/dl on postoperative day 5.Repeat imaging showed no abnormalities. Indwelling catheter was removed with minimal residual volumes. Review of the medications administered during the hospitalization revealed the patient was started on Entereg on post op day 1. Discontinuation of the medication improved the urinary retention. Unfortunately the medication was restarted during the same hospitalization with concomitant urinary retention and rise in creatinine. The agent was discontinued resulting in resolution of urinary retention and creatinine improvement to 1.0mg/dl.

Conclusions: Entereg is a peripherally acting μ-opioid receptor antagonist which achieves selective gastrointestinal opioid antagonism without reversing the central analgesic effects of μ-opioid agonists. Entereg is used to speed recovery of intestinal motility after gastrointestinal surgery, and prevent adverse effects caused by narcotics. Most common adverse effects of Entereg undergoing bowel resection are anemia, dyspepsia, hypokalemia, back pain, and urinary retention. Entereg is a new medication associated with urinary retention leading to post renal AKI. Prompt discontinuation of the medication may reverse this process.

FR-PO1111

Is Buttonhole Cannulation Associated Bacteremia Under-Reported?

Sabyasachi Roy,[1] Sarabjit S. Bhalla,[2] Priya Radhakrishnan,[1] Rajiv Poduval.[2] *[1]Internal Medicine, St Joseph Hospital & Medical Center, Phoenix, AZ; [2]Nephrology, Southwest Kidney Institute, Phoenix, AZ.*

Background: AV fistulas (AVF) remain the access of choice for hemodialysis (HD) patients. In recent years, the buttonhole (BH) technique for AVF cannulation has been gaining popularity. However BH cannulation (BHC) associated infection remains under-reported. We report 2 cases of bloodstream infection in patients who underwent BHC.

Methods: Case 1. 69-year-old diabetic HD patient, cannulated using BH technique was evaluated for persistent Staphylococcus lugdunensis bacteremia. Inpatient workup with Chest X ray, urine & sputum cultures, & echocardiogram failed to identify any source of infection. Patient was treated with IV Vancomycin for 4 weeks but surveillance cultures showed recurrence of bacteremia. Patient was treated with 2 more weeks of Vancomycin and switched from BH to sharp cannulation. Subsequent surveillance cultures were negative.

Case 2. 53-year-old diabetic HD patient, also cannulated by the BH technique, developed Methicillin Sensitive Staphylococcus Aureus (MSSA) bacteremia. Workup with sputum and urine cultures, Chest X ray and Echocardiogram was negative. Patient was treated with 2 weeks of IV Vancomycin, with resolution of bacteremia. Patient continues HD using BHC, but with improved technique and stringent sterile precautions. Surveillance cultures remain negative.

Conclusions: While the incidence of bacteremia is fairly common in patients undergoing HD, the actual incidence with BHC is unknown. Staphylococcus lugdunensis and MSSA are natural skin commensals. As BHC involves blunt needle introduction through the same fistula site repeatedly, non-healed infected skin and scab overlying the BH can potentially lead to introduction of skin flora into the bloodstream causing bacteremia, sepsis, increased morbidity and healthcare expenditures. Recognising BH associated infections, careful patient selection for this technique, training HD staff adequately regarding aseptic scab removal and proper angulation techniques for needle insertion can help minimize such infections. Further prospective studies will be required to examine the association of infections with BHC.

FR-PO1112

Treatment of Thyroid Storm Using Charcoal Hemoperfusion

Asish Thakkar, Rose Marie Shim, Christopher Valentine, Samir Parikh. *Department of Nephrology, Wexner Medical Center at the Ohio State University, Columbus, OH.*

Background: Untreated hyperthyroidism can be a devastating condition that may lead to thyrotoxicosis. Complications can be severe and include development of congestive heart failure, arrhythmias, psychosis, stupor, coma, and hepatic failure. Traditional treatments include beta-blockers, propylthiouracil, glucocorticoids, and iodine. In refractory cases, thyroidectomy is treatment of choice, however in unstable patients surgery may not be possible and non-traditional therapies must be considered. We report a case of thyrotoxicosis treated with charcoal hemoperfusion at our institution.

Methods: 27 year old female with history of untreated hyperthyroidism presented at 21 weeks gestation with new onset atrial fibrillation and stupor. She was found to be in severe thyroid storm and cardiogenic shock (EF-20%). On initial labs her TSH was <0.008uIU/mL and free T4 was 4.43ng/dl. She was started on hydrocortisone, PTU, propanolol, and SSKI. Unfortunately, her fetus did not survive. Despite five days of aggressive treatment her thyroid hormone levels continued to rise with Total T4>30ug/dl, free T4 >6ng/dl, total T3 >8.0ng/ml. She remained hemodynamically unstable and a poor surgical candidate. Given her refractory course, we attempted charcoal hemoperfusion for thyroid hormone removal. Results are shown in table.

Thyroid Function

Hemoperfusion Tx#	Total T4(ug/Dl)	Free T4(ng/dl)	Total T3(ng/ml)
One	>30.0	>6.00	>8.00
Two	27.9	>6.00	5.67
Three	24.1	>6.00	3.79
Four	19.7	>6.00	3.49
Five	16.4	>6.00	2.98

Conclusions: Hemoperfusion is an uncommon therapy for thyrotoxicosis but has been reported to be successful, particularly after L-thyroxine overdose. The absorbent particle in hemoperfusion can vary from charcoal to other resins which are effective in removing substances that are highly lipid soluble or protein bound. In our patient, hemoperfusion successfully removed thyroid hormone, improved clinical parameters and allowed for successful thyroidectomy. Charcoal hemoperfusion is an effective therapeutic tool for severe thyrotoxicosis and should be considered in patients' refractory to traditional therapies.

FR-PO1113

Persistent Left Superior Vena Cava

Niama Huda, Jian Li, Lalathaksha Murthy Kumbar. *Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: A significant proportion of patients start dialysis with a temporary or tunneled cuffed catheter (TCC) inserted by anatomical landmarks or ultrasound (U/S) guidance. U/S guidance reduces the complications of dialysis catheter insertions.

Methods: A 76-year-old man with a history of end stage renal disease (ESRD) on hemodialysis through a right TCC presented with chest pain and respiratory distress requiring intubation. A contrast enhanced computed tomography (CT) scan of chest revealed septic emboli. The TCC was removed. A left internal jugular vein (IJV) CVC was inserted under U/S guidance at bedside after failed femoral vein catheterization. Post placement chest X ray showed the catheter along the left para-mediastinal region. The CT scan showed a persistent left superior vena cava (PLSVC).

Conclusions: CVC placement is a common procedure. Awareness and recognition of anatomic anomalies of the great vessels of the neck and chest is important. Differential diagnoses for a catheter following a left-sided course include subclavian or carotid artery, pericardium, mediastinum, superior intercostal vein, the left internal mammary vein or PLSVC placement. Arterial insertion can be ruled out through blood gas analysis and waveform transduction, while nonvascular site placement may fail to yield blood upon aspiration. Embryologically, the cardinal veins are the main venous drainage system. The superior vena cava (SVC) is formed by the right common and the right anterior cardinal vein. Left-sided SVC (PLSVC) is the most common thoracic venous anomaly (0.3– 0.5% of the population). PLSVC is caused by the persistence of the left anterior cardinal vein and obliteration of the common cardinal and proximal part of the anterior cardinal veins. Two types of PLSVC reported: 1.PLSVC connecting to the right atrium via coronary sinus-90%. 2. PLSVC connects to the left atrium-10%. Right-sided SVC often coexists with a PLSVC, and CVCs are more commonly placed in the right IJV, hence PLSVCs often go unnoticed. The presence of a PLSVC should be considered when CVC placement is difficult, as it may result in serious complications. Catheter tip manipulation in the coronary sinus may cause angina, arrhythmias or cardiac arrest.

FR-PO1114

Hemodialysis for Treatment of Severe Accidental Hypothermia

Tripti Singh, Kenneth R. Hallows. *Renal Electrolyte Division, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: Severe hypothermia is defined as a core body temperature <28°C. Delays in rewarming and slower rates of rewarming are the most important prognostic factors associated with increased mortality. Consensus exists about the use of cardiopulmonary bypass as a treatment of choice for active internal rewarming of patients. We describe a case where hemodialysis was used to treat severe accidental hypothermia.

Methods: A 49-year old male was found down unresponsive in his garage. On presentation to a regional hospital at 6pm his core temperature was measured at 23.5°C. He was promptly intubated and given warm air along with warm crystalloids intravenously. On arrival at our center, his core temperature was 25.9°C, heart rate 58 bpm, BP 160/90 mm Hg and respiratory rate of 19 on the ventilator. Admission laboratory results included: hemoglobin 4.4g/dl, white blood cell count $2.2\times10^3/\mu l$, serum BUN 44 mg/dl, creatinine of 2.5 mg/dl, Na^+ 138 mmol/L, K^+ 4.4 mmol/L and lactate 10 mmol/L. Arterial blood gas on admission showed a pH <6.8, PaCO2 38mmHg, PaO2 90mmHg and HCO_3^- 5mmol/L which improved to pH 7.01, $PaCO_2$ 33, PaO_2 193, HCO_3^-8 after initial resuscitation. Serum and urine toxicology screens were negative. His core temperature increased to 28.5°C at 11.30pm when hemodialysis was initiated through a right femoral catheter. A large surface area Rexeed 18 dialyzer was used with dialysate flow rate of 800ml/min and a blood flow rate of 400ml/min. The dialysate composition was Na^+ 139mmol/L, K^+ 4mmol/L, Ca^{2+} 2.5mmol/L and HCO_3^- 40mmol/L. No heparin was used. The initial dialysate temperature was 36°C and increased to 38°C after 3 hours. Hemodialysis was continued for a total of 4hours until the core temperature of the patient had increased to 33.4°C. The average rate of rewarming achieved during hemodialysis was 1.25°C/hr.

Conclusions: Hemodialysis can be used as an effective method for active internal rewarming for patients with accidental hypothermia and hemodynamic stability. It is more widely available, less invasive and less expensive than cardiopulmonary bypass and can correct associated acidosis and electrolyte disturbances commonly seen in patients with severe hypothermia.

FR-PO1115

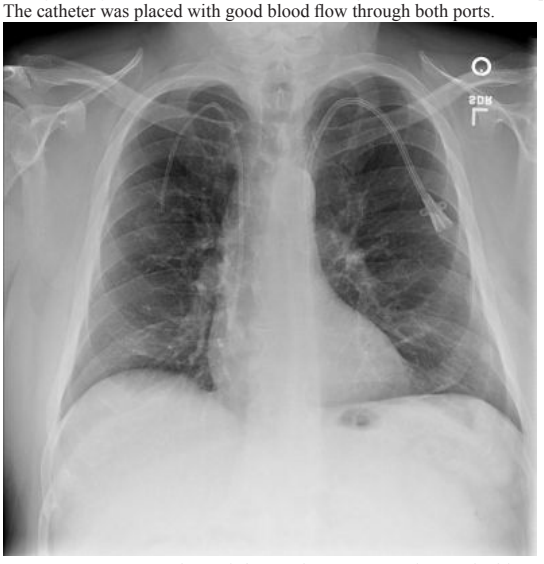
Bilateral Superior Vena Cava: An Interesting Anatomic Variation

Don N. Chang, Jamie L. Ross. *Division of Nephrology, Univerisity of California Davis, Sacramento, CA.*

Background: Chest x-rays are often used for confirmation of successful central venous catheter placement in the internal jugular or subclavian vein. Clinicians should be aware of normal variations in venous anatomy that will change the position of dialysis catheters when viewed on confirmatory x-rays.

Methods: A 59 year old male with a history of diffuse large cell lymphoma was seen in the interventional nephrology suite for placement of an apheresis catheter. He was diagnosed with lymphoma in 1999 and completed a course of chemotherapy with complete remission until November 2011, when he developed back pain and was found to have recurrent disease. He had a port-a-cath placed via his right internal jugular vein 3 months prior to presentation to initiate salvage therapy with R-ICE, with the plan to proceed with autologous stem cell transplant using apheresis to collect the cells. Under flouroscopy, cannulation of his left internal jugular vein resulted in the guidewire persistently staying straight down

and to the left. There was initial concern that the guidewire was in the hemiazygous vein. Contrast was injected through the left internal jugular demonstrated drainage to the heart through a large, left sided venous structure, diagnostic of a left-sided superior vena cava. The catheter was placed with good blood flow through both ports.



Conclusions: Persistent left superior vena cava has an incidence of 0.3% in the general population and 4.3% in those with congenital heart disease. It is the most common anomaly of venous circulation resulting from a failure of the left anterior cardinal vein to obliterate with drainage normally to the coronary sinus. After ruling out placement in the hemiazygous vein, practitioners without the benefit of fluoroscopy should be aware of this anomaly and include it in the differential when a left-sided central venous catheter does not travel along its usual course.

FR-PO1116

Role of Hemodialysis after Disruption of Blood Brain Barrier Following Intra-Arterial Contrast Jingyin Yan, Medha Airy, Venkataraman Ramanathan. *Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Osmotic disruption of blood brain barrier (BBB) and subsequent subarachnoid contrast extravasation and cerebral edema have been rarely reported after intra-arterial contrast use. We report an end stage renal disease (ESRD) patient who developed such a complication following intra-arterial contrast and who also had brisk clinical improvement and complete resolution of intraventricular contrast enhancement after emergent hemodialysis (HD). We propose a potential role for HD in those patients.

Methods: A 63 year old man, with history of left orbital apex syndrome and ESRD on HD, was admitted with massive epistaxis. Pseudo aneurysm in left internal carotid artery (ICA) was diagnosed and cerebral arteriogram was performed with subsequent embolization. 910 mL of intra-arterial contrast (iodixanol) was used during the procedure. Following the procedure, the patient developed severe altered mental status and was intubated. CT was done immediately and it showed new bilateral subarachnoid hyperattenuation and diffuse cerebral edema. The patient was emergently dialyzed, within 4 hours after contrast exposure, with high-flux dialyzer, followed by another HD session in 24 hours. His mental status improved remarkably following contrast removal with HD and was extubated the next day with no residual neurologic deficits. Repeat CT done in 24 and 72 hours showed partial and complete resolution of both subarachnoid hyperattenuation and brain edema, respectively.

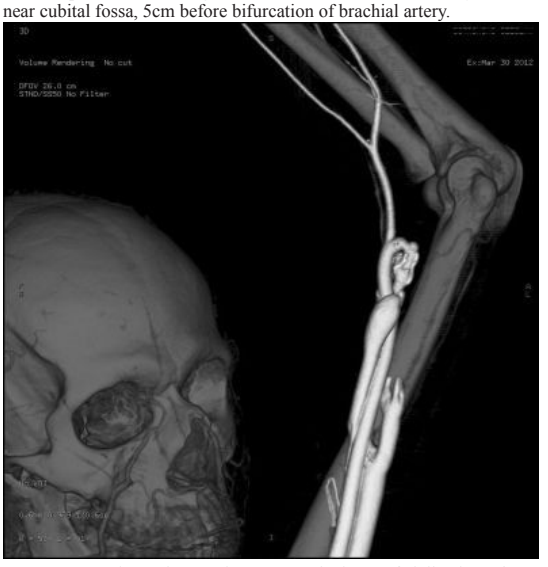
Conclusions: Iodinated contrast can be removed with HD and blood concentration can be quickly reduced. This is partly due to its size, water solubility and low protein binding properties. Even though ~80% of contrast is removed with a 4 hr HD session, the utility of immediate post-contrast HD in ESRD patients is always questioned. But this case represents a unique situation. Large volume of intra-arterial contrast used in neurologic interventional procedures is associated with disruption of BBB and severe cerebral edema. Prompt removal of contrast with HD lead to significant clinical recovery. To our knowledge, this is the first case to describe a potential therapeutic role for prompt HD in such patients.

FR-PO1117

Congenital Arteriovenous Fistula of Left Brachial Vessels Used for Hemodialysis after Superficialization Mariusz Kusztal,[1] Krzysztof Letachowicz,[1] Tomasz Golebiowski,[1] Ewa Watorek,[1] Przemyslaw Szyber,[2] Maciej Guzinski,[3] Katarzyna Madziarska,[1] Magdalena Krajewska,[1] Jerzy Garcarek,[3] Waclaw Weyde,[1] Marian Klinger.[1] *[1]Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland; [2]General and Vascular Surgery, Wroclaw Medical University; [3]Radiology, Wroclaw Medical University.*

Background: Vascular abnormalities (more often in ADPKD) may produce difficulties in vascular access creation.

Methods: We report on a 20-year-old men with a history of ADPKD and currently failing 5-year kidney transplant that presented with congenital arteriovenous fistula (AVF) in the left arm. At the age of 14y due to the lack of suitable veins on both upper extremities for AVF creation and asymptomatic "vascular anomaly" on the left arm he started peritoneal dialysis. Currently, following physical examination, Doppler ultrasonography, venogram, CT-angiography (figure) the diagnosis of a brachial artery-to-brachial vein AVF was made. The oblique communicating channel between the brachial artery and brachial vein mapped near cubital fossa, 5cm before bifurcation of brachial artery.



Results: The patient underwent surgical superficialization of concomitant vein as described by Weyde et.al (Kidney Int. 2002;61:1170, J Vasc Access. 2006;7:74), used for deeply located arterialized vein. Four weeks after the procedure the AVF was successfully cannulated for hemodialysis. Doppler ultrasonography showed AVF blood flow of 1292ml/min, brachial artery diameter of 6.1 and 4mm at pre and post communicating channel section respectively. The vein transposed underneath the skin had 7mm in diameter. Plethysmography (PVL, Biomedix, MN) performed pre and post operatively showed no ischemia up to fingers level on both extremities.

Conclusions: To our knowledge, this is the first reported case of congenital AVF converted to vascular access for hemodialysis.

Funding: Government Support - Non-U.S.

FR-PO1118

Acute Kidney Injury as the Initial Manifestation of Non-Hodgkin Lymphoma Mana Dissadee, Andres Serrano. *Department of Medicine Mount Sinai Hospital, RFUMS/CMS, Chicago, IL.*

Background: Bilateral infiltration of the kidneys from lymphoma is a rare entity. We report a case of diffuse large B-cell lymphoma presenting as acute kidney injury (AKI).

Methods: An 18-year-old man with no significant past medical history, presented with two months history of right-sided headache. He was taking 20 tablets of Ibuprofen per day for pain control. The review of systems was positive for generalized weakness, anorexia and 15 kg weight loss in 3 months. Physical exam showed right temporal scalp swelling and mild bilateral costovertebral tenderness. Laboratory data revealed a BUN of 64 mg/dl and a creatinine of 9.18 mg/dl. Urinalysis showed protein of 30 mg/dl, negative glucose, RBC of 1/HPF, WBC 1/HPF and no casts. Patient was admitted with diagnosis of AKI secondary to NSAIDs abuse. Ibuprofen was discontinued and he was started on IV fluids. Two days later, his renal function failed to improve. Renal ultrasound revealed bilateral enlarged kidneys with hydronephrosis. Urology was consulted and bilateral ureteral stents were placed, but his renal function did not change. Renal biopsy showed diffuse infiltration by intermediate-to-large mononuclear cells with prominent nucleoli. Immunophenotype studies were CD20+, Bcl-6+, MUM-1+, CD10-, TdT-. This is consistent with diffuse large B-cell lymphoma. Subsequent staging work-up was initiated. Bone marrow aspiration and biopsy were negative for malignancy. MRI of the brain showed thickening of the dura with lesion within the calvarium. Bone scintigraphy showed lytic lesion in the right ilium. Patient was treated with hemodialysis and chemotherapy. A dramatic improvement of renal function was observed after 2 weeks with complete recovery in 4 weeks.

Conclusions: This is an unusual presentation of extranodal lymphoma. Overall understanding of this disease is limited to 20 case reports in the literature. Prognosis is poor with 1-year mortality rate of 75%. Chemotherapy with CHOP plus Rituximab can increase complete response rates and prolong disease-free survival.

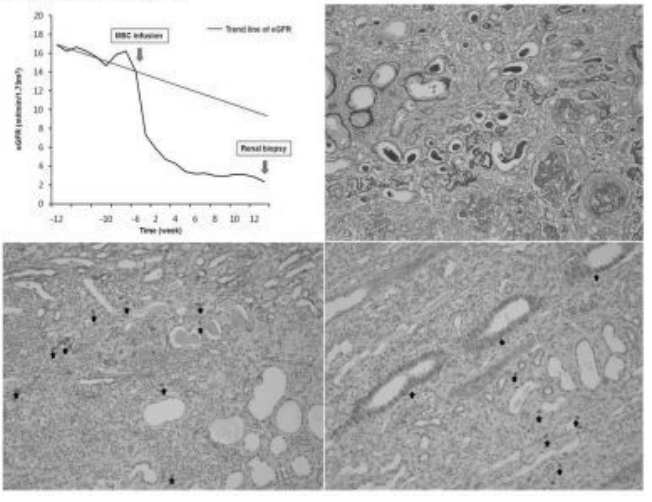
FR-PO1119

Rapid Deterioration of Preexisting Renal Insufficiency after Autologous Mesenchymal Stem Cells Therapy Jun-Seop Kim,[1,2] Owen Kwon,[1,2] Ji-Young Choi,[1,2] Jang-Hee Cho,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Yong-Jin Kim,[3] Yong-Lim Kim.[1,2] *[1]Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, Republic of Korea; [2]Clinical Research Center for ESRD in Korea, Republic of Korea; [3]Department of Pathology, Yeungnam University College of Medicine, Daegu, Republic of Korea.*

Background: Autologous mesenchymal stem cell (MSC) administration has shown to improve renal function and histologic finding in acute kidney injury (AKI) models. However, whether MSC can contribute to the regression of renal fibrosis and regeneration of nephron in chronic kidney disease (CKD) is unclear, especially in clinical setting. Moreover, efficacy of homing mechanism and safety of transdifferentiation are not established in late CKD patient. Here, we report a case of CKD patient with focal segmental glomerular sclerosis treated by intravenous infusion of autologous MSC derived from adipose tissue in a foreign country.

Methods: The renal function of patient had been stable for several years before MSC administration. One week after the autologous MSC infusion, the preexisting renal insufficiency was rapidly aggravated without any evidences of AKI. Two months later, eGFR was markedly reduced from 16 to 7.3 ml/min/1.73m^2. Hemodialysis was started at 3 months after MSC administration. The renal biopsy findings at the time of dialysis showed severe interstitial fibrosis and inflammatory cell infiltration with a few cells expressing CD34 and CD117, surface markers of stem cell.

Figure 1. Clinical course and renal biopsy findings of patient with MSC therapy. (a) Clinical course. (b) Histologic finding with PAS staining. (c) Immunohistochemical staining with CD34. (d) Immunohistochemical staining with CD117.



Conclusions: This case emphasizes the potential nephrotoxicity of autologous MSC therapy in CKD patients although positive reports about the efficacy of MSC therapy in experimental CKD model are increasing.

Funding: Government Support - Non-U.S.

FR-PO1120

A Case of Lymphocytic Hypophysitis with Normal Imaging Adeel A. Siddiqui,[1] Aqeel A. Siddiqui,[1] Anshul Kumar.[2] *[1]Nephrology, Lankenau Medical Center, Wynnewood, PA; [2]Internal Medicine, Lankenau Medical Center, Wynnewood, PA.*

Background: There have been over 350 cases of Lymphocytic Hypophysitis identified so far. Among these, several had positive MRI findings. We report a case of Lymphocytic hypophysitis with normal MRI.

Methods: The patient is a 31-year-old female 5 months postpartum with no significant PMH presented with 1 day history of confusion.She was brought to the ER and intubated for airway protection. For the last 2 weeks, she noticed decrease lactation. BP 130/78, HR 71, temp 98.9 F.Lab data revealed Na 121 mEq/L, BUN < 5mg/dl, Creatinine 0.6 mg/dl ,Uric acid 1.4mg/dl, S. Osmolality 280 mOsm/kg, Urine Osmolality 106 mOsm/kg, Urine Na 69 mEq/L/day. Initially, she was treated with NSS and her Na increased to 137 in a span of 16 hours, then she was given DDAvP and her Na dropped to 131. In the mean time, she developed hypotension and was started on Nor Epinephrine drip. CT of the brain and LP was unremarkable.Euvolemic hyponatremia due to SIADH, glucocorticoid deficiency, or hypothyroidism was considered. MRI of the brain was performed to R/O destructive pituitary lesion that showed a normal pituitary gland.TSH and Free T4 were normal,ACTH was low at <5 pg/ml with low cortisol level 2.5 mcg/dl .Prolactin level was normal at 58.3 ng/ml.With hypotension and low ACTH, she was started on IV hydrocortisone. After glucocorticoid replacement, the patient's symptoms were dramatically resolved. However, 2 days after glucocorticoid replacement, her Na level increased to 139 meq/L with urine osmolality of 54. Patient was started on DDAvP which later tapered off as an outpatient. She was continued on hydrocortisone with stable Na.

Conclusions: Among the reported cases of lymphocytic hypophysitis,our patient had a very unique presentation.The pathophysiological process was well shown in our patient who developed water diuresis and hypernatremia after glucocorticoid therapy.Characteristics of lymphocytic hypophysitis seen on brain MRI include stalk thickening, diffuse enlargement of the pituitary gland and homogeneous enhancement of gadolinium contrast medium.Our patient had a normal MRI.

FR-PO1121

Healthy Young Male with Left-Lower Quadrant (LLQ) Abdominal Pain Huma S. Hasnain, Susanne B. Nicholas. *David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Renal artery dissection (RAD) is a rare condition that can lead to renovascular hypertension and renal mass loss. The following is a rare presentation of acute isolated spontaneous bilateral RAD.

Methods: A 52-year-old gentleman presented to the Emergency Department for evaluation of the sudden onset of sharp, LLQ abdominal pain lasting 2 hours, associated with nausea and emesis. His past medical history was significant for a similar episode of right-lower quadrant pain two years prior that prompted an appendectomy for presumed appendicitis. The pathology report showed a normal appendix, but renal angiogram done 2 weeks later noted vascular occlusion of the right renal artery with renal infarctions and focal cortical loss. His occupation as an air-traffic controller involved unusual physical exertion and stress with rapid twisting of the abdomen and periods of "bearing down." He had no known history of hypertension, and was normotensive on the recent presentation. Laboratory findings noted a serum creatinine level of 1.4mg/dl, urea nitrogen level of 14mg/dL; and an unremarkable urinalysis. CT scan noted a new, left renal infarct with decreased perfusion to the lateral aspect of the left kidney. Extensive evaluation for an underlying cause was negative: skin biopsy revealed no collagen abnormalities, echocardiogram was normal and genetic testing revealed no prothrombotic mutations. Management of this patient's bilateral RAD was non-operative, without anticoagulation. CT scan obtained 1 year later showed normal renal arteries without evidence of dissection; cortical loss was seen in 2-3 areas of the right kidney and 2 areas of the left kidney. Iothalamate renal clearance studies showed an improvement in GFR from 57ml/min to 73ml/min.

Conclusions: This is a rare presentation of isolated spontaneous RAD. These types of dissections can occur as a result of unusual physical exertion and are temporally related to the activity. Mechanisms may include the sudden increase in intra-abdominal pressure or blood pressure via the Valsalva maneuver. Management is mostly non-operative, with improvement within 6-12 months of anticoagulation and blood pressure control.

FR-PO1122

Dabigatran Induced Acute Kidney Injury Deepak Kadiyala,[1] Ursula C. Brewster,[1] Gilbert W. Moeckel.[2] *[1]Nephrology, Yale University, New Haven, CT; [2]Pathology, Yale University, New Haven, CT.*

Background: Dabigatran is a newly approved oral direct thrombin inhibitor that does not require frequent blood monitoring of levels. We describe a patient who developed AKI in the setting of its initiation and a renal biopsy that revealed the mechanism.

Methods: A 67 year-old caucasian male presented with AKI and 1 week of gross hematuria. He denied flank pain or respiratory tract symptoms. Past medical history included coronary artery disease, paroxysmal atrial fibrillation, and benign prostatic hypertrophy. He had no prior renal disease. Medications included aspirin, sotalol, omperazole, lisinopril and dabigatran. Coumadin was changed to dabigatran 1 week prior to presentation. BP was 140/76mmHg. Physical examination was unremarkable; no rashes or edema. Laboratory data: normal CBC, BUN 57 mg/dl, creatinine 5.5 mg/dl, autoimmune and viral serologies were negative, complements were normal. Renal biopsy showed extensive tubular injury with cytoplasmic reabsorption droplets containing eosinophilic proteinaceous material and RBC casts in the areas of tubular injury. Immunohistochemistry showed strong, diffuse positivity for CD163 in proximal tubular cells. Light microscopy, IF and EM of the glomeruli were consistent with mild underlying IgA nephropathy with no hypercelluarlity. His serum creatinine stabilized at 6.0 mg/dl without need for dialysis and slowly declined over a 2-month period to 2.7 mg/dl. Final diagnosis was subclinical IgA Nephropathy that led to glomerular bleeding with Dabigatran.

Conclusions: AKI in IgA-nephropathy can be due glomerular bleeding leading to tubular injury, Heme molecule is tubulo-toxic by causing lysosomal overload, inflammation, apoptosis, oxygen radical formation and endothelin-1 induction causing ischemic injury. Dabigatran should be used cautiously in patients with known kidney disease or with evidence of hematuria. Monitoring for hematuria might be prudent for an early diagnosis of subclinical nephropathy as in our patient.

Kveder, R AKI in IgA nephropathy: potential role of macroscopic hematuria and acute tubulointerstitial injury. Ther Apher Dial, 13: 273-277, 2009.

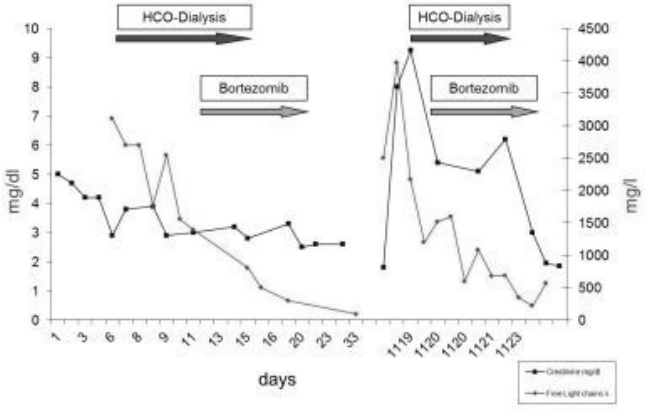
FR-PO1123

Twice Successful Treatment of Multiple Myeloma-Induced Acute Renal Failure with High-Cutoff Dialysis Daniel Zickler, Achim Joerres, Ralf Schindler. *Nephrology and Intensive Care, Charite University Medicine.*

Background: Multiple Myeloma with excessive free light chain production can lead to Cast-nephropathy with impairment of renal function. In cast nephropathy kidney damage is induced via the toxic effects of the free light chains. Early removal of FLC correlates with chances of renal recovery. The High-cutoff-filter HCO 1100 is a new dialysis-membrane which allows the elimination of molecules with a size of up to 60-70 kd. We report a 63

year-old female patient diagnosed with Multiple Myeloma Type Lambda developing Cast-nephropathy with consecutive acute renal failure. The treatment of the first episode, as well as the relapse was treated with HCO-Dialysis in addition to Chemotherapy.

Methods: Case Description Initially the 63 year-old female patient presented to the ER with back pain. Laboratory values revealed acute renal failure and 120-fold-increased lambda FLC. Kidney biopsy showed Cast nephropathy. Thus, chemotherapy with Bortezomib, Dexamethasone and Cyclophosphamide was started. Acute renal failure required hemodialysis therapy. To enhance FLC removal, six dialysis sessions with the High-cutoff-filter HCO 1100 were performed. Lambda FLC have a molecular size of 45 kd. Kidney function improved to a non-dialysis dependent level. Three years later, after high-dose melphalan and preceding stemmcell apheresis a relapse with excessive FLC production occurred, again causing dialysis dependent acute renal failure. Once more, dialysis with High-cutoff-filters was started, again causing significant reduction of FLC, as well as improvement of renal function.



Conclusions: High cutoff dialysis may provide a therapy option not only for newly diagnosed cast nephropathy but may also be of interest in therapy of relapsing multiple myeloma with high levels of free light chains.

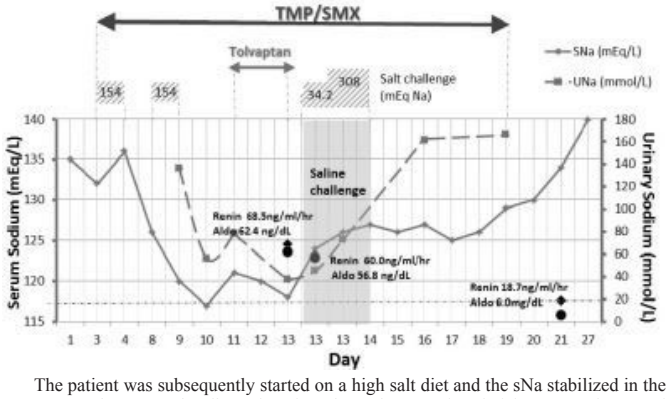
FR-PO1124

Trimethoprim/Sulfamethoxazole Associated Renal Salt Wasting

Revekka Babayev, Sofia Fayngold, Subani Chandra, Jai Radhakrishnan, Sumit Mohan. *Department of Medicine, Columbia University Medical Center, New York, NY.*

Background: Renal salt wasting (RSW) is rare and difficult to differentiate from SIADH clinically. Here we report a case of a 28 year old man with HIV and Pneumocystis pneumonia (PCP) who developed hyponatremia while receiving 16mg/kg/d trimethoprim-sulfamethoxazole (TMP/SMX).

Methods: Serum Na (sNa) on admission was 135mEq/L (with no prior history of hyponatremia) and dropped gradually to 117mEq/L by day 7 of TMP/SMX. In a setting of unclear volume status and PCP, he was initially treated for SIADH with fluid restriction and tolvaptan without improvement in sNa. A diagnosis of renal salt wasting secondary to TMP was subsequently confirmed with clinical hypovolemia and high renin 68.5ng/mL/hr (normal 0.2-4ng/mL/hr), aldosterone 62.4ng/dL (normal ≤31ng/dL), urinary Na 45mmol/L, FEphosphate 76% (normal <20%) and FEurate 13.6% (normal ≤10%). Tolvaptan was discontinued and a challenge of 2L 0.9%NaCl was given with improvement in sNa to 126mEq/L, and reductions in renin and aldosterone to 18.7ng/mL/hr and 6ng/dL respectively confirming the diagnosis of RSW.



The patient was subsequently started on a high salt diet and the sNa stabilized in the 126-129mEq/L range. After discontinuation of TMP/SMX on hospital day 19, sNa improved further into the normal range and was 140mEq/L on last follow up.

Conclusions: TMP/SMX related RSW is probably underdiagnosed and often mistaken for SIADH since both have low serum uric acid levels. RSW should be considered for patients on high dose TMP/SMX and can be differentiated from SIADH by the presence of high FEurate and FEphosphate and clinical hypovolemia (confirmed by high renin and aldosterone levels) (Maesaka et al, Kid Intl, 71, 2007). RSW from TMP can be treated with Na supplementation to offset ongoing urinary losses if the TMP/SMX cannot be stopped.

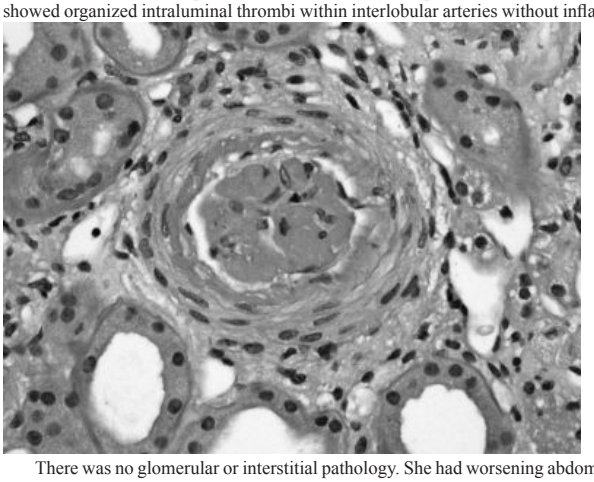
FR-PO1125

Bilateral Renal Artery Thrombosis Associated with Acute Necrotizing Pancreatitis

Bijin Thajudeen, Pooja Budhiraja. *Nephrology, University of Arizona College of Medicine, Tucson, AZ.*

Background: Renal artery thrombosis is a rare, but serious and often under diagnosed, condition. Prevalence is 0.002% based on case series of emergency department patients.

Methods: A 66-year old Caucasian female presented with abdominal pain and oliguria. She was diagnosed with acute renal failure 2 weeks ago due to acute pancreatitis which resolved with hydration. The only significant finding on physical examination was epigastric tenderness. Serum chemistry revealed elevated BUN, Creatinine and LDH. Urine analysis showed hematuria and 1+ proteinuria without any dysmorphic RBCs or casts. Renal biopsy showed organized intraluminal thrombi within interlobular arteries without inflammation.



There was no glomerular or interstitial pathology. She had worsening abdominal pain, hypotension and CT of the abdomen with contrast was done which showed renal infarction and bilateral renal artery thrombosis in addition to possible bowel perforation. Emergent laprotomy showed necrosed pancreas. Hemodialysis and anticoagulation were initiated. Doppler studies showed deep vein thrombosis of bilateral lower extremity and internal jugular vein thrombosis. Workup for hypercoagulability and vasculitis were unremarkable. There was no past medical or family history of hypercoagulable state. Final impression was acute kidney injury secondary to bilateral renal artery thrombosis and eitiology of renal artery thrombosis thought to be due to hypercoagulability of acute necrotizing pancreatitis.

Conclusions: Renal infarction from bilateral renal artery thrombosis secondary to hypercoagulable state of necrotizing pancreatitis has never been reported before. Possible etiologies increase in factor VIII, fibrinogen ,factor V activity,DIC. A high degree of suspicion is thus needed when symptoms arise acutely in the setting of a predisposing condition.

FR-PO1126

Gitelman's Syndrome in Pregnancy: Challenges in Management

Rajan Kapoor, Tarun Chugh, Savneek S. Chugh, Sreedhar R. Adapa, Maureen E. Brogan. *Department of Nephrology, New York Medical College, Valhalla, NY.*

Background: Gitelman's syndrome (GS) is a rare autosomal recessive disorder, caused by inactivating mutation of NaCl cotransporter in DCT, leading to hypokalemia and hypomagnesemia. GS in pregnancy is associated with further worsening of serum electrolyte making management of GS in pregnancy a challenge.

Methods: We present a case of 23 yo female with GS, diagnosed 2 yrs ago, when we worked her up for persistent hypokalemia and hypomagnesemia following a seizure episode with no apparent neurological cause. She was started on spironolactone, potassium chloride (KCl) and magnesium oxide (MgO). Since then her K^+ stayed 3 -3.5meq/L and Mg^{2+} 1.5 – 2.4mg/dl with no seizures. Now, she presents during her second pregnancy at 6 weeks gestation. Her first pregnancy was in Peru (records not available). She was on prenatal vitamins and above supplements except spironolactone (stopped 4 weeks back). Vitals were stable, BP 90-96/ 46-50 mm Hg. LABS: K^+ 2.7meq/L, Mg^{2+} 1.2mg/dl, HCo_3 26meq/L. She was started on Eplerenone and KCl and MgO doses were increased. Despite increasing medication doses (max doses- KCl 80meq Q4hr, MgO 800mg Q4hr, eplerenone 25mg BID), her K^+ remained 2.5 – 3.0 meq/L and Mg^{2+} 0.8 – 1.5 mg/dl and hence, she required three hospitalizations by 24th week for intravenous (IV) supplements. To minimize hospitalizations, she initially got intramuscular (IM) Mg^{2+} twice weekly and later K^+ and Mg^{2+} through PICC line/IV(thrice weekly) along with oral supplements. At 37th week, she delivered a 2420 g child vaginally with no complications. Post delivery, her serum K^+ and Mg^{2+} levels improved and oral supplement doses were decreased.

Conclusions: Obstetric literature supports the association of GS with development of oligohydramnios and IUGR in pregnancy. Given our patient's history of seizures and risk of developing above complications, she required a very close weekly follow up. Literature shows good outcomes in these patients, with aggressive electrolyte repletion (even multiple hospitalizations for IV supplements), as reciprocated in our case too. We propose an outpatient management approach with IV or IM supplements in such high risk patients.

FR-PO1127

Electrolyte Abnormalities of the Ectopic ACTH Syndrome Rebecca Kurnik Seshasai, John S. Barbieri, Amos J. Shemesh, Mina Sedrak, Brenda B. Hoffman. *Univ of Pennsylvania, Philadelphia, PA.*

Background: We describe a patient who presented with severe hypertension, hypokalemia and metabolic alkalosis with a rare diagnosis of ectopic ACTH syndrome secondary to thymic carcinoid tumor.

Methods: A 61 year old man with history of stable hypertension presented with lower extremity edema, dyspnea, 16kg weight loss, new onset diabetes, and worsening hypertension. He denied vomiting and diarrhea. Home medications were ramipril 10mg, furosemide 20mg, valsartan/hctz 320/25mg, amlodipine 10mg and metformin. Exam was notable for BP 184/120 and 3+ lower extremity edema. Labs were notable for Na 140 mmol/L, K 1.7 mmol/L, Cl 95 mmol/L, HCO_3 40 mmol/L, BUN 26 mg/dL, creat 1.3 mg/dL, Mg 1.6 mg/dL, and arterial pH 7.63. CTA showed patent renal arteries and bilateral pulmonary emboli. Lower extremity doppler showed an acute DVT. Renin activity and aldosterone levels were low: renin 0.2ng/ml/hr and aldosterone <1.6ng/dL. Cortisol was 47.9 µg/dL (normal 6-23 µg/dL) and 24 hr urine cortisol was 2960 µg/day (normal <60 µg/day). After 1mg dexamethasone suppression, cortisol was elevated at 91.6 µg/dL. ACTH was 185 pg/ml (normal 7-69 pg/ml). These findings were consistent with ACTH dependent Cushing syndrome. PET-CT showed a 3.7 x 5.5cm mass in the anterior mediastinum and bilateral adrenal hyperplasia. The patient received carvedilol 25mg bid, hydralazine 10mg tid, valsartan 320mg daily, spironolactone 100mg daily, ramipril 10mg daily, amiloride 5mg bid and >200mEq KCl daily, yet he remained hypokalemic. His TTKG was 17.84 demonstrating significant urinary potassium wasting, and his BP remained elevated. The tumor was removed and pathology showed a typical carcinoid tumor of the thymus which stained positively for ACTH, confirming ectopic ACTH syndrome. His cortisol, ACTH, glucose and potassium all normalized post-operatively.

Conclusions: Most patients with the ectopic ACTH syndrome have severe hypokalemic metabolic alkalosis due to cortisol binding the mineralocorticoid receptor. This syndrome is rarely seen in Cushing disease with pituitary secretion of ACTH. Thymic carcinoid tumors are rare and aggressive with a poor prognosis, and those patients with Cushing syndrome have the lowest survival rates.

FR-PO1128

IgA Nephropathy Following TNF-alpha Antagonists for Ankylosing Spondylitis and Crohns Disease Muhammad Omer, Cybele Ghossein. *Nephrology/Internal Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Background: We report an unusual case of IgA nephropathy in a patient treated with Tumor Necrosis Factor (TNF) alpha antagonists for Ankalosing Spondilitis (AS) and Crohns Disease (CD).

Methods: A 22 year old man with AS diagnosed in 2006 presented for nephrology consultation for an elevated creatinine obtained on routine screening. His AS was initially treated with non steroidals and Etanercept until 2008 when he was diagnosed with CD and etanercept was switched to Adalimumab. Both AS and CD remained in remission with improvement in back pain, joint stiffness, diarrhea and functional status. Laboratory data on presentationrevealed a serum creatinine was 1.73 mg/dl. His urinalysis showed 3+ protein and > 100 RBCs/HPF and his urine spot protein / creatinine ratio was 2.6. Serological work up including ANA and ANCAwas negative on presentation. A Kidney biopsy was consistent with IgAN HAAS grade III.

Conclusions: Both AS and CD have been associated with IgAN (1, 2). Frequent flares usually secondary to respiratory and gastrointestinal infections, leading to antibody production by the hyperactive mucosal immune system is considered to be the underlying etiology in these patients (3). Our case is unique in that our patient was in remission, with no recent flares. The most likely etiology of his IgAN, is the use of Adalimumab. This effect by Adalimumab, a human monoclonal antibody against TNF, is the result of cytokine imbalance, caused by a switch of the cytokine response from T-helper type 1 to T-helper type 2. This switch can result in the production of antibodies by activated B cells which can potentially cross-react with relatively elevated circulating IgA1 in patients with AS and Crohns disease producing IgA1 immune complexes and leading to IgAN. Frequent and close monitor of renal function and urinalyses in patients on TNF alpha antagonists may lead to the detection of the glomerular disease process in its earliest form.

1. Bruneau et al*Semin Arthritis Rheum*.1986;15:179 –184
2. Peeters et al *Ann Rheum Dis*. 1990;49:638–640.
3. Bene et al Am J Kidney Dis. 1988;12:406-9.

FR-PO1129

A Case Report of Noncirrhotic Portal Fibrosis in a Renal Transplantation Recipient Hae Min Lee, Keunsuk Yang, Jeong Gwan Kim, Myung Hyun Lee, Byung Ha Chung, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim, Bum Soon Choi. *Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, the Catholic University of Korea, Seoul, Korea.*

Background: Noncirrhotic portal fibrosis (NCPF) can be a cause of portal hypertension in non-cirrhotic patients, and it can lead to serious complications such as a variceal bleeding. Although bacterial infection and immune abnormalities have been proposed as possible etiologies of NCPF, there is a limited understanding of the disease process. We describe a rare case report of NCPF in renal transplantation recipient which first manifested as a variceal bleeding then treated successfully.

Methods: A 40-year-old man who underwent living donor kidney transplantation 7 months ago visited our emergency room because of syncope. He had diabetes and history of CMV DNAemia. He had a tachycardia at the time of visiting. His hemoglobin level declined to 5.1 g/dL from 9.8 g/dL within 2 weeks. He was performed emergency esophagogastroduodenoscopy (EGD). We found active bleeding on gastric cardiac varices then stopped bleeding by endoscopic variceal ligation (EVL). We reviewed his past EGD before transplantation, but there was no evidence of variceal change. On his abdominal imaging, we could find intra-hepatic arteriovenous shunt without liver cirrhosis nor splenic thrombosis, then performed embolization successfully. He had a liver biopsy that shows he has mild portal fibrosis. 6 months later, we performed transjugular intrahepatic portosystemic shunt (TIPS), because cardiac varices had been growing on follow up EGD despite additional EVLs and adequate dose of beta blocker. 7 months later, he had no additional bleeding and the patency of TIPS stent is intact.

Conclusions: NCPF can occur in renal transplantation recipients because they use immunosuppressant and are vulnerable to infection. The most important point of managing NCPF is to prevent life-threatening variceal bleeding. EVL and beta blockers are common therapy for the primary prophylaxis. But if variceal bleeding recurred frequently in patients with NCPF despite adequate primary prophylaxis, TIPS can be considered to reduce the portal flow.

FR-PO1130

Improvement of Aplastic Anemia after Kidney Transplantation and Immunosuppressive Therapy Jeong Gwan Kim, Hae Min Lee, Myung Hyun Lee, Keunsuk Yang, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Department of Internal Medicine, Seoul Saint Mary's Hospital, the Catholic University of Korea, Seoul, Korea.*

Background: Usually treatment of aplastic anemia (AA) is bone marrow transplantation (BMT) or immunosuppressive (IS) therapy. And common IS agents are anti-thymocyte globuline (ATG) plus cyclosporine A (CsA). Several investigators tried to deliver an intensified IS by adding a third IS agent. However, this strategy did not show results in a substantial benefit.

Methods: A 35-years-old female patient was diagnosed with stage V chronic kidney disease (CKD) and severe AA. And she had been treated chronic kidney disease by continuous ambulatory peritoneal dialysis. For the treatment of AA, we used ATG in five days, but there was no response. So we planed kidney transplantation (KT) and BMT. At first KT and IS therapy were begun. That IS agents were tacrolimus, MMF and prednisolone. Laboratory finding was improved gradually after KT. Recently laboratory findings show that her AA status is not indication of BMT. So we will decide a need for BMT after further IS therapy.

Conclusions: On the basis of our clinical experience, we suggest that kidney transplantation in AA patients may be success, and tacrolimus may be an effective IS agent used to treat AA.

FR-PO1131

An Exploration of Falsely Elevated Tacrolims Concentrations in a Kidney Transplant Patient: A Case Report Sahoko Yamamura, Yudo Tanno, Hiroshi Hayakawa, Hiroyasu Yamamoto, Keitaro Yokoyama, Tatsuo Hosoya. *Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan.*

Background: Affinity column mediated immunoassay (ACMIA) is a more simple and rapid way of measuring tacrolimus (TAC) levels compared to previous methods such as chemiluminescence immunoassay (CLIA). Although a good correlation in the results has been reported, there have been some reported cases with a discrepancy between the two sets of results.

Methods: We report a case of a 61 year old woman who underwent renal transplantation 3 years ago. After transplantation, her baseline serum creatinine level remained around 1.3mg/dl with a stable trough TAC level of around 5ng/ml measured using CLIA. However, when her TAC level was measured using ACMIA, it showed a sudden rise to 58.1ng/ml. Thereafter, it continued to yield consistently high levels when using ACMIA with no correlation with the results obtained from CLIA. Furthermore, levels of TAC in the serum, which should have been undetectable also demonstrated abnormally high readings using ACMIA. Thereby, instead of using whole blood samples, samples containing only washed erythrocytes (without serum) were used to assess if factors in the serum were playing a role. In this test, same results were obtained using the two methods (ACMI and CLIA). This suggested that factors in the serum were somewhat responsible for giving falsely high TAC levels in ACMIA. Furthermore, in samples tested after removal of human IgG, IgA, IgM, correct levels of TAC were only obtained in those containing no IgG. This indicated that false readings were due toIgG antibodies in the serum. Effects of human anti-mouse antibody and anti-β-galactosidase antibody on TAC levels, which had been reported to give high readings were also assessed. However, this yielded negative results.

Conclusions: In our case, IgG subclass was found to be responsible for the falsely high TAC levels. In such cases, whole blood samples should be re-tested if available, using other methods such as CLIA. Otherwise, in order to avoid false results it is sufficient to use ACMIA, provided washed erythrocytes without serum are used for testing.

FR-PO1132

Reactivation of Whipple's Disease in a Prosthetic Aortic Valve after Kidney Transplant Jiwan K. Thapa, Neha Garg, Titte Srinivas. *Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Background: Whipple's disease (WD) is a bacterial infection with multi-system manifestations caused by gram positive bacterium Trophyrema Whipplei. Here, we describe a rare presentation of reactivation of WD as prosthetic valve endocarditis (PVE) after initiating immunosuppression for renal transplant.

Methods: We present a case of 72 yr old male diagnosed with WD 14 years prior when he had presented with polyarthritis involving large joints for past 7 years unresponsive to treatment. Diagnosis was made after Lt Hip arthroplasty when PCR (in blood and synovial tissue) and PAS positive staining (in synovial tissue) showed T. Whipplei. Duodenal tissue PCR was negative for T.Whipplei. His symptoms resolved with IV Ceftriaxone followed by oral Bactrim for 1 year. Rt hip arthroplasty done 2 years later was negative for T. Whipplei by PCR.

2 yrs ago, he underwent Aortic Valve Replacement (AVR) with Carpentier Edwards # 23 valve for Aortic Regurgitation. 1 year ago, he received deceased donor kidney transplantation (CMV D/R-, EBV D-/R-) for hypertensive nephrosclerosis. He had Simulect induction and was maintained on Tacrolimus/ Cellcept/Prednisone with stable allograft function (S.Cr 1.5 mg/dl).

Over last 1 year, he developed progressive fatigue & weight loss. Evaluation showed 3-4 + Aortic Insufficiency & he underwent redo- AVR with Trifecta prosthesis # 25. A large tear in Left Cusp and small perforation in Right cusp of unclear etiology was found. Pathology of ex-planted valve showed no gross vegetation but granular deposits on the ventricular side of leaflet and no inflammatory infiltrate on microscopy. PCR on the ex-planted valve was positive for T.Whipplei. Treatment for PVE was initiated with IV Ceftriaxone followed by lifelong bactrim therapy.

Conclusions: Tissue invasion by T.Whipplei is characterized by lack of immunologic response and inflammatory infiltrates. Although host immune deficiency has been implicated in the past, WD has not been demonstrated to reactivate in transplant patients on mmunosuppressants. Our case demonstrates that immunosuppression used for solid organ transplant might pose a risk for reactivation and atypical presentation of this elusive infection.

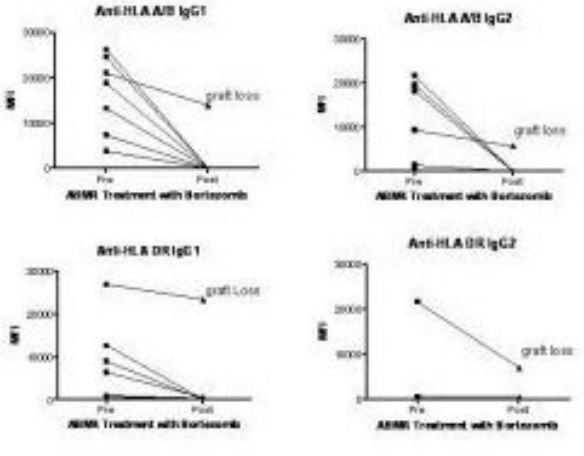
FR-PO1133

Donor Specific Antibody IgG Subtypes AND Responsiveness to Bortezomib Based Therapy for Acute Antibody Mediated Rejection (ABMR) Dinesh Kannabhiran,[1] Matthew J. Everly,[2] Blanca Mae Ponce,[1] Vijay K. Sharma,[1] Manikkam Suthanthiran,[1] Darshana Dadhania.[1] *[1]Nephrology, Cornell Med. Center, NY, NY; [2]Terasaki Foundation.*

Background: Donor Specific Antibodies (DSA) are routinely measured at the time of ABMR. Class I DSAs are more likely to decline following treatment for ABMR compared to Class II and the presence of Class II DSAs correlates with poor allograft survival. We investigated the reduction in IgG subtypes for Class I & Class II DSAs following Bortezomib based therapy for ABMR.

Methods: We analyzed serum obtained at the time of biopsy (pre-Bortezomib therapy) and 2-6months following treatment of ABMR with Bortezomib (post-Bortezomib therapy) from 7 kidney recipients with biopsy confirmed ABMR (2-Class I, 3-Class II, 2-ClassI&II DSAs) had both Class I and II DSA). Sera were diluted 1:3 and tested for anti-HLA IgG using LABScreen single antigen beads (SAB). Importantly, sera were tested for IgG subclass (1, 2, 3, and 4) using SAB with an IgG subclass specific secondary antibody. The cutoff for single antigen positivity was 1000 mean fluorescence intensity (MFI).

Results: DSAs against HLA- class I A, B (N=7) and DSAs against HLA- class II DR (N=7) were studied. The predominant IgG subtypes of the DSAs directed at HLA-A and/ or B were IgG1 & IgG2 (1 patients had Class I-IgG3) and for HLA-DR, the predominant subtype was IgG1 (1 had IgG2). Bortezomib therapy reduced both IgG1 & IgG2 DSA directed at HLA A, B & DR except in the two patients who lost their grafts.



Conclusions: Our data suggest that successful reversal of ABMR with Bortezomib is associated with reduction in both IgG1 & IgG2 DSA whereas failure to reduce IgG1 DSA is associated with graft loss. Mechanistic basis for persistently elevated levels of both IgG1 & IgG2 remains to be resolved and may represent a significantly larger and/or resistant plasma cell clone.

FR-PO1134

Very Early Onset De Novo Crescentic Nephropathy with IgA Predominance in a Kidney Transplant Recipient Karthik Karanam, Muhammad Ahmad Mujtaba. *Indiana University Hospital, Indianapolis, IN.*

Background: It is reported that 20% patients of IgA nephropathy have some degree of crescents. Further de novo IgA with crescents is very rare. Recurrent or denovo IgA with crescents has been seen as early as 5 months after transplant. We report a case of de novo IgA with crescents diagnosed very early at 3 weeks post transplant.

Methods: A 23 year old white male with ESRD due to congenital dysplasia of kidneys underwent living related kidney transplant in 1987; the kidney lasted for less than a week for technical reasons. He was on CAPD; in 1990 had a deceased donor transplant that worked till year 2000, biopsy revealed chronic transplant glomerulopathy. He was on intermittent HD and then underwent a deceased donor 3 antigen mismatch kidney transplant from a 18 year old female Caucasian in 2009. He was highly sensitized & was given IVIG. He was on thymoglobulin, steroids, mycophenolate and tacrolimus. He was discharged with a downward trending creatinine in low 2's. Post op day 14 creatinine increased to 3.4mg/dl with worsening proteinuria of 7 grams & RBC's. Transplant kidney biopsy showed acute glomerulonephrritis (GN) and acute cellular rejection; BANFF 2A. Immunological work up for complements, anti-GBM antibodies, ANA, anti-dS DNA, RF, hepatitis C, ANCA's were negative. Pulse steroids were given and creatinine improved but proteinuria persisted. A repeat biopsy was done on 34th Post op day for acute renal failure, proteinuria 13 grams and dysmorphic RBC. It showed Immune complex GN with crescents (2/11) and tubular necrosis, IgA predominant. The mate kidney from the same donor was followed but the other recipient of the paired kidney was doing fine. Patient underwent plasmapheresis, received Rituximab but his renal function deteriorated. Post op day 84 another biopsy showed DPGN with 1 crescent and segmental sclerosis. His renal function continued to worsen with fluid overload. He was initiated on intermittent HD.

Conclusions: Patient's history of congenital kidney, lack of IgA in previous transplant and no features of IgA in the potential donor with a normal mate kidney makes the possibility of de novo disease. We suggest that de novo IgA can arise in post transplant kidneys very early.

FR-PO1135

A Case of Posterior Reversible Encephalopathy Syndrome during Eculizumab Therapy Sajan Thomas. *Nephrology, Goldcoast Hospital, Gold Coast, Queensland, Australia.*

Background: This is a case of Eculizumab related complication in a patient treated for hemolytic uremic syndrome.

Methods: This is a 24 year old male who presented with five day history of diarhoea to the Emergency department.On examination he was found to be dehydrated but hemodynamically stable.His investigation revealed acute renal failure with a creatinine of 1430umol/L,hemolytic anemia with Hb of 94g/L,thrombocytopenia with platelet count of 89*10^{9}L,raised LDH 1130U/L,low haptoglobin 0.03g/L and fragmented red cells on peripheral smear.A diagnosis of Hemolytic uremic syndrome was made later confirmed by a renal biopsy. He was initiated on Plasma exchange and haemodialysis. He had five sessions of daily plasma exchange and on day six of his hospital admission he was started on eculizumab. He was dialysis dependent for further three weeks. His renal functions has progressively improved. His main complication during eculizumab therapy was hypertension related PRES. He is currently on three antihypertensive agents with well controlled blood pressure and stable renal functions. He continues to be on fortnightly doses of eculizumab.

Funding: Pharmaceutical Company Support - ALEXION

FR-PO1136

Olanzapine Induced Hypercalcemia Deep Adhikari, Eric Iida. *Nephrology, University of Massachusetts Medical Center, Worcester, MA.*

Background: Olanzapine is an atypical antipsychotic used to treat schizophrenia and bi-polar disorder. We describe a case of olanzapine induced hypercalcemia in a patient with CKD.

Methods: A 67 y.o. female developed new hypercalcemia with a total calcium of 13.3 mg/dL and an ionized calcium of 6.8 mg/dL. Lithium had been discontinued ten years previously due to CKD with Cr 2.5 mg/dL and nDI. She was not on thiazides, vitamin D or calcium supplements. iPTH was 8 pg/mL and 25 vitamin D was 19 ng/mL. CXR, SPEP, UPEP, TSH and PTH-rp were all normal. The only new medication was olanzapine, started 20 days earlier. She was treated with IVF, furosemide and pamidronate. She had a similar presentation 9 weeks later. Shortly after, olanzapine was discontinued for psychiatric reasons. Calcium remained normal for 15 months until olanzapine was restarted. Hypercalcemia ensued fifteen days later. Again, iPTH and 25 vitamin D were unremarkable. A 1, 25 vitamin D was 105 pg/mL but was normal 3 days later. CXR, CT chest, CBC, Vitamin A level and total body bone scan were unrevealing. Hypercalcemia resolved with IVF, furosemide, calcitonin and a weekly bisphosphonate. She remained on olanzapine. She presented 4 months later with hypercalcemia. In the interim, she had been on cholecalciferol and had self-discontinued the bisphosphonate. Repeat CT chest showed lung

nodules suggestive of metastatic calcium deposits. CBC, PFTs, and 1, 25 Vitamin D were normal. She was treated as previously. Discontinuation of olanzapine is being considered.

Conclusions: Olanzapine is an atypical antipsychotic classified as a thienobenzodiazepine. Although hypocalcemia has been reported in the setting of pancreatitis, our patient developed hypercalcemia with a temporal association to olanzapine. Lithium may cause hypercalcemia even after discontinuation, but not with a suppressed PTH. The single elevated 1, 25 Vitamin D level was not reproducible.

A mechanism for olanzapine causing hypercalcemia is obscure. Its effects are mediated through serotonin, dopamine, and other receptors, none of which explain hypercalcemia. To our knowledge, this is the first report of olanzapine induced hypercalcemia.

FR-PO1137

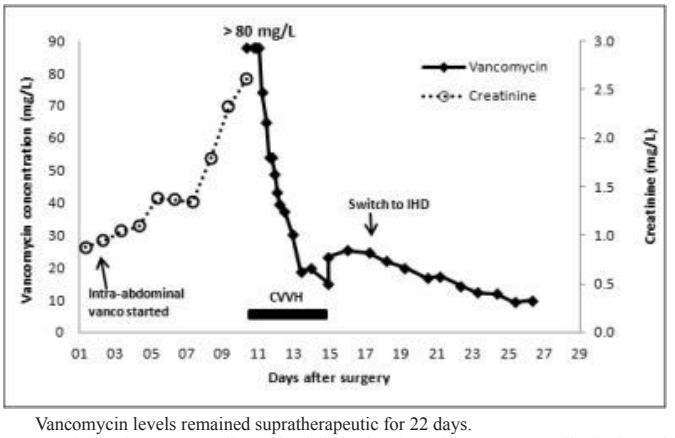
Vancomycin-Associated Nephrotoxicity Joelle Mardini,[1] Karine Mardini,[3] Marc Ghannoum,[3] Josee Bouchard,[2] Valery Lavergne.[2] *[1]Mcgill University, Montreal, QC, Canada; [2]Sacre-Coeur Hospital, Montreal, QC, Canada; [3]Verdun Hospital, Montreal, QC, Canada.*

Background: Vancomycin-induced AKI remains a controversial entity. Current literature suggest that etiology of AKI is often clouded by other comorbidities. We present a patient who developped AKI when vancomycin overdosage was the only risk factor.

Methods: A 73 year-old man, known with type 2 diabetes and normal kidney function, was admitted following abdominal surgery for ampulloma.

Cultures from the Jackson-Pratt grew MRSA. High dose intraabdominal vancomycin (2g q 12h) was purposely started on day 2 following surgery to counter possible exudation of the antibiotic. Acute kidney injury progressively ensued although no etiology was identified. Vancomycin level was not monitored until day 11 post-surgery, when it was found to be > 80mg/L. No other associated vancomycin-related complication were evident, namely ototoxicity, hemodynamic instability or cardiac conduction defects. Vancomycin was discontinued immediately.

In order to remove vancomycin, high-dose CVVH (4L/h) was started on day 11 and continued until day 15. Intermittent HD was began on day 17 and continued for 2 more weeks.



Vancomycin levels remained supratherapeutic for 22 days.

Hospitalization was complicated by delirium but the patient was eventually discharged 2 weeks later with some degree of renal impairment (eGFR = 40 mL/min/1.73 m^2), which persisted on follow-up 2 months later.

Conclusions: AKI can occur in the setting of isolated severe vancomycin toxicity.

Vancomycin T1/2 under CVVH was 148h, which is considerably longer than what is reported by DelDot et al, 2004 (15.6h). This suggests ongoing absorption from the abdominal cavity.

Disposition of intra-abdominal vancomycin shortly after surgery is erratic and requires closer monitoring.

FR-PO1138

All that Is Natural Is Not Innocuous Connie Rhee, Li-Li Hsiao. *Renal Division, Brigham and Women's Hospital, Boston, MA.*

Background: Aristolochic Acid (AA) is a known cause of tubulointerstitial disease and urothelial cancer. In the absence of herbal medicine regulation, US consumers are at increased risk of exposure.

Methods: A 54 year-old white male was referred to the clinic for stage 3 chronic kidney disease. 3 years prior, he presented to an outside nephrologist with an elevated creatinine (1.6 mg/dl) and microalbumin/creatinine ratio (54 mcg/mg) in the context of heavy herbal use (16 pills/day). Two renal biopsies showed interstitial fibrosis/tubular atrophy (IFTA) and a renal papillary neoplasm fragment for which he was referred to urology. Serial imaging studies were negative for malignancy. His creatinine rose to 2.0 mg/dl, and he was referred to a second nephrologist.

By this time, he had stopped all supplements and was on lisinopril 10 mg daily for hypertension. Examination demonstrated a fit male with normal blood pressure. His creatinine was 1.9 mg/dl and urine sediment was bland. Review of prior biopsies revealed a gradient pattern of IFTA (outer cortex>inner cortex>medulla). Ingredient labels from his supplements did not list any nephrotoxins, but evaluation at an outside laboratory revealed low amounts of AA (240-279 ppm) in 2 bottles, pointing to AA as the cause of his nephropathy and neoplasm.

Conclusions: Herbal medicine use is highly prevalent in the US (17.7% in a 2007 survey), with sales estimated at $4.8 billion annually. Although some herbals confer health benefits, AA is a nephrotoxin and carcinogen characterized by proximal tubular defects, rapid renal function decline, and urothelial malignancy. AA nephropathy is recognized by its gradient pattern of hypocellular IFTA and glomerular sparing.

The 1994 Dietary Supplement Health and Education Act exempts supplement manufacturers from the safety, efficacy, and quality regulations that apply to prescription and over the counter drugs. Consumers may be subject to toxin exposure due to 1) inaccurate labeling, 2) contamination, 3) substitutions, and 4) herb-drug interactions. The 2007 Good Manufacturing Practices aim to improve standards, but criteria remain lenient. It is imperative that physicians routinely ask patients about herbal use and that rigorous regulation of supplements be implemented.

Funding: NIDDK Support

FR-PO1139

Methotrexate-Induced Nephrotoxicity Abdur R. Baig,[1] Gustavo Westin-figueiredo,[2] Ahmed A. Waheed,[1] Dollie F. Green,[1] Oliver Lenz.[1] *[1]Nephrology and Hypertension, University of Miami, Miller School of Medicine, Miami, FL; [2]Medicine, University of Miami, Miller School of Medicine, Miami, FL.*

Background: Methotrexate (MTX) is one of the most widely used anticancer medications. Despite aggressive hydration, urinary alkalinization and LV rescue therapy, nephrotoxicity remains a major concern.

Methods: A 51-Year-old male with Mantle Cell Lymphoma and hyper IgE syndrome received high dose intravenous (IV) MTX therapy (3000 mg/m^2) as part of his R-MACLO (IV rituximab, doxorubicin, vincristine, cyclophosphamide, methotrexate with LV rescue therapy) chemotherapy regimen. He had previously received methotrexate 1200 mg/m^2 without adverse reactions. On admission his serum creatinine was 1 mg/dl. He developed acute renal failure, initially non-oliguric and later oliguric, within less than 12 hours of MTX infusion. Renal function continued to worsen, and his serum creatinine peaked at 5 mg/dl. Maximum blood MTX level was 26 mcmol, and he was given bicarbonate infusions and was started on LV rescue as per his initial chemotherapy protocol. On the following day, MTX level was 10.55 mcmol. After administration of glucarpidase, MTX level improved to 0.69 mcmol. There was no indication for dialysis, and his renal function slowly improved.

Conclusions: High-dose MTX (≥ 1000 mg/m^2) is used for the treatment of variety of malignancies, including lymphoma. Nephrotoxicity has been observed since the 1970s and is caused by tubular precipitation. Although leucovorin rescue, volume expansion, and urinary alkalinization, have decreased complication rates significantly, nephrotoxicity remains a major concern. Carboxypeptidase-G_2 (glucarpidase) metabolizes circulating MTX to the inactive metabolite diamino methylpteroic acid, providing an alternate route for elimination. Glucarpidase administration in combination with thymidine and LV is highly effective. Within 15 minutes of administration plasma MTX concentration decrease by 95%–99%. Rebounds <10% in plasma MTX concentrations were reported in only 60% of patients. Both charcoal hemoperfusion in combination with hemodialysis or high-flux hemodialysis have been shown to effectively remove MTX.

FR-PO1140

Acute Interstitial Nephritis and Cocaine: An Unusual Presentation for Acute Kidney Injury Narender Goel,[1] James M. Pullman,[2] Maria Coco.[1] *[1]Nephrology, Albert Einstein College of Medicine, Bronx, NY; [2]Pathology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Acute interstitial nephritis (AIN) is a well-recognized cause of acute kidney injury (AKI). Most AIN cases are caused by medication. However, possible relationship of AIN with Cocaine is unusual and seldom reported.

Methods: A 49 years old male with history of Diabetes Mellitus, Hypertension, chronic Hepatitis C and prior substance abuse, presented with nausea, vomiting and decreased oral intake for 1 week. He stated to have taken ibuprofen for last 2 weeks and later admitted to recent intravenous cocaine use. Initial physical examination was unremarkable.

Initial labs were serum Sodium 131 meq/L, Potassium 6.1 meq/L, Blood Urea Nitrogen/ Creatinine (BUN/Cr) 110/12 mg/dl, WBC count 12500/mm^3 with 3% eosinophil, Creatinine Phosphokinase (CPK) 45 U/L. Urinalysis showed specific gravity 1.014, large blood but no protein, with large hematuria, leukocyturia and eosinophils but no casts. Urine toxicology was positive for Cocaine. Renal sonogram showed 14 cm size kidneys and mildly increased echogenicity. Hemodialysis was initiated. Antineutrophilic cytoplasmic antibody, Anti-GBM antibody, rheumatoid factor, serum/urine protein electrophoresis and HIV were negative. Serum complements were normal. Kidney biopsy showed AIN. Patient was started on Prednisone 60 mg daily with gradual renal recovery. He was discharged home with serum Cr 3.2 mg/dL but presented 1 week later with anuria for 1 day after recurrent cocaine use. Chemistries at that time showed serum Potassium 7 meq/L, BUN/Cr 230/12 mg/dL and CPK 34 U/L. Hemodialysis was resumed. Course was further complicated by acute bacterial endocarditis requiring aortic valve replacement.

Conclusions: Literature reports 3 cases of cocaine induced AIN. Rhabdomyolysis, glomerular microcirculation vasoconstriction, malignant HTN and renal infarcts are primarily defined mechanisms of cocaine induced AKI. Diagnosis of AIN may be difficult due to lack of classical allergic symptoms such as rash, fever and eosinophilia. In our case ibuprofen may have been contributory, but his 2nd presentation with recurrent AKI following another cocaine exposure suggests cocaine as etiology of AIN.

FR-PO1141

Plasmacytoma Masquerading as Acute Kidney Injury with Hematuria Jagannath H. Saikumar,[1] Mazen El Atrache,[2] Mona Vekaria,[2] Pablo Buitron de la Vega,[2] Kausik Umanath.[1] *[1]Nephrology, Henry Ford Hospital, Detroit, MI; [2]Internal Medicine, Henry Ford Hospital, Detroit, MI.*

Background: Acute kidney injury (AKI) and hematuria have many etiologies ranging from pre-renal to glomerulonephritides and nephrolithiasis with obstruction.Plasma cell dyscrasias can affect the kidneys in many ways. We present a unique case of plasmacytoma causing mechanical obstruction and hematuria.

Methods: A 65 y.o. Hispanic woman presented to the emergency room (ER) with left sided flank pain and gross hematuria.She had a history of IgG Kappa multiple myeloma (MM) diagnosed one year prior that was treated with 4 cycles of bortezomib, dexamethasone and autologous stem cell transplant with good response.In the ER,she was found to have AKI with a serum creatinine (SCr) of 2.9 mg/dL.Renal ultrasound showed left sided hydronephrosis.A subsequent noncontrast CT scan of the abdomen/pelvis showed a large left retroperitoneal hemorrhage surrounding the left kidney extending into the renal pelvis and presacral region, compressing the left external iliac vessels.A left nephrostomy tube was placed after failure to position stents via cystoscopy.This was inadvertently removed and her kidney function declined prompting placement of a right nephrostomy tube. Worsening serum immunoglobulins led to repeat bone marrow biopsy that showed MM relapse. Transurethral bladder biopsy revealed a plasmocytoma infiltrating the bladder mucosa.The patient was treated again with chemotherapy as well as 10 sessions of palliative radiation targeting the bladder without response. An MRI of the retroperitoneal hemorrhage showed infiltrative tissue surrounding the left kidney.Biopsy of this mass was also consistent with plasmacytoma. She was then started on daunorubicin, vincristine and dexamethasone therapy which was complicated by tumor lysis syndrome and AKI requiring dialysis.Her renal function eventually recovered with a SCr of 1.4.

Conclusions: Most extramedullary plasmacytomas are in the lung but can rarely occur in the abdomen. They present with end-organ damage leading to anemia, AKI, hypercalcemia or lytic bone lesions. This case highlights the complexity and range of renal involvement of MM.

FR-PO1142

A Case Report: Severe Neurotoxicity Induced by Isoniazid and Ethambutol in a Peritoneal Dialysis Patient Hui Peng, Meirong Zhong, Yan-ru Chen, Yuanqing Li, Sujay Dutta Paudel, Tan-qi Lou. *Division of Nephrology, Department of Medicine, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: Patients with end stage kidney disease are vulnerable to toxic effects of anti-tuberculous drugs. However, concurrence of severe peripheral neuropathy, retrobulbar neuritis and recurrent laryngeal nerve injury induced by isoniazid and ethambutol was rarely reported in peritoneal dialysis patient with active tuberculosis. Here, we present a patient of such case.

Methods: A 36-year-old man was diagnosed with CKD (stage 5) as well as infiltrative pulmonary tuberculosis on admission in April 2011. CAPD was initiated soon. And anti-tuberculosis treatment was begun with isoniazid 200 mg/day, ethambutol 750mg every other day, rifapentine 600 mg/week and VitB6 30mg/d. After four months, the patient gradually experienced numbness and weakness of lower extremities and finally he became unable to stand on his own. Electromyogram indicated peripheral nerve injury. Simultaneously, he noticed numbness of the fingertips and declined vision. Given the suspicion of side effect of anti-tuberculosis drugs, isoniazid and ethambutol were discontinued. Levofloxacin, Pyrazinamide combined with rifapentine were then used to anti-tuberculosis. Large dose of vitamin B6 and vitamin B12 were administered. However, there was no improvement in one month. Subsequently he displayed blurred vision, hoarseness and drink water to cough. Ophthalmological examination revealed blurred borders of both optic disks. Visual acuities were finger counting at 0.5 meter both eyes. Laryngoscopy found glottic dysraphism, therefore, recurrent laryngeal nerve injury associated with isoniazid was considered.

Three months after withdrawal, weakness and numbness of both extremities as well as hoarseness disappeared. Four months later, he was able to walk with a crutch, and his eyesight restored to near normal as well.

Conclusions: Severe neurological toxic effects associated with isoniazid and ethambutol may occur in patients undergoing chronic dialysis. Early discontinuation of anti-tubercolosis drugs and intensified neurotrophic therapy may be effective in dealing with neurological complications.

Funding: Government Support - Non-U.S.

FR-PO1143

Severe Rhabdomyolysis Caused by Red Yeast Rice with Concomitant Use of Diltiazem and Warfarin Dhwanil Vyas, Anuja Vyas, Mamta Shah. *University of Connecticut.*

Background: Red Yeast Rice (RYR) is cooked rice fermented with Monascus purpureus (Red Yeast). It is widely used in China as a coloring agent as well as a dietary supplement. In recent years, it has been shown to successfully reduce LDL, total Cholesterol and triglyceride levels. Monacolin K, amongst other constituents of RYR, is thought to be primarily responsible for this effect. Its molecular structure resembles Lovastatin. The mechanism of action is similar, hepatic 3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibition. Despite its increasing use, RYR is not regulated by FDA. Also, the safety profile and drug interactions are not well studied. We describe a case of rhabdomyolysis caused by RYR and possibly its interaction with Warfarin and Diltiazem.

Methods: A 77 year-old man with a history of Simvastatin-induced-myopathy and atrial fibrillation on stable doses of Diltiazem and Warfarin, presented with 4 weeks' history of generalized muscle aches predominantly in bilateral thighs and shoulders. He was found to have rhabdomyolysis and acute kidney injury with creatinine kinase level of 42,705 U/L and creatinine of 1.6 mg/dl. His complete medication list that was later obtained from the pharmacy included supplemental Red Yeast Rice 1200mg/day. Evidently, it was started 4 weeks prior to the beginning of his symptoms. No other potential causes of Rhabdomyolysis could be identified in this patient. He improved clinically with supportive care and avoidance of RYR.

Conclusions: Literature search yielded 5 reports of Rhabdomyolysis caused by RYR. Studies suggest that there is a higher incidence of milder forms of myopathy with RYR. Also, since many constituents of RYR are metabolized by cytochrome P450 isoenzyme 3A4 (CYP450 3A4) in the liver, there is a risk for drug interactions. We hypothesize that CYP450 3A4 inhibition by Diltiazem and Warfarin led to high levels of monacolin K in our patient, predisposing him for getting rhabdomyolysis. Basic science research to delineate drug interactions and large clinical studies to assess incidence of adverse effects of RYR as compared to statins are warranted.

FR-PO1144

A Rare Case of Voriconazole Induced Acute Kidney Injury Alin A. John, Gautham Viswanathan, Ronald D. Perrone. *Division of Nephrology, Tufts Medical Center, Boston, MA.*

Background: Acute Kidney Injury (AKI) in kidney transplant recipients is common. Voriconazole is a potent anti-fungal for which there is limited data regarding nephrotoxicity. A case of Voriconazole associated AKI is reported below.

Methods: A 52 year old male underwent living unrelated kidney transplantation for ESRD secondary to diabetic nephropathy, receiving Tacrolimus, Mycophenolate Mofetil and Prednisone for immunosuppression. His post-transplant course was complicated by acute allograft glomerulopathy at 3 months treated with high dose steroids with excellent response. Serum creatinine had decreased to 1.25 mg/dL from a nadir creatinine of 2.5 mg/dL. 6 months post transplant, a draining ulcer on the patient's left ear lobe was noted which grew Fusarium. He was initially started on Posaconazole, and then switched to Voriconazole 300 mg twice daily once sensitivities returned; tacrolimus level at this time was 20.7. One week later he presented with lower extremity edema, dyspnea, weight gain, and a peak creatinine of 4.46 mg/dL with a protein/creatinine ratio of 2g/g, tacrolimus level was 5.4. A repeat biopsy showed no evidence of rejection, but revealed severe tubular injury. Voriconazole levels returned elevated at 5.3 mcg/mL (nl < 2.1 mcg/mL). The AKI was thought to be secondary to voriconazole which was discontinued. Off Voriconazole, creatinine steadily improved over 2 weeks to 1.19 mg/dLwith significantly reduced proteinuria.

Conclusions: This patient had biopsy proven severe tubular injury in the setting of a supratherapeutic voriconazole level, suggesting this medication as the causative agent. The data regarding acute kidney injury secondary to Voriconazole is limited. Voriconazole is a potent anti-fungal agent which is commonly used in immunosuppressed patients, including those with kidney transplants. This case indicates that closer monitoring of renal function may be indicated.

Funding: Other NIH Support - T32 Training Grant

FR-PO1145

Warfarin Related Nephropathy in a Patient with Normal Renal Function Banshi M. Rathi, Praveen N. Chander, Stephen Adler. *New York Medical College, Valhalla, NY.*

Background: Warfarin was introduced as an oral anticoagulant in 1950 and has been the most often prescribed anticoagulant ever since. Warfarin related nephropathy (WRN) is a recently described complication of warfarin anticoagulation associated with significant morbidity and possible mortality. The risk factors associated with WRN are chronic kidney disease (CKD), diabetes, diabetic nephropathy, hypertension and heart failure. We report an interesting case of WRN in a patient without risk factors and with normal baseline renal function.

Methods: A 66 year old female with history of multiple myeloma and peripheral stem cell transplant was consulted for 4 weeks of hematuria, bilateral flank pain and acute kidney injury. She did not have a history of CKD, proteinuria, diabetes mellitus, heart failure, nephrolithiasis, trauma or hypertension. There was no history of non-steroidal anti-inflammatory drug intake or intravenous contrast exposure. Outpatient work-up with non-contrast computed tomography scan and cystoscopy were unrevealing. The patient had been on warfarin for deep venous thrombosis for one year, with therapeutic prothrombin time and international normalized ratio (INR). Urinalysis revealed hematuria (3+, 30-50 red blood cells, dysmorphic) without proteinuria. A kidney biopsy was performed and revealed tubules blocked with fresh or hemolyzing erythrocyte consistent with WRN. The patient did not recover renal function with conservative management and was initiated on hemodialysis for worsening renal function and uremia.

Conclusions: WRN has been associated with supra-therapeutic INR, nephrotic syndrome, and drugs causing glomerular hypertension. Our patient did not have CKD by clinical, laboratory or radiologic criteria, and did not meet the above mentioned risk factors. WRN frequently leads to a progressive decline in renal function terminating in end stage renal disease. The clinical course of our patient is consistent with the currently opined natural history of this condition. Our report emphasizes that WRN can also occur in patients considered at low risk for this disorder. Prospective multicenter studies are needed to better define the clinical features and natural history of this entity.

FR-PO1146

Beta HCG Producing Poorly Differentiated Carcinoma Causing Acute Kidney Injury

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Background: A case of beta HCG producing poorly differentiated carcinoma infiltrating the kidney causing acute kidney injury requiring dialysis.

Results: A 63 year old white male with systemic hypertension and transient ischemic attack presented to the hospital with nausea, vomiting, and upper abdominal pain. He was found to have microscopic hematuria, proteinuria of 500 mg/g of creatinine and an elevated serum creatinine of 2.8 mg/dL, and bilateral exudative pleural effusions. Thoracentesis yielded an exudative effusion with negative cytology. Serum complement levels and screening for ANA, double-stranded DNA, ANCA, anti-GBM, hepatitis B and C, and HIV were unremarkable. A cystoscopy revealed a WHO grade 2 of 3 non-invasive papillary urothelial carcinoma of the urinary bladder. The renal failure progressed, requiring renal replacement therapy, and he underwent a kidney biopsy, which revealed a poorly differentiated carcinoma in all cores with a negative p63 stain. It was histologically different from the papillary urothelial carcinoma of the bladder. Multiple tumor markers were normal except for Ca19-9 of 50 unit/ml and beta-HCG level of 777 mlU/ml. Further tissue diagnosis was sought with a CT guided biopsy of a pancreatic lesion which revealed poorly differentiated carcinoma similar to that seen on the kidney biopsy. CT scan of the chest revealed pleural effusion with pleural thickening, septal thickening, mediastinal lymphadenopathy and partial collapse of right middle lobe. A scrotal ultrasound did not show any mass. A diagnosis of beta HCG producing poorly differentiated carcinoma was made.

Conclusions: The frequency of metastases to the kidney in cancer patients is 7% - 13 % in large autopsy series. The most common primary malignancy to involve the kidney is bronchogenic carcinoma, then breast and gastrointestinal cancers. A literature search for beta HCG producing poorly differentiated carcinoma metastases to the kidney was performed. No such cases were readily found with the search.

SA-PO001

Long Term Impact of Preventing AKI with Off-Pump Coronary Artery Bypass Surgery: A Risk-Adjusted Propensity-Matched Cohort Study
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Background: Off-pump (OPCAB) surgery should protect kidneys from On-Pump (ONCAB) CABG related AKI. The long-term impact of OPCAB AKI prevention on hard outcomes needs to be established.

Methods: All adult patients submitted to CABG surgery in INCC-IMPASA between 1/1/2000 and 12/31/2008 were enrolled (n=4384; 1747 OPCAB (39.8%), 2637 ONCAB (60.2%)). Patients were propensity-matched if included into a mean ± 2 SD logistic regression probability of receiving OPCAB (logistic EuroSCORE (ESC), bypass number and diabetes). ESRD patient were excluded. Demographics, comorbidities, intraoperative and postoperative (PO) variables were prospectively-collected. AKI was defined according to R+I+F criteria. Long-time survival (up to 10 year) was obtained by a systematic telephone survey, minimum 2-year follow-up, and ESRD status from the Uruguayan Dialysis Registry. eGFR was assessed by Cockroft-Gault formula. ***Statistical analysis***. t test, Mann-Whitney test, ANOVA, $\chi 2$, Kaplan-Meier curves, long-rank test and Cox regression for survival analysis. $p<0.05$ (*).

Results: 785 OPCAB and 1377 ONCAB patients included into analysis: age 64.7±9.4 yrs (31-91). No differences between groups in: diabetes, ESC, previous stroke, hemodinamic instability, LVEF, AMI, sex, bypass number, age, preoperative (PreO) SCr and eGFR. In PO (OPCAB vs ONCAB): SCr: 1,27 mgrs/dl vs.1,47 mgrs/dl*, AKI: 25,6% vs 38,5%*; RRT: 0.1% vs 1,5%*. PreO eGFR-stratified RRT (stages: I, II, IIIa, IIIb, IV, V): 0%; 0%; 0%, 0%, 11,1% vs 0%; 0,4%, 1.1%; 2,9%, 10,4%, 50%*; 33% higher mortality in ONCAB due to mortality in AKI patients (6,7% vs 1,5%*), mainly in eGFR stages IIIb, IV and V*. The long-term 5-year composite end point (ESRD and mortality) survival was higher in OPCAB (0.89 vs 0,84*) due to less events in non-AKI patients (0.89 vs 0,81*). In Cox model, age, AKI, eGFR, ESC and ONCAB were associated with long-term mortality and ESRD*.

Conclusions: Lower incidence of AKI and a 10-fold reduction of PO RRT were observed in OPCAB, resulting in a higher 5-year event free survival. Few other preventive strategies described in the literature demonstrated such impact on long-term PO AKI related events.

Funding: Private Foundation Support

SA-PO002

In-Hospital and Long Term Management and Outcomes of Acute Kidney Injury
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Background: Recent studies have shown that management of patients with AKI is variable and subject to significant gaps in diagnosis, investigations and follow up contributing to sub-optimal care. We conducted a retrospective case-control study to evaluate the quality of inpatient management and assess short and long-term outcomes of patients with and without AKI at an academic medical center. We hypothesized that AKI patients would have worse outcomes and their care would be associated with gaps in recognition, diagnosis, management and follow-up.

Methods: We reviewed charts of 100 patients discharged with ICD-9 diagnosis codes 584.4-9 for AKI (ICD-9+) and 190 matched controls (ICD-9-). AKI was confirmed by a modification of the AKIN criteria (Standard: increase in serum creatinine (sCR) or Modified: decrease in sCR of > 0.3 mg/dl over 48 hrs. We evaluated the in-hospital management and post-discharge events within one year to determine the follow up care and outcomes including repeat AKI, re-hospitalizations, ESRD, and mortality.

Results: All ICD-9+ patients were confirmed to have AKI but only 38% met the standard criteria, while 25.8% of the ICD-9- group had AKI (8.9% by standard criteria). AKI patients experienced more complicated hospital stays and had worse outcomes than non-AKI with volume overload (49% vs. 8.4%, p<0.001), sepsis (32% vs. 5.3%, p<0.001), need for dialysis (15% vs. 0%, p<0.001), and mortality (18% versus 1.1%, p<0.001). Within the first year, ICD-9+ patients had a greater frequency of subsequent AKI (59.4% versus 22.3% in ICD-9-, p=0.005), had a significantly increased rate of progression to ESRD (7.8% versus 0.6%, p=0.007), while rates of readmission and mortality were not different. Only 23.4% of ICD-9+ patients followed up with a nephrologist post-discharge.

Conclusions: Our results are consistent with prior studies highlighting the need for continued improvement in the recognition and management of AKI patients both during and following hospitalization. Focused attention is required to improve outcomes from this disease.

Funding: NIDDK Support

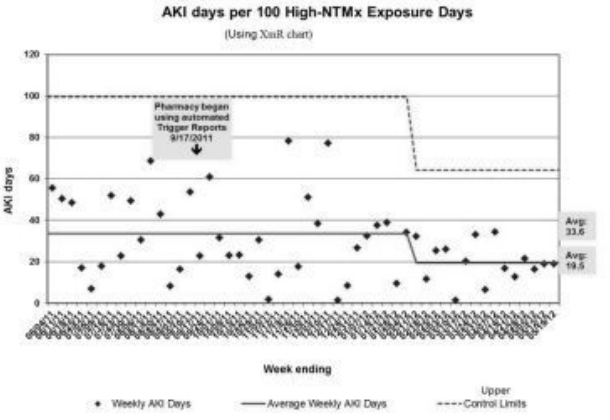
SA-PO003

Electronic Health Record (EHR) Identification of Nephrotoxic Medication (NTMx) Exposure Can Guide Systematic Kidney Function Assessment, Detect Acute Kidney Injury (AKI) Reliably, and Lead to Decreased AKI Rates and Intensity
Stuart Goldstein, Eric S. Kirkendall, Hovi Nguyen, Joshua K. Schaffzin, Stephen E. Muething. *Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Nephrotoxic medications are a common cause of AKI (NTMx-AKI) in hospitalized children. A portion of NTMx-AKI goes unnoticed from lack of reliable kidney function surveillance in exposed patients. We developed an HER screening process to quantify NTMx-exposure and AKI rates in non-critically ill patients over a ten month period.

Methods: High NTMx exposure was defined as ≥3 NTMx exposure at once OR an IV aminoglycoside for ≥3 days. AKI was defined by pRIFLE criteria, based on serum creatinine (SCr) values. We recommended daily SCr in high NTMx-exposed pts. Outcome metrics were 1) High NTMx admissions (adm) per 1000 hospitalized patient days (pt-days) 2) AKI rates per High NTMx adm (%) and 3) Days in AKI per 100 high NTMx days.

Results: 71,794 pt-days resulted from 24,913 adm (19,942 unique patients). Of these, 663 unique pts comprised 773 adm (5,857 pt-days) with high NTMx exposure (11 High NTMx adm per 1000 pt-days). Daily SCr monitoring occurred in >99% high NTMx days. Pts with high NTMx exposure had 1,746 AKI-days (1,135, 482, 129 days in pRIFLE R, I and F). AKI rates per high NTMx adm decreased from 35% to 28.8% in the last 4 months of study. Days in AKI per 100 days of high NTMx exposure decreased by 42%, potentially avoiding 880 annual AKI days.



Conclusions: We successfully implemented an EHR-based daily NTMx-AKI screening program. We found a 1) high rate of AKI in non-critically ill children with high NTMx-exposure and 2) decrease in AKI rates and duration with only recommendations for daily SCr monitoring. We suggest improvements resulted from increased awareness and earlier identification of AKI with daily SCr screening.

Funding: Other NIH Support - Agency for Healthcare and Research Quality

SA-PO004

Comparative Renal Safety of Statin Formulations by Dose and Potency
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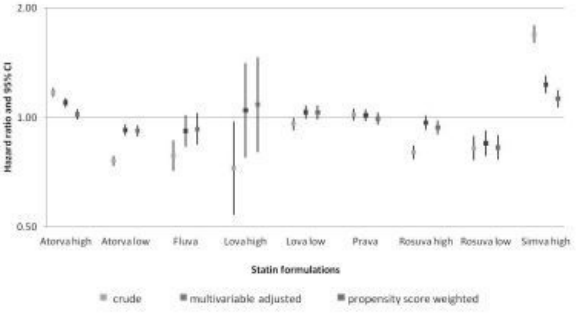
Background: Higher-potency statins effectively lower lipoprotein cholesterol, but may also have greater risk of adverse events. Limited information is available on the effect of these agents on acute kidney injury (AKI). We examined the association of higher-potency formulations and doses with AKI in a large US insurance claims database, years 2000-2010.

Methods: We identified statin initiators following a six-month washout period, and categorized them into potencies by formulation and dosage. Diagnosis codes during a one-year follow-up were searched for AKI. We estimated hazard ratios (HR) and 95% confidence intervals (CI) for the risk of AKI in all higher-potency vs. lower-potency users. Individual formulations were also compared to lower-dosage simvastatin. Covariates included cardiovascular and renal risk factors, medications, and other claims and diagnoses. Analyses were repeated with propensity score methods.

Results: We identified 4,146,507 eligible statin initiators (73.8% lower-dosage formulations). As a class, higher-dosage statins showed no increased risk of AKI over lower-dosage statins: HR=1.09 (95% CI: 1.07-1.12). Some individual formulations conveyed a higher risk of AKI: higher-dosage atorvastatin, HR=1.09 (1.07-1.13); and high-dosage simvastatin, HR=1.23 (1.17-1.30). Formulations with lower AKI risk were: lower-dosage atorvastatin, HR=0.92 (0.89-0.96); lower-dosage rosuvastatin, HR=0.85 (0.79-0.92).



Conclusions: While the risk of AKI in most statin formulations is comparable to lower-dose simvastatin, some higher-intensity formulations are associated with higher risk of AKI. Residual confounding by disease severity may partially explain the observed effects, but modern adjustment techniques reduce this possibility.

Funding: NIDDK Support

SA-PO005

The Incidence, Risk Factors, and Prognosis of Warfarin-Related Nephropathy in a Tertiary Hospital in Korea

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Background: Warfarin-related nephropathy(WRN) is a recently described disease entity, where excessive warfarinization(international normalized ratio(INR)>3.0) causes acute kidney injury. Hence we analyzed the incidence, risk factors, and prognosis of WRN by the retrospective analysis in a single tertiary hospital in Korea.

Methods: During the period March 2003 to December 2011, a total of 1297 warfarin-treated patients who had at least one event of INR>3.0 and also had serum creatinine(sCr) levels measured within 1week after INR>3.0 and within 6months before INR>3.0 were identified. WRN was defined as more than 50% or more than 0.3mg/dL elevation of sCr level measured within 1week after INR>3.0 over sCr level measured within 6months before INR>3.0. Chronic kidney disease(CKD) was defined as eGFR<60ml/min/1.73m^2.

Results: WRN developed in 19.3% of entire patients. The incidence was higher in CKD group(24.0%) than non-CKD group(17.4%). The majority cases of WRN(82.2%) occurred within 1year after the initiation of warfarin therapy. In laboratory findings after INR>3.0, patients with WRN had higher INR and lower hemoglobin and hematocrit level than those without WRN. In multivariate analysis, the risk of WRN increased as basal serum albumin level decreased and was strongly associated with coronary artery disease and congestive heart failure. The presence of CKD or basal eGFR were not independent risk factors for WRN. Despite of no significant difference in basal sCr level, sCr level was higher in patients with WRN than those without WRN after follow-up. The mortality rates at 1,2,and 5years were also higher in patients with WRN than those without WRN(33.0% vs 15.6%; 41.7% vs 23.3%; 56.8% vs 45.1%).

Conclusions: WRN developed in 19.3% of patients having excessive warfarinization. Lower basal serum albumin level and comorbidities such as coronary artery disease and congestive heart failure were associated with the occurrence of WRN. The development of WRN adversely affected the renal and patient outcomes.

SA-PO006

Incidence and Outcomes of Acute Kidney Injury in a Large NHS Hospital

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Background: There are few published data on incidence and outcome of acute kidney injury (AKI) in the United Kingdom since the widespread use of RIFLE and AKIN criteria. We constructed and analyzed a four year retrospective database of AKI for Nottingham University Hospitals NHS Trust (NUH), one of the largest acute NHS hospitals in the U.K.

Methods: For every patient >16 years admitted to hospital and requiring an overnight stay between 31 March 2007 and 31 March 2011, we scrutinized all serum creatinine results during the admission using algorithms operating within the hospital computer systems. Primary analyses used AKIN criteria to detect and stage AKI, taking baseline creatinine to be the lowest serum creatinine from 7-365 days prior to admission. Where no baseline creatinine existed, a theoretical value was applied (based on GFR 75mL/min). Only the highest stage of AKI episode during an admission was counted. For secondary analyses we applied RIFLE and KDIGO criteria; we also compared the use of different baselines, including average creatinine from 7-365 days prior to admission. Main outcome measures were in-hospital mortality, length of stay and fall in GFR from baseline with follow-up of >1 year post-discharge. We are undertaking subset analyses for different specialties and demographic groups.

Results: Mean number of qualifying admissions per year was 99,571 (95,475-103,913). Using AKIN criteria, incidence of AKI was 5.20% stage 1, 1.45% stage 2, 0.95% any stage 3; 0.15% required acute renal replacement therapy (RRT). Percentage of each AKIN stage detected from admission creatinine (i.e. 'community acquired') was 69.8% of stage 1, 87.8% of stage 2, 79.9% of stage 3. Average length of stay: 14 days (stage 1), 15 days (stage 2), 16 days (all stage 3), 30 days (stage 3 with RRT). In-hospital mortality for AKIN stage 1,2,3 was 16.3%, 29.5% and 36.1% respectively.

Conclusions: We have compiled and analyzed one of the largest single centre datasets of AKI in the U.K. In-hospital mortality and length of stay increase with AKIN stage. Most AKI was 'community acquired' but 30% of stage 1 AKI developed after admission.

Funding: Private Foundation Support

SA-PO007

Low Preoperative Hepcidin Concentrations in Urine and Plasma as Risk Factors for Mortality after Cardiac Surgery: A Pilot Study

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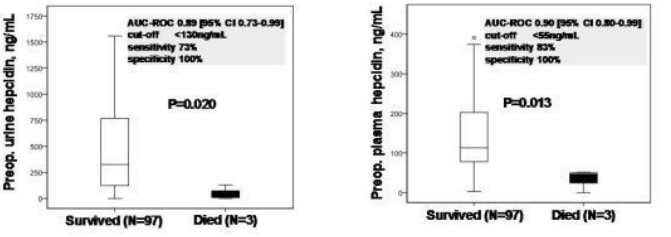
Background: Hepcidin regulates iron absorption and recycling and is central to host defence, protection from reactive iron species, and is a biomarker of iron-related pathophysiology. Here, we assess the value of hepcidin measured preoperatively for the prediction of in-hospital mortality and renal outcomes.

Methods: We studied 100 adult patients undergoing cardiac surgery in the control arm of a randomized controlled trial. Plasma and urine were sampled before induction of anesthesia and hepcidin-25 was quantified by cELISA. Renal outcomes were acute kidney injury (AKI, defined by RIFLE) and acute dialysis. Variables potentially influencing hepcidin expression were investigated.

Results: Low preoperative hepcidin concentration in urine (15.3 [0-129.1] ng/mL) and in plasma (49.2 [0-52.2] ng/mL) were observed in patients who died in the hospital (Figure 1). Preoperative urine and plasma hepcidin predicted in-hospital mortality (Figure 1 - Box). However, preoperative serum creatinine did not predict mortality (AUC-ROC 0.50 [0.10-0.94]). Further, at this time point, urine and plasma hepcidin as well as serum creatinine did not distinguish patients with acute dialysis from those without (urine: AUC-ROC 0.62 [0.38-0.86], plasma: AUC-ROC 0.63 [0.34-0.91], creatinine: AUC-ROC 0.61 [0.22-0.99]). Preoperative renal function and hemoglobin did not correlate with hepcidin indices whereas plasma markers of inflammation did (plasma IL-6, CRP).

Conclusions: Low preoperative hepcidin may be a risk factor for in-hospital mortality. Findings should be validated in larger patient cohorts with greater number of events.



Funding: Pharmaceutical Company Support - Intrinsic LifeSciences

SA-PO008

Non-Steroidal Anti-Inflammatory Drugs Associated with Pediatric Acute Kidney Injury

Mark D. Poirier, Kevin C. Abbott. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Non-steroidal anti-inflammatory (NSAID) medications can induce two (2) different forms of acute kidney injury: hemodynamically mediated and acute interstitial nephritis. It has been shown previously in adults that volume depletion, in the setting of taking NSAIDs can lead to acute kidney injury. The studies done to date involve adults and to the best of our knowledge there has not been any description of NSAIDs in the pediatric population to date. We hypothesized that NSAIDs are associated with pediatric acute kidney injury.

Methods: We conducted a retrospective study of the entire Department of Defense (DoD) database in which we identified patients less than 18 years of age receiving a prescription for an NSAID between the dates of September 1, 2001 through December 31, 2009 in an outpatient setting. A separate search for pediatric acute kidney injury (pAKI), defined as a 50% increase in the serum creatinine above the baseline, in a cohort of patients less than 18 years of age was also conducted n the same DoD database between the dates January 1, 2011 through January 1, 2011. We excluded patients with no serum creatinine measurements, evidence of AKI without a known baseline, neonates or history of prescriptions written for NSAIDs.

Results: A total of 10,065 outpatient prescriptions for NAIDs were identified in the pediatric cohort. There were also a total of 5,540 (55%) cases of AKI associated with pediatric acute kidney injury with all of these receiving the NSAID prescription within 90 days of the pAKI. The odds ratio for developing pAKI was 3.373 (95% CI 1.74-6.55) and a p value < 0.0001. In a separate sub-population analysis, African Americans and males prescribed NSIADs had odds ratios of 2.22 (95% CI 1.23-3.91, p value <0.0001) and 2.33 (95% CI 1.32-4.11) respectively.

Conclusions: In a pediatric cohort identified from a DoD database, NSAIDs were associated with acute kidney injury and the association was ore pronounced in African American males. Our results suggest that NSAIDs, currently known to be nephrotoxic for certain adult populations, may also have similar toxic effects in children and therefore may need to be prescribed with more caution.

SA-PO009

Fluid Balance during Renal Replacement Therapy Affects Mortality in Critically Ill Patients Marzena Wojewodzka,[1,2] Jie Teng,[1,3] Dehua Gong,[1,4] Claudio Ronco,[1] Dinna N. Cruz.[1] *[1]IRRIV, St Bortolo Hospital, Italy; [2]Medical University, Poland; [3]Fudan University, China; [4]Jinling Hospital, China.*

Background: Among critically ill patients with severe AKI, fluid overload (FO) of >10-20% of body weight at RRT initiation is associated with poor outcomes in many studies. However, fluid balance during RRT itself may be equally important, and few studies have addressed this aspect. Our aim was to evaluate the impact of the sum of fluid balance before and during RRT on hospital mortality.

Methods: We studied 99 patients (mean age 64 yrs; 78%M) with severe AKI treated with continuous RRT in the ICU. We recorded cumulative fluid balance (CFB) from ICU admission to RRT initiation (A), and during the entire duration of RRT (B). Total FO was calculated as (A+B)/weight x 100%. Patients were divided into 2 groups: total FO<20% and FO≥20%. Hospital mortality was compared using survival curve analysis. Multivariable analysis was performed using Cox regression adjusting for sepsis and CKD.

Results: There were 63 patients in the FO≥20% group, and 36 in FO<20%. The FO≥20% group had a higher proportion of sepsis (27% vs 8%, p=0.04) and CKD (70% vs 50%, p=0.06). This group had significantly higher CFB both before and during RRT (Fig. 1). Notably, the FO<20% group had negative CFB during RRT. The total FO≥20% group had significantly higher 28-day hospital mortality (p=0.046, Fig. 2). This remained significant after adjusting for sepsis and CKD (adjusted HR 2.23, 95%CI 1.08-4.60). Similarly, FO≥20% during RRT was associated with higher mortality (adjusted HR 2.89, 95%CI 1.11-4.32).

Fig.1. Cumulative fluid balance: total, pre- and during RRT

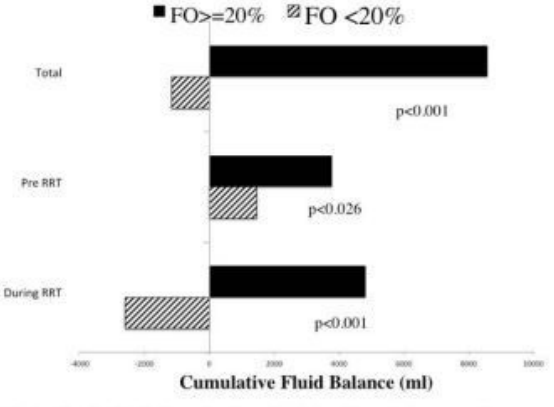
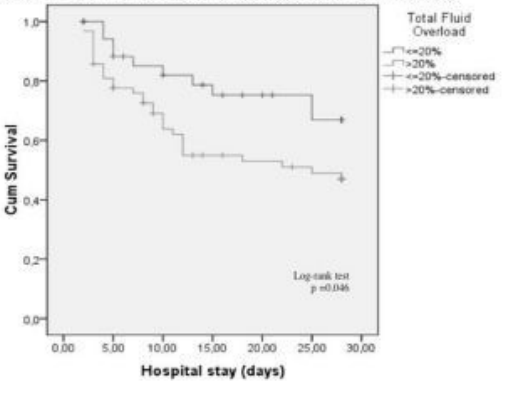


Fig.2. Total Fluid Overload and 28 day hospital mortality



Conclusions: This is the first study examining the effect of fluid overload both before and during RRT. More fluid overload, both in total and during RRT, is associated with increased hospital mortality. This highlights the importance of adequate fluid removal during RRT, in addition to timely RRT initiation.

SA-PO010

AKI Biomarkers Remain Elevated 5-8 Years Following AKI due to Cardiac Surgery in Children Donna J. Claes, David S. Cooper, Michael R. Bennett, Qing Ma, Catherine D. Krawczeski, Stuart Goldstein. *Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Novel biomarkers can predict AKI after cardiac surgery (CPB-AKI). Emerging evidence shows AKI increases risk for future CKD, yet no studies assess novel urinary biomarkers long-term after CPB-AKI.

Methods: We assessed for CKD and urinary NGAL, IL-18, KIM-1 & L-FABP in 14 pts (mean age 8.6±1.6yr) who had pRIFLE-I (n= 10) or –F (n=4) (SCr-based) within 24 h post-CPB from 2004-2007 (mean follow-up 6.5 +/-0.8 yr) and compared baseline characteristics to pts with pRIFLE-I or –F from the same AKI study who were not readily available for follow-up.

Results: Study pts who had history of pRIFLE-I were older (median age 1.3 vs 0.5 yrs, p=0.037), had higher peak SCr (median 0.75 vs 0.6 mg/dl, p=0.0004), and longer CPB time (164 vs 100 min, p=0.04) than non-study pRIFLE-I pts. At follow-up, study pts had normal urine microalbumin to creatinine ratios (median 10.53 mcg/mg, IQR 4.92-17.03), Schwartz eGFR (median 114 ml/min/1.73m2, IQR 95-137) & were normotensive (mean SBP 100, SD 6.67; mean DBP 59, SD 10). 4 pts were on anti-hypertensive meds for afterload reduction. We compared follow-up biomarkers to pre-CPB & 24 hour urinary biomarker values of an independent cohort previously reported in the literature (median and IQR are provided unless noted):

	NGAL (ng/ml)	IL-18 (pg/ml)	KIM-1 (pg/ml)	LFABP (ng/ml)
Baseline, no AKI (n=160)	8.5 (4, 12.5)	1.2 (0, 8)	193.9 (83.9, 301.4)	7.3 (2.3, 27.4)
Baseline, AKI (n=54)	16 (8, 23)	0.9 (0, 12)	250 (84, 399)	8.8 (3.7, 31)
24 h, no AKI (n=160)	12 (5, 30)	10 (0, 29.3)	373.42 (150.5, 753.7)	77.1 (16, 234.4) n=150
24 h, AKI (n=54)	99 (35, 206)	50 (27, 100)	1010 (668, 1589)	244.1 (132.7, 590.6) n=50
5-8 yrs later, AKI (n=14)	9.13 (5.37, 20.73)	56.9 (41.1, 77.3)**	473.43 (256.6, 686.6)***	8.87* (3.68)

*mean, SD,** p< 0.0001, ***p = 0.0192 as compared to Baseline AKI (Wilcoxon Rank Sum)

Conclusions: In this follow-up of a small cohort of children with post-CPB AKI, we observed 1) persistent urinary IL-18 & KIM-1 elevation and 2) minimal evidence of classic CKD signs. We suggest novel urinary biomarkers could serve as a more sensitive marker of chronic kidney injury.

SA-PO011

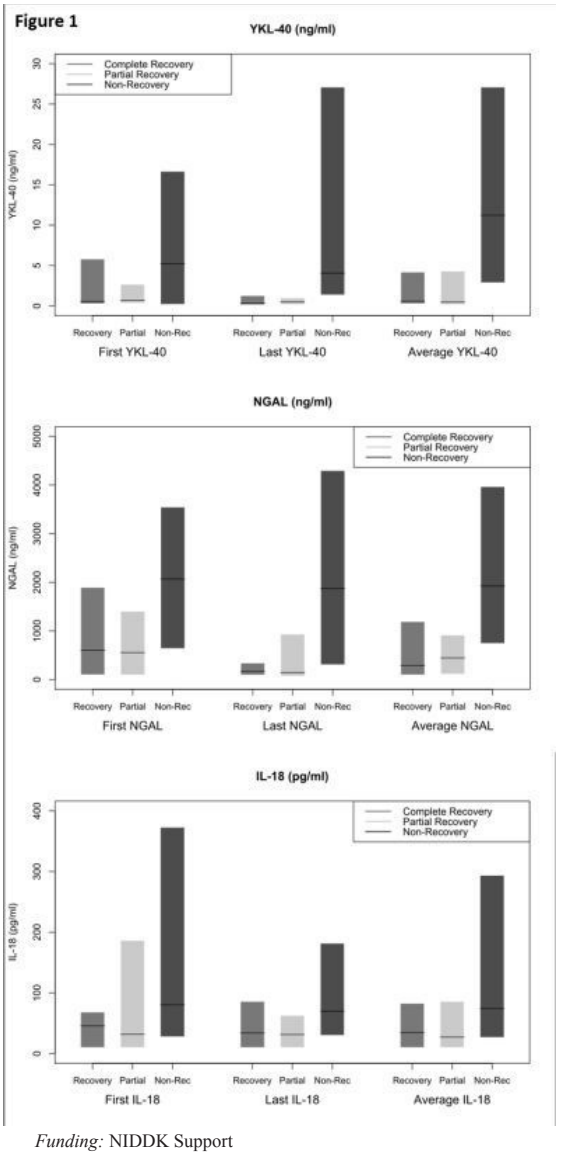
Urinary Biomarkers and Association with Recovery from Severe AKI Rahul Agarwal, Divakar Jammalamadaka, Bita Fakhri, Chirag R. Parikh, Steven G. Coca. *Yale School of Medicine, New Haven, CT.*

Background: Few studies have examined the ability of urinary biomarkers to associate with recovery from AKI. We hypothesized that urinary biomarkers of kidney injury (NGAL, IL-18) or repair (YKL-40) would be associated with renal recovery in patients with severe AKI.

Methods: We prospectively enrolled 48 patients admitted at Yale New Haven Hospital with severe AKI (AKIN Stage 3). Daily morning urine samples were collected (until recovery up to max of 9 days). Levels of urinary YKL-40, NGAL, and IL-18 were measured. The primary exposures were the concentrations of the biomarkers on the first, last, and average daily values of the biomarker during enrollment. The primary outcome was renal recovery at discharge and was classified as "complete" if SCr ≤1.4 mg/dL, "partial" if 1.4 < SCr < 4 mg/dL and "non-recovery" if SCr ≥ 4mg/dL or death.

Results: Sixteen patients recovered completely, 18 recovered partially, and 14 did not recover. Mean time to partial recovery from the first sample collection was 6.7 ± 10 days and mean time to complete recovery was 10 ± 9 days. Median values of only the last and average YKL-40 (p=0.01 and 0.02) and NGAL (p=0.02 and 0.007) were significantly higher in patients that did not recover from AKI, compared to those that experienced partial or complete recovery (Figure 1). The upper tertiles of the last (RR 8, 95% CI 1.1-56.8) and average (RR 9, 95% CI 1.3-63.0) YKL-40 values were associated with a greater risk of non-recovery, as were the upper tertiles of the last (RR 9, 9% CI 1.3-63.0) and average (RR 8, 95% CI 1.1-56.8) NGAL values. The AUCs for first, last and average YKL-40 for non-recovery were 0.61, 0.77, and 0.77 respectively, 0.76, 0.78, and 0.79 for NGAL, and 0.62, 0.66, and 0.67 for IL-18.

Conclusions: High values of YKL-40 and NGAL appear to be associated with non-recovery from severe AKI. Larger studies will need to confirm these findings.



Funding: NIDDK Support

SA-PO012

Outcomes of Dialysis after Continuous Flow Left Ventricular Assist Device (LVAD) Implantation John J. Dillon,[1] Salil V. Deo,[2] Lyle Joyce,[2] Amy W. Williams,[1] Robert C. Albright,[1] Margaret M. D'Uscio,[1] Richard C. Daly,[2] Tal Hasin,[3] Michelle M. O'Shaughnessy,[1] Sudhir S. Kushwaha,[3] John Stulak,[2] Barry A. Boilson,[2] Soon J. Park.[2] *[1]Division of Nephrology & Hypertension, Mayo Clinic; [2]Division of Cardiovascular Surgery, Mayo Clinic; [3]Division of Cardiology, Mayo Clinic, Rochester, MN.*

Background: Continuous flow LVADs, such as the HeartMate II, are now an established treatment option for patients with severe heart failure. The FDA approved the continuous-flow HeartMate II LVAD as a bridge to transplantation in 2008 and as destination therapy in 2010. Acute or chronic renal failure, requiring dialysis, may occur after LVAD implantation.

Methods: One hundred twenty-eight patients underwent HeartMate II implantation at our institution between February 2007 and June 2011. We report the outcomes of those who required dialysis. Follow up was through December 2011.

Results: Twenty-one patients (16%) required dialysis after LVAD implantation. Three of these had received CVVH for a median of 2 (range 2-7) days prior to LVAD implantation. The initial post-LVAD dialysis was CVVH for 19 patients, hemodialysis for 2. Although continuous flow LVAD patients have little or no pulse, this was not a barrier to dialysis. The median time from LVAD implantation to dialysis was 3 (range 0-1048) days. Among 10 patients (48%) who recovered renal function, 8 (80%) survived to last follow up with a median survival > 536 (range 39-1184) days. Among 11 who did not recover renal function, 2 (18%) survived to last follow with a median survival of 36 (range 1-915) days (P<0.01 vs those who recovered renal function.) Among those who recovered, the median time to renal recovery was 17.5 (range 1-220) days. Only one patient recovered renal function after 45 days. Five patients required outpatient dialysis and 4 developed ESRD.

Conclusions: Renal failure, requiring dialysis, is not uncommon after LVAD implantation. We demonstrate that this can be done successfully despite the continuous flow nature of current devices. Almost half of our patients recovered renal function, most with good outcomes. When renal recovery occurred, it usually happened within 45 days. Nineteen % of those requiring dialysis developed ESRD.

SA-PO013

Delayed AKI Referral and Mortality: A Single Centre UK Post NCEPOD-AKI Audit Azharuddin Mohammed, Simon Fletcher, Sunil Daga. *Nephrology, University Hospital Coventry and Warwickshire, Coventry, United Kingdom.*

Background: 2009 UK NCEPOD AKI National Clinical Enquiry into Patient Outcome and Death-Acute Kidney Injury) report identified delayed referral (LR) as an area of concern and early renal referral (ER) recommended (NICE CG 50).We looked into change in practice pattern of AKI referral post NCEPOD-AKI and mortality outcome.

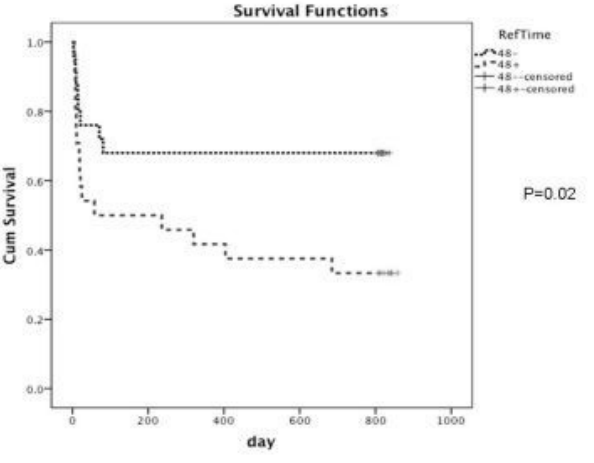
Methods: All inpatient AKI nephrology referrals at a single UK university hospital over 6 weeks studied. Data collected using 1) referrer interview, clinical assessment 2) CRRS(IT)-lab results and clinic letters. Only new referrals with raised creatinine (Cr) and not under renal team included. We excluded all CKD patients under nephrologists, referrals for electrolyte/acid base disorders, dialysis and kidney transplantation patients. Referrals grouped into ER<48 hours and LR >48 hours. Mortality analysed.

Results: 49(n=) referrals included, M:F at 63.3 Vs 36.7%. Median age 65.5 years (16-95). 52% had baseline CKD, 44% HTN, 24% DM, 18% proteinuria >500mg/d, 68% septic and hypovolemic. Mean referral Cr was 362 (umol/L). Fisher exact test used for categorical and t-test for continuous data. Overall 30 day mortality was 34.6% with ER: LR at 24 Vs 46 % (RR=1.9). Mortality groups showed significant differences in length of hospital stay, referral time and mean referral Cr, despite equal RF scores.

Demographics of AKI mortality group

	< 48 hours	> 48 hours	p=
Median Age	68.3	75.1	NS
Mean AKI risk score	3.8	4.4	NS
Mean length of hospital stay	1	9.4	<0.01
Mean days for AKI referral	0.6	6.3	0.01
Mean referral Cr	305	380	0.02
Overall mortality%(n) 30-day	24(6)	46(11)	0.13
2 years	66(14)	66(14)	0.02

30-day mortality was high in LR but not statistically significant (small n=). Long term survival in LR is significantly poor at 2 years (p=0.02).



Conclusions: LR has significant long term mortality. ER may allow treatment optimization, reduce complications and risk of death. Electronic prompt system may improve AKI care.

SA-PO014

A Collaborative Approach to Kidney Biomarker Qualification Joe Keenan,[1] Patrick T. Murray,[2] Frank Dieterle,[3] Ralf Schindler,[4] Scott H. Adler.[5] *[1]SAFE-T Consortium, Argutus, Dublin, Ireland; [2]SAFE-T Consortium, UCD Mater Hospital, Dublin, Ireland; [3]SAFE-T Consortium, Novartis AG, Basel, Switzerland; [4]SAFE-T Consortium, Charite Hospital, Berlin, Germany; [5]SAFE-T Consortium, Astrazeneca, Wilmington, DE.*

Background: A European consortium (SAFE-T) was established in 2009 consisting of 12 pharmaceutical companies, 3 SMEs and a number of hospitals with the objective of providing a data submission package to the Health Authorities to allow for the qualification of biomarkers to monitor drug induced kidney injury. The working plan of the consortium is to perform a first phase exploratory study of a larger list of candidate biomarkers through a number of healthy volunteer and drug induced kidney injury (DIKI) clinical cohorts. Following statistical assessment of these exploratory studies SAFE-T will dismiss certain candidates that do not meet performance criteria and then perform a larger confirmatory DIKI study, the results of which are intended to serve as a convincing submission package for the qualification of a number of key kidney markers with the health authorities.

Methods: SAFE-T have established a candidate list of 22 most promising kidney biomarkers assembled from an extensive literature search and graded accordingly. Assays have been validated at 4 sites for all of the 22 markers (some markers using dual technology i.e. both microtitre ELISA and Luminex). A healthy volunteer cohort of samples has already been assessed. Clinical samples from four studies are being collected. 1. Cisplatin Nephrotoxicity 2. Acute Glomerulonephritis (GN) 3. Contrast Media 4. Transplantation study.

Results: Healthy volunteer data will be presented. Normative ranges of the markers will be presented. Performance of all 22 markers in an interim analysis of the key Cisplatin, acute GN and Contrast Media study cohorts will be assessed, presented and discussed. SAFE-T will discuss critical aspects of this biomarker qualification, such as using an adjudication committee to compensate for imperfect gold standards to diagnose kidney injury. Furthermore, the decision process for advancing successful biomarkers as well as failing unsuccesful ones will be discussed.

Funding: Government Support - Non-U.S.

SA-PO015

A Need for New Acute Kidney Injury Classification in Patients with Pre-Existing Chronic Kidney Disease Nitin V. Kolhe, Richard J. Fluck, Chris W. McIntyre, Nigel Lawson, Nicholas M. Selby. *Department of Renal Medicine, Royal Derby Hospital, Derby, Derbyshire, United Kingdom.*

Background: Current AKIN classification for diagnosing AKI performs less well in patients with pre-existing chronic kidney disease (CKD). Using a subset of patients with background CKD from a large group of prospectively identified patients with AKI we aimed to test different creatinine thresholds for AKI staging and correlate these with mortality, to help guide future strategies for AKI classification.

Methods: All episodes of AKI occurring in the period September 2010 - October 2011 in Royal Derby Hospital were identified by a hospital wide, real-time electronic reporting system. We extracted cases of AKI who had abnormal baseline creatinine values along with demographic, outcome and hospital coding data. AKI was then reclassified using several different methods, testing both absolute increase and percentage increase over baseline. The different AKI models were then compared with current AKIN criteria with respect to the strength of the associations between increasing AKI stage and mortality.

Results: During the study period we identified 999 patients with AKI who had pre-existing CKD (1123 episodes of AKI in total). 198 died during their index admission (17.6% mortality). The mean age at onset of AKI was 78yrs and the mean baseline creatinine was 168μmol/L. AKIN classification resulted in very few patients in AKI stage 2 and proportionally more in stage 3; the higher AKI stages were not differentiated by higher mortality rates. A model using absolute rises in creatinine over baseline (table 1) produced improved associations between increasing AKI stage and increasing mortality rates.

Creat. rise from baseline	No. of patients	In hospital mortality	30 day mortality
27-44μmol/L (stage 1)	301 (26.8%)	24 (8%)	29 (9.6%)
45-88μmol/L (stage 2)	405 (36.1%)	56 (13.8%)	64 (15.8%)
≥89μmol/L (stage 3)	417 (37.1%)	118 (28.3%)	122 (29.3%)

Table 1

Conclusions: The optimal method to classify AKI in patients with background CKD remains uncertain although it appears that the current AKIN staging may need to be adapted. Methods that employ an absolute rise in creatinine rise over baseline may be more predictive of outcomes in this group.

SA-PO016

Validation of KDIGO AKI Definition in AKI Population with Various Renal Parenchymal Injuries Rong Chu, Gang Liu, Cui Li, Li Yang. *Renal Division, Peking University First Hospital, Beijing, China.*

Background: The current definition for acute kidney injury (AKI) mainly comes from the ICU patients and reflects the course of acute tubular necrosis (ATN). This study aimed to investigate whether the KDIGO AKI definition is applicable for the AKI population with various acute renal parenchymal injuries.

Methods: Patients who were pathologically defined as diffuse renal parenchymal acute injuries throughout 2011 were enrolled in the study. The pathological changes include cellular crescentic glomerulonephritis (CCGN), ATN, acute interstitial nephritis (AIN), and acute thrombotic microangiopathy (TMA). Changing profile of serum creatinine (Scr) and urine volume throughout the disease course was analyzed in each individual case. 2012 KDIGO AKI definition was applied to recognize patients with AKI, acute kidney disease (AKD), and non-AKD.

Results: One hundred and thirty-nine patients, 77 men and 62 women, aged 45±16y, met the enrollment criteria, with CCGN in 44, ATN in 33, AIN in 53, and TMA in 9 cases. One hundred and twenty-six patients (91%) conformed to AKD, among which 82 (59%) were defined as AKI, with 20 cases at stage 1 (24%), 4 at stage 2 (5%), and 58 at stage 3 (71%). There was no difference of gender or age among cases with AKI, non-AKI-AKD and non-AKD. AKI patients had the highest level of Scr at biopsy ($p<0.001$) and the longest hospitalization time (33±19, 18±9 and 17±8 days, $p<0.001$) among the three groups. AKD definition recognized 91% of CCGN, 94% of ATN, 94% of AIN, and 56% of TMA. AKI was diagnosed in 66% of CCGN, 70% of ATN, 52% of AIN and 22% of TMA. Non-AKI-AKD took more part in AIN and TMA than the other two groups. For those who did not meet the AKI definition, 63% had less rapid Scr increase than the proposed criteria, 37% already reached the peak of renal dysfunction while the first Scr information was collected.

Conclusions: KDIGO AKD definition detects most of the patients that have diffuse acute renal parenchymal histological injuries, while the AKI definition recognizes patients who have more severe renal dysfunction and need longer time for hospitalization treatment. The applicable capacity of AKI definition varies with different pattern of histological injuries.

Funding: Government Support - Non-U.S.

SA-PO017

The Use of Urine Dipstick and Lab Urinalysis within 24 Hours of Admission in the Emergency Department in Three Irish Teaching Hospitals Limy Wong,[1] Frank J. O'Brien,[2] Sean F. Leavey,[2] Marie Patricia Boyle.[2,3] *[1]Department of Nephrology, Beaumont Hospital, Dublin 9, Ireland; [2]Department of Nephrology, Waterford Regional Hospital, Waterford, Ireland; [3]Department of Emergency Medicine, Mater Misericordiae University Hospital, Dublin, Ireland.*

Background: The UK Renal Association, KDOQI and KDIGO guidelines recommend AKI risk assessment (serum biochemistry, urine dipstick and lab urinalysis). We audited guideline adherence in three large Irish teaching hospitals.

Methods: Serum creatinine, recent creatinine trends and performance of urine dipstick and lab urinalysis within 24 hours of admission was collected prospectively for 94 randomly selected medical patients in all three hospitals.

Results: Hospital 1

Mean age was 63.5 years (17-93). Mean plasma creatinine 107 μmol/l (28-929). Sixty-nine had normal renal function, 20 had AKI and acute on chronic kidney disease (CKD) and 5 had stable CKD. Urine dipstick was performed in 1 patient, lab urinalysis in 39 patients.

Hospital 2

Mean age was 64.5 years (19-96). Mean plasma creatinine 121 μmol/l (42-769). Sixty-nine had normal renal function, 17 had AKI and acute on chronic kidney disease (CKD) and 8 had stable CKD. Urine dipstick was performed in 24 patients, lab urinalysis in 43 patients.

Hospital 3

Mean age was 66.3 years (27-98). Mean plasma creatinine 96 μmol/l (36-693). Seventy-five patients had normal renal function, 4 had AKI and acute on chronic kidney disease (CKD) and 14 had stable CKD. Urine dipstick was performed in 2 patients, lab urinalysis in 65 patients.

Conclusions: Both urine dipstick and lab urinalysis were underutilised: urine dipstick–mean 5.6% (range 0–11.7%) and lab urinalysis–mean 38.4% (range 35.3–40.0%). Our results demonstrate suboptimal guideline adherence. A pilot clinical pathway was introduced in hospital 2 whereby the medical admission proforma was altered to formally document abnormal renal function and the performance of urine dipstick and lab urinalysis. Following clinical pathway introduction, 87 patients (93%) had urine dipsticks (25% initially) and 66 patients (70%) had lab urinalysis (46% initially). Our study suggests structured documentation proformas may facilitate adherence to good standards of clinical practice.

SA-PO018

Do Urine Eosinophils Have a Role in the Diagnosis of Acute Interstitial Nephritis? Angela K. Muriithi,[1] Samih H. Nasr,[2] Nelson Leung.[1] *[1]Division of Nephrology, Mayo Clinic; [2]Division of Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Background: Urine eosinophils (UE) were shown to correlate with acute interstitial nephritis (AIN) but the three largest series that investigated the test characteristics did not use kidney biopsy as the gold standard. This study was undertaken to determine the ability of UE to diagnose AIN in patients with a renal biopsy.

Methods: We conducted a retrospective study of adult patients with biopsy-proven diagnoses from 1994 to 2011 and who had UE as part of their work-up. UE were tested using Hansel's stain. Results were compared using the 1% and 5% eosinophils of all urinary white blood cells cutoffs.

Results: Of the 566 patients, 322 were male. Median age was 61 years (range 18-91). Pyuria was defined as one or more white cell/HPF and was detected in 467 of 566 patients. AIN was diagnosed in 91 patients of whom 73 had drug-induced. UE were found in a variety of kidney diseases (Figure 1). Moving the cutoff from 1% UE to 5% UE decreased the sensitivity but increased the specificity (Table 1). Presence of pyuria did not affect the diagnostic characteristics. UE was no better at distinguishing AIN from acute tubular necrosis (ATN) as compared to other kidney diseases (Table 1).

Figure 1: Eosinophiluria in Kidney Disease

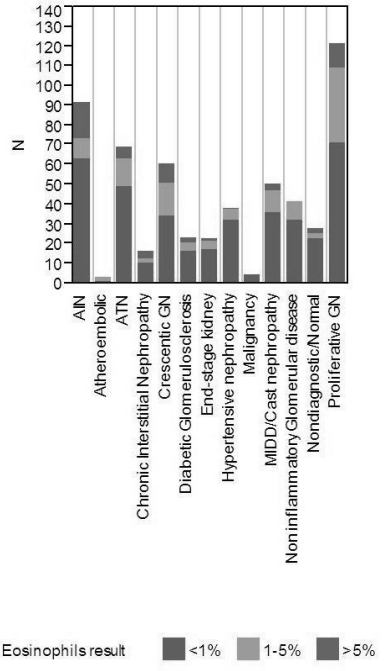


Table 1: AIN compared to all other diagnoses and to ATN alone

	Cutoff of UE	Sensitivity	Specificity	PPV	NPV
AIN compared to all other diagnoses					
All patients (566)	>1%	30.8%	68.2%	15.6%	83.7%
	>5%	19.8%	91.2%	30.0%	85.6%
Patients with Pyuria only (467)	>1%	38.4%	61.7%	15.6%	84.4%
	>5%	24.7%	89.3%	30.0%	86.5%
AIN compared to ATN					
All patients (160)	>1%	30.8%	71.0%	58.3%	43.8%
	>5%	19.8%	91.3%	75.0%	46.3%
Patients with Pyuria only (128)	>1%	38.4%	63.6%	58.3%	43.8%
	>5%	24.7%	89.1%	75.0%	47.1%

Conclusions: UE were found in a variety of kidney diseases besides AIN. Even at a cutoff of 5%, UE had poor sensitivity, specificity, PPV and NPV distinguishing AIN from ATN or other kidney diseases.

SA-PO019

Acute Interstitial Nephritis: A Current Perspective Angela K. Muriithi,[1] Nelson Leung,[1] Lynn D. Cornell,[2] Mary E. Fidler,[2] Sanjeev Sethi,[2] Samih H. Nasr.[2] *[1]Division of Nephrology, Mayo Clinic; [2]Division of Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Background: Acute Interstitial Nephritis (AIN) is an important cause of Acute Kidney injury (AKI), especially in hospitalized patients. We conducted this study to provide a current perspective on its causes, outcome, and predictors of response to steroids.

Methods: We retrospectively studied 136 patients with biopsy-proven AIN seen at our institution between 1994-2011.

Results: The median age was 58 years with M:F ratio of 1. In 54% of patients, AIN occurred during hospitalization. AIN was suspected in 46% prior to biopsy. 28% had leukocytosis and 18% had eosinophilia. Only 6% had the "classical triad" of fever, rash and eosinophilia, all with drug-induced AIN. 47% of patients had pyuria, 33% eosinophiluria, 87% some proteinuria, 30% hematuria and 14% oliguria. Peak serum creatinine was 5.4 mg/dL and 20% required dialysis. 86% of patients were treated with steroids. Causes of AIN were drugs in 70%, autoimmune diseases in 20% (Sarcoidosis, Sjögren's, TINU and IgG4 disease), infections in 3%, other conditions in 3%, and unknown in 4%. Drug-induced AIN was due to antibiotics in 47% (most commonly penicillins and fluoroquinolones), proton pump inhibitors (PPIs), particularly Omeprazole, in 13%, NSAIDs in 10%, and other drugs in the rest. Of the 118 patients with available follow up, 39% had complete recovery, 42% partial recovery, and 19% no recovery by 3 months from renal biopsy. The mean time to recovery was 7.7 weeks. Of the 82 patients with drug-induced AIN treated with steroids, 38% achieved complete recovery, 41% partial recovery and 21% did not recover by 3 months. Drug-induced AIN was less likely to recover at 3 months compared to other types of AIN. For patients with drug-induced AIN treated with steroids, predictors of complete recovery were a shorter exposure to the culprit drug and a shorter interval between the development of AKI and start of steroid therapy.

Conclusions: The etiology of AIN is shifting: PPIs are emerging as an important contributor to this disease as are autoimmune diseases. Prompt discontinuation of the culprit drug and initiation of steroid therapy are essential in the management of drug-induced AIN.

SA-PO020

Long-Term Prognosis and Affecting Factors of Acute Interstitial Nephritis Cui Li, Tao Su, Rong Chu, Xiaomei Li, Li Yang. *Renal Devision, Peking University First Hospital, Beijing, China.*

Background: Acute interstitial nephritis (AIN) has abrupt response to prednisone treatment and is thought to have optimal prognosis in most cases. This study aimed to disclose the long-term outcomes of AIN and investigate the potential affecting factors.

Methods: Seventy-two patients, 17 men and 55 women, aged 47±13y, clinical-pathologically diagnosed as AIN from 2001 to 2011, were enrolled in this study. These patients were regularly followed up for 3 (1~11) years after renal biopsy and the etiology of AIN was re-evaluated and confirmed. Detailed clinical data was collected. Semi-quantitative scores for interstitial inflammation, fibrosis, tubulitis, and tubule atrophy were developed. The outcome of AKI was determined at 3m, 6m and 12m post biopsy by the assessment of eGFR, and the affecting factors were analyzed.

Results: Fifty patients had drug-induced AIN (DAIN), 22 developed Tubulointerstitial Nephritis and Uveitis syndrome (TINU), among which 13 (59.1%) were diagnosed as DAIN at the time of biopsy, but developed uveitis within one year after. Prednisone was prescribed to all the cases and cyclophosphamide was administered in 29% of the patients. Scr decreased acutely within one month after prednisone initiation, and reached relatively stable levels in 82% of patients by three months. One year after biopsy, 12.1% of the cases were with normal kidney function, 36.2% at CKD stage 2, 48.3% at stage 3, and 3.4% at stage 4. Correlation analysis showed that eGFR at 12m was correlated with age (r=-0.572, p=0.000), levels of peak Scr (r=-0.409, p=0.001), accompanied thyroid diseases (r=-0.289, p=0.028), and tubule atrophy (r=0.281, p=0.038). Patients with TINU tended to have worse outcome, with 19/22 (86.4%) developed CKD stage 3 and 4 [OR=7.4 (1.614-34.291), p=0.01].

Conclusions: AIN leads to CKD in 87.9% of the patients, which urges re-evaluation of the treatment strategy, and reinforces the importance of long-term follow up of the patients. Patients with TINU tend to have worse outcome and are easy to be miss-diagnosed since half of them develop uveitis months after AIN. Age, Scr peak, accompanied thyroid diseases, and tubule atrophy are related to the worse outcome of AIN.

Funding: Government Support - Non-U.S.

SA-PO021

Long Term Outcomes in Biopsy Proven Acute Interstitial Nephritis Treated with Steroids Maria Prendecki, Anisha Tanna, Alan D. Salama, Frederick W.K. Tam, Tom Cairns, David Taube, H. Terence Cook, Neill D. Duncan, Charles D. Pusey. *Imperial College, London, United Kingdom.*

Background: There are no prospective randomised control trials describing the outcome of Acute Interstitial Nephritis (AIN) treated with steroids and retrospective studies are limited. AIN is a common pathology found in 9.5% of native renal biopsies at our centre in 2000-2010.

Methods: All patients with an acute interstitial inflammatory infiltrate on native renal biopsy were identified. Patients on maintenance steroids were excluded. Treated patients received oral prednisolone or IV methylprednisolone then oral prednisolone; dose and duration of treatment depended on clinician choice. Data were collected retrospectively and outcomes analysed according to treatment.

Results: 164 patients were steroid treated, 89 male, age 51.5 (range 16.4-85.3) years, follow up 41.8 (0.2-164) months. 31 were not treated, 20 male, age 55.2(19.2-87.8) years, follow up 31.3 (0.2-138) months. There was no difference in median eGFR at time of biopsy, 22.5(2-110) ml/min in the steroid group, 24.0(4-59) ml/min in untreated group (p=0.423 Mann-Whitney U test). 12.1% of steroid treated and 19.3% of untreated patients required renal replacement therapy (RRT) at the time of biopsy (p=0.32, χ^2).

Steroid treated patients showed greater improvement in eGFR.

Median Change in eGFR (ml/min)from biopsy to	Steroid treated	Untreated	p (Mann-Whitney U test)
4-6 months	+9(n=133)	+0.5(n=24)	0.0002
10-14 months	+9(n=124)	+3(n=22)	0.002
30-36 months	+11(n=77)	+2(n=11)	0.02
Last follow up	+11(n=164)	+1(n=31)	0.0001

Fewer patients were dependent on RRT at last follow up in the steroid group, 11.56% vs 38.71% (p=0.0004, χ^2).

46/56 patients with drug induced AIN were steroid treated, proportionately more than those with non-drug induced. The median change in eGFR at 6 months was +21 ml/min in the steroid group and -1ml/min in the untreated group (p=0.002 Mann-Whitney U test) and at 12 months, +24 ml/min in the steroid group and -4 ml/min in the untreated group (p=0.009 Mann-Whitney U test).

Conclusions: This retrospective study suggests a benefit of steroids in the treatment of AIN with improvement in eGFR and fewer patients progressing to end stage renal disease.

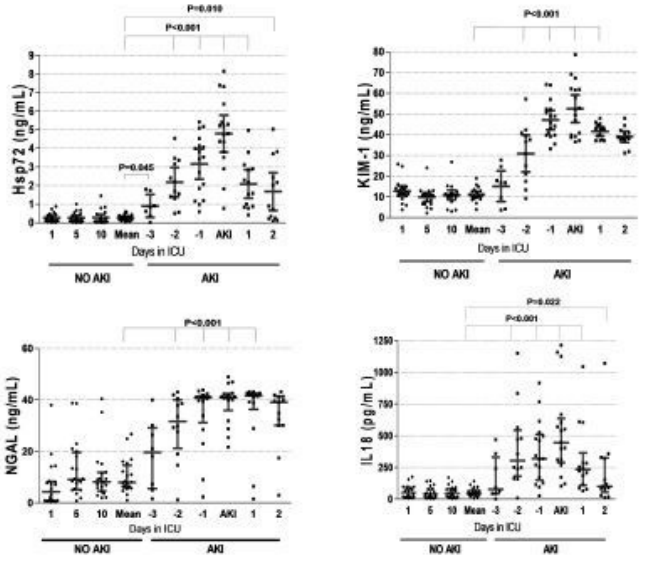
SA-PO022

Heat Shock Protein 72 Is a Novel Biomarker to Predict Acute Kidney Injury in Critical Care Patients Omar Israel Salas-Nolasco,[1] Jonatan Barrera-Chimal,[1,2] Gustavo Alejandro Casas-Aparicio,[1] Irizar S. Sergio Saul,[1] Ricardo Correa-Rotter,[1] Norma Bobadilla,[1,2] Luis E. Morales-Buenrostro.[1] *[1]Nephrology & Mineral Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición S.Z., D.F., Mexico; [2]Molecular Physiology, Instituto de Investigaciones Biomédicas, UNAM, D.F., Mexico.*

Background: Several biomarkers have been proposed for early diagnosis of AKI in specific and predictable situations; however their performance in the intensive care unit (ICU) setting is unknown. Previously, we showed that Heat Shock Protein 72 (Hsp72) is an early and sensitive biomarker of AKI. This study was designed to evaluate the performance of urinary Hsp72 compared to NGAL, KIM-1, and IL-18 to predict AKI in ICU patients.

Methods: We recruited 37 critically ill patients with eGFR >60 ml/min/1.73 m2. Urine samples were collected every day during ICU hospitalization. AKIN criteria were used to define AKI. Urinary Hsp72, KIM-1, NGAL, and IL-18 were measured by ELISA in 17 patients with AKI since 3 days before and until two days after AKI development and 20 patients without AKI (urine from days 1, 5 and 10 after admission). Using ROC curve, we analyzed the biomarkers sensitivity and specificity at 2 and 1 days before AKI development.

Results: The Figure depicted the biomarkers behavior in ICU patients. The Table shows the biomarkers sensitivity and specificity.



Biomarker	48 hrs before (-2) AKI			24 hrs before (-1) AKI		
	Cut-off Value	AUC	Sensitivity/ Specificity(%)	Cut-off Value	AUC	Sensitivity/ Specificity(%)
Hsp72 (ng/mL)	0.5	0.98	100/90	1	0.99	94/100
KIM-1 (ng/mL)	16	0.91	83/95	26	1	100/100
NGAL (ng/mL)	20	0.89	83/90	20	0.91	88/90
IL-18 (pg/mL)	150	0.92	92/100	120	0.93	88/95

AUC= Area Under Curve

Conclusions: The earliest urinary biomarker was Hsp72 (48-h before AKI) and it was similar to KIM-1 (24-h before AKI). Moreover, Hsp72 and KIM-1 exhibited a better performance than NGAL and IL-18 to predict AKI development in ICU patients.

Funding: Government Support - Non-U.S.

SA-PO023

Urinary Glutathione S-Transferase Does Not Predict Acute Kidney Injury in Patients Undergoing Cardiac Surgery Shay Mcguinness,[1] Rachael L. Parke,[1] Rinaldo Bellomo,[2] Frank Van Haren,[3] Michael J. Bailey,[4] Anja Haase-Fielitz,[5] Michael Haase.[5] *[1]ICU, Auckland City Hospital, New Zealand; [2]Intensive Care, Austin Health, Melbourne, Australia; [3]ICU, Canberra Hospital, Australia; [4]ANZIC Research Center, Monash University, Melbourne, Australia; [5]Nephrology, Otto-von-Guericke University, Magdeburg, Germany.*

Background: Previously, it was shown that alpha-GST (Glutathione S-transferase) - a proximal tubular damage marker - and pi-GST, a potential marker of distal tubule damage, may be useful in the prediction of acute kidney injury (AKI) progression. However, best predictive values were reported at the time of AKI diagnosis.

Methods: Here, we assessed the predictive value of early postoperatively measured alpha- and pi-GST concentrations in urine for the prediction of RIFLE-AKI, acute dialysis and in-hospital mortality in 474 adult patients undergoing cardiac surgery (NCT 00878956). Urine and plasma samples were collected at the time of ICU admission at three Australian and New Zealand hospitals between July 2009 and June 2011. Urinary alpha-GST and pi-GST levels were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Results: 161 (34%) subjects developed AKI. Patients developing AKI required more dialysis (10% vs 1%, P<0.001) and had a higher in-hospital mortality rate (6% vs 2%, P=0.031) compared to patients without AKI. At ICU arrival, urinary alpha-GST and pi-GST were not able to predict subsequent development of AKI, the need for acute dialysis or mortality (Table 1). Also, serum creatinine measured at the same time had no value in the prediction of these clinical outcomes.

At 24 hours postoperatively, findings remained essentially unchanged for all assessed endpoints except for creatinine (AUC for dialysis: 0.78).

Conclusions: Urinary GST seems not to be useful for prediction of renal outcomes or mortality after cardiac surgery in adults.

	At ICU admission	24 hrs postoperatively
	AUC-ROC (95% CI)	AUC-ROC (95% CI)
RIFLE-AKI		
Alpha-GST	0.56 (0.51-0.62)	0.44 (0.38-0.49)
Pi-GST	0.57 (0.52-0.63)	0.54 (0.48-0.60)
Serum creatinine	0.57 (0.51-0.63)	0.68 (0.62-0.73)
Acute Dialysis		
Alpha-GST	0.62 (0.48-0.77)	0.45 (0.34-0.57)
Pi-GST	0.69 (0.54-0.84)	0.66 (0.53-0.79)
Serum creatinine	0.65 (0.52-0.78)	0.78 (0.64-0.91)
In-hospital mortality		
Alpha-GST	0.58 (0.45-0.72)	0.55 (0.40-0.71)
Pi-GST	0.55 (0.39-0.72)	0.64 (0.48-0.79)
Serum creatinine	0.49 (0.34-0.64)	0.60 (0.43-0.78)

Funding: Clinical Revenue Support

SA-PO024

Maximum Dose of N-Acetylcysteine and Prevention of Renal Dysfunction in Patients with Chronic Kidney Disease Undergone Surgical Coronary Intervention: A Prospective, Randomized, Double-Blind, Controlled Trial Eduesley Santana Santos,[1] Luiz Antonio Machado Cesar,[1] Ludhmila Abrahão Hajjar,[1] Jose Jayme Galvão De Lima,[1] Valéria A. Costa-hong,[1] Maria Heloisa M. Shimizu.[2] *[1]Hypertension Unit/Intensive Unit Care Cardiovascular Surgery, Heart Institute - School of Medicine University of Sao Paulo, Sao Paulo, Brazil; [2]LIM-12, School of Medicine University of Sao Paulo, Sao Paulo, Brazil.*

Background: The role of N-acetylcysteine (NAC) for the prevention of renal damage in experimental models as well as in diverse clinical settings is controversial. Disparate results may be caused by differences in dosing and route administration.

Methods: We test the safety and efficacy of maximum IV dose of NAC, as recommended for the management of acetaminophen overdose, on the incidence of renal dysfunction, in 50 patients with GFR <60/≥15 ml/min, undergone elective surgical coronary intervention (CI). Patients were randomized to receive either IV saline (n=26) or NAC (n= 24) in equal volume of saline (150 mg/Kg followed by 50 mg/kg) during surgery. End-points: renal dysfunction (AKIN criteria) during the first 72-h post-operation; serum NGAL and TBARS, need of dialysis and death (30-days post-operation).

Results: NAC was not associated with significant side-effects. There was one death in the NAC group (cardiac arrest, 48-h after operation) and none among controls and no patient needed dialysis. Baseline age, gender, race, diabetes, dyslipidemia, smoking, comorbidities, GFR, Euroscore, and number of grafts, on-pump surgery, transfusions, vasoactive drugs and diuresis (during and after operation) did not differ between groups. Renal dysfunction was observed in 64% (controls) and 27% (NAC) patients (p= 0.02) and no use of NAC was the only independent predictor of renal dysfunction (OR= 4.86, p=0.02). NGAL and TBARS were higher in patients not receiving NAC (=< 0.05).

Conclusions: Maximum IV dosing of NAC was well tolerated and was associated with reduction on the incidence of renal dysfunction in patients with CKD undergone surgical coronary intervention. More work is needed to determine the optimal dosing of NAC in this setting.

Funding: Government Support - Non-U.S.

SA-PO025

Factors Predicting Acute Renal Failure after Heart Surgery Bernhard M.W. Schmidt,[1] Katharina A. Foerster,[1] Jan T. Kielstein,[1] Tobias Schilling,[2] Axel Haverich,[2] Hermann G. Haller,[1] Tim Kaufeld.[2] *[1]Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Heart, Thoracic, Transplant and Vascular Surgery, Hannover Medical School, Hannover, Germany.*

Background: Acute kidney injury (AKI) following cardiac surgery is a frequent complication and several risk factors increasing its incidence have already been characterized. The aim of this study was to compare the relative importance of patient factors and surgery associated factors to predict AKI after cardiac surgery.

Methods: 247 consecutive patients who underwent elective coronary artery bypass grafting, valve replacement/ repair or combined bypass and valve surgery were prospectively analyzed. Primary endpoint was the incidence of AKI as defined by the AKIN criteria.

Results: 30 (12.1%) of the 247 patients developed postoperative AKI. In univariate analysis BMI >30 kg/m² (OR 3.220, p=0.004) and CABG (OR 0.418, p=0.049), preoperative serum creatinine (OR 4.509, p<0.001), preoperative uric acid (OR 4.680, p=0.001), chronic kidney disease (OR 3.938, p=0.001) and diabetes (OR 2.609, p=0.017) were significantly associated with the incidence of AKI. Intraoperative parameters as operation time >300 minutes (OR 4.466, p=0.002), cardiopulmonary bypass (CPB) time >90 minutes (OR 4.360, p=0.001), aortic cross clamp (ACC) time above the median (OR 2.502, p=0.036) and the number of intraoperative transfused fresh frozen plasma (FFP) (OR 2.776, p=0.012) go along with a higher risk for postoperative AKI.

In the multivariate analysis uric acid levels above the median (OR 5.497, CI 95% 1.772; 17.054, p=0.003), cardiopulmonary bypass (CPB) time >90 minutes (OR 4.595, CI 95% 1.587; 13.305, p=0.005), body mass index (BMI) >30 kg/m² (OR 3.208, CI 95% 1.202; 8.562; p=0.02), and preoperative elevated serum-creatinine levels (OR 1.015, CI 95% 1.001; 1.029, p=0.04) were independently associated with postoperative AKI.

Conclusions: Serum uric acid is the most important risk marker for AKI after cardiac surgery. Notably, elevated uric acid levels are associated with a higher odds ratio than creatinine levels.

SA-PO026

Acute Kidney Injury Following Cardiac Surgery: A Prospective Observational Study to Assess Incidence, Severity, Risk Factors, and Mortality at a Single Center in India

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Background: Acute kidney injury (AKI) occurs in up to 30% of patients undergoing cardiac surgeries and is associated with increased morbidity and mortality. There is paucity of such data in our country. Hence this study was performed to assess the incidence of AKI following cardiac surgery and its associated risk factors, in the Indian context.

Methods: All patients age>18yrs undergoing cardiac surgeries were enrolled prospectively from July to October 2011. Patients on dialysis, renal transplants or with pre-existing AKI were excluded. Incidence and severity of AKI was defined by AKIN criteria. NKF/KDOQI definition was used to define CKD. Prevalence of risk factors such as age, sex, BMI, DM, HTN, dyslipidemia, CKD, past CVA, PVD, prior MI, periop AMI, use of cardiopulmonary bypass (CPB) and prolonged ventilation were assessed.

Results: 1,040 patients were enrolled with these baseline characteristics: 83.5% were males, mean age 58.2±10. 4yrs, mean BMI 25.6±4.3, DM 48.2%, HTN 57.5%, dyslipidemia 70%, CVA 36%, CKD 6.3%, PVD 0.9%, peri-op AMI 0.6% and use of CPB 24%. Overall incidence of AKI was 23.3% and 71.2% in patients with CKD. AKI was more severe in patients with higher CKD stages. Stage 3 AKI occured only in those with CKD3 or 4. Overall need for dialysis was 1.1% and 4.9% in AKI group. 8.5% of patients with CKD needed dialysis as compared to 4.1% without CKD. In multivariate risk factor analysis HTN (p=0.01), peri-op AMI (p=0.03), prolonged ventilation (p=0.01), CVA (p=0.01) and CKD (p<0.001) were found to be significantly associated with AKI whereas DM (p =0.12), PVD (p=0.13), use of CPB (p=0.4), prior AMI (p=0.07) were not significant. Overall mortality was 0.67% and 2.9% in AKI group. All mortalities were associated with AKI.

Conclusions: Incidence of AKI (23.3%) and mortality (0.67%) in cardiac surgery patients at our center in India is similar to other reported studies.We found that underlying CKD is the most significant risk factor for AKI in these patients in addition to HTN, past CVA, prolonged ventilation and pre-op AMI. Use of CPB did not increase the incidence of AKI.

SA-PO027

Risk Models of Acute Kidney Injury (AKI) in Cardiac Surgery

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Background: AKI is a frequent complication of cardiac surgery. A large number of novel postoperative biomarkers have been proposed to assess the risk of AKI. However, there are neither preoperative biomarkers nor robust validated risk models that predict AKI. Endogenous Ouabain (EO) is an adrenal stress hormone with hemodynamic and renal effects. Our group have already reported that higher pre-operative EO levels are associated with a worse renal outcome after cardiac surgery. Our aim is to develop a new risk model of AKI using both clinical aspects and levels of EO as biomarker.

Methods: The primary outcome was AKI according to AKIN stage II. We built predictive risk model (CLIN-AKI) considering clinical variables (age, sex, preoperative ejection fraction, basal eGFR, surgery type, hypertension, diabetes, redo-intervention). A further risk score (CLIN-EO-AKI) was developed including preoperative EO values in CLIN-AKI score. These models were tested on 407 patients admitted for elective cardiac surgery and in a validation population of 219 patients.

Results: In both populations EO levels were confirmed strongly associated with incidence of AKI and clinical complication (total ICU stay and in-hospital mortality). AKI incidence according to EO tertiles were 2.8%, 8.3%, 20.3% and 0%, 5.9%, 8.2% in the two populations(p<0.001). To calculate CLIN-AKI risk score we considered β coefficients of logistic regression analysis for AKI. CLIN-AKI model has a good predicting power for AKI per se (AUC 0.81 – CI 95% 0.75-0.88). Adding the preoperative EO level to the clinical model AUC increased to 0.85 (CI 95% 0.79-0.91). We confirmed all these results in the validation cohort. Cumulative AUC were 0.79 (CLIN-AKI) and 0.85 (CLIN-EO-AKI). Inclusion of EO provided improved risk prediction over the clinical models alone (AUC difference 0,065, CI 95% -0.011 to 0.015, p <0.01).

Conclusions: Preoperative EO level is a powerful biomarker of AKI. Since elevated plasma EO levels are related to target organ damage (heart and kidney), incorporation of preoperative values in clinical model significantly improves predictive risk score.

SA-PO028

Mortality and Causes of Death in Patients with Acute Kidney Injury

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Background: High mortality rates are well recognised in acute kidney injury (AKI). The exact mode of death in AKI remains under-studied, particularly in general hospitalised populations who represent the majority of those affected. We aimed to report causes of death in a large group of prospectively identified patients with AKI that may guide future strategies to improve patient outcomes.

Methods: A hospital wide, real-time electronic reporting system for AKI has been in place at Royal Derby Hospital since 2010. This system has good diagnostic accuracy (false positive rate 1.7%, false negative rate 0.2%). It generated a prospective database of all cases of AKI collecting demographic, outcome and hospital coding data automatically. Between Sept 2010 - Oct 2011 we extracted causes of death as recorded on the death certificate for those who died during hospital admission.

Results: During the study period there were 4949 episodes of AKI in 3930 patients. 861 died during their index admission (21.9% mortality). Median age was 80 (IQR 16) and 51% were male. Median Charlson co-morbidity score was 2 (IQR 2). Distribution across the AKIN stages were: 62% stage 1 AKI, 20.8% stage 2, 17.4% stage 3. 65% of AKI was acquired in the community. 7.5% were elective admissions.

Cause of death could be identified in 802 cases (93.4%). Three causes of death accounted for three quarters of mortality; sepsis (41%), cardiovascular disease (19.2%) and malignancy (12.9%). AKI was the primary cause of death in only 3% of cases. The median creatinine value prior to death was 168mmol/l (IQR 116). 76.2% of patients had not recovered renal function by the time of death (defined as serum creatinine less than 26μmol/l above baseline value). 88.1% of death certificates did not mention AKI in any part.

Conclusions: Mortality associated with AKI remains high, although this occurs due to concurrent acute illness. By identifying the major causes of death, it may be possible to target specific interventions to improve outcomes aimed not just at treating AKI but also the co-existing conditions. Examples would include care bundles for pneumonia with AKI and focussing on cardiac patients with AKI.

SA-PO029

COMT Gene Variants in Patients with Severe Sepsis or Septic Shock: Results from the VISEP Trial#

Michael Haase,[1] Michael Oppert,[2] Anja Haase-Fielitz,[1] Melanie Naether,[3] Duska Dragun,[2] Wolf-hagen Schunck,[4] Michael Kiehntopf,[5] Frank Martin Brunkhorst.[6] *[1]Nephrology, OvG University, Magdeburg, Germany; [2]Nephrology and Intensive Care Medicine, Charite Campus Virchow, Berlin, Germany; [3]Knauer GbmH, Berlin, Germany; [4]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [5]Clinical Chemistry and Laboratory Medicine, Friedrich-Schiller-University Jena, Germany; [6]Anesthesiology and Intensive Care Medicine, Friedrich-Schiller University Jena, Germany.*

Background: Adrenergic gene variants contributing to catecholamine-refractory septic shock and acute kidney injury (AKI) have not been investigated, yet. Catecholamin-O-methyltransferase (COMT) is a ubiquitous enzyme with co-dominant inheritance which is involved in systemic and renal catecholamine degradation. Recently, COMT LL carriers, coding for thermolabile COMT variant Val158Met with low enzyme activity, were identified to be at increased risk for vasodilatory shock and AKI in two independent cardiac surgery cohorts.

Methods: We hypothesized that in septic shock patients, COMT LL genotype occurs more frequently and affects outcomes. Data from patients with septic shock enrolled in the VISEP trial [1] (n=430) were analyzed. COMT genotyping was performed using PCR-based RFLP-assay.

Results: Distribution of COMT genotypes in septic shock patients (LL 30.0%, HL 47.2%, HH 22.8%) was different from that expected basing on pooled data from two independent cardiac surgery cohorts (LL 23.9%, HL 51.2%, HH 24.9%), P<0.05. However, LL carriers were not different from HL or HH carriers with regard to demographics, cause/severity of septic shock, dose and type of vasopressors, renal outcomes and 90day mortality, all P>0.2.

Conclusions: LL genotype seems to predispose to septic shock, however not to affect outcomes within a septic cohort.

#for the German Study Group Competence Network SepNet

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E. Kuhnt (stat. analysis)

1. Brunkhorst FM et al. NEJM 2008;358:125-39.

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SA-PO030

NGAL Predicts Postoperative Fluid Overload after Cardiac Surgery

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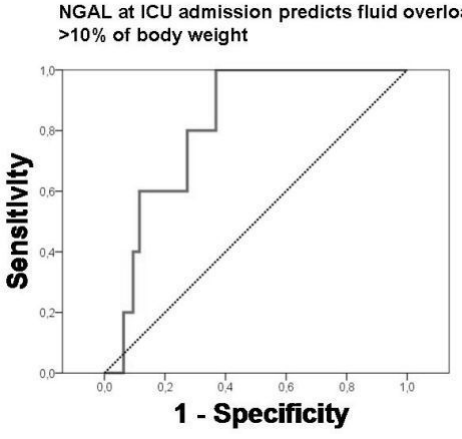
Background: Neutrophil gelatinase-associated lipocalin (NGAL), early measured after cardiac surgery, has been demonstrated to predict postoperative acute kidney injury (AKI). Fluid overload is a typical postoperative complication associated with increased mortality. Fluid overload potentially masks a subsequent acute renal function loss through dilution of creatinine and maintenance of urine output just above AKI-defining criteria.

Methods: We investigated the early postoperative value of NGAL - a marker of tubular damage - versus that of simultaneously measured serum creatinine to predict subsequent fluid overload. We studied 100 adult cardiac surgery patients in the control arm of a RCT. Severe postoperative fluid overload was defined as positive fluid balance>10% of preoperative body weight within 48hrs after surgery. Urine NGAL (ARCHITECT) and creatinine were sampled immediately after ICU admission.

Results: Severe postoperative fluid overload was present in 5% of patients with a mean positive fluid balance of 15.8±9.5L. Body weight-adjusted fluid balance predicted mortality (N=3) with AUC 0.91 (95%CI 0.76-0.99). Fluid overload correlated with length of stay in ICU (corr. coeff. 0.43; P<0.001). At ICU admission, urine NGAL predicted severe fluid overload (AUC-ROC 0.82 [95%CI 0.70-0.94]) and mortality (AUC-ROC 0.88 [95%CI 0.78-0.97]). Serum creatinine measured at the same time did not predict severe fluid overload (AUC-ROC 0.52 [95%CI 0.26-0.79] or mortality (AUC-ROC 0.61 [95%CI 0.16-0.99]).



Conclusions: Early NGAL-guided adjustments to fluid management may reduce organ edema after cardiac surgery. Our findings should be validated in larger patient cohorts.

Funding: Private Foundation Support

SA-PO031

Cystatin C at Admission in the ICU Predict Mortality among Elderly Patients

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Background: Cystatin C has been used in the critical care setting in the evaluation of renal function. It has also been found to correlate with mortality. It is not clear whether this association is due to the occurrence of Acute Kidney Injury (AKI) or to some other mechanism. To evaluate whether serum cystatin C at intensive care unit (ICU) entry predicts AKI occurrence and mortality in critically ill elderly patients.

Methods: This was a prospective cohort study of critically illelderly patients (> 60 years old) without AKI at admission in the ICU (initially normalserum creatinine levels)**.** We evaluated 400 consecutive ICU patients, of whom 234 (58%) were selected, 45 (19%) developed AKI during the ICU stay according to AKIN criteria. We also evaluated the demographic and clinical characteristics of patients according to normality for serum cystatin C at ICU admission (≤ 0.96 and > 0.96 mg/dL).

Results: We observed that those patients on mechanical ventilation at admission were more prone to develop AKI (35.5%). Higher serum levels of cystatin C were more prevalent in patients longer ICU stay (6±16 vs. 4±6; p = 0.04). Interestingly, higher levels of cystatin C did not discriminate AKI occurrence (1.05±0.48 vs 0.94±0.36; p = 0.1). However, in logistic regression analysis, we observed that a higher cystatin C level was an independent predictor of mortality H.R. = 6.16; (95% CI 1.46 – 26.00; p = 0.01). In contrast, AKI was not associated with death. Additionally, in the ROC curves, cystatin C also provided a moderate and significant area (0.67; p = 0.03) compared to AKI (0.47; p = 0.6) to detect death.

Conclusions: We demonstrated that a higher cystatin C level is an independent predictor of mortality in critically ill elderly patients in the ICU, indicating that it could be used as a marker of poor prognosis in this population.

Funding: Government Support - Non-U.S.

SA-PO032

TNF-α Plus IL-10 Low Producer Gene Predicted AKI and Death in Acute Kidney Injury (AKI)

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Background: Patients in ICU with SIRS have a high risk to development acute renal failure (AKI) and death.

Aim: To investigate whether genetic polymorphism of –308 TNF-α, -174 G>C IL-6 and 1082 G>A IL-10 gene may to predispose ICU patients with SIRS to development AKI and death.

Methods: In a prospective nested case-control study, we enrolled 672 ICU patients. Of them, 220 (33%) developed AKI (n = 220/ 33%) and 452 (67%) had not AKI, according to AKIN criteria. We measured serum creatinine, CRP, albumin and accessed genetic polymorphism to TNF-α, IL-6 e IL-10 in leukocytes.

Results: Patients who developed AKI had a higher APACHE score (p=0.0001), CRP levels (p=0.02) lower albumin (p=0.001) and were on mechanical ventilation (MV) (p=0.001). In the regression analysis, only APACHE (O.R. 1.07 C.I. 1.04 – 1.10; p=0.0001) and MV (O.R. 0.53 C.I. 0.31 – 0.90; p=0.02) remained as markers of risk for AKI. Patients who had higher APACHE and lower albumin had significantly death rates. We did no observe significant differences in the frequency of genotypes of TNF-α, IL-6 and IL-10 in respect to AKI or no AKI or death. To phenotypes, we observed 54% to low of TNF-α, 59% to intermediate of IL-10 and 75% to high producer of IL-6 gene in both groups. When we stratified by genotype combinations to the TNF-α and IL-10, we observed that TNF-α plus IL-10 low producer gene was more prevalent in patients whose occurred AKI and/ or death (54.3% vs. 33.3%; p=0.02). In regression analysis, adjusted by overall variables, TNF-α plus IL-10 low producer gene remain as risk factor for these outcome (O.R. 3.03, C.I. 1.34 – 6.86; p=0.008).

Conclusions: Although the phenotype of low of TNF-α and high producer of IL-6 in overall patients may lead to poor outcome and a pro-inflammatory state, these isolated polymorphisms did not predict AKI or death in our population. However, the combination of TNF-α plus IL-10 low producer gene predicted AKI and/or death in our population.

Funding: Government Support - Non-U.S.

SA-PO033

Hypoxia-Inducible Factor-1 alpha (HIF-1α) Haplotypes and Acute Kidney Injury (AKI) Following Cardiopulmonary Bypass (CPB)

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Background: HIF-1α is a transcription factor regulating several genes in response to ischemia-reperfusion injury, a common cause of AKI. We examined whether 3 polymorphisms in the HIF-1α gene and their respective haplotypes predict development of severe AKI (>2-fold ↑ sCr or dialysis) or in-hospital death in patients undergoing CPB.

Methods: We tested this hypothesis in a prospective cohort study of 283 adults undergoing CPB at 4 acute care facilities. Genomic DNA was extracted from leukocytes. Using the HapMap genome browser for European populations, 3 SNPs (rs12435848, rs4899056 and rs2057482) were selected and genotyped with the TaqMan system. Haplotypes were imputed using the PLINK software.

Results: Tests for Hardy-Weinberg equilibrium showed no deviation from expected frequencies. Baseline characteristics did not significantly differ across genotypes, with a few exceptions. Of the 5 haplotypes identified, A-C-T, the second most frequent (12%) haplotype, was associated with development of severe AKI (>2-fold ↑ sCr or dialysis).

	>2-fold ↑ sCr or dialysis		>2-fold ↑ sCr, dialysis, or in-hospital death	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
HIF-1α A-C-T haplotype (per 1-copy increase)				
- Unadjusted	2.23 (0.87, 5.72)	0.095	1.75 (0.78, 3.93)	0.173
- Adjusted for age, sex and race	3.74 (1.39, 10.01)	0.009	2.47 (1.09, 5.63)	0.031
- Adjusted for age, sex, race, heart failure, surgery status, and CPB time	3.30 (1.23, 8.81)	0.017	2.29 (0.97, 5.39)	0.058

Conclusions: This study identifies a common HIF-1α A-C-T haplotype derived from 3 SNPs as a risk marker for post-CPB severe AKI. Additional studies are needed to confirm these findings.

Funding: NIDDK Support

SA-PO034

Perioperative Risk Factors and Clinical Predictive Score for Acute Kidney Injury Associated with Cardiac Surgery Nan Ye, Yan Zhang, Hong Cheng, Yi-Pu Chen. *Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China.*

Background: To develop a clinical score to predict the acute kidney injury (AKI) after cardiac surgery by incorporating the effects of all of its major risk factors.

Methods: a total of 3500 patients receiving cardiac surgery were divided into two groups, AKI and non-AKI group, based on AKI due to AKIN definition. The univariable analysis and logistic regression model were used to establish the predictive score.

Results: The derivation cohort consisted of 2385 patients and the validation consisted of 1115 patients. In derivation cohort, the rate of AKI was 40.5%, while rate of ARF requiring dialysis was 2.5%. In the validation cohort, the rate of AKI was 39.6%, while rate of ARF requiring dialysis was 1.4%. In the derivation cohort, the mortality rate was 5.6% for AKI patients, while 45.8% for ARF requiring dialysis. In the validation cohort, the mortality rate was 4.3% for AKI patients, while 68.8% for ARF requiring dialysis. Variables selected for the logistic regression model and then predictive score were the following: male (2 points), older age (increased 1 point with every 5 years increment from 60), diabetes mellitus (2 points), preoperative use of ACEI/ARB (1 point), eGFR (increased 1 point with every 10 decrements from 90 ml/(min•1.73m^2)), NYHA class 4 (3 points), CPB time>120min (2 points), intraoperative hypotension time>60min (2 points), postoperative hypotension time>60min (3 points), postoperative use of loop diuretics 60-100mg per day (2 points), postoperative use of loop diuretics>100mg per day (3 points), postoperative mechanical ventilation time>24h (2 points). The stratification of risk factors is as follows:low-risk (within 0-5 point), intermediate-risk (6-11 point), and high-risk (≥12 point). The frequencies of AKI were 20.7%, 44.3%, and 83.6% respectively. The score showed adequate discrimination for the derivation dataset and the validation sample as well as adequate calibration (P=0.305).

Conclusions: we developed a clinical predictive score for AKI after cardiac surgery. This predictive score presented good discrimination and calibration. It would help the clinicians to make decision for preventive intervention.

Funding: Government Support - Non-U.S.

SA-PO035

Decreased Baseline GFR Is a Predictor of AKI after Off-Pump Cardiac Surgery Rachita Sethi Reddy, Michael F. Michelis, Nirav C. Patel, Maria V. DeVita. *Lenox Hill Hospital, New York, NY.*

Background: Acute kidney injury (AKI) is a serious complication of cardiothoracic surgery (CTS), with a 7-28% incidence increasing hospital length of stay, infections, dialysis and mortality. We previously reported on AKI in off-pump CTS patients, showing greater incidence in those with diabetes and postoperative complications. Since prior studies lacked a standard definition of AKI, we reanalyzed our data using the AKI Network definition. Our study is the first to specifically investigate risk factors for AKI in patients undergoing off-pump CTS, and the association between preoperative GFR and AKI as defined by established guidelines.

Methods: Using database entry points for The Society of Thoracic Surgeons Adult Cardiac Surgery Database, we performed a single-center, retrospective analysis on all off-pump CTS patients at Lenox Hill Hospital from June 2009 to January 2011. Extracted data included: age, sex, race, preoperative serum creatinine (SCr), postoperative peak SCr, comorbidities (diabetes, dyslipidemia, hypertension, peripheral arterial disease, cerebrovascular disease), urgent versus elective surgery, complications during postoperative period, use of ACEI/ARBs, mortality.

Results: Of the 441 patients in the database, 22% had AKI. Patients were grouped by baseline GFR ranges similar to CKD stages. Groups 1-4 had AKI rates of 21%, 15%, 31%, 53%, respectively. There were no CKD5 patients. Chi-squared analyses compared risk factors between patients with and without AKI. Patients with AKI were older ($p<0.01$), more likely to be diabetic ($p<0.01$), undergo urgent vs. elective surgery ($p<0.05$) and have postoperative complications ($p<0.02$), mainly atrial fibrillation and prolonged ventilation. The presence of multiple risk factors compounded a patient's risk of AKI ($p<0.01$). All other data points had no significant difference in predicting risk of kidney injury.

Conclusions: Incidence of AKI was linearly related to baseline GFR. Patients with AKI after off-pump CTS were older, had urgent surgery and other postoperative complications. Diabetics were twice as likely to have AKI. Finally, multiple risk factors compounded the risk of AKI.

SA-PO036

Risk Factors of Acute Kidney Injury after Coronary Artery Bypass Grafting and Mortality Nara Shin,[1] Sejoong Kim,[2] Ho Jun Chin,[2] Dong Wan Chae,[2] Ki Young Na.[2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Internal Medicine, Seoul National University Bundang Hospital, Republic of Korea.*

Background: Acute kidney injury (AKI) after coronary artery bypass grafting (CABG) has been proved as an important and independent predictor of morbidity and mortality. The purpose of our study was to investigate the risk factors of AKI and the mortality associated with AKI in the patients who received CABG.

Methods: We conducted the retrospective observational study with electronic medical records. Patients undergoing elective CABG from 2004 to 2010 in Seoul National University Bundang Hospital were studied. The outcomes were the risk factors of AKI after CABG, and mortality.

Results: AKI by the definition of AKIN classification occurred in 304 patients among a total of 819 patients. After multivariate regression, male sex (OR 1.866, 95% CI 1.188-2.933, p=0.007), use of IABP (OR 2.278, 95% CI 1.285-4.039, p=0.005), combined valve operation (OR 1.831, 95% CI 1.056-3.175, p=0.031) and on-pump operation (OR 3.281, 95% CI 2.210-4.870, p<0.001) were the risk factors. In-hospital mortality (non-AKI group vs. AKI group=1.4% vs. 12.5%, p<0.001) and long-term mortality (10.1% vs. 28.6%, p<0.001) were high in the AKI group. There were differences between the survival group and deceased group in valve operation, emergency, use of IABP, except on-pump operation. After multivariate Cox proportional hazard model analysis, older age (OR 1.056, 95% CI 1.029-1.084, p<0.001), male sex (OR 1.920, 95% CI 1.181-3.121, p=0.008), high CRP (OR 1.081, 95% CI 1.024-1.142, p=0.005), and AKI (OR 1.928, 95% CI 1.249-2.976, p=0.003) affected the long term mortality. There were no meaningful relationship between albuminuria, CKD and long-term mortality.

Conclusions: Our study showed the independent risk factors of AKI and high mortality were as above. In-hospital mortality and long-term mortality were high in AKI group. After multivariate regression analysis, older age, male, high CRP and postoperative AKI affected the long term mortality. AKI is the important risk factor in long-term mortality in patients undergoing CABG after adjustment with CKD, albuminuria and other variables.

SA-PO037

The Additive EuroSCORE Predicts the Risk of Acute Kidney Injury Post Cardiac Surgery David A. Ferenbach,[1] Sharleen Hill,[2] David C. Kluth,[1] Jeremy Hughes.[1] *[1]Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom; [2]Cardiothoracic Surgery, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.*

Background: The aetiology of Acute Kidney Injury (AKI) following surgery is complex. This study sought to identify the incidence and factors predicting post-operative AKI in cardiac surgery patients.

Methods: Demographic data, surgical procedure, medical co-morbidities and clinical course of 4651 cardiac surgery patients at the Royal Infirmary of Edinburgh between April 2006 and March 2011 was analysed. Pre-op and peak post-op creatinine levels were used to determine the presence and severity of AKI using the Acute Kidney Injury Network (AKIN) scoring system. The additive EuroSCORE was available for all patients as a validated scoring system for predicted operative mortality risk.

Results: Incidence & effect of AKI: 1862 of 4651 had a pre-op eGFR<60ml/min. The incidence of AKI stage I-III was 9.3% with dialysis required in 1.5% of cases. Any post-op AKI was associated with increased mortality (2%Þ18.9%, RR 9.4, p<0.0001) & hospital stay (9.6±0.3Þ18.6±1.3 days, p<0.0001)

Factors associated with AKI on univariate analysis were:Increasing Age, Diabetes, NYHA IV dyspnoea, Baseline eGFR<60ml/min, Hypertension, Female sex, Extra-cardiac atherosclerosis, LVEF<30%, Urgent surgery and procedures other than CABG alone. Of these Age (RR 1.022/additional year), Diabetes (RR 1.6), NYHA IV dyspnoea (RR 1.9) & Urgent surgery (RR 2.7) were all significant after multivariate analysis.

Additive EuroSCORE: Patients with AKI had higher pre-op EuroSCOREs (Additive EuroSCORE 4.9±3.2 vs 6.8±3.6, no AKI vs AKI, p<0.05). The additive EuroSCORE outperformed any single demographic factor in predicting AKI, with each additional point resulting in a 15% increase in the rate of AKI on multivariate analysis (p<0.0001).

Conclusions: Post-op AKI in cardiac surgery patients is associated with both increased mortality and use of health resources. The additive EuroSCORE may usefully highlight patients at high risk of AKI and thus assist in both clinical management (fluid, drugs etc) and future trial design aimed at improving outcomes.

Funding: Government Support - Non-U.S.

SA-PO038

Development of a Risk Score for Prediction of Contrast Induced Nephropathy after Coronary Angiography and Intervention in China Yu-mei Gao, Di Li, Hong Cheng, Yi-Pu Chen. *Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China.*

Background: This study is to develop and validate a score to contrast induced nephropathy (CIN) baseed on cumulative effect of multi-risk factors.

Methods: A total of 3,957 patients undergoing coronary angiography and percutaneous coronary intervention were randomly assigned to a development and a validation dataset. Several baseline clinical and procedural characteristics of 2,773 patients in the development dataset were considered as candidate univariate predictors of CIN (increase ≥ 0.5 mg/dL in serum creatinine levelwithin 72 hours following the procedure vs. baseline). Multivariate logistic regression was then used to identify independent predictors of contrast induced nephropathy. Based on the odds ratio and clinical characteristic, the independent predictors were assigned a weighted integer and a risk score system was derived. The risk score was validated in a second cohort of 1,184 patients.

Results: CIN occurred in 4.4% of patients (176/3957). In the development dataset, the following factors were independent predictors of CIN: age ≥65years(OR=1.98), acute myocardial infarction(OR=2.30), 103μmol/L < baseline serum creatinine≤177μmol/L(OR=1.52), baseline serum creatinine >177μmol/L(OR=3.39), Periprocedural heart failure(OR=2.11), Periprocedural intra-aortic balloon pump(OR=3.86), pre-procedural ACEI or ARB therapy(OR=1.69), 200mL<contrast media≤300mL(OR=1.49), contrast media >300mL(OR=3.02). The risk score system based on these variables, the patients in development cohort were further categorized into three groups: low risk (0~3 points), the incidence of CIN was 2.3%; moderate risk (4~6 points), 8.0%; high risk (>6 points), 21.6% *P<0.001).* The incidence of CIN in the validation cohort was close to those in the development cohort inside each of the three risk groups. In the validation dataset, the risk score system demonstrated good discriminative power (ROC 0.73, 95% CI 0.66-0.8).

Conclusions: We developed and validated a clinical prediction tool based on to determine which patients are at high risk for CIN. Use of this risk score system may help physicians perform targeted intervention to reduce this risk.

Funding: Government Support - Non-U.S.

SA-PO039

An Audit on the Preventative Measures against Contrast-Induced Nephropathy in Patients Undergoing Contrast-Enhancing Imaging Studies

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Background: Contrast induced nephropathy (CIN) is a common form of hospital acquired acute kidney injury with a mortality rate of up to 64% in high-risk individuals. It is an easily preventable condition. Royal College of Radiologists guidelines recommend that if IV contrast studies are unavoidable, sufficient hydration needs to be given with stoppage of any nephrotoxic drugs. The aim of this audit is to benchmark CIN preventative measures at Broomfield Hospital, Chelmsford, England.

Methods: It was a retrospective review of patients who had IV contrast-enhanced imaging over a 2-week period (10/10/11 – 24/10/11). We determined how many of these patients had assessment of their renal function pre- and post-contrast study. Using an eGFR of <60 ml/min, we reviewed the case notes of patients who fell into this category and audited the CIN preventative measures that were instituted.

Results: 243 patients were eligible for analysis; 64 inpatients, 141 outpatients, and 38 cardiac angiography patients. 19 inpatients (30%) did not have a post-contrast eGFR. 8 of 45 patients (18%) who had a pre- and post-contrast eGFR, showed a significant decrease in eGFR in keeping with the definition of CIN. Of the outpatient studies, 75/141(53%) did not have a pre- or post-contrast renal function test. Only 7 patients (5%) had a post-contrast renal function test. 32/38 (84%) angiography patients did not have a post-angio eGFR. 4/7 patients (57%) with a pre-contrast eGFR of <60 did not have a post-contrast eGFR. Of the 14 case notes reviewed, 6 (43%) did not have sufficient hydration and in 11 patients (79%) nephrotoxic drugs were not stopped prior to the study.

Conclusions: A significant number of patients undergoing contrast-enhanced studies did not have their renal function assessed peri-procedure and were not identified as at risk of developing CIN, and therefore not given the necessary preventative measures. Even amongst the group of patients who had reduced eGFR (<60ml/min), a large majority did not receive preventative measures as recommended by current guidelines.

SA-PO040

"Pseudo Contrast Induced Nephropathy": Creatinine Elevation after CT without Iodinated Contrast Use

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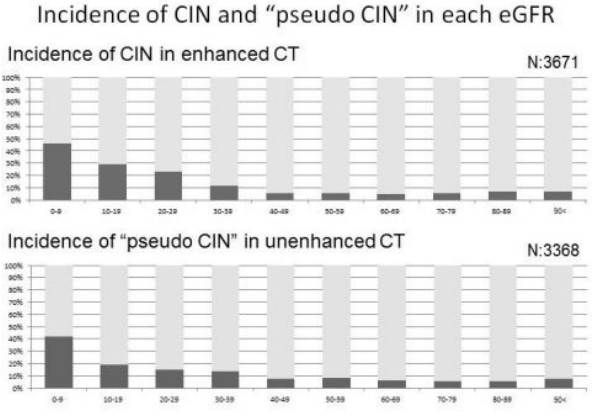
Background: Most studies in contrast induced nephropathy (CIN) lack controls to distinguish it from nephropathy from other causes. The purpose of this study was a retrospective comparison of the incidence of CIN in patients undergoing enhanced CT and the incidence of AKI in patients undergoing unenhanced CT (a condition we have termed "pseudo CIN").

Methods: Serum creatinine (sCr) was evaluated for 7039 patients who had had, either enhanced CT (Exposure, n=3671) or unenhanced CT (Comparison, n=3368), at St. Luke's international hospital in Tokyo between 2003 and 2010. Rates of CIN in exposure group and "pseudo CIN" in control group, defined as a 0.5 mg/dL increase in sCr or a 25% or greater decrease in eGFR within 7 days after CT, were compared among groups stratified according to baseline sCr and eGFR.

Results: 7.2% of patients in exposure group developed CIN and 9.5% of patients in comparison group developed "pseudo CIN". In both groups, the incidence of sCr elevation was increased with worsening baseline sCr.



The incidence of "pseudo CIN" is higher than that of CIN for all baseline sCr values and all stages of CKD (P<0.05 by Wilcoxon test).

Conclusions: We identified a high incidence of " pseudo CIN" among control group undergoing unenhanced CT. These findings suggest that the additional risk of AKI accompanying administration of contrast medium may be overstated and that much of the sCr elevation in patients developing CIN is attributable to underlying disease, condition or treatment. A more accurate assessment of the risk of CIN could lead to wider contrast use, more accurate diagnoses, and better clinical treatment.

SA-PO041

Development and Validation of a Prognostic Risk Score for Acute Renal Injury in Patients Hospitalized for Acute Decompensated Heart Failure

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Background: This study is to develop and validate a score of acute renal injury(AKI) in patients hospitalized for acute decompensated heart failure(AHDF).

Methods: A total of 1709 hospital medical records of AHDF cases were reviewed. AKI was defined as a increase of SCr(≥26.4 μmol/l or 50%) in 48 hours**.** A multivariate logistic regression analysis was undertaken to develop a prediction score. The area under the ROC curve and the H-L goodness-of-fit statistic were calculated to assess the discrimination and calibration of the score.

Results: A risk scoring system was developed based on the β coefficients of each identified risk factors retained in prediction model as follows:2 points were assigned to worsen heart functional class on admission, admission serum Na <130mmol/L and positive urinary protein on admission; 3 points were assigned to age≥70 years, history of heart failure more than 3 times and intravenous furosemide dose 80~159 mg/d; 4 points were assigned to admission systolic blood pressure <90 mmHg; 5 points were assigned to serum creatinine on admission 104~176umol/L; 7 points were assigned to serum creatinine on admission 177~264umol/L; 8 points were assigned to intravenous furosemide dose ≥160mg/d; and 10 points were assigned to serum creatinine on admission ≥265umol/L. The rate of AKI increased gradually with increasing risk score. A risk score of 8 points was identified as the optimal diagnostic cut-off point, with sensitivity of 70.0 %, specificity of 70.6 %, positive-predictive value of 55.1% and negative-predictive value of 82.0 %, respectively. Patients with a score of ≥8 would be considered at high risk for developing AKI(55.1% compared to 18% in those <8 patients, P<0.001). The score showed adequate discrimination for both the derivation dataset and the validation sample (ROC curve 0.760 and 0.761, respectively) as well as adequate calibration (H-L statistic 0.985 and *P*=0.133, respectively).

Conclusions: Newly developed and validated clinical prediction scores may allowsearly identification of patients at high risk of AKI, and may support decision making for early protective kidney treatment.

Funding: Government Support - Non-U.S.

SA-PO042

Transient Acute Kidney Injury and Acute Kidney Injury on Admission Are Associated with Increased Readmission and Mortality in Acute Decompensated Heart Failure

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Background: Acute Kidney Injury (AKI) influences therapy and outcomes in Acute Decompensated Heart Failure (ADHF). Little is known regarding the impact of timing [admission (AKI-A) vs hospital-acquired (AKI-HA)] or duration [transient AKI (TA) vs sustained AKI (SA)] on outcomes.

Methods: We studied 15,296 patients from a 70-hospital cohort with a primary discharge diagnosis of ADHF and use of a diuretic during an index hospitalization (2008-10). Exclusions were age < 18, ESRD, length of stay < 1 day or < 3 serum creatinines (Scr). AKI-HA, defined as a 0.3 mg/dl or 1.5 times increase in Scr from admission, was categorized as TA (Scr returned to < 10% of admission value within 72 hrs) or SA (>72 hours). AKI-A had a 0.3 mg/dl or 1.5 times decline in Scr from admission. Using regression models, AKI groups were compared to no AKI for hospital mortality (including hospice), length of stay (LOS) (log-transformed) and 30-day readmission rate (censored for death/hospice).

Results: The sample was 51% female, 75% Caucasian, with a mean age 73.8 years. 35% had diabetes mellitus, and 62% received an ACE inhibitor/Angiotensin receptor antagonist. Crude (not shown) and adjusted estimates predict poor outcomes across most AKI groups. Of patients with TA and AKI-A, 31% and 67% respectively, reached a nadir Scr <1.3 mg/dL.

Multivariable Adjusted Outcomes

Group	N (%)	Mortality %, RR (95% CI)	30-Day Readmission %, RR (95%CI)	LOS Mean Days
No AKI	6,630(46)	3.7%, referent	17.6%, referent	4.5
AKI-A	2,608(18)	5.5%, 1.48(1.21-1.81)	19.0%, 1.08(0.97-1.19)	6.8
TA	1,310(9)	6.7%, 1.80(1.43-2.27)	21.2%, 1.21(1.07-1.36)	7.5
SA	3,935(27)	8.2%, 2.21(1.87-2.61)	21.3%, 1.21(1.11-1.32)	6.1

Conclusions: AKI-A, TA and SA are associated with increased risk of hospital mortality in ADHF; TA and SA increase the risk of readmission. Regardless of improvement in Scr, AKI in ADHF predicts poor outcomes.

SA-PO043

Antihypertensive Medication Use before and after Acute Kidney Injury in General Medicare Patients Areef Ishani,[1,2] Ghaziuddin Qadri,[3] Craig Solid.[1] *[1]USRDS Coordinating Center, MMRF, Minneapolis, MN; [2]Minneapolis VAMC, Minneapolis, MN; [3]HealthPartners.*

Background: Acute kidney injury (AKI) is a common antecedent to ESRD. Many patients have their reno-protective medications stopped during the AKI episode. We aimed to determine if these medications, specifically antihypertensive medications, are then restarted within the next year.

Methods: We used the Medicare 5% Random Sample to identify patients aged 65+ who had an Acute Kidney Injury (AKI) during 2008. Medicare Part D enrollment and medication information was used to identify prescription medications that were filled before and after the AKI. Followup periods included the first 2 months immediately following the AKI event, as well as the period of time 10 to 12 months after the AKI.

Results: We identified a total of 10,294 patients with AKI in 2008. Of these, the percent with Part D claims for medications prior to the AKI were as follows: Beta Blocker: 44%; Calcium Channel Blocker: 21%; Loop diuretic: 38%; ACE Inhibitor: 36%; ARB 18%. During followup, of patients who received the medication prior to their AKI, the percent of patients who had another claim for a Beta Blocker were 78% (0-2 months after AKI) and 73% (10-12 months after), for another Calcium Channel Blocker were 69% and 59%, and for another ACE/ARB were 61% and 51%.

Conclusions: Many antihypertensive medications are stopped during an AKI episode and are not restarted up to one year after the AKI episode. It is unclear if medication discontinuation may account for some of the poor outcomes after an AKI episode.

Funding: NIDDK Support

SA-PO044

Recognition, Diagnosis and Management of Acute Kidney Injury Remains Poor Despie Recent Initiatives Christopher Lawrence, Peter Hill. *Department of Renal Medicine, The Hillingdon Hospital NHS Trust, Uxbridge, Middlesex, United Kingdom.*

Background: Acute Kidney Injury (AKI) is a common feature of patients presenting to hospital as medical emergencies. AKI is associated with increased morbidity and mortality (3-5 fold), longer length of stay and higher healthcare costs. The aim of this study was to assess our practice in light of the National Confidential Enquiry into Patient Outcomes and Deaths (NCEPOD) report 'Acute Kidney Injury: Adding Insult to Injury' (2009) and assess the knowledge of clinicans caring for acutely unwell patients with respect to these guidelines.

Methods: KDIGO AKI stage (delta creatinine) was assessed for all patients admitted to hospital during a one week period. Care was assesed against a standard taken from national guidelines. At the end of the week an electronic survey was sent to clinicians.

Results: 22/201 (11%) patients admitted as a medical emergency had AKI (Stage 1 n=16, Stage 2 n=5, Stage 3 n=1). All patients had electrolytes (UEs) checked on admission but only 12 (55%) had UEs rechecked within 24 hours. Although case review identified 7 patients who required imaging of the renal tract because of severity of AKI or failure of AKI to resolve, no patient had been imaged before 24hrs. 26 physicians (all grades) answered a web based survey. The majority of clinicians correctly identified, or over estimated, the incidence of AKI (21/26, 81%) and the associated mortality rates (22/26, 85%) but clinicians were either unaware of the KDIGO classification for AKI (19/26, 73%) or were not confident in its application (7/26, 27%). Only 11/26 (42%) clinicans correctly identified the target of obtaining renal tract imaging within 6 hours for patients suspected of having an obstructed and infected kidney. Clinicians felt that automated reporting of AKI stage with serum creatinine (19/26, 73%), and electronic ordering of diagnostic imaging (20/26, 76%) would improve management of patients with AKI.

Conclusions: AKI is a common feature of acutely unwell medical patients but depsite recent national initiatives (NCEPOD 2009) remains under recognised and poorly managed. Clinicans need further education and superior informatics to facilitate improved care for patients with AKI.

SA-PO045

Acute Kidney Injury in Reykjavik, Iceland 1993-2011 Þórir E. Long,[1] Martin I. Sigurdsson,[2] Gisli H. Sigurdsson,[1,2] Olafur S. Indridason.[3] *[1]Faculty of Medicine, University of Iceland; [2]Department of Anesthesia and Intensive Care; [3]Division of Nephrology, Landspitali - National University Hospital of Iceland, Reykjavik, Iceland.*

Background: The epidemilogy of acute kidney injury (AKI) has not been extensively studied. The aim of this study was to determine the incidence of AKI in Landspitali-The National University Hospital of Iceland (LUH), and examine renal recovery following AKI.

Methods: The laboratory at LUH serves the hospital and primary care facilities in the Reykjavik area. We searched its database for every serum creatinine (SCr) value measured from May 1993 to end of 2011. We identified the highest SCr value for each person, searched for a baseline value within the 6 preceding months and used it to categorize patients into risk (R), injury (I) and failure (F) groups using the RIFLE criteria. Subsequent SCr measurements for up to 12 months were used to examine renal recovery. The population of the Reykjavik area aged above 18 years was used for incidence calculations and time trends examined using regression analysis.

Results: A total of 1,642,295 SCr values were found for 215,000 subjects, of whom 54,814 had a baseline SCr available. AKI occurred in 12,561 (22.9%), with 12.5%, 5.4% and 5.1% in the R, I and F groups, respectively. AKI incidence increased with age and those in the older age-groups were more likely to have I or F than the younger ones (p<0.001). There were more females in the R and I groups, and more males in the F group (p< 0,001). The total AKI incidence increased steadily over time, from 414 to 662 per 100,000 per year (p<0.001) from the first to the last year. The rise in incidence was 55.3% in the R group (p<0.001), 55.1% in the I group (p=0.001) and 80.9% in the F group (p=0.01). Of those surviving AKI with creatinine available at 12 months, 8.9% of the R group, 26.1% of the I group and 26.2% of the F group had average SCr above 1.5x the baseline SCr.

Conclusions: The incidence of AKI seems to be rising in Reykjavik during the last two decades. The reasons are probably multifactorial, including higher age and more use of medications with effect on renal blood flow. A considerable proportion of survivors remain with impaired kidney function long term.

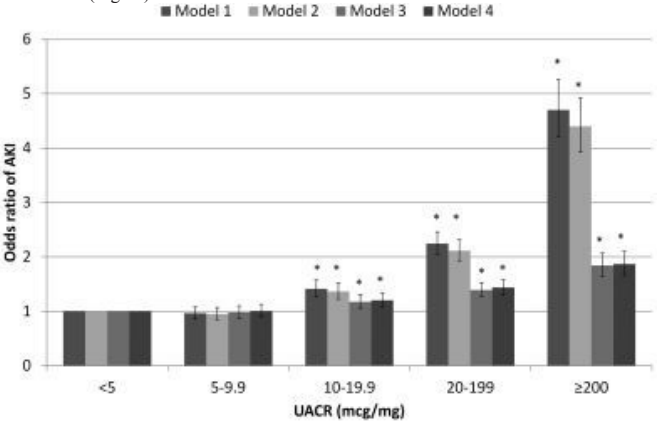
SA-PO046

Independent Association of Proteinuria with Acute Kidney Injury in a Large National Cohort of US Veterans Csaba P. Kovesdy,[1,2] Miklos Zsolt Molnar,[3] Jun Ling Lu,[4] Jennie Z. Ma,[5] Leigh Darryl Quarles,[2] Kamyar Kalantar-Zadeh.[3] *[1]Memphis VA Medical Center, Memphis, TN; [2]University of Tennessee Health Science Center, Memphis, TN; [3]Harold Simmons Center at Harbor-UCLA, Torrance, CA; [4]Salem Research Institute, Salem, VA; [5]University of Virginia, Charlottesville, VA.*

Background: Lower GFR predicts an increased risk of acute kidney injury (AKI), but it is unknown if the severity of proteinuria is also associated with AKI independent of kidney function.

Methods: We examined the association between the amount of urine microalbumin/creatinine ratio (UACR) and AKI (based on ICD-9 codes) during 2005-2006 in a national cohort of 299,180 US veterans, using crude (Model 1) and multivariable adjusted random effects logistic regression. Multivariable models were adjusted sequentially for age, gender, race (Model 2), plus comorbidities (Model 3), plus blood pressure, use of ACE-inhibitors, and estimated GFR (Model 4). UACR was examined both as a continuous variable and as a categorical variable with pre-defined cutoffs.

Results: 4,140 patients (1.4%) developed AKI, with 357 patients (0.1%) having experienced more than one episode during a median follow-up period of 1.0 year. Higher levels of UACR were associated with increased incidence of AKI, independent of confounders (Figure). A one-unit higher UACR in log-scale was associated with odds ratios (95% confidence interval [CI]) of AKI of 1.41 (1.38-1.43) in unadjusted and 1.15 (1.12-1.17) in fully adjusted models. Patients with UACR <10 mcg/mg were at the lowest risk for AKI (Figure).



Conclusions: Severity of UACR is associated with the incidence of AKI independent of the level of kidney function. Assessment of proteinuria should be considered along with measurement of kidney function when risk-stratifying patients for AKI.

Funding: NIDDK Support, Veterans Administration Support

SA-PO047

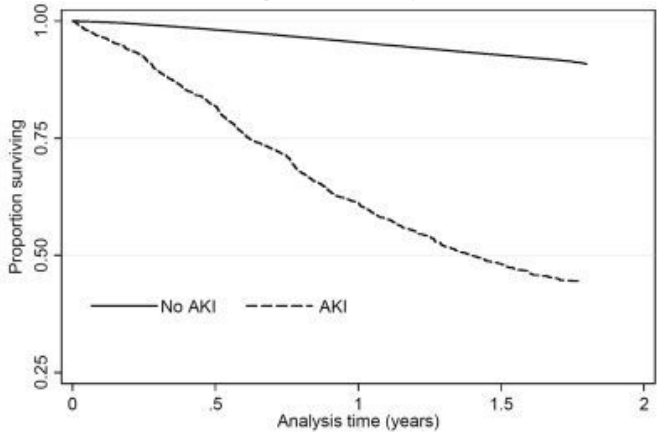
Predictors of Acute Kidney Injury and Its Association with Mortality in a Nationally Representative Cohort of US Veterans Csaba P. Kovesdy,[1,2] Miklos Zsolt Molnar,[3] Jun Ling Lu,[4] Jennie Z. Ma,[5] Leigh Darryl Quarles,[2] Kamyar Kalantar-Zadeh.[3] *[1]Memphis VA Medical Center, Memphis, TN; [2]University of Tennessee Health Science Center, Memphis, TN; [3]Harold Simmons Center at Harbor-UCLA, Torrance, CA; [4]Salem Research Institute, Salem, VA; [5]University of Virginia, Charlottesville, VA.*

Background: The incidence of acute kidney injury (AKI), its predictors, and its association with mortality in the wider population of CKD is unclear.

Methods: We examined the incidence of AKI, its predictors, and its association with all-cause mortality in a national cohort of 615,591 US veterans with CKD stages 1-5. Predictors of AKI were determined from logistic regression. The association of AKI with mortality was examined in time-dependent Cox models with adjustment for age, gender, race, hospitalizations, BP, ACE-inhibitor use, comorbidities, and laboratory variables.

Results: The incidence of AKI was 18.7/1000 patient-years (95%CI: 18.4-19.1), with a linear increase with more advanced stages of CKD. Older age, black race, higher comorbidity burden, lower SBP, eGFR, albumin, bicarbonate and hemoglobin, and higher

WBC were associated with increased risk of AKI. During a median follow-up of 1.0 year 30,572 patients died. AKI was associated with significantly higher crude mortality rate (HR, 95% CI: 9.8, 9.2-10.4, Figure), which remained significant but was attenuated substantially after multivariable adjustment (adjusted HR, 95% CI: 1.33, 1.24-1.43). The mortality risk associated with AKI was similar in patients with all stages of CKD.



Conclusions: AKI is common in CKD, especially in those with advanced stages. AKI is associated with a 10-fold higher risk of death, but most of this is due to characteristics that predispose to AKI. The risk of mortality associated with AKI is similar in all stages of CKD.

Funding: NIDDK Support, Veterans Administration Support

SA-PO048

Incidence of Major Acute Kidney Events (MAKE) and Major Acute Renal-Cardiovascular Events (MARCE) after an Episode of Acute Kidney Injury: A Study of US Veterans

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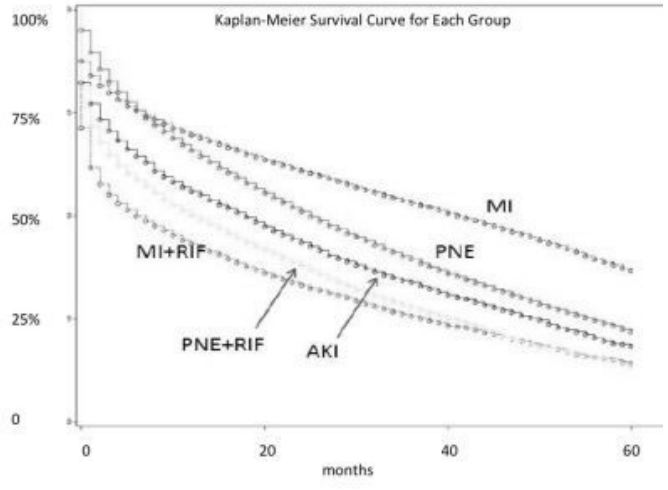
Background: NIH & AKI Consensus groups have proposed MAKE as a composite endpoint in clinical trials of AKI. The incidence of MAKE & MARCE are not well characterized.

Methods: We assessed patients admitted with a primary diagnosis indicating AKI (ARF ICD9- 584.xx), myocardial infarction(MI; codes 410.xx), and pneumonia(PNE; codes- 486. xx). PNE and MI patients who developed AKI during their hospitalization (defined by RIFLE) were designated PNE-RIF and MI-RIF, respectively. Patients with a pre-existing eGFR < 60 ml/min were excluded. From this dataset we generated 5 exclusive groups: AKI, MI, PNE, PNE-RIF, MI-RIF. Our endpoints were MAKE, defined as the composite of death, need for dialysis, or permanent loss of GFR (a persistent decrement of 25% from baseline eGFR). Our other endpoint was MARCE, defined as MAKE, plus a diagnosis of MI, CHF, or stroke after the index hospitalization.

Results: 67,878 subjects were assessed: AKI (5,352), MI (16,671), PNE (29,940), MI-AKI (6,362), and PNE-AKI (9,552). The likelihood of reaching the composite endpoint of MARCE was higher in patients with AKI (MI+RIF 75.9%, PNE+RIF 72.7%, AKI 62.6%), compared with patients admitted for PNE or MI without AKI (61.3% and 48.0%, respectively). Patients with MI and PNE were significantly less likely to reach the composite endpoint than all other groups, and the three groups with AKI were the most likely to reach this endpoint throughout the next 5 years (log-rank < 0.001 for all groups).



Conclusions: The occurrence of AKI magnifies adverse outcomes in hospitalized patients. AKI survivors are at risk for both renal and cardiovascular complications after their index hospitalization. Future clinical trials AKI should capture the composite outcomes of MAKE and MARCE; this will enhance power of AKI trials.

Funding: Other NIH Support - Paul Kimmel Works for NIDDK

SA-PO049

Long-Term Outcomes of Community-Acquired (CA-AKI) versus Hospital-Acquired Acute Kidney Injury (HA-AKI)

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Background: Medical literature on CA-AKI is limited compared to HA-AKI, especially in relation to clinically relevant outcomes. Our hypothesis: renal survival and mortality would be better for patients suffering CA-AKI vs HA-AKI.

Methods: Retrospective cohort of 718 cases of AKI and 2320 cases without AKI (NA) among hospitalized U.S. veterans from 2004-5. AKI defined by RIFLE creatinine criteria. Outcomes were assessed 3y after discharge from index hospitalization. Primary: doubling of the serum creatinine, end-stage renal disease (ESRD), death, and composite of the three. Secondary: de novo chronic kidney disease (CKD), recovery of renal function, and re-admission rate.

Results: CA-AKI (>50% rise from baseline creatinine on admission) was identified in 78% of patients with AKI. CA-AKI had better baseline renal function (mean estimated glomerular filtration rate 71.3 vs. 61.1 mL/min/1.73 m^2, P<0.05), and a lower percentage of chronic kidney disease (42.3 vs. 51.9%, P=0.03) than HA-AKI. No other significant differences in demographics and clinical characteristics. More patients with CA-AKI than HA-AKI met failure RIFLE criteria (43.8 vs. 29.1%, P<0.001). In contrast, patients with HA-AKI had an average length of stay twice as long (22.6 vs. 11.4 days, P<0.001), were more than twice as likely to require ICU stay or mechanical ventilation (45.6 vs 20.5%, P<0.001), and had a higher in-hospital mortality rate (26.6 vs. 19.6%, P=0.06). Need for inpatient dialysis was similar (19.6 vs. 26.6%, P>0.05). By 3y, the composite end point occurred in 74.3% patients with CA-AKI vs 74.1% patients with HA-AKI (P=0.66). Similarly, no differences were found for the other primary and secondary outcomes tested (all P>0.05).

Conclusions: CA-AKI was found to be considerably more common than HA-AKI. In contrast to our hypothesis, we found that long-term consequences in CA-AKI were similar to HA-AKI. Prospective analyses will be needed to identify measures to prevent AKI from developing in the outpatient setting.

SA-PO050

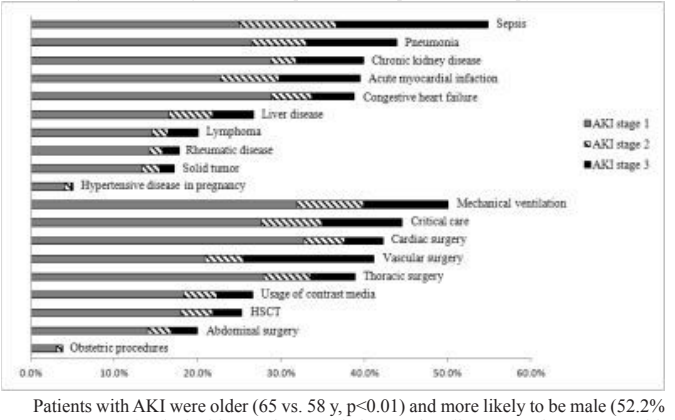
The Epidemiology of Acute Kidney Injury According to the KDIGO Criteria

Xiaoxi Zeng, Sushrut S. Waikar. *Renal Division, Brigham and Women's Hospital, Harvard Medical School.*

Background: Acute kidney injury (AKI) is a common complication in hospitalized individuals. We undertook this study to update the epidemiologic profile of AKI in hospitalized patients using the newly released KDIGO consensus definition and staging system.

Methods: Adult patients hospitalized at Brigham and Women's Hospital in 2010 with at least one inpatient serum creatinine (SCr) value were included. We excluded patients with end-stage renal disease and those who received kidney transplantation. We defined and staged AKI according to SCr-based KDIGO criteria (increase in SCr ≥ 0.3 mg/dl in 48 hours or to ≥ 1.5 times baseline in 7 days). Baseline was defined as mean outpatient SCr within 7 to 365 days prior to admission. We estimated the association between AKI and mortality, length of stay (LOS), and costs using logistic or linear regression analyses, adjusting for demographic and clinical confounders.

Results: Among 18,814 patients, the incidence of AKI was 16.9% (75.5% stage 1, 13.3% stage 2, 14.2% stage 3; 4.7% required renal replacement therapy).



Patients with AKI were older (65 vs. 58 y, p<0.01) and more likely to be male (52.2% vs. 40.3%, p<0.01). The incidence of AKI varied across baseline eGFR, from 15.3% with baseline eGFR ≥ 90 to 45.8% with baseline eGFR < 30. In-hospital mortality was higher in patients with AKI (11.3% vs. 0.9%, p<0.01) and increased with the severity of AKI (stage 1: 6.3%, stage 2: 13.4%, stage 3: 34.4%). Increasing severity of AKI was associated with adjusted increased odds of mortality: stage 1, 3.4 (95%CI 2.6 to 4.4); stage 2, 5.3 (95%CI

3.6 to 7.7); stage 3, 17.9 (95%CI 12.8 to 25.1). AKI was associated with 38.7% increase LOS (p<0.01) and 34.4% increase in cost (p<0.01).

Conclusions: Approximately 1 in 6 hospitalized patients developed AKI as defined by the KDIGO definition. AKI is strongly associated with in-hospital mortality, increased LOS and increased costs.

SA-PO051

Comparison of Creatinine-Based Definitions of Acute Kidney Injury
Xiaoxi Zeng, Joseph V. Bonventre, Sushrut S. Waikar. *Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: A number of definitions have been proposed to standardize the diagnosis and staging of acute kidney injury (AKI). We compared the incidence, mortality, and predictive values of AKI according to 4 distinct definitions: KDIGO, AKIN, RIFLE, and a definition we previously proposed which others have termed the Waikar-Bonventre (WB) definition.

Methods: Adult patients hospitalized at Brigham and Women's Hospital in 2010 with at least one inpatient serum creatinine (SCr) measurement were included. We excluded patients with end-stage renal disease and those who received kidney transplantation. We defined AKI using inpatient SCr values according to KDIGO (increase in SCr ≥0.3 mg/dl in 48 hours or to ≥ 1.5 times baseline in 7 days), AKIN (increase in SCr ≥0.3 mg/dl or ≥ 50% in 48 hours), RIFLE (increase in SCr to ≥ 1.5 times baseline in 7 days), and WB (increase in SCr ≥ 0.3 mg/dl in 24 hours or ≥ 0.5 mg/dl in 48 hours) definitions, and calculated incidence and in-hospital mortality. Using standard metrics of diagnostic accuracy, we then tested the ability of the 4 definitions to identify the eventual need for renal replacement therapy (RRT).

Results: Among 18,814 patients, AKI incidence was 15.0% as defined by KDIGO, 12.4% by AKIN, 10.8% by RIFLE and 8.8% by WB. Mortality was highest in patients with AKI defined by WB (19.0%), followed by RIFLE-AKI (16.0%), AKIN-AKI (15.0%) and KDIGO-AKI (13.2%). Prior to the initiation of RRT (n = 150), the diagnosis of AKI was made in 79.3% by RIFLE, and 92.0% by KDIGO, AKIN and WB. Among those meeting criteria for AKI, RRT was eventually required in 4.9% for KDIGO, 5.9% for AKIN and RIFLE, and 8.4% for WB.

Diagnostic performance characteristics of AKI definitions for the identification of RRT

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
KDIGO	92.0%	85.7%	4.9%	99.9%
AKIN	92.0%	88.3%	5.9%	99.9%
RIFLE	79.3%	89.8%	5.9%	99.8%
WB	92.0%	91.9%	8.4%	99.9%

Conclusions: The estimated incidence and mortality of AKI varies according to the definition used. The highest incidence was observed with the KDIGO definition. The Waikar-Bonventre definition had the highest positive predictive value for the eventual need for RRT.

SA-PO052

Prognostic Value of Kidney Biopsy in Myeloma Cast Nephropathy: A Retrospective Study of 69 Patients from a Single Institution
Laure Ecotiere,[1] Céline Debiais-delpech,[2] Sebastien Delbès,[1] Estelle Desport,[1] M. Guy Touchard,[1] Frank Bridoux.[1] *[1]Nephrology, Dialysis and Renal Transplantation, CHU Poitiers, Poitiers, France, Metropolitan; [2]Pathology, CHU Poitiers, Poitiers, France, Metropolitan.*

Background: Myeloma cast nephropathy (MCN) is the major cause of severe renal failure in multiple myeloma (MM), which significantly impacts survival.

Methods: The prognostic value of renal pathological findings on renal and vital outcomes was retrospectively evaluated in 69 patients with biopsy-proven MCN and MM. Patients were categorized in 2 groups according to the achievement (RR group) or not (NRR group) of renal response (RR), defined by estimated glomerular filtration rate (eGFR) value ≥ 30 ml/min/1.73m^2 and/or dialysis independence at 3 months.

Results: Compared with the NRR group, the RR group had lower baseline median serum creatinine (605 vs 328 µmol/l, P=0.024) and higher median eGFR value (7 vs 15 ml/min/1.73m^2, respectively, P=0.009). Hematological response (HR) rate (30% vs 91%, P<0.0001) and median percentage of light chain (LC) reduction at day 21 (13% vs 92%, P=0.025) were higher in the RR group. The extent of interstitial fibrosis (evaluated semi-quantitatively and by automated analysis), tubular atrophy and interstitial inflammation were not significantly associated with RR. By multivariate analysis, the median number of casts per 10 fields (13.5 vs 25, P=0.005) were significantly lower in the RR group among patients who achieved HR.

Conclusions: The achievement of RR is closely associated with the baseline eGFR and the HR. The presence of numerous casts (>20) is associated with poor renal and vital prognosis.

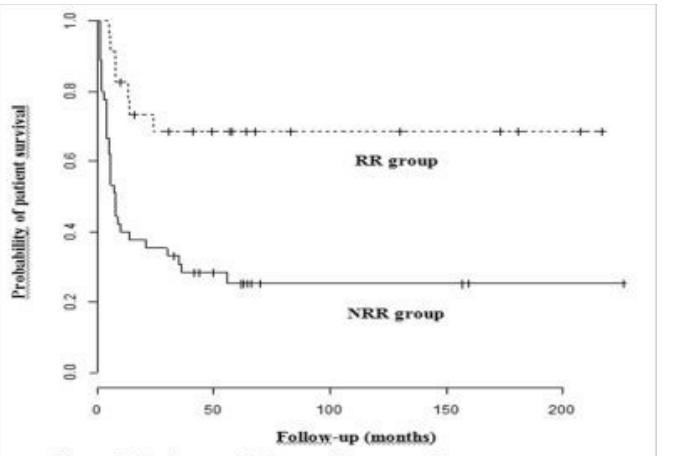


Figure 1: Patient survival according to renal response

Moreover, it is an independant predictor of RR in patients who achieved HR, in whom rapid removal of circulating LC through high cut-off dialysis or plasma exchanges should be considered in addition to efficient chemotherapy to improve outcomes.

SA-PO053

Acute Kidney Injury Is Associated with Increased Morbidity after the Arterial Switch Operation and Underestimated by Failure to Correct for Fluid Balance
Rajit K. Basu, Catherine D. Krawczeski, Derek Wheeler, Stuart Goldstein. *Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Acute kidney injury (AKI) is common and carries significant morbidity after cardiopulmonary bypass (CPB) in children. Few studies examine the impact of AKI in lesion specific – surgery specific populations.

Methods: We examined the impact of AKI in 92 consecutive patients (pts) after arterial switch operation (ASO).

Results: The AKI rate by KDIGO criteria (Kidney Disease - Improving Global Outcomes) was 18/92 (20%). Patient age, weight and CPB time did not correlate with AKI development. AKI was associated with: higher post-operative day 1 (POD_1) net fluid balance, higher inotrope scores (POD_1 and POD_2), and longer: post-operative intensive care unit (ICU) length of stay (LOS), overall ICU length of stay, and post-operative hospital LOS. Time to peak creatinine for AKI pts was between POD_1 and POD_2. Correction of creatinine for fluid balance increased the population defined as severe AKI (KDIGO Stage II-III) and strengthened the association of AKI with post-operative morbidity.

	UNCORRECTED			CORRECTED		
VARIABLE	No AKI or Stage I	Stage II-III	p value	No AKI or Stage I	Stage II-III	p value
Post-op ventilation (hours)	58 (36,114)	99 (44,129)	0.19	56 (34.5, 113.3)	99 (62, 161)	0.033
POD 1 Inotrope Score	13.5 (10,16)	13 (10,16)	0.87	13.5 (10,16.2)	15 (12, 18.6)	0.06
POD 2 Inotrope Score	10 (7.5,14)	10.6 (7.6,14)	0.58	10 (7.5, 13.9)	13 (8, 16.3)	0.05
Post-op ICU LOS (days)	5 (4,7.3)	7 (5,12.3)	0.056	5 (4,7)	8 (6, 14)	0.005
ICU LOS (days)	9 (8,12.3)	14 (7,19)	0.12	9 (8,12)	13 (10,16)	0.05
Post-op hospital LOS (days)	8 (7,13)	11 (8.5,16.8)	0.07	8 (7,12)	13 (9,20)	0.006

Impact of AKI after ASO and the effect of correction of creatinine for fluid overload.
AKI = acute kidney injury, POD = post operative day, LOS = length of stay

Conclusions: We conclude AKI following the ASO is associated with increased morbidity. In this single center, single population, and homogenous cohort of patients, the development of AKI was associated with prolonged duration of ventilation and hospitalization. Notably, failure to correct serum creatinine for fluid balance underestimates the prevalence and impact of AKI. Finally, even with corrected creatinine, the delayed time to diagnosis of AKI underscores the need to utilize more time-sensitive biomarkers of kidney injury.

SA-PO054

Acute Kidney Injury Diagnosis Delayed by Intravenous Fluids

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Background: A positive fluid balance produced by fluid resuscitation may dilute creatinine concentration and delay acute kidney injury (AKI) diagnosis or result in the underestimation of its severity. The extent of dilution will depend on fluid type and timing of sampling following infusion.

Methods: The study combined a two-compartment creatinine kinetic model with a two-compartment volume kinetic model to quantify dilution during AKI taking into account fluid type, and the rate of fluid input and urine output. Creatinine kinetics incorporated dynamic changes in the volume of distribution of each compartment and changes in glomerular filtration rate (GFR). Volume kinetics simulated the distribution and elimination of infused fluids. A simulated patient received 1000 ml/h for 6 hours of crystalloids or colloids under four GFR scenarios corresponding to RIFLE criteria AKI severity stages: no-change, 33.3%, 50%, and 66.7% loss. The model was applied to four critically ill patients to estimate GFR change.

Results: During fluid infusion, creatinine concentration decreased irrespective of fluid type or extent of GFR change. AKI diagnosis was delayed by about 8 hours. Colloids produced a greater reduction in creatinine concentration. The model provided a more accurate estimate of the loss of GFR in a patient with sustained AKI, and accounted well for the changes in creatinine in two of three others. In the fourth patient, the fall in creatinine concentration was greater than predicted; hyperfiltration could explain this outcome.

Conclusions: Combining creatinine and volume kinetics allowed quantitation of dynamic changes in GFR in the critically ill, facilitated earlier diagnosis, provided insight into progression and more accurate estimates of AKI severity.

Funding: Government Support - Non-U.S.

SA-PO055

Soluble CD25 Is Increased in Patients with Septic Acute Kidney Injury

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Background: Sepsis has been shown to induce the expansion of suppressive CD25+CD4+ regulatory T cells (Tregs) and this paradoxical immune suppression has also been suggested to be closely associated with the development of sepsis induced organ dysfunction. The purpose of this study was to investigate the possible link between immune suppression and the development of septic AKI.

Methods: We prospectively enrolled patients older than 18 years with the diagnosis of sepsis with or without AKI and also patients with non-septic AKI from Jan 2010 to Dec 2011. AKI was diagnosed according to RIFLE criteria, and serum and urine were collected to measure NGAL, cytokines, and soluble CD25 (sCD25).

Results: Of 82 patients enrolled, 44, 18 and 20 patients were classified into septic AKI, sepsis-no AKI and non-septic AKI. There was no difference in baseline characteristics including age, sex, and the prevalence of chronic kidney disease among these groups. The severity of AKI was also not different between septic and non-septic AKI. Patients with septic or non-septic AKI had significantly higher APACHE II score and FeNa compared to patients with sepsis-no AKI. Serum levels of proinflammatory cytokine IL-6, IL-1β, and serum and urine NGAL levels were significantly elevated in patients with septic AKI compared to those with sepsis-no AKI or non-septic AKI. Finally, the level of serum sCD25, the marker of suppressive Tregs, was significantly increased in patients with septic AKI (18.8±14.4 vs. 11.2±10.1 vs. 8.3±6.1 ng/ml, septic AKI vs. non-septic AKI vs. sepsis-no AKI, $p<0.05$), suggesting the possible association of paradoxical immune suppression and the development of septic AKI.

Conclusions: These results might suggest that immune suppression in sepsis is closely linked to the development of AKI and can also propose that sCD25 might be useful for a novel biomarker of septic AKI.

SA-PO056

Fluid Overload and Mortality: A Problem beyond Acute Kidney Injury

Catarina Teixeira,[1] Francesco Garzotto,[1] Nicola Brienza,[2] Claudio Ronco,[1] Pasquale Piccinni,[1] Dinna N. Cruz.[1] *[1]St Bortolo Hosp; [2]Univ of Bari.*

Background: Fluid overload (FO) has been associated with poor clinical outcomes in selected critically ill populations, particularly AKI and acute respiratory distress syndrome. Few studies have addressed the impact of FO in a general intensive care unit (ICU) population. Our aim was to evaluate the effect of mean fluid balance (MFB) on ICU mortality, and compare its effect in patients with and without AKI.

Methods: We performed a secondary analysis of data from a prospective multicenter ICU cohort (NEFROINT study). AKI was defined by renal SOFA score (sCr>3.5mg/dL or urine output (UO)<500mL/d). Oliguria was defined as a UO<500mL/d. MFB and mean urine output (MUO) were the mean of all daily values in ICU. Diuretic use was noted daily. Multivariable analysis was performed by Cox regression; variables included MFB, MUO, age, sex, co-morbidities, sepsis, SOFA and diuretic use.

Results: Of 573 patients (63 yrs; 60%M), 132 had AKI. In AKI vs non-AKI group, MFB was higher (0.75 vs 0.45 L/d; P=0.008) and MUO was lower (1.81 vs 2.26 L/d; P<0.001). Oliguric AKI patients had higher MFB (P<0.001) and had higher mortality (P<0.001). Overall, non-survivors had higher MFB; this was seen in both AKI and non-AKI groups (Fig 1). MUO was lower in non-survivors as a whole and in AKI group, but not in non-AKI. On multivariable analysis, MFB and MUO were independent predictors of mortality in all patients and in AKI (Tab 1). In non-AKI, MUO was no longer a predictor. Of interest, diuretic use was associated with lower mortality in all groups.

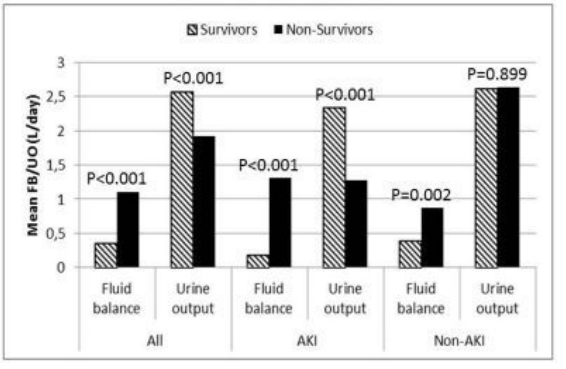
Conclusions: Fluid balance was an independent predictor of ICU mortality in AKI and non-AKI patients while UO was significant only in AKI. These results suggest that despite preserved UO in non-AKI patients, attention to fluid intake and prevention of FO are also fundamental in these patients.

Table 1: Independent predictors of mortality

	All	AKI	Non-AKI
	Adjusted Hazard Ratio		
Mean fluid balance	1.48 (1.29-1.70)**	1.67 (1.33-2.09)**	1.38 (1.10-1.72)*
Mean urine output	0.66 (0.52-0.86)*	0.47 (0.33-0.67)**	1.08 (0.72-1.61)
Diuretic use	0.31 (0.20-0.48)**	0.25 (0.12-0.52)**	0.25 (0.13-0.47)**
Non-renal SOFA	1.11 (1.04-1.18)*	1.04 (0.96-1.14)	1.15 (1.05-1.26)*

*P<0.01; **P<0.001

Figure 1: Mean fluid balance and mean urine output in survivors and non-survivors



SA-PO057

Adverse Mortality Effect of Fluid Overload Can Be Applicable to All Critically Ill Patients with Acute Kidney Injury Receiving Continuous Renal Replacement Therapy?

Il Young Kim, Soo Bong Lee, Dong Won Lee, Harin Rhee, Byeong Yun Yang, Eun Young Seong, Ihm Soo Kwak. *Pusan National University School of Medicine, Busan, Republic of Korea.*

Background: Fluid overload (FO) has known to be associated with increased mortality in critically ill patients with acute kidney injury (AKI). In this study, we investigated whether adverse mortality effect of FO could be applicable to all patients with AKI receiving continuous renal replacement therapy (CRRT).

Methods: We analyzed 171 AKI patients who received CRRT in intensive care units of our university hospital from 2007 to 2009. The presence of FO was defined as ≥10% increase in body weight over the baseline. Demographics, comorbid diseases, clinical data, severity of illness (the sequential organ failure assessment (SOFA) score, number of vasopressors, diagnosis of sepsis, use of ventilator) at ICU admission, FO status were reviewed from medical records.

Results: Of total subjects (n=171), patients with FO≥10% (n=59) from 3 days before CRRT initiation to 3 days after it showed significant higher mortality compared to patients with FO<10% (n=112) (30-day mortality: 71.2% vs. 43.7%, Log rank p<0.001). The adjusted hazard ratio for mortality associated with FO≥10% was 1.70 (95% CI: 1.09-2.66). We classified all patients into sepsis group (n=101) and non-sepsis group (n=70). The sepsis group showed the patients with FO≥10% had the higher mortality over 30 days compared to those with FO<10% (30-day mortality: 72.5% vs 40.9%, Log rank p<0.001). However, the non-sepsis group did not. Next, we divided all patients into high SOFA score group (≥13, n=87) and low SOFA score group (<13, n=84) according to severity of illness. In high SOFA score group, there was statistically significant difference of survival between patients with FO≥10% and FO<10% (30-day mortality: 86.5% vs 66.0%, Log rank p=0.002), but not in low SOFA score group.

Conclusions: The result of this study shows FO is independently associated with mortality in AKI patients receiving CRRT. However, our study demonstrates adverse mortality effect of FO could not be applicable to some subgroups of AKI patients receiving CRRT such as non-sepsis group or low SOFA score group.

SA-PO058

Bioelectrical Impedance Analysis: An Useful Tool as Prognosis in Acute Kidney Injury Francisco Javier Lavilla, Nuria Garcia-Fernandez. *Nephrology, Clinica Universidad de Navarra, Pamplona, Navarra, Spain.*

Background: Bioelectrical impedance analysis (BIA) is a simple, cheap and non-invasive tool for monitoring nutritional and hydration status in several diseases. The aim of this prospective study was to evaluate BIA in the prognosis of Acute Kidney Injury (AKI).

Methods: In a cohort of 96 patients (mean age: 64 years-old, SD: 1.57, 76 males) with a diagnosis of AKI, a bioimpedance analysis was made. Bioelectrical parameters: Phase angle (PA), Total Body Water, Extracellular/Intracellular water ratio (EC/IC), Na/K exchange rate (Na/K); acute clinical index: individual severity (ISI), multiorgan failure index (MOFI); chronic clinical index: Charlson and Karnofsky; biochemical parameters: C-reactive protein, prealbumin, albumin and peak creatinine; and mortality were evaluated. The mortality rate was 14%.

Results: In Table 1 correlation results are shown among different variables. According to mortality, the PA provided the highest prognostic information with an Area Under the curve (AUC) of 0.78 (95%CI: 0.65-0.91) and the cutoff was 3.2º (Sensitivity: 78%, Specificity: 67%). Respect to survival, the Na/K exchange showed an AUC of 0.81 (IC95%CI: 0.61-1.00), the cutoff was 1.39 (Sensitivity: 83%, Specificity:67%). Using a stepwise multivariate logistic regression analysis including all the bioelectrical parameters, an independent relationship was found between Na/K exchange and mortality (OR 15.38; 95% CI: 1.61-146.4).

Table 1. Correlations among different studied variables.

	Phase angle (º)	Phase angle (º)	EC/IC	EC/IC	Na/K exchange rate	Na/K exchange rate
	r	p	r	p	r	p
ISI	- 0.22	0.035	0.23	0.03	ns	ns
MOFI	- 0.25	0.035	- 0.32	0.002	0.34	0.014
C-reactive protein (mg/dL)	- 0.42	0.005	0.40	0.001	ns	ns
Prealbumin (mg/dL)	0.38	0.025	- 0.48	0.004	- 0.43	0.049
Albumin (mg/dL)	0.32	0.011	- 0.39	0.002	ns	ns
Karnosfky	0.44	0.005	- 0.54	0.005	- 0.32	0.025
Charlson	ns	ns	ns	ns	ns	ns

ISI: individual severity index, MOFI: multi organ failure index EC/IC: Extracellular/Intracellular water ratio

Conclusions: Bioelectrical impedance analysis can be used as a parameter in the prognosis of AKI. High Extracellular water volume, and moreover decreased phase angle and elevated Na/K are related to a worst prognosis in the patient with AKI.

SA-PO059

Biomarkers and Renal Ultrasound in the Diagnosis of Cirrhosis' Acute Kidney Injury (AKI) Belen Ponte, Laurent Spahr, Grégory Berra, Pierre-Yves F. Martin. *University Hospitals of Geneva.*

Background: AKI is frequent in patients with cirrhosis. Pre-renal, acute tubular necrosis (ATN) or hepatorenal syndrome (HRS) have different prognosis but are difficult to differentiate. As plasmatic creatinin is not helpful we aimed to study new biomarkers as well as renal artery resistive indexes (RI) to distinguish AKI etiologies.

Methods: We included in a prospective study adults presenting with cirrhosis and ascitis. Exclusions' criteria were: multifocal hepatocellular carcinoma, acute hemorrhage, severe chronic kidney disease (eGFR<15ml/min/1.73m2 or dialysis) and renal or hepatic transplant. AKI diagnosis was made according to AKIN criteria. Urine and blood were taken as soon as possible from the admission to analyze cystatin c, NGAL and KIM1. Renal doppler ecography was performed within the next days. The follow-up period was 1 month.

Results: In an intermediate analysis 77 patients were included, mostly men (66.2%) aged 58.3±10.2 years old. AKI occured in 50.6% cases: 76.9% pre-renal, 15.4% ATN and 7.7% HRS. Seven patients were admitted to intensive care unit (ICU) and 6 died. RI, age, sex, infection, ICU admission or death were similar in the 3 etiological groups. New biomarkers were significantly useful to differenciate ATN from pre-renal not from HRS.

Table 1: Comparison beween AKI causes

Variables	Pre-renal (n=30)	ATN (n=6)	HRS (n=3)	p
Chronic kidney disease (%)	2(5)	3(7.7)	0	0.012
Creatinin mimol/l	72 (64.8-141)	224.5 (128.3-300.3)	189 (121-200)	0.07
Plasmatic NGAL ng/ml	90.8 (67.8-229-2)	216.4(167.6-672.3)	261.3(207.5-371.3)	0.023
Urinary NGAL ng/ml	70.5(25.5-101)	134(114.8-171.7)	82.5(72.3-344.5)	0.002
Urinary/Plasmatic NGAL	6.2(3.9-9.1)	12.5(9.9-50.8)	7.5(7.1-15.9)	0.02
Urinary KIM-1 ng/ml	0.12 (0.05-0.23)	0.38(0.31-0.69)	0.55(0.53-0.73)	0.04
Serum Cystatin C	1.5(1.2-2.0)	2.4(2.0-2.7)	2.1(1.8-2.5)	0.028
Renal artery resistive index	0.78(0.63-0.9)	0.83(0.77-0.87)	0.79(0.75-0.87)	0.162

Variables are expressed as median and 25th-75th percentiles

In the ROC analysis, AUC for ATN was 0.93 (0.78-100) using urinary NGAL.

Conclusions: Novel biomarkers can help to differentiate ATN from pre-renal AKI but not from HRS. Renal artery RI are not useful to distinguish AKI etiology. Urinary NGAL seems to be the better predictor to diagnose ATN.

SA-PO060

Factors Impacting the Development of Acute Kidney Injury (AKI) after a Liver Transplantation (OLT) Hugues Bouchard, Veronique Lapointe, Louise Roy, Marie-Noelle Pepin. *Nephrology, Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada.*

Background: The aim of this retrospective study was to compare the characteristics of patients developing AKI (RIFLE stages 1, 2, 3) within the first week post liver transplantation and patients not developing AKI.

Methods: From 2004 to 2008, 125 patients with cirrhosis and receiving a first OLT were included in the study. Among them, 48 patients developed no AKI during the first week post transplant (controls (C)), 28 patients developed an AKI stage 1, 18 patients AKI stage 2 and 31 patients AKI stage 3. Factors studied were baseline demographics factors, complications of cirrhosis, baseline renal function and biochemical values; factors occurring during surgery and after transplantation.

Results: All four groups had similar baseline clinical and biochemical characteristics (including renal function) except for MELD score, hyponatremia and hospitalization awaiting OLT. Other factors were also significantly different between controls and AKI patients Results are Mean ± SEM; *p≤0.05 vs AKI; # p≤0.01 vs AKI (proportional odds ratio).

	Controls	AKI 1	AKI 2	AKI 3
MELD score	11.5±6.0 #	13.4±5.1	16.6±5.9	14.6±7.2
% pts with Na <130	10% #	7,7%	27,8%	35,5%
Hosp before OLT	35.4% *	46.4%	61.1%	56.7%
Blood loss during OLT (ml)	546±446 *	1352±972	1193±900	1095±752
BP drop during OLT	60.7%#	78.6%	88.9%	87.1%
INR post OLT	1.07±0.09 #	1.13±0.14	1.25±0.17	1.28±0.36
Bilirubin post OLT(mm/l)	52.8±50.2 #	53.3±50.9	89.9±115.6	129.7±126.0
Hb (g/l) 1 week post OLT	89±21 #	85±19	78±15	78±20
Total tacolimus dose during 1st week (mg)	57±19 *	62±27	53±39	38±26
Serum creatinine (umol/L) at 1 month	87.6 ± 26.2 #	95.8 ± 32.3	107.5 ± 31.8	136.3 ± 81.2

Results are Mean ± SEM; *p≤0.05 vs AKI; # p≤0.01 vs AKI

More patients were on dialysis at 1 month, although it did not reach statistical significance (C: no patient, AKI-1: no patient, AKI-2: 5.6%, AKI-3: 16.1%).

Conclusions: Other factors than baseline renal function affect the risk of developing AKI post-OLT such as liver function and hemoglobin. The outcome of AKI is not as good as shown by higher creatinine levels at one month. This study could be the basis for a randomized control trial evaluating new renal-sparing protocols.

Funding: Clinical Revenue Support

SA-PO061

Use of the Acute Kidney Injury Criteria Facilitates Earlier Detection of Renal Dysfunction in Hospitalized Cirrhotics David A. Foxwell,[1] Andrew D. Yeoman,[1] Gareth Roberts,[2] Marek A. Czajkowski.[1] *[1]Hepatology, Royal Gwent Hospital, Newport, United Kingdom; [2]Nephrology, University Hospital of Wales, Cardiff, United Kingdom.*

Background: Acute Kidney Injury (AKI) is common in hospitalized cirrhotics and portends a poor prognosis. Anecdotal data suggest that AKI is poorly recognised and under treated, and is frequently mislabelled as hepatorenal syndrome (HRS). The AKI network (AKIN) criteria is sensitive for the detection of early renal dysfunction. The aims of this study were to determine if use of AKIN criteria would be a more sensitive and rapid indicator of acute renal dysfunction in cirrhotics and determine how occurrence and severity of AKI relate to in-patient mortality.

Methods: A prospectively collated cohort of 120 hospitalized cirrhotic patients was retrospectively analysed. The AKIN criteria and HRS creatinine criteria (>133 µmol/l / 1.5 mg/dl) were utilised.

Results: Mean baseline creatinine was 61 µmol/l (0.69mg/dl) in females and 65 µmol/l (0.74mg/dl) in males. At admission, 38% met AKI whilst 17% met HRS criteria and cumulative incidences were 54% and 35% respectively. Inpatient mortality was 35% for those meeting AKI criteria versus 7% in those without AKI (p=<0.0001). Mortality was not significantly higher in patients who met AKI criteria during their admission than at presentation (53% versus 28% p=0.06). Amongst patients with AKI, 51% were stage 1, 14% stage 2 and 35% stage 3. Corresponding mortality was 12%, 44% & 65% respectively. 20 patients who met AKI stage 1 but did not progress had similar mortality (12%) to patients with no AKI (7%) (p=0.9). Of 26 patients who met criteria for AKI but not HRS at admission, 14 later met HRS criteria at a mean interval of 4.3 days with an in-patient mortality of 50%.

Conclusions: AKI is extremely common in hospitalized cirrhotics and has a high mortality dependent on stage. Most AKI (70%) is present at time of admission. The use of AKIN criteria rather than HRS criteria doubles the detection rate of renal dysfunction. Of greater significance, AKIN criteria ensures earlier detection of renal failure, so that basic therapeutic interventions can be delivered, potentially limiting progression and improving outcomes.

SA-PO062

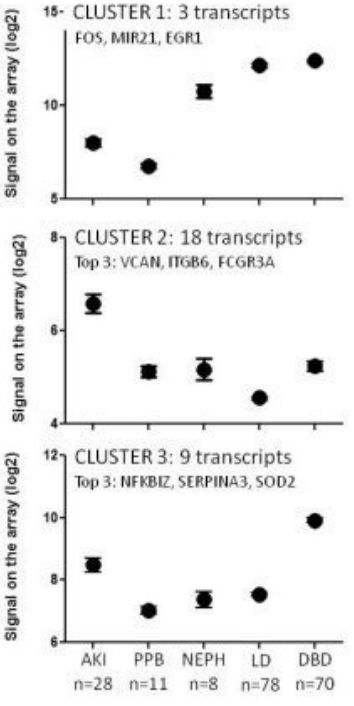
The Injury-Repair Response Reflects General Stress, Acute Kidney Injury and the Effect of Brain Death at the Time of Implantation Konrad S. Famulski,[1] Chatchai Kreepala,[2] Jessica Chang,[2] Philip F. Halloran.[2] *[1]Laboratory Medicine and Pathology, University of Alberta; [2]Medicine, University of Alberta.*

Background: We previously reported that human kidney transplants with early acute injury express transcripts indicating injury repair process (IRRATs). Expression of IRRATs correlated with delayed graft function and future recovery. The present study investigated expression of IRRATs at the time of implantation. Our hypothesis was that IRRATs will reflect various injuries of implanted kidneys.

Methods: Expression of IRRATs was measured by microarrays in biopsies from grafts with early AKI, early stable grafts (PPB), nephrectomies (NEPH), living donors (LD) and donation after brain death (DBD). K-means clustering identified expression patterns across conditions.

Results: We found that cluster 1 had highest expression of IRRATs in both LD and DBD biopsies, whereas in cluster 2 highest expression was in grafts with AKI. Cluster 3 had highest expression of IRRATs in DBD biopsies. Clusters 2 and 3 had the best predictive value for early delayed graft function of DBD kidneys.

Figure 1. Transcriptional profiling



Prediction of delayed graft function (AUC)		
Cluster 1	**Cluster 2**	**Cluster 3**
0.72	**0.81**	**0.81**

We identified other transcripts with highly similar profiles to clusters 1-3.

Genes highly similar to clusters 1-3

Similar to cluster	Gene Ontology p<0.001	Transcription factor / regulator* IPA
Cluster 1 (n=573)	DNA binding (n=140)	n=127
Cluster 2 (n=419)	Cell adhesion (n=55)	n=104
Cluster 3 (n=170)	none (p>0.05)	n=13

*significant difference between clusters 1-2 and 3

Cluster 1 and 2-like genes represented different transcriptional regulation, while cluster3-like genes had no prevailing function.

Conclusions: Thus in the implant biopsies expression of IRRATs reflects general stress, acute injury and the effect of brain death. Each of these processes has different functional and transcriptional programs. Prediction of early delayed graft function at the implantation by molecular test will aid management post-transplant.

Funding: Government Support - Non-U.S.

SA-PO063

Molecular Signal of Reversible Acute Kidney Injury Predicts Failure of Scarred and Inflamed Grafts Konrad S. Famulski,[1] Chatchai Kreepala,[2] Philip F. Halloran.[2] *[1]Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada; [2]Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: We previously reported that human kidney transplants with early acute injury express transcripts indicating injury repair process – the acute kidney injury (AKI) signal. The AKI signal correlated with depression of function and future recovery. The present study investigated significance of this signal in transplants with progressive diseases, late post transplant. Our hypothesis was that kidneys have one fundamental response to injury or diseases, the injury-repair response.

Methods: Expression of 30 transcripts constituting the AKI signal was measured by microarrays. For each biopsy, we summarized their expression as the AKI signal.

Results: High injury signal correlated with increased frequency of graft failure, particularly in fibrotic and inflamed grafts, but not with fibrosis alone.

Distribution of failures in non-rejecting grafts according to their histology status and the strength of AKI signal

	Strength of AKI signal		
	Low signal	Moderate signal	High signal
No fibrosis and inflammation*	2	1	1
Fibrosis only	0	0	1
Fibrosis and inflammation*	2	4	20

* indicates significant difference in the distribution among AKI tertiles (Chi-square test)

In late biopsies (>1 year) the AKI signal predicted future graft loss, similar to a published molecular Risk Score previously derived in late kidneys (Figure 1) and shared many individual transcripts with the Risk Score e.g. ITGB6, VCAN, NNMT.

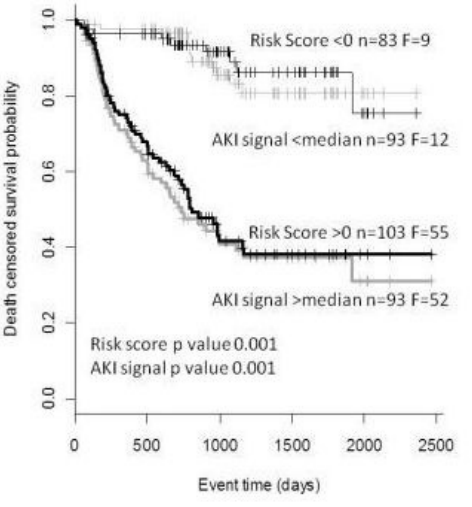


Figure 1. Survival curves of kidney grafts based on the AKI signal and the molecular Risk score.

F - failed, n – number of transplants.

In late transplants, the AKI signal was a better predictor of future graft loss than fibrosis, inflammation, or expression of collagen genes.

Conclusions: Thus the AKI signal is present in progressive diseases and reflects parenchymal distress. Progression in chronic diseases is not due to fibrosis but to parenchymal injury from diseases, with secondary inflammation and fibrosis (wound healing).

Funding: Government Support - Non-U.S.

SA-PO064

Central Venous Oxygen Saturation as Predictor of Azotemia and Adverse Clinical Outcomes in Cardiovascular Surgical Patients Jonathan Chavez,[1] Jaime Briseño,[2] Yajaziel Azpeitia,[3] Guillermo G. Garcia.[1] *[1]Nephrology, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico; [2]Internal Medicine, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico; [3]Cardiology, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico.*

Background: Close to 30% of patients undergoing cardiac surgery develop transient elevation of serum creatinine that does not meet AKIN definition (< 0.3 mg/dl). However, this elevation has been associated to adverse clinical outcomes. Predictive models of acute kidney injury after cardiovascular surgery don't consider central venous oxigen saturation (CVO_2S) as a risk factor for the development of AKI or azotemia

We determined the association between CVO_2S and the development of azotemia and adverse clinical outcomes in CV surgical patients.

Methods: We measured perioperative CVO_2S and other adverse prognostic variables and outcomes in 42 consecutive patients undergoing cardiovascular surgery at our center. A $CVO_2S < 68\%$ prior, during, or 12 h after surgery was considered low. Azotemia was defined by the presence or absence of any rise in serum creatinine < 0.3 mg/dl postoperatively. t test, ANOVA, and multivariate regression analysis where used when appropriate. A p value < 0.05 was considered significant.

Results: Patients' age was 57 ± 11.6 years (range 24-76 years); 52% were diabetics. Mean CVO_2S was lower in patients with azotemia (75.5 ± 8.86) than those without it (82.0 ± 7.51), p < 0.01. The most common surgical procedure was coronary revascularization (25%). 73% had a low CVO_2S that was associated with the development of azotemia, use of vasopressors, and death (table 1).

HR (95% CI) of azotemia and adverse outcomes associated with low CVO2S

		RR (95% CI)	p value
Azotemia (%)	22 (52.4)	2.66 (1.4-6.18)	0,02
AKI (%)	6 (14.2)	1.18 (0.92-1.50)	0.24
S Lactate >2.5 mmol/l (%)	8 (19.0)	1.6 (0.89-2.8)	0.12
Vasopressors (%)	21 (50.0)	4.0 (2.10-7.59)	0.01
Death (%)	9 (21.4)	1.36 (1.02-1.83)	0.05

AKI, acute kidney injury

Conclusions: We conclude that a low perioperative CVO_2S in patients undergoing CV surgery is associated with the development of azotemia and adverse clinical outcomes.

SA-PO065

Mild Elevation of Urinary Biomakers in Pre-Renal Acute Kidney Injury: Distinct Responses of Urinay L-FABP and NGAL Kent Doi,[1,2] Daisuke Katagiri,[1] Kousuke Negishi,[1] Yoshifumi Hamasaki,[1] Masaomi Nangaku,[1] Takehiro Matsubara,[2] Naoki Yahagi,[2] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Emergency and Critical Care Medicine, University of Tokyo, Tokyo, Japan.*

Background: Pre-renal acute kidney injury (AKI) has been regarded as reversible renal function loss without structural damage. Although pre-renal and renal AKI frequently co-exist in clinical situations, serum creatinine and urine output provide no information to support their differentiation. Because recently developed biomarkers reflect tubular epithelial injury sensitively, we evaluated urinary biomarker levels in patients who had been clinically evaluated as pre-renal AKI and animals that showed transient BUN elevations by volume depletion.

Methods: Urinary biomarkers of L-FABP, NGAL, IL-18, NAG, and albumin at ICU admission were evaluated with an adult mixed ICU cohort consisted of 337 patients. Pre-renal AKI was defined as showing recovery within 48 hr and FENa <1%. We further conducted a proof-of-concept animal experiment to examine urinary L-FABP and NGAL excretion in pre-renal AKI induced by volume depletion.

Results: Modest but significant increases of urinary L-FABP, NGAL, IL-18, NAG, and albumin were observed in AKI patients who had been clinically defined as pre-renal AKI. Animal experiment revealed that volume depletion caused modest secretion of L-FABP and NGAL into urine compared with cisplatinum and ischemia reperfusion models. Of note, urinary L-FABP responded more sensitively than NGAL did. Although no histological evidence of structural damage was observed using light microscopy, partial hypoxia in the kidney was demonstrated by pimonidazole incorporation in the volume depletion model.

Conclusions: Mild increases of urinary AKI biomarkers were detected in AKI patients who were regarded clinically as pre-renal. Animal experiments indicated that renal hypoxia is associated with urinary secretion of L-FABP in pre-renal AKI. These data suggest that new AKI biomarkers, especially L-FABP, can detect mild but significant renal tubular injury that occurs in pre-renal AKI.

Funding: Government Support - Non-U.S.

SA-PO066

Bedside Tool for Predicting the Risk of Acute Kidney Injury after Cardiac Surgery in Chinese Elderly Patients Penghua Hu, Xinling Liang, Wei Shi, Zhilian Li, Fen Jiang. *Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: The purpose of this study was to provide a risk score for predicting acute kidney injury (AKI) after cardiac surgery in Chinese elderly patients.

Methods: A total of consecutive 848 elderly patients (age≥60 years old) undergoing cardiac surgery with cardiopulmonary bypass between January, 2005 and July, 2010 was evaluated. The clinical outcome was AKI according to the serum creatinine criteria of the RIFLE clssification during the first 7 days postoperatively. Patients were excluded if they had an end stage renal disease, or experienced renal replacement therapy. Those who had missing data were also excluded. In randomly selected 682 patients of the total cohorts, multivariate logistic regression analysis was used to develop a new prediction score based on clinical characteristics and perioperative variables of patients. The new score was validated in the remaining patients.

Results: The incidence of AKI in the derivation cohort was 62.3% (425/682), while in the test cohort was 59.6% (99/166). Eight variables were included in the predictive index and each was assigned a number of points based on its standardized regression coefficients. Variables and points of each variable in score were an estimated glomerular filtration rate ≤ 60 ml/min (2 points), male (2 points), hypertension (2 points), New York Heart Association above stage 2 (2 points), total red blood cell transfusions above 625 ml (2 points), cardiopulmonary bypass time above 113 minutes (3 points), duration of ventilator-assisted respiration during postoperative above 24 hours (3 points), previous cardiac surgery (1 points). The area under the receiver operating characteristic curve, judging the discrimination of the score, was 0.798 (95%CI 0.764 to 0.832) in the derivation, which in the validation set was 0.804 (95%CI 0.739 to 0.870). The calibration of the score assessed using the Hosmer-Lemeshow statistic in derivation and validation set were 0.362, 0.221, respectively.

Conclusions: We provide a new and simple score, based on data from Chinese elderly subjects, to predict AKI after cardiac surgery.

Funding: Government Support - Non-U.S.

SA-PO067

Biomarkers of Acute Kidney Injury in Children Treated with Cisplatin, Carboplatin, and Ifosfamide Maya Harel-Sterling,[1] Julie Ho,[3] Ang Gao,[3] Melissa Piccioni,[1] Michael Pizzi,[1] Zubaida Al-Ismaili,[1] Michael R. Bennett,[2] Prasad Devarajan,[2] Michael Zappitelli.[1] *[1]McGill Univ Health Cent, Montreal, Canada; [2]Cincinnati Children's Hosp Med Cent; [3]Section of Nephrology, Univ of Manitoba, Canada.*

Background: Cisplatin (Cis), Carboplatin (Carb) and Ifosfamide (Ifos) are commonly used nephrotoxic chemotherapies. Biomarkers (BioM) of tubular injury may allow for early acute kidney injury (AKI) diagnosis, treatment (Rx) and chronic injury prevention.

Methods: We prospectively measured serum creatinine (SCr), urine alpha-glutathione S-transferase (a-GST, µg/L, proximal tubule) and pi-GST (µg/L, distal tubule), from children receiving Cis/Carb and Ifos Rx. AKI definition: ≥50% rise from pre-Rx. We compared peak AKI *vs.* non-AKI BioM levels (Mann-Whitney) and characterized BioM changes over consecutive Rx days (ANOVA) in patients with and without AKI. In a pilot study, we measured hepcidin-25 (HEP-25, ng/ml) and liver-type fatty acid-binding protein (L-FABP, ng/ml) in a subset of patients.

Results: We studied 42 children (24 Ifos;18 Cis/Carb), mean[SD] age was: 9.9[4.0] yrs; 41% boys; 33% Cis/Carb-AKI; 54% Ifos-AKI. a-GST rose significantly on Ifos Rx (Day 1: mean[SD] 4.3[9.1]; Day 5: 9.5[20.5], p=0.0035); pi-GST rose on Cis/Carb (Day 1: 3.7[6.5]; Day 5: 21.5[30.3], p=0.0034) and Ifos Rx (Day 1: 3.5[2.9]; Day 5: 9.2[10.8], p=0.03). a-GST and pi-GST on Cis/Carb Rx only rose significantly in AKI patients (both p=0.002); with Ifos, pi-GST only rose in AKI (p<0.01) but a-GST rose with and without AKI (both p<0.05). Peak Rx alpha-GST and pi-GST levels were not significantly different for the AKI *vs.*non-AKI patients for both drugs. Peak HEP-25 was higher (p=0.0108) in *non*-AKI patients on Ifos (as per previous research). Peak L-FABP was higher in AKI vs.non-AKI with Ifos Rx, but without statistical significance (p=0.08).

Conclusions: This is the first evaluation of these BioM in pediatric Cis/Carb and Ifos-AKI. The a-GST and pi-GST excretion pattern is consistent with tubular damage from these drugs. As in other study populations, HEP-25 is higher in non-AKI patients. These BioM show promise for early AKI diagnosis and possibly for predicting renal tubular damage severity.

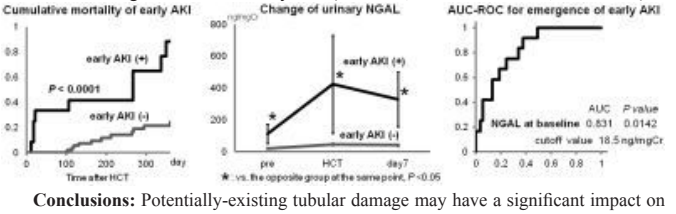
SA-PO068

Potentially-Existing Tubular Damage Is Associated with Early Emergence of Acute Kidney Injury Following Hematopoietic Stem Cell Transplantation Taku Morito,[1,2] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical Hospital, Shinjuku-ku, Tokyo, Japan.*

Background: Acute kidney injury (AKI) in the early stage of hematopoietic stem cell transplantation (HCT) is often fatal. Prediction of such AKI may contribute to the improvement of prognosis.

Methods: A 1-year prospective study was conducted in 67 patients receiving allogeneic myeloablative HCT. Urinary neutrophil gelatinase associated lipocalin (NGAL) levels were consecutively measured as a marker of tubular damage before conditioning therapy (baseline), and at days 0 (the morning of HCT) and 7. "Early AKI" defined as AKI developing before stem cells are engrafted in the bone marrow. Cumulative mortality was analyzed by the Kaplan–Meier method. Discriminative ability of NGAL for emergence of early AKI was evaluated using area under the receiver operating characteristic curve (AUC-ROC). Multivariate Cox hazards regression analysis was performed to evaluate the association of NGAL level and the emergence of early AKI, adjusting for known risk factors.

Results: Incidence of early AKI was 18% (Risk: 1.5%, Injury: 6.0%, and Failure: 10%). The Kaplan-Meier estimate of the group with early AKI was significantly higher. While serum Cr remained statistically constant, in early AKI group, urinary NGAL level manifested the peak at HCT and the transient decrease at day 7. Notably, the AKI group already had significantly higher NGAL level at baseline, which had good discriminative ability of AKI and was a significant risk of early AKI (HR: 4.14, 95%CI: 1.31-13.2, P = 0.0164).



Conclusions: Potentially-existing tubular damage may have a significant impact on the development of early AKI, leading to poor prognosis.

SA-PO069

Outcomes of Acute Kidney Injury Patients with and without Cancer: A Single Center Study Yeon Soon Jung, Ye Na Kim, Ho Sik Shin, Hark Rim. *Internal Medicine, Kosin University College of Medicine, Busan, Republic of Korea.*

Background: The risk of AKI was 18% during the 1st year after cancer diagnosis. Up to 50% of cancer patients experience AKI while in the ICU. Few studies have examined the outcomes of AKI in patients with and without cancer (esp. solid cancers). Therefore, we evaluated these outcomes. The purposes of this study were to evaluate and compare the characteristics and outcomes of cancer and non-cancer patients, to determine the impact of cancer diagnosis on hospital mortality; and to compare outcome predictors between the two groups of patients.

Methods: We conducted a retrospective cohort study in Kosin University Gospel Hospital during 12 years. The patients were divided into two major groups:1,360 AKI patients without cancerand 851 AKI patients with cancer. Predictors of all-cause death were examined using Kaplan-Meier and Cox proportional hazards analyses in both groups.

Results: The main contributing factors of AKI were sepsis (31.1%) and ischemia (52.7%). AKI was multifactorial in 78% of patients with cancer and in 71% of patients without cancer. Hospital mortality rates were higher in patients with cancer (42.8%) than in patients without cancer (22.5%) (P = 0.014). In multivariate analyses, diabetes mellitus (DM) and cancer diagnosis were associated with hospital mortality. Cancer diagnosis was independently associated with mortality [odds ratio = 3.010 (95% confidence interval, 2.340-3.873), P = 0.001]. Kaplan-Meier analysis revealed that subjects with DM and cancer (n = 146) had lower survival rates than subjects with DM and without cancer (n = 687) (log rank test, P = 0.001).

Table 1. Main patient characteristics and outcomes

Variables	All patients (n = 2211)	Non-cancer patients (n = 1360, 61.5%)	Cancer patients (n = 851, 38.5%)	P-value
Age (years)	61.1 ± 14.1	61.4 ± 15.3	60.7 ± 12.1	0.221
Male gender	1356 (61.3%)	789 (58%)	567 (66.6%)	0.001
DM	833 (37.7%)	687 (50.5%)	146 (17.1%)	0.001
Sepsis	687 (31.1%)	442 (32.5%)	245 (28.7%)	0.001
Hospital mortality	671 (30.3%)	306 (22.5%)	365 (42.8%)	0.001
ICU admission	523 (23.7%)	385 (28.3%)	138 (16.2%)	0.001
ICU mortality	340 (66.0%)	230 (59.7%)	110 (79.9%)	0.001
Inotropics	607 (27.4%)	441 (30.2%)	166 (19.5%)	0.001
Mechanical ventilator	480 (21.7%)	385 (28.3%)	95 (11.2%)	0.001
Chronic hepatitis B	211 (9.5%)	93 (6.8%)	118 (13.8%)	0.001
Chronic hepatitis C	185 (8.4%)	114 (8.4%)	71 (8.3%)	0.512
Cause of death				
cancer	234 (34.9%)	NA	234 (64.2%)	
cerebrovascular disease	58 (8.6%)	51 (16.7%)	7 (1.9%)	0.001
heart disease	49 (7.3%)	45 (14.7%)	4 (1.0%)	0.001
DM	1 (0.2%)	1 (0.3%)	0	0.001
infection	273 (40.8%)	160 (52.3%)	113 (31.0%)	0.001
liver diease	53 (7.7%)	46 (15.0%)	7 (1.9%)	0.001
hypertensive disease	3 (0.5%)	3 (1.0%)	0	0.001
Type of cancer				
Locoregional solid tumor	189 (8.5%)		189 (22.2%)	NA
Metastatic solid tumor	604 (27.4%)		604 (71.0%)	
Hematological malignancy	58 (2.6%)		58 (6.8%)	
Cancer status				
controlled	143 (6.4%)	NA	143 (16.8%)	
uncontrolled/newly diagnosed	81 (3.6%)	NA	81 (9.5%)	
uncontrolled/recurrence progression	627 (28.3%)	NA	627 (73.8%)	

Conclusions: Hospital mortality rates were higher in cancer patients than in non-cancer patients. DM and cancer were associated with hospital mortality, and cancer diagnosis was independently associated with mortality.

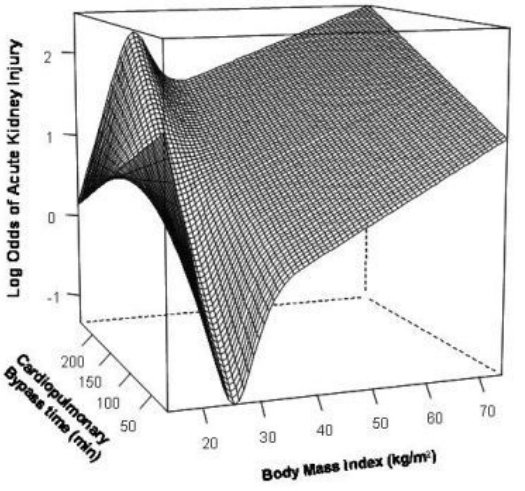
SA-PO070

Body Mass Index Is an Independent Risk Factor for Acute Kidney Injury Following Cardiac Surgery Sevag Demirjian,[1] Sankar D. Navaneethan,[1] Jean-pierre Yared,[2] Allen Bashour.[2] *[1]Nephrology & Hypertension, Cleveland Clinic; [2]Cardiothoracic Anesthesiology, Cleveland Clinic.*

Background: Obesity is a risk factor for diabetes, hypertension and other illnesses, however mortality following cardiac surgery is lower in overweight and obese patients compared to normal (obesity paradox). We examined the effect of body mass index (BMI) on the incidence of acute kidney injury (AKI) following cardiac surgery.

Methods: 21,502 subjects who underwent valve and/or coronary artery bypass graft surgery at Cleveland Clinic between April 2000 and January 2008 were included. AKI was defined as doubling of serum creatinine or need for dialysis. The multivariable logistic regression model adjusted for demographics, comorbidities, baseline renal function, surgery type, and cardiopulmonary bypass (CPB) time.

Results: Median age was 66 years; 68% were male, and 89% white. The median height, weight, and BMI were 172 cm, 81 kg, and 27 kg/m^2, respectively. There were 8188 valve only, and 4923 combined surgeries; the median CPB time was 94 minutes (see Table). There were 258 (1.2%) subjects with BMI< 18.5, 6144 (29%) with BMI between 18.5 – 24.99, 8362 (39%) with BMI between 25 – 29.99, and 6731(31%) with BMI ≥ 30. BMI had an independent and nonlinear association (p<.0001) with AKI in the multivariable model; the latter association was modified by the length of CPB time (p=.005).



Conclusions: BMI has nonlinear and independent association with AKI following cardiac surgery. Obesity was associated with higher incidence of AKI compared to subjects in normal and overweight categories when CPB time was less than 150 minutes. With longer CPB times, the incidence of AKI was comparable in normal, overweight and obese groups.

SA-PO071

Long-Term Survival among Acute Kidney Injury Patients Was the Worse in Group of Patients Experiensed Sepsis Mai Ots-Rosenberg, Jana Uhlinova. *Internal Medicine, University of Tartu, Tartu, Estonia.*

Background: Acute kidney injury (AKI) is a serious risk factor which impairs patients (pts) outcomes. In pts with pre-existing chronic kidney disease (CKD), renal recovery after AKI is less likely and end-stage kidney disease frequently develops. The aim of our study was to investigate etiology and long-term outcome of only dialysis requiring AKI at university clinic as well as to determine the quantity of acute-on-chronic started dialysis.

Methods: The report is based on retrospective data from patients' records in 3 year period starting from January 1, 2009. A Kaplan-Meier analysis was used to determine 3-year survival and separately in septic and diabetic pts groups.

Results: The study was comprised of 318 dialysis requiring AKI pts. In 86 pts pre-existing CKD was known and dialysis was started acutely. During the hospitalization period 106 pts died. Among those 33% were septic and 31% diabetic pts and all together 212 survived. Renal function was completely recovered in a half of the survivors. CKD developed or progressed in 110 pts (52%) from the survival group and after the hospital discharge 30% of CKD pts remained dialysis dependent. Long-term analysis revealed that the overall 3-year survival in the study population was 45% and 3-year survival among discharged pts was 67%. The Kaplan-Meier curve showed significant overall survival difference between sepsis and patients' group without sepsis (P=0.039). There was not a statistically significant difference in long-term survival between diabetics and non-diabetics.

Conclusions: Our study demonstrated that almost one third of AKI survivors stayed dialysis dependent. Acute-on-chronic start dialysis pts formed only 8% of all the AKI population. But, among chronic dialysis incidence pts at our centre acute-on-chronic pts formed 33%. AKI in septic and diabetic pts formed the biggest part of all the AKI population at our hospital and these conditions were associated with high in-hospital mortality. Long-term survival was worse especially in patients with sepsis.

SA-PO072

Cerebral Blood Flow during Hemodialysis in Acute Kidney Injury and Liver Cirrhosis Giuseppe Regolisti,[1] Carola Cademartiri,[1] Umberto Maggiore,[1] Aderville Cabassi,[1] Riccardo Antoniotti,[1] Santo Morabito,[2] Alberto Caiazza,[3] Enrico Fiaccadori.[1] *[1]Department of Internal Medicine and Nephrology, University of Parma, Parma, Italy; [2]Division of Nephrology, Policlinico Umberto I, Rome, Italy; [3]Department of Medicine and Diagnostics, AUSL, Parma, Italy.*

Background: Hemodialysis (HD) can decrease cerebral blood flow (CBF) in patients with acute kidney injury (AKI). Moreover, since regulation of cerebral hemodynamics may be impaired in liver cirrhosis (LC), patients with AKI and LC may be at increased risk of cerebral hypoperfusion during HD.

We studied CBF during the first HD session in patients admitted for oliguric AKI, with or without LC.

Methods: CBF was examined by measuring middle cerebral artery mean flow velocity (MCAmfv) with transcranial Doppler (posterior temporal window, duplicate measurements) at baseline, at midtime and at the end of the first 4-h HD session *(Qb 200 ml/min, Qd 300 ml/min, parallel flows)* in 11 patients with both AKI and LC (median age 69 yrs, range 40-87, 7 males). Eleven patients with AKI without LC (median age 77 yrs, range 69-92, 6 males) served as controls. Data were analyzed using mixed models for repeated measurements.

Results: At the start of the HD session there was a trend, albeit not statistically significant, toward a higher MCAmfv in patients with LC compared to controls (mean MCAmfv 33.8 cm/sec [SEM 4.9] vs 25.4 cm/sec [SEM 2.4], P=0.14). Also, we observed

a marginally smaller relative blood volume (RBV) decrease (hemoglobinometry) during the HD session in the patients with LC than in the controls (-0.6% [SEM 1.2] vs -5.5% [SEM 2.6], P=0.05). The change in mean arterial pressure at end HD was similar between the 2 groups (P=0.70). MCAmfv decreased significantly during the HD session in both groups (P=0.01). However, after adjusting for baseline MCAmfv values and the difference in RBV decrease, the change in MCAmfv during the HD session did not differ between the patients with LC and the controls (time-averaged difference between groups: P=0.99; group-by-time interaction: P=0.07).

Conclusions: HD induces a decrease of CBF in patients with AKI. Patients with LC do not show a more pronounced fall of CBF during the course of the HD session.

SA-PO073

Urine Output on Continuous Renal Replacement Therapy Predicts Survival in Pediatric Acute Kidney Injury Heather A. Lesage-Horton,[1] Rebecca M. Lombel,[1] Theresa Mottes,[1] Kassandra L. Messer,[3] Michael Heung,[2] Neal B. Blatt.[1] *[1]Pediatrics-Nephrology, University of Michigan, Ann Arbor, MI; [2]Internal Medicine-Nephrology, University of Michigan, Ann Arbor, MI; [3]Biostatistics, University of Michigan, Ann Arbor, MI.*

Background: Acute kidney injury (AKI) remains a common and serious medical condition in intensive care unit patients with mortality rates approaching 50%. Currently, the indications for starting Continuous Renal Replacement Therapy (CRRT) and the timing of when to initiate and discontinue CRRT are clinician- and hospital-dependent.

Methods: Retrospective chart review of pediatric patients who received CRRT in the C.S. Mott Children's Hospital from July 1, 2006, through September 30, 2010. Statistical comparisons were made via unpaired t-tests for continuous variables and chi-square or Fisher's exact test for categorical variables. Logistic regression was used to look for associations with survival for the whole cohort and for renal recovery or dialysis for survivors.

Results: 84 patients with AKI had 107 sessions of CRRT during the study period. Looking only at the last circuit of the first CRRT session, 43% (n=36) had renal recovery, 30% (n=25) required intermittent hemodialysis or peritoneal dialysis, and 27% (n=23) died. Significant differences between survivors and non-survivors were found for initial fluid overload (FO, 15 vs. 25%, p=0.02), final FO (6 vs. 19%, p<0.01), and urine output (UO, mean 0.8 vs. 0.1 mL/kg/hr, p <0.01). Each 1 mL/kg/hr increase in UO increased the odd ratio for survival by 14.9-fold. Survivors with renal recovery had increased use of diuretics (43 vs. 16%, p=0.03) compared to those with dialysis dependence.

Conclusions: Pediatric survivors of severe AKI requiring CRRT show increased UO at CRRT discontinuation. Survivors have less FO at both CRRT initiation and discontinuation compared to non-survivors. These findings suggest that readily available clinical characteristics may help predict long-term outcomes for children on CRRT. We are analyzing additional patient characteristics with the hope of developing a model that may be used to predict which patients can be successfully discontinued from CRRT.

SA-PO074

Pulmonary Artery Pressure Is a Strong Predictor of Acute Kidney Injury after Lung Transplantation Jiwan K. Thapa, Edgard I. Wehbe, Marie M. Budev, Andra E. Duncan, Brian R. Stephany. *Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Pulmonary hypertension (PH) is common in patients awaiting lung transplantation. The impact of pulmonary artery pressure on the risk of perioperative AKI remains unknown.

Methods: Using the Cleveland Clinic lung transplant registry, we identified 354 patients who underwent lung transplantation between 1997 and 2009 and who had pre-operative assessment of pulmonary artery pressure. PH was defined as mean pulmonary artery pressure (mPAP) >25 mmHg. We further categorized the severity of PH as - mild: mPAP 25-34 mmHg (n=133), moderate: mPAP 35-44 mmHg (n=39), severe: mPAP ≥ 45 mmHg (n=20). AKI was defined by absolute rise in creatinine by >= 0.3 mg/dl according to the AKIN classification.

Results: We identified 192(54%) patients with pre-operative PH. Of those, 136(70%) developed AKI compared to 84(52%) who developed AKI in the non PH cohort (P=0.0002). The rate of AKI increased as the severity of PH increased (mild 66%, moderate 74%, severe 95%, P=0.001). In multivariate logistic regression, controlling for known risk factors for AKI, PH was significantly associated with post-operative AKI (OR 1.76, 95% CI 1.08-2.9, OR 2.41, 95% CI 1.08-5.7 and OR 13, 95% CI 2.5-23) for mild, moderate and severe PH respectively). When mPAP was examined as a continuous measure, every 10-mmHg increase in pulmonary pressure was associated with 67% increase in risk for AKI (OR 1.67, 95% CI (1.28,2.24).

Conclusions: In patients undergoing lung transplantation, pre-operative pulmonary hypertension is a strong risk factor for perioperatve acute kidney injury.

SA-PO075

Acute Kidney Injury Is Under-Recognized in Pediatric Presentations of Sepsis Marie-Carmelle Elie-Turenne, Azra Bihorac, Tezcan Ozrazgat Baslanti, Mohammad G. Abu-farsakh, Mark S. Segal. *University of Florida, Gainesville, FL.*

Background: The incidence of acute kidney injury in severe sepsis (sAKI) ranges from 23-67% in adults. Since the incidence is not clearly defined, the objective was to characterize sAKI in pediatric patients.

Methods: This is a 10 year retrospective study from a single academic hospital of sepsis patients ≤19 years admitted from 2001 to 2011. Patients with a history of chronic kidney disease or long-term dialysis were excluded. Demographics, laboratory, and outcome data were collected.

Results: 405 subjects were identified of whom 45% met criteria for AKI. 2.5% (10/405) of subjects had a documented diagnosis of AKI by their physician. Using pRIFLE criteria, 137/405 (33%) were R, 18/405 (4%) I, and 26/405 (6%) F. When height data was available (157/405), the Schwartz equation estimated a higher creatinine clearance 141 (130, 153) compared to the Shull equation 102 (98, 107) p <.0001. However, among all patients with sAKI, there was no difference between the calculations rendered by the equations 57.9 (51, 64) 62.8 (53.1, 72.5) p=0.163. Of subjects under 1 year of age, 84% (133/158) had AKI.

Characteristics of Patients with and Without Sepsis Related AKI

	No AKI (n=224)	AKI (n=181)	p-value
Median (Q1, Q3)	17.5 (15.5, 21.2)	16.4 (14.4, 18)	0.016
BMI	17.5 (15.5, 21.2)	16.4 (14.4, 18)	<0.0001
Age (months)	92 (30, 173)	2 (1, 14)	0.2241
Anion Gap	16 (14, 18)	16 (14, 19)	0.2165
C Reactive protein	32 (8.7, 68.9)	34.7 (13.1, 108.6)	0.3708
Sedimentation rate	32 (17, 56)	30 (7, 52)	0.0974
Hemoglobin	11.6 (10.2, 12.7)	11 (9.7, 12.5)	0.0009
BAND	9.5 (5, 19)	5.5 (2, 12)	0.9585
WBC	12 (7.1, 16.1)	11.5 (7.9, 15.9)	0.0299
Day1 Lactate	2.1 (1, 2.7)	2.6 (1.8, 4.8)	0.8829
Day 1 Cr	0.4 (0.3, 0.6)	0.3 (0.3, 0.5)	<0.0001
Day 1 Cr Clearance	117.2 (98.4, 135.2)	78.7 (61.7, 78.7)	0.045
Day2 Lactate	1.2 (0.9, 1.7)	1.9 (1.1, 3.1)	0.3931
Day 2 Cr	0.5 (0.3, 0.7)	0.4 (0.3, 0.9)	0.0587
LOS	2 (1, 4)	2 (2, 6)	0.0055
ICU_LOS	0 (0, 0)	0 (0, 0)	0.1588
Female Gender, n(%)	92 (41%)	87 (48%)	0.0137
ICU, n(%)	31 (14%)	43 (24%)	0.0137
In-hospital mortality, n(%)	0 (0%)	5 (3%)	0.0173

Conclusions: AKI is highly prevalent among septic pediatric children. Increased recognition is needed early in the course of presentation.

SA-PO076

Acute Kidney Injury in Pregnancy: Experience from a Large Tertiary Care Referral Centre Alexandra Mihalache, Oier Ateka, Inês Palma Reis, Kate Harding, Catherine Nelson-piercy, Anita Banerjee. *Women's Services, St Thomas' Hospital, London, United Kingdom.*

Background: Acute Kidney Injury (AKI) in pregnant women can be due to several causes such as preeclampsia, sepsis and volume depletion. The causes and outcomes of AKI in pregnancy have not been explored in detail. This study investigated the clinical features, aetiology, and perinatal outcomes of de novo AKI and AKI in patients with CKD.

Methods: Data on clinical features and outcome were collected on 39 patients with AKI. AKI was defined as a serum creatinine (sCr) >90 µmol/L during any time of hospital admission. Data on previous CKD before pregnancy were also collected.

Results: The clinical features of the patients were as follows: age 32±5 (mean±SD) years and BMI 27±5 kg/m^2. Peak sCr was 121±35 µmol/L (range 90-263), peak potassium was 4.9±0.6 mmol/L (range 4.1-7.1). AKI in pregnancy was commonest in the third trimester (73%). Preeclampsia was the cause in 65% of cases and 20% of cases were due to postpartum haemorrhage. AKI in pregnancy was not recognised in 43.5% of cases. There was no significant difference in peak creatinine between patients in whom the AKI was and was not recognised. Birth weight was also similar in both groups. 72% of cases required either high-dependency unit or intensive care unit support. Five patients had peak sCr >150 µmol/L and these women had similar maternal age and birth weight to the rest of the cohort. There was no association between peak sCr and birth weight. Prior known CKD was present in 9/39 (25%) of the cases. Those with CKD had similar age, birth weight but higher peak sCr than those without (162±41 µmol/L vs. 109±21 µmol/L; p<0.005). Causes of CKD included focal segmental glomerulosclerosis, reflux nephropathy, previous nephrectomy, Sjogrens Syndrome and tubulointerstitial nephritis. 60% of women with AKI required an emergency Caesarean section.

Conclusions: AKI in pregnancy is worse in women with prior CKD and 43% cases were not recognised. A large proportion of patients were managed in high-dependency and intensive care and required emergency Caesarean section.

SA-PO077

Acute Kidney Injury in Pediatric Idiopathic Nephrotic Syndrome without Hypovolemia Yoshinobu Nagaoka,[1] Kenji Ishikura,[1] Riku Hamada,[1] Tomoyuki Sakai,[1] Yuko Hamasaki,[2] Hiroshi Hataya,[1] Masataka Honda.[1] *[1]Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Tokyo, Japan; [2]Pediatric Nephrology, Toho University Omori Medical Center, Ota-Ku, Tokyo, Japan.*

Background: Acute kidney injury (AKI) is an occasional complication in idiopathic nephrotic syndrome (NS). Hypovolemia accompanied with hypoalbuminemia had previously been considered the major cause of AKI in NS. On the contrary, several recent reports documented AKI in NS without hypovolemia. However, these studies contained small number of patients and no mention of frequency.

Methods: We analyzed retrospectively 156 children (102 boys, 54 girls) with idiopathic NS managed in our hospital from 2000 to 2011. AKI was defined as prerenal and renal states having more than the twice the baseline serum creatinine value, with prerenal AKI as having more than +5% of the baseline hematocrit level.

Results: AKI was observed 42 times in 29 (18.6%) of 156 patients. Among them, 9 times in 6 patients (boy/girl ratio 1.3) were prerenal AKI, and 33 times in 27 patients (boy/girl ratio 3.1) were renal AKI. Comparisons of mean value (prerenal vs renal) were as follows: age at AKI, 4.1+/-1.4 years vs 5.4+/-0.7 years [p=0.37, t-test]; difference from the 50th percentile systolic blood pressure (by age and height), 17.7+/-4.0 mmHg vs 20.4+/-2.1 mmHg [p=0.60]; cardiothoracic ratio, 44.6+/-2.5% vs 48.9+/-1.2% [p=0.26]; fractional excretion of sodium (FENa), 0.03+/-0.06% vs 0.11+/-0.03% [p=0.045]; urea nitrogen, 32.1+/-8.7 mg/dl vs 47.9+/-4.6 mg/dl [p=0.11]; and uric acid, 6.0+/-1.1 mg/dl vs 9.5+/-0.5 mg/dl [p=0.004].

Conclusions: In pediatric idiopathic NS cases, about 20% were complicated with AKI, and most of them (approximately 80% of AKI) were renal AKI. In renal AKI, decrease of FENa and increase of urea nitrogen or uric acid were observed, which appeared similar to hypovolemia and different from acute tubular necrosis. Hypertension or enlargement of cardiothoracic ratio were observed, which seemed likely to cause increase in circulating plasma volume. It is important that clinicians are alert to the possibility of hypervolemia, and should perform infusion of crystalloid or albumin preparations with care.

SA-PO078

Acute Kidney Injury after Antibiotic Spacer Implantation for Infected Joint Prostheses

Renu Bansal, Andrew Luu, Madhumathi Rao. *Nephrology, Tufts Medical Center, Boston, MA.*

Background: Acute kidney injury (AKI) is an under-recognized complication after antibiotic spacer placement for two-stage arthroplasty for infected joint prostheses, progressing to dialysis in 2-6% patients. Antibiotic impregnated spacers produce high and sustained joint antibiotic levels exceeding the MIC of many potential organisms. Two stage arthroplasty is standard of care and performed at an increasing rate in older and higher risk patients. Between August 2007 and July 2011 this procedure comprised 4% of joint surgeries at this center (national average 1%).

Methods: We performed a retrospective study of 42 patients undergoing 2-stage arthroplasty who received antibiotic spacers containing tobramycin (3.6 g to 10.8 g) and vancomycin, and examined the incidence and risk factors of AKI. AKI was defined as doubling of serum creatinine from baseline, anytime between implant and explant of spacer.

Results: The mean age was 61 years, a third each had diabetes or vascular disease and 20% had CKD. AKI developed in 50% of patients in three clinical patterns – early rise in serum creatinine followed by recovery (50% of AKI), delayed rise in serum creatinine (20% of AKI) and AKI without recovery (30% of AKI). Three patients needed dialysis and one patient died. Patients who developed AKI were found to be older, with poorer kidney function and vascular disease.

Table 1

Variable	HR	95% CI	p value
Age (10 years)	1.4	1.03-1.91	0.03
CVD	4.84	1.9-12.5	0.002
CKD 1 (reference)			0.16
CKD 2 (eGFR 60-90 ml/min)	3.2	1.10-9.0	0.032
CKD 3-4 (eGFR <60 ml/min)	4.6	1.5-13.8	0.006

For every 0.1 mg increase in serum creatinine the risk of AKI increased by 9 % (95% CI 4 to 14%), p=0.001 and for every 10 ml increase in baseline eGFR the risk of AKI decreased by about 30% (95%CI 14-34%), p=0.001.

Conclusions: In summary, at a referral center, after 2-stage arthroplasty with antibiotic spacers, the incidence of AKI was 50%, cumulative toxicity appeared to occur and specific risk factors were identified including even minor declines in baseline eGFR. We therefore recommend careful selection of patients, close monitoring and long term follow up.

SA-PO079

Electronic Results Reporting of Acute Kidney Injury in Cirrhotics

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Background: Current Acute Kidney Injury Network (AKIN) criteria for defining AKI perform well in general hospitalised patients but have not been validated in certain subgroups. Liver disease is increasing, disproportionately affecting younger patients with reduced body mass. We aimed to apply AKIN definitions of AKI to a population of cirrhotic patients and correlate this with outcomes in a 1000-bedded acute teaching hospital with a Tier 2 hepatology centre.

Methods: A hospital wide, real-time electronic reporting system based on the AKIN diagnostic criteria for AKI has been in place at Royal Derby Hospital since 2010. We prospectively identified all hepatology patients with AKI over an 18 month period. They were screened for radiological or histological evidence of cirrhosis and compared to a control group with evidence of cirrhosis and no evidence of AKI identified from the same time period and clinical setting.

Results: 162 cirrhotic patients were identified. They were predominantly young (56.8 +/- 13.6), male (65.4%) and had alcoholic liver disease (78.4%). 110 cirrhotics had AKI: 44 stage 1, 32 stage 2 and 34 stage 3. They were well matched in terms of age, sex and liver severity with 52 cirrhotics without AKI. There were important differences in diabetes and baseline creatinine between those with and without AKI. AKI was significantly associated with increased mortality in cirrhotics (31.8% vs 3.8%, p<0.000). Mortality increased with increasing AKI stage; 3.8% in cirrhotics without AKI, 13.5% in stage 1, 37.8% in stage 2 and 43.2% in stage 3. Increasing severity of liver disease (Child-Pugh class) correlates with increased mortality (p=0.006 for trend). AKI was associated with an increased length of stay (p<0.000). Multivariable analysis was performed for independent factors in predicting mortality (table 1).

Factor	Significance	Hazard Ratio	95% Confidence Intervals
AKI	0.02	10.6	2.4 - 47.0
Child Pugh B	0.04	8.1	1.0 - 65.4
Child Pugh C	0.016	13.1	1.6 - 106.0

Table 1

Conclusions: AKIN diagnostic criteria does correlate and associate with increased mortality in patients with cirrhosis. Severe liver disease and AKI appear to be independent variables in predicting death in cirrhotic patients.

SA-PO080

Urinary Angiotensinogen Predicts Outcomes of AKI Patients in the ICU

Joseph Alge,[1,2] Nithin Karakala,[1,2] Michael G. Janech,[1,2] John M. Arthur.[1,2] *[1]Medical University of South Carolina; [2]Ralph H. Johnson VAMC.*

Background: We have identified urinary angiotensinogen as a prognostic biomarker of acute kidney injury (AKI) after cardiac surgery. However, AKI can be the result of many other etiologies, and it is necessary to qualify angiotensinogen in other settings.

Methods: Urinary angiotensinogen was measured by ELISA in urine samples from ICU patients with AKI of diverse causes including both ischemic and non-ischemic etiologies (n=40; Table 1). The ability of the ratio of angiotensinogen-to-urine creatinine (uAnCR) to predict the following outcomes was evaluated using the area under the ROC curve (AUC): worsening of AKI, AKIN stage 3, need for renal replacement therapy (RRT), AKIN stage 3 or death, and RRT or death.

Results: Median uAnCR was markedly elevated in patients who met the primary outcome of RRT or death compared to those who did not (133.3 ng/mg versus 11.4 ng/mg), and uAnCR was a strong predictor of this outcome (AUC=0.79). It was also a modest predictor of the composite outcome AKIN stage 3 or death (AUC=0.71). Finally, patients with high concentrations of uAnCR (*i.e.* ≥median value) had increased median length of stay in the hospital compared to those with low uAnCR (22 days versus 7 days; p=0.03), and uAnCR was a strong inverse predictor of hospital discharge ≤7 days from sample collection (AUC=0.79).

Conclusions: These data confirm the potential of angiotensinogen as a prognostic AKI biomarker, and this is the first report to demonstrate that it is predictive of outcomes in the setting of AKI secondary to causes other than cardiac surgery.

Table 1. Characteristics of ICU Patients with AKI

	No RRT/Survived	RRT/Death	p
n	19	21	
Age (yrs)[a]	63.2±17.4	53.8±16.4	0.06
Caucasian[c]	68% (13)	67% (14)	0.83
Male[c]	58% (11)	67% (14)	0.81
Time of Collection (days)[a]	0.84±1.10	0.96±1.14	0.53
Baseline sCr (mg/dL)[a]	1.2±0.6	1.3±0.5	0.59
sCr at Collection (mg/dL)[a]	2.1±0.9	2.5±0.8	0.11
Max sCr (mg/dL)[a]	2.4±1.3	4.3±2.4	0.004
MAP[a,b]	78.2±12.0	73.4±10.5	0.26
AKIN Stage 1 at Collection[c]	95% (18)	76% (16)	0.19
ACE Inhibitors[c]	53% (10)	24% (5)	0.12
HTN[c]	89% (17)	48% (10)	0.01
DM[c]	53%(10)	24% (5)	0.12
RRT[c]	0% (0)	57% (12)	<0.001
Death[c]	0% (0)	76% (16)	<0.001

[a]mean±SD; [b]24 hr average MAP on day of sample collection; [c]percentage (n)

Funding: NIDDK Support, Veterans Administration Support

SA-PO081

Serum and Urine NGAL after Partial Nephrectomy: No Greater Renal Injury in Warm Robotic versus Cold Open Techniques

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Background: There is concern that advantages of minimally invasive robotic partial nephrectomy (RPNTX) could be outweighed by arterial cross clamping causing renal injury: "warm" ischemia, compared to "cold" ischemia used during open partial nephrectomy (OPNTX).

Methods: We measured the serum and urine renal ischemia biomarker neutrophil gelatinase-associated lipocalin (NGAL, at time 0, 4, 8, 12, 24 and 48 hrs post-op) to test the hypothesis that renal injury is avoided in RPNTX by virtue of brief renal hilar arterial clamp times. 32 pts underwent RPNTX for tumors, excluding pts with solitary kidneys: 61.3 ± 10.9 years old, 55% male, BMI 29.8 ± 5.4 kg/m^2, eGFR 79.4 ± 25.7 ml/min, tumor size 2.7 ± 1.0 cm, warm ischemia time 25.4 ± 5.2 min, and blood loss 96.1 ± 57.5 ml. A contemporaneous group of 12 pts underwent OPNTX: some of higher BMI (34.7 ± 12.4, p=ns); trend for larger tumor size (3.4 ± 1.3, p=0.06); longer (cold) ischemia time (38.2 ± 9.6, p=0.0007); and more blood loss (206 ± 103 ml, p=0.004).

Results: With RPNTX mean peak serum NGAL was 213 ng/ml (range 77 to 934). Mean peak urine NGAL was 101 ng/ml (range <25 to 1462), with 28% of pts having values <25 ng/ml at all time points. OPNTX peak serum NGAL was 194 ng/ml (65 to 437) and urine NGAL was 111 ng/ml (<25 to 666), with 17% of pts remaining <25 ng/ml at all times. Neither serum or urine peak NGAL correlated with ischemia time, tumor size, blood loss, BMI or age: for RPNTX, OPNTX or across all pts. There were no significant differences in NGAL or changes in eGFR between groups. While most of the maximal NGAL levels occurred between 2-12 hrs, for many pts the serum and urine values had a second (or delayed) peak at 24 or 48 hrs: occurring in 45.5% and 47.3% of all pts, respectively.

Conclusions: In conclusion, RPNTX with warm ischemia time of no more than approx 30 minutes has risk for renal injury that appears relatively low and comparable to that of the more invasive OPNTX performed with cold ischemia. Delayed or second peaks of serum and urine NGAL are worrisome for reperfusion injury, and warrant further study.

SA-PO082

Incidence and Outcomes of Acute Kidney Injury Complicating Hospitalization with Acute Nonvariceal Upper Gastrointestinal Bleeding in the U.S. (1999-2008) Juyeh Yang,[1,2] Tsung-chun Lee,[3] Maria E. Montez-Rath,[2] Glenn M. Chertow,[2] Wolfgang C. Winkelmayer.[2] *[1]Far Eastern Memorial Hospital, New Taipei City, Taiwan; [2]Stanford University School of Medicine, Palo Alto, CA; [3]National Taiwan University Hospital, Taipei, Taiwan.*

Background: Kidney failure is an established risk factor for mortality after upper gastrointestinal bleeding (UGIB). However, little is known about the incidence and outcome of acute kidney injury (AKI) among patients hospitalized with UGIB.

Methods: From the U.S. Nationwide Inpatient Sample, we identified patients admitted with acute nonvariceal UGIB (ANVUGIB) between 1999 and 2008. We analyzed trends in the incidence of diagnosed AKI and AKI requiring dialysis (AKI-D) and assessed their associations with in-hospital mortality using survey methods to obtain national estimates. Analyses were conducted using multivariable log-Poisson regression models to estimate adjusted relative risks (RR).

Results: We identified 3,814,416 hospitalizations with a diagnosis of ANVUGIB of which 321,878 (8.4%) were complicated by AKI (AKI-D: 34,797; 0.9%). Adjusted percents of AKI (AKI-D) among ANVUGIB patients increased from 4.3% (0.7%) in 1999 to 15% (1.2%) in 2008. The overall in-hospital mortality of ANVUGIB with AKI (AKI-D) was 18.8% (31.1%) and declined from 27.9% (39.0%) in 1999 to 14.7% (25.1%) in 2008 after adjustments. Compared with ANVUGIB patients without diagnosed AKI, the adjusted mortality risk ratios for AKI and AKI-D were 1.88 (95% CI: 1.84-1.93) and 1.45 (95% CI: 1.39-1.52), respectively. Between 1999 and 2008, adjusted mortality decreased among ANVUGIB episodes without or with AKI or AKI-D, but the decrease was significantly more pronounced for patients with AKI (RR=0.53) compared with ANVUGIB patients without AKI (0.62; p-interaction=0.008. For AKI-D: RR=0.64; p-interaction=N.S.).

Conclusions: In summary, the incidences of AKI and AKI-D among patients with ANVUGIB increased, while the in-hospital mortality declined between 1999 and 2008. Overall, AKI and AKI-D were associated with higher mortality in patients with ANVUGIB. Reductions in mortality over the past decade were more pronounced among patients whose ANVUGIB episode was complicated by AKI than in those without it.

SA-PO083

Fluid Overload Is an Independent Risk Factor for Worse Outcome in ICU Patients Pedro Fidalgo,[1] Silvia Coelho,[1] Bruno Rodrigues,[1] Luis Inchaustegui,[1] Ana Luisa Papoila,[2] Fernando Liano,[3] Karina Soto.[1] *[1]Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal; [2]Biostatistic, Faculty of Medical Sciences, Lisbon, Portugal; [3]Nephrology, Hospital Universitario Ramon y Cajal, Madrid, Spain.*

Background: The beneficial effect of Early Goal-Directed Therapy approach was a hallmark in the standard of care of critically ill patients. However, there is a growing concern about the threshold beyond which fluid therapy determines adverse outcomes. The aim of the present study was to determine whether the fluid balance influences worse outcomes in critically ill patients.

Methods: A prospective study of 128 consecutive ICU patients over 6 months was developed. AKI was defined as >Risk, according to RIFLE criteria. Daily intake and output fluids were recorded and fluid balance (FB) was calculated. Cumulative fluid balance (CFB) was calculated by the sum of daily fluid balance. The Percentage of CFB (PCFB) was calculated according to formula:PCFB= [$\sum$ daily FB/admission body weight (kg)]*100. Mann-Whitney test was used for continuous variables. Odds Ratios were calculated by logistic regression.p value <0.05 was considered significant.

Results: Patients were median age 66y, 61.7% male, 85.2% non-black and 35.2 % of them developed AKI. Median SAPS II and APACHE II scores were 57 (CI 50-58) and 24 (CI 22-26), respectively. Crude mortality was 31.3% and 70% of deaths were AKI patients. Median CFB was 7.6 L (P_{25}3.7-P_{75}14.2), median PCFB was 10.1% of body weight (P_{25}4.8-P_{75}18.3), and both were significantly higher in patients who died than in survivors (p=0.04 in ICU, p=0.02 in-hospital mortality). Higher fluid overload was significantly associated with the length of ICU stay (p<0.001) and with mechanical ventilation (p<0.001). In a multivariable analysis, septic shock, MOF, APACHE II and PCFB were determinants of ICU and in-hospital mortality. For each 5% increase in the PCFB, there was an increase of 24.6% and 19.7% in the odds of in-hospital and ICU dead, respectively.

Conclusions: There is a strong association between fluid overload and mortality in ICU patients. Even after adjustment for known risk factors, it remains an independent risk factor for mortality.

SA-PO084

Dietary Intake of Creatinine Can Mimic Acute Kidney Injury Sha Liu, Cory Iverson, David L. Hyndman, David T. Hagerty, Kimberly J. Manhard, Jeffrey N. Miner, Barry D. Quart. *Ardea Biosciences, San Diego, CA.*

Background: During the conduct of a phase 1 clinical trial, significant elevations in serum creatinine (sCr) were observed during the pre-dose and post-dosing periods in both placebo and compound treated subjects at one of two clinical research units (CRU). The elevations were very large (often >0.5 mg/dL), transient, not associated with urinalysis or renal function changes, and were temporally associated with meals. Heat is known to convert the creatine found in meat into creatinine and prolonged exposure to heat results in a significant rise in creatinine concentration in the meat and meat juices. Cases of elevated sCr due to meat consumption have been reported sporadically, but this issue is not widely appreciated and was not highlighted in published recommendations.

Methods: Careful evaluation of the two CRUs revealed that the CRU where elevations were observed served meat that had been cooked for extended periods (longer than 45 minutes) and sometimes included the resulting meat juice as well. To confirm that the elevations were not due to a problem with the serum enzymatic creatinine assay, additional samples were assayed by both the Jaffe and LC-MS/MS methods, and the same intra-day creatinine elevations were observed.

Results: Serial measurements of plasma cystatin C confirmed no change in renal function. Ingestion of long cooked meat resulted in the largest sCr elevations. Simultaneous determination of both plasma creatine and creatinine revealed a strong association between meal time and creatine/creatinine peaks. Meals with shorter meat cooking times were associated with larger creatine peaks but smaller creatinine peaks, while meals with longer cooking times were associated with equal size creatine and creatinine peaks. First morning blood samples collected under fasting conditions were the most stable and provide an easy means of eliminating the effect of dietary creatinine.

Conclusions: We conclude that meat cooked for prolonged periods may cause very large and transient increases in sCr. We recommend that for patients with non-fasting, unexpectedly large increases in sCr, the potential effect of diet should be considered.

SA-PO085

Colistin (Colistimethate Sodium) Induced Nephrotoxicity: A Single Center Experience Mamdouh N. Albaqumi, Lutfi Alkorbi. *Medicine, KFSHRC.*

Background: The emergence of multidrug-resistant, gram negative bacteria has resulted in significant increase in the use of colistin, which had previously fallen out of favour because of reports of nephrotoxicity. Rates of nephrotoxicity have ranged from around 50% in older studies to almost no toxicity in recent reports. Our objective in this study is to determine the incidence of colistin-associated nephrotoxicity at our center and to assess possible associated risk factors.

Methods: This retrospective study was designed to identify predictors of acute kidney injury (AKI) associated with intravenous (i.v.)colistin treatment. From July 2004 to Jan 2010, 327 adult patients receiving colistin for at least 72 h were enrolled. AKI was defined using Acute Kidney Injury Network (AKIN) criteria. We excluded all patients who have estimated glomerular filtration rate (eGFR) of 15 ml/min or less on the first day of using colistin.

Results: A total of 327 patients were included in the study. AKI developed in 144 patients (44.04%).In the AKI group, AKIN stage I developed in 74 patients (51.39%), stage II in 43 patients (29.86%), and stage III in 27 patients (18.75%).

Patients with AKI secondary to colistin were older (mean of 50 y in AKI group vs. 42.5 y in those without AKI), received higher cumulative dose, and longer duration of colistin therapy. Aminoglycosides and Diuretics use were more common in those who developed AKI while use of ACEI/ARBS, Amphotericine, NSAIDs, and Vancomycin was comparable in both groups.

Patients who didn't develop colistin-induced AKI had a better 1 year survival in comparison to those who had AKI (33.9% vs. 20.4%, respectively).

Conclusions: AKI was a common complication of colistin treatment. Age, cumulative dose, duration of treatment, and use of Aminoglycosides and diuretics were associated with higher risk of colistin-induced AKI.

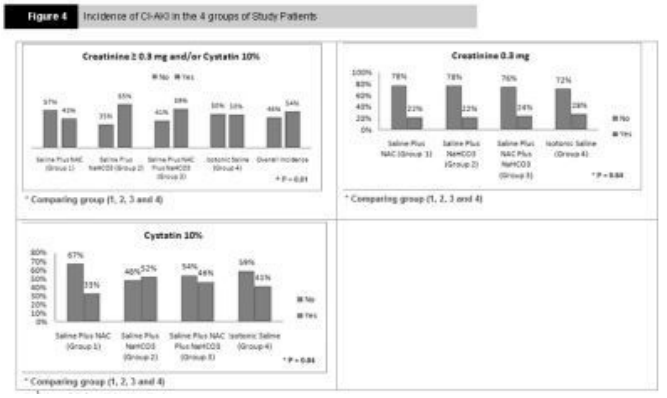
SA-PO086

Intravenous High Dose of N-Acetylcysteine and Sodium Bicarbonate for the Prevention of Contrast-Induced Acute Kidney Injury: A Randomized Controlled Trial Antonio Jose Inda-Filho,[1] Adriano Caixeta,[2] Marcia Manggini,[4] Fabio Santos,[3] Nestor Schor.[1] *[1]Nephrology, Federal University of Sao Paulo, Sao Paulo, Brazil; [2]Cardiology, Hospital Israelita Albert Einstein, Sao Paulo, Brazil; [3]Internal Medicine, Hospital das Forças Armadas, Brasilia, Distrito Federal, Brazil; [4]Cardiology, Instituto de Cardiologia de Brasilia, Brasilia, Distrito Federal, Brazil.*

Background: Contrast-induced nephropathy (CI-AKI) has become an important cause of iatrogenic acute renal impairment. Prior studies using N-acetylcysteine (NAC) or sodium bicarbonate ($NaHCO_3$) in the prevention of CI-AKI have found conflicting results.

Methods: Randomized, controlled study enrolling 500 patients undergoing coronary angiography at a single center using high-osmolar contrast media. The patients were allocated: IV NAC+saline(group 1), IV $NaHCO_3$+saline (group 2), IV NAC+$NaHCO_3$+saline (group 3), IV saline (group 4). All solutions were given 1h before contrast in doses: NAC 150 mg/kg, $NaHCO_3$ 3.5 ml/Kg/h, saline 1ml/Kg/h. During and 6h after procedure doses were: NAC 50 mg/kg, $NaHCO_3$ 1.18 ml/Kg/h, saline 1 ml/Kg/h. Cr and CyC were measured before procedure and at days 1, 2, 3. The end point was CI-AKI based on the Cr (≥0.3 mg/dL) and/or CyC (≥10%) increase between day 0 and 72h after contrast administration.

Results: 75 (15%) patients were excluded. Median patient age was 59.8 ± 11.6 years. The end point was met in 20.0% of the NAC group, 30.4% of the $NaHCO_3$ group, 26.5% of the NAC plus $NaHCO_3$ group, and 23.0% of the saline group (P = 0.01).



Multiple logistic regression model (dependent variable: nephropathy; independent variables: group, diabetes, medications, total volume infused) did not show a significant relationship with nephropathy.

Conclusions: This study suggests that high dose IV NAC was effective to prevent CI-AKI.

Funding: Government Support - Non-U.S.

SA-PO087

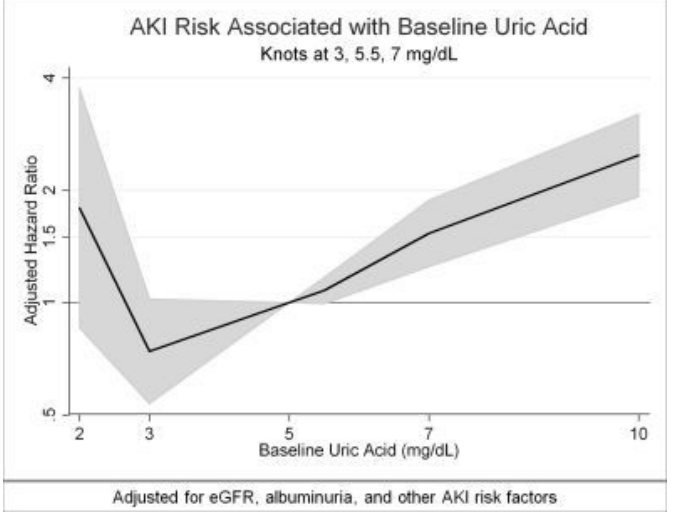
Uric Acid, Gout, and the Risk of Acute Kidney Injury: The ARIC Study

M. Grams, Mara Mcadams, Janet W. Maynard, Michelle M. Estrella, Rugmini P. Warrier, Wen Hong Linda Kao, Josef Coresh. *JHU.*

Background: Urate levels above 5.5 mg/dL associate with AKI after cardiac surgery. Whether this association extends to all AKI over the full range of serum urate is unknown.

Methods: Visit 4 (when urate and gout were assessed) ARIC participants were included (N= 11,487). Participants with previous AKI or eGFR<15 were excluded (N=50). Urate levels were modeled as splines (knots at 3, 5.5, and 7 mg/dL). Cox regression estimated associations with hospitalized AKI, adjusting for race, sex, age, hypertension, diabetes, coronary heart disease, log-albuminuria, and eGFR splines. Additional adjustment in European-Americans included the genetic urate score, or the difference in expected urate level for a given individual compared with an individual homozygous for the major alleles at 8 susceptibility loci and expressed per 100 µmol/L (1.68 mg/dL).

Results: Mean urate was 5.6 mg/dL (SD: 1.5 mg/dL); mean follow-up was 12 years. At levels > 3 mg/dL, urate displayed a nearly linear relationship with AKI in adjusted analysis.



An increase in 1 mg/dL of urate above levels of 3 mg/dL was associated with a 20% increase in AKI risk (adjusted hazard ratio [aHR] 1.20, 95% CI: 1.14-1.25, p<0.001). A history of gout was significantly associated with AKI (aHR 1.29, 95% CI: 1.02-1.64, p=0.03); however, this association was no longer significant with adjustment for urate (aHR 1.10, 95% CI: 0.87-1.41, p=0.4), while the coefficients for urate were unchanged. In European-Americans, genetic urate score was not associated with AKI in crude or adjusted analysis (aHR 0.99, 95% CI: 0.94-1.04, p=0.6).

Conclusions: Higher urate levels above 3 mg/dL were independently and continuously associated with increased risk of AKI in a population-based cohort. A genetic urate score was not associated with AKI: perhaps the relationship between urate and AKI represents latent confounding rather than causality.

Funding: NIDDK Support, Private Foundation Support

SA-PO088

Annualized Change in Estimated GFR Associates with Acute Kidney Injury

M. Grams,[1] Kunihiro Matsushita,[1] Brad C. Astor,[2] Josef Coresh.[1] *[1]JHU; [2]U Wisconsin.*

Background: Reduced eGFR is a potent risk factor for acute kidney injury (AKI). It is unknown, however, whether antecedent rates of kidney function decline provide additional AKI risk information.

Methods: Data from 11,432 participants in the Atherosclerosis Risk in Communities (ARIC) Study were used to evaluate the relative hazard of hospitalized AKI associated with antecedent 9-year annualized changes in eGFR, as calculated using the CKD-Epi equation. Participants with CKD stage 5, a history of AKI, or missing eGFR measures at ARIC enrollment or year 9 were excluded. Cox proportional hazards analyses were adjusted for race, sex, age, hypertension, diabetes, coronary heart disease, log-albuminuria, and eGFR at the last time point (year 9, or ARIC visit 4).

Results: Median follow-up was 12 years after visit 4. There were 763 incident AKI hospitalizations (1.5 events per million person-years). Median eGFR change was -1.0 ml/min/ 1.73m^2/year (IQR: -2.0 to +0.4 ml/min/year) over the antecedent 9 years. The relationship between annualized change in eGFR and AKI appeared U-shaped, although the association of increasing eGFR and AKI was not statistically significant. In categorical analysis, annual eGFR loss more than 3 ml/min/1.73m^2 was significantly associated with AKI, independent of either first or last eGFR.

Longitudinal changes in kidney function and their association with AKI

9-year annualized change in eGFR	N (%)	Crude HR	Adjusted HR (+ first eGFR)	Adjusted HR (+ last eGFR)
≥ 3 ml/min/1.73m2 yearly decline	1,195 (10.5%)	2.8 (95% CI: 2.3-3.5)	2.3 (95% CI: 1.8-2.8)	1.5 (95% CI: 1.2-2.0)
1-3 ml/min/1.73m2 yearly decline	4,374 (38.3%)	1.2 (95% CI: 1.0-1.4)	1.1 (95% CI: 0.9-1.4)	1.0 (95% CI: 0.9-1.3)
0-1 ml/min/1.73m2 yearly decline	3,861 (33.8%)	Reference	Reference	Reference
Increase in eGFR over time	2,002 (17.5%)	1.1 (95% CI: 0.9-1.4)	0.9 (95% CI: 0.7-1.2)	1.1 (95% CI: 0.9-1.4)

Coronary heart disease, older age, diabetes, hypertension, male sex, black race, and first or last eGFR were also significantly associated with AKI in fully adjusted analysis (p<0.02 for all).

Conclusions: Long-term antecedent changes in eGFR provide additional risk information over traditional AKI risk factors.

Funding: NIDDK Support, Private Foundation Support

SA-PO089

Determinants of Renal Function at Hospital Discharge of Patients Treated with Renal Replacement Therapy in the Intensive Care Unit

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Background: Acute kidney injury (AKI) requiring renal replacement therapy (RRT), is a frequently seen complication in the critically ill and constitutes a risk factor for chronic kidney disease (CKD) and increased mortality after hospital discharge. Identification of patients at risk for poor recovery of renal function is important for the development of renal protective strategies.

Methods: We conducted a retrospective cohort study in a large academic hospital, evaluating all patients admitted to the intensive care unit (ICU) suffering from AKI requiring RRT in the period January 1994 to April 2010. Clinical characteristics and potential risk factors for poor renal recovery, were documented in the patients that survived their hospital stay. The outcome variables were estimated glomerular filtration rate (eGFR-MDRD) and dialysis dependency at hospital discharge.

Results: Of the 423 patients in our cohort, 75 (18%) patients had documented CKD before admission to the ICU. Only 18.4 % of the patients were discharged from the hospital with an eGFR > 90 ml/min/1.73m^2. An eGFR ≤ 60 ml/min/1.73m^2 at hospital discharge occurred in 64.8% of cases and 12.1% of the cases left the hospital dialysis dependent. Multivariate logistic regression showed that age (OR = 1.04, p < 0.001), a medical history of CKD (OR = 29.77, p = 0.001), serum creatinine concentration at start of RRT (OR = 1.003, p < 0.001) and administrations of iodine-containing contrast (OR =0.82, p = 0.007) were significantly associated with an eGFR ≤ 60 ml/min/1.73m^2 at hospital discharge. In contrast, potential risk factors such as diabetes mellitus and hypertension lost their significance within a multivariate analysis. Only a medical history of CKD (OR = 9.20 p < 0.001) was associated with dialysis dependency.

Conclusions: The majority of critically ill patients surviving an episode of AKI requiring RRT have an impaired renal function at hospital discharge. The elderly and in particular the patients with pre-existent CKD, have a high risk of reaching end-stage renal disease.

SA-PO090

Long-Term Outcome of Patients Followed by Nephrologist after an Acute Tubular Necrosis Episode Daniela Ponce, Germana Alves Brito, Juliana Abrao, Pasqual Barretti, André Balbi. *Internal Medicine, Botucatu School of Medicine - University Sao Paulo State, Botucatu, Sao Paulo, Brazil.*

Background: Acute Kidney Injury is associated with high mortality in-hospital. However, data from long-term survival and recovery of renal function are controversial. The aims of our study were to analyze the long-term survival and recovery of RF in patients surviving an acute tubular necrosis (ATN) episode followed by the same nephrologist and determines factors associated with long-term mortality.

Methods: We performed a prospective cohort study from October 2004 to May 2011 that evaluated the long term outcome of patients surviving an ATN episode. Variables were analyzed at the time of the acute episode and during follow-up.

Results: We followed 212 patients, of whom 34.4% were diabetics, 39.1% had previous CKD, 38.6% had cardiovascular disease, and age was 59.2 years (48-71) Mortality at the end of follow-up was 24.5% and 19.3% lost the follow-up. Fifty percent of the patients had a follow-up of 24.4 months (9-39). The probability of these patients being alive 5 years after discharge was 55%. During the follow-up, 4.7% of patients needed for chronic dialysis. Only patients with glomerular filtration rate < 60mL/min at the first assessment progressed to dialysis after 12 months. Univariate analysis showed that previous CKD (*log rank* = 9.14, p = 0.0079), cardiovascular disease *(log rank* = 8.18, p = 0.019), older than 60 years (*log rank* = 1.05, p <0.0001) and higher SCr baseline (*log rank* = 2.18, p = 0.001) after 12 months (*log rank* = 1.43, p = 0.0015) and 36 months of follow-up (*log rank* = 1.58, p = 0.004) were predictors of long-term mortality. In multivariate analysis, older age (HR = 6.4, CI 95% = 1.2-34.5, p = 0.02) and higher SCr after 12 months of follow-up (HR = 2.1, 95% CI 95%= 1.14- 4.1, p = 0.017) were identified as risk factors associated with long-term mortality.

Conclusions: Fifty five percent of patients surviving an ATN episode were still alive and less than 5% required chronic dialysis 60 months later and age and increased Scr after 12 months of follow-up were identified as risk factors associated with late death. Finally, the survivors of AKI deserve a careful and long-term medical follow-up.

SA-PO091

A Randomized Clinical Trial of High Volume Peritoneal Dialysis versus Extended Daily Hemodialysis for Acute Kidney Injury Patients Daniela Ponce, Germana Alves Brito, Juliana Abrao, Bianca Albino Balarin, Pasqual Barretti, André Balbi. *Internal Medicine, Botucatu School of Medicine - University Sao Paulo State, Botucatu, Sao Paulo, Brazil.*

Background: Acute kidney injury (AKI) requiring dialysis in critically ill patients is associated with an in-hospital mortality rate of 50 to 80%. Extended daily hemodialysis (EHD) and high volume peritoneal dialysis (HVPD) have recently emerged as alternative modalities.

Methods: A double center, randomized, controlled trial was conducted comparing EHD vs. HVPD for the treatment of AKI in the intensive care unit (ICU). One hundred forty three patients were randomized and analyzed. Principal outcome measures were hospital mortality, recovery of renal function and metabolic and fluid control.

Results: There was no difference between the two groups in relation to median ICU stay (11 (5,7-20) vs. 9 (5,7-19)), recovery of kidney function (26.9 vs 29.6%, p= 0.11), need for chronic dialysis (9.7 vs 6.5%, p=0.23) and hospital mortalitiy (63.4 vs. 63.9%, p=0.94). The groups were different in metabolic and fluid control. Blood urea nitrogen (BUN), creatinine and bicarbonate levels were stabilized faster in EHD group than in HVPD group. Delivered Kt/ V and ultra filtration were higher in EHD group. Despite randomization, there were significant differences between the groups in some covariates, including age, pre dialysis BUN and creatinine levels, biased in favor of the EHD. Using logistic regression to adjust for the imbalances in group assignment, the odds of death associated with HVPD was 1.4 (95% CI, 0.7 to 2.4, p=0.19). A detailed investigation of the randomization process failed to explain the marked differences in patient assignment.

Conclusions: Despite faster metabolic control and higher dialysis dose and ultra filtration with EHD, this study provides no evidence of a survival benefit of EHD compared with HVPD. This study did not control for other supportive management strategies as nutrition support and timing of initiation that might influence outcomes in AKI.

SA-PO092

A Risk Prediction Tool to Predict Death or Dialysis after Admission with Severe Rhabdomyolysis Gearoid M. McMahon, Sushrut S. Waikar. *Nephrology, Brigham and Women's Hospital, Boston, MA.*

Background: Rhabdomyolysis occurs due to damage to muscle and can cause severe acute kidney injury (AKI). The epidemiology of rhabdomyolysis is not well characterized, and risk stratification of patients is not commonly performed. We aimed to develop a simple risk prediction tool to identify patients at risk for poor clinical outcomes.

Methods: We obtained laboratory and clinical data from the medical records of all patients at two teaching hospitals in Boston, MA with CPK levels >5000 U/L within 3 d of admission between 2000 and 2011. We excluded patients <18 years old or with end stage renal disease on admission. We determined the etiology of rhabdomyolysis by review of discharge summaries. Using multivariable logistic regression modeling, we developed a prediction model for the composite outcome of AKI requiring dialysis or in-hospital death. We used data from Massachusetts General Hospital (MGH) as the derivation cohort and data from Brigham and Women's Hospital (BWH) as the validation cohort.

Results: The final dataset included 2,910 patients (1,713 MGH, 1,197 BWH). Mean age was 52±18 yrs and 74.6% were male. 55.2% were surgical patients. 44% developed AKI as defined by the Acute Kidney Injury Network criteria while death or dialysis occurred in 19.3%. The commonest clinical settings were cardiac disease (26.5%) followed by trauma (21.4%) and immobilization (14.7%). The lowest risks of death or dialysis were noted in patients presenting following exercise (3.3%), myopathies (5.3%) or seizures/ syncope (6%). The final prediction model included age, sex, etiology and quintiles of initial calcium, phosphate, creatinine and bicarbonate. The C-statistic for the model was 0.82 in the MGH cohort and 0.86 in the BWH cohort. The model was well calibrated according to the Hosmer-Lemeshow test (p=0.17 and p=0.12 respectively). Based on the model, a score was generated for each patient. A score of <1 identified patients with a risk of <1.5% compared to 68% for a score >4.

Conclusions: We developed and validated a simple risk calculator using commonly available clinical and laboratory variables to accurately predict the risk of death or dialysis in patients admitted with severe rhabdomyolysis.

SA-PO093

The Effect of Renin-Angiotensin-Aldosterone System Blockade on Contrast-Induced Nephropathy in Patients with Chronic Kidney Disease Jisuk Han, Jwa-kyung Kim, Myung Jin Choi, Min-Gang Kim, Young Rim Song, Sung Gyun Kim. *Internal Medicine, Hallym University Sacred Heart Hospital, Kidney Research Institute, Republic of Korea.*

Background: The aim of the present study was to assess the influence of renin-angiotensin-aldosterone system (RAAS) blockade on the development of contrast-induced nephropathy (CIN) in patients with chronic kidney disease (CKD).

Methods: A retrospective analysis was conducted on a total of 239 patients with CKD stage 3 and 4 who underwent contrast-enhanced computed tomography from May 2011 to May 2012. Patients were stratified according to pre-contrast ACEI or ARB therapy. CIN was defined as an increase in serum creatinine (SCr) of at least 25% or 0.5mg/dL from baseline value within 72 hours of radio-contrast exposure.

Results: Of the total patients, 101 (43.0%) were treated with RAAS blockade: 17 with ACEI (7.1%), 83 with ARB (34.7%) and 1 with both (0.4%). There were no significant differences in age (p=0.068), creatinine (p=0.309), gender (p=0.174), diabetes mellitus (p=0.093), use of prophylactic N-acetylcysteine (p=0.857) between patients with or without RAAS blockade. However, patients with RAAS blockade had higher prevalence of statin use (p=0.048) and previous history of coronary artery disease (CAD, p=0.026) and hypertension (p<0.001). CIN occurred in 16 patients (6.7%) and the incidence was significantly higher in patients with RAAS blockade than without (10.7% vs. 1.3%, p=0.020). Post contrast SCr of patients with RAAS blockade were higher than patients without (1.49±0.09 vs. 1.28±0.06, p=0.069). Patients treated with RAAS blockade had 9.3-fold increased risk for CIN and the risk was higher for patients at CKD stage 4 than those at stage 3. Multivariate analysis showed that history of CAD [odds ratio (OR) 3.37, 95% confidence interval (CI) 1.14-9.94], left ventricular systolic dysfunction (ejection fraction<50 %) (OR 2.62, 95% CI 2.10-5.22), nonuse of N-acetylcysteine (OR 10.55, 95% CI 6.18-33.12), and pre-contrast RAAS treatment (OR 6.36, 95% CI 1.09-70.45) were significant risk factors for the development of CIN.

Conclusions: The use of RAAS blockade before contrast exposure was associated with the development of CIN in patients with CKD.

SA-PO094

KDIGO Acute Kidney Injury Classification and Outcomes Ligia Costa Battaini, Liliany P. Repizo, Janaina De Almeida Mota Ramalho, Lilian P.F. Carmo, Etienne Macedo. *Nephrology, University of São Paulo, São Paulo, SP, Brazil.*

Background: The two current criteria for diagnosing and staging AKI, RIFLE and AKIN, are widely used and validated; however, they are not uniformly applied across studies. Discrepancy in the determination of baseline and reference SCr and different time frames to detect changes in SCr cause inconsistency in the classification. A new consensus definition has emerged from KDIGO. This definition will help to identify the largest number of AKI patients and provide early intervention. The aim of our study is to diagnose and classify patients according to the new consensus staging and to assess its prognostic value.

Methods: We performed a retrospective study of patients with AKI who were followed by nephrologists in a tertiary center. They were categorized by the SCr criterion of the KDIGO AKI staging at the moment of nephrologist consultation and later at peak SCr or dialysis initiation. Reference SCr was defined as recorded SCr in the period of 6 months before admission or the lowest during hospitalization.

Results: Baseline characteristics and patients outcomes according to maximum KDIGO are shown in table 1. We identified progressive AKI in 63 (39%) patients and observed a higher mortality in this group (63%x41% p0.006). DeltaCr, percentage of elevation of creatinine or maximum creatinine was no different between survivors and non-survivors. MaxKDIGO was associated with increased mortality across stages.

Conclusions: MaxKDIGO category was associated with hospital mortality which was not observed with changes in creatinine.

Table 1

	Maximum KDIGO			
	1(n=28)	2(n=30)	3(n=108)	p
Age, yrs	68.5 (55-72)	69 (51– 78)	56 (46 -71)	
Baseline creatinine	1.55 (0.98-1.85)	1.14 (0.78-1.62)	0.9 (0.66-1.30)	
Gender, male n (%)	14 (50)	19 (63)	66 (61)	0.621
Mechanical ventilation, n (%)	8 (28.6)	8(26.7)	73 (67.6)	<0.001
RRT*, n (%)	0	0	73 (67.1)	<0.001
In-hospital mortality, n (%)	11 (40.7)	9 (31)	59 (56.7)	0.031
Initial KDIGO 1 mortality , n (%)	11 (36)	5 (16)	15 (48)	< 0.001
Initial KDIGO 2 mortality, n(%)	0	4 (17)	20 (83)	< 0.001
Initial KDIGO 3 mortality, n (%)	0	0	24 (44.5)	< 0.001
Maximium Creatinine	2.35 (1.30-3.30)	2.23 (1.75-3.65)	3.56 (2.67-4.68)	

SA-PO095

Acute Kidney Injury Associated with Spice Use: A New Clinical Entity Denyse Thornley-Brown, Gautam K. Bhanushali, Gaurav Jain. *Division of Nephrology, Department of Medicine, Univeristy of Alabama at Birmingham, Birmingham, AL.*

Background: Spice or K2 encompasses preparations of synthetic cannabinoids marketed as incense products, bath additives and air fresheners and used for recreational purposes. These preparations are usually smoked for their cannabis-like effects. They are of increasing popularity as drugs of abuse since they do not appear on routine urine toxicology screens. Geographic clusters of acute kidney injury (AKI) have been reported in the lay press; however, little is known about the clinical features of the disorder.

Methods: This was a retrospective review of patients presenting to University of Alabama Hospital with AKI and a history of spice ingestion over the 12 week period from March 1 to May 31, 2012.

Results: All patients were male, and all were from the same community in Northeast Alabama. Clinical features are summarized in Table 1.

table1 Clinical findings of spice users with AKI

Case	Age (yrs)	Clinical features	Admission creatinine	Peak creatinine	Discharge creatinine	biopsy findings	Urine drug screen
1	20	nausea, vomiting	11.3	13.7	2.9	ATN	negative
2	23	nausea, vomiting	8.2	15.2	11.5	ATN	negative
3	26	nausea, vomiting, diarrhea	11.1	13.3	8.6	ATN	negative
4	30	nausea, vomiting, diarrhea	2.9	2.9	2.4	N/A	negative

Serum creatinine, in mg/dL; ATN, acute tubular necrosis

Initially, all were suspected of having pre-renal azotemia; however their kidney function did not improve with the administration of intravenous fluids. Three of the patients had renal biopsies; all of which showed acute tubular necrosis. Two of the biopsies had the additional finding of calcium oxalate crystals.

Conclusions: Synthetic cannabinoids are associated with AKI, as indicated in this report. Given the widespread use of these agents and the geographic clustering of these cases, it is likely that a nephrotoxin was present in the preparation. Further studies are needed to identify the nephrotoxin(s). Spice nephropathy should be included in the differential diagnosis of unexplained AKI.

Funding: Clinical Revenue Support

SA-PO096

Acute Kidney Injury in Patients with Acute Liver Failure: A Cohort Analysis Maria Joao Melo,[1] Jose Lopes,[1] Marta Pereira,[1] Paula Alexandrino,[2] Jose Velosa,[2] Antonio Gomes da Costa.[1] *[1]Department of Nephrology and Renal Transplantation, HSM; [2]Department of Gastrenterology, HSM.*

Background: The epidemiology of acute kidney injury (AKI) in patients with acute liver failure (AFL) has not been extensively studied.

Methods: The objective of this study was to analyze the incidence, risk factors and prognosis of AKI in a cohort of patients with ALF hospitalized in the Gastrenterology and Hepatology Intensive Care Unit (ICU) of the Hospital de Santa Maria (Lisbon, Portugal) between January 1992 and December 2011. Exclusion criteria were chronic kidney disease on renal replacement therapy, requirement for renal replacement therapy prior to and/or in the first 48 hours after ICU admission, less than 48 hours of ICU stay and/or less than two determinations of serum creatinine (SCr). AKI was defined by an increase in baseline serum creatinine (SCr) ³50% and/or an increase in SCr ³0.3 mg/dl within 48h during ICU stay. Comparisons between patients with and without AKI were performed using the Student's t-test or the χ2-test. Multivariate logistic regression method was used to determine predictors of AKI and in-hospital mortality. A two-tailed P value <0.05 was considered significant.

Results: A total of 73 patients were analyzed. Forty-six patients (63%) had AKI. Patients with AKI were more likely to be Caucasian (P=0.018) and had higher bilirubin levels (P=0.002) at ICU admission. Sepsis was more frequent among patients with AKI (P=0.045) and these patients were more likely to receive vasopressor support (P=0.012) and mechanical ventilation (P=0.019). Bilirubin (adjusted odds ratio (OR) 1.1; 95% confidence interval (CI) 1.03-1.21; P=0.006) and vasopressor support (adjusted OR 4.7; 95% CI 1.03-20.9; P=0.045) were associated with increased risk for AKI. Overall mortality was 47.9% (N=35). Twelve patients (16.4%) underwent hepatic transplantation and none died. Patients with AKI had higher in-hospital mortality than those patients without AKI (82.9% versus 44.7%, P=0.001). AKI was independently associated with in-hospital mortality (adjusted OR 3.9, 95% CI 1.2-13.7, P=0.028).

Conclusions: AKI is a common complication in patients with ALF and it is associated with increased mortality.

SA-PO097

Outcomes of Obstetric Acute Kidney Injury at a University Medical Center of a Developing Country Syed Rizwan Bokhari,[1] Hafiz I. Ahmad,[1] Sara Saeed,[1] Amtullah Zarreen,[1] Sabin Nasir,[1] Ayesha Mahmood Malik,[1] Arif Asif.[2] *[1]Department of Nephrology, Allama Iqbal Medical College, Lahore, Punjab; [2]Division of Nephrology, University of Miami School of Medicine, Miami, FL.*

Background: Acute kidney injury (AKI) continues to be a cause of increased morbidity and mortality in pregnant women. While there have been studies on the incidence and etiology of this complication the current study focused on the outcomes of obstetric AKI.

Methods: Fifty six patients admitted with obstetric AKI (May 2011 to April, 2012) were included in this prospective study. Patients were followed for a period of three months postpartum. Fifteen patients were lost to follow-up and as such were excluded from study. The diagnosis of AKI was based on the classification of the Acute Kidney Injury Network group.

Results: The mean age of the patients was 26±6 years. Twenty-two (53%) patients were multipara and 19(46%) were primigravida. Twenty (48%) patients did not receive any antenatal care, 13(31%) were visited by a traditional birth attendant and only 8(19%) had adequate antenatal care by a physician/gynecologist. Of the 41 patients with AKI, seven presented before 28-weeks and 34 patients after were seen after 28-weeks of gestation. Four patients were found to be in stage I, 4 in stage II and 33 in stage III AKI during hospitalization. The cause of AKI included sepsis in 32 (78%), intrauterine death (IUD) in 24(60%), postpartum hemorrhage in 17(41%), preeclampsia/eclampsia in 7(17%), shock in 15(36%) and coagulopathy in 3(7%) patients. Twenty-eight (68%) patients received hemodialysis therapy during their hospital stay. Three-month follow-up showed complete resolution of AKI in 14 (34%) patients, partial resolution in 7 (17%), ESRD in 10 (24%) and death in 10 (24%) patients.

Conclusions: The current study indicates that a great majority of patients with obstetric AKI require dialysis. Residual renal dysfunction and ESRD are common at a 3-month follow-up. Incidentally, sepsis and IUD were the most common causes. Increased awareness and appropriate antenatal/obstetrical care can have a major positive impact on minimizing morbidity and mortality.

SA-PO098

Regional Citrate Anticoagulation (RCA) for Continuous Venovenous Hemodialysis (CVVHD) in Acute Kidney Injury (AKI) Cancer Patients in the Intensive Care Unit (ICU) Veronica T. Costa e Silva,[1] Juliana Silva Bezerra,[1] James Hung,[1] Henrique Palomba,[1] Elerson Costalonga,[1] Ludhmila Abrahão Hajjar,[2] Ana Paula Leandro Oliveira,[1] Luciane Oikawa,[1] Cilene Muniz Soares,[1] Luis Yu,[1] Emmanuel A. Burdmann.[1] *[1]Nephrology Division, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Intensive Care Department, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Background: The safety and efficacy of RCA have not been studied in AKI cancer ICU pcts.

Methods: We prospectively analyzed all CVVHD performed in AKI adult cancer pcts in the Sao Paulo State Cancer Institute ICU from January 2010 to December 2011. RCA for CVVHD was utilized according to an adapted protocol published by Mehta et al. CVVHD was performed with a Diapact machine (BBraun) with polysulphone hemofilter.

Results: A total of 9303 hours of CVVHD therapy (319 filters) were performed in 152 AKI pcts. Pcts' characteristics were age 60 +/- 15 y, 60% male, 71.5% on vasopressors and 45% on mechanical ventilation. Most (76.3%) patients had solid cancer, 35.7% with metastatic disease (MD) and 29.6% with previous chemotherapy (CT). Sepsis was the most important AKI etiology factor (71.1%). Hospital mortality was 80%. Venous access was temporary triple lumen catheter (11 Fr) in 97% (70% femoral and 27% internal jugular veins). Blood flow was 180 (150 -180) ml/min and citrate dose was 20.4 mmol/hr. Dialysis dose was 29 (21 – 31) ml/Kg/hr. Systemic ionized calcium (SCai) was 4.35 (4.10 – 4.70) mg/dL and hypocalcemia (SCai < 4.0 mg/dL) was observed in 19% of procedures. There was a satisfactory metabolic control: Na 142 (138 – 147) mEq/L; K 4.00 (3.70 – 4.50) mEq/L; Bic 25.2 (21.9 – 29.3) mEq/L. Median Total systemic calcium (CaT)/SCai ratio was 1.88 (1.75 – 2.04). Median filter patency was 24.0 (11.0 – 43.00) hr despite of post-filter ionized calcium level of 1.63 (1.40 – 190). There were no differences in CaT/SCai (P: 0.846) or Bic (P:0.915) between pcts with international normalized ratio above or under 2.0.

Conclusions: RCA is a safe method for CVVHD therapy in AKI ICU cancer pcts. Short filter patency needs to be better evaluated.

SA-PO099

Early Initiation and Optimal Indications of Continuous Renal Replacement Therapy in Acute Kidney Injury Patients with Early Renal Recovery and Early Death Hiroo Kawarazaki. *Department of Nephrology and Hypertension, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.*

Background: The role and/or necessity of short term continuous renal replacement therapy (CRRT) in acute kidney injury (AKI) patients with early renal recovery and early death is equivocal. The objective of this study was to characterize the following AKI patients; (1) those who had recovered renal function within 48 hours of CRRT and (2) those who died within 48 hours of CRRT.

Methods: The study was a multicenter retrospective study of AKI patients hospitalized in 12 different Japanese intensive care units (ICU) who were initiated on CRRT from January, 2010 to December, 2010. Patients under 18 years of age, on chronic dialysis and those who had RRT before ICU admission were excluded. (1) Patients who recovered from AKI after CRRT for less than 48 hours (Early Recovery group; ER) were compared to the rest of the cohort, and (2) patients who died within 48 hours of CRRT (Early Death group; ED) were compared to the rest of the cohort.

Results: Total of 343 AKI patients (65.9% male) were included. Median age was 69 (59-77) years, sequential organ failure assessment (SOFA) score was 11 (8-13) and simplified acute physiology score II was 53 (40-68). There were 52 ER patients and 52 ED patients. Multivariate stepwise regression analysis of parameters at the time of CRRT initiation showed that; (1) early initiation of CRRT (p=0.01), low serum lactate (p=0.002) and high urinary output (p<0.001) were independently associated with early renal recovery and, (2) high SOFA score (p=0.04), low urinary output (p=0.03), low serum albumin (p=0.01) and high serum lactate (p<0.001) were independently associated with early death.

Conclusions: AKI patients with high urinary output and low serum lactate may not necessitate early initiation of CRRT. Furthermore, AKI patients with high SOFA scores, low urinary output, low serum albumin and high serum lactate may have extremely high mortality in spite of CRRT. Appropriate timing and indication for such AKI patients need reassessment.

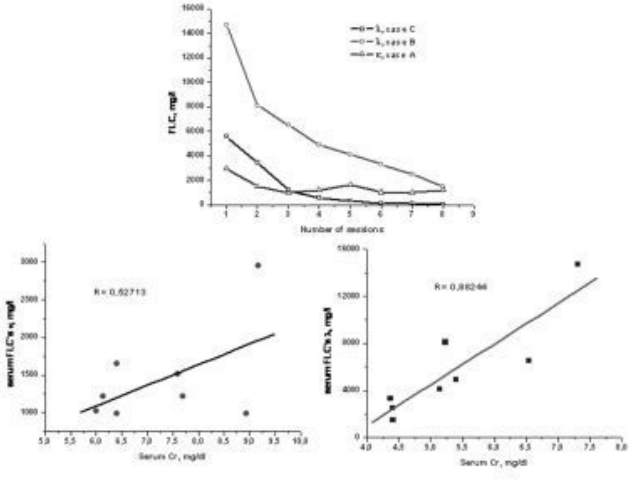
SA-PO100

The Combination of Extracorporeal Free Light Chains Removal and Chemotherapy in Myeloma Acute Kidney Injury: New Therapeutic Options and Old Prognostic Factors Sonia Pasquali,[1] Mattia Corradini,[1] Silvia Mattei,[1] Achiropita Bovino,[1] Francesco Iannuzzella,[1] Danio Somenzi,[1] Alfredo Stefani,[1] Marialuisa Caiazzo,[2] Giuseppe Palladino.[3] *[1]ASMN-IRCCS, Reggio Emilia, Italy; [2]UNIMORE, Italy; [3]Bellco, Italy.*

Background: The combination of high cut-off membranes extracorporeal treatments (ET) and chemotherapy allows sustained reductions in circulating free light chains (FLC) and a high rate of renal function (RF) recovery in patients affected by Acute Renal Failure (ARF) due to Myeloma (M). These ET are well tolerated, but require albumin replacement in about 80% of the cases. We studied a new therapeutic approach with adsorbent resins in order to evaluate its efficacy.

Methods: We treated 3 patients, affected by dialysis-dependent ARF due to biopsy proven FLC M cast nephropathy (λ in 2 cases and k in the remaining case). The M was newly diagnosed in all patients. Within 7 days after the onset of ARF, each patient underwent Bortezomib chemotherapy associated to ET with SUPRA HFR technique, that utilizes separated convection by a high cut-off membrane, diffusion and adsorption with a styrenic resin. The ET, lasting 4 hours each, were repeated for 8 consecutive days and followed every other day.

Results: All the patients showed a significant reduction of serum FCL and a complete recovery of RF after 21, 30 and 44 days from the beginning of the therapy. Serum FLC and creatinine correlations are shown in Figure 1.



The albumin values maintained stable in all the patients.

Conclusions: We confirm the close association between FLC reduction and RF recovery. ET with adsorbent resins represent an effective therapeutic strategy that does not require albumin replacement. In agreement with the literature, timely initiation of the therapy, newly diagnosed M and histological picture of M cast nephropathy represent good prognostic factors, correlated with the reversibility of RF.

SA-PO101

Recurrent Acute Kidney Injury and the Risk of ESRD or Death Bipin R. Bista,[1] Craig Solid,[2] Eric D. Weinhandl,[2] Areef Ishani.[2,3] *[1]University of MN, Minneapolis, MN; [2]USRDS Coordinating Center, MMRF, Minneapolis, MN; [3]Minneapolis VAMC, Minneapolis, MN.*

Background: In patients with acute kidney injury (AKI), the risk of AKI recurrence and long-term implications in terms of ESRD and mortality has not been studied in a large patient population. In this study, we aimed to determine the risk of recurrences of acute kidney injury and the subsequent risk of ESRD and mortality in that patient population.

Methods: We identified 70,710 Medicare beneficiary patients with 85,902 AKI claims in 2005-2006. A cohort of 41,002 eligible elderly patients aged >66 years, discharged with a diagnosis of AKI and without ESRD, were followed for one year.

Results: In this cohort, 22.31% had recurrent AKI within 1 year following the hospital discharge. The risk of development of ESKD within one year of AKI was significantly higher (3.63%) in patients with recurrent AKI as compared to those without (2.44%), with RR 1.50[CI 95%(1.32-1.70); P<0.0001]. The patients with recurrent AKI were also found more likely to die within one year (total incidence of death of 49.5% as compared with 37.0% in patients without recurrence) with RR 1.33[CI 95%(1.32-1.70); P<0.0001]. There were no significant differences in the rate of recurrence among different age groups in this study. However, the risk of recurrence was found to be higher in patients with diabetes [RR 1.35(95% CI 1.30-1.39)], hypertension [RR 1.31(95% CI 1.25-1.37)] and more in CKD patients [RR 1.62 (95% CI1.56-1.68)].

Conclusions: In summary, there was a significant risk of recurrence of acute kidney injury and was associated with worse outcome in terms of ESRD and mortality in a one-year period.

Funding: NIDDK Support

SA-PO102

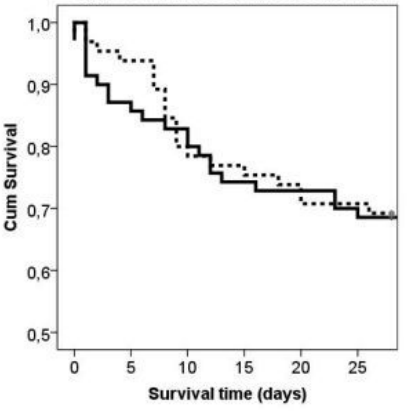
Citrate Anticoagulation versus Systemic Heparinisation in Continuous Venovenous Hemofiltration in Critically Ill Patients with Acute Kidney Injury: A Multicentered Randomized Clinical Trial Louise Schilder, Shaikh Azam Nurmohamed, Marc G. Vervloet, Albertus Beishuizen, Pieter M. Ter Wee, Johan Groeneveld. *VU Medical Center.*

Background: During continuous venovenous hemofiltration (CVVH) in critically ill patients with acute kidney injury, anticoagulation is often administered. The aim was to compare mortality and renal function recovery between systemically administered unfractionated heparin and regional anticoagulation with citrate buffered replacement solution in CVVH. Secondary endpoints were safety and efficacy.

Methods: Adults without a high bleeding risk admitted to the ICU requiring CVVH, were randomly assigned to heparin-CVVH or citrate-CVVH in a predilution fashion. Primary endpoints were patient mortality after 28 days and renal function recovery defined as independence from dialysis after 28 days. Safety was defined as the absence of adverse events necessitating discontinuation of the study anticoagulant. For efficacy, among other variables, survival times of the first hemofilter were studied.

Results: Of the 139 patients, 73 were randomized to heparin-CVVH and 68 to citrate-CVVH. Mortality rates were similar; 35% for heparin-CVVH versus 33% for citrate-CVVH (P=0.92).

Figure 1. Survival times. Dotted line represents citrate.



Renal function recovery did not differ; 66% in heparin-CVVH versus 68% in citrate-CVVH (P=0.85). Heparin was discontinued in 15 patients whereas citrate was discontinued in 4 patients (P=0.001). Manifest bleeding occurred more in the heparin group (P=0.06). Circuit survival times were superior for citrate-CVVH (46 hrs vs 32 hrs, P=0.001), as were the number of filters used (P=0.002) and the offtime within 72 hours (P=0.002).

Conclusions: Patient mortality and renal function recovery did not differ between heparin and citrate anticoagulation in CVVH in the critically ill patient with acute kidney injury. However, citrate-CVVH appeared superior in terms of safety and efficacy.

SA-PO103

Pattern of Acute Kidney Injury(AKI) in a Tertiary Hospital in Sri Lanka Dinith Prasanna Galabada, Abdul Latiff Nazar. *Nephrology, National Hospital of Sri Lanka, Colombo, Western, Sri Lanka.*

Background: Observational studies of acute kidney injury (AKI) and acute-on-chronic renal failure are surprisingly sparse and confounded by differences in definition. There is limited data on the pattern of acute kidney injury in Sri Lanka. It is crucial to know the etiology, prognostic factors and outcome of AKI to promote preventive strategies and to provide adequate resources for the management of AKI.

Methods: Prospective observational study of 201 patients with AKI presented between June to November 2011.

Data was collected using an interviewer administered questionnaire and through a review of medical records.AKI was defined according to RIFLE criteria.

Results: Mean age was 52.8± 21 (range 13 to 91) years, 70% were males. 25% of patients were managed in intensive care units.15% had hospital acquired AKI. Acute on chronic renal failure was present in 18.4%. Sepsis was the most common cause of AKI (37%), followed by leptospirosis (26%). Urosepsis(38%) was the commonest aetiology for sepsis. Commonest co-morbidities were hypertension (32.8%),diabetes (25.9%). 10% of acute kidney injuries were related to post surgical reasons. Hypotension was present in 34%. 69% required renal replacement therapy.

Hospital mortality was 29.5%. 66.5% of patients were dialysis independent on discharge.ICU mortality was significantly higher than the ward mortality.(53.1% vs 18.5%, P <0.0001).ICU deaths were most frequently associated with sepsis(77%).

According to the RIFLE criteria, 83.8 % was in the Failure category. There was nearly linear increase in mortality from Risk to Failure (Risk, 11.1%; Injury, 21.7%; and Failure, 28.3%).

Age, maximum RIFLE category, and hypotension were independent predictive factors of mortality.

Age, hypotension, acute heart failure, disseminated intravascular coagulation and hypertension were independent predictive factors of need for intensive care.

Conclusions: Sepsis and leptospirosis were the leading causes for AKI. Hospital mortality was 29.5%. Majority of patients were in Failure category according to RIFLE criteria. Increasing age, maximum RIFLE category, and presence of hypotension were independent predictors of poor outcome.

SA-PO104

Variation of *NEDD4L* Is Associated with Hypertension in Chronic Kidney Disease Patients Jun Zhang, Cheng Wang, Hui Peng, Zengchun Ye, Tan-qi Lou. *Department of Nephrology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou.*

Background: Neural precursor cell expressed developmentally down-regulated 4-like(NEDD4L), a regulator of the epithelial sodium channel (ENaC), is suggested a candidate gene for essential hypertension. Carriers of an NEDD4L rs4149601 (G/A) AA genotype which result a frameshift mutation of *NEDD4L*, has been shown to be associated with ambulatory BP. However, there is no study to investigate whether polymorphism of NEDD4L rs4149601 contributes to hypertension in patients with chronic kidney disease. The purpose of the current study was to investigatethe relationship between the variation of NEDD4L rs4149601 and hypertension in Chronic Kidney Disease (CKD) patients.

Methods: A total of 307 CKD patients were eligible (aged 18–70 years) and enrolled in our study. The rs4149601 polymorphism was genotyped using real-time PCR based techniques. All patients underwent ambulatory BP monitoring. Clinical data were also collected and eGFR were assessed by simplified MDRD equation. Multivariate logistic regression analysis was used to identify the relationship between polymorphisms and hypertension.

Results: 277 patients carried GG/GA genotype and 30 carried AA genotype. Chi-squared test revealed that rs4149601 AA genotype carriers had significantly higher rate of hypertension than GG/GA genotype carriers(73.3% vs 42.2%, P = 0.02). AA genotype carriers also had a higher day (142±15 vs 133±18, P=0.029) and night (135±21 vs 125±21, P=0.037) mean systolic blood pressure when compared with GG/GA genotype carriers. Multivariate logistic regression models revealed that AA genotype [OR= 3.28, 95% CI (1.09–9.85)], eGFR decline [OR=0.98, 95% CI (0.97–0.99)], old age [OR=1.02, 95% CI (1.01–1.04)] were independently associated with hypertension in CKD patients.

Conclusions: Polymorphism of NEDD4L may be associated with hypertension in CKD patients.

SA-PO105

Renal Denervation Using Standard Irrigated Cardiac Ablation Catheter Is Effective in the Management of Refractory Hypertension in Chronic Kidney Disease Patients Jocemir R. Lugon,[1] Maria A. Carreira,[1] George M. Maia,[2] Tetsuaki Kiuchi,[2] Miguel Luis Graciano,[1] Marcio Galindo Kiuchi.[1] *[1]Medicine, Universidade Federal Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]Surgery, Hospital Regional Darcy Vargas, Rio Bonito, Rio de Janeiro, Brazil.*

Background: Target blood pressure (BP) levels may not be achieved in severe forms of hypertension. Recently, the transcatheter renal sympathetic denervation procedure (TRD) has proved effective in lowering BP in refractory cases. We evaluated the safety and efficacy of TRD using standard irrigated cardiac ablation catheter (SICAC) and its impact on estimated glomerular filtration rate (eGFR) and albuminuria in chronic kidney disease (CKD) patients with refractory hypertension.

Methods: Twelve patients with CKD (NKF stages 2, 3 and 4) and refractory hypertension underwent bilateral TRD with SICAC (Biotronik, Germany). Duplex ultrasound of renal arteries was performed before the procedure. Office BP measurements, eGFR (simplified MDRD equation), plasma renin activity (PRA), serum levels of catecholamines and microalbuminuria were obtained at baseline. The first 30 days of follow-up are reported.

Results: Baseline data (Mean±SEM) were: BP 181.6±4.4 / 108.4±3.4 mmHg; eGFR 58.0±6.4 ml/min/1.73m^2. BP values at 7 and 30 days after the procedure fell to 131.5±2.8 / 84.7±1.6 mmHg and 131.9±2.6 / 85.3±2.3 mmHg, respectively (P<0.0001 for every comparison). There was a mean reduction of 2.9±0.3 (P=0.0028) in the number of BP drugs per patient. Except for bleeding at the site puncture requiring blood transfusion in one patient, no other complication was observed with the procedure. eGFR increased significantly from baseline (58.0±6.4 ml/min/1.73m2) to 30 days (87.3±11.0 ml/min/1.73m2), P=0.0014. No significant change was found in PRA, serum levels of catecholamines, or in microalbuminuria along the evaluation period.

Conclusions: TRD may be performed safely in CKD patients with refractory hypertension using SICAC resulting in better control of blood pressure, reduction in the number of antihypertensive drugs, and a short term improvement in eGFR. Although encouraging, data are preliminary and need to be confirmed in the long term.

Funding: Pharmaceutical Company Support - Biotronik

SA-PO106

The Effect of the Amorphous SDD Formulation of Bardoxolone Methyl on Blood Pressure George L. Bakris,[1] Pablo E. Pergola,[2] Melanie Chin,[3] Susan Potts,[3] Sudarshan Hebbar,[3] Colin John Meyer,[3] Paul Audhya.[3] *[1]ASH Comprehensive Hypertension Center, University of Chicago Medicine, Chicago, IL; [2]Renal Associates PA, San Antonio, TX; [3]Reata Pharmaceuticals, Irving, TX.*

Background: Bardoxolone methyl (BARD) is a synthetic triterpenoid that induces Nrf2 and suppresses NF-κB and has been shown to increase GFR in patients with diabetes and stages 3b/4 CKD. A multicenter, randomized, open-label, adaptive dose-ranging study was conducted to investigate the effects of the amorphous spray-dried dispersion (SDD) formulation of BARD in type 2 diabetic patients with stages 3b/4 CKD. Additional analyses were conducted to assess the effect on systolic and diastolic blood pressures (SBP and DBP).

Methods: Patients (n = 131) were assigned to receive 2.5, 5, 10, 15, or 30 mg of BARD once daily for 12 weeks. Antihypertensives were adjusted by investigators per their standard of care. SBP and DBP values over time were analyzed in the combined and individual dose groups.

Results: Baseline mean (SD) SBP and DBP in the combined dose group was 130.0 (12.1) mmHg and 69.2 (8.9) mmHg respectively; these were similar across individual dose groups. After 12 weeks of treatment, mean SBP and DBP were unchanged from baseline for the combined and each individual dose group.

Change in SBP and DBP (Mean ± SD)

	2.5 mg	5 mg	10 mg	15 mg	30 mg	All Doses
	N = 14	N = 25	N = 28	N = 50	N = 14	N =131
Week 12 SBP Change (mmHg)	0.1 ± 15.59	-1.5 ± 11.46	-2.4 ± 15.31	1.1 ± 15.59	7.1 ± 21.64	0.4 ±15.48
p -value*	0.99	0.54	0.45	0.63	0.28	0.78
Week 12 DBP Change (mmHg)	0.2 ± 6.72	-1.4 ± 7.58	0.3 ± 6.46	-1.0 ± 8.37	3.2 ±7.60	-0.3 ± 7.61
p-value*	0.91	0.38	0.80	0.42	0.18	0.71

* p-value calculated from two-sided t-test comparing Week 12 change to zero.

Conclusions: In this study, treatment with BARD at doses between 2.5 and 30 mg per day had no measurable effect on SBP or DBP in any dose group or timepoint during the study. Further assessments of the effects of BARD on blood pressure will be conducted in the BEACON trial (using 20 mg per day of BARD;NCT01351675), including an ambulatory blood pressure monitoring substudy.

SA-PO107

Bardoxolone Methyl and Albuminuria in Type 2 Diabetes Patients with CKD Peter Rossing,[1,2] Melanie Chin,[3] William M. Deen,[4] Sudarshan Hebbar,[3] Susan Potts,[3] Paul Audhya,[3] Colin John Meyer,[3] Hans-Henrik Parving.[2,5] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]HEALTH, Aarhus University, Aarhus, Denmark; [3]Reata Pharmaceuticals, Irving, TX; [4]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA; [5]Department of Endocrinology, University Hospital, Copenhagen, Denmark.*

Background: Bardoxolone methyl (BARD) is a synthetic triterpenoid that promotes the resolution of inflammation by inducing Nrf2 and suppressing NF-κB. A phase 2 trial (BEAM) of BARD in patients with type 2 diabetes and CKD (eGFR of 20-45 mL/min/1.73m^2), reported an increase in eGFR and urinary albumin creatinine ratio (UACR). In this post-hoc analysis, ratios of UACR/eGFR were analyzed to further characterize the relationship between changes in eGFR and urinary albumin excretion.

Methods: Patients (n=227) were randomized to placebo or BARD at a target dose of 25, 75 or 150 mg for 52 weeks. UACR and eGFR (by MDRD) were measured every 4 weeks. ANCOVA analysis was used to compare UACR and UACR/eGFR changes throughout the active treatment period in the combined (all 3 BARD groups) ITT population versus the placebo group.

Results: The mean (SD) eGFR at baseline was 31.2 (0.8) and 32.7 (0.5) ml/min/1.73 m^2 and UACR geometric mean was 62.4 and 77.4 mg/g in the placebo and BARD groups respectively. At week 52, the mean (SD) change from baseline in eGFR was -1.0 (1.0) and +7.5 (1.0) ml/min/1.73 m^2 and the change in UACR (ratio of week 52/baseline (95%CI)) was 1.3 (1.0 to 1.8) and 1.9 (1.6 to 2.3) for placebo and BARD groups, respectively. Adjusting for baseline values, ANCOVA analysis noted a 44% (11 to 87) increase in UACR for treatment with BARD relative to placebo (p<0.01). In contrast, change in UACR/eGFR with BARD was not significantly different from placebo: 15% (-12 to 49) (p=0.30).

Conclusions: Treatment with BARD increases eGFR and UACR. UACR/eGFR ratios were not statistically different between BARD-treated and placebo patients. This suggests that the increase in UACR is potentially due to increased filtration (eGFR).

SA-PO108

Current Status of Anti-Hypertensive Treatment in Hospitalized Chinese Patients with Chronic Kidney Disease: A Multicenter Analysis Shuwen Liu, Guangyan Cai, Xiang-Mei Chen, Ying Zheng. *Department of Nephrology, State Key Laboratory of Kidney Diseases, Chinese PLA General Hospital, Beijing, China.*

Background: Hypertension is the most common comorbidity in patients with chronic kidney disease (CKD), which associate with progression of CKD and the risk of cardiovascular diseases. Currently, there is no nation-wide investigation on the therapy status of hypertension in Chinese CKD patients.

Methods: From November 2009 to January 2010, we performed a multi-center cross-sectional study on hospitalized patients with CKD in 61 hospitals among 31 provinces in Mainland China. The demographic, socioeconomic and clinical data were collected. Blood pressure level, classifications and numbers of antihypertensive agents were recorded. Blood pressure goal was set as less than 130/80mmHg. Multivariable logistic regression analysis was used to explore risk factors associated with uncontrolled and resistant hypertension.

Results: Overall, 6826 eligible patients were analyzed, including 57.4% male. The mean age was 51.0±17.1 years. Calcium channel blockers (CCBs) were the most common used antihypertensive agents in Chinese CKD patients, which account for 78%. The second type was angiotensin II receptor blockers (ARB), which account for 42.2%. Only 16.6% patients took diuretics. 34.7%, 33.3%, 21.1% and 10.9% of patients took one, two, three or more than four kinds of antihypertensive agents, respectively. The prevalence of resistant hypertension was 15.3%. After multivariable adjusted, CKD3 stage (OR 1.3; 95%CI 1.1-1.7), CKD4 stage (OR1.9; 95%CI 1.4-2.4), CKD5 stage (OR 2.7; 95%CI 2.2-3.4), obesity (OR1.4; 95%CI 1.1-1.8), high-salt diet (OR1.2; 95%CI 1.1-1.4), current smoking (OR1.2 95%CI 1.0-1.8), diabetes (OR1.4; 95%CI 1.2-1.7), cardiovascular disease (OR1.5 95%CI 1.3-1.9), cerebrovascular disease(OR1.4; 95%CI 1.0-1.8), and proteinuria (OR1.2; 95%CI 1.0-1.5) were independently associated with resistant hypertension.

Conclusions: In China, blood pressure control rate in CKD patients was suboptimal. Antihypertensive agents with diuretics or combination therapy were low. Adequate antihypertensive treatment and life-style change will improve the control of hypertension in CKD patients.

Funding: Private Foundation Support

SA-PO109

ApoAI Modifications of HDL in Patients with End-Stage Renal Disease Results in a Reduction of Cholesterol Efflux Capacity Mirjam Schuchardt, Tao Huang, Vera Jankowski, Markus Tolle, Markus van der Giet. *Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany.*

Background: The endogenous high density lipoprotein (HDL) particle has pleiotropic protective properties. In patients compositional changes of HDL could be detected and are related to reduced protective function of HDL. The aim of this study was to investigate the apolipoprotein composition and cholesterol efflux capacity of HDL from patients with end-stage renal disease (ESRD) in comparison to healthy subjects.

Methods: HDL was isolated from serum using ultracentrifugation. Apolipoprotein content were measured via Luminex™ technology. HDL protein and lipids were separated using isopropanol/hexane. The protein fraction were separated using high pressure liquid chromatography and the apoAI fraction was analyzed via mass spectrometry.

Results: The apolipoprotein concentration of HDL is different in ESRD patients compared to healthy controls. The patients have an increased level of apoCII and apoCIII. The apoAI content is not significantly different in both investigated groups. The apoB content were measured as control and could not be detected in all investigated HDL samples. A main function of HDL is the cholesterol efflux capacity. Compared to healthy subjects, the patients had a significantly reduced cholesterol efflux capacity. Furthermore, the enzyme activity of lecithine-cholesterol-acyltransferase (LCAT) is significantly reduced in patients with ESRD. ApoAI is the main apolipoprotein of HDL and has a major function in cholesterol efflux and as cofactor for LCAT. Mass spectrometric analysis of the apoAI protein fraction shows oxidative modifications on three amino acids in the apoAI sequence in patients with ESRD. These oxidative modifications could be induced in HDL from healthy controls using hypochloric acid. The modifications are associated with a reduced cholesterol efflux capacity.

Conclusions: ApoAI from ESRD patients has oxidative-modified amino acids. These may be associated with a reduced functionality of HDL, e.g. as seen in this study for cholesterol efflux capacity. The molecular mechanisms has to be further investigated.

SA-PO110

Biologically Active Osteopontin Is an Accurate Biomarker of Type 2 Diabetic Nephropathy in Mexican Americans Susanne B. Nicholas,[1,2] Satyesh K. Sinha,[2] Keith C. Norris,[1,2] Kamyar Kalantar-Zadeh,[3] Dulcie Kermah.[2] *[1]Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Research, Charles R. Drew University of Medicine and Science, Lynwood, CA; [3]Medicine, Harbor- UCLA LA Biomed, Carson, CA; [4]Family Investigation of Nephropathy and Diabetes (FIND) Investigators, UCLA.*

Background: Ethnic minorities have ~2-4-fold higher incidence of type 2 diabetic nephropathy (T2DN), more rapid progression to end stage renal disease, and significantly higher rates of premature cardiovascular mortality compared to non-Hispanic whites. Several factors contribute to disparities in the diagnosis and management of T2DN. Identification of a highly sensitive biomarker that accurately reflects the mechanisms of intrinsic glomerular disease in T2DN could be an important tool to reduce these disparities. We have shown that full-length osteopontin (flOPN) is critical in the development of T2DN in OPN-null mice. Here, we investigate the mechanisms of a biologically active, thrombin-cleaved N-terminal fragment of OPN (ntOPN) in primary mesangial cells (MC), and as a clinical biomarker of T2DN in a subset of Mexican Americans (Mex Am) from the FIND study.

Methods: MC were grown in normal (4mM) and high glucose (25mM) and treated with thrombin (0-7.5U/ml), and an arginine-glycine-aspartate (RGD) peptide, which blocks the RGD-motif of ntOPN and activates signal transduction through integrin-binding, for Western blot analyses. We quantified ntOPN and flOPN levels in plasma and urine of Mex Am subjects (N=101).

Results: Expression of ntOPN is increased with thrombin in high glucose, RGD peptide (100μg/ml) down-regulated TGF-β, ERK/MAPK, AKT, STAT3, fibronectin, collagen I and collagen IV. Plasma ntOPN did not change, but ntOPN-to-creatinine ratio (ntOCR) was 3-fold higher in T2DN compared to diabetics or healthy controls (p<0.001). The area under the receiver operating characteristic curve for ntOCR was 0.9. The true positive and false positive rates were 0.75 and 0.02, respectively. Urine flOPN did not indicate the presence of T2DN in diabetics. ntOCR may accurately diagnose T2DN in Mex Am.

Conclusions: Thus, ntOCR may accurately diagnose T2DN in Mex Am.

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SA-PO111

MicroRNA-21 Modulates Renal Fibrogenesis by Regulating the Expression of Smad7 Phillip Kantharidis,[1] Radko Komers,[2] Mark E. Cooper,[1] Aaron D. McClelland,[1] Karin Jandeleit-Dahm.[1] *[1]Diabetic Complications, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; [2]Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, OR.*

Background: Diabetic nephropathy (DN) is characterised by the accumulation of extracellular matrix (ECM) proteins in the kidney. TGF-β1 is the main pro-fibrotic driver of this process. A number of microRNAs modulated by TGF-b1 have been shown to be important in renal fibrogenesis. Here we demonstrating a role for miR-21 in promoting fibrosis by regulating the TGF-β1 signalling.

Methods: Proximal tubular epithelial cells were analyzed for changes in ECM gene and miRNA expression following exposure to TGF-β1. miR-21 levels were modulated to determine the effect of this miRNA on fibrogenesis. The effect of miR-21 on ECM gene expression, SMAD signalling and in particular SMAD7 was also assessed. The relationship between diabetic nephropathy and miR-21 was also examined in the kidney from streptozotocin-induced diabetic SD rats.

Results: TGF-β1 treatment of PTCs resulted in the typical EMT-like changes. Significanlty increased expression was observed for collagen I and IV, fibronectin and PAI-1.

Realtive Gene Expression in NRK52E Cells

	Collagen I	Fibronectin	PAI-1
Control	1	1	1
TGF-β1	2	3	7
miR-21	1.6	2	1.8
miR-21+TGF β1	4	7	13

TGF-β1 also increased miR-21 expression 4 fold. Ectopic expression of miR-21 also elevated expression of these genes but to a lesser extent than TGF-β1. Interestingly, miR-21 further enhanced the fibrotic effects of TGF-β1. miR-21 also induced SMAD7 mRNA expression, a negative regulator of TGF-β1 signalling. In contrast SMAD7 protein levels

were reduced, consistent with the SMAD7 3'UTR data demonstrating that miR-21 targets SMAD7. Furthermore, miR-21 also increased SMAD3 activity by 50%. Inhibition of miR-21 attenuated the fibrotic action of TGF-β1. miR-21 was also elevated in the kidney of diabetic rats in association with increased ECM gene expression, a finding that is consistent with the in vitro observations.

Conclusions: These data demonstrate an important mechanism by which miR-21 regulates the TGF-β1 signalling pathway and therefore fibrosis, by targeting SMAD7.

SA-PO112

A New Mechanism of Insulin Resistance in Chronic Kidney Disease: Actions of SIRP-α, an Unexplored Phosphatase Sandhya S. Thomas, Liping Zhang, Yanjun Dong, John Kent Lin, William E. Mitch. *Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Insulin resistance in CKD begins at a creatinine ≤2.4 mg/dL blunting anabolic responses to insulin which is identified by a low p-Akt activating proteases and leading to muscle protein breakdown. Although inflammation is an inciting factor, the mechanism leading to impaired insulin signaling is unknown.

Methods: In muscles of CKD mice (BUN>80 mg/dL) vs pair-fed control mice and in myotube studies, defects in insulin function were based on microarray, RT-PCR, immunofluorescence, and western blot.

Results: Microarray analysis revealed upregulation of inflammatory cytokines($P<0.05$), and signal regulatory protein (SIRP) (5.2-fold increase; $P<0.05$ vs. control mice). We hypothesized that this membrane-bound tyrosine phosphatase impairs insulin signaling. 1. SIRP-α in myofibers membranes from CKD mice was substantially greater vs that in muscles of pair-fed control mice. 2. The SIRP-α promoter contains NF-kB binding sites, and a combination of IL-6, TNF-α, INF-γ and LPS increased NF-kB activity in muscle cells. This led to increased SIRP-α mRNA and protein ($P<0.05$) levels. 3. p-Akt was reduced 3-fold($P<0.05$), leading to increased proteolysis via upregulation of E3 ubiquitin ligases (Atrogin-1 & MuRF-1, initiators of proteolysis in the ubiquitin-proteasome system). 4. SIRP-α in myotubes was suppressed($P<0.05$) by transfection of a silencing RNA(SiRNA) vs responses to scrambled siRNA, which increased p-Akt, reducing the expression of E3 ligases($P<0.01$), despite cytokine exposure. 5. SIRP-α overexpression in myotubes lowered p-Akt($P<0.001$) and enhanced proteolysis($P<0.05$). 6. SIRP-α upregulation was also found in db/db mice, another model of muscle atrophy and insulin resistance.

Conclusions: Thus, CKD activates SIRP-α in muscle and impairs insulin signaling. SIRP-α blockade not only improves insulin signaling but also suppresses cytokine-induced muscle protein losses. This novel mechanism may be the pathway linking CKD, inflammation and insulin resistance to muscle wasting. Targeting SIRP-α could prevent muscle wasting in CKD and improve insulin action in other catabolic conditions with impaired insulin signaling.

Funding: Other NIH Support - T32 DK-07656

SA-PO113

Diabetic Nephropathy in Meprin Deficient Mice Elimelda Moige Ongeri. *Biology, North Carolina A&T State University, Greensboro, NC.*

Background: Diabetic nephropathy (DN) is the single most common cause of end stage renal disease (ESRD), and is associated with an increased risk for cardiovascular disease and mortality. Pathological changes seen in DN are a direct consequence of accumulation of extracellular matrix (ECM) proteins, suggesting that the rate of ECM production exceeds removal. Meprins are metalloproteinases that are abundantly expressed in the brush border membrane (BBM) of proximal kidney tubules. Expression of meprins has also been documented in podocytes and leukocytes such as monocytes and macrophages. Meprins are capable of degrading several ECM proteins, and could play a role in reversing the ECM imbalance observed in DN. Meprin β gene polymorphism is also associated with human DN in the Pima Indians, a United States group with an extremely high incidence of type 2 diabetes and subsequent ESRD. Meprin levels in human kidneys are much lower compared to those in mouse models commonly used to study DN. Compared to humans, common mouse models for DN such as the db/db mouse,and STZ models develop modest elevations in albuminuria, serum creatinine, and kidney histological lesions. The objective of this study was to determine if meprin deficiency makes mice more susceptible to DN.

Methods: STZ was used to induce type 1 diabetes in 8-week old male WT and meprin KO mice on a C57BL/6 background. Blood and urine samples were collected at 7, 10, and 18-weeks post STZ-injection. Samples were analyzed for BUN, and the albumin to creatinine ratio determined by ELISA.

Results: Diabetic meprin αβ KO mice had a significantly higher mortality rate compared to WT and meprin α KO counterparts. An important finding was a significantly higher albumin/creatinine ratio in diabetic meprin αβ KO mice when compared to diabetic WT mice. Diabetic meprin αβ KO mice also had higher BUN levels at 7 and 10 weeks post STZ injection, compared to non-diabetic controls. This trend was not observed in WT diabetic mice.

Conclusions: Our data imply that meprin αβ double KO mice have more severe diabetic kidney damage compared to WT mice with normal meprin expression levels. High meprin levels may thus confer partial resistance to diabetic kidney damage in mice.

SA-PO114

Indoxyl Sulfate Exacerbates Renal Anemia by Induction of Hemolysis Following Methemoglobin Formation in Rats Junya Hirata, Hirobumi Asai, Sayaka Mizukami, Misaki Miyamoto, Kazuya Hirai, Mie Watanabe-akanuma. *Kureha Corporation, Tokyo, Japan.*

Background: Renal anemia is common among patients with chronic kidney disease (CKD). Recently, uremic toxins are known to be involved in hypoxia and erythropoietin (EPO) production. In the present study, we investigated the effect of indoxyl sulfate (IS), a representative uremic toxin, on the progression of renal anemia.

Methods: First, male Wistar rats were fed a diet containing 0.3% adenine (w/w) for 4 weeks to establish CKD model. Rats were then given IS in drinking water at 0.025%, 0.05% and 0.1% (w/v) for 4 weeks. Red blood cell (RBC) parameters and serum EPO level were examined periodically. Second, IS was given at 0.1% and 0.25% for non adenine-treated rats as well to clarify the mechanism of IS-induced anemia. The previous parameters, reticulocyte ratio and methemoglobin (Met-Hb) were examined periodically and histopathology of the spleen was performed.

Results: S-Cr and BUN levels significantly increased and the RBC parameters and serum EPO level significantly decreased in adenine-induced CKD model rats. Under CKD condition, RBC parameters decreased further by IS administration, which highly correlated with the serum IS level. These hematological effects of IS were observed in non adenine-treated rats as well. Further investigations revealed that IS increased Met-Hb level in the RBCs. Other than this, congestion and deposition of brown pigments in the spleen were observed in IS-treated rats, indicating an increase of hemolysis. On the other hand, increases in reticulocyte ratio and serum EPO level in IS-treated rats indicated enhanced erythropoiesis.

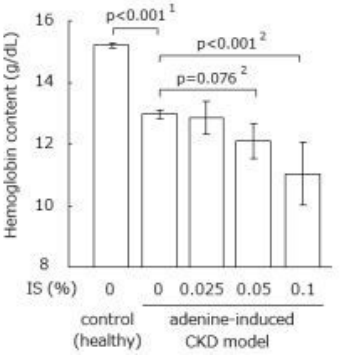

Figure 1.
Hemoglobin content at 1 week after IS administration in adenine-induced CKD model rats.
Values are mean±SD (n=6).
[1], t-test; [2], Dunnett's test.

Conclusions: IS exacerbates renal anemia by induction of hemolysis in the spleen following Met-Hb formation in the RBCs. Therefore, IS could contribute to progression of renal anemia in CKD patients whose serum IS level is increased by the renal dysfunction.

SA-PO115

Urinary Angiotensinogen Is a Significant Predictor of the Need for Erythropoiesis-Stimulating Agent Therapy in Patients with Chronic Kidney Disease Keiichi Takiue,[1] Hitoshi Sugiyama,[1] Hiroshi Morinaga,[1] Masashi Kitagawa,[1] Ayu Ogawa,[1] Toshio Yamanari,[1] Yoko Kikumoto,[1] Tatsuyuki Inoue,[1] Shinji Kitamura,[1] Shigeru Akagi,[1] Yohei Maeshima,[1] Akira Nishiyama,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Pharmacology, Kagawa Medical University, Kita-gun, Kagawa, Japan.*

Background: Patients with chronic kidney disease (CKD) exhibit anemia and the progression of renal dysfunction as a consequence of reduced production of erythropoietin in the kidneys. However, little is known about the associations between urinary biomarkers that predict the progression of anemia and the need for treatment with erythropoiesis-stimulating agents (ESAs) in CKD patients. Therefore, we investigated the relationship between urinary angiotensinogen (AGT) levels and the use of ESA therapy in patients with CKD.

Methods: Using sandwich ELISA analyses specific for human angiotensinogen, we determined the levels of urinary AGT in spot urine samples of patients with CKD (n=90). The associations between the levels of urinary AGT, levels of hemoglobin, estimated glomerular filtration rate (eGFR) and the use of ESA therapy were analyzed during an observation period of two years.

Results: Urinary AGT to creatinine ratios (AGT/Crs) at baseline were significantly higher in patients who received ESA therapy during the two-year period than in those who did not receive ESA therapy ($P = 0.0156$). Urinary AGT/Crs at baseline showed a significant correlation with subsequent decreases in hemoglobin levels, but not with changes in eGFR. Higher levels of urinary AGT at enrollment significantly predicted the need for ESA therapy during the follow-up period ($P = 0.0234$). In a multivariate analysis, urinary AGT/Crs were found to be significant predictors of monthly decreases in hemoglobin levels, even after adjusting the initial levels of hemoglobin and proteinuria ($P = 0.0004$).

Conclusions: These data suggest that urinary AGT levels are significantly associated with the progression of anemia in CKD patients. AGT levels may become a novel biomarker for predicting the need for ESA therapy in patients with CKD.

Funding: Private Foundation Support

SA-PO116

Intravenous Ferric Carboxymaltose versus Standard Medical Care in the Treatment of Iron Deficiency Anemia in Patients with Chronic Kidney Disease: A Randomized, Active-Controlled, Multi-Center Study
Chaim Charytan,[1] Marializa Bernardo,[2] Todd Koch,[3] Angelia Butcher,[3] David D. Morris,[4] David B. Bregman.[3] *[1]New York Hospital Medical Center, NY; [2]Southwest Houston Research, TX; [3]Luitpold Pharmaceutticals, PA; [4]WebbWrites, LLC, NC.*

Background: Ferric carboxymaltose (FCM) is a stable, non-dextran iron formulation developed for rapid intravenous (IV) administration in high doses with controlled delivery of iron into target tissues. The objective of this study was to evaluate the safety of FCM compared to standard medical care (SMC) in hemodialysis (HD) and non-dialysis dependent (NDD) chronic kidney disease (CKD) patients.

Methods: Patient with CKD ages 18 to 85 were enrolled in this study. Iron was administered to NDD-CKD patients (n=204) as an undiluted IV dose of FCM (15 mg/kg to a maximum of 1000 mg) and to HD-CKD patients (n=50) as an undiluted IV push of 200 mg approximately 30 to 60 minutes into the dialysis session. Patients randomized to the SMC group (n=259) received either oral iron, IV iron, or no iron, as determined by the investigator.

Results: Single doses of 200 mg FCM in HD-CKD patients and up to 1000 mg in NDD-CKD patients were well tolerated. Incidences of treatment-emergent adverse events were similar between groups: 30.3% (77/254) in the FCM group and 32.8% (85/259) in the SMC group. Incidences of serious adverse events were higher in the SMC group (8.9% vs. 3.5%. p<0.02). In patients receiving FCM, the most commonly reported related adverse event was nausea and vomiting in NDD-CKD patients, and muscle spasm and hypotension in HD-CKD patients. For the total study population, which consisted of both HD and NDD patients, 25.7% of patients in the FCM group had an increase of ≥ 1 g/dL in hemoglobin vs. 22.1% in the SMC group. This difference was not statistically significant.

Conclusions: FCM was well tolerated, with an adverse event profile, which is in line with previous studies and were expected in this chronically ill patient population. There were no statistically significant differences between the FCM and SMC groups with regard to efficacy endpoints.

Funding: Pharmaceutical Company Support - Luitpold Pharmaceuticals, LLC

SA-PO117

Induction of Erythropoiesis in Anemic Patients by Prolylhydroxylase Inhibitor in a Repeat Dose, Randomized Placebo Controlled Trial
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Background: GSK1278863A is a novel small molecule that has demonstrated *in vitro* and *in vivo* inhibition of the hypoxia-inducible factor prolyl hydroxylases.

Methods: Study PHI112844 was a randomized, single-blind, placebo-controlled, parallel-group study conducted in two parallel populations to evaluate the safety, pharmacokinetics (PK), and efficacy of 28-day repeat oral doses of GSK1278863A. Population 1 subjects were anemic non-dialysis, Stage 3, 4 and 5 chronic kidney disease (CKD) patients. Population 2 subjects were anemic hemodialysis (HD)-dependent Stage 5 CKD patients. Subjects in both populations were dosed once daily for 28 days with doses of 10, 25, 50 and 100 mg (Population 1) or 10 and 25 mg (Population 2) GSK1278863A or placebo.

Results: Oral administration of GSK1278863A was generally well tolerated with most AEs occurring at higher doses. A two compartment first-order oral absorption model with lag time was used to describe the PK data. The only significant PK covariate was a difference in apparent central volume of distribution between Population 1 and 2. Dose-dependent changes across hematologic and iron metabolism biomarkers were observed. The changes include increased erythropoietin, reticulocyte count, and hemoglobin with increasing GSK1278863A dose. Across all GSK1278863A treatment groups, multiple subjects were discontinued (early termination) due to high hemoglobin rate of rise or high absolute hemoglobin concentration per discontinuation criteria. Hepcidin was observed to decrease from baseline in a dose dependent manner. VEGF pre-dose and post-dose mean changes from baseline patterns were inconsistent with large variability and no clear separation between placebo and the GSK1278863A groups.

Conclusions: These data indicate that GSK1278863A was able to induce erythropoiesis in CKD patients with anemia.

Funding: Pharmaceutical Company Support - GlaxoSmithKline

SA-PO118

Modulation of Hepcidin by Prolylhydroxylase Inhibitor in Randomized Trials
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Background: Increases in hypoxia-inducible factor can suppress the production of hepcidin. GSK1278863A is a novel small molecule that has demonstrated *in vitro* and *in vivo* inhibition of the HIF prolyl hydroxylases.

Methods: Study PHI112844 was a randomized, single-blind, placebo-controlled, parallel-group study conducted in two parallel populations to evaluate the safety, pharmacokinetics, and efficacy of 28-day repeat oral doses of GSK1278863A. Both anemic non-dialysis, Stage 3, 4 and 5 chronic kidney disease (CKD) patients and anemic hemodialysis (HD)-dependent Stage 5 CKD patients were studied. Subjects in both populations were dosed once daily for 28 days with doses of 10 to 100 mg GSK1278863A or placebo. Plasma samples were collected both pre- and post-dose on days 1, 15 and 22 and on days 29 and 57 for hepcidin analysis.

Results: A dose dependent decrease in hepcidin from baseline was observed without any diurnal variation (40-93% decrease). In contrast, the placebo groups were unchanged, also with no diurnal variation. The anemic HD population had higher baseline hepcidin compared to the anemic non-dialysis population. This was consistent with data observed previously with HD and non-dialysis (stage 3 and 4) subjects in study PHI112843, asingle dose, 2-period crossover study. CKD patients had higher baseline hepcidin than matched healthy subjects (HS) in study PHI112843 and healthy males in study PHI112842, a 14-day placebo controlled repeat dose study. Data in the two previous studies also indicate that a single dose of GSK1278863A suppressed hepcidin by 24 hours post-dose with a greater magnitude observed at higher doses in all populations and also with greater magnitude in CKD patients versus HS.

Conclusions: These data indicate that GSK1278863A was able to induce hepcidin suppression in patients with anemia.

Funding: Pharmaceutical Company Support - GlaxoSmithKline

SA-PO119

Hepcidin Determinants and Its Relation with Anemia in Chronic Kidney Disease
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Background: Hepcidin plays a central role in iron metabolism and its level is markedly altered in chronic kidney disease (CKD). We studied its determinants and relation with CKD anemia in 199 CKD stage 2-5 patients, not treated by erythropoietin stimulating agent nor by IV iron.

Methods: All had glomerular filtration rate measured by ^{51}Cr-EDTA renal clearance (mGFR) together with measurements of erythropoietin (Quantitine IVD Epo double-antibody sandwich ELISA method, Minneapolis, MN) and hepcidin (immunochemical assay, Amgen, Thousand Oaks, CA). In all analyses, hepcidin was square-root transformed to meet normality criteria.

Results: Hepcidin levels varied from 0.2 to 193 ng/mL (median 27.9 ng/mL IQR 16.4-45.7). Women under 55 years had lower values (n=35, 15.6ng/mL IQR 8.8-40.2). Hepcidin increased from 23.3 ng/mL [8.8-28.7] to 36.1 ng/mL [14.1-92.3] with mGFR decline from ≥60mL/min/1.73m² to <15mL/min/1.73m²(p=0.02). Higher body mass index, albuminemia, C-reactive protein, ferritin,TSAT levels and oral iron therapy were associated with elevated hepcidin, while WHO-anemia, lower EPO and transferrin iron binding capacity (TIBC) values were associated with a decreased level. Absolute iron deficiency (ID) defined by TSAT <20%, TIBC≥50µmol/L, ferritin <40 ng/mL was associated with profound hepcidin collapse (5.0 ng/mL [0.7-11.7], n=12). In multivariate analysis, women under 55 years, absolute ID and higher EPO levels were associated with decreased hepcidin levels, while higher albuminemia, CRP and mGFR were associated with increased levels. Each ng/mL increase of hepcidin was associated with 0.01 ± 0.003 g/dL decrease in Hb (p=0.005), independent of diabetes, gender, mGFR and iron status.

Conclusions: Several factors including iron profile, EPO level, mGFR, nutrition and inflammation may affect hepcidin levels in CKD. Despite increasing level with mGFR decline, profound hepcidin collapse remains in patients with absolute iron deficiency. This study also showed a lack of down-regulation of hepcidin with Hb decrease suggesting a causal role of hepcidin in CKD anemia.

Funding: Government Support - Non-U.S.

SA-PO120

Neutrophil Gelatinase-Associated Lipocalin in Chronic Heart and Renal Failure Correlates with Endogenous Erythropoietin Levels and Decreases in Response to Low-Dose Erythropoietin Treatment
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Background: In two recent studies, Neutrophil-gelatinase associated lipocalin (NGAL) levels were associated with disordered iron metabolism in haemodialysis patients. Iron metabolism plays a pivotal role in the pathophysiology of combined chronic kidney disease (CKD) and chronic heart failure (CHF). We investigated whether serum NGAL levels reflect iron metabolism in patients with CHF/CKD and whether treatment with erythropoietin (EPO) modulates serum NGAL levels.

Methods: In the EPOCARES trial (ClinTrialsNCT00356733) serum NGAL, hepcidin-25, transferrin saturation (TSAT), reticulocyte hemoglobin content (Ret-He) and endogenous EPO levels were measured in 56 patients with combined CKD (Cockroft-Gault 36±15 ml/min) and CHF. Subjects were randomized to receive EPO 50IU/kg/week (n=37) or not (n=19).

Results: Serum NGAL levels correlated positively with cystatin C (r=0.767, p<0.001) and showed an inverse correlation with Cockcoft-Gault (r=-0.571, p<0.001) and baseline EPO levels (r=-0.395, p=0.003). There was no correlation with TSAT (r=-0.316, p=0.32), Ret-He (r=-0.119, p=0.39) and hepcidin-25 levels (r=0.147, p=0.28). After two weeks, NGAL levels decreased in the EPO group (182 [122-219] vs baseline 189 [133-265] ng/mL,

p=0.01), while there was no change in the no-EPO group (256 [111-357] vs baseline 238 [129-313] ng/mL, p=0.31). The magnitude in NGAL decrease in the EPO group correlated with baseline EPO levels (r=0.431, p=0.01).

Conclusions: In combined CKD and CHF, elevated serum NGAL levels do not correlate with parameters of iron metabolism, as assessed by TSAT, hepcidin-25 and Ret-He, and hence might reflect tubular damage. NGAL levels inversely correlated with baseline EPO levels and decreased in response to short-term low-dose EPO treatment.

Funding: Pharmaceutical Company Support - Roche Pharmaceuticals, the Netherlands; Alere Inc., San Diego, CA, USA, Government Support - Non-U.S.

SA-PO121

The Erythropoietic Effect of Low Fixed Dose Erythropoietin and Red Cell Turnover Are Related to Quality of Life and Cardiac Function in Cardiorenal Patients Carlo A. Gaillard,[1] Mireille E. Emans,[2] Maarten Jan M. Cramer,[2] Adry Diepenbroek,[1] Karien Van der Putten,[3] Pieter Doevendans,[2] Branko Braam.[4] *[1]Nephrology, VU Medical Centre, Amsterdam, Netherlands; [2]Cardiology, UMC Utrecht, Netherlands; [3]Nephrology, LUMC, Leiden, Netherlands; [4]Nephrology, University of Alberta, Canada.*

Background: The EPOCARES study was designed to differentiate erythropoietic from non-erythropoietic effects of low fixed dose erythropoietin (EPO) in combined chronic heart and kidney failure (CHF/CKD). Here we report cardiac function, quality of life (QoL) and exercise capacity.

Methods: Fifty-six anemic patients (median age 74 years) with CKD (Cockroft-Gault 36±15 ml/min) and CHF, on iron supplementation, were randomized into three groups: two groups received 50 IU/kg/week EPO during 26 weeks. In one group hemoglobin levels (Hgb) were allowed to increase (EPO-Hgb-rise); in the other group Hgb-levels were maintained at baseline level by phlebotomy (EPO-Hgb-stable). The control group did not receive EPO. The area under the curve (AUC) for the Hgb-change in time was calculated to assess the Hgb-response.

Results: The AUC after 26 weeks in the EPO-Hgb-rise group was 19.8 ± 17.1, in the EPO-Hgb-stable group 5.9 ± 9.6 and in the control group -2.2 ± 9.4 (rise vs stable group, p=0.009). Only the EPO-Hgb-rise group demonstrated an increase in QoL (RAND36 from 54.9 ± 13.8 to 63.6 ± 15.3, p=0.03) and ejection fraction (from 42 ± 8 to 45 ± 10%, p= 0.048) and a decrease in NT-proBNP levels (from 1373 [524-2151] to 941 [452-1721] pg/mL, p= 0.04). There was no significant change in the EPO-Hgb-stable and the control group. RDW levels, a measurement of anisocytosis, which may reflect red cell turnover, at baseline correlated negatively with QoL and exercise capacity (RAND-36 r=-0.363, p=0.008; pVO_2/kg r = -0.586, p< 0.001) and increased in both EPO groups. No significant changes were detected in parameters of inflammation.

Conclusions: In combined CHF/CKD low fixed dose EPO-induced improvement of cardiac function and QoL occurred only in patients in which Hgb-levels were allowed to increase. These data support the contention that low dose EPO to cardiorenal patients may have a positive impact on heart function and QoL.

Funding: Pharmaceutical Company Support - Roche Pharmaceuticals, the Netherlands, Government Support - Non-U.S.

SA-PO122

Management of Renal Anemia in Patients with CKD Stages 3-5 Not on Dialysis Treated with C.E.R.A. in Catalonia Nephrology Units (The MICENAS II Study) Alberto M. Martinez-Castelao,[1] Aleix Cases,[2] Joan Fort,[3] Jordi Bonal,[4] Xavier Fulladosa,[1] J.M. Galceran,[5] J. V. Torregrossa,[2] Elisabeth Coll.[6] *[1]H. Bellvitge, Spain; [2]H. Clínic Barcelona, Spain; [3]H. Vall Hebron, Spain; [4]H. Germans Trias i Pujol, Spain; [5]Fundacio Althaia, Spain; [6]Fundacio Puigvert, Spain.*

Background: Therapeutic management of renal anemia with CERA in patients with CKD stages 3-5 not on dialysis in Catalonia is unknown.

Methods: Observational retrospective study conducted in Catalonia Nephrology Units. CKD anemic patients stages 3-5 not on dialysis, or 5-T, aged≥18years, treated with CERA during at least 6 months, who gave informed consent were recruited. Ferropenia (ferritin<100ng/mL or TSAT<20%) and Hb target at 6 months (Hb≥10.5g/dL) were assessed.

Results: 331 patients were recruited by 15 investigators, 267 valid for analysis. 52.1% were women, mean age 66.6±17.4years, 38.5% were kidney transplants. CKD stage distribution was 3:40.4%; 4:46.8% and 5:12.7%. 51.3% had been previously treated with ESA (naïve:48.7%); most common previous ESA was epoetin-beta(56.0%). At the beginning of CERA, mean Hb were 10.2±0.9 and 11.5±1.2g/dL for naïve and conversion, respectively(p<0.05); mean CERA initial dose/month 85.4±36.5 and 87.2±34.8µg and at 6 months 81.2±44.4 and 91.5±46.8µg (p>0.05). Likewise, no differences were found in mean CERA doses among transplanted and non transplanted. Regarding previous ESA and respective ranks of weekly dose administered:<8000UI;8000-16000UI;>16000UI; CERA dose administered monthly in conversion were 72.5±18.4; 79.5±22.7; 105.0±49.1µg, respectively. 50.0% of naïve patients started treatment at dose recommended by CERA Summary of Product Characteristics(SPC) (1.2±0.6µg/kg/month). At initiation with CERA 25.1% had ferropenia, only 49.3% of whom received iron. Mean Hb after 6 months with CERA showed a significant increase in naïve (1.5±1.5g/dL;p<0.05) and stable values in conversion (0.2±1.5g/dL;p>0.05). At 6 months 77.8% reached target Hb regardless of previous use of ESA (naïve:78.3%;conversion:77.4%).

Conclusions: Monthly use of CERA corrects anemia in ESA naïve CKD patients. In conversion lower CERA doses than those recommended by SPC were required to achieve target Hb.

Funding: Pharmaceutical Company Support - Roche Farma, S.A.

SA-PO123

Anemia Management in Chronic Kidney Disease Patients Not on Dialysis in Clinical Practice Following the Recommendations from the Anemia Working Group of European Renal Best Practice (ERBP) Alberto M. Martinez-Castelao,[1] Aleix Cases,[2] Javier Torralba-iranzo,[3] Alberto Torre-carballada,[4] Daniel Toran,[5] Carmen Bernis,[6] Fernando Vallejo Carrión.[7] *[1]H Bellvitge, Spain; [2]H Clinic, Spain; [3]H Alicante, Spain; [4]H La Paz, Spain; [5]H Jerez, Spain; [6]H La Princesa, Spain; [7]H Puerto Real, Spain.*

Background: According to the TREAT study results, the Anemia Working Group of ERBP recommends maintaining Hb levels within 11-12g/dL, not intentionally exceeding 13g/dL and considering doses of ESA therapy to achieve the Hb target range. This study was designed to evaluate the impact of this statement in clinical practice.

Methods: Multicenter cross-sectional observational study in patients with anemia secondary to chronic kidney disease (CKD) not on dialysis who initiated anemia treatment (naïve) or converted from other ESAs from January 2011. Preliminary results at baseline after correction and conversion are presented.

Results: 182 patients were evaluated (naïve 59.3%;conversion 40.7% [mean ESA treatment duration 18.9±16.6months]). Mean age 72.9±12.6years. CKD 3/4/5 29.4%/44.6%/23.2%. Predominant etiologies 34.1% vascular and 23.6% diabetes. At baseline, 33.7% of naïve patients attained Hb range of 11-12g/dL(40.3% Hb<11g/dL,26% Hb>12g/dL) with mean Hb of 11.3±1.4g/dL vs starting treatment(9.9±0.7g/dL); 38.1% of those converted maintained Hb levels within the target range(14.3% Hb<11g/dL,47.6% Hb>12g/dL). Prior to study initiation, 91% of naïve patients had started treatment with Mircera®(mean monthly dose 58.7±26.3µg) and 67% of those on maintenance had converted to Mircera®(mean monthly dose 84±37.9µg) being a less frequent administration the main reason for ESA change(65.8%). At baseline, 89% naïve and 84.5% converted patients were on Mircera®(mean monthly dose 52.3±24.5µg, 79.8±40.8µg) and also oral iron therapy(72.7%,80%,respectively). 2 naïve patients required transfusions during the correction period.

Conclusions: An appropriate anemia management in CKD patients not on dialysis can be obtained by the achievement Hb levels of 11-12g/dL according to the ERBP Group recommendations. Hb target range is attained and maintained in most patients receiving once-monthly Mircera®.

Funding: Pharmaceutical Company Support - Roche Farma, S.A.

SA-PO124

Decisive Indicator for Gastrointestinal Workup in Anemic Patients with Non-Dialysis Chronic Kidney Disease Eun Oh Kim,[1] Youn Mi Song,[2] Eun Sil Koh,[1] Sungjin Chung,[1] Sangju Lee,[1] Yoon-Kyung Chang,[1] Suk Young Kim,[1] Hyeonseok Hwang.[1] *[1]Division of Nephrology, Departments of Internal Medicine, College of Medicine, the Catholic University of Korea, Daejeon, Korea; [2]Division of Gastroenterology, Departments of Internal Medicine, College of Medicine, the Catholic University of Korea, Seoul, Daejeon, Korea.*

Background: Anemia and iron deficiency are universal problems in patients with chronic kidney disease (CKD). However, decisive indicators to guide the diagnosis of bleeding-related gastrointestinal (GI) lesions have not been determined.

Methods: This diagnostic test study included 104 anemic patients with nondialysis-dependent CKD stages 3–5 (38 patients at stage 3, 26 patients at stage 4, and 40 patients at stage 5), who were naïve for iron and erythropoiesis-stimulating agents. Lesions causing GI blood loss were identified by esophagogastroduodenoscopy and colonoscopy. Hemoglobin, serum ferritin,transferrin saturation (TSAT), mean corpuscular volume (MCV), and corrected reticulocyte count data were collected to evaluate their diagnostic utility for bleeding-related GI lesions.

Results: Bleeding-related GI lesions were found in 55 (52.9%) patients, and patients with stage 5 CKD had a higher prevalence of gastric lesions than patients with CKD stage 3 or 4 (all $P < 0.05$). The areas under the receiver operating characteristic curves used to predict bleeding-related lesions were 0.69 for TSAT ($P = 0.002$) and 0.61 for serum ferritin ($P = 0.09$). Hemoglobin, MCV, and corrected reticulocyte levels had no significant diagnostic utility. The sensitivity and specificity of a cutoff value for TSAT of < 20% were 59% and 74%, respectively. On multivariable logistic regression, the chance of GI lesions increased by 6% for each 1% reduction in TSAT and increased 4.1-fold for patients with CKD stage 5 (all $P = 0.01$).

Conclusions: Half the anemic patients with CKD stages 3–5 had bleeding-related GI lesions, and TSAT was a useful decisive indicator of the GI workup. Stage 5 CKD was independently associated with GI bleeding-related lesions and TSAT should be used cautiously in these patients.

SA-PO125

Pathologic Endothelial Response and Impaired Function of Circulating Angiogenic Cells in Patients with Fabry Disease Johan M. Lorenzen,[1] Christoph Wanner,[2] Hermann G. Haller,[1] Thomas Thum.[1] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Nephrology, Würzburg University, Würzburg, Germany.*

Background: Fabry disease is an X-chromosomal recessive deficiency of the lysosomal hydrolase alpha-galactosidase A. This results in an accumulation of globotriaosylceramide (GL-3) in a variety of cells and functional impairment. The impact of Fabry disease on the biology of circulating angiogenic cells (CACs) and endothelial response to transient ischemia was investigated.

Methods: Untreated patients with Fabry disease (n=26), patients after initiation of enzyme replacement therapy (ERT, n=16) and healthy controls (n=26) were investigated. Endothelial function was assessed by the EndoPAT2000 device. CAC numbers were assessed by flow-cytometry, CAC function by a modified Boyden chamber assay. The effect of supernatant of cultured CACs on endothelial cells was assessed by a tube formation and Boyden chamber assay.

Results: Fabry patients showed a pathologic endothelial response, which normalized after enzyme replacement therapy. CACs were increased in number, but functionally impaired. Immunofluorescence and electron microscopy identified an accumulation of GL-3 in Fabry CACs. ERT attenuated CAC dysfunction in Fabry patients via a reduction in GL-3 accumulation in vitro and in vivo. Silencing of alpha-galactosidase A in healthy CACs impaired their migratory capacity underlining a key function of this enzyme. Fabry CAC supernatant impaired angiogenesis and migratory capacity of HUVECs.

Conclusions: Fabry patients show a dysfunction of CAC and a pathologic endothelial response. Enzyme replacement therapy improves CAC and endothelial function and thus may attenuate development of cardiovascular disease in the long-term in this patient population.

Funding: Government Support - Non-U.S.

SA-PO126

Paroxysmal Nocturnal Hemoglobinuria and the Kidney: An Underestimated Complication Julie Sohier Attias,[2] Marie-noelle Peraldi,[1] Martin Flamant,[2] Gérard Socié,[1] Régis Peffault delatour.[1] *[1]Hopital Saint-Louis, Université Paris 7, Paris, France; [2]Hopital Bichat, Université Paris 7, France.*

Background: Paroxysmal nocturnal hemoglubinuria (PNH) is a rare acquired stem-cell disorder characterized by an increased sensitivity of the erythrocytes to complement-mediated intravascular haemolysis. Renal manifestations are recognized since the 1950s, but their prevalence is still unknown. A potential effect of Eculizumab on kidney function is expected but is still not described.

Methods: Renal functional studies were performed in 7 patients suffering classic-PNH. For each patient, Glomerular Filtration Rate (GFR) was measured and tubular function tests were performed both before and after Eculizumab treatment.

Results: Before Eculizumab treatment, six out of the 7 patients had abnormal renal function: one had glomerular hyperfiltration and 5 chronic kidney disease (median GFR of the whole cohort: 78,1 ml/min/1,73m^2; range: 54,6- 133,2). Among the patients with chronic kidney disease, 2 had significant microalbuminuria. Three patients had proximal tubular dysfunction and one had a distal tubular defect. According to the hematologists, Eculizumab therapy was totally effective for 4 patients and partially effectve for 3 patients, with a residual hemolysis. After a median treatment duration of 9 months (range: 5-11 months), 3 patients had a decline of renal function (minus 5,1 ml/min/1,73m^2). The GFR of one patient with no microalbuminuria improved. Two of the 3 patients with persistant hemolysis developed a proximal tubulopathy. The proximal tubular dysfunction disappeared in only one case.

Conclusions: Renal failure in PNH seems to be more common than expected. The pathogenesis may be compared with other hemolytic anaemia such as Sickle Cell Disease. Over a short-time period, Eculizumab does not appear to have major beneficial effects on renal functions.

Funding: Government Support - Non-U.S.

SA-PO127

Complications of Renal Artery Embolization in Patients with Renal Angiomyolipoma: A US National Retrospective Cohort Study John J. Bissler,[1] Peter Sun,[2] Zhimei Liu,[3] Hearns Charles,[4] John C. Hulbert.[5] *[1]University of Cincinnati, Cincinnati, OH; [2]Kailo Research Group, Fishers, IN; [3]Novartis Pharmaceutical Corporation, East Hanover, NJ; [4]Langone Medical Center, New York University, New York, NY; [5]Urologic Physicians, PA, MN.*

Background: This study aims to examine the prevalence rates of complications from renal artery embolization (RAE) in US patients with renal angiomyolipoma (AML).

Methods: Retrospective cohort study design was used with three US national health claims databases (Years: 2000-2010; Covered Population: 60+ millions). Patients with sporadic AML(ICD9-CM: 223.0 but no 759.5) or with tuberous sclerous complex (TSC) related AML (ICD9-CM: 223.0 and 759.5), who had minimal two years of health plan enrollment (one year prior and one year after first observed RAE) were included in the analysis. Based on a literature review and experts' opinions, more than 25 clinical conditions were predefined as potential complications related to RAE. Prevalence rates of these conditions in the post-RAE year were estimated for and compared between the two cohorts using two-sample t-test for proportions.

Results: Patients (N=9,901) had mean age of 49.9 years at their first observed RAE. In the post-RAE year, 5.2% of patients had flank pain, 4.3% had renal insufficiency, 4.0% had fever, 3.9% had nephrotic syndromes, and 5.9% had other diseases related to kidney or ureter. A higher proportion of patients in the TSC AML subgroup had renal insufficiency (5.1% vs. 3.6%; $P<0.05$), while the sporadic AML group had higher occurrence of flank pain (5.5% vs. 4.9%, $P<0.05$) and other diseases related to kidney or ureter (6.4% vs. 5.4%, $P<0.05$). Among all AML patients who had RAE, 4.4% underwent a partial nephrectomy and 9.6% received a complete nephrectomy within the post RAE year.

Conclusions: Flank pain, renal insufficiency, nephrotic syndromes, fever and other kidney or ureter related diseases were among the most common RAE related complications. Future analysis with control for pre-existing conditions and with objective of identify feasible approaches to improve RAE outcomes is warranted.

Funding: Pharmaceutical Company Support - The Study Was Sponsored by Novartis Pharmaceutical Corporation

SA-PO128

Complications of Nephrectomy in Patient with Renal Angiomyolipoma: A US National Retrospective Cohort Study John C. Hulbert,[1] Peter Sun,[2] Zhimei Liu,[3] Judith A. Prestifilippo,[3] Hearns Charles,[4] John J. Bissler.[5] *[1]Urologic Physicians, PA, MN; [2]Kailo Research Group, Indianapolis, IN; [3]Novartis Pharmaceutical Corporation, NJ; [4]New York University, NY, NY; [5]University of Cincinnati, Cincinnati, OH.*

Background: This study aimed to estimate the prevalence rates of nephrectomy related complications in patients with sporadic renal angiomyolipoma (AML) or TSC-associated AML in the United States.

Methods: Our study used a retrospective study design and three US national health claims databases (Years: 2000-2010; Covered Population: 60+ millions). Based on their TSC claim history patients with health insurance for a period one year preceding and one year following their first claim of nephrectomy for AML were selected into TSC-AML cohort or sporadic AML cohort. More than 50 clinical conditions were identified as nephrectomy related complications based on a literature review and expert opinions. Prevalence rates of these conditions in the first post-nephrectomy year were estimated for each cohort and compared across the two cohorts using two- sample t-tests for proportions.

Results: The study included 15,381 AML patients (TSC-AML: 5,191; sporadic AML: 10,190) with a mean age of 52.2 years at the newly observed nephrectomy. Within the post-nephrectomy year, prevalence rates were: digestive system complications, 7.4% (paralytic ileus: 5.9%, others: 3.9%); tension headache, 6.5%; acute posthemorrhagic anemia, 4.0%; incisional hernia, 3.3%; postoperative hemorrhage, 3.0%; paresthesia, 2.8%; diaphragmatic hernia, 2.4%; as well as cerebrovascular diseases and pancreatitis, 1.3%. Some of these rates were slightly higher in the sporadic AML cohort than in TSC-AML cohort.

Conclusions: The most common nephrectomy complications were digestive system complications (including postoperative ileus), tension headache, acute posthemorrhagic anemia, incisional hernia and postoperative hemorrhage. Further research is warranted to identify approaches to improve the clinical outcome of nephrectomy in patients with angiomyolipoma.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

SA-PO129

Pregnancy in Women with Chronic Pyelonephritis/Vesico-Ureteric Reflux (CPN/VUR): Fetal and Maternal Outcomes Sajeda Youssouf,[1] Matt Hall,[2] Liz Lightstone,[3] Graham Graham Lipkin,[4] Nigel J. Brunskill,[1] Sue Carr.[1] *[1]The John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; [2]Nottingham City Hospital, Nottingham, United Kingdom; [3]Imperial College Kidney and Transplant Institute, London, United Kingdom; [4]Queen Elizabeth Hospital, Birmingham, United Kingdom.*

Background: Pregnancy is associated with adverse fetal and maternal outcomes in women with advanced CKD. There is little evidence that the etiology of maternal renal disease has an effect on outcomes but most information is based upon retrospective, historical data. In this study we analyzed fetal and maternal renal outcomes in pregnancy in women with a diagnosis of chronic pyelonephritis/vesico-ureteric reflux at the time of conception.

Methods: Prospective data from three specialist renal obstetric services has been collected since 2003. In this analysis we identified those with a diagnosis of chronic pyelonephritis, recurrent UTI or vesicoureteric reflux at the time of conception and reviewed pregnancy outcomes and maternal renal outcomes in this group.

Results: We identified 57 pregnancies in 48 women aged 16-40. Follow-up post-partum ranged from 6-78 months. CKD stage at time of conception is shown in the table below.

CKD stage	Number of pregnancies	%
1-2	30	53
3a	8	14
3b	4	7
4	2	4
Unknown	13	22

56 pregnancies (98%) resulted in a live birth; there were two early neonatal deaths.

17 pregnancies (30%) had at least one adverse event:

- Low birth weight 24%
- Admission to neonatal intensive care 19%
- Preterm delivery 30%

4 women (7%), had a decline in renal function in pregnancy, including one who developed ESRD during pregnancy. 4 women (7%) had persistent loss of >25% of renal function postpartum, and two developed ESRD; both had CKD 3b at the time of conception. Three women with CKD 1-2 (10%) experienced a decline in renal function post partum, but none developed ESRD.

Conclusions: Fetal outcomes in women with chronic pyelonephritis are better than reported in historical case series of pregnancy in women with CKD, but adverse event rates remain high. There is no evidence that CPN/VUR has an impact on maternal renal dysfunction post-partum, except in those with pre-existing advanced CKD.

SA-PO130

Kidney Disease Is Associated with a Higher Risk of Adverse Fetal Outcomes in Pregnancy Shailendra Sharma,[1] John R. Holmen,[2] M. Chonchol,[1] Gerard John Smits,[1] Jessica B. Kendrick.[1] *[1]University of Colorado School of Medicine, Aurora, CO; [2]Intermountain Healthcare, Salt Lake City, UT.*

Background: Pregnant women with kidney disease have an increased risk of adverse fetal outcomes, however, the degree of this risk is unclear. We tested the hypothesis that pregnant women with kidney disease have an increased risk of adverse fetal outcomes independent of other comorbid conditions.

Methods: Using the Intermountain Healthcare Data Warehouse we identified 646 women with kidney disease who gave birth between 2000 and 2011. 62,757 pregnancies from women without kidney disease were randomly selected for comparison. Kidney disease was defined by ICD9 code. Fetal outcomes were defined as low birth weight (< 2500 gm), number of admissions to the neonatal intensive care unit (NICU) and infant death. We used multivariate logistic regression to examine the association between kidney disease and fetal outcomes.

Results: In the whole study population the mean (SD) birth weight was 3324 ± 578 gm and there were 2,629 admissions to the NICU and 81 infant deaths. Of the women with kidney disease, the mean (SD) age and gestational age at delivery were 28 ± 5 years and 37 ± 3 weeks, respectively. Women with kidney disease had a higher prevalence of diabetes (11.9% vs. 1.4%) and chronic hypertension (27.5% vs. 7.1%) than women without kidney disease. There was a higher rate of infant mortality in women with kidney disease compared to those without kidney disease (12.5% vs. 4.1%). After adjustment for age, race, diabetes, chronic hypertension, liver disease and connective tissue disorders, infants born to women with kidney disease had a higher odds of low birth weight (OR 1.66, 95% CI 1.11-2.48), number of admissions to the NICU (OR 1.97, 95% CI 1.31-2.97) and infant death (OR 1.93, 95% CI 1.28-2.90) compared to infants born to women without kidney disease.

Conclusions: In this cohort study, kidney disease during pregnancy was an independent predictor of low birth weight, admission to the NICU and infant mortality. Further studies are needed to determine if early detection and appropriate management of kidney disease can improve fetal outcomes.

Funding: NIDDK Support

SA-PO131

Immunogenicity of Investigational HEPLISAV Compared with Licensed Hepatitis B Vaccine (Engerix-B) in Patients with Chronic Kidney Disease (CKD) and Additional Factors that Reduce Immune Responses to Vaccine Robert Janssen, Sophia Rahman, Hamid Namini, William Heyward, Tyler Martin. *Dynavax Technologies Corporation.*

Background: CKD patients are commonly hypo-responsive to hepatitis B vaccines. Additional factors such as diabetes mellitus, male sex, older age, and high BMI further reduce immune responses. A multicenter, observer-blind, phase 3 study was conducted among 521 patients 18-75 years of age with CKD (GFR ≤ 45 mL/min/1.73 m^2), comparing 3 doses of HEPLISAV™ (H: 20 mcg rHBsAg + 3000 mcg 1018 ISS adjuvant, a toll-like receptor 9 agonist) given at 0, 1, and 6 months to 4 double-doses of Engerix-B® (EB: 2x20 mcg rHBsAg+500 mcg alum) given at 0, 1, 2, and 6 months. The peak geometric mean concentration (GMC) of antibodies to hepatitis B surface antigen (anti-HBs) was significantly higher in subjects who received H (587.1 mIU/mL) than those who received EB (156.5 mIU/mL). Peak GMC correlates with duration of seroprotection.

Methods: Peak GMCs at Week 28 were analyzed by diabetes status, age, sex, and BMI. Anti-HBs serum levels were measured using the Ortho Vitros® assay.

Results: The table presents peak GMCs at Week 28 in the modified intent-to-treat population (H: 227; EB: 242).

	GMC (mIU/mL) (95% CI) (N)		Ratio of GMCs H/EB (95% CI)[a]
	HEPLISAV	Engerix-B	
Diabetes status			
Diabetes	448.2 (271.4, 740.1) (153)	109.3 (63.1, 189.1) (150)	4.10 (1.96, 8.59)
No Diabetes	1025.6 (483.0, 2178.1) (74)	280.8 (153.1, 515.2) (92)	3.65 (1.41, 9.43)
Age			
40 – 55 years	967.3 (390.8, 2394.4) (51)	159.1 (62.8, 402.9) (48)	6.08 (1.69, 21.90)
56 – 75 years	512.8 (318.2, 826.5) (174)	136.3 (84.7, 219.5) (185)	3.76 (1.92, 7.37)
Sex			
Women	1582.5 (917.8, 2728.5) (81)	217.5 (115.9, 408.1) (99)	7.27 (3.12, 16.97)
Men	338.7 (193.6, 592.4) (146)	124.5 (72.0, 215.4) (143)	2.72 (1.25, 5.93)
BMI			
≥ 30 kg/m²	524.1 (303.7, 904.7) (137)	94.7 (54.1, 165.7) (143)	5.53 (2.54, 12.06)
< 30 kg/m²	697.6 (361.0, 1348.0) (90)	323.1 (180.1, 579.5) (99)	2.16 (0.90, 5.16)

[a]The lower limit of the 95% CI greater than 1.0 indicates a statistically significant difference in GMCs.

Conclusions: In CKD patients with additional factors that reduce their immune response to hepatitis B vaccines, HEPLISAV induced significantly higher GMCs than Engerix-B.

Funding: Pharmaceutical Company Support - Dynavax Technologies Corporation

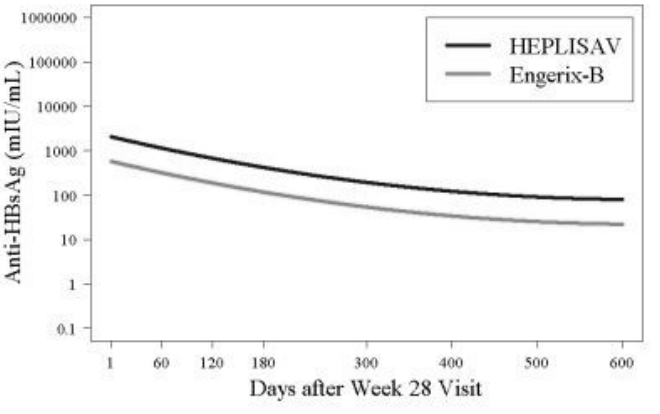
SA-PO132

Antibody Decay up to 1.5 Years after Last Dose of Investigational HEPLISAV Compared with Licensed Hepatitis B Virus (HBV) Vaccine (Engerix-B) in Patients with Chronic Kidney Disease (CKD) Robert Janssen, Fang Xie, William Heyward, Tyler Martin. *Dynavax Technologies, Berkeley, CA.*

Background: Because CKD patients have impaired immune responses, long-term protection against HBV depends on maintenance of antibodies against HBV surface antigen (anti-HBs).

Methods: A multicenter, phase 3 study among 521 adult CKD patients (GFR ≤ 45 mL/min/1.73 m^2) compared 3 doses of HEPLISAV™ (H: 20 mcg rHBsAg+3000 mcg 1018 ISS adjuvant, a toll-like receptor 9 agonist) to 4 double-doses of Engerix-B® (EB: 2x20 mcg rHBsAg). Anti-HBs serum levels were measured using the Ortho Vitros® assay at Weeks 28, 36, 44 and 52. A subset of 78 unselected subjects had one additional antibody level between 0 and 60 weeks after Week 52. After Week 28, antibody decay curves for individual subjects showed a generally linear pattern with antibody levels in log10 scale against time. Linear models using antibody levels in a log scale as the dependent variable, vaccine group as a factor, and time (days from Week 28) as a covariate were constructed with quadratic and interaction terms.

Results: 39 subjects in each vaccine group with anti-HBs ≥ 10 mIU/mL at Week 28 were included in the analysis. Vaccine group and time interaction were not significant variables, indicating a constant decay rate for both vaccine groups. The fitted model was T_i= 3.31-0.555*vaccine-0.00451*t+0.00000359*t^2, where T is the antibody level, t is time (in days) after Week 28 visit, and vaccine=0 for H and 1 for EB. Both vaccine group and time were statistically significant (<0.0001) in the model. The negative estimate for the vaccine term indicates that the superior antibody level of H in comparison to EB is constant across the decay period.



Conclusions: In CKD patients, antibody levels declined at the same rate for H and EB after Week 28, maintaining the superiority of the antibody response to H compared to EB.

Funding: Pharmaceutical Company Support - Dynavax Technologies Corporation

SA-PO133

Strong Ion Gap Associates with Glomerular Filtration Rate Decline in Chronic Kidney Disease Akashi Togawa,[1] Satoko Uyama,[1] Seiko Takanohashi,[1] Takehiko Miyaji,[2] Hiroyuki Endo.[3] *[1]Nephrology, Shizuoka Saiseikai General Hospital, Shizuoka, Japan; [2]Miyaji Clinic, Shizuoka, Japan; [3]Tanpopo Clinic, Shizuoka, Japan.*

Background: Metabolic acidosis is known to accelerate the progression of chronic kidney disease (CKD). However, whether undetermined anions indicated from strong ion gap (SIG) associate with estimated glomerular filtration rate (eGFR) decline in patients with CKD is not known.

Methods: In retrospective, 22 patients with CKD (base line eGFR15 to 60 ml/min per 1.73m^2) were examined. Based on Stewart approach, SIG was determined from the gap between apparent strong ion difference (SIDa) and effective strong ion difference (SIDe) (Kellum et al, J Crit Care 1995).

Results: The average baseline eGFR was 27.5 ± 13.0 ml/min/1.73m^2 and the average eGFR decline rate per 6 months (ΔeGFR) was8.8 ± 22.2 %. Anion gap and SIG were 11.7 ± 1.70 and 6.29 ± 1.84, respectively. As previously reported, ΔeGFR was correlated with urinary protein-creatinin ratio (UPCR) (r=0.592, P<0.005), serum albumin concentration (r= - 0.534, p<0.01). ΔeGFR was positively associated with SIG(r=0.586, p<0.005) but not with anion gap(r=0.119, p=0.597). Furthermore, in multivariable linear regression analyses, SIG remained significantly associated with ΔeGFR (ß=0.61, P<0.05) after controlling for age, baseline eGFR, serum albumin concentration, UPCR, and HCO_3^- concentration.

Conclusions: These data suggest that SIG appears to be associated with the progression of CKD. Focusing on SIG during the course of CKD may be important for developing new therapeutic strategy to prevent CKD progression.

SA-PO134

Comparative Study of Matrix Metaloproteinase 9/Tissue Inhibitor of Metalloproteinase 1 System in All Stages of Chronic Kidney Disease
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Background: The matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) may play a key role in chronic kidney disease (CKD) atherosclerosis by their impact on extracellular matrix (ECM) accumulation. The MMP9/TIMP1 system received special emphasis because its disturbances has been discovered within the atherosclerotic plaques. In this study we demonstrated the analysis of MMP9/TIMP1 system in CKD and in patients with cardiovascular disease (CVD) but without kidney dysfunction.

Methods: To the study we enrolled 80 patients divided into 4 groups: CKD1-2 - the initial stage of CKD, CKD3-4 - pre-dialyzed, CKD5 - hemodialyzed, CVD – CVD patients without kidney dysfunction and 20 healthy volunteers (HV). All participants were sex- and age-matched and free of diabetes mellitus. Serum levels of MMP9 and TIMP1 were measured by an ELISA method and the carotid artery intima-media thickness (CA-IMT) was assessed by high-resolution ultrasonography.

Results: Compared to HV, CKD patients showed a significant TIMP1, MMP9 and CA-IMT increase. CKD1-2 and CKD5 differed in TIMP1 concentration, but no differences in MMP9 and CA-IMT were found between CKD groups. TIMP1/MMP9 ratio was higher in CKD5 than in other CKD groups. CVD patients showed higher MMP9, lower TIMP1, and lower TIMP1/MMP9 ratio if compared to CKD and HV. CA-IMT was comparable in all groups of patients. The positive correlations between TIMP1 and CA-IMT, MMP9 and HD vintage, cardiovascular disease and hypertension, and the negative correlations between TIMP1 and eGFR and HDL-cholesterol and between MMP9 and HDL-cholesterol were observed.

Conclusions: Our results showed that, on the one hand, CKD is characterized by MMP/TIMP disturbances, partially aggravated by the progression of CKD. On the other hand, CVD patients without kidney dysfunction showed also the propensity to this impairment. It seems that renal dysfunction is important but not crucial for the MMP9/TIMP1 system imbalance.

SA-PO135

High Plasma Levels of Fibroblast Growth Factor 23 Are Associated with Pulmonary Hypertension in Chronic Kidney Disease Patients
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Background: Pulmonary hypertension (PAH) is characterized by increased pulmonary vascular resistance and is a cardiovascular (CV) complication in advanced chronic kidney disease (CKD) patients. High fibroblast growth factor 23 (FGF23) levels have been associated with impaired vasoreactivity and CV events in CKD. We hypothesized that high plasma FGF23 levels might be related to pulmonary hypertension in advanced CKD.

Methods: We identified 100 patients with advanced CKD (45 CKD and 55 ESRD) from the Homocysteine in Kidney and End Stage Renal Disease study who had echocardiograms performed for clinical indications during the study follow-up. C-terminal FGF23 levels were measured in stored plasma samples. Multivariate regression analyses were performed to evaluate whether higher plasma FGF23 levels were associated with a higher pulmonary artery pressure (PAP) and PAH (defined as PAP >40 mmHg) independently of other covariates.

Results: Participants had a mean age of 64±12 years, 45% were black, and 60% were smokers. Mean eGFR among those not requiring dialysis was 18±5 ml/min/1.73m^2. The median [IQR] plasma FGF23 level and mean estimated PAP at baseline were 1398 [589-6537] RU/mL and 43±14 mmHg, respectively. Median follow-up time was 1.9 years. After adjustment for age, gender, race, smoking, hypertension, diabetes, baseline PAP, body mass index, CKD status, albumin, and 1,25-dihydroxyvitamin D level, every $\log_{10}$ increase in FGF23 was associated with a PAP increase of 9.5 mmHg (p=0.01) during the follow-up period. Higher plasma FGF23 levels were also independently associated with an almost 4-fold increased risk of PAH in multivariate analysis (odds ratio 3.7, 95% CI 1.50 to 9.40; p=0.005).

Conclusions: Higher plasma FGF23 levels were independently associated with increased PAP and a greater risk for PAH in patients with advanced CKD during study period. These findings suggest a novel relationship between FGF23 and PAP that warrant further investigation.

Funding: NIDDK Support

SA-PO136

cFGF23 Levels in Patients with Iron Deficiency Anemia and Chronic Kidney Disease at High Risk of Cardiovascular Events
Myles S. Wolf,[1] David B. Bregman,[2] Angelia Butcher,[2] Todd Koch,[2] Andy He,[2] David D. Morris.[3] *[1]University of Miami, FL; [2]Luitpold Pharmaceuticals, PA; [3]WebbWrites, LLC, NC.*

Background: Fibroblast growth factor 23 (FGF23) is a hormone that regulates phosphate homeostasis. Elevated levels of C-terminal FGF23 (cFGF23) have been associated with greater risk of cardiovascular disease (CVD) events and mortality in patients with chronic kidney disease (CKD). Recent data suggest that iron deficiency (ID) is associated with elevated cFGF23 levels but there are limited data on the effect of iron replacement therapy on cFGF23 levels in ID patients with CKD.

Methods: We measured cFGF23 levels (Immutopics, San Clemente, CA) in a subset of 799 of 2561 subjects in a randomized trial (REPAIR-IDA) that compared the effects on hemoglobin (Hb) and iron indices of intravenous (IV) ferric carboxymaltose (Injectafer; 2 x 750 mg on Days 0 and 7) versus IV iron sucrose (Venofer; 5 x 200 mg on Days 0-14) in ID anemic CKD patients (hemoglobin [Hb] ≤ 11.5 g/dL) at high CVD risk due to low glomerular filtration rate (GFR) or other Framingham risk factors. Subjects underwent 120 days of follow-up for CVD events. cFGF23 levels were measured at baseline in all 799 subjects, and repeated on Days 7, 14, 28, and 56 in 37 of the 799 subjects (15 Injectafer; 22 Venofer).

Results: Baseline characteristics of the cFGF23 subset were similar to those of the overall study population. The mean baseline GFR was 32 mL/min/1.73 m^2, mean Hb was 10.4 g/dL and mean ferritin was 73.5 ng/mL. Mean baseline cFGF23 levels were 2-fold higher among subjects who developed a composite endpoint of death, myocardial infarction, stroke, unstable angina, congestive heart failure, or arrhythmia compared to those who did not (993 ± 1381, n=28 vs. 443 ± 671, n=771 Relative units [RU]/mL; P = 0.0001). In the 37 participants with repeated measurements, cFGF23 decreased from baseline to Day 56 (364 to 181 RU/mL; P = 0.0008).

Conclusions: These data confirm that elevated cFGF23 levels are associated with increased risk of adverse CVD events, and demonstrate for the first time that treatment of ID anemia using IV iron may be a novel approach to reduce markedly elevated cFGF23 levels in patients with CKD.

Funding: Pharmaceutical Company Support - Luitpold Pharmaceuticals

SA-PO137

Urinary Phosphate Excretion and Left Ventricular Hypertrophy in Stage 4 and 5 CKD
Muhammad Salman Siddiqui, Jennifer C. Rodrigues, Sameena Z. Iqbal, Paul E. Barre, Istvan Mucsi, Ahsan Alam. *Division of Nephrology, McGill University Health Centre, Montreal, Canada.*

Background: Left ventricular myocardial hypertrophy (LVH) is an important complication in advanced CKD. Fibroblast growth factor-23 (FGF-23) has been shown to induce cardiac hypertrophy, and also regulates renal phosphate excretion. We hypothesized that phosphaturia may be used as a surrogate for the phosphaturic effect of FGF-23, and examined its association with LVH in a cohort of patients with stage 4-5 CKD.

Methods: Single-centre, cross-sectional study of consecutive patients with stage 4-5 CKD referred for pre-dialysis assessment. Twenty four hour urine phosphate excretion, corrected for creatinine excretion, (PO4:Cr) was tabulated. Left ventricular diastolic mass index (LVMI) was assessed by 2D echocardiography. Moderate or severe LVH was determined using gender-based LVMI cut-offs according to American Society of Echocardiography criteria. The association of phosphaturia with LVMI or LVH was examined using linear and logistic regression analyses, respectively.

Results: 129 patients (mean age 65 ± 16 years; 57% male; 50% diabetic; 68% hypertensive; median eGFR 18 ± 9 ml/min/1.73 m2) had both a 24-hour urine collection and an echocardiogram, and were included in the final analysis. The mean hemoglobin was 113 ± 18 g/L with 27% on ESA therapy. Moderate or severe LVH was detected in 23% of patients. There was no association detected between PO4:Cr and either LVMI or LVH in our main effects model; however a significant interaction was found with gender for both LVMI (p=0.01) and LVH (p=0.03). After adjusting for other determinants of LVMI in univariate analysis (underlying heart disease, serum albumin, hemoglobin, ESA and diuretic use, and log-transformed CRP), males had a positive association for PO4:Cr with LVMI, while females showed an inverse relationship.

Conclusions: In patients with stage 4-5 CKD, urinary phosphate excretion was significantly associated with LVMI and LVH after accounting for the interaction of gender. Whether urinary phosphate excretion indeed correlates with FGF-23 levels remains to be determined. Gender may be an important effect modifier for the development of LVH in patients with CKD.

Funding: Private Foundation Support

SA-PO138

Can Parathyroidectomy Reverse Decreased Heart Rate Variability in End Stage Renal Disease Patients?
Jing Zhang,[1] Chang Ying Xing,[1] Xiaoming Zha,[2] Kejiang Cao,[3] Ningning Wang.[1] *[1]Department of Nephrology, First Affiliated Hospital with Nanjing Medical University, Nanjing, Jiangsu, China; [2]Department of General Surgery, ; [3]Department of Cardiology, .*

Background: Reduction of heart rate variability (HRV) in end stage renal disease (ESRD) patients implies high risk of cardiovascular disease (CVD). Secondary hyperparathyroidism (SHPT) is positively correlated with incidence of CVD. However, the relationship between mineral metabolism and HRV is obscure. It is also unclear whether the impaired HRV can be reversed by parathyroidectomy (PTX) in severe SHPT.

Methods: Eighty ESRD patients were received 24-hour Holter analysis to evaluate HRV.

Baseline characteristics of the subjects (N=80)

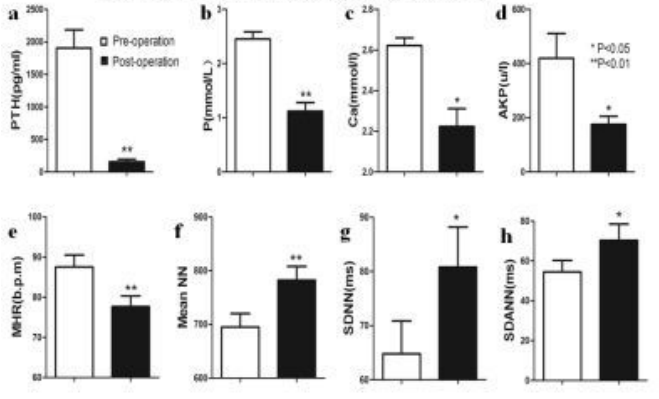
Variables	Value
Age(yr)	52.0±13.6
Male/female	40/40
Duration of dialysis(m)	36.0(96.0-9.0)
Hemoglobin(g/l)	95.2±22.8
Glucose(mmol/l)	5.1±2.0
Albumin(g/l)	37.2±5.6
Ca(mmol/l)	2.3±0.3
P(mmol/l)	2.0±0.6
ALP(u/l)	103.6(176.8-80.2)
PTH(Pg/ml)	442.3(1183.3-193.2)

Mean values ± SD, or median (IQR), as appropriate. PTH=Parathyroid hormone, ALP= Alkline phosphatase.

Eleven calcitriol resistant SHPT patients were followed for median of 4.5 months after total PTX with forearm autotransplantation.

Results: Serum PTH level was correlated with SDNN (standard deviation of normal RR intervals) and SDANN (standard deviation of the averages of 5-min normal RR intervals over 24h) (r=-0.293 and r=-0.305, P<0.01); Serum ALP was correlated with Mean 24h HR (Heart rate) (r=0.227, P<0.05) and SDNN (r=-0.237, P<0.05); Serum P was correlated with pNN50 (proportion of adjacent RR intervals with a difference by >50ms over 24h) (r=0.277, P<0.05). Improvement of HRV was noted in severe SHPT patients after PTX.

Figure 1. Comparison of mineral metabolism and HRV indices in severe SHPT patients before and after PTX



Conclusions: Disorders of mineral metabolism are important risk factors for sympathetic hyperactivity in ESRD. Successful PTX may reverse this CVD risk, as assessed by HRV in severe SHPT patients.

SA-PO139

Evaluation of Some Gene Polymorphisms Coding Selected Cytokines and Urinary Excretion of This Cytokines as Independent Risk Factors for the Progression of Primary Chronic Glomerulonephritis

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Background: The aim of the study was to estimate whether: 1/ carrying the genotype of the IL-6 gene G(-174)C, TGFβ1 gene C (-509)T, MCP-1 gene A(-2518)G, and TNFa gene G(-308)A polymorphism is associated with urinary excretion of some cytokines in patients with new recognized primary glomerulonephritis (GN); and 2/whether carrying some genotype or urinary IL-6, TGFβ1, MCP-1, TNFR1 and EGF excretion at baseline predict the progression of GN in prospective observation.

Methods: 150 Caucasian patients were included in the study. The identification of the SNP was carried out using PCR-RFLP with specific starters. Urinary cytokines excretions were measured using the ELISA methods. After 4-year follow-up, patients were divided into two groups: with loss of eGFR ≥ 5ml/min/y –PG and with loss of eGFR<5ml/min/y – NPG. The influence of traditional risk factors (TRF) and urinary excretions of cytokines (UEC) on eGFR and ΔeGFR calculated as the difference between eGFR measured at the end of follow-up and basal eGFR in multiple linear regression analysis were evaluated.

Results: No association between cytokine gene polymorphism and urinary excretions of correspondence cytokines were found. No differences in genotype distribution between PG and NPG patients were observed. UEC were significantly higher but U EGF excretion was significantly lower in PG when compared with the NPG. The comparison of the influence of UEC and TRF on eGFR showed that age (p< 0,0001), UPE (p<0,0001), UTNFR1 (p<0,003) and UEGF(p<0,003) were most significant factors. The most significant association of ΔeGFR were found with the initial measured: UPE, UEGF and UMCP-1.

Conclusions: The determination of UTNFR1, UEGF and UMCP-1 independent on genetic factors may serve as prognostic markers of deterioration of renal function in patients with primary GN.

SA-PO140

Prevalence of Bone Diseases and/or Soft Tissue Calcifications and Its Correlation with Se-iPTH Level in Dialyzed Patients

Zoltan Kiss, Csaba Ambrus, Andras Szabo, Dr.szegedi János, Jozsef Balla, Marietta Török, Botond Csiky, Erzsebet Ladanyi, Otto Arkossy, Sándor Túri, Imre Kulcsar, Istvan Kiss. *CKD-MBD Working Group, Hungarian Society of Nephrology, Budapest, Hungary.*

Background: One of the most life threatening consequences of CKD-MBD are bone disease (BD) and soft tissue calcification (STC) including vascular calcification. These diseases have several common pathomechanisms. Therefore, our aim was to examine the characteristics of these co-morbidities in dialyzed patients (CKD-5D).

Methods: It was a country wide observational, multicentre, retrospective, cross-sectional study among CKD-5D patients. From the enrolled 5008 patients we excluded pts with incomplete data and parathyroidectomy. We analyzed 4904 patient's data statistically. Bone disease (BD) was defined any fracture or any bone pathology detecting any imaging technique. All calcification in the body was defined as soft tissue calcification (STC). We allocated the pts into groups based on existence of their BD and/or STC (group I) and neither BD nor STC (group II).

Results: There was 2883 pts in group I (58,79 %), including the biggest population who has BD and STC concurrently. Between the I and II groups significant difference were in age (65,5±13,0 vs. 61,0±15,1), in se-Ca level (2,26±0,18 vs. 2,24±0,19), dialysis modality and prevalence of DM (35,9% vs. 29,5%). We revealed an "U" shaped significant association between se-iPTH level and number of pts in group I. The lowest prevalence (55,3 % and 55,1 %) occurred in groups with iPTH 151-300 and 301-500 pg/ml. The prevalence of group I increased significantly with increasing age. Significant and independent factors of co-morbidities are: age, DM, dialysis modality, se-PTH.

Conclusions: Among CKD-5D pts BD and/or STC occurred in more than a half of the pts. There is an "U" shape association between se-iPTH and co-morbidities (BD / STC). Depending on se-iPTH level the lowest prevalence of BD / STC are in the PTH target range recommended by the KDIGO guideline.

SA-PO141

Possible Contribution of Anemia to Brain Atrophy in Predialysis Patients with Chronic Kidney Disease (CKD)

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Background: Brain atrophy has been reported to be correlated with kidney dysfunction (Yakushiji Y, et al. Hypertens Res, 2010); however, its mechanism remains to be elucidated. In dialysis patients, brain atrophy was demonstrated to be associated with anemia and longer dialysis vintage (Kamata T, et al. Am J Nephrol, 2000). These findings suggest that anemia might be a major factor contributing to brain atrophy in CKD patients. Thus, in the present study, we investigated the association of hemoglobin (Hb) level and brain volume (BV) determined by magnetic resonance imaging (MRI) in predialysis CKD patients.

Methods: Eighty-five predialysis CKD patients (46 men, 39 women) aged 37–79 (63±10) years without history of cerebrovascular disease were recruited and underwent MRI scanning for this study. T1-weighted MRI images were analyzed with statistical parametric mapping software. Total gray matter (GM), total white matter (WM), and cerebrospinal fluid were segmented and each volume was quantified using MRI voxel-based morphometry. BV was normalized as a percentage of intracranial volume to adjust for variations in head size. The dependent variable was normalized BV (%GM and %WM), and the independent variables were Hb and other clinical parameters. Patients are divided into quartiles according to Hb level. Linear regression analysis was used to examine the association of Hb level with %GM and %WM.

Results: Hb level is positively associated with %GM, but not %WM. The association between Hb level and %GM remained significant even after adjustment of age, gender, eGFR, underlying kidney disease, history of smoking, diastolic blood pressure, and LDL-cholesterol. Jonckheere-Terpstra trend test showed a significant dose-response relationship (P for trend = 0.009) which remained significant in the multivariate analysis.

Conclusions: The present study demonstrates the association of Hb level with BV, suggesting that anemia might contribute to brain atrophy independent of aging in CKD patients.

SA-PO142

Relationship of Aortic Pulse Wave Velocity to Cognitive Function in Chronic Kidney Disease: The Chronic Renal Insufficency Cohort Study

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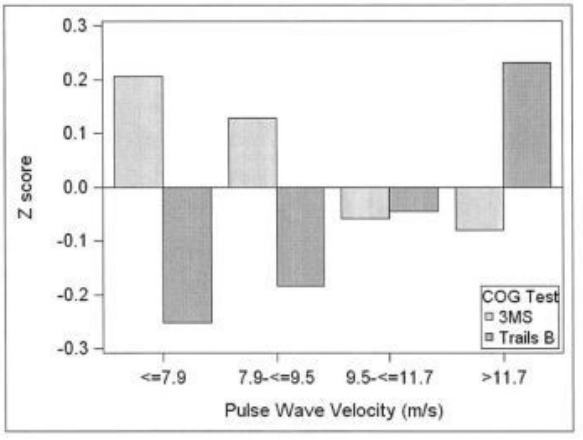
Background: Cognitive function is impaired in older patients with CKD and aortic stiffness may be involved. We evaluated the relationship of aortic (carotid-to-femoral) Pulse Wave Velocity (PWV) in 697 participants aged 55 years or more in the Chronic Renal Insufficiency Cohort (CRIC) at year 2 visit.

Methods: Participants were 66(+/-6) years old, half male, most white (50%) or AA (47%) with 3% Hispanic. Estimated GFR of 42(+/-14) mL/min. Cognitive function included Trails A/B, Modified-MMSE (3MS), BUSHKE A, Boston Naming, Verbal

Fluency and Recall. Carotid-femoral PWV was performed by tonometry of the carotid/femoral arteriesand divided into quartiles. Z-scores were used for each cognitive function test, analyzed by PWV quartile.

Results: Global cognition (3MS) decreased with increasing quartiles of PWV ($p = 0.013$). Increasing PWV showed decreased performance on Trails A ($p = 0.008$), Trails B ($p < 0.001$), verbal (category) fluency ($p < 0.001$), and the delayed recall component of the Buschke ($p < 0.015$). Immediate recall of the Buschke and Boston Naming Test did not differ by quartiles of PWV ($p > 0.2$). The graph shows results representative of frontal lobe function (Trails B, negative = better cognition) and global cognitive function (3MS, positive = better cognition).



Conclusions: PWV was associated with decreased performance on tasks of attention (Trails A), cognitive flexibility (Trails B), and verbal fluency, and deficits in memory and global cognition (3MS). These suggest PWV is associated with an impairment of frontal-executive functions in CKD.

Funding: NIDDK Support

SA-PO143

Percutaneous Coronary Intervention for Acute Myocardial Infarction in the Elderly Patients with Renal Dysfunction: Results from the Korea Acute Myocardial Infarction Registry Eun Hui Bae, Chang Seong Kim, Soo Wan Kim. *Internal Medicine, Chonnam National University Hospital, Gwanju, Korea.*

Background: The question as to whether percutaneous coronary intervention (PCI) benefits for acute myocardial infarction (AMI) in elderly aged ≥ 75 years patients with renal dysfunction, is unresolved.

Methods: As part of the Korea Acute Myocardial Infarction Registry (KAMIR), 1,458 AMI patients with renal dysfunction (glomerular filtration rate [GFR] < 60 mL/min) received either medical (n = 439) or PCI (n = 1,019) therapy. Major adverse cardiac events (MACE) at 1-month and 1-year were compared between these 2 groups.

Results: On comparison with the medical therapy group, the PCI group showed a significantly lower incidence of in-hospital mortality. Moreover, the short term and long term MACE rate was significantly higher for the medical therapy group than for the PCI group (36.8% vs. 20.7%; 55.5% vs. 33.0%, $p < 0.05$), and this difference was mainly attributed to cardiac death (30.8% vs. 17.4%; 43.8% vs. 22.7%, $p < 0.05$). The MACE-free survival time after adjustment was also higher in PCI group in short term (hazard ratio (95% confidence interval) 0.67 (0.45-0.98) p=0.037) and long term (hazard ratio (95% confidence interval) 0.61 (0.45-0.83) p=0.002) follow up.

Conclusions: In elderly aged ≥ 75 AMI patients with renal dysfunction, PCI therapy has a favorable in-hospital and short-term and long-term MACE -free survival.

Funding: Government Support - Non-U.S.

SA-PO144

Association of Cancer with Moderately Impaired Renal Function at Baseline in a Large, Representative, Population-Based Cohort Followed for up to 30 Years Anders G. Christensson. *Nephrology and Transplantation, Clinical Sciences, Malmö, Malmö, Sweden.*

Background: Epidemiologic studies have found that the prevalence of chronic kidney disease (CKD) in the United States is over 10%. higher incidence of malignancies in patients with chronic renal failure has been reported. There are also reports implicating increased risk of cancer in patients with earlier stages of CKD. We evaluated whether moderately impaired renal function at baseline influenced risk of cancer or cardiovascular disease during long-term follow.

Methods: The cohort included 33,346 subjects, aged 26-61 years at baseline, in a representative, population-based study enrolling subjects from 1974 to 1992. Median follow-up time was 28 years. Incident cases of cancer were identified from the Swedish Cancer Registry. To account for the unique sampling design, participants were divided by sex and age at baseline into 1,132 older men (age 60), 14,254 younger men (age 40–52), 7,498 older women (age 47–57) and 1,688 younger women (age 35–43). Glomerular filtration rate (GFR) was estimated using the CKD-EPI formula. Patients were classified as having either normal to mildly impaired kidney function (eGFR≥60 mL/min/1.73m^2), or moderate kidney dysfunction (eGFR<60 mL/min/1.73m^2). We calculated the risk of cancer using competing risks regression.

Results: Overall, 6,595 participants were diagnosed with cancer, and 854subjects (3.5%) had moderately impaired renal dysfunction at baseline. There was a nominal significant association between moderately decreased GFR at baseline and cardiovascular events in younger men and older women but no association with long-term cancer risk. The previously described association between low baseline GFR and subsequent risk of kidney cancer was confirmed in younger men (hazard ratio, 3.38; 95% CI, 1.48 to 7.71; *P*=0.004).

Conclusions: In conclusion, few studies, including ours, show an increased risk of kidney cancer among younger men with eGFR less than 60 mL/min/1.73m^2. These findings should be explored further to discover possible pathological mechanisms of carcinogenesis and identify more specific high-risk groups. Surveillance for kidney cancer may be appropriate for high-risk groups.

SA-PO145

High Prevalence of Sticky Platelet Syndrome in Patients with Chronic Kidney Disease Anja Susanne Muhlfeld,[1] Eray Yagmur,[2] Dario Frank,[3] Jürgen Floege.[1] *[1]Division of Nephrology and Immunology, RWTH Aachen University, Aachen, Germany; [2]Medizinisches Versorgungszentrum Stein und Kollegen, Mönchengladbach, Germany; [3]Department of Internal Medicine, Sankt Antonius Hospital Eschweiler, Eschweiler, Germany.*

Background: Sticky platelet syndrome (SPS) is a platelet disorder associated with arterial and venous thromboembolic events. It is defined as maximal aggregation of platelets in response to the inducers ADP and/or epinephrine despite decreasing doses. Patients with chronic kidney disease (CKD) have a high risk for thromboembolic complications. The prevalence of this syndrome in the normal population versus CKD patients has not been described to date.

Methods: Citrate blood samples were assessed for platelet aggregation using light transmission aggregometry in healthy volunteers and CKD patients. ADP and epinephrine in 4 different concentrations were used as stimuli for platelet aggregation. SPS type I was defined as platelet hyperaggregability in response to epinephrine and ADP. Hyperaggregability with only epinephrine indicated sticky platelet syndrome type II.

Results: Platelet aggregation was assessed in 34 healthy volunteers. Of these only one had increased platelet aggregation defined as SPS type II. In a population of 30 patients on hemodialysis 67% were positive for SPS type II with similar results before and after hemodialysis excluding mechanical platelet alteration. The effect of partial restoration of renal function in such patients was assessed in 34 renal transplant patients. 82 percent of these patients were positive for SPS II independent of their CKD stage. The dosage and type of immunosuppressive medication did not influence platelet aggregation. There was a trend towards more thromboembolic events in patients with SPS.

Conclusions: CKD patients exhibit a hitherto unappreciated high prevalence of platelet hyperaggregability. This may contribute to the high rate of vascular complications in patients with CKD.

SA-PO146

Hospitalization and Mortality in Elderly Iron Deficient, Anemic Patients with CKD and Heart Failure Receiving Intravenous Iron Therapy: A Five Year Follow-Up from a Pilot Study Jorge E. Toblli, Federico Di Gennaro. *Nephrology, Hospital Aleman, Buenos Aires, Argentina.*

Background: In a previous pilot, double-blind, placebo-controlled study (JACC 2007; 50:1657-65), intravenous (i.v) iron without erythropoeisis stimulating agents (ESA) substantially preserved LVEF(%), exercise capacity, NT-pro-BNP, CRP, renal function, and quality of life in iron deficient, anemic patients with CKD and heart failure (HF). The present study evaluates hospitalization and mortality in the same group of patients after a follow-up of 1 and 5 years.

Methods: 40 patients with CKD and HF (mean age >74years) from the original pilot study were evaluated after a follow-up of 1 and 5 years. Inclusion criteria: hemoglobin (Hb g/dl) <12.5 for men and <11.5 for women; TSAT<20%; ferritin<100ng/ml; CrCl <90ml/min and left LVEF% <35%. Group-A: isotonic saline solution (ISS); Group-B: Iron Sucrose Complex (ISC). Patients received a weekly infusion of ISS or 200mg ISC during 5 weeks. Patients did not receive ESA before or during the study.

Results:

Mean ± SD	Group-A # Baseline	Group-B # Baseline	Group-A 1 year	Group-B 1 year	Group-A 5 years	Group-B 5 years
Hb (gr/dl)	10.2 ± 0.5	10.3 ± 0.6	9.6 ± 0.6 *	11.4 ± 0.5	9.3 ± 0.5 *	11.0 ± 0.4
Ferritin (ng/ml)	70.6±21.4	73.0±29.9	83.9±16.3 *	220.6 ± 36.3	172.6 ± 58.0	166.5 ± 30.8
TSAT (%)	20.0±1.0	20.0±1.0	19.9±1.3*	23.2±1.8	19.3±1.5*	21.1±1.4
CrCl (ml/min)	37.7±10.2	39.8±10.1	27.0±10.2*	39.6±10.9	23.7±4.0*	34.6±7.5
LVEF (%)	30.8±1.7	31.3±3.7	28.5±1.8*	34.9±3.6	23.9±2.1*	34.4±2.4
NYHA	2.9±0.6	2.9±0.7	3.3±0.5*	2.3±0.6	3.6±0.5**	2.8±0.8
Hospitalization (%)	--	--	10 (50)*	2 (10	17 (85)*	4 (20)
Mortality (%)	--	--	4 (20)	1 (5)	11 (55)**	4 (20)

*p<0.01 vs. group-B. **p<0.05 vs. group-B. # Results of the pilot study.

Conclusions: Despite the small number of patients, the present data suggest that i.v. iron therapy without ESA may reduce hospitalization and mortality in iron deficiency, anemic CKD patients with HF.

SA-PO147

Combination Treatment of Primary Chronic Glomerulonephritis Using General Acteoside of Rehmanniae Leaves and Angiotensin Receptor Blocker: A Randomized Controlled Trial Ping Fu, Hongyu Qiu. *Nephrology Department, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: This study aims to assess the efficacy and safety of general acteoside in Rehmanniae leavesused in combinationwith the angiotensin receptor blocker irbesartan to treat primary chronic glomerulonephritis.

Methods: This was a prospective, randomized, controlled study. A total of 479 patients diagnosed with primary chronic glomerulonephritis were recruited from outpatient clinics. Participants were randomly assigned to the treatment group (general acteoside of Rehmanniae leaves, two 200-mg tablets, bid; and irbesartan, one 150-mg tablet, qd) or the control group (irbesartan, one 150-mg tablet, qd). The primary outcome was 24-h urinary protein. Secondary outcome measures included blood pressure (BP), evaluated glomerular filtration rate (eGFR), erythrocyturia, serumalanine aminotransferase (ALT), aspartate transaminase (AST) and electrolytes. All outcome measures were assessed at baseline, 4 weeks, and 8 weeks over the 8-week study period, except BP, serum ALT, AST and electrolytes, which were assessed at baseline and 8 weeks.

Results: After 8 weeks of treatment, the treatment group showed a mean reduction in 24-h proteinuria of 36.42% compared to baseline, which was significantly higher than the mean reduction from baseline of 27.97% in the control group (P=0.0278). Neither group showed obvious changes between baseline and 8 weeks in eGFR, erythrocyturia, BP or electrolytes.In addition, adverse drug reactions occurred at a similar low rate in the treatment group (0.4%) and control group (1.2%, P = 0.3724).

Conclusions: In the treatment of chronic glomerulonephritis, the combination of general acteoside of Rehmanniae leaves and irbesartan can reduce proteinuria more effectively than irbesartan alone, and it can improve renal function and clinical symptoms to a similar extent as irbesartan by itself. In addition, general acteoside of Rehmanniae leaves is well tolerated.

Funding: Pharmaceutical Company Support - Meidakang

SA-PO148

Serum Soluble Secreted α-Klotho Levels Decrease during Intensification of Antiproteinuric Therapy in Non-Diabetic Chronic Kidney Disease Patients Charlotte A. Keyzer,[1] Marc G. Vervloet,[2] Pieter M. Ter Wee,[2] Jan-luuk Hillebrands,[3] Gerjan Navis,[1] Martin H. De Borst.[1] *[1]Nephrology, University Medical Center Groningen; [2]Nephrology, Free University Medical Center Amsterdam; [3]Pathology, University Medical Center Groningen, on Behalf of NiGrAm, Netherlands.*

Background: Reduction of blood pressure (BP) and proteinuria (UP) by RAAS-blockade is the cornerstone of chronic kidney disease (CKD) management. Dual RAAS-blockade and dietary sodium restriction enhance the short-term effects on BP and UP. Preclinical data suggest that loss of renal and circulating Klotho plays a role in progressive CKD and its cardiovascular complications. We evaluated the effect of different RAAS-blockade-based treatments on soluble α-Klotho in the clinical setting.

Methods: A post-hoc analysis was performed on an RCT in 52 non-diabetic CKD patients (age 51±13 yrs) receiving lisinopril 40 mg/day (ACEi) and either valsartan 320 mg/day (ARB) or placebo, combined with either low sodium (LS; 106 mmol Na^+/d) or regular sodium (RS; 184 mmol Na^+/d) diet. Soluble secreted α-Klotho was measured in serum by ELISA (IBL). Paired t-tests were used to determine the effects of treatment on α-Klotho. Data are presented as mean±SD or median [interquartile range] where appropriate.

Results: At baseline (during ACEi+RS) UP was 1.9 [0.9-3.4] g/d and creatinine clearance (CrCl) was 69 [50-110] mL/min. During intensification of therapy, UP decreased to 0.7 [0.4-1.4] g/d and CrCl to 59 [42-81] mL/min during ACEi+ARB+LS (both p<0.001). Serum α-Klotho at baseline was 636±133 pg/mL, was reduced during ACEi+ARB+RS (625±141 pg/mL, p=NS), was reduced further during ACEi+LS (597±112 pg/mL, p<0.001 vs baseline) and was lowest during ACEi+ARB+LS (586±108 pg/mL, p<0.001 vs baseline). The decrease in α-Klotho correlated with the decrease in CrCl (β=0.321, p<0.05), but not with the decrease in UP.

Conclusions: Intensification of antiproteinuric therapy is associated with reduced serum α-Klotho levels in CKD patients. Considering the alleged protective effects of Klotho, this adjunct effect could potentially limit the therapeutic benefits of intensive treatment regimens in CKD, and warrants further investigation.

Funding: Government Support - Non-U.S.

SA-PO149

L/N-Type Calcium Channel Blocker, Cilnidipine, Ameliorates Albuminuria and Uric Acid Production in CKD Patients (J-CIRCLE Study) Shunya Uchida,[1] Hideki Kato,[1] Masayuki Tanemoto,[1] Takafumi Ito.[2] *[1]Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan; [2]Nephrology, Shimane University School of Medicine, Izumo, Japan.*

Background: Cilnidipine, an L/N-type calcium channel blocker (CCB), shows a renoprotective effect as well as a blood pressure-lowering effect. We examined effects of cilnidipine on blood pressure, urine albumin excretion, and uric acid metabolism after switching from an L-type CCB amlodipine.

Methods: We enrolled 71 hypertensive patients with albuminuria > 30 mg/g creatinine who had been receiving amlodipine for three months or longer. Methods: Blood pressure, urinary albumin-creatinine ratio (ACR), serum uric acid (UA), urinary uric acid creatinine ratio (UA/Cr), and fractional excretion of UA (FEUA) were measured before and three months after cilnidipine treatment.

Results: Three months of cilnidipine treatment significantly reduced urinary ACR (p=0.032) whereas blood pressure were unchanged. On the other hand, neither serum UA, urinary UA/Cr ratio, nor FEUA significantly changed in all patients. In the patients whose urinary UA/Cr ratio exhibited 0.5 or greater, urinary UA/Cr ratio and FEUA significant decreased respectively (p=0.038 and p=0.013).

Conclusions: Urinary albumin excretion was significantly reduced by cilnidipine treatment as compared with amlodipine. A novel finding is that urinary uric acid excretion was dramatically reduced in response to cilnidipine. These findings are probably due to amelioration of sympathetic hyperactivity through inhibition of N-type calcium channel by cilnidipine.

SA-PO150

Increased Urinary Albumin Level within the Normal Range Has an Impact on Adverse Outcomes in HIV-Infected Individuals Naoki Yanagisawa,[1,2] Minoru Ando,[1,2] Atsushi Ajisawa,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Infectious Diseases and Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Microalbuminuria poses a risk of developing adverse outcomes; however, clinical significance of albumin excretion within the normal range has not been considered in an HIV-infected population.

Methods: A 3.5-year prospective cohort study was undertaken to test an association of low-grade albumin excretion and incidence of composite outcomes, including all-cause mortality, cardiovascular disease (CVD), and a decrease in renal function. A total of 515 subjects with urinary albumin-to-creatinine ratio (ACR) <30mg/g were enrolled. Albumin excretion within the normal range was arbitrarily classified into 3 grades according to ACR: 0-9, 10-19 and 20-29mg/g. The cumulative incidence of outcomes was depicted by the Kaplan-Meier curves, stratified by the 3 normal ACR grades. Univariate Cox proportional hazards regression analysis was used to determine clinical variables associated with developing such outcomes, adjusted for known risk factors.

Results: The frequencies of ACR of 0-9, 10-19 and 20-29mg/g were 59, 31, and 10%, respectively. Of 515, 41 (8.0%) developed at least one of the outcomes. A decrease in renal function, all-cause mortality and CVD occurred in 29(71%), 7(17%) and 5(12%) subjects, respectively. The Kaplan-Meier estimate of ACR 20-29 mg/g was significantly higher than that of ACR 0-9 mg/g (P=0.03), but that of ACR 10-19 mg/g was not (P=0.26). The Cox analysis showed that ACR 20-29mg/g, as well as presence of diabetes mellitus and CD4 cell count, was associated with developing adverse outcomes.

Variates	HR (95% CI)	P value
ACR 20-29 mg/g	2.44 (1.01-5.39)	0.0484*
ACR 10-19 mg/g	1.48 (0.73-2.94)	0.2711
ACR 0-9 mg/g (reference)	1	-
Age, per year	1.02 (0.99-1.05)	0.1331
Hypertension (+)	0.85 (0.29-1.98)	0.7314
Diabetes mellitus (+)	4.70 (1.41-11.7)	0.0158*
Log CD4 cell count, μL	0.16 (0.09-0.32)	<.0001*
Log HIV-RNA level, copies/mL	1.23 (0.94-1.56)	0.1199

Conclusions: The high level within the normal range of albuminuria likely carries the risk for the development of adverse outcomes in HIV-infected individuals.

SA-PO151

Plasma Proprotein Convertase Sutilisin-Kexin Type 9 Is Elevated in Proteinuric Subjects: Relationship with Lipoproteins Response to Antiproteinuric Treatment Arjan J. Kwakernaak,[1] Gilles Lambert,[2] Maartje C.J. Slagman,[1] Femke Waanders,[1] Francine Petrides,[2] Bert D. Dikkeschei,[3] Gerjan Navis,[1] Robin P.F. Dullaart.[4] *[1]Dept. of Nephrology, University Medical Center Groningen, Groningen, Netherlands; [2]The Heart Research Institute, Sydney, Australia; [3]Dept. of Clinical Chemistry, Isala Clinics, Zwolle, Netherlands; [4]Dept. of Endocrinology, University Medical Center Groningen, Groningen, Netherlands.*

Background: The proprotein convertase subtilisin–kexin type 9 (PCSK9) pathway plays a key role in lipoprotein metabolism by promoting LDL receptor degradation. Its contribution to atherogenic lipoprotein abnormalities in proteinuric states is unknown. We studied whether plasma PCSK9 is elevated in proteinuria, and determined the relationships of PCSK9 with lipoproteins and their responses to proteinuria reduction.

Methods: Thirty-nine CKD patients (eGFR 61±29 ml/min/1.73m^2, proteinuria 1.9 [0.9-3.3] g/day; 19 on statin treatment) were studied during 2 randomized double-blind 6-week periods on either lisinopril (40 mg/day) and a regular sodium diet (194±49 mmol Na^+/day; baseline treatment) or lisinopril and valsartan (320 mg/day) and a low sodium diet (102±52 mmol Na^+/day; maximal treatment), and compared to age- and gender-matched controls. Maximal treatment decreased proteinuria to 0.5 [0.3-1.1] g/day (P<0.001).

Results: Plasma PCSK9 was increased at baseline in proteinuric subjects (213 [161-314] vs. 143 [113-190] μg/L in controls, P≤0.001), irrespective of statin use, eGFR and BMI. PCSK9 correlated with proteinuria at baseline (R=0.449) and at maximal proteinuria reduction (R=0.434, both P=0.007). PCSK9 did not decrease during proteinuria reduction (P=0.841). However, individual changes in total cholesterol, non-HDL cholesterol, LDL cholesterol and apolipoprotein B (all P≤0.01) related significantly to individual PCSK9 responses. PCSK9 at baseline independently predicted total/HDL cholesterol ratio response to treatment (P=0.041).

Conclusions: Plasma PCSK9 levels are elevated in proteinuria, predict lipoprotein responses to proteinuria reduction but remain unaffected by reduction of proteinuria. Inhibition of the PCSK9 pathway may provide a novel treatment strategy in proteinuric subjects.

SA-PO152

Accelerated Thrombopoiesis in CRF Rat Megakaryocytes due to Compensatory Thrombopoietin Production from the Liver and Bone Marrow Itsuro Kazama. *Physiology I, Tohoku University Graduate School of Medicine, Sendai, Miyagai, Japan.*

Background: Decreased thrombopoiesis has been ascribed a role in the pathogenesis of uremic bleeding in chronic renal failure (CRF). However, serum thrombopoietin (TPO) levels are usually elevated in CRF patients, suggesting increased thrombopoiesis. The aim of this study was to determine the thrombopoietic activity in CRF.

Methods: Male Sprague-Dawley rats that underwent 5/6 nephrectomy were used as the model of CRF. Age-matched sham-operated rats were used as controls. Single megakaryocytes were isolated from the rat bone marrow, and their size distribution was examined. Megakaryocyte membrane invaginations were monitored by confocal imaging of di-8-ANEPPS staining, and patch-clamp whole-cell recordings of membrane capacitance. TPO gene expression was assessed in various tissues.

Results: Circulating platelet counts and the number of large megakaryocytes in the bone marrow were significantly increased in CRF rats. Massive di-8-ANEPPS staining and increased membrane capacitance per cell surface area in large megakaryocytes (12.16±0.91 vs. 9.19±0.22 uF/cm2 in intermediate megakaryocytes, P<0.05) demonstrated increased membrane invaginations. Unaffected Kv1.3-channel currents per cell surface area (0.92±0.08 in large vs. 0.98±0.06 mA/cm2 in intermediate megakaryocytes, P<0.05) demonstrated unaltered channel densities. TPO transcription was decreased in the renal cortex but significantly increased in the liver and bone marrow of CRF rats.

Conclusions: Increased thrombopoiesis in CRF was thought to be a reactive mechanism to platelet dysfunction. Increased TPO production from the liver and bone marrow compensated for the decreased production from damaged kidneys.

Funding: Government Support - Non-U.S.

SA-PO153

Electrolyzed Water with High Dissolved Hydrogen (H_2) Ameliorates Aged Cardiorenal Injury by Improving Anti-Oxidant Effect in Dahl Salt Sensitive Rat Wan-jun Zhu,[1] Masaaki Nakayama,[3] Shigeru Kabayama,[2] Takefumi Mori,[1] Sadayoshi Ito.[1] *[1]Center for Advanced and Integrated Renal Science, Tohoku University, Sendai, Japan; [2]Medical Device, Nihon Trim Co, Ltd., Osaka, Japan; [3]Fukushima Medical University, Fukushima, Japan.*

Background: Electrolyzed water (EW) exhibits high dissolved H2 (DH). We recently reported that EW protects kidney and heart tissue from renal ischemia reperfusion injury. Oxidative stress and inflammation play a crucial role for chronic kidney injury by aging. The present study aims to test the effect of EW drinking on the development of cardio-renal tissue injury by aging.

Methods: Dahl salt sensitive male rats (n=90) were divided into three groups: filter water (FW; DH 0.0mmol/L), de-gas EW (DW; DH 0.0 mmol/L), and EW (DH 0.35 mmol/L) for ad lib drinking (n=30 each). They were fed with 0.5% salt diet during the study. Blood pressure (BP) were measured by tail cuff method every 4weeks. Echocardiography, and tissue samplings of kidney and heart, were performed at 16th, 24th, and 48th week.

Results: There were no differences during the study in body weight, water and food consumption among the groups, but the BP was the lowest in EW (p<0.05). Regarding the test parameters, no differences were found at 16th, 24th week, but the following parameter levels or changes(increase ratio) (vs. 16th week) were significantly less in EW as compared to counterparts at 48th week (p<0.05); (Heart) left ventricle posterior wall thickness: FW 14.4%, DW 12.7%, EW 6.3%; cardiomyocyte size: FW 43.2%, DW 14.0%, EW 0%; heart tissue fibrosis: FW 300.4%, DW 155.2%, EW 25.1%; ED1 staining (number/slice): FW 28.1±1.46, DW 20.5±1.29, EW 12.1±2.41, Kidney: ED1 cells in cortex (number/slice): FW 113.5±13.11, DW 88.2±11.85, EW: 75.3±3.04; MDA staining in cortex (%/field): FW 60.5±1.18, DW 66.2±1.48, EW 57.2±1.20. Nrf2 and SOD2 protein expression in heart tissue: FW 1.02±0.35, DW 1.28±0.46, EW 2.42±0.63, (Nrf2) ; FW 2.90±0.39, DW 2.13±0.56, EW 4.35±0.39, (SOD2).

Conclusions: Ad lib drinking of high H2 water could suppress the development of cardiorenal tissue injury of Dahl Salt sensitive rat by aging, by upregulate anti-oxidant effect.

Funding: Government Support - Non-U.S.

SA-PO154

Altered Arginine Methylation, Oxidative Stress, and Vascular Dysfunction Contribute to Accelerated Atherosclerosis in an Animal Model of Chronic Kidney Disease Lixia Zeng, Anna V. Mathew, Jaeman Byun, Kevin B. Atkins, Frank C. Brosius, Subramaniam Pennathur. *Department of Medicine, Division of Nephrology, University of Michigan, Ann Arbor, MI.*

Background: Atherosclerotic cardiovascular disease is the leading cause of death in chronic kidney disease (CKD) patients. However, the molecular mechanisms underlying this increased risk remain poorly understood.

Methods: We developed a mouse model of CKD-accelerated atherosclerosis and investigated the role of oxidative stress and altered arginine methylation by liquid chromatography / mass spectrometry (LC/MS). Six week old male LDL receptor deficient mice were subjected to sham (CTL) or 5/6 nephrectomy (CKD) surgery. Subsequently, the mice were maintained in low (LFD) or high fat diet (HFD) for 24 weeks.

Results: As anticipated, the CKD mice had significantly higher plasma creatinine, lower hematocrit, decreased body weight and higher mortality. Quantification of lesions revealed that both LFD and HFD CKD mice had significantly elevated aortic plaque area fraction, necrotic core, fibrosis and greater luminal narrowing consistent with accelerated atherosclerosis compared with CTL mice. Additionally, cholesterol content and macrophage infiltration were elevated in the aortic lesions of the CKD mice. HFD accentuated these changes and reduced endothelium-dependent relaxation in isolated aortic rings in the CKD mice. LC/MS analysis of oxidation markers (nitrotyrosine and dityrosine) showed marked elevation in the aortic lesions of the CKD mice consistent with enhanced oxidative stress. Targeted LC/MS analysis of arginine methylation in plasma including asymmetric dimethyl arginine (ADMA), symmetric dimethyl arginine (SDMA), N-mono-methylarginine (NMMA), homoarginine, arginine and its catabolites (citrulline and ornithine) was performed. No significant changes were noted for NMMA, homoarginine and ornithine between groups. Although elevated plasma levels of ADMA and SDMA were found in the CKD mice, only higher ADMA levels correlated with aortic lesion area.

Conclusions: These findings strongly implicate the contribution of altered arginine methylation, oxidative stress and vascular dysfunction in CKD-accelerated atherosclerosis.

Funding: NIDDK Support

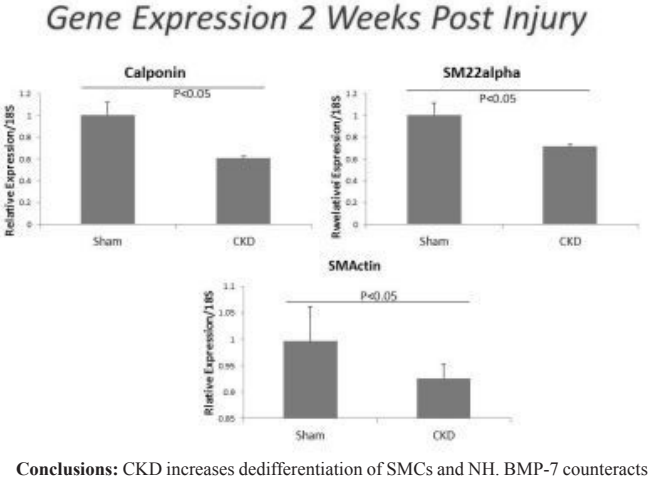
SA-PO155

Smooth Muscle Dedifferentiation after Vascular Injury in Chronic Kidney Disease Senthil Nathan Jayarajan,[1] M. Alexandra Monroy,[1] Jianhua Fang,[1] Catherine Loveland-jones,[1] Shrenik Pankaj Shah,[1] Keith A. Hruska,[2] Eric T. Choi.[1] *[1]Department of Surgery, Temple University, Philadelphia, PA; [2]Department of Pediatrics, Washington University School of Medicine, St. Louis, MO.*

Background: Neointimal hyperplasia (NH) is accelerated in chronic kidney disease (CKD). Administration of bone morphogenetic protein -7 (BMP-7), an endogenous protein, reduces NH in animal models of vascular injury. SMC dedifferentiation (contractile to synthetic phenotype conversion) leading to cell proliferation and migration is required for NH. The purpose of this study was to investigate the effect of BMP-7 on SMC dedifferentiation after vascular injury in CKD.

Methods: Human aortic SMC treated with CKD patient serum were used to identify phenotypic changes in vitro. Left kidney ablation and right nephrectomy in mice created CKD. Carotid artery ligation caused vascular injury. Dedifferentiation was characterized by reduction of SM Actin, SM22α and calponin expression, gene markers of contractile phenotype, using real-time PCR.

Results: In cell culture, CKD reduced expression of contractile markers, SM Actin, calponin, and SM22α (p<0.05). BMP-7 increased calponin and SM22α mRNA expression in SMC exposed to CKD serum by 33% (p<0.05). SM Actin, SM22α and calponin mRNA expressions were measured at 1 and 2 weeks after carotid artery ligation injury in sham (no CKD) vs. CKD mice. At 1 and 2 weeks, the expression of SM Actin, calponin, SM22α was reduced when comparing CKD to sham (p<0.05). BMP-7 and vehicle alone were administered to CKD mice. At 1 week, BMP-7 increased expression of SM Actin when compared to the CKD vehicle mice (p<0.05). At 2 weeks, BMP-7 increased mRNA expression of SM22α (p=0.023) and calponin (p=0.022).



Conclusions: CKD increases dedifferentiation of SMCs and NH. BMP-7 counteracts the effect of CKD, reducing dedifferentiation and attenuates NH development.

SA-PO156

Effect of Lowering ADMA on Vascular Pathology in Atherosclerotic ApoE-Deficient Mice with Reduced Renal Mass Johannes Jacobi,[1] Renke Maas,[2] Nada Cordasic,[1] Karl F. Hilgers.[1] *[1]Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; [2]Clinical Pharmacology, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Asymmetric dimethylarginine (ADMA) is a marker of cardiovascular disease but its causal role for vascular pathology remains largely unknown. We tested the hypothesis that lowering endogenous ADMA levels ameliorates atherosclerosis in ApoE-deficient (ApoE -/-) mice with reduced renal mass.

Methods: To reduce ADMA levels, a transgene overexpressing the ADMA degrading enzyme dimethylarginine dimethylaminohydrolase (DDAH) was introduced into the ApoE -/- line by crossbreeding. Male DDAH transgenic ApoE -/- (N=15) and non-transgenic ApoE -/- mice (N=15) underwent 5/6 nephrectomy (SNX, ablation) at 3 months of age. Nine months later, animals were sacrificed and plaque formation was evaluated in the entire aorta (Sudan IV stained en-face preparations). Sections of the truncus brachiocephalicus were evaluated histologically. Male ApoE -/- controls with normal kidney function were also studied (N=15 DDAH-transgenic and N=15 non-transgenic), as were SNX mice with a normal ApoE genotype (N=13 DDAH-transgenic and N=10 non-transgenic).

Results: At 12 months of age, plasma ADMA measured by LC-MS was significantly lower in DDAH-transgenic ApoE -/- than in non-transgenic ApoE -/- mice after SNX (0.41±0.02 vs. 0.62±0.03 µmol/L). Plaque burden of the entire aorta was reduced in SNX ApoE -/- DDAH-transgenic mice, compared with SNX ApoE -/- non-transgenic animals (12.5±1.0% versus 15.8±1.2% of aortic surface). In non-SNX ApoE -/- mice of the respective genotype, the DDAH transgene afforded a rather greater extent of protection (7.6±2.1% versus 12.4±1.9%). The DDAH transgene did not affect the extent of macrophage infiltration or calcification of the vascular wall. In contrast, wall-to-patent-lumen-ratio was reduced in SNX ApoE -/- mice with the DDAH transgene (0.77±0.05) compared to transgene-negative animals (0.94±0.12).

Conclusions: In summary, lowering ADMA reduced vascular remodeling and aortic plaque burden to some extent in ApoE -/- mice. However, the data do not support a major role in this model of atherosclerosis and reduced renal mass.

Funding: Government Support - Non-U.S.

SA-PO157

Regression of Heart Fibrosis in CKD Is Accompanied by Decrease in Plasma Marinobufagenin Grzegorz Piecha,[1] Nadezda Koleganova,[2] Marie-Luise Gross-Weissmann,[2] Eberhard Ritz.[3] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Institute of Pathology, University of Heidelberg, Heidelberg, Germany; [3]Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany.*

Background: Marinobufagenin is a causal factor in the genesis of uremic cardiomyopathy; it induces fibrosis, and its production is stimulated by the renin-angiotensin system. The purpose of the present study was to examine whether in subtotally nephrectomized rats reversal of cardiac hypertrophy and fibrosis by Losartan and Spironolactone is paralleled by changes in plasma marinobufagenin concentrations.

Methods: Seventy-two Sprague-Dawley rats were subjected to subtotal nephrectomy (SNX) or sham operation. Eight weeks after surgery, they were either euthanized or treated orally with vehicle, losartan (250 mg/kg/day), spironolactone (15 mg/kg/day), and their combination for the subsequent 4 weeks (n=9-12 per group). Heart morphology was evaluated by stereology in tissues obtained using pressure-controlled perfusion fixation. Concentration of marinobufagenin in plasma was measured by an immunoassay.

Results: Systolic blood pressure was significantly higher in SNX (173±18 mmHg) compared with sham-operated animals (116±8) and decreased in all treatment groups (losartan: 134±18; spironolactone: 133±26; combination: 116±18). The capillary density and fibrosis in untreated SNX deteriorated significantly compared with sham-op. Both parameters were improved in SNX treated with losartan+spironolactone, but not with spironolactone alone. Losartan alone reduced fibrosis but failed to improve capillary density. In parallel, 12 weeks after surgery, plasma marinobufagenin levels were elevated 8-fold in untreated SNX vs. sham-op, and lower compared to untreated SNX in the SNX treated with losartan, spironolactone, and particularly losartan+spironolactone.

Conclusions: The study documents in subtotally nephrectomized rats major regression of heart remodeling of after combined treatment with losartan and spironolactone. Whether the change in marinobufagenin concentration is the cause or the consequence of the cardiac changes is examined in ongoing studies.

SA-PO158

The Role of Bone Marrow Derived Cells in Heart Capillary Rarefaction due to Chronic Kidney Disease (CKD) Yvonne Riedl, Katharina Bibl, Christoph Daniel, Kerstin U. Amann. *Nephropathology, University Erlangen-Nuremberg, Erlangen, Germany.*

Background: Mortality rate is still very high in patients with CKD. CKD patients show characteristical cardiovascular structural alterations. Present data suggest that impaired angiogenesis plays an important pathophysiological role. Using a well-established animal model we investigated if angiogenesis in CKD is associated with reduced recruitment of hematopoietic stem cells.

Methods: To investigate the impact of bone marrow derived cells (BMDC) in animals with moderate stable CKD, bone marrow from human placental alkaline phosphatase (hPAP) transgenic F344 rats was transplanted into wildtype F344 rats. Ten weeks after irradiation and bone marrow transplantation, reconstituted chimeric recipients were randomly assigned to two groups and subtotally nephrectomized (SNX) or sham operated (sham). The experiment was terminated 12 weeks after SNX and BMDC were subsequently analysed in hearts and kidney by confocal microscopy.

Results: In comparison to sham-operated animals, SNX rats showed increased proteinuria, serum creatinine and urea. Renal structural changes as determined by glomerulosclerosis and tubulointerstitial damage index were significantly higher in SNX rats. Capillary density was significantly decreased in both kidneys and hearts of SNX rats in comparison to sham-operated animals. Interestingly, the number of BMDC was 80% higher in hearts from SNX rats compared to sham-operated controls indicating higher influx of these cells into the heart muscle. However, endothelial cells from bone marrow origin were rarely found in hearts from rats with CKD as well as in healthy rats. In contrast, many hPAP positive cells were also positive for markers of inflammatory cells like macrophages and leucocytes. Further characterization of hPAP-positive cells is ongoing.

Conclusions: CKD is associated with cardiac hypertrophy and lower capillary supply leading to impaired cardiac blood flow and oxygen supply. BMDC seem not to be a substantial source for heart endothelial cells. Further characterization of hPAP-positive cells is needed to detect potential qualitative cell differences in hearts from CKD rats versus healthy controls.

Funding: Government Support - Non-U.S.

SA-PO159

Increased Wave Reflections Are Related to Capillary Density in Skin in Patients with Advanced Chronic Kidney Disease Camiel L.M. De Roij van Zuijdewijn, Muriel Grooteman, Menso Jan Nube. *Department of Nephrology, VU University Medical Center, Amsterdam, Noord-Holland, Netherlands.*

Background: Arterial stiffness contributes to the risk of cardiovascular events in patients with chronic kidney disease (CKD). Heart rate-adjusted augmentation index (AIx75) can be used as a measure of both wave reflections and arterial stiffness. In patients with advanced CKD we recently showed baseline capillary rarefaction and reduced capillary density after both arterial (functional impairment) and venous occlusion (structural impairment). In the present study we analyzed whether alterations in the macro-vasculature are accompanied by changes in the microcirculation.

Methods: AIx75 was measured in 26 healthy subjects and 54 patients with CKD stages 4 and 5. In both patients and controls, capillary density in skin was measured by nailfold microscopy at baseline, after post-occlusive hyperemia and after venous occlusion. A p-value ≤ 0.05 was considered significant.

Results: Baseline number of capillaries was 47±20/mm^2 in healthy subjects and 43 ± 15/mm^2 in CKD patients (controls versus patients p=0.05). Aix75 was 20 ± 11 in healthy controls and 25 ± 10 in advanced CKD ($p < 0.05$). In healthy controls, no correlations were found between AIx and capillary density, neither at baseline nor after arterial and venous occlusion. In patients with advanced CKD, AIx was positively correlated with the number of capillaries at baseline (p=0.008), after post-occlusive hyperemia (p=0.17) and after venous occlusion (p=0.04).

Conclusions: Measurements of AIx and capillary density in healthy controls were considerably different from patients with advanced CKD. In healthy individuals no correlation was found between arterial stiffness and wave reflections and microcirculatory function, as measured by capillary density in skin. In patients with advanced CKD however, Aix was positively related to the functional and structural number of capillaries in skin. As AIx depends not only on central arterial stiffness, but also on the reflective properties of the arterial tree, our data may indicate that capillary integrity in CKD patients is maintained to some extent by a transmitted forward pressure in the microcirculation.

Funding: Government Support - Non-U.S.

SA-PO160

Use of Beta-Blockers Is Independently Associated with Arterial Stiffness in Patients with Advanced CKD Camiel L.M. De Roij van Zuijdewijn, Muriel Grooteman, Menso Jan Nube. *Department of Nephrology, VU University Medical Center, Amsterdam, Noord-Holland, Netherlands.*

Background: Carotid-femoral pulse wave velocity (PWV) is the gold standard for the measurement of arterial stiffness and an independent predictor of cardiovascular disease (CVD). Both age and chronic kidney disease (CKD) are positively associated with PWV. The aim of the present study was to assess clinical, laboratory and treatment-related determinants of PWV in patients with advanced CKD.

Methods: PWV was measured in 28 healthy subjects and 59 patients with CKD stages 4 and 5. In CKD patients, the plasma levels of phosphorus (P), FGF23, calcium, IL-6, hsCRP, 25-hydroxyvitaminD3 and 1,25 dihydroxyvitaminD3 were assessed. Antihypertensive medication was adopted from the patient files. Determinants of PWV in CKD patients were analyzed in a multi-linear regression model. A p-value of < 0.05 was considered statistically significant.

Results: Mean age in healthy subjects and CKD patients was 53 ± 15 and 62 ± 13 years, respectively. In these subjects, mean PWV was 7.3 ± 1.8 m/s and 10.1 ± 3.2 m/s, resp. Multivariate regression analysis of the total group of participants showed that both age (p= 0.000) and CKD (p= 0.01) are independent determinants of PWV. In patients with advanced CKD, multivariable linear regression analysis showed that only IL-6 (log Il-6: p=0.04) and β-blockers (p=0.009) are independent and positively correlated with PWV.

Conclusions: Interleukin-6 and especially the use of β-blockers are independently associated with arterial stiffness in patients with CKD. Whether the latter correlation is causal or confounded by indication cannot be concluded from this cross-sectional analysis.

Funding: Government Support - Non-U.S.

SA-PO161

Comparative Efficacy and Adverse Effects of the Addition of Ezetimibe to Statin versus Statin Titration in Chronic Kidney Disease (CKD) Patients Hiromichi Suzuki, Tsuneo Takenaka, Hirokazu Okada, Tsutomu Inoue. *Department of Nephrology, Saitama Medical University, Iruma Gun, Saitama, Japan.*

Background: A recent SHARP trial has clearly demonstrated that reduction of LDL cholesterol [with] and a daily regimen of simvastatin plus ezetimibe safely reduced the incidence of major atherosclerotic events in patients with CKD. **Aim:** The aim was to compare the efficacy and adverse effects of statin up-titration vs. statin in combination with ezetimibe because only a few studies have been conducted bearing on this question.

Methods: This was a randomized, open-blind multi-center trial that included 286 patients with CKD whose LDL cholesterol levels were not reduced below 120 mg/dL in spite of statin therapy. Patients received double doses of statin or ezetimibe 10 mg daily. Observation period was one year. During the study, patients were checked on regular visits to the clinic for adverse effects as well as usual laboratory examinations. The key prespecified outcome was the incidence of adverse effects, which included skeletal muscle complaints, myalgia, muscle weakness, muscle cramps with and without elevated CK levels. Increases of ALT or AST levels <1.5 times the upper limit of normal (ULN) were considered as clinically significant adverse effects.

Results: The occurrence rates of all adverse events were 9/145 in the ezetimibe group and 24/141 in the statin up-titration group (P<0.01). Moreover, in patients with CKD of stages 3 to 5, the rates were 6/58 vs. 20/52 (P<0.01). No serious adverse effects such as rhabdomyolysis were noted in both groups. Serum creatinine levels remained essentially unchanged in both groups except in CKD stages 4 and 5. Reductions of LDL-cholesterol were similar between the two groups at the start of and at the end of the study. During the study, no atherosclerotic events were reported in both groups.

Conclusions: When statin up-titration produced adverse effects such as myopathy, combination therapy with ezetimibe is recommended instead of statin alone.

SA-PO162

Cessation of Cigarette Smoking Compared to Continued Smoking Preserves eGFR in Stage 2 Chronic Kidney Disease due to Hypertensive Nephropathy Bethany Kirkpatrick,[1] Jan Simoni,[2] Chanhee Jo,[3] Donald E. Wesson.[1,4] *[1]Internal Medicine, Texas A&M HSC College of Medicine, Temple, TX; [2]Surgery, Texas Tech University HSC, Lubbock, TX; [3]Biostatistics, Scott and White Healthcare, Temple, TX; [4]Internal Medicine, Scott and White Healthcare, Temple, TX.*

Background: Cigarette smoking might worsen eGFR in some nephropathies but whether its cessation preserves eGFR in hypertensive nephropathy is not clear.

Methods: We recruited 108 smoking and 108 non-smoking adults with chronic kidney disease (CKD) due to hypertensive nephropathy, stage 2 eGFR (60-90 ml/min), and urine albumin (mg)-to-creatinine (g) ratio (alb/cr) >200 mg/g in a spot specimen. Smokers received 12 weekly sessions of substance abuse counseling along with 4 weeks of nicotine patch and oral bupropion to encourage quitting determined by reduction of urine cotinine to pre-specified levels at 24 weeks. Non-smokers (NS, n = 108), continued smokers (S, n = 83), and quitters (Quit, n = 25) were followed 5 years on angiotensin converting enzyme inhibitors with yearly eGFR (CKD-EPI equation), urine alb/cr, and urine isoprostane 8-isoprostaglandin $F_{2\alpha}$ (a measure of oxidative stress) in a spot specimen, factored by g creatinine (8-iso/cr).

Results: There was no difference in entry eGFR (p=0.58) and urine alb/cr (p=0.20) but entry 8-iso/cr was higher (p<0.0001) in S (4.3 ug/cr) and Quit (4.1 ug/cr) than NS (1.6 ug/g). Systolic blood pressure was not different among groups at entry (p=0.29) and at 5 years (p=0.22). At 5 years, eGFR was lower (p<0.0001) in S (54.9 ml/min) than NS (66.8 ml/min) and Quit (64.1 ml/min) and was lower in Quit than NS (p=0.043). Urine alb/cr at 5 years was higher (p<0.0001) in S (627.9 mg/g) than NS (504.9 mg/cr) and Quit (415.0 mg/g) but was not different between Quit and NS (p=0.059). Urine 8-iso at 5 years was higher (p<0.0001) in S (3.7 ug/g) than NS (1.6 ug/g) and Quit (1.5 ug/g) but was not different between Quit and NS (p=0.928).

Conclusions: Continued smoking compared to non-smoking worsens eGFR in stage 2 hypertensive nephropathy. By contrast, smoking cessation compared to continued smoking preserves eGFR, possibly mediated through reduced oxidative stress.

Funding: Private Foundation Support, Clinical Revenue Support

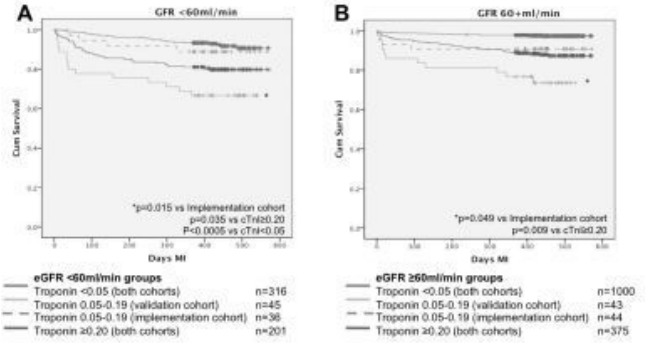
SA-PO163

Interpretation of a Sensitive Troponin Assay in Patients with Chronic Kidney Disease and Suspected Acute Coronary Syndrome David A. Ferenbach,[1] Wendy Metcalfe,[2] Bryan Conway,[1] Nick Mills.[1] *[1]University of Edinburgh, Edinburgh, United Kingdom; [2]Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.*

Background: The significance of low level positive Troponin I (TnI) in patients with chronic kidney disease (CKD) remains controversial. We examine the impact of CKD on patient management and outcome of TnI +ve acute coronary syndrome (ACS), before and after implementation of a high sensitivity TnI assay.

Methods: We identified all consecutive patients presenting to Edinburgh Royal Infirmary with suspected ACS in two 6-month cohorts: Validation (V) (n=1019) and Implementation (I) (n=1043). These cohorts spanned a change in diagnostic threshold from >0.2 (V) to >0.05ng/L (I) for TnI, with figures below 0.2ng/ml reported as 'negative' to clinicans in the (V) cohort, but available for analysis. Data was collected on co-morbidity, inpatient management, discharge medication and 12-month outcome.

Results: CKD (eGFR<60ml/min) patients were older with more vascular co-morbidity than those with normal renal function (NRF). Reducing the ACS diagnostic threshold to TnI<0.05ng/ml resulted in an increase in ACS rates of 23% and >40% in NRF and CKD patients respectively (p<0.001). Patients with unreported TnI of 0.05-0.19 in the (V) cohort had rates of recurrent MI of 33.3% and 25.6% in CKD and NRF patients (p=0.43). This was reduced to 11.1% and 9.1% in CKD and NRF groups in the (I) cohort (p=0.015 and p=0.049 vs (V) cohort respectively).



Patients with CKD had significantly lower rates of inpatient revascularisation and less use of secondary preventative medication (aspirin, clopidogrel, β-blocker, ACE inhibitor or statin, all p<0.05).

Conclusions: This study validates high sensitivity TnI assays for risk stratifying ACS in the CKD population. Factors underlying altered prescribing and invasive management rates in CKD require further study.

Funding: Government Support - Non-U.S.

SA-PO164

Lower Platelet Inhibition to Aspirin in CKD Nishank Jain,[1,2] Beverley Adams-huet,[3] Ravi Sarode,[3] Robert D. Toto,[1] Susan Hedayati.[1,2] *[1]Nephrology Division, UT Southwestern; [2]Veterans Affairs; [3]UT Southwestern.*

Background: High residual platelet aggregability (RPA) to antiplatelet agents (APA) is a novel modifiable risk factor predicting future cardiovascular (CV) events. Despite excess CV events, little is known about RPA in CKD. We hypothesized that compared to patients without CKD, those with CKD (eGFR <60 mL/min/1.73 m^2) have lower platelet aggregability at baseline and respond poorly to aspirin-induced inhibition of platelet aggregation.

Methods: Whole blood platelet aggregation (WBPA) to 0.5 mM arachidonic acid was measured at baseline in 63 participants of the Chronic Kidney Disease Antidepressant Sertraline Trial, a randomized trial to assess the safety and efficacy of sertraline vs. placebo in CKD patients with major depression, and assess sertraline effect on platelet aggregability. Patients were classified into on aspirin and not on aspirin groups. WBPA was compared between groups using Wilcoxon rank sum or Student's t-test. Percent of participants who had inhibition of WBPA <17 Ω was compared between groups using Fisher's Exact test.

Results: There were 19 CKD patients not on aspirin and 44 on aspirin. Mean (SD) age was 68 (11) years and eGFR was 35 (10) mL/min/1.73m^2. 63% were diabetic and 44% African American. Percent with inhibition of WBPA was not different in the not on aspirin vs. on aspirin groups (79% vs. 83% respectively, p =0.7). There was no difference in WBPA whether or not CKD patients were on aspirin (p =0.07). In those not on aspirin, WBPA was significantly lower in CKD patients than reported in normal controls (p<0.001). In the aspirin group, WBPA in CKD patients was higher compared to controls (p<0.001).

Whole Blood Platelet Aggregation (WBPA) in CKD patients and normal controls

Group	N	WBPA Not on Aspirin, Mean (SD), Ω	WBPA on Aspirin, Mean (SD), Ω
Normal controls	106	12 (4)	1 (4)
CKD	63	8.3 (8.7)	5.6 (9.9)

Conclusions: CKD patients vs. controls have lower platelet aggregability at baseline but respond poorly to aspirin, potentially explaining the increased CV events in those with CKD on APA. Future studies need to investigate the use of newer APA with better aggregability profiles in CKD patients.

Funding: Other NIH Support - 1R01DK085512, Veterans Administration Support

SA-PO165

The Prothrombotic State in Predialysis and Hemodialysis Is Related to an Impaired Fibrinolysis

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Background: As CKD is associated with an increased risk of both thrombosis and bleeding, hemostasis was investigated with a method which investigates overall fibrin formation and fibrin degradation. We also measured PAI-1 antigen and TAFI activity in plasma.

Methods: 15 hemodialysis, 23 predialysis patients (CKD 4; mean eGFR 17 ml/min) and 13 healthy controls were studied. We used a global hemostasis assay developed by our group to determine fibrin formation and fibrin degradation in plasma over time when recombinant tissue factor, purified phospholipids, calcium and rt-PA were added to the sample. The fibrin turbidity was determined kinetically to obtain Coagulation activation profile (Cp) and Fibrinolysis activation profile (Fp). The Overall Hemostasis Index (OHI) is the ratio of Cp to Fp, reflecting the balance between coagulation and fibrinolysis.

Results: OHI was higher in the hemodialysis group; median 52.4 (range 17.8-364; $p<0.04$), and in the predialysis group 46.4 (2.5-124; $p<0.02$), compared to controls 27.9 (16.0-46.9) indicating a prothrombotic state. Cp was 17.1 (7.7-82.5) in the hemodialysis group and 16.8 (6.7-28.3) in controls (ns). Fp was lower in the hemodialysis group; 0.24 (0.15-1.18; $p<0.02$) compared to controls 0.58 (0.31-0.74), despite lower PAI-1 antigen ($p<0.05$ compared to controls). TAFI activity in plasma did not differ significantly between groups. Fibrinogen and von Willebrand factor levels in plasma were higher, and plasma albumin levels were lower in both CKD groups.

Conclusions: The prothrombotic state of severe renal impairment is characterized by impaired fibrin degradation despite low or normal plasma levels of PAI-1 and TAFI activity.

Funding: Government Support - Non-U.S.

SA-PO166

Mild Renal Dysfunction Is Linked to Low HDL Cholesterol Level, Monocytosis, and Atherosclerosis in the Malmö Diet and Cancer Study

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Background: Defects in HDL-mediated cholesterol efflux have recently been linked to increased production of monocytes in animal models. CKD patients have been shown to have monocytosis, which has been proposed to increase CV risk. We hypothesized that individuals with mild renal dysfunction would have reduced HDL levels, increased blood monocyte counts, and increased carotid IMT.

Methods: 5067 individuals (enrolled 1991-1994) without CV disease participated in the MDC-CV Cohort, designed to investigate the epidemiology of carotid artery disease. 4757 individuals had plasma cystatin C (cysC) measured at baseline. We divided these subjects into 5 cysC quintiles.

Results:

cysC quintile (mg/L)	cysC range (mg/L)	cysC mean (mg/L)	eGFR mean (ml/min/1.73m²)	HDL level (mg/dL)	Monocyte count (million cells/mL)	No. of subjects
1	0.28-0.67	0.62±0.002	81.5±0.50	57.6±0.47	0.48±0.005	1002
2	0.68-0.73	0.71±0.001	78.0±0.47	54.7±0.48	0.50±0.006	927
3	0.74-0.79	0.77±0.001	75.0±0.48	52.8±0.46	0.51±0.006	932
4	0.80-0.87	0.83±0.001	73.0±0.46	51.1±0.44	0.51±0.005	904
5	0.88-3.29	0.99±0.006	68.8±0.47	49.4±0.44	0.54±0.006	992
p-value				<0.0001	<0.0001	

Within quintile 5 cysC, HDL level was inversely related to monocyte count ($r= -0.2$, $p<0.001$). Among all 4757 subjects, via multivariate logistic regression analysis adjusted for age, diabetes, and quintiles 1-4 cysC, quintile 5 cysC independently increased the odds of being in the top quartile monocyte count (OR 1.6, 95% CI 1.207-2.006, $p=0.001$) and HDL level and female gender independently decreased the odds (OR 0.6, 95% CI 0.443-0.728, $p<0.001$ and OR 0.6, 0.529-0.738, $p<0.001$, respectively). Via multivariate linear regression analysis, age, diabetes, monocyte count, and quintile 5 cysC each independently were associated with increased carotid IMT and HDL level and female gender independently with decreased carotid IMT at baseline ($p<0.05$).

Conclusions: Subjects with mild renal dysfunction had significant monocytosis at baseline, associated with reduced HDL levels, and monocytosis was predictive of increased carotid atherosclerosis cross-sectionally. Reduced HDL levels may give rise to monocytosis and accelerated atherogenesis in subjects with mild renal dysfunction.

Funding: Government Support - Non-U.S.

SA-PO167

Renoprotective Effects of Statin in Patients with Chronic Kidney Disease

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Background: The reduction of accumulated uremic toxins in patients with chronic kidney disease (CKD) protects against the development of cardiovascular diseases. We have revealed that 1) the overexpression of human kidney specific uremic toxin transporter SLCO4C1 in rat kidney promoted renal excretion of uremic toxins and reduced hypertension, cardiomegaly, and inflammation in renal failure, 2) statins up-regulated SLCO4C1 to enhance the uremic toxin clearance in renal failure rats (JASN 2009). We estimated renoprotective effects of statin by assessment of statin treatment on patients with CKD.

Methods: 41 patients with different stages of CKD received statin (pitavastatin 2mg/day). Serum creatinine (S-Cr), serum lipid profiles, and urine protein (Up) were measured before and three months after statin treatment started. Uremic toxins in blood were also measured by liquid chromatography mass spectrometry (LC-MS).

Results: S-Cr and estimated GFR (eGFR) were not significantly improved after statin treatment. T-chol, LDL-chol, and triglyceride were significantly decreased, however, HDL-chol was not changed. Up was significantly decreased. LC-MS revealed that representative uremic toxins, guanidinosuccinate (GSA) and asymmetric dimethylarginine (ADMA) significantly decreased.

Conclusions: After the statin treatment, eGFR was not improved, but Up excretion and plasma uremic toxins were also reduced. The reduction of plasma uremic toxins might be mediated by promoted tubular excretion through enhanced SLCO4C1.

Funding: Government Support - Non-U.S.

SA-PO168

Ventricular Arrhythmia in Chronic Kidney Disease: Prevalence and Associated Factors

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Background: Cardiovascular mortality in chronic kidney disease (CKD) has shown to be substantially elevated even in early stages of the disease. Complex ventricular arrhythmia (VA) has been pointed as one of the etiologies of sudden death. The aim of the present study was to evaluate the prevalence of VA and to investigate the factors associated with their occurrence in non-dialyzed CKD patients.

Methods: This is a cross-sectional study that evaluated one hundred and eleven patients with non-dialyzed CKD (57 ± 11.4 years, 60% male, eGFR: 34.7 ± 16.1 mL/min per 1.73m², 24% diabetics). Patients underwent laboratory tests, 24h electrocardiogram, 24-h ambulatory blood pressure monitoring (ABPM), echocardiogram and multi-slice computed tomography.

Results: Sixteen percent of the patients had VA. Non controlled hypertension was observed in 21% of the patients while absence of systolic decency in 29%. Left ventricular hypertrophy was found in 27% of the patients and heart failure in 20%. Coronary artery calcification was observed in 52 patients, from which 46% had severe calcification. Comparing to the patients without VA, the VA group was older ($p = 0.008$) and had higher eGFR ($p = 0.018$) and hemoglobin ($p = 0.007$). This patients had lower iPTH ($p = 0.049$) and triglycerides ($p = 0.003$) levels. The VA group also had higher left ventricular mass index ($p = 0.001$), coronary calcium score ($p= 0.025$) and lower ejection fraction ($p = 0.002$). In the multiple logistic regression analysis, age and ejection fraction were independently associated with the presence of VA.

Conclusions: VA is prevalent in non-dialyzed CKD patients, especially in the older ones. The factors associated with its occurrence were mainly related to alterations in cardiac structures, such as left ventricular hypertrophy, coronary calcification and heart failure.

SA-PO169

Increased NO Activity during Statin Treatment in Patients with Non-Diabetic Nephropathy

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Background: We investigated the effects of short term atorvastatin treatment on blood pressure (DBP and SBP), GFR and fractional excretion of sodium (FE-Na) during inhibition of nitric oxide synthase (NOS) in patients with non-diabetic nephropathy.

Methods: Twenty-five patients with chronic kidney disease stage IV-V (eGFR 90-30 ml/min/m²) were examined in a randomised, placebo-controlled, double-blinded, cross-over study. All subjects were given Atorvastatin 80 mg per day or placebo 5days prior to an examination day. During the treatment period subjects were on a standardized diet. ^{51}Chrom-EDTA clearance was used for GFR measurements and the nitric oxide (NO) synthase inhibitor, L-NMMA, were administered as a 4.5mg/kg bolus injection followed by 3.0 mg/kg/hour IV infusion for one hour. SBP, DBP and heart rate (HR) were repeatedly measured. Blood and urine samples were collected every 30 minutes during the 90 min baseline period, during L-NMMA infusion and 60 minutes after cessation of L-NMMA infusion. Differences are presented in means ± SD or medians with interquartile.

Results: IV infusion of L-NMMA increased both DBP and SBP and decreased HR (p less than 0.001). There were no differences in change in SBP (12±8 mmHg vs. 12 ± 8 mmHg), DBP (9 ± 3 mmHg vs. 9 ± 3 mmHg), and HR (-3± 2vs. -4±1) between groups. GFR (49.8 ± 16.0 ml/min vs. 49.3 ± 16.9 ml/min)and FE_{Na} (1.59 (0.72;3.41)% vs. 1.57 (0.70;3.08)%)were similar at baseline. GFR decreased dignificantly during L-NMMA infusion (-5.2±5.7 ml/min vs. -4.8±6.9 ml/min), but there were no differences between groups (p=0.818). L-NMMA infusion caused a significant reduction in FE_{Na} in both groups (-0.61±0.61% vs. -0.80±0.82%, p=0.001 in both groups) and the reduction was similar (p=0.224). In the post-LNMMA period FE_{Na} the decrease was more pronounced in atorvastatin group compared to placebo (-0.45±0.69% vs. -0.79±0.94%, p=0.024).

Conclusions: Short term Atorvastatin treatment significantly increased the reduction in FE-Na during NOS inhibition. Our results suggest that atorvastatin increases the bioavailability of renal NO and may be an explanation of the pleiotropic effects of statins.

Funding: Government Support - Non-U.S.

SA-PO170

Increased Arterial Thrombo-Embolic Events and Major Bleeding in Patients with Atrial Fibrillation and Chronic Kidney Disease on Vitamin K-Antagonist Treatment Judith Kooiman, Bas Spaans, Wilke R. van de Peppel, Koen van Beers, Felix Van der Meer, Suzanne Cannegieter, Ton J. Rabelink, Menno V. Huisman. *Leiden University Medical Center, Netherlands.*

Background: To analyze the risk of arterial thrombotic events(ATEs), major bleeding and its predictors in patients with chronic kidney disease(CKD) compared with non-CKD patients, treated with vitamin K-antagonists(VKAs) for atrial fibrillation(AF).

Methods: Medical records of 417 CKD and 300 non-CKD(GFR>60ml/min) patients starting VKA therapy between 1997-2005 were searched for ATEs and major bleeding. ATE was defined by myocardial infarction, stroke/transient ischemic attack, claudicatio intermittens, unstable angina, carotid or peripheral bypass graft/angioplasty. Major bleeding was defined by bleeding being fatal, causing a drop in Hb level≥1,24mmol/L, requiring transfusion of≥2 units of whole blood/red cells, or being symptomatic in a critical area/organ.

Results: ATEs occurred in 108/737(14.7%,95%CI12.3-17.4) patients. Patients with a GFR<30ml/min were at increased risk of ATE compared with non-CKD patients(hazard ratio(HR)3.4(95%CI2.1-5.6). ATEs occurred as frequent in patients with GFR30-60ml/min as in non-CKD patients(HR1.1,95%CI0.7-1.7). Major bleeding occurred in 115/737 patients(15.6%,95%CI13.2-18.4). The risk of major bleeding increased in patients with a GFR<30ml/min compared with non-CKD patients(HR1.8,95%CI1.1-3.0). Major bleeding risk was not increased in patients with a GFR 30-60ml/min compared with non-CKD patients(HR0.9,95%CI0.6-1.4). Low GFR levels were associated with high variability within a patients INR values (correlation term-0.17,P=<0.001) and low serum albumin levels (correlation term-0.13,P0.03). Both INR variability>0.5(HR1.5,95%1.0-2.4) and serum albumin<34g/L(HR1.7,95%CI1.0-3.0) increased the major bleeding risk.

Conclusions: Patients with a GFR<30ml/min are at increased risk of ATEs and major bleeding compared with non-CKD patients. The increased bleeding risk may be due to low serum albumin levels and high INR variability, which is a marker of suboptimal VKA therapy.

Table 1.

	N	%
Mean age(SD)	75	10
Male	415	56
Mean duration VKA therapy in years(SD)	3.5	3.1
eGFR in ml/min		
>60	300	41
30-60	294	40
<30	143	19

Funding: Government Support - Non-U.S.

SA-PO171

The Safety and Efficacy of Endovascular Treatment of Peripheral Artery Disease in Chronic Kidney Disease Patients Aris Q. Urbanes, Gerald A. Beathard, Terry Litchfield. *Lifeline Vascular Access, Vernon Hills, IL.*

Background: Over 10 million Americans have Peripheral Artery Disease (PAD) with only 25% being diagnosed and treated. PAD is very common in the CKD population, with prevalence rates reported of 15% to 48%. Patients with impaired renal function have a greater than two-fold risk for developing PAD. KDOQI recommends at the time of dialysis initiation, all patients should be evaluated for the presence of PVD. A large system of outpatient vascular centers developed and implemented a coordinated care approach to PAD including outpatient endovascular interventions in the CKD population.

Methods: A coordinated program including nephrologists; dialysis units; podiatrist; primary care physicians and interventional center was observed. The program included patient identification; education of providers and patients; and treatment if indicated. Non invasive testing was performed and were conducted in accordance with TASC II guidelines. In 2011, 771 PAD interventions were performed in CKD patients, with outcomes entered into an electronic health record. Data were analyzed in SPSS.

Results: Technical success rate for the seven hundred and seventy one (771) cases was 93% with a complication rate of 1.9% with 2 major complications; 1 related to an expanding hematoma at the site where the closure device was deployed and the other related to embolization. Most of the minor complications were related to closure devices. The case mix was fairly complex with Angioplasty-55%; Atherectomy-32% and Stent placement-13%. Demographics were consistent with USRDS data.

In CKD 4 and 5 patients, special attention to contrast use (mean contrast volume of 46.2 ml) including adherence to renal preservation techniques yielded only 2 cases requiring dialysis within 30 days of the intervention.

Conclusions: P.A.D. is undertreated in the CKD population, which leads to amputation and other morbid events. Diagnosis and endovascular treatment can be successfully and safely performed in a controlled outpatient system. Patient outcomes and quality of life can be optimized in these patients using a collaborative approach.

SA-PO172

Utility of Cardiac Troponin I-Ultra as an Assessment of Myocardial Injury in Renal Insufficiency Prachi Aggarwal, Anubhav Kaul, Sonia Agarwal, Thara Basavaiah, Frantz M. Duffoo, Shitij Arora. *Internal Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.*

Background: Evidence depicts the elevation of cardiac troponins(TnI) in patient with renal insufficiency, without the presence of myocardial ischemia. Patients with chronic kidney disease (CKD) are susceptible to silent ischemia and interpretation of ongoing myocardial ischemia is challenging using TnI assays. Numerous studies have discussed TnI elevation in hemodialysis patients; however, the data is insufficient regarding patients with CKD. Our study aims to evaluate the rise in TnI levels with respect to GFR.

Methods: In a retrospective design, 33 CKD patients were designated into two groups based on the GFR. CKD stages 2/3 were assigned as mild/moderate and stages 4/5 as the severe/fail group. Patients on hemodialysis, of age > 90, impaired ejection fraction or a history of coronary artery disease, myocardial infarction, coronary artery bypass surgery/stent were excluded. Ultra-sensitive troponin I test was used in the patients. Data was tabulated and analyzed using SPSS statistical analysis software.

Results: Group 1 (CKD stage 2 and 3; n=17) and Group 2 (CKD stage 4 and 5; n=16) were assessed with group statistics and comparison of means using the independent t-test. The average GFR was 52.9 ± 4.06 for Group 1 and 18.6 ± 1.49 for Group 2; the difference is significant (p = 0; sig. 1-tailed). The average troponin value for Group 1 was 3.66 ± 1.97 and 2.62 ± 0.763 for Group 2; the difference is not significant (p= 0.315 sig. 1-tailed). Using the Pearson correlation model for bivariate analysis, there is no significant correlation between GFR and troponin levels when assessing Group 1 and Group 2 collectively, with a correlation of -0.109 (p = 0.273 sig. 1-tailed).

Conclusions: Based on our results, elevated TnI in CKD patients are not dependent on the GFR. It is unlikely that the rising TnI in CKD are a result of decreased clearance by the kidney. Furthermore, if the theory of cardiac troponin re-expression in uremic skeletal muscle holds true, CKD stage 3/4 should have higher troponins levels as opposed to early CKD. Thus, the elevation of troponins in CKD patients remains unclear.

SA-PO173

Analysis of the Basal Timepoint in the Cohort of the Multicenter Prospective and Observational Study of Atheromatosis in CKD in Spain (NEFRONA Study): Data on Prevalence of Plaque Angels Betriu,[1] Montserrat Martinez-alonso,[1] Jose M. Valdivielso,[1] Elvira Fernandez.[1] *[1]IRBLleida.*

Background: There are no previous prospective studies assessing the impact of CKD on atheromatosis development, stratified by gender and age. The NEFRONA study is a multicenter prospective observational study monitoring, in patients CKD 3 to 5D, the changes in atherosclerosis, and their relationship with biochemical and genetic biomarkers.

In the present abstract we report the prevalence of atheromatous plaque in the baseline time point of the NEFRONA cohort (2455 patients without previous CV event, at different stages of CKD), compared with a control population of 559 individuals without CKD.

Methods: Data on presence of plaque (% of patients) was obtained with carotid ultrasound by the same team and assessed by one reader in a blinded fashion. Images from 8 different carotid territories were obtained (bulb, common, internal and external carotid in both sides). Plaque was defined as IMT≥1.5 mm. We analyze the trend according to degree of CKD stratified by age and sex using logistic regression analysis for the presence of carotid plaque. Moreover we also analyze the effect of sex stratified by degree of CKD and age group.

Results: 2455 patients were enrolled of which 61% are male, 25% diabetics and a population of 559 controls of which 53% are male and 11% diabetics. 98% Caucasian in both groups.

Age (y)	No CKD (n=559)		CKD3 (n=944)		CKD4-5 (n=821)		CKD 5D (n=690)		Trend p value along CKD stage	
	M	W	M	W	M	W	M	W	M	W
25-35	5.6	9.1	4	0	4.2	0	17.1	23.1	.097	.136
35-45	7.7	5.3	17	14.3	20.8	20.9	35.8	30.6	.001	.003
45-55	48	32*	49.3	34.7	44	38.5	66.7	42.6*	.035	.170
55-65	65.7	39.5*	65.9	43.2*	70.3	59.6	79.1	75.8	.017	< .001
65-75	70.5	54.3	80.2	64.9*	80.5	66.4*	88.9	81.8	.017	.004

Conclusions: The results show that the presence of plaque is lower in women than in men at all ages and stages of CKD and increases progressively with advancing CKD, being higher than in controls from CKD 3. There is a variable percentage of patients in every stage, age and sex who have not developed plaque. Prospective analysis of the study will identify the risk and protective factors for progression of atherosclerotic disease, distinguished by gender, age and stage.

Funding: Government Support - Non-U.S.

SA-PO174

Use of Secondary Preventative Therapies in Individuals with Acute Coronary Syndrome and Renal Dysfunction: An Analysis of the Myocardial Ischaemia National Audit Project (MINAP) Database Catriona Shaw,[1] Cornelia Junghans,[1] Sapna Shah,[1] Donal O'Donoghue,[2] Claire C. Sharpe.[1] *[1]Department of Renal Medicine, Kings College London, United Kingdom; [2]Department of Renal Medicine, Salford Royal NHS Foundation Trust.*

Background: CKD is a recognised risk factor for acute coronary syndromes (ACS) and mortality. Our aim was to assess the association between renal dysfunction and the use of secondary preventative therapies.

Methods: Data on 54,062 individuals with NSTEMI and 34,733 individuals with STEMI were included in univariable logistic regression models to investigate the association between eGFR and utilisation of secondary preventative therapies: aspirin, statins, ace inhibitors and cardiac rehabilitation. A multivariable model was developed to assess the association between eGFR and cardiac rehabilitation. An eGFR>90ml/minute was used as the baseline category.

Results: The overall prevalence of eGFR <60ml/minute was 49% in the NSTEMI population, and 30% in the STEMI population. As eGFR reduced, the odds of receiving aspirin, a statin, an ace inhibitor or cardiac rehabilitation declined compared with individuals with an eGFR>90ml/minute in both populations (p<0.0001). In the NSTEMI population, at an eGFR of 15-29ml/minute the OR of receiving aspirin was 0.57(95% CI 0.51-0.64), an ace inhibitor 0.35 (95% CI 0.32-0.38) and a statin 0.66 (95% CI 0.59-0.73). In the STEMI population an eGFR 15-29ml/minute was associated with an OR of receiving aspirin of 0.51 (95% CI 0.42-0.61), an ace inhibitor of 0.27 (95% CI 0.23-0.31) and a statin of 0.56 (95%CI 0.46-0.67). After adjustment for major co-morbidity, the adjusted OR of being offered cardiac rehabilitation decreased as eGFR declined (p<0.0001). With an eGFR 15-29ml/minute the adjusted OR for cardiac rehabilitation were 0.76 (95% CI 0.67-0.86) in the NSTEMI population and 0.68 (95% CI 0.54-0.84) in the STEMI population.

Conclusions: The substantial burden of cardiovascular disease in renal impairment is well documented. Data from this contemporary cohort suggests that at time of hospital discharge, individuals with renal impairment receive fewer secondary preventative therapies post ACS compared with individuals with normal renal function.

SA-PO175

Periodontal Disease Is Independently Associated with Vascular Stiffness in Patients with Progressive CKD Stephanie J. Stringer,[1,2] Praveen Sharma,[1,3] Khai Ping Ng,[1,2] Mark David Jesky,[1,2] Mary Dutton,[2] Charles Ferro,[1,2] Iain Chapple,[1,3] Paul Cockwell.[1,2] *[1]Immunity and Infection, University of Birmingham, Birmingham, United Kingdom; [2]Nephrology, University Hospital Birmingham, United Kingdom; [3]Dentistry, University of Birmingham, United Kingdom.*

Background: Periodontal disease is the commonest chronic inflammatory disease. Chronic diseases including CKD are associated with vascular stiffness, vascular stiffness is a determinant of poor outcomes. We hypothesised that periodontitis is independently associated with vascular stiffness in people with CKD.

Methods: Patients enrolled into a prospective observational cohort of progressive CKD underwent a full periodontal assessment (carried out by a dentist and a hygienist), arterial stiffness (PWV), AGEs and measurement of highly sensitive c-reactive protein (hsCRP) and polyclonal serum free light chains (FLC) kidney function, proteinuria and demographics.

Results: 196 patients with progressive CKD were included, 60% were male, the mean age was 61.6 years(+/-16.5) and the median eGFR was 23 (7-90) mL/min. Periodontal disease was classified using clinical attachment loss and probing depths; 3% were classified as healthy, 37% as moderate periodontitis, 46% as severe periodontitis and 14% were edentulous (these patients were removed from the analysis as it is impossible to comment on periodontal health in these individuals). In a univariate analysis AGEs**, PWV**, cystatin C* and eGFR* (** p <0.001, * p <0.05) correlated with severity of periodontal disease, hsCRP and sFLC did not. In the multivariate analysis only PWV was significantly associated with the severity of periodontal disease (p = 0.028).

Conclusions: In this prospective bioclinical study of progressive CKD the severity of periodontitis was the only independent risk factor associated with increased vascular stiffness. There was no association with serum hsCRP or polyclonal FLC, indicating that these markers are not sufficiently sensitive systemic biomarkers for this association or other pathophysiological pathways link these processes. Clinical trials to address the hypothesis that treatment of periodontitis improves outcomes in people with CKD are required.

Funding: Private Foundation Support

SA-PO176

Cardiac Troponin T Predicts All-Cause and Cardiovascular Mortality in Predialysis Non-Diabetic Patients Terumasa Hayashi,[1] Akira Suzuki,[1] Kodo Tomida,[1] Tatsuya Shoji,[1] Noriyuki Okada,[2] Yoshiharu Tsubakihara.[1] *[1]Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [2]Clinical Laboratory, Osaka General Medical Center, Osaka, Japan.*

Background: Asymptomatic elevation of cardiac troponin T (cTnT) is associated with higher mortality and cardiovascular events in patients on hemodialysis. Furthermore, recent analysis of TREAT (Trial to Reduce Cardiovascular Events With Aranesp Therapy) revealed that cTnT was predictive for cardiovascular events and mortality in patients with predialysis chronic kidney disease (CKD) and diabetes mellitus (DM). However, little is known about predictive value of cTnT in non DM patients with predialysis CKD.

Methods: We measured cTnT in 265 consecutive patients before the start of chronic dialysis treatment between March 2005 and October 2010. The relationship between cTnT level and all-cause and cardiovascular mortality was analyzed using Cox proportional hazards models.

Results: Median age was 68 (male gender, 58%) and the prevalence of DM was 39.6%. Detectable cTnT (>0.01ng/ml) was present in 80% of patients. 51 patients died (14 from cardiovascular disease) during the median follow-up period of 32 months. Asymptomatic elevation of cTnT (cTnT>0.01ng/ml) was observed in 248 patients and was associated with all-cause (HR1.433, 95%CI 1.056-1.946) and cardiovascular mortality (HR 1.991, 95%CI 1.128-3.516) after adjustment for age, sex, diabetes, cardiovascular history, smoking, C-reactive protein, albumin, hemoglobin and estimated glomerular filtration rate. Sensitive analysis in non DM group showed similar and significant association between cTnT and all-cause (HR 1.659, 95%CI 1.114-2.469) and cardiovascular mortality (HR 2.617, 95%CI 1.085-6.311).

Conclusions: In predialysis patients, regardless of DM or non DM, asymptomatic elevation of cTnT is strongly predictive for all-cause and cardiovascular mortality. These findings suggest ways to improve cardiovascular risk stratification of patients with predialysis CKD.

SA-PO177

Study of Vascular Calcification Progression in Elderly Patients with and without CKD Stephen G. John,[1] Paul J. Owen,[1] Jane H. Youde,[2] Chris W. McIntyre.[1,3] *[1]Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Medicine for the Elderly, Royal Derby Hospital, Derby, United Kingdom; [3]School of Graduate Entry Medicine and Health, University of Nottingham, Derby, United Kingdom.*

Background: Chronic kidney disease (CKD) is highly prevalent in older people and is associated with changes in cardiovascular (CV) function and elevated CV risk. Vascular calcification (VC) predicts CV risk and mortality in CKD, but the contribution of CKD in older people and linkage to other markers is incompletely understood. We performed a prospective, controlled study of VC and CV function, body composition, functional capacity and falls in older (>70 yrs) non-diabetic patients with and without CKD 3/4.

Methods: We recruited 61 subjects (including non-CKD controls). Blood pressure (BP) was controlled to 130/80mmHg. Baseline assessment included VC (superficial femoral artery CT), CV function (carotid-femoral pulse wave velocity (PWV), baroreflex sensitivity (BRS), central haemodynamics), skin AGE, body composition (bioimpedance analysis) and function (Timed get Up and Go test (TUG)), bloods (inc. PTH, Ca, PO_4, Vitamin D), proteinuria and falls diaries. Assessment was repeated at 1 year (FU).

Results: Mean age was 76±4yrs, mean eGFR (CKD group) 42±14ml/min/1.73m^2, mean BP 128/69 mmHg, significant proteinuria in 18%. 50% had significant VC. Median VC (12±71units [0-1397]) and mean PWV (12.4±2.7m/s) were similar in CKD and non-CKD. VC was higher in men (28±11;81±57units;p=0.044), correlated with TUG (r=0.297;p=0.034) but not PWV, BRS or any other variable. VC increased at FU (12±71;28±125units;p<0.001), whilst PWV did not (12.4±2.7;12.4±2.5m/s;p=0.753). VC increased similarly (p=0.336) in both CKD (13±162;28±190units;p=0.001) and non-CKD (8±61;28±92units;p=0.026) and was independent of any other variable.

Conclusions: Over 1 year, with controlled BP, VC increased but there was no change in other markers of vascular health. VC is associated with falls risk. Although overall VC load was similar to that previously reported by our group, this cohort had earlier CKD but advanced age. Furthermore, as VC was similar in non-CKD and CKD groups this suggests a greater contribution from ageing than renal dysfunction in CKD 3/4.

SA-PO178

Ankle Brachial Index Predicts Cardiovascular Disease Risk and Mortality among Patients with Chronic Kidney Disease: A Prospective Analysis from the CRIC Study Jing Chen, L. Lee Hamm, Dawei Xie, Raymond R. Townsend, Jackson T. Wright, Matthew Jay Budoff, Lisa C. Nessel, Qiang Pan, Susan P. Steigerwalt, Pranav S. Garimella, Jiang He. *Tulane University.*

Background: Cross-sectional studies suggest that the proportion of individuals with an ankle brachial index (ABI) at the high or low extremes was increased among patients with chronic kidney disease (CKD).

Methods: We investigated the prospective relationship between baseline ABI and subsequent risk of cardiovascular disease (CVD) events and total mortality among 3,627 CKD patients who participated in the Chronic Renal Insufficiency Cohort (CRIC) Study and did not have a history of upper or lower extremity revascularization or amputation at

baseline examination. Peripheral arterial disease (PAD) and other CVD events [myocardial infarction (MI), congestive heart failure (CHF), and stroke] were adjudicated by an assessment committee.

Results: The event rates per 100 person-years for ABI<0.90, 0.90-0.99, 1.00-1.39, and ≥1.40 were 1.12, 0.54, 0.20, and 1.05 for PAD; 2.18, 1.81, 0.69, and 2.54 for MI; 4.70, 2.59, 1.91, and 2.89 for CHF; 6.66, 4.55, 2.80, and 5.02 for total CVD other than PAD; and 4.51, 2.78, 1.55 and 2.28 for total mortality. Compared to those with ABI of 1.00-1.39, those with ABI<0.90, 0.9-0.99, and ≥1.40 had hazard ratios (HR, 95% CI) of 4.03 (2.03, 7.97), 2.39 (1.11, 5.17) and 5.46 (1.59, 18.7) for PAD, p<0.001; 2.01 (1.33, 3.06), 2.42 (1.59, 3.68) and 2.81 (1.28, 6.21) for MI, P<0.001; and 1.49 (1.12, 1.99), 1.40 (1.02, 1.92) and 1.00 (0.44, 2.28) for total mortality, P=0.03, respectively, after adjustment for traditional CVD risk factors. The multivariable-adjusted HR for CHF and total CVD other than PAD was not statistically significant.

Conclusions: These data indicate that both higher and lower ABI was associated with higher risk of PAD and MI events, while lower ABI was associated with higher total mortality. In conclusion, our study suggested that ABI may be a useful index for risk stratification for PAD, MI and mortality among CKD patients. Furthermore, ABI cut points for diagnosis of PAD may need to be further defined in CKD patients.

Funding: NIDDK Support

SA-PO179

Subclinical Left Atrial Enlargement in Stage 3-5 Chronic Kidney Disease

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Background: Left atrial enlargement (LAE) is a marker of diastolic dysfunction & predicts adverse outcomes in end-stage renal disease. However, little is known of its prevalence & factors associated with subclinical LAE in earlier stages of chronic kidney disease (CKD).

Methods: We prospectively recruited 261 stage 3–5 CKD patients without symptomatic cardiovascular disease(mean eGFR:34±17ml/min per1.73m^2)& performed 2D-echocardiography to determine left atrial volume index (LAVi)& other cardiac parameters.

Results: 109 patients (41.8%) had LAE of which it was mild[LAVi≥29-34ml/m^2],moderate[LAVi≥34-40ml/m^2] & severe[LAVi≥40ml/m^2] in 45, 28 & 36 patients,respectively. Mild & moderate/severe LAE were observed in 22.9% & 41.3% of patients with left ventricular hypertrophy (LVH) (n=109) *vs* 13.2% & 12.5% of patients without LVH (n=152), respectively(P<0.001). On univariate analysis, other than increasing age, systolic blood pressure (SBP), worsening anemia, serum albumin, phosphorus & declining eGFR being significantly associated with LAE, a significant increase in plasma sodium level was observed across patients with increasing severity of LAE (P=0.001). In the multiple stepwise logistic regression, plasma sodium level emerged as one of the most significant factors associated with LAE [adjusted odds ratio(OR), 1.31, 95% CI, 1.15–1.49; P<0.001]. Its significance was maintained [adjusted OR, 1.24, 95% CI, 1.07–1.44; P=0.004] when including LV mass & volume index & N-terminal pro-brain natriuretic peptide in the model while SBP and hemoglobin were displaced.

Conclusions: This is the first study to demonstrate an extremely high prevalence of subclinical LAE & a novel,positive association between plasma sodium level & subclinical LAE in stage 3–5 CKD patients. Longitudinal study is needed to determine whether high plasma sodium may be causally linked to LAE in CKD & the underlying mechanism. More attention is needed to target subclinical LAE in CKD.

Funding: Pharmaceutical Company Support - Unrestricted Grant from Genzyme

SA-PO180

Advanced Glycation End-Products and Arterial Stiffness in Diabetic and Non-Diabetic Patients with Chronic Kidney Disease

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Background: The formation of advanced glycation end-products (AGEs) is increased in diabetic patients. Impaired renal function also elevates AGEs. Pulse wave velocity (PWV) is a measure of arterial stiffness and valuable prognostic parameter. The association between AGEs and arterial stiffness was found in hypertensive patients and in hemodialysed patients. The study investigated the relationship between plasma AGEs concentration and arterial stiffness in diabetic (DN) and non-diabetic (Non-DN) patients with chronic kidney disease (CKD).

Methods: The study population consisted of 60 CKD patients: 24 with DN, and 36 with Non-DN, and 19 controls. Carotid-femoral PWV was measured using Complior device. To assess plasma AGEs concentration fluorescence spectra were recorded with Fluoroscan Ascent FL Labsystems (excitation 355 nm/emission 460 nm). AGEs measurements were taken in duplicate, averaged, and expressed in arbitral units (AU/ml). Systolic (SBP), diastolic (DBP) blood pressure and estimated glomerular filtration rate (eGFR) were also investigated.

Results:

	Controls	Non-DN	DN
Age (years)	46±13	51±15	53±14
Male gender (%)	11(58%)	21(58%)	14(58%)
SBP (mmHg)	125±10	137±21[1]	148±24[2]
DPB (mmHg)	79±6	84±10[1]	80±10
eGFR (ml/min/1,73m^2)	81±15	33±13[2]	32±14[2]
AGEs (AU/ml)	7,8±1,2	12,3±3,1[2]	21,1±6,8[2,3]
PWV (m/s)	8,4±1,6	10,1±2,4[1]	13,7±4,3[2,3]

[1]– p<0,05 vs controls; [2]– p<0,001 vs controls; [3]– p<0,001 vs Non-DN. Significant correlation was found between AGEs and PWV in the whole study population (r=0,49;p<0,001;n=79), as well as in CKD patients (r=0,39;p<0,01;n=60), but not separately in DN (r=0,18;NS) and Non-DN (r=-0,06;NS) patients. In multiple regression analysis PWV was independently associated with age, diabetes and SBP, but not with eGFR and AGEs (R^2 for the model = 0,53).

Conclusions: Accumulation of AGEs and arterial stiffness are increased in CKD, particularly in diabetic CKD patients. However our results are not sufficient to confirm the role of AGEs accumulation in arterial stiffening in CKD patients.

SA-PO181

Left Ventricular Mass and Chronic Kidney Disease: A Cardiac MRI Study in Hypertensive Patients with Marked Cardiovascular Risk: A Substudy of SMART

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Background: Increased left ventricular mass (LVM) is known to predict cardiovascular morbidity and mortality. Left ventricular mass is high in ESRD patients. Previous studies using echocardiography showed that in patients with mild to moderate chronic kidney disease (CKD) left ventricular mass is already increased. Our aim was to study the relationship between renal function and left ventricular mass with cardiac MRI in a population of patients at high risk for cardiovascular disease (CVD).

Methods: Cardiac MRI was performed in 529 hypertensive patients with manifest arterial disease or marked risk factors for CVD. All subjects had been screened for cardiovascular risk factors in a standardized way as part of SMART, a prospective single-centre cohort study. Multivariable linear regression was used to study the relationship of both eGFR (using the MDRD formula) and presence of albuminuria with left ventricular mass. Presence of albuminuria was defined as a urinary albumin/creatinin ratio above 2,5 mg/mmol in men and 3,5 mg/mmol in women.

Results: Mean LVM was 121 g for men (SD 26) and 87 g for women (SD 20). Mean eGFR was 82 ml/min/1.73m² (SD 19). 78% of the patients had stage I to III CKD, 22% had an eGFR >90 ml/min/m² and normoalbuminuria. 75 patients (14,1%) had albuminuria. After adjusting for known determinants of LVM (height, weight, sex and age) eGFR did not relate to LVM while presence of albuminuria did (B for eGFR 0.066, 95%CI -0.045 to 0.178, p=0.243, B for albuminuria 8.684, 95%CI 3.234 to14.135, p=0.002). Additional adjustment for systolic blood pressure did not change results (B for eGFR 0.039, 95%CI -0.072 to 0.150, p=0.490, B for albuminuria 7.453, 95%CI 2.029 to12.877, p=0.007).

Conclusions: In our MRI study in hypertensive patients with high vascular risk decreased eGFR was not related to increased LVM. Presence of albuminuria was related to increased LVM even after adjustment for systolic blood pressure.

SA-PO182

Fat Depots, Cardiovascular Disease Risk Factors, and Coronary Artery Calcification in Non-Dialysis Dependent Chronic Kidney Disease Patients

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Background: Specific fat deposition may be associated with variable metabolic risk. We sought to evaluate the correlations between pericardial fat, visceral fat and subcutaneous fat accumulation with anthropometric parameters, metabolic risk factors, surrogates of inflammation and the presence of coronary artery calcification (CAC) in non-dialysis dependent chronic kidney disease patients (NDD-CKD).

Methods: We cross-sectionally evaluated 234 NDD-CKD stages 3–5 patients (61 [52–67] years, 146 men). Pericardial, visceral and subcutaneous fat, as well as CAC were assessed by computed tomography.

Results: All studied fat depots showed significant positive correlations (although varying in strength) with BMI, waist circumference, LDL cholesterol, triglycerides, HOMA-IR index white blood cell count and C3, whereas they correlated negatively with HDL cholesterol. Pericardial and visceral fat, but not subcutaneous, positively correlated with age, 24h urine creatinine clearance (CrCl), serum albumin and CAC score (rho=0.19 and 0.20 for pericardial and visceral fat, respectively; P<0.01). Furthermore, only visceral fat correlated with CRP (rho=0.14; P<0.05). In a series of logistic regression analyses with specific fat depots and which included confounders such as age, sex, CrCl, diabetes, smoking, BMI, HDL, triglycerides and calcium-phosphorus product, visceral fat was the only fat compartment that remained significantly associated with the presence of CAC [CAC score ≥10 Agatston] (OR per 0.1 cm^2 of increase in visceral fat: 1.05; 95% CI=1.01–1.10).

Conclusions: Excess fat deposition in NDD-CKD patients correlates with multiple metabolic risk factors. Among the fat depots studied, visceral proved to be independently associated with the presence of CAC. Given its metabolic activity, our data altogether emphasizes the need for strategies to reduce visceral fat stores among NDD-CKD individuals.

Funding: Government Support - Non-U.S.

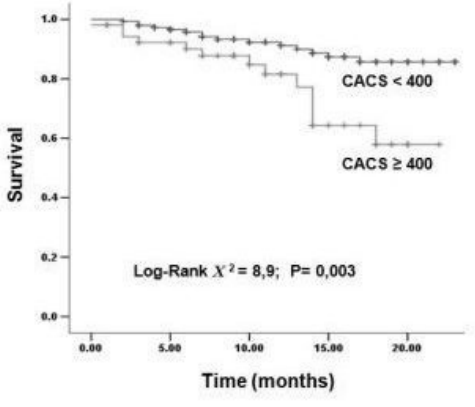
SA-PO183

Coronary Artery Calcium Score Predicts Adverse Events in Non-Dialysis Dependent Chronic Kidney Disease Patients Antonio C. Cordeiro,[1] Bengt Lindholm,[2] Antonio T. Paladino Filho,[1] Marcela Perini,[1] Celso Amodeo,[1] Ibraim M.F. Pinto,[1] Fernanda C. Amparo,[1] Amanda G.M.R. Sousa,[1] Juan Jesus Carrero.[2] *[1]Dante Pazzanese Institute of Cardiology, Sao Paulo, Brazil; [2]Divisions of Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden.*

Background: Coronary artery calcium score (CACS) is a valuable cardiovascular (CV) risk stratification tool in the general population. However, its applicability in chronic kidney disease (CKD) patients is controversial, since these patients are already at high CV risk. We aimed to evaluate the prognostic power of CACS in non-dialysis dependent (NDD) CKD patients.

Methods: We cross-sectionally evaluated 203 NDD-CKD stages 3–5 patients (60 [52–67] years, 123 men). CACS (in Agatston) was assessed by computed tomography. All patients were followed-up for 12 [7 – 18] months, and a composite endpoint of mortality and cardiovascular events was registered.

Results: CACS was correlated with age (Rho=0.60; P<0.001), total cholesterol (TC, Rho=-0.16; P=0.01), HDL-cholesterol (HDL, Rho=-0.15; P=0.02) and PTH (Rho=-0.17; P=0.01). Patients were divided in two groups according to CACS (<400 [n=149] and ≥ 400 [n=54]). Patients with higher CACS were significantly older (68 vs. 57 years; P<0.001) and presented higher prevalence of diabetes (70 vs. 41%; P<0.001), as well as lower levels of TC and HDL. During the follow-up period, there were a total of 28 events (15 deaths), and patients with a high CACS presented an increased rate of events (**Figure**).



The crude hazard ratio for the composite endpoint was 2.94 (95% CI: 1.39–6.19) for patients with high CACS. This remained true after correcting for age, sex and diabetes (adjusted HR: 3.03; 95 % CI: 1.23–7.46).

Conclusions: Elevated CACS was predictive of adverse events in NDD-CKD patients. This data is suggestive of the potential utility of CACS as a risk stratification tool in this patient population.

Funding: Government Support - Non-U.S.

SA-PO184

Is Plasma beta-2 Microglobulin a Better Predictor of Cardiovascular Outcomes than Traditional Risk Factors in Chronic Kidney Disease Patients? Sophie Liabeuf,[1 2] Aurélie Terrier Lenglet,[1 2] Lucie Desjardins,[1 2] Nathalie Neirynck,[3] Griet L.R.L. Glorieux,[3] Horst-Dieter Lemke,[4] Raymond C. Vanholder,[3] Momar Diouf,[2] Gabriel Choukroun,[1 5] Ziad Massy.[1 2 5] *[1]INSERM U-1088, Amiens, France; [2]Clinical Research Centre - Division of Clinical Pharmacology, Amiens University Hospital, Amiens, France; [3]Nephrology Dialysis Transplantation Department, Department of Internal Medicine, University Hospital, Gent, Belgium; [4]EXcorLab GmbH, EXcorLab GmbH, Obernburg, Germany; [5]Division of Nephrology, Amiens University Hospital, Amiens, France.*

Background: As beta-microglobulin (B2M) a surrogate marker for middle molecular weight uremic toxins and the major protein component in dialysis-related amyloidosis, B2M is frequently studied in dialysis patients. However, it is not known whether or not B2M has an impact in chronic kidney disease (CKD) patients not yet on dialysis.

Methods: We decided to perform a prospective, observational study of the impact of plasma B2M levels on clinical and cardiovascular outcomes in 142 patients at different CKD stages (mean age ± SD: 67 ± 12 years; 8% at CKD stage 2, 26% at stage 3, 26% at stage 4, 8% at stage 5 and 32% at stage 5D).

Results: B2M levels increased with CKD stage and thus were highest in hemodialysis patients. Baseline B2M levels were associated with vascular calcification but not with arterial stiffness or bone density. During the follow-up period (mean: 969 ± 374 days), 44 patients died and 49 presented a cardiovascular event. Higher B2M levels were independently associated with overall and cardiovascular mortality and cardiovascular events in the whole cohort. Moreover, B2M appears to be a better predictor than well known factors associated with outcomes in this population including eGFR (only for predialysis patients), inflammation biomarkers and others factors included in a propensity score.

Conclusions: In conclusion, our study confirmed the strong relationship between B2M levels and the estimated glomerular filtration rate. More importantly, we confirmed the power of the B2M level for predicting overall and cardiovascular mortality and cardiovascular events in patients at different CKD stages.

SA-PO185

Chronic Kidney Disease (CKD) in Children Impairs Normal Protective Functions of High Density Lipoprotein (HDL) Ryohei Kaseda, Kathy L. Jabs, Tracy E. Hunley, Deborah P. Jones, Valentina Kon. *Pediatric Nephrology, Vanderbilt University, Nashville, TN.*

Background: Trials to increase HDL levels have shown disappointing clinical effects and have given rise to the concept of dysfunctional HDL. Renal impairment can affect the composition of HDL, which has been revealed to be a key modulator in diverse biologic activities relevant to CKD complications. Recently, we showed that HDL of hemodialyzed adults has impaired cholesterol acceptor and anti-inflammatory capacities. Whether moderate CKD and end stage renal disease (ESRD) requiring dialysis in childhood disrupts the newly appreciated functions of HDL is unknown.

Methods: Inflammatory and chemotactic effects were assessed in human macrophage-like THP-1 cells exposed to HDL isolated from children with ESRD on peritoneal dialysis (ESRD-PD, n=5), children with CKD (stage III-IV, n=6) and normal kidney function (Control, n=4). Cytokine response of LPS-activated THP-1 macrophages (LPS 50 ng/ml x 4h) ± HDL (18 ug/ml) from the 3 groups was assessed by mRNA expression of inflammatory markers. Chemotaxis of THP-1 cells to monocyte chemoattractant protein-1 (MCP-1) was assessed in a modified Boyden transwell chamber.

Results: Compared with Control, HDL of CKD and ESRD-PD elicited a heightened inflammatory cytokine response: TNF-a Control:0.087±0.017, CKD-0.187±0.018*, ESRD-PD-0.157±0.019*; MCP-1: Control:1.136±0.203, CKD:2.509±0.146*, ESRD-PD:2.040±0.172*; IL-1b: Control:1.157±0.207, 2.439±0.566*, 2.905±1.237*, (*p<0.05 vs Control). Control HDL reduced chemotaxis of THP-1 cells while HDL from CKD or ESRD-PD had no effect (MCP-1alone:44.6±2.2, MCP-1+Control:12.6±1.2* MCP-1+CKD 44.2±2.6#, ESRD-PD:29.6±1.9#, *p<0.05 vs MCP-1 alone, #p<0.05 vs Control).

Conclusions: Compared to children with intact kidney function, HDL of children with CKD and ESRD-PD has dramatically impaired capacity to abrogate LPS-induced inflammatory response together with reduced anti-chemotactic ability. These results suggest that HDL of children with reduced renal function has compromised protective functions and may, instead, be harmful.

Funding: NIDDK Support

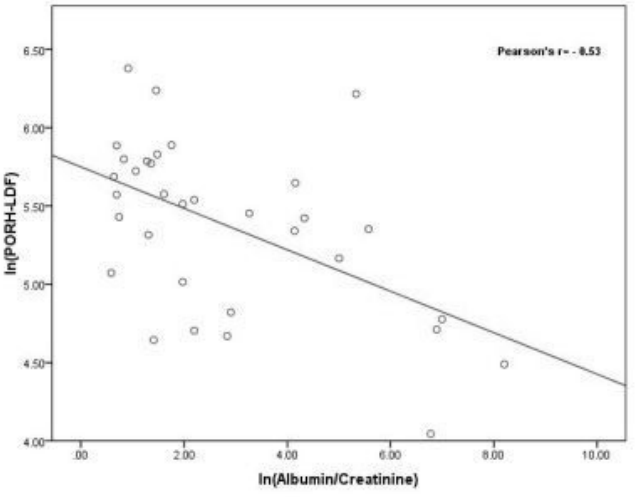
SA-PO186

Non-Invasive Measures of Cutaneous Microcirculatory Endothelial Function Are Associated with CKD and Albuminuria in Older Adults Afshin Parsa,[1] Shabnam Salimi,[1] Leslie I. Katzel,[1,2] Jamie Giffuni,[2] Stephen L. Seliger.[1] *[1]Medicine, U Maryland, Baltimore, MD; [2]GRECC, Baltimore VA Medical Center, Baltimore, MD.*

Background: Prior studies of endothelial dysfunction in CKD have mainly examined conduit-artery responses. However, micro-circulatory function may also be a significant determinant of the development and progression of albuminuria, CKD and associated CVD outcomes. We measured cutaneous microcirculatory endothelial function using laser Doppler flowmetry (LDF) to determine the association with albuminuria and CKD.

Methods: Study participants were older men (age 60-85) from the Baltimore VA Medical Center. Endothelial function was quantified with LDF measures of post occlusive reactive hyperemia (PORH) and thermal hyperemia (TH) responses in 17 CKD subjects and 18 age matched hypertensive non-CKD controls. Linear regression was used to estimate the association of CKD and urinary albumin to creatinine ratio (ACR) with LDF measures, adjusting for age, diabetes, and mean blood pressure.

Results: Mean age was 70 years, 50% had diabetes, median eGFR among CKD subjects was 44 mL/kg/1.73m^2. Peak PORH-LDF response was significantly lower in our CKD vs. non-CKD group at 260.7 ±156.8 vs 315.3 ±161.6 perfusion units, respectively (adjusted standardized β=-0.48, p=0.02). Peak PORH-LDF response also negatively correlated with ACR (standardized β=-0.69, p=0.001; Figure). TH LDF measures were not significantly lower in CKD subjects, but first TH peak response was negatively associated with ACR (standardized β=-0.64, p=0.004).



Conclusions: Among older adults with HTN, LDF measures of cutaneous microcirculatory endothelial function are significantly lower in CKD and in those with greater albuminuria. These findings suggest that cutaneous LDF measures may provide a practical and useful proxy of CKD related disturbances in microcirculatory endothelial function.

Funding: Other NIH Support - NIA, Veterans Administration Support

SA-PO187

Differences in Progression to End-Stage Renal Disease between Black and White Incident Patients on Pre-Dialysis Care in a Universal Health Care System Moniek C.M. de Goeij,[1] Tessa O. Van den Beukel,[1,2] Carl E.H. Siegert,[2] Friedo W. Dekker,[1] Nynke Halbesma.[1] *[1]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Sint Lucas Andreas Hospital, Amsterdam, Netherlands.*

Background: Black CKD patients, mainly stages I-IV, have a faster progression to ESRD than whites. However, it is unknown whether this difference is also present in patients on pre-dialysis care in a universal health care system. Therefore, we investigated whether the rate of starting renal replacement therapy (RRT) and the decline in renal function was different between black and white patients on pre-dialysis care in the Netherlands.

Methods: In the Dutch observational multicenter PREPARE study, 1049 incident patients on pre-dialysis care (CKD stages IV-V) were included. Patients were followed until the start of RRT, death, or end of 2 years follow-up. Black and white ethnicity was assessed by the medical staff. A Cox proportional hazard regression analysis was used to estimate the hazard ratio (HR) for starting RRT and a linear mixed model to compare the rate of decline in renal function between blacks and whites. To explore mechanisms, adjustments were made for patient characteristics at the start of pre-dialysis care.

Results: 945 patients were white, 49 were black, and the 55 patients with another ethnicity were excluded. At the start of pre-dialysis care, blacks were younger, had more diabetes, more proteinuria, and a higher eGFR than whites (19.1 vs. 14.6 ml/min/1.73 m^2). In the crude analysis, blacks had a comparable rate of starting RRT as whites, HR 1.07 (95%CI, 0.74-1.54). After adjustment for eGFR the HR increased to 1.55 (95%CI, 1.04-2.32). During pre-dialysis care, blacks had a faster decline in renal function than whites, 0.20 ml/min/1.73 m^2/month (95%CI, 0.05-0.35). Further adjustment for patient characteristics did not change these results.

Conclusions: We demonstrated that black patients on specialized pre-dialysis care in a universal health care system show a faster progression to ESRD than white patients. Future studies should focus more on biological explanations and explore whether guidelines for pre-dialysis treatment should take data on ethnicity into consideration.

Funding: Pharmaceutical Company Support - AMGEN BV. (Funded a Part of the PREPARE Study)

SA-PO188

Renal Function in Familial Longevity: The Leiden Longevity Study Moniek C.M. de Goeij,[1] Nynke Halbesma,[1] Friedo W. Dekker,[1] Ton De Craen.[2,3] *[1]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [2]Gerontology and Geriatrics, Leiden University Medical Center, Leiden, Netherlands; [3]Netherlands Consortium for Healthy Ageing, Leiden University Medical Center, Leiden, Netherlands.*

Background: Studying renal function in subjects with a familial propensity for longevity might provide new targets for treatment. We investigated whether renal function in middle aged individuals with a familial propensity for longevity is different compared to environment matched controls.

Methods: In the Leiden Longevity Study, middle aged offspring of nonagenarian siblings and their partners as environment matched controls were included. Information on life style, medical history, medication use, and a non-fasting blood sample were collected. Renal function was estimated with the Chronic Kidney Disease epidemiology collaboration (CKD-epi) formula. Analyses were stratified for sex. To account for familial dependencies within the offspring, a linear mixed model was used.

Results: A total of 1300 offspring (47% males, mean ± SD age 59.3 ± 6.5 years) and 596 partners (43% males, mean ± SD age 58.6 ± 7.1 years) were available for analyses. Female offspring and female partners had a similar eGFR, age adjusted difference of 0.44 (SE, 0.72; p=0.54) ml/min/1.73 m^2. Male offspring had a higher eGFR compared to male partners, age adjusted difference of 1.78 (SE, 0.78; p=0.022) ml/min/1.73 m^2. Further adjustment for known risk factors for decline in renal function such as history of cardio- and cerebrovascular disease, diabetes mellitus, anti-hypertensive and anti-diabetic medication changed the point estimates to 0.05 (SE, 0.74; p=0.94) ml/min/1.73 m^2 for females and 1.79 (SE, 0.79; p=0.024) ml/min/1.73 m^2 for males. Adjustment for blood parameters or lifestyle factors somewhat diluted the difference in males (p=0.048 and p=0.051 respectively).

Conclusions: Middle aged males, and not females, with a propensity for longevity have better renal function compared to environment matched controls. The better renal function in males could not be explained by differences in main known risk factors for renal dysfunction.

Funding: Government Support - Non-U.S.

SA-PO189

Association of GFR and Albuminuria with Mortality and End-Stage Renal Disease across Asians, Whites, and Blacks: A Collaborative Analysis of 45 Cohorts (for the CKD-PC Collaborators) Chi Pang Wen, Kunihiro Matsushita, Josef Coresh, Kunitoshi Iseki, Muhammad Islam, Ronit Katz, William M. McClellan, Carmen A. Peralta, Haiyan Wang, Dick de Zeeuw, Brad C. Astor, Ron T. Gansevoort, Andrew S. Levey, Adeera Levin. *CKD Prognosis Consortium.*

Background: Global interest in CKD has been increasing, but whether the impact of low GFR or high albuminuria, on clinical outcomes differs by race/ethnicity is yet unsettled.

Methods: We studied 1,102,660 individuals (827,089 Asians, 227,821 whites, and 47,750 blacks) from 45 cohorts inthe CKD Prognosis Consortium (CKD-PC). The hazard ratios (HRs) of all-cause mortality and ESRD for estimated GFR (eGFR) by the CKD-EPI equation and albuminuria (ACR or dipstick) were quantified in each race. Most Asian studies used dipstick, while others more frequently used ACR. Meta-regression analysis was used to compare HRs across races.

Results: For Asians, whites and blacks, mean age was 46, 63, and 59 years, and proportion with diabetes was 5%, 12% and 24%, respectively, with Asians having higher GFR and less albuminuria than others. Adjusted HR of mortality for eGFR and albuminuria compared to the reference group was similar in Asian, whites, and blacks (for example, for individuals with eGFR 30-44 ml/min/1.73m^2, the respective HR [95% CI] was 1.7 [1.5-2.0], 1.4 [1.3-1.6] and 2.0 [1.5-2.7] and for microalbuminuria [ACR 30-299 mg/g or dipstick 1+] 1.6 [1.4-1.8], 1.7 [1.5-1.9] and 1.8 [1.7-2.1], respectively). (Figure) Meta-regression comparisons were generally not significant and similar results were seen for ESRD.

Conclusions: Despite wide variability in clinical characteristics among cohorts and lower risk profile in Asians, the adjusted relative risks for mortality and ESRD according to low eGFR or high albuminuria were similar among Asians, whites and blacks.

Relative risk of mortality and ESRD according to eGFR and ACR/dipstick categories in whites, Asians, and blacks in general population cohorts

		All-cause mortality					ESRD				
		ACR/Dipstick					ACR/Dipstick				
	eGFR	<10 / Dip "-"	10-29 / Dip "±"	30-299 / Dip "1+"	300+ / Dip "≥2+"		<10 / Dip "-"	10-29 / Dip "±"	30-299 / Dip "1+"	300+ / Dip "≥2+"	
Asian	≥105	1.1	1.7	3.6	5.4	1.2			54.8	47.5	1.0
	90-104	REF	1.6	1.8	3.4		REF		8.3		
	75-89	1.0	1.3	1.6	2.5	0.9	1.8		5.4	72.1	2.1
	60-74	1.0	1.3	1.7	2.1	1.0	3.8	24.1	19.9	130	7.1
	45-59	1.3	2.0	1.8	2.8	1.3	17.2	64.6	116	577	27.6
	30-44	1.9	3.0	2.7	3.6	1.7	115	113	631	1426	93.6
	15-29	3.4	4.1	6.0	8.9	3.3	625	3813	2709	8170	526
	<15	8.2	8.1	4.7	11.8	4.1		20599	48789	37298	1545
			1.4	1.6	2.2			2.7	7.4	24.8	
White	≥105	1.2	2.0	2.9	7.6	1.3	3.7	17.4	27.4	54.3	8.6
	90-104	REF	1.6	1.7	4.0		REF	3.3	4.3	57.2	
	75-89	0.9	1.4	1.6	2.1	0.9	1.2	6.2	6.6	25.7	1.9
	60-74	1.0	1.5	1.8	2.5	1.0	3.9	5.8	17.4	43.0	4.1
	45-59	1.1	1.6	1.9	2.9	1.1	10.2	10.5	40.0	156	11.2
	30-44	1.5	2.2	2.6	3.9	1.4	46.5	37.6	265	512	50.8
	15-29	3.2	3.4	3.0	5.8	2.1	501	296	725	857	116
	<15	3.8	4.4	6.2	9.7	3.7		4132	1561	4680	375
			1.4	1.7	2.4			1.3	4.0	10.2	
Black	≥105	1.4	1.6	2.1	3.6	1.2	1.3	1.5	2.5	27.9	0.4
	90-104	REF	1.4	1.9	3.7		REF	2.6	7.3	26.5	
	75-89	1.1	1.5	2.1	3.2	1.0	0.8	3.8	8.5	50.6	1.2
	60-74	1.2	1.8	2.3	3.4	1.2	2.0	5.3	5.7	53.6	1.6
	45-59	1.3	2.4*	2.5	4.3	1.3	3.8	29.1	25.4	104	4.1
	30-44	2.3	2.0	4.3	5.9	2.0*	69.7	34.9	101	392	12.4
	15-29	2.3	5.1	4.9	5.3	2.0	238	159	395	692	26.1
	<15		21.3	14.8	11.7	4.4			2142	1439	44.3
			1.4	1.8	2.7			1.9	5.6	20.4	

Each number represents a pooled hazard ratio from meta-analysis adjusted for covariates and compared with the reference cell (REF) within each race. Bold numbers indicate statistical significance at $P<0.05$. Green, yellow, orange, and red colors indicate quartiles of risk from low to high, respectively. All hazard ratios for blacks and Asians are compared with those for whites for interaction using meta-regression, and stars (*) indicate a significant interaction at $P<0.05$. Colors reflect ranking of relative risk.

Funding: Private Foundation Support

SA-PO190

Prior Contact with a Nephrologist and Outcomes in Adults with Chronic Kidney Disease: The Chronic Renal Insufficiency Cohort (CRIC) Study

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Background: Pre-dialysis nephrology care for adults with advanced chronic kidney disease (CKD) is associated with improved outcomes. Less is known about the effects of nephrologist management in earlier stages of CKD.

Methods: We conducted cross-sectional analyses of baseline data from CRIC enrollees to assess the association between CKD management and self-reported prior contact with a nephrologist, which was elicited by answering "yes" to the question "Have you ever seen a nephrologist or a kidney doctor?" We also examined the association between prior contact with a nephrologist and outcomes ascertained up to 4 years of follow-up [CKD progression (≥50% eGFR loss or ESRD), incident cardiovascular disease (CVD), and death] using Cox-proportional hazards models.

Results: Of 3861 participants, 67% reported prior contact with a nephrologist. Enrollees who were older, female, of Hispanic ethnicity, had less than high school education, higher eGFR, and lower urine protein were less likely to report prior contact with a nephrologist ($p<0.05$). CKD management according to prior contact with a nephrologist in participants with eGFR 30-60 ml/min/1.73m² are in Table.

CKD Management Parameters by Prior Contact with a Nephrologist

	Prior Contact with Nephrologist	
Management Parameter, %	Yes (n=1760)	No (n=943)
Systolic BP	58	56
ACE-i/ARB use	74*	67
HbA1C <7%	36	34
Phosphate 2.7-4.6 mg/dl	89	91
LDL <100 mg/dl	53	50
iPTH, pg/ml, median	54*	45
Hemoglobin, g/dl, mean	12.7	12.7

*p<=0.01

After adjusting for demographic and clinical factors, prior contact with a nephrologist was not significantly associated with CKD progression, incident CVD or death ($p>0.05$).

Conclusions: One-third of CRIC participants reported they had not seen a nephrologist before enrollment, and this prior contact was subject to age, sex, and ethnic-related disparities. Self-reported prior contact with a nephrologist was not associated with an improvement in long-term adverse outcomes.

Funding: NIDDK Support, Veterans Administration Support

SA-PO191

ACE Inhibitor and Angiotensin Receptor Blocker Use and Mortality in Patients with CKD

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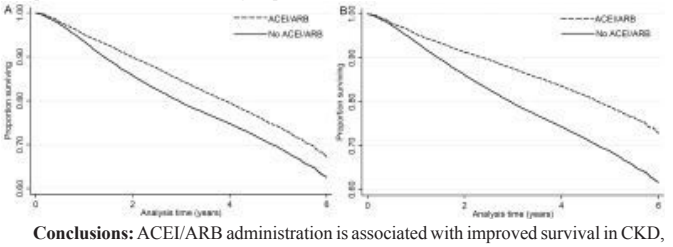
Background: There is insufficient evidence about the association of angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) with mortality in CKD.

Methods: We assessed the association of ACEI/ARB use with all-cause mortality in 141,413 US veterans with CKD stages 1-5 previously unexposed to ACEI/ARB treatment. Logistic regression analysis was used to calculate the propensity of ACEI/ARB initiation. The association of ACEI/ARB with mortality was examined in patients matched by propensity scores, using the Kaplan-Meier method and Cox models in "intention-to-treat" analyses, and a generalized linear model with binary outcomes and inverse probability treatment weighing (IPTW) in "as-treated" analyses.

Results: Over a median follow-up of 4.7 years there were 5,028 deaths (25%, mortality rate 22.6 [22.0-23.2]/1000 patient-years) in the treated group and 6,450 deaths (32%, 26.5 [25.9-27.2]/1000 patient-years) in the untreated group in the propensity score-matched cohort. ACEI/ARB use was associated with lower mortality in both intention-to-treat (HR, 95%CI: 0.81, 0.78-0.84, panel A) and in as-treated models (OR, 95%CI: 0.37, 0.34-0.41, panel B).(Figure) The association of ACEI/ARB treatment with lower risk of mortality was present in all examined subgroups.



Conclusions: ACEI/ARB administration is associated with improved survival in CKD, suggesting that ACEI/ARB may hold benefits beyond renoprotection in this vulnerable population.

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SA-PO192

Association between Variability in eGFR and Mortality

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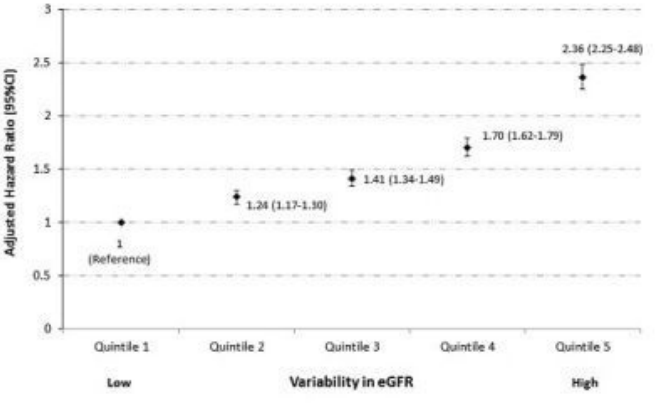
Background: Change in kidney function, in particular variability in eGFR, and risk of death is not well understood. We aimed to investigate the association between variability in eGFR and risk of mortality among a community based population.

Methods: We included 529,312 adults from a province-wide laboratory registry in Alberta, Canada between 2002-2008, who had at least 3 outpatient eGFR measurements over a 4 year accrual period. Variability in eGFR was defined using the coefficient of variation(CV) across measurements for each participant during the accrual period. Variability was categorized by quintile, with the lowest quintile representing patients with the least variability (referent) and increasing quintiles reflecting greater degrees of variability. Cox proportional hazard model (adjusting for baseline covariates, kidney function, proteinuria, and rate of change in eGFR over time) was used to estimate the HRs of all-cause mortality associated with increasing quintiles of variability, with follow-up to March 31 2009.

Results: Among the participants (mean age 55.6 years, 41.9% male) there were 32,372(6.1%) deaths over a median follow-up of 2.5 years. Compared with the referent (lowest quintile), patients in the highest quintile of eGFR variability had more than two-fold increased risk of death.

Figure 1 Association between Variability in eGFR and All-Cause Mortality



Increasing magnitude of eGFR variability was associated with an increased risk of death (trend $p=0.04$). Similar patterns were observed when results were stratified by stages of baseline kidney function levels.

Conclusions: Our results demonstrate an independent and graded association between variability in kidney function and mortality. These results suggest that overall variation in eGFR over time may be an important prognostic marker.

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Renal Insufficiency, as Well as Diabetes Mellitus, Is a Risk Factor for Bacterial Infection

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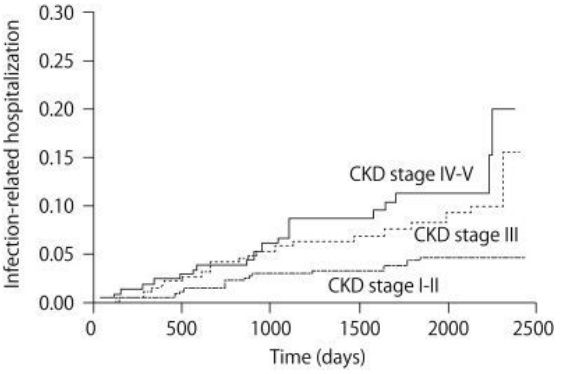
Background: It has recently been shown that renal insufficiency is associated with increased morbidity from bacterial infections. The aim of this study was to evaluate the relative risk of renal insufficiency for bacterial infection in comparison with other known risk factors.

Methods: Nominal logistic regression analysis and a Cox proportional-hazards model were used for multivariate analysis. The event investigated was "bacterial infection making hospitalization necessary", and the investigation items consisted of age, diabetes mellitus, the use of statins and immunosuppressive agents, and several laboratory values. Subjects included all patients visiting our outpatient clinic between January and December 2005 for whom complete medical records were available for at least 3 years from 2005. Patients receiving renal replacement therapy were excluded.

Results: A total of 836 patients were surveyed. The mean period of observation was 4.87 ± 1.39 years. CKD stage 1-2 was observed in 36.1% of subjects in the infected group, and in 55.0% of those in the non-infected group. Nominal logistic regression analysis demonstrated that a CKD stage of 3 or more, the use of immunosuppressive agents, and diabetes mellitus were statistically significant risk factors for bacterial infection-related hospitalization, with relative risks (RR) of 2.533, 2.529, and 2.272, respectively. The RR for statin use was 0.475, and it appeared to decrease morbidity from bacterial infection-related hospitalization. A Cox proportional-hazards model also showed that advanced CKD stage was a statistically significant independent risk factor.



Conclusions: Renal insufficiency, the use of immunosuppressive agents, and diabetes mellitus were important risk factors for bacterial infection-related hospitalization, whereas the use of statins may be linked to a reduced risk.

SA-PO194

The Impact of Renal Dysfunction on Mortality after Coronary Artery Bypass Grafting Surgery in Contemporary Practice Piangwarin Phaosawasdi, Tonga Nfor, Kambiz Shetabi, Fengyi Shen, Molla Teshome, Samia Salih, Paul G. Jenkins. *Internal Medicine, Aurora Health Care, Milwaukee, WI.*

Background: Preoperative renal dysfunction (RD) has been known to affect outcomes following coronary artery bypass grafting (CABG) but with current advancements in medical care it is unclear how much impact it has. In this study we investigated the independent effect of RD on clinical outcomes in patients undergoing CABG.

Methods: We analyzed a retrospective cohort of 1036 patients who had CABG at a tertiary care center in 2009 and 2010. Multivariate logistic regression was used to assess the effect of RD on outcomes adjusting for demographics, baseline comorbidities and Society of Thoracic Surgeons (STS) surgical risk score.

Results: We categorized patients into CKD stage 1-5 based on their preoperative MDRD eGFR. The distribution was 16.4%, 48.2%, 28.6%, 4.7% and 2.1% for stage 1, 2, 3, 4/5 and chronic dialysis respectively. Table 1 shows 30-day and 1-year mortality by CKD stage. There was a progressive increase in mortality from CKD stage 1 to stage 4/5 and chronic dialysis, p for linear trend <0.001. In multivariate analysis the odds ratio of death increased with more advanced CKD. Days spent in the hospital and ICU were longer with worsening preoperative GFR. The need for post-CABG dialysis was 27.5% of CKD stage 4/5 compared to none in stage 1 patients. Actual 30-day mortality was nearly twice that of STS predicted mortality in stage 4-5 CKD (18.3% vs. 10.3% predicted) and dialysis patients (18.2% vs. 8.72% predicted).

CKD stage	stage 1	stage 2	stage 3	stage 4/5	Chronic dialysis	p
Patients	170	499	296	49	22	
30-day mortality	0.6 %	1.2 %	3.7 %	18.4 %	18.2 %	<0.001
OR (95% CI)	Ref	3.9 (0.2 to 5.6)	10.1 (0.4 to 13.6)	20.8(1.2 to 45.7)	32.2 (2.3 to 52.9)	
1-year mortality	2.9 %	2.4 %	8.4 %	28.6 %	45.5 %	<0.001
OR (95% CI)	Ref	1.0 (0.3 to 3.5)	2.47 (0.7 to 8.6)	7.4 (1.8 to 30.5)	14.6 (5.6 to 37.4)	
CKD chronic kidney disease; OR adjusted odds ratio compared to CKD stage 1 ; CI confidence interval						

Conclusions: Preoperative RD is a strong independent predictor of post-CABG mortality. STS score may underestimate mortality at tertiary care centers in patients with GFR < 30 ml/min.

SA-PO195

Extreme Low Blood Pressure *Is a Risk* for Cardiovascular Disease in the Japanese Population: The Gonryo Study Tae Yamamoto,[1] Mariko Miyazaki,[1] Masaaki Nakayama,[2] Toshinobu Sato,[3] Hiroshi Sato,[1] Sadayoshi Ito.[1] *[1]Tohoku University, Sendai, Japan; [2]Fukushima Medical University, Fukushima, Japan; [3]Sendai Shakaihoken Hospital, Sendai, Japan.*

Background: Controlled blood pressure in chronic kidney disease (CKD) is important to decrease a risk of developing *cardiovascular disease (*CVD). However, studies of lower blood pressure for predicting CVD risk in CKD is limited.

Methods: 2,656 CKD patients, the mean age 60 ± 16 years and males 53%, recruited from 11 outpatient nephrology hospitals and followed for two years. They were divided by systolic blood pressure (sBP) to 6 groups, <110 mmHg (group1, n=197), 110 to 119 (group2, n=360), 120 to 129 (group3, n=676), 130 to 139 (group4, n=687), 140 to 149 (group5, n=419) and ≥ 150 (group6, n=317), and group 2 used as reference. Risks of CVD incidence including events such as ischemic heart disease, congestive heart failure, brain disease and death due to CVD, as well as inducting renal replacement therapy (RRT) were examined.

Results: During the observation time, 170 patients progressed RRT and 95 patients occurred CVD events and CVD related death. The mean eGFR of all patients was 55.3 ± 29.5 ml/min and 74% were treated by anti-hypertensive drugs or/and diuretics. As expected, sBP correlated positively with age (ρ= 0.216, p<.0001), and negatively with eGFR (ρ= -0.174, p<.0001). RRT increased significantly in over 140 mmHg (group 5 and 6), while CVD incidence increased significantly in over 130 mmHg (group 4, 5 and 6) and also in under 110mmHg (group1) by logistic regression analysis. The significant high risks for CVD incidence remained in multivariate regression analysis adjusted for age, sex, diabetes, hemoglobin and eGFR levels. The relative risk was 5.36 with 95%CI (1.81 – 19.6) in group 1, 2.85 (1.09 – 9.76) in group 4, 2.83 (1.03 – 9.94) in group 5 and 2.83 (1.01 – 10.0) in group 6 compared to group 2.

Conclusions: We concluded that systolic blood pressure lower than 110 mmHg was an independent predictor of CVD, as well as the not fully controlled high blood pressure.

SA-PO196

Longitudinal Changes in Hematocrit in Hypertensive Chronic Kidney Disease Teresa K. Chen,[1] Michelle M. Estrella,[1] Brad C. Astor,[2] Tom Greene,[3] Xuelei Wang,[4] Lawrence J. Appel.[1] *[1]Johns Hopkins University School of Medicine, Baltimore, MD; [2]University of Wisconsin School of Medicine, Madison, WI; [3]University of Utah School of Medicine, Salt Lake City, UT; [4]Case Western Reserve University School of Medicine, Cleveland, OH.*

Background: Anemia is common in chronic kidney disease (CKD) and is associated with worse outcomes. In cross-sectional studies, lower estimated glomerular filtration rates (eGFR) have been associated with higher prevalence of anemia; however, how hematocrit changes as eGFR declines and what factors impact this longitudinal association are unclear.

Methods: We used mixed-effects models to analyze 12 years of follow-up data from 1094 African-Americans with hypertensive CKD in the African-American Study of Kidney Disease and Hypertension (AASK). Primary exposure was annual eGFR; primary outcome was change in hematocrit. Covariates included gender, age at randomization, baseline eGFR, baseline proteinuria and randomized drug group.

Results: At baseline, mean hematocrit was 39%, and 441 (40%) individuals had anemia (hematocrit <40.5% for men; <36% for women). In longitudinal analyses, hematocrit decline per 10 ml/min/1.73 m² decline in eGFR was steeper for eGFR values <40 than ≥40 ml/min/1.73 m² (-2.2% versus -0.2%, respectively; p<0.01). For an eGFR less than 40 ml/min/1.73 m², there was a greater decrease in hematocrit per unit change in eGFR among those who were male (p-interaction=0.02), had baseline proteinuria (p-interaction=0.006) or were randomized to receive ramipril (p-interaction=0.04).

Table 1: Longitudinal analyses of change in hematocrit per change in eGFR, by gender, baseline proteinuria, and randomized drug group

	Subgroup	Absolute change in Hct (%) per 10 ml/min/1.73 m² decline in eGFR	95% CI	p-interaction
Time-Varying eGFR	**Gender***			
<40 ml/min/1.73 m²	Male	-2.4	(-2.6, -2.1)	0.02
	Female	-2.0	(-2.2, -1.7)	
≥40 ml/min/1.73 m²	Male	-0.3	(-0.5, -0.1)	0.05
	Female	-0.03	(-0.2, 0.2)	
Time-Varying eGFR	**Baseline Proteinuria***			
<40 ml/min/1.73 m²	No	-2.0	(-2.2, -1.7)	0.006
	Yes	-2.5	(-2.8, -2.2)	
≥40 ml/min/1.73 m²	No	-0.2	(-0.3, -0.1)	0.82
	Yes	-0.2	(-0.6, 0.1)	
Time-Varying eGFR	**Randomized Drug***			
<40 ml/min/1.73 m²	Ramipril	-2.5	(-2.8, -2.2)	0.04
	Metoprolol	-2.1	(-2.4, -1.8)	
≥40 ml/min/1.73 m²	Ramipril	-0.2	(-0.4, -0.003)	0.60
	Metoprolol	-0.2	(-0.4, -0.05)	

Each model was adjusted for age at randomization, gender, baseline proteinuria (defined as urine protein-to-creatinine ratio greater than 0.22), baseline eGFR, and time; Abbreviations: eGFR, estimated glomerular filtration rate; Hct, hematocrit; CI, confidence interval.
*From baseline to end of cohort phase; ^From baseline to end of trial phase.

Conclusions: Hematocrit decreases as eGFR declines, more so when eGFR falls below 40 ml/min/1.73 m² particularly in men, persons with baseline proteinuria and those assigned to ACE-inhibitors. Results suggest that anemia screening should be more individualized, with more intensive and frequent testing in these subgroups of patients with CKD.

Funding: NIDDK Support

SA-PO197

Association of Serum Bicarbonate with Risk of Renal and Cardiovascular Outcomes in Chronic Kidney Disease: A Report from the Chronic Renal Insufficiency Cohort (CRIC) Study Mirela A. Dobre,[1] Wei (Peter) Yang,[2] Jing Chen,[2] Paul E. Drawz,[1] L. Lee Hamm,[2] Edward J. Horwitz,[1] Thomas H. Hostetter,[1] Bernard G. Jaar,[2] Claudia M. Lora,[2] Lisa C. Nessel,[2] Akinlolu O. Ojo,[2] Julia J. Scialla,[2] Susan P. Steigerwalt,[2] Valerie L. Teal,[2] Myles S. Wolf,[2] Mahboob Rahman.[1] *[1]Case Western Reserve University; [2]CRIC Study Group.*

Background: The purpose of this study is to evaluate serum bicarbonate as a risk factor for renal outcomes, cardiovascular events and mortality in patients with chronic kidney disease (CKD).

Methods: Data from a prospective study of 3939 participants with CKD stages 2-4 who enrolled in the Chronic Renal Insufficiency Cohort (CRIC) between June 2003-December 2008 was analyzed. Renal outcomes were defined as end-stage renal disease (either initiation of dialysis or kidney transplant) or a 50% decline in eGFR. Adverse cardiovascular events were defined as myocardial infarction, congestive heart failure, stroke or peripheral arterial disease. Multivariable Cox proportional hazards models including serum bicarbonate modeled with linear splines were used to test the association between baseline serum bicarbonate and clinical outcomes.

Results: The mean (SD) eGFR was 42.8 (13.5)mL/min/1.73m^2, and the median serum bicarbonate was 24 mmol/L (interquartile range 22-26 mmol/L). During a median follow-up of 3.9 years, 374 patients died, 762 had a renal outcome, and 604 experienced a cardiovascular event. In adjusted analyses, the risk of developing a renal endpoint was 3% higher per 1mmol/L decrease in serum bicarbonate (hazard ratio [HR] 1.03; 95% confidence interval [CI], 1.01-1.06, p=0.01). The risk of adverse cardiovascular outcomes increased by 10%(HR 1.10, 95%CI 1.01-1.19) per 1mmol/L increase in serum bicarbonate over 24 mmol/L (p=0.03). Serum bicarbonate was not independently associated with all-cause mortality (HR 0.97;95%CI 0.93-1.00, p=0.08).

Conclusions: In a cohort of patients with CKD stages 2 to 4, low serum bicarbonate was an independent risk factor for renal disease progression. The risk of cardiovascular events was higher at the upper and lower extremes of serum bicarbonate. There was no significant association between serum bicarbonate and all-cause mortality.

Funding: Other NIH Support - 5T32DK007470

SA-PO198

Association of Serum Bicarbonate Level with Incident Functional Limitation

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Background: Cross-sectional studies have found that low serum bicarbonate (HCO_3) is associated with slower gait speed and quadriceps strength. CKD patients are at increased risk of functional impairment and acidosis.Whether HCO_3 levels independently predict the development of functional impairment has not been previously studied.

Methods: We assessed whether HCO_3 levels predicted incident lower extremity functional limitation in the Health, Aging and Body Composition Study, a cohort study of older individuals without functional limitation at baseline. HCO_3 levels were measured using arterialized venous blood gas. CKD was defined as eGFR < 60 ml/min/1.73m^2 using the CKD-EPI formula and functional limitation as difficulty walking 1/4 mile or climbing 10 steps on 2 consecutive reports in the same domain, 6 months apart.

Results: Of the 1634 individuals with complete data, the mean age was 75.5, 52% male, 25% Black, 14% CKD; 501 developed functional limitation over a median 4.4 yrs. Individuals with low HCO_3 (<23 meq/L, 9.7%) were more likely to be diabetic and have CKD and less likely to be on diuretics. Analyzed as a continuous variable with Cox proportional hazards analysis, each 1 meq/L lower HCO3 was associated with a 6% higher risk of functional impairment (HR 1.06 95% CI 1.02, 1.11, p=0.006), adjusting for age, race, sex, eGFR, BMI, blood pressure, smoking, obstructive lung disease, diabetes and gait speed. Categorized as < 23,≥ 23 meq/L, there was a CKD*HCO3 interaction (p=0.049) with an adjusted HR in CKD of 2.02 (1.23, 3.34) vs. 1.07 (0.74, 1.53) in individuals without CKD.

Conclusions: Low HCO_3 levels are associated with an increased risk of functional impairment, with a stronger relationship in CKD. Whether or not HCO_3 therapy decreases the risk of functional impairment requires further study.

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SA-PO199

Renal Dysfunction and Long-Term Risk of Ischemic and Hemorrhagic Stroke Following Coronary Artery Bypass Grafting

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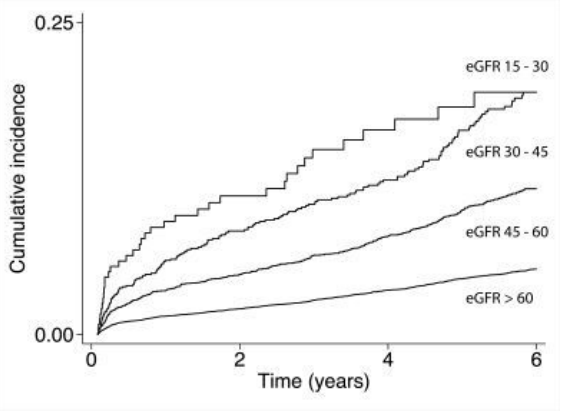
Background: Renal dysfunction is associated with increased long-term mortality and incidence of myocardial infarction following coronary artery bypass grafting (CABG). There is little information on the relationship between renal dysfunction and long-term risk of stroke following CABG.

Methods: All 29 602 patients who underwent primary isolated CABG 2000 to 2008 in Sweden, with no myocardial infarction within 14 days before surgery and no prior stroke were included from the SWEDEHEART registry. Cox proprotional hazards regression was used to calculate hazard ratios for first hospitalization for ischemic, hemorrhagic or unspecified stroke.

Results: During 4 years of follow-up there were 2836 (76%) ischemic, 300 (8%) hemorrhagic and 598 (16%) unspecified strokes. Glomerular filtration rates (eGFR) were estimated using the MDRD study equation and hazard ratios (HR) with 95 % confidence intervals (CI) were calculated for stroke. Adjusted HR with 95% CI for all stroke among patients with eGFR 45 to 60, 30 to 45 and 15 to 30 mL/min/1.73 m^2 were; 1.16 (1.02 to 1.32), 1.49 (1.22 to 1.81) and 1.72, (1.16 to 2.56), respectively, compared to patients with eGFR > 60 mL/min/1.73 m^2. Similar associations were find for ischemic and hemorrhagic stroke. There was a stronger relationship between eGFR 15 to 45 mL/min/1.73 m^2 and stroke for women than for men. The relative risk for stroke associated with renal dysfunction was smaller for elderly patients compared to younger patients. The cumulative incidence of all stroke increased gradually with decreasing eGFR during follow-up (Figure1).



Conclusions: Renal dysfunction is a long-term predictor of both ischemic and hemorrhagic stroke after primary isolated CABG both among men and women. The impact of renal dysfunction on risk for all stroke is greater for younger than for older patients.

Funding: Private Foundation Support

SA-PO200

Renal Artery Calcium, Cardiovascular Risk Factors and Renal Function

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Background: Observational studies show an association between atherosclerotic renovascular disease and coronary artery disease. Recent data suggests renal artery calcium (RAC) is associated with hypertension above and beyond traditional cardiovascular disease (CVD) risk factors. However, limited data exists focusing on the association of RAC, CVD risk factors and measures of renal function.

Methods: We examined 2618 Framingham Heart Study participants (mean age 60.3 years, 50.7% women) who were part of the multidetector computed tomography substudy (2008 to 2011). We applied a multivariable logistic regression model to evaluate the associations between RAC, CVD risk factors and renal function. Risk factors included age, sex, BMI, smoking, hypertension, diabetes, triglycerides, HDL cholesterol, total cholesterol, chronic kidney disease (CKD), and microalbuminuria. RAC was defined as an Agatston score greater than zero. CKD was defined as an eGFR less than 60mL/min/1.73m^2. Microalbuminuria was defined as ACR ≥17mg/g in men and ≥25mg/g in women.

Results: The prevalence of RAC was 28.8% in the study population and was similar in women (29.3%) and men (28.2%). RAC was associated with age (OR=4.1, 95% CI 3.6-4.7, p=<0.001), BMI (OR=1.1, 95% CI 1.0-1.3, p=0.03), smoking (OR=2.5, 95% CI 1.6-3.7, p=<0.001), hypertension (OR=2.2, 95% CI 1.7-2.8, p=<0.001), and microalbuminuria (OR=1.9, 95% CI 1.3-2.8, p=0.002). Results of the logistic regression model showed that individuals with RAC have a higher risk of hypertension (OR=2.1, 95% CI 1.7-2.7, p=<0.001), diabetes (OR=1.6, 95% CI 1.2-2.3, p=0.004), and microalbuminuria (OR=1.9, 95% CI 1.3-2.8, p=<0.001). The association between RAC and CKD was not statistically significant (OR=0.9, 95% CI 0.6-1.4, p=0.58).

Conclusions: RAC is associated with hypertension, diabetes, and microalbuminuria but not CKD. The prevalence of RAC increases with age and may be a marker of increased cardiovascular disease.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-PO201

Cardiac Geometry Is Associated with Kidney Function and Markers of Inflammation: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study

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Background: Left ventricular hypertrophy (LVH) is highly prevalent in patients with chronic kidney disease (CKD) and is an important predictor of mortality. We examined the relationship between biomarkers of inflammation and LVH in 3939 CRIC study participants with varying levels of kidney function.

Methods: Baseline serum/plasma levels of inflammatory cytokines (IL-6, IL-1RA, IL-1β, TNF-α and TGF-β) and acute-phase proteins (hs-CRP, fibrinogen and albumin), and baseline echocardiographic findings were used for this analysis. LVH was defined as LV mass index (LVMI) ≥103 g/m^2 for men and ≥89 g/m^2 for women. Associations between log-transformed values of individual biomarkers and LVMI (continuous) and LVH (binary) were investigated using linear and logistic regression models adjusted for age, sex, race/ethnicity, smoking status, diabetic status, blood pressure and albuminuria.

Results: LVH was present in 57.2% of subjects. eGFR was negatively associated with LVMI [coefficient β = -0.28 (95% Confidence Limits -0.35, -0.22), p < 0.0001] and LVH [Odds Ratio (OR) = 0.98 (0.97, 0.99), p < 0.0001]. Among biomarkers, IL-6, IL-1β and TNF-α were positively associated with LVMI [β = 1.71 (0.74, 2.70), p = 0.005; β = 0.21 (0.06, 0.35), p = 0.04 and β = 2.0 (0.8, 3.24), p = 0.01 respectively], whereas the association of serum albumin with LVMI was in the opposite direction [β = –17.30 (–25.05, –9.54), p < 0.0001]. Serum albumin was also negatively associated with LVH [OR = 0.29 (0.13, 0.62), p = 0.01].

Conclusions: In patients with CKD, inflammation was positively associated with LVMI and decreased kidney function with LVMI and LVH. Each 10 percent decrease in serum albumin was associated with 1.7 unit increase in LVMI.

Funding: NIDDK Support

SA-PO202

Predictors of Subclinical Myocardial Injury in Chronic Kidney Disease Patients: The Chronic Renal Insufficiency Cohort Ruth F. Dubin,[1,2] Alan S. Go,[3] Martin Keane,[4] Christopher deFilippi,[5] Rakesh Mishra,[2] Myles S. Wolf,[6] Michael Shlipak.[2] *[1]Nephrology, University of California San Francisco, San Francisco, CA; [2]Medicine, San Francisco VAMC, San Francisco, CA; [3]Clinical Research, Kaiser Permanente, Oakland, CA; [4]Cardiology, University of Pennsylvania, Philadelphia, PA; [5]Cardiology, University of Maryland Medical Center, Baltimore, MD; [6]Nephrology, University of Miami, Miami, FL.*

Background: Chronic kidney disease (CKD) is a strong, independent risk factor for cardiovascular disease (CVD). Predictors of subclinical myocardial injury, as measured by highly sensitive cardiac troponin T (hs-TnT), have not been previously identified in a CKD cohort. We hypothesized that cystatin-based estimated glomerular filtration (eGFRcys) and urine albumin-creatinine ratio (ACR) would be independently associated with higher hs-TnT.

Methods: We evaluated cross-sectionally the associations of renal and non-renal factors with hs-TnT in 2464 participants without self-reported CVD in the multi-ethnic Chronic Renal Insufficiency Cohort (CRIC). Predictors of log hs-TnT were investigated using multivariable tobit regression, and coefficients were exponentiated to yield percentage effects of expected hs-TnT attributable to each predictor.

Results: Hs-TnT was detectable in 81% of subjects, and the median (IQR) hs-TnT was 9.4 pg/ml (4.3-18.3.) After multivariable adjustment lower eGFRcys was associated with higher expected hs-TnT; participants with eGFRcys<30 ml/min/1.73m^2 had 200% higher expected hs-TnT compared to subjects with eGFRcys>60. Compared to those with ACR<30 mg/g, subjects with ACR>1000 had 50% higher expected hs-TnT. In contrast, hemoglobin was not independently associated with hs-TnT. Independent of the renal predictors, older age, male gender, black race, diabetes, higher blood pressure, LV mass index≥100 g/m$^{2.7}$ and EF≤35% were all associated with higher expected hs-TnT.

Conclusions: Among CRIC participants without CVD, eGFRcys, ACR, age, male gender, black race, diabetes, higher blood pressure, LV mass index and ejection fraction were independently associated with higher hs-TnT.

Funding: NIDDK Support

SA-PO203

Clinical Outcomes in Patients Admitted with Acute Myocardial Infraction and Chronic Kidney Disease in an Australian Setting Sradha S. Kotwal, Isuru Ranasinghe, Alan Cass, Martin P. Gallagher. *Renal and Metabolic Division, The George Institute for Global Health, Sydney, NSW, Australia.*

Background: International data suggests patients with CKD have a higher morbidity and mortality following AMI and are less likely to receive revascularisation. However, the impact of CKD on longer term clinical outcomes and health resource usage remains unclear.

Methods: New South Wales (NSW) is Australia's most populous state with an estimated population of 7.2 milllion, representing 32.3% of the total Australian population. The NSW admitted patient data collection (NSW APDC) is an administrative coding dataset collecting data on all admissions to NSW hospitals. Coding uses the International Classification of Disease 10 Australian Modification (ICD10 AM). All patients admitted to a NSW hospital with a principle diagnosis of AMI (ICD10 codes: I21.0-I21.3 and I21.4) between 2004 and 2008 were extracted from NSW APDC and linked to the NSW Registry of Births Deaths and Marriages. We analysed outcomes of 30 day, 1 year and medium term (median follow up) all cause mortality, length of stay (LOS) and revascularisation crude rates based on the presence or absence of a diagnostic code of CKD (N18, N19, Z49).

Results: A total of 40,482 patients with AMI were analysed, including 1565 with a CKD code, to a median follow up 3.5 years. Unadjusted analyses of patients coded as having CKD showed higher 30 day mortality (15% versus 7%, p<0.001), 1 year mortality (40% versus 14.5%, p<0.001), 3.5 year mortality (67% versus 25%, p<0.001), LOS (mean 10.4 days versus 7.5 days, p < 0.001) and lower revascularisation rates (14% versus 35%, p< 0.001) compared to those without a CKD code.

Conclusions: Patients admitted to NSW hospitals with an AMI and coding history of CKD have lower rates of intervention, despite, significantly higher mortality and length of stay. CKD coding was used infrequently and further research is required to explore the utilisation of CKD diagnostic codes and the poorer outcomes they portend.

SA-PO204

Association of Metabolic Syndrome and Renal Insufficiency with Clinical Outcome in Acute Myocardial Infarction Chang Seong Kim,[1] Joon Seok Choi,[1] Eun Hui Bae,[1] Seong Kwon Ma,[1] Soo Wan Kim.[1] *[1]Department of Internal Medicine, Chonnam National University Hospital, Gwangju, Republic of Korea; [2]Department of Physiology, Chonnam National University Hospital, Gwangju, Republic of Korea.*

Background: Metabolic syndrome (MS) is an independent risk factor for chronic kidney and cardiovascular diseases. However, few studies have examined the combined effects of MS and renal insufficiency after acute myocardial infarction (AMI). We examined the effect of MS on clinical outcomes in patients with AMI in the presence or absence of renal insufficiency.

Methods: From November 2005 to September 2008, 11,462 patients with AMI were enrolled in the prospective Korean Acute Myocardial Infarction Registry. Participants were analyzed according to presence or absence of MS and renal insufficiency, defined by a low estimated glomerular filtration rate (eGFR). The primary endpoints were major adverse cardiac events (MACE), including a composite of all cause-of-death, myocardial infarction, target lesion revascularization, and coronary artery bypass graft during the 1-year follow-up period.

Results: MS was higher in AMI patients with lower eGFR. In-hospital death and primary endpoints at 1-month were significantly higher in MS than in non-MS, regardless of renal insufficiency. However, composite MACE scores at 1-year were significantly higher only in those with MS and renal insufficiency. Multivariate analysis showed that old age, multi-vessel involvement, high levels of inflammation, diabetes and MS were associated with 1-year composite MACE in renal insufficiency. After adjusting for multiple covariates, the 1-year mortality rate was higher in MS with renal insufficiency than in MS without renal insufficiency, or in non-MS.

Conclusions: MS is associated with poor clinical outcomes and increases the mortality in patients with AMI, especially in association with renal insufficiency.

SA-PO205

Insulin Resistance Is Not Related to Development of Renal or Atherosclerotic Cardiovascular Outcomes or Death in Non-Diabetic Chronic Kidney Disease: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study Amanda Hyre Anderson,[1,4] Wei (Peter) Yang,[1,4] Raymond R. Townsend,[1,4] Jing Chen,[2,4] L. Lee Hamm,[2,4] Bruce M. Robinson,[3,4] Harold I. Feldman.[1,4] *[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [2]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA; [3]Arbor Research Collaborative for Health, Ann Arbor, MI; [4]Chronic Renal Insufficiency Cohort (CRIC) Study Group.*

Background: Metabolic and inflammatory abnormalities including insulin resistance are common in chronic kidney disease (CKD), but their relationships to CKD progression, cardiovascular disease (CVD), and death in this setting remain unclear.

Methods: Data from a subset of CRIC Study participants with non-diabetic CKD (N=1,742, average follow-up 4 years) were used in Cox proportional hazards models to examine the association of homeostasis model assessment-estimated insulin resistance (HOMA-IR) and the risk of halving of estimated glomerular filtration rate (eGFR) or ESRD (Renal1), atherosclerotic CVD (ASCVD; myocardial infarction, stroke, peripheral arterial disease), and death with adjustment for demographics, anthropometrics, comorbidities, and health-related factors.

Results: Median (interquartile range) HOMA-IR was 3.1 (2.1-4.5). Mean (standard deviation) eGFR was 48.4 (17.8) mL/min/1.73m^2. Crude event rates for Renal1, ASCVD, death, and ASCVD plus death were 36, 15, 16, and 30 per 1000 person-years, respectively. HOMA-IR, whether examined continuously on the log scale or across quartiles, was not associated with any examined outcome (each P value > 0.2) in unadjusted or adjusted models, with and without additional adjustment for level of high-sensitivity C-reactive protein.

Conclusions: Previously reported associations of insulin resistance with renal/ASCVD events and death were not detectable in this study of individuals with reduced levels of kidney function. Variation in effects by level of eGFR merits further study.

Funding: NIDDK Support, Other NIH Support - CTSA and GCRC Grants

SA-PO206

Oral Disease in People with Chronic Kidney Disease: A Systematic Review and Meta-Analysis of Cohort Studies Suetonia Palmer,[1] Marinella Ruospo,[2] Mariacristina Vecchio,[3] Letizia Gargano,[2] Fabio Pellegrini,[3] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Diaverum Medical Scientific Office; [3]Mario Negri Sud Consortium; [4]University of Sydney.*

Background: Oral disease includes a wide spectrum of clinical abnormalities affecting the mouth including mucosa, teeth, periodontal tissue and salivary function. While observational data for oral and dental diseases are available in people with chronic kidney disease (CKD), existing published information has not yet been systematically evaluated. We aimed to summarize the overall prevalence of oral diseases in people with CKD and explore associations between oral disease and mortality in this clinical setting.

Methods: We conducted a systematic review and meta-analysis of observational studies reporting prevalence or clinical outcomes of oral disease in people with CKD. English-language studies were identified from systematic searching MEDLINE through April 2010. Multiple reviewers extracted details on participant characteristics, tools used to measure

oral disease, details of statistical analyses including adjustments for confounding. Estimates of prevalence, mean score, or risk of mortality were summarized using random-effects meta-analysis and expressed as rates or means and 95% confidence intervals (CI). Effects of severity of CKD on estimates were analyzed using subgroup analysis.

Results: 112 studies (150 cohorts) including 18 339 people with CKD and 16 310 controls were analyzed. 103 cohorts were in people on dialysis, 22 cohorts were in earlier stages of CKD and 25 cohorts were in kidney transplant recipients (15.6%). The mean decay/missing/filled teeth (DMFT) index in people with CKD was 13.7 and number of teeth was 19.4. Nearly 40% of people with CKD had enamel hypoplasia and over half had periodontitis. Overall, the mean plaque index was 1.62 and periodontal pocket depth (PPD) was 2.30 mm. Approximately 25% of people with CKD reported never brushing.

Conclusions: Data evaluating the prevalence and severity of oral disease in people with CKD are sparse and incomplete. Large longitudinal studies of the prevalence and clinical associations with oral disease in CKD are now needed.

SA-PO207

Meta-Analysis: Effects of Targets for Serum Phosphorus, Parathyroid Hormone and Calcium on Mortality in Chronic Kidney Suetonia Palmer,[1] Valeria Maria Saglimbene,[2] Jonathan C. Craig,[3] Mariacristina Vecchio,[2] Marinella Ruospo,[4] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Consorzio Mario Negri Sud; [3]University of Sydney; [4]Diaverum Medical Scientific Office.*

Background: International treatment guidelines suggest achieving target levels of serum phosphorus, parathyroid hormone (PTH) and calcium in people with chronic kidney disease (CKD). We evaluated the evidence that specifically targeted biomarker levels (phosphorus,parathyroid hormone, and calcium) improved clinical outcomes in people with CKD.

Methods: We conducted a systematic review of randomized controlled trials (RCT) that reported achievement of serum targets for phosphorus, PTH, and calcium in adults with CKD and that reported clinical outcomes (mortality, fracture, kidney function, and withdrawal due to treatment toxicity). We systematically searched Cochrane and Embase databases. We included RCT of interventions that reported biochemical for phosphorus, PTH, and calcium at study end. We summarized the effects of specific biochemical targets on specified outcomes using random-effects models.

We also summarized the non-randomized associations between the proportion of participants achieving biomarker targets and clinical outcomes using two-dimensional arrow plots. Subgroup and meta-regression analyses to evaluate the effects of patient and intervention characteristics on treatment effects were not possible due to insufficient data.

Results: We included 158 trials (24,965 people) evaluating interventions on serum biomarker values. No consistent associations between the proportion of participants achieving a biomarker target and clinical outcomes were observed. No consistent associations between biomarker values at study end and clinical outcomes were observed. An increasing proportion of participants achieving a serum PTH target with treatment was not consistently associated with lower mortality but may be associated with increased withdrawal from treatment due to adverse events.

Conclusions: Targeting specific levels of phosphorus, PTH, and calcium is not consistently associated with improved clinical outcomes in adults with CKD, but may be associated with treatment-related toxicity.

SA-PO208

Are Statin Effects Different Based on Stage of Chronic Kidney Disease: A Meta-Analysis Suetonia Palmer,[1] Jonathan C. Craig,[2] Sankar D. Navaneethan,[3] Marcello Tonelli,[4] Fabio Pellegrini,[5] Giovanni F.M. Strippoli.[2,5,6] *[1]University of Otago; [2]University of Sydney SPH; [3]Cleveland Clinic; [4]University of Alberta; [5]Consorzio Mario Negri Sud; [6]Diaverum Medical Office.*

Background: Cardiovascular events are the most frequent cause of death in people with chronic kidney disease (CKD). Statins lower cardiovascular events. Benefits of statins may depend on severity of CKD. Individual randomized trials may have insufficient power to determine treatment effects according to stage of CKD. We summarize the effects of statins on mortality, major cardiovascular events, and toxicity based upon stage of CKD.

Methods: We conducted a systematic review and meta-analysis of randomized controlled trials including adults with CKD and that compared statins with placebo or no treatment. We systematically searched Cochrane and EMBASE databases. Two reviewers extracted details on participant characteristics, interventions, and risk of bias. We summarized treatment effects on mortality, major cardiovascular events, and withdrawal of treatment using a random-effects model and explored the effects of stage of CKD on treatment effects using subgroup analysis.

Results: 80 trials (51 099 participants) included 48 comparisons with earlier stages of CKD, 21 comparisons on dialysis and 17 in kidney transplant recipients. Statin treatment reduced death in people with earlier stages of CKD (relative risk [RR] 0.81 [CI 0.74-0.88]), but had little effect in people on dialysis (RR 0.96 [0.88-1.04]) and uncertain effects in kidney transplant recipients (RR 1.05 [0.84-1.31]). Treatment effects of statins on major cardiovascular events also differed significantly based on stage of CKD. Statins prevented major cardiovascular events in people with earlier stages of CKD (RR 0.76 [0.73-0.80]), but had little effect in people on dialysis (RR 0.95 [0.87-1.03]) and were uncertain in transplant recipients (RR 0.84 [0.66-1.06]). Statins had little or no effect on risks of withdrawal from therapy.

Conclusions: Statins lower mortality and major cardiovascular events in people with CKD not yet on dialysis but have little effect in people on dialysis and efficacy is uncertain in kidney transplant recipients.

SA-PO209

Association between Diabetes, se-iPTH, Bone Disease and Vascular Calcification in Patients with ESRD Csaba Ambrus, Zoltan Kiss, Andras Szabo, Dr.szegedi János, Jozsef Balla, Marietta Török, Erzsebet Ladanyi, Botond Csiky, Otto Arkossy, Sándor Túri, Imre Kulcsar, Istvan Kiss. *CKD-MBD Working Group, Hungarian Society of Nephrology, Budapest, Hungary.*

Background: The association between diabetes and bone disease is still debated in the general population. However, in patients with ESRD, both relative hypoparathyroidism and low bone turnover have been associated with the presence of diabetes. If this association is mediated by PTH or diabetes has an independent effect on bone is not clear. We aimed to analyze this relationship in a large cohort of ESRD patients in Hungary.

Methods: In a country-wide, retrospective, cross-sectional survey, data was collected from 5334 patients. In addition, information about the presence of bone abnormality (BA) and soft tissue calcification (STC) were collected, both defined as abnormality detected on X-ray. Data are reported as mean±SD or median; interquartile range, as appropriate.

Results: Data from 5008 patients were eligible for analysis. Patients with diabetes were older (66±12 vs 62±15, p<0.001), had higher BMI (28.9±5.9 vs 25.5±5.3, p<0.001) and had lower PTH (151;230 vs 194;327pg/ml, p<0.001). Diabetes and PTH were highly correlated. In a multivariate model, this association was independent from age, gender, BMI, albumin and treatment modality. The odds ratio for PTH<150pg/ml in diabetes was 1.486 (95%CI 1.310-1.686).

BA and STC were more prevalent in diabetes (42.2% vs 36.9% (p<0.001) for BA and 56.1% vs 48.1% (p<0.001) for STC). The relationship between PTH and the prevalence of BA followed a U-shaped curve in both subgroups (46.2%, 33%, 40.9% vs 38.4%, 29.2%, 44.9% for PTH groups and DM vs non-DM groups, respectively). While similar U-shaped association was found between PTH and STC in patients without diabetes (48.3%, 44.1%, 55%), the prevalence of STC was not different between PTH levels in patients with diabetes.

Conclusions: In this large cross sectional cohort we confirmed that bone disease and soft tissue calcification are highly related and share common risk factors in patients with ESRD. Diabetes seems to have a distinct effect on bone independent from PTH.

SA-PO210

The Association between Parathyroid Hormone Levels and Hemoglobin in Diabetic and Non-Diabetic Participants in the National Kidney Foundation-Kidney Early Evaluation Program (KEEP) Imran A. Memon,[1] Carmen A. Peralta,[3] Suying Li,[7] Claudine T. Jurkovitz,[5] Peter A. McCullough,[6] Shu-cheng Chen,[7] Andrew S. Bomback,[4] Georges Saab.[2] *[1]University of Michigan, Ann Arbor; [2]Metrohealth Cleveland; [3]University of California, San Francisco; [4]Columbia University, New York; [5]Christiana Care Health System; [6]St. John Providence Health System; [7]Chronic Disease Research Group.*

Background: Parathyroid Hormone (PTH) has been speculated to contribute to anemia of chronic kidney disease.

Methods: The relationship between PTH and hemoglobin (Hgb) levels was investigated in 10,770 participants in the Kidney Early Evaluation Program with estimated GFR (eGFR) < 60 ml/min/1.73m^2.Multivariate linear regression was used to examine the effect of increasing iPTH on Hgb. Multiplicative interaction terms were generated to evaluate for effect modification of diabetes (DM).

Results: The mean age of the participants was 70.7 years and 32.4% were male. The majority were white (68.7%), followed by blacks (22%) and other races (9.3%). 47.3% had DM while the mean eGFR was 47.4 ml/min/1.73m^2. In unadjusted analysis, higher PTH levels were associated with lower Hgb levels. However, after multivariate adjustment for age, race, gender, smoking status, education, cardiovascular disease, hypertension, cancer, albuminuria, BMI, baseline eGFR, calcium and phosphorus, the direction of association changed. That is, higher PTH levels were associated with higher hemoglobin levels. Furthermore, significant effect modification for DM was seen (p for interaction = 0.0003). Among those with DM, each standard deviation increase in natural log transformed PTH was associated with a 0.10 (95% CI: 0.054-0.138, p <0.0001) g/dl in increase Hgb, while no association was seen among those without DM. Similarly, among those with DM, as compared to the first PTH quartile, Hgb levels were 0.15 mg/dl (95% CI 0.03-0.26), 0.22 mg/dl (95% CI 0.1-0.33) and 0.28 mg/dl (95% CI 0.16-0.41) higher for the second, third and fourth quartile respectively while no association was seen among those without DM.

Conclusions: There is a small positive association between iPTH and Hgb among diabetics but not non-diabetics.

SA-PO211

Bisphenol A Exposure and Low-Grade Urinary Albumin Excretion in US Children Howard Trachtman, Leonardo Trasande. *Pediatrics, NYU Langone Medical Center, New York, NY.*

Background: Bisphenol A (BPA) is an industrial chemical used in an array of consumer products with a consequent exposure to humans. In a recent study of 3,055 Chinese adults in the Shanghai area aged ≥40 years, urinary BPA was an independent determinant of the urinary albumin:creatinine ratio (ACR). There was a significantly increased risk of low-grade albuminuria with an adjusted odds ratio of 1.23 for the highest compared to the lowest BPA concentration quartiles. It is unknown whether there is a relation between BPA exposure and low-grade albuminuria in pediatric patients.

Methods: A cross-sectional study of a nationally representative sample of US children, the National Health and Nutrition Examination Survey (NHANES) data collected during 2009-2010, was performed. Patient gender, race/ethnicity, BMI, BP, cholesterol, and

HOMA-IR (in a subsample) were recorded. Patients were included if they had low grade albuminuria defined as an ACR <30 mg/g and had a measurement of BPA excretion. ACR and BPA excretion were determined using standard methods. Multivariate regression analysis was performed to determine the relationship betwen BPA excretion and ACR.

Results: In the total cohort of 10,253 children and adolescents (range 6-19 yr), 667 participants had determination of both ACR and BPA excretion. The mean age was 12±4 yr with 355 M and 312 F. The racial distribution was 34% White, 19% Black, 39% Hispanic/Mexican, and 8% other. The mean ACR was 1.87±0.58 mg/g and BPA excretion 3.83±10.9 ng/mL. After controlling for age, gender, BMI category, socioeconomic status, presence of prehypertension, hypercholesterolemia, urinary cotinine excretion, urinary concentration, and insulin resistance, patients in the highest quartile of BPA excretion had an increment in the ACR of 1.19 mg/g, a 23% difference *versus* those in the lowest quartile (P<0.03).

Conclusions: These findings suggest that, similar to adults, exposure to BPA independently increases low grade urinary albumin excretion in pediatric patients. These observations support efforts to limit exposure to this chemical from early childhood. Further studies are required to determine whether this is a sign of generalized endothelial dysfunction or intrinsic renal disease.

Funding: NIDDK Support

SA-PO212

Progression to End-Stage Kidney Disease in Children with CKD: A Nation-Wide Cohort Study in Japan Kenji Ishikura, Osamu Uemura, Shuichi Ito, Naohiro Wada, Motoshi Hattori, Yasuo Ohashi, Yuko Hamasaki, Ryojiro Tanaka, Koichi Nakanishi, Tetsuji Kaneko, Masataka Honda. *The Pediatric-CKD Study Group in Japan.*

Background: In 2010, we conducted a nationwide epidemiologic survey of children (3 months to 15 years old) with pre-dialytic CKD (stage 3-5) in Japan, and 447 children (mean age 8.7 years, 271 male; 316 in stage 3, 104 in stage 4, and 27 in stage 5) participated in our cohort (FR-PO1478 in ASN 2011). Longitudinal follow-up has been required to explore their outcome and risk factors of disease progression.

Methods: We conducted a prospective cohort study, and investigated progression to end-stage kidney disease (ESKD) and death, and its risk factors. Progression of CKD stage was also evaluated.

Results: Follow-up data in 409 of 447 children with CKD as of April 1, 2011 were analyzed. Three patients died and 49 patients (11.0%; 9 from stage 3, 26 from stage 4, 14 from stage 5) developed to ESKD, including 1 death in 1-year follow-up. Renal replacement therapy was peritoneal dialysis (PD) in 28, preemptive kidney transplantation in 12, kidney transplantation after PD in 3, hemodialysis (HD) in 3, and PD after HD in 2, and death occurred after HD in 1 patient. Age at the observation (odds ratio, 1.13; 95% CI, 1.01-1.26), urine protein/creatinine ratio (1.37; 1.17-1.59), hypertension (3.59; 1.34-9.62), and CKD staging (odds ratio of stage 4 to 3, 14.7; 95% CI, 5.1-42.9; odds ratio of stage 5 to 3, 162; 95% CI, 25.5-1029.4) were independent risk factors for developing ESKD or death among several putative risk factors, including sex, background disease [CAKUT, non-CAKUT], syndromal stigmata, and premature delivery in multiple logistic analyses. Forty-one and 11 (9) of 316 children in stage 3 progressed to stage 4, and 5 (5D), respectively, and 34 (25) and 2 of 104 in stage 4 developed to stage 5 (5D) and death, respectively.

Conclusions: This study revealed that more than 10% of children with non-dialytic CKD (stage 3-5) in our cohort progressed to ESKD in 1-year follow-up. Proteinuria and hypertension are significant risk factors for developing to ESKD, suggesting necessity of anti-proteinuric intervention and anti-hypertensive drugs in children with CKD.

Funding: Government Support - Non-U.S.

SA-PO213

Baseline Characteristics of Korean Pediatric Patients with Chronic Kidney Disease: Pediatric Subcohort of KNOW-CKD Hye Jin Chang,[1] Hee Gyung Kang,[1] Jiwon L. Lee,[1] Yo Han Ahn,[2] Sang Taek Lee,[1] Min Hyun Cho,[3] Kook-Hwan Oh,[4] Hye Won Park,[5] Young Seo Park,[6] Curie Ahn,[4] Hae Il Cheong,[1] IL-Soo Ha.[1] *[1]Departments of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Center for Pediatric Oncology, National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea; [3]Department of Pediatrics, Kyungpook National University Hospital, Daegu, Republic of Korea; [4]Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [5]Health Promotion Center, Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Republic of Korea; [6]Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea.*

Background: In Korea, nation-wide school urinary screening system has led to early detection of CKD; now, to assess the characteristics and relevant risk factors of CKD and its progression, KNOW-CKD (KoreaN cohort study for Outcome in patients With Chronic Kidney Disease) was launched in 2011, with pediatric subcohort as one of its five subcohorts.

Methods: Pediatric subcohort of KNOW-CKD enrolled children (younger than 20 years) with CKD stage I~V (pre-dialysis) from four major pediatric nephrology centers in Korea and collected medical and social data.

Results: Total 186 patients (M:F 123:63) were enrolled in 2011. Average age was 10.4 years (yrs). Etiology of CKD were congenital renal dysplasia (43%), reflux nephropathy/chronic pyelonephritis (25%), primary glomerular diseases (17%), secondary glomerular diseases (6%), and others. One third had histories of UTI and 10% had developmental delay. Growth delay was observed even in early stage of CKD stage; younger patients (< 6yrs) with advanced CKD (IV and V) had more profound growth delay. Deficiency of 25-hydroxyvitamin D3 (<20 ng/ml) and anemia were common (53% and 43%, respectively). LVH was observed in 11% of the patients.

Conclusions: This prospective, multicenter cohort study aiming to improve CKD outcome implies that our current management of pediatric CKD was suboptimal. We expect to obtain more information on pediatric CKD with this ten-year project KNOW-CKD, which can be translated to better management for this population.

Funding: Government Support - Non-U.S.

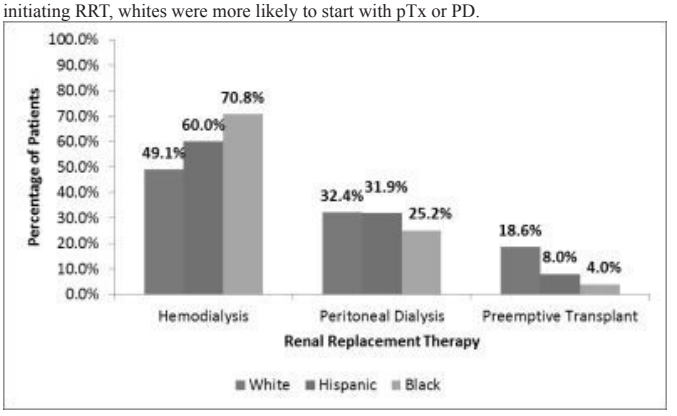
SA-PO214

Racial Disparities in Renal Replacement Therapy in the Pediatric End Stage Renal Disease Population Roshan P. George,[1] Laurence A. Greenbaum,[1] Allan Kirk,[2] Nancy G. Kutner,[3] Rachel E. Patzer.[2,4] *[1]Pediatric Nephrology, Emory Univ & Children's of Atlanta; [2]Surgery, Emory Transplant Center; [3]USRDS Rehabilitation/QoL Special Studies Center, Emory Univ; [4]Epidemiology, Rollins School of Public Health & Emory Univ, Atlanta, GA.*

Background: Limited data exists on racial disparities in the choice of renal replacement therapy (RRT) in pediatric ESRD patients. Blacks are more likely to receive hemodialysis (HD) vs. peritoneal dialysis (PD) or preemptive transplant (pTx). Ethnic differences and access to pre-ESRD nephrology care were not examined in prior studies.

Methods: Retrospective cross-sectional study of patients < 20 yrs, using USRDS from 01/01/05-09/30/09. Generalized logit models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) of RRT choice by race/ethnicity. We adjusted for differences in age, sex, SES (zip code and insurance status), OPO region and access to nephrology care.

Results: Among 5,623 patients (43.3% whites, 30.3% blacks, and 26.4% Hispanics) initiating RRT, whites were more likely to start with pTx or PD.



Compared to whites, blacks were less likely to have pTx (OR=0.15; 95% CI: 0.11-0.20) or PD (OR=0.54; 95% CI: 0.47-0.63) vs. HD. Among Hispanics, pTx (OR=0.35; 95% CI: 0.29-0.44) or PD (OR=0.81; 95% CI: 0.70-0.93) vs. HD was lower than whites. More (67.4%) whites had access to pre-ESRD nephrology care than Hispanics (52.5%) or blacks (59.1%) (p< 0.0001). After adjusting for demographic, clinical, and SES differences, racial disparities were attenuated among Hispanics; however, compared to HD, pTx was 75% lower (OR=0.25; 95% CI: 0.18-0.35) and PD 22% lower (OR=0.78; 95% CI: 0.64-0.94) for blacks vs. whites.

Conclusions: Blacks and Hispanics are less likely to receive PD or pTx, compared to whites. SES, access to care and demographic factors explain this racial disparity among Hispanics, but not blacks.

SA-PO215

Hepcidin (Hepc) and Anemia in Children with CKD: Longitudinal Data from the Chronic Kidney Disease in Children (CKiD) Cohort Study Meredith A. Atkinson,[1] Christopher B. Pierce,[2] Cindy N. Roy,[1] Bradley A. Warady,[3] Colin T. White,[4] Alison G. Abraham,[2] Susan L. Furth.[5] *[1]Johns Hopkins University; [2]Johns Hopkins Bloomberg School of Public Health; [3]The Children's Mercy Hospital; [4]BC Children's Hospital; [5]The Children's Hospital of Philadelphia.*

Background: The iron-regulatory protein Hepc mediates iron-restricted erythropoiesis, is increased in both adults and children with CKD, and is a potential target for anemia treatment.

Methods: We examined baseline Hepc and 1) change in Hgb over time with linear mixed effects modeling (max follow-up time 2.5 yrs) and 2) incident anemia (defined as Hgb < 5th percentile for age/sex OR initiation of an ESA) using Cox proportion hazard models. Hepc was analyzed by quartile (≤ 35, 36-60, 61-130, and ≥ 130 ng/mL). Follow-up Hgb obtained annually. GFR measured by plasma iohexol disappearance.

Results: Baseline characteristics (median or percent) in 132 children: age 13 yrs, 58% male, GFR 43 ml/min/1.73m^2, 20% with glomerular dz, Hgb 12.8 g/dL, Hepc 58.3 ng/mL. Baseline Hepc level did not differ by age, sex, or race.

Baseline Characterstics by Hepc Quartile

	Baseline Hepc (ng/mL)				
Median [IQR] or %	≤35	36-60	61-130	≥130	p-value
iGFR, ml/min/1.73m2	51 [40, 58]	45[38, 56]	42 [31, 51]	34 [27, 50]	<0.001
Hgb (g/dL)	13.4 [11.8, 14.2]	13.2 [11.9, 13.7]	12.6 [11.9, 13.3]	12.4 [11.4, 13.4]	0.02
Ferritin (ng/mL)	20 [13, 29]	38 [29, 50]	45 [33, 54]	98 [59, 254]	<0.001
TIBC (µg/dL)	350 [327, 366]	303 [289, 325]	303 [281, 326]	271 [255, 302]	<0.001
ESA use	0	6	11	29	<0.001
Fe use	20	21	43	66	<0.001

Adjusting for anemia treatment, sex, race, age, GFR, and glomerular dz, baseline Hepc was not associated with Hgb change in follow-up. 23 subjects developed incident anemia; cumulative incidence of anemia by Hepc quartile was 12%, 29%, 56%, and 33% respectively (p=0.03 for trend). Multivariate Cox modeling revealed hazard ratio 1.20 (95% CI 0.91, 1.57, p=0.20) for risk of incident anemia per doubling of baseline Hepc.

Conclusions: Higher Hepc values are associated with lower GFR and Hgb, higher ferritin, and increased prevalence of anemia treatment. Baseline Hepc quartile was not independently associated with change in Hgb, but a trend for increased anemia incidence was noted.

Funding: NIDDK Support

SA-PO216

Urinary Tract Infection Characteristics in Children with Vesicoureteral Reflux: RIVUR Baseline Data Myra A. Carpenter,[1] Tej K. Mattoo,[2] Alejandro Hoberman,[3] Russell W. Chesney,[4] Marva M. Moxey-Mims,[5] Ranjiv I. Mathews,[6] Saul P. Greenfield.[7] *[1]Biostatistics, University of North Carolina, Chapel Hill, NC; [2]Children's Hospital of Michigan, Detroit, MI; [3]Children's Hospital of Pittsburgh, Pittsburgh, PA; [4]Le Bonheur Children's Medical Center, Memphis, TN; [5]NIDDK, NIH, Bethesda, MD; [6]Johns Hopkins School of Medicine, Baltimore, MD; [7]Women's and Children's Hospital of Buffalo.*

Background: Vesicoureteral reflux (VUR) is the most common congenital abnormality of the urinary tract in children. It is frequently undiagnosed until after a child has a urinary tract infection (UTI). VUR has been reported in approximately 30% to 40% of children with a UTI.

Methods: The Randomized Intervention for Children with Vesicoureteral Reflux (RIVUR) trial is designed to determine if antimicrobial prophylaxis is superior to placebo in preventing recurrent UTI in children with VUR grade I-IV enrolled following a first or second UTI (index UTI). Follow-up is on-going, but enrollment was completed in May 2011.

Results: RIVUR enrolled 607 children aged 2-71 months from 19 sites in the US. Participants were predominately female (558, 92%), white (482, 81%), grade II (254, 42%) or III (230, 38%) VUR, and without renal scarring (561, 96%) at baseline. The pre-enrollment (index) UTI required (1) presence of fever or urinary tract symptoms, (2) urinalysis-based pyuria, and (3) culture-proven infection with a single organism. Most children (554, 91%) were enrolled following their first UTI; the median age at enrollment was 12 months. The index UTI was symptomatic in 83 (14%), febrile in 197 (32%), and both febrile and symptomatic in 326 (54%) children. Escherichia was the infection organism in 541 (89%) of the index UTIs. In comparison with those having Escherichia infections, children with non-Escherichia infections had a higher grade of VUR (p=0.002), a non-febrile index UTI (p=0.01) and renal scarring (p=0.05). Findings are limited due to the small numbers of non-Escherichia infections (N=66), children with renal scarring (N=21), and children with grade IV VUR (N=50).

Conclusions: RIVUR participants are well-suited for evaluating benefits and risks of antimicrobial prophylaxis in children with VUR. Outcome data for 2 –year follow-up will be available in 2013.

Funding: NIDDK Support

SA-PO217

Disease Progression in Children with Chronic Kidney Disease Bradley A. Warady,[1] Alison G. Abraham,[2] George J. Schwartz,[2] Alvaro Munoz,[2] Craig S. Wong,[2] Mark Mitsnefes,[2] Frederick J. Kaskel,[2] Laurence A. Greenbaum,[2] Robert H. Mak,[2] Joseph T. Flynn,[2] Marva M. Moxey-Mims,[2] Susan L. Furth.[2] *[1]Nephrology, Children's Mercy Hospital, Kansas City, MO; [2]CKiD Investigator.*

Background: Few studies have evaluated the progression of CKD in children and factors influencing progression. The Chronic Kidney Disease in Children (CKiD) observational cohort study has evaluated risk factors for GFR decline in 492 children.

Methods: Analyses were anchored at the 2nd annual study visit. Baseline clinical and demographic factors were assessed as predictors of progression, the composite event defined as halving of iohexol or estimated glomerular filtration rate (ieGFR) or initiation of renal replacement therapy (RRT). Proteinuria was defined as Up/c >2 and elevated blood pressure (eBP) as systolic or diastolic BP >90th percentile. Parametric failure time models were used to explore adjusted associations between baseline levels of covariates and the composite event.

Results: Of 492 patients (pts), 93/396 (23%) with non-glomerular (NG) and 40/96 (42%) with glomerular (G) disease achieved the composite event. At baseline, NG pts with events compared to event-free pts (N=303) had a lower median GFR (31[24,40] vs 51[39,60] ml/min/1.73m2), higher proportion with Up/c > 2 (30% vs 3%) and a higher proportion with eBP (34% vs 23%). G pts with events compared to event-free pts (N=56) had a lower GFR (36[25,47] vs 53[43,72], a higher proportion with Up/c >2 (45% vs 0%) and a higher proportion with eBP (40% vs 16%). Subjects with ieGFR <30 at baseline achieved events faster: 82% and 91% faster in NG and G pts, respectively, compared to pts with an ieGFR ≥30, adjusted for rate of GFR decline (p<0.001). In stratified analyses, among NG pts, adjusting for baseline GFR, times to an event were significantly faster in pts with Up/c >2 (77% faster), eBP (44% faster), male sex (33% faster) and older age (5% faster/yr); in G pts, Up/c >2(91% faster), eBP (64% faster) and African American race (60% faster) were significant factors.

Conclusions: In both NG and G patients, proteinuria and elevated blood pressure are modifiable factors that significantly hasten the time to RRT/50% decline in GFR in children with CKD.

Funding: NIDDK Support, Other NIH Support - NHLBI, NINDS

SA-PO218

Tripterigium for the Treatment of Chronic Kidney Disease: A Systemic Review and Meta-Analysis Bin Zhu,[1,2] Ying Wang,[1] Meg J. Jardine,[1] Min Jun,[1] Jicheng Lv,[1,3] Alan Cass,[1] Yongjun Wang,[1] Vlado Perkovic.[1] *[1]Renal & Metabolic Division, The George Institute for Global Health, University of Sydney, Sydney, Australia; [2]Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China; [3]Department of Nephrology, Peking University First Hospital, Beijing, China.*

Background: Tripterygium preparations are widely used for the treatment of people with chronic kidney disease (CKD) in some parts of the world, especially in China. The objective of this meta-analysis is to define the efficacy and safety of tripterygium in treating CKD.

Methods: We systematically searched Medline, Embase, Cochrane Library, VIP, Wanfang, CNKI and CBM for prospective, randomized, controlled trials assessing the effects of tripterygium compared to placebo, standard care or other treatment in CKD. Study quality was evaluated using Cochrane quality criteria and Jadad scale. Weighted mean difference and summary estimates of relative risk (RR) reductions were calculated with a random effects model. Outcomes analyzed included change in proteinuria, serum creatinine(Scr), as well as remission and relapse rate and drug-related adverse events.

Results: We identified 75 trials involving 4386 participants. Compared to control regimens, tripterygium therapy reduced proteinuria by 0.63g/d (95% CI:-0.74,-0.52) and Scr by 9.75µmol/L(15.12,-4.38)(P<0.001), with significant heterogeneity in the magnitude of the effect (both P<0.001). Meta-regression analysis suggested that the heterogeneity was partly explained by baseline proteinuria for proteinuria(P=0.046), baseline Scr(P<0.001) or age for Scr(P=0.035). Tripterygium increased the likelihood of complete remission by 56%(CI :32-85%, P<0.001), and complete or partial remission by 23%(CI:16-31%, P<0.001) while reducing relapse by 58% (CI:42-69%, P<0.001). It increased the likelihood of liver function abnormalities(RR:4.00, CI:1.48-10.78, P=0.006) and altered menstruation(RR: 24.37, CI:3.65-162.58, P=0.001). Overall study quality was suboptimal, meaning the results should be interpreted with caution.

Conclusions: Tripterygium is a promising nephroprotective agent, but high quality trials are required to reliably determine the balance of benefits and risks.

SA-PO219

Effect of Lowering LDL-Cholesterol on Kidney Function: Meta-Analysis of Data from 120,000 Participants in 21 Randomized Trials David A. Lewis, Jonathan R. Emberson, Lisa J. Blackwell, Rory Collins, Colin Baigent. *On Behalf of the Cholesterol Treatment Trialists' (CTT) Collaboration, Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), University of Oxford, Oxford, United Kingdom.*

Background: Randomized trials have shown that lowering LDL-cholesterol (LDL-C) reduces the risk of occlusive vascular events among a wide range of individuals. Lowering LDL-C might also slow the progression of kidney disease, but previous studies have been inconclusive.

Methods: We undertook meta-analyses of individual participant data from randomized trials comparing a statin vs control or a more intensive statin regimen vs a standard regimen which involved at least 1000 participants and at least 2 years' treatment duration. For participants with creatinine measurements at randomization, about 1 year follow-up and/or at study end (21 trials; 121,038 individuals; median follow-up 4.5 years), we used linear regression to assess individual rates of change in estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI equation.

Results: At randomization, 73% were male, 20% diabetic and 58% had prior vascular disease. Mean eGFR was 71 mL/min/1.73m^2 and 25% had chronic kidney disease (CKD) as defined by eGFR <60 mL/min/1.73m^2. Allocation to active therapy yielded a highly significant reduction in the annual progression rate of about one quarter (absolute annual rate of change [mL/min/1.73m^2 per year]: -0.67 [SE 0.01] active vs -0.87 [0.01] control, weighted mean difference 0.22 [0.02]; p<0.00001). Among 91,100 participants without CKD at randomization, statin therapy produced a significant 10% reduction in the incidence of new CKD during follow-up (8377 [18.4%] vs 9006 [19.8%], RR 0.90, 95% CI 0.88-0.93, p<0.0001) with similar RRs seen across a wide range of individuals.

Conclusions: In addition to reducing the risk of occlusive events, lowering LDL-C also slows the progression of kidney disease in a wide range of individuals without CKD or with only modest renal impairment. The renal benefits of LDL-lowering therapy are small relative to the cardiovascular benefits but may be important in patients at low risk of cardiovascular disease but high risk of developing kidney disease.

Funding: Pharmaceutical Company Support - Most of the trials in this report were supported by research grants from the manufacturers of statins (Astra Zeneca, Bristol Myers Squibb, Merck, Novartis and Pfizer), but no support from the Pharmaceutical Industry Has Been Received for This Meta-Analysis Work

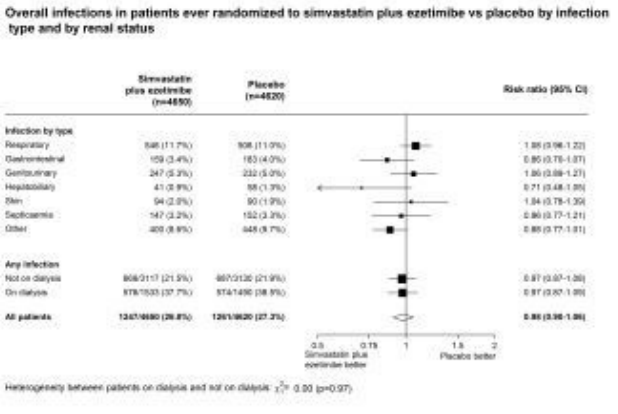
SA-PO220

Effect of Lowering LDL-Cholesterol with Simvastatin Plus Ezetimibe on Infection in Patients with Chronic Kidney Disease: Results from the Study of Heart and Renal Protection (SHARP) Christina A. Reith. *On Behalf of the SHARP Collaborative Group, CTSU, University of Oxford, Oxford, United Kingdom.*

Background: The Study of Heart and Renal Protection (SHARP) was a randomized controlled trial of LDL cholesterol-lowering in patients with chronic kidney disease (CKD) which showed that simvastatin plus ezetimibe reduced vascular events with no significant adverse effects on muscle, hepatobiliary outcomes or cancer. Infection is common in patients with CKD and is a major cause of morbidity. Low cholesterol is associated with infection in some cohorts, but other studies have suggested that statins may reduce the risk of infection.

Methods: 9270 patients with CKD were randomized, 6247 of whom were not on dialysis at baseline. All infectious serious adverse events were routinely recorded post-randomization. Post-hoc intention-to-treat analyses were performed of the effect of allocation to simvastatin plus ezetimibe on time to first infection overall, by infection type, by outcome (fatal or non-fatal) and by renal status.

Results: During 4.9 years median follow-up, similar numbers experienced infections in the two groups: 1247 [26.8%] in those on simvastatin plus ezetimibe *vs* 1261 [27.3%] in those on placebo (rate ratio 0.98, 95% CI 0.90-1.06; p=0.57). There were no significant differences in fatal or non-fatal infections or infection by system of disease between the treatment groups. Effects were similar in those on dialysis and those not (χ^2_1= 0.00; p=0.97).



Conclusions: In the SHARP cohort, combination therapy with simvastatin 20mg plus ezetimibe 10mg had no effect, beneficial or hazardous, on the incidence of infection, either overall, or by infection type, outcome or renal status.

Funding: Pharmaceutical Company Support - The SHARP Study Was Funded Mainly by Merck/Schering-Plough Pharmaceuticals (North Wales, PA, USA), Government Support - Non-U.S.

SA-PO221

Effect of Statin Therapy on Cardiovascular and Renal Outcomes in Patients with Chronic Kidney Disease: A Systematic Review and Meta-Analysis Wanyin Hou,[1] Jicheng Lv,[1] Vlado Perkovic,[2] Lihong Yang,[3] Na Zhao,[1] Meg J. Jardine,[2] Alan Cass,[2] Hong Zhang,[1] Haiyan Wang.[1] *[1]Renal Division, Peking University First Hospital, China; [2]The George Institute for Global Health, University of Sydney, Australia; [3]Guangdong Hospital of Traditional Chinese Medicine, China.*

Background: Guidelines provided uncertain evidence about the statin roles in patients with chronic kidney disease. We undertook a systematic review and meta-analysis to investigate the effects of statin on major clinical outcomes with cardiovascular event, renal outcomes included.

Methods: The study conducted according to the PRISMA guidelines for analyses. Relative risk (RR) reductions were calculated with a random effects model.

Results: We identified 31 trials with 48,429 patients with CKD, including 6690 major cardiovascular events and 6653 deaths. Statin produced a 23% RR reduction (16 to 30) for major cardiovascular events (p<0.001), an 18% RR reduction (8 to 27) for coronary events, and 9% (1 to 16) reduction in cardiovascular or all cause deaths,no significant effect on stroke (21%, –12 to 44) or kidney failure events (5%, -1 to 10). Adverse events were not significantly increased by statins including hepatic (RR 1.13, 95%CI 0.92 to1.39) or muscular disorders (RR 1.02, 95%CI 0.95 to1.09). Meta regression analysis showed an association between cardiovascular event and LDL–reduction among non-dialysis and dialysis patients. Subgroup analysis demonstrated the relative effects of statin therapy in CKD were significantly reduced in people with advanced CKD(p<0.001), (Figure 1) but the absolute risk reductions were comparable.

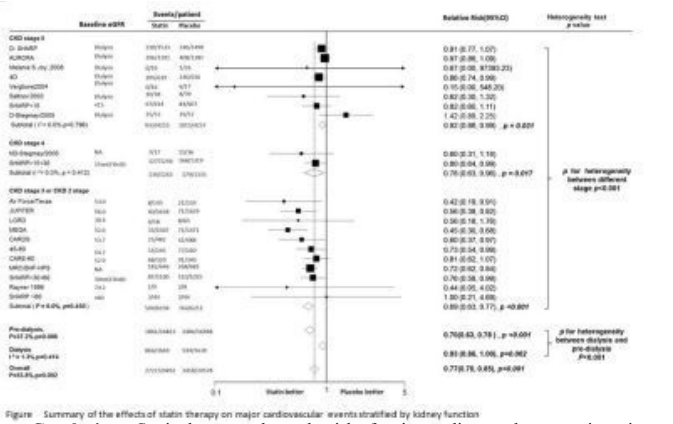


Figure Summary of the effects of statin therapy on major cardiovascular events stratified by kidney function

Conclusions: Statin therapy reduces the risk of major cardiovascular events in patients with chronic kidney disease including those receiving dialysis. Alternative approaches to reducing the substantial excess of cardiovascular events in people with advanced CKD are required.

SA-PO222

Impact of Phosphate Binder Choice on Outcomes among Pre-Dialysis Chronic Kidney Disease Patients in the Netherlands Edith M. Heintjes,[1] Jetty Overbeek,[1] Elizabeth Dunn,[2] Fernie Penning-van Beest,[1] Ron Herings.[1,3] *[1]PHARMO Institute, Utrecht, Netherlands; [2]Genzyme Corporation, Cambridge, MA; [3]Erasmus MC, Rotterdam, Netherlands.*

Background: Sevelamer has been shown to slow the progression of coronary artery calcification (CAC) relative to calcium based binders (CBB) in pre-dialysis CKD patients. Whether sevelamer's effects result in reduced progression to renal replacement therapy (RRT) or an overall survival benefit compared with calcium based binders (CBB) in this population is less understood. This study compared the risk of RRT and mortality between pre-dialysis CKD patients using sevelamer and those using CBB.

Methods: The PHARMO database network including clinical laboratory, outpatient pharmacy and hospitalization records of approximately 1.5 million residents in the Netherlands was linked to the Renine foundation dialysis registry data. From this database all pre-dialysis patients with hyperphosphatemia and CKD stage 3-5 were selected. Patients using sevelamer only were matched to patients using CBB only on age, phosphate level, eGFR, albuminuria severity and propensity score. Multivariate Cox proportional hazards regression modeling was performed to compare the incidence rate (IR) of RRT and all-cause mortality between sevelamer users and CBB users.

Results: The source population included 653 pre-dialysis patients with hyperphosphatemia and CKD stage 3-5 who also received phosphate binders. After applying the matching procedure, 94 patients using sevelamer only and 94 patients using CBB only were included in the study. Patients treated with sevelamer had a 50% reduced risk of progression to RRT compared to patients treated with CBB (crude IRs 22 and 28 per 100 person years (py) respectively, adjusted hazard ratio (HR) 0.5 (95%CI: 0.3-0.9)). Crude IRs for mortality were 14 (sevelamer) and 19 (CBB) per 100 py and the adjusted HR was 0.9 (95%CI: 0.5-1.6).

Conclusions: In this study of community dwelling stage 3-5 CKD pre-dialysis patients in the Netherlands, the risk of RRT for patients using sevelamer was significantly lower (50% reduction) than that of patients using CBB. No significant difference was found for all-cause mortality.

SA-PO223

Paricalcitol Induced Reduction of Proteinuria in Non-Dialysis Chronic Kidney Disease Patients Robert Ekart,[1] Nina Hojs,[2] Breda Pecovnik Balon,[1] Sebastjan Bevc,[2] Radovan Hojs.[2] *[1]Clinic for Internal Medicine, Department of Dialysis, University Clinical Centre Maribor, Maribor, Slovenia; [2]Clinic for Internal Medicine, Department of Nephrology, University Clinical Centre Maribor, Maribor, Slovenia.*

Background: Proteinuria is an independent predictor for chronic kidney disease (CKD) progression and cardiovascular disease. Existing treatment of proteinuria is not sufficient to halt the CKD epidemic. Therefore the aim of our study was to evaluate the effect of paricalcitol on proteinuria in non-dialysis CKD patients.

Methods: 41 non-dialysis CKD patients with secondary hyperparathyroidism (iPTH>65 pg/mL), serum calcium <2.6 mmol/L, serum phosphate <1.8 mmol/L and with proteinuria (>150mg/day) were treated with paricalcitol 1 µg/day. Most were treated for 6 months, with the exception of three patients having iPTH below 30 pg/mL after 3 months of treatment, in whom the therapy after 3 months was stopped. All patients were followed for 6 months. Fixed doses of ACE inhibitors and/or ARBs and/or statins were kept for 3 months before and during the study.

Results: 41 patients (30 men, 11 women; age 62.44±11.93 years, range 31-84 years) with different primary causes of CKD were enrolled in the study. Urinary albumin/creatinine ratio (UACR), 24-hour urinary albuminuria (24hUA) and 24-hour quantitative proteinuria (24hUQP) were measured. Values at 0 and 6 months of these parameters were log-transformed for statistical analysis. After treatment with paricalcitol statistically

significant reduction (paired t-test) in 24hUA (mean 5.99; p<0.01) and 24hUQP (mean -0.34; p<0.0001) were found. The difference in UACR was not significant (mean 5.49; p=0.074). In the observational period no statistically significant reduction in 24-hour ambulatory systolic (mean 135.4 vs mean 133.2 mmHg) and diastolic (mean 76.6 vs mean 76.5 mmHg) blood pressure was found.

Conclusions: Treatment with 1 µg paricalcitol daily according to clinical practice in non-dialysis CKD patients with secondary hyperparathyroidism and proteinuria significantly reduces 24-hour urinary albuminuria and 24-hour quantitative proteinuria without significant change in 24-hour blood pressure.

SA-PO224

Effect of Iron Isomaltoside 1000 on Phosphate Levels in Patients with Non-Dialysis Dependent Chronic Kidney Disease Philip A. Kalra,[1] Lars L. Thomsen.[2] *[1]Department of Renal Medicine, Salford Royal Hospital, Salford, United Kingdom; [2]Pharmacosmos A/S, Holbaek, Denmark.*

Background: Low serum (*s*)-phosphate values have been reported following treatment with some parenteral iron products, the nadir occurring approximately 2 weeks after dosing. When defined as a *s*-phosphate < 2 mg/dL, hypophosphatemia has been noted with an incidence ranging from 3.8 to 70.1 %. The objective of this study was to investigate, in an interim analysis of a randomized clinical trial (RCT), whether patients with non-dialysis dependent chronic kidney disease (NDD-CKD) treated with intravenous (IV) iron isomaltoside 1000 (Monofer®) developed low *s*-phosphate.

Methods: In an ongoing RCT, 350 NDD-CKD patients with renal-related anaemia are being randomised 2:1 to either IV iron isomaltoside 1000 (group A; 234 subjects) or oral iron sulphate (group B; 116 subjects). The patients in group A are equally divided into A1 (IV infusion of maximum 1000 mg or 20 mg/kg iron isomaltoside 1000 over 15 minutes; full iron replacement achieved by up to 2 doses at a weekly interval) and A2 (IV bolus injections of 500 mg iron isomaltoside 1000 administered over 2 minutes once weekly until full replacement dose is achieved). Group B are treated with 200 mg oral iron sulphate daily for 8 weeks. *S*-phosphate is measured prior to iron administration and at 1,2,3,4, and 8 weeks. The current analysis is a per protocol interim analysis including data from 100 patients treated with iron isomaltoside 1000.

Results: Two patients (2 %) who were infused with 750-1000 mg of iron isomaltoside 1000 experienced a decrease in *s*-phosphate below 2 mg/dL. One had *s*-phosphate of 1.2 and the other 1.8 mg/dl (baseline values 3.5 and 2.8 mg/dL, respectively) three weeks after dosing. In both patients, *s*-phosphate had returned to the normal range at the following visit. The low *s*-phosphate was not associated with any clinical symptoms. Furthermore, no drug related adverse events were observed in these patients.

Conclusions: Treatment with iron isomaltoside 1000 has not been associated with clinically significant hypophosphatemia in patients with NDD-CKD. In the two patients (2 %) with a transient low *s*-phosphate, this had normalised by the following visit.

Funding: Pharmaceutical Company Support - Pharmacosmos A/S

SA-PO225

The Effect of Febuxostat on Uric Acid Level and Renal Function Nara Shin,[1] Jiwon Ryu,[1] Hajeong Lee,[1] Sejoong Kim,[2] Dong Wan Chae,[2] Ki Young Na,[2] Suhnggwon Kim,[1] Ho Jun Chin.[2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Internal Medicine, Seoul National University Bundang Hospital, Republic of Korea.*

Background: Allopurinol has been the classic treatment of hyperuricemia. But when using allopurinol, we have to take renal function and adverse effect into account. Febuxostat, a new agent of a non-purine xanthine oxidase inhibitor has been identified as a safe and efficacious alternative. Therefore, we conducted a retrospective study to investigate the benefits of febuxostat treatment, based on the change of uric acid level and renal function and suggest recommended dosage.

Methods: A retrospective observational study was performed based on the outpatient clinic. The patients who started febuxostat and did regular follow-up were enrolled. All patients' serum uric acid, creatinine, glomerular filtration rate level and other laboratory data were collected.

Results: We excluded the patients under 18 year-old, who had gout attack and had missing value. A total of 69 patients was selected. We devided into two groups by the target uric acid level (serum uric acid <6.0mg/dl or not). Male sex, younger age and high BMI were associated with lower uric acid change. In Allopurinol group and Steroid group, uric acid level was less decreased .In multivariate logistic regression, male sex (OR 7.803, 95% CI 1.143-53.27, p=0.036) and allopurinol group (OR 6.316, 95% CI 1.214-32.85, p=0.028) were the significant factors related to less controlled uric acid level. In comparison of the group of eGFR<30 and eGFR≥30, there was no difference about the variation of uric acid and renal function. Compared with the variation of uric acid by febuxostat dose, there was no difference of uric acid variation between 20mg, 40mg and 80mg of febuxostat in two groups.

Conclusions: Our study showed that febuxostat is effective in improving serum uric acid. Male sex and allopurinol group were the significant factors related to less controlled uric acid level. In our study, low dose(20mg) for the group of eGFR under 30mg/dl/m2 was no inferior to high dose (40mg, 80mg). Large group and long-term follow-up studies are required to investigate the long term effect of febuxostat.

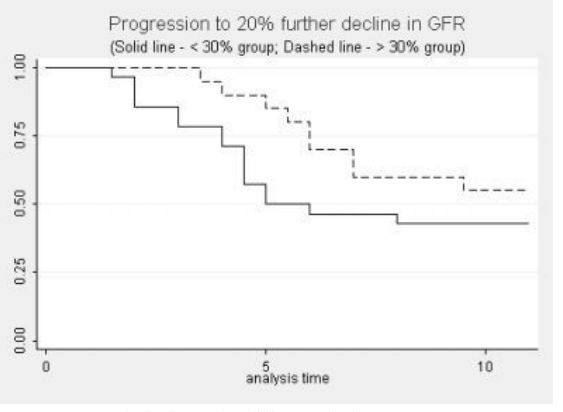
SA-PO226

Long-Term Outcomes in CKD Patients Treated with Renin-Angiotensin Inhibitors (RASI) Who Experience an Increase in Serum Creatinine Greater than 30% Sheldon M. Hirsch,[1] Jackie Hirsch,[1] Udayan Y. Bhatt,[2] Brad H. Rovin.[2] *[1]Lakeside Nephrology, Chicago, IL; [2]Nephrology, The Ohio State University, Columbus, OH.*

Background: RASIs slow progression of CKD. However, they may lead to an initial decline in eGFR. Current recommendations suggest tolerance of an initial increase in serum creatinine (sCr) only up to 30%. This may limit aggressive use of RASI. The purpose of this study was to evaluate the long-term follow-up of CKD subjects who experienced an initial increase in sCr, with RASI, greater than 30%, and in whom RASI was not reduced.

Methods: 48 patients at a community-based nephrology clinic were followed longitudinally. Patients were aggressively treated with RASI with goals of SBP ≤ 125mmHg and a dipstick urine protein of ≤ 30 mg/dl. Those whose sCr increased > 30% were compared to those whose sCr increased ≤ 30% using survival and longitudinal analysis.

Results: Patients in the group with sCr increase > 30% had a long-term decline in eGFR of 0.52cc/min/1.73m^2 compared to a decline of 1.8cc/min/1.73m^2 in the ≤ 30% group. The decline in eGFR was not different in the groups in a multivariate (MV) model (p=0.228). Cox proportional hazards regression was used to compare the time to a further 20% decrease in eGFR (after the initial decline associated with RASI maximization, Figure).



MV Cox analysis showed no difference in the groups (HR=0.60, 95% CI 0.26, 1.35). The incidence of End-Stage Renal Disease and death was also similar in the two groups, and, along with the slow decline in eGFR, compared favorably with previous large-scale CKD studies.

Conclusions: Important outcomes in patients who experienced sCr increase > 30% were not significantly different than those whose sCr remained unchanged or increased ≤ 30%. This suggests that an initial increase in sCr should not limit RASI dosing, contrary to current recommendations.

SA-PO227

Renin-Angiotensin-Aldosterone System Inhibition Is Associated with Lower Plasma Fibroblast Growth Factor 23 Levels and Decreased Mortality in Patients with Advanced Chronic Kidney Disease: A Propensity Score Analysis of the HOST Study Anna Jeanette Jovanovich,[1] Jessica B. Kendrick,[1] Alfred K. Cheung,[2,4] James S. Kaufman,[3] Tom Greene,[2,4] M. Chonchol.[1] *[1]University of Colorado; [2]VASLCHCS; [3]VA Boston HCS; [4]University of Utah.*

Background: Evidence suggests that the renin-angiotensin-aldosterone system (RAAS) intersects with vitamin D-FGF23-Klotho axis. Increased angiotensin II lowers renal klotho expression, thus increasing fibroblast growth factor 23 (FGF23) levels. We investigated whether FGF23 levels differ and modify the risk of outcomes in response to RAAS inhibition (ACEI/ARB usage) in advanced chronic kidney disease (CKD).

Methods: We studied 1753 patients with advanced CKD (1099 CKD not yet on dialysis, eGFR=18±6 ml/min/1.73m^2; and 654 ESRD) who participated in the Homocysteine in Kidney and End Stage Renal Disease study. Outcome measures were plasma C-terminal FGF23 levels and all-cause mortality in all participants and progression to ESRD in those with CKD not yet on dialysis. Linear and Cox regression models adjusted for important confounding variables and propensity score analysis were used to assess the association of ACEI/ARB usage with FGF23 levels, all-cause mortality, and dialysis initiation.

Results: Average age was 66±12 years, 38% were black, with 870 (50%) taking ACEI/ARB. Over a mean follow-up of 3 years, there were 714 (41%) deaths and 615 patients (56%) initiated chronic dialysis. In adjusted linear regression models, ACEI/ARB users had significantly lower plasma FGF23 levels (difference, -332 RU/mL; 95% CI -184 to -459; p=0.0009) compared with ACEI/ARB nonusers. Those treated with ACEI/ARB had significantly lower all-cause mortality with an adjusted hazard ratio of 0.81 (95% CI 0.69-0.94, p=0.005) and lower dialysis initiation 0.84 (95% CI 0.72-0.99, p=0.03) in those with CKD not yet on dialysis. Higher FGF23 levels did not attenuate the protective effect of ACEI/ARB use.

Conclusions: ACEI/ARB use was associated with lower plasma FGF23 levels and was protective for all-cause mortality and dialysis initiation in patients with advanced CKD, suggesting that RAAS inhibition may have an FGF23-mediated protective effect.

Funding: NIDDK Support

SA-PO228

Reno-Protection with Renin-Angiotensin-System (RAS) Blockers in Type 2 Diabetes Mellitus (T2DM) and Chronic Kidney Disease (CKD): Examining the Implementation Gap

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Background: Several meta-analyses and a recent report from U.S. Preventive Task Force indicate that randomized controlled trials (RCT) of RAS blockade reduced the risk of end stage renal disease in diabetics with CKD, but do not support cardiovascular risk reduction. We examined the implementation gap between patients enrolled in RCT's and those seen in practice.

Methods: We searched MEDLINE (English) to include all RCT's examining reno-protective effects of RAS use in T2DM and cross referenced with citations in relevant systematic reviews. Also included were RCT's referenced in the appropriate KDOQI practice guideline. Eight RCT's were identified. Key baseline characteristics from the pooled RCT's were compared, using Chi-square tests, to a validated Veterans Affairs (VA) cross-sectional cohort of 2,889 ambulatory care patients with T2DM and CKD (one outpatient estimated glomerular filtration rate < 60 ml/min/1.73m2). Age estimates in pooled RCT's were derived from standard deviations (SD).

Results: Baseline characteristics of the pooled RCT cohort were: mean age 58 (SD 6.4), mean systolic blood pressure (SBP) 153 mm of Hg (SD 17), mean serum creatinine (SCr) of 1.8 mg/dl (SD, 0.5), and mean HbA1c 9.2 (SD 1.7). These were significantly different in our VA cohort: mean age 70 (SD, 10.3), mean SBP 134 mm of Hg (SD, 15), mean SCr 1.7 mg/dl (SD, 1.1), and mean HbA1c of 7.1 (SD, 1.6); [p value for each parameter < 0.0001]. RAS usage in VA cohort was 77%. RCT's had only 3% of patients > 71 years old, 26% with heart disease, and 12% with lipid disorders compared to 53%, 61%, and 74% respectively in the VA cohort (p value of <0.0001 for all).

Conclusions: The study outlines a wide implementation gap between characteristics of RCT participants and those encountered in practice; in practice patients were older, had a higher frequency of heart disease, lipid disorders, and different levels of blood pressure and glucose control. Reno-protective effect of RAS blockers in older adults is unclear; in contrast to RCT's their cardiovascular benefits may change with age and other co-morbidities.

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SA-PO229

Combination Therapy with Renin-Angiotensin System Inhibitors and an Oral Adsorbent, AST-120 Can Delay the Initiation of Dialysis

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Background: An oral adsorbent, AST-120 (AST) can slow the progression of chronic kidney disease (CKD). Renin angiotensin system inhibitors (RASi) can prevent the progression of CKD as well. We therefore examined whether combination therapy with RASi and AST-120 has enhancing effects in delaying the initiation of dialysis.

Methods: The study was a multicenter cross-sectional study. The subjects were 2,335 CKD patients who were initiated on hemodialysis. The patients were divided into four groups (RASi-/AST-, RASi+/AST-, RASi-/AST+, RASi+/AST+) and we compared the parameters between the four groups. Multiple linear regression analysis was conducted to clarify the factors for the timing of initiation of dialysis.

Results: The results were shown in **Table 1**.

The comparison of parameters between four groups

	RASi-/AST- N=835	RASi+/AST- N=1092	RASi-/AST+ N=119	RASi+/AST+ N=289	p value
Age	67.0±14.5	66.3±13.1	67.9±15.2	64.8±13.1	0.102
Female	294	342	47	78	0.039
Cardiovascular disease	211	310	36	76	0.299
Duration of nephrologist care	826±1261	1056±1054	1040±1391	1294±1118	0.146
systolic blood pressure	149±28	153 ± 26	148 ± 28	152 ± 25	0.009
Cardiothoracic ratio	55.2 ± 7.7	54.2 ± 7.9	54.7 ± 7.0	52.8 ± 7.9	0.001
BUN	91.8 ± 32.0	86.4 ± 28.2	90.1 ± 23.9	93.6 ± 28.7	<0.0001
Creatinine	9.22 ± 3.91	8.85 ± 3.25	9.17 ± 3.17	9.52 ± 3.31	0.008
eGFR	5.87 ± 4.22	5.72 ± 2.67	5.20 ± 2.13	5.24 ± 2.07	0.014
Phosphorus	6.3 ± 2.0	6.0 ± 1.7	6.0 ± 1.6	6.1 ± 1.7	0.009
Albumin	3.17 ± 0.65	3.19 ± 0.61	3.28 ± 0.64	3.28 ± 0.58	0.032
Hemoglobin	8.6 ± 1.7	8.6 ± 1.5	8.6 ± 1.4	8.5 ± 1.5	0.888
C-reactive protein	2.57 ± 4.61	1.69 ± 3.83	1.91 ± 3.53	1.17 ± 3.51	<0.0001
Ferritin	257 ± 328	212 ± 255	181 ± 171	178 ± 162	<0.0001

Multiple linear regression analysis revealed a significant association of the use of AST-120 with the initiation of dialysis in patients who were administered RASi.

Conclusions: In conclusion, the above results suggested that the combination therapy with RASi and AST-120 delayed the initiation of dialysis by anti-inflammatory effects.

SA-PO230

Subgroup Analyses in Nephrology Randomized Controlled Trials

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Background: Subgroup analyses are commonly used in randomized controlled trials (RCT) to examine for heterogeneity of treatment effects. While they may be useful, when overused or inappropriately applied they can result in misleading conclusions. The purpose of our study was to describe how subgroup analyses are used and reported in Nephrology RCTs.

Methods: We used a search strategy that yielded RCTs from nephrology and major medical journals published between July 1, 2010 and June 30, 2011. Articles were excluded if they were from phase 1 trials, secondary analyses of previously published RCTs or appeared only as a letter to the editor. Abstraction was performed for all articles and then a secondary review of each was performed by a separate investigator. Meetings were held to reconcile differences in abstraction.

Results: In total, 83 articles were reviewed (Table 1). Subgroup analyses were reported in 31 articles (37.3%), and had a mean of 1.5 ±1.0 outcomes 4.6 ±4.0 subgroups per analysis. Only 7 of 31 subgroup analyses were prespecified (22.6%). The appropriate statistical method (test of interaction) was conducted in only 11/31 subgroup analyses (35.3%). Positive results of subgroup analyses were reported in 16/31 articles (51.6%). When results were positive, claims were made in the discussion section in 14/16 (87.5%) cases. Predictors of use of subgroup analyses included studies of dialysis, larger sample size and multicenter studies.

Conclusions: In conclusion, subgroup analyses are frequently used in nephrology trials, and the methodology is often suboptimal.

Characteristics of Subgroup Analyses

	n (%)
Number of Articles with Subgroup Analysis	31 (37.3)
Analysis Prespecified:	
Yes	7 (22.6)
No	24 (77.4)
Mean Number of Outcomes Reported	1.45±1.00
Mean Number of Analyses per study	4.6±4.4 (range 1-25)
Test of Interaction used to Analyze Subgroups	11 (35.3)
Positive Claims regarding Subgroups Analyses in Discussion	87.5%

Funding: Clinical Revenue Support

SA-PO231

Efficacy of Tacrolimus in the Treatment of Children with Focal Segmental Glomerulosclerosis

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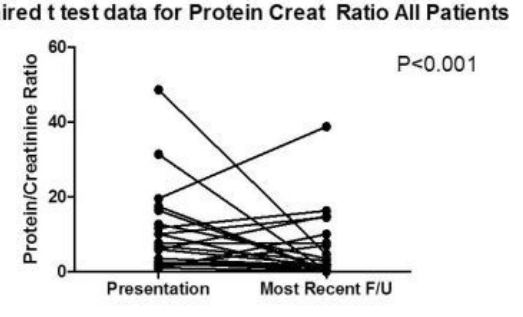
Background: Focal segmental glomerulosclerosis (FSGS) is the most common glomerular condition leading to end stage renal disease (ESRD) and the third most common cause of ESRD in pediatric patients.

Methods: This is a retrospective study consisting of 22 pediatric patients with FSGS and heavy proteinuria. After demonstrating steroids resistance, patients were treated with tacrolimus, targeting a trough level 5-8 ng/ml. The primary outcome is the induction of proteinuria remission. Secondary outcomes are preservation of renal function and the incidence of tacrolimus toxicity.

Results: Thirteen patients (59%) achieved remission (complete in 31.8% vs. partial in 27.2%, P<0.001) (Figure 1) and 12 had stable or improved renal function over an average of 2.9 years (0.2-7) of follow up (P<0.0053).



Average eGFR and urine protein creatinine ratio

Parameter	Presentation	Most resent follow up	P value
eGFR	92.3 (31.3-138)	70.13 (9.3-161)	<0.053
P/Cr ratio	10.1 (0.86-48.64)	5.96 (0.05-38.8)	<0.001

Abbreviations: eGFR, estimated glomerular filtration rate (cc/min/1.73 m2), P/Cr ratio: Protein/ creatinine ratio (mg/mg). Data are presented as mean with the range in parenthesis.

Non- compliance with treatment was an important factor in 4 of the 9 non-responsive patients (44%). None of the patients had any significant side effects related to the use of tacrolimus. The repeat biopsies did not demonstrate an increase in interstitial fibrosis. The best renal outcome was for patients who achieved complete remission. Partially responsive patients had improved renal survival compared to resistant patients.

Conclusions: Tacrolimus is an effective and relatively safe option in the treatment of children with idiopathic steroid resistant FSGS.

SA-PO232

Agricultural Work, *Lija* and Water Consumption Associated with Chronic Kidney Disease in León, Nicaragua Jill Lebov,[1] Douglas Morgan,[1] Edgar M. Pena,[3] Scott Leonard Sanoff,[2] Yichun Hu,[1] Romulo E. Colindres,[1] Susan L. Hogan.[1] *[1]UNC, Chapel Hill, NC; [2]UVA, Charlottesville, VA; [3]Centro de Investigación e Intervenciones en Salud, León, Nicaragua.*

Background: Recent studies have suggested a non-traditional chronic kidney disease (CKD) etiology in Pacific coastal Central America, including possible influences from agricultural work, consumption of unregulated homemade alcohol (*lija*), and exposure to toxins in water and food. However, results across studies have been inconsistent and findings are based on small or non-population-based samples.

Methods: Using a population-based sample of residents of León, Nicaragua, we conducted a cross-sectional study of CKD and associated factors. Blood sample collection and structured interviews were conducted in participants' homes. Glomerular filtration rate was estimated (eGFR) using the MDRD equation, with CKD defined as eGFR<60 ml/min/1.73m^2. We used multivariate logistic regression to estimate prevalence odds ratios (POR) and 95% confidence intervals (CI), adjusted for sex, age, and self-reported hypertension and diabetes.

Results: In adjusted models, self-reported diagnosis of hypertension, rural residence, more years of agricultural work, *lija* consumption, and higher levels of average daily water consumption were significantly associated with CKD. Notably, self-reported diabetes was not associated with CKD in adjusted models.

Risk Factor	Number (%)	Adjusted POR (95% CI)
Hypertension		
No	1921 (87)	*1.0*
Yes	284 (13)	1.89 (1.24, 2.90)
Zone		
Urban	1585 (71)	*1.0*
Rural	655 (29)	1.80 (1.24, 2.63)
Agricultural Work (# years)		
None	1331 (60)	*1.0*
0-4	292 (13)	1.20 (0.65, 2.21)
5-9	164 (7)	1.51 (0.79, 2.88)
10-14	143 (6)	2.19 (1.19, 4.05)
15+	275 (12)	2.39 (1.50, 3.80)
Lija consumption (# of times/week)		
0	2101 (95)	*1.0*
≥1	285 (13)	2.05 (1.08, 3.87)
Number of glasses of water/day		
0-4	653 (30)	*1.0*
5-8	755 (34)	0.86 (0.52, 1.44)
9-12	452 (21)	1.26 (0.74, 2.16)
13-52	344 (16)	1.90 (1.08, 3.36)
Diabetes		
No	2105 (95)	*1.0*
Yes	100 (5)	1.16 (0.64, 2.10)

Conclusions: Our findings confirm the likely contribution of non-traditional risk factors to this burgeoning regional CKD epidemic in Nicaragua.

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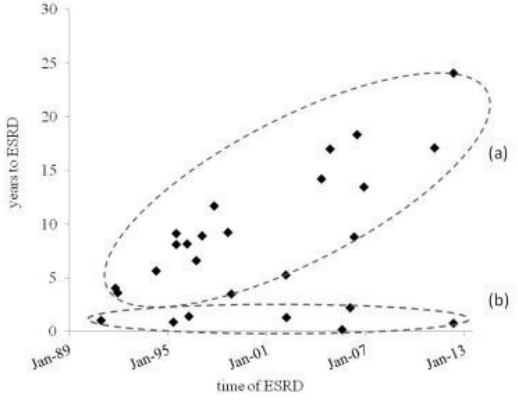
SA-PO233

End-Stage-Renal-Disease after Hematopoietic Stem Cell Transplantation Eric P. Cohen, Manpreet Bedi, William Drobyski. *Medicine, Medical College of Wisconsin, Milwaukee, WI.*

Background: Hematopoietic stem cell transplantation (HSCT) is often complicated by acute and chronic kidney disease. End-stage-renal-disease (ESRD) has a 16-fold higher incidence after HSCT compared to the age-matched general population. It has multiple causes and a variable time-of-occurrence after HSCT.

Methods: 2264 subjects have undergone HSCT at our center since 1986. In 26 cases of ESRD, we portrayed time from HSCT to ESRD as a function of the date of occurrence of ESRD, to obviate bias of follow-up time.

Results:



Conclusions: This shows two forms of ESRD after HSCT. The first (a) occurs at several or more years after HSCT, and its time to occurrence appears to be at ever increasing times after HSCT. This could result from changes in HSCT practice, e.g. less-intense conditioning regimens, or from improved care of patients with chronic kidney disease (CKD). The second form of ESRD (b) occurs at a lesser interval after HSCT, and its time of occurrence has not changed over time. 9 of the 19 type (a) ESRD cases had BMT nephropathy as their underlying diagnosis. 1 of the 7 type (b) ESRD cases had BMT nephropathy as their underlying diagnosis. BMT nephropathy can be treated and mitigated (Int J Radiat Onc Biol Phys, 2008). Other causes of CKD after HSCT require investigation for improved patient care. Mitigation of CKD after HSCT is important because it may occur in 15% of all HSCT, and evolution to ESRD is costly and has a poor prognosis.

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SA-PO234

Long-Term Renal Outcome in Patients with APRT Deficiency and 2,8-Dihydroxyadeninuria Vidar O. Edvardsson,[1,2] Hrafnhildur Linnet Runolfsdottir,[1] Inger Maria Agustsdottir,[2] Runolfur Palsson.[1,2] *[1]University of Iceland – National University Hospital of Iceland, Reykjavik, Iceland; [2]University of Landspitali – National University Hospital of Iceland, Reykjavik, Iceland.*

Background: Adenine phosphoribosyltransferase (APRT) deficiency is an autosomal recessive disorder of purine metabolism leading to excessive urinary excretion of the poorly soluble 2,8-dihydroxyadenine (DHA). The majority of reported patients have developed radiolucent kidney stones but the prevalence of chronic kidney disease (CKD) and end-stage kidney failure (ESKF) has not been well established. The aim of this study was to assess the long-term renal outcome in patients with this disorder.

Methods: The medical records of all 45 patients listed in the APRT Deficiency Registry of the Rare Kidney Stone Consortium were reviewed for clinical features including kidney stones, significant unilateral kidney damage, reduced kidney function and age at onset of renal replacement therapy. Data are presented as median and range.

Results: Of the 45 patients in the Registry, 20 were males. Median age at diagnosis was 27.3 (0.6-62.8) years. Clinical features included kidney stones in 24 patients (53%), CKD in 18 patients (40%) and reddish-brown diaper stain in 5 (11%) infants. Fifteen patients (33%) were asymptomatic at the time of diagnosis. Seven patients (16%) developed ESKF at a median age of 37 (15-54) years. In 4 of these patients, the diagnosis of APRT deficiency was not made until 4.8 (0.2-5.1) years after the development of ESKF. Severe unilateral kidney damage was observed in 4 additional patients leading to unilateral nephrectomy in 3 cases. Overall, a delay in diagnosis of 6.7 (0.4-39.2) years occurred in 24 patients. Causes for this delay included confusion of DHA calculi with uric acid stones and of renal histopathological findings with other forms of crystalline nephropathy. In addition, urinary crystals were not identified correctly in several patients.

Conclusions: CKD is a common manifestation of APRT deficiency in our series of patients. Unfortunately, a substantial proportion of patients progress to ESKF due to lack of timely diagnosis and treatment. Increased awareness of this disorder among both clinicians and pathologists is important.

Funding: NIDDK Support, Other NIH Support - ORD, RDCRN

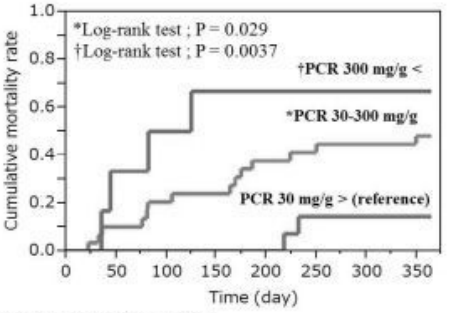
SA-PO235

Proteinuria with Increased Serum Hepcidin-25 Level Is a Sign of Poor Prognosis in Patients with Non-Hodgkin Lymphoma Masaki Hara,[1] Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Proteinuria is associated with mortality. Highly elevated serum hepcidin-25 levels may imply existence of strong inflammation related to cancer. An association between proteinuria and serum hepcidin-25 level was studied with special reference to mortality in non-Hodgkin lymphoma (NHL) patients.

Methods: A 1-year prospective cohort study was conducted in 54 NHL patients (male, 66.6%). Proteinuria was defined as protein to creatinine ratio (PCR) > 30 mg/g. Serum hepcidin-25 level was measured by mass spectrometry. Mortality rate was analyzed by the Kaplan-Meier method. Multivariate Cox's analysis was used to calculate hazard ratio (HR) with its 95% confidence interval (CI) of proteinuria for mortality, adjusted for gender, estimated glomerular filtration rate (eGFR) and international prognostic index (IPI) scores for lymphoma. Multiple linear regression analysis was employed to identify factors associated with presence of proteinuria, incorporating gender, IPI scores, hemoglobin, eGFR, serum hepcidin-25, serum c-reactive protein, serum ferritin and serum albumin.

Results: Prevalence of proteinuria was 25.9%. The mortality rate and HR were significantly increased according to increasing grades of PCR. The median [interquartile range] serum hepcidin-25 level was 10-fold higher than that in healthy controls (50.5 [22.6-97.7] vs 5.7 [1.6-12.7] ng/ml). serum hepcidin-25, but not other variables, was identified as a significant factor related to the presence of proteinuria (standardized $\beta = 0.43$, $P = 0.0016$).



Multivariate Cox's analysis for mortality

Variable	HR	95% CI	*P*
PCR 30 mg/g >	-	-	-
PCR 30–300 mg/g	**6.88**	1.75-47.46	**0.0036**
PCR 300 mg/g <	**13.97**	2.35-119.93	**0.0039**

Conclusions: Mortality of NHL patients is significantly associated with the presence of proteinuria, which might be induced by cancer-related inflammation.

SA-PO236

Determining Factors of Kidney Size and Its Association with Kidney Function Ylian S. Liem,[1] Peter J. Blankestijn,[1] Frank L.J. Visseren.[2] *[1]Department of Nephrology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Department of Vascular Medicine, University Medical Center Utrecht, Utrecht, Netherlands.*

Background: Reports on factors that determine kidney size are conflicting. The aim of this study was to assess which factors are associated with kidney size in a population of patients with manifestations of atherosclerotic vascular disease. In addition, we assessed mean values of kidney size for different groups.

Methods: In a population with clinically manifest vascular disease, included in the Second Manifestations of ARTerial disease (SMART) study (n=5,840), kidney length was measured in cm on ultrasound, and the mean of the left and right kidney was calculated. We examined the association between age, gender, height, weight, smoking, blood pressure, presence of diabetes, hyperlipidemia, estimated GFR (eGFR), type of vascular disease, and extent of vascular disease with kidney length, using univariable and multivariable (corrected for age and gender) linear regression analysis. We excluded patients with known renal disease and patients with partial or complete agenesis of one kidney.

Results: Increasing age (B per year -0.021, 95% CI -0.023 to -0.019) and female gender (B -0.637; 95% CI -0.689 to -0.585) were associated with shorter kidney length. In the multivariable analyses, increasing height (B per m 3.302, 95% CI 2.975 to 3.629), weight (B per kg 0.025, 95% CI 0.023 to 0.027), and higher eGFR (B per ml/min 0.014, 95%CI 0.013 to 0.016) was associated with greater kidney length. The presence of diabetes mellitus was also associated with greater kidney length (B 0.257, 95%CI 0.197 to 0.316). Mean kidney length for age groups <55yr, 55-65yr and >=65yr were 11.6, 11.5 and 11.1cm for males and 11.0, 10.8 and 10.5cm for females. For eGFR groups <60, 60-90 and >= 90 ml/min, mean kidney lengths were 10.6, 11.2 and 11.7cm.

Conclusions: Increasing age, female gender, shorter stature, lower weight and lower eGFR are associated with shorter kidney length. In addition, diabetes mellitus is associated with greater kidney length; hyperfiltration – present in diabetes mellitus – might explain for this greater kidney length.

SA-PO237

Determinants and Heritability of Kidney Length: A Family-Based Population Study Menno Pruijm,[1] Belen Ponte,[2] Daniel Ackermann,[3] Philippe Vuistiner,[4] Ute Eisenberger,[3] Michel Burnier,[1] Pierre-Yves F. Martin,[2] Felix J. Frey,[3] Murielle Bochud.[4] *[1]Nephrology, University Hospital (CHUV), Lausanne, Switzerland; [2]Nephrology, University Hospital (HUG), Geneva, Switzerland; [3]Nephrology, University Hospital, Bern, Switzerland; [4]Institute of Social and Preventive Medicine (IUMSP), Lausanne, Switzerland.*

Background: Kidney length is an important parameter in renal clinical decision making, yet large population-based studies are sparse, and the heritability of kidney size is unknown. The aim of this study was to assess the heritability and some other determinants of kidney length in a family-based study.

Methods: The SKIPOGH study (Swiss Kidney Project on Genes in Hypertension) is a cross-sectional examination survey exploring the role of genes and kidney hemodynamics in blood pressure regulation and hypertension. Anthropometric parameters and renal ultrasound measurements were assessed in a randomly selected sample of index subjects and at least one first degree relative. The ASSOC program in SAGE (Statistical Analysis in Genetic Epidemiology) was used to estimate the age, sex, body weight and height, eGFR (CKD-EPI) and center-adjusted narrow sense heritability.

Results: In total, 793 participants coming from 205 nuclear families were included. Mean (±SD) kidney length in men (n=374, age 47±18 years, BMI 26.2±4 kg/m², eGFR 98±18 ml/min/1.73m²) was 11.4±0.8cm, as compared with 10.7±0.8cm in women (aged 48±17y, BMI 24.5±5, eGFR 95±17). There was no difference in length between the right and left kidney. In multilevel adjusted linear regression analysis, body height, weight and eGFR were positively associated with kidney length, whereas gender, diabetes and hypertension were not. There was a non linear association between age and kidney length. The heritability (h^2) of kidney length, adjusted for all mentioned confounders, was 52.4±8 %, p<0.001.

Conclusions: This study suggests that kidney length is an inherited trait, independently of other important determinants such as age, estimated kidney function, body height and weight.

Funding: Government Support - Non-U.S.

SA-PO238

Evidence of Altered Perception Causing Severe Fatigue in ANCA-Associated Vasculitis Andrew McClean,[1] Neil Basu,[2] Matthew David Morgan,[1] David A. Jones,[1] Jos A. Bosch,[1] Lorraine Harper.[1] *[1]University of Birmingham, United Kingdom; [2]University of Aberdeen, United Kingdom.*

Background: Up to 70% of people with ANCA-associated vasculitis (AAV) report severe fatigue, the main cause of reduced quality of life in this group. However, no studies have previously determined whether the mechanism for this fatigue is cardiovascular, neuromuscular, or central.

Methods: We recruited 46 patients with AAV in remission, and 28 matched healthy controls. Baseline fatigue was measured using the Multidimensional Fatigue Index (MFI-20). Participants underwent a submaximal graded exercise test; heart rate (HR) and VO2 measurements were used to estimate VO2max (cardiovascular fitness), and perception of exertion was assessed using the Borg scale. A DEXA scan was used to assess muscle mass. Quadriceps muscle strength and voluntary muscle activation were measured by repeated maximal voluntary contractions (MVC) with super-imposed stimulation. Stamina was assessed with a sustained 50% MVC; super-imposed stimulations allowed assessment of muscular reserve at the point of subjective exhaustion.

Results: The AAV group reported much more severe fatigue than the control group in all dimensions of the MFI-20 (all p<.001). During exercise, estimated VO2max was similar in both groups (p=.216), but perception of exertion was significantly greater in the AAV group (mean gradient of Borg score against heart rate=0.184 [SE=0.02] vs 0.106 [SE=0.01] p=.002). Muscle mass was not significantly different between groups (p=.737), and neither were muscle strength (p=.439) or percentage voluntary muscle activation (p=.096). The AAV group appeared to have less stamina than the controls, as assessed by time to volitional muscular fatigue (mean=73.68s [SE=5.58] vs 96.3s [SE=6.41], p=.011), but importantly they had more residual muscular reserve (mean=73.7% [SE=3.3] vs 61.25% [SE=3.6], p=0.018), suggesting perception of fatigue was more important than actual muscular fatigue.

Conclusions: This evidence suggests that fatigue in AAV is not due to reduced muscular or cardiovascular capacity, but is the result of altered perception of cardiovascular and muscular exertion. Future strategies to improve fatigue should target this mechanism.

Funding: Clinical Revenue Support

SA-PO239

Severe Fatigue and Psychological Morbidity in ANCA-Associated Vasculitis Andrew McClean,[1] Neil Basu,[2] Matthew David Morgan,[1] David A. Jones,[1] Jos A. Bosch,[1] Lorraine Harper.[1] *[1]University of Birmingham, United Kingdom; [2]University of Aberdeen, United Kingdom.*

Background: Patients with ANCA-associated vasculitis (AAV) report fatigue as a main debilitating symptom, but little systematic research has been done to assess its impact. We aimed to characterize the severity of this fatigue, and its influence on patients' well-being and psychological morbidity.

Methods: We recruited 149 patients with AAV, 68 CKD disease controls, and 57 healthy controls to this cross-sectional study. Participants completed a questionnaire comprising the Multi-Dimensional Fatigue Index (MFI-20), and a number of other symptom rating scales including the Hospital Anxiety and Depression Scale (HADS).

Results: Both the AAV and CKD groups reported significantly higher levels of fatigue than the healthy group (Table 1, p<.001 multivariate), with scores comparable to the norm levels in chronic fatigue syndrome (CFS) patients. Both also reported that fatigue is a major impairment to professional, family, and social life (p<.001 for all, compared to healthy controls). Moreover, 27% of AAV patients had clinically significant anxiety or depression; this was comparable to the values found in the CKD group, and three-fold higher than in the healthy group (p<.001). Stepwise regression analysis showed that individual differences in fatigue explained differences in anxiety and depression, but not vice-versa, suggesting a causal association.

Table 1

	AAV	CKD	Healthy
MFI-20:General	14.27 (.355)	12.87 (.542)	7.71 (.481)
MFI-20:Physical	13.48 (.4)	13.39 (.572)	7.18 (.418)
MFI-20:Activity	11.95 (.384)	12.59 (.589)	6.73 (.467)
MFI-20:Motivation	10.54 (.317)	11.00 (.506)	6.63 (.374)
MFI-20:Mental	10.39 (.382)	9.66 (.567)	7.20 (.414)
HADS-Anxiety	6.90 (.384)	6.32 (.530)	4.49 (.466)
HADS-Depression	5.64 (.291)	5.60 (.490)	2.15 (.369)

Mean scores with SE for the 5 dimensions of fatigue (MFI-20), anxiety and depression (HADS)

Conclusions: Fatigue in AAV is comparable to the levels seen in CFS and CKD, and is associated with major role impairments. High levels of anxiety and depression are also seen in this group, possibly secondary to fatigue. This indicates the need for further research into the determinants and treatment of severe fatigue in AAV.

Funding: Clinical Revenue Support

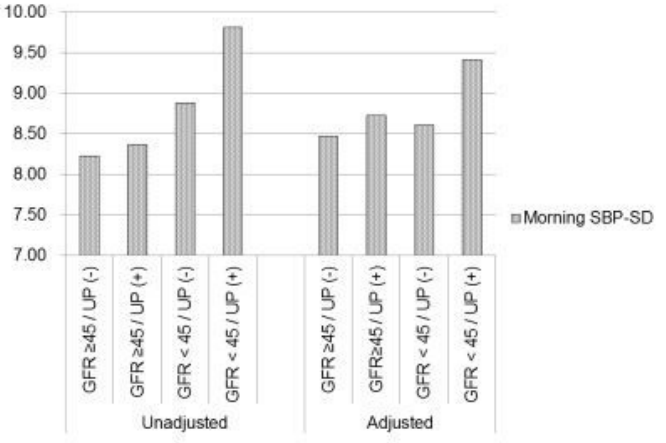
SA-PO240

The Presence of Chronic Kidney Disease Is Related to Higher Day-by-Day Blood Pressure Variability: The Ohasama Study Hiroyuki Terawaki,[1] Masaaki Nakayama,[1] Masahiro Kikuya,[2] Takayoshi Ohkubo,[2] Yutaka Imai.[2] *[1]Department of Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan; [2]Department of Planning for Drug Development and Clinical Evaluation, Tohoku University Graduate School of Pharmaceutical Sciences, Sendai, Japan.*

Background: Both day-by-day blood pressure variability, defined as within-subject SDs of home measurement, and chronic kidney disease (CKD) are known to be linked with an increased risk of cardiovascular disease, however, their relationship remains unclear. The present study was aimed to evaluate the relationship between renal dysfunction, proteinuria and day-by-day blood pressure variability.

Methods: We recorded self-measured morning blood pressure (BP) at home in 1,383 individuals (mean 63.2 years old; males, 32.0%; mean glomerular filtration rate [GFR], 46.1 mL/min/1.73 m^2; positive proteinuria, 13.4%) and classified the subjects into four groups: higher GFR without proteinuria (GFR≥45/UP(-), n=637), higher GFR with proteinuria (GFR≥45/UP(+), n=69), lower GFR without proteinuria (GFR<45/UP(-), n=561), and lower GFR with proteinuria (GFR<45/UP(+), n=116).

Results: Compared with the GFR≥45/UP(-) group, the GFR<45/UP(+) group exhibited a significantly higher SD of systolic BP.



Conclusions: The presence of CKD is related to higher day-by-day blood pressure variability.

SA-PO242

Timing and Determinants of Low Urinary Creatinine Excretion in Chronic Kidney Disease Elena Tynkevich,[1] Jean-philippe Haymann,[3] Pascal Houillier,[4] Benedicte Stengel.[1] *[1]CESP U1018 INSERM, Villejuif, France; [2]U702 INSERM, Tenon Hospital, Paris, France; [3]U872 INSERM, HEGP, Paris, France.*

Background: Reduced muscle mass is common in patients with end-stage CKD, but less is known about its timing and determinants in early stage CKD.

Methods: After excluding patients with incomplete urine collection, we studied 24-hr urinary creatinine excretion (UCrV), a marker of muscle mass, in 1087 (726 men/361 women) non dialysis patients with all-stage CKD. All of them had glomerular filtration rate measured (mGFR) by ^{51}Cr-EDTA renal clearance. We used gender specific thresholds to define low creatinine excretion as a value < the 10th percentile of UCrV distribution in patients with mGFR ≥60 ml/min/1.73m^2, i.e., 0.13 mmol/kg/24h in men and 0.12 mmol/kg/24h in women.

Results: Mean mGFR was 42.4 ml/min/1.73m^2 in men and 40.1 ml/min/1.73m^2 in women; mean UCrV was 0.17 mmol/kg/24h and 0.14 mmol/kg/24h, respectively. In both genders, the percentage of low UCrV increased with increasing age and body mass index (BMI), was negatively associated with protein intake assessed by 24-hr urinary urea excretion and was lower in African patients. In men, this percentage was higher in patients with elevated serum prealbumin level, but lower in those with high serum fibrin level. After adjusting for age, ethnicity, BMI, prealbumin, serum fibrin and protein intake, the OR (95% CI) of low UCrV associated with decreasing mGFR from 30-44 to 15-29 and <15 compared to >=45 ml/min/1.73m^2 were 1.8 [1.0-3.4], 5.5 [3.0-10.0] and 6.2 [2.2-17.7] in men and 1.5 [0.7-3.5], 1.5 [0.7-3.4] and 3.1 [0.8-11.6] in women.

Conclusions: The observed decrease in UCrV associated with mGFR decline suggests that a reduction in muscle mass may appear early in CKD patients. An increase in extra-renal excretion of creatinine via the gut cannot be ruled out to explain this association, even though it is usually considered only significant in late stage CKD.

Funding: Pharmaceutical Company Support - Amgen, Roche, Government Support - Non-U.S.

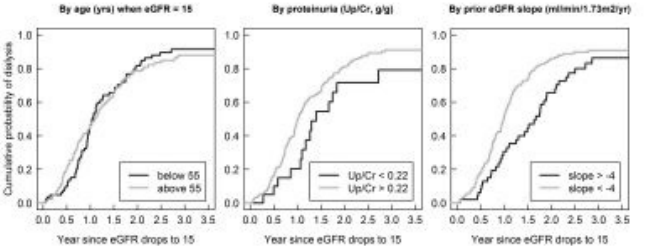
SA-PO243

Time-to-Dialysis when Estimated GFR (eGFR) Drops below 15 ml/min/1.73m2: Results from the African American Study of Kidney Disease (AASK) Study Liang Li,[1] Lawrence J. Appel,[2] Brad C. Astor,[3] Michael S. Lipkowitz,[4] Jackson T. Wright,[5] Tom Greene.[6] *[1]Cleveland Clinic; [2]Johns Hopkins University; [3]University of Wisconsin-Madison; [4]Georgetown University; [5]Case Western Reserve University; [6]University of Utah.*

Background: Preparation for renal replacement therapy is essential as GFR declines towards 15 ml/min/1.73m^2 (CKD stage V), but the time to subsequent initiation of the therapy and its relationship with patient conditions at first reaching eGFR of 15 have not been established.

Methods: Bayesian penalized splines were used to estimate the eGFR trajectory for each AASK patient with at least 3 yrs follow-up. From these trajectories, we identified pts whose eGFR declined to 15 ml/min/1.73m^2. The time from eGFR reaching 15 to dialysis was analyzed, with adjustment for competing risks by death and stratification by age, urine protein/creatinine ratio (Up/Cr) when eGFR dropped below 15, and the mean eGFR slope over the prior 3 yrs.

Results: An eGFR decline to 15 ml/min/1.73m^2 occurred in 175 of 846 (21%) of AASK pts with at least 3 yrs follow-up. Compared to those whose eGFR did not decline to 15, these pts were more likely female (50.3% vs. 36.4%), had lower eGFR (39 vs. 52.5 ml/min/1.73m^2) and higher Up/Cr (0.556 vs. 0.156 g/g) on average at enrollment. Of the 175 pts, 46% started dialysis within a year, 80% within 2 yrs, and 90% within 3 yrs. Longer gap between eGFR reaching 15 and dialysis was not significantly associated with younger age (p=0.6) and lower proteinuria (p=0.1) but was associated with less steep prior eGFR slope (p=0.02).



Conclusions: This study demonstrates considerable heterogeneity in the timing of dialysis among CKD patients whose eGFR drops to 15 ml/min/1.73m^2. These results highlight the importance of developing prediction models to guide personalized planning for initiation of dialysis.

Funding: NIDDK Support

SA-PO244

Using Machine-Learning to Predict Progression of Chronic Kidney Disease from Electronic Health Record Data Herbert S. Chase. *Departments of Medicine and Biomedical Informatics, Columbia University, New York, NY.*

Background: The individual rate of progression of Stage 3 Chronic Kidney Disease (CKD) varies considerably. Current calculators provide reasonable probability estimates of progression but require manual data input and cannot be used if data is missing. The purpose of this study was to use machine-learning tools to predict CKD progression using data from the electronic health record (EHR), even if incomplete.

Methods: Using EHR data, patients with Stage 3 CKD were identified as having MDRD-eGFR < 60 ml/min/1.73m2 consistently and for at least 90 days. Two cohorts of patients were identified: Progressors (P) (eGFR declined > 3 ml/min/1.73m2/year) and Non-Progressors (NP) (eGFR declined < 1 ml/min/1.73m2). The data from 2 years prior to 2 years after Stage 3 entry was collected for each patient, if available, and yearly averages were calculated. ICD-9 codes for co-morbid conditions and demographic information on each patient were extracted. Various machine-learning algorithms (Logistic regression, Naïve Bayes, and J-Rip) were used to develop a model predicting disease progression.

Results: The following attributes differed between the two arms and were used for machine learning models: serum albumin, calcium, phosphorous, hemoglobin, hemoglobin A1C, HCO3, Na, K and urine dipstick protein, diabetes mellitus, acute kidney injury, congestive heart failure, glomerulonephritis and African-American race. A minority of patients had all lab values recorded; over 30% lacked urine protein and phosphorous measurements. Of the three machine-learning models, Naïve Bayes provided the best results: sensitivity and positive predictive value were 55% and 56%, respectively; specificity and negative predictive value were 86% and 85%, respectively. The area under the ROC curve was 0.77.

Conclusions: Even when data is missing, machine-learning tools that utilize EHR data can differentiate with reasonable accuracy patients who are likely to proceed from stage 3 to stage 4 CKD from non-progressors. Accuracy should improve when patients receive all appropriate tests. The prediction tool could function automatically by extracting EHR data, without manual entry.

SA-PO245

Differential Risk of Dialysis versus Death in Black versus White Veterans Is Attenuated by Older Age and Lower Levels of Kidney Function

Jessica W. Weiss,[1] Thy P. Do,[2] Adam J. Batten,[2] Yoshio N. Hall,[2] Ann M. O'Hare.[2] *[1]Div. Nephrology, Oregon Health and Science Univeristy, Portland, OR; [2]Div. Nephrology, University of Washington, VA Puget Sound Healthcare, Seattle, WA.*

Background: Recent studies have reported a higher risk of progression to end stage renal disease (ESRD) as compared with death among Black patients versus White patients in the U.S. However, the relative risk of ESRD versus death may be modified by level of kidney function.

Methods: We conducted a retrospective cohort study of a national cohort of VA patients aged 18-100 years, who were either Black (n=225,942) or White (n=1,127,362) and with at least one outpatient measurement of serum creatinine, blood pressure, and body mass index from 10/1/2000 - 9/30/2001. We calculated incidence rate ratios (IRRs) to describe the time to ESRD versus time to death by race, age and estimated glomerular filtration rate (eGFR).

Results: Black-White differences in risk of ESRD vs. death were attenuated in older patients and those with lower levels of kidney function. With decreasing kidney function, progression to ESRD was more common as compared with death in both black and white patients. However, racial disparities in the relative incidence of these two outcomes were amplified by decreasing kidney function and increasing age. In cohort members age 60-74, Black patients with severe CKD experienced a relatively greater risk of ESRD than death (IRR 1.72), whereas the risk of death exceeded that of ESRD in White patients in this age group with any level of eGFR.

Conclusions: Racial differences in the relative frequency of ESRD and death vary as a function of age and kidney function.

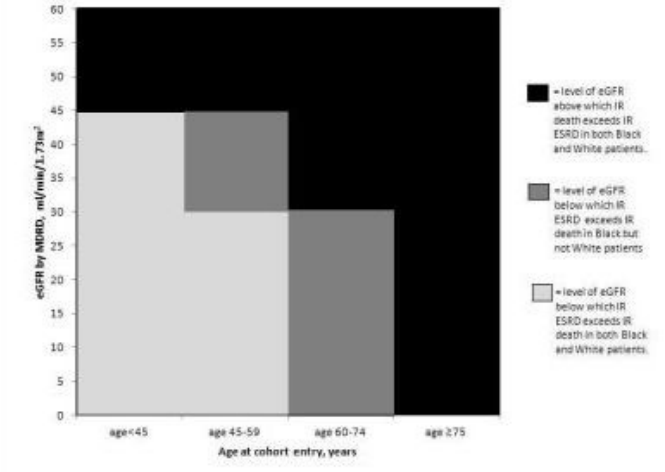


Figure 1: The threshold level of eGFR at which incidence rate (IR) of ESRD exceeded that of death differed for Black and White patients.

Funding: Veterans Administration Support

SA-PO246

The Relationship between Blood Pressure and Mortality in Older Adults with Chronic Kidney Disease Is Modified by Age and Renal Function

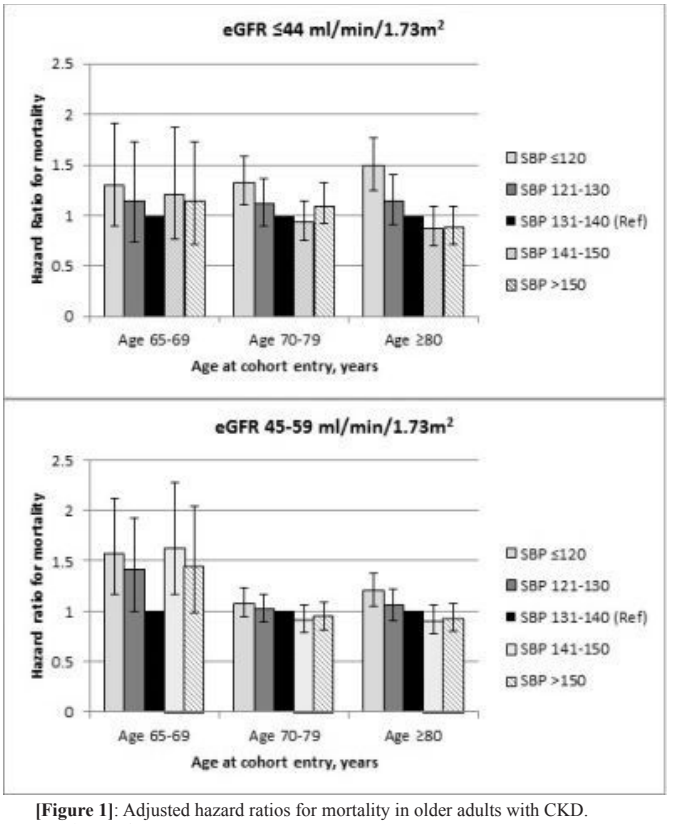
Jessica W. Weiss,[1] Dawn Peters,[2] Xiuhai Yang,[3] Amanda F. Petrik,[3] David Smith,[3] Eric S. Johnson,[3] Micah L. Thorp,[3] Cynthia Morris,[4] Ann M. O'Hare.[5] *[1]Div. Nephrology, Oregon Health and Science Univ. (OHSU), Portland, OR; [2]Div. Biostatistics, OHSU, Portland, OR; [3]Science Program, Center for Health Research, Kaiser Permanente NW, Portland, OR; [4]Medical Informatics & Clin. Epi., OHSU, Portland, OR; [5]Div. Nephrology, Univ. of Washington, VA Puget Sound Healthcare, Seattle, WA.*

Background: Few studies have evaluated the impact of renal function on age-related differences in the relationship between blood pressure and mortality.

Methods: We conducted a retrospective cohort study of older adults with CKD within a health maintenance organization (Kaiser Permanente Northwest) between 1999-2010 (n=23,511). We classified patients by baseline systolic blood pressure (SBP) in 10 mmHg bands (≤120…>150), severity of CKD (estimated glomerular filtration, eGFR, ≤44 versus 45-59 ml/min/1.73m^2), and age (65-69, 70-79, 80+ years). The relationship between SBP and mortality was measured with Cox Proportional Hazards regression, stratified by age group and level of renal function.

Results: In the youngest cohort members, there was a u-shaped relationship with SBP, with higher mortality at SBP levels both above and below the referent (SBP 131-140 mmHg); this difference was significant among those with higher baseline eGFR. In older cohort members (ages 70+), mortality was highest for those with the lowest levels of SBP, whereas mortality at the highest level of SBP was similar to the referent. This increase in mortality with low SBP in the older cohort was greatest for those age 80+ with low eGFR.

Conclusions: The relationship between blood pressure and mortality in older adults with CKD may vary with age and renal function.



[**Figure 1**]: Adjusted hazard ratios for mortality in older adults with CKD.

Funding: Other U.S. Government Support

SA-PO247

Albumin:Creatinine Ratio or 24 Hour Albumin Excretion for Albuminuria Staging?

Lieneke E. Scheven, Hiddo Jan Lambers Heerspink, Paul E. de Jong, Dick de Zeeuw, Ron T. Gansevoort. *Nephrology, University Medical Center Groningen, Netherlands.*

Background: New guidelines advocate the use of albumin:creatinine ratio (ACR) instead of 24h-urinary albumin excretion (UAE) for staging albuminuria. Concern has been expressed that this may result in misclassification because of interindividual differences in the urinary creatinine excretion. We examined the number of subjects that are reclassified and their cardiovascular (CV) outcome.

Methods: Included were subjects participating in the PREVEND study (N=5367) and the RENAAL study (N=701) of whom data on 24h-UAE, ACR (in a first morning void as well as 24h-urine sample) and fractional UAE were available at baseline. For categorization 3 albuminuria classes as advocated by KDIGO were used (<30, 30-300, >300 mg/24h and mg/g and corresponding fractional UAE classes).

Results: When using ACR in the first morning void instead of UAE 89% of subjects were classified in corresponding albuminuria categories. 234 (3.9%) participants were classified to a higher and 426 (7.0%) to a lower category. When 24hr ACR was used instead of the first morning void ACR the number of reclassified subjects was significantly lower (n=47 [0.8%] up and n=280 [4.6%] down, resp., both p<0.001), indicating that reclassification is for a large part due to (diurnal) variation in urinary albumin rather than creatinine concentration. Using data on fractional UAE it showed that 44/47 (94%) of upward reclassified subjects were correctly reclassified, suggesting that errors in 24hr urine collection also partly explain reclassification. In line, Cox regression analyses showed that upward reclassification when using first morning void ACR instead of UAE was associated with a tendency for increased CV risk, whereas downward reclassification was associated with lower risk: e.g. upward reclassification from UAE normo- to first morning void ACR microalbuminrua (n=203 or 3.4%) was associated with a Hazard rate (95%CI) of 1.71 (1.14-2.57) unadjusted and 1.48 (0.98-2.24) age and gender adjusted.

Conclusions: Our results indicate that although there is reclassification when using first morning void ACR instead of 24hr UAE, reclassification is often correct and indicative for prognosis.

SA-PO248

Is Albuminuria Associated with an Increase in Tubular Reabsorption of Uric Acid? Lieneke E. Scheven, Michel M. Joosten, Paul E. de Jong, Stephan J.L. Bakker, Ron T. Gansevoort. *Nephrology, University Medical Center Groningen, Netherlands.*

Background: In several studies serum uric acid (SUA) concentration has been found to be associated with albuminuria. This association has been explained as SUA causing vascular damage, resulting in an increase in albuminuria. We studied whether the opposite relation may also be true, i.e. whether albuminuria can be a cause for an increase in SUA, and -if so- what the underlying mechanism would be.

Methods: Included were 7,573 participants of the PREVEND Study (an observational general population based cohort study) with data on SUA and 24hr urinary UA excretion available, who were not using drugs that may influence SUA. Linear regression analyses were used to test associations of albuminuria with SUA and uric acid load (calculated as 24h UA excretion) and tubular UA reabsorption (calculated as (100-fractional UA excretion) %). Cox regression analyses were used to study the association of SUA and albuminuria with incident cardiovascular (CV) events.

Results: Albuminuria was positively associated with SUA, crude, as well as in models adjusted for potential confounders such as age, gender, alcohol consumption, BMI, eGFR and 24hr UA excretion (both p<0.001). When tubular UA reabsorption was added to the model, the association between albuminuria and SUA lost significance (p=0.79). In separate analyses albuminuria was found to be positively associated with tubular UA reabsorption, again crude, as well as in adjusted models (both p<0.001). During a median follow-up of 10.5 yrs 704 CV events occurred. After adjustment for CV risk factors CV event rate was predicted by albuminuria (HR=1.10 (95%CI 1.04-1.18) per SD, p=0.003) as well as SUA (HR=1.20 (1.11-1.31) per SD, p<0.001). When both were entered together into the Cox model a negative interaction between these variables was found (p=0.04), indicating that a high SUA has less predictive value for CV events when a high ACR is present, and vice versa.

Conclusions: Based on these data we conclude that albuminuria may cause an increase in SUA, by enhancing tubular UA reabsorption. This phenomenon may explain in part why albuminuria is associated with CV outcome.

SA-PO249

Prevalence of Vitamin D Deficiency According to Degrees of Albuminuria Yun Jung Oh,[1] Hajeong Lee,[1] Hayne C. Park,[1] Jeonghwan Lee,[1] Yon Su Kim,[1,2] Dong Ki Kim.[1] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Kidney Research Institute Medical Center, Seoul National University Hospital, Seoul, Republic of Korea.*

Background: Both albuminuria and vitamin D deficiency are common health problems associated with cardiovascular and kidney diseases. Although prevalence of albuminuria is increased with decreasing serum vitamin D levels, there is no clear threshold level of albuminuria that predicts vitamin D deficiency or changes in serum 25-hydroxyvitamin D (25OHD) levels.

Methods: We analyzed data from 7,709 adultswho had a voluntary routine health checkup at Seoul National University Hospital(Seoul, Korea) from Jan 2008 to Mar 2012. Vitamin D deficiency was defined as a serum 25OHD≤15 ng/ml, and a degree of albuminuria was evaluated with urinary albumin-to-creatinine ratio(ACR) from untimed urine samples and categorized as normal (ACR <10), mildly increased (ACR 10-29), moderately increased (ACR 30-299), and severely increased albuminuria (ACR >300 mg/g).

Results: The overall prevalence of vitamin D deficiency was 19.2%, and the mean serum 25OHD levels were22.4±8.2 ng/ml. The mean ACR was 23.2±180.3 mg/g, and the prevalence of normal, mildly increased, moderately increased, and severely increased albuminuria were 75.4%, 17.0%, 6.6%, and 1.0%, respectively. In univariate analysis, a stepwise increase inthe levels of 25OHD (P for trend 0.019) and the prevalence of vitamin D deficiency(P for trend 0.001) were observed with increasing degrees of albuminuria.The multivariate analysis with the use of dichotomized ACR levels adjusted for age, sex, season, vitamin D supplementation, hypertension, diabetes, anemia, and glomerular filtration rate, showed a significant increased risk of vitamin D deficiency, beginning at ACR ≥10 mg/g (Odds ratio, 1.204; 95% confidential interval, 1.018-1.423).

Conclusions: Even mildly increased albuminuria may have association with vitamin D deficiency in Korean adult population.

SA-PO250

Prevalence, Predictors and Outcomes Associated with Dysnatremia in CKD Sang-Woong Han,[1,2] Anca Tilea,[2] Brenda W. Gillespie,[2] Fredric O. Finkelstein,[3] Margaret A. Kiser,[4] George Eisele,[5] Peter Kotanko,[6] Rajiv Saran.[2] *[1]Hanyang University; [2]University of Michigan; [3]Metabolism Associates; [4]University of North Carolina; [5]Medical College of Albany; [6]Renal Research Institute.*

Background: The epidemiology of dysnatremia in patients with advanced pre-dialysis chronic kidney disease (CKD) in the ambulatory setting has not been systematically investigated.

Methods: This prospective cohort study enrolled 2,183 CKD patients (55% male, 65% white, baseline mean age 64 years, 86% hypertensive, 43% diabetic) followed at 78 US sites for an average of 1.8 years. Linear regression was used to find predictors of serum sodium (SNa) at baseline or over time. Associations between SNa and time to death, and time to ESRD in baseline and time-dependent Cox models were examined, adjusting for age, gender, race, diabetes, blood pressure and eGFR. SNa was categorized as 4 indicator variables in the models (≤135, 135<SNa≤140, 140<SNa<145[ref], ≥145 mEq/L).

Results: The mean±SD estimated GFR was 25±11 mL/min/1.73 m^2, and mean baseline SNa was 140.5±3.1 mEq/L. The prevalence of hyponatremia (≤135 mEq/L) and hypernatremia (≥145 mEq/L) were 6% and 9%, respectively. Higher baseline SNa was significantly associated with male sex, higher systolic blood pressure, BMI and serum albumin, and lower eGFR and BUN, with a stronger effect associated with the same variables in time-dependent models. Significant relationship between baseline SNa and mortality was observed, with mortality risk significantly greater at SNa≤135mEq/L, (hazard ratio, HR=2.09, p=0.01) and ≥145 mEq/L (HR=1.83, p=0.02) compared with 140<SNa<145 mEq/L. Risk for ESRD was significantly higher among patients with SNa≤135 mEq/L compared with 140<SNa<145 mEq/L in time-dependent models (HR=1.48, p=0.04).

Conclusions: Dysnatremia was seen in 15% of patients with CKD in the outpatient setting. CKD patients with SNa≤135mEq/L were at higher risk for progression to ESRD and both hyponatremic and hypernatremic CKD patients were at higher risk of mortality. Greater attention to sodium disorders in routine nephrology care may help to improve patient outcomes.

Funding: Pharmaceutical Company Support - Renal Research Institute

SA-PO251

Hyponatremia Associates with Mortality in Virologically Suppressed HIV Infected Patients Fabrice Gankam Kengne,[1] Henning J. Drechsler,[2] Naim M. Maalouf,[1] Roger Bedimo.[2] *[1]Internal Medicine, UTSW Medical Center, Dallas, TX; [2]Internal Medicine, North Texas VA Hospital, Dallas, TX.*

Background: Hyponatremia is the commonest electrolyte disorder in HIV infected patients. It is associated with increased mortality in hospitalized HIV patients with low CD4 count, but its significance in HIV infected but virologically suppressed ambulatory patients is unclear.

Methods: We assessed the association between baseline and incident time-updated outpatient hyponatremia and all cause mortality in HIV-infected US veterans who maintained virologic control after starting HAART. We used data from the VA Clinical Case Registry to build a Cox regression model adjusting for time-updated comorbidity parameters of the VACS score and restricted index (age, Hgb, eGFR, FIB4-liver fibrosis score, HCV coinfection, CD4 count, and viral load). Additionally we controlled for HAART adherence as calculated from the patients medication refill history.

Results: We identified 15714 patients with follow-up time >1.5 years after starting HAART who remained virologically suppressed for a median time of 3.3 years (IQR 2.0-5.8). 1249 (7.93%) patients had hyponatremia at cohort entry (baseline). We observed 1139 (7.2%) deaths during the follow up time. Incident mild (Na 130-135 mEq/L) and severe (Na <130 mEq/L) hyponatremia was associated with an increased risk of death both in univariate analysis and after adjusting for the VACS and restricted index covariates (HR 1.62, CI 1.39-1.90 and 2.64, CI: 1.91-3.64 respectively, p< 0.0001 for both in multivariate analysis).

Conclusions: Incident hyponatremia carries an independent significant association with mortality in successfully treated HIV-infected patients. Further studies are needed to better understand the nature of this association and if therapies directed toward preventing the occurrence of hyponatremia will affect mortality in HIV-infected patients.

Funding: Other U.S. Government Support

SA-PO252

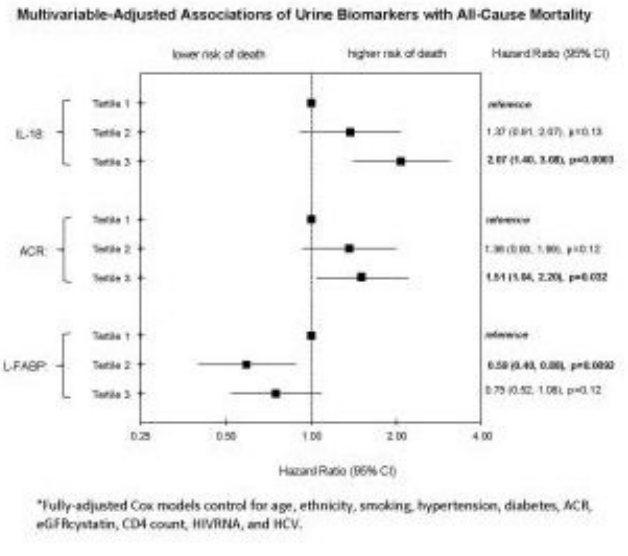
Urinary Biomarkers of Kidney Injury Are Associated with Death in the Women's Interagency HIV Study (WIHS) Carmen A. Peralta,[1,2] Rebecca Scherzer,[1,2] Carl Grunfeld,[1,2] Alison G. Abraham,[3] Phyllis Tien,[1,2] Prasad Devarajan,[4] Michael R. Bennett,[4] Mark J. Sarnak,[5] Chirag R. Parikh,[6] Michael Shlipak.[1,2] *[1]UCSF; [2]SFVAMC; [3]JHU; [4]Cincinnati Children's Hospital; [5]Tufts; [6]Yale.*

Background: Urinary markers of kidney injury have been associated with kidney disease risk in both HIV-infected and uninfected populations. CKD is associated with death. We investigated whether these markers are associated with risk for death among HIV infected women.

Methods: We determined the association of urine biomarkers with 10-year, all-cause death in 908 HIV-infected women. Biomarkers measured from stored baseline urine included interleukin-18 (IL-18), liver fatty acid binding protein (L-FABP), albumin-to-creatinine ratio (ACR), kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL).

Results: Mean (SD) age was 41 ± 8 years;mean eGFRcys was 88 ± 21 ml/min/1.73m^2 at baseline. Participants in the highest IL-18 tertile had an age-adjusted mortality rate of 32%, compared with 21% and 15% in middle and lowest tertiles. After multivariable adjustment for demographic, traditional, HIV-related factors, and eGFRcys, higher IL-18 and higher ACR were independently associated with increased mortality. We found a U-shaped association between levels of L-FABP with death, as persons in the middle tertile had the lowest risk for death (Figure). Persons in the highest tertile of KIM-1 and NGAL were at higher risk for death compared to lowest in demographic adjusted models, but associations were attenuated after full adjustment: KIM-1 HR 1.41 (0.99, 2.02), NGAL HR 1.05 (0.74, 1.51).

Conclusions: Among HIV-infected women, novel urine markers of kidney injury are associated with risk for death, independently of kidney function and albuminuria. These markers represent potential tools to identify high risk HIV-infected persons with early kidney injury.



Funding: NIDDK Support, Other NIH Support - NIA

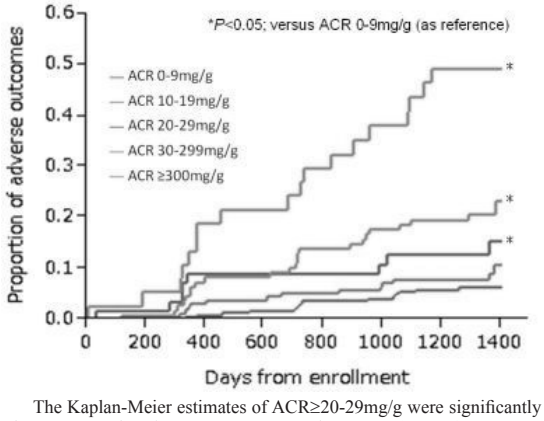
SA-PO253

Low-Grade Levels of Albumin Excretion Should Be Considered Significant in an HIV-Infected Population Naoki Yanagisawa,[1,2] Minoru Ando,[1,2] Atsushi Ajisawa,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Infectious Diseases and Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Albuminuria is a risk factor of overt kidney disease, mortality, and cardiovascular disease (CVD). A cut-off value that is relevant to such outcomes could be less than the conventional threshold in an HIV-infected population.

Methods: A 3.5-year prospective cohort study was conducted in 661 HIV-infected individuals (598 men, 63 women) to test a new cut-off relevant to the incidence of composite outcomes, including all-cause mortality, CVD, and a decrease in renal function. A decrease in renal function was defined as greater than 25% decrement of estimated glomerular filtration rate from baseline. Albumin excretion levels in spot urine were classified into 5 grades according to urinary albumin-to-creatinine ratio (ACR); 0-9, 10-19, 20-29, 30-299, and ≥300mg/g. The cumulative incidence of outcomes was analyzed by the Kaplan-Meier method, stratified by the grades of albumin excretion.

Results: All patients completed the follow-up period. The frequencies of ACR of 0-9, 10-19, 20-29, 30-300, and >300mg/g were 45.7, 23.9, 8.3, 16.5 and 5.6% respectively. Of 661, 82 (12.4%) developed at least one of the outcomes. A decrease in renal function, all-cause mortality, and CVD occurred in 60 (73%), 16 (19.5%), and 16 (19.5%), respectively. The cumulative incidence of outcomes increased with increasing grades of ACR.



The Kaplan-Meier estimates of ACR≥20-29mg/g were significantly higher than that of ACR 0-9 mg/g, whereas ACR 10-19mg/g was not (P=0.26).

Conclusions: The conventional cut-off value of ACR 30mg/g likely underestimates the number of HIV individuals at risk for developing adverse outcomes. Lowering the threshold of ACR to 20mg/g should be considered in medical care of HIV-infected persons.

SA-PO254

The Relationship between Ethnicity and Mortality within a Multi-Ethnic Population with Chronic Kidney Disease Mark David Jesky,[1,2] Paul Cockwell,[1,2] Andrew Felix Burden.[3] *[1]Nephrology Department, University Hospitals Birmingham NHS Foundation Trust, United Kingdom; [2]Department of Infection and Immunity, University of Birmingham, United Kingdom; [3]Birmingham and Solihull NHS Cluster of CCGs, United Kingdom.*

Background: People of South-Asian and Black ethnicity with chronic kidney disease (CKD) have more rapid progression to end stage kidney disease but better survival on dialysis than people of White ethnicity. However there are limited data on mortality differences by ethnicity in pre-dialysis CKD. We therefore assessed the relationship between ethnicity and mortality in a multi-ethnic, primary-care population with CKD stages 3 and 4.

Methods: Laboratory and clinical data are collated centrally in Heart of Birmingham Teaching Primary Care Trust (UK) for individuals 40 years of age or older in 63 of 74 primary-care practices. Using the May-June 2008 dataset, individuals with an estimated Glomerular Filtration Rate (eGFR) of 15-59 ml/min and a reported Albumin-Creatinine Ratio (ACR) within the previous 12 months were identified. Data on all deaths until February 2011 was obtained from the Office of National Statistics. Cox proportional hazards regression was used to analyse the association between ethnicity and mortality.

Results: 1909 individuals met criteria for analysis (1098 [57.5%] South Asian, 484 [25.4%] White and 327 [17.1%] Black). There were 224 (11.7%) deaths and a higher proportion of Whites died (White 15.5%, South Asian 10.6%, Black 10.1%, p=0.012). After adjusting for known risk factors, South Asians (hazard ratio [HR] of 0.728, 95% confidence interval [CI] 0.532 – 0.997, p=0.048) and Blacks (HR 0.642, 95% CI 0.42 – 0.98, p=0.04) had a lower risk of death than Whites. An increased risk of death was also independently associated with high ACR (above 30mg/g), decreased eGFR (below 30ml/min), age (above 80 years), male sex, current smoking status and the presence of ischaemic heart disease and heart failure.

Conclusions: In people with non-dialysis dependent CKD, individuals of South Asian and Black ethnicity have a lower risk of death than those of White ethnicity. This difference remains after correction for known risk factors.

Funding: Private Foundation Support, Clinical Revenue Support

SA-PO255

Estimated Glomerular Filtration and Proteinuria in the Moroccan Population Marc E. De Broe,[1] Zamd Mohamed,[2] Monique M. Elseviers,[3] Abdelali Belghiti Alaoui,[4] Mohammed El Hassane Trabelssi,[4] Benyounès Ramdani,[4] Bayahia Rabia,[2] Mohammed Benghanem Gharbi.[2] *[1]International Society of Nephrology; [2]Moroccan Society of Nephrology, Morocco; [3]Epidemiology Research, University of Antwerp, Wilrijk, Belgium; [4]Ministry of Health, Morocco.*

Background: MaReMar (Maladies Rénales Chroniques au Maroc) is an epidemiological study estimating the prevalence of CKD, hypertension, diabetes type I and II in a representative randomized sample (n=10524) of the adult population of Morocco (26-70 y/age), identifying subjects at risk to develop CKD, establishing an intervention follow-up/program over 5 yrs.

Methods: The study was performed in 2 middle sized towns (Khemisset, El Jadida) with resp. a rural and industrialized character. We report on the eGFR (MDRD equation), microproteinuria (mPR), macroproteinuria (MPR). Serum creatinine (Scr) was determined using a methodology traceable to the Scr reference system (NIST) (GC-IDMS), microproteinuria (mPR) using the Hemocue method, MPR using dipstick (++ or more) on two morning midstream urine samples.

Results: 1.6% of the adult population of Morocco has a eGFR <60 ml/min/1.73m². In the age categories 26-40, 41-55, and 56-70 yrs of age, an eGFR<60ml/min/1.73m² was found in respectively 0.5%, 1.3%, and 6.6% of the subjects. In those with a eGFR below 60ml/min/1.73m², 63.7% had no proteinuria while 23.8% had mPR, 12.5% had MPR. 90% of them are categorized in the CKD3 class.

Glomerular Filtration Rate (MDRD)

		N	p5*	p25*	p50*	p75*	p95*	GFR<60
MALE	Total:	5122						3,0%
	age 26-29	466	83,7	102,1	114,4	129,8	165,1	0,4%
	age 30-39	1086	77,6	94,9	107,8	121,1	158,3	0,7%
	age 40-49	1026	73,5	89,1	101,9	116,3	143,3	0,9%
	age 50-59	1525	66,2	83,4	95,7	110,4	136,3	2,6%
	age 60-69	1019	51,1	73,8	87,3	99,8	122,9	9,1%
FEMALE	Total:	5402						2,5%
	age 26-29	411	87,9	104,6	118,3	134,5	176,0	0,0%
	age 30-39	1197	78,5	98,7	112,1	128,3	160,6	0,5%
	age 40-49	1393	73,1	89,6	102,2	117,1	150,4	0,6%
	age 50-59	1445	64,6	84,3	96,9	110,6	138,8	3,2%
	age 60-69	956	55,0	74,5	89,0	104,2	131,0	7,7%

* 5th, 25th, 50th, 75th and 95th percentile of the distribution within the gender and age category

Conclusions: The age and gender specific reference values of the eGFR in the Moroccan population is not substantially different from values found in other populations except that the median eGFR values are higher. 2/3 of the subjects with eGFR <60ml/min/1.73m² have no mPR nor MPR, and most likely not related to any form of renal disease. Follow up of MaReMar over the next 5 yrs will help in the unraveling of the clinical relevance of the KDOQI staging of CKD.

Funding: Government Support - Non-U.S.

SA-PO256

Decline in Kidney Function in the General Population: Results from the Reykjavik Study Anna Andrea Kjeld,[1] Runolfur Palsson,[1,2] Thor Aspelund,[1,3] Hrefna Gudmundsdottir,[1,2] Margret B. Andresdottir,[1] Vilmundur Gudnason,[1,3] Olafur S. Indridason.[2] *[1]Faculty of Medicine, University of Iceland; [2]Division of Nephrology, Landspitali - National University Hospital of Iceland; [3]Icelandic Heart Association, Reykjavik, Iceland.*

Background: The purpose of this study was to examine the change in kidney function over time among individuals with and without chronic kidney disease (CKD).

Methods: For subjects aged below 75 years who participated the Reykjavik Study, between 1967 and 1995, we retrieved all available measurements of serum creatinine from entry into the study until March 2011. Estimates of glomerular filtration rate (eGFR) were obtained using the MDRD Study equation and the slope of the change in eGFR in mL/min/1.73 m²/10 years calculated. Regression analysis was used to assess the relationship between risk factors (history of hypertension, diabetes and smoking, and measured blood pressure and cholesterol levels) and eGFR slope.

Results: Of 17,748 subjects with baseline data, 13,038 (73.4%) had at least one follow-up SCr value, of whom 5,818 (41.6%) were men. At baseline, 51.8% had hypertension, 3.0% had diabetes and 43.3% were smokers. Median (interquartile range) follow-up time was 29.4 (23.5-35.7) years.

Slope of eGFR in subgroups based on proteinuria and eGFR

Kidney function		≥ 60 mL/min/1.73 m²		< 60 mL/min/1.73 m²	
		No proteinuria	Proteinuria	No proteinuria	Proteinuria
Men	N	5418	133	238	29
	Slope*	-4.8 (-5.0 - -4.6)	-9.4 (-11.4 - -7.3)	-0.9 (-2.5 - 0.7)	-9.3 (-17.5 - -4.8)
Women	N	6347	48	806	19
	Slope	-3.7 (-3.9 - -3.6)	-8.1 (-11.4 - -4.8)	-0.4 (-1.2 - 0.3)	-7.6 (-14.2 - -1.1)

*in mL/min/1.73 m²/10 years (95% confidence interval)

The slope was steeper among diabetics and those with hypertension (P<0.05), and was associated with measured systolic blood pressure and smoking in both genders (P≤0.01). In men, higher cholesterol also associated with a steeper slope (P=0.006).

Conclusions: Our preliminary analysis indicates that the decline in kidney function is slow in this population-based cohort but is aggravated by proteinuria and traditional CVD risk factors. Smoking cessation and careful treatment of hypertension, diabetes and hypercholesterolemia might delay the progression of CKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO257

Overview of BC Patients with Chronic Kidney Disease Registered from 2003 to 2007 Gabriela Espino-Hernandez,[1] Lee Er,[1] Ognjenka Djurdjev,[1] Monica C. Beaulieu,[1,2] Adeera Levin.[1,2] *[1]BC Renal Agency, Vancouver, BC, Canada; [2]Division of Nephrology, UBC, Vancouver, BC, Canada.*

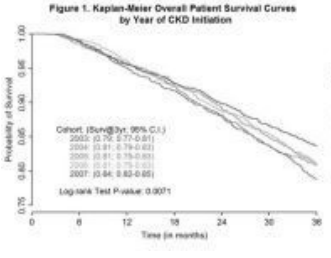
Background: British Columbia (BC) is the only Canadian province with formal registration and full data collection of patients with Chronic Kidney Disease (CKD) followed by nephrologists. This complete f/u permits accurate assessment of outcomes from 2003.

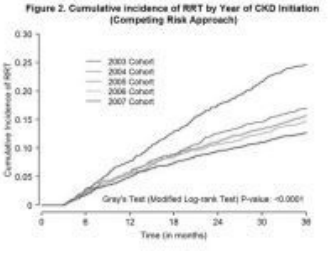
Methods: We created a cohort of 10,111 CKD patients registered in the provincial database from 2003 to 2007, with at least 3 yrs of f/u. Outcomes of interest: patient survival and Renal Replacement Therapy (RRT) event rate by year of CKD initiation, at 3 yrs, as well as eGFR at dialysis start.

Results: About 2,022 new CKD patients were registered each year. Cohorts remained similar: mean age 68 yrs, 44% female, 66% Caucasian, 38% diabetics, and 79% hypertensive. However, mean eGFR at entry went from 33mL/min in 2003 to 36mL/min in 2007. From the patients who reached RRT, 95% started dialysis, and 5% underwent pre-emptive transplant. Selection of PD vs HD as RRT remained at about 26% vs 74%, respectively.

The log-rank test indicated patient survival curves varied by CKD year (Figure 1). Based on a Cox proportional hazards model, after adjusting for age, gender, race, eGFR, uACR, and dialysis, the estimated hazard ratios went from 96% in 2004 to 76% in 2007. Figure 2 presents the cumulative incidence curves of RRT by year of CKD initiation, accounting for death as a competing risk factor. The Gray's test indicated differences in the RRT incident rate by CKD year. The adjusted hazard ratios decreased from 81% in 2004 to 65% in 2007.

Conclusions: This unique cohort serves the basis for ongoing evaluation of outcomes for clinical, CQI, and research purposes. Over time, there appears to be an improvement in the overall patient survival, and a delay in the progression of the disease. Reasons for this improvement warrant further study, and are currently under investigation.





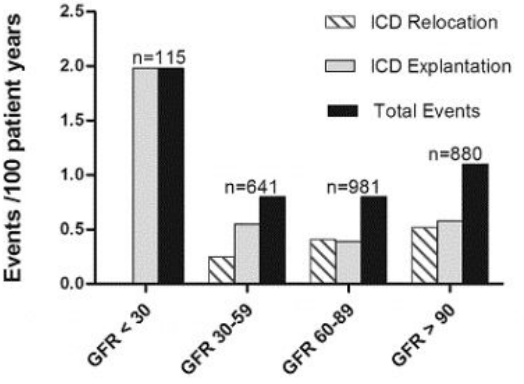
SA-PO258

Severe Renal Disease Increases the Risk for Device Related Complications in Patients with an Implantable Cardioverter Defibrillator Maurits S. Buiten,[1] Mihaly K. De Bie,[1] Joep Thijssen,[1] Joris I. Rotmans,[2] J. H.M. Groeneveld,[3] Ton J. Rabelink,[2] J. W. Jukema,[1] Martin J. Schalij,[1] L. Van Erven.[1] *[1]Cardiology, Leiden University Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [3]Nephrology, Medisch Centrum Haaglanden, Den Haag, Netherlands.*

Background: The implantable cardioverter defibrillator (ICD) has become a well-accepted therapy for the prevention of sudden cardiac death. However, serious comorbidities are influencing the ICD's positive effects. One of the most important comorbidities is renal disease, which might increase the risk for ICD related complications. In this study, the association between kidney function and the occurrence of ICD related complications was assessed.

Methods: All patients implanted with an ICD in the LUMC, the Netherlands, between '96 and 2011 were included. Patients were stratified according to the American Kidney Foundation stages of renal disease: GFR ≤30, 30-59, 60-89 or GFR≥90 mL/min. During follow-up all ICD explantations and relocations were monitored. Event rates were noted per 100 pt years.

Results: During follow-up (48±39 months) 3148 patients received an ICD (79% male, 62±13 yrs). In 97 (3%) patients, at least one ICD related complication occurred (43 relocations, 54 extractions). When stratified for GFR, the event rate was 2.0 per 100pt/years (95% CI 0.8–4.4) in patients with severe kidney disease (GFR≤30) and approximately 0.9(95% CI 0.7–1.1) in all other patients. When comparing severe kidney disease to patients with a GFR>30, the calculated rate ratios are 2.2 (95% CI 0.9–5.4, P=0.09) for all monitored complications and 4.0(95% CI 1.6–10.1, P=0.003) for ICD explantations.



Conclusions: This study demonstrates that severe kidney disease, is associated with an event rate for ICD related complications almost two times that of patients with a GFR≥30 mL/min.

SA-PO259

Blood Manganese Level, Diabetes and Chronic Kidney Disease Eun Sil Koh, Sungjin Chung, Hyun Chul Whang, Seok Joon Shin, Cheol Whee Park, Yong-Soo Kim, Yoon-Sik Chang. *Department of Internal Medicine, The Catholic University of Korea College of Medicine, Seoul, Korea.*

Background: The purpose of this study is to evaluate the association between blood manganese level and the prevalence of chronic diseases in Korean population.

Methods: Based on the Korean National Health and Nutrition Examination Survey (KNAHNES), a cross sectional study was done to analyze the association between blood manganese level and five chronic diseases, such as chronic kidney disease, hypertension, diabetes, ischemic heart disease and stroke. The study comprised 3996 participants aged ≥ 20, whose blood manganese had been measured. We defined renal dysfunction as eGFR < 65 ml/min/1.73m² and diabetes as fasting blood glucose ≥ 126 mg/dL.

Results: Serum manganese level was significantly decreased in renal dysfunction group than in normal renal function group (1.28±0.03 μg/L vs 1.35±0.01 μg/L, respectively; p=0.036). The level of serum manganese was also significantly lower in diabetes group than in non-diabetes group (1.26±0.02 vs 1.35±0.01 μg/L, respectively; p=0.001). There was no significant association with blood manganese level according to the presence of hypertension, ischemic heart disease or stroke. As results of multivariate logistic regression analysis adjusting for age, sex, and body mass index, the odds ratio for renal dysfunction was 0.589 (95% CI 0.394-0.881) and the odds ratio for diabetes was 0.649 (95% CI 0.478-0.879) when comparing the high quartile Q_{2-4} with the low quartile Q_1 of blood manganese level. The prevalence of renal dysfunction was 8.6% in low quartile Q_1, compared with 4.6% in high quartile Q_{2-4} (p=0.0148). Similarly, the prevalence of diabetes was 10.7% in low quartile but 7.4% in high quartile (p=0.0045).

Conclusions: The prevalence of renal dysfunction and diabetes was decreased in subjects with high blood manganese level, suggesting that blood manganese may have some role in renal function and glucose homeostasis.

SA-PO260

Association of Non-Alcoholic Fatty Liver Disease with Chronic Kidney Disease in the U.S. Population: Data from the National Health & Nutrition Examination Survey Jeffrey C. Sirota,[1] Kim Mcfann,[1] Giovanni Targher,[2] M. Chonchol,[1] Diana I. Jalal.[1] *[1]Division of Renal Diseases & Hypertension, University of Colorado, Aurora, CO; [2]Section of Endocrinology and Metabolism, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy.*

Background: Chronic kidney disease (CKD) is independently associated with non-alcoholic fatty liver disease (NAFLD) in other countries. This relationship has not been evaluated in the United States, where racial, ethnic, and dietary factors differ. We hypothesized that greater severity of NAFLD is associated with higher odds of CKD.

Methods: Cross-sectional analysis of 11,368 U.S. adult participants of the National Health and Nutrition Examination Survey 1988-1994. NAFLD, the exposure variable, was defined by ultrasonographic detection of steatosis in the absence of other liver diseases. CKD, the outcome, was defined as an estimated glomerular filtration rate (eGFR, by Modification of Diet in Renal Disease calculation) of ≤ 60mL/min/1.73m^2 or the presence of albuminuria in subjects with an eGFR of >60mL/min/1.73m^2.

Results: 2,891 (25.4%) patients in the cohort had CKD. NAFLD was associated with CKD in univariate regression analysis (OR 1.47, 95% C.I. 1.29-1.67, p <0.0001). Adjustment for demographics and metabolic syndrome components attenuated this relationship (adjusted OR 1.04, 95% C.I. 0.88-1.23, p=0.64). Moderate and severe NAFLD were increasingly associated with prevalent CKD in unadjusted analysis only.

Odds ratios for CKD (95% CI)

	mild NAFLD	moderate NAFLD	severe NAFLD
unadjusted model	1.11 (0.92-1.35, p=0.28)	1.64 (1.38-1.94, p<0.0001)	1.96 (1.53-2.51, p<0.0001)
adjusted model*	1.02 (0.81-1.27, p=0.89)	1.01 (0.82-1.24, p=0.92)	1.17 (0.83-1.66, p=0.38)

*adjusted for age, race, gender, history of hypertension or diabetes, systolic blood pressure, waist circumference, triglycerides, HDL-C, and HOMA-IR

Conclusions: Ultrasound-diagnosed NAFLD is associated with prevalent CKD among US adults in univariate analysis. This association is most likely due to the shared cardio-metabolic risk factors, as the relation is attenuated after adjusting for components of metabolic syndrome.

SA-PO261

Creatinine Excretion Rate and Mortality in Type 2 Diabetes and Nephropathy Arjan J. Kwakernaak,[1] Steef Jasper Sinkeler,[1] Stephan J.L. Bakker,[1] Gerjan Navis,[1] Hiddo Jan Lambers Heerspink,[2] Dick de Zeeuw.[2] *[1]Nephrology, University Medical Center Groningen, Groningen, Netherlands; [2]Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands.*

Background: Creatinine excretion rate (CER) is associated with premature mortality in the general population and renal transplant recipients, possibly due to its close relationship with muscle mass. Whether CER has prognostic impact in diabetes is unknown, we therefore investigated whether CER is a determinant of mortality in diabetic nephropathy.

Methods: The RENAAL and IDNT trial databases were used for analysis. In total, 1.872 subjects (58% of the overall cohort) with type 2 diabetes and nephropathy with 24-hour urinary creatinine excretion data available were included. Primary endpoint for this analysis was all-cause mortality.

Results: Mean age was 60±8 years and median CER was 1400 mg/day (range 366–3400 mg/day). Body Surface Area, hemoglobin levels, non-Caucasian race, and albuminuria were independent, positive determinants of CER, while age, female gender, smoking and diabetes duration were independent negative determinants of CER. After a median follow-up of 36 (29-45) months, 300 subjects died. A Kaplan Meier analysis using sex-stratified tertiles of CER showed that each tertile of lower CER was associated with increased risk for mortality. In a multivariable Cox regression analysis, lower CER (as a continuous variable) was associated with higher risk for mortality (HR=0.45 (0.34 – 0.59), P<0.001) independent of other patient characteristics including age, gender and body weight. In sensitivity analyses with exclusion of subjects deviating more than 50% or 30% from estimated CER, as calculated by anthropometric data, the association of CER with mortality did not change materially.

Conclusions: Lower CER was strongly associated with increased mortality in patients with type 2 diabetes and nephropathy. As CER can be considered a proxy for muscle mass, this places renewed emphasis on physical condition in this population.

SA-PO262

Renal Artery Embolization, Nephrectomy and Repeated Procedures in Patients with Renal Angiomyolipoma: A US National Retrospective Cohort Study Hearns Charles,[1] Peter Sun,[2] Zhimei Liu,[3] Judith A. Prestifilippo,[3] John C. Hulbert,[4] John J. Bissler.[5] *[1]NYU Langone Medical Center; [2]Kailo Research Group; [3]Novartis Pharmaceuticals Corporation; [4]Urologic Physicians; [5]University of Cincinnati.*

Background: This study aimed to estimate the prevalence rates of newly observed and repeated renal artery embolization (RAE), partial and complete nephrectomy, in patients with sporadic renal angiomyolipoma (AML) or tuberous sclerosis complex (TSC) related AML in the United States.

Methods: We used a retrospective cohort study design and three US national health claims databases (2000-2010). Inclusion criteria were:1) diagnosis of sporadic AML (ICD9-CM: 223.0 but no 759.5) and AML associated with TSC (ICD9-CM: 223.0 and 759.5); 2) minimum data availability of one year prior to and 5 years following newly observed AML diagnosis. Prevalence rates of the first and repeated RAE, partial and complete nephrectomy for each cohort (TSC AML versus sporadic AML) were estimated. Cross cohort differences in these prevalence rates were examined using two-sample t-tests for proportions.

Results: Patients (N=2,813) meeting the inclusion criteria had a mean age of 51.5 years at the newly observed AML diagnosis; 47.1% were male. Within 5 years after the newly observed AML diagnosis, more TSC AML patients than sporadic AML had embolization (90.7% versus 30.5%, P<0.05), partial nephrectomy (68.3% versus 21.4%, P<0.05), and complete nephrectomy (77.2% versus 68.7%, P<0.05). Rate of repeated embolization was higher for sporadic AML (10.5% versus 2.6% P<0.05,) but rates of repeated partial nephrectomy (3.2% versus 2.0%) and complete nephrectomy (1.5% versus 1.4%) were similar between the two groups. In the TSC AML subgroup, more than half of the patients had at least two procedures (embolization, partial nephrectomy or complete nephrectomy) within 5 years after the newly observed AML diagnosis.

Conclusions: The prevalence rates of RAE and nephrectomy were relatively high and differed between TSC AML and sporadic AML patients. The prevalence rates of repeated RAE and nephrectomy also differed between the two groups. Future research to understand their impact on clinical outcomes is needed.

Funding: Pharmaceutical Company Support - Novartis Pharmceuticals Corporation

SA-PO263

Predictive Value of the HAS-BLED Score in Patients with Atrial Ffibrillation and Chronic Kidney Disease Judith Kooiman,[1] Koen van Beers,[1] Bas Spaans,[1] Jonna R. Bank,[1] Gregory Y. Lip,[2] Felix Van der Meer,[1] Suzanne Cannegieter,[1] Ton J. Rabelink,[1] Menno V. Huisman.[1] *[1]Leiden University Medical Center, Netherlands; [2]City Hospital, University of Birmingham for Cardiovascular Sciences, United Kingdom.*

Background: The HAS-BLED score enables a risk estimate of major bleeding in patients with atrial fibrillation (AF) on vitamin K-antagonist (VKA) treatment, but has never been validated in patients with chronic kidney disease (CKD). We analyzed the predictive value of the HAS-BLED score in CKD compared with non-CKD patients.

Methods: Medical records of 416 CKD(GFR0-30 ml/min, 30-60 ml/min) and 300 non-CKD patients starting VKA treatment for AF between 1997-2005 were searched for items on the HAS-BLED score (hypertension; renal or liver disease; stroke; major bleeding or anemia; labile INR, (time within therapeutic range<60%), age>65 years; use of NSAIDs, anti-platelet therapy or alcohol) and major bleeding events. Areas under the curves (AUC) of the receiver operating characteristic (ROC) were calculated for the total population and CKD patients.

Results: Mean HAS-BLED score in CKD patients was 3.1 versus 2.6 in non-CKD patients(P<0.01). Major bleeding occurred in 115/716(6.1%,95%CI12.8-19.9%) patients. AUC of the ROC analysis in the total population was 0.50(95%CI0.44-0.56); 0.53(95%CI0.43-0.62) in patients with a GFR30-60 ml/min, and 0.35(95%CI0.21-0.49) in patients with a GFR<30 ml/min. In a Cox regression analysis performed in all patients, renal impairment, labile INR and age>65 years were predictive of major bleeding, hazard ratios 2.5(95%CI1.2-5.2), 2.2(95%CI1.2-3.7) and 5.2(95%CI1.6-16.8), respectively.

Conclusions: Performance of the HAS-BLED score was limited in the total population and further reduced in patients with a GFR<30ml/min. Further research is needed before the HAS-BLED score can be used in CKD patients.

Table 1

	N	%
Mean age(SD)	74	10
Male gender	403	56
Mean duration VKA therapy in years(SD)	3.8	3.3
Renal failure*	100	14
Hypertension	457	64
Liver disease	3	0.4
Prior stroke	140	20
Prior major bleeding or anemia	222	31
Labile INR	357	50
Age>65 years	598	84
Alcohol use	118	17
Use of antiplatelet therapy	81	11

*Serum creatinine>200µmol/L, on dialysis or after renal transplantation

Funding: Government Support - Non-U.S.

SA-PO264

Incident Atrial Fibrillation and Risk of End-Stage Renal Disease in Adults with Chronic Kidney Disease Nisha Bansal,[1] Chi-yuan Hsu,[1] Gregory Marcus,[1] Alan S. Go.[1,2] *[1]UCSF; [2]KPNC.*

Background: Atrial fibrillation (AF) frequently occurs in patients with chronic kidney disease (CKD). However the long-term impact of AF on the risk of adverse renal outcomes is unknown. In a large, diverse community-based population with CKD, we examined the association between incident AF and risk of end-stage renal disease (ESRD).

Methods: We studied adults with CKD (defined as $eGFR_{CKD-EPI}$ <60 ml/min/1.73 m^2) enrolled in Kaiser Permanente Northern California (identified between 2002-2010) who did not have prior ESRD or previously documented AF. Incident AF was identified using primary hospital discharge diagnoses and/or two or more outpatient visits for AF based on validated ICD-9 codes. Incident ESRD was ascertained from a healthplan registry for chronic dialysis and renal transplant. We identified demographic characteristics, comorbidity and medication use based on validated algorithms from healthplan databases.

Results: Among 206,229 adults with CKD, 16,463 developed incident AF. During a mean follow-up of 5.1± 2.5 years, 343 cases of ESRD occurred after development of incident AF (73 per 1000 person-years) compared with 6495 cases of ESRD during periods without AF (64 per 1000 person-years, P<0.001). In multivariable extended Cox regression analysis, incident AF was associated with a twofold increase in rate of ESRD(Table).

Conclusions: Incident AF independently increases the risk of developing ESRD in adults with CKD.

Table. Association between incident AF and subsequent risk of ESRD among adults with CKD

	HR (95% CI)
Unadjusted	1.16 (1.04-1.29)
Adjusted for participant characteristics*	1.96 (1.75-2.19)
Adjusted for participant characteristics, cardiovascular risk factors and medication use*†	2.03 (1.75-2.34)

* Age, sex, race, income and educational attainment. † eGFR, albuminuria, hemoglobin, diabetes, hypertension, coronary heart disease, ischemic stroke, transient ischemic attack, heart failure, peripheral arterial disease, dyslipidemia, chronic lung disease, chronic liver disease, hyperthyroidism and medication use (β-blockers, ACE inhibitors/ARBs, calcium channel blockers, diuretics, statins, other lipid lowering agents, warfarin, antiplatelet agents)

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-PO265

Kidney Protection by Anti-β1-Adrenergic Receptor Autoantibodies in CKD Patients Masaya Yamato,[1] Keiko Yasuda,[2] Koichi Sasaki,[1] Terumasa Hayashi,[1] Hiromi Rakugi,[2] Yoshitaka Isaka.[2] *[1]Nephrology, Rinku General Medical Center, Izumisano, Japan; [2]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Japan.*

Background: It has been reported that autoantibodies against the β1-adrenergic receptor (β1AR) induce progressive development of DCM. Recently, the existence of autoantibodies against the β1AR was reported in rats with adenine-induced CRF. Therefore, we examined clinical significance of anti-β1AR antibodies in CKD patients.

Methods: We prospectively enrolled 258 patients (males 71.3% and mean age 66.4 years) with CKD stage3-5 not on dialysis, from a single department of nephrology between February 2005 and September 2011. Mean estimated glomerular filtration rate (eGFR) was 23.5 mL/min/1.73m^2. Mean left ventricular ejection fraction (EF) was 62.5%. Among 111 patients (43.0%), the primary cause of CKD was diabetic nephropathy. Circulating anti-β1AR autoantibodies were detected in 127 patients (49.2%) by ELISA. Relationships between autoantibodies and kidney end point (defined as doubling of serum creatinine or initiation of maintenance dialysis) were measured using Kaplan-Meier analysis and Cox models for case-mix and laboratory variables.

Results: During a median follow-up period (1.73 years, IQR 0.63-3.37), 137 patients (53.1%) reached the kidney end point. The value of anti-β1AR antibodies was not correlated with EF. Kaplan-Meier analysis revealed that autoantibody-negative patients more frequently reached the kidney end point than autoantibody-positive patients (Log-rank test P=0.026). After adjustment for age, gender, diabetes mellitus, systolic/diastolic blood pressure, EF, eGFR, Hb, Alb, Ca, IP, iPTH, urinary protein, use of RAS inhibitors and use of β-blockers, multivariable Cox regression analysis identified autoantibody-negative as an independent predictor of kidney end point (adjusted HR 1.64 [95%CI, 1.15-2.34], P=0.006).

Conclusions: These results suggest that anti-β1AR autoantibodies might play a self-protective role in CKD progression. Our data support the hypothesis that these autoantibodies shifted the renal β1AR into the agonist-coupled high-affinity state, thereby leading to these receptors down-regulation and ultimately kidney protection.

SA-PO266

Meta-Analysis of over 130,000 Individuals of European Ancestry Reveals New Loci Associated with Creatinine-Based Glomerular Filtration Rate in the CKDGen Consortium Matthias Olden. *Framingham Heart Study, National Heart, Lung, and Blood Institute, Framingham, MA.*

Background: Reduced levels of glomerular filtration rate (GFR) and chronic kidney disease are major health issues associated with end stage renal disease and cardiovascular mortality and morbidity. We conducted a large-scale meta-analysis of genome-wide association studies (GWAS) for creatinine-based GFR (eGFRcrea) with the goal of uncovering additional new loci for renal function.

Methods: A total of 133,728 individuals of European descent from 50 population-based studies were included. Each participating study performed a GWAS of ~2.5 million single nucleotide polymorphisms on log transformed eGFRcrea, estimated using the 4-variable Modification of Diet in Renal Disease formula, adjusting for age and sex. The results were then meta-analyzed using an inverse-variance weighted fixed effects model. Statistical significance threshold was set to 5E-8.

Results: We identified a total of 46 loci for eGFRcrea that exceeded genome-wide significance, with 16 loci being novel. The strongest signal was rs13329952 near *UMOD* (P-value=9E-44), which has been previously reported. The strongest novel signals were located in or near *CYP24A1* locus at 20q13 (P-value=3E-11) and at the *MANBA* locus at 4q22-q25 (P-value=5E-10). *CYP24A1* encodes a protein involved in vitamin D metabolism, synthesis of cholesterol and other lipids, while mutations in *MANBA* are associated with lysosome storage disease. We also confirmed 30 loci previously identified in association with eGFRcrea.

Conclusions: Large-scale meta-analyses continue to uncover new loci for renal function. While our findings require independent replication, they advance our understanding of the biologic mechanisms of kidney function.

Funding: Other NIH Support - Contract No. N01-HC-25195

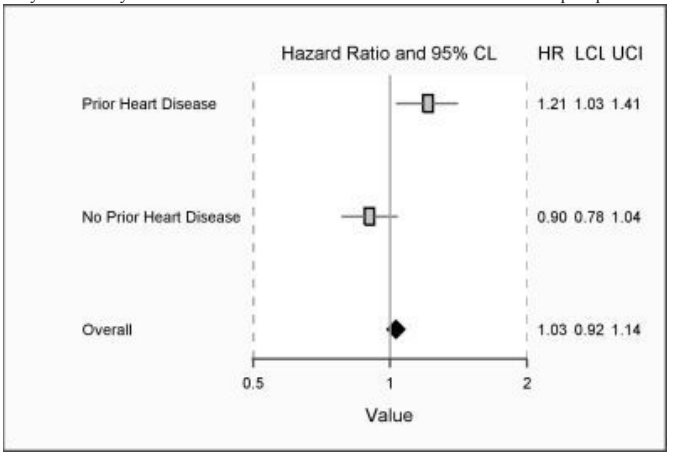
SA-PO267

CKD, Prevalent Cardiovascular Disease, and the Risk of Death among Female Patients Prescribed Bisphosphonates Robert M. Perkins,[1] H. Lester Kirchner,[1] Kunihiro Matsushita,[2] Ion D. Bucaloiu,[1] Evan Norfolk,[1] James E. Hartle.[1] *[1]Geisinger Clinic; [2]Bloomberg School of Public Health.*

Background: Whether bisphosphonates impact cardiovascular morbidity and mortality is controversial. We have previously shown that among female patients with CKD and without clinically manifest cardiovascular disease (CVD), treatment with bisphosphonates is associated with a modest all-cause mortality risk reduction.

Methods: We tested the hypothesis that, among patients with CKD, prevalent CVD (prior MI, CHF, stroke, or TIA) would modify the effect of bisphosphonates on the risk of death. Adult female patients receiving primary care through Geisinger Clinic, an integrated health care system in central PA, between January 1, 2004 and March 31, 2011, with stage 3 or 4 CKD but without a history of advanced malignancy, ESRD, or solid-organ transplantation were eligible for the study. Subjects were followed through September 30, 2011 for the outcome of death. For non-bisphosphonate users at entry, we treated such therapy as a time-dependent exposure, and prevalent (prior to January 1, 2004) CKD status was accounted for using left-truncation in a multivariate-adjusted Cox proportional hazard model. The primary analysis examined the multivariate-adjusted mortality hazard associated with bisphosphonate treatment and its interaction with baseline CVD status.

Results: 6,756 females (2,173 with history of prior CVD) were followed for a median 4.3 years. Nearly 40% of those with and without CVD were treated with bisphosphonates.



Conclusions: Among females with CKD in this cohort, bisphosphonates were associated with increased mortality if CVD pre-existed but not if there was no history of CVD. The test of interaction was significant (interaction p-value = 0.004). Bisphosphonate treatment may increase the risk of death among those with CKD and pre-existing CVD, and warrants further investigation.

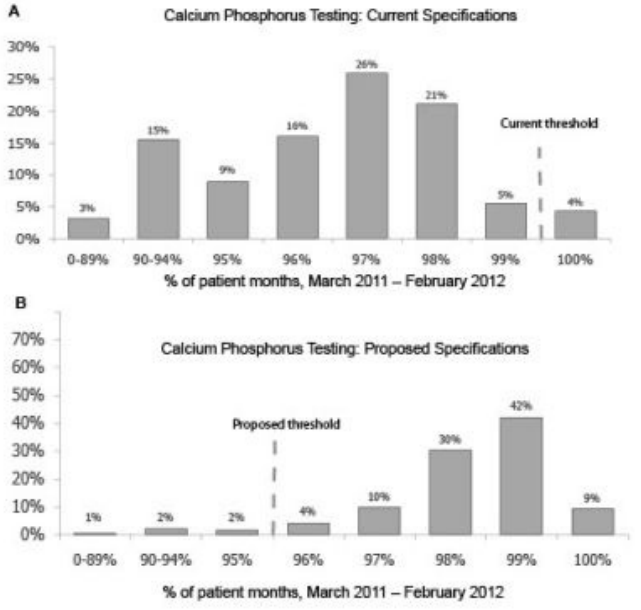
SA-PO268

Analysis of the 2014 Quality Improvement Program Reporting Measures for Calcium Phosphate in Dialysis Patients Michael Phillips, Andrew Barba, Mahesh Krishnan, Allen R. Nissenson. *DaVita Inc., Denver, CO.*

Background: The aim of the End-Stage Renal Disease (ESRD) Quality Improvement Program (QIP) is to promote patient health by encouraging renal dialysis facilities to deliver high-quality patient care. This incentive program will result in a payment reduction up to 2% if a facility does not meet or exceed the minimum Total Performance Score as set forth by CMS. For the 2014 QIP, calcium and phosphorus testing contribute 3.3% of the total score. Currently, a facility must test every Medicare patient in every month to receive the points associated with this metric. The revised specifications for the calcium and phosphorus metric presented here will report the percentage of eligible months in which ESRD Medicare patients were tested for calcium and phosphorus. Each provider/facility would be required to test 95% of eligible patient-months to attest to completion of the measure.

Methods: The measurement duration is a 12-month period. Under the proposed specifications, only ESRD patients with at least 4 months of eligible Medicare claims are included. Claims are included if at least 7 hemodialysis equivalent treatments were reported. Calcium and phosphorus lab results were extracted from the central laboratories of a large dialysis organization to determine which patients received a test in each of the 12 months being measured. The numerator for the facility measure was eligible Medicare patient months with a lab result. The denominator for the facility measure was total eligible Medicare patient months.

Results:



Conclusions: Only 4% of facilities would be able to attest to the current standard, as seen in Figure 1A. Ninety five percent of facilities would be able to attest to the proposed standard, as seen in Figure 1B. The proposed standard is designed to align with clinical practice and the inclusion criteria of other QIP metrics.

Funding: Pharmaceutical Company Support - DaVita Inc.

SA-PO269

The Correlation between Pathological Findings and Independent Clinical Prognostic Parameters in IgA Nephropathy Ryuta Sato,[1] Kensuke Joh,[2] Hideki Wakui,[3] Shigeaki Muto,[1] Eiji Kusano.[1] *[1]Division of Nephrology, Department of Internal Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan; [2]Department of Pathology, Sendai Shakaihoken Hospital, Sendai, Miyagi, Japan; [3]Department of Hematology, Nephrology and Rheumatology, Akita University Graduate School of Medicine, Akita, Japan.*

Background: Pathological prognostic findings of IgA nephropathy (IgAN) were shown in Oxford study. However, clinical parameters were not included. Therefore, the present study was to elucidate the correlation between pathological findings and clinical parameters such as proteinuria (PU), estimated glomerular filtration rate (eGFR) and mean atrial blood pressure (MAP) in IgAN.

Methods: 203 patients with IgAN were collected since 1981 to 2001 in Akita University Hospital. Clinical data and renal biopsy specimens were obtained from these patinets. Patients were 47% male, median 42 y/o age, and median follow up period was 11.7 years. Progression of end-stage renal disease or 50% drop in eGFR was defined as a first endpoint in multivariate Cox regression analysis. Annual decline of eGFR was defined as a dependent variable in multivariate linear regression analysis. Clinical parameters were also analyzed by two regression models. The correlation between pathological findings and clinical parameters such as PU, eGFR and MAP was investigated by multivariate linear regression analysis.

Results: 1g/day≤ PU (HR 2.1, P=0.01), eGFR <60ml/min./1.73m^2(HR 3.2, p<0.0001) and 100mmHg≤ MAP (HR 1.8, p=0.02) were shown to be independent risks in multivariate Cox regression analysis. 1g/day≤ PU significantly promoted decline of eGFR in multivariate linear regression analysis. High PU values were significantly correlated with endocapillary hypercellularity (ENDO) and acute glomerular lesions (AL: cellular crescent, fibro-cellular crescent or tuft necrosis). There were positive correlations among the drop of eGFR, the increment of MAP and the severity of tubular atrophy/interstitial fibrosis (TA/IF).

Conclusions: Our study indicated that ENDO, AL and TA/IF were potential risk factors for the disease progression of IgAN since these findings strongly correlated with the changes of PU, eGFR and MAP.

SA-PO270

10-Year Incidence of Tuberculosis in Chronic Kidney Disease Patients of South Asian Origin in Lancashire Laurence R. Solomon,[1] John S. Cheesbrough,[3] Reuben Roy,[1] Kate Platt,[2] Sharmila Bandyopadhay.[1] *[1]Renal Unit, Lancashire Teaching Hospitals, Preston, United Kingdom; [2]Health Protection Agency, Chorley, United Kingdom; [3]Dept Microbiology, Lancashire Teaching Hospitals, United Kingdom.*

Background: Patients with chronic kidney disease (CKD) on renal replacement therapy (RRT) have a high risk of active tuberculosis (TB), but it is unknown whether prophylaxis of at risk patients is warranted. In the UK, migrants from South Asia (SA) have high incidences of TB and RRT. All cases of TB are notified to the Health Protection Agency (HPA). Our aim was to determine the incidence of tuberculosis in CKD and whether prophylaxis in SA patients is justified.

Methods: Names of all patients on our renal unit database up to 2012 were correlated with TB notifications to the HPA between 1998 and 2010. The database contained 13579 patients and all 2166 patients who were on or started RRT from a population of 1.5 million in Lancashire. SA patients were identified by declared ethnicity or typical name. Dates of presentation, start of RRT and TB notification were noted.

Results: 199 SA patients received RRT, of whom 27 were notified with TB. 170 received dialysis only, 22 of whom developed TB. 1 patient was diagnosed post-mortem and 2 within 3 months of death. A cumulative incidence function indicated that 10% of patients on dialysis for 5 years developed TB. 29 received a kidney transplant. 3/25 (12%) patients who received prophylaxis with isoniazid after renal transplant were notified compared to 2/4 (50%) patients who did not receive this (P=0.07).

Conclusions: Most cases of TB in UK RRT patients occur in South Asians. The data indicate a NNT of 10 (95% CI 6, 15) for fully effective prophylaxis of TB at the onset of RRT to prevent one case in 5 years. This greatly exceeds the risk of isoniazid hepatitis. Treatment might have avoided the need for prophylaxis in a further 14% of patients who had transplants reducing risk of drug interaction. Isonaizid prophylaxis substantially reduced the incidence but did not eliminate TB, but newer drug regimes may have a higher therapeutic index. Prophylaxis for TB is indicated in SA patients starting RRT and may be warranted in other high risk populations.

Funding: Clinical Revenue Support

SA-PO271

The Size of Palatine Tonsils Cannot Decide the Indication of Tonsillectomy for IgA Nephropathy Mitsuhiro Sato,[1] Mika Adachi,[2] Hideyuki Kosukegawa,[1] Toshinobu Sato,[1] Yoshio Taguma.[1] *[1]Nephrology, Sendai Shakaihoken Hospital, Sendai, Miyagi, Japan; [2]Otorhinolaryngology, Sendai Shakaihoken Hospital, Sendai, Miyagi, Japan.*

Background: We previously reported clinical remission (CR: disappearance of hematuria and proteinuria) could be obtained by the treatment using tonsillectomy with steroid pulse (TSP) in IgA nephropathy (IgAN, Hotta O et al. Am J Kidney Dis 38, 2001). However, the relationship between the indication of tonsillectomy and the size of palatine tonsils (PT) in patients with IgAN has not yet been reached.

Methods: To clarify the influence of the size of PT on the histopathological findings and the effect of TSP, we performed the retrospective cohort study dividing the patients into two groups according to the weight of their excised PT : (1) hypertrophic group (HG), PT weight≥2.85g (n=30), and (2) non-hypertrophic group (NHG), PT weight<2.85g (n=31). We compared clinical parameters, therapeutic parameters in addition to TSP, histopathological findings of PT, and the endpoints, CR and 50% increase of serum creatinine from baseline, between the two groups.

Results: No significant differences in age, serum creatinine, GFR, proteinuria, hematuria, serum IgA, presence of hypertension, use of anti-plt. drugs, use of ACE-inhibitor, and use of ARB were observed between the two groups. There were also no significant differences in the presence of the macroscopic pusplug on the surface of PT and the distribution of bacterial flora which was detected from the core of the crypt between the two groups. Microscopically, pusplug in the crypt was observed in all specimens. Moreover, the unmatured folliculi and the enlarged interfollicular area that are considered to be specific pathological findings to PT of IgAN were observed in all specimens. In the mean follow-up term of 43months, no significant difference in the incidence of the endpoints was observed between the two groups (HG vs. NHG: CR rates, 83.3 vs. 80.6% ; 50% increase of serum creatinine, 0 vs. 3.2%).

Conclusions: These results indicate that there are the same histopathological findings and the equal treatment effect regardless the hypertrophy; therefore the size of PT cannot decide the indication of tonsillectomy for IgAN.

SA-PO272

The Long-Term Prognosis of IgA Nephropathy Patients Treated by Tonsillectomy Plus Steroid Pulse Therapy Takayuki Toyoyama, Daigo Nakazawa, Junya Yamamoto, Naoko Matsuoka, Akiko Sato, Tasuku Nakagaki, Yasunobu Ishikawa, Sekiya Shibazaki, Saori Nishio, Tatsuya Atsumi. *Department of Medicine 2, Hokkaido University, Sapporo, Japan.*

Background: It has been reported that tonsillectomy plus steroid pulse therapy (T/S) is effective for IgA nephropathy (IgAN). However there is a little report observed for long time. In this study we analyzed long-term renal prognosis and remission rate of IgAN treated by T/S.

Methods: Between 1995 and 2012, we retrospectively followed 108 patients who treated by T/S based on the diagnosis of IgAN by renal biopsy at Hokkaido University hospital. All patients received intravenous methylprednisolone pulses of 0.5 g/day for three consecutive days, followed by oral prednisolone at an initial dosage of 0.5 mg/kg per day. The oral prednisolone was gradually tapered by 5 mg every 2 month, then discontinued by 12~18 month after the initial therapy. We recorded their age, gender, serum-Cre (S-Cre), proteinuria, hematuria, blood pressure, and histological severity at initial therapy. We evaluated the remission rate (disappearance of both hematuria and proteinuria) and 8-year renal survival rate ([1] renal death and [2] impaired renal function;1.5 fold increase of S-Cre or S-Cre≥2 mg/dl). And we analyzed the risk factors for impaired renal function by the univariate and multivariate regression analysis.

Results: Patients findings at initial therapy were the following; Age 32 years, S-Cre 0.9 mg/dl, proteinuria 1.31 g/g•Cre (these data show average), men/women 46/62.

Remission rate at 1 year after treatment was 74 %. 8 year survival rate was 97 % ([1]renal death), and 78 % ([2]S-Cr 1.5 fold or ≥2.0 mg/dl). By cox hazard model risk factors for impaired renal function were male, age, histological severity. Furthermore, in recalcitrant cases (at 1 year after treatment) odds risk for impaired renal function was 5.8 fold compared to remission cases.

Conclusions: The long-term prognosis of IgA nephropathy patients treated by tonsillectomy plus steroid pulse therapy was extremely favorable, due to high rate of clinical remission.

SA-PO273

Characteristics and Burden of Renal Angiomyolipomas in Patients with Tuberous Sclerosis Complex: Results of a Patient and Caregiver Survey

Anne M. Rentz,[1] Chris Leo Pashos,[1] Sarrit Kovacs,[1] Zhimei Liu,[2] Corey Pelletier,[2] Judith A. Prestifilippo,[2] Jo Anne Nakagawa,[6] James Wheless,[5] Michael Frost,[3] David W. Dunn.[4] *[1]United BioSource Corporation, Bethesda, MD; [2]Novartis Pharmaceuticals Corporation, East Hanover, NJ; [3]Minnesota Epilepsy Group, PA, St. Paul, MN; [4]Riley Hospital for Children, Indianapolis, IN; [5]Le Bonheur Children's Medical Center, Memphis, TN; [6]TS Alliance, Silver Spring, MD.*

Background: Patients with tuberous sclerosis complex (TSC), a genetic disorder characterized by benign tumor growth in multiple organs, may experience non-cancerous kidney tumors known as renal angiomyolipomas (AML). This analysis aimed to assess the prevalence, presentation, and associated impact of AMLs in patients with TSC in the United States (US).

Methods: An Institutional Review Board-approved Web-based survey of US TSC patients and their caregivers obtained information on prevalence of different TSC manifestations, their treatment and management, and epidemiological, clinical, economic and health-related quality of life (HRQL) burden impact on patients and caregivers. This analysis focused on the prevalence, presentation, and management of AMLs.

Results: The online survey is on-going. Of 380 TSC patients whose data have been collected to date, 59% were women and mean age was 30.4 years (median: 32.5, range: 0-70). Twenty-three percent of patients had been diagnosed with AMLs. Patients with AMLs also experienced skin lesions (75.9%), seizures (74.7%), and cognitive concerns (57.5%). Seventy-five percent of TSC-AML patients were bilaterally affected with lesions on both kidneys. Within the population experiencing involvement of only one kidney, 22.7% had multiple tumors within the affected kidney. Almost three-quarters of AML patients (72.4%) had some type of invasive procedure including but not limited to embolization and nephrectomy.

Conclusions: Initial survey data have demonstrated that TSC-AML is associated with significant epidemiological and clinical burden. The complete survey data will provide further insights on the clinical profile and economic and HRQL impact of TSC-AML.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

SA-PO274

Rosuvastatin Reduces Urinary Protein Excretion in Patients with Chronic Glomerulonephritis Compared to the Pravastatin Use: A Crossover Trial

Hirofumi Ikeda, Yoriko Ura, Masaru Nakayama. *National Kyushu Medical Center Hospital, Division of Nephrology and Clinical Research Institute, Department of Internal Medicine, National Kyushu Medical Center Hospital, Fukuoka City, Fukuoka Pref, Japan.*

Background: Experimental evidence suggests that lipid abnormalities contribute to the progression of kidney disease. However, very few studies have evaluated the effect of statins on the proteinuria reduction in patients with glomerulonephritis. The purpose of this study was to determine the effect of rosuvastatin on the proteinuria reduction in patients with chronic glomerulonephritis compared to the pravastatin use.

Methods: Seventeen stable chronic glomerulonephritis patients with proteinuria (urinary protein excretion (UPE)> 0.2g/gCr) and hypercholesterolemia (LDL-cholesterol(C)>120mg/dl) were randomized to receive rosuvastatin 5mg/ day or pravastatin 10mg/day for 6 months, 4-8 weeks wash-out, and then the opposite drug for 6 months. There were no changes in medications (doses of ACEi/ARB and steroids) and food intake. Serum(s) LDL-C, sHDL-C, sMalondialdehyde-Modified LDL (MDA-LDL), as a marker of oxidized LDL, s-creatinine, and UPE were measured at baseline and after each 6-months treatment.

Results: All patients completed the study. As compared to pravastatin treatment, rosuvastatin significantly decreased %changes in both LDL-C (-17.7% vs. -46.7%, $p<0.05$) and %changes in MDA-LDL (-32.1% vs. -44.2%, $p<0.05$). In contrast, in both treatment groups, there were no significant changes in HDL-C and eGFR throughout the study. Rosuvastatin treatment significantly reduced UPE (1.36+/-1.04 to 1.02+/-0.62 g/gCr, $p<0.05$), whereas pravastatin treatment did not reduce UPE (1.07 +/- 0.72 to1.42 +/- 0.96g/gCr). A modest correlation between %changes in LDL-C and in UPE was also present ($R^2=0.21$, $p<0.05$).

Conclusions: In the present study, rosuvastatin reduces UPE in chronic glomerulonephritis patients, together with a remarked reduction in LDL-C, compared to the pravastatin use. This result suggests that aggressive lipid-lowering therapy with rosuvastatin might be a useful treatment for patients with chronic glomerulonephritis.

Funding: Pharmaceutical Company Support - Astra Zeneca

SA-PO275

Study on the Transcription Factor C/EBPα in Primary Glomerulonephritis

Fang Zhong, Weiming Wang, Hong Ren, Jingyuan Xie, Qiuhua Huang, Nan Chen. *Ruijin Hospital, Shanghai Jiao Tong University, Shanghai, China.*

Background: Early diagnosis of CKD by the biological markers of blood or urine has a high practical value.

Methods: Forty-five patients with primary glomerulonephritis (PG): IgA nephropathy (IgAN, n=15), focal segmental glomerulosclerosis (FSGS, n=15) and membranous nephropathy (MN, n=15) were enrolled. We enrolled patients had proteinuria (> 0.5g / day) and GFR> 15ml/min/1.73m^2. Patients had immunosuppressant before renal biopsies were excluded. 15 healthy subjects were used as a control group. The samples were removal of high abundance proteins using the Multiple Affinity Removal LC Column, desalined, quantitied and marked.

Results: The male:female ratio is 7:8 in all three groups. Average age is 29.6 ± 10 ys, 39.8 ± 13.4 ys and53.2 ± 12.6 ys, 24-hr urine protein output is 1491 ± 752 mg/d, 877 ± 640 mg/d and 3988 ± 2332 mg/d, and Scr is 112 ± 56 μmol/L, 121 ± 50 μmol/L and 91 ± 55 μmol/L in the IgA group, FSGS group and MN group, respectively. 1956 proteins were identified with mass spectra (MS). Compared with controls, 447 differentially expressed protein peaks were found in serum from PG patients. Compared with the controls, 68, 48 and 120 proteins in FSGS, IgAN and MN patients were significantly different respectively. In the expression profile, there are some differences in the expression of specific proteins. Through bioinformatic analysis, we studied the protein modification sites, sub-cellular localization and interactive network of the proteins and one of the target proteins C/EBPα (CAAT/enhancer binding proteins, C/EBP) was identified. We found that serum C/EBPα decreased while urine C/EBPα increased in patients with primary glomerulonephritis, compared with normal control group. Urine C/EBPα level was significantly different between MN group and IgA group ($P<0.05$) by ELISA method.

Conclusions: There are differences in serum protein expression profiles in primary glomerulonephritis compared with normal healthy people; There are also differences among the different protein expression profiles in different types of PG. These findings suggested that C/EBPα might serve as a biomarker of renal inflammation.

SA-PO276

Analysis of the Genomic Profile Associated with the Development of a Vascular Phenotype in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Sandro Rossetti, Maria V. Irazabal, Katharina Hopp, Vickie J. Kubly, Jamie L. Sundsbak, Marie C. Hogan, Vicente E. Torres, Peter C. Harris. *Mayo Clinic, Rochester, MN.*

Background: The development of intracranial aneurysms (IAs) and subarachnoid hemorrhage (SAH) is the most life threatening phenotype in ADPKD. ADPKD patients have a prevalence of IAs of ~8%, with SAH and death occurring in ~6%. Familial clustering and intrafamilial variability of IAs/SAH in ADPKD pedigrees suggests the involvement of genetic factors other than the *PKD1*/*PKD2* germline mutation.

Methods: We identified 30 ADPKD pedigrees where at least one individual developed IAs/SAH. In each pedigree, the severity of the vascular phenotype was graded as a quantitative trait based on clinical and imaging data (presence of familial clustering, rupture, requirement for surgery and IA size). In each proband, we captured exons from 116 genes (RainDance method) encoding proteins involved in the structure/maintenance of the arterial wall, receptors for structural proteins, or candidate genes identified by genome-wide association study (GWAS). Sequencing was performed using the Illumina HiSeq2000 and ultra-rare variants were filtered and classified for likely pathogenicity in each pedigree.

Results: We identified 46 ultra-rare variants in 25 pedigrees in 31 genes. Overall, genes with a structural role (collagens, laminins and elastin) accounted for ~50% of these variants (22/46). We identified likely recessive or dominant hypomorphic pathogenic variants in genes playing key roles in: arterial wall structure (*ELN*, *FBN1*, *LAMA* and *LAMB* genes, *COL4A4*, *COL18A1*), associated with IA syndromes (*COL4A1*, *LAMA5*, *TGFBR2*), mapped by GWAS (*STARD13*), encoding receptors for structural proteins (integrins), and involved in extracellular matrix maintenance (MMP genes). Known pathogenic variants were found in *BCAM*, *COL18A1*, *COL5A1*, and *SERPINA1* in 8 pedigrees.

Conclusions: The finding of ultra-rare variants in mutation characterized *PKD1/PKD2*-IAs patients suggests that the co-inheritance of these variants promotes the development of the vascular phenotype on the background of the inherited ADPKD mutation, hence, working as genetic modifiers of the vascular disease presentation.

Funding: NIDDK Support

SA-PO277

Characterization of the Genomic Origin of PKD1 Gene Conversions in Autosomal Dominant Polycystic Kidney Disease (ADPKD) Sandro Rossetti,[1] Terry J. Watnick,[2] Ellis D. Avner,[3] Akio Koizumi,[4] Peter C. Harris.[1] *[1]Mayo Clinic, Rochester, MN; [2]John Hopkins University, Baltimore, MD; [3]Medical College of Wisconsin, Milwaukee, WI; [4]Kyoto University, Kyoto, Japan.*

Background: ADPKD is caused by mutations at two genes, *PKD1* and *PKD2*. *PKD1* is duplicated on chromosome 16 (*PKD1P1-P6)*. Gene conversions (GCs) transfer sequence variants from segmental duplications into the master gene. GCs have been previously suspected in ADPKD, but their genomic origin and extension have not been characterized.

Methods: We developed a strategy for the genomic characterization of *PKD1* GCs. By deep sequencing locus-specific amplicons (specificity in *PKD1* intron 1, exon 15 and single copy area) using the Illumina HiSeq2000, detailed sequence data are generated. Careful comparison with the *PKD1P1-P6* sequence validates GCs and traces their genomic origin.

Results: We deep sequenced 5 previously suspected GCs identified in ADPKD pedigrees. In pedigree P150, used to develop the methodology, we characterized an 8.5 Kb GC with *PKD1P6* (Rossetti 2012). In pedigree M525, we identified 6 intronic and 3 exonic variants, all matching *PKD1P1* and tracing the GC over 5.8 Kb of contiguous sequence (IVS16-ex23). In pedigrees JHU086 (Watnick 1997) we identified 8 intronic and 5 exonic variants, while in pedigree JHU273 (Watnick 1997) we identified 11 intronic and 6 exonic variants: in both pedigrees these variants were all matching *PKD1P1* and traced the 2 GCs over 6.9 Kb of contiguous sequence (IVS16-IVS24 for both). In pedigree C (Inoue 2002) we identified 3 intronic and 4 exonic variants, all matching *PKD1P6* and tracing the GC over 624 bp of contiguous sequence (IVS22-ex23). These GCs introduce likely disease-causing changes and suggest that *PKD1P1-P6* serve as a reservoir of mutations in ADPKD.

Conclusions: Deep sequencing of ADPKD pedigrees with suspected GCs will help prove that they are genuine GCs, determine their genomic origin and precisely define their extension, thereby emphasizing their disease association. GCs may be an underappreciated cause of *PKD1* mutations, particularly in mutation negative cases, and may be missed due to the locus-specific primers employed to screen the *PKD1* duplicated portion.

Funding: NIDDK Support

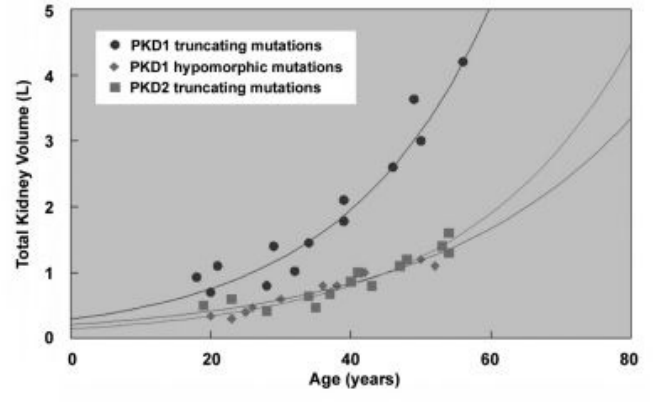
SA-PO278

Hypomorphic *PKD1* Mutations Are a Common Cause of Mild ADPKD Young-Hwan Hwang,[1] John Conklin,[2] Karen Campbell,[1] Ning He,[1] Kairong Wang,[1] Jamie L. Sundsbak,[3] Christina M. Heyer,[3] Masoom Haider,[2] Peter C. Harris,[3] York P. Pei.[1] *[1]Divisions of Nephrology and Genomic Medicine, University Health Network and University of Toronto, Toronto, ON, Canada; [2]Department of Medical Imaging, University Health Network and University of Toronto, Toronto, ON, Canada; [3]Division of Nephrology, Mayo Clinic, Rochester, MN.*

Background: Comprehensive *PKD1* and *PKD2* mutation screening in large clinical cohorts has identified protein-truncating (PT) mutations in ~2/3 of cases (JASN 18:2143-60, 2007). By contrast, non-synonymous mutations/small in-frame deletions comprised most of the remaining cases and their clinical significance is not well defined. Emerging data suggest that some of the latter mutations may function as "hypomorphic alleles" associated with mild ADPKD (Kidney Int 81:412-7, 2012).

Methods: In this single center study, we examined the genotype-phenotype correlation of 228 probands from unrelated families ascertained through a PKD Clinic in Toronto between 2005-2012. All study patients underwent a detailed reveiw of their family history, a standardized protocol to quantify their renal disease severity, and for most, a comprehensive *PKD1* and *PKD2* mutation screen.

Results: Overall, 82% of our probands were of European descent and 46%, male. The mean (±SD) age and eGFR at their 1st clinic visit were 43±13 yrs and 80±28 ml/min, respectively. At least 19% of them had an affected relative who remained renal sufficient or developed ESRD ≥70 years of age. Preliminary results show that 40%, 21%, 27%, and 12% of 151 probands who completed their mutation screen had PT *PKD1* mutations, hyomorphic *PKD1* mutations, PT *PKD2* mutations, and no mutation detected, respectively. Renal disease severity as measured by total kidney volume associated with hypomorphic *PKD1 and* PT *PKD2* mutations appears similar.



Conclusions: Hypomorphic *PKD1* mutations are a common cause of mild ADPKD.

Funding: NIDDK Support

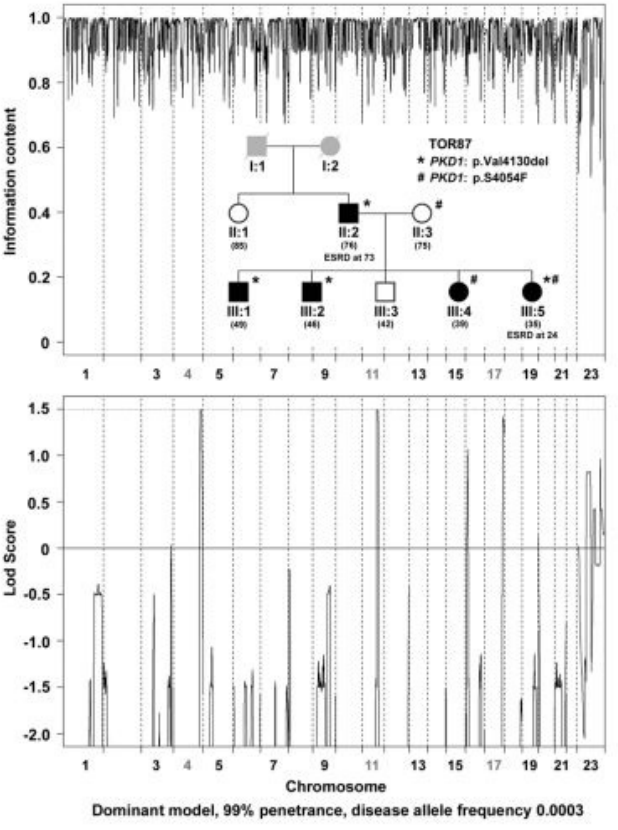
SA-PO279

Complex Genetic Inheritance Underpinning a Family with Putative PKD3 Young-Hwan Hwang,[1] Kairong Wang,[1] Karen Campbell,[1] Alessia C. Borgo,[1] Jamie L. Sundsbak,[2] Christina M. Heyer,[2] Nicole M. Roslin,[3] Andrew D. Paterson,[3] Peter C. Harris,[2] York P. Pei.[1] *[1]Divisions of Nephrology, University Health Network and University of Toronto, Toronto, ON, Canada; [2]Division of Nephrology, Mayo Clinic, Rochester, MN; [3]Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada.*

Background: The documentation of several families unlinked to both *PKD1* and *PKD2* has led to the speculation for the presence of at least one rare gene (PKD3) (Kidney Int 54:1759-61, 1998) whose identification has remained elusive.

Methods: We ascertained a family with ADPKD (TOR87) unlinked to both *PKD1* and *PKD2* in which complications such as DNA mix-up, genotyping errors, and non-paternity had been rigorously excluded. We performed a genome-wide linkage scan in 8 available family members including 5 affected using the Illumina Linkage-24 SNP arrays and screened 2 key affected members (II:2 and III:4) for *PKD1* and *PKD2* mutations.

Results: Our genome scan revealed 3 suggestive linkage signals with multipoint lod-scores ~1.5 on chr. 4, 11, and 17.



Mutation screening in II:2, however, identified a de-novo *PKD1* mutation (p.V4130del) that segregated in 3/4 affected children (III:1, III:2, III:5). By contrast, mutation screening in III:4 identified a 2nd *PKD1* mutation (S4054F; scored 0.988 by Polyphen-2) originating from II:3 (who was assumed to be unaffected at age 75 with normal renal function). With the exception of III:5, all family members affected with one of the *PKD1* mutations had very mild disease. By contrast, III:5, who inherited both mutations *in-trans*, had early and severe disease.

Conclusions: Complex genetic inheritance can give rise to a disease segregation pattern mimicking the presence of a novel gene by genome-wide linkage scan. Interaction of 2 putative hypomorphic *PKD1* mutations *in-trans* can modify renal disease severity in ADPKD.

Funding: NIDDK Support

SA-PO280

Genetic and Phenotypic Characteristics of Subjects with Autosomal Dominant Polycysitic Kidney Disease in the Japanese Mahiro Kurashige,[1,2] Kazushige Hanaoka,[1] Yoshindo Kawaguchi,[1,3] Toshio Hasegawa,[1,3] Minako Imamura,[2] Shiro Maeda,[2] Tatsuo Hosoya.[1] *[1]Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University, School of Medicine, Minato, Tokyo, Japan; [2]Laboratory for Endocrinology and Metabolism, RIKEN Center for Genomic Medicine, Yokohama, Kanagawa, Japan; [3]Department of Medicine, Kanagawa Prefectural Shiomidai Hospital, Yokohama, Kanagawa, Japan.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is common hereditary kidney disease, and most of its heritability could be explained by mutations in two genes, *PKD1* and *PKD2* in Caucasian populations. However, little is known about genetic and/or phenotypic characteristics for Japanese ADPKD patients.

Methods: We screened the entire coding regions for the *PKD1* and *PKD2* by direct sequencing using 100 Japanese ADPKD patients from 91 unrelated families. Regarding novel candidate mutations found in the present ADPKD patients, their frequencies in ~1,500 individuals from Japanese general population were determined by using multiplex PCR invader assay. We also investigated correlation between eGFR decline, mutated genes and plasma arginine vasopressin (ADH) by multiple linear regression analysis.

Results: Among 91 families examined in the present study, we identified 54 mutations within 66 families (72.5%), including 44 *PKD1* mutations (24 truncated, 20 missennse) and 10 *PKD2* mutations (8 truncated, 2 missennse). Twenty-nine out of the 54 mutations have not been reported previously; these novel mutations were not detected in the Japanese general population, except for two missense mutations found in 1 of ~1,500 individuals. Decline of eGFR was significantly slower in patients with *PKD2* mutation than those in patients with *PKD1* mutation or patients who were not detected causal mutation (-1.97, -3.21, -3.55 ml/min/year in *PKD2*, *PKD1*, unknown respectively, p=0.038). Plasma ADH concentrations were significantly associated with eGFR decline in patients with *PKD1* mutation (r=-0.433, p=0.018).

Conclusions: Our present finding suggests that patients with *PKD2* mutation have milder clinical manifestations, and that plasma ADH concentrations affects the eGFR decline in patients with *PKD1* mutation.

SA-PO281

Clinical Course and Genetic Analysis of 39 Japanese Children with Autosomal Recessive Polycystic Kidney Disease Akinori Miyazono,[1] Yuriko Yoneda,[2] Takuya Fujimaru,[1] Mai Sato,[1] Masao Ogura,[1] Koichi Kamei,[1] Hirotomo Saitsu,[2] Shuichi Ito.[1] *[1]National Center for Child Health and Development, Tokyo, Japan; [2]Yokohama City University, Yokohama, Japan.*

Background: Autosomal recessive polycystic kidney disease (ARPKD) is characterized by bilateral kidney cysts and hepatic fibrosis or Caroli's disease. Although *PKHD1* mutations cause ARPKD, the clinical course varies widely in each case. Geographical and ethnic differences regarding mutation sites have not been well investigated.

Methods: Thirty-nine Japanese children clinically diagnosed with ARPKD were enrolled in this study. The clinical course was examined by a questionnaire. Analysis of *PKHD1* mutations was performed by high-resolution melting analysis (HRM) and multiplex ligation-dependent probe amplification (MLPA). Some patients without mutations detected by HRM and MLPA were additionally examined by microarray analysis and next-generation sequencing.

Results: The mean age was 5.8±6.0 years. Fifteen patients (39%) needed mechanical ventilation in the neonatal period, 22 (57%) developed hypertension, and 25 (64%) had hepatic manifestations. Ultrasound showed swollen kidneys (n=29, 76%), cystic kidneys (n=23, 61%), and bright kidneys (n=24, 63%). Eight patients (21%) progressed to end-stage renal disease at a mean age of 1.8 months. Overall, 23 patients (59%) had compound heterozygous mutations or one heterozygous mutation accompanied by one deletion and 6 (15%) had one heterozygous mutation in *PKHD1*. We found 27 novel mutations. Four of these mutations were common to several patients, suggesting unique mutations among Japanese. Interestingly, some patients with the same mutations showed different clinical manifestations and courses. In addition, one patient without *PKHD1* mutations but with typical clinical manifestations had an *MKKS* gene mutation responsible for Bardet–Biedl syndrome.

Conclusions: We found no correlations between mutation sites and clinical manifestations. However, most of the detected mutations had not previously been reported, and 4 novel mutations could be unique and common among Japanese. It is important to distinguish between *PKHD1* and *MKKS* mutations, because some patients showed similar clinical manifestations.

SA-PO282

Mutation Analysis of PKD1 and PKD2 Genes Reveals Novel Mutations and Evidence for Hypomorphic Alleles in Taiwanese Patients Ming-Yang Chang,[1] Yah-huei Wu-chou.[2] *[1]Kidney Research Center and Department of Nephrology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan; [2]Human Molecular Genetics Laboratory, Chang Gung Memorial Hospital, Taoyuan, Taiwan.*

Background: ADPKD is a common genetic disease caused by mutations in the PKD genes. Mutational analysis for ADPKD has become increasingly important in recent years. However, interpretation of the pathogenicity for identified missense mutations remains a challenge. Recent studies suggest that multiple hypomorphic alleles of *PKD1* may occur simultaneously and these mutations could modify disease progression of ADPKD.

Methods: Here we describe our experience in a Taiwanese patient cohort using direct sequencing for all PKD1 and PKD2 exons.

Results: We found 118 sequence variants in 46 unrelated patients who had the ultrasonographic diagnosis of ADPKD. Among these variants, fifteen are definite pathogenic mutations and 15 are missense mutations with highly likely pathogenicity. Overall, 21 patients are PKD1, six are PKD2, and 19 remain undetermined although some of them carry non-pathogenic PKD1 variants. Seven definite pathogenic mutations and 10 pathogenic missense mutations were novel. Furthermore, we found two PKD1 hypomorphic alleles, R2477H and R3439W, in one family. The two mutations are segregated in two affected sisters who developed small renal cysts in the age of 20s, but their parents, each carries only one mutation, had no cysts in their age of 50s. None of the 21 PKD1 patients had family history of dialysis after 70 years old compared to two in six PKD2 patients. The mean ESRD age of PKD1 was 49.0±6.0 (n=5) and PKD2 was 65.5±2.1 (n=2).

Conclusions: Our data support the existence of PKD1 hypomorphic alleles in patients with atypical presentations. The results suggest that missense mutations need to be carfully interpreted for making a correct diagnosis of ADPKD using mutational analysis.

Funding: Private Foundation Support

SA-PO283

Revisiting the "PKD3" Gene in ADPKD Binu M. Paul,[1] Moonnoh Ryan Lee,[1] Mark B. Consugar,[1] Jamie L. Sundsbak,[1] Christina M. Heyer,[1] Sandro Rossetti,[1] Vickie J. Kubly,[1] Vicente E. Torres,[1] Eliecer Coto,[2] Daniel G. Bichet,[3] Maurizio Clementi,[4] Peter C. Harris.[1] *[1]Nephrology & Hypertension, Mayo Clinic, Rochester, MN; [2]HUCA, Spain; [3]Univ of Montreal, Canada; [4]Univ. of Padova, Italy.*

Background: Mutations in two different genes, *PKD1* (16p13.3) and *PKD2* (4q21) are associated with autosomal dominant polycystic kidney disease (ADPKD). However, a number of ADPKD families unlinked to either of these genes have been described, suggesting a third locus, *PKD3*. Our previous work revealed a *PKD1* mutation in a formerly unlinked Portuguese family by microsatellite marker analysis and direct sequencing (JASN 16:358A).

Results: Here, we report *PKD1* mutations in two other described unlinked families. The mother and son in a two-generation Italian family were described with multiple bilateral renal cysts at 46 and 21 yrs, respectively (Turco 1996). The son progressed to ESRD at 51 yrs, whereas the mother had no additional cysts by 75 yrs. Direct sequencing showed a *PKD1* mutation (R4228X) in the son; however, the mother was negative for this mutation in blood, urine and buccal smear DNA. A *de novo* mutation in the son and simple cysts in the mother seems the most likely explanation, but low-level mosaicism in the mother cannot be ruled out. Re-sampling and direct sequencing of the previously reported French-Canadian family with six affected members (Daoust 1995) showed the *PKD1*: D3782_V3783insD mutation in four of them. Repeat ultrasounds in two mutation negative cases previously defined as affected, indicated that they were misdiagnosed. A sample mix-up between an affected and unaffected case further confounded the linkage analysis. Direct sequencing of the unlinked Spanish case (Ariza 1997) did not identify a *PKD1* or *PKD2* mutation. However, the PKD phenotype is atypical, with mild disease and an atrophic kidney in one affected individual.

Conclusions: In conclusion, three of the published "PKD3" cases now are PKD1, questioning the existence of a third gene for ADPKD. Nevertheless, consistent findings of ~10% mutation negative cases in sequenced ADPKD cohorts leaves open the possibility of a further locus that candidate gene and/or whole exome analysis may help identify.

Funding: NIDDK Support

SA-PO284

Identification of *TRIM21* as a Novel Disease Gene in Autosomal Dominant Glomerulocystic Kidney Disease Monica Tucci Cramer,[1] Howard Wiener,[2] Hemant Tiwari,[2] Lisa M. Guay-Woodford.[3] *[1]Pediatrics, UAB, Birmingham, AL; [2]Biostatistics, UAB, Birmingham, AL; [3]Center for Translational Science, CNMC, Washington, DC.*

Background: Glomerulocystic kidney disease (GCKD), a rare form of polycystic kidney disease, has both sporadic and familial occurrence. In previous studies, we have identified a five generation African-American family in which GCKD segregates as an autosomal dominant trait. The disease locus is distinct from the ADPKD genes, *PKD1* and *PKD2*, as well *BICC1*, the human orthologue of the mouse *jcpk* gene. We propose that the disease-susceptibility gene in this family is a novel PKD gene that likely has glomerular function.

Methods: Using the Illumina Cyto-SNP12 assay, we genotyped 19 individuals (13 affected; 6 unaffected) for 294,655 SNPs and performed a genome wide association study (GWAS). We further analyzed these data for evidence of linkage. Whole exome sequencing was performed in 4 individuals (3 affected;1 unaffected). Results were filtered as follows: 1) pathogenic variants present on one allele; and 2) variants present in exons or involving splice sites. Novel variants (not present in dbSNP Database) were further prioritized based on: 1) localization to candidate chromosomal intervals; and 2) gene expression pattern in the GUDMAP database.

Results: Using renal ultrasound or renal biopsy, we classified 13 family members as affected. GWAS analysis identified 6 putative intervals of interest on chromosomes 4, 6, 9, 11 and 18. Whole exome sequencing identified 19 candidate genes within these intervals. One chromosome 11 gene, *TRIM21*, harbored a predicted pathogenic missense variant (Q315P) in all affected individuals. This variant was absent in all unaffected family members, as well as 91 African-American controls. Linkage analysis supported disease locus linkage to this candidate interval (LOD score >3). *TRIM21* is mammalian-specific gene expressed in renal vesicles and the S-shaped body of the developing mouse kidney (GUDMAP database).

Conclusions: We have identified *TRIM21* as a novel disease-susceptibility gene in this GCKD family. We are currently evaluating the function of this mammalian-specific gene in glomerular development and differentiation.

Funding: Private Foundation Support

SA-PO285

A Functional Variant of Angiopoietin-1 Is Associated with Hypertension and Severity of Renal Structural Damage in Patients with ADPKD

Wei Wang, M. Chonchol, Robert W. Schrier, Berenice Y. Gitomer. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

Background: Angiogenesis has been implicated in promotion of renal cyst growth in autosomal dominant polycystic kidney disease (ADPKD). Angiopoietin 1 (Ang-1), an angiogenic growth factor functions to stabilize blood vessels but overexpression has been associated with increased angiogenesis. We previously reported that serum Ang-1 level in ADPKD patients is significantly correlated with structural changes in both the kidney and heart. Recently a functional variant in the 3'-UTR of Ang-1 (rs2507800) was reported to interfere with binding of microRNA-211 (miR-211). We hypothesized that this variant might be associated with severity of cardiovascular complications and renal structural changes in ADPKD.

Methods: We genotyped DNA from 274 ADPKD patients using Taqman SNP assays from Applied Biosystems. Clinical data for all subjects was collected during a 2-day inpatient evaluation at our clinical center.

Results: Variant rs2507800 is an A to T change. The characteristics of the population were as follows: mean age 35.5±12.7 years, 44.3% males, 72.9% hypertensive, 35.7% had reached ESRD, and eGFR60.6 ml/min/1.73m^2 (55.6-66.00, geometric mean and 95CI). There were 34 patients (12.4%) with AA genotype, 138 patients (50.4%) with AT genotype and 102 patients (37.2%) with TT genotype. Age did not differ between genotype groups. Significantly less hypertension were observed in the AA group than in other two groups (51.6% vs 78.5% in AT and 72.2% in TT respectively, p=0.02). Renal volumes were not different among the three groups when males and females were combined. However, when genders were analyzed separately, renal volume differed significantly in female patients based on genotype. The AA group had significantly lower renal volume than the other genotype groups without age differences between groups (483±303mm^3, n=34 vs 792±54mm^3 n=138 in ATand 712±470mm^3, n=102 in TT, p<0.001).

Conclusions: The AA genotype of Ang-1 variant rs2507800 is associated with less severe cardiovascular disease and renal phenotype in female ADPKD patients. This may be due to suppression of Ang-1 expression by facilitated miR-211 binding.

Funding: NIDDK Support, Private Foundation Support

SA-PO286

Autosomal Dominant Polycystic Kidney Disease Patients Express a Distinct Cardiovascular Risk Profile

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic cause of renal failure. The commonest cause of death in this disease is cardiovascular. In addition to hypertension, insulin resistance has been reported. We investigated whether ADPKD patients could express a distinct vascular risk profile compared to other patients with chronic kidney disease (CKD).

Methods: In a cross sectional study on 59 ADPKD and 41 chronic kidney disease (CKD) patients, vascular risk was assessed by clinical data, oral glucose tolerance test and arterial stiffness (pulse wave velocity, PWV). Fasting samples were assayed for biomarkers including highly sensitive C-reactive protein (hsCRP), E-selectin, insulin and nitrate. Insulin resistance was calculated using the HOMA index.

Results: Both groups were comparable in age, blood pressure, body mass index, estimated glomerular filtration rate (eGFR), smoking status and glucose tolerance. PWV was lower in the ADPKD group (9.5 v 11.3 m/s; p<0.001) and was associated with 8-fold higher serum nitrate (78.5 v 9.3 µmol/l; p<0.001) but lower hsCRP (3229 v 7512 ng/ml; p=0.029), E-selectin (32.8 v 45.7 ng/ml; p=0.002) and insulin (58.6 v 96.7 pmol/l; p=0.034). In an overall multivariate model for PWV, ADPKD (p=0.002), age (p<0.001), systolic (p<0.001), diastolic blood pressure, 2 hour plasma glucose, E-selectin and triglyceride were significant factors. When analysing the 2 study groups separately for PWV, age was a significant factor in both; blood pressure was significant only in ADPKD.

Conclusions: ADPKD patients had lower vascular stiffness with a biomarker profile indicative of better endothelial function and a less inflammatory phenotype indicating a different vascular risk profile than in CKD. There was no difference in glucose tolerance categories or HOMA index between ADPKD and CKD patients despite differences in fasting insulin. The results highlight the importance of blood pressure control in reducing vascular risk in ADPKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO287

Prevalence of Cardiovascular Events in Patients with Autosomal Dominant Polycystic Kidney Disease

Imed Helal, Berenice Y. Gitomer, Pamela Mettler, Kim Mcfann, Oleksandra O. Tkachenko, Xiang-Dong Yan, Robert W. Schrier. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: Cardiovascular problems are a major cause of morbidity and mortality in patients with autosomal dominant polycystic kidney disease (ADPKD). This study evaluates the prevalence of cardiovascular events in several stages of chronic kidney disease in ADPKD patients.

Methods: We distributed surveys to 1439 subjects from our ADPKD research database. 426 subjects with ADPKD (30%) completed and returned surveys. Seven of 426 surveys returned were children and were excluded from the study.

Results: ADPKD patients responding were likely to be female (63.2%), non-Hispanic (88.1%) and white (93.6%). The mean age of total group was 53.2 ± 13.7 years. 82.8% had a family history of ADPKD and 32.5% had reached end-stage renal disease. Among respondents analysis of cardiovascular risk factors demonstrated that 86.6% had hypertension with mean age of diagnosis of 36.9 ± 12.9 years with significantly higher prevalence in males, 19.6% were obese, 20.8% were smokers, 8.7% had diabetes, 45.7% had high cholesterol and 17.8% were sedentary. The most prevalent self reported cardiovascular events were arrhythmia (25.9%) with mean age of diagnosis of 43.3 ± 16.4 years, evidence of peripheral vascular disease (16.5%) with mean age of diagnosis of 45 ± 13 years, heart valve problem (14.4%) with mean age of diagnosis of 41.2 ± 16.5 years, cardiac enlargement (9.5%) with mean age of diagnosis of 42.6 ± 13.9 years, stroke or cerebral bleeding (7.5%) with mean age of diagnosis of 50.8 ± 13.4 years, myocardial infarction (6%) with mean age of diagnosis of 53.4 ± 9.6 years and brain aneurysm (5.0%) with mean age of diagnosis of 43.4 ± 13.7 years. The most commonly used antihypertensive medications were angiotensin converting enzyme inhibitors with 46.8% of hypertensive ADPKD patients.

Conclusions: These findings confirm the high prevalence of cardiovascular risk factors and events in ADPKD patients and associate risk for mortality. Due to the prevalence and early onset of cardiovascular risk factors in the ADPKD population, early diagnosis and clinical intervention are recommended.

Funding: Private Foundation Support

SA-PO288

Changing Pattern of End-Stage Renal Disease Treatment in Autosomal Dominant Polycystic Kidney Disease Patients over Time

Imed Helal, Berenice Y. Gitomer, Kim Mcfann, Oleksandra O. Tkachenko, Xiang-Dong Yan, Robert W. Schrier. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: This study was undertaken to determine whether there has been an improvement in the treatment of end-stage renal disease (ESRD) in autosomal dominant polycystic kidney disease (ADPKD) patients over two separate periods, before and after 2000.

Methods: We distributed surveys to 1439 subjects from our ADPKD research database. 426 subjects with ADPKD (30%) completed and returned surveys. 133 ADPKD patients who ESRD were included in descriptive analyses, 33 were reached ESRD during or before the year 2000 (Period A) and 100 were reached ESRD after the year 2000 (Period B).

Results: The mean age of ESRD in the total group was 51.5 ± 9.1 years, the majority of ADPKD patients (68.4%) received kidney transplant after less than one year on dialysis. Of them, 49 (37.1%) underwent preemptive transplantation. The majority of patients (97.7%) have hypertension at time of onset of ESRD. 16.2% had enlarged heart, 18 % have heart valve problem, 12.2% had heart attack, 9.3% had brain aneurysm and 15% had stroke or cerebral bleeding. There were no differences in gender, ethnic or race distributions between the two periods; however, those in Period B were younger than those in Period A at the time of the survey. Family history of ADPKD and BMI were the same in the two periods. Mean ESRD age was significantly younger in Period A than Period B (45.2 ± 7.8 versus 53.5 ± 8.5, p < 0.0001) and more patients in Period A underwent dialysis than in Period B. Patients in period B received a kidney transplant at an earlier age or underwent preemptive transplantation (p < 0.0001).There were no differences in distribution of cardiovascular events between the 2 periods, but those in Period B underwent heart surgery at an earlier age than those in Period A (54.3 ± 7.4 versus 65.0 ± 1.0, p = 0.0007), respectively.

Conclusions: This study demonstrates a significant improvement in patient management and treatment of end stage in ADPKD over time. In fact, these patients reach ESRD at older age, receive kidney transplantation at an earlier age or undergo preemptive transplantation.

Funding: Private Foundation Support

SA-PO289

Endothelial Dysfunction and Vascular Inflammation in ADPKD

Jelena Klawitter,[2] Jacek Klepacki,[2] Alexander T. Pennington,[2] Jost Klawitter,[2] Robert W. Schrier,[1] M. Chonchol,[1] Berenice Y. Gitomer,[1] Uwe Christians.[2] *[1]Department of Medicine, University of Colorado Denver, Aurora, CO; [2]Department of Anesthesiology, University of Colorado Denver, Aurora, CO.*

Background: We have reported that autosomal dominant polycystic kidney disease (ADPKD) patients with normal GFR have higher levels of 9-HODE and 13-HODE suggesting an increase in lipid peroxidation and oxidative stress. They had an abnormal clearance of asymmetric (ADMA) and symmetric dimethylarginine (SDMA) and higher levels of homocysteine (HCy), indicating a role of endothelial dysfunction in ADPKD. We hypothesized that the above established markers will change in ADPKD patients with poorer eGFR and thus may be used as markers of disease progression.

Methods: We analyzed serum from 49 ADPKD patients with eGFR < 65 mL/min. Several targeted multi-analyte tandem mass spectrometry assays were used to analyze markers of oxidative stress and endothelial dysfunction.

Results: See table 1.

Changes in pro-inflammatory, oxidative stress and endothelial dysfunction markers in serum of ADPKD patients.

	ADPKD (n=5) eGFR: 68-134 TKV<800ml	ADPKD (n=18) eGFR:56-114 TKV>1500ml	ADPKD (n=49) eGFR: 29-65	Normal range
9-HODE9ng/ml)	25.3±11.4	43.1±14.7	120.5±114.9	8-24
13-HODE (ng/ml)	21.4±9.9	37.6±13.3	74.2±67.4	3-18
HCy(μM)	11.4±3.2	14.9±4.5	16.4±2.0	3.7-12.9
SAM (μM)	0.36±0.13	0.34±0.11	0.12±0.05	0.07-0.13
SAH (μM)	0.17±0.03	0.20±0.0	0.60±0.09	0.01-0.04
ADMA ((μM)	0.81±0.14	0.98±0.21	0.92±0.16	0.4-0.77
SDMA (μM)	1.38±0.29	2.26±1.13	1.96±0.62	0.31-0.55

S-adenosylhomocysteine (SAH), S-adenosylmethionine (SAM), total kidney volume (TKV); eGFR in mL/min/1.73m2. All markers, except ADMA, significantly inversely correlated with the eGFR in the 29-65 mL/min group.

Conclusions: Higher serum levels of 9-HODE and 13-HODE in ADPKD patients with higher TKV suggest an increase in lipid peroxidation and oxidative stress. The increased serum ADMA, SDMA, Hcy and SAH levels indicate high cardiovascular risk in these patients. Interestingly, all these markers showed more pronounced changes with disease progression. Future studies will evaluate if this marker panel can serve as a clinical monitoring tool for disease progression and indicator of adverse cardiovascular events.

Funding: NIDDK Support, Private Foundation Support

SA-PO290

The Relation between Uric Acid Level and Endothelial Dysfunction in Patients with Early Autosomal Dominant Polycystic Kidney Disease

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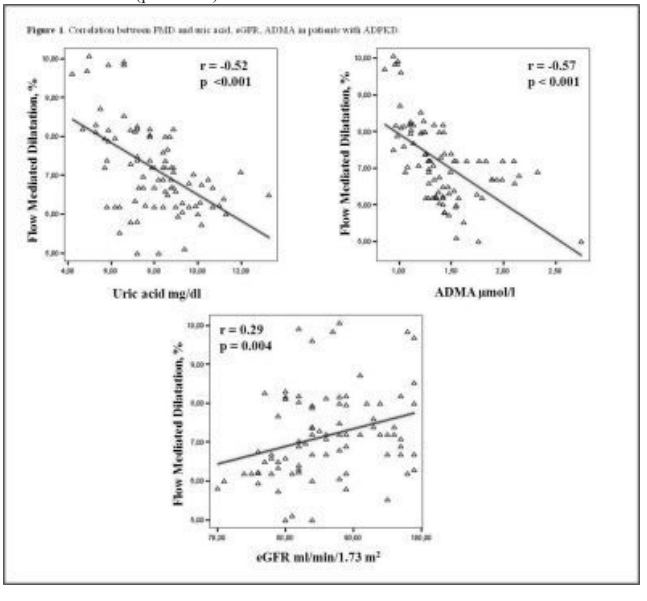
Background: We investigated the association of serum uric acid levels with endothelial dysfunction as determined by measuring the asymmetric dimethylarginine (ADMA) and the flow mediated dilation (FMD) in patients with early autosomal dominant polycystic kidney disease (ADPKD).

Methods: 91 early ADPKD patients (patients with normal kidney function and stage 1-2 chronic kidney disease) were included. Endothelial dysfunction was assessed by flow mediated dilatation measurement and serum ADMA levels.

Results: Early ADPKD patients with higher serum uric acid levels had higher ADMA levels (p = 0.018), higher selective C reactive protein (hs-CRP) levels (p < 0.001) and lower FMD rates (p < 0.001).



Multiple regression analysis demonstrated that uric acid (β = -0.32, p < 0.001), ADMA (β = -0.33, p < 0.001), hs-CRP (β = -0.30, p < 0.001) and estimated glomerular filtration rate (eGFR) (β = 0.30, p < 0.001) were statistically significant predictors of the FMD.

Multiple regression model predicting of flow mediate dilatation in all study subjects (n=91).

Variables	β	p
Uric acid mg/dl	-0.32	< 0.001
ADMA µmol/l	-0.33	< 0.001
hs- CRP mg/dl	-0.30	< 0.001
eGFR ml/min/1.73 m2	0.30	< 0.001

hs-CRP, high sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; FMD, flow mediated dilatation; ADMA, asymmetric dimethyl arginine. Adjusted r2=0.57 for FMD

Conclusions: Uric acid level is associated with endothelial dysfunction in patients with early ADPKD patients.

SA-PO291

Phosphorus and Disease Progression in Autosomal Dominant Polycystic Kidney Disease

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Background: A high phosphorus diet aggravates the progression of polycystic kidney disease in PCK rats, more severely in females (Wang et al, this meeting). Here we use data from CRISP to inquire whether the phosphorus content of the diet might aggravate the progression of human ADPKD.

Methods: CRISP is a prospective, observational, longitudinal, multicenter study of 241 ADPKD adults who had preserved renal function at enrolment. A multiple regression model to ascertain the effect of urine volume and excretion of sodium, potassium, phosphorus, calcium, oxalate and citrate on the slope of log transformed total kidney volume (lnTKV) in female and male CRISP participants during the first six years of the study, without and with adjustment for gender, height and baseline lnTKV was used.

Results: Without gender or height adjustment, urine sodium (p=0.001) and phosphorus (p=0.02) excretions were positively correlated with lnTKV slope. Adjusting for height, phosphorus excretion was positively (p=0.02) and calcium excretion was negatively (p=0.03) correlated with lnTKV slopes in females. No significant correlations between phosphorus or calcium excretions and lnTKV slopes were detected in males. Adjusting for baseline lnTKV (with or without height) has the same effect as adjusting for height, where phosphorus becomes non-significant in males, but is still significant within females. We also tested the same models with the outcome of GFR slopes and found no significant associations, or specific trends by gender, between phosphorus excretion and GFR slope.

Conclusions: Together with the animal study results, these observations suggest a possible effect of dietary phosphorus on the progression of ADPKD, particularly in females.

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SA-PO292

Comparison of Total Kidney Volume (TKV) Measurements by MRI or CT to Predict Disease Progression in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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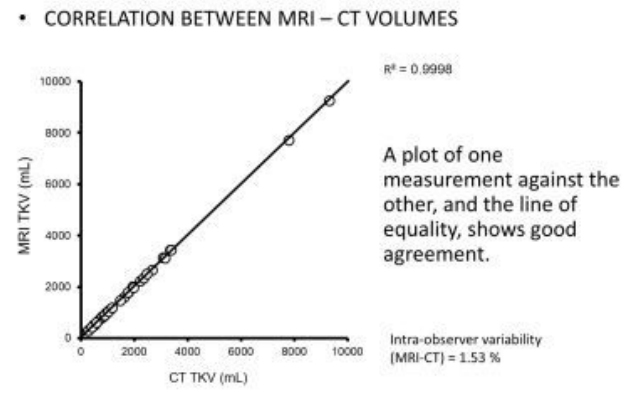
Background: Previous studies have shown TKV measured by MRI to be an excellent surrogate for disease progression in ADPKD. However, MRI may be contraindicated or not available in some patients. To date, no comparison between measurements by both methods is available. The purpose of this study is to assess the degree of agreement between TKV measured by MRI and CT.

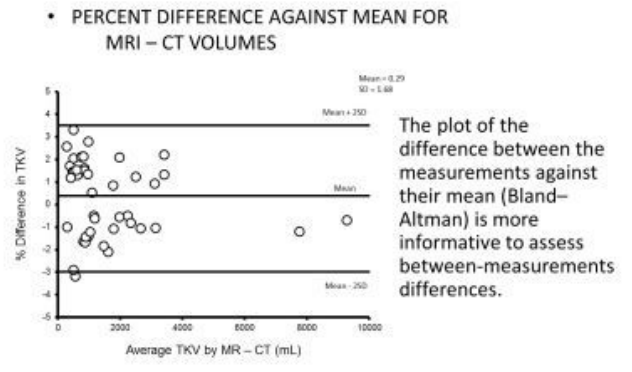
Methods: We identified from our ADPKD database 43 patients (16M/27F;45±13 yo at imaging) who had abdominal CT and MRI obtained within 15 days. Patients with images with incomplete coverage of the kidneys, interventional procedure/acute event in between images or images acquired after ESRD were excluded. Axial non-contrast CT/MR images, of 3-5mm, were used to calculate TKV by stereology method, using Analyze software.

Results: At imaging, SCr=1.5±1 mg/dL. Median TKV=974 (273-9,282) mL. CT plotted against MR TKV are close to the identity line and show an excellent correlation (FigA). A plot of the % difference between the measurements against their mean is more informative to assess between-measurements differences (FigB). The mean % difference in TKV=0.29±1.68, and 42/43 measurements lay within the mean±2SD. The combined % measurement error=1.53 (0.79 MR;0.83 CT;p=NS). The probability of seeing a ≥5.3% increase in TKV in a single patient in the absence of a true change, due to switching MRI or CT, is 0.0008.

Conclusions: Difference in TKV (MRI-CT) is within 3.1% below to 3.6% above true TKV (MRI,CT mean). This is less than the annual mean increase of 5.3% reported in ADPKD. The probability that an MRI-CT switch could account for a change of this magnitude is extremely small (0.0008). This data suggests that MRI and CT could be used interchangeably if necessary to measure TKV.



A



B

SA-PO293

UMOD Gene Polimophysms Affect GFR Decay in ADPKD Maria Teresa Sciarrone Alibrandi,[1] Marina Nuzzo,[1] Simona Delli Carpini,[1] Lino Merlino,[1] Elena Brioni,[1] Laura Zagato Villa,[1] Marco Simonini,[1] Rodolfo F. Rivera,[2] Paolo Manunta.[1] *[1]San Raffaele Scientific Institute, Milan, Italy; [2]San Gerardo Hospital, Monza, Italy.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenetic nephropathy and an important cause of ESRD, accounting for about 5% of patients requiring dialysis. The number of cysts and their complications are variable among single patients as well as the renal outcome. Phenotypic variability is due to the genetic heterogeneity, with the more common PKD1. However, in ADPKD, eGFR decay (Δ) is related to other "gene modifier" involved in Na handlyng and hypertension. Uromodulin (Tamm-Horsfall glycoprotein) is the most common protein excreted in the urine of healthy individuals, yet its function remains unclear. Mutations in the UMOD gene result associated to CKD and hypertension. Aim of the study is to evaluate the influence of UMOD in renal outcome in APKD.

Methods: 108 pts with APKD regularly followed in outpatient clinic of Nephrology Division of our Hospital, DGFR was evaluated at the follow up. Statistical analysis has been performed by GLM adjusted for sex, age, blood pressure, comorbid conditions, therapy and previous renal function.

Results: ADPKD patients carrying UMOD CC genotype present a significant (p=0.013) loss of renal function vs TT ones, after 18 months of average follow-up. Recoding patients in two groups (UMOD CC+GC vs TT) data were further confirmed (ΔGFR -25.5 ml/min vs -6.05 ml/min p=0.004).

Conclusions: Mutations in the UMOD gene result in a marked decrease in the synthesis of uromodulin, as well as the accumulation of abnormal uromodulin in tubular cells, leading to tubular cell death. This phenomena may play a key role in the worsening of renal failure in APKD.

SA-PO294

Affected Parent Gender and Severity of ADPKD Berenice Y. Gitomer, Kim Mcfann, Xiang-Dong Yan, Wei Wang, Imed Helal, Godela M. Brosnahan, Robert W. Schrier. *Department of Medicine, University of Colorado Denver, Aurora, CO.*

Background: Autosomal dominant polycystic disease (ADPKD) is the most common potentially lethal disorder. Morbidity and mortality associated with this disorder represents a significant economic burden both in the US and worldwide. Understanding the factors that impact disease severity and progression is therefore of crucial importance. While ADPKD is a genetic disorder there is considerable variability in severity even among members of the same family all of whom carry the same germ line mutation. This variability has been attributed to the effects of modifier genes or environmental influences. We hypothesized that ADPKD affected children born of an affected mother may have more severe disease influenced by the *in utero* environment.

Methods: We identified 897 PKD1 patients from our clinical database, with known affected parent gender and available clinical information. Subjects were stratified according to gender of the affected parent. Chi Square test of independence or Fisher's Exact tests were used to test the differences in distribution of categorical variables. Kaplan-Meier survival analysis and Log-Rank statistic were used to test the difference in survival times to onset of end-stage renal disease or hypertension.

Results: Children born of an ADPKD affected mother were more likely to have very early onset disease (VEO) ADPKD defined as onset of clinical symptoms within the first 18 months of life compared to those with an affected father, 28/246 versus 4/206, P <0.0001. Male children with an affected father developed ESRD and at an earlier age compared to those with an affected mother, 52 (49-54) years and 54 (51-57), p= 0.0005. Likewise children with an affected father developed hypertension at an earlier age compared to those with an affected mother 32 (28-34) years and 33 (30-36), p = 0.0036.

Conclusions: Affected parent gender does affect severity of ADPKD. Children born of an affected mother are more likely to develop VEO. Surprisingly, affected male but not female offspring with an affected father develop ESRD at an earlier age. This may be related to earlier development of hypertension in male offspring with an affected father.

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SA-PO295

Tubular Creatinine Secretion in Autosomal Dominant Polycystic Kidney Disease: Consequences for Cross-Sectional and Longitudinal Performance of Renal Function Estimation Equations Edwin M. Spithoven, Esther Meijer, Wendy E. Boertien, Steef Jasper Sinkeler, Hilde Tent, Paul E. de Jong, Gerjan Navis, Ron T. Gansevoort. *Nephrology, UMC Groningen, Netherlands.*

Background: ADPKD is characterised by renal tubular cell proliferation and dedifferentiation, which may influence tubular creatinine secretion. Therefore, we investigated tubular creatinine secretion in ADPKD patients and controls and studied consequences for the performance of GFR estimation equations.

Methods: GFR was measured in ADPKD patients and healthy controls as ^{125}I-iothalamate clearance (mGFR), simultaneously with creatinine clearance (CrCl). Tubular creatinine secretion was defined as difference between CrCl and mGFR. CKD-EPI and MDRD equations were used to estimate GFR (eGFR).

Results: In 121 ADPKD patients (56% males, age 40±11y) and 215 controls (48% males, age 53 ± 10y), mGFR was 78±30 and 98±17mL/min/1.73m^2 and tubular creatinine secretion 16±11 and 11±11mL/min/1.73m2, resp.(p<0.001). The higher tubular creatinine secretion in ADPKD patients remained significant after adjustment for covariates and appeared to be dependent on mGFR, with a difference observed only at high-normal mGFR. mGFR correlated highly with CKD-EPI and MDRD eGFR (R=0.95 and 0.93, both p<0.001). Values for bias, precision and accuracy were similar or slightly better than in controls (table). In addition, change in mGFR during 3 years of follow-up in 45 ADPKD patients correlated well with change in CDK-EPI and MDRD eGFR (R=0.73 and 0.71, both p<0.001).

Conclusions: Tubular creatinine secretion in ADPKD patients is increased compared to controls, but this effect is limited to subjects with high-normal mGFR. Consequently, the CKD-EPI and MDRD equations perform relatively well to estimate GFR as well as change in GFR in ADPKD patients.

Performance of CKD-EPI and MDRD equations to estimate GFR.

	ADPKD	Controls	p-value
eGFR CKD-EPI	75 ± 30	90 ± 14	<0.001
-Bias	2.7	8.0	<0.001
-Precision	9.6	13.4	
-Accuracy	99	97	0.16
eGFR MDRD	72 ± 28	89 ± 15	<0.001
-Bias	6.2	9.3	0.028
-Precision	10.8	14.4	
-Accuracy	97	97	0.98

Bias = mGFR minus eGFR. Precision = one SD of bias. Units are ml/min/1.72m2. Accuracy = percentage of eGFR values within 30% of corresponding mGFR value.

Funding: Clinical Revenue Support

SA-PO296

A New Metric to Predict Autosomal Dominant Polycystic Kidney Disease (ADPKD) Progression: Cyst Parenchyma Surface Area (CPSA) Joshua D. Warner,[1] Maria V. Irazabal,[1] Bradley J. Erickson,[1] Bernard F. King,[1] Kyong Tae Bae,[2] Jared J. Grantham,[3] Arlene B. Chapman,[4] Michal Mrug,[5] Doug Landsittel,[2] Michael F. Flessner,[6] William M. Bennett,[7] Vicente E. Torres.[1,8] *[1]Mayo Clinic, Rochester, MN; [2]U. Pittsburgh; [3]U. Kansas; [4]Emory U; [5]U. Alabama Birmingham; [6]NIDDK/NIH; [7]Legacy Good Samaritan; [8]For the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP).*

Background: Cyst development and growth cause renal enlargement and destruction of parenchyma due to mechanical compression and release of inflammatory mediators, eventually leading to renal failure. Although total kidney volume (TKV) is a strong predictor of GFR decline, cases where large TKV are determined by a relatively small number of large cysts weaken the strength of the association. We hypothesize that CPSA which depends on the number, volume and location of the cysts will have a stronger association with GFR decline.

Methods: Ten Mayo CRISP participants with rapid GFR decline (rapid progressors,RP) and 10 patients with stable GFR (slow progressors,SP) over 6 years of follow-up were randomly selected. Four additional CRISP participants were selected because their baseline MRI showed atypical large kidneys with small number of cysts (atypical cases,AC). T2 MRI TKV were measured using stereology.CPSA was obtained by tracing cyst surface area and removing borders not in contact with parenchyma.

Results: TKV was higher in RP (1707±614), compared to SP (671±291) but not AC (1625±742 mL), whereas CPSA was higher in RP (111609±52662) compared to both SP (31211±19500) and AC (40829±6720 u). GFR declined faster in RP (-4.53±1.47), compared to both SP (1.47±0.55) and AC (-1.68±1.92 mL/min/1.73 m^2). Baseline lnCPSA (r -0.733,p <0.0001) correlated better than lnTKV (r – 0.38,p=0.0008) with year 6 eGFR when the atypical cases were included. In the absence of the atypical cases both metrics performed equally well (r -0.724 p=0.0003 and r -0.723 p=0.0003 respectively).

Conclusions: CPSA is a better predictor of GFR decline than LnTKV in atypical ADPKD patients with large TKV and a relatively small number of cysts. In the majority of cases, both metrics perform equally well.

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SA-PO297

Diagnostic Utility of Magnetic Resonance Imaging in Autosomal Dominant Polycystic Kidney Disease York P. Pei,[1] Young-Hwan Hwang,[1] John Conklin,[2] Jamie L. Sundsbak,[3] Christina M. Heyer,[3] Kairong Wang,[1] Ning He,[1] Mostafa Atri,[2] Peter C. Harris,[3] Masoom Haider.[2] *[1]Division of Nephrology, University Health Network and University of Toronto, Toronto, ON, Canada; [2]Department of Medical Imaging, University Health Network and University of Toronto, Toronto, ON, Canada; [3]Division of Nephrology, Mayo Clinic, Rochester, MN.*

Background: The clinical utility of ultrasonography (US) in autosomal dominant polycystic kidney disease (ADPKD) is currently limited by its reduced diagnostic sensitivity (SEN~80%) in at-risk subjects younger than 30 years of age (JASN 20:205-212, 2009). With increased spatial resolution for detecting smaller renal cysts, magnetic resonance imaging (MRI) may provide improved diagnostic capability.

Methods: In this single center study, we compared the diagnostic performance of MRI vs. US in a prospective cohort of 115 subjects between 17-40 years of age who were born with a 50% risk of ADPKD. All these subjects underwent a standardized renal US and MRI as well as mutation and/or linkage-based genetic testing to define their disease status. Concurrently, 44 age-matched healthy control subjects without a family history of ADPKD underwent the same imaging protocol to provide specificity data.

Results: Our preliminary results were derived from 91 at-risk subjects from 81 families (including 14 with *PKD1* hypomorphic mutations and 16 with *PKD2* mutations) whose disease status was unequivocally defined genetically ("gold standard") and 44 presumably unaffected control subjects. Using "a total of ≥ 3 renal cysts" as a test criterion, we found that US provided a higher than expected diagnostic SEN (>95%) but reduced specificity (SPEC~93%) particularly in the 30-40 age group. By contrast, using "a total of ≥ 5 renal cysts" as a test criterion, MRI provided a high SEN and SPEC both at ~98%.

Conclusions: Pending confirmation by the complete analysis of our study cohort, these data suggest that improved diagnostic SEN can be achieved in an experienced center with modern US scanners but at the expense of modest reduction of SPEC. By contrast, MRI may provide improved diagnostic accuracy by maintaining both high SEN and SPEC.

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SA-PO298

Is There Any Role of Urogenital Cysts on Semen Parameters in Autosomal Dominant Polycystic Kidney Disease? Sami Uzun,[1] Savas Ozturk,[1] Meltem Gursu,[1] Abdullah Sumnu,[1] Egemen Cebeci,[1] Serhat Karadag,[1] Zeki Aydin,[1] Rumeyza Kazancioglu.[2] *[1]Department of Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey; [2]Department of Nephrology, Bezmialem Vakif University Medical Faculty, Istanbul, Turkey.*

Background: Autosomal dominant polycystic kidney disease(ADPKD) is a systemic disease with cysts in many organs including the urogenital tract. The aim of the study is to evaluate relationship between urogenital cysts, semen pathologies and infertility in ADPKD.

Methods: Male ADPKD patients aged 18-60 with creatinine clearance higher than 60ml/min were included. All patients had MRI of the urinary system and pelvis; and scrotal Doppler ultrasonography besides sperm analysis. The results were compared with those of a healthy control group.

Results: 27 patients and 17 volunteers were included. Seminal vesicle and prostate cysts were detected in four(15%) and six(22%) patients, respectively. Five of the 23 married patients(21%) had infertility which was more frequent than in control group(p=0.044). Ratio of sperms with normal morphology and progressive motility were lower; and the rate of hypospermia, oligozoospermia, azospermia, asthenozoospermia and teratozoospermia were higher in patient group.

Comparison of semen analysis of the patient and the control groups.

	Patient group	Control group	p
Semen volume (ml)	2.4±1.4	2.9±1.4	0.26
Number of sperms (X106 /mm3)	20 ±17	47±19	<0.0001
Total sperm count (X103)	55±65	155±12	<0.0001
Liquefaction time (min)	18±6	18±7	0.85
Normal morphology (%)	11±14	17±10	0.008
Abnormal morphology (%)	89±14	83±10	0.008
Progressive motility (%)	29±20	54±15	<0.0001
Immobile (%)	62±23	38±16	0.001
Nonprogressive motility (%)	8.1±3.7	7.2±2.7	0.37
Hypospermia[n (%)]	14 (52)	4 (22)	0.063
Asthenospermia [n (%)]	20 (74)	7 (41)	0.005
Oligozoospermia [n (%)]	13 (48)	0 (0)	<0.0001
Teratozoospermi [n (%)]	21 (78)	8 (47)	0.036

There was no significant difference between patients with/without urogenital cysts regarding seminal pathologies.

Conclusions: Seminal abnormalities and infertility are more frequent in patients with ADPKD. Defects in spermatogenesis and sperm motility as well as ciliary pathologies may be responsible.

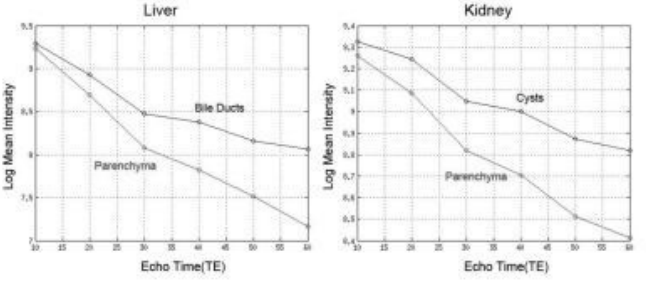
SA-PO299

T2 Relaxation Magnetic Resonance Imaging to Assess Kidney and Liver Disease in an Autosomal Recessive Polycystic Kidney Disease Model Ying Gao,[1,2] Huaiqiang Sun,[2] Lan Lu,[1] David L. Wilson,[1,2] Christopher A. Flask,[1,2] Katherine MacRae Dell.[3,4] *[1]Dept of Radiology; [2]Dept of Biomedical Engineering; [3]Dept of Pediatrics; [4]CWRU Center for the Study of Kidney Disease and Biology, Case Western Reserve University, Cleveland, OH.*

Background: ARPKD causes significant morbidity and mortality in affected children. There are currently no methods for measuring disease severity or progression, severely limiting clinical trial development. Quantitative MRI techniques may provide these important methods. The aim of this study was to develop T_2 relaxation assessments of renal cysts and biliary dilatation in the PCK rat, while limiting variable effects of tissue perfusion.

Methods: Axial kidney and liver images were obtained from 3 PCK rats using a 7T Bruker Biospec MRI scanner with a respiratory-gated,multiecho RARE acquisition. T_2 relaxation maps were generated via linear least squares fitting of conventional monoexponential decay models using all 6 echo images (TE=10-60ms) or the last 4 (TE=30-60ms). A region of interest (ROI) analysis was used to identify biexponential decay effects suggestive of tissue perfusion. Histogram analysis was used to establish thresholds, generate separate compartments and calculate mean T_2 values for normal kidney/cysts and normal liver/dilated bile ducts.

Results: Kidney and liver data showed biexponential T_2 decay characteristics suggestive of tissue perfusion.



Histogram analysis of T_2 maps from the TE=30-60ms data showed consistent segmentation thresholds for kidney (130ms) and liver (100ms). Mean T_2values (ms) were: normal kidney/cysts=83/184;normal liver/dilated bile ducts=51/175.

Conclusions: Initial *in vivo* MRI results in PCK rats suggest that quantitative T_2 assessments can provide a rigorous, high resolution characterization of ARPKD kidney and liver disease severity while correcting for confounding effects of tissue perfusion.

Funding: NIDDK Support

SA-PO300

Systematic Analysis of Polycystic Liver Disease in the ADPKD HALT: A Cohort Marie C. Hogan,[1] Kaleab Z. Abebe,[2] Arlene B. Chapman,[3] Robert W. Schrier,[4] Theodore I. Steinman,[7] Kyong Tae Bae,[2] Michael F. Flessner,[6] William E. Braun,[8] Franz Winklhofer,[9] Godela M. Brosnahan,[3] D. Miskulin,[4] Frederic F. Rahbari-Oskoui,[2] Ronald D. Perrone,[5] Vicente E. Torres.[1] *[1]Mayo Clinic; [2]U Pittsburgh; [3]U Emory; [4]U Colorado; [5]Tufts U; [6]NIH/NIDDK; [7]Beth Israel; [8]Cleveland Clinic; [9]U Kansas; [10]HALT PKD Study Group.*

Background: Few studies have analyzed the clinical correlates of polycystic liver disease (PLD) in large cohorts of ADPKD patients. HALT-PKD study A includes 558 ADPKD patients (51% male) systematically studied with standardized abdominal MRI.

Methods: Correlation and multiple regression cross-sectional analyses to determine associations of baseline clinical, laboratory and QOL variables with log transformed height (ht), adjusted total liver (htTLV) & cyst volumes (htLCV) & with combined ht(TLV+TKV).

Results: Mean (±std) age, TLV, LCV, and eGFR were 37±8yo, 1963±807ml, 292±816ml and 92±8ml/min/1.73 m2. 5.36% had severe PLD (defined as htTLV>1800ml/m) (2.70% in males; 7.98% in females; p=0.007). In the multivariate analysis in females,weight, alk phos & lower eGFR, albumin and platelets associated with lnhtTLV and lnhtLCV associated with age, weight, lnhtTKV, alk phos and lower platelets. LnhtTLV and lnhtLCV univariately associate with various biochemical, hematologic and QOL parameters (Table). These associations were stronger for females. Lnht (TKV+TLV) strongly correlated with back pain in males.

	Ln HtTLV			Ln HtLCV			Ln Ht (TKV+TLV)		
Variable	N	R	P	N	R	P	N	r	P
AST (U/L)	522	0.09514	0.0298*	385	0.07785	NS	513	0.04230	NS
ALT (U/L)	522	0.16271	0.0002*	385	0.03109	NS	513	0.11387	0.0098*
Alk Phos (U/L)	522	0.12525	0.0042*	385	0.11301	0.0266*	513	0.03183	NS
sAlbumin (mg/dL)	519	-0.13125	0.0027*	384	-0.14312	0.0050*	510	-0.09272	0.0363*
Platelets (Cells/µL)	518	-0.11142	0.0112*	382	-0.07382	NS	509	-0.08735	0.0489*
QOL: RP	512	-0.13856	0.0017*	378	-0.10202	0.0478*	503	-0.10504	0.0185*
QOL: GH	514	-0.11327	0.0102*	378	-0.02952	NS	505	-0.05974	NS
QOL: PCS	511	-0.12307	0.0053*	376	-0.04215	NS	502	-0.05853	NS

Table: Pearson correlations. (RP, GH are SF36 QOL subdomains for physical role, general health. PCS is physical component score.)

Conclusions: This study confirms the association of female gender with severity of PLD and reveals that the degree of liver enlargement is associated with the presence of biochemical and hematologic abnormalities, pain and impaired QOL.

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SA-PO301

Increased Prevalence of Renal Cysts in Patients with Sickle Cell Disease
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Background: The availability and use of abdominal ultrasound and CT has led to frequent detection of simple renal cysts. Though generally regarded as benign and incidental, some develop complications of haemorrhage, infection, rupture and malignant transformation. Our aim was to investigate whether the presence of Sickle Cell Disease (SCD) is associated with an increased prevalence of simple renal cysts.

Methods: We identified 198 adults with SCD and normal renal function (eGFR<60 ml/min) who had undergone abdominal US or CT within the past 5 years. The number of reported cysts, location and diameter were recorded. We then re-examined the abdominal CT scans of the 43 patients who had been imaged within the previous 5 years and specifically looked for the presence of cysts. In addition, abdominal CT scans were examined from 180 trauma patients from similar ethnic background (Black African or Black Caribbean) and age who had been scanned within the same time period to act as a control group. All CT scans were examined by 2 radiologists who came to a consensus opinion.

Results: Standard CT reports were approximately twice as likely to mention the presence of renal cysts than ultrasound reports. On re-examination of the CT scans, approximately twice as many cysts were present as had been previously reported. 58% of the SCD patient group had cysts compared with 20% of the black control population (OR 5.4 (CI 2.6-11.0), RR 2.8 (CI 1.9-4.2)). Of those with SCD 28% had cysts bilaterally compared with 5% of the control group. The average number of cysts in the SCD population was 3.76 compared with 1.94 in the control population.

Conclusions: CT is a more sensitive method of detecting simple renal cysts than ultrasound scanning. Unless specifically requested, the presence of simple cysts is not always mentioned in imaging reports. When compared with an ethnically matched control population, patients with sickle cell disease are much more likely to have simple renal cysts, which are often multiple and bilateral.

SA-PO302

A Risk-Stratified Approach to Screen for Intracranial Aneurysms in ADPKD Patients and Its Potential Implications
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Background: Intracranial aneurysms (ICA) are found in 8% of patients (pts) with autosomal dominant polycystic kidney disease (ADPKD). Although rupture is an uncommon event, its high mortality justifies presymptomatic screening (PS) in high-risk pts. Apart from classical risk factors, such as family history (FH) of subarachnoidal hemorrhage (SAH)/ ICA, little is known about the impact of other risk factors on screening.

Methods: A retrospective study was conducted in an ADPKD referral center from 2007 to 2012. From 181 pts, 66 were evaluated for ICA based on symptoms (group A, 13 pts, several imaging methods) or based on risk factors (group B, 53 asymptomatic pts evaluated with magnetic resonance angiography, MRA).

Results: In group A, pts with severe, new-onset headache or focal neurological deficit presented more abnormal findings (6 in 7 pts; 5 ICA, 1 arterial dissection) than those with worsening headache (2 in 6 pts; 1 ICA, a non ADPKD-related diagnosis, NR). In group B, 18 pts displayed 19 abnormal findings: 7 ICA, 3 arachnoidal cysts (AC), 4 vascular ectasias (VE) and 5 NR. PS performed as follows: FH of SAH/ICA (16 pts: 3 ICA, 2 AC, 2 VE); possible FH of SAH/ICA (16 pts: 1 ICA, 3 NR); absent/unknown FH of ADPKD (9 pts: 2 ICA); need for anticoagulation (2 pts: 1 NR); previous to major surgery (4 pts: 1 ICA, 1 AC, 1 NR); imaging for other neurologic disorders (2 pts, 1 VE); without identifiable risk (4 pts, all normal). In total, 13 pts with a mean age of 42 ys had 18 ICA with a mean diameter of 5.36 mm (1.6-11.0), most in the middle cerebral artery (61.1%). Rupture occurred in 3 pts. Pts with ICA presented with lower eGFR (MDRD) than those with normal MRA (55.1 vs 80.5 mL/min/1.73m^2, p<0.05). There were no significant differences regarding age, gender, tobacco use, diagnosis of hypertension or dyslipidemia.

Conclusions: Our results show that 19.7% of risk-stratified pts had ICA, being this group associated with lower GFR. In addition, nonaneurysmal but ADPKD-related manifestations were detected in 12.1% of the pts. While PS in the presence of definite risk factors such as FH of SAH/ICA is strongly advised, these data suggest that alternative risk factors, such as absent/unknown FH of ADPKD, should be also considered.

Funding: Government Support - Non-U.S.

SA-PO303

High-Throughput Mutation Analysis in Patients with Nephronophthisis-Associated Ciliopathies by Multiplex PCR and Next-Generation Sequencing
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Background: To identify disease-causing mutations within coding regions of 11 known NPHP genes (*NPHP1-NPHP11*) in a cohort of 192 patients diagnosed with nephronophthisis-associated ciliopathies.

Methods: Mutation analysis was carried out using PCR-based Access Array microfluidic technology (Fluidigm™) with consecutive next-generation sequencing. We applied a 10-fold primer multiplexing approach allowing PCR-based amplification of 475 amplicons (251 exons) for 48 DNA samples simultaneously. After 4 rounds of amplification followed by indexing all 192 patient-derived products with different barcodes in a subsequent PCR, 2x100 paired-end sequencing was performed on one lane of a HiSeq2000 instrument (Illumina™). Bioinformatics analysis was performed using "CLC Genomics Workbench™" software. Potential mutations were confirmed by Sanger sequencing, shown to segregate with the affected status, and to be absent from 96 control individuals.

Results: NGS sequencing and bioinformatics analysis resulted in 62.1 million mapped reads with a median exon coverage depth of 449-fold per patient. Furthermore, 168/192 DNA samples (87.5%) and 134/251 exons (93.2%) consistently showed sufficient coverage depth above 30-fold. We identified pathogenic mutations in 34/192 patients (18%) and discovered 21 novel mutations in the genes *NPHP3* (7), *NPHP4* (3), *IQCB1* (4), *CEP290* (5), *RPGRIP1L* (1), and *TMEM67* (1). Additionally, we found 40 different single heterozygous missense variants of unknown significance.

Conclusions: We conclude that the combined approach of array-based multiplexed PCR-amplification on a Fluidigm™ Access Array platform followed by NGS is highly cost-efficient and strongly facilitates diagnostic mutation analysis in broadly heterogeneous Mendelian disorders.

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SA-PO304

Mutation of the Renal Magnesium Transporter *SLC41A1* Causes a Nephronophthisis-Like Phenotype
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Background: Nephronophthisis-related ciliopathies (NRCs) are the leading genetic cause of end-stage renal failure in the first three decades of life. To identify novel causative mutations for NRCs, we performed whole exome resequencing in a consanguineous Italian family with an apparent NRC phenotype.

Methods: An Affymetrix Genome-Wide Human SNP Array 6.0 was utilized to perform homozygosity mapping on two affected siblings. Exome capture was performed using the Nimblegen SeqCap EZ Human Exome Library v2.0 followed by resequencing on an Illumina HiSeq2000 platform.

Results: An NPHP diagnosis on two affected sibs of family F438 was based on clinical findings of polyuria, renal failure and increased echogenicity by RUS. Histological analysis of renal biopsies revealed the typical NPHP triad of tubular ectasia, tubular basement membrane disruption and tubularinterstitial infiltrations. Homozygosity mapping in the two affected sibs yielded 8 homozygosity peaks. Whole exome capture revealed a homozygous splice acceptor site mutation (c.698G>T) in the renal Mg^{2+} transporter *SLC41A1*, resulting in skipping of exon 6 of *SLC41A1* leading to an in frame deletion. Analysis of Mg^{2+} efflux on cells transfected with either wild type or DExon6 SLC41A1 revealed that deletion of exon 6 completely blocked the Mg^{2+} transport function of SLC41A1. Finally, we demonstrate that endogenous SLC41A1 localizes to renal tubules situated at the corticomedullary boundary consistent with the region of the kidney where the increased echogenicity was observed.

Conclusions: We have identified by whole exome capture a novel causative mutation in the Mg^{2+} transporter *SLC41A1*. This data suggests that defects in maintenance of renal Mg^{2+} homeostasis may lead to tubular defects that phenocopy nephronophthisis analogous to *CLDN16* mutation in a bovine model of nephronophthisis.

Funding: NIDDK Support

SA-PO305

Genetic Diagnosis of Nephronophthisis of Korean Children Using Traditional and New Methods: Stratified Sanger Sequencing and Targeted Exome Sequencing Hee Gyung Kang,[1] Su Jung Park,[1] Jiwon L. Lee,[1] Hye Jin Chang,[1] Hye Won Park,[3] Hae Il Cheong.[1] *[1]Pediatrics, Seoul National University Children's Hospital, Seoul, Korea; [2]Pediatrics, Center for Pediatric Oncology, National Cancer Center, Korea; [3]Pediatrics, Seoul National University Bundang Hospital, Seong Nam-Si, Korea.*

Background: Nephronophthisis (NPHP) is the most common genetic cause of end stage renal failure with insidious onset in childhood and adolescence. Recently, genetic diagnosis of this disease became available; Gene locus heterogeneity, allelism, and modifier genes are considered to govern genotype-phenotype correlation of NPHP. Here we report our result of genetic diagnosis in Korean children with NPHP.

Methods: Using Sanger sequencing, all the patients with clinical diagnosis of NPHP (n=71, M:F 38:33) were screened for total homozygous deletion of *NPHP1*. Other known genes of NPHP were tested after stratification; *NPHP2/INVS* for infantile type (n=7), *NPHP5* for those (n=12) with retinitis pigmentosa, *NPHP6/CEP290* for those (n=8) with cerebellar involvement, and *MKS/TMEM67* for those (n=7) with hepatic fibrosis. Then, exome sequencing, targeting 112 ciliopathy-related genes, was done for patients whose genetic diagnosis was not obtained by traditional method (n=59).

Results: Stratified Sanger sequencing of five genes revealed 4 juvenile cases with total deletion of *NPHP1*, 3 cases (Senior-Løken syndrome) with *NPHP5* mutations, one case (Joubert syndrome) with *NPHP6* mutations, and 4 cases (Meckel-Gruber syndrome) with *MKS3* mutations. Targeted exome sequencing confirmed mutations of ciliopathy-related genes in additional 8 patients; single cases of *NPHP1, NPHP3, NPHP4, NPHP12/TTC21B, SDCCAG8, PLXDC2,* and two cases of *PKHD1* mutations. In addition, a heterozygous mutation in single gene, in 2 genes and in 3 genes were detected in 22, 10 and 5 cases, respectively.

Conclusions: In total, 20 (28%) of 71 NPHP patients were genetically diagnosed either by traditional Sanger sequencing or by targeted exome sequencing. In addition, the latter method revealed candidate genes in 37 (52%) additional patients. Further studies on candidate genes found in exome capture would improve our understanding of NPHP.

SA-PO306

Retinal Macular Degeneration: A Pathognomic Finding of Nephronophthisis Type 1 with *NPHP1 Total Deletion?* Hee Gyung Kang,[1] Yo Han Ahn,[2] Su Jung Park,[1] Hye Jin Chang,[1] Jiwon L. Lee,[1] Sang Taek Lee,[1] Hyewon Park,[3] IL-Soo Ha,[1] Hae Il Cheong.[1] *[1]Depts of Pediatrics, Seoul National University Children's Hospital, Seoul, Republic of Korea; [2]Center for Pediatric Oncology, National Cancer Center, Goyang, Republic of Korea; [3]Health Promotion Center, Seoul National University Bundang Hospital, Seongnam, Republic of Korea.*

Background: Nephronophthisis (NPHP) is the most common genetic cause of end-stage renal disease of childhood and adolescence. More than thirteen genes are known to cause NPHP, among which *NPHP1* of nephrocystin-1 is the most common causative gene. While extrarenal symptoms, such as retinal pigmentosa, cerebellar vermis aplasia, and oculomotor apraxia, are commonly found in NPHP patients, NPHP type 1 is generally known as of isolated kidney involvement.

Methods: Genetic analysis and clinical correlation of NPHP type 1 patients.

Results: Five patients (M:F 4:1) with chronic kidney disease (CKD) were diagnosed as NPHP1 by genetic analysis, revealing homozygous total deletion of *NPHP1*. Their presentation of CKD was typical of NPHP at 7-15 years of age with nocturia or fatigue. Kidney biopsy findings were also compatible with NPHP in 4 patients. Renal function was lost between ages of 9 to 20. All patients had progressive decrease of visual acuity with various onset age (age 2-17) and one complained of night blindness. Ophthalmologic examination revealed the macular degeneration in 4 patients (age 14-23), which is quite compatible with Stargardt macular dystrophy; findings of one representative case was as follows. Fundoscopic examination showed parafoveal retinal pigment epithelium atrophy and degeneration. Electroretinography showed decreased amplitude of cone and rod responses. Fluorescein angiography revealed multiple flecks with choroidal silence.

Conclusions: While 6-10% of NPHP1 patients have been reported to have eye involvement, all of our patients with total deletion of *NPHP1* had progressive degenerative condition of eye, especially Stargardt disease in four out of five cases. These cases suggest that the possibility of *NPHP1* total deletion should be considered in CKD patients with progressive disturbance of visual acuity with typical findings of Stargardt disease.

SA-PO307

RITAZAREM: An International, Open Label, Randomized, Controlled Trial Comparing Rituximab with Azathioprine as Maintenance Therapy in Relapsing ANCA-Associated Vasculitis David R.W. Jayne,[1] Rona M. Smith,[1] Peter A. Merkel.[2] *[1]Vasculitis Office, Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Division of Rheumatology, University of Pennsylvania, Philadelphia, PA.*

Background: Rituximab is an established induction agent in ANCA-associated vasculitis (AAV), especially for those with relapsing disease. Its role as a maintenance agent is less clear. 50% of patients with AAV will pursue a chronic relapsing course, despite standard maintenance immunosuppression. Therefore, there is unmet need in the prevention of relapses. Pilot data suggests that fixed interval repeat dose rituximab reduces relapse rate and may lead to sustained remission after a two-year treatment period. T his needs to be confirmed in a prospective, randomized controlled trial.

Methods: RITAZAREM is a multi-center, international, open label, randomized controlled trial in relapsing AAV. Patients with granulomatosis with polyangiitis (Wegener's) (GPA) and microscopic polyangiitis (MPA) will be recruited at the time of relapse, and all will receive induction therapy with rituximab (375mg/m^2/week x 4). At four months patients who have achieved remission will be randomized, 1:1 to receive maintenance therapy with either fixed-interval, repeat dosing of rituximab (1g every 4 months (5g in total)) or azathioprine. Glucocorticoid therapy will be protocolized and fully withdrawn by month 20.

Results: This study has three phases: i) four months induction; ii) 20 months maintenance; and iii) 24 months follow-up. The primary outcome is time from remission to relapse. Centers have been selected from the European Vasculitis Study Group (EUVAS; www.vasculitis.org) and the Vasculitis Clinical Research Consortium (VCRC). Recruitment will commence in summer 2012. Results are anticipated in late 2016.

Conclusions: This RITAZAREM study will address the efficacy and safety of fixed-time interval, repeat therapy with rituximab as a maintenance strategy in AAV. The outcome of this trial will help define optimal therapy for AAV.

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SA-PO308

Long Term Outcome of Low Dose Cyclophosphamide (CYC) and Plasma Exchange (PLEX) Induction in ANCA Associated Vasculitis (AAV) Wladimir M. Szpirt,[1] Elizabeth Krarup,[2] Martin Egfjord.[1] *[1]Nephrology, Rigshospitalet; [2]Herlev Hospital, Copenhagen, Denmark.*

Background: Adverse events to the treatment of AAV have a greater threat for 1 year's outcome than the active vasculitis. The use of PLEX in AAV is not commonly accepted in patients (pts.) with plasma creatinine <500 µmol/L (<500), despite evidence of the involvement of ANCA and complement in the pathogenesis of vasculitis. We combined low dose of CYC and PLEX in order to minimize the infection/sepsis rate which mostly is due to the immunsuppression.

Methods: A prospective cohort study of all AAV pts. referred to our centre between 2000-2010 was performed. All pts. had AAV based on positive ANCA and a compatible clinical syndrome. The use of PLEX was decided on severity of renal biopsy and ANCA titres. Immunsuppression consisted of prednisolone 1 mg/kg/day and a low dose of daily oral CYC (100 mg/day in pts. <65 years and 50 mg/day in pts. >65). AZA/MMF was given for maintenance of remission.

Results: 132 pts. were admitted and 118 followed for a mean of 5.7 years (range 0.2-12.3; 676 patient years). 57% were male, 47% were MPO-ANCA positive. 36% had high creatinine >500. 40% were aged >65. 105 pts. received PLEX (mean 7 (5-11)). Only 5 pts (4%) died during CYC treatment (2 sepsis, 2 AMI, 1 lung haemorrhage, all on HD). In total 11 pts. (9%) died within 1 year and 14 more pts. died < 5 years. The total number of deaths was 33 (28%) with an expected 12 years patient survival of 62% (Kaplan Meier). 27 pts. (23%) developed ESRD, with an expected 12 years kidney survival of 75% (K.M). 43 pts. (36%) died or developed ESRD during the study, resulting in an expected 12 years dialysis free patient survival of 62% (K.M.). Pts. aged <65 or <500 had significantly better dialysis free survival. 29 pts. (23%) had infection <first 4 months, 15 (13%) being leucopenic. 41 (34%) relapses occurred during the 12 years of follow up.

Conclusions: The use of low dose CYC and PLEX for induction resulted in a high survival rate and good preservation of renal function together with low rate of complications. As the septicaemia and mortality was low, the combination of PLEX and low CYC seems to be less toxic, than the conventional AAV induction treatment regimen.

SA-PO309

ANCA-Negative Pauci-Immune Crescentic Glomerulonephritis: Clinical Characterestics and Outcome Amit Gupta, Narain Prasad, Anita Saxena, Raj K. Sharma. *Department of Nephrology, SGPGIMS, Lucknow, UP, India.*

Background: There is paucity of data on clinical characteristics and outcome of the ANCA negative pauci immune crescentic Glomerulonephritis(PCrGN). This analysis was done to compare the clinical profiles and outcome of ANCA negative (-ve) and ANCA positive (+ve) PCrGN.

Methods: The renal and extra-renal clinical characteristics, histological evidences and outcomes of all ANCA +ve and -ve patients seen from January 2006 to June 2011 were compared. The criteria for the PCrGN was the intensity of glomerular immunoglobulin staining (scale 0 to 4+) by direct immunofluorescence assay. The renal remission (complete- serum creatinine ≤1.4 mg/dl; partial- serum creatinine>1.4 mg/dl and dialysis free) between the two groups were compared.

Results: Of the 102 patients with PCrGN, 41(40%) were ANCA -ve and 61(60%) +ve (c- ANCA=28,p-ANCA=33). The extra renal manifestations were significantly high in ANCA +ve patients compared to ANCA -ve patients. The differing clinical characteristics of ANCA +ve versus -ve PCrGN were age (47±12 vs 40±8 years=0.04); fever (70% vs 44%, p=0.01); fatigue(82%vs56%,p=0.007),weight loss (41% vs 24.40%,p=0.09); arthralgia (46%vs 12%,p=0.001); muscle pain(36% vs 10%,p=0.003); gross hematuria (15% vs 32%,p=0.05); thrombocytosis (18% vs 2.4%,p=0.025); nephrotic range proteinuria (18% vs 41.40%,p=0.005). GIT, skin, sinus, eye involvement & BP similar in both groups. Although the glomerulosclerosis was significantly higher in ANCA -ve (27%) compared to ANCA +ve (11%),p=0.04 patients, the grade of interstitial fibrosis and tubular atrophy were similar(p>0.05). Complete or partial renal recovery was observed in 42/61(69%)ANCA +ve and 29/41(71%) in ANCA -ve patients.19/61(31%) ANCA +ve patients progressed to ESRD while 12/41(29.29%) ANCA -ve patients progressed to ESRD (p>0.05).

Conclusions: The ANCA negative PCrGN patients are relatively younger. Athough the extra renal manifestations are significantly higher in ANCA positive compared to ANCA negative PCrGN, the renal outcome is similar in both groups.

SA-PO310

Efficacy of Reduced Dose Intravenous Cyclophosphamide Induction Regime on Relapse Rates of ANCA Associated Vasculitis: A Single Centre Experience Nileshkumar Shah, Yadullah Syed, Fiona E. Harris, David Makanjuola. *Renal Unit, St. Helier Hospital, Carshalton, Surrey, United Kingdom.*

Background: Anti neutrophil cytoplasmic antibody associated systemic vasculitis (AASV) remains an important cause of renal disease. The aim of treatment is to induce a stable remission with minimum toxicity. We adapted our local protocol from published studies but reduced the duration of intravenous (i.v) cyclophosphamide treatment to minimise total exposure.

Intravenous Cyclophosphamide dosing schedule

Age (years)	Creatinine (µmol/L) <300	Creatinine (µmol/L) >300			
<60	15 mg/kg/pulse	12.5 mg/kg/ pulse			
>60 - <70	12.5 mg/kg/ pulse	10 mg/kg/pulse			
>70	10 mg/kg/pulse	7.5 mg/kg/pulse			
Dose 1	Dose 2 (2/52)	Dose 3 (4/52)	Dose 4 (7/52)	Dose 5 (10/52)	Dose 6 (13/52)

The aim of this study was to see whether the reduced dose at induction therapy increased the possibility of relapses of AASV.

Methods: We reviewed 90 patients with AASV diagnosed between June 2004 and Jan 2011 Patients were treated with i.v cyclophosphamide, Maintenance therapy was with azathioprine (1.5mg/Kg) in 68 (75%) patients and with other agents including Mycophenolate in the rest. We collected data on ANCA, creatinine and eGFR at the end of induction treatment (4 months), 12 months and at most recent follow up.

Results: 87/90 were Caucasian, M:F = 46:44, mean age was 64yrs; mean duration of follow up was 1317 days. 18 (20%) patients relapsed during follow up, 7 patients within the first 12 months, 11 between 12 - 96 months (PR3 positive n=11, MPO positive n=7) 14 of the relapsers were on Azathioprine and 4 were on Mycophenolate as maintenance treatment.

Conclusions: 7.7% of our patients had a relapse in the first 12 months. This compares favourably with 15.5% in the CYCAZAREM study, despite Cyclophosphamide exposure at induction in our patients being much less. We also found relapse to be more common in patients with PR3 positivity.

Our data suggest that our regime is equipotent at inducing remission and maintaining remission, without increased incidence of relapses. The lower cumulative Cyclophosphamide dose would be expected to cause fewer infectious complications and less gonadal toxicity.

SA-PO311

Reduced Dose Intravenous Cyclophosphamide Regime in Induction of ANCA Associated Vasculitis: A Single Centre Experience Yadullah Syed, Nileshkumar Shah, David Makanjuola, Fiona E. Harris. *Renal Unit, St. Helier Hospital, Carshalton, United Kingdom.*

Background: Anti neutrophil cytoplasmic antibody associated vasculitis (AASV) remains a significant cause of renal disease. There is evidence to support the use of intravenous (i.v) Cyclophosphamide for treatment. There is a constant desire to reduce treatment related toxicity in the care of such patients, whilst still seeking to induce and maintain a successful remission. We adapted our local protocol from published studies, but our regimen gives a reduced total dose of cyclophosphamide.

i.v Cyclophosphamide dosing schedule

Age (years)	Creatinine <300µmol/L	Creatinine >300µmol/L			
<60	15 mg/kg/pulse	12.5 mg/kg/ pulse			
>60-<70	12.5 mg/kg/ pulse	10 mg/kg/pulse			
>70	10 mg/kg/pulse	7.5mg/kg/pulse			
Dose 1	Dose 2 (2/52)	Dose 3 (4/52)	Dose 4 (7/52)	Dose 5 (10/52)	Dose 6 (13/52)

Methods: We reviewed patients with AASV diagnosed between June 2004 and Jan 2011; median follow up was 1323 days. We collected data at the end of induction treatment (4 months), 12 months and most recent follow up. Changes in creatinine and eGFR were defined as improved (>25% improvement), stable </= 25% change, or worse (>25% deterioration) from renal function at presentation.

Results: 87/90 were Caucasian; M:F = 46:44; mean age was 64.06±14.80. 35 (39%) needed dialysis on admission (Group A). 55 (61%) did not (Group B). 82 (91%) patients went into remission at the end of induction; 32(35.55%) in group A and 50 (55.55%) in group B. 5 (5.55%) patients did not achieve remission 1(1.11%) in group A and 4 (4.44%) in group B. (3.33%) died during the induction phase, 1 of whom did not achieve remission.

At the end of induction, 14(40%) patients in group A became dialysis independent. In group B, renal function improved in 33(60%), was stable in 16 (29.09%) and worse in 3(5.45%) patients at the end of induction.

Conclusions: 91 % of our patients went into remission at the end of 4 months of induction therapy. This is comparable to the remission rates in the CYCLOPS study (88%), in spite of the fact that the cumulative dose in our patients was less. We believe that our regime limits Cyclophosphamide exposure and maintains efficacy with regard to inducing remission.

SA-PO312

Gestational Rituximab Exposure in Women with Vasculitis William Franklin Pendergraft,[1-3] Martina M. McGrath,[1,3] Andrew P. Murphy,[3] Patrick Murphy,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Div. of Nephrology, MGH, Boston, MA; [3]Vasculitis Clinic, MGH, Boston, MA.*

Background: Historically, cyclophosphamide (CYC) has been the mainstay of therapy for ANCA vasculitis. Rituximab (RTX) has proven to be an effective alternative to CYC in women of child-bearing age, but little is known about fetal effects of RTX exposure during pregnancy.

Methods: While being treated with RTX, women were counseled to avoid or plan pregnancy. Urine hCG was checked before each dose. Among pregnancies, patients and fetuses were monitored for recurrent disease and complications associated with RTX and immunosuppression. Where possible, maternal and fetal cord blood was tested for CD20+ B cells at delivery.

Results: 157 women (age range 16-93yrs, mean [SD] 58.9yrs [17.3]), including 22 women under 40yrs, were treated with RTX from 2002-present. Only one patient (40yrs) who desired pregnancy was unable to conceive. Six women achieved eight pregnancies (see Table), four planned and four unplanned. Patient 3a had progressive airway disease despite absence of B cells, ANCA and other features of active vasculitis. Patient 2b had a miscarriage at 15 wks. Remaining pregnancies were uneventful. Maternal CD20+ B cells were absent at delivery in most patients; however, B cells were at normal levels in fetal cord blood.

Characteristics of patients treated with RTX who achieved pregnancy

PATIENT	AGE	ANTENATAL RTX EXPOSURE (months)	WEEKS GESTATION	CHILD SEX/ APGAR	CHILD WT (g)	MATERNAL B CELLS (%)	FETAL B CELLS (%)
1	31	8	31	M/8,9	1625	<0.01	NR
2a	25	16.5	41	F/9,9	3790	2.79	NR
2b	27	7.5•	miscarriage 15wks	NR	NR	<0.01	NR
3a	20	8.3	40	M/9,9	2945	<0.01	3
3b	22	0.5*	TBD	M/TBD	TBD	<0.01#	TBD
4	29	13.5	38	F/8,9	3270	<0.01	5
5	40	9	38	M/8,10	3515	<0.01	NR
6	32	2.8*	TBD	F/TBD	TBD	<0.01#	TBD

•=months b/f D&C @ 15wks, *=months b/f estimated conception, #=trimester 3

Conclusions: Most women were able to achieve and complete pregnancy after RTX treatment, and fetal CD20+ B cells do not appear to be affected. Further study is needed to determine perinatal safety of RTX, especially as it is replacing CYC as the mainstay of therapy.

Funding: Clinical Revenue Support

SA-PO313

Characterization of Drug-Induced ANCA Vasculitis at Massachusetts General Hospital (MGH): 1989-2012 William Franklin Pendergraft,[1,2,3] Martina M. McGrath,[1,3] Andrew P. Murphy,[3] Patrick Murphy,[3] Karen A. Laliberte,[3] John Niles.[2,3] *[1]Joint Nephrology Fellowship Program, Massachusetts General Hospital (MGH) and Brigham and Women's Hospital, Boston, MA; [2]Division of Nephrology, MGH, Boston, MA; [3]Vasculitis Clinic, MGH, Boston, MA.*

Background: The etiology of vasculitides associated with anti-neutrophil cytoplasmic autoantibodies (ANCA) remains largely unknown; however, drug-induced forms of disease exist. Reported culprits include hydralazine, minocycline, penicillamine, propylthiouracil (PTU), sulfasalazine and as recently described by our group, levamisole in the presence of cocaine. Our group has been the main clinical ANCA laboratory in New England and evaluation of positive patients is needed to identify exposures to known and potential culprit drugs.

Methods: We conducted a systematic retrospective analysis of patients from 1989-2012 with an initial positive ANCA test by indirect capture ELISA directed against either MPO and/or PR3. For each new ANCA-positive patient, a limited discussion was held with the ordering clinician to review implications of the positive test and to identify exposure to any known culprit drugs (discussions noted in MGH ANCA test registry were used for initial data analyses).

Results: A total of 2257 patients with positive ANCA tests were identified: 1428 (63%) were MPO-ANCA, 793 (35%) PR3-ANCA, and 38 (2%) dual MPO- and PR3-ANCA. 139 (6%) patients were exposed to drugs known to cause disease: 46 (33%) received hydralazine, 45 (32%) levamisole and cocaine, 33 (24%) PTU, 12 (9%) minocycline, 2 (1%) sulfasalazine and 1 (<1%) penicillamine.

Conclusions: This study represents the first and largest of its kind. The true number of drug-induced cases is likely underrepresented given that medications in use at the time of a positive ANCA test, in many cases, were unknown. Most importantly, the number of cases of ANCA vasculitis associated with many of these drugs is of concern given their ubiquitous use in the general population. Based on these results, measures should be implemented to limit hydralazine and propylthiouracil use and counsel against cocaine use.

Funding: Clinical Revenue Support

SA-PO314

Treatment and Outcome of Elderly Patients with ANCA-Associated Systemic Vasculitis Maria Weiner,[1] Aladdin Mohammad,[2] Zdenka Hruskova,[3] Kerstin W. Westman,[2] Per Eriksson,[1] Vladimir Tesar,[3] Alan D. Salama,[4] Marten Segelmark.[1] *[1]Medical and Health Sciences, Linköping University, Linköping, Sweden; [2]Clincial Sciences, Lund University, Lund, Sweden; [3]Nephrology, Charles University, Prauge, Czech Republic; [4]UCL Centre for Nephrology, Royal Free Hosptial, London, United Kingdom.*

Background: Age is a well known risk factor for patients with ANCA-associated vasculitis (AAV), but as few elderly patients have been included in clinical trials and there is little evidence concerning their optimal treatment.

Methods: In this multicenter study we analyzed baseline data and treatment during the first three months and related that to outcome between 3 months and 2 years in patients aged 75 years or more. We included consecutive patients with a clinical diagnosis of AAV according to Chapel Hill nomenclature from Linköping, Lund, Malmö, Prague and London diagnosed between 1997 and 2009.

Results: Data on treatment were available for 87 patients with a mean age at diagnosis of 79.8 years. During the first three months 16 patients died and 20 patients died between three months and two years giving a mean survival of 545 days. Mortality was significantly higher among those above 80 years (mean survival 475 days vs. 598; p=0.045). 51 patients were MPO-ANCA positive and 28 PR3-ANCA positive; serology did not affect survival. Among the 71 patients surviving the first 3 month Cyclophosphamide was given to 61 patients with a median cumulative dose of 3350 mg, 7 patients received Retuximab and 23 plasma exchange. Overall there were no significant differences in survival related to therapy or dose. However, when comparing patients who received less intense therapy (<3g of Cyclophosphamide and no Retuximab) with standard therapy, the standard therapy group did better (mean survival 712 vs 573 days, p=0.47).

Conclusions: Mean survival for AAV patients >75 years of age was 18 months, being significantly worse for those above 80 years. Insufficient treatment may contribute to mortality, but we cannot rule out that patients with adverse prognosis were treated less intense.

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SA-PO315

Small Vessel Vasculitis with Renal Involvement in Patients over 75 Years: A 24-Year Retrospective Study Helena Marco,[1] Emilia Corica,[2] Montserrat Picazo,[1] Yolanda Arce,[3] Jm Llobet,[2] Montserrat M. Diaz Encarnacion,[1] Jose Ballarin.[1] *[1]Nephrology, Fundacio Puigvert, Spain; [2]Rheumatology, Hospital Sant Pau, Spain; [3]Pathological Anatomy, Fundacio Puigvert, Spain.*

Background: Recent studies have shown a late onset of small vessel vasculitis (SVV), reaching up to 75 years or more. However, the differences in disease presentation and outcome between older and younger patients remain controversial. In our study we compare a group of very elderly patients (≥75 years) versus a group of younger patients (<75 years) with SVV and renal involvement.

Methods: We performed a single center retrospective review of 111 cases of SVV (1985-2009). We evaluated clinical and laboratory variables at diagnosis, presence of ANCA, kidney biopsy, immunosuppression therapy and renal/patient survival.

Results: Diagnoses were Wegener Granulomatosis(9%), Microscopic Polyangiitis(85%), Goodpasture's syndrome(6%). Patients ≥75 years old were 21%. The median age of all patients was 68(12-89)years: 79(75-89)years in very elderly patients and 65(12-74)years in patients <75 years. Creatinine at the treatment onset was 449(+/-211)µmol/L in the first group and 453(+/-267)µmol/L in the second.

95% of all patients received immunosuppressive induction treatment, 91% of the first group and 97% of the younger group. 72% of the oldest patients received Cyclophosphamide versus 93% of the youngest patients (p=0.01) and 14% of the oldest patients received Mycophenolate Mofetil versus 2% of the youngest patients (p=0.05).

The amount of patients that received definitive dialysis was similar (47% and 49% respectively). 30% of the elderly and 38% of patients <75 years had leukopenia. We observed similar number of infections in both groups (50% and 43% respectively).

The median follow up was 39.5(1-201) months, the actuarial patient survival was 70% in very elderly patients and 81% in the younger group at 24 months of follow-up (p=0.17).

Conclusions: The proportion of patients ≥75 years old with SVV is high (21%). Oldest patients received less induction treatment with Cyclophosphamide although there were no statistically significant differences in terms of complications, renal survival and patient survival with the younger patients.

Funding: Private Foundation Support

SA-PO316

Clinical Characteristics and Outcome of Pauci-Immune Glomerulonephritis in African Americans Duvuru Geetha,[1] Caroline Jennette Poulton,[2] Yichun Hu,[2] Philip Seo,[1] JulieAnne G. McGregor,[2] Patrick H. Nachman,[2] Susan L. Hogan.[2] *[1]Johns Hopkins University; [2]University of North Carolina, Chapel Hill.*

Background: Pauci-immune glomerulonephritis is rare in African Americans (AA) and the clinical presentation and treatment outcomes of vasculitis have not been well described.

Methods: We identified patients 2 to 92 years of age between 1983 and 2011 with a diagnosis of biopsy proven pauci-immune glomerulonephritis (GN) at any point during their disease course. Comparing AA to Caucasian patients, we examined demographics, clinical features at presentation, treatment and outcomes of relapse, end stage renal disease (ESRD) and death.

Results: Of the 701 patients, 79 were AA with the remainder Caucasian. Compared to Caucasians, AA disease onset was at an earlier age (52 vs. 57 yrs, p=0.04), was more often MPO-ANCA positive (70% vs 53%, p=0.01), and trended toward being more frequent in females (56% vs 45%, p=0.07). Median (Interquartile range (IQR)) follow-up in months was 26 (5, 54) in AA and 28 (11, 59) in Caucasian patients. AA patients were less likely to have renal disease at diagnosis (92% vs 98%, p=0.02), but extra renal manifestations were similar. Induction drug treatment regimens were similar. Median eGFR was similar at presentation (23 vs. 21 ml/min/m^2). AA were less likely to have a renal relapse (20% vs 31%, p=0.07). Overall, controlling for age, race, MPO vs PR3-ANCA, there was no difference in reaching ESRD by race over the entire time of follow-up. There were no differences in relapse or death rates by race.

Conclusions: AA patients with vasculitis are younger and more often MPO-ANCA positive compared to Caucasians. There were no differences in ESRD, relapse or death rates by race over the entire time of follow-up.

SA-PO317

BK Virus Replication in Patients with Granulomatosis with Polyangiitis and Microscopic Polyangiitis Duvuru Geetha, Stuart M. Levine, Rebecca Manno, Alex Valsamakis, Philip Seo. *Johns Hopkins University.*

Background: Current immunosuppressive drug treatment regimens to induce and maintain remission of vasculitis in patients with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are associated with substantial morbidity, including opportunistic infections. BK virus (BKV) is an important cause of renal dysfunction in renal transplant (TX) recipients and the intensity of overall immunosuppression is a major risk factor for BKV replication in these patients. The prevalence of BKV replication in immunosuppressed vasculitis patients without transplant is not known.

Methods: We prospectively screened 30 consecutive patients with a diagnosis of GPA or MPA who were on immunosuppressive drug treatment for induction or maintenance of remission for BKV replication. We collected data on demographics, disease duration, organ involvement, renal function and immunosuppressive drug use. Plasma BKV load was measured by quantitative PCR. We compared BKV replication in this non-transplant vasculitis cohort to BKV replication in a historical cohort of vasculitis kidney TX recipients.

Results: Thirty patients (20 GPA, 10 MPA) had mean disease duration of 77 months. Mean age was 51 yrs, 53% female. The mean time from vasculitis onset to BKV testing was 33 months, with 15/30 patients tested within 24 months of induction therapy. At the time of BKV testing, 70% were on prednisone (P) with azathioprine, mycophenolate mofetil (MMF), methotrexate or leflunomide. 30% were on P monotherapy. None of the non-transplanted vasculitis patients had BKV replication in plasma. 5/33(15%) transplanted GPA/MPA patients had BKV replication in plasma or allograft on surveillance testing or when tested to evaluate allograft dysfunction at a mean of 15 months post TX (P=0.05). These patients were on maintenance therapy with MMF, P and tacrolimus.

Conclusions: In immunosuppressed patients with GPA/MPA without renal TX, we found no evidence of BKV replication in the plasma. However, BKV replication was common in GPA/MPA patients after renal transplantation suggesting that BKV replication may be related to specific immunosuppressive agents, alloimmune activation or differences in host defense mechanisms.

Funding: Private Foundation Support

SA-PO318

Prognosis of ANCA-Associated Glomerulonephritis (ANCA-GN) Requiring Dialysis at Presentation Taewoo Lee, Vimal K. Derebail, Caroline Jennette Poulton, Sophia Lionaki, JulieAnne G. McGregor, Susan L. Hogan, J. Charles Jennette, Ronald J. Falk, Patrick H. Nachman. *UNC Kidney Center, University of North Carolina.*

Background: ANCA-GN commonly presents with a rapidly progressive course and severe renal failure requiring dialysis. In such cases, treatment with corticosteroids, cyclophosphamide and plasmapheresis is recommended. This study aims at determining predictive factors of response to therapy and outcomes of patients who required dialysis at presentation.

Methods: The rate of renal recovery, patient and renal survival at 12 months and causes of death were assessed. To determine the prognostic factors of renal survival at 12 months, clinical and pathologic variables at presentation were evaluated as covariates in a binary logistic regression model. The primary outcome was dialysis independence for ≥3 months.

Results: Of 116 patients (mean age 60.4 ± 19.4 yrs; 85% white; 26% treated with plasmapheresis), 54 (46%) recovered renal function in a median of 4.4 weeks [IQR 2 -8.7]; median GFR at recovery 28.9 ml/min/1.73m^2 [IQR 21.9-36.9]. Of these 4 relapsed leading to ESRD. At 12 months, 50 patients (43%) were dialysis-free, 51 (44%) were dialysis-dependent and 21 (13%) had died. The main causes of death were cardiovascular events (n=8, 38%), pulmonary hemorrhage (n=5, 24%) and infections (n=5, 24%). In those who recovered renal function, patient and renal survival rates were 94% and 89% respectively at 12 months and 70% and 61% respectively at 36 months. Compared to patients who recovered renal function, those who did not had significantly greater degree of glomerular sclerosis, interstitial fibrosis, arteriosclerosis, and higher frequency of MPO-ANCA than PR3- ANCA. In a multivariable model, arteriosclerosis (≥ moderate) was an independent predictor of lack of renal recovery after adjusting for the other variables (Odds ratio 0.35, P-value 0.026).

Conclusions: The likelihood of recovery of renal function in patients with ANCA-GN requiring dialysis is about 50%, and is associated with measures of histologic chronicity at presentation, in particular, arteriosclerosis. Renal recovery is associated with a sustained independence from dialysis.

Funding: NIDDK Support

SA-PO319

ANCA-Associated Vasculitis: A Comparison of Patients Presenting to Renal and Rheumatology Services Bairbre A. McNicholas,[1] Tomas Patrick Griffin,[1] Louise Ryan,[1] John Joseph Carey,[2] Louise Giblin,[1] David Lappin,[1] Donal N. Reddan,[1] Matthew D. Griffin.[1] *[1]Nephrology Centre, Galway University Hospital, Galway, Ireland; [2]Rheumatology Centre, Galway University Hospital, Galway, Ireland.*

Background: ANCA associated vasculitis is a life-threatening disease but patients are cared for by different specialities depending on presentation. The aim of this study was to compare patients with ANCA-associated vasculitis under the care of the renal and rheumatology services at a tertiary referral centre.

Methods: A retrospective study of two cohorts with a diagnosis of ANCA positive vasculitis between 1993 and 2012. Cases were identified from biopsy reports and chart review. Presentation, induction and maintenance treatment, relapses, mortality and incidence of major treatment complications between cohorts was compared.

Results: 51 subjects met study eligibility: 30 renal, 19 rheumatology, with mean follow-up time of 41 months and 49 months respectively.

Feature	Renal	Rheumatology	P Value
Mean Age (SD)	63 (10)	52 (12)	0.01
% Female	42	46	0.5
Mean ANCA titre (AU/L)	67	24	0.01
c-ANCA %	40	63	0.14
Mean C-reactive Protein	112	54	0.03
ENT Manifestations (%)	40	73	0.04
% Relapsed	30	57	0.22
Mortality	5(22%)	1(5%)	0.22
% Infection	33	15	0.19
% Cancer	16	0	0.14

All renal patients had biopsy proven glomerulonephritis and 6 rheumatology patients had renal involvement. Renal patients were older, more likely to be p-ANCA positive, and had fewer ENT manifestations. Renal patients were more likely to be treated with IV steroids, IV cyclophosphamide, and plasmapheresis, but less likely to be treated with methotrexate. Renal patients had a trend towards being less likely to relapse but towards increased mortality. Treatment related complications were more common among renal patients.

Conclusions: Patients with ANCA-associated vasculitis represent a heterogenous group who may receive primary care from different medical subspecialties. Compared to rheumatology patients, renal patients were older and more likely to be p-ANCA positive, had lower relapse rates but also higher number of treatment related complications. Differences in speciality based cohorts should be considered in clinical trial design and interpretation.

Funding: Government Support - Non-U.S.

SA-PO320

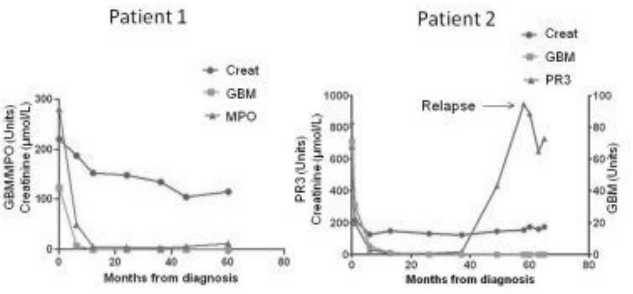
Long Term Outcomes in Patients with Both ANCA and Anti-GBM Antibodies Anisha Tanna,[1] Jeremy B. Levy,[1] Stephen Paul McAdoo,[1] Alan D. Salama,[2] Charles D. Pusey.[1] *[1]Imperial College, London, United Kingdom; [2]UCL, London, United Kingdom.*

Background: Anti-Neutrophil Cytoplasmic Antibodies (ANCA) have been reported in up to 40% of patients with anti-glomerular basement membrane(anti-GBM)disease whilst anti-GBM antibodies are present in 5-10% of patients known to have ANCA positivity. The impact of "double positivity" on long term renal and patient outcome has yet to be fully ascertained.

Methods: A search was conducted on records of patients currently being followed up at our centre with dual antibody positivity. Information on outcomes was collated using both electronic and paper records.

Results: 9 patients were identified, 3 male and 6 female with mean age at presentation of 54 years. 55% of patients were Caucasian,44% Indoasian. 5 patients were positive for both GBM and MPO antibodies and 3 for GBM and PR3. One patient was GBM, PR3 and MPO positive. 8/8 biopsies revealed crescentic glomerulonephritis. 1 patient was too unwell for biopsy. 4 patients were dialysis dependent on presentation. All patients had cyclophosphamide induction and 7 received plasma exchange. The mean follow up was 84 months. 4 patients experienced clinical relapses of their disease (2 GBM/MPO and 2 GBM/PR3). All relapses were associated with recurrence of ANCA not GBM.

Figure 1



7/9 patients currently maintain independent renal function, including 2/4 patients who presented dialysis dependent. The patient with "triple positivity" presented and remained dialysis dependent throughout. Of note, 5 patients developed interstitial lung disease during follow up.

Conclusions: Patients with "dual positivity" are reported to experience poorer outcomes than those with an isolated ANCA. Despite this, 50% of our patients presenting at end stage have maintained independent renal function for at least 5 years. It is important to test for both antibodies on a regular basis in these patients and to tailor treatment accordingly.

SA-PO321

Cardiovascular Outcomes in ANCA-Vasculitis Patients Claudia Yuste,[1] Alina L. Casian,[2] Cristina Jironda,[3] David R.W. Jayne.[2] *[1]Nephrology, Gregorio Marañón, Madrid, Spain; [2]Lupus and Vasculitis, Addenbrooke's Hospital, Cambridge, United Kingdom; [3]Nephrology, Carlos Haya, Malaga, Spain.*

Background: ANCA associated vasculitis (AAV) (granulomatosis with polyangiitis (GPA), and microscopic polyangiitis (MPA)) is associated with an increased frequency of cardiovascular events (CVE). **Objectives:** To identify predictors, including the role of vasculitis therapies, for CVE in AAV.

Methods: A single centre retrospective review of 307 AAV patients (173 GPA, 134 MPA, 47% male, 12% diabetic; mean age 53 [±17] years with follow-up 6.1 (±5.3) years. The primary end-pointy was CVE defined as acute coronary syndrome, new onset angina, symptomatic peripheral vascular disease, stroke or transient ischemic attack, or death. Potential predictive factors were assessed by Cox proportional regression analysis.

Results: 51 CVE occurred in 42 patients (13.6%) with 28 (9%) deaths and the end-point was more frequent in MPA (16.4% and 14.9% respectively) than in GPA patients (11.6% and 4.6%) (p= 0.003). It was associated with lower PR3-ANCA levels in PR3-ANCA patients and higher MPO-ANCA levels in MPO-ANCA patients, assessed at diagnosis (p= 0.011). Independent predictors for the end-point were: maintenance prednisolone dose >5mg/day (hazard ratio (HR) 0.047 (95% CI 0.005-0.418), cumulative cyclophosphamide dose <10g (HR 0.08 [1.5 -7.6]), haemoglobin level at the end of follow up (HR 0.6[0.378-0.96]) and history of prior CVE (HR 2.84 [1.07-7.49]). A cumulative RTX dose <6g was associated with higher prednisolone dose (p=0.016).

Conclusions: Patients with MPA have higher risk of CVE or death than GPA. ANCA level at diagnosis carried different associations in PR3-ANCA and MPO-ANCA patients. A higher maintenance prednisolone dose and lower cumulative cyclophosphamide or RTX dose were also predictors for CVE and death.

SA-PO322

Computerized Instersitial Fibrosis Is the Most Powerful Histological Predictor of Renal Outcome in ANCA-Associated Vasculitis Charlene Levi,[1] Vannary Meas-yedid,[2] Cristina Daniliuc,[1] Luc Mouthon,[3,4] Jean-christophe Olivo-marin,[2] Elsa Guiard,[1,4] Melanie Roland,[1] Loïc Guillevin,[3,4] Christian H. Jacquot,[1,4] Alexandre Karras,[1] Eric Thervet.[1,4] *[1]Nephrology, Hopital Europeen G.Pompidou, Paris, France; [2]Image Analysis, Pasteur Institute, Paris, France; [3]Internal Medicine, Hopital Cochin, Paris, France; [4]Universite Paris Descartes, Paris, France.*

Background: Renal involvement in ANCA-associated vasculitis (AAV) is characterized by necrotizing crescentic glomerulonephritis(GN) and can result in severe renal dysfunction. Although a new pathologic classification of the glomerular lesions has been recently proposed, its predictive value is unknown compared to interstitial fibrosis (IF) or renal function.

Methods: We retrospectively included 78 patients with recently diagnosed AAV and biopsy-proven renal involvement between 2001 and 2011. All renal biopsies were analyzed and classified according to the recently defined classification and divided in focal GN (fGN≥50% normal glomeruli), crescentic GN (cGN≥50% cellular crescent), mixed GN (mGN) or sclerotic GN (sGN≥50% globally sclerotic). Among this population, computerized interstitial fibrosis was analyzed in 62 patients using a specific software already described. To summarize, a cortical section was analysed by a program of colour segmentation imaging, which automatically extracts green colour areas characteristic of IF.

Results: Median follow-up was 22 months. Median creatinine at presentation was 212µmol/l (45 to 1448). Renal prognosis, defined as the proportion of patients with CKD stage 4/5 at 12 months was not statistically different according to the initial glomerular classification. The mean IF was 29±11,6% (range 9-58%). There was no correlation between the glomerular score and computerized IF. Compurized IF % correlated significantly with renal function (eGFR) at baseline and at 12 months ($p<0.001$) and with the risk of CDK 4/5 at 12 months. Baseline GFR also correlated with 1-year GFR ($p<0.001$).

Conclusions: In our cohort of patients, the glomerular classification of renal AAV doesn't predict the 12-months renal outcome. Initial renal function and computerized IF quantification are better prognostic markers than glomerular lesions in AAV.

Funding: Clinical Revenue Support

SA-PO323

Renal Histopathological Classification of ANCA Associated Glomerulonephritis: A Validation Study of 92 Patients

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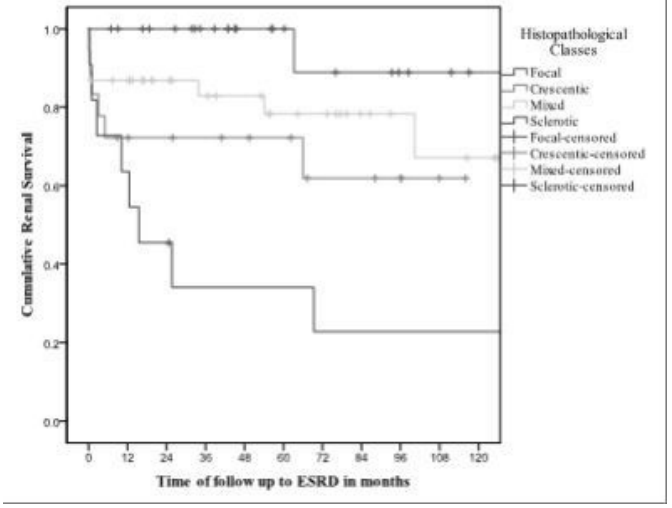
Background: A novel histopathological classification was proposed for ANCA associated glomerulonephritis (GN): focal (>50% normal glomeruli), crescentic (affecting >50% of glomeruli), mixed and sclerotic (>50% sclerosed glomeruli). We performed a validation study to determine the prognostic value of the classification.

Methods: 92 renal biopsies with ANCA+ GN were classified retrospectively, with a mean number of 18 glomeruli. eGFR was corrected for baseline eGFR. Age,sex,ANCA subtype, baseline eGFR and histological category were covariates in the Cox regression model. Renal survival was assessed by Kaplan-Meier analysis, comparing histological classes using the log rank test.

Results: Mean follow-up 62 months. Median age 61.9 years, 53.3% male, 61.9% microscopic polyangiitis vs 38% granulomatosis with polyangiitis. 26% of biopsies were classified as focal, 20% crescentic, 14% sclerotic, 40% mixed. Renal survival at 1 year was 100% in the focal class, 86% in mixed, 72% in crescentic and 61% in sclerotic class (p=0.002).



Median Baseline eGFR (ml/min)	Focal 50.0 ± 30.4	Crescentic 12.7 ± 7.8	Mixed 15.5 ± 17.2	Sclerotic 16.54 ± 11.2
Median eGFR at 5y (ml/min)	50.4 ± 26.1	51.7 ± 28.4	35.6 ± 17.8	16.75 ± 15.84

The sclerotic group displayed a higher mortality (38%) than average (23%)($p<0.05$).

Conclusions: Similar to the previous study the focal group did best, and the sclerotic group worst in terms of ESRD. In contrast, our crescentic group had higher ESRD rates than the mixed group, which we associate with lower baseline eGFR. However, those crescentic patients without ESRD had better recovery than the mixed group, due to the predominance of reversible acute lesions. Renal histology in conjunction with baseline eGFR predicted renal outcome better than eGFR alone.

SA-PO324

New Histopathologic Classification of Anti-Neutrophil Cytoplasmic Antibody Associated Pauci-Immune Glomerulonephritis: Correlation with Renal Outcome

Carla L. Ellis, Rebecca Manno, Lorraine C. Racusen, Duvuru Geetha. *The Johns Hopkins Hospital and School of Medicine, Baltimore, MD.*

Background: Renal biopsy is of diagnostic and prognostic value in ANCA associated glomerulonephritis and a new classification for prognostication of pauci-immune glomerulonephritis (GN) based on four categories (Mixed, Crescentic, Sclerotic and Focal) was proposed by an international working group of renal pathologists (IWGRP). The goal of our study was to apply the proposed classification system to a United States cohort of vasculitis patients and correlate IWGRP class to the estimated glomerular filtration rate (eGFR) at baseline, six months and one year.

Methods: Cases of pauci-immune glomerulonephritis diagnosed from 1995 to 2011 were identified and clinical data were collected. Histology was reviewed by a pathologist and classified according to the new classification. MDRD formula was used to calculate e-GFR. We correlated IWGRP class to renal function at presentation, at six months and at one year. X^2, one way ANOVA and linear regression analysis were performed as appropriate.

Results: Seventy-six cases of pauci-immune glomerulonephritis were identified; mean age: 58 years, 57% males, 82% Caucasian. 82 %were ANCA positive. Seventy-four patients were treated with induction therapy. Renal biopsies were categorized as focal: n=21, crescentic: n=18, mixed: n=27, sclerotic: n=10. The baseline e-GFR was lowest in the crescentic class and highest in the focal class. Table 1 shows the renal outcome according to class. In linear regression analysis investigating e-GFR at 6 months and 1 year; age, baseline e-GFR and renal biopsy category were independent predictors of e-GFR at both time points.

Conclusions: The e-GFR at diagnosis and the IWGRP class were predictors of e-GFR at 1 year, making both values important predictors of long term renal outcome in ANCA associate vasculitis patients.

Table 1

Class	Baseline e-GFR - mean (SD)	1 year e-GFR - mean (SD)
Focal (n=21)	50 (62)	69 (38)
Crescentic (n=18)	16 (10)	38 (26)
Mixed (n=27)	27 (23)	36 (23)
Sclerotic (n=10)	25 (15)	27 (17)

SA-PO325

Renal Function and Sclerotic Glomerular Injury Predicts Poor Prognosis in Patients Presenting with Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis

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Background: In ANCA associated vasculitis (AAV) treatment regimes are universally applied; no laboratory or histological variables have proven prognostic value. An international collaborative pathology group has published a predictive glomerular classification which requires validation. We sought to determine predictors of mortality and renal outcome in AAV.

Methods: 113 consecutive AAV patients (1993-2011) who had adequate renal biopsies and clinical information from a single centre were studied. Demographics, laboratory results and outcomes were collected. Kidney biopsies were classified, by three pathologists blinded to clinical data, into the patterns: focal, mixed, crescentic and sclerotic. The primary outcome was time to end stage renal disease (ESRD) or all cause mortality modelled using Cox regression analysis.

Results: Sclerotic injury and reduced GFR at presentation predicted poor outcomes. Median age was 66 years (IQR 56-74) with follow up of 3.9 years, 58% were male. Biopsies were: focal (26%), mixed (29%), crescentic (29%) and sclerotic (16%). No difference was seen amongst the groups for: age, sex or ANCA subtype. Compared to sclerotic injury, focal (HR 0.29, 95% CI 0.12-0.68, P<0.01), mixed (HR 0.37, CI 0.17-0.84, P<0.05) and crescentic (HR 0.46, CI 0.21-0.99, P<0.05) injury had a lower risk of ESRD/death, after age adjustment. Median GFR at presentation was different amongst biopsy groups (focal 35mls/min, mixed 22mls/min, crescentic 9mls/min and sclerotic 9mls/min $p < 0.001$). The addition of baseline GFR, the strongest predictor of ESRD/death (HR 0.96 per 1ml/min increase in GFR, CI 0.93-0.98 $p = 0.001$) attenuated the prediction of outcome by biopsy classification. Interestingly, crescentic injury was associated with a short duration of symptoms and increased prevalence of lung involvement (P<0.05).

Conclusions: Reduced presenting GFR and a sclerotic pattern of glomerular injury predict poor patient and renal survival. Treatment protocols should be adjusted accordingly.

Funding: Government Support - Non-U.S.

SA-PO326

Anti Neutrophil Cytoplasmic Antibody Vasculitis in a Single Centre in North West UK and Outcomes Following Rituximab or Cyclophosphamide

Mrityunjay Hiremath, Noshaba Naz, Harsha Wodeyar, Victoria Stewart, Neville Nicholas, Yaser Shah, Anindya Banerjee. *Wirral University Hospital NHS Foundation Trust.*

Background: The annual incidence of ANCA associated systemic vasculitis is around 20/million in UK.We noticed high number of patients with ANCA positivity in our centre. Some were treated for systemic vasculitis with Rituximab as first line while rest were treated with Cyclophosphamide.

Methods: Local incidence of ANCA positive systemic vasculitis.Renal outcome in each treatment arm (Rituximab vs Cyclophosphamide) -Retrospective analysis of patients with ANCA-associated systemic vasculitis between 2007-2011. Data was collected using Hospital Electronic record system.We analysed data for relapse, creatinine levels, ESRD leading to dialysis and deaths.

Results: 77 patients were identified to have ANCA positivity between 2007-2011,an incidence of 140/million population over 5 years. -45 patients with renal failure and systemic features were treated.Incidence of ANCA associated systemic vasculitis requiring immunosuppressions at our centre ranged between 7/million in 2007 to 23/million in 2010. Age of 70 or more with creatinine higher than 550 micromols/L on presentation predicted ESRD requiring dialysis. Most relapses occurred more than 2 years after initial treatment with Rituximab, Only 1 patient relapsed in Cyclophosphamide group.There was no significant difference in relapse rates, deaths or ESRD between 2 groups.

Conclusions: Rituximab is not superior to Cyclophosphamide as an induction agent for treating ANCA associated systemic vasculitis. We had similar incidence rates of systemic ANCA vasculitis as rest of UK.

(Table 1)sets out results in 45 patients

Patient Characteristics	Rituximab (n=31)	Cyclophosphamide(n=14)	p -value
Age(years)	67.6	72.4	0.21
Sex	17 M ; 14 F	8 M ; 6 F	
Creatinine(micromoles/ litre)	501	554	0.60
Plasma Exchange	6	0	
Lung Haemorrhage	3	0	
Relapse	7 (22%)	1 (7%)	0.4
Pts. requiring dialysis	9 (29%)	2 (14.28%)	0.3
Dialysis dependant on presentation	12 (38.7%)	2 (14.28%)	0.37
Dialysis dependant at 3 months	9 (29%)	2 (14.28%)	0.3
Survival 1 year	22(70.96%)	12(85.71%)	0.19
Death	9 (29.04%)	2 (14.29%)	0.3
Initial therapy	Methyl prednisolone	Methyl prednisolone	

SA-PO327

Vaccine Responses in ANCA Vasculitis Patients Matthew David Morgan, Alex Richter, Constantina Paulo Yiannakis, Lorraine Harper. *University of Birmingham.*

Background: Serious infection is a significant cause of morbidity and mortality in immunosuppressed patients with antineutrophil cytoplasm antibody associated vasculitis (AAV). Vaccination is recommended to reduce the incidence of infection. This study explores AAV patient responses to routine vaccination and factors associated with infection and poor vaccine responses.

Methods: All patients attending a tertiary centre vasculitis clinic were offered vaccination with conjugate vaccines against pneumococcus, haemophilus and meningococcus as routine care if in remission for more than 6 months, not pregnant and not within 6 months of rituximab or cyclophosphamide therapy. Functional IgG antibodies were measured at baseline, 4, 8 and 16 weeks post vaccination. Serum IgG and peripheral blood lymphocyte subsets were measured at baseline. Clinical data was collected for cumulative steroid and cyclophosphamide exposure, previous relapse rates and infective episodes. Patients were followed for 2 years post-vaccination. Ethics committee approval and patient informed consent was obtained for the study.

Results: 92 patients (median age 65, median 6 years post diagnosis, 52 men, 55 granulomatosis with polyangiitis, 22 microscopic polyangiitis, 11 other) participated. 22% had serum IgG<6g/L, 55% had lymphopenia and 73% low B cell counts at baseline. Serum IgG correlated with B cell count (correlation coefficient (cc)=0.3; p<0.01), CD4 count (cc=0.3; p<0.01) and maintenance immunosuppression.

Improvement in functional antibody titres following vaccination correlated with serum IgG (cc=0.4; p<0.01), B cell count (cc=0.35; p<0.01) and withdrawal of immunosuppression (no immunosuppression vs azathioprine or mycophenolate p=0.04). No increase in annual relapse rate was seen following vaccination (0.15/year pre vs 0.12/year post vaccination; p>0.05). Infection rate correlated with cumulative steroids (cc=0.24; p=0.02), lymphocyte count (cc=-0.23; p=0.04), serum IgG (cc=-0.2; p=0.06) and continued immunosuppression.

Conclusions: AAV patients on continued immunosuppression with impaired immune function have increased infection rates and poor responses to routine vaccination.

Improved strategies are needed to reduce infection risk in this group of patients.

SA-PO328

Infection and Mortality in Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis Roberto Negrete, JulieAnne G. McGregor, Caroline Jennette Poulton, Yichun Hu, Patrick H. Nachman, Ronald J. Falk, Susan L. Hogan. *Division of Nephrology and Hypertension, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Infectious complications of immunosuppression impacts patient outcomes in anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV).

Methods: An inception cohort of 225 patients diagnosed with AAV from 2000-2011 and treated with immunosuppressive therapy (methylprednisolone pulses 72%, prednisone 96%, intravenous (IV) cyclophosphamide 67%, oral cyclophosphamide 51%, azathioprine 42%, mycophenolate mofetil 44% and rituximab 22%) was studied. Number of infections, severe infections (requiring IV antibiotics or intensive care unit [ICU] hospitalization), leukopenia, number of relapses, diabetes mellitus (DM), use of prophylactic antibiotic therapy (trimethoprim/sulfamethoxazole, atovaquone, dapsone), and death were recorded. Patients were analyzed based on number of infections: 0, 1 to 2 or >3. Statistical comparison between the groups were done with Fisher's exact test or Kruskal-Wallis test.

Results: A total of 417 infectious episodes were recorded over a median follow-up of 26 months (IQR 13,52). Most frequent infections found were bronchopulmonary infections (34%). There was no difference with respect to age at the time of diagnosis, sex, or ANCA specificity between the 3 groups. See table for comparisons across the groups.

Outcomes with Respect to Infection Occurrence

	0 Infections (N=66)	1 to 2 Infections (N=137)	≥3 Infections(N=22)	p values
PR3/C-ANCA positivity	26(40%)	60(44%)	10(45%)	0.87
ICU Hospitalizations	0(0%)	14(10%)	7(32%)	<0.0001
IV Antibiotics	0(0%)	53(39%)	13(60%)	<0.0001
DM at time diagnosis	4(6%)	11(8%)	1(5%)	0.92
New onset DM	21(32%)	50(37%)	11(50%)	0.29
Prophylactic Antibiotic Therapy	28(42%)	56(41%)	12(55%)	0.5
Leukopenia	20(30%)	64(47%)	12(55%)	0.04
Death	1(2%)	14(10%)	2(9%)	0.05
Number of Relapses	0.3±0.6	0.7±1.2	1.45±1.3	0.0001

Conclusions: In AAV, leukopenia and relapses were associated with an increased number of infections. Infections were also associated with a greater mortality risk. Higher number of infections increased the likelihood of severe infections. Broncopulmonary infections were the most common found in our cohort.

SA-PO329

Audit of Infective Complications of Immunosuppressive Therapy in Vasculitis and Relationship with Lymphocyte Count Memuna Hussain, Ajay Prabhakar Dhaygude. *Renal Medicine, Royal Preston Hospital, Preston, United Kingdom.*

Background: Cyclophosphamide (CYP) has transformed ANCA associated systemic vasculitis (AASV), from a potentially fatal disease to a relapsing remitting condition. However, treatment-related toxicity remains a major concern and recently published meta-analysis of four EUVAS trials suggest that infection has overtaken active vasculitis as the leading cause of death during induction therapy, accounting for majority of deaths in the first 12 months. Cyclophosphamide, an alkylating agent, interferes with DNA transcription and replication and predominantly causes lymphopenia. In this retrospective study we analyzed the relationship between lymphocyte counts and major infections in AASV patients.

Methods: Data regarding demographics, renal function, details of CYP treatment, serial total and differential nadir white cell count and infective complications was collected for 71 consecutive patients with AASV treated between 2006 and 2010. Major infections were defined as those requiring hospitalization and/ or intravenous antibiotics.

Results: Median age was 62 years. Twenty-two episodes of major infections occurred in 21 patients. There was a significant difference in the mean lymphocyte count between patients who had major infections and patients who did not have major infections. Average lymphocyte count for patients with major infection group was 0.76 and for patients with no infection group it was 1.21. (p=0.0005 assuming unequal variances).

Conclusions: Patients with lower lymphocyte counts during cyclophosphamide treatment were more likely to have major infections. Current protocol suggests adjustment of cyclophosphamide dose with respect to the age, renal function and total white cell count. Adjusting the dose with respect to the nadir lymphocyte count may reduce the infective complications in this patients.

References: Long-term patient survival in ANCA-associated vasculitis. Flossmann O et al. Ann Rheum Dis.2011 Mar;70(3):488-94. Epub 2010 Nov 24.

SA-PO330

Increased Prevalence of Antineutrophil Cytoplasmic Antibodies Associated Renal Vasculitis (AAV) in Immigrant Central & South American Patients (CSA) Sudhanshu Jain, George N. Coritsidis, Salwa Rhazouani. *Elmhurst Hospital Center, New York, NY.*

Background: AAV is uncommon with most epidemiologic studies arising from Europe, United States and Australia. Data from South America is limited. The US 5-year period prevalence rate (PPR) is 26/million and 30/million in New York City (NYC). Elmhurst and Queens Hospital Centers serve the most ethnically diverse county in the US, with a large CSA population. Noting an increased incidence of AAV in CSA, we were interested in assessing their course and prevalence.

Methods: Review of all renal biopsies (2001 – 2011) was undertaken assessing: age, gender, proteinuria (gms/d), MDRD glomerular filtration rate (eGFR, ml/min/1.73m^2), renal outcomes and histology [granulomatosis polyangiitis (GPA) vs microscopic polyangiitis (MPA)] at presentation. Data presented as average ± standard error of mean. PPR for 2006-11 was calculated using our demographic data from Queens's community district 4 of the NYC department of city planning.

Results: Of 165 biopsies reviewed: AAV represented 8% (n= 13); 16% of all CSA biopsies (n=71) and 2% of all non-CSA biopsies (n=94). In CSA, AAV was the second most common glomerulonephritis after SLE (29.7% vs. 45%). Among 11 CSA with AAV, 55% (n=6) had MPA and 45% (n=5) GPA. CSA age was 54.4 ± 5.5 years with females (n=6) presenting older (60.8± 8.2 vs 47± 6.3). Proteinuria and eGFR were 4.4 ± 1.1 and 11.4 ± 2.1, respectively. Pulmonary symptoms were present in 77% (n=7). In addition to standard treatment with cyclophosphamide and prednisone, two patients received plasmapharesis. 45% (n=5) presented with eGFR<8 and were the only dialysis dependent (DD) patients. MPA were older (60.5 ± 8.2 vs. 47.4± 6.5 yrs), female (83% vs 20%) and had better renal outcomes (DD 33% vs 60%) when compared to GPA patients. Local Queens PPR of AAV was 42/million and for CSA 80/million.

Conclusions: PPR of AAV in CSA was almost 4 times greater than that of the US and almost 2 times greater than non-CSA Queens' patients. Unlike other populations, CSA had worse renal function and were more likely to become DD. This may be related to aggressive disease or delayed access to medical care. CSA with MPA were older, female and had better outcomes than GPA.

SA-PO331

The Deleterious Impact of Anti-Neutrophil Cytoplasmic Antibody on the Outcome of Lupus Nephritis: A Case-Control Study Vivian Lumi Onusic, Maria Julia C.L.N. Araujo, Ligia Costa Battaini, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, School of Medicine University of Sao Paulo, Sao Paulo, Brazil.*

Background: Few studies have analyzed the impact of anti-neutrophil cytoplasmic antibody (ANCA)on the outcome of lupus nephritis (LN). The aim of this study was to evaluate the influence of ANCA Seropositivity in the renal outcome of LN.

Methods: A retrospective analysis was carried out on all SLE patients (345) submitted to a kidney biopsy between 1999-12. Patients that fulfilled ACR lupus criteria and tested for ANCA were enrolled. Positive ANCA patients (POS) were randomly matched to ANCA seronegative patients (NEG) according to the type of LN and baseline clearance(MDRD simplified formula). Clinical and laboratory data were collected at baseline, after one year and at the end of follow up. Treatment was decided by the clinical staff based on conventional literature protocols.

Results: We included 128 patients (32 POS/96 NEG). Perinuclear ANCA was detected in 87,5% (n=28) of POS patients. At baseline, POS and NEG groups were similar regarding age, complement level, ANA, anti-DNA antibody, eGFR (46±36vs44±29ml/min/1.73), proteinuria (3.4±2.6 vs 4.6±4.4 g/day), WHO LN classes, histological activity index, chronicity index, vascular lesions and follow up time. Interestingly, after one year of follow up, Pos group was significantly associated with a lower serum C3 (86,7±22vs106,5±32mg/dl p=0,01) and positive Anti-dsDNA (66%vs31% p<0,01). At the end of follow up, the POS group showed a tendency to have a lower eGFR (56±37vs71±36ml/min/1.73 p=0.09) as well as more patients with eGFR<60ml/min(56,3%vs33,3% p=0,03). Finally, logistic regression analysis showed that ANCA is an independent predictor of eGFR<60ml/min during follow up, even after adjustments for inicial eGFR and chronicity index (table 1).

Logistic Regression Analysis

	OR	95%IC	p
ANCA	3,62	1,3-9,96	0,013
Inicial eGFR	0,98	0,97-1,0	0,064
CI	1,4	1,1-1,7	0,002

Conclusions: In our study, positive ANCA was significantly associated with a worse renal outcome of LN when compared to matched negative ANCA patients.

Funding: Government Support - Non-U.S.

SA-PO332

Rituximab in Lupus Nephritis: Analysis of Clinical Variables Associated with Renal Response in African-Americans in the LUNAR Trial Kajal Rao,[1,2] Maria Dallera,[3] David Wofsy,[3] Brad H. Rovin,[4] Romeo Maciuca,[1] Paul Brunetta.[1] *[1]Genentech, South San Francisco, CA; [2]Nephrology, UCSF, San Francisco, CA; [3]Rheumatology, UCSF, San Francisco, CA; [4]Nephrology, Ohio State University, Columbus, OH.*

Background: LUNAR was a randomized trial evaluating the efficacy of add-on therapy with rituximab (RTX) to MMF and prednisone in patients (pts) with active class III-IV (+/- V) lupus nephritis. The overall renal response (ORR) endpoint was not met (56.9% and 45.8%, p=0.18). But, in a pre-specified analysis of African-Americans (AA), week 52 ORR was higher with RTX (70%) vs. placebo (Pbo, 45%) (p=0.20). This difference was driven by a higher partial response rate (35% versus 5%). Our aim was to characterize the clinical parameters that might have accounted for this difference.

Methods: We undertook an exploratory post-hoc analysis of the 40 AA pts from LUNAR, comparing RTX versus Pbo, and comparing those with ORR to non-response (NR).

Results: Baseline characteristics were similar between the Pbo and RTX arms (data not shown). Patients with NR (vs. ORR) had higher serum Cr, proteinuria, and BP.

Table 1: Baseline Characteristics of African-Americans

Baseline Values	Non-response (n=17) (placebo n=11; rituximab n=6)	Overall Renal Response (n=23) (placebo n=9; rituximab n=14)
Serum Cr[a]	1.27 (.96-1.58)	0.95 (.81-1.10)
Proteinuria (UPCR)[a]	4.4 (2.87-5.94)	3.0 (2.26-3.81)
SBP (mmHg)[a]	142 (130-153)	124 (115-132)
DBP (mmHg)[a]	88 (80-96)	77 (72-83)
HTN [b]	65% (11/17)	17% (4/23)

[a]Values reported as means with Confidence intervals. [b]HTN defined as either SBP >= 140 or DBP >= 90

None of the 8 pts with partial renal response had hypertension (HTN), and 7 of these 8 pts were in the RTX arm. Over 70% of pts without HTN (79% RTX, 73% Pbo) achieved a renal response versus only 27% of those with HTN (3/6 RTX, 1/9 Pbo).

Conclusions: AA pts with HTN, high proteinuria or high serum Cr had markedly lower renal response rates in LUNAR regardless of treatment arm. This however was not seen consistently in the non-AA subgroup in LUNAR (data not shown). Thus, the interpretation of the results, and its implications regarding treatment with RTX are uncertain, particularly in the context of the small size of the AA subgroup.

Funding: Pharmaceutical Company Support - Genentech

SA-PO333

Abstract Withdrawn

SA-PO334

Mycophenolate Sodium (MPS) versus Cyclophosphamide (CY) in Lupus Nephritis (LN): Histopathology after Induction Ana Valeria Malvar Perrin,[1] Bernarda Fazzini,[1] Bruno Jorge Lococo,[1] Paola Pirruccio,[1] Cecilia Aleman Cuestas,[1] Ricardo M. Heguilen,[1] Valeria Gabriela Alberton,[2] Brad H. Rovin.[3] *[1]Nephrology, Hospital Fernandez, B. Aires, Argentina; [2]Pathology, Hospital Fernandez, B. Aires, Argentina; [3]Nephrology, Ohio State University, Columbus, OH.*

Background: Mycophenolic acid has been shown to be an alternative for the treatment of LN.The purpose of this study was to compare the histopathological response to treatment with ivCY or MPS evaluated through the changes in the activity(AI) and chronicity(CI) indices in paired renal biopsies.

Methods: All biopsies performed in patients with LN between January 2008 and December 2011were reviewed.The samples were categorized according to the ISN/RPS classification and the AI and CI were calculated by a pathologist blinded to induction therapy.Thirty nine Latin patients with class IV LN were included. Mean age was 30±6 and GFR was ≥75 ml/min.Induction therapy was ivCY(1 gm/mo X 6) or MPS(1440 mg/dX 6 mos).Mixed class V and crescentic LN were excluded.Complete remission (CR) was considered a decrease in proteinuria to ≤ 0.5 gm/day and GFR ≥75 ml/min. Data are expressed as mean±SD or median (range).

Results:

Results are presented in the table.

	sex M/F	Proteinuria 1st Bx gm/day	Proteinuria 2nd Bx gm/day	AI 1st Bx median	AI 2nd Bx median	CI 1st Bx median	CI 2nd Bx median
ivCY(n=23)	1/22	3,5 ± 2	1 ± 1,1	10(3-16)	4(0-9)	3(1-6)	4(2-6)
MPS(n=16)	2/14	3,1 ± 1,5	0,8 ± 0,7	9(6-16)	4(1-9)	3(0-5)	4(3-6)

After 6 months the fall in AI and the changes in the CI were not different between both therapies. Eleven patients who received ivCY and 7 who received MPS achieved CR. The AI in the remitters was similar to that of the non-remitters (R: ivCY: 4(0-8) MPS: 4(2-6); NR: ivCY: 4(0-8) MPS: 4(2-9)). Also, there was no difference in the CI of remitters and non-remitters.

Conclusions: MPS and ivCY induction therapy achieved the same level of AI reduction after 6 months, and the same number of patients attained CR. Despite achieving CR based on proteinuria and GFR, remitters still had considerable histologic activity in their biopsies, suggesting that clinical markers alone are insufficient to follow response to therapy in LN.

SA-PO335

Mycophenolate Mofetil (MMF) versus Cyclophosphamide (CY) as Induction Therapy in Severe Lupus Nephritis (LN) Brad H. Rovin,[1] Samir Parikh,[1] Lee A. Hebert,[1] Daniel Tak Mao Chan,[2] Chi Chiu C. Mok,[3] Ellen M. Ginzler,[4] Lai Seong Hooi,[5] Paul Brunetta,[6] Romeo Maciuca,[6] Neil Solomons.[7] *[1]Internal Medicine, The Ohio State Medical Center, Columbus, OH; [2]University of Hong Kong, Hong Kong; [3]Tuen Mun Hospital, Hong Kong; [4]State University of New York Downstate Medical Center, Brooklyn, NY; [5]Sultanah Aminah Hospital, Johor Bahru, Malaysia; [6]Genetech, Inc, San Francisco, CA; [7]Aspreva International, LTD, Victoria, BC, Canada.*

Background: Clinical trials have shown that MMF compares favorably to CY for remission induction of mild-moderate LN, but its role in severe LN is unclear.

Methods: A systematic review of outcomes after treating severe LN with MMF or CY was done using published or personal data. Severe LN was defined as indicated in table 1. Partial and complete remissions were determined and long-term outcomes compared.

Results: The pooled results suggest MMF and CY are equally effective in inducing remission (Table1). Long-term data, available from 4 studies, showed CY was associated with fewer LN relapses and less risk for ESRD (Table2).

Conclusions: MMF and CY appear equal in inducing remission for severe LN, but over time renal function may be better preserved with CY.

Table 1

Criteria of Severe LN	Treatment	N	PR (%)	CR (%)	Response Time (Mo)
Class IV; >50% crescents; SCr ≥1.24mg/dl	MMF IVCY	26 23	19.2 43.5	53.8 26.1	12 12
Class IV; >60% cap necrosis; 20% crescents; Mean SCr 1.51mg/dl	MMF IVCY	9 11	29 27	44 0	6 6
Class IV; Mean SCr 1.2mg/dl; persistent/relapsing LN	MMF	3	0	0	3-15
Class III/IV; Mean SCr 1.87 mg/dl; persistent/relapsing LN	MMF	5	20	40	4-16
Class III/IV LN; Mean SCr 1.69 mg/dl	MMF	29	34.5	17.2	12
Class IV LN; Mean SCr 1.3mg/dl	IVCY POCY	22 21	14 21	59 57	24 24
Class IV LN; SCr>1.2mg/dl	IVCY or POCY	78	46	40	6
Proliferative LN; SCr ≥1.14 mg/dl	MMF	14	29	57	6
Class III/IV LN; mean SCr 1.52mg/dl for MMF 1.46mg/dl for POCY	MMF POCY	11 10 20 16	55 20 55 50	9 40 25 38	6 12 6 12
Proliferative LN; mean SCr 1.48mg/dl for MMF, 1.69mg/dl for IVCY	MMF IVCY	13 8	50 50	13 0	6 6
Proliferative LN with CrCl < 60ml/min	MMF IVCY	6 8	33 38	0 0	6 6

Table 2

Study et al	Treatment	N	Relapse	Median Relapse Time (mo)	ESRD Risk (%)	F/U (mo)
Koo et al	MMF, IVCY	20, 51	15%, 4%	NA	20%, 0%	60
Chan et al	MMF, IVCY-AZA	32, 30	28%, 26.6%	20.2±13.4, 32.7±17.9	0%, 6.7%	63
Chan et al	MMF, POCY	21, 36	50% Combined	35±19, 62±26.7	NA	72
Dooley et al	IVCY + MMF/AZA, MMF + MMF/AZA	107, 120	19.2%, 30.2%	NA	NA	36

SA-PO336

Therapeutic Effect of FK506 on Murine Lupus Nephritis and Its Mechanisms Haiyan Tu, Jianghua Chen. *Zhejiang University, The First Affiliated Hospital, College of Medcine, Zhejiang University, Hangzhou, Zhejiang, China.*

Background: To systematically evaluate the clinical effects of FK506, which are calcineurin inhibitors, on progression of renal damage and its immunopathology in MRL-lpr/lpr lupus-prone mice and to explore the related mechanisms.

Methods: We treated MRL/lpr mice with FK506 intraperitoneally at dose of 2mg/kg/day, starting at week 8 of age and continuing through week 12.We evaluated level of proteinuria, urinary creatinine, serum anti-dsDNA antibody, and kidney histopathology changes. We also determined the expression and localization of calcineurin with synaptopodin in addition of the effect of FK506 on them.

Results: After 4 weeks of FK506 therapy, significant reduction in proteinuria was achieved compared with vehicle-treated control mice (0.29 ±0.20 g/24h vs 0.91±0.24g/24h., p< 0.01). In parallel, a significant decrease in urinary creatinine is found (1.23±0.72 umol vs 3.01±0.11 umol, p< 0.01). Meanwhile, the serum anti-dsDNA antibody is lower in FK506 treatment group than the control mice (73.5±31.7 u/ml vs 119.9 ±16.4 u/ml, p <0.01) and histopathology renal lesions are also less severe. Double immunofluorescent staining shows calcineurin colabeling with synaptopodin. Calcineurin increased expression in lupus-prone mice compared to controls. In contrast, synaptopodin diminished and distributed discontinuously. However, we observed decreased expression of calcineurin, accompanied by the increased expression of synaptopodin after FK506 treatment.

Conclusions: FK506 suppressed the development of kidney disease in MRL/lpr lupus-prone mice. Mechanistically, decreased glomerular expression of calcineurine which might protect synaptopodin from degradation may partly explain the antiproteinuric effects of FK506 in our experiment. We propose that FK506 may represent a valuable treatment for patients with lupus nephritis.

SA-PO337

Outcome of Long-Term Treatment with Mycophenolate Mofetil in Patients with Severe Lupus Nephritis Desmond Y.H. Yap, Maggie Kam Man Ma, Daniel Tak Mao Chan. *Department of Medicine, Queen Mary Hospital, Hong Kong, China.*

Background: Corticosteroids with mycophenolate mofetil (MMF) has proven efficacy when used as induction or maintenance immunosuppression for proliferative lupus nephritis, but data on long-term mycophenolate mofetil treatment is lacking.

Methods: This was a single-centre retrospective study of patients with Class III/IV±V lupus nephritis who have received prednisolone and MMF continuously as treatment in the early phase and the long-term maintenance phase.

Results: 65 patientswere included. 31 patients were treated with only prednisolone and MMF throughout (Group I). 23 patients had their MMF substituted with azathioprine (AZA)(Group II) and 11 patients with calcineurin inhibitors (CNI)(Group III) at some point during the maintenance phase. The follow-up was 91.9±47.7 months. The 10-year patient and renal survival rates were 91% and 86% respectively, with no difference between the three groups. Relapse-free survival was higher in Group I than Group II and Group III (76% vs 56% vs 43% respectively at 5-year; 69% vs 32% vs 0% respectively at 10-year; I vs II, p=0.049; I vs III, p=0.019; II vs III, p=0.490). Patients who received MMF for more than 24 months showed better relapse-free survival than those treated for shorter durations (88% vs 48% respectively at 5-year; 81% vs 28% respectively at 10-year; p<0.001). Serum creatinine was lower in Group I at 10 years. Apart from more anemia with MMF treatment, the side-effects profile was similar in patients during MMF, AZA, or CNI treatment.

Conclusions: Continuous treatment with corticosteroids and MMF as both induction and long-term maintenance immunosuppression is associated with favorable long-term outcome and possibly a lower risk of disease flare compared with other maintenance immunosuppressive medications.

SA-PO338

Efficacy and Safety of Multitarget Therapy by Combination with Tacrolimus, Mycophenolate Mofetil and Steroid in Patients with Active Lupus Nephritis Hidekazu Ikeuchi, Keiju Hiromura, Satoshi Takahashi, Keiichiro Mishima, Noriyuki Sakurai, Toru Sakairi, Yoriaki Kaneko, Akito Maeshima, Yoshihisa Nojima. *Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: In a previous study, we reported that tacrolimus (TAC) was effective as induction therapy for active lupus nephritis (LN). The complete remission (CR) rate was 84.6% (11/13 patients), which was achieved at 7.7 months in average (Mod Rheumatol 2011). In this study, we retrospectively analyze the efficacy and safely of multitarget therapy using TAC, mycophenolate mofetil and prednisone as induction therapy for active LN.

Methods: All 16 consecutive patients were included, who were treated with the multitarget therapy for active LN between 2009 and 2011 in our department.

Results: The mean patient age was 33.7 years (range, 21-50). Eight patients were treated for LN flares. The mean serum creatinine level and urinary protein/creatinine ratio before treatment were 0.73 mg/dl (0.45-1.36) and 4.6 g/gCr (1.21-10.79), respectively. The renal biopsy was performed on all patients; III (n=1), III+V (n=1), IV (n=6), IV+V (n=6), V (n=2), according to the ISN/RPS2003 classification. All patients achieved CR at 4.1 months in average (Figure). There was one serious adverse event; gastric ulcer due to cytomegalovirus infection, which was successfully treated by anti-viral therapy and proton pump inhibitor. Cytomegalovirus antigenemia was observed in 5 patients without signs of serious organ damage expect for thrombocytopenia, which was also recovered by anti-viral therapy.

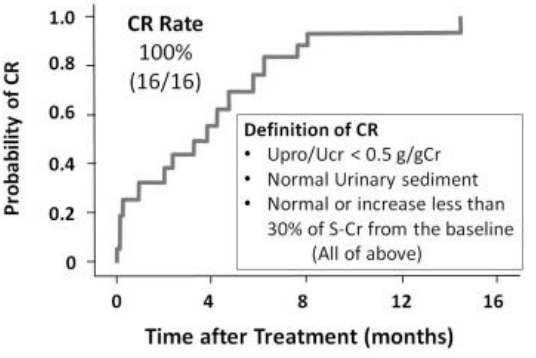


Figure. Probability of Complete Remission (CR)

Conclusions: The multitarget therapy was effective as induction therapy for active LN to achieve early remission and high remission rate. Although this therapy was generally well-tolerated, the potential risk of cytomegalovirus infection should be taken into consideration.

Funding: Pharmaceutical Company Support - Astellas Pharma Inc, Mitsubishi Tanabe Pharm, MSD, Takeda Pharmaceutical Company Limited, Kyowa Hakko Kirin Co., Chugai Pharmaceutical Co., Teijin Pharma Limited, Daiichi Sankyo Co., Eizai Co., Torii Pharmaceutical Co., Asahi Kasei Co., Government Support - Non-U.S.

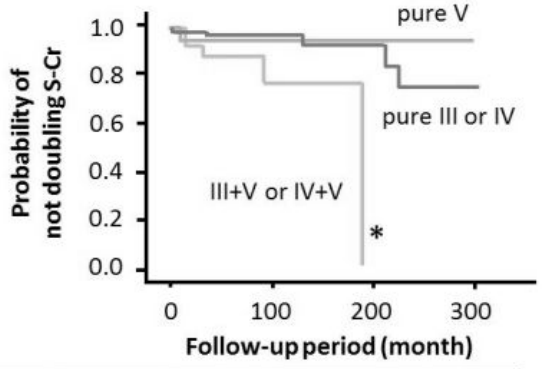
SA-PO339

Mixed Lesion of Membranous (Class V) and Proliferative (Class III/IV) Lupus Nephritis Predicts Poor Renal Prognosis Hidekazu Ikeuchi, Keiju Hiromura, Satoshi Takahashi, Keiichiro Mishima, Noriyuki Sakurai, Toru Sakairi, Yoriaki Kaneko, Akito Maeshima, Yoshihisa Nojima. *Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: We have previously reported that glomerular chronic lesions are associated with poor renal outcome among patients with diffuse global lupus nephritis (LN) (Rheumatology 2008). In this study we sought to examine the effect of membranous lesions on renal prognosis in LN.

Methods: We retrospectively analyzed 119 LN patients, 18 male and 101 female, who had class III (focal LN), IV (diffuse LN), V (membranous LN) by ISN/RPS2003 classification in our hospital between 1986 and 2011. Renal endpoint was defined as the doubling serum creatinine (S-Cr) or end-stage renal disease.

Results: The mean patient age at renal biopsy was 36.8 years (range, 12-68) and the mean observation period was 104.9 months (0.8-301). The mean S-Cr level and urinary protein/creatinine ratio were 0.84 mg/dl (0.30-2.94) and 4.1 g/gCr (0.6-21.3), respectively. The relative frequency of each class was as follows; III 18%, IV 66%, and V 16%. The combination of class V was observed in 15% of class III and 37% of class IV. Kaplan-Meier analysis showed that patients with mixed lesions (III+V or IV+V) had poor renal outcome compared to patients with proliferative lesions alone (pure III or IV) (P=0.030, Figure). Multivariable analysis indicated that S-Cr at the time of biopsy and the combination of membranous and proliferative lesions are the independent risk factor for poor renal prognosis, with hazard ratios of 8.08 (95% CI 2.04-32.07, P<0.003) and 19.33 (95%CI 2.79-133.86, P<0.003), respectively.



pure V	20	12	8	5	3	2	0
pure III or IV	80	58	40	22	12	5	1
III+V or IV+V	19	10	4	3	0	0	0

Figure. Kaplan-Meier curves for renal survival

* P=0.030, vs pure III or IV

Conclusions: This study demonstrated that mixed lesion of membranous and proliferative LN by ISN/RPS2003 classification is associated with poor renal prognosis.

Funding: Government Support - Non-U.S.

SA-PO340

Reproductive Factors and Response to Induction Therapy in Systemic Lupus Erythematosus with Nephritis

Elizabeth W. Dehmer, Keisha L. Gibson, Susan L. Hogan, Mary Anne Dooley. *University of North Carolina, Chapel Hill, NC.*

Background: Systemic lupus erythematosus (SLE) predominantly affects women during their reproductive years, and estrogen may play a pathogenic role. We evaluated the association between baseline reproductive factors, a proxy for estrogen exposure, and response to induction therapy in female SLE nephritis.

Methods: Women enrolled in a clinical trial of mycophenolate mofetil vs. cyclophosphamide completed a menstrual history questionnaire. Response to therapy, defined as complete (return to ≤10% of normal values of serum creatinine, proteinuria, and urine sediment) or partial (improvement of 50% in all abnormal renal measurements) remission at 24 weeks, was examined via logistic regression adjusted for age, race, body mass index (BMI) and treatment, with odds ratios (OR) and 95% confidence intervals (CI) reported.

Results: N=120 females (57% African American, 20% Hispanic, 23% Caucasian or other race) participated and had a mean age and BMI of 31.9 ± 5.0 years (range 15-58) and 27.7 ± 6.6 kg/m^2 (range 15-59) respectively. Mean baseline serum creatinine was 1.07 ± 0.52 mg/dl, proteinuria was 4.43 ± 3.15 grams/day. No measures were statistically associated with response to therapy (see table). The magnitude of effect suggests breastfeeding may influence response to therapy (OR=2.35, 95% CI=0.86, 6.41). This association persisted after adjustment for age, race, BMI and treatment (OR=2.51, 95% CI=0.72, 8.77).

Odds of treatment response based on reproductive factors

Predictor Variable	N (%)	Adjusted OR (95% CI)
Pregnancies		
0	24 (24)	1.
1-2	40 (39)	1.16 (0.33, 4.05)
>2	38 (37)	1.75 (0.44, 6.95)
Pregnancy Losses		
0	57 (59)	1.
1-2	34 (35)	0.65 (0.23, 1.82)
>2	5 (5)	2.06 (0.24, 18.09)
Breastfeeding		
No	35 (51)	1.
Yes	33 (49)	2.51 (0.72, 8.77)
Oral Contraceptive Use		
No	93 (80)	1.
Yes	24 (20)	1.05 (0.37, 2.97)
Menopause		
No	103 (87)	1.
Yes	16 (13)	0.93 (0.21, 4.17)

Conclusions: A prior history of breastfeeding may be associated with response to induction therapy in SLE nephritis. More detailed evaluation and analysis of reproductive history, especially breastfeeding, is warranted in future trials.

SA-PO341

Urine Vitamin-D Binding Protein Levels Distinguish Class III/IV Proliferative, from Class V Membranous Lupus Nephritis

Michael R. Bennett, Rina Mina, Shannen Nelson, Jessica Hummel, Prasad Devarajan, Hermine Brunner. *Cincinnati Children's Hospital.*

Background: Nearly 80% of childhood-onset SLE patients have lupus nephritis (LN). The ISN/RPS Morphologic Classification of LN reports on histological features that differentiate between various forms of LN, most notably between Diffuse Proliferative Class III/IV and pure Membranous Class V lesions. There is a lack of non-invasive biomarkers that can discriminate LN subtypes so that appropriate therapies can be planned. Using liquid chromatography tandem mass spectrometry proteomics, we identified Vitamin-D Binding Protein (VDBP) to discriminate between Class III/IV and Class V LN. We set out to validate this finding in an independent cohort on a clinically applicable platform.

Methods: Urine was collected from 53 patients with LN. Renal biopsies had classified these patients as having either Class III (10), Class IV (17), or Class V (26) LN. VDBP was measured using a commercially available ELISA. Urine creatinine (UCr) was measured by modified Jaffe reaction on an automated clinical analyzer. Results were reported as medians and analyzed both raw and normalized to UCr using a non-parametric Mann-Whitney rank sum analysis.

Results: Median urinary VDBP levels were significantly higher (p=0.046) in Class III/IV LN (90.9 ng/ml, IQR 28.9 - 948.4) than Class V LN (33.5 ng/ml, IQR 21.3 - 124.1). The results remained significant (p=0.045) when corrected for UCr - Class III/IV LN (22.2 ng/mg, IQR 29.0 - 1822.9), Class V (44.1 ng/mg, IQR 14.8 - 139.5).

Conclusions: We validated our proteomic finding that urinary VDBP can distinguish Class III/IV proliferative, from Class V membranous LN. Class III/IV and Class V LN require different therapeutic approaches, and therapies must be initiated early to be most effective. A kidney biopsy is currently considered the "gold standard" for the diagnosis of SLE nephritis. However, biopsies cannot be done serially within a short period of time to screen for pathological changes in LN in a given patient. The discovery of a non-invasive biomarker that could possibly be used to screen for such changes could have a tremendous impact on treatment strategies for patients with LN.

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SA-PO342

Outcome of Treatment of Lupus Nephritis in Older People

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Background: The outcomes of treatment of lupus nephritis (LN) in older patients are not well documented. We compared outcomes of patients aged >60 with LN treated with Rituximab induction & MMF maintenance therapy with a control group <60yrs.

Methods: Patients aged >60, with active Class III or IV &/or V LN**,** were treated with 2 doses of Rituximab 1g plus methylprednisolone 500mg iv and MMF maintenance therapy +/- low dose oral steroids. Complete (CR) or Partial (PR) renal response, where CR = urine protein:creatinine ratio (PCR) <50mg/mmolno & no >15% rise in creatinine, and PR = ≥50% improvement in proteinuria and non-nephrotic & no >15% rise in creatinine, were compared at 6 months & 1 year with control patients <60 years matched for gender, ethnicity & class of LN.

Results: 12 patients >60 were treated (9 Class V, 3 III/IV) vs. control group: Mean age 67 (range 60-74) vs 39 (31-52); mean creatinine at biopsy 139µmol/l (73-383) vs 103µmol/l (56-250) and mean PCR 605mg/mmol (112-1944) vs 551mg/mmol (98-1555). By 6 months: CR achieved in 3/12(25%) & PR in 4/12(33%) of patients >60 vs. CR 5/12 (42%) & PR 4/12(33%) in controls. By 1yr CR in 6/12(50%) & PR in 6/12(50%) of those >60 vs. 8/12(67%) & 2/12(17%) in controls (all: p=ns). In those >60yrs, proteinuria fell from a mean PCR of 605 to 121mg/mmol at 1 year, similar to controls (from 551 to 59 mg/mmol at 1 year). Median time to CR was similar in both groups (267 vs 279 days, p=0.4). There were 2 deaths in the >60 group. One patient initially achieved PR. 3 years later she developed CMV colitis with associated renal relapse, progressed to dialysis & died 18/12 later age 78. The other patient was initially a non-responder but achieved PR with Rituximab re-dosing. He died suddenly at home 10 months later age 71.

Conclusions: These data suggest that older patients with LN treated with Rituximab + MMF achieve remission rates comparable with younger patients. The elderly do well but may have lower rates of CR, possibly reflecting more chronic damage. Treatment related adverse events were infrequent but further studies in this subgroup of patients are warranted to optimise greatest efficacy with least toxicity.

SA-PO343

Cytomegalovirus Infection in Patients with Lupus Nephritis Lei Zhang,[1] Jianling Tao,[1] Yubing Wen,[1] Li Li,[2] Xueyi Wu,[1] Xuemei Li,[1] Xuewang Li.[1] *[1]Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Nephrology, Traditional Chinese Medicine of Fangshan Hospital, Beijing, China.*

Background: Cytomegalovirus infection in patients with lupus nephritis has been paid more attention to in recent years. However, very few reports are available on cytomegalovirus infection in lupus nephritis, and the clinical features and therapeutic strategy for this group of patients are still elusive.

Methods: Cases occurring within the last 10 years were collected, and detailed clinical and laboratory data were analyzed.

Results: A total of 40 cases were included in this study, and positive antigenemia (pp65) was detected in all the patients. The main cytomegalovirus symptoms of these patients were fever, raised transaminases, hematological disorders and pulmonary disease. Infectious complications other than cytomegalovirus were observed in 15 cases. High SLEDAI score (16±5), low C3 (0.46±0.26g/L), low C4 (0.10±0.10g/L), a moderate amount of proteinuria and hematuria, normal creatinine clearance (77.82±25.19ml/min), hypertriglyceridemia (3.16±2.57mmol/L), and strong immunosuppressive therapy history were observed as the main characteristics for these 40 lupus nephritis patients. Besides, renal histological classes of the 40 cases were identified, of which classIV was the most common type (37.5%). Ganciclovir was administered to 33 patients, and the need for early anti-viral treatment was observed among the symptomatic ones. Additionally, the doses of steroids and immunosuppressants were not reduced after the diagnosis of cytomegalovirus infection in this study. All the 40 patients survived, and the symptoms of cytomegalovirus infection alleviated satisfactorily.

Conclusions: Our results suggest that the symptoms of cytomegalovirus infection are characteristic in lupus nephritis. There are certain clinical features of patients with lupus nephritis which may indicate the infection of cytomegalovirus: active systemic lupus erythematosus, hypertriglyceridemia, classIV lupus nephritis, and strong immunosuppressive therapy history. The symptomatic patients need early anti-viral treatment.

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SA-PO344

Systemic Flares of Lupus Are Common in Patients with End Stage Renal Disease Secondary to Lupus Nephritis Whether on Dialysis or Transplanted Daniel M. McGuinness,[1] Christopher Wincup,[1] Adam Mclean,[1] Liz Lightstone.[1,2] *[1]Imperial College Healthcare NHS Trust Renal and Transplant Centre, Hammersmith Hospital, London, United Kingdom; [2]Renal Section, Department of Medicine, Imperial College London, London, United Kingdom.*

Background: A proportion of patients with lupus nephritis (LN) progress to End Stage Renal Disease (ESRD). Systemic lupus flares are said to be rare once patients are on dialysis (Dx) or transplanted (Txp). Experience tells us otherwise. We retrospectively reviewed the presence of flares in a patient cohort with ESRD due to LN.

Methods: Retrospective notes review of the 53/72 ESRD patients alive in Jan '11 with ESRD due to LN for whom notes were available. Patients were either on Dx or Txp & their notes interrogated from first presentation up to Jan '12.

Results: 45F & 8M patients; median age at ESRD 37yrs (range 14-79). Median time from lupus diagnosis to ESRF-36 mths (0-316). Median f/up post ESRD 7.6yrs (1.8-30.5). At Jan '12 census point, 34 had functioning Txp, 15 on Dx (of whom 8 had failed Txp) & 4 had died (3 HDx, 1 Txp). Systemic lupus flares were common: overall, 1 or >1 flares in 23/53 (43.4%) & more common in Dx patients (11/18, 61%) vs. Txp patients (12/35, 34.2%). Most patients had multiple symptoms: arthralgias (in 7 Dx & 8 Txp), skin (5 & 5), haematological (5 & 2), cerebral (1 & 1) & other (3 & 9). Markers of lupus activity: low complement in 23/53, raised dsDNA abs in 22, thrombocytopenia in 34 & lymphopaenia in 39. Of those with low complement **plus** high dsDNA abs (16), only 5 had no flares. Immunosuppression (IS) was very different in the 2 groups: 13/18 Dx patients were on oral steroids & rarely on other agents. We use a steroid sparing Txp protocol & of those with Txp who flared, 5 were on tacrolimus alone (+/- low dose maintenance steroid) & 4 also on MMF. Two grafts were lost to recurrent LN.

Conclusions: ESRD does not protect from systemic lupus flares which can be severe. Flares are more common in Dx than in Txp patients, probably reflecting IS differences. Lupus markers may predict flares. Future work will include morbidity related to lupus flares in RRT patients & explore the apparently high mortality.

SA-PO345

Disease Activity, Health-Related Quality of Life, and Transition/Self-Management in Adolescents with Systemic Lupus Erythematosus May Doan,[1] Maria E. Ferris,[1] Keisha L. Gibson,[1] Nicole M. Fenton.[2] *[1]Nephrology, University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: SLE has a >95% five-year survival rate among adolescents. Several factors such as disease activity, health-related quality of life (HRQOL), and mood may interact and contribute to successful transition into adult-focused care. We examined the relationships among disease activity, HRQOL, mood, and transition/self-management in adolescents with SLE.

Methods: Youth ages 12-25 years with SLE completed the UNC TR$_x$ANSITION Scale™ (health-provider administered, 32 questions) to assess transition/self-management skills, and the PedsQL to assess HRQOL. Disease activity was measured using the SELENA SLEDAI Scale. Relationships were determined using Pearson's correlation, and comparative analyses were performed between lupus patients with and without nephritis. Significance levels were set at $p<.10$.

Results: Of 25 patients, 64% were female, 52% were African American, and the mean age at assessment was 16.8±3.1 years old. Mean age at diagnosis was 13.2±3.7 years. Disease activity did not significantly correlate with total UNC TR$_x$ANSITION Scale™ scores, but did correlate with domains of Nutrition knowledge (r=.43, p=0.03) and Ongoing support (r=.36, p=0.08). Disease activity was not associated with physical, emotional, social, or cognitive QOL, and was not associated with mood. Patients with SLE nephritis (N=20) had more knowledge about reproductive issues (mean 0.63 vs. 0.25, p=.056) and better mood (mean 4.1 vs. 2.6, p=.09) than those without SLE nephritis.

Conclusions: In this ongoing study, there seems to be a trend that patients with greater disease activity have more skills managing their own disease, particularly regarding nutrition and future disease self-management. Further, patients with SLE nephritis have more knowledge about reproductive issues and report better moods than lupus patients without nephritis.

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SA-PO346

Intrarenal Expression of Toll-Like Receptors in Lupus Nephritis Ying Xiang,[1] Shang Guo Piao,[1,4] Hong Bin Zou,[2] Bi Hu Gao,[3] Chul Woo Yang,[4] Can Li.[1] *[1]Nephrology, YanBian University Hospital, YanJi, JiLin, China; [2]Nephrology, The First Affiliated Hospital of JiLin University, ChangChun, JiLin, China; [3]Nephrology, The Affiliated Zhong-Shan Hospital of DaLian University, DaLian, LiaoNing, China; [4]Nephrology, The Catholic University of Korea, Seoul, Korea.*

Background: Recent studies demonstrated that the expression of toll-like receptors (TLRs) is upregulated in lupus mice models. However, the expression of TLRs and the role of innate immunity in human lupus nephritis has yet to be illustrated. The aim of this study was to investigate the expression of TLRs and its ligands in human biopsy specimens with lupus nephritis (LN).

Methods: We studied 14lupus nephritis patients with renal biopsy, and 14 patients with nephrectomy for renal cancer served as controls. Clinical characteristics were recorded, and intrarenal expression of TLRs (TLR2 and TLR4) and MYD88 was examined by immunohistochemistry.

Results: The intrarenal expression of TLR2 was significantly increased in the glomerulus of the LN group compared with the controls (55.07 ± 0.29%/mm^2 vs. 30.92 ± 5.34%/mm^2, $P<0.01$), whereas TLR4 expression was decreased (49.13 ± 5.75%/mm^2 vs. 128.99 ± 6.62%/mm^2, $P<0.01$).This trend of TLRs expression was accompanied by suppressing MYD88 expression (59.39 ± 7.60%/mm^2 vs. 104.83 ± 12.64%/mm^2, $P<0.01$). Correlation analysis revealed that TLRs expression was correlated with the 24h urinary protein excretion and the eGFR.

Conclusions: These findings suggest that adifferent expression in TLR2 and TLR4 is closely associated with lupusnephritis, and that innate immunity may be one of important molecular mechanisms in lupusnephritis.

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SA-PO347

MYH9 and APOL Gene Polymorphisms: CKD Progression in Lupus Nephritis in a Highly Admixtured Population Vinícius Sardao Colares,[1] Silvia M. Titan,[1] Patrícia Malafronte,[1] Rui Toledo Barros,[1] Alexandre Costa Pereira,[2] Viktoria Woronik.[1] *[1]Nephrology, Sao Paulo University, Sao Paulo, Brazil; [2]Laboratorio de Cardiologia Molecular, Instituto do Coração, Hospital das Clinicas, Sao Paulo University, Sao Paulo, Brazil.*

Background: Polymorphisms of MYH9 have been recently associated with CKD progression in non-diabetic chronic kidney disease, FSGS and HIV nephropathy patients. However, the association of MYH9 E-1 haplotype with CKD seems to be confounded by the strong linkage disequilibrium with apolipoprotein(APOL) genes, as shown in certain populations. In our study, we have analysed the effect of MYH9 and APOL 1 and 3 gene polymorphisms on CKD risk in Brazilian lupus nephritis patients.

Methods: Retrospective analysis of 196 patients with LN followed in our outpatient glomerular disease service from January 1999 to December 2010. Clinical and laboratorial data were retrieved and genotypying of MYH9 (rs4821480, rs2032487, rs4821481 and rs3752462- E1 haplotype) and APOL1 (rs73885319 and rs71785313) polymorphisms were performed. Primary outcome (PO) was defined as doubling of serum creatinine and/or end stage renal disease (ESRD).

Results: The mean follow-up time was 6.1 years, with an initial mean creatinine of 1.6 g/dL and mean proteinuria 3.9 g/day. Of the initial 196 patients, 62 (31.6%) presented the PO. The four MYH9 polymorphisms segregate as an haplotype, according to the Hardy-Weinberg model. Analysing each polymorphism, only TT/CT genotype from rs3752462 polymorphism was associated with the PO (p= 0.01). MYH9 E1 haplotype was associated with PO (OR 1.85, 95%CI 1,09 - 3,24, p=0,02; haplotype presence in 29,7% of cases and 18,5% of controls), whereas APOL 1 and 3 gene polymorphisms were not.

Conclusions: MYH9 E1 haplotype, and not APOL1 polymorphisms, is associated with a worse renal prognosis in lupus nephritis patients. Future studies should focus on the possible mechanisms underlying this association.

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SA-PO348

"Class II" Lupus Nephritis with Nephrotic Syndrome: A Podocyte Disease

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Background: Lupus podocytopathy, a newly recognized but not well defined renal lesion of lupus nephritis(LN), is still in the classII of LN. In this study, we analyzed the clinical and histological features of class II LN with nephrotic syndrome(NS) and further explored the association of podocyte lesion with nephrotic syndrome in LN class II.

Methods: 120 patients with class II LN were included and were divided into the NS group and non-NS group based on the amount of urinary protein excretion. The degree of mesangial proliferation was graded as mild(M1),moderate(M2) and severe(M3). Diffuse foot process effacement was defined as more than 50% of the glomerular capillary loops showing glomerular foot process effacement by EM. Clinical, immunological and pathological features were compared between the two groups.

Results: 26 patients(21.7%) were in the NS group, 94 patients in the non-NS group. No significant differences in gender, age, LN duration, the levels of serum autoantibodies and complements were found between the two groups. Compared to non-NS group, the patients in NS group had a much higher incidence of renal manifestations as the first onset symptom(50% vs 19%, P<0.05) and AKI (22% vs 0, P<0.01), but much lower incidence of malar rash(48% vs 67%,P<0.05), fever(22% vs 61%, P<0.05), arthritis(39% vs 72%, P<0.05) and hematuria(0 vs 45%,p<0.01). In renal histology, the NS group had less severe mesangial proliferation(M2+M3: 9% vs 100%, P<0.01), but significantly higher incidence of diffuse foot process effacement(82% vs 3%, P<0.01) than that in non-NS group. Three patients in NS group had no glomerular immune deposition by IF. 19 patients (86.4%) in the NS group achieved complete remission and 3 patients partial remission after the steroid treatment for 1-9 months(median 2 months), but 9 of them(41%) relapsed during the 6-124 months (median 56 months) follow-up.

Conclusions: This study indicates that the nephrotic "class II" LN is a podocyte disease which should be differentiated from the class II LN with mesangial proliferation and be described as minimal change disease(MCD)-like LN.

Funding: Government Support - Non-U.S.

SA-PO349

Long-Term Outcome of Patients with IgG4-Related Kidney Disease

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Background: In the kidney lesion associated with IgG4-related disease [IgG4-related kidney disease (IgG4-RKD)], steroid therapy has been shown to elicit rapid amelioration. However, few data on long-term outcome are available.

Methods: We retrospectively analyzed the long-term clinical course in 43 patients diagnosed as having definite IgG4-RKD (median follow-up 32 months).

Results: Forty of the 43 patients were treated with steroids, and 95% of them were still being maintained on steroid at the last review. Before treatment, the estimated glomerular filtration rate (eGFR) had decreased to less than 60 ml/min (31.6 ± 16.0 ml/min) in 24 of these 40 patients. At 1 month after treatment, it was significantly improved (43.5 ±14.0 ml/min, P<0.01), and renal function was maintained at a similar level at both 12 months and the last review, although maintenance hemodialysis became necessary in one patient. Among the patients whose eGFR exceeded 60 ml/min, there was no difference between the eGFR before therapy and that at the last review. Renal radiologic abnormalities characteristic of IgG4-RKD were present in 28 of the 40 patients, and improvement was evident at 1 month after treatment in all of them. Although renal atrophy was not evident in any of 40 patients before treatment, it had developed in 11 of 25 (44%) evaluated patients (2/9 in the eGFR ≥60 group and 9/16 in the eGFR <60 group) at the last review. Relapses of IgG4-RKD and extra-renal organ involvement were evident in 3 and 5 patients, respectively.

Conclusions: IgG4-RKD shows rapid improvement with steroid therapy and the recovered renal function persists for a long time with maintenance steroid therapy, although renal atrophy progresses in many patients.

SA-PO350

Immunosuppressive Therapy in Fibrillary Glomerulonephritis

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Background: Non-amyloid fibrillary glomerulonephritis (FGN) is a rare disorder with poor renal prognosis in the absence of efficient therapy. The aim of the present retrospective study was to evaluate the effect of immunosuppressive regimens on long-term renal outcome.

Methods: Sixteen men and ten women (median age: 60 years) were included, according to the following criteria: 1) presence of glomerular extra-cellular Ig deposits 2) Congo-red-negativity 3) fibrillary organization at the ultrastructural level. Patients with immunotactoid GN and cryoglobulinemic GN were excluded. Clinicopathological characteristics and renal outcome were evaluated in 13 untreated patients (control group) and in 13 patients who received at least 1 of the following regimens: steroids (2 cases), steroids plus rituximab (7 cases)/cyclosporine (4 cases)/cyclophosphamide (3 cases) or bortezomib (1 case).

Results: At baseline, all patients had proteinuria (median: 2.9 g/day) associated with nephrotic syndrome (38%), hematuria (69%), renal insufficiency (58%) and hypertension (69%). Eight patients had a history of auto-immune disease but none had haematological malignancy. Light microscopic studies showed mesangial GN (73%), atypical membranous GN (15%) and atypical membranoproliferative GN (12%). By immunofluorescence, deposits stained for C3 (96%) and IgG in all cases (IgG4 14/14, IgG1 9/14). IgG deposits were polyclonal in 92% of cases. Serum IgG subclasses distribution was normal in 7 patients tested. After a mean follow-up of 62 months, renal response occurred in 6/13 patients with cyclophosphamide (1 case) or rituximab (5 cases). Median eGFR was 74 and 42 ml/min/1.73m2 at the time of treatment introduction, in responding and non-responding patients, respectively (p = 0.079). By contrast, CKD progressed in 12 control patients, 10 reaching end-stage renal disease.

Conclusions: The present data indicate that early introduction of immunosuppressive drugs may improve renal prognosis of FGN. Efficacy of rituximab remains to be confirmed in larger collaborative studies.

SA-PO351

Renal and Survival Outcome of Patients with Combined Renal Light Chain Deposition Disease (LCDD) & Myeloma Cast Nephropathy (MCN)

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Background: LCDD is due to over production of monoclonal light chains and typically associated with multiple myeloma. At the time of biopsy, between 16-46% of patients with LCDD also have evidence of MCN. The clinical and renal outcome of patients with both LCDD and MCN is not well-established.

Methods: We retrospectively reviewed 69 patients with pure LCDD, LCDD + MCN, & MCN alone at Mayo Clinic Rochester and compared their clinical, renal, and survival outcome.

Results: Between 1999 and 2011, there were 26 patients with LCDD, 30 MCN and 13 had both LCDD and MCN on renal biopsy. Patient characteristics were shown in Table 1. Creatinine at time of biopsy was significantly lower in LCDD group compared to the other 2 groups, but no difference in the need for dialysis at time of biopsy. In LCDD group, 69% had diagnosis of multiple myeloma compared to 100% in the other two groups. There was no difference in the type of therapy amongst the three groups (Bortezomib vs IMiD-based vs stem cell transplantation). The median patient survival was significantly lower in MCN and LCDD + MCN compared to LCDD, but no difference when comparing MCN to MCN + LCDD group. The median renal survival was higher in LCDD group compared to MCN and LCDD + MCN (p=0.005). The death-censored renal survival, however, was not different amongst the three groups.

Patient demographics and outcomes

Characteristics	LCDD (n=26)	MCN (n=30)	LCDD+MCN (n=13)	p value
Gender (M/F)	18/8	17/13	7/6	NS
Age (yr) at time of biopsy	56.4±2.47	64.2±2.30	60.0±3.49	0.07
Creatinine at time of biopsy	3.5±0.44	5.0±0.43	5.1±0.63	0.03
Dialysis at time of biopsy	5	9	4	NS
Multiple myeloma at time of biopsy	15	30	12	0.0006
Progression to ESRD	7	14	7	NS
Death	9	21	9	0.01
Median patient survival (m)	97	12	39	0.007
Median renal survival (m)	86	4	6	0.005
Death-censored median renal survival (m)	not reached	23	39	0.2

Conclusions: Patient with both LCDD and MCN on renal biopsy are similar to those with MCN alone as opposed to LCDD alone with a trend towards older age and higher creatinine at time of renal biopsy, higher mortality rate and lower renal survival (not censored for death).

SA-PO352

Bortezomib for Heavy Chain Deposition Disease: A Report of Three Cases

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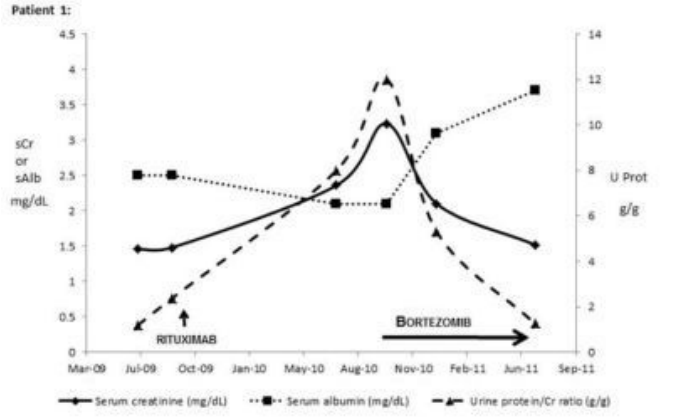
Background: Heavy chain deposition disease (HCDD) is a plasma cell dyscrasia in which monoclonal heavy chains deposit in glomerular and tubular basement membranes. Literature describing effective therapies is sparse, and historically prognosis has been very poor.

Methods: We describe 3 cases of HCDD with biopsy proven glomerular involvement but without myeloma on bone marrow biopsy who were successfully treated with bortezomib (BTZ), a proteasome inhibitor.

Results: Case Histories: Case 1: 60 yo man with nephrotic syndrome (NS) & rising serum creatinine (sCr), refractory to RAAS inhibition, diuretics, and rituximab. He began BTZ/dexamethasone (DEX). In 6 mos sCr improved from 3.23 to 1.52 mg/dl, proteinuria (UPCR) from 12 to 1.26 g/g.



Peripheral neuropathy led to cessation of BTZ at 7 mos. He began lenalidomide and remains stable 1yr later. Case 2: 75 yo man with refractory NS, rising sCr, and transfusion-dependent anemia. BTZ/DEX over 6 mos led to sCr improving from 3.0 to 1.3 mg/dl, UPCR from 4.2 to 1.9 g/g, and NS and anemia resolved. At 9 mos he developed peripheral neuropathy which stabilized with decreasing BTZ frequency. He remains stable on q2wk BTZ 20 mos later. Case 3: 56 yo man with refractory NS and rising sCr. He received cyclophosphamide/BTZ/DEX x4 cycles, with sCr 2.7 to 2.0 mg/dl and proteinuria 7.4 to 0.6 g/24h. He remained stable at 1yr with sCr 1.8, proteinuria 80 mg/24h.

Conclusions: In these HCDD cases, BTZ-based therapy resulted in sustained resolution of NS and improvement in renal function. Treatment was well-tolerated, but 2/3 cases developed peripheral neuropathy.

SA-PO353

Autologous Stem Cell Transplantation in 18 Patients with Primary AL Amyloidosis: Long-Term Outcome Masahiro Kawada, Kenmei Takaichi, Yoshifumi Ubara, Junichi Hoshino, Tatsuya Suwabe, Noriko Hayami, Keiichi Sumida, Koki Mise. *Nephrology, Toranomon Hospital, Tokyo, Minato-ku, Japan.*

Background: AL amyloidosis shows a wide spectrum of organ involvement and is well known to have a poor long-term prognosis. High-dose melphalan and autologous stem cell transplantation (HDM/ASCT) have been reported to prolong the survival of patients with AL amyloidosis. Induction with VAD therapy (vincristine, doxorubicin, and dexamethasone) before HDM/ASCT has been proposed to reduce the risk of relapse and improve the response rate. In Japan, this treatment is only performed for patients who meet certain eligibility criteria with regard to their general condition and complications. The long-term outcome of VAD with HDM/ASCT was investigated in this study.

Methods: In 18 patients with AL amyloidosis, HDM/ASCT was carried out after one or two courses of VAD therapy (performed according to the criteria and regimen of Gono) between 2004 and 2010.

Results: The median follow-up time was 43 months (21-85). The patients had a mean age of 52 years (6 women), mean serum Cr of 0.86 mg/dL, serum Alb of 2.23 g/dL, and proteinuria of 7.11 g/day before treatment. Three patients died (2 of AL amyloidosis and 1 of infection), and 15 patients remained alive. Proteinuria declined to <0.1 g daily in 2 patients after 1 year and in 7 patients after 2 to 3 years. It also decreased to <2 g daily in 2 patients, but proteinuria persisted in the other 6 patients. There was no treatment-related mortality. Overall survival (OS) was 84.7% at 3 years and 63.5% at 5 years. Renal reaction was seen in 47%. Compared with patients who showed mild to moderate amyloid deposition in the glomeruli, patients who had severe amyloid deposition were refractory to treatment (p=0.083).

Conclusions: VAD + HDM/ASCT may be an effective treatment for patients with AL amyloidosis who meet the eligibility criteria.

Funding: Government Support - Non-U.S.

SA-PO354

Tacrolimus in the Treatment of HBV Associated with Membranous Nephropathy: A Prospective Study Kailong Li, Yani He. *Department of Nephrology, DaPing Hospital, Third Military Medical University, ChongQing, China.*

Background: Hepatitis B virus-associated membranous nephropathy(HBVMN)is the major pathological types of hepatitis B virus-associated glomerulo- nephritis(HBV-GN), and now, there is no specific treatment for the adult HBVMN. We conducted a prospective randomized trial evaluating the combination therapy of tacrolimus, corticosteroids and entecavir to achieve complete or partial remission in adult patients with biopsy-proven HBVMN.

Methods: Thirty-five patients were diagnosed as having chronic glomerulonephritis associated with chronic hepatitis B. Histopathological findings of the renal biopsy specimen from all the patients indicated membranous nephropathy.Seventeen of the patients received combination therapy of tacrolimus (0.05 mg/kg/day), corticosteroids(0.5mg/kg/day)and entecavir (0.5mg/day)over 12 months with a 6-month taper to tacrolimus, whereas eighteen of the patients were in the control group(entecavir monotherapy 0.5mg/day).

Results: The probability of remission in the treatment group was 82.4,and 100% after 6 and 12 months but only 11.1and 22.2%, respectively in the control group. The decrease in proteinuria was significantly greater in the treatment group. entecavir was started for the treatment of hepatitis in all patients, which caused the disappearance of serum hepatitis B virus DNA and the normalization of ALT and AST level in 2 months. Notably, three patients in the control group and no patient in the treatment group reached the secondary end point of a 50% increase in their serum creatinine. No patient in the tacrolimus group showed a relapse during the taper period. Nephrotic syndrome reappeared in almost 1/4 of the patients who were in remission by the 18th month after tacrolimus withdrawal.

Conclusions: We conclude that the combination therapy of tacrolimus,corticosteroids and entecavir is a very useful therapeutic option for patients with HBVMN and preserved renal function. The majority of patients experienced remission with a significant reduction in the risk for deteriorating renal function and Hepatitis B virus replication.

SA-PO355

Treatment of Refractory Idiopathic Membranous Nephropathy with Steroid and Tacrolimus Ping Fu, Tao Ye, Xiao Xiao Yu, Jin Qiaoling, Mian Wei. *Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: To investigate the efficacy and safety of steroid and tacrolimus (FK506) in the treatment of refractory idiopathic membranous nephropathy.

Methods: Fifteen patients with idiopathic membranous nephropathy (IMN) who failed the previous therapy including steroid and cyclophosphamide or mycophenolate mofetil(MMF) and other immunosuppressive treatments from May 2009 to September 2011 in our hospital were treated with steroid and tacrolimus(FK506). The initial dose of prednisone was 30-40mg/d.The dose continued 4-6 weeks and then tapered gradually to 10mg/d.The dose of FK506 was 1mg/d.At the same time, Wuzhi capsule or Diltiazem was used to adjust the blood concentration of FK506.The target blood concentration was 5-8ng/mL and would be increased to 8-12ng/mL if the clinical parameters shwoed no improvement after three months.The blood concentration of FK506, urine protein,liver and renal function,blood glucose,blood lipids and infection were recorded in the regular follow-up.

Results: After 6 months of treatment,seven(46.7%)patients achieved complete remission(CR), three(20%) achieved partial remission(PR), while five(20%) failed this treatment.After 1 year of treatment, nine patients (60%)obtained CR, two patients(13.3%) obtained PR and four patients(26.7%) had no response. Four patients developed glucose intolerance,One developed respiratory infection, and three experienced gradual increasing in the serumcreatinine.One patient withdrew the sdudy, and no patient died because of the side effects.

Conclusions: The therapy with steroid and small dose of FK506 is an effective and safe therapy for refractory inpatients with IMN. However, futher sdudy is necessary to conducted to research the optimum blood concentration and course of treatment.

SA-PO356

Long-Term Survival after Dialysis Therapy in Patients with IgA Nephropathy Hiroyuki Komatsu,[1] Akihiro Fukuda,[1] Masao Kikuchi,[1] Yuji Sato,[1] Shouichi Fujimoto.[2] *[1]First Department of Internal Medicine, University of Miyazaki Hospital, Miyazaki, Japan; [2]Department of Hemovascular Medicine and Artificial Organ, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.*

Background: Little is known about how dialysis affects the survival of patients with biopsy-proven IgA nephropathy. The present long-term cohort study quantifies the survival rates and incidence of cardiovascular events among such patients in Japan.

Methods: Fifty-two of 433 patients with IgA nephropathy who had reached end-stage kidney disease underwent renal replacement therapy (RRT) between 1981 and 2010 and were followed-up for 11.3 ± 6.4 years. The overall survival rate and incidence of cardiovascular events in these 52 patients were evaluated during the follow-up.

Results: Initial RRT in 46 and 6 patients comprised hemodialysis and peritoneal dialysis, respectively. The mean age at starting RRT was 42.8 ± 13.3 years. Only seven patients died during follow-up and Kaplan-Meier analysis revealed favorable survival rates of 93.3% and 65.1% at 10 and 20 years, as compared with that of patients with glomerulonephritis who required RRT in Japanese Society for Dialysis Therapy registry (49.0% and 27.8% at 10 and 20 years). Cardiovascular disease and malignancy were causes of death at 3.9 ± 1.3 and 13.6 ± 4.8 years respectively after starting RRT. Cardiovascular events developed in 15 patients (incidence, 2.7 per 100 person-years) and acute coronary syndrome and cerebral hemorrhage developed relatively soon after starting RRT. Cox proportional hazards models revealed that age at the time of starting RRT was a significant factor affecting the onset of cardiovascular events.

Conclusions: Although the survival of patients with IgA nephropathy is favorable after dialysis, the onset of cardiovascular events during the early phase of dialysis should be carefully monitored.

SA-PO357

Prospective Study to Compare Efficacy of Tacrolimus versus Cyclyophosphamide in Inducing Remission in Idiopathic Membranous Nephropathy Sanjay K. Agarwal, Pavitra Manu Dogra, Sanjay Gupta, Dipankar M. Bhowmik. *Department of Nephrology, All India Institute of Medical Sciences, New Delhi, Delhi, India.*

Background: Steroid alone is not useful for Idiopathic Membranous nephropathy (IMN). There are reports on use of Tacrolimus (Tac) in IMN. However, none has compared it with Modified Ponticelli regimen (MPR). This study has prospectively compared efficacy of Tac with MPR in IMN.

Methods: In this, prospective study adult patients of IMN (eGFR > 30 ml) and nephrotic syndrome inspite of adequate Telmisartan were included. Patients were randomized in 1:1 ratio. In group-A, Tac (0.1 mg/Kg/day for 6 months, then taper off in next 6 months) and Prednisolone (0.5 mg/Kg/day till remission, then tapered by 5 mg/week with minimal maintenance dose) was used. In group-B, MPR was used. Duration of therapy was 12 months in Gr-A and 6 months in Gr-B. Primary end point was any remission (AR) at 6 month, secondary end points was partial remission (PR) at 6 month, AR and renal survival at 12 months. Written informed consent was taken from each patient. Study was ethically approved and registered with Clinical Trials Registry of India (CTRI/2010/091/000231).

Results: A total of 41 patients included. Mean age (38 years), mean duration of edema (8 months), number of female (7), degree of blood pressure was similar in both groups. Gr-A had Stage II histology in 90% and stage III in 10%. In Gr-B, all had stage II disease. Primary endpoint was seen in 100 % patients of Gr-A and 85% in Gr-B (p=0.04). PR at 6 months was seen in 95% patients in Gr-A and 85% in Gr-B. AR at 12 months was obtained in 94.4% patients in Gr-A (33.3% PR + 61.1% CR) as against 85% in Gr-B (35% PR + 50% CR), (p=0.52). 1 patient in each group expired due to infection. Remission was more rapid and statistically significant in Gr-A. At any given time, proteinuria reduced more in Gr-A than Gr-B with a mean difference of 1.24 g/day (p=0.012). There was minor reduction seen in eGFR (6 ml/min) in both groups, at the end of follow-up.

Conclusions: Our study shows that Tacrolimus with steroid is a good option for management of idiopathic Membranous Nephropathy. This drug when compared with Modified Ponticelli protocol has a rapid reduction in proteinuria.

SA-PO358

Comparison of Treatment Strategies in Idiopathic Membranous Nephropathy Jan A.J.G. van den Brand,[1] Alfonso Segarra,[2] Manuel Praga,[3] Jack F. Wetzels.[4] *[1]On Behalf of the Study Group; [2]Servicio de Nefrología, Hospital 12 de Octubre, Madrid, Spain; [3]Nefrologia, Hospital Vall d'Hebron, Barcelona, Spain; [4]Nephrology, Radboud University Medical Centre, Nijmegen, Netherlands.*

Background: Treatment of patients with idiopathic membranous nephropathy is debated, and various regimens are used. We compared two strategies consisting of supportive treatment with anti-proteinuric drugs and statins in low risk patients, and cyclophosphamide (CP) in high risk patients (Therapy I); the latter of supportive treatment, calcineurin inhibitors (CNIs) and CP in low, intermediate and high risk patients, respectively (Therapy II).

Methods: We merged datasets from prospective cohorts of patients from Spain and the Netherlands, including adult patients with biopsy proven iMN who presented with the nephrotic syndrome. We defined the end-point as doubling of serum creatinine, end stage renal disease (ESRD) or mortality. To achieve pseudo-randomization we used a logistic regression model to create 0.05-wide propensity score strata. Subsequently, we calculated a Mantel-Haenszel odds ratio (OR) to compare both strategies, excluding strata for which no comparison could be made.

Results: Table 1 shows confounder balance achieved by the propensity score stratification. During a median follow-up of 81 months (IQR 39 - 115), 50 patients reached the end-point, 18 of whom died, 15 developed ESRD and 17 showed a doubling of serum creatinine. The OR for the Therapy II compared to Therapy I was 0.94 (95%CI 0.46 to 1.91).

population characteristics

	Therapy I	**Therapy II**
n (% male)	188 (66)	89 (64)
age	52 (14)	50 (17)
year of biopsy	2000 (6.0)	1998 (7.8)
mean arterial pressure	97 (15)	99 (20)
eGFR	70 (23)	75 (34)
serum albumin	25 (6.4)	24 (5.3)
serum cholesterol	7.7 (2.6)	8.3 (2.1)
proteinuria	9.5 (5.2)	9.1 (4.4)
ACEi/ARBs (%)	68	67
statin (%)	48	61
Therapy (%)		
none or steroids	100 (53)	40 (45)
CP	87 (46)	17 (19)
CNI	1 (0.5)	32 (36)

Data are presented as mean (SD) unless stated otherwise

Conclusions: We observed no statistically significant difference in mortality, ERSD or doubling of serum creatinine between the two therapeutic strategies. Therefore, treatment choice may be determined by patient characteristics and potential side effects.

Funding: Private Foundation Support

SA-PO359

Maliginancy Risk after Cyclophosphamide Treatment in Membranous Nephropathy Jan A.J.G. van den Brand,[1] Peter R. Van Dijk,[2] Julia M. Hofstra,[1] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Medical Centre, Nijmegen, Netherlands; [2]Diabetes Centre, Isala Clinics, Zwolle, Netherlands.*

Background: Cyclophosphamide treatment improves renal survival in patients with membranous nephropathy (MN). However, its use is associated with late malignancy. We determined the incidence and risk of cancer after cyclophosphamide treatment in MN patients.

Methods: We included 269 biopsy proven MN patients in a prospective cohort since 1995. We defined outcome as incident malignancy or death, and calculated exposure time as the time between start of cyclophosphamide therapy and either outcome or end of follow-up. Conversely, for patients not receiving therapy, exposure time was calculated from biopsy until outcome or the end of follow-up, respectively. We then calculated incidence densities and relative risks, and subsequently stratified incidence by exposure time to estimate the latency period from cyclophosphamide exposure to outcome. Finally, we used a poisson regression model to correct for confounding by gender, smoking and age at biopsy.

Results: We observed 19 cases over 685 personyears in the cyclophosphamide treated group, compared to 7 cases over 1138 personyears in the untreated group. The table shows the overall unadjusted relative risk and relative risks by exposure time. The overall adjusted relative risk was 3.6 (95% confidence interval [CI] 1.5 - 8.7).

Unadjusted relative risk of cancer or death

Exposure time (years)	RR	95%CI
0-3	4.3	0.9 - 20
3-6	8.5	1.0 - 70
6-9	6.9	0.3 - 143
9-12	2.7	0.3 - 24
Total	4.5	1.7 - 17

Conclusions: Cyclophosphamide therapy was associated with malignancy and mortality. We observed an increased risk directly after the start of treament. Cyclophosphamide is unlikely to be causative for these early malignancies, as studies in other patient groups have shown a five year latency period for cancer to occur. We hypothesize that early unrecognized malignancies may be the cause of MN, and contribute to progressive disease, necessitating the use of cyclophosphamide. This explains the association of cyclophophamide with early malignancies, which means the association may be inflated.

Funding: Private Foundation Support

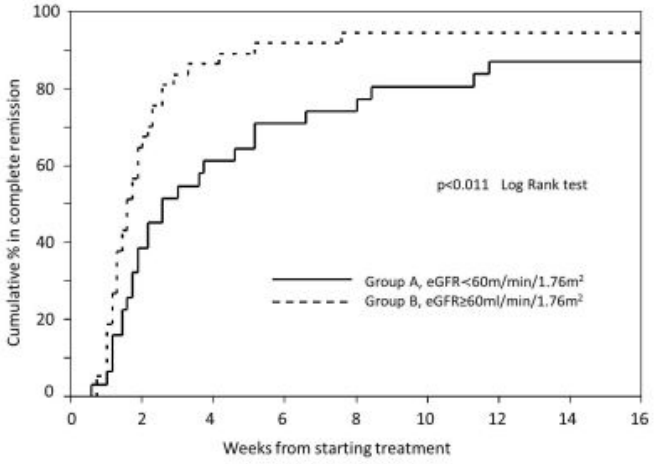
SA-PO360

Renal Function and Age at Onset Are Independently Associated with Subsequent Induction of Complete Remission in Adult Minimal Change Nephrotic Syndrome Daisuke Komukai, Yoshihiko Inoue, Fumihiko Sasai, Mamiko Takayasu, Ashio Yoshimura. *Div. of Nephrology, Showa University Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

Background: Renal insufficiency is a common feature of minimal change naphrotic syndrome (MCNS) at onset especially in adults, however its impact on subsequent steroid-responsiveness and the rate of complete remission (CR) are still unclear. We analyzed factors at onset affecting clinical course of adult MCNS patients.

Methods: 68 patients with MCNS (41 male and 27 female with a mean age of 43.6 years, range:16-76), all have been diagnosed by kidney biopsy, were studied retrospectively. Clinical data at onset including age, gender, serum creatinine, serum albumin, daily urinary protein excretion and the time to CR were studied. To compare the cumulative rates of CR between the groups of decreased eGFR (<60 ml/min/1.73m^2, group A) and preserved eGFR (≥60 ml/min/1.73m^2, group B), we used life-table analysis with Kaplan-Meier method. Cox proportional-hazards model was used to determine independent effects of clinical factors.

Results: The mean follow-up period was 43.2 months (median 41.2, range:1.3-269.0). The decrease in eGFR was shown in 31 patients (group A). All patients were treated with oral prednisolone (mean 0.72 mg/kg/day) and 33 patients (48.5%) received methylprednisolone pulse therapy (500 mg iv. for 3 days) followed by oral prednisolone. CR was achieved 54.4% of patients at 2 weeks after the start of treatment and 86.8% at 8 weeks. The time to CR in group A was significantly longer.



In Cox analysis, higher age and the decrease in renal function were both independently associated with longer duration necessary for CR after adjustment for gender, serum albumin and urinary protein.

Conclusions: Renal function and patient age at onset were independently associated with the rate of CR in adult MCNS.

SA-PO361

Long-Term Outcome of Primary and Secondary Membranous Nephropathy: Single Center Experience Mi Hyun Jang, Eun-Ah Hwang, Sung Bae Park. *Internal Medicine, Keimyung University Kidney Institute, Daegu, Korea.*

Background: Membranous nephropathy (MN) is common cause of adult onset nephritic syndrome and one of leading cause of renal failure within glomerulonephritis group. MN may be associated with other diseases such as hepatitis virus infection and lupus. This study addressed the long-term outcome of MN. We compared the rate of renal function decline according to primary or secondary MN and renal outcome among primary MN patients with a partial remission (PR), complete remission (CR) and no remission (NR) of proteinuria.

Methods: From 1985 to 2011, a total of 3648 cases of renal biopsy was performed in our center. Among them, 208 patients were diagnosed MN and followed up at least 6 months. We reviewed the medical records of these patients and compared long-term outcomes according to etiologies. The impact of treatment responses was evaluated.

Results: The mean age of studied patients was 45.6±15.6 years and M:F ratio was 1.1:1. 147 patients(70.7%) were diagnosed of primary MN and remaining 61 patients(29.3%) were secondary MN. The most common cause of secondary MN was lupus(52.5%), followed by Hepatitis B infection(45.9%). Within the patients with primary MN, 34 patients(23.1%) were received only steroids and 29 patients(19.7%) were received steroids combined with cyclophosphamide. Remaining 80 patients(54.4%) were received conservative treatment such as ACE inhinbitor and antiplatelet agents. During the follow up (average 91 months), 25% of primary MN patients and 42.6% of secondary MN patients reached renal failure. The 5 years and 10 years of renal survival rate of primary MN was 86.1% and 71%, respectively. In primary MN patients treated with steroids and/or cyclophosphamide, 39.5% of the patients had a CR, 21.8% had a PR and 12.9% had a NR. 10 years renal survival rate of patients with CR or PR was significantly higher than that of patients with NR(100% vs. 47%, p<0.000).

Conclusions: In our study, the long-term outcome of primary MN was better than secondary MN. In primary MN, patients with CR or PR showed excellent renal outcome compared to patients with NR. In primary MN patients, treatment with steroid or cytotoxic agents for reducing proteinuria can improve renal outcome.

SA-PO362

Renal Flare (RF) as a Biomarker of Incident and Progressive Chronic Kidney Disease (CKD) in Patients with Lupus Nephritis (LN) Samir Parikh, Alison Marie Mckinley Neal, Brad H. Rovin. *Internal Medicine, Ohio State University Medical Center, Columbus, OH.*

Background: Acute kidney injury (AKI) leads to CKD. In patients with LN, RF is an AKI equivalent.

Methods: To determine whether RF frequency and duration can be used as a marker of new CKD or progression of established CKD, we correlated RF with starting and ending serum creatinine (SCr) levels in the Ohio SLE Study (OSS) cohort.

Results: New onset CKD occurred in 12/41 patients over a median follow up of 4.5 years. The CKD group had more RF events than the non-CKD group: 31 (2.59/pt) vs 17 (0.59/pt), respectively, and spent more time in RF (Table 1). Only 8% of the CKD group versus 59% of the non-CKD group had no RF. In those OSS patients with established CKD, those who progressed had more RF events than non-progressors: 13 (1.63/pt) vs. 2 (0.29/pt), respectively. In the non-progressor group 71% had no RF, compared to 37.5% of progressors. Progressors had a significant change in SCr over the study period (p=0.0078). Differences in number of RF and RF duration were not significant between the 2 groups but tended to be higher in the progressors (Table 2).

Conclusions: In patients with LN, the frequency and duration of RF are biomarkers of new CKD and progression of existing CKD. As new LN therapeutic regimens are developed, targeting RF prevention will be an important consideration.

Table 1

	No CKD (n=29)	New CKD (n=12)	P-Value
Age	31.3	34.3	NS
% Male	14%	0%	-
% African American	28%	50%	-
Mean starting SCr (mg/dl)	0.85±0.15	0.75±0.15	NS
Mean end SCr(mg/dl)	0.83±0.12	1.78±1.95	0.0001
Median time in flare (mo)	0	20	0.0003
New RF/year	0.14	0.72	0.0001
Median time in renal health (mo)	52	30.4	0.0038

Table 2

	Non-progressors (n=7)	Progressors (n=8)	P-Value
Age	39.1	40.5	NS
% African American	14%	62.5%	-
Mean starting SCr(mg/dl)	1.94±0.83	2.15±0.81	NS
Mean end SCr(mg/dl)	1.56±0.87	4.0±2.45	0.0093
Mean new RF per year	0.10±0.19	0.41±0.42	NS
Median time in Renal Health (mo)	48	25	0.0560

SA-PO363

Immunosuppression Induced Partial Remission in Idiopathic Membranous Nephropathy Durga A.K. Kanigicherla, Paul E. Brenchley, Milind Nikam, Colin Short, Michael Venning. *Nephrology and Transplantation, Central Manchester University Hospitals, Manchester, United Kingdom.*

Background: About 2/3 of patients with IMN achieve Partial Remission (PR), yet 15-40% progress to renal insufficiency. PR is associated with lower incidence of ESRD/death. It is unclear how immunosuppression induced PR (PR-IS) compares with spontaneous PR (PR-S).

Methods: 1. Biopsy proven IMN followed for ≥1 year

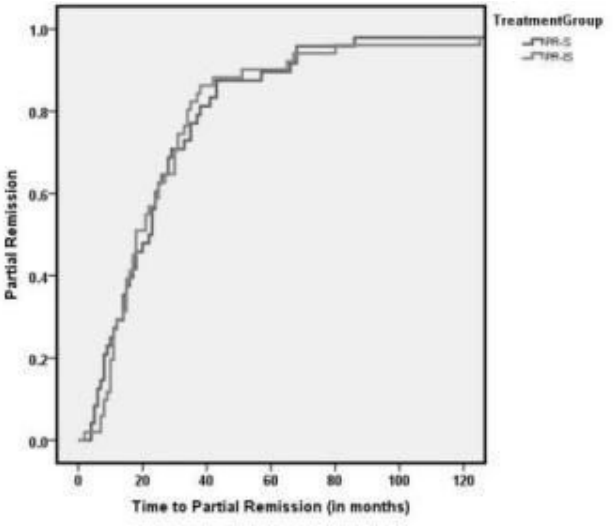
2. IS used for standard indications.

3. PR: 50% proteinuria reduction & 0.3-<3.5gm/24hrs, CR: Proteinuria <0.3gm/24hrs & stable eGFR, Relapse: proteinuria ≥3.5gm/24hrs.

Results: Of 128 patients followed for a median of 9.5 years, 100 (78%) achieved PR, and 28 had persistent active disease (PAD).

51/100 achieved PR with IS treatment (PR-IS) & 49 spontaneously (PR-S). IS regimes included steroids (n=18), CsA (7), Cyclophosphamide (23) & Rituximab (3). IS was initiated at 6 months (IQR5-11) after biopsy. Age, DM, use of ACEi/ARBs, statins was not different. At presentation, proteinuria was higher (10.6 v 6.6, p<0.001) and albumin was lower (24 v 30, p<0.0001) in PR-IS cf. PR-S. In PR-IS eGFR was lower at treatment than at presentation (54 v 70mL/min, p<0.001) In PR-IS, eGFR slope improved from -0.9 pre-IS to +0.39 ml/min/month post-IS treatment. After PR, eGFR was similar in PR-IS and PR-S (-0.02 Vs -0.02 ml/min/month).

	PR-S	PR-IS	p
Time to PR (months)	22	18	0.92
CR (%)	65	51	0.16
Relapse (%)	22	31	0.37
Relapse after PR (months)	101	21	0.002*
Doubling of creatinine	3	7	0.32



Doubling of creatinine was seen in 2/73 of non-relapsers cf. 8/27 relapsers (p=0.02) / ESRD in 0/73 non-relapsers cf. 3/27 relapsers.

Of 28 patients with PAD, 18 (64%) reached ESRD of whom 7 died.

Conclusions: PR-IS is associated with similar outcomes as those with PR-S despite more severe disease. Relapse, however, with worse outcome, occurs sooner in PR-IS than in PR-S. 29% of patients who achieved PR did so in 1 year, and 82% in 3 years.

SA-PO364

Respiratory Manifestations of ANCA-Associated Vasculitis Kerry Greenan,[1] Darren Green,[2] Smeeta Sinha.[2] *[1]University of Manchester; [2]Department of Renal Medicine, Salford Royal NHS Foundation Trust, United Kingdom.*

Background: The prevalence of pulmonary manifestations of ANCA-associated vasculitis (AAV) is not well understood. This study assesses the prevalence of respiratory complications of AAV in patients presenting to a tertiary renal centre with primarily renal disease, not initially requiring dialysis; evaluates whether such complications are recognised and investigated appropriately, and examines the relationship between pulmonary vasculitis and mortality.

Methods: 58 patients with AAV were identified from the Chronic Renal Insufficiency Standards Implementation Study database, which is a prospective epidemiological study of patients with CKD stages 3-5, not requiring dialysis. Patients were identified as having AAV between 2000 and 2012. Presentation data was only available for 38 patients.

Results: A chest x-ray on presentation was available for 36 out of 38 patients, of which 16 (44.4%) had changes that could be associated with vasculitis. Only 31.3% of these were followed up with pulmonary function tests, a CT scan or respiratory review. 14 patients from the entire cohort of 58 had had a CT thorax during the course of their disease: 100% of these showed abnormalities, the most common being fibrosis (71.4%) and bronchiectasis (64.3%). 15 of 58 patients (25.9%) had confirmed pulmonary vasculitis, either as documented after respiratory review or as demonstrated by changes associated with vasculitis on a CT scan. There was no evidence of a link between pre-existing respiratory disease and this diagnosis (Fisher's Exact Test: p=0.103). The relationship between survival and pulmonary vasculitis was analysed using Cox-regression (correcting for age, smoking status, pre-existing respiratory disease and diabetes) and was not found to be statistically significant (p=0.086), although there was a trend towards increased mortality in those with pulmonary vasculitis.

Conclusions: Pulmonary manifestations of ANCA-associated vasculitis occurred in a significant proportion of patients. Only a small proportion of patients had had thorough respiratory investigations, even after an abnormal chest x-ray, suggesting that the pulmonary manifestations of AAV may be under-diagnosed.

SA-PO365

Anti-PLA2R-Associated Membranous Nephropathy in Adolescents and Young Adults Laurence H. Beck,[1] Rivka Ayalon,[1] Howard Trachtman,[2] David J. Salant.[1] *[1]Medicine, Boston University Medical Center; [2]Pediatrics, New York University Langone Medical Center.*

Background: Primary membranous nephropathy (MN) is most prevalent in Caucasian males aged 30-60 years. It is an uncommon cause of MN in pediatric cases which tend to be associated instead with secondary causes such as SLE or hepatitis B, or rarely with antibodies to bovine serum albumin (BSA) in younger children. We have assessed the prevalence of anti-PLA2R-associated MN in subjects whose disease was manifest prior to the age of 30.

Methods: A database of 275 subjects with MN, tested for anti-PLA2R at our institution, was queried for those whose initial disease presented at an age < 30 yr. We excluded those whose MN was associated with secondary causes, such as SLE (n=6), NSAIDs (n=1), or Hg exposure (n=1). We tested sera by western blot against PLA2R as well as neutral and cationic BSA.

Results: 21/29 cases (72%) were positive for anti-PLA2R antibodies. The age of disease onset in this group was 19.4±4.6 yr, 71% were male (15M:6F) and two cases exhibited symptoms as early as 12 years of age. At the time of sample collection, the mean age was 23.1±6.0, and values for serum Cr, albumin, and urine protein were 1.1±0.6 mg/dl, 2.8±0.9 g/dl, and 6.3±4.5 g/g Cr, respectively. In the remaining 8/29 (28%) who had no antibodies to PLA2R at the time of sample collection, age of disease onset was 15.9±6.2 and 75% were male (6M:2F). In this group, age at sample collection was 18.1±6.9, and mean values for serum Cr, albumin, and urine protein were 0.7±0.3 mg/dl, 3.1±1.4 g/dl, and 0.8±1.1 g/g Cr, respectively. None of the anti-PLA2R-negative cases had antibodies to neutral or cationic BSA.

Conclusions: Primary anti-PLA2R-associated MN is not limited to older adults and the prevalence in a pre-teen, adolescent, and young adult (< 30) age group is similar to that found in adults. Primary MN should be strongly considered in this age group. Although antibodies to PLA2R (or BSA) were not detected in 28%, this group had much lower proteinuria at the time of sample collection, suggesting that some of them might have already entered immunological remission. Further research is required to elucidate this possibility and other potential antigens in this group.

Funding: NIDDK Support, Private Foundation Support

SA-PO366

Anti-Phospholipase A_2 Receptor Antibodies: Correlation between Indirect Immunofluorescence and Western Blot Immunoassay with Clinical Status in Idiopathic Membranous Nephropathy Nuria S. Pérez,[1] Rivka Ayalon,[2] Carola Arcal Cunillera,[1] Laurence H. Beck,[2] Josep Maria Campistol Plana,[1] David J. Salant,[2] Luis F. Quintana.[1] *[1]Nephrology and Renal Transplantation, Hospital Clinic, Barcelona, Spain; [2]Nephrology and Renal Transplantation, Boston University Medical Center, Boston, MA.*

Background: Cumulative data from serum samples have shown that 70-80% of patients with idiopathic membranous nephropathy (IMN) have anti-PLA_2R antibodies that are reactive with native glomerular extract using a western blot (WB). Levels of circulating anti-PLA_2R show correlation with disease activity and may provide a tool for monitoring treatment efficacy. An alternative assay using an indirect immunofluorescence test (IIFT) enables the easy detection of anti-PLA_2R antibodies in serum.Using IIFT, anti-PLA_2R antibodies were reported in 52% of patients with biopsy-proven IMN, but in none of those with secondary MN . This discrepancy could be associated with different sensitivities of the two methods and requires evaluation in the same cohort. To do so we assessed the prevalence of anti-PLA_2R in a Spanish cohort of IMN patients by WB and IIFT in order to establish the correlation between these two methods for detecting anti-PLA_2R.

Methods: Anti-PLA_2R was assayed by WB and IIFT in serum samples from 30 patients with IMN collected at various stages of disease activity and correlated with other parameters.

Results: WB detected anti-PLA_2R in 50.0% of IMN patients in our cohort whereas the IIFT was positive in 46.7% suggesting similar sensitivity of the two tests. However, discrepant results were found in three cases. One might have been a false positive IIFT reaction because of the presence of anti-mitochondrial antibodies in a patient with primary biliary cirrhosis but the other two have no clear explanation. The antibody levels measured by IIFT in these patients correlated strongly with proteinuria (P =0.02).

Conclusions: This study reveals the limitation of existing methodologies–high specificity but limited ability to quantify anti-PLA_2R levels by WB and potential false-positive reactions with IIFT–and highlights the need for an antigen-specific quantitative immunoassay.

SA-PO367

Autoantibody against Phospholipase A_2 Receptor in Korean Patients with Membranous Nephropathy Yun Jung Oh,[1] Seung Hee Yang,[2] Hajeong Lee,[1] Jung Pyo Lee,[3] Dong Ki Kim,[1] Yon Su Kim.[1,2] *[1]Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea; [2]Kidney Research Institute Medical Center, Seoul National University Hospital, Seoul, Republic of Korea; [3]Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Republic of Korea.*

Background: Membranous nephropathy (MN) is an autoimmune disease and a common cause of nephrotic syndrome in adults. Since the M-type phospholipase A_2 receptor (PLA_2R) was identified as a target autoantigen in adult idiopathic MN, the prevalence of autoantibodies against PLA_2R has been reported in Caucasian, African American, and Chinese patients recently. However, the prevalence is unknown among Korean patients with MN.

Methods: We explored the prevalence of autoantibodies against PLA_2R in Korean patients with idiopathic MN using Western blot immunoassay. Western blotting was performed in 65 patients with idiopathic MN who were in various stage of clinical disease. We isolated glomeruli from normal kidney tissue obtained after radical nephrectomy for renal cell cancer with graded sieving. Human glomerular protein extraction was electrophoresed under nonreducing condition, and human serum was used as the primary antibody at a dilution of 1:100 and 1:25 in case of negative results. We assessed the reactivity of serum samples to PLA_2R blindly without knowledge of the clinical status.

Results: 25(67.6%) patients had detectable autoantibodies against human PLA_2R among 37 patients with nephrotic ranged proteinuria(>3.5g/day). Anti-PLA_2R was negative in all patients(n=17) who had entered clinical remission after treatment. Proteinuria was more severe in MN patients with anti-PLA_2R antibody(Ab) than those without (7,300 mg/g versus 97mg/g, P<0.001). However, the anti-PLA_2R Ab level was not significantly correlated with the severity of proteinuria.

Conclusions: A majority of Korean patients with idiopathic MN had autoantibodies against PLA_2R. These results confirmed the role of PLA_2R as a target antigen in idiopathic MN in Korean patient cohort.

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SA-PO368

Prevalence of Autoantibody against PLA2R1 in Patients with Membranous Nephropathy in Japan Shin'ichi Akiyama,[1] Takenori Ozaki,[1] Shoichi Maruyama,[1] Hitoshi Yokoyama,[2] Gerard J. Lambeau,[3] David J. Salant,[4] Seiichi Matsuo,[1] Enyu Imai.[1] *[1]Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Nephrology, Kanazawa Medical University, Kanazawa, Japan; [3]Institute of Molecular and Cellular Pharmacology, CNRS and University of Nice, France; [4]Medicine, Boston University.*

Background: Membranous nephropathy (MN) is the leading cause of the nephrotic syndrome in adults. The M-type phospholipase A2 receptor (PLA2R1) was recently identified as a major target antigen of idiopathic MN (iMN), but the prevalence of anti-PLA2R1 immunoglobulin G (anti-PLA2R1) is unknown among Japanese patients with MN. In this study, we determined the prevalence of anti-PLA2R1 from Japanese patients with MN.

Methods: We studied 109 patients with biopsy-proven iMN and 49 patients with secondary MN, and as controls, 5 each of patients with IgA-N, MPGN, MCNS and 5 healthy volunteers. Plasma samples were collected before treatment. Anti-PLA2R1 was analyzed in plasma by Western blotting under non-reducing condition with human glomerular extract (HGE) or recombinant internal fragment, 21-633 aa of PLA2R1 protein (rPLA2R1) as antigen. The HGE was made from human glomeruli after removing contaminating endogenous IgG. rPLA2R1 was produced in HEK293 cells transfected with a plasmid DNA vector coding for thePLA2R1 sequence.

Results: Anti-PLA2R1 was only found in MN, and was not detected in any of the patients with other glomerular diseases or healthy volunteers. The anti-PLA2R1 was detected in 54 out of 109 patients with iMN and 3 of 49 patients with secondary MN. Causes of 3 cases of secondary MN with presence of anti-PLA2R1 were one bucillamine-induced nephrotic syndrome, and 2 other diseases. The major subclass of anti-PLA2R1 was IgG4.

Conclusions: The prevalence of anti-PLA2R1 in Japanese patients with idiopathic and secondary MN was49.5% and 6.1%, respectively. The prevalence of iMN caused by anti-PLA2R1 may be lower in Japanese iMN compared with those in Caucasian and Chinese. Some types of secondary MN might be linked to the presence of anti-PLA2R1.

SA-PO369

How to Distinguish between Idiopathic and Malignancy-Associated Membranous Nephropathy Jennie Lönnbro-widgren,[1] Jenny C. Nystrom,[3] Johan C. Molne,[2] Borje Haraldsson.[1] *[1]Sahlgrenska Academy, University of Gothenburg, Institute of Medicine, Gothenburg, Sweden; [2]Sahlgrenska Academy, University of Gothenburg, Institute of Biomedicine, Gothenburg, Sweden; [3]Sahlgrenska Academy, University of Gothenburg, Institute of Neuroscience and Physiology, Gothenburg, Sweden.*

Background: Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults, and presents either as idiopathic (I-MN) or secondary to other diseases. Approximately 5-20% of the cases of MN are malignancy-associated. Previous studies have suggested that deposition of glomerular IgG4, as well as PLA2-receptor antibodies, may play an important role in the pathogenesis of I-MN. The clinical and renal pathological manifestations of I-MN and malignancy-associated membranous nephropathy (M-MN) are similar, and the diagnostic tools to distinguish between I-MN and M-MN are not clearly validated, but of great importance.

Methods: In this retrospective study, 41 patients were included, 27 with I-MN and 14 patients with M-MN diagnosed between 2000 and 2011. Demographic and clinical data were analyzed. Furthermore, we analyzed the histopathological pattern of IgG4 by immunohistochemistry.

Results: The histopathological pattern, with positive IgG4 was present in 15/27 patients with I-MN, but only in 3/14 patients with M-MN. ($p<0.001$). The patients with M-MN were significantly older (63±10 years) compared to those with I-MN (45±14 years) $p<0.001$. However, serum albumin and the degree of albuminuria did not differ between the two groups.

Conclusions: By combining parameters in a new algorithm, we were able to discriminate patients with M-MN from those of idiopathic disease with even higher precision. We conclude that it is possible to identify patients with MN more likely to have an underlying malignant disease based on the absence of IgG4 in the renal biopsy.

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SA-PO370

Subendothelial Electron-Dense Deposits May Provide a Clue to the Diagnosis of Malignancy-Associated Membranous Nephropathy Kana Iwazu, Yoshitaka Iwazu, Shin-ichi Takeda, Tetsu Akimoto, Yoshiyuki Morishita, Wako Yumura, Taro Sugase, Eiji Kusano. *Division of Nephrology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

Background: Cancer patients occasionally develop renal disorders independently of tumor invasion into the kidneys. However, it is often unclear in such cases whether there is a direct relationship to the pre-existing malignancies or not. We recently reported that tumor-bearing rats showed features of glomerulopathy (Nephrol Dial Transplant 27: 1786, 2012). Of particular interest is that subendothelial electron-dense deposits (EDDs) were observed in glomeruli of this animal model, whereas such ultrastructure is usually located in the subepithelial area among patients with idiopathic membranous nephropathy (I-MN). **Aim:** To elucidate distinctive characteristics of malignancy-associated MN (M-MN) from the perspectives of clinical use.

Methods: 7 patients who have malignancies in addition to membranous nephropathy (MN; group M) and 35 patients with I-MN (group I) were included in this study. The clinical and pathological profiles in group M were compared with those in I-MN (group I). The localization of EDDs and immunohistochemical IgG3-affinities were investigated, respectively.

Results: Subendothelial EDDs were evident exclusively in group M (3 out of 7 patients), whereas only one case in 35 I-MN cases in which the electron micrograms were available. Although EDDs were observed also in the mesangial area, there was little difference in the degrees between the two groups. The IgG3 score was significantly greater in group M (n=7) than in group I (n=18).

Conclusions: The present study indicates that subendothelial EDDs and IgG3 deposition in glomeruli are the prominent features of M-MN. These findings might provide new insights into the clinical management of malignancy-associated nephropathies.

SA-PO371

Kidney Injury Molecule-1 and Neutrophil Gelatinase-Associated Lipocalin as Prognostic Markers in Idiopathic Membranous Nephropathy Rutger J. Maas,[1] Femke Waanders,[2] Julia M. Hofstra,[1] Hilde P. Peters,[1] Esther Meijer,[2] Harry Van Goor,[2] Jan A.J.G. van den Brand,[1] Jack F. Wetzels.[1] *[1]Nephrology, Radboud University Nijmegen Medical Center, Netherlands; [2]Kidney Center, University Medical Center Groningen, Netherlands.*

Background: Urinary excretion of beta 2-microglobulin (β2m) and alpha 1-microglobulin predict progression of idiopathic membranous nephropathy (iMN) with reasonable accuracy (±80%). KIM-1 and NGAL are novel urinary biomarkers of tubular damage. We investigated if these markers can improve the prediction of progression in iMN.

Methods: We measured KIM-1 and NGAL concentrations in frozen stored urine samples of a previously described cohort of patients with iMN, nephrotic proteinuria and normal kidney function (CJASN 2011;6:2846). Progression was defined as a serum creatinine rise >50%, a rise in serum creatinine of >25% and an absolute value of ≥135µmol/l, or a clinical decision to start immunosuppressive therapy. Remission was defined as proteinuria <3.5 g/d and >50% reduction.

Results: 69 urine samples were available for measurement of KIM-1 and NGAL. Baseline patient characteristics were comparable to the entire cohort. Median survival time was 31 months (IQR 17-58 months). Progression occurred in 30 patients (44%), and spontaneous remission occurred in 36 patients (52%). In univariate analysis both KIM-1 and NGAL were predictors of progression (Table). In multivariate analysis, β2m was the strongest independent predictor. Combining β2m with KIM-1 and NGAL improved specificity, at the expense of sensitivity (Table).

Conclusions: KIM-1 and NGAL predict progression of iMN, and may be used in combination with β2m to increase specificity of outcome prediction.

Prognostic accuracy of urinary proteins in progression of iMN

	ROC-AUC (95% CI)	Optimal cut-off	Sensitivity (%)	Specificity (%)
β2m*	0.80 (0.69-0.91)	1.0 µg/min	75	78
KIM-1	0.75 (0.62-0.87)	1.6 ng/min	77	67
NGAL	0.74 (0.62-0.87)	30 ng/min	67	82
β2m + KIM-1 + NGAL		combined tresholds	54	92
β2m		4.5 µg/min**	46	92

* β2m concentration could not be determined in four samples because of a urinary pH < 6.0
** Elevated cut-off value with specificity equal to combined tresholds of β2m, KIM-1, and NGAL

SA-PO372

Clinical and Histological Prognostic Factors in Idiopathic Membranous Nephropathy: Long Term Evaluation Antonello Pani, Floris Matteo, Patrizia Melis, Maura Conti, Riccardo Cao, Matteo Floris, Andrea Angioi, Valeria Matta. *Nephrology, G Brotzu Hosp, CA, Italy.*

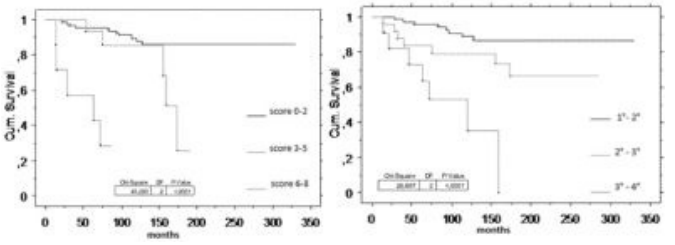
Background: We review our cumulative series of pts (>20yrs) with IMN and Nephrotic Syndrome (NS) in order to evaluate the long term outcome, the role of therapy in influencing remission, the histological prognostic factors and the clinical course of relapses.

Methods: Our report includes 114 pts (M=70, F=44) with biopsy-proven IMN and NS at presentation treated with IS therapy (80.7% Ponticelli's Scheme; 18.4 steroids alone; 0.9 Cyclosporin). Histological lesions (HL) were graded according to: **a**) glomerular stage: from 1 to 4; **b**) percentage of glomerular sclerosis (GS); **c**) tubulo-interstitial damage: from 0=absent to 3=severe; **d**) arteriolar lesions: from 0=absent to 3= severe**.** 1 year after the beginning of therapy**,** response was evaluated as: complete (**CR** proteinuria ≤0.3), partial (**PR**≤3.5 and>0.3), and non remission (**NR**>3.5).

Results: At diagnosis eGFR was below 60 in 29% of pts. At the end of follow up ESRD occurred in 20.1% and 25.4% had died. The censored renal survival rates at 5 and 20 yrs were 91.1 and 71.1%; we observed 53 relapses in 31 pts. Cumulative survival at 5 and 20 yrs was 90.9 and 66.5%. We obtained the histological score by summing the scores of parameters listed in **tab1** and then performing a correlation with long-term renal survival.

Parameter	Score (%)			
	0	1+	2+	3+
Tubulo-interstitial damage	65.4	26.1	6.5	2.8
Arterial-arteriolar lesion	73.8	18.6	6.5	0.9
Glomerular sclerosis		<15%	15-30%	>30%
		87.8	6.5	5.6
Glomerular stage		1° - 2°	2° -3°	3° - 4°
		67.5	22.5	9.9

Tab.1: Biopsy findings at presentation



Severity of lesions and glomerular stage predicted lower renal survival. No significant differences were observed in renal outcome between pts who had 1 or more relapses and those who never relapsed.

Conclusions: An overall response rate (**CR**+**PR**) of 63% was obtained,with a favourable trend in PR compared to NR (all in ESRD after 15 yrs). Pts with severe HL at presentation had worse prognosis, and glomerular stage statistically correlated to lower renal survival. The main prognostic negative factors at presentation were elevated SCr and low GFR. GS has been identified as an independent prognostic factor.

SA-PO373

Efficacy and Safety of Rituximab Plus Cyclosporine in Idiopathic Membranous Nephropathy: Initial Report of an Ongoing Prospective Trial

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Background: Cyclosporine (Csa) reduces proteinuria in idiopathic membranous nephropathy (IMN) but partial remissions(PR) rather than complete remissions(CR) are common & relapse upon drug withdrawal is problematic. Extending treatment may increase remissions & reduce relapses but potential for nephrotoxicity is a concern. Rituximab (RTX) has shown promise in IMN but PRs are more common & effect on proteinuria tends to be delayed for many mos exposing pts to the effects of nephrotic syndrome (NS) for longer periods. We are conducting a prospective trial (NCT00977977) in IMN pts to investigate whether "induction"with RTX +Csa followed by "maintenance" RTX achieves greater reduction in proteinuria than either agent alone, increases % CRs & reduces relapse rate. Here we report the interim data.

Methods: Patients with high grade proteinuria despite conservative treatment for minimum(min) 6 mos & eGFR≥40 ml/min/1.73 m^2 receive RTX (1 gm day 1,15) +Csa x6 mos (then tapered); 2nd course of RTX given after min of 6 mos *and* evidence of B cell recovery.

Results: To date 8 pts have been treated. All pts showed progressive rise in proteinuria (± decline in renal fxn) during conservative treatment. Mean eGFR:57 ml/min/1.73 m^2(range 37 -90); Proteinuria 11.2 g/24 hrs (8-14). 5 pts completed a min of 6 mos f/u. At 6 mos,cumulative remission rate: 80%; 3 (60%) PR(def: proteinuria ≤3.5g/d+ ≥ 50% reduction);1(20%)CR (def: ≤0.3 g/d). No relapses. Regimen was well tolerated. 1 pt developed late onset neutropenia.

Table: Trend in Proteinuria

Pt #	Baseline	2 mos	3 mos	6 mos	9 mos	12 mos	15 mos
1	11.2	8.9*	10.9	10.8	14	5.7	1.5*
2	10.5	0.8*	1.1	0.2	0.3	0.1	0.2
3	14.1	-	6.4	3.4	2.1		
4	14	1.7*	1.1	0.9	0.35		
5	8.0	2.7	1.9	1.2			
6	9.8	8.2	7.2				
7	9.6	0.5					
8	12.6	4.1					

24 hr urine collections (g/24 hrs) unless indicated; * spot pr/cr ratio (mg/mg)

Conclusions: "Induction" with combination RTX + Csa followed by "maintenance" RTX may be an approach for IMN to achieve more rapid remission of the NS & may obviate the need for long term immunosuppression. It appears to be well tolerated. Enrollment continues. Long term f/u is needed.

Funding: NIDDK Support

SA-PO374

Intravenous Methylprednisolone Therapy Accelerated Complete Remission of Proteinuria in Adult Patients with Minimal Change Nephrotic Syndrome

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Background: The effects of intravenous methylprednisolone therapy (mPSL) on complete remission (CR) of urinary protein in patients with minimal change nephrotic syndrome (MCNS) are controversial because of insufficient sample size study. The present study aimed to clarify the effects of mPSL on CR in the largest cohort of adult patients with new-onset MCNS in the world.

Methods: The present Japanese multicenter retrospective cohort study included 125 patients who were ≥15 years, diagnosed as new-onset MCNS by renal biopsy in 5 major nephrology centers between 2000 and 2009, and initiated corticosteroid therapy. Predictors of CR were identified using Log-rank test and univariate and multivariate Cox proportional hazards models. To control confounding by indication, we used propensity score (PS), an estimated probability of use of mPSL.

Results: Baseline characteristics of 125 patients before initiated corticosteroid therapy were age 40(23-62) yr (median (interquartile)), male 64%, creatinine 0.9(0.7-1.3)mg/dL, and urinary protein 8.4(4.0-14.5)g/day. Time to CR was significantly shorter in 65 patients with initial mPSL than 60 patients without initial mPSL (11[8-20] vs. 19[12-37] days, P<0.001). Patients with mPSL had significantly higher cumulative probability of CR, compared with those without mPSL (P=0.006). Use of mPSL was identified as predictor of early CR in multivariate Cox proportional hazards model (HR1.54 [95%CI 1.05-2.26], P=0.026) and PS-stratified model (1.47[1.01-2.14], P=0.042).

Conclusions: In adult patients with new-onset MCNS, mPSL accelerated complete remission of proteinuria.

SA-PO375

Rituximab in Steroid-Dependent or Multirelapsing Nephrotic Syndrome of Adults and Children: Results from the NEMO Trial

Piero Ruggenenti,[1,2] Barbara Ruggiero,[1] Paolo Cravedi,[1] Marina Vivarelli,[3] Laura Massella,[3] Maddalena Marasa',[1] Annalisa Perna,[1] Antonietta Chianca,[1] Nadia Rubis,[1] Mauro Abbate,[1] Francesco Emma,[3] Giuseppe Remuzzi.[1,2] *[1]Mario Negri Institute for Pharmacological Research, Bergamo, Italy; [2]Ospedali Riuniti di Bergamo, Italy; [3]Bambino Gesù Children Hospital, Roma, Italy.*

Background: Treatment of steroid dependent or multirelapsing minimal change disease (MCD) and focal-segmental glomerulosclerosis (FSGS) is burdened by major side effects.

Methods: In this prospective, sequential, open-label, multicentre trial, 24 patients (10 children) with steroid-dependent or multirelapsing MCD (n=16) or FSGS on remission while on maintenance immunosuppression, received one (n=22) or two (n=2) 375 mg/m^2 rituximab (RTX) infusions to deplete B cells. Concomitant treatment back-titration up to withdrawal was planned over 6-9 months. Primary aim was to compare intra-patient incidence of NS relapses 1 year post vs pre RTX.

Results: Over 1 year post-RTX there were 19 relapses compared to 72 in the year before [p<0.0001]. Event reduction was similar in children vs adults and MCD vs FSGS.

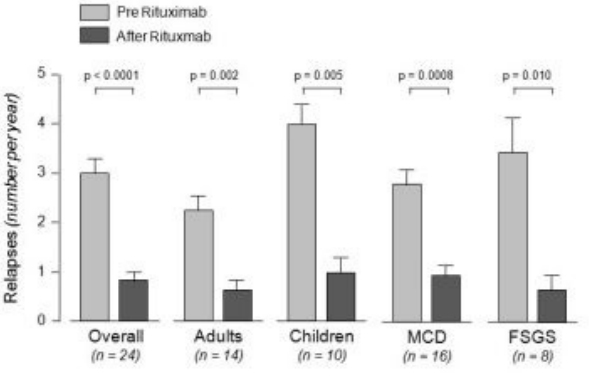


Figure1. Incidence of NS relapses before and after rituximab in the study group as a whole (Overall) and according to age and diagnosis

Eleven patients never relapsed after RTX and 14 were in remission at 1 year post-RTX without any immunosuppression. In parallel with reduced cumulative doses of steroids, calcineurin inhibitors and anti-proliferative agents (p=0.005, p<0.01, p<0.005 vs pre RTX, respectively), hypertension and dyslipidemia improved in all patients. Children resumed normal growth. There were no RTX-related side effects.

Conclusions: RTX is a valuable option to safely maintain remission and avoid /limit chronic immunosuppression in steroid-dependent or multirelapsing MCD and FSGS, in adults as well in children.

Funding: Government Support - Non-U.S.

SA-PO376

Impact of Rituximab on Growth, Weight and Bone in Children with Intractable Steroid Dependent Nephrotic Syndrome Mai Sato, Akinori Miyazono, Takuya Fujimaru, Masao Ogura, Koichi Kamei, Shuichi Ito. *Division of Nephrology and Rheumatology, National Center for Child Health and Development, Tokyo, Japan.*

Background: Treatment for childhood steroid-dependent nephrotic syndrome (SDNS) is still challenging. Patients often suffer a lot of serious adverse events due to steroid such as growth retardation, obesity and osteoporosis. These adverse events impair the adherence and also cause behavioral and psychological difficulties. There is increasing evidence of the efficacy of rituximab (RTX) against intractable SDNS. We examined the impact of RTX on growth retardation, obesity and osteoporosis in SDNS.

Methods: Fourteen patients with SDNS, who has been intractable despite of multiple immunosuppressive agents, were treated with RTX. RTX infusion was given 1 to 7 times and the mean follow-up after the first RTX infusion was 2.4 years. We compared height (standard deviation), obesity index and bone mass density (BMD) between before RTX infusion and the last visit. We assessed improvement of these items before and after RTX use.

Results: Number of relapses and the cumulative dose of prednisone were significantly decreased by RTX. Thirteen (92.8%) of the 14 patients had significantly improved height SD (n=14, before vs after: -1.28 SD vs -0.69SD; paired t- test. P=0.03) and obesity index (n=14, 23.6% vs 9.19%: paired t-test, p=0.026). All patients had significantly improved BMD z-scores (n=5, -3.52 vs -1.94; paired t-test, p=0.03). There were no severe adverse events except for treatable agranulocytopenia in one patient.

Conclusions: RTX could confer significant improvement of growth, obesity and osteoporosis in children with intractable SDNS, which may contribute to in improvement of activities of daily living and quality of life.

SA-PO377

Immune Modulatory Mechanism of Rituximab in Steroid Refractory Heavy Proteinuria Ching-Yuang Lin.[1,2] *[1]Clinical Immunology Center, China Medical University Hospital, Taichung, Taiwan; [2]College of Medicine, China Medical University, Taichung, Taiwan.*

Background: Heavy proteinuria was a risk factor for chronic kidney disease in later life. Rituximab has been suggested is a one of the therapeutic options. We hypothesized that rituximab may induce remission of heavy proteinuria and preserve $CD8^+FoxP3^+$ regulatory T (Treg) cell function, decrease IL-17 secreting T cell (T17) cell and serum IL-17A level.

Methods: We studies 66 refractory heavy proteinuria patients with open-label randomized controlled study with or without rituximab treatment for 6 months. All of them received renal biopsy. 33 of whom were treated with rituximab. $CD4^+FoxP3^+$, $CD8^+FoxP3^+$, $CD4^+T17$ and $CD8^+T17$ cells in peripheral blood mononuclear cells and renal biopsy specimens, suppressive activity and granzyme B expression of $CD8^+FoxP3^+$ Treg cells and serum IL-17A level were studied before and after rituximab treatment.

Results: Rituximab treatment was associated with induction of remission and recovery of circulating T17/Treg ratio in patients with IgM nephropathy (IgMN) and focal segmental glomerular sclerosis (FSGS). Treg cells were rare and T17 cells were abundant in renal tissue from FSGS and IgMN patients. After rituximab therapy, T17 cells almost disappeared and Treg cells were noted particalarly in renal tissue of responsive cases. The decreased population of T17 cells were concurrent with decreased plasma IL-17A levels and suppressive activity in $CD8^+FoxP3^+$ Treg cells after rituximab treatment. $CD8^+T17/CD8^+$ Treg ratio in PBMCs were correlated with Δ dailyurine protein in rituximab responsive cases. Rituximab was no effectiveness in IgA nephropathy, idiopathic crescentic glomerulonephritis.

Conclusions: Our study suggests that treatment of refractory heavy proteinuria in patients with IgMN or FSGS by rituximab might induce remission by modulating Treg/T17 cell number and function.

SA-PO378

Longterm Effects of LDL Apheresis for the Drug Resistant Nephrotic Syndrome Evidenced in a Prospective Observational Cohort Study (POLARIS) Eri Muso,[1] Takao Saito.[2] *[1]Nephrology and Dialysis, Kitano Hospital, the Tazuke Kofukai Medical Research Institute, Osaka, Japan; [2]Nephrology and Rheumatology, Fukuoka University Hospital, Fukuoka, Japan.*

Background: LDL apheresis (LDL-A) has been applied for drug-resistant nephritic syndrome (NS) as an alternative therapy to induce remission via improving hyperlipidemic condition. A multicenter prospective study, POLARIS (Prospective Observational Survey on the Long-Term Effects of the LDL-Apheresis on the Drug Resistant Nephrotic Syndome) was conducted and final data were analyzed under the preliminary results of nearly half of enrolled patients being provided early relief from NS (ASN 2009).

Methods: Refractory nephrotic syndrome patients who had been resistant to primary medication for at least 4 weeks were prospectively recruited to the study and treated with LDL-A (2 sessions/week, max. 12 sessions). The clinical efficacy was evaluated based on the rate of the patients who achieved remission of nephrotic state defined as proteinuria (UP) level of <1.0g/day after 2years follow-up. Parameters before start or at the completion of LDL-A that affect clinical efficacy were also examined.

Results: 58 refractory NS patients were recruited to the POLARIS study and treated with LDL-A in 40 facilities. Two-year follow-up data were obtained in 44 patients and 21 (47.8%) had preferable outcome achieving UP level <1.0g/day. Moreover 11 (25.0%) maintained remission state throughout follow-up period without any relapse. The UP level at the completion of the LDL-A in these patients ware significantly lower than the others (1.68±1.75 g/day vs 6.18±3.54 g/day, p<0.001). The improvement rate of UP, serum albumin, and serum creatinine during LDL-A also affected clinical efficacy.

Conclusions: Nearly half of drug-resistant NS showed remission even 2 years after LDL-A. Prognosis of patients who responded LDL-A and exerted an improvement of nephrotic parameter are likely to be favorable.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO379

Plasmapheresis in Steroid-Resistant FSGS Tej K. Mattoo,[1] Mark Mentser,[2] Frederick J. Kaskel,[3] William E. Smoyer,[2] Lena Peschansky.[1] *[1]Pediatrics, Children's Hospital of Michigan, Detroit, MI; [2]Pediatrics, Nationwide Children's Hospital, Columbus, OH; [3]Pediatrics, The Children's Hospital at Montefiore, Bronx, NY; [4]Pediatrics, Nationwide Children's Hospital, Columbus, OH; [5]Pediatrics, Children's Hospital of Michigan, Detroit, MI.*

Background: FSGS causes end-stage renal failure in about 15% of children and 5% of adults. Only about 30%-50% of patients with FSGS respond to any therapeutic intervention. Experimental findings support the possibility of a circulating factor as the cause for FSGS.

Methods: The primary objective of this randomized, prospective study was to evaluate the role of plasmapheresis with and without tacrolimus in patients with steroid-resistant native kidney FSGS. A total number of 14 patients were randomized to tacrolimus only (Group I) or tacrolimus with 15 sessions of plasmapheresis (Group II). The primary study end-point point was the number of patients in complete remission (urine protein/creatinine ≤ 0.2) at 6 months.

Results: The results of the study primary end-point for group I and 2 are shown in the table. No significant differences existed in age (13 ± 4.2 Vs 13.6 ± 2.7), gender and racial distribution of the patients in the two groups. The mean serum creatinine (1.15 ± 1.1 Vs 1.4 ± 0.8), mean serum albumin (3.4 ± 0.8 Vs 3.8 ± 0.4) and the mean serum tacrolimus (6.9 ± 1.0 Vs 6.7 ± 3.3) levels between group 1 and 2 at the time of study conclusion were also not significantly different. Additional data were collected in patients who continued follow-up at 12 months after randomization.

	Mean Urine Pr/Cr Ratio				Number of Patients in Remission At 6 Months	Extended Follow-UP At 12 Months	
	Baseline	6 Weeks	3 Months	6 Months		Urine Pr/Cr	Patients in Remission
Group 1 (n=8)	6.8±5.7	4.4±5.3	4.2±4.4	2.0±2.5	1 (12.5%)	3.9±7.8 (n=5)	2
Group 2 (n=6)	5.1±2.8	2.3±2.0	1.8±2.1	1.3±1.1	2 (33%)	4.4± 6.8 (n=6)	1*
P Value	<0.5	<0.3	<0.2	<0.5			

*The second patient became non-compliant with medication and follow-up

Conclusions: The study did not reveal any significant difference in proteinuria at 6 months. The major limitation of the study was the sample size; invasive nature of plasmapheresis made patient recruitment difficult.

Funding: NIDDK Support

SA-PO380

Role of Immunosuppressive Therapy in Renal Survival in Collapsing Focal Segmental Glomerulosclerosis (FSGS) Louis-Philippe Laurin,[1] JulieAnne G. McGregor,[1] Vimal K. Derebail,[1] Susan L. Hogan,[1] Caroline Jennette Poulton,[1] Adil M.H. Gasim,[2] J. Charles Jennette,[2] Ronald J. Falk,[1] Patrick H. Nachman.[1] *[1]Division of Nephrology and Hypertension, University of North Carolina, Chapel Hill, NC; [2]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.*

Background: Idiopathic collapsing FSGS has historically been associated with poor renal prognosis. Minimal clinical data exists on the efficacy of immunosuppressive therapy, specifically calcineurin inhibitors.

Methods: Inception cohort study of biopsy-proven collapsing FSGS patients diagnosed between 1989 and 2012. Statistical analysis performed with Mann-Whitney or Fisher's exact tests. Time to end-stage renal disease (ESRD) was reported with Cox regression hazard ratio (HR) with 95% confidence interval (CI).

Results: 42 patients studied: age 34±16 years; 64% Black; and 52% female. Baseline eGFR 53±29mL/min/1.73m^2; proteinuria 12±7g/d; serum albumin 2.5±1.0g/dL; and follow-up time 37±30mo. 32 patients (76%) treated with immunosuppression (7 with steroids alone and 23 with calcineurin inhibitors). Treated patients had a trend towards a higher baseline eGFR (55±29 vs. 43±24mL/min/1.73m^2, p=0.2). More treated patients had either complete (proteinuria <0.3g/d) or partial (decrease in proteinuria >50% and <3.5g/d) remission [18/29 (62%) (treated) vs. 1/7 (14%) (untreated), p=0.04]. 17 patients (40%) reached ESRD. Patients receiving immunosuppressors were less likely to reach ESRD [9/32 (28%) vs. 8/10 (80%), p=0.008]. In univariate analyses, immunosuppression (HR 0.38, 95%CI 0.14-0.99, p=0.049) and higher baseline eGFR (HR 0.97, 95%CI 0.948-0.995, p=0.02) were associated with a better renal survival. In multivariate analysis, baseline eGFR predicted better renal survival (HR 0.97, 95%CI 0.948-0.997, p=0.03), and effect

of immunosuppression on renal survival persisted after controlling for baseline eGFR, but was not statistically significant (HR 0.61, 95%CI 0.2-1.8, p=0.4).

Conclusions: Immunosuppression may be associated with an improved renal survival in collapsing FSGS. A larger cohort of patients is needed to better define response to therapy.

SA-PO381

Treatment of Resistant Primary Focal Segmental Glomerulosclerosis (FSGS) with Adrenocorticotropic Hormone (ACTH) Gel Jonathan J. Hogan, Andrew S. Bomback, Pietro A. Canetta, Maya K. Rao, Gerald B. Appel, Jai Radhakrishnan. *Department of Internal Medicine, Division of Nephrology, Columbia University Medical Center, New York, NY.*

Background: Immunosuppression-resistant FSGS often leads to ESRD. ACTH has shown efficacy in resistant nephrotic syndrome, but data are limited with ACTH treatment of FSGS.

Methods: In this retrospective case series, 12 patients with primary FSGS and nephrotic syndrome resistant to previous immunosuppression were administered ACTH gel (median dose 80 units subcutaneously SC twice weekly) for a median of 26 (range 12-56) weeks of therapy. Complete remission was defined as stable/improved serum Cr and a sustained fall in proteinuria to <500 mg/day by last follow-up, and partial remission as stable/improved serum Cr with 50% reduction proteinuria to 500-3500 mg/day by last f/u.

Results: Median age was 41 (range 18-66) years and median time from diagnosis to treatment was 30 (range 2-276) months. Median eGFR was 33 (range 17 to 116) mL/min/1.73m^2 and median proteinuria 6100 (range 1600 to 16,800) mg/day. FSGS subtypes were tip lesion (n=6), NOS (n=4) and cellular (n=2). All patients had previously received prednisone and had failed or relapsed after at least 2 (median 3) prior immunosuppressive therapies, and 10 patients had failed at least 3 therapies. Five of 12 patients experienced partial remission at last follow-up (median follow-up time 58 (range f/u 4-82) weeks after stopping ACTH).

Serum Creatinine and Proteinuria for Patients Experiencing Partial Remission with ACTH

Pre-ACTH Cr (mg/dL)	Pre-ACTH Proteinuria (mg/day)	Post-ACTH Cr (mg/dL)	Post-ACTH Proteinuria (mg/day)	Cr at last f/u (mg/dL)	Proteinuria at last f/u (mg/day)	F/U (wks) after stoping ACTH
2.3	4000	1.3	963	1.3	1297	11
1.1	5200	0.7	1500	0.7	960	28
1.2	2100	1.4	272	1.3	641	82
1.6	10,300	1.2	5380	1.6	1277	56
3.6	15,200	1.3	891	1.1	1810	4

The median time-to-remission was 6 (range 6-24) weeks. No severe adverse events were reported. No clinical or biopsy characteristics predicted response to ACTH therapy.

Conclusions: ACTH gel is a useful treatment option for some patients with nephrotic syndrome due to immunosuppression-resistant primary FSGS, with partial remissions observed in 5 of 12 patients.

SA-PO382

Long-Term Follow Up of Rituximab Treatment for Fibrillary Glomerulonephritis Jonathan J. Hogan, Michaela Restivo, Pietro A. Canetta, Jai Radhakrishnan, Gerald B. Appel, Andrew S. Bomback. *Department of Internal Medicine, Division of Nephrology, Columbia University Medical Center, New York, NY.*

Background: Approximately 50% of patients with fibrillary glomerulonephritis (GN) progress to ESRD within 2 yrs of diagnosis, and no standard therapy exists. A previous case series reported short-term disease remission in 3 pts treated with rituximab, but data on larger cohorts with longer f/u are lacking.

Methods: We retrospectively reviewed all cases of fibrillary GN at Columbia University Medical Center treated with rituximab. Nonprogression of disease was defined as stable/improved serum Cr with a minimum of 1 yr of f/u for those who did not reach ESRD.

Results: Eleven pts (8 women, all caucasian) with fibrillary GN were treated with rituximab (1 gm IV X 2 doses or 375 mg/m^2 X 4 doses); 4 received concomitant oral steroids. All were on ACEI or ARB therapy. Median age at diagnosis was 57 yrs (range 38-68). Median time from diagnosis to treatment was 5 mo (range 1.2-83). Median Cr at rituximab initiation was 2.1 mg/dl (range 0.7-3.0) and median proteinuria 4497 mg/day (range 210-7400). Light microscopy findings were mesangioproliferative GN(n=2), MPGN(n=7), endocapillary proliferative GN(n=1), and diffuse proliferative/crescentic GN (n=1). Four pts had received immunosuppression before rituximab and 9 received immunosuppression after rituximab, with 4 pts receiving a second rituximab course. At last f/u, 2 pts were non-progressors; 5 progressed to more advanced CKD and 4 reached ESRD.

Detailed Data for Nonprogressors

Age, Gender	Light Microscopy Finding	Pre-Rituximab Cr (mg/dL)	Pre-Rituximab Proteinuria (mg/day)	Last Cr (mg/dL)	Last Proteinuria	F/U time (mo) after Rituximab (mg/day)	Immunosuppression after Rituximab
46, Female	MPGN	0.7	3000	0.7	4065	76	Tacrolimus, *Rituximab
67, Female	MPGN	2.3	1300	2.0	135	26	Steroids, *Rituximab

*Both pts received a second course of rituximab

Median f/u after rituximab for non-ESRD pts was 33 mo (range 24-76). No significant adverse events were reported.

Conclusions: In the largest case series describing long-term f/u of fibrillary GN after rituximab, 2/11 pts had nonprogression of disease, 5/11 progressed to more advanced CKD and 4/11 reached ESRD.

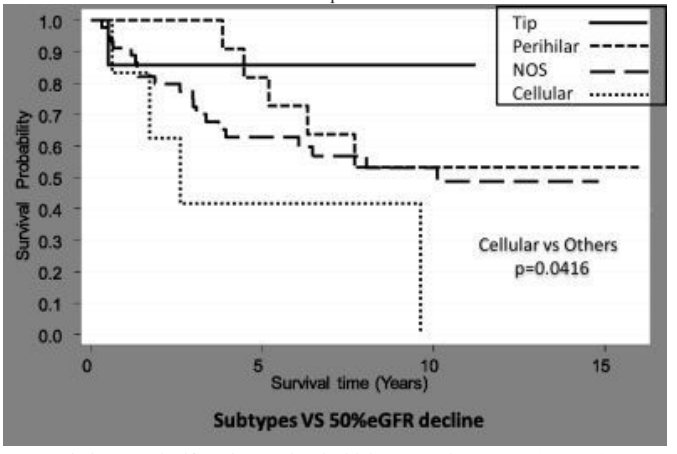
SA-PO383

Impact of Pathological Subtypes on Long-Term Outcomes of Focal Segmental Glomerulosclerosis Montira Assanatham,[1] Panas Chalermsanyakorn,[2] Suchin Worawichawong,[2] Vasant Sumethkul,[1] Pinkaew Klyprayong,[1] Chagriya Kitiyakara.[1] *[1]Renal Division, Department of Internal Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Department of Pathology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*

Background: It has been proposed that primary Focal Segmental Glomerulosclerosis (FSGS) can be divided into different histopathological subtypes. Initial data from western population show that different subtypes may have different characteristics and outcomes. To date, there is no long-term outcome data in Asian populations. Our main goal is to define the frequency and long-term outcome for each subtypes in Thai patients.

Methods: A retrospective analysis of primary FSGS diagnosed at Ramathibodi Hospital from 1993 to 2006 was conducted. FSGS subtypes were classified by 2 experienced renal pathologists. Duration from diagnosis to remission (complete and partial), 50% GFR decline and end stage renal disease (ESRD) was documented. Log-rank survival and multiple logistic analysis was performed.

Results: The median follow-up was 79 months for all FSGS (n=84). The frequency of each subtype was: NOS 56%, perihilar 26.2%, tip 9.5%, cellular 8.3%, and collapsing 0%. Baseline demographic characteristics for each subtype were similar. Tip variant had a better presenting renal function and higher remission rates. By multivariate analysis, cellular variant was associated with more rapid GFR decline.



Remission was significantly associated with better renal outcomes (p<0.001).

Conclusions: In our cohort, NOS was the most common subtype. In contrast to western studies, the collapsing variant was not found. Tip variant tended to had better renal outcomes while cellular variant was independently associated with rapid GFR decline. Finally, remission was associated with better renal outcomes regardless of subtypes.

SA-PO384

Clinical and Laboratorial Findings of 26 Patients with Collapsing Glomerulopathy: A Single Center Experience Andrea C.E.P. Valenca, Maria Carolina N.R. Neves, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil.*

Background: Collapsing glomerulopathy (CG) is an aggressive form of kidney disease. Is characterized by heavy proteinuria associated with rapidly progressive renal failure and poor renal outcome. Originally described as a condition associated with HIV infection, it is known that there are several etiological factors involved with this histological finding. Here we describe an experience of clinical series followed in our institution.

Methods: We retrospectively analyzed 26 patients with biopsy proven CG from 1998-2012; all of them had at least one glomerulus with defining features: glomerular capillary tuft collapse and overlying podocyte hypertrophy and hyperplasia.

Results: Epidemiological, clinical manifestations and outcomes are described below. Etiological classification showed that the idiopathic form was most common 17 cases, followed by: HIV 3; lupus 2; interferon 1; anabolic steroids 1; lymphangioleiomyomatosis 1; pamidronate 1.

Demographics, Clinical Presentation and Outcomes

	N=26
Age (mean)	34 ± 14.4
Female (%)	61.5
Black (%)	7.6
Nephrotic syndrome (%)	69.2
HTN (%)	38.5
MAP (mmHg)	96 ± 17.6
sCr (mg/dl) (mean)	2.96 ± 2.6
sCr > 1.2 mg/dL (%)	88
Albumin (g/dL)	2.13 ± 0.75
Proteinuria (g/day)	11.2 ± 5.7
Follow up (mean)	37.8 ± 42
ESRD 12-months follow up (%)	46
ESRD (during follow up) (%)	69.2
Time to ESRD (mean)(months)	10.5 ± 8.8
Steroids therapy (%)	73

HTN Hypertension ;MAP=mean arterial pressure;ESRD= End Stage Renal Disease; Numbers are means ± or percentages; sCr= Serum Creatinine

Conclusions: We described series of cases of CG. We found heavy proteinuria and high proportion of patients developing ESRD. These findings are consistent with the results of other studies. Some etiological factors were identified, although the most common was idiopathic.

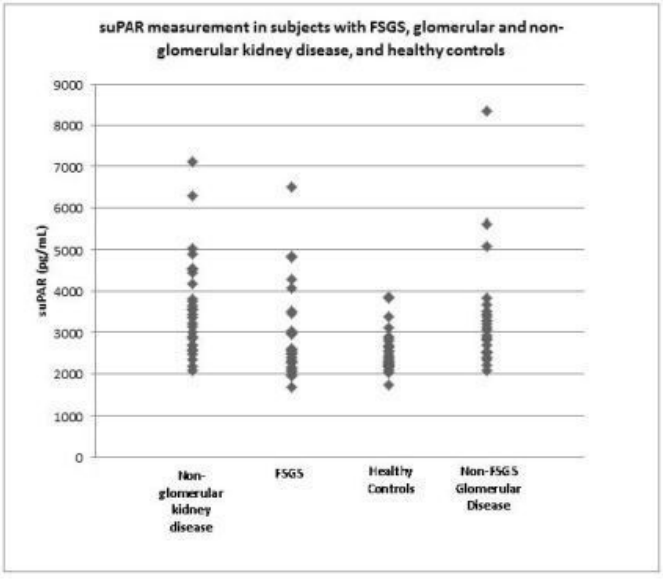
SA-PO385

Circulating Serum Soluble Urokinase Receptor (suPAR) Is an Unlikely Cause of Childhood Focal Segmental Glomerulosclerosis Margret E. Bock, Heather E. Price, Craig B. Langman. *Pediatrics, Northwestern University, Feinberg School of Medicine, Chicago, IL.*

Background: Focal segmental glomerulosclerosis (FSGS) is the primary cause of childhood nephrotic syndrome leading to end-stage kidney disease. Permeability factors, recently including circulating serum soluble urokinase receptor (suPAR), have been postulated as putative causes in adults with primary FSGS. Similar results have yet to be proven in children.

Methods: We investigated the pathogenic role of suPAR in children with FSGS or other glomerular and non-glomerular kidney disease.

Results: We studied 110 subjects, aged 1-21 years, of which 28 patients had FSGS, 25 had non-FSGS glomerular disease, 28 had non-glomerular chronic kidney disease, and 29 subjects were healthy controls. Age, gender and race were similar across study groups (p>0.05). suPAR levels did not differ in subjects with FSGS, non-FSGS glomerular disease and healthy controls (p>0.05). However, suPAR levels (mean ± SD) were higher in children with non-glomerular chronic kidney disease (3607±1201 pg/mL) as compared to healthy controls (2522 ±507 pg/mL; p = 0.001). Notably, only in African American (AA) children were suPAR concentrations higher in those with FSGS as compared to AA children with other kidney diseases (p = 0.01); this trend was not found in Latino or Caucasian patients. Males with nephrotic range-proteinuria (UPr/Cr >2), independent of primary kidney disease, had higher suPAR levels than those without significant proteinuria (4434 ±1916 vs. 3245±1012, respectively, p = 0.015). This trend was not seen among females, and suPAR levels in all female subjects were lower than in males, p=0.036.



Conclusions: Although we found an interaction with ethnicity and gender in suPAR, we cannot implicate its levels as a relevant biomarker for all childhood FSGS. The mechanisms for the high suPAR in AA children with FSGS remain uncertain.

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SA-PO386

Human Ultrastructure Changes of Podocyte Foot Processes during the Remission Phase of Minimal Change Disease Gang Liu, Xiaojing Liu, Jing Huang, Yi-miao Zhang, Su-xia Wang. *Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Background: To observe the ultrastructure changes of podocyte foot processes and its relationship with the amount of the proteinuria in the remission phase of patients with minimal change disease (MCD).

Methods: Electron micrographs of glomerular capillaries were randomly taken from 36 adult cases with MCD, including 14 cases with nephrotic syndrome, 17 cases in partial remission (PR) and 5 cases in complete remission (CR). The "ridges", projections from "fused" podocyte foot process, were classified into two groups by the ratio of the height to basal width (0.5 or ≥1.0). Similarly, the foot processes were classified into three groups by the ratio (0.5-1, 1-2 and ≥2). The foot process width (FPW), the number of "ridge" and the number of foot processes per 10 μm of glomerular basement membrane (GBM) were measured. Twelve age- and gender-matched renal tissues, obtained from the nephrectomized specimens of renal tumors, were enrolled as controls.

Results: There were statistical differences in the mean FPW among the nephrotic group (1598.4±417.3nm), PR group (997.5±225.3nm), CR group (1031.5±224.8nm) and normal controls (471.9±51.8nm) (*P<0.001*). The mean FPW is positively related with the amount of proteinuria (r=0.51, *P=0.001*). For the height-to-width ratio ≥0.5, the number of "ridges" per 10 μm GBM was significantly greater in the CR group than that in the nephrotic group (1.66±0.35 vs 0.87±0.49, *P=0.001*) and normal controls (1.66±0.35 vs 0.84±0.4, *P=0.001*). As for the "ridges" with height-to-width ratio ≥1, the results were similar (the CR group vs the nephrotic group: 1.17±0.22 vs 0.52±0.36, *P<0.001*; the CR group vs normal controls: 1.17±0.22 vs 0.68±0.36, *P=0.003*).

Conclusions: In patients with MCD, as the amount of proteinuria decreased, the mean FPW decreased and the number of "ridges" and foot processes increased. Especially in CR phase, more and higher "ridges" occurred in the still "fused" foot processes before mature foot processes recovered.

Funding: Government Support - Non-U.S.

SA-PO387

Role of Serum Level of Soluble Urokinase Receptor in Various Biopsy-Proven Kidney Diseases and Staging Effect in Diabetic Nephropathy Jin-Shuen Chen,[1] Yuh-feng Lin,[1] Wei-ya Lin,[2] Kuo-hsiung Shu,[3] Chi-Hung Cheng,[3] Han-hsiang Chen,[4] Chih-Jen Wu,[4] Chwei-Shiun Yang,[5] Tzu-ling Tseng.[2] *[1]Tri-Serv General Hosp, Taiwan; [2]ITRI; [3]Taichung Veterans General Hosp; [4]Mackay Memorial Hosp; [5]Cathay General Hosp, Taiwan.*

Background: To date, the role of soluble urokinase receptor (suPAR) in various biopsy-proven kidney diseases, including nephrotic, nephritic, and tubulointerstitial kidney diseases, and staging effect in diabetic nephropathy is still unknown.

Methods: First, serums were collected from groups including healthy control/HC, nephrotic cases (minimal change disease/MCD, membranous nephropathy/MN, FSGS, DN), nephritic cases (IgA nephropathy/IgAN, lupus nephropathy/LN) and tubulointerstitial cases/CIN at medical centers of the **Taiwan Renal Biomarker Consortium**. More than 140 cases of disease groups were proved by renal biopsy. Second, serums from different stages of diabetic nephropathy were also studied. Soluble uPAR ELISA kits were used to study all serum samples. Also, the expression of uPAR in renal tissues was tested by immunohistochemistry. Finally, the relationship among serum suPAR, renal function (represent by estimated glomerular filtration rate, eGFR) and the amount of daily protein loss (DPL) was investigated.

Results: As for the first part, the serum level of suPAR in various kidney diseases was CIN>FSGS, DN, MN, LN, IgAN>MCD, HC, suggesting group CIN had highest suPAR level. Furthermore, correlation between the serum level of suPAR of all kidney diseases and eGFR and DPL was calculated. We demonstrated that suPAR is significantly negative related to the eGFR (r = -0.67) and positive related to the amount of proteinuria (r = 0.26). Finally, the expression of uPAR in renal tissue from all kidney diseases showed MCD>MN, FSGS>CIN. Regarding the second part,the level of serum suPAR was as significantly increased as the staging development of DN and amount of proteinuria.

Conclusions: suPAR had the highest serum level and lowest expression in renal tissue in group CIN when compared to those samples with other kidney diseases. In addition, serum suPAR had a good correlation with stages of diabetic nephropathy, eGFR and amount of proteinuria.

SA-PO388

Serum Levels of Soluble Prorenin Receptor and Prorenin Are Modulated in Chronic Kidney Disease Patients Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Toru Kagawa,[1] Masayuki Ishihara,[1] Kenji Yuasa,[2] Atsuhiro Ichihara,[3] Yoshio Terada.[1] *[1]Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Naokoku, Kochi, Japan; [2]Kochi-Takasu Hospital, Kochi, Japan; [3]Department of Endocrinology and Hypertension, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Prorenin is a precursor of renin and recent years it has been attracting attention that prorenin may be bound to the prorenin receptor (PRR) and prorenin may have a physiological activity. Also it has been elucidated that ligand binding sites of PRR are cleaved and present in the serum as soluble PRR. However dynamics of serum prorenin and soluble PRR in patients with CKD are unclear. We therefore examined the

clinical significance of measurement of the concentration of prorenin and soluble PRR in CKD patients.

Methods: Subjects are 315 CKD patients in our hospitals. After ethics committee approval of each facility, with informed consent, we measured the concentration of soluble serum PRR and the concentration of serum prorenin and general indicators of renal damage such as serum Cr and concentration of serum αKlotho which is known to be regulated by RAS.

Results: Concentration of soluble serum PRR was significantly higher in CKD stage 3-5 patients compared with stage 1-2 and they were positively correlated with serum Cr, BUN and UA ($p<0.0001$). On the other hand serum concentration of prorenin was significantly higher in patients with diabetes (n=46) than non-diabetic patients (n=268) ($p<0.0001$). Also in the group complicated with hypertension (n=169), serum prorenin concentration was higher than non-hypertension group (n=145). There was positive correlation between prorenin level and serum Cr, BUN and UA.

Conclusions: We demonstrate that concentration of soluble serum PRR is significantly higher in CKD stage 3-5 and it is suggested that soluble serum PRR may be involved in organ damage in patients with CKD. We also demonstrate that serum levels of prorenin is significantly higher in patients with diabetes and hypertension in patients with CKD. Therefore it is suggested that serum prorenin may be involved in CKD pathogenesis induced by diabetes and hypertension.

SA-PO389

Expression of Podocyte-Associated Molecules in Proteinuric Glomerulopathies Francisco José Veríssimo Veronese, Patrícia Garcia Rodrigues, Jonathan Fraportti do Nascimento, Gabriel Joelsons. *Nephrology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Background: Podocyte proteins may be a marker of kidney damage in proteinuric glomerulopathies (GP). This study investigated the mRNA profile of podocyte-associated molecules in GP.

Methods: Seventy-five adult patients were included; 35 with nonproliferative GP (NPG): FSGS, membranous, minimal change and diabetic nephropathy; and 41 with proliferative glomerulonephritis (PG): lupus nephritis, IgA nephropathy, membranoproliferative and crescentic nephritis. Eighteen controls (C) were included. Nephrin (nephr), podocin (pod), podocalixin (pdx), synaptopodin (syn) and alpha-actinin 4 (actin4) mRNA were quantified by real time PCR in kidney tissue and urinary sediment (at biopsy, 6 and 12 months). Standard immunosuppression and/or ACEi/ARB were used. mRNA was log transformed (median/IIQ).

Results: Tissue and urinary mRNA were presented in table 1. In tissue, all genes (except syn) were significantly decreased in both NPG and PG groups as compared to C. Urinary mRNA at biopsy was higher in the PG group (table 1), and gene transcripts of both PG and NPG groups did not differ from C at 6 and 12 months ($p>0.05$). Urinary mRNA correlated with proteinuria ($p<0.05$), but not with renal function.

mRNA tissue and urinary profile of podocyte molecules

Tissue mRNA	Controls	NPG	PG	P
Nephr	2.00(1.07-2.56)	0.87(0.02-1.10)	0.86(0.51-2.08)	0.009
Pod	2.04(1.43-3.04)	0.89(0.24-1.60)	1.12(0.05-1.85)	0.007
Pdx	1.86(1.21-2.53)	0.76(0.38-1.22)	0.96(0.39-1.75)	0.031
Actin4	1.70(1.39-2.57)	0.66(0.40-1.47)	1.01(0.41-1.60)	0.019
Urinary mRNA (at biopsy)				
Nephr	2.29(0.64-2.58)	2.13(1.69-3.17)	2.87(2.16-3.64)	0.015
Pod	1.92(0,85-3,05)	2.85(1.97-3.64)	4.82(4.15-5.52)	<0.001
Pdx	2.33(0.90-3.01)	2.98(2.41-3.94)	3.97(3.63-4.42)	<0.001
Actin4	1.42(1.11-2.23)	2.38(1.87-2.99)	2.42(2.05-3.16)	0.011

Conclusions: Expression of podocyte molecules were reduced in the kidney (for both NPG and PG) and increased in the urine (for PG), suggesting podocyte damage in tissue and podocyturia, as evidenced by lower and higher amounts of their mRNA in these compartments, respectively. Overall, after six months of immunosuppressive treatment, urinary mRNA in proteinuric GP did not differ from controls.

SA-PO390

Osteopontin (Opn) and Transforming Growth Factor Beta 1 (TGFb1) in Steroid Resistant Primary Focal Segmental Glomerulosclerosis (FSGS), FSGS-Clinical Trial (FSGS-CT) Experience Robert Woroniecki,[1] Zhongfang Du,[2] Milena Radeva,[4] Jennifer J. Gassman,[4] Debbie S. Gipson,[3] Howard Trachtman,[5] Aaron L. Friedman,[6] Frederick J. Kaskel.[1] *[1]Pediatric Nephrology, New York-Presbyterian, Columbia University Medical Center, New York, NY; [2]Pediatric Nephrology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; [3]Michigan Institute for Clinical and Health Research, University of Michigan, Ann Arbor, MI; [4]Cleveland Clinic, Cleveland, OH; [5]Pediatric Nephrology, NYU Langone Medical Center, New York, NY; [6]University of Minnesota, Minneapolis, MN.*

Background: Urine and renal tissue Opn levels correlate with response to steroids (high Opn in minimal change disease (MCD), low in FSGS), and urinary TGFb1 level is increased in children with FSGS, but does not predict steroid response. We hypothesized that in steroid resistant subjects with FSGS, who do subsequently respond to other immunosuppressive therapy (RS), urinary Opn and TGFb1 levels will be lower than in those who do not respond to treatment (nRS).

Methods: Urine samples were obtained from 24 random subjects out of 138 recruited in the FSGS-CT (http://clinicaltrials.gov) that evaluated effectiveness of 2 arms of immunosuppressive treatment (IT); Cyclosporine/Prednisone(CsP) vs. MMF/dexamethasone (MD) in steroid resistant primary FSGS at 0, 26, 52, and 78 weeks. Opn and TGFb1 were measured by immunoassay (R&D). RS was defined as urine protein/Cr (UPC) ≤0.2 and nRS as UPC >0.2 during the 52 weeks after randomization.

Results: We analyzed 23 subjects (1 had missing data at week 52) in both arms of treatment (results at week 52, Table).

Subjects (N=23)	RS (N=6)	nRS (N=17)	p value
Age (years)	16.2 ± 12.1	21.4 ± 10.7	0.33t
BMI	25.5 ± 5.4	27.3 ± 7.2	0.58t
Caucasians	4 (66.7%)	8 (47%)	0.83F
Male	3 (50%)	11 (64.7%)	0.63F
in CsP arm	5 (83.3%)	7 (41.2%)	0.15F
GFR 0	140.9 ± 45.9	115 ± 48.8	0.27t
GFR 52	121.1 ± 41.3	103.4 ± 58.6	0.50t
TGFb1 (pg/ml)	5167 ± 589	5459 ± 701	0.37t
Opn (ng/ml)	8.4 ± 1.2	8.1 ± 3.4	0.82t

F-fisher exact, t-t test

Conclusions: We found no difference in Opn and TGFb1 and no significant correlation between Opn, or TGFb1 and eGFR at 52 weeks of IT.

Funding: Private Foundation Support

SA-PO391

Increased Urinary Biomarkers of Inflammation, Angiogenesis, and Epithelial Adhesion in HIV+ African Americans with and without CKD Mark A. Kraus,[1,2] Krishnamurthy P. Gudehithlu,[1,4] Kathleen Weber,[3] Ashok K. Singh,[1,4] Audrey French.[2,3] *[1]Nephrology, John H Stroger Jr Hospital of Cook County, Chicago, IL; [2]Medicine, Rush University, Chicago, IL; [3]Infectious Disease, Ruth M Rothstein CORE Center/Cook County Health & Hospitals System, Chicago, IL; [4]Hektoen Institute of Medicine, Chicago, IL.*

Background: Despite improved viral suppression with highly active antiretroviral therapy, HIV+ adults remain at high risk for chronic kidney disease (CKD) with a reported two-fold increased risk of developing end stage renal disease over the general population. This study tested for changes in discrete urine biomarkers hypothesized to contribute to this increased risk.

Methods: We collected random urine samples from African American (AA) HIV+ and age matched HIV uninfected (HIV-) women with eGFR>60ml/min (n=9 & 15) and HIV+ AA with eGFR<60ml/min (HIV+CKD, n=5) from the Chicago Women's Interagency HIV Study (WIHS) cohort and the HIV Nephrology clinic at our institution. Using ELISA kits, we analyzed urine for the presence of biomarkers of inflammation, hypoxia, angiogenesis, monocyte activation, and cell adhesion. Biomarker concentrations were normalized to urine creatinine concentrations.

Results: Fetuin-A concentration was 2.3 fold higher in HIV+ ($p=0.007$) and 6 fold higher in HIV+CKD ($p=0.05$) groups vs. HIV- controls. E-Cadherin rose 2 fold in HIV+ ($p=0.02$) and 6.3 fold in HIV+CKD from HIV- levels. Angiopoietin-2 concentrations were comparable in HIV- and HIV+ groups and increased 19.8 fold in HIV+CKD ($p=0.02$) vs. HIV- controls. NGAL rose 2.2 fold in HIV+ ($p=0.02$) and 16.6 fold in HIV+CKD ($p=0.02$) groups vs. HIV- controls. Although detectable, there were no differences among the groups in TGF-beta, MCP-1, and IL-8 concentrations. In all groups, HIF-1alpha, TNF-alpha, FGF, IL-6, IL-10, and MMP-9 were not detectible.

Conclusions: These results are the first to test the involvement of discrete biological systems in the development of CKD in HIV+ AA. Our data support a possible role of angiogenic factors (angiopoietin-2), inflammation mediators (fetuin-A), endothelial adhesion (E-cadherin), and ischemia (NGAL) in the pathogenicity of CKD in the setting of HIV.

Funding: Other NIH Support - Women's Interagency HIV Study

SA-PO392

Renin Angiotensin System Modulates Sclerotic but Not Collapsing Lesions in HIV-Associated Nephropathy Andrei Plagov,[1] Partab Rai,[1] Dileep Kumar,[1] Ashwani Malhotra,[1] Guohua Ding,[1] Praveen N. Chander,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Background: Although collapsing glomerulopathy has been considered a hallmark of HIV-associated nephropathy (HIVAN) but significant numbers of glomeruli in HIVAN patients also display sclerotic lesions. We hypothesize that both collapsing and sclerosing lesions are manifestation of HIVAN and their display is dependent on associated host factors. We studied the role of the activation and down regulation of renin-angiotensin system (RAS) on the frequency of sclerotic and collapsing lesions in a mouse model of HIVAN.

Methods: To have the activated RAS, HIV transgenic (Tg26) mice were genetically engineered to have variable copies of angiotensinogen (Agt). Twelve weeks old Tg26 mice having two (Agt-2), or four copies (Agt-4) of Agt in groups of six were evaluated for number of sclerotic or collapsing glomeruli in their renal cortices. To down regulate the RAS, four weeks old Tg26 mice were treated with either saline, captopril (angiotensin converting enzyme inhibitor, 50 mg/Kg), aliskiren (renin activity inhibitor, 50 mg/Kg), aliskiren + captopril, telmisartan (Ang II blocker, 50 mg/kg, or aliskiren + telmisartan for 4 weeks. Subsequently, renal cortices were studied for number of sclerotic and collapsing glomeruli.

Results: Tg26/Agt-2 mice displayed greater number of sclerotic glomeruli vs. collapsing glomeruli. In Tg26/Agt-2, one out of 7 glomeruli showed sclerotic lesion; whereas, one out of 33 glomeruli displayed collapsing lesion. Tg26/Agt-4 mice showed higher number of sclerotic glomeruli (one out four glomeruli) when compared to Tg26/Agt-2 mice. Interestingly, all treatment groups predominantly displayed decreased percentages of sclerotic glomeruli.

Conclusions: Sclerotic lesions are more common in HIVAN and are correlated with the activation of the RAS. Since majority of biopsy specimen often may not have adequate number of glomeruli to display one collapsing glomerulus but may display sclerotic one, both sclerotic and collapsing glomerulus should be considered the manifestation of HIVAN.

Funding: NIDDK Support

SA-PO393

Minimal Change Disease due to Mercury-Containing Skin Lightening Cream: Clinical and Pathological Analysis Yan-yan Wang,[1] Hong Cheng,[1] Guo-qin Wang,[1] Hong-rui Dong,[1] Chang-jiang Wang,[2] Yan-ling Tang,[3] Yi-Pu Chen.[1] *[1]Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China; [2]The Central Hospital of Jiamusi City, Heilongjiang, China; [3]Tang Shan People's Hospital, Hebei, China.*

Background: To investigate the clinicapathological characteristics and the treatment of minimal change disease (MCD) due to mercury-containing skin lightening cream.

Methods: 5 cases with mercury-induced MCD were enrolled in this study. The feature of mercury toxicity, mercury in urine and skin lightening cream, the clinical and pathological change and the treatment were analyzed.

Results: 1. All cases were female (mean age 37.6) and had a history of continuous use of mercury containing skin lightening cream from 3 to 5 months. 2. All cases had edema, heavy proteinuria, hypoalbuminemia, hyperlipidemia and no hematuria. 2. Renal biopsy showed no changes of glomeruli on light microscopy. The immunofluorescence was negative. On electron microscopy, there is a characteristic fusion of epithelial foot. They were diagnosed as MCD. Case 5 had renal tubular epithelial cell lysosome increase and some microvilli fall off. Renal tubules and interstitial had no obvious lesions in the other 4 cases. 3. The urinary mercury concentration was more than 2.2-75 times the upper limit of normal value. 3 cases' cosmetic cream was found to contain mercury levels of 1.20×10^3-1.56×10^3mg/kg. 4. All cases ceased application of the cream, and were treated with prednisone and chelation therapy. 3 cases had started to use prednisone before admission and they already had complete remission before chelation therapy. The other 2 cases took prednisone while using chelation therapy. They all had complete remission within 1-2months. The urinary mercury concentration of case 1 and case 4 had normalized after two course of chelation therapy. However, the other 3 cases' urinary mercury is still above the normal level after one or three course of chelation therapy. All cases are stable and on a reducing dose of prednisone.

Conclusions: The clinicapathological presentation and the response to prednisone of MCD due to mercury-containing skin lightening cream are like that of primary MCD.

SA-PO394

HIV Immune Complex Kidney Disease: Clinical Features and Impact of Anti-Retroviral Therapy Matthew Foy,[1] Gregory Lucas,[2] Faryal Tahir,[3] Michelle M. Estrella,[1] Derek M. Fine,[1] Mohamed G. Atta.[1] *[1]Division of Nephrology, The Johns Hopkins Hospital, Baltimore, MD; [2]Division of Infectious Disease, The Johns Hopkins Hospital; [3]Dow University of Health Sciences.*

Background: HIV-associated nephropathy (HIVAN) is well-described, but the clinical features of HIV-immune complex kidney disease (HIVICK) have not been well-established. Our study evaluated the clinical features associated with HIVICK and the impact of antiretroviral therapy (HAART) on end-stage renal disease (ESRD).

Methods: This was a nested case-control retrospective study of HIV-infected individuals followed in the Johns Hopkins HIV Clinical Cohort from 1/1996 to 6/2010. Groups were compared using X2 test or rank-sum analysis. Conditional logistic regression was used to estimate odds ratios for HIVICK. Incidence of ESRD for HIVICK/HIVAN and ESRD with/without HAART exposure were calculated using Kaplan-Meier analysis.

Results: Of 139 patients examined, 83 had HIVICK and were predominantly African-American (92%). Comparing HIVICK to HIVAN: HIVICK had more HAART exposure, lower HIV viral loads, and higher CD4 counts and estimated GFR. Adjusting for race, injection drug use, increased HIV RNA, CD4 < 200, HCV infection, diabetes, and hypertension, those with higher HIV RNA (OR=1.56, 95%CI 1.13-2.14), diabetes (OR=2.59, 95%CI 1.04-6.4) and hypertension (OR=2.32, 95%CI 1.19-4.53) had higher odds of HIVICK versus 332 matched controls. ESRD was less common in the HIVICK group versus HIVAN (30% vs. 82%, p<0.001). While HAART was associated with lower ESRD incidence in the HIVAN group (44% vs. 72%, p=0.023), it was not in the HIVICK group.

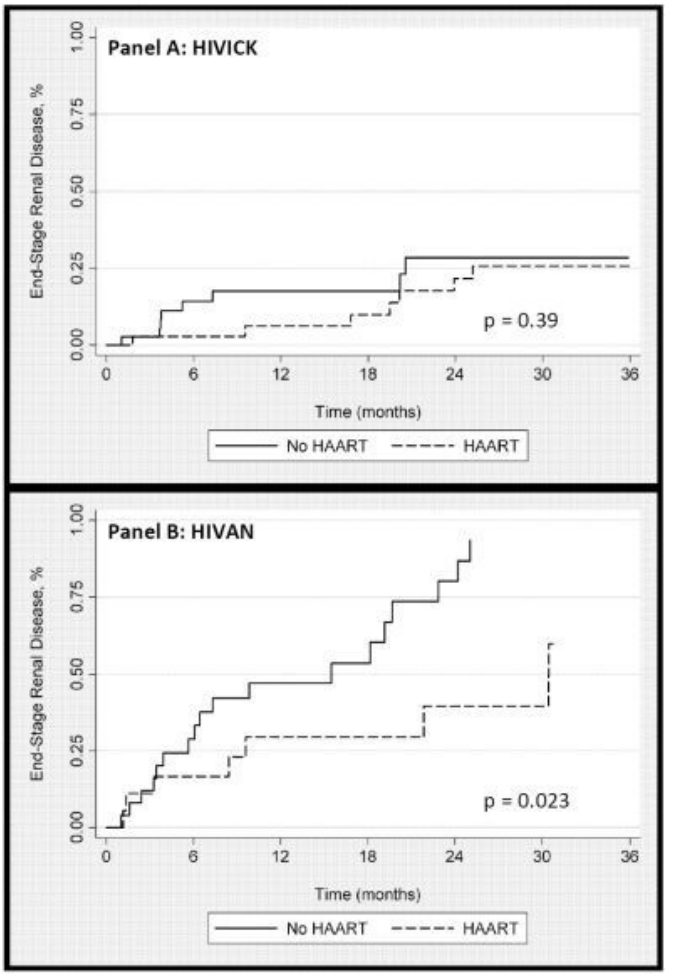


Figure 1. Incidence of ESRD among patients with HAART vs. no HAART exposure.

Conclusions: HIVICK occurs commonly in African-American patients. These patients are more likely to have higher HIV RNA. ESRD incidence is lower in HIVICK patients compared to HIVAN. Unlike HIVAN, HAART does not impact incidence of ESRD in HIVICK.

Funding: Other NIH Support - This Research Was Supported by the National Institute of Health (R01-DA11602)

SA-PO395

Renal Safety Profile of Lopinavir/Ritonavir-Based Regimens in the PROGRESS Study Boris Renjifo, Christos Argyropoulos, Roxann Stubbs, Dilek Arikan, Roger N. Trinh, Angela M. Nilius. *Abbott Laboratories, Abbott Park, IL.*

Background: There is an increasing need to identify alternative antiretroviral (ARV) combinations for HIV(+) patients that avoid Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI) to minimize long term organ toxicity. The PROGRESS study (NCT00711009) compared the novel NRTI-sparing regimen LPV/r + raltegravir (RAL) to LPV/r + TDF and emtricitabine (FTC) in ARV-naïve patients. Through 48 weeks of follow up LPV/r + RAL was noninferior in efficacy and exhibited comparable safety and tolerability profiles compared to LPV/r + TDF/FTC. As a component of a secondary endpoint, we evaluated the renal safety profile associated with each ARV regimen during the entire follow up time.

Methods: Creatinine clearance (CrCl) was estimated by the Cockroft-Gault equation, and changes from baseline (BL) were assessed by one way ANOVA. Subjects were followed through 96 weeks or until discontinuation from the study based on the investigator's best clinical judgment.

Results: Study participants (n=172, LPV/r + TDF/FTC = 90) were 39.9±10.7 years old, 88.4% White, 11.6% women, BL CrCl of 120.3±34.9 ml/min, and 28.9 % had a diagnosis of hypertension. There were no differences in BL demographics between arms. Mean change from BL to week 96 in CrCl was statistically significantly greater with LPV/r + TDF/FTC compared to LPV/r + RAL (Δ -7.3 versus -1.5 ml/min, p=0.035). Four subjects experienced a renal serious adverse event (SAE) for which clinical course, renal biopsies, laboratory abnormalities, concomitant comorbidities and medications will be presented.

Age in years	33	42	52	55
Gender	M	F	M	M
Race	W	B	W	B
ARV regimen	LT	LR	LT	LT
Renal diagnosis	Obstructive stone	AKI	Fanconi	AKI with tubulointerstitial nephritis (biopsy)
Outcome	Ureteral Stent Placed	Dialysis dependent	Persistent	Persistent

M=Male, F=Female, W=White, B=Black, LR: LPV/r + RAL, LT: LPV/r + TDF/FTC

Conclusions: At week 96 the majority of patients had a CrCl in the normal range. Decline in CrCl was larger with LPV/r + TDF/FTC. Renal SAEs were temporally related to exposure to non ARV meds, except for one Fanconi case considered by the investigator to be related to LPV/r + TDF/FTC exposure.

Funding: Pharmaceutical Company Support - Abbott Laboratories

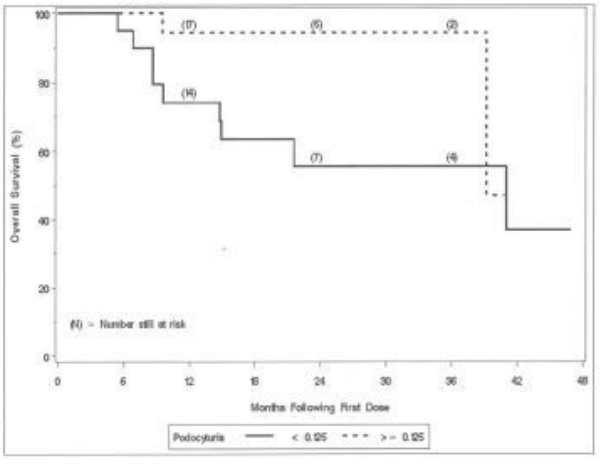
SA-PO396

Podocyturia May Predict Response and Survival in Patients with Solid Organ Tumors Treated with Anti-VEGF Medications Juan C. Calle,[1] Suzanne R. Hayman,[2] Joseph P. Grande,[3] Vesna D. Garovic.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Hematology and Oncology, Mayo Clinic, Rochester, MN; [3]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Background: Anti-angiogenic agents targeting VEGF are being used increasingly for solid organ tumors, particularly in advanced disease. The two most common renal side effects are proteinuria and hypertension, thought to be secondary to the removal of VEGF trophic effects by these agents. Proteinuria has recently been shown by our group to be associated with increased levels of podocyturia (excretion of viable podocytes in the urine) in patients treated with anti-VEGF medications.

Methods: In forty patients treated with anti-VEGF medications urinary podocyte culture was performed to detect viable podocytes.

Results: Among the 40 patients, 11 died during the period of the study with a median time to death of 9.6 months (range, 5.4 – 41.0 months). Among the remaining 29 patients, the median duration of follow-up was 20.3 months (range, 5.1 – 69.5 months). Overall survival was better in those who developed podocyturia (cutoff ≥ 0.125 cell/mg of creatinine, p=0.05).



Conclusions: Previous studies have shown that treatment-related hypertension may predict response to anti-angiogenic treatments. Our data indicate possible predictive roles of early proteinuria and podocyturia in overall survival in those patients treated with anti-VEGF medications.

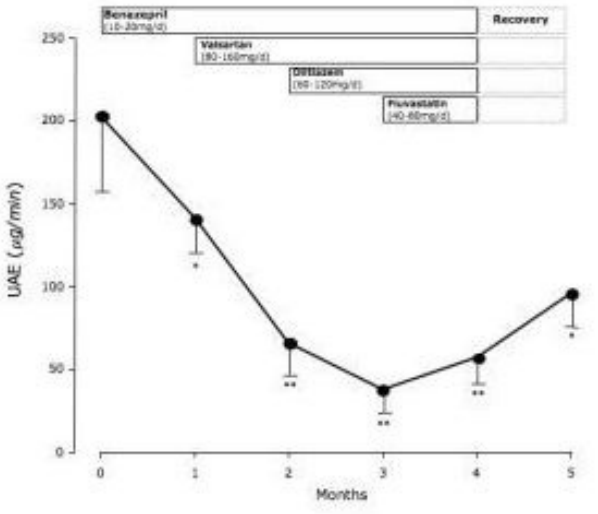
SA-PO397

Effects of the Remission Clinic Approach in Alport Syndrome: Results from a Prospective, Sequential Cohort Study Erica Daina,[1] Sara Gamba,[1] Piero Ruggenenti,[1,2] Mirella Alpa,[3] Dario Roccatello,[3] Paolo Cravedi,[1] Flavio Gaspari,[1] Giuseppe Remuzzi.[1,2] *[1]Mario Negri Institute, Bergamo, Italy; [2]Ospedali Riuniti di Bergamo, Italy; [3]Ospedale S.G. Bosco, Torino, Italy.*

Background: Combined use of ACE inhibitors, ARBs, ndCCBs and statins (the Remission Clinic approach) reduced proteinuria and halted progression to ESKD in patients with non-diabetic proteinuric nephropathies (JASN,2008;19:1213). We tested the effects of this strategy in Alport syndrome, a disease orphan of specific treatment so far.

Methods: After 1-month wash-out from RAS inhibitor therapy, 9 consecutive patients (3 males) aged 33.0±15.2yrs with diagnosis of Alport syndrome and cr. cl. >20ml/min/1.73m^2 entered a 4-month sequential, add-on treatment period with benazepril, valsartan, diltiazem, and fluvastatin followed by 1 month wash-out (recovery). Drugs doses were up-titrated as already described (JASN,2008;19:1213). Albuminuria (primary outcome) and other parameters were evaluated at baseline and monthly thereafter. GRF and RPF were directly measured by Iohexol and PAH clearances at baseline, and at the end of the treatment and recovery periods.

Results: Albuminuria significantly declined over 1-month benazepril therapy and further progressively decreased over the treatment period. After washout it again tended toward baseline values.



Over the treatment period mean arterial BP and LDL/HDL cholesterol ratio progressively declined ($p<0.05$) from 102.2±10.9 to 87.5±10.2mmHg and from 2.5±0.9 to 1.9±0.4, respectively. They increased to 96.6±9.9mmHg and 2.4±0.8 after recovery. GFR, RPF and serum potassium levels were stable throughout the study. Treatment was well tolerated.

Conclusions: The Remission Clinic approach safely reduced albuminuria and BP, and ameliorated dyslipidemia in patients with Alport syndrome. Whether these effects may translate into long-term renoprotection is worth investigating.

Funding: Pharmaceutical Company Support - Novartis Farma S.p.A., Italia

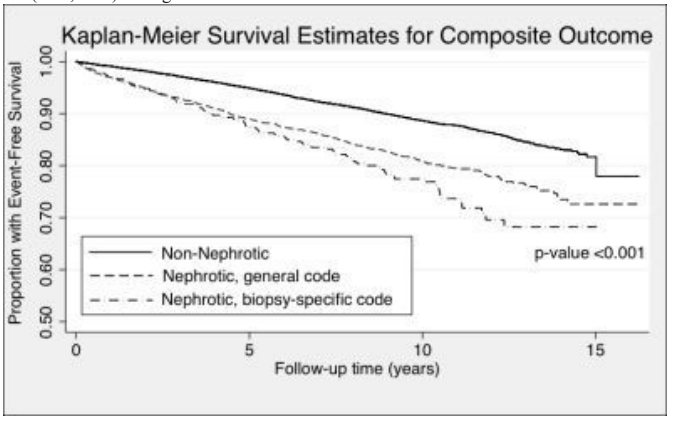
SA-PO398

Risk of Cardiovascular Disease in Primary Nephrotic Syndrome: A Population Based Cohort Study Laura H. Mariani, Michelle Denburg, Kevin Haynes, Mary B. Leonard. *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.*

Background: Cardiovascular disease (CVD) is the major morbidity for patients with chronic kidney disease (CKD). The magnitude of risk and impact of specific risk factors in the subset of CKD patients with primary nephrotic syndrome (NS) is unknown. The Health Improvement Network (THIN) is a primary care database of >9 million patients in the United Kingdom and allows for population-based studies of rare diseases.

Methods: We conducted a retrospective cohort study of 4,729 NS patients in THIN and 23,585 unexposed patients who were matched based on age, gender and practice location from 1994-2010. Nephrotic patients were identified by diagnostic code and those with prior diabetes, other glomerular diseases and CVD were excluded. Survival analysis was performed using Cox proportional hazards multivariable regression with a composite outcome of myocardial infarction, revascularization procedure, stroke and death.

Results: 838 patients had a biopsy specific diagnosis of minimal change disease, focal segmental glomerulosclerosis or membranous nephropathy. An additional 3,891 had a general code for nephrotic syndrome. Mean (SD) age at diagnosis was 34 (24) yr. 53.4% were male. The unadjusted HR (95%CI) was 2.33 (1.93, 2.82) for patients with a biopsy-specific code and 1.88 (1.70, 2.08) for a general code as compared to non-nephrotic patients (Figure 1). Adjusting for age, sex, hyperlipidemia, hypertension, smoking and obesity, the HR (95%CI) was 1.98 (1.63, 2.42) for those with a biopsy-specific code and 2.1 (1.88, 2.33) for a general code.



Conclusions: Primary NS is associated with an increased risk of CVD. Adjusting for traditional CVD risk factors did not eliminate the increased risk. Further study is needed to identify non-traditional, modifiable risk factors in this subset of patients with CKD who are at high risk of CVD.

Funding: NIDDK Support

SA-PO399

An Observational Study of ACTH in Combination with Cyclosporine for Treatment of Membranous Nephropathy Anna-lena Berg, Anneli Jonsson, Sten-erik Bäck. *Lund University, Lund, Sweden.*

Background: We previously reported that synthetic $ACTH_{1-24}$ (Synacthen Depot; ACTH) had both lipid-lowering and antiproteinuric effects in patients with nephrotic syndrome due to membranous nephropathy (MN). We now report our observational data adding low dose immunosuppression with cyclosporine (CsA) to ACTH in a subset of MN patients who achieved partial remission of proteinuria on ACTH monotherapy.

Methods: CsA (maximal dose 100 mg twice daily) was added to ACTH in 10 patients with MN who had partial remission of proteinuria on ACTH monotherapy. The median age of the patients was 73.5 yr (range 31-81). Median time from initial diagnosis of MN was 6 months (range 0.25-300). All patients were treated with standard of care, including ACE-inhibitors/ARB, statins and other symptomatic therapies. Treatment with ACTH started at a dose of 1 mg weekly and was increased to a maximum dose of 1 mg twice weekly. The median duration of ACTH monotherapy was 9 months (range 4-24), and ACTH/CsA combination therapy continued for an additional median duration of 9 months (range 4-18). All patients achieved partial remission (proteinuria reduced by 50% and < 2000 mg/L) on ACTH monotherapy. After the addition of low dose CsA, 8 of 10 patients achieved complete remission (proteinuria < 200 mg/L). Urinary albumin decreased from a mean (±SE) pre-ACTH value of 9389±1921 mg/L to 1076±190 mg/L with ACTH monotherapy, and remained improved (130±57 mg/L) after combination therapy with ACTH/CsA ($p \leq 0.0001$ at both time points compared with baseline). Serum albumin increased from a pre-ACTH mean (±SE) of 21.2±1.9 g/L to 29.2±1.7 g/L with ACTH monotherapy ($p<0.01$), and further increased with ACTH/CsA combination therapy to 36.5±1.4 g/L ($p<0.0001$ compared with baseline and ACTH monotherapy). Serum creatinine and eGFR did not change significantly after ACTH alone, or after the combination of ACTH/CsA.

Conclusions: These data suggest that the combination of ACTH and a low dose immunosuppressant may be beneficial for inducing complete remission of proteinuria in patients with MN who are only partially responsive to ACTH monotherapy. Controlled clinical trials will be required to confirm the findings from this observational cohort.

Funding: Pharmaceutical Company Support - Queatcor Pharmaceuticals Inc, Government Support - Non-U.S.

SA-PO400

Identification of *TRAP1* as a Novel CAKUT-Causing Gene by Whole Exome Capture and Next Generation Sequencing Pawaree Saisawat,[1] Ethan Douglas Sperry,[1] Alina Christine Hilger,[1,5] Heon Yung Gee,[1] Daw-yang Hwang,[1] Stefan Kohl,[1] Velibor Tasic,[2] Iris Van Rooij,[3] Radovan Bogdanovic,[4] Heiko M. Reutter,[5,6] Friedhelm Hildebrandt.[1,7] *[1]Department of Pediatrics, University of Michigan, Ann Arbor, MI; [2]Department of Pediatric Nephrology, University Children's Hospital, Skopje, Macedonia, the Former Yugoslav Republic of; [3]Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; [4]Medical Faculty, University of Belgrade, Belgrade, Serbia; [5]Department of Human Genetics, University of Bonn, Bonn, Germany; [6]Department of Neonatology, University of Bonn, Bonn, Germany; [7]Howard Hughes Medical Institute, Chevy Chase, MD.*

Background: Congenital abnormalities of the kidney and urinary tract (CAKUT) account for 50% of all chronic kidney disease in children. Although there are several mouse models of recessive non-syndromic CAKUT, only a few genes are known to cause CAKUT in humans.

Methods: To identify new CAKUT genes we performed homozygosity mapping and whole exome resequencing using NimbleGen's SeqCaP EZ Exome™ protocol in 13 CAKUT families (27 patients). NextGen sequencing was performed using Illumina Genome Analyzer II.

Results: We detected a homozygous missense mutation (R469H) in *TNF Receptor Associated Protein 1 (TRAP1)* in two unrelated CAKUT families. The R469 residue was conserved down to bacteria and R469H was predicted to be 'damaging' by PolyPhen algorithm. The mutation segregated in the families and it is not present homozygously in the Exome Variant Server database. Additionally, we screened 384 more CAKUT patients for mutations in *TRAP1* in a Cel1 restriction enzyme assay, in which we found another affected carrying a compound heterozygous mutation (R469H and R13C).

Conclusions: *TRAP1* is a likely new cause of recessive CAKUT in humans.

Funding: NIDDK Support

SA-PO401

Glucose Transport in the Proximal Tubule: Disruption of Urate Transport in Familial Renal Glucosuria and Report on SGLT2 Expression in Normal and Pathological Kidney Ines Aires,[1,2] Ana Rita Santos,[2] Gurkan Genc,[3] Roberto Bogarin,[4] Joaquim T. Calado.[1,2] *[1]Department of Genetics-Faculty of Medical Sciences, Universidade NOVA de Lisboa, Lisbon, Portugal; [2]Division of Nephrology, Hospital de Curry Cabral, CHLC, Lisbon, Portugal; [3]Department of Pediatric Nephrology, Ondokuz Mayis University Faculty of Medicine, Samsun, Turkey; [4]Department of Pediatric Endocrinology, Hospital Nacional de Niños, San José, Costa Rica.*

Background: Mutations in SGLT2, the major Na^+-glucose cotransporter of the proximal tubule, underlie Familial Renal Glucosuria (FRG). We aimed at characterizing the molecular and phenotype findings of a FRG cohort and detailing the SGLT2 expression in the kidney.

Methods: Direct sequencing for the identification of SGLT2 mutations, as well Western blotting and immunofluorescence for the expression of SGLT2. In the absence of renal biopsies from FRG individuals, we selected nucleoside analogs induced proximal tubular toxicity as a model for glucosuric nephropathies.

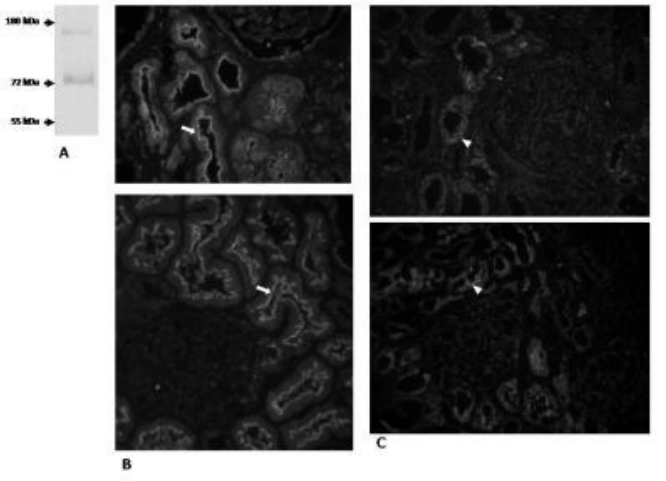
Results: Six novel SGLT2 mutations are reported. We describe hyperuricosuria with associated hypouricemia as part of the the most severe FRG phenotypes.

Phenotype evaluation and mutational analysis

proband	glucosuria (g/1.73m2/24h)	uric acid (serum; mg/dl)	uric acid (urine; mg/1.73m2/24h)	genotype
1	87.8	1.9	1242	R345fsX108/ R345fsX108
2	43.7	3.5	990	W649X/wt
3	30	na	na	Q448L/IVS7+5g>a
4	25	na	na	C522X/C522X
5	14	na	na	T191P/T191P
6	4.5	na	na	G492V/wt

na: not available

SGLT2 expression is localized to the brush-border of the proximal tubular epithelia cell A, B.



This normal pattern is disrupted in cases of nucleoside analogs induced tubulopathy C.

Conclusions: We present 6 novel SGLT2 mutations and describe hyperuricosuria/hypouricemia as part of the FRG phenotype. The disruption of the normal SGLT2 brush-border expression pattern may underlie the glucosuria seen with the use of adefovir and tenofovir.

SA-PO402

The Metabolic Basis of Primary Hyperoxaluria Type III Yaacov Frishberg,[1] James Pitt,[2] Ruth Belostotsky.[1] *[1]Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel; [2]Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Melbourne, Australia.*

Background: Primary hyperoxaluria type III (PHIII) is a recently identified type of childhood kidney stone disease caused by mutations in *HOGA1* (formerly DHDPSL). HOGA1 encodes a mitochondrial 4-hydroxy-2-oxoglutarate (HOG) aldolase which cleaves HOG to pyruvate and glyoxylate in the 4-hydroxyproline catabolic pathway. Paradoxically, glyoxylate is an immediate precursor of oxalate.

Methods: Hydroxyproline metabolites in the urine of patients with PHIII were measured using gas chromatography – mass spectroscopy (GC-MS). This was complemented by GC-MS and biochemical studies in a human hepatocyte cell line (SK-HEP-1).

Results: Significant increases in concentrations of HOG, its reduced product 2,4-dihydroxyglutarate and 4-hydroxyglutamate, the immediate precursor of HOG, were found in the urine of PHIII patients compared to carriers of the corresponding mutations or healthy controls. These data confirm that *HOGA1* mutations result in loss of function and that accumulating HOG can exit the mitochondria. *In vitro* studies using a non-PHIII human hepatocyte cell line demonstrated significant HOG aldolase activity in mitochondrial and cytosolic fractions. No HOGA1 protein was detected in the cytosolic fraction indicating that it contains other enzyme(s) with HOG aldolase activity. Aldolase enzymes are known

to have broad substrate specificity and several cytosolic aldolases could fill this role. We propose a model for PHIII whereby accumulating HOG exits the mitochondria and is partially metabolized to glyoxylate and oxalate in the cytosol.

Conclusions: Our data provide a diagnostic tool for screening larger cohorts for PHIII and shed light on glyoxylate metabolism and the pathogenesis of the primary hyperoxalurias.

Funding: Clinical Revenue Support

SA-PO403

Molecular Effects of Four Mutations Identified in the ATP Binding Domain on Transporter Activity of hClC-5 in Japanese Patients with Dent's Disease

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Background: Dent's disease, characterized by low-molecular-weight proteinuria, hypercalcinuria, and nephrolithiasis, is caused mainly by inactivating mutations in the human chloride channel 5 (hCLC-5) gene. Binding of ATP to the ATP binding domain (ABD) of hClC-5 is known to play an important role in regulating the transport activity of hClC-5. The X-ray crystal structure of the cytoplasmic domain of hClC-5, including the ABD, has been established, thus making it possible to construct a model of this domain and examine the effect of its mutations.

Methods: We examined four missense mutations in the hClC-5 ABD that were included among 29 such mutations identified in 114 Japanese patients with Dent's disease.

Results: Only four mutations-F703S, L706P, C711W and K725E-were identified as ABD mutation points (ASN 2011 FR-PO1508). Structural mapping indicated that K725 might interactively affect ATP binding via electrostatic interaction between ATP and the ABD, due to the charge inversion resulting from K725E mutation. Two L706 side-chains provided mutual stability for each other in the dimer structure, and F703 and C711 were positioned on the line between L706 and K725. We virtually simulated these mutation structures in the molecular model, and confirmed that the K725E mutation could weaken the binding of ATP. F703S and C711W, on the other hand, appeared to change the volume and shape of the hydrophobic core of the ABD, and L706P was considered to change the dimer interaction between ABDs.

Conclusions: We postulate that the above residues have an important relationship to intermolecular signaling upon ATP binding, and that their mutations might result in failure of channel function, in view of the predicted changes in interaction between the ABD and trans-membrane domain that would result from ATP binding.

SA-PO404

LMX1B Mutation with Residual Transcriptional Activity as a Cause of Nail-Patella-Like Renal Disease

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Background: Nail-patella syndrome (NPS) is an autosomal dominant disorder caused by mutations of the LMX1B gene. Dysplasia of the nails, absence or hypoplasia of the patellae, and the kidney disease are cardinal features. Patients with typical renal lesions of NPS without skeletal nor nail findings were also reported known as nail-patella-like renal disease.It is unknown whether nail-patella-like renal disease is also caused by LMX1B mutation.

Methods: The subject was a 6-year-old girl with proteinuria and hematuria. There was no familial history. She had neither skeletal nor nail abnormalities. Renal biopsy showed ultrastructual changes of the irregularly thickened glomerular basement membrane consisted of fibrillar material. We diagnosed her as nail-patella-like renal disease. We analyzed the LMX1B gene from peripheral leukocyte genome DNA of the subject and her parents. For examining the functional consequences of the detected mutation, wild-type and mutant LMX1B were transiently overexpressed in Cos-1 cells, and the transcriptional activity was analyzed by luciferase reporter assay.

Results: A novel heterozygous mutation (R223Q) was identified. R223Q was not found in the parents. R223Q is not registered in SNP databases, and the absence of each DNA sequence abnormality in 100 alleles from 50 unrelated normal individuals indicated R223Q was a mutation .Transcriptional activity of R223Q were significantly lower than that of wild-type.However, it was higher than that of other mutation found in a patient with typical NPS.

Conclusions: This the first report a LMX1B gene mutation was identified in a patient with nail-patella-like renal disease. As R223 is located in the homeodomain and well conserved region, R223Q would have the passibility tha function of LMX1B was damaged. Functional analyses revealed R223Q transcriptional activity was impaired but remained. It is suspected that nail-patella-like renal disease may be caused by LMX1B mutations with residual transcriptional activity.

Funding: Government Support - Non-U.S.

SA-PO405

The Rennin Producing Cell Recruitment in Patients with Gitelman Syndrome

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Background: This study is trying to observe the clinical pathologic features and the local RAS activation of the kidney in Gitelman syndrome (GS) patients.

Methods: 33 patients with a clinical suspicion of GS were recruited. Genomic DNA was isolated from peripheral blood and used for PCR amplification of individual exon of the SLC12A3gene. Direct sequencing was performed. The general clinical data of genetic diagnosed GS patients was collected. Plasma renin activity (PRA), angiotensin II and aldosterone levels were detected by radioimmunoassay. 14 GS patients did renal biopsy. Renin and α-actin immunofluoescense (IF) double staining and COX2 immunohistochemistry (IHC) were performed.

Results: The average onset age was 23.4±11.8 (3-47)years old of the 27 GS patients with 22 SLC12A3 gene mutants, including 4 novel mutants. The upper respiratory tract infection, strenuous exercise, emotion change, diarrhea were the common incentives. Episodes of hypokalemia (1.2-2.8mmol/L) was the universal presentation of all patients, companied with fatigue (88%), convulsion (36%), numbness (32%), palpitation (32%) and flaccid paralysis(24%). Metabolic alkalosis occurred in 87.5% patients, urine Ca^{2+} to Cr ratio decreased in 94% patients. Hypomagnesaemia (80.9%), elongation of QT interval of ECG (54.2%) and hypochloremia (40.9%) were also observed in GS patients. Significantly increasing of PRA (35%), Ang II(85.7%) and Aldosterone(64%) levels were observed in GS patients even when serum potassium level was approaching normal. Almost GS patients (13/14) showed amplification of juxtaglomerular apparatus(JGA) in kidney. Renal local renin and COX2 expression were significantly higher in JGA .than normal control. Renin and α-actin co-expression was observed in both microartery and JGA of GS patients by confocal laser scanning microscopy.

Conclusions: After a physiologic challenge of Na^+ and Cl^- wasting, induced the transformation of arteriolar smooth muscle cells into renin-expressing cells may result the local RAS activation in GS patients.

SA-PO406

Identification of Novel Mutations in the SLC12A3 Gene in Chinese Patients with Gitelman Syndrome

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Background: Gitelman syndrome(GS) is an autosomal recessive disorder characterized by hypokalaemic metabolic alkalosis, significant hypomagnesemia and low urinary calcium. The GS phenotype is usually caused by mutations in the SLC12A3 gene. Hundreds of mutations have been reported in the literature, but very few of these mutations were identified in Chinese. The aim of this study is to investigate the features of SLC12A3 gene mutations in Chinese GS patients.

Methods: From Feb 2009 to Apr 2011, 28 unrelated patients suspected of having GS were enrolled in this study. 100 healthy persons were used as controls. Biochemical parameters were measured and documented. We analyzed DNA samples of these patients with a clinical suspicion of GS by direct sequencing of all 26 exons of the SLC12A3 gene. Reverse-transcription polymerase chain reaction and complementary DNA sequence analysis were performed to confirm deletion or splicing variants.

Results: In these 28 patients(18 men and 10 women),21 patients suffered from severe symptoms interfering with daily activities, such as paralysis, tetany or cramp. All patients had hypokalaemia, metabolic alkalosis, hypomagnesaemia and normal blood pressure. 15 patients had hypocalciuric and six patients were found to have proteinuria. All patients showed normal hearing and serum calcium. For the genotype, 20 patients were identified to carry two SLC12A3 mutant alleles,while only one mutant allele was found in the other 8 patients.In total, 24 different mutations were identified,10 of which have not been reported before. These novel mutations include one insertion, seven missense, one splice site and one nonsense mutation.Thr60Met mutation was found to have a high frequency in the cohort, two patients carried homozygous T60M mutation,while 5 other patients carried heterozygous T60M mutation.Phenotype-genotype correlation was difficult to build in our cohort.

Conclusions: We have totally identified 24 mutations, including ten novel mutations in the SLC12A3 gene in 28 patients with GS. T60M is the most frequent variant in our patients. There was no significant correlation between genotype and phenotype in our patients.

SA-PO407

The Fabrazyme®, Angiotensin Receptor Blocker and ACE Inhibitor Treatment Study in Fabry Nephropathy (for the FAACET Investigators)

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Background: Patients with Fabry nephropathy lose kidney function despite treatment with enzyme replacement therapy (ERT). The FAACET study (NCT00446862) examined control of urine protein/creatinine ratio (UPCR) with antiproteinuric therapy in patients receiving agalsidase beta (1 mg/kg every two weeks). Ten international study sites participated.

Methods: Adults with confirmed Fabry disease, treated with agalsidase beta at 1 mg/kg every two weeks were included if baseline estimated GFR (eGFR) was <60 ml/min/1.73 m² and UPCR >0.5 g/day, or baseline eGFR <125 ml/min/1.73 m² and UPCR >1 g/day. ACE inhibitor or ARB therapy was titrated during 3 monthly visits to a UPCR ≤0.5 g/day, and the patients were then followed with visit every 3 months for the next 18 months. The primary objective was reduction of first morning UPCR to < 0.5 gram/gram. The primary outcome measure was the regression slope of estimated GFR with time in years.

Results: Twenty four patients (9 females/15 males) completed the protocol. Average age was 44±9(SD) yrs, with 1.8±0.2 yrs follow up. Thirteen achieved target UPCR ≤0.5 g/day, and 6 had averaged UPCR >0.5 and ≤1.0 g/day.

eGFR Slopes in Groups Stratified by Achieved UPCR

		Initial Baseline Values		Averaged	Active Treatment Period	
Number	Gender (F/M)	eGFR	UPCR	UPCR Strata	eGFR Slope	Averaged UPCR
13	6 F/7 M	70.6 (27.0)	0.6 (0.5)	≤0.5	–1.0 (6.1)	0.3 (0.1)
6	1 F/5 M	62.4 (26.3)	0.8 (0.4)	>0.5 & ≤1.0	–4.5 (7.0)	0.7 (0.2)
5	2 F/3 M	77.7 (24.4)	0.8 (0.7)	>1.0	–6.6 (11)	1.5 (0.5)

UPCR (g/day (SD)), eGFR Slope (ml/min/1.73 m²/year (SD)

Conclusions: The eGFR slope was associated with achieved UPCR in adult patients with Fabry nephropathy treated with ERT and antiproteinuric therapy. The majority of patients could be titrated to UPCR ≤0.5 g/day, and had eGFR slopes not different from zero. Effective antiproteinuric therapy is an important adjunct to ERT in treating patients with Fabry nephropathy.

Funding: Pharmaceutical Company Support - Genzyme Corporation

SA-PO408

Impact of Agalsidase beta on Renal Function in 60 French Patients with Fabry Disease Morgane Wetstein,[1] Claire Trivin,[2] Bruno Moulin,[3] Gabriel Choukroun.[1] *[1]Nephrology and Transplantation, CHU Amiens - Hopital Sud, Amiens, France; [2]Nephrology, HEGP, Paris, France; [3]Nephrology and Transplantation, Hopitaux Civils, Strasbourg, France.*

Background: Fabry disease is responsible for the progressive accumulation of Gb3 in the lysosomes of many cell types, causing significant cardiac, renal and neurological morbidity and mortality. Treatment with enzyme replacement therapy by agalsidase beta has been available for over 10 years, thus an evaluation of its efficacy on renal function is warranted.

Methods: We conducted a retrospective study in 24 Nephrology centres to assess the impact of algasidase beta at a dose of 1 mg/kg every 2 weeks on renal function in 60 patients (46 men).

Results: Mean age was 48±13 yrs and mean duration of treatment was 84±35 mo. Treatment was started on average at 40±13 yrs. In the 47 patients who were not on dialysis at the start of ERT, the eGFR was 79±43 ml/min and proteinuria averaged 1.1±1.6 g/d. Twenty-four patients were receiving antihypertensive therapy, including 12 on RAAS blocking agents. At the initiation of ERT, SBP was 130±17 mmHg and DBP was 75±10 mmHg. Calculating the slope of eDFG on the duration of follow-up demonstrates a stabilization of renal function with a loss of GFR averaged -0.59 ml/min/yr. An analysis conducted on populations stratified according to sex and stage of CKD at the time of the introduction of the ERT shows an average slope of eDFG in men and women at CKD 1-2, of -0.28 ml/min/year and 0.62 ml/min/year respectively (proteinuria <1.0 g/24 h in both populations). For men treated by ERT at a more severe stage (CKD 3 and proteinuria of 1.75 g/24 h before ERT), the calculated average slope was more pronounced (eDFG decline of -3.80 ml/min/yr). However, these rates were lower than that observed in the natural history of Fabry disease. At the end of follow-up, 4 patients progressed to ESRD, including 3 patients who were already in stage 5 before starting treatment; 4 patients died.

Conclusions: This study shows that treatment with agalsidase beta can significantly slows the progression of renal failure in patients with Fabry disease. This benefit is more pronounced if the treatment can be started early.

SA-PO409

Structural-Functional Relationships of Fabry Nephropathy Michael Mauer,[1] Chester B. Whitley,[1] Einar Svarstad,[2] Camilla Tøndel,[2] Marie-Claire Gubler,[3] Michael L. West,[4] Behzad Najafian.[5] *[1]Pediatrics and Medicine, University of Minnesota, Minneapolis, MN; [2]Medicine, Haukeland University, Bergen, Norway; [3]Pathology, Université René Descartes, Paris, France; [4]Medicine, Dalhousie University, Halifax, Canada; [5]Pathology, University of Washington, Seattle, WA.*

Background: There is a clear need for discovery of biomarkers to predict renal dysfunction and risk in Fabry disease. Renal biopsy structural parameters would be promising biomarker candidates. We studied which parameter(s) correlate best with glomerular filtration rate (GFR), urine albumin creatinine (UACR) and protein creatinine ratios (UPCR) in patients with Fabry disease.

Methods: Renal biopsies from 46 (M/F=1.9) treatment naive patients with Fabry disease, age 25 [4-63] years with UPCR 51 [0-290] ug/min and GFR 103±27 ml/min/1.73 m² were studied. Foot process width (FPW), fractional volume of globotriaosylceramide (GL3) inclusions per podocytes [Vv(Inc/PC)], mesangial [Vv(Inc/Mes)], and endothelial cells [Vv(inc/Endo)] were estimated using electron microscopy stereology.

Results: UACR correlated with Vv(Inc/PC) (r=0.52, p=0.048) and Vv(Inc/Mes) (r=0.51, p=0.04). UPCR correlated with Vv(Inc/Mes) (r=0.47, p=0.03). Multiple regression analysis (MRA) in all Fabry patients showed that 37% of UACR variability was explainable by Vv(Inc/PC), Vv(Inc/Mes) and Vv(Inc/Endo) (p=0.04) with Vv(Inc/PC) and Vv(Inc/Mes) being independent predictors of UACR. However, MRA with same predictor variables in male patients, explained up to 76% of UPCR variability (p=0.007).

GFR inversely correlated with age (r=-0.48, p=0.004). With MRA the combination of age and UPCR explained 31% and age, UPCR and FPW explained up to 47% of GFR variability in all patients with Fabry disease.

Conclusions: These studies for the first time show that glomerular structural parameters alone or in combination with clinical data can explain a substantial portion of early renal dysfunction in patients with Fabry disease. These studies will help us better identify pathologic lesions that closely correlate with early GFR decline and likely play a causal role in progression of Fabry renal disease.

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SA-PO410

High-Throughput Screening Identified Disease-Causing Mutants and Functional Variants of α-Galactosidase A Gene in Japanese Male Hemodialysis Patients Kent Doi,[1] Eisei Noiri,[1] Tomoko Ishizu,[1] Kousuke Negishi,[1] Yoshifumi Hamasaki,[1] Masaomi Nangaku,[1] Hitoshi Sakuraba.[2] *[1]Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [2]Analytical Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan.*

Background: Fabry disease is a genetic disorder caused by deficient activity of lysosomal enzyme α-galactosidase A (GLA) and end-stage renal disease (ESRD) will be present after accumulation of glycosphingolipids within the kidney. Undiagnosed atypical variants of Fabry disease, which are limited to renal involvement, were found in several ESRD patient populations. On the other hand, unexpectedly high frequencies of male subjects having the p.E66Q showing low α-GLA activity have been reported on Japanese and Korean screening for Fabry disease. However, several evidences indicate the p.E66Q is not a pathogenic mutation but is a functional polymorphism.

Methods: Serum α-GLA activity of 1080 male Japanese ESRD patients treated by hemodialysis was measured with 96-well based four-hour assay and confirmation by leukocyte α-GLA activity measurement and genetic analysis were subsequently conducted.

Results: Serum screening identified 10 patients with low enzyme activity (<1.5 nmol/h/ml). Leukocyte α-GLA activity measurement confirmed defect of enzyme activity in two patients and eight other patients had residual enzyme activity. Genetic analysis of the two Fabry disease patients revealed two genetic mutation of p.G195V in exon 4 and p.M296I in exon 6. All of the other eight patients had a functional polymorphism of p.E66Q. Notably, serum α-GLA activity measurement distinguished two disease-causing mutations from the p.E66Q. Functional analysis revealed p.E66Q leads to instability of the enzyme protein and subjects harboring the E66Q enzyme exhibited no increased level of plasma globotriaosylsphingosine in these subjects.

Conclusions: Our high-throughput serum enzyme assay distinctly identified two disease-causing mutants and functional variants of α-galactosidase A gene in Japanese male hemodialysis patients. In addition, functional analysis clearly demonstrated p.E66Q is not a pathogenic mutation but is a functional polymorphism.

Funding: Government Support - Non-U.S.

SA-PO411

Increased Platelet Count and Aggregability due to Urinary Loss of PACAP in Congenital Nephrotic Syndrome Benedicte Eneman,[1] Kathleen Freson,[2] Chris Van Geet,[2] Elena N. Levtchenko.[1] *[1]Development and Regeneration, Catholic University (KUL), Leuven, Belgium; [2]Department of Molecular and Vascular Biology, Catholic University (KUL), Leuven, Belgium.*

Background: Thrombotic complications occurring in up to 15% of patients represent a severe burden in nephrotic syndrome (NS). The underlying mechanisms are mainly unraveled in regard of venous thrombosis, while elevated blood platelet count and hyperaggregability increase the risk of arterial thrombosis.

A role of the neuropeptide PACAP (pituitary adenylate cyclase-activating polypeptide) as an inhibitor of megakaryocyte maturation and platelet function has recently been established. PACAP interferes with the regulation of apoptosis in megakaryocytes, via stimulation of NFκB signaling. We assumed that urinary losses of PACAP bound to ceruloplasmin in NS might lead to PACAP deficiency, leading to thrombocytosis and increased platelet reactivity.

The aim of this study was to investigate plasma PACAP levels in relation to blood platelet counts and aggregability in patients with congenital NS (CNS).

Methods: Four patients with CNS of the Finnish type, aged 0.5-19 months were tested. Plasma and urinary levels of PACAP were measured semi-quantitatively by western blot.

Results: All patients had plasma PACAP deficiency (14-40% of control, p<0.001) and excessive urinary PACAP excretion. In one patient aged 19 months both kidneys were removed as a routine treatment of CNS. Plasma PACAP levels progressively increased during the first days after nephrectomy and blood platelet count normalized.

In analogy to PACAP deficient mice, an increased platelet aggregation response to collagen was found in patients in nephrotic state, while platelets after bilateral nephrectomy showed normal reactivity towards collagen.

Conclusions: Our observation provides new insights on the mechanisms of arterial thrombosis in NS and indicates that PACAP deficiency exists in CNS. In analogy to mice, PACAP deficiency seems to play an important role in the thrombocytosis and platelet hyperaggregability in CNS. When confirmed in larger studies, PACAP replacement or stimulation of PACAP receptors might become a valuable therapeutic option for prevention of arterial thrombosis in CNS.

Funding: Government Support - Non-U.S.

SA-PO412

Clinical Analysis of the Largest Number of Fabry Patients in China
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Background: Our study is to evaluate the clinical features of patients with Fabry disease (FD).

Methods: We enrolled 47 FD patients in this survey which is the largest cohort in China.

Results: Among all participants, 31 were male and 16 were female, the average age was 28.89±11.80 years (12 to 64 years). The mean onset age was 16.85±12.53 years. Correct diagnosis was made by 1 to 30 years' delay. First symptoms included acroparesthesia and angiokeratoma, the incidence rate of 40.4% and 23.4%, respectively. 27.4% of patients has proteinuria, including five cases (10.6%) of ESRD, 3 cases had renal transplantation. Hypertension and stroke presented in 19.1% and 10.3% of all patients, respectively. Echocardiography showed left ventricular hypertrophy, valve involvement in 41.7% of patients .17 patients (13 males and 4 females) had presumably ischemic lesions on brain MRI .Compare to female patients, males had higher proteinuria ($p<0.05$), more hypertension ($p<0.05$) and lower MDRD-GFR ($p<0.01$), five cases of ESRD were males. Additionally, according to the Clinical classification of FD, our patients are divided into three groups: 31cases are classic type,12 cases are renal variant type, 4 cases are cardiac variant type, eGFR did not show significant statistical differences among three groups ($P> 0.05$).In contrast, proteinuria ($P <0.01$) and serum creatinine ($P <0.05$) has significant differences in the groups. Finally, patients with blood type AB and B were associated with higher serum creatinine ($p<0.01$), more proteinuria ($p<0.05$) and lower eGFR ($p<0.05$) when compare to patients with type A and O.

Conclusions: As the first study of the largest number of Chinese FD patients, it defines the clinical features in patients with FD, in order to improve early intervention. The classic phenotype is the most common in China, and the other types are also confined. Hypertension and proteinuria were associated with worse renal outcome, therefore they should be treated more intensively and it is necessary to establish long-term follow-up system to manage those patients.

SA-PO413

Cystinuria: A Large French Cohort
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Background: Cystinuria is an autosomal recessive disorder and is the cause of 1% and 8% of stones in adults and children respectively. Objectives of our study were to determine epidemiological and clinical characteristics, medical and surgical treatments and co morbidities among cystinuric patients.

Methods: We conducted a retrospective cohort study in France involving 47 centers of adult and pediatric nephrology and urology.

Results: We collected data from 442 cystinuric patients (sex ratio = 1, median age at last follow-up = 32.5 years [range:0.3 – 86.6]). Median age at first symptoms was 16.7 years [0.3 – 72.1]. The main diagnosis modality was a renal colic in 60.6% of cases. Diagnosis, which lagged behind first symptoms by 1.3 years [0.0 – 45.7], was based on stone analysis in 57.7% of cases and on cystinuria assessment in 27.9%. Ninety one percent of patients were treated medically. Cystine chelators were prescribed in 55.3% of patients and induced side effects in 25% of cases. Eighty two percent of patients had at least one urological procedure. After 2000, flexible ureteroscopy was more frequently used than rigid ureteroscopy. In patients followed for over 10 years, the median annual rate of surgical procedures per patient was 0.2 [0.0 – 2.0]. Thirty one percent of adult patients (>16 years) followed for over 10 years had impaired renal function (MDRD estimated glomerular filtration rate < 60 mL/min/1.73 m^2) and 27% of adult patients had hypertension. In univariate analysis, impaired renal function was significantly associated with age, obesity, hypertension and a past history of partial or total nephrectomy.

Conclusions: We report the largest cohort of cystinuric patients. The burden of urological interventions in this disease is high. In particular, chronic renal failure is an underestimated consequence of cystinuria and hypertension prevalence is surprisingly high considering the young age of this population.

SA-PO414

Schimke Immunoosseous Dysplasia Caused by Segmental Paternal Uniparental Disomy for Chromosome 2
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Background: Schimke immunoosseous dysplasia (SIOD) is an autosomal recessive multisystem disorder caused by mutations in the *SMARCAL1* gene. The main clinical findings are spondyloepiphyseal dysplasia (SED), nephropathy, and T-cell deficiency.

Methods: We describe a 7-year-old boy with SIOD. Genetic analysis of *SMARCAL1* was performed in the child as well as his parents.

Results: Molecular analysis showed that the child was homozygous for the mutation, c.1930C>T (p.R644W) in the exon 12 of*SMARCAL1* gene. Carrier testing on the parental samples revealed that the father was heterozygous for the mutation whereas the mother did not carry the mutation. Seven microsatellite markers of chromosome 2 and 12 single-nucleotide polymorphisms around the mutation in the *SMARCAL1* gene were selected to analyse the origins of the patient's chromosome2. Copy-number variants of the exon 12 of *SMARCAL1* gene in the patient and his parents were identified by real time PCR. The results showed that the child has segmental paternal uniparental disomy.

Conclusions: This is the first case of SIOD resulting from uniparental disomy and discloses an alternate mechanism of SIOD. It is also very important for the genetic counseling of the family.

SA-PO415

Uromodulin Mutations Hamper Trafficking of Urate Transporters
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Background: Point mutations in the uromodulin (UMOD) are the cause for the uromodulin associated kidney disease (UAKD). The earlieast sign of UAKD is a hyperuricemia in adolescents. The retention of mutated UMOD in the secretory pathway leads to a reduced expression and secretion at the plasma membrane. It is known that mutated UMOD interferes with the proper function of ROMK. We postulate that disturbed trafficking of UMOD mediates a retention of renal uric acid transporters such as GLUT9, ABCG2 and OAT4 in the secretory pathway.

Methods: HEK293T-cells transiently expressing uric acid transportes (GLUT9, ABCG2, OAT4) and UMOD are lysed and coimmunoprecipitations are performed. To examine cell surface expression of UMOD and its mutant P236L non permeabelized cells are labeled with UMOD antisera. Susequent immunoprecipitation of UMOD is done. Cells expressing transiently GLUT9, UMOD-WT or UMOD P236L are homogenized in a sucrose buffer. A subcellular fractionation is performed to visualize the distribution of UMOD and urate transporters. By immunofluorescence cos7 cells expressing GLUT9 and either UMOD-WT or UMOD P236L are used for visualization of their subcellular localization of UMOD and the uric acid transporters.

Results: UMOD co-immunoprecipitates with GLUT9, ABCG2 and OAT4. The UMOD mutant P236L shows a considerably reduced cell surface expression compared to UMOD WT. The coexpression of UMOD P236L with GLUT9deltaN results in an augmented retention within the secretory pathway. UMOD P236L and GLUT9 are coexpressed a reduced GLUT9 staining at the plasma membrane and an increased GLUT9 staining in the cytoplasm are seen.

Conclusions: The urate transporters GLUT9, ABCG2 and OAT4 are interaction partners of UMOD. Proper trafficking of UMOD to the plasma membrane is essential for cell surface expression of GLUT9. Furthermore, UMOD mutations impair the cell surface expression of GLUT9.

SA-PO416

Uromodulin Mutation P236R Is Associated with Long-Segment Bilateral Renal Artery Stenosis
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Background: Uromodulin (UMOD) associated kidney disease (UAKD) is associated with juvenile hyperuricemia, followed by progressive interstitial fibrosis leading to endstage renal disease with about 20 years after disease onset. A minority of the effected individuals with this autosomal dominant disease present renal cysts. Here we present a polish-german family with 39 members out of 3 generations with UAKD. Within this pedigree we found a long-segement bilateral renal artery stenosis as a novel phenotype.

Methods: The members of the polish-german family received a physical examination, doppler ultrasound of the kidneys and lab tests for renal function, RAAS und urine analysis. 8 family members (WT and UMOD P236R) had contrast enhanced renal artery imaging by angio CT or MRI done. Genotyping was done by PCR and sequencing.

Results: In the polish-german family with 39 members 11 family members carried the mutation which. All family members having bilateral renal artery stenosis carried the UMOD mutation P236R (c707C>G). Overall 11 family members carried the mutation. Statistical analysis showed a strong correlation (r=0,84) between the reduced renal artery caliber and decreased eGFR. The earliest symptom is hyperuricemia in childhood.

Conclusions: The uromodulin mutation c.707C>G results in a single aminoacid exchange p.P236R. This is closely correlated with long-segment bilateral renal artery stenosis. Hyperuricemia might cause proliferation of vascular smooth muscle cells within the renal artery wall causing the stenosis.

SA-PO417

Inflammation Is an Early Event of Pathogenesis in Uromodulin-Associated Kidney Disease Celine Schaeffer,[1] Matteo Trudu,[1] Michela Riba,[2] Paola Brambilla,[2] Ilenia Bernascone,[1] Sylvie Janas,[3] Masami Ikehata,[4] Antonio Amoroso,[5] Gian Marco Ghiggeri,[5] Francesco Scolari,[5] Filippo Martinelli Boneschi,[2] Maria Pia Rastaldi,[4] Olivier Devuyst,[6] Luca Rampoldi.[1,5] *[1]Division of Genetics and Cell Biology, Dulbecco Telethon Institute c/o San Raffaele Scientific Institute, Milan, Italy; [2]San Raffaele Scientific Institute, Milan, Italy; [3]Université Catholique de Louvain, Brussels, Belgium; [4]Fondazione D'Amico per la Ricerca Sulle Malattie Renali, Milan, Italy; [5]MCKD Consortium, Italy; [6]University of Zurich, Zurich, Switzerland.*

Background: Uromodulin-associated kidney disease (UAKD) is an autosomal dominant disorder caused by mutations in *UMOD*, the gene encoding uromodulin. We previously demonstrated that transgenic mice expressing mutant uromodulin ($Tg^{UmodC147W}$) are a remarkable model of UAKD that recapitulates the main features of the disease, i.e tubulo-interstitial nephritis and progressive renal failure.

Methods: To gain insight into the disease pathogenesis, we performed microarray profiling, qRT-PCR and biochemical analysis on kidneys from $Tg^{UmodC147W}$ mice and from expression-matched transgenic mice for the wild-type protein.

Results: Kidney microarray analysis performed on young pre-symptomatic mice (4 and 8 weeks) showed significant upregulation of pathways related to inflammation and fibrosis. Interestingly, some key pro-inflammatory signals (eg IL-6, TNFa) were already upregulated as early as at 1 week of age, well before the presence of any sign of renal damage but soon after first detection of the accumulation of misfolded mutant uromodulin in the endoplasmic reticulum (ER). Uromodulin ER retention was never accompanied by induction of the unfolded protein response or apoptosis.

Conclusions: These data suggest that chronic inflammation plays a key role in the onset and progression of UAKD. Uromodulin mutations seem to exert a non cell-autonomous proteotoxic effect, mediated by the induction of pro-inflammatory signals. The identification of such signals downstream of mutant uromodulin ER retention could represent a significant step towards therapeutic intervention and might have broad relevance for tubulo-interstitial nephritis and other conformational diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO418

Two New Mutations in *NPHS2* Represent an Egyptian Founder Mutation in Nephrotic Syndrome Virginia Vega-Warner,[1] Neveen Soliman Elshakhs,[2,3] Hanan Fathy,[4] Heon Yung Gee,[1] Pawaree Saisawat,[1] Svjetlana Lovric,[1] Shazia Ashraf,[1] Gil Chernin,[1] Friedhelm Hildebrandt.[1,5] *[1]Pediatric and Human Genetics, University of Michigan, Ann Arbor, MI; [2]Department of Pediatrics and Center of Pediatric Nephrology & Transplantation, Cairo University, Cairo, Egypt; [3]Egyptian Group for Orphan Renal Diseases (EGORD), Cairo, Egypt; [4]Pediatric Nephrology Unit, Alexandria University, Alexandria, Egypt; [5]Howard Hughes Medical Institute, Ann Arbor, MI.*

Background: Mutations in *NPHS2* (podocin), a recessive gene, cause nephrotic syndrome (NS) in children and rarely in adults. Kidney biopsies reveal focal segmental glomerulosclerosis (FSGS) or diffuse mesangial sclerosis. In general, NS resulting from *NPHS2* mutations is resistant to steroid treatment (SRNS).

Methods: Blood samples of children with NS from Egypt were received between 2006 and 2012. Genomic DNA was extracted using Puregene sytem (Qiagen). Mutation analysis was performed by direct Sanger sequencing of all 8 exons of *NPHS2*.

Results: During this period 132 Egyptian families (175 individuals) were studied. Most of the families had SRNS and kidney biopsies showed FSGS. In 20 families (15%), we detected both causative *NPHS2* mutations. Among these, 18 families had homozygous and 2 families showed compound heterozygous mutations. Two mutations were novel: Ex1:c.1A>T(H); p.M1L and Ex 5:c.596dupA(H); p.N199K*fs*14. These were present in 6 and 4 families, respectively. One of these mutations was presented in families from different cities in Egypt. Both mutations were absent from 90 healthy controls and from 1,732 families with other ethnicities received in the same period. Interestingly, in one of the families with multiple consanguinities, both founder mutations segregated in different members.

Conclusions: Given the concentration of these novel mutations in the Egyptian population and the absence from patients of other ethnicities, we conclude that both are founder mutations in the Egyptian population allowing for more directed mutation analysis.

Funding: NIDDK Support

SA-PO419

The Spectrum of Genes Causing Nephrotic Syndrome in Saudi Arabia Mohamed Al-hamed,[1] John Andrew Sayer,[1] Essam A. Alsabban,[2] Abbas Alabbad,[2] Naffaa Nafe Alharbi,[2] Ibrahim A. Alhassoun,[2] Eissa Ali Faqeih,[3] Brian F. Meyer.[2] *[1]Insitute of Genetic Medicine, Newcastle University, Newcastle, Tyne and Wear, United Kingdom; [2]King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [3]King Fahad Medical City, Saudi Arabia.*

Background: Nephrotic Syndrome (NS) is a renal disease characterized by heavy proteinuria, hypopalbuminemia, odema and hyperlipidemia. Its presentation within the first 3 months of life (congenital NS) and in multiple family members suggests an underlying inherited cause. Inherited NS is genetically heterogeneous and to date defects in at least ten genes have been recognized. Proteins encoded by these genes influence the function of podocytes and the slit diaphragm. The Saudi Arabian population has a tribal structure and the overall rate of consanguineous marriage is reported to be over 55%. In such a population, the identification of mutations in known disease genes causing inherited NS is a robust method to allow appropriate diagnosis and clinical management.

Methods: To determine the frequency of inherited NS, sixty six cases (representing 53 families with NS) from Saudi Arabia were screened for mutations in the following genes: *NPHS1, NPHS2, PLCE1, LAMB2,* and *CD2AP.*

Results: We detected likely causative mutations in 25 out of 53 families studied. In 15 families where familial NS was suspected, we found mutations in 12 families (80%). We found that the most common genetic cause of NS in our cohort was a homozygous mutations in the *NPHS2* gene, found in 10 of the 53 families (19%). Mutations in the *NPHS1* and *PLCE1* genes allowed a molecular genetic diagnosis in 13% and 7% of families, respectively. Mutations in *LAMB2* and *CD2AP* remained rare. We detected 11 novel sequence variants in this study and their pathogenicity was analysed by in silico tests and by genetic screening of control populations.

Conclusions: This is the first report describing the molecular genetics of NS in an Arabian peninsula population.

Funding: Government Support - Non-U.S.

SA-PO420

The Use of Exome Sequencing in Families with Focal and Segmental Glomerulosclerosis to Obtain Genetic Diagnoses Moumita Barua,[1] Giulio Genovese,[1,2,3] Michael Fischereder,[4] Katrin Uhlig,[5] Andrea Uscinski Knob,[1] Martin R. Pollak.[1] *[1]Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [2]Department of Genetics, Harvard Medical School, Boston, MA; [3]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; [4]Nephrology Division, Department I of Internal Medicine, University Hospital Munich-Campus Grosshadern, Ludwig-Maximilians-University, Munich, Marchioninistrasse 15, Germany; [5]Division of Nephrology, Department of Medicine, Tufts Medical Center, Boston, MA.*

Background: Advances in sequencing technology now makes it feasible to sequence the entire coding region of an individual's genome, termed exome sequencing. The majority of Mendelian disease-causing mutations have been found in the exome.

Methods: We aim to sequence the exome of two related individuals from autosomal dominant families with focal and segmental glomerulosclerosis (FSGS) to identify known and novel disease-causing genes.

Results: 29 individuals from 15 families with nephrologist reported proteinuria and/or a pathologic diagnosis of FSGS had exome sequencing performed. Each individual yielded 33 to 92 novel heterozygous and homozygous non-synonymous (NS) variants after filtering. Affected family members shared 1 to 46 novel NS variants. Capture of the known renal disease-causing genes was heterogeneous though adequate for most genes, with the notable exception of INF2. To illustrate the clinical utility of exome sequencing, we provide the example of uncovering a novel WT1 mutation, p.P382T, in one family with FSGS, when this diagnosis had not been previously suspected. The mutation was subsequently confirmed by Sanger sequencing in four affected family members while absent in nine unaffected members. Affected individuals presented during childhood with isolated nephrotic syndrome in the absence of associated syndromic features of WT1 disease.

Conclusions: Exome sequencing is powerful technology with the promise to identify known and novel disease causing genes. Its main limitation is heterogeneous capture of the exome.

Funding: NIDDK Support, Government Support - Non-U.S.

SA-PO421

Whole Exome Sequencing Diagnoses NPHP3 in a Consanguineous Family Originally Diagnosed with FSGS Moumita Barua,[1] Giulio Genovese,[1,2,3] Andrea Uscinski Knob,[1] Martin R. Pollak.[1] *[1]Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [2]Department of Genetics, Harvard Medical School, Boston, MA; [3]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA; [4]Clinical Study Group Iran, IR of Iran Academy of Medical Sciences, Tehran.*

Background: Accurate diagnoses can be difficult given the overlapping clinical and histopathologic features of many kidney disorders. We show here the benefit of genetic testing in obtaining an accurate diagnosis of a consanguineous family originally diagnosed with FSGS but found to have NPHP3 disease with whole exome sequencing.

Methods: We enriched and sequenced the exome of one affected individual using the Nimblegen 2.1M Human Exome kit and Illumina HiSeq 2000, respectively. For validation, interesting variants were sequenced in 3 affected and 11 unaffected individuals by Sanger method. Phenotypic data were collected from review of medical records.

Results: Family FG-CR is an Iranian consanguineous family with autosomal recessive inheritance. A marriage between related individuals resulted in 9 children, 3 of whom were affected. The exome of individual FG-CR 6 yielded 165 novel non-synonymous variants but only 2 that were homozygous. Given its known association with kidney disease, we selected the NPHP3 gene for further study. The variant p.R301T in the gene NPHP3 segregated with disease when Sanger sequenced in the 14 available samples. All 3 affected individuals presented with nephrotic range proteinuria and developed end-stage renal disease. Individual FG-CR 6 had a native kidney biopsy, which was interpreted to be consistent with FSGS.

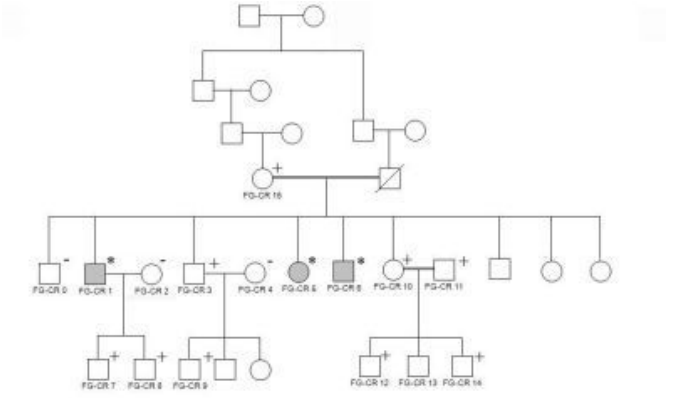

Figure 1. Pedigree of family FG-CR. Affected individuals are shaded grey. Individuals with the NPHP3 p.R301T variant who are homozygous are denoted with * to the upper right while those who are heterozygous are denoted with +. Individuals without the variant are indicated with -. The DNA sample belonging to FG-CR 13 was not tested.

Conclusions: Exome sequencing is a useful clinical diagnostic tool to arrive at accurate diagnoses in inherited kidney disease.

Funding: Government Support - Non-U.S.

SA-PO422

A Novel Mutation outside of the Candidate Region for Diagnosis in the Formin Gene INF2 Causes Focal Segmental Glomerulosclerosis Not Associated with Charcot-Marie-Tooth Neuropathy

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Background: Focal and segmental glomerulosclerosis (FSGS) is a histological pattern derived from several etiologies, including genetics. The autosomal dominant (AD) form of FSGS is a heterogenic disease with three known genes: α-actinin 4 (*ACTN4*), canonical transient receptor potential 6 (*TRPC6*) and inverted formin 2 (*INF2*), which was recently discovered. More recently, *INF2* mutations have also been attributed to Charcot-Marie-Tooth neuropathy associated with FSGS.

Methods: In this study, we performed direct sequencing, histological characterization and functional studies in a cohort of families with AD-FSGS.

Results: We detected a novel mutation in exon 6 of *INF2* gene. This mutation is predicted to alter a highly conserved amino acid residue within the 17th α-helix of the diaphanous inhibitory domain (DID) of the protein. A long-term follow-up of this family showed that all patients were diagnosed in adulthood and progressed to end-stage renal disease (ESRD) at different times without clinical or electrodiagnostic evidence of neuropathy.

Conclusions: This is the first study to describe a mutation in *INF2* linked non-syndromic FSGS outside of the candidate region used for rapid diagnosis (exons 2 to 4), indicating the necessity for full gene sequencing if no mutation was found in this region of the gene.

Funding: Government Support - Non-U.S.

SA-PO423

Highly Parallel Mutation Analysis in 21 Known Nephrotic Syndrome Genes by Next-Generation Sequencing

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Background: In nephrotic syndrome (NS), more than 21 single-gene causes are known, but mutation analysis in these genes is time and cost intensive. Here we develop a new method for mutation analysis using a PCR-based microfluidic technology (48.48 Access Array System from Fluidigm™) with consecutive next generation sequencing (NGS).

Methods: As a proof of principle, we evaluated 48 patients with confirmed homozygous or heterozygous mutations in various NS genes. We applied a 10-fold primer multiplexing approach allowing PCR-based amplification of 499 amplicons (424 exons) for 48 DNA samples simultaneously. The maximal length of each PCR Product was 290 bp. In our analysis we included the recessive NS genes: *NPHS1*, *NPHS2*, *LAMB2, PLCE1, COQ6, SMARCAL 1,COQ2, PDSS2, CD2AP, SCARB2, CFH, CUBN, PTPRO, MYO1E, NEIL1, ITGA3* and the dominant NS genes *ACTN4*, *WT1*, *LMX1B, TRPC6 and INF2.* In a second PCR we indexed all 48 patient-derived PCR-products uniquely with different barcodes. Bidirectional (150 bases) sequencing was performed on one lane of a GA II instrument (Illumina™). Bioinformatics analysis was performed using "CLC Genomics Workbench™" software.

Results: NGS and bioinformatics analysis resulted in 15.2 million mapped reads with a median exon coverage depth of 374-fold per patient. All DNA samples (100%) and 468/499 exons (93.78%) consistently showed sufficient coverage depth above 25-fold. We identified the disease causing mutation in 42/48 patients (87.5%) of our cohort.

Conclusions: We conclude that the combined method of array-based multiplexed PCR amplification on a Fluidigm™ Access Array platform followed by NGS is a novel highly efficient approach towards mutation analysis in the heterogeneous single-gene forms of NS.

Funding: NIDDK Support

SA-PO424

A Recurrent Homozygous Missense Mutation in *TTC21B* Encoding the Cilia Protein IFT139 Unexpectedly Causes Nephrotic Syndrome/FSGS

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Background: To identify novel genes involved in hereditary steroid-resistant nephrotic syndrome (SRNS), we performed exome sequencing in consanguineous families.

Results: We found a recurrent pathogenic homozygous missense mutation, P209L, in *TTC21B* in2 families with SRNS and focal and segmental glomerulosclerosis (FSGS). Further screening revealed the same homozygous mutation in 5 additional families with SRNS/FSGS. *TTC21B* encodes the retrograde intraflagellar transport protein IFT139 and intriguingly, this mutation was previously reported to cause isolated nephronophthisis (NPH). Indeed, 3 unrelated patients diagnosed as NPH and carrying the homozygous P209L mutation also have proteinuria and FSGS. All patients display hypertension,glomerulopathy with proteinuria (1-7 g/24h), FSGS, and severe tubular basement membrane anomalies characteristic of NPH. End-stage renal failure occurred at a mean age of 23 (15-34 years). All patients were from North Africa or Portugal and shared a common haplotype at the locus. The P209L variation was not found in 161 ethnic controls. We found that IFT139 was expressed by podocytes and distal tubules in fetal and adult human kidneys. Developing podocytes in fetal kidney and undifferentiated podocytes in culture have a cilium, whereas it was absent in differentiated podocytes. While IFT139 localizes at the cilia base in undifferentiated podocytes, its staining follows the extended microtubule network in non-ciliated differentiated cells.

Conclusions: Our results point to a critical function of IFT139 in podocytes in addition to tubular epithelial cells, likely via dysregulation of ciliary transport and microtubule network essential for cell polarity and proper organization/maintenance of intercellular junctions, including the slit diaphragm.

Funding: Government Support - Non-U.S.

SA-PO425

Comparison of the Clinical Features in X-Linked and Autosomal Recessive Alport Syndrome

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Background: X-linked Alport syndrome accounts for 85% patients and recessive disease for 15% but the distinction is important because of the different implications for their families. We have compared clinical features in adults with the two modes of inheritance.

Methods: Patients with X-linked disease were identified on the basis of a mutation in the COL4A5 gene, and those with recessive disease with two COL4A3 or COL4A4 mutations. All patients were examined by the same nephrologist and ophthalmologist.

Results: Patients included 18 males and 24 females from 24 families with X-linked Alport syndrome, and 15 patients from 10 families with autosomal recessive disease. Most men with X-linked disease had renal failure (14/18, 78%), hearing loss (17/18, 94%), lenticonus (11/17, 65%) and the central (9/17, 53%) or peripheral (12/15, 80%) retinopathy. Most females with X-linked disease had an affected male relative (15/19, 79%), but renal failure (3/24, 13%), and central retinopathy (4/24, 17%) were uncommon, and lenticonus did not occur. However hearing loss (10/24, 42%) and peripheral retinopathy (6/15, 40%) were frequently present. With recessive Alport syndrome, males (8/15, 53%) and females (7/15, 47%) were affected equally often, and another family member with renal failure was uncommon (2/10, 20%). Most patients had proteinuria (15/15, 100%), renal failure (14/15, 93%), hearing loss (15/15, 100%), lenticonus (12/15, 80%) and the central (10/14, 71%) or peripheral (9/11, 82%) retinopathy. End-stage renal failure developed at 20 years (median, range 14 – 50) in males with X-linked disease, and at 25 (range 15 – 35) in recessive disease. Mutations resulting in a stop codon occurred in 46% (11/24) patients with X-linked disease and 80% (8/10) with recessive disease.

Conclusions: Autosomal recessive Alport syndrome should be considered in all patients, especially from consanguineous families, where the father has haematuria, and in females with proteinuria, renal impairment, hearing loss, lenticonus or a central retinopathy.

SA-PO426

Copper Deficiency in Cystinosis Patients Martine T. Besouw,[1] Jerry A. Schneider,[2] Francesco Emma,[3] Francois Nobili,[4] Fransiska Malfait,[5] Flemming Skovby,[6] Sofie Symoens,[5] Anne De Paepe,[5] Elena N. Levtchenko.[1] *[1]Development and Regeneration, Catholic University (KUL), Leuven, Belgium; [2]Pediatrics, University of California, San Diego, CA; [3]Nephrology and Urology, Bambino Gesù Children Hospital and Research Institute, Rome, Italy; [4]Pediatric Nephrology, University Hospital, Besançon, France; [5]Medical Genetics, University Hospital, Ghent, Belgium; [6]Clinical Genetics, Juliane Marie Centre, University Hospital, Copenhagen, Denmark.*

Background: Cystinosis is an autosomal recessive disorder, marked by intralysosomal cystine accumulation in various tissues, causing renal Fanconi syndrome (FS). The disease is treated by cysteamine. Recently, 8 cystinosis patients were reported with skin lesions and some - with severe bone and muscular weakness, caused by cysteamine. Electron microscopy of skin biopsies showed "cauliflower" collagen fibres caused by decreased collagen cross-linking. We hypothesized that cysteamine might interfere with this process in analogy to D-penicillamine. Furthermore, since the generation of aldehydes required for cross-linking is catalyzed by the enzyme lysyl oxidase, which needs copper as a co-factor, we measured serum copper and ceruloplasmin levels and urinary copper excretion in cystinosis patients, since copper deficiency might contribute to cysteamine toxicity.

Methods: 35 cystinosis patients were included: 21 with FS (including 6 with cysteamine toxicity), 12 with renal graft, 1 was on hemodialysis and 1 with ocular cystinosis.

Results: Mean ± SD serum copper and ceruloplasmin levels were lower in patients with cysteamine toxicity (63±7 vs 98±27 µg/dL and 0.20±0.02 vs 0.31±0.08 g/L respectively; $p<0.01$), urinary copper excretion was increased in patients with FS (169±75 vs 35±20 µg/g; $p<0.01$).

Conclusions: Increased urinary copper excretion leading to copper deficiency might decrease lysyl oxidase activity resulting in decreased aldehyde formation. Subsequent administration of cysteamine can block the remaining aldehydes, further diminishing collagen cross-linking. This mechanism is likely to be responsible for cysteamine toxicity in cystinosis patients, therefore, copper suppletion might prevent cysteamine toxicity.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO427

Role of the Renin-Angiotensin System in TSC Renal Angiomyolipoma Progression Brian J. Siroky,[1] Ryan J. Reichert,[2] Lu Lu,[1] Robert Lou,[1] Ej Kathman,[1] John J. Bissler.[1] *[1]Nephrology & Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Medicine, Northeast Ohio Medical University, Rootstown, OH.*

Background: Tuberous Sclerosis Complex (TSC) is a multi-system hamartomatous disease that is genetically linked to disruptions of *TSC1* or *TSC2*. TSC lesions are highly vascularized and associated to aberrant signaling of mTOR. Previous studies have focused on the blockade of the mTOR pathway for disease management. It has been suggested clinically that disease progression is also decelerated when the hypertensive manifestations of TSC are managed with renin-angiotensin system (RAS) blockade. In this study, we utilized a renal cell model of TSC to investigate the effects of angiotensin II (ANG II) on intracellular Ca^{2+} levels, and also VEGF release. Intracellular Ca^{2+} is a known signaling effector of ANG II mediated AT_1 G protein-coupled receptor activation. VEGF release, which has been linked to ANG II signaling, is elevated in TSC cells, and is an important factor in angiomyolipoma vascularization.

Methods: We measured changes in intracellular Ca^{2+} levels using fura-2/AM, and VEGF secretion by ELISA in *TSC2*-deficient human renal angiomyolipoma cells (TRI102), and in cells in which TSC2 has been re-expressed (TRI103).

Results: We found that TRI102 cells had higher baseline Ca^{2+} levels, and that ANG II (1 µM) produced a greater increase in intracellular Ca^{2+} in TRI102 cells compared to TRI103. We also observed greater baseline VEGF release in TRI102 compared to TRI103 cells. mTOR inhibition (20 nM RAD001, 24 hrs) lowered VEGF release in TRI102 cells while TRI103 cells were not affected. ANG II (10 and 100 nM, 24 hrs) increased VEGF release in TRI102 and not TRI103 cells.

Conclusions: These results suggest hypersensitivity of *TSC2*-deficient angiomyolipoma cells to ANG II, which may contribute to aberrant cellular activity. Furthermore, these findings support a possible role of RAS signaling in TSC disease progression, and suggest the potential of utilizing RAS blockade for tumor/patient management in TSC.

Funding: Private Foundation Support

SA-PO428

Sporadic Renal Angiomyolipoma and Tuberous Sclerosis Complex Related Renal Angiomyolipoma in the United States Peter Sun,[1] Zhimei Liu,[2] Judith A. Prestifilippo,[2] Hearns Charles,[3] John C. Hulbert,[4] John J. Bissler.[5] *[1]Kailo Research Group; [2]Novartis Pharmaceuticals Corporation; [3]NYU Langone Medical Center; [4]Urologic Physicians; [5]University of Cincinnati.*

Background: This study aimed to assess the proportion of sporadic renal Angiomyolipoma (AML) versus tuberous sclerosis complex (TSC) related AML in the United States.

Methods: A retrospective cohort study was conducted using three US national healthcare claims databases (Years 2000-2010, 60+ million population). Patients with an AML diagnosis (ICD9-CM 223.0) and a minimal 2year health plan enrollment (one year prior to and one year following newly observed AML diagnosis) were included. AML patients with a TSC diagnosis (ICD9-CM 759.5) were grouped as TSC AML patients; the rest were grouped as patients with sporadic AML. Proportions for each group, as stratified by age, health plan types, were compared using two-sample t-tests.

Results: Patients (N=18,146) had a mean age of 55.4 years at newly observed AML diagnosis; 47.1% were male. In all AML patients, 26.1% were TSC related and 73.9% were sporadic AML. These rates were different across age groups (TSC-AML: 26.8-53.6%; sporadic AML: 46.4-73.3%), and varied by data sources. The proportion of TSC AML was much higher than sporadic AML in the Medicaid database (88.6% versus 11.4%), but was lower than sporadic AML in the commercial databases (database# 1: 42.2% versus 57.8%; database# 2: 1.3% versus 98.7%).

Conclusions: The proportions of patients with sporadic AML or TSC related AML varied by age groups and data sources. This should be taken into account when analyzing outcomes related to AML treatment.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

SA-PO429

Measurement of 2,8-Dihydroxyadenine Excretion in Patients with APRT Deficiency Using UPLC-MS/MS Runolfur Palsson,[1,2] Baldur Bragi Sigurdsson,[3] Margret Thorsteinsdottir,[2,3] Vidar O. Edvardsson.[1,2] *[1]Landspitali - National University Hospital of Iceland; [2]University of Iceland; [3]Arctic Mass, Reykjavik, Iceland.*

Background: Adenine phosphoribosyltransferase (APRT) deficiency results in excessive urinary excretion of poorly soluble 2,8-dihydroxyadenine (DHA), causing recurrent kidney stones and/or chronic kidney disease (CKD) in affected patients. Treatment with allopurinol has proved effective but reliable methods for therapeutic monitoring are lacking. We evaluated a new ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-MS/MS) method for measuring urinary DHA excretion in patients with APRT deficiency.

Methods: UPLC-MS/MS was used to measure the concentration of DHA and adenine in urine samples from 2 patients with APRT deficiency, before and during treatment with allopurinol 300 mg/day and the alternative agent, febuxostat 80 mg/day. Urine samples from 4 healthy subjects were used as controls. Aliquots of urine were collected immediately after the urine container had been inverted several times to suspend settled particles. The pH of the aliquots was adjusted to 10 using 20 mM NH_4OH to dissolve all precipitates before testing. The urinary excretion of DHA and adenine are expressed as DHA-to-creatinine and adenine-to-creatinine ratio (mmol/mmol).

Results: In 4 healthy controls, the average DHA-to-creatinine ratio was 0.08 mmol/mmol and the adenine-to-creatinine ratio was below a quantifiable level (<2.96 mmol/mmol). In the patient treated with allopurinol, the DHA-to-creatinine ratio decreased from 34.0 to 11.0 mmol/mmol and the adenine-to-creatinine ratio increased from 4.0 to 24.0 mmol/mmmol. In the patient treated with febuxostat the DHA-to-creatinine ratio decreased from 22.0 to 3.0 mmol/mmol and the adenine-to-creatinine ratio increased from 7.0 to 21.0 mmol/mmol.

Conclusions: Marked reduction in urinary excretion of DHA was observed after pharmacotherapy was initiated. However, both patients continued to excrete substantial amounts of DHA on conventional doses of both drugs. These preliminary data suggest that the novel UPLC-MS/MS assay will greatly enhance monitoring of pharmacotherapy in patients with APRT deficiency.

Funding: NIDDK Support

SA-PO430

LRRC6 Identified as a Novel Single-Gene Cause of Primary Ciliary Dyskinesia by Homozygosity Mapping and Whole Exome Resequencing Katrina A. Diaz,[1] Heon Yung Gee,[1] Moumita Chaki,[1] Toby W. Hurd,[1] Edgar Otto,[1] Jan Peter Halbritter,[1] Maimoona A. Zariwala,[3] Michael R. Knowles,[3] Friedhelm Hildebrandt.[1,2] *[1]Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI; [2]Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI; [3]Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina, Chapel Hill, NC.*

Background: Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disorder with a complex phenotype caused by impaired motility of cilia. The disease is characterized by abnormal function and/or ultrastructural defects of the ciliary mechanism. Mutations in genes encoding for parts of the ciliary ultrastructure have been recently implicated in the pathogenesis of the disease. The objective of this study was to identify novel single-gene causes of PCD by whole exome resequencing (WER).

Methods: To identify novel PCD-causing genes, homozygosity mapping was performed as well as WER in a cohort of 44 families with the disease. Homozygous recessive mutations were further evaluated for candidacy.

Results: A homozygous frameshift mutation (p.W210CfsX12) in the gene *LRRC6* was identified in a Pakistani family with two affected siblings. This mutation segregated with the affected status of each individual in the family and was confirmed with Sanger sequencing. *LRRC6* is a gene involved in ciliary assembly and maintenance similar to *LRRC50*, a gene with known association with inner and outer dynein arm ultastructural defects. This is congruent with the phenotype of the affected individuals. Additionally, there is a *D. rerio* homolog model (*Lrrc6l*) as well as a *D. melanogaster* homolog model (*tilB*) available for *LRRC6* that exhibit dynein arm defects and impaired ciliary motility.

Conclusions: There is strong evidence that *LRRC6* is associated with inner and outer dynein arm defects and is a novel single-gene cause of PCD.

Funding: Other NIH Support - Health and Human Services

SA-PO431

***GSTT1* Gene Abnormality in Minimal Change Nephrotic Syndrome with Hyper Immunoglobulin E** Kohei Miyazaki, Shinsuke Fujita, Hitomi Nishi, Keisuke Sugimoto, Mitsuru Okada, Tsukasa Takemura. *Pediatrics, Kinki University School of Medicine, Osakasayama, Osaka, Japan.*

Background: The cause of minimal change nephrotic syndrome (MCNS) is considered to involve abnormalities of cellular and humoral immunity such as increases in T-helper (Th) 2 cytokines reflecting a Th2-dominant state among T lymphocytes, as well as hypersensitivity of $CD8^+$ cells to antigenic stimuli. Imbalance between Th1 and Th2 lymphocytes and effects of reactive oxygen species (ROS) upon glomerular capillary walls have been implicated in MCNS. In addition, Oxidant/antioxidant imbalance has been reported to be involved in development of the nephrotic syndrome (NS).

Methods: Among 63 pediatric patients with MCNS at our institution, we identified 29 children with MCNS accompanied by allergic disease. Among the 29 patients, 15 who experienced remission without immunosuppressant treatment including corticosteroids, were enrolled in the present study. By polymerase chain reaction and comparative genomic hybridization, we evaluated mutations of the GSTT1 gene (*GSTT1*), a member of the glutathione S-transferase (GST) supergene family associated with both protection of cells from ROS and control of allergic reactions and serum IgE.

Results: Among 15 children with MCNS, IgE elevation (over 2000 IU/L) and *GSTT1* abnormality (complete deletion of *GSTT1* identified by Array CGH) was found in 2 who showed severe allergic symptoms. Serum ROS concentrations in these 2 patients were significantly higher than in healthy controls or other MCNS patients. The serum dROM/ROS ratio was significantly low in this patient with *GSTT1* abnormality (2.07 in patient 1, 2.48 in patient 2) compared with that in MCNS patients with no *GSTT1* abnormality (median, 3.54) or that in healthy control subjects (3.47), indicating deficient ROS degradation. In addition, IL-4 in both patients was very high, indicated that a Th2 shift caused by increased serum IL-4 was observed.

Conclusions: These results suggest presence of a *GSTT1* abnormality in some children with MCNS having marked serum IgE elevations and various allergic complications. Defective ROS degradation and Th1/Th2 imbalance caused by *GSTT1* abnormality could initiate proteinuria leading to MCNS.

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Mitochondrial Stability Is Important to High Glucose Induced Podocyte Injury Dae Eun Choi,[1] Jin Young Jeong,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, Chungnam National University Hospital, Daejeon, Republic of Korea; [2]Internal Medicine, Daejoen Saint Mary Hospital, Daejeon, Republic of Korea.*

Background: Podocyte injury plays a major role in diabetic glomerulosclerosis. High glucose induces oxidative stress, the changes of actin dynamics, and apoptosis in podocyte. Our previous report showed mitochondrial injury of podocyte induces massive albuminuria, glomerular sclerosis. Using crif1 deletion in podocyte. we evaluated that mitochondrial injury aggravate high glucose induced podocyte injury.

Methods: We used a conditionally immortalized mouse podocyte cell line and podocyte specific crif1 half deletion mouse (crif1 (Δ/+)). Crif1 siRNA in podocyte was used for inducing mitochondrial injury. We compared high glucose (25mmol/L) to low glucose (5.5mmol/L) in Crif1 siRNA treated podocyte. We make the phenotype of crif1 (Δ/+) in streptozotocin (75mg/kg, i.p., 3 days) induced diabetes mice. Using confocal microscopy, we observed actin and mitochondria in immortalized podocyte. In mice, we evaluated renal histology and metabolic finding including blood glucose, urine albumin and creatinine.

Results: High glucose with Crif1 siRNA treated podocyte showed decrease of actin to cell ratio comparing to low glucose with Crif1 siRNA and high glucose with wild type podocytes. In mice, it showed normal range of albuminuria, normal renal histologic finding and normal mitochondria morphology in both control mice and podocin specific half deletion of crif1 (crif1 (Δ/+)). After 10 weeks streptozotocin treatment, podocin specific heterozygous deletion of crif1 (crif1 (Δ/+)) showed massive albuminuria. However, Control mice showed normal range of albuminuria.

Conclusions: In conclusion, damage and instability of mitochondria induced by podocyte crif1 deletion aggravate high glucose induced podocyte injury.

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P2X7 Receptors and the Oxidative Stress in the Kidneys of Diabetic Rats Submitted to Aerobic Training Elisa M.S. Higa,[1,2] Adelson M. Rodrigues,[1] Cassia T. Bergamaschi,[3] Guilherme B. Nogueira,[1] Fabiane R. Maciel,[1] Giovana R. Punaro,[1] Marcello Franco,[1] Maria José S. Fernandes.[4] *[1]Nephrology, UNIFESP; [2]Emergency Division, UNIFESP; [3]Cardiovascular, UNIFESP, Brazil; [4]Neurology, UNIFESP.*

Background: Previous studies in our Laboratory showed the role of oxidative stress and the favorable effects of exercise training, on the progression of diabetic nephropathy (DN) in rats. $P2X_7$ receptors ($P2X_7$-R), in pathological conditions, are significantly up-regulated, increasing the levels of oxidative stress. The aim of this study was to evaluate the $P2X_7$-R and the oxidative stress in the kidneys of diabetic rats submitted to aerobic training.

Methods: Diabetes mellitus (DM) was induced in adult Wistar rats, with streptozotocin (60mg/kg, i.v.); control animals (CTL) received its vehicle. The animals were submitted to aerobic training on treadmill at a work rate of 16m/min for 60 min/day, 5 days a week/8weeks (DM+EX and CTL+EX); DM+SE and CTL+SE were kept resting on the treadmill at the same schedule (n=12 for each group). The animals all groups were treated with N-acetylcysteine (NAC). Data = mean ±SEM; one way ANOVA; significance at $P<0.05$.

Results: DM+SE vs CTL+SE increased the urinary excretion and renal tissue thiobarbituric acid reactive substances (TBARS), all $P<0.05$; there was also a reduced urinary excretion and renal tissue nitric oxide (NO). NAC reduced TBARS and increased NO in urine and the kidney, in DM+SE vs CTL+SE; exercises had similar effects. Immunohystochemistry analysis of $P2X_7$-R in the kidneys showed a significant increase in DM+SE vs CTL+SE (43.3±5.5 vs 0.6±0.1;%). In DM rats, all these effects were attenuated by NAC (34.9±4.3%) or by aerobic training (25.6±3.4%). However, the association of NAC with exercises significantly reduced the concentration of these receptors (3.8±0.6%).

Conclusions: Our data show that the routine of exercise associated with NAC expressively decrease the activation of $P2X_7$-R in the kidneys of diabetic rats. The attenuation of oxidative stress through this mechanism, which also resulted in the increase of NO bioavailability, suggests that the control of these receptors' up-regulation could be useful to delay the progression of diabetic nephropathy.

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Rosuvastatin Activates Transcription Factor Nrf2 and Protects Prevents Albuminuria through Preservation of the Glomerular Endothelial Integrity in Akita Diabetic Mice Chieko Ihoriya, Minoru Satoh, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Statins also have been shown to reduce urinary albumin excretion and to maintain glomerular filtration rate in diabetic kidney disease;however, the mechanism is not fully elucidated. The renoprotective effects of statins are independent of lipid-lowering effect and presumably involve their pleiotropic effects, especially to anti-oxidant activity. Nuclear factor-erythroid 2-related factor 2 (Nrf-2) is a basic leucine zipper transcription factor that binds to antioxidant response element (ARE) sequences in the promoter regions of a series of genes in response to oxidative stress. The aim of this study is to investigate possible involvement of Nrf2 in statin-mediated effects on the glomerular permselectivity and development of albuminuria in diabetic mice.

Methods: Male wild type (WT) and Akita diabetic mice (AKITA) were treated with rosuvastatin for 4 weeks. Using in vivo imaging technique with laser microscopy, alterations in glomerular permeability of macromolecules were was examined. Nrf2- ARE activity was measured in human umbilical vein endothelial cell (HUVEC) with with luciferase assay after transfection of reporter plasmids containing AREs. The expressions of Nrf2- regulated genes (NADPH/quinone oxidoreductase1, heme oxygenase-1, and glutamate cysteine ligase catalytic subunit) were also examined.

Results: The amount of lectin-stained glomerular endothelial surface layer important for permselectivity in vascular wall was significantly reduced in AKITA mice. Increased urinary albumin excretion in AKITA mice was significantly reduced by rosuvastatin treatment. Furthermore, in vivo-imaging study revealed massive leakage of 70-KDa dextran in AKITA mice and suppression of this hyperfiltration of macromolecules by rosuvastatin. Rosuvastatin significantly increased the transcriptional activity of the AREs and subsequent expressions of Nrf2- regulated genes in HUVEC.

Conclusions: These data indicate that rosuvastatin has anti-oxidative effect through activation of Nrf2, thereby, restores glomerular endothelial dysfunction and prevents development of albuminuria in diabetes.

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P66shc-Mediated Mitochondrial Dynamic Alterations Contribute to Tubular Oxidative Injury in Human Diabetic Nephropathy Ming Zhan, Li Xiao, Xun Zhou, Shikun Yang, Fuyou Liu, Lin Sun. *Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China.*

Background: Renal tubular injury is an early characteristic of diabetic nephropathy (DN) that is related to mitochondrial injury and oxidative stress. We investigate mitochondrial dynamic changes and reactive oxygen species(ROS) production in DN patients, and determine the role of the adaptor protein p66Shc in mitochondrial regulation and oxidative injury during high-glucose(HG) treatment of renal tubular cells .

Methods: Renal biopsy specimens from recruited patients, and HK-2 cells were studied. Mitochondrial dynamics was measured by electron and confocal microscopy, the expression of mitochondria-shaping proteins and p66Shc were evaluated by immunohistochemistry, immunoflurescence, PCR and western blot assays. Cellular and mitochondrial ROS production was detected. In addition, p66Shc wide-type or siRNA plasmid was transfected in vitro.

Results: Filamentous mitochondria became fragmented mainly in renal tubules of patients with DN. Compared with the control group, Drp1 was significantly increased and Mfn1 was decreased in DN tissues. These changes were accompanied by p66Shc elevation and ROS overproduction. In HK-2 cells, the percentage of cells with mitochondrial fragmentation and Drp1 expression were both elevated with HG treatment in a time- and dose-dependent manner. Drp1 inhibitor mdivi-1 attenuated mitochondrial fragmentation and also ROS overproduction, whereas ROS scavenger did not alter mitochondrial dynamics. Notably, siRNA knockdown of p66Shc down-regulated HG-induced Drp1 expression, alleviated mitochondrial fragmentation and ROS production, whereas over-expression of p66Shc could promote these changes under low-glucose condition.

Conclusions: This study provides first evidence of mitochondrial fragmentation in human diabetic nephropathy. Alteration in mitochondrial dynamics is mediated by p66Shc through its interaction with Drp1, which may serve as an upstream trigger of mitochondrial ROS overproduction that result in tubular oxidative injury under hyperglycemic conditions.

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Relevance of Myo-Inositol Oxygenase (MIOX) in Pathogenesis of Tubulo-Interstitial Injury in the Context of Diabetic Nephropathy Yashpal S. Kanwar,[1] Fu-You Liu,[2] Lin Sun.[1,2] *[1]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [2]Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan Province, China.*

Background: MIOX is a renal cortical tubular enzyme that channels glucose intermediaries, regulates osmoregulation, and modulates the activity of Redox-sensitive transcription factor Nrf2 (JBC 2011).

Methods: In view of the above we investigated if it plays any role in the generation of reactive oxygen species (ROS) in tubular compartment with ensuing perturbation in mitochondrial homeostasis and tubulo-interstitial injury under high glucose ambience while accelerating the events of Glucuronate-Xylulose (GX) pathway.

Results: Our initial observations indicated a concomitant increased expression of MIOX and NOX4 in tubules of patients with diabetic nephropathy. Transfection of MIOX pCDNA into LLCPK1 cells resulted in increased expression of MIOX/activity, perturbation of NADPH:$NADP^+$ and NAD^+:NADH ratios in the GX pathway, and an increase in DCF and Monobromobimane (MBB) staining under high glucose ambience. This increased ROS generation was associated with early adaptive changes in the scavenging system, where an increased expression of SOD1, SOD2, NOX 4, HO-1 and Catalase was observed. The redox imbalance was accompanied with hyperacetylation of cellular proteins and decreased expression of NAD^+-dependent SIRT1/SIRT3 enzymes and transcription factor PGC1-alpha, increased mitochondrial DNA fragmentation, mitochondrial fission, leakage of cytochrome C, perturbation in membrane potential and apoptosis. In addition, a marked increase in fibronectin expression, attenuated by MIOX-siRNA, was observed. Increased expression of MIOX with redox imbalance and apoptosis in proximal tubules was also observed in CD1 mice with STZ-induced diabetes and AKITA mice that exhibited upregulation of glutathione S-transferase. The increased expression of fibronectin in the diabetic mice was inhibited by ZOPOL, an inhibitor of MIOX/ALR1 complex.

Conclusions: These data suggest that MIOX plays a role in pathogenesis of tubulo-interstitial injury/fibrosis while generating ROS in diabetic nephropathy.

Funding: NIDDK Support

SA-PO437

Is Oxidized Albumin, a Mediator and a Biomarker of Systemic Inflammation in Diabetic Nephropathy? Batya Kristal,[1,3] Shifra Sela,[2,3] Teuta Zeitun,[1] Galina Shapiro,[2] Yoav Fridman,[2] Ronit Geron,[1] Regina Michelis.[2] *[1]Nephrology Department, Western Galilee Hospital, Nahariya, Israel; [2]Eliachar Research Laboratory, Western Galilee Hospital, Nahariya, Israel; [3]Faculty of Medicine in the Galilee, Bar Ilan University, Zefat, Israel.*

Background: Hypoalbuminemia, Oxidative Stress (OS) and systemic inflammation are common in patients with diabetic nephropathy (DN). Oxidative modification of serum albumin was shown to impair its quantification by the bromocresol-green assay (BCG) in patients on hemodialysis therapy, linking OS to hypoalbuminemia. In this study we examined whether hypoalbuminemia in DN is also due to impaired quantification of modified albumin and assessed if modified albumin activates neutrophils to release ROS and proteases, accelerating systemic OS and inflammation.

Methods: Blood samples from a cohort of 19 DN patients and 15 healthy controls were used to determine "albumin-detection index", defined as the ratio between the albumin read-out by BCG assay to the albumin concentration measured by optical density (OD) at 280 nm after albumin purification according to its size. This index was correlated with: sera albumin levels, various OS and inflammatory markers and with eGFR. Activation of separated neutrophils by glycoxidized-modified albumin was assessed by the release of neutrophil gelatinase associated lipocalin (NGAL) and myeloperoxidase (MPO).

Results: The albumin-detection index of DN patients was significantly lower compared to healthy controls, correlating positively with serum albumin and kidney function, and negatively with glycoxidized albumin and inflammatory markers. Glycoxidized-albumin had a direct role in neutrophil activation, resulting in NGAL and MPO release.

Conclusions: Hypoalbuminemia in DN patients, partially results from the low detection efficacy by BCG of modified/oxidized molecules, as expressed by a low albumin detection index. Modified/oxidized molecules initiate reciprocal process by activating neutrophils, commencing a vicious cycle a of accelerated OS and inflammation. We imply that albumin detection index, a new marker of OS, may also serve as a biomarker, correlating to the severity of diabetic nephropathy.

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The Antioxidant Silybinin Prevents High Glucose-Induced Production of Superoxide and Apoptosis in Podocytes, Both *In Vitro* and *In Vivo* Khaled Khazim,[1,3] Yves C. Gorin,[1] Hanna E. Abboud,[1,2] Paolo Fanti.[1,2] *[1]Nephrology, Univ. of Texas Health Science Center, San Antonio, TX; [2]Nephrology, ALM VA Memorial Hospital, San Antonio, TX; [3]Nephrology, W. Galilee Hospital, Nahariya, Israel.*

Background: Damage and loss of podocytes is associated with proteinuria in patients with diabetic nephropathy (DN) and in animal models of diabetes. Oxidative stress participates in the pathogenesis of DN, including podocyte injury and alteration of the glomerular filtration barrier. Here, we explored the potential protective effect of the antioxidant silybinin, a polyphenolic flavonolignans from silybum marianum (milk thistle), against diabetes-induced podocyte injury.

Methods: *In vitro*, we exposed conditionally immortalized mouse podocytes to high glucose concentration (HG) and measured intracellular superoxide (O_2^-)generation by confocal laser scanning microscopy and by high performance liquid chromatography, with dihydroethidium (DHE) as a probe; NADPH oxidase (Nox) activity by chemiluminescence; Nox4 protein expression by Western blot; and cell apoptosis by DNA fragmentation. *In vivo*, we treated OVE26 type 1 diabetic mice with silybinin or vehicle for 6 weeks and counted the glomerular podocyte number adopting confocal immunohistochemistry of podocyte-specific synaptopodin and of GBM-specific type-IV collagen. We used FVB mice as non-diabetic controls.

Results: Exposure of podocytes to HG for 24 h resulted in a robust increase in intracellular O_2^-production and NADPH-dependent O_2^-generation, along with increased Nox4 protein expression and apoptosis. These effects of HG were all blocked by pretreatment of the cells with 10 μM silybinin. In addition, administration of silybinin to OVE26 mice significantly reduced the glomerular loss of podocytes and the decrease of synaptopodin expression.

Conclusions: We confirm that HG induces oxidative stress, Nox4 expression, and cell apoptosis in podocytes. Moreover, we report the novel finding that the flavonolignan silybinin protects against HG-induced oxidative stress and podocyte injury, both *in vitro* and *in vivo*. Silibynin may act by blocking the expression of Nox4, and O_2^- generation known to play a key role in podocyte injury.

Funding: Other NIH Support - NCCAM, Veterans Administration Support, Clinical Revenue Support

SA-PO439

Role of Renal Hypoxia in the Progression of Experimental Diabetic Nephropathy Naoki Takahashi,[1] Hideki Kimura,[1] Kazuko Kamiyama,[1] Seiji Yokoi,[1] Yoshinari Yokoyama,[1] Daisuke Mikami,[1] Kenji Kasuno,[1] Haruyoshi Yoshida,[2] Masayuki Iwano.[1] *[1]Division of Nephrology, Fukui University, Fukui, Japan; [2]Department of Internal Medicine, Sugita Genpaku Memorial Obama Municipal Hospital, Obama, Japan.*

Background: Renal hypoxia has been proposed to be one of the important pathophysiologic features of diabetic kidney disease in experimental diabetic mice. Previously, we have reported that chronic hypoxia exacerbated diabetic glomerulosclerosis in db/db mice. In this study, to disclose the molecular mechanisms of the chronic hypoxia-induced progression of experimental diabetic glomerulosclerosis, we examined and compared glomerular mRNA levels of various cytokines with proinflammatory, angiogenic and sclerotic functions in db/db mice.

Methods: Eight w.o. male db/db mice were bred in a normobaric hypoxic chamber (12%O_2). The mice in hypoxia were kept in this chamber for 4 weeks (n=11) or 16 weeks (n=11) and the mice in normoxia were bred in room air for 4 weeks (n=12) or 16 weeks (n=12). Mice were sacrificed at 12 and 24 w.o. in order to evaluate histological changes and extracted mRNA (VEGF-A, VEGF-R1, VEGF-R2, PAI-1, MCP-1, TGF-β1, CTGF, eNOS, CD34, Nephrin, Ang-1 and Ang-2) from 50-150 glomeruli/mouse obtained by laser capture microdissection.

Results: No significant differences in blood pressure and s-Cr levels were found between normoxic mice (NM) and hypoxic mice (HM). Urinary albumin levels were consistently increased in the 10, 12, 16, 24 w.o. HM and severe glomerulosclerosis was observed in 24 w.o. HM, as compared with NM. The number of glomerular F4/80+macrophages in HM was significantly greater than that in NM. Real time PCR analysis showed significant increases in glomerular mRNA levels of MCP-1 in HM at 12 w.o. and 24 w.o. and a significant increase in glomerular mRNA levels of TGF-β1, CTGF and eNOS in HM at 24 w.o. as compared with in NM. However, glomerular mRNA levels of VEGF-A, VEGF-R1 and VEGF-R2 were not significantly increased in HM at 12 w.o. and 24 w.o.

Conclusions: The development of diabetic glomerulosclerosis associated with chronic hypoxia in db/db mice may result from the increased macrophage infiltration via glomerular MCP-1 induction, but not from VEGF-A axis.

Funding: Other U.S. Government Support

SA-PO440

Metallothionein Deficiency Accelerates Diabetic Nephropathy by Inducing Oxidative Stress and Inflammation in Proximal Tubular Epithelial Cells Hiromi Tachibana, Daisuke Ogawa, Naoto Terami, Chikage Sato-horiguchi, Jun Eguchi, Jun Wada, Kenichi Shikata, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Oxidative stress and inflammation play an important role in diabetic complications, including diabetic nephropathy. We recently reported that metallothionein (MT) is induced in proximal tubular epithelial cells as an antioxidant in diabetic kidney (Exp Diabetes Res, 2011), however, the role of MT in renal function still remains unclear. Therefore, we investigate whether deficiency of MT accelerates DN through oxidative stress and inflammation.

Methods: Diabetes was induced by injection of streptozotocin into MT deficient ($MT^{-/-}$) and $MT^{+/+}$ mice. We analyzed urinary albumin excretion, histological changes, and gene expression of inflammatory mediators in the kidney at 12 weeks after inducing diabetes. Mitochondrial reactive oxygen species (ROS) were measured using MitoTrackerRed. In addition, we performed siRNA experiments to further elucidate the role of MT in high glucose condition. mProx24 cells, a murine proximal tubular epithelial cell line, were transfected with MT siRNA or scrambled siRNA.

Results: Despite equivalent hyperglycemia, albuminuria, mesangial matrix accumulation, and glomerular hypertrophy, along with markers for inflammation including MCP-1, TGF-β, and osteopotin were more accelerated in diabetic $MT^{-/-}$ mice than in diabetic $MT^{+/+}$ mice. Macrophage infiltration, mitochondrial ROS production, and interstitial fibrosis were exacerbated in diabetic $MT^{-/-}$ mice compared with diabetic $MT^{+/+}$ mice. Electron microscopy revealed that impaired mitochondrial morphology in proximal tubular epithelial cells was also observed in diabetic $MT^{-/-}$ mice. In vitro studies demonstrated that knockdown of MT by siRNA enhanced the high glucose-induced oxidative stress and inflammation in mProx24 cells.

Conclusions: These findings provide previously unrecognized evidence that high glucose-induced ROS and subsequent inflammation exacerbates diabetic nephropathy and that MT plays an important role in protecting kidney from diabetic stress as an antioxidant protein.

SA-PO441

Effects of Streptozotocin-Induced Diabetes Mellitus on Glutamine Transport and Metabolism in the Rat Kidney Hassane Amlal, Rashma Faroqui, Ali Al-fraihat, Xiaoyi Lin. *Internal Medicine, University of Cincinnati, Cincinnati, OH.*

Background: Total ammonia ($NH_4^+ + NH_3$) causes renal cell hypertrophy, and diabetes mellitus (DM) is associated with increased kidney mass. However, whether DM alters glutamine transport and metabolism and stimulates ammoniagenesis in the kidney is poorly understood.

Methods: Rats were housed in metabolic cages and had free access to food and water. After adjustment, rats were injected with vehicle or streptozotocin (STZ) to induce DM and were sacrificed after 6 days (6d) or 2 weeks (2wk) later. At the end of treatment, blood composition was analyzed, urinary NH_4^+ and protein excretion were measured and the expression of various genes involved in glutamine transport and metabolism was examined.

Results: STZ-treated rats exhibited massive polyuria, polydipsia and significant hyponatremia with normal serum [HCO_3^-] vs. vehicle. Kidney mass (kidney weight/BW x 1000) significantly increased from 3.75 to 6.21 and 6.58 (gm/gm) in vehicle vs. 6d and 2wk STZ, respectively. This correlates with massive proteinuria and significant increase in urinary NH_4^+ excretion (mmol/day) from 1.05 to 4.12 and 5.87 in vehicle vs. 6d and 2wk STZ, respectively. The expression of glutamine transporters BoAT1 and SNAT3 increased significantly by 99 and 28 % and by 200 and 32% after 6d and 2wk STZ vs. vehicle, respectively. Ammoniagenic enzymes glutaminase (GA) and glutamate dehydrogenase (GDH) both increased significantly by 75 and 93% and by 87 and 119% after 6d and 2wk STZ vs. vehicle, respectively. The gluconeogenic enzyme phosphoenolpyruvate carboxy kinase (PEPCK) also increased significantly by 130 and 207% after 6d and 2wk STZ vs. vehicle, respectively.

Conclusions: STZ-induced DM is associated with the stimulation of ammoniagenesis, which results from increased glutamine transport and metabolism in the proximal tubule cells. The stimulation of ammonia production is not mediated by acidosis and correlates with increased renal mass and proteinuria, indicating that ammoniagenesis likely contributes to the development of renal hypertrophy and kidney disease during diabetes mellitus.

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SA-PO442

A Murine Model of Diet-Induced Insulin Resistance and Metabolic Syndrome Tomasz A. Wietecha, Kelly L. Hudkins, Jinkyu Kim, Antonio Haw Sta. Teresa, Kristina M. Sorg, Kevin D. O'Brien, Charles E. Alpers. *Pathology, Univ of WA.*

Background: BTBR is an inbred mouse strain that is susceptible to development of diabetes and advanced diabetic nephropathy in the presence of the leptin-deficiency mutation (ob/ob) (JASN. 2010; 21:1533-42). Heterozygous BTBR mice (BTBR ob/+) are not hyperglycemic on chow diet, but have abnormal insulin and glucose tolerance tests. We tested whether dietary modification could induce diabetes and diabetic nephropathy (DN), and whether the glucose lowering drug Metformin reduced diabetic complications in this model.

Methods: At 4 weeks of age, female BTBR ob/+ mice were given Chow or DD (diet high in fat and refined sugar) diet. At 14 weeks of age, the DD group was further divided: both groups continued on the DD diet, but half of the mice also received Metformin in drinking water for 6 weeks.

Results: Oral glucose tolerance tests (OGTTs) and fasting serum glucose (FSG) were performed at 8, 12, 16, and 20 weeks of age. At all time points the DD group had significantly impaired OGTT responses compared with Chow fed mice ($P<0.001$) in addition to increases in FSG ($P=0.001$, Chow vs. DD at 20 weeks). Introduction of Metformin did not significantly lower OGTTs and FSG in DD-fed mice. Increased body weight in DD diet group compared to Chow fed mice ($P=0.001$) was only slightly reduced by Metformin. Increased kidney weight in the DD group ($P=0.019$) was significantly reduced by Metformin treatment. Kidney function (albumin/creatinine ratio) was unchanged despite intervention. Histologically, the DD group had significantly increased matrix accumulation compared to Chow fed mice. Introduction of Metformin in DD fed mice lowered the Col IV positive glomerular matrix area to the Chow control group levels (18.5, 11.9, 11.4 % in DD, DD+Metformin, and Chow respectively, $P<0.01$). In all groups, the level of Mac-2 positive cells in glomeruli remained unchanged. Mild glomerular hypertrophy in the DD group was only slightly reduced with Metformin ($P>0.05$).

Conclusions: These results characterize early changes of diet-exacerbated insulin resistance, and present the possibility that an intact insulin-leptin signaling axis retards the development of DN in BTBR mice.

Funding: NIDDK Support

SA-PO443

Distinct Metabolomic Profiles Are Associated with Severe Diabetic Nephropathy in Mice Susan B. Gurley, Svati H. Shah, Damian M. Craig, Natalie Mattocks, Christopher B. Newgard, Thomas M. Coffman. *Department of Medicine, Duke University Medical Center, Durham, NC.*

Background: Diabetic nephropathy (DN) is a leading cause of ESRD worldwide but the pathophysiology of DN has not been clearly defined. One limitation to deciphering DN pathogenesis has been the lack of animal models recapitulating the characteristics of human DN. We have found that activation of the renin-angiotensin system (RAS) with a constitutively active renin transgene (RenTg) superimposed on a genetic model of type 1 DM (Akita mouse) causes exaggerated kidney injury resembling human DN. Thus, RAS activation and diabetes have synergistsic effects to promote glomerular disease on a permissive strain background. We hypothesized that the combination of diabetes and RAS activation may trigger specific metabolic pathways in the kidney resulting in enhanced disease.

Methods: To test this, we performed MS-based targeted metabolomic profiling (80 individual metabolites) of tissue homogenates from 129 Akita-RenTg mice compared to 129 mice with diabetes alone, RAS activation alone, or WT mice.

Results: 129-Akita mice with a transgene expressing renin under control of the albumin promoter develop pronounced hyperglycemia, hypertension, marked albuminuria, and glomerulosclerosis, whereas 129 mice with the Akita mutation or RenTg alone develop only modest renal injury. Kidneys from 129-Akita-RenTg mice had increased levels of the branched-chain amino acids (BCAA), valine (28.1±2 vs 23.9±1 μM, $p<0.01$) and leucine/isoleucine (26.7±1.9 vs. 23.1±0.8 μM, $p<0.01$) compared to controls. This pattern was also observed in liver, skeletal muscle, heart, and plasma and has been associated with increased risk of metabolic disease and coronary artery disease in humans. In addition, hexanoyl (C6) acylcarnitine levels in kidney were significantly reduced in 129-Akita-RenTg mice ($p=0.004$) compared to controls.

Conclusions: Thus, we have identified distinct metabolic signatures associated with accelerated nephropathy in diabetic mice, and these resemble profiles that have been observed in cardiometabolic disease in humans. Further understanding of these pathways may lead to new strategies for diagnosis and treatment of DN.

Funding: NIDDK Support, Other NIH Support - AMDCC, U01 DK076136-5, Private Foundation Support

SA-PO444

The Role of Potent Lipid Second Messenger Sphingosine 1-Phosphate in the Development of Diabetic Nephropathy Dania Yaghobian,[1] Anthony Don,[2] Sarina Yaghobian,[1] Xinming Chen,[1] Sonia Saad,[1] Carol A. Pollock.[1] *[1]Medicine, Kolling Institute of Medical Research, University of Sydney, Sydney, NSW, Australia; [2]Medicine, Lowy Cancer Insitute, University of New South Wales, Sydney, NSW, Australia.*

Background: Hyperglycaemia and all TGFβ isoforms are involved in the development of diabetic nephropathy. S1P is derived from sphingosine by sphingosine kinase (SPHK-1/2) and is considered a key mediator of biological processes mimicking TGFβ signalling. However the role of SPHK1 in inducing fibrotic and inflammatory responses in diabetic nephropathy is unknown. The aim is to determine the role of the potent lipid second messenger sphingosine 1-phosphate (S1P) in the development of diabetic nephropathy.

Methods: Human proximal tubular cells (HK2) were exposed to 5mM, 30mM D-glucose or 0.5ng/ml TGFβ 1,2,3 in the presence or absence of a SPHK inhibitor (SKI-II; 2μM) or SPHK1/2 siRNA (30nM) for 72 hours. Exposure to S1P (0.1-5μM) demonstrated the biological effect of SPHK activation. Wild type (WT) and $SPHK1^{-/-}$ mice with or without 24 weeks of diabetes were studied. In both in-vitro and in-vivo models, mRNA and protein expression of fibrotic and inflammatory markers were determined. Sphingolipid pathway metabolites were measured by mass spectrometry (MS).

Results: HK2 cells exposed to high glucose or TGFβ1,-2,-3 independently increased SPHK1 enzymatic activity, mRNA and protein expression. TGFβ1,-2,-3 and S1P induced fibronectin, collagen IV, chemokine ligand 2, vimentin and downstream phospho-p44/42 expression, which were reversed by both SKI-II and SPHK1/2 siRNA. WT diabetic mice exhibited a lower creatinine clearance, higher urinary albumin excretion, increased renal cortical fibronectin, collagen IV and phospho-p44/42 mRNA and protein expression compared to $SPHK1^{-/-}$ diabetic mice. MS demonstrated an increased in S1P in the kidneys of WT diabetic mice.

Conclusions: This study suggests limiting the formation of S1P inhibits renal inflammation and fibrosis in diabetic mice. This may be due to the reduced S1P receptor activation or indirectly due to inhibition of the p42/44 MAP kinase pathway.

Funding: Government Support - Non-U.S.

SA-PO445

Fructose Production and Fructokinase Activation in the Proximal Tubule Mediate Tubular Injury in Ciabetic Nephropathy Christina Cicerchi, Carlos Alberto Roncal-jimenez, Takuji Ishimoto, Yoshifuru Tamura, Katsuyuki Tanabe, Nanxing Li, Takahiko Nakagawa, Richard J. Johnson, Miguel A. Lanaspa. *Medicine, University of Colorado Denver, Aurora, CO.*

Background: Fructose consumption has been shown to induce and exacerbate features of metabolic syndrome including type 2 diabetes. Besides dietary intake, fructose is endogenously produced from glucose by the activation of the polyol pathway in the cortex of the kidney in diabetic states. The aim of this study is to determine the specific role of

this endogenous fructose and its metabolism by fructokinase (KHK) in the pathogenesis of diabetic nephropathy.

Methods: Diabetes was induced by injection for 5 consecutive days of a low-dose of streptozotocin (STZ) in 8 week old wild type or littermate KHK knockout (KHK-KO) mice with similar body weights. Diabetic nephropathy was assesed by determination of parameters of renal function, and kidney tubular injury was assessed by NGAL urinary excretion, histology, collagen III and macrophage infiltration.

Results: STZ-induced diabetes significantly elevated serum glucose in both wild type and KHK-KO mice with no significant difference between groups (330±46 vs 322+56 mg/dl, p>0.05). Activation of the polyol pathway in the kidney cortex was confirmed in both conditions by the up-regulation of aldose reductase (AR), the first and rate-limiting enzyme of this pathway and increased accumualtion of sorbitol and fructose. Diabetic KHK-KO mice demonstrated reduced phosphate and urate fractional excretion (75±3 and 68±13 % reduction respectively, p<0.01) and lower albuminuria levels (3.2±1.5 vs 0.7±0.2 μg/mg of creatinine, p<0.01) as compared to diabetic wild type mice suggesting improved reabsorption ability. This data was corroborated by decreased NGAL urinary concentration (17.3±2.8 vs 12.4±3.6 ng/ml, p<0.05), urinary excretion (72±10 % reduction, p<0.01), and improved tubulointerstitial injury.

Conclusions: In summary, this data demonstrate that endogenous fructose generated in proximal tubules during diabetic nephropathy may have a deleterious role in the pathogenesis of diabetic nephropathy. Therefore, KHK-KO mice are partially protected against diabetic nephropathy.

Funding: NIDDK Support

SA-PO446

Liver X Receptor Agonist Ameliorates Diabetic Nephropathy by Inhibiting High Glucose-Induced Osteopontin Expression in Proximal Tubular Epithelial Cells Daisuke Ogawa, Hiromi Tachibana, Jun Wada, Jun Eguchi, Chikage Sato-horiguchi, Naoto Terami, Kenichi Shikata, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Osteopontin (OPN) is a proinflammatory cytokine implicated in the chemoattraction of monocytes and the development of diabetic nephropathy. Synthetic agonists for the Liver X Receptor (LXR), prevent the development of atherosclerosis by regulating cholesterol homeostasis and suppressing inflammatory gene expression, however, the role of LXR in diabetic nephropathy is poorly understood.

Methods: We administered LXR agonist T0901317 (10 mg/kg/day) to STZ induced-diabetic mice for 8 weeks after inducing diabetes, and evaluated the effects for diabetic nephropathy. The mechanism of OPN expression was also analyzed in mProx24 cells, a mouse renal proximal tubular epithelial cell line, stimulated with high glucose medium and pretreated with T0901317. OPN promoter activity was analyzed by transient transfection experiments with deletion series, heterologous promoter assays, and chromatin immunoprecipitation assays.

Results: The LXR agonist T0901317 ameliorated albuminuria, glomerular mesangial expansion, and interstitial fibrosis without affecting blood glucose and triglyceride levels in STZ-induced diabetic mice. T0901317 treatment markedly decreased the expression of OPN, macrophage infiltration, and the expressions of inflammatory genes, including MCP-1, TNF-α, and TGF-β, in the diabetic kidney. Furthermore, in vitro experiments revealed that high glucose-induced OPN expression in proximal tubular epithelial cells is dependent on AP-1 binding to the proximal OPN promoter. T0901317 inhibits high glucose-induced OPN expression and negatively interferes with AP-1 binding to the proximal OPN promoter by inhibiting c-Fos and phospho-c-Jun protein expressions.

Conclusions: These findings uncover a previously unrecognized mechanism for the inhibition of renal OPN expression by activation of LXR and support the concept that LXR agonists may offer a novel therapeutic approach for the treatment of diabetic nephropathy.

SA-PO447

Nuclear Hormone Receptor Expression in Mouse Kidney and Cell Lines Naoto Terami, Daisuke Ogawa, Hiromi Tachibana, Chikage Sato-horiguchi, Jun Eguchi, Jun Wada, Kenichi Shikata, Hirofumi Makino. *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Nuclear hormone receptors (NHRs) are transcriptional factors that regulate carbohydrate and lipid metabolism, immune response, and inflammation. Although several NHRs have shown a renoprotecitve effect in the context of diabetic nephropathy, the expression and role of NHR in kidney are still unclear. In this study, we have comprehensively analyzed the expression of all 49 of the NHR superfamily in mouse kidney and cell lines in both normal and high glucose condition.

Methods: We used normal and streptozotocin-induced diabetic C57BL/6 mice, db/m (control) and db/db (diabetic) mice, and renal cell lines including mesangial cells (MES13), podocytes (MPC), proximal tubular epithelial cells (mProx24), and collecting duct cells (mIMCD3). RNA was isolated from tissue samples or cultured cells. To evaluate the mRNA expressions of NHR in the kidney and cultured cells, quantitative real-time PCRwas performed using TaqMan® Array Fast Plates (Applied Biosystems).

Results: 25 NHR members were expressed in the kidney of normal C57BL/6 and db/m mice, and 6 NHRs were not expressed in mouse kidney.(Figure1).

Figure 1 The Composition of Nuclear Hormone Receptors in Kidney

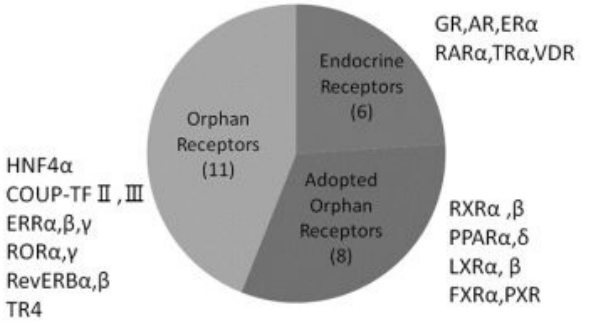
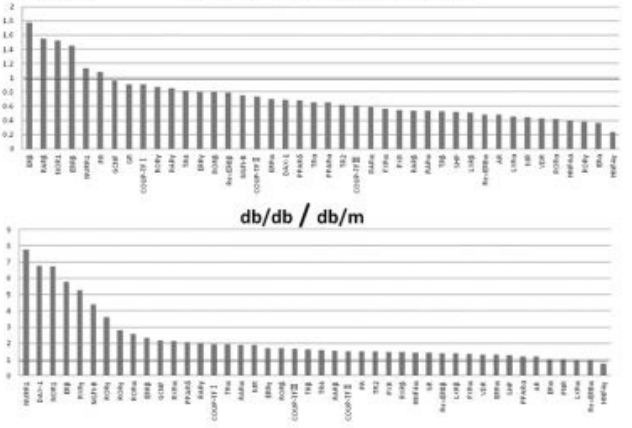


Figure 2 C57BL6 (DM) / C57BL6 (control)



In addition, 5 NHRs were upregulated in diabetic C57BL/6 and db/m mice (Figure 2). In cell lines, PPARδ was highly expressed in mesangial and proximal tubular epithelial cells, and COUP-TF II/III was highly expressed in podocytes and collecting duct cells. The expression profile of NHR was different between under normal and high glucose conditions. NGFI-B was highly upregulated in mesangial and collecting duct cells by high glucose stimulation.

Conclusions: These findings identify NHR present in mouse kidney and cultured cell lines and suggest potential therapeutic targets in kidney for the treatment of diabetic nephropathy.

SA-PO448

Alteration of Renal Lipid Deposition and Gene Expression Induced by Fasting and High-Fat Diet Feeding Keita P. Mori, Kiyoshi Mori, Masashi Mukoyama, Hideki Yokoi, Masato Kasahara, Takashige Kuwabara, Hirotaka Imamaki, Yoshihisa Ogawa, Tomoko Kawanishi, Akira Ishii, Kenichi Koga, Yukiko Kato, Naohiro Toda, Shoko Ohno, Akira Sugawara, Kazuwa Nakao. *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.*

Background: Renal triglyceride (TG) contents increase by high-fat diet (HFD) and also by fasting, but their detailed mechanism remains elusive. In this study, we investigated whether increase of renal TG contents induced by fasting or by HFD is correlated with extrarenal energy-supplying factors, such as blood levels of non-esterified fatty acid (NEFA) and insulin, or with intrarenal lipid-regulating factors, such as peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element-binding protein (SREBP)-1.

Methods: Male C57BL/6J mice were fed either normal diet (ND) or HFD for 5 weeks. Mice were killed under ad libitum-fed or 12 h-fasted conditions.

Results: HFD resulted in acceleration of weight gain, elevation of serum glucose, insulin, and total cholesterol levels, and mild increase of renal and hepatic TG contents. Overnight fasting in ND-fed mice caused significant reduction of serum glucose and insulin levels, elevation of serum NEFA levels, and marked increase of renal (3-fold) and hepatic TG contents (9-fold). Fasting in HFD-fed mice, however, showed diminished changes in serum glucose, insulin and NEFA levels. Notably, renal TG contents did not increase, and hepatic TG contents increased slightly by fasting in HFD conditions. Both in the kidney and liver, PPAR-alpha gene expression was elevated and SREBP-1a,c was reduced by fasting as shown by real-time RT-PCR, which were not influenced by HFD.

Conclusions: Fasting induces marked elevation of renal TG contents, but pretreatment with HFD blunts this elevation potentially by diminished changes in serum glucose, insulin, and NEFA levels. Changes in gene expression of PPAR-alpha and SREBP-1a,c both in kidneys and livers appear not to be the causes of tissue TG deposition but rather the results or compensatory changes.

SA-PO449

LXR-Dependent Gene Expression in Human Diabetic Nephropathy Michal Herman-Edelstein,[1] Ana Tobar,[1] Pnina Scherzer,[1] Moshe Levi,[2] Uzi Gafter.[1] *[1]Department of Nephrology, Rabin Medical Center, Petah Tikva, Israel; [2]Division of Renal Diseases and Hypertension, University of Colorado, Denver, CO.*

Background: Liver X receptors (LXR) α and β are nuclear receptors that are master regulators of lipid and cholesterol metabolism and also have anti-inflammatory effects. In the current study, we investigated the expression levels of LXRs and their target genes of lipid metabolism, lipid accumulation and inflammation in kidneys biopsies from DN patients.The purpose of this study was to investigate the protective role of LXRs in the pathogenesis of human DN.

Methods: Renal lipid contents, inflammation and genes involved in cholesterol and fatty acid metabolism and most of the known LXRs target genes, were studied in RNA isoleated from formalin-fixed, paraffin-embedded (FFPE) renal biopsy from patients with established DN (n=32) versus normal kidneys (n=12).

Results: The DN kidney exhibited a significant inverse relationship between LXR expression and the degrees of glomerulosclerosis; tubulointerstitial injury; heavy lipid accumulation and inflammation. We studied LXRα and LXRβ target genes expression in the kidneys. We found up-regulation of modified low-density lipoprotein receptors: lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), scavenger receptor A1 (SR-A1), and CD36, whereas lipid efflux ATP binding cassette transporters ABCA-1 and ABCG-1were down-regulated. Furthermore we found down-regulation of factors involved in fatty acid oxidation (PPAR-α, CPT1 and L-FABP). There was an inverse correlation between LXRs expression and fibrotic markers, macrophage load, lipid and cholesterol accumulation, eGFR, and renal outcomes.

Conclusions: We showed for the first time that renal LXR expression is inversely and significantly associated with lipid accumulation, pro-inflammatory markers, and the profibrotic molecules in kidney biopsies from patients with DN. Our results suggest that decreased expression of LXR is an important feature of DN**.**These results support the concept that LXRs and related downstream pathways may be important in modulating diabetic lipid burdenand inflammation and suggest LXR agonists as a novel therapeutic target for human diabetic nephropathy.

SA-PO450

Role of Bile-Acid-Activated Receptors TGR5 and FXR in Human Diabetic Nephropathy Michal Herman-Edelstein,[1] Pnina Scherzer,[2] Ana Tobar,[1] Renana Eshet-leon,[1] Moshe Levi,[3] Uzi Gafter.[1] *[1]Felsenstein Medical Research Center and Department of Nephrology, Rabin Medical Center, Petah Tikva, Israel; [2]Nephrology, Hadassah, Jerusalem, Israel; [3]Division of Renal and Hypertension, Med Center and University of Colorado Health Sciences Center, Denver, CO.*

Background: Bile acids (BA) act as signaling molecules that activate BA receptors, which regulate glucose, lipid homeostasis and energy expenditure. Two major receptors for BA have been identified: (1) the nuclear receptor, farnesoid X receptor (FXR), and (2) the membrane-bound, G protein-coupled receptor (TGR5). The expression of TGR5 and its function are distinct from FXR.TGR5 is a key factor in energy expenditure by activating the mitochondrial biogenesis regulatorPPARγ coactivator (PGC), which enhances metabolic pathways, such as fatty acid oxidation, and also increases antioxidant defense mechanisms. The purpose of the present study was to determine the expression and role of BA receptors and signaling pathways in human diabetic nephropathy (DN) kidney biopsies.

Methods: Renal lipid contents, FXR, TGR5, and BA receptor targets were studied by immunofluorescence staining and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) renal biopsies from patients with established DN (n=32) versus normal kidneys (n=12), who have been treated in our department.

Results: TGR5 and FXR protein and mRNA abundance were significantly decreased in DN kidneys. FXR and TGR5 expression negatively correlated with disease progression and lipid accumulation. We found in DN decreased expression of transcription factors, nuclear receptors (ERRα,Rev-Erbα/β, NRF-1, NRF-2, PPARα,and PPARγ) and members of the PGC-1 family of regulated coactivators (PGC-1α, PGC-1β). Furthermore, we showed the expression of gluconeogenic enzymes phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) in diabetic kidneys.

Conclusions: As yet there is little information focused specifically on TGR5, PGC1-α, and ERRα in the regulation of mitochondrial energy metabolism in the diabetic kidney. The available data indicate that these pathways may become promising areas for study in the modification of renal disease and diabetic nephropathy.

Funding: Private Foundation Support

SA-PO451

ER Stress-Mediated Unfolded Protein Response Protects Renal Proximal Tubular Cells against Free Fatty Acid-Mediated Damage Naoko Takeda,[1] Shinji Kume,[1] Daisuke Koya,[2] Shin-ichi Araki,[1] Keiji Isshiki,[1] Masakazu Haneda,[3] Takashi Uzu,[1] Hiroshi Maegawa.[1] *[1]Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan; [2]Diabetes and Endocrinology, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan; [3]Medicine, Asahikawa Medical University, Asahikawa, Hokkaido, Japan.*

Background: Free fatty acid (FFA)-bound albumin is filtered via glomeruli and reabsorbed in proximal tubular cells. FFA-mediated tubular damage is strongly associated with progressive renal dysfunction, although the underlying mechanism has not been determined. ER stress-mediated unfolded protein responses (UPRs) is known as cellular adaptive mechanisms. However, the relationship between FFA-induced tubular damage and UPR is unclear. The objective of this study was to elucidate the role of UPR in FFA-induced apoptosis and inflammation in cultured proximal tubular cells (PTCs).

Methods: To induce apoptosis and inflammation, cultured PTCs were exposed to palmitate (150μM), a saturated FFA. Apoptosis and inflammation were determined by caspase 3 and PARP cleavages, and *MCP-1* mRNA expression, assessed by western blotting and real-time PCR, respectively. UPRs were determined by PERK phosphorylation, ATF6 cleavages, XBP1 splicing, and BiP expression level (a stress-inducible molecular chaperone).

Results: In cultured PTCs, palmitate increased apoptosis and *MCP-1* expression together with UPRs. Pretreatment with the exogenous chemical chaperone, tauroursodeoxycholic acid (TUDCA 1mM), inhibited palmitate-induced apoptosis and *MCP-1* expression with a reducing UPRs. To clarify the role of UPRs in tubular damage, we examined the effects of the silencing three independent key genes of UPR signaling, IRE1, PERK and ATF6, by siRNA. Gene silencing of each UPR signaling pathway exacerbated the palmitate-induced apoptosis and *MCP-1* expression and reduced the palmitate-induced BiP expression. Meanwhile, TUDCA ameliorated the UPR-deficiency-mediated-exacerbation of palmitate-induced apoptosis and *MCP-1* expression.

Conclusions: These results indicate that UPRs play protective roles against palmitate-induced tubular cell damage, and that the refilling of molecular chaperones may be a new therapeutic strategy in FFA-mediated tubular injury.

SA-PO452

Bilirubin Regulates the Expression of SREBP-1, LXRα, and PPARγ in Liver and Kidney of Type I DM Animal Model Jianwei Xu, Shin-young Ahn, Ho Jun Chin. *Internal Medicine, Seoul National University Bundang Hospital, Seong Nam, Kyeong ki do, Korea.*

Background: The abnormal lipid metabolism was proposed as an important mechanism in the pathogenesis of diabetic nephropathy. Bilirubin was reported to have inverse relationship with prevalence of diabetes and diabetic nephropathy in human. We investigated the role of bilirubin on lipid metabolism in type I DM model.

Methods: The SD rats were injected with streptozotocin through intra-peritoneum (IP) route and checked the serum glucose level to define diabetes with the criteron of more than 300 mg/dL at 3 days after injection. We injected unconjugated bilirubin dissolved in DMSO (60 mg/kg, IP) every other day at 1 day after induction of diabetes and continued for 4 weeks. Serum glucose level was maintained less than 600 mg/dL with insulin every other day. We sacrificed rats of control (group 1, 5 rats), diabetes (group 2, 8 rats), and diabetes injected with bilirubin (group 3, 5 rats).

Results: At scarification, the amount of food and water intake and the levels of fasting glucose and HbA1c were not different between group 2 and 3. The weight of kidney was not different but the liver weight was lower in group 3 than group 2 (606±143 vs 418±120 mg, p=0.030). The levels of serum total cholesterol (111.5±30.7 vs 73.2±10.4 mg/dL, p=0.033), triglyceride (791.0±297.0 vs 162.6±69.8 mg/dL, p=0.002), and creatinine (0.71±0.22 vs 0.45±0.05 mg/dL, p=0.012), were lower in group 3 than group 2. The fold changes of protein expressions, compared to that of control, in liver were significantly improved in group 3 than group 2 (SREBP- 1, 3.28±1.25 vs 0.94±0.56, p=0.002 ;LXRα, 1.63±0.30 vs 1.07±0.32, p=0.008; PPARγ, 0.20±0.07 vs 0.79±0.14 folds, p<0.001). The same trends of expressions of SREBP-1, LXRα, and PPARγ were also notified in the kidney. The precent of area of mesangium to glomerulus was lower in group 3 compared to group 2 (16.5±0.78 vs 15.2±0.68 %, p=0.012).

Conclusions: Bilirubin improved dyslipidemia through decrease in expressions of SREBP-1 and LXRα and increased PPARγ expression in liver and kidney of Type I DM animals. Normalization of dyslipidemia by bilirubin was associated with preservation of kidney function and improvement of renal pathology.

Funding: Private Foundation Support

SA-PO453

Genetic Deletion of Growth Differentiation Factor 15 Augments Renal Damage in a Type 2 Model of Diabetes Magdalena Mazagova, Hendrik Buikema, Maria Sandovici, Dick de Zeeuw, Robert H. Henning, Leo E. Deelman. *Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Background: Growth differentiation factor 15 (GDF15) is emerging as a valuable biomarker in cardiovascular disease and diabetic kidney disease. Also, GDF15 represents an early response gene induced after tissue injury and studies performed in GDF15 knockout mice suggest that GDF15 plays a protective role after injury. In the current study, we investigated the role of renal GDF15 in type 2 diabetes.

Methods: The progression of diabetic nephropathy was monitored in male db/db GDF15-/- (diabetic GDF15 ko), db/db GDF15+/+ (diabetic wt) and in db/+ GDF15-/- control mice (non-diabetic GDF15 ko) from an age of 6 weeks onwards. Body weight and blood pressure were measured weekly. Mice were placed in metabolic cages for 24 hours when aged 6, 8, 10, 12, 14, 16 and 18 weeks, and food and water intake were measured. Urine samples were collected. At an age of 18 weeks mice were anesthetized with isoflurane (2%) and blood samples were collected. Kidney sections were frozen for isolation of mRNA and protein. Remaining kidney sections were fixed in paraformaldehyde and examined by immunohistochemistry.

Results: Genetic deletion of GDF15 in a model of type 2 diabetes resulted in more renal damage in GDF15 ko mice over wt mice, as demonstrated by increased urinary glucose loss and increased urine production. These changes were accompanied by increased levels of renal damage markers in the GDF15 ko mice. Kidneys from type 2 diabetic GDF15

ko mice showed increased KIM-1 mRNA and KIM-1 protein expression over diabetic wt control, indicating more tubular damage in the diabetic GDF15 ko mice. Furthermore, plasma creatinine levels were increased only in the diabetic GDF15 ko mice, indicating impaired kidney function in the diabetic GDF15 ko mice.

Conclusions: These data indicate that GDF15 both protects the kidney from diabetic damage and maintains renal tubular function in type 2 diabetic mice model.

Funding: Government Support - Non-U.S.

SA-PO454

Aggravation of Inflammatory and Fibrotic Alterations of Diabetic Nephropathy in Mice Lacking Vasohibin-1 Norikazu Hinamoto,[1] Yohei Maeshima,[1] Daisuke Saito,[1] Masaru Kinomura,[1] Hiroko Yamasaki,[1] Hiroyuki Watatani,[1] Haruyo Ujike,[1] Hitoshi Sugiyama,[1] Yasufumi Sato,[2] Hirofumi Makino.[1] *[1]Medicine and Clinical Science, Okayama University, Okayama, Japan; [2]Vascular Biology, Tohoku University, Sendai, Miyagi, Japan.*

Background: Diabetic nephropathy is the leading cause of end-stage renal disease, and the involvement of proangiogenic factors such as VEGF had been reported. We previously reported the protective role of exogenous Vasohibin-1 (VASH-1), a negative feedback regulator of angiogenesis, in mouse models of diabetic nephropathy. In the present study, we aimed to examine the mechanisms involved in the potential protective role of endogenous VASH-1 in diabetic nephropathy.

Methods: Type 1 diabetes was induced in male VASH-1 heterozygous knockout mice ($VASH1^{+/-}$) or wild-type ($VASH1^{+/+}$) littermates (C57/BL6J) by intraperitoneal injections of streptozotocin (STZ, 50 mg/kg) for 5 consecutive days. Mice were sacrificed on week 16 after inducing diabetes. Immunohistochemistry, real-time PCR and immunoblot were performed.

Results: Glomerular alterations were not observed in non-diabetic $VASH1^{+/-}$ mice. Although hyperglycemia, blood pressure or glomerular hyperfiltration were not altered, renal/glomerular hypertrophy, albuminuria, glomerular accumulation of type IV collagen and mesangial matrix, glomerular nephrin/ZO-1 redistribution, glomerular monocyte/macrophage infiltration, GBM thickness, foot process density (TEM) and glomerular $pp65^{+}$ (active NF-kB) cellularity were significantly exacerbated in the diabetic $VASH1^{+/-}$ mice compared with diabetic $VASH1^{+/+}$ mice. Renal levels of transforming growth factor (TGF)-beta1, VEGF-A, angiopoietin-2, pIk-Bα (immunoblot) and MCP-1 (PCR) were significantly increased, and the levels of angiopoietin-1, Total Ik-Bα (immunoblot) and arginase-1 (PCR) were decreased in the diabetic $VASH1^{+/-}$ mice.

Conclusions: These results suggest that endogenous VASH-1 may possess renoprotective effects in type 1 diabetic nephropathy, via suppressing inflammation and fibrosis, thus implicating its potential use to serve as a novel therapeutic reagent for diabetic nephropathy.

SA-PO455

P2X4 Promotes Renal Tubulointerstitial Inflammation in Diabetic Nephropathy through Activating NLRP3 Inflammasome Kehong Chen, Jinhua Zhang, Jurong Yang, Yani He. *Department of Nephrology, DaPing Hospital, Third Military Medical University, Chongqing, China.*

Background: NLRP3 inflammasome has been implicated in the pathogenesis of type 2 diabetes mellitus. NLRP3 inflammasome activation can be triggered by membrane P2X receptors leading to maturation of IL-1β and IL-18. In this study, We investigated the involvement of P2X4 in NLRP3 inflammasome activation and renal tubulointerstitial inflammation in type 2 diabetic nephropathy (DN).

Methods: Human proximal tubular epithelial cell line (HK-2) cells were incubated to normal (5mM) or high glucose (35mM, 48h) with and without the presence of apyrase (an enzyme that degrades extracellular ATP), suramin (P2 receptor inhibitor), TNP-ATP (P2X receptor inhibitor), or 5-BDBD (P2X4 receptor inhibitor). HK-2 cells were transfected with P2X4 siRNA. P2X4 activity was detected by measuring intracellular K+ and Ca2+. 30 patients with type 2 DN and 10 renal hamartoma patients subjected to nephrectomy were recruited in this study. P2X4, NLRP3, IL-1β and IL-18 in renal biopsies were detected by immunohistochemistry and immunofluorescence.

Results: The expression of NLRP3, IL-1β and IL-18 in HK-2 cells under high glucose stimulation increased significantly. NLRP3 inflammasome activation was completely blocked by apyrase which implied that high glucose-associated NLRP3 inflammasome activation was dependent on extracellular ATP. 5-BDBD prevented NLRP3 inflammasome activation through blocking P2X4 open. Silencing P2X4 diminished the high glucose-induced expression of NLRP3, IL-1β and IL-18. Meanwhile, P2X4, NLRP3, IL-1β and IL-18 in tubular epithelial cells were upregulated in type 2 DN patients. P2X4 was co-expressed with NLRP3, IL-1β and IL-18 in the tubular epithelial cells. P2X4, as well as NLRP3 expression was correlated directly with pathological scores of tubulointerstitial inflammation, renal interstitial fibrosis, tubular atrophy respectively.

Conclusions: These results demonstrate that the ATP-P2X4 signaling axis is indispensable for high glucose-induced NLRP3 inflammasome activation. P2X4 and NLRP3 may be exploited for renal tubulointerstitial inflammation in diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO456

The Effects of Fibroblast Growth Factor 21 on db/db Mouse Model of Type 2 Diabetic Nephropathy Hyunwook Kim,[1] Ji Eun Lee,[1] Jin Joo Cha,[2] Young Youl Hyun,[3] Jung Eun Kim,[2] Mihwa Lee,[2] Hye Kyung Song,[2] Kum Hyun Han,[4] Sang Youb Han,[4] Young Sun Kang,[2] Nam Ho Kim,[5] Dae R. Cha.[2] *[1]Department of Internal Medicine, Wonkwang University College of Medicine Sanbon Hospital, Gunpo-si, Kyunggi-do, Korea; [2]Department of Internal Medicine, Korea University College of Medicine Ansan Hospital, Ansan-si, Kyunggi-do, Korea; [3]Department of Internal Medicine, Sungkyunkwan University School of Medicine Kangbuk Samsung Hospital, Seoul, Korea; [4]Department of Internal Medicine, Inje University College of Medicine Ilsan Baik Hospital, Koyang-si, Kyunggi-do, Korea; [5]Department of Internal Medicine, Chonnam University College of Medicine, Gwangu-si, Jeollanam-do, Korea.*

Background: Fibroblast growth factor 21 (FGF21) is an emerging metabolic hormone assumed to have an important role in regulating energy balance by exerting adaptive starvation responses. However, there has been no study exploring its direct effects on renal changes in diabetic milieu. Therefore, we tested the effects of exogenous FGF21 administration on db/db mouse model of type 2 diabetic nephropathy.

Methods: db/db mice were injected with FGF21 intraperitoneally once daily for 12 weeks from the age of 8 weeks and compared to control db/db mice with vehicle treatment or lean normal mice.

Results: At 20 weeks of age, FGF21-treated db/db mice, compared to control db/db mice, showed significantly improved lipid profiles and insulin tolerance test results. Further, serum adiponectin level was significantly higher, whereas serum insulin level, serum and urinary 8-isoprostane levels, and the homeostatic model assessment (HOMA) index were significantly lower in the FGF-treated db/db mice than control db/db mice. In addition, epididymal adipose tissue of the FGF21-treated mice presented more small-differentiated features with relevant changes in mRNA expressions of adipokines. Furthermore, FGF21 administration significantly reduced both mesangial expansion and expression of fibrotic markers, such as TGF-β, collagen-IV, and plasminogen activator inhibitor-1 in renal pathology as well as urinary albumin excretion.

Conclusions: These results suggest that FGF21 is a potential candidate for therapeutic strategy for obesity-related diabetic nephropathy.

SA-PO457

FGF23 and Klotho Expression in the Kidney of Zucker Diabetic Fatty (ZDF) Rats and Modulation by ACE Inhibitor Carlamaria Zoja,[1] Cristina Zanchi,[1] Monica Locatelli,[1] Daniela Corna,[1] Susanna Tomasoni,[1] Giuseppe Remuzzi,[1,2] Ariela Benigni.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Ospedali Riuniti, Bergamo, Italy.*

Background: Fibroblast growth factor (FGF) 23 is a phosphaturic hormone mainly produced by bone that acts in the kidney after binding to FGF receptor in the presence of the cofactor Klotho. In CKD, circulating FGF23 increases likely to counteract phosphate retention, and serum FGF23 is an independent predictor of renal outcome in patients with DN. The kidney apparently produces FGF23 at very low level, if any, both in control and CKD animals. However, data in animals with chronic renal disease only derive from 5/6 nephrectomy model that does not necessarily reflect the most common clinical condition. Here we investigated renal expression of FGF23 in a model of human type 2 diabetes.

Methods: Renal expression of FGF23 and Klotho was assessed by real time PCR and immunohistochemistry in ZDF and lean rats at 2,4,6,8mo of age (n=5/each group). To evaluate whether the renoprotective effect of ACEi in type 2 diabetes was associated with changes in FGF23 and Klotho, ZDF rats received ramipril from 4mo of age, when proteinuric, until 8mo.

Results: ZDF rats developed progressive proteinuria, glomerulosclerosis and interstitial inflammation. FGF23 mRNA was not detectable in the kidney of lean rats, nor in ZDF rats at 2mo but became measurable at 4mo and further increased thereafter. Focal expression of FGF23 protein was found in distal and proximal tubules of ZDF rats at 6 and 8mo. Concomitantly, renal Klotho mRNA progressively decreased. ACEi limited the signs of renal injury and attenuated FGF23 upregulation while normalized Klotho expression. Serum P levels were similar in ZDF and lean rats. Total urinary P content and fractional excretion of P were increased ($p<0.05$) in ZDF vs lean rats at 8mo (U_P: 23±3 vs 4±0.5 mg/d; FE_P: 4.5±1.1 vs 0.7± 0.1 %). After ACEi, U_P was reduced by 36%, FE_P by 23%.

Conclusions: These data suggest that the kidney contributes to FGF23 production that is limited but not abrogated by ACE inhibition, and may offer new clues to understand how to interfere with the delicate balance of FGF23 and phosphorus in diabetes with potential implications in clinics.

Funding: Government Support - Non-U.S.

SA-PO458

Vitamin D Receptor Activation Prevents ER Stress, Oxidative Stress, and Lipid Accumulation in db/db Mice with Type 2 Diabetes Xiaoxin Wang, Liru Qiu, Hannah Danielle Santamaria, Moshe Levi. *Medicine, University of Colorado, Aurora, CO.*

Background: Decreased serum concentration of 1,25-dihydroxyvitamin D, the hormonally active form of vitamin D, and of its precursor 25-hydroxyvitamin D are reported with increasing frequency in subjects with obesity and type 1 or type 2 diabetes. Treatment with vitamin D receptor (VDR) agonists has been proposed to play an important role in

the prevention of diabetic nephropathy. The purpose of these studies was to determine the effects of Paricalcitol in db/db mice with type 2 diabetes mellitus.

Methods: We treated db/m and db/db mice with vehicle or Paricalcitol for 12 weeks.

Results: We found that VDR agonist treatment (Paricalcitol) decreased urinary albumin excretion, and prevented podocyte injury, mesangial expansion, tubulointerstitial fibrosis, and macrophage infiltration. Treatment with Paricalcitol also prevented renal lipid accumulation and the increased expression of the lipid droplet proteins Plin4 and Plin5. In addition treatment with Paricalcitol decreased endoplasmic reticulum stress as determined by decrease in CHOP, a transcription factor that controls genes encoding components involved in apoptosis. Consistent with this Paricalcitol restored the expression of spliced X-box binding protein 1 (XBP1s) and glucose-regulated protein 78 (GRP78). Furthermore Paricalcitol also prevented expression of Cyp4a10 of the cytochrome P450 of the 4A family (CYP4A) associated with ROS and restored glutathione peroxidase GPX2. These effects suggest a role of Paricalcitol in regulation of oxidative stress.

Conclusions: Our data, thus, indicates a novel and an important role for VDR activation for prevention of lipid accumulation, ER stress, and oxidative stress, which are important mechanisms for prevention and treatment of diabetic nephropathy.

Funding: NIDDK Support, Veterans Administration Support, Pharmaceutical Company Support - Abbott

SA-PO459

Novel Effects of the G Protein Coupled Receptor TGR5 Activation in db/db Mice with Type 2 Diabetes and in Human Podocyte Cells Liru Qiu, Xiaoxin Wang, Hannah Danielle Santamaria, Moshe Levi. *Medicine, University of Colorado, Aurora, CO.*

Background: Diabetic nephropathy is the most common cause of chronic renal failure in the world. In spite of aggressive glucose and blood pressure control the incidence of diabetic complications including nephropathy are on the rise. The purpose of the present study was to determine the effects of the G Protein Coupled Receptor TGR5 activation in db/db mice with type 2 diabetes.

Methods: We treated db/m and db/db mice with vehicle or the selective TGR5 agonist INT-777 for 12 weeks.

Results: We found that treatment of db/db mice with INT-777 decreased proteinuria, podocyte injury, mesangial expansion, and tubulointerstitial fibrosis. Urinary H2O2 and TBARS levels which were increased in db/db mice, were significantly decreased after INT-777 treatment. The decrease in oxidative stress induced by INT-777 was further demonstrated by reduced total protein carbonylation in diabetic kidneys. Treatment with INT-777 also increased renal phosphorylated AMPK protein level as well as PGC-1α and ERRα protein abundance, which are master regulators of mitochondrial biogenesis and inhibitors of oxidative stress. We also performed additional studies in human podocytes grown in culture. We found that palmitic acid complexed to BSA (FFA) in concentration of 250 uM resulted in increased activity of caspase 3/7. The pretreatment of the podocytes for 24 hours with INT-777 prevented the FFA induced apoptosis. FFA also induced increased ROS generation. INT-777 decreased ROS generation as determined with MITOSOX staining. In addition INT-777 also increased PGC-1α and ERRα protein expression which are master regulators of mitochondrial biogenesis.

Conclusions: These data suggest a novel role for TGR5 in preventing oxidative stress and inducing mitochondrial biogenesis, which indicate a potential important role for TGR5 in preventing kidney injury in diabetes.

Funding: NIDDK Support, Veterans Administration Support, Pharmaceutical Company Support - Intercept

SA-PO460

Inhibition of Apoptosis Signal-Regulating Kinase 1 Prevents Progressive Diabetic Nephropathy in Mice Haichun Yang,[1] John Liles,[2] David G. Breckenridge,[2] Agnes B. Fogo.[1] *[1]Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN; [2]Gilead Sciences, Inc., Foster City, CA.*

Background: Apoptosis Signal-Regulating Kinase 1 (ASK1), a ubiquitously-expressed MAPKKK, is implicated inapoptosis,differentiation, and inflammation. ASK1 is activated by oxidative stress and high glucose in mesangial cells and endothelial cells in vitro. We aimed to investigate effects of a selective ASK1 inhibitor (GS-444217) on progressive development of diabetic nephropathy in db/db eNOS$^{-/-}$ mice.

Methods: By 10 weeks of age, db/db eNOS$^{-/-}$ mice developed early diabetic injury with proteinuria, mild glomerulosclerosis, and hyperfiltration (Baseline, n=8). db/db eNOS$^{-/-}$ mice received normal chow (Placebo, n=14) or chow with 0.3% GS-444217 by weight (GS-444712 group, n=9) from 10 until 18 weeks. Renal function and histopathology at week 18 in Placebo and GS-444217 were compared to 10 weeks in Baseline.

Results: GFR (inulin clearance) decreased significantly in untreated db/db eNOS$^{-/-}$ mice from 10 to 18 weeks, which was attenuated by GS-444217 (Baseline 10.3±0.9, Placebo 4.4±0.5 vs. GS-444217 6.2±0.6 µl/min/g BW, p<0.05). GS-444217 also decreased albuminuria (Baseline 4022.6±752, Placebo 4796.3±610, GS-444217 3145.6±452 µg/mg) and reduced glomerular injury (Baseline injury score 0.6±0.1, Placebo 1.5±0.2 vs. GS-444217 0.7±0.1, 0-4 scale, p<0.05). Db/db eNOS$^{-/-}$ mice treated with GS-444217 had significantly decreased glomerular collagen IV immunostaining, decreased glomerular and tubulointerstitial apoptosis (Baseline 0.3±0.1, Placebo 0.7±0.1 vs.GS-444217 0.3±0.1 TUNEL^{+} cells/HPF, p<0.05), lower HbA1C, and lower blood pressure (Baseline 102.0±3.0, Placebo 130.5±3.4 vs. GS-444217 114.9±6.8 mmHg, p<0.05) compared to Placebo. Kidney Phospho-p38, a downstream mediator of ASK1 signaling, was reduced by GS-444217 treatment.

Conclusions: Our data demonstrate that an ASK1 inhibitor markedly slows progression of diabetic nephropathy in mice as assessed by GFR, and halts progressive glomerulosclerosis and apoptosis in the kidney. The data support the ASK1 pathway as a therapeutic target in diabetic nephropathy with the potential to ameliorate or slow disease progression in patients.

Funding: Pharmaceutical Company Support - Gilead Sciences, Inc.

SA-PO461

Protective Effect of PPARalpha against Diabetic Nephropathy through Inhibition of the Canonical Wnt Pathway Rui Cheng, Xuemin He, Jianxing Ma. *Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Peroxisome proliferator-activated receptor-α (PPARα) plays a crucial role in the regulation of lipid metabolism. PPARα deficiency has been shown to aggravate diabetic nephropathy by increasing extracellular matrix formation and inflammation. Recently, we have reported that aberrant activation of the canonical Wnt pathway plays a pathogenic role in diabetic nephropathy. The purpose of the present study is to investigate whether PPARα ameliorates diabetic nephropathy via inhibiting the Wnt pathway.

Methods: Expression levels of PPARα and activity of the Wnt pathway were measured in the renal cortex of streptozotocin (STZ)-induced diabetic rats. Fenofibrate, a specific agonist of PPARα, was administrated in the diabetic model. To identify the protective role of PPARα under diabetes conditions, PPARα was over-expressed using adenovirus in primary human renal proximal tubular epithelial cells (HRPTCs).

Results: In HRPTCs, both high glucose and Wnt3a activated the Wnt pathway and up-regulated expression of CTGF and fibronectin, target genes of the Wnt pathway, which were attenuated by over-expression of PPARα. Similarly, Fenofibrate suppressed the activation of the Wnt pathway and over-expression of CTGF and fibronectin in a concentration- and time-dependent manner. Compared to non-diabetic rats, PPARα expression was down-regulated in the kidney of STZ-induced diabetic rats, which is negatively correlated with Wnt signaling activity. Fenofibrate reduced albuminuria and decreased collagen IV and fibronectin expression in the kidney of the diabetic rats. Fenofibrate also attenuated the over-renal expression of inflammatory factors ICAM-1 and TNF-α, target genes of the Wnt pathway, in the diabetic rats. Furthermore, Fenofibrate decreased renal levels of LRP6 and β-catenin in the diabetic rats.

Conclusions: These results suggest that diabetes-induced down-regulation of PPARα in the kidney plays an important role in diabetic nephropathy. PPARα has a protective effect against diabetic nephropathy through inhibiting the canonical Wnt pathway, which renders a new therapeutic target for diabetic nephropathy.

Funding: Other U.S. Government Support

SA-PO462

Aquaporin 11 Insufficient Mice Are Predisposed to the Development of Diabetic Kidney Disease Elena E. Tchekneva,[1] Elena N. Atochina-vasserman,[2] Elena Abramova,[3] Liberty Foye,[1] Andrew J. Gow,[3] Raymond C. Harris.[1] *[1]Vanderbilt University Medical Center; [2]University of Pennsylvania; [3]Rutgers University.*

Background: Accumulating evidence suggests proximal tubules (PT) as a primary target in diabetic kidney disease. The factors predisposing PT to hyperglycemia-induced injury and initiating diabetic kidney disease remain uncertain. In the kidney, the water channel, aquaporin 11 (Aqp11), is exclusively expressed in PT, resides in the ER and maintains cytosolic and/or vesicle osmolality. Loss-of-function of Aqp11 resulted in PT-specific injury and kidney failure in *Aqp11* deficient mutant mice with *sudden juvenile death syndrome (sjds/sjds)* mutation. *Aqp11* insufficient mice (*sjds/+)* were phenotypically normal (JASN 10:1955, 2008).

Methods: The *sjds* mutation was crossed with *Ins2Akita* mice, which develop type I diabetes.

Results: *Sjds* carriers showed significantly higher levels of ROS in renal cortex than +/+ littermates. In *sjds/+* mice repetitive injections with high glucose significantly increased BUN that was abrogated by antioxidant sulforaphane. We hypothesize that *sjds/+* mice are susceptible to hyperglycemia-induced PT-specific injury and predisposed to diabetic kidney disease. By the age 5 months, *sjds/Ins2Akita*mice exhibited severe PT injury, renal hypertrophy, tubulointerstitial fibrosis and glomerular mesangial expansion compared with wild type *+/Ins2Akita* littermates. The histopathologic changes were associated with significantly increased BUN (40.48±7.56 *vs*. 28.04±7.08mg/dl; p<0.05), ROS (3.02±0.29 *vs*.2.3±0.04 AU/ug; p<0.05), and albumin/creatinine ratio (ACR) (135±35 *vs*.40±6ug/mg; p<0.005) in *sjds/Ins2Akita* progeny compared to control diabetic *+/Ins2Akita* littermates. Non-diabetic *sjds/+*mice exhibited a compensatory 40% higher Aqp11 expression than +/+ mice. Importantly, hyperglycemia also increased the Aqp11 expression in diabetic *+/Ins2Akita*, but expression was not further increased in diabetic *sjds/Ins2Akita* mice.

Conclusions: We showed that Aqp11 insufficiency in PT is a novel risk factor for the development of diabetic kidney disease. ROS-mediated PT-specific injury induced by hyperglycemia triggers diabetic kidney disease in mice with Aqp11 insufficiency.

Funding: NIDDK Support

SA-PO463

Null Mutations at the p66 and Bradykinin 2 Receptor Loci Induce Divergent Phenotypes in the Diabetic Kidney Himanshu Vashistha,[1,3] Pravin C. Singhal,[2] Ashwani Malhotra,[2] Krzysztof Reiss,[3] Leonard G. Meggs.[1,3] *[1]Nephrology, Ochsner Clinic Foundation, New Orleans, LA; [2]Nephrology, Feinstein Research Institute, New York, NY; [3]Neurological Cancer Research, SSSCC, LSUHSC, New Orleans, LA.*

Background: Candidate genes have been identified that confer increase risk for diabetic glomerulosclerosis (DG). Mice heterozygous for the Akita ($Ins2^{+/C96Y}$) diabetogenic mutation with a second mutation introduced at the bradykinin 2 receptor ($B2R^{-/-}$) locus, express a disease phenotype that approximates human DG. The p66 longevity gene controls mitochondrial metabolism and cellular responses to oxidative stress, aging and apoptosis.

Methods: We generated p66-null Akita mice to test whether loss of function mutations at the p66 locus will rescue kidneys of Akita mice from disease causing mutations at the Ins2 and B2R loci. Here we show null mutations at the p66 and B2R loci interact with the Akita ($Ins2^{+/C96Y}$) mutation, independently and in combination, inducing divergent phenotypes in the kidney.

Results: The $B2R^{-/-}$ mutation induces detrimental phenotypes, as judged by increased systemic and renal levels of oxidative stress, histology and urine albumin excretion, whereas the p66-null mutation confers a powerful protection phenotype. Furthermore, we provide a rationale for the protection phenotype in p66-null Akita mice, by revealing previously unrecognized crosstalk between p66 and the redox sensitive transcription factor p53, that controls hyperglycemia-induced ROS metabolism, transcription of p53 target genes (angiotensinogen, angiotensin II type-1 receptor; bax), angiotensin II generation and apoptosis. Finally, protein levels of p53 target genes were upregulated in kidneys of Akita mice, but unchanged in p66-null Akita mice.

Conclusions: We speculate p66 is the most promising molecular target for therapeutic intervention in DG, since the discovery of ACEI and ARB.

Funding: NIDDK Support

SA-PO464

Diabetes-Associated SORCS1 Gene Regulates Water Balance in Mice Melkam Kebede,[1] Brendan J. Floyd,[1] Angie Oler,[1] Arjang Djamali,[2] Alan Attie.[1] *[1]Biochemistry, University of Wisconsin-Madison, Madison, WI; [2]University of Wisconsin School of Medicine and Public Health, Madison, WI.*

Background: Genome Wide Association Studies in humans and an F2 mouse cross in our laboratory have linked the *sortilin-related receptor CNS expressed 1* (*SorCS1*) gene to type 2 diabetes mellitus and its complications, including retinopathy, neuropathy, and nephropathy. SorCS1 is a member of a family of five proteins (sortilin, SorLA and SorCS1–3) that contain a vacuolar protein sorting-10 (VPS10) domain. It is highly expressed in the brain, heart and kidney and at low levels in other tissues such as pancreatic islets. To identify the direct role of *SorCS1* gene in diabetes susceptibility and complications, we have generated a whole-body *SorCS1* knockout (KO) mouse on a C57BL/6 background with and without the *Leptin^{ob}* mutation.

Methods: Urine collection: Mice were acclimated to metabolic cages with food and water, and then transferred to a fresh cage without food for urine collection over 8 hours during the light cycle. Values are adjusted to 24 hours.

Urine analysis: Osmolality was measured by vapor pressure osmometry (Vapro) and chemistry was measured at Marshfield Laboratories, Marshfield, WI.

Results: The most notable phenotype observed in the female *SorCS1* KO mice was a reduction in urine volume both in the lean and Ob background. Lean SorCS1 KO mice showed a ~50% reduction in urine output compared to their WT littermates (173 ± 44 vs. 380 ± 80, $p<0.05$). When in the Ob background, the WT mice increased their urine output by two-fold compared to their lean counterparts (685 ± 106 vs. 380 ± 80, $p<0.05$). Interestingly, knockout of the SorCS1 gene abolishes the polyuria phenotype of $Leptin^{ob/ob}$ mice, despite glucosuria, and hyperglycemia.Further urine analysis revealed that urine from SorCS1 KO animals has elevated [Na^+], [K^+], and [Cl^-], but reduced Na^+/creatinine and Cl^-/creatinine ratios compared to WT controls. Both KO strains have reduced blood urine nitrogen, a potential sign of serum dilution. In addition, SorCS1 KO urine has lower pH compared to their WT littermates (5.56± 0.18 vs. 6.09 ± 0.15, $p<0.05$).

Conclusions: Taken together, these results demonstrate that SorCS1 is an important regulator of water and electrolyte balance in mice and studies are now underway to identify the mechanism(s) of such regulation.

SA-PO465

Serum Amyloid A and Inflammatory Cytokine Expression in Kidney Cells Exposed to Diabetes-Like Conditions Rick L. Meek,[1] Robert J. Anderberg,[1] Sheryl K. Cooney,[1] Katherine R. Tuttle.[1,2] *[1]Providence Sacred Heart Medical Center and Children's Hospital, Spokane, WA; [2]Nephrology Division, University of Washington School of Medicine, Spokane, WA.*

Background: Inflammation is increasingly recognized as a pathogenic mechanism in diabetic kidney disease. Serum amyloid A (SAA) is a potent cytokine that binds pro-inflammatory receptors including the receptor for advanced glycation end products (RAGE). We recently discovered that kidney SAA expression is increased in mouse models of types 1 and 2 diabetes. The study aims were to assess: 1) SAA expression in podocytes, mesangial cells and tubular cells exposed to AGE, aberrant metabolic factors characteristic of diabetes; 2) effects of SAA on inflammatory cytokine expression in these kidney cells; 3) impact of RAGE on expression of SAA and caspase 4, another novel cytokine, in podocytes.

Methods: Mouse podocytes and mesangial cells (MES-13),and human proximal tubule cells (HK-2) were cultured with AGE, SAA or control conditions. SAA protein (ELISA) and mRNA (real-time PCR) and mRNA for other inflammatory mediators (caspase 4; monocyte chemotactic protein-1: MCP-1; inducible nitric oxide synthase: iNOS) were also measured. Blocking antibody for RAGE was used as an inhibitor of receptor activation.

Results: Both SAA and AGE induced expression of SAA, caspase 4, MCP-1, and iNOS in podocytes and mesangial cells. SAA induced autocrine expression of itself as well as expression of caspase 4 in HK-2 cells. Anti-RAGE antibody inhibited expression of AGE-induced SAA, caspase 4, MCP-1, and iNOS mRNA in podocytes.

Conclusions: SAA expression is up-regulated by AGE in cells derived from both glomerular and tubular compartments. SAA also induces expression of inflammatory mediators, including an *autocrine effect on its own expression. RAGE may be a key receptor for induction of novel cytokines including SAA and caspase 4 in podocytes.*

Funding: Private Foundation Support

SA-PO466

Serum Amyloid A and Podocyte Apoptosis in Mouse Models of Diabetic Kidney Disease Rick L. Meek,[1] Sheryl K. Cooney,[1] Robert J. Anderberg,[1] Charles E. Alpers,[2,3] Kelly L. Hudkins,[3] Katherine R. Tuttle.[1,4] *[1]Providence Sacred Heart Medical Center and Children's Hospital, Spokane, WA; [2]Mouse Metabolic Phonotyping Center; [3]Pathology Department; [4]Nephrology Division, University of Washington School of Medicine, Spokane and Seattle, WA.*

Background: Serum amyloid A (SAA) is a pro-inflammatory cytokine and ligand for the receptor for advanced glycation end products (RAGE). Activation of RAGE induces podocyte apoptosis. The potential contribution of SAA to kidney damage is unknown. The specific aims of this study were to determine if SAA expression is increased in the diabetic kidney and whether SAA induces podocyte apoptosis via RAGE.

Methods: SAA mRNA (real-time PCR) and/or protein (immunocytochemistry) expression were examined in kidneys of streptozotocin-injected C57BL/6 mice (mRNA and protein after 20 weeks) and BTBR obob mice (protein after 24 weeks), models of types 1 and 2 diabetes, respectively. Mouse podocytes were cultured with SAA, AGE, or control conditions. Levels of mRNA for SAA and caspase 4 (indicator of early phase apoptosis) were quantified by real-time PCR. Later phase apoptosis was measured by TUNEL staining. Blocking antibody for RAGE was used as an inhibitor of receptor activation.

Results: Expression of SAA mRNA was elevated (~4-fold) in kidney cortex of type 1 diabetic mice compared to non-diabetic controls. SAA protein was greater in the proximal tubules of both mouse models compared to kidneys of their respective non-diabetic control mice. Exposure of cultured podocytes to AGE induced expression of SAA mRNA. Similarly, in podocytes exposed to SAA, production of SAA mRNA and protein increased along with caspase 4 mRNA and TUNEL staining. RAGE inhibition prevented induction of apoptosis in response to SAA.

Conclusions: SAA expression is up-regulated in kidneys of mouse models of types 1 and 2 diabetes as well as in cultured podocytes exposed to AGE. Notably, SAA directly promotes its own expression and apoptosis in podocytes. RAGE activation may be a pathway for increasing expression of SAA as well as in mediating autocrine effects of SAA in podocytes.

SA-PO467

A Novel Glycosaminoglycan Formulation Prevents Mesangial Expansion, Albuminuria, Macrophage Infiltration and Attenuates Podocyte Loss in Experimental Diabetic Nephropathy Conrado Rodrigues Gomes, Carolina Venturotti, Cristina Leão, Andre Barreira, Roberto Fonseca, Christina Maeda Takiya, Paulo Mourão, Alvimar Delgado, Maurilo Leite. *Internal Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.*

Background: Diabetic nephropathy (DN) is characterized by thickening of glomerular basement membrane, mesangial matrix (MM) expansion, albuminuria (ALB) and progressive kidney damage. Glycosaminoglycan (GAG) have been shown to decrease ALB in DN. This study used fucosylated condroitin sulfate (FuCS), a novel GAG from marine invertebrate, and a low molecular weight heparin (LMWH), in experimental DN.

Methods: Diabetes Mellitus (DM) was induced in 15 rats by streptozotocin (65mg/Kg) via the caudal vein. They were divided into 4 groups: 1) control, 2) DM without GAG, 3) DM + FuCS, 8mg/kg, 4) DM + LMWH, 4 mg/kg. G1 received citrate buffer. After week 8 (G:3 and 4), the GAGs were given SC daily for 6 weeks, and then on alternate days for other 6 weeks and then the groups were sacrificed.

Results: There was an increase in ALB of G2: 16.38±4.3 compared to G1: 3.04±0.98, G3: 5.35±3.05; G4: 5.06±3.07 (mg/24hs, $p<0.002$). We observed expansion of MM in the DM group compared to the other groups (G2: 14.19±3.01; G1: 9.5± 2.8; G3: 10.2±2.6 and G4: 8,19±2.5 (%mesangial area, $P<0.001$), and an increase in macrophages in gomeruli of G2 compared to other groups (G2: 0.12±0.09 x G1:0.04±0.05, G3:0.05±0,05 and G4:0.05±0.06, %ED-1-stained area/mm^2, $P<0.001$). There was a reduced immunoreactivity to nestin antibody in podocytes of G2, compared to G1 (2.8±0.3 vs 7.6±0.5 % glomerular area, $P<0.05$), which was prevented in G3 and G4 groups (6.7±0.36 and 5.6±0.34 %, $P<0.05$,compared with G2). TGF-β1 stained area in glomeruli (%) was increased in G2 (4.6±0.3) compared to treated groups (G3: 1.2±0.15 and G4: 1.9±0.2, $P<0.05$) and G1 (0.7±0.1, $P<0.05$).

Conclusions: Both GAGs prevented ALB, MM expansion, macrophage infiltration, and promoted attenuation of TGF-β1 expression in diabetic rats. Also, podocytes were preserved by the treatment with GAGs. We conclude that FuCS may be a useful option for the prevention of DN.

SA-PO468

Interferon Beta Dramatically Reduces the Development of Albuminuria in Strepzotocin-Induced Diabetic DBA2J RccHSD Mice

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Background: We have previously described the anti-proteinuric actions of interferon beta (IFNβ) in 3 different rat models of glomerulonephritis and have shown that IFNβ has direct effects on cells of the glomerular filtration barrier. The aim of the current study was to determine whether IFNβ is effective in inhibiting proteinuric diabetic renal disease.

Methods: Eight week old male DBA2J-RccHSD mice were made diabetic via a 5 day regimen of 40mg/kg STZ *i.p*. This produced a robust hyperglycaemia in all animals with similar starting plasma glucose values (sham 28.8mmol/l +/-3.9 vs. 26.3+/-2.4). After four weeks of hyperglycaemia and prior to production of albuminuria, animals were randomised into two groups with one group given daily *i.p*. injection of 1000 units of IFNβ the other group were given an equivalent volume of saline. The study was carried out with no insulin supplementation.

Results: Weekly analysis of individual animal weights showed no significant difference between the two treatments (Sham 21.1 +/-1.5g vs. IFNβ 20.14+/-1.86g P<0.198). Albuminuria increased in sham control animals from week 2 of treatment with the albumin: creatinine ratio (ACR) attaining a 9 fold increase over baseline by week 3 (3236.4µg/mmol +/-194.7 vs. own baseline 347.02+/-229.6.) and reaching a 84 fold increase 28983.3 µg/mmol+/- 2905.3 at culling (week 5). By week three IFNβ treated animals had 9-fold less albumin excretion than sham controls (373.5 µg/mmol +/- 148.6 vs. 3236.4 +/- 194.7 P<0.001), with IFNβ treatment continuing to retard albumin excretion to culling (Sham 28983.3 +/-2905.3 vs. IFNβ 3942.3+/416.1 P<0.001).

Conclusions: IFNβ treatment therefore significantly inhibited the diabetes mediated increase in ACR with treated animals displaying 90% less albumin excretion than saline treated animals at culling. IFNβ and the glomerular cellular pathways it modulates present a potential target for therapy in proteinuric renal disease including diabetic nephropathy.

SA-PO469

P2X7 Deficiency Attenuates Renal Inflammation and Pancreatic Beta Cell Injury in Experimental Diabetes

John W. Booth,[1] Jill T. Norman,[1] Frederick W.K. Tam,[2] Robert J. Unwin.[1] *[1]Centre for Nephrology, University College London; [2]Imperial College London.*

Background: Inflammation is a key pathogenic mechanism in both diabetes and diabetic nephropathy (DN). The P2X7 receptor is an ATP-gated cation channel with roles in inflammation and cell death; it is expressed in immune cells and also resident renal cells and pancreatic islets. We investigated the role of P2X7 in early DN and pancreatic injury using a mouse model of type 1 diabetes as well as human mesangial cells (HMC) cultured in a diabetic milieu.

Methods: Low dose (50mg/kg) streptozotocin injections (x5) were administered to wildtype (WT) and two strains of P2X7 knockout mice with differing profiles of residual receptor expression: Glaxo (GSK) and Pfizer (PF). Random blood glucose (BG) and pancreatic insulin staining were assessed at 3 weeks. Renal CD68 staining was assessed at 12 weeks in persistently diabetic mice. HMCs were grown for 2 days in 4mM or 30mM glucose media. Secreted MCP-1 was measured by ELISA, and the effect of the selective P2X7 antagonist A438079 (10µM) and the P2X7 agonist BzATP (0.1M) were tested.

Results: 20/22 (91%) WT and 13/18 (72%) GSK achieved a BG >16mM at 3 weeks. In a separate experiment, 6/7 (86%) WT and 1/6 (17%) PF achieved this. Islet insulin staining was relatively preserved in PF (p=0.036 vs WT). At 12 weeks, renal macrophage accrual was reduced in GSK in both glomerular (p<0.0001 vs WT) (Figure 1) and interstitial (p=0.01 vs WT) compartments; urine albumin excretion was not increased at this time.

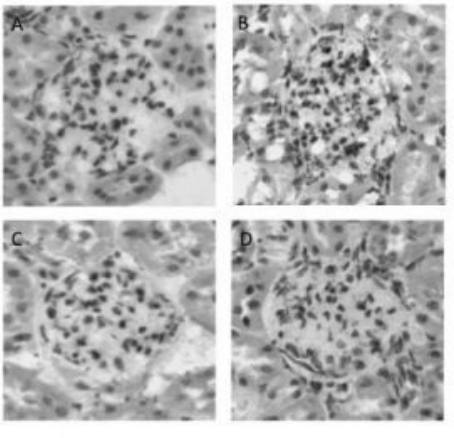

Figure 1. Glomerular CD68 macrophage immunohistochemistry at 12 weeks. A: WT vehicle-injected control; B: WT diabetic animal; C: GSK vehicle-injected control; D: GSK diabetic animal.

In vitro, hyperglycemia enhanced MCP-1 secretion from HMCs, which was reduced 51-100% by A438079. BzATP further augmented glucose-induced MCP-1 release (p=0.028 vs glucose alone).

Conclusions: P2X7 contributes both to early renal inflammation and beta cell injury in experimental diabetes. P2X7 appears to regulate glucose-induced MCP-1 release from HMCs which may, in part, explain the renal findings.

SA-PO470

Macrophage-Mediated Glucolipotoxicity Contributes to Progression of Diabetic Nephropathy through MRP8/TLR4 Signaling

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Background: We have identified that toll-like receptor 4 (TLR4) and its endogenous ligand myeloid-related protein 8 (MRP8) are upregulated in diabetic nephropathy (DN) using glomerular cDNA microarray. We have also observed that high-fat diet (HFD) feeding upon streptozotocin (STZ)-treated mice results in further upregulation of glomerular MRP8/TLR4 along with doubling of albuminuria. Here, we investigated the pathophysiological role of MRP8/TLR4 signaling in DN.

Methods: In vivo, STZ-treated wild-type (WT) and TLR4 knockout (KO) mice fed with HFD for 6 weeks were analyzed. In vitro, effects of high-glucose, fatty acid and MRP8 were examined in macrophages (Mφ) derived from WT and KO bone marrow. In 65 human biopsy samples, MRP8 expression was evaluated with immunostaining.

Results: In STZ-treated WT and KO mice, addition of HFD similarly enhanced hypertriglyceridemia and renal lipid accumulation. Although HFD aggravated DN in STZ WT mice, as indicated by increase of albuminuria, mesangial expansion and inflammatory and fibrotic gene upregulation, effects of HFD upon renal lesions were almost completely abolished in STZ KO mice. MRP8 positive cells were abundantly found in glomeruli of STZ-HFD-treated WT mice and colocalized with Mφ marker MAC2. Recombinant MRP8 markedly induced expression of IL-1β and TNFα as potently as LPS, and triggered auto-induction of MRP8 in Mφ. Palmitate amplified MRP8 expression only under high-glucose conditions in Mφ from WT, but not in Mφ from KO. In human DN, punctate MRP8 signals were observed in glomeruli and tubulointerstitium. Glomerular MRP8-positive cell counts were strongly correlated to extent of proteinuria.

Conclusions: These findings suggest that hyperlipidemia complicated with diabetes activates TLR4 on Mφ, induces MRP8, and deteriorates DN. We propose "Mφ-mediated glucolipotoxicity" through MRP8/TLR4 signaling as a novel mechanism of DN progression.

SA-PO471

The Role of Cellular Inflammation and STAT3-SOCS3 in Early Obesity-Related Nephropathy in the Zucker Rat

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Background: Inflammation, regulated by STAT3 signaling, contributes to renal damage in obesity/metabolic syndrome/diabetes. STAT3 signaling is negatively regulated by suppressor of cytokine signaling 3 (SOCS3). The aim of the study was to assess cellular inflammation and relative STAT3/SOCS3 expression in the kidneys of Zucker rats with early obesity related nephropathy.

Methods: 14 and 18 week old male Obese Zucker rats (OZR) were compared to lean Zucker rats (LZR) with regard to weight, proteinuria, cellular inflammation and expression of STAT3 and SOCS3 in the kidney. Analyses included histology, immunohistochemistry (IHC) and multi-color flow cytometry of kidney cell suspensions.

Results: By 18 weeks OZR were heavier (555±16 vs 391±17g p=0.005) with increased albuminuria (391±89 vs 27.6±9mg/dl p=0.03). Light microscopy showed no overt glomerular or tubulinterstitial abnormalities of OZR kidney. Flow cytometry for total kidney myeloid and T cell subset numbers were similar for OZR vs LZR. However IHC revealed increased glomerular $CD68^+$ macrophages in OZR (3.3±0.3 vs 1.5±0.25 cells/glom p=0.002). At both 14 and 18 weeks, there was increased distal tubular STAT3 expression in OZR (4.4±0.07 vs 2.3±0.05 %area/HPF p=0.05) associated with clusters of $p\text{-}STAT3^+$ cells. SOCS3 was widely expressed in tubular epithelium of OZR and LZR while OZR glomeruli had increased staining for SOCS3 (73±0.05 vs 17±0.05 %+ve glom p=0.001). Active glomerular injury in OZR was confirmed by increased desmin expression (14±2 vs 9±1%area/glom p=0.03) which was higher in $SOCS3^+$ vs $SOCS3^-$ glomeruli (27±2 vs 18±2%area/glom p=0.02).

Conclusions: Overt cellular inflammation is absent in the OZR model of obestiy-related nephropathy to 18 weeks. OZR glomeruli have increased macrophages and desmin expression accompanied by increased SOCS3. Increased tubular STAT3/pSTAT3 is also accompanied by widespread tubular SOCS3 expression. SOCS3 counterregulation of STAT3-mediated inflammatory events may be critical for renal integrity in obesity/metabolic syndrome.

Funding: Government Support - Non-U.S.

SA-PO472

Irbesartan Attenuates Inflammation in Diabetic Nephropathy through Modulation of TLR4/MyD88 Pathway Jinlei Lv, Hong-bo Xiao, Qinkai Chen. *Nephrology, The First Affilliated Hospital of NanChang University.*

Background: The pathogenesis of Diabetic nephropathy(DN) is not completely understood so far. Some researchers prefer that DN is an "innate immune disease", Toll-like receptor 4(TLR4) modulate immune responses and inflammatory diseases, but its role in DN remains to be under active consideration. The aims of this study were to reveal the possible role of TLR4 and its signal pathway in DN model and in vitro, and to explore the novel mechanisms of Irbesartan in the treatment of diabetic nephropathy.

Methods: In vivo, rats were randomly assigned to four groups, which are N(normal control group), M(DN model group), ARB(irbesartan treated group) and SiRNA group. Biochemical indicators were measured at the end of 8W. Morphological changes of renal tissue were observed by HE,Masson and PASM stain. RealTime-PCR were performed to investigate the mRNA expression of TLR4/MyD88, Immunofluorescence method was used to observe the protein expression of TLR4,MCP-1 and IL-1; In vitro, we use SiRNA transfection and Real-time Polymerase Chain Reaction(RT-PCR) technique to analyze TLR4 signal under high glucose condition, identifying the relationship between TLR4 signal and inflammation factor.

Results: In this study, we found upregulated expression of TLR4/MyD88 in the renal tubules and mesangial cell of diabetic nephropathy compared with expression of TLR4/MyD88 in normal kidney. The intensity of tubular TLR4/MyD88 expression correlated directly with interstitial macrophage infiltration as well as MCP-1,IL-1, and inversely with irbesartan treated group. In vitro, high glucose induced TLR4 /MyD88 expression in a dose-dependent manner, resulting in upregulation of MCP-1,IL-1 IL-6 expression in NRK-52E cell. Silencing of TLR4 with small interfering RNA attenuated high glucose-induced TLR4 /MyD88 activation, inhibited the downstream synthesis of MCP-1,IL-1. We observed similar effects using irbesartan.

Conclusions: These data suggest that TLR4/MyD88 pathway may promote tubulointerstitial inflammation in diabetic nephropathy, Irbesartan attenuates inflammation in DN through modulation of this pathway.

Funding: Government Support - Non-U.S.

SA-PO473

Impaired Glomerular Capillary Repair with Persistent Glomerular Inflammation in Streptozotocin Induced Diabetic Nephropathy Hideki Takano,[1,2] Taiko Yoshida,[1,2] Yukinari Masuda,[1] Akira Shimizu.[1] *[1]Department of Pathology, Nippon Medical School, Tokyo, Japan; [2]Division of Nephrology, Tokyo Teishin Hospital, Tokyo, Japan.*

Background: Microvascular injury is considered the essential precipitating factor in diabetes mellitus (DM) nephropathy, the mechanism of glomerular capillary repair after microvascular injury in DM nephropathy is still uncertain. We assessed the process of glomerular healing in streptozotocin (STZ)-induced DM rats, with particular focus on glomerular capillary regeneration and maturation.

Methods: DM was induced in rats by STZ (DM group). Three days later (day 0), glomerular capillary injuries were induced in both DM and non-DM groups by OX-7 antibody injection. Glomerular healing variables including glomerular capillary repair; changes in expression of vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2); and renal function were assessed until day 28.

Results: Following OX-7 antibody injection, both groups developed diffuse mesangial cell lysis on day 1, followed by segmental glomerular capillary destruction by days 3 to 7. In non-DM control rats, glomerular healing with mesangial cell proliferation by day 14. Glomerular capillary regeneration and maturation developed with increased expression of VEGF and Ang-1, most glomeruli recovered by day 28. In DM rats, in contrast, capillary regeneration ($P<0.01$) and endothelial cell proliferation ($P<0.05$) were rare in damaged glomeruli, which showed decreased expression of VEGF on days 7 (VEGF/actin ratio: non-DM 3.95±0.90 versus DM 1.28±0.21, $P<0.01$). DM rats also showed impaired glomerular capillary maturation, with decreased expression of Ang-1 and increased expression of Ang-2 (Ang-2/actin ratio: non-DM 1.20±0.78 versus DM 3.77±0.74, $P<0.01$). Mesangial proliferation and desmin+ glomerular epithelial damage remained on day 28, accompanied by glomerular infiltration of ED1+ macrophages.

Conclusions: Glomerular healing, especially glomerular capillary repair, was impaired after glomerular injury in STZ-induced DM rats. The development of DM nephropathy may be associated with not only glomerular injury but also subsequent impaired glomerular healing with the persistence of glomerular inflammation.

SA-PO474

The Prostaglandin E2 EP1 Receptor Promotes Glomerular and Tubular Dysfunction in OVE26 Diabetic Mice Jean-Francois Thibodeau,[1,2] Anthony Carter,[2] Chris R. Kennedy.[2] *[1]Cellular and Molecular Medicine, Univerisity of Ottawa, Ottawa, ON, Canada; [2]Kidney Research Center, Ottawa Hospital Research Institute, Ottawa, ON, Canada.*

Background: Cyclooxygenase (COX)-derived prostanglandin E2 (PGE2) synthesis and downstream EP receptor activation contributes to diabetic nephropathy (DN). COX inhibition lowers albuminuria and renal damage in human DN and experimental models. Given that pharmacological EP1 antagonism is beneficial in diabetic rats, we hypothesized that the Gq-coupled EP1 receptor promotes glomerular and/or tubular damage in DN.

Methods: Our prior studies revealed that gene-targeted EP1 knockout mice (EP1-/-), subjected to the low-dose streptozotocin (STZ) model of type 1 diabetes (T1DM), were significantly less albuminuric than wild-type (WT) mice at 16 weeks. Despite comparable glomerular damage, EP1-/- mice were protected from megalin downregulation, a marker of proximal tubule (PT) injury. We backcrossed EP1-/- mice onto the OVE26 transgenic model of T1DM (OVE26EP1-/-) and measured various functional and structural parameters.

Results: At 8 and 26 weeks, albuminuria was exacerbated in the OVE26 cohort compared to the OVE26EP1-/- mice (8 weeks: OVE26, 865±119 vs. OVE26EP1-/-, 508±66 μg/24 hrs, $p<0.001$; 26 weeks: OVE26, 2762±1067 vs. OVE26EP1-/-, 1022±395 μg/24 hrs, $p<0.05$) mirroring our STZ-study findings (WTstz, 1546±282 vs. WT, 525.8±110 μg/24 hrs, $p<0.001$). EP1 receptor deletion reduced the extent of glomerular hypertrophy (OVE26, 9447±396vs. OVE26EP1-/-, 7397±315 μm^2, $p<0.001$) as well as mesangial expansion (OVE26, 35.07±1.19vs. OVE26EP1-/-, 26.34±1.01% of glomerular area, $p<0.001$) while dramatically increasing survival rates. Semi-quantitative PCR revealed that OVE26EP1-/- mice were protected from DN-induced megalin downregulation, while nephrin mRNA decreased in both diabetic groups.

Conclusions: These results implicate the EP1 receptor in DN, downstream of the classic COX2/ PGE2 pathway. Our data are consistent with the idea that EP1 activation may exacerbate filtration barrier damage in DN by increasing podocyte and/or mesangial cell damage while promoting dysfunctional post-glomerular albumin processing via the PT's megalin/ cubilin complex.

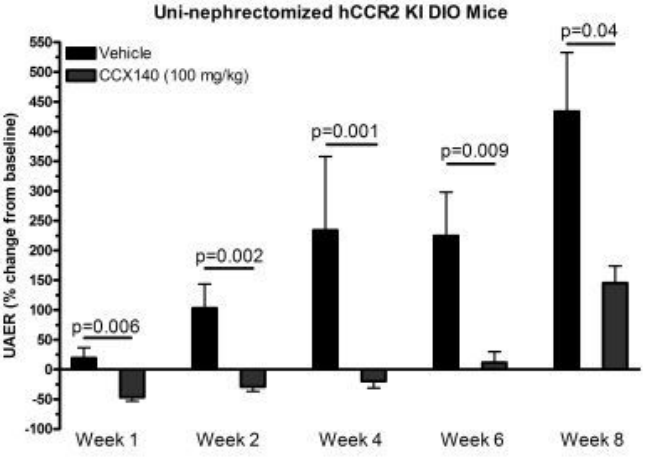
SA-PO475

The CCR2 Chemokine Receptor Antagonist CCX140 Improves Renal Function in Diabetic Mice Expressing Human CCR2 Tim Sullivan, Zhenhua Miao, Robert D. Berahovich, Jay P. Powers, Trageen Baumgart, Linda Ertl, Shichang Miao, Thomas J. Schall, Juan C. Jaen. *ChemoCentryx, Inc., Mountain View, CA.*

Background: Diabetic Nephropathy is a major consequence of uncontrolled diabetes. The chemokine receptor CCR2 has been implicated in the recruitment of blood monocytes into kidney in response to hypertension and hyperglycemia. In addition, parenchymal renal cells are thought to upregulate CCR2 under those pathological conditions. We set out to assess the therapeutic benefit of CCR2 antagonism in a mouse model of diabetic nephropathy utilizing CCX140, which is currently being studied in two Phase 2 diabetic nephropathy clinical studies.

Methods: Given the high selectivity of CCX140 for human CCR2, gene knock-in mice were generated which express human CCR2 in place of mouse CCR2 (hCCR2 KI). CCX140 was dosed daily to male human CCR2 knock-in (hCCR2) db/db mice (age 12-19 weeks) or to uni-nephrectomized human CCR2 knock in mice placed on a high fat/ high protein diet for 36 weeks (hCCR2 DIO). Assessments included body weight, fasting plasma glucose, serum clinical chemistry, and 24 hr urinary volume and output of albumin, creatinine and glucose.

Results: Treatment with CCX140 significantly reduced urinary albumin excretion (UAER) and albumin:creatinine ratio (ACR) in both hCCR2 db/db mice as well as uni-nephrectomized hCCR2 DIO mice. Statistically significant improvements in UAER and urinary ACR were noted as early as 5 days after initiation of CCX140 treatment. The benefits seen on renal function preceded significantly reduced fasting plasma glucose levels.



Conclusions: Robust and rapid improvements of albuminuria and hyperglycemia were seen following pharmacological intervention with the clinical molecule CCX140 in two rodent models of diabetic nephropathy. These results support the continued clinical evaluation of CCX140-B for the treatment of diabetic nephropathy.

Funding: Pharmaceutical Company Support - ChemoCentryx, Inc.

SA-PO476

An Oral Adsorbent AST-120 Decreased Proteinuria and Albuminuria in Metabolic Syndrome/Diabetes Rats Ryoko Tateoka, Yoshiharu Itoh, Fujio Sekine, Kaori Kikuchi, Hideyuki Yamato. *Kureha Corporation, Tokyo, Japan.*

Background: An oral sorbent AST-120 is clinically used in Japan for the treatment of chronic kidney disease(CKD) patients to slow the progression of CKD. There is little clinical evidence when AST-120 should be prescribed for subjects with early stage overt diabetic nephropathy. In this research, we examined the effect of AST-120 on the early stage of nephropathy using SHR/NDmc-cp, a rat model of metabolic syndrome/ type 2 diabetes. In addition, we applied the metabolomic analysis in the serum of normal and SHR/NDmc-cp rats with or without AST-120 by capillary electrophoresis mass spectrometry with time-of -flight(CE-TOFMS).

Methods: Male SHR/NDmc-cp(SHR/ND) rats, aged 7 weeks, were administered AST-120 with the chow containing 0%, 8% for 16 weeks. WKY rats were used as a normal. Every 4 weeks, serum and 24-hour urine samples were collected for biomedical studies. We analyzed the serum metabolites in normal and SHR/ND rats with or without AST-120 for 8 weeks by CE-TOFMS and applied CE-TOFMS data to principal component analysis(PCA).

Results: AST-120-administered SHR/ND rats showed significantly lower level of urinary albumin excretion and urinary protein excretion as compared with SHR/ND rats. PCA score plot showed clear separation among three groups(Normal, SHR/NDand AST-120-administered SHR/ND). Normal group and SHR/ND group could be distinguished by a line of PC1. SHR/ND group and AST-120-administered SHR/ND group could be distinguished by a line of PC2. The PC1 and PC2 were supposed to reflect the effect of disease and AST-120, respectively. In addition, we could detect 40 metabolites which accumulated in the serum of SHR/ND rats, and their serum levels were reduced by administration of AST-120. In these metabolites, o-hydroxybenzoic acid, hippuric acid and indole-3-acetic acid had the highest loading on PC2.

Conclusions: AST-120 decreased proteinuria and albuminuria in SHR/ND rats. It indicated that the administration of AST-120 at an early stage has a protective effect on the progression of diabetic nephropathy. We could detect 40 metabolites of which serum levels were increased in SHR/ND rats as compared with normal rats, and were reduced by administration of AST-120.

SA-PO477

The Role of Adenosine Generation and Signaling in Diabetic Nephropathy Douglas Ridyard, Eunyoung Tak, Almut Grenz. *Anesthesiology, UC Denver, Denver, CO.*

Background: DN is the leading cause of end-stage renal failure in developed countries. Only limited therapeutic approaches are available, including dialysis or kidney transplantation. Therefore, novel therapeutic approaches to prevent or at least treat DN are presently an area of intense investigation. Adenosine represents an endogenous signaling molecule to balance inflammatory reactions under different pathophysiological conditions. Extracellular adenosine is derived mainly via phosphohydrolysis of adenosine 5'-monophosphate (AMP) by the ecto-5'-nucleotidase (CD73). Once generated into the extracellular space, adenosine can activate four individual adenosine receptors (A1AR, A2AAR, A2BAR and A3AR). At present, the role of adenosine generation and signaling in DN is unknown.

Methods: At 8 weeks of age, mice received daily streptozotocin injections intraperitoneally (50 mg/kg, made fresh in 0.1 M citrate buffer, pH 4.5) for 5 consecutive days. Vehicle-injected mice served as controls. The fasting level of blood glucose was examined weekly with a B-Glucose Analyzer. The mice were considered diabetic if the blood glucose concentration (BGC) was above 500 mg/dl.

Results: Sixteen weeks after induction of STZ-induced diabetes histological evaluation by PAS staining showed significantly more glomerular sclerosis in CD73-/- compared to WT controls. Furthermore we could show a selective induction of the renal A2B adenosine receptor transcript and protein 16 weeks following STZ-induced diabetes. Treatment with an A2BAR agonist (BAY60-6583) via Alzet pumps in wild type STZ-induced mice ameliorated the severity of apoptosis thereby indicating a protective role of extracellular adenosine by stimulating the A2BAR. Interestingly we could show an increase of the A2BAR in renal vessels during STZ-induced diabetes mellitus in an A2BAR reporter mouse by utilizing XGal staining.

Conclusions: In summary we show that adenosine generation by CD73 and stimulation of the endothelial A2BAR ameliorates renal damage occurring during diabetic nephropathy. We hope these studies will lay the groundwork for novel and specific therapeutic approaches in the treatment of DN, which are urgently needed for improving the outcome of Type 1 diabetic patients.

SA-PO478

High Glucose Mediates Endothelial-to-Chrondrocyte Transition in Human Aortic Endothelial Cells Rining Tang, Min Wu, Hong Liu, Xiaoliang Zhang, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University, Nanjing, Jiangsu, China.*

Background: Vascular calcification is the common complications in diabetes mellitus. Many studies showed high glucose (HG) could cause cardiovascular calcification, but its underlying mechanism is not understood. Recent studies showed that conversion of endothelial-to-mesenchymal transition (EndMT) into mesenchymal stem cells (MSCs) could be triggered into chondrocytes. Our previous research indicated that HG induced EndMT in human aortic endothelial cells (HAECs).In this study, we firstly addressed the questions of whether HG-induced endothelial mesenchymal transition (EndMT) could be transitioned into chondrocytes and be involved in vascular calcification.

Methods: HAECs were divided into three groups: a normal glucose (NG) group, HG group (30mmol/L), and mannitol (5.5 mM NG + 24.5 mM) group. Immunofluorescence staining was performed to detect the co-expression of CD31 and fibroblast markers, such as fibroblast-specific protein 1 (FSP1). The expressions of FSP1 and α-SMA were detected by PCR and Western blot. Endothelial-derived MSCs were grown in MSC medium for one week. The expressions of MSCs marker STRO-1, CD44 and chondrocyte marker SOX9 were detected by immunofluorescence staining and Western blot. Chondrocyte expression was detected by alcian blue staining.

Results: The incubation of HAECs exposure to HG resulted in a fibroblast-like phenotype, wherein increased microfilamentation and a roughened endoplasmic reticulum structure were observed in the cytoplasm. Double staining of the HAECs indicated a co-localization of CD31 and FSP1. The expressions of FSP1 and α-SMA were significantly increased in the HG group and the undergoing EndMT cells also showed expression of the STRO-1, CD44 and SOX9 comparing to controls($P<0.05$). And alcian blue staining in HG group was positive comparing to NG group. Specifically, we showed that HG-induced EndMT is accompanied by activation of the canonical snail pathway.

Conclusions: Our study demonstrated that HG could induce endothelial cell transdifferentiation into chondrocyte-like cells via EndMT, which is mediated in part by activation of the snail signaling pathway.

Funding: Government Support - Non-U.S.

SA-PO479

Hepatocyte Growth Factor Switch the Balance M1/M2 Inducing Renal Repair in a Mouse Model of Diabetic Nephropathy Maria Flaquer,[1] Marcella Franquesa,[1] August Vidal,[2] Nuria Bolanos,[1] Joan Torras,[1] J. Grinyo,[1] Josep M. Cruzado.[1] *[1]Experimental Nephrology, IDIBELL, Hospitalet de Llobregat, Barcelona, Spain; [2]Pathology Service, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain.*

Background: Experimental studies have demonstrated the involvement of two different subtypes of macrophages (MØ) in kidney disease. M1 is classically activated by pro-inflammatory cytokines and can induce renal fibrosis. On the other hand, M2, induced by anti-inflammatory cytokines, has been associated with kidney repair and regeneration. The aim of our study was to evaluate whether an HGF-induced anti-inflammatory environment influences the presence of MØ subtypes and tissue regeneration in a mouse model of diabetic nephropathy.

Methods: Db/db mice received bone marrow transplantation (BMT) from C57BL/6J–EGFP+ transgenic donor mice and were divided in different groups (n=10-12/group):db/db–BMT (diabetic animals with BMT);db/db+HGF (diabetic animals with BMT and HGF treatment);db/db-G-CSF (diabetic animals with BMT and G-CSF treatment);db/db+HGF+G-CSF (diabetic animals with BMT,HGF and G-CSF treatment). We used db/-(non-diabetic animals) and db/db (diabetic animals) as age-matched control groups.After a follow-up of 4 weeks,renal samples were collected for MØ, M1, M2 and Bowman's Capsule parietal epithelial cells(PECs) histological analysis. Blood samples were also collected and pro-inflammatory and anti-inflammatory serum cytokine expression was analyzed by cytometric bead array.

Results: HGF reduced pro-inflammatory cytokines (IFNγ, TNFα, IL-6) and enhanced anti-inflammatory cytokines (IL-4, IL-10) in peripheral blood. After HGF treatment, we found MØ mainly around glomeruli and some in the interstitial area. Nearly all of the MØ expressed $EGFP^+$, which means that these cells derived from bone marrow. Moreover, M2 periglomerular MØ were enhanced by HGF. There was also a small number of $EGFP^+$ PECs in HGF-treated groups, suggesting MØ-PECs cell fusion. Altogether, HGF was associated with glomerulosclerosis reduction and podocyte preservation.

Conclusions: HGF induces an anti-inflammatory environment that promotes M2 MØ differentiation which may participate in renal repair in diabetic nephropathy.

SA-PO480

Association between Major Depression and Chronic Kidney Disease in an Outpatient Diabetic Population Margaret K. Yu,[1] Bessie A. Young.[1,2] *[1]Nephrology, University of Washington, Seattle, WA; [2]Nephrology, VA Puget Sound Healthcare System, Seattle, WA.*

Background: Depression is an independent predictor of mortality in end-stage renal disease. Little is known about the impact of depression in a general primary care population with diabetes and chronic kidney disease (CKD) who are not yet on dialysis. The objective of this study is to evaluate the association between major depression and CKD in an outpatient diabetic cohort.

Methods: The Pathways Study is a prospective cohort of ambulatory, diabetic patients from a large managed care population in Seattle, WA. Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI equations. CKD was defined as an eGFR <60 ml/min/1.73 m^2 or microalbuminuria (urine albumin/creatinine >30 mg/g). Patients with Patient Health Questionnaire (PHQ-9) scores ≥10 including either depressed mood or loss of interest were considered to have major depression. Logistic regression was used to determine the association between major depression and CKD, adjusting for age, gender, race, marital status, education, hemoglobin A1c, smoking, and body mass index ≥30 kg/m^2.

Results: 546 of the 4,757 total subjects met criteria for major depression (11.5%). Subjects with major depression tended to be younger, were more likely to be female, and less likely to be married compared to subjects without depression. Major depression was associated with smoking, higher hemoglobin A1c, diabetes complications, and obesity. Patients with CKD had a higher prevalence of major depression than patients without CKD (12.9% vs. 10.5%, p=0.03). Major depression was associated with 35% greater odds of

CKD (95% CI 1.06-1.71), which was primarily associated with microalbuminuria and not eGFR <60 ml/min/1.73 m^2 (microalbuminuria OR 1.48, 95% CI 1.19-1.84; eGFR <60 ml/min/1.73 m^2 OR 1.17, 95% CI 0.86-1.60).

Conclusions: The prevalence of major depression in patients with pre-dialysis CKD was 12.9% in this study. Major depression was associated with greater odds of CKD, which is primarily accounted for by the strong association between depression and microalbuminuria. Further investigations into the effect of depression on CKD progression in diabetes are warranted.

Funding: NIDDK Support, Other NIH Support - NIMH, Veterans Administration Support

SA-PO481

First Ever 24-Hour Central Blood Pressure in Patients with Type 1 Diabetes Simone Theilade,[1] Maria Lajer,[1] Christel Joergensen,[1] Frederik I. Persson,[1] Gudbjörg Andrésdóttir,[1] Henrik Reinhard,[1] Stine Nielsen,[1] Peter S. Lacy,[2] Bryan Williams,[2] Peter Rossing.[1,3] *[1]Steno Diabetes Center, Denmark; [2]Department of Cardiovascular Sciences, University of Leicester, United Kingdom; [3]Aarhus University, Aarhus, Denmark.*

Background: We investigated the association between 24-hour ambulatory central blood pressure and diabetes duration, albuminuria and complications in patients with type 1 diabetes (T1DM).

Methods: Cross-sectional study including 86 controls (C), 69 patients with short diabetes duration (<10 years), normoalbuminuria (<30mg/24-hour) and not receiving antihypertensive treatment (SN); 211 with longstanding diabetes (≥10 years) and normoalbuminuria (LN); 163 with microalbuminuria (30-300mg/24-hour) (Mi) and 186 with macroalbuminuria (>300mg/24-hour) (Ma). 24-hour central aortic systolic pressure (CASP) was measured with tonometry (Bpro, HealthStats, Singapore), previously validated according to ESH and AAMI. Central dipping (CD) was percentage decrease in BP from day- to night time (12pm-6am).

Results: For the groups (C, SN, LN, Mi, Ma): mean±SD age was 49±12; 39±13; 57±11; 58±13 and 55±10 years; diabetes duration: nil; 6±3; 38±11; 35±15 and 38±11 years; and eGFR; 96±16; 107±21; 90±20; 85±26; and 62±29 ml/min/1.73m^2; (p<0.001 for all). Mean 24-hour CASP was 114±17; 115±13; 121±13; 119±16 and 122±14mmHg; (p<0.001). CD was 10.0±5.9; 11.1±7.9; 8.7±6.1; 8.9±6.6 and 6.6±6.6%; (p<0.001). Following multivariate adjustment (age, gender, 24-hour mean arterial pressure, 24-hour heart rate, eGFR) 24-hour CASP increased and CD decreased with diabetes duration and albuminuria (p<0.001 and =0.013). Furthermore, 24-hour CASP was higher in patients with CVD, LVH, retinopathy and autonomic neuropathy (AN), and CD was lower in patients with CVD and AN, independent of covariates (p<0.05 for all).

Conclusions: 24-hour CASP measurements were feasible in T1DM. 24-hour CASP increased and CD decreased with diabetes duration and albuminuria. Furthermore, 24-hour CASP increase while CD decrease in presence of diabetic complications, independently of covariates.

SA-PO482

Arterial Stiffness Is Associated with Cardiovascular, Renal, Retinal and Autonomic Disease in Type 1 Diabetes Simone Theilade, Maria Lajer, Frederik I. Persson, Christel Joergensen, Peter Rossing. *Steno Diabetes Center, Denmark.*

Background: In patients with type 1 diabetes, we investigated the association between arterial stiffness and diabetic complications.

Methods: Cross-sectional study including 676 Caucasian patients with type 1 diabetes (349(55%) men, mean±SD age 54±13 years) and 51 non-diabetic controls (28(55%) men, age 47±13 years).

Aortic pulse wave velocity (PWV) was measured with SphygmoCor (AtCor Medical, Australia) on 635 patients and all 51 controls.

Results: PWV (mean±SD) in patients and controls were 10.4±3.4 and 7.6±1.9 m/s, respectively (p<0.001).

Following multivariate adjustment, PWV correlated with age, diabetes duration, urinary albumin excretion rate, heart rate and blood pressure (p<0.05 for all). ANCOVA was used for comparisons between groups, adjusted for gender, age, eGFR, heart rate, HbA_{1c} and 24-hour mean arterial pressure.

PWV in normo-, micro- and macroalbuminuric patients was 9.5±3.2; 11.0±3.6 and 11.4±3.0 m/s, respectively (adjusted p<0.001). PWV in patients with *vs.* without previous cardiovascular disease was 12.1±3.5 *vs*. 10.0±3.2 m/s, respectively (adjusted p<0.001). PWV in patients with high (≥140/90 mmHg) *vs.* intermediate (130-40/80-89 mmHg) and low (<130/80 mmHg) blood pressure was; 11.8±3.6; 10.0±3.0 and 9.8±3.3 m/s, respectively (adjusted p<0.001). Furthermore, PWV increased with increasing degree of retinopathy 8.0±2.5 (nil); 10.0±2.8 (simplex); 12.1±3.5 (proliferative) and 12.7±2.4 (blind) m/s, respectively (adjusted p<0.001). Finally, PWV increased with abnormal heart rate variability 11.5±3.3 *vs.* 10.1±3.1 (borderline) and 8.1±2.1 (normal) m/s, (adjusted p=0.027).

Conclusions: Arterial stiffness increased with presence and duration of type 1 diabetes. Furthermore, PWV increased with all the investigated diabetic complications (cardiovascular, renal, retinal and autonomic disease) independently of other risk factors.

SA-PO483

Ultrasonographic Resistive Index Could Noninvasively Predict Renal Outcome in the Patients with Diabetic Nephropathy Koji Harada,[1] Kouichi Sumida,[1] Hiroshi Shintani,[1] Yukinari Yamaguchi,[1] Yasuhiro Akai.[2] *[1]Department of Nephrology, Rakuwakai-Otowa Hospital, Kyoto, Japan; [2]Center for Postgraduate Training, Nara Medical University, Kashihara, Nara, Japan.*

Background: Performing renal biopsy in the patients with diabetic nephropathy (DMN) is important because it could demonstrate the tubulointerstitial and vascular damages, which are pivotal markers for the renal and overall outcome, as well as definite histological diagnosis. But performing renal biopsy is not feasible for all the patients with DMN. Ultrasonographic marker of resistive index (RI) is reported be useful in noninvasively detecting renal histological damages. We conducted this study to evaluate the significance of RI in detecting tubulointerstitial and vascular injuries in diabetic patients with proteinuria/renal impairment using renal biopsy as a standard.

Methods: Forty-five patients with type 2 diabetes mellitus (DM) and proteinuria/renal impairment were enrolled in this study. Renal biopsy was performed for all the patients in order to obtaining definite renal diagnosis.

Results: Renal biopsy revealed that 32 patients (71.1%) had diabetic lesions and were diagnosed as DMN. Patients with DMN had severer tubulointerstitial (p=0.0281) and vascular (p=0.0003) injuries than those without histological diabetic changes. To evaluate the significance of RI, we examined the correlation between histological severity index of tubulointerstital and/or vascular damages and RI. The severity of vascular damage was significantly correlated with RI (Spearman rank correlation [r_s] = 0.322; p = 0.0325), irrespective of diabetic renal changes. Notably, RI was significantly correlated with the severity index of tubulointerstitial (r_s = 0.388; p = 0.0308) and vascular (r_s= 0.525; p = 0.0035) injuries in the patients with DMN.

Conclusions: RI could reflect the tubulointerstitial and vascular damages and could serve as a useful index to determine the renal and overall prognosis in the patients with DMN.

SA-PO484

Albuminuria and Arterial Stiffness in Type 2 Diabetics with Well-Preserved Renal Function Lee Ying Yeoh, Xiaowei Ng, Su-chi Lim. *Medicine, Khoo Teck Puat Hospital, Singapore.*

Background: Objective of this study is to determine if various cardiovascular risk factors and measures of arterial stiffness were associated with the degree of albuminuria in type 2 diabetes patients (T2DM) with preserved renal function.

Methods: This is a cross-sectional study which recruited T2DM clinic patients from July 2011 to April 2012. Pulse wave velocity (PWV) was measured by applanation tonometry (SphymoCor®) to estimate aorto-iliac arterial compliance. Albuminuria was quantified using spot urine albumin creatinine ratio. The patients were stratified into 3 groups based on albuminuria: normo-albuminuria (< 30ug/mg), micro-albuminuria (30-300 ug/mg) and macro-albuminuria (>300ug/mg). Univariate analysis using ANOVA and multivariate using linear regression model were performed.

Results: There were 635 T2DM patients, 50.2% male, 51.7% Chinese, mean age was 56.7 ±11.5 years and mean GFR (MDRD equation) was 92.9 ± 37.0 ml/min/1.73m^2. Higher pulse pressures (PP), $HbA1_C$, total cholesterol, triglyceride, PWV and augmentation index were associated with increasing albuminuria.

	Normo-albuminuria (N=332) 10.3 ± 8.3ug/mg	Micro-albuminuria (N=113) 98.0 ± 72.1ug/mg	Macro-albuminuria (N=102) 1364 ± 1190 ug/mg	p (ANOVA)
PP (mmHg)	56.7 ± 14.2	59.3 ± 15.2	74.0 ±18.0	< 0.001
HbA1c (%)	7.7 ± 1.3	8.0 ± 1.3	8.1 ± 1.3	0.002
Total cholesterol (mmol/l)	4.41 ± 0.91	4.42 ± 0.84	4.85 ± 1.15	< 0.001
Triglyceride (mmol/l)	1.48 ± 0.68	1.76 ± 1.31	2.16 ± 2.15	< 0.001
PWV (m/s)	9.27 ± 2.63	10.00 ± 2.64	12.59 ± 14.14	< 0.001
Augmentation index	24.07 ± 26.51	26.17 ± 29.11	26.76 ± 31.47	0.003

PP, PWV, total cholesterol and triglyceride remained significantly associated with albuminuria using multivariate analysis (p< 0.05). For each 1 mmHg increase in PP and each 1m/s increase in PWV, there was a 10.5 ug/mg (95%CI: 2.5-18.6) and 10.7ug/mg (95%CI: 7.5-13.9) increase in albuminuria respectively.

Conclusions: In T2DM patients with normal renal function, measures of arterial stiffness such as PP and PWV, as well as more the traditional risk factor cholesterol, were significantly associated with the amount of albuminuria. Vascular effects on albuminuria seem to precede the decline in GFR.

SA-PO485

Adipokines: The Cinderella of Cardiovascular Risk in Type 2 Diabetic Patients with Nephropathy Ana Paula Silva, André Fragoso, Anabela Malho, Cláudia Silva, Nélio Santos, Marilia Faisca, Pedro Neves. *Nefrologia, Hospital de Faro, Portugal.*

Background: Epidemiologic studies have demonstrated that the carotid intima media thickness (CIMT) is a strong, independent predictor of cardiovascular events in both the general population and among those with renal disease.

Recently, it was also showed that several adipokines, oxidative stress, inflammation and abnormalities of mineral metabolism are associated with arterial media calcification. The purpose of this study was to determine the relationship between these new risk factors with the carotid intima-media complex thickness.

Methods: In this cross- sectional study we included 78 type 2 diabetic patients (f = 30, m = 48), with a mean age of 61 years and a mean estimated glomerular filtration rate (MDRD) of 43.5 ml / min, followed in our outpatient nephrology clinic. The right and left common carotids and internal carotids were measured using high-resolution-B-mode ultrasonography.

We analyzed several laboratory parameters, such as: interleukin 6 (IL6), adipokines (visfatin, resistin, adiponectin (APN), oxidative stress (oxLDL), mineral metabolism (PTH, phosphorus, FGF-23, 25 (OH)D3), renal function as well as the carotid intima media thickness.

Results: In a simple linear regression model the CIMT was positively correlated with, age (r=0.113 p=0.006), phosphorus (r=0.592 p=0.0001), PTH (r=0.601 p=0.0001), FGF-23 (r=0.663 p=0.0001), IL6 (r=0.763 p=0.0001), oxLDL (r=0.819 p=0.0001), resistin (r=0.944 p=0.0001), visfatin (r = 0.912 p=0.0001), and inversely with eGFR (r= - 0.388 p=0.0001), APN (r= - 0.842 p=0.0001), 25 (OH) D3 (r= - 0.907 p=0.0001).

In a multiple regression model, resistin (r=0.415 p=0.0001), visfatin (r=0.200 p=0.0015), 25 (OH)D3 (r= - 0.206 p=0.006) and APN (r= - 0.162 p= 0.003), independently influenced the CIMT (R^2 =0.890 p=0.0001).

Conclusions: In our study we found that vitamin D and adipokines are new biomarkers preditors of the carotid intima media thickness in type 2 diabetic with mild to moderate kidney disease.

Even in the early stages of diabetic nephropathy, several are the factors associated with CIMT, such as inflammation, oxidative stress, adipokines and the mineral metabolism.

Funding: NIDDK Support

SA-PO486

Risk Factors of Subclinical Atherosclerosis in Type 2 Diabetic Patients with Nephropathy André Fragoso, Ana Pinho, Anabela Malho, Cláudia Silva, Nélio Santos, Ana Paula Silva, Pedro Neves. *Nephrology Department, Hospital de Faro, Faro, Portugal.*

Background: Carotid Intima-Media Thickness (CIMT) is an early marker of atheromatous disease and a strong predictor of cardiovascular (CV) events. Recently, the disturbances of the mineral metabolism and oxidative stress were also related to CV disease, even in the early stages of CKD. The purpose of this study was to determine the relationship between these new risk factors with the CIMT.

Methods: In a cross-sectional study, we included 78 type 2 diabetic patients (61.5% males) with CKD stages 3 and 4. The mean age was 66 years and the mean eGFR was 43,53ml/min. We analyzed mineral metabolism (PTH, 25(OH)D3, FGF23, phosphorus, calcium), inflammation (IL-6), oxidative stress (oxLDL) and lipid profile. We measured the CIMT using the high-resolution B-mode ultrasonography and our population was divided in two groups according to that parameter: G-1(CMIT<0,9mm n=42) and G-2 (CMIT≥0,9mm n=36). In the analysis we used for comparison between groups the Student's t-test. To evaluate the factors that influenced the CIMT we used a multiple regression model and the receiver operating characteristic (ROC) curve analysis.

Results: We found that G-2 patients showed higher FGF23 (327,59 vs 58,07ng/ml p=0,0001), PTH (204,84 vs 77,56 µg/ml p=0,0001), Phosphorus (5,08 vs 3,66mg/dl p=0,0001), oxLDL (65,01 vs 38,05mg/dl p=0,0001), IL6 (9,19 vs 3,42pg/ml p=0,0001) and lower 25(OH)D3 (11,56 vs 26,21ng/ml p=0,0001) and eGFR (33,43 vs 52,19ml/min p=0,0001). In a multiple regression model, IL6 (r=0.288 p=0.003), PTH (r=0.289 p=0.004), 25(OH)D3 (r=-0.294 p=0.018) and oxLDL (r=0.294 p=0.001) independently influenced the CIMT (R^2=0.856). ROC curve analysis showed that PTH (AUC=0,915; p=0.0001), IL6 (AUC=0,942; p=0.0001) and oxLDL (AUC=0,984; p=0.000) are predictors of CIMT (cut-off=0.9mm).

Conclusions: Our results confirm that the pathophysiology of atherosclerosis is complex. Even in the early stages of diabetic nephropathy, several are the factors associated with CIMT, such as inflammation, oxidative stress and the mineral metabolism. Further studies are needed to validate the clinical significance role of these new markers in the pathogenesis of atherosclerosis.

SA-PO487

Insulino Resistance, Inflammation and Vitamin D: New Predictors of Left Ventricular Hypertrophy André Fragoso, Ana Pinho, Anabela Malho, Nelson Almeida Tavares, Marilia Faisca, Ana Paula Silva, Pedro Neves. *Nephrology Department, Hospital de Faro, Faro, Portugal.*

Background: Left ventricular hypertrophy (LVH) is an independent predictor of cardiovascular disease in type2 diabetics with CKD. Although the etiologies for altered myocardial structure and function in CKD are multiple, there is a bulk of evidence suggesting that disturbances of mineral metabolism, inflammation, oxidative stress and insulin resistance may play important roles. The aim of this study was to evaluate how these new players are related to LVH.

Methods: In this cross-sectional study, we included 92 type2 diabetic patients (m=54), with a mean age of 62years and a mean eGRF of 42ml/min. At baseline, several laboratory parameters were analyzed: insulin resistance (HOMA-IR), inflammation (IL-6), mineral metabolism (phosphorus(P), calcium, PTH, vitaminD, FGF23), oxidative stress (oxLDL), adiponectin and albumin-creatinine ratio. We evaluated the mean arterial pressure (MAP) and the left ventricular mass index (LVMI) was calculated. Our population was divided in two groups: G-I (n=44) without LVH and G-II (n= 48) with LVH stratified according to gender. For comparison between groups we used the Student's t-test and a multiple regression model was used to determine predictors of risk for LVH.

Results: We found that G-II was older (65*vs*59 p=0.0001) and showed higher levels of P (5.1 *vs* 3.7mg/dL p=0.0001), PTH (212.5 *vs* 77.08mg/dL p=0.0001), FGF23 (306.1 *vs* 62.9 Ru/mL p=0.0001), IL6 (8.7 *vs* 3.1pg/mL p=0.0001), oxLDL (59.3 *vs* 28.1U/L p=0.0001), HOMA-IR (3.5 *vs* 0.69 p=0.0001), albumin-creatinine ratio (265.5 *vs* 119.9 p=0.0001) and MAP (96.0 *vs* 90.4mmHg p=0.032); G-II also showed lower levels of 25(OH)D3 (12.4 *vs* 25.9 ng/mL p=0.0001) and adiponectin (12.8 *vs* 43.6 p=0.0001). In the multiple regression model we found that HOMA-IR (r=0.280 p=0.009), IL6 (r=0.208 p=0.0 44) and 25(OH)D3 (r=-0.263 p=0.020) independently influenced the LVMI (R^2=0.870 p=0.0001).

Conclusions: In our study inflammation, insulin resistance and insufficiency/deficiency of vitaminD are independent predictors of LVH. Further prospective studies are required to clarify if the control of these players is associated with the regression of the LVH in CKD.

SA-PO488

Insulin Resistance: Marker or Mediator of Increased Cardiovascular Risk in Diabetic Nephropathy? André Fragoso, Ana Pinho, Anabela Malho, Nelson Almeida Tavares, Marilia Faisca, Ana Paula Silva, Pedro Neves. *Nephrology Department, Hospital de Faro, Faro, Portugal.*

Background: Cardiovascular disease is the main risk factor of morbidity and mortality in Chronic Kidney Disease (CKD) patients and its relevance increases when diabetes is the etiology of the renal disease. Insulin resistance is associated with the progression of atherosclerosis and has been reported to be a strong risk factor for cardiovascular disease. The purpose of this study was to examine the usefulness of insulin resistance as a predictor of cardiovascular mortality.

Methods: We followed during a period of 56 months 119 type 2 diabetic patients in stage 3 and 4 of CKD without history of cardiovascular disease at the beginning of the study. Several laboratory parameters were analyzed: hemoglobin, albumin, estimated glomerular filtration rate (MDRD), mineral metabolism, markers of inflammation (interleukin-6), insulin resistance (HOMA-IR) and the left ventricular mass index (LVMI). According with the presence or absence of cardiovascular mortality our population was divided in two groups: G-1 with cardiovascular mortality (n=48) and G-2: survivors (n=71).

Results: We found that G-1 patients showed higher age (67,08 vs 59,85 years, p=0,002), iPTH (204,41 vs 83,78pg/ml, p= 0,0001), phosphorus (4,92 vs 3,92mg/dl, p= 0,0001), Interleukin-6 (8,27 vs 2,83pg/ml, p= 0,0001), HOMA-IR (3,79 vs 0,77, p= 0,0001), LVMI (130,08 vs 93,52g/m, p= 0,0001) and lower Hg (12.32 vs 12,99g/dl, p=0,047), Albumin (4,05 vs 4,30g/dl, p=0,016), eGFR (33,66 vs 52,45ml/min, p=0,0001). In multivariate Cox proportional hazard stepwise LR to identify independent risk factors of cardiovascular mortality iPTH, HOMA-IR and LVMI were found to predict patient survival, (HR= 0.4, 95% CI, 0.1 to 0.6, p=0.015), (HR= 38, 95% CI, 8.7 to 75.3, p=0.008) and (HR=4.7, 95% CI, 2.4 to 7, p=0.0001), respectively.

Conclusions: In our study, insulin resistance is an important risk factor for cardiovascular mortality in diabetic patients in stage 3 and 4 of chronic kidney disease. Further studies are needed to better understand the mechanisms that link insulin resistance to cardiovascular morbidity e mortality.

SA-PO489

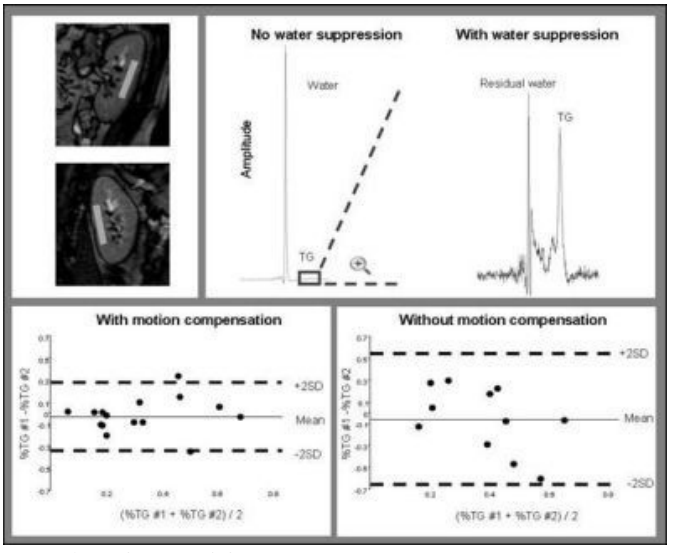
Metabolic Renal Imaging Using Proton Magnetic Resonance Spectroscopy (1H-MRS) In-Vivo Aiko P.J. De Vries,[1] Sebastiaan Hammer,[2] Ton J. Rabelink,[1] Hildo Lamb.[2] *[1]Nephrology; [2]Radiology, Leiden University Medical Center, Netherlands.*

Background: Lipid accumulation in kidney (*Fatty Kidney*) is a putative biomarker for obesity-associated renal disease. Clinical research is hampered, however, by the lack of a non-invasive method to assess Fatty Kidney. We aimed to develop a protocol for quantification of renal triglyceride (TG) content by 1H-MRS in-vivo, and test feasibility, reproducibility, and necessity of respiratory motion compensation.

Methods: Measurements were performed on a 1.5T MR scanner (Philips, Netherlands) using a 10cm surface coil. Spectral quality was quantified as the 'Full Width at half Maximum' (FWHM) of the unsuppressed water signal. Cortical TG content of left kidney was measured using single voxel 1H-MRS in 15 healthy young subjects. Water suppressed & unsuppressed spectra with(out)compensation for respiratory motion using navigator echoes were collected. Subjects were removed and repositioned to determine reproducibility. Spectra were fitted using dedicated software. TG content at 1.3 ppm was expressed as a percentage of the water signal.

Results: Spectral quality without compensation was 10.8±0.4 Hz (mean±SE) and improved to 10.2±0.4 Hz with use of respiratory motion compensation (P<0.05). **Mean TG content with use of compensation was 0.34±0.05%, and 0.31±0.05% after repositioning (P>0.05).** Mean TG content without respiratory motion compensation was 0.42±0.08%, and 0.36±0.05% after repositioning, P>0.05 compared to navigator measurements (4 spectra without motion compensation could be no be fitted due to insufficient quality). Use of respiratory motion compensation showed more narrow limits of agreement in Bland-Altman analyses.

Conclusions: In-vivo assessment of *Fatty Kidney* by 1H-MRS is feasible to non-invasively study its role as a biomarker for obesity-related renal disease. Respiratory motion compensation improves spectral quality and reproducibility.



Funding: Private Foundation Support

SA-PO490

Early Renal Function Decline in Non-Proteinuric Patients with Type 1 Diabetes Estimated by Serial Measurements of Serum Cystain C and Creatinine Jan Skupien,[1] Monika A. Niewczas,[1] Adam Smiles,[1] Rita R. Holak,[1] Kevin Patrick Mcdonnell,[1] John H. Eckfeldt,[2] James Warram,[1] Andrzej S. Krolewski.[1] *[1]Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA; [2]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.*

Background: Renal function decline can be observed when patients have normo- or microalbuminuria. We compared renal function decline estimated using cystatin C or creatinine.

Methods: We followed 230 patients with normoalbuminuria and 325 with microalbuminuria for median 6.4 years with annual determinations of serum cystatin C and creatinine. We estimated rates of renal function decline (linear decline expressed as annual percent change) from glomerular filtration rate (eGFR) calculated with Stevens (cystatin C) and CKD-EPI (creatinine) formulas. To assess effects of clinical predictors on slopes we used mixed effects models.

Results: Median renal function decline of creatinine-eGFR was 1.25% per year and 2.25% per year of cystatin C-eGFR. Using a threshold 3.3% decline per year, 22% of patients were decliners with creatinine slopes and 38% with cystatin C slopes. There was also only moderate rank correlation between the two slopes, 0.47. A linear mixed effects model in which both creatinine and cystatin C eGFR decline were modeled simultaneously eliminated within-patient inconsistency of rates of decline. Cystatin C-based eGFR decline was, however, systematically steeper than the creatinine-based by 1.0% ($p<0.0001$). Impact of clinical predictors on two slopes is shown in Table. We compared mean rate of decline between 1st and 4th quartile of predictors.

Predictor	Creatinine Slope	Cystatin C Slope	P for difference between two slopes
ACR	2.4% (p<0.0001)	3.3% (p<0.0001)	0.015
HbA1c	2.4% (p<0.0001)	2.8% (p<0.0001)	0.22
TNFR1	2.0% (p=0.0006)	1.2% (p=0.044)	0.05

Conclusions: Renal function decline estimated from cystatin C eGFR is faster than estimated from creatinine eGFR by 1%. Using linear mixed effect model we can eliminate discrepancies in rates of decline that arise from random variation of creatinine and cystatin C measurements. Certain clinical predictors show stronger association with either cystatin C or creatinine-based decline.

Funding: NIDDK Support

SA-PO491

Poor Glycemic Control Is Significantly Associated with Overestimation of Glomerular Filtration Rate (GFR) Assessed by Estimated GFR (eGFR): An Inulin Clearance (C_{in}) Study in Patients with Chronic Kidney Disease (CKD) Akihiro Tsuda, Eiji Ishimura, Yoshiteru Ohno, Mitsuru Ichii, Shinya Nakatani, Shinsuke Yamada, Katsuhito Mori, Masaaki Inaba. *Osaka City University Graduate School of Medicine, Osaka, Japan.*

Background: Serum creatinine levels are lower in CKD patients with diabetes mellitus (DM) than in their non-DM counterparts, leading to possibly higher values of eGFR in the former than in the latter. The extent of and the factors associated with the higher evaluation of eGFR in predialysis DM-CKD patients is not well known. The aim of the present study is to explore the factors associated with the dissociation of eGFR and C_{in} in CKD patients.

Methods: Forty eight CKD patients (age 57.7±2.1 years; 25 males and 23 females; 31 DM and 17 non-DM) were enrolled. eGFR was calculated from serum creatinine and age (Matsuo, et al. Am J Kidney Dis 2008). C_{in} was measured with 90-min. continuous infusion technique of inulin (Horio, et al. Clin Exp Nephrol 2009). Creatinine clearance (C_{cr}) was estimated by the formula of Horio et al (Clin Exp Nephrol 1997). $eGFR/C_{in}$ ratio was assessed as the dissociation, and C_{cr}/C_{in} ratio was evaluated as a parameter of tubular secretion of creatinine.

Results: Between eGFR and C_{in}, DM-CKD showed a weaker correlation ($r=0.567$, $p=0.0007$) than non-DM-CKD ($r=0.936$, $p<0.0001$). $eGFR/C_{in}$ ratio was significantly higher in DM-CKD than in non-DM-CKD (1.169±0.368 vs. 0.943±0.040, $p<0.05$), indicating higher eGFR in the former. There were significant and positive correlations between $eGFR/C_{in}$ ratio and hemoglobin A1c (HbA1c) ($r = 0.471$, $p = 0.0006$) and serum glycated albumin (GA) ($r = 0.471$, $p = 0.0025$), indicating that dissociation of eGFR and C_{in} was associated with poor glycemic control. There were significant and positive correlations between C_{cr}/C_{in} ratio and HbA1c ($r=0.426$, $p=0.0023$) and GA ($r=0.469$, $p=0.0019$), suggesting increased tubular secretion of creatinine in patients with poor glycemic control.

Conclusions: eGFR overestimated GFR as glycemic control became poorer in predialysis CKD patients. It is suggested that a factor of overestimation of eGFR in CKD patients is increased tubular secretion of creatinine affected by poor glycemic control.

SA-PO492

Effect of Diabetic Cystopathy on Decline of Renal Function in Patients with Diabetes Mellitus Sun Ryoung Choi, Youngki Lee, Min-Gang Kim, Myung Jin Choi, Jwa-kyung Kim, Soo Jin Kim, Taejin Park, Young Rim Song, Sung Gyun Kim, Ja-Ryong Koo, Hyung Jik Kim, Jung-woo Noh. *Department of Internal Medicine, Hallym University College of Medicine, Seoul, Korea.*

Background: Diabetic cystopathy is one of autonomic complications in diabetes. The identification of bladder dysfunction is critical complication of this disease. Diabetic cystopathy is characterized with impaired sensation, decreased contractility, and impaired emptying, which result in overflow incontinence. Until now, the effect of diabetic cystopathy on the progression of diabetic nephropathy is unclear. Therefore, we performed this study to evaluate the effect of post-voiding residual urine volume (PVR) on the decline of glomerular filtration rate (GFR) in type 2 diabetic patients.

Methods: We enrolled type 2 diabetes patients who were treated for more than 1 year. Exclusion criteria were as follows; cerebrovascular disease, parkinson's disease, benign prostate hyperplasia, disc herniation, and urinary incontinence, which resulted in bladder symptoms. We measured PVR by urodynamic evaluation. A PVR of greater than 50mL was defined as abnormal PVR group. We evaluate whether PVR is the risk factor for the decline of GFR.

Results: 97 patients were enrolled. Male was 34(35%). The mean age was 63.0±10.6 years. The duration of diabetes was 12.7±8.2. The portion of patients with abnormal PVR was 35.1% (n=34). The GFR was significantly lower at baseline (50.6±27.1 vs 64.3±28.1 mL/min/1.73m2, P=0.02) and had a trend to more rapid decline over 1 year (7.0±13.3 vs 2.3±11.6 mL/min/1.73m2/y, P=0.07) in abnormal PVR group than in normal PVR group. However, PVR had no relationship between duration of diabetes, hemoglobin A1c, hypertension, and proteinuria. But, there was significant correlation between PVR and decline of GFR (r=0.31, P=0.002). In the multivariate analysis, PVR was independent risk factor for decline of GFR (P=0.02).

Conclusions: Diabetic cystopathy was independent risk factor of the decline of GFR. Therefore, the active diagnosis and treatment of diabetic cystopathy may help to ameliorate the progression of GFR in T2DM.

SA-PO493

Assessment of Glomerular Filtration Rate in Chinese Patients with Type 2 Diabetes Using a Radial Basis Function Neural Network Xun Liu,[1,2] Chenggang Shi,[1] Linsheng Lv,[3] Cailian Cheng,[1] Hua Tang,[1] Xiaoming Wu,[2] Tan-qi Lou.[1] *[1]Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China; [2]College of Biology Engineering, South China University of Technology, Guangzhou, Guangdong, China; [3]Operating Room, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China.*

Background: In the previous study, a radial basis function (RBF) neural network had been established in a group of 327 chronic kidney disease (CKD) patients. In this study, we verified the accuracy of the RBF model in a group of Chinese type 2 diabetic patients.

Methods: 207 type 2 diabetic patients in the third affiliated hospital of Sun Yet-sun University, China, were enrolled. GFR was estimated by the RBF network, reexpressed 6-variable MDRD equation and reexpressed 4-variable MDRD equation.

Results: On the Bland–Altman plot, the precision of the RBF network was much less than those of the reexpressed MDRD equations. However the mean of difference and the median of difference showed that the eGFR of the RBF network more overestimated the sGFR. R-square from simple linear regression of eGFR vs. sGFR were 0.715, 0.714 and 0.689, and intra-correlation coefficients were 0.869, 0.880 and 0.895 for reexpressed 4-variable MDRD equation,reexpressed 4-variable MDRD equation and the RBF network, respectively. All agreements of the prediction models reach almost perfect. The ROC curve analysis showed that the maximum diagnostic accuracy of all the equations for the diagnosis of moderate renal failure was not statistically significant. The 30% accuracy of the RBF network was significantly higher compared with the other prediction models. The absolute differences of the RBF network was less than the other prediction models, and the 15% accuracy and 50% accuracy of the RBF network were higher than those of the other prediction models, but all without statistical significance.

Conclusions: The performances of this simple RBF network model is at lease not wore than the reexpressed MDRD equations. Especially in terms of accuracies and agreements, this RBF network performed better than the reexpressed MDRD equations.

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SA-PO494

Glycated Albumin (GA), Not Hemoglobin A1c (HbA1c), Predicts Cardiovascular Hospitalizations and Length of Stay (LOS) in Dialysis Patients with Diabetes Mariana Murea,[1] Gregory B. Russell,[2] Anthony J. Bleyer,[1] Barry I. Freedman.[1] *[1]Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Pathology and Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC.*

Background: Relative to glycated hemoglobin (HbA1c), glycated albumin (GA) more accurately predicts mortality and all-cause hospitalizations in diabetics on dialysis. We evaluate here relationships between GA, HbA1c, and casual plasma glucose (PG) with cause-specific hospitalization rates and length of stay (LOS).

Methods: Prevalent dialysis patients with type 1 (n=38) or type 2 (n=406) diabetes, mean±SD diabetes duration 18.5±10.8 years and dialysis vintage 2.9±2.6 years, were followed for 2.33 years. Monthly PG, quarterly GA, and random HbA1c were included. Hospitalization rates and LOS for cardiovascular (CV), infectious disease (ID), and vascular access (VA) complications occurring within 17days and 30 days of glycemic testing were evaluated. Best-fit, time-dependent Cox models with incremental adjustments were constructed. Fully adjusted model included age, sex, race, BMI, diabetes duration, dialysis vintage, Hb, albumin, and phosphorus.

Results: In the fully adjusted models, CV hospitalization rates were significantly associated with increasing GA (Hazard Ratio [HR] 1.32; 95%CI 1.11-1.57; P=0.002 at 17 days; HR 1.21; 95%CI 1.02-1.43; P=0.02 at 30 days) and PG (HR 1.10; 95%CI 1.02-1.17; P=0.01 at 17 days; HR 1.07; 95%CI 1.00-1.14; P=0.03 at 30 days), not with HbA1c (HR 1.24; 95%CI 0.89-1.73; P=0.21 at 17 days; HR 1.26; 95%CI 0.96-1.65; P=0.10 at 30 days). LOS for CV hospitalization positively associated with GA (HR 1.18; 95%CI 1.01-1.39; P=0.03), not PG (HR 1.04; 95%CI 0.99-1.10; P=0.15) or HbA1c (HR 1.03; 95%CI 0.92-1.15; P=0.21). ID and VA admission rates and their associated LOS did not correlate with these assays.

Conclusions: Improved glycemic control in dialysis based on GA predicted CV hospitalizations and CV-related LOS. HbA1c fails to predict any cause-specific hospitalization or LOS in dialysis. Prospective trials targeting various GA and fasting PG concentrations should be performed in patients with diabetes on dialysis to determine optimal glycemic targets to reduce CV morbidity.

SA-PO495

Relationship between HbA1c or Glycated Albumin and Circadian Blood Glucose Using Continuous Glucose Monitoring in Diabetic Patients Undergoing Hemodialysis Izumi Nyumura, Tetsuya Babazono, Michino Takagi, Naoshi Yoshida, Ko Hanai, Nobue Tanaka, Junnosuke Miura, Yasuko Uchigata. *Department of Medicine, Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.*

Background: Both glycated hemoglobin (A1C) and glycated albumin (GA) are routine markers of glycemic control in diabetic populations; however, the credibility may be undermined in uremic patients with anemia for A1C and in those with hypoalbuminemia for GA. Continuous glucose monitoring (CGM) can provide more information regarding circadian glycemic variability than intermittent self-monitoring of blood glucose. We conducted this study to compare efficacy of A1C and GA as a measure of circadian blood glucose using CGM in diabetic hemodialysis patients.

Methods: A total of 93 diabetic patients undergoing hemodialysis were studied. There were 35 type 1 and 58 type 2 diabetic patients, 44 women and 49 men, and the mean (± SD) age and duration of diabetes were 56 ± 13 years and 22±10 years, respectively. Among type 2 diabetic patients, 40 patients were treated with insulin. Interstitial glucose concentrations were monitored at least 48 hours including a 4-hour hemodialysis session. Mean glucose, standard deviation (SD) of glucose, coefficient of variation (CV) of glucose, duration of hyperglycemic time (≥ 10 mmol/l), and mean amplitude of glycemic excursions (MAGE) were calculated.

Results: The mean GA and HbA1c values were 6.4±1.3% and 22.8±7.0%, respectively. The levels of GA were significantly associated with the mean glucose (r=0.44, p<0.001), SD (r=0.53, p<0.001), CV (r=0.36, p<0.001), duration of hyperglycemia time(r=0.47, p<0.001) and MAGE (r=0.40, p<0.001). Similar association was observed between A1C and the mean glucose (r=0.28, p=0.008), SD (r=0.39, p<0.001), CV (r= 0.31, p=0.004), duration of hyperglycemia time(r=0.32, p=0.003) and MAGE (r=0.34, p=0.003); however, all these parameters were correlated more strongly with GA than HbA1c.

Conclusions: GA may more accurately reflect mean glycemic control and glycemic variability than A1C in diabetic patients undergoing dialysis.

SA-PO496

Developing a New Hemoglobin A1C Based Equation to Estimate Blood Glucose in Maintenance Dialysis Patients Junichi Hoshino,[1,2] Miklos Zsolt Molnar,[1,3] Kunihiro Yamagata,[4] Yoshifumi Ubara,[2] Kenmei Takaichi,[2] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Nephrology Center, Toranomon Hospital, Tokyo, Japan; [3]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [4]Department of Nephrology, University of Tsukuba, Ibaraki, Japan; [5]University of Tennessee, Memphis, MN.*

Background: Hemoglobin A1c (A1c) has been widely used as a clinically important assessment tool for outcome analyses related to glycemic control. However, because of special conditions in dialysis patients including the uremic milieu, there is no A1c-blood glucose (BG) equation formula specific for patients on dialysis.

Methods: We examined associations between A1c and random serum BG over time in a contemporary cohort of diabetic patients with dialysis treated in DaVita dialysis clinics. We identified 12,836 patients (63±12 years old and 50% male) with 74,330 paired measurements of A1c and BG over 5 years (2001-2006). Bootstrapping method was used to estimate average BG and corresponding A1c. The association was adjusted by patient factors using linear regression.

Results: Linear regression analyses yielded the following three regression equations, BG=52.2+29.2·A1c-18.7·Alb (model 1, R^2=0.480), BG=97.7+29.5·A1c-16.3·Alb-4.7·Hb (model 2, R^2=0.484), and BG=77.1+30.4·A1c-14.5·Alb-5.4·Hb+0.3·Age+Race (model 3, R^2=0.488). All our models showed stronger association than previous equation models (R^2=0.467 in the DCCT and ADAG equations).

Model	Formula	Adjusted R^2
DCCT	BG=35.6 HbA1c-77.3	0.467
ADAG	BG=28.7 HbA1c-46.7	0.467
Model 1 (+Alb)	BG=52.2+29.2 HbA1c-18.7 Alb	0.480
Model 2 (+Alb,Hb)	BG=97.7+29.5 HbA1c-16.3 Alb-4.7 Hb	0.484
Model 3 (complex)	BG=77.1+30.4 HbA1c-14.5 Alb-5.4 Hb+0.3 Age +Race	0.488

* Race: +0.0 if Caucasian, +2.1 if Asian, -11.5 if African American, -2.8 if Hispanic

Conclusions: The association between A1c and BG in dialysis patients is different from patients with normal kidney function. Our analysis suggests that equations including serum albumin and/or hemoglobin are better for dialysis patients.

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SA-PO497

Diabetes Mellitus Increases Cancer Specific Mortality in Patients with Renal Cell Carcinoma: Role of OGG1 and Tuberin S. Simone,[1] M. Cariello,[1] Antonio Vavallo,[1] Monica Rutigliano,[1] E. Ranieri,[2] M. Battaglia,[1] P. Ditonno,[1] Francesco Paolo Schena,[1] Loreto Gesualdo,[1] Giuseppe Grandaliano,[2] G. Pertosa.[1] *[1]Dept. of Emergency and Organ Transplantation, Univ of Bari, Bari, Italy; [2]Dept. of Medical and Surgical Sciences, Univ of Foggia, Foggia, Italy.*

Background: Cancer has been recognized as one of the main cause of mortality in diabetes mellitus (DM) patients (pts). 8-oxoG-DNA glycosylase (OGG1) is a specific DNA repair enzyme and its deficiency may increase the risk of cancer. In an animal model of DM, hyperglycemia leads to phosphorylation/inactivation of tuberin and downregulation of OGG1 via a redox-dependent Akt pathway activation in renal tubular epithelial cells. Aims of the study were to investigate whether DM may influence the survival in pts with renal cell carcinoma (RCC) and to explore the hypothesis that the diabetic milieau may lead to increased tumor aggressiveness through tuberin inactivation and subsequent OGG1 downregulation.

Methods: We enrolled 462 pts treated with radical nephrectomy between 1979 and 2009 for unilateral sporadic RCC with (Group I, n=76) and without DM (Group II, n=386) with a median follow-up of 43 months.

Results: At the time of surgery, Group I showed a larger tumor size (p=.02), a higher % of necrosis (<.001) and metastases (<.001) compared with Group II. Multivariate analysis proved that independent predictors of death from RCC included DM, TNM stage grouping, tumor size and UISS stage system. Interestingly, DM was an independent predictor of progression-free survival in pts with RCC (HR 4.1; 95% CI; 2.6-6.4, p<.0001). A significant increase (p<.05) in tuberin and S6p70 (p=.04) phosphorylation and a downregulation of OGG1 protein expression (p=.002) was observed in renal tissue samples (13) from Group I compared to Group II (immunoblotting, immunohistochemical/immunofluorescence analysis). Both normal kidney and tumor tissue samples of DM pts showed a striking increase in nuclear 8-oxo-dG levels (immunofluorescence analysis), a marker of DNA oxidation.

Conclusions: DM may significantly reduce overall and cancer specific survival in pts with RCC and this observation might be at least partially explained by the reduced expression of OGG1.

SA-PO498

Diabetes and Risk of Renal Cell Carcinoma Samy L. Habib,[1,2] Deepika Sirohi,[3] Sherry L. Werner.[1,3] *[1]Geriatric Research, Education, and Clinical Center, South Texas Veterans Healthcare System, San Antonio, TX; [2]Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX; [3]Pathology, University of Texas Health Science Center, San Antonio, TX.*

Background: Epidemiological evidence indicates that individuals with diabetes are at higher risk of cancer. Poor glycemic control in diabetic patients may play a role in increased cancer risk. We showed a strong association between diabetes and renal cell carcinoma in a cohort of kidney cancer patients. Whether renal cell cancer (RCC) and the nonneoplastic tissue morphology in diabetic and nondiabetic patients differ has not been evaluated.

Methods: A retrospective analysis of 78 patients who underwent nephrectomy for renal cell cancer was performed. Diabetic RCC and nondiabetic RCC patients were screened for age, gender, HgA1C and glucose levels. RCC subtypes were classified, Fuhrman nuclear grading was used and tumors were staged based on standard TNM system. Nonneoplastic tissue around the tumor of diabetic and nondiabetic RCC cases was assessed for morphology.

Results: Of the 78 cases with RCC, 26 (33.3%) had a history of diabetes. diabetes. Most of cases of RCC alone were male while cases of RCC+diabetes were female. There were more subjects between 50-59 years old (61.5%) in RCC+diabetes compared to subjects with RCC alone (52%). All RCC+diabetes cases showed clear cell type of RCC. In both groups, Furman nuclear grade of 2-3 predominated. The majority of RCC+diabetes showed tumor size 1-5cm (57.7%) and tumor size 5-10cm (30.1%). HgA1C values showed a negative correlation with tumor size in RCC+diabetes subjects. Importantly, tumors in nondiabetics were well-circumscribed tumors, whereas tumors in diabetics showed an infiltrative pattern. Nonneoplastic tissue in both groups showed mild to severe interstitial fibrosis and mild to severe vascular disease. Glomeruli in the diabetic patients were normal or showed basement membrane.

Conclusions: Our findings suggest that diabetes predisposes to RCC with an infiltrative pattern and that the degree of glomerular, interstitial and vascular disease was not significantly different in diabetic and nondiabetic patients.

Funding: Veterans Administration Support

SA-PO499

A Dose-Response Study to Determine the Minimal Dose of Sevelamer Carbonate that Reduces Cardiovascular Disease Risk Factors in Type 2 Diabetic Hemodialysis Patients Gary E. Striker,[1] Elena M. Yubero-Serrano,[1] Helen Vlassara.[1] *[1]Geriatrics and Medicine, Mount Sinai School of Medicine, New York, NY; [2]Epidemiology, Johns Hopkins University, Baltimore, MD.*

Background: Morbidity and mortality in hemodialysis patients (HD) due to cardiovascular disease (CVD) is directly associated with elevated inflammation and reactive oxygen species (infl/ROS). Cytopathic advanced glycation endproducts (AGEs) increase infl/ROS in T2D, and restriction of food AGEs reduces them. Sevelamer carbonate (SC) binds AGEs in food, reducing AGEs and CVD risk factors in. Calcium carbonate reduced phosphorous levels, but does not affect infl/ROS or AGEs. Ouraim was to determine the relationship(s) between increasing dose(s) of SC and cytopathic AGEs (CML) and methylglyoxal derivatives (MG), lipids, and phosphorus in T2D HD patients.

Methods: We studied serum samples from T2D HD hyperphosphatemic patients enrolled in a randomized, double-blind, placebo-controlled, dose-ranging study. After a 2 week washout of all phosphate binders patients were given a daily dose of 2.4, 4.8 or 7.2 grams of SC with meals for 3 weeks. Cytopathic AGEs, lipids, ROS and inflammation were measured as previously reported.

Results:

Serum AGEs

	Baseline	Placebo	Sevelamer (2.4 gm/d)	Sevelamer (4.8 gm/d)	Sevelamer (7.2 gm/d)
#		74	16	23	19
CML (U/ml)	30.1±14.5	30.1±9.3	30.7±7.7	22.8±8.1	22.9±8.9
p vs. baseline		N.S	N.S.	0.012	0.04
MG (nMol/ml)	3.3±1.4	3.2±1.1	3.2±1.9	2.3±1.1	2.4±0.6
p vs. baseliine		N.S.	N.S.	0.002	0.007

There was also a dose-dependent decrease (trend) for serum phosphate ($p<0.0001$), total cholesterol and LDL-cholesterol ($p=0.0001$).In addition, VCAM1 was decreased ($p=0.002$) at 7.2 gm/day. Tests for trend by dose: HDL 0.76; TC $p<0.0001$; LDL $p<0.0001$; Trigs $p=0.06$.

Conclusions: The high serum levels of cytopathic AGEs and lipids in T2D HD patients can be decreased by sevelamer carbonate within 3 weeks. Since AGEs raise risks for CVD, sevelamer carbonate treatment may be efficacious against this complication. Since 2.4 g/day did not reduce cytopathic AGEs, assuring that patients reach the 4.8 g/day dosage level may represent an important therapeutic goal.

Funding: Pharmaceutical Company Support - Sanofi/Genzyme Corporation

SA-PO500

Positive Association of Serum Levels of Dipeptidyl Peptidase-4 with Advanced Glycation End Products in Humans Kumiko Kaifu,[1] Nobuhiro Tahara,[1] Seiji Ueda,[1] Sho-ichi Yamagishi,[2] Miyuki Yokoro,[1] Nana Obara,[1] Ryotaro Ando,[1] Yosuke Nakayama,[1] Yusuke Kaida,[1] Masayoshi Takeuchi,[3] Kei Fukami,[1] Seiya Okuda.[1] *[1]Department of Medicine, Kurume University School of Medicine, Kurume, Japan; [2]Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Japan; [3]Department of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Kanazawa, Japan.*

Background: Dipeptidyl peptidase-4 (DPP-4) is an enzyme which mainly degrades incretins such as glucagon like peptide-1 (GLP-1) and is altered in a variety of inflammatory disorders. DPP-4 and GLP-1 receptor are abundantly expressed in the kidney cells. We have recently found that DPP-4 inhibitors ameliorate vascular injury in diabetic rats by blocking the harmful effects of advanced glycation end products (AGEs). However, how circulating DPP-4 level is regulated remain unknown.

Methods: We examined which anthropometric and metabolic valuables, including AGEs are independently correlated with circulating DPP-4 levels in 432 consecutive outpatients. We further investigated the effects of AGEs on DPP-4 in cultured human renal proximal tubular epithelial cells (RPTECs).

Results: Mean serum AGEs and DPP-4 levels in our patients were 8.96±2.57 U/mL and 520±33.9 ng/mL, respectively. Multiple regression analysis revealed that female ($p<0.001$), HDL-cholesterol ($p<0.001$), HbA1c ($p<0.001$), and serum AGEs ($p<0.03$) were independent determinants of DPP-4. *In vitro*, AGEs significantly increased DPP-4 expression in RPTECs and stimulated the secretion of soluble DPP-4, both of which were completely blocked by an anti-oxidant N-acetylcysteine.

Conclusions: These present results suggest that AGEs may stimulate renal DPP-4 expression via oxidative stress, which could partly explain for the positive association between serum DPP-4 and AGEs.

SA-PO501

Pre-Operative Clinical Assessment of Insulin Resistance and Pancreatic beta Cell Reserve (Insulin Sensitivity) in Renal Allograft Recepients: Prediction of Post Transplant Diabetes Mellitus Pratik Das, Soumava Gupta, Debmalya Sanyal. *Nephrology, Manjula Ben Mehta Kidney Hospital, Kolkata, West Bengal, India.*

Background: Post-transplant diabetes mellitus (PTDM) has negative impact on renal allograft survival, cardiovascular risk and patient survival. Pre-tansplant evaluation may alter the outcome.

Methods: The predictive value of a fasting plasma glucose (FPG) level, fasting serum insulin, fasting serun C peptide, HbA1C, BMI and Waist-hip ratio 2 days before transplantationon (before initiation of immunosuppression) were prospectively evaluated in 100 *de novo* nondiabetic renal allograft recipients. The data were subjected to Homa scale for insulin resistance and sensitivity (beta cell reserve). Retransplantation, cadaver transplant and patients with hepatitis B and C seropositivity were excluded. PTDM was defined as the uninterrupted need for glucose-lowering medication for at least 3 months.

Results: 15 patients (15%) developed PTDM (follow-up 18.25 ± 9.8 months). Recipient age, body mass index (BMI), biopsy-proven acute rejection (BPAR), early graft function and proteinuria, tacrolimus-based therapy, cumulative corticosteroid dose and thiazide diuretics were associated with PTDM (univariate analysis). Multivariate logistic regression analysis identified age [OR (odds ratio): 1.08 (95% confidence interval: 1.023–1.099)], BMI [OR: 1.05 (1.015–1.172)] and insulin resistance on Homa scale[OR: 1.31 (1.022–2.29)], BPAR [OR: 1.92 (1.223–4.604)] as independent risk factors for PTDM while high insulin sensitivity and beta cell reserve on Day 2 pre-transplantation was associated with a strongly reduced risk for PTDM [OR: 0.04 (0.007–0.152)]. High insulin sensitivity and beta cell reserve had the best sensitivity (89.6%) and specificity (68.99 %) with a high negative predictive value (94.32%).

Conclusions: Pre-transplant insulin resistance and beta cell reserve are independent predictors of PTDM that can be used for identifying recipients at high or reduced risk for PTDM, other than the impact of independent clinical risk factors like age, BMI and BPAR (treatment). This information can help clinicians to modify therapeutic plan to improve graft and patient survival after transplantation.

SA-PO502

Diabetes Occuring Post Transplant Has a Different Course Depending on Ethnic Background when Using a Steroid Sparing Protocol Georgina H. Aldous, Vaarisan Ganeshalingam, Dawn Goodall, Jack Galliford, Adam Mclean, David Taube, Andrew H. Frankel. *Imperial College Renal & Transplant Centre.*

Background: Few studies describe the natural history of new onset diabetes after transplant (NODAT). We describe the natural history of NODAT using a steroid sparing regime.

Methods: NODAT was diagnosed upon treatment of diabetes and diet-controlled diabetes used ADA criteria. Follow-up was from diagnosis to one year. Treatment and HbA_{1c} at 3, 6, 9 & 12 months were recorded. They were compared to 90 patients with pre-existing Type 2 diabetes (T2DM) [75M, 15F; mean age 58.1±9.9 yrs]. Patients received monoclonal induction and our Tacrolimus based steroid sparing protocol. Rejection was treated with Methylprednisolone and oral Prednisolone.

Results: 73/778 [9.4%] patients developed NODAT and 12/778 [1.5%] diet-controlled diabetes [51M, 34F; mean age 51.1±11.1 yrs]. 25/85 [29.4%] Caucasian and 38/85 [44.7%] Asian. Asian patients had worse glycaemic control than Caucasian patients from 6 months [Table 1], with HbA_{1c} similar to preexisting T2DM [6.9%, 7.1%, 7.9% & 8.1% at 0, 1, 6 & 12 months].

HbA1c levels of Asian & Caucasian NODAT/diet-controlled DM patients compared using Mann Whitney U Test.

Months from diagnosis	Asian HbA1c (%)	Caucasian HbA1c (%)	p-value
0	7.6	7.3	0.643
3	6.6	6.7	0.831
6	7.3	6.8	0.050*
9	7.5	6.6	0.024*
12	7.7	6.5	0.026*

Asian and Caucasian patients had similar initial BMI [27.4 vs 29.4kg/m^2, p=0.19] with no significant difference in weight gain. 10 Asian and 11 Caucasian patients had rejection [p=0.15]. Prednisolone use was greater in Caucasians at diagnosis [52% vs 22%, p=0.02]. There was no significant difference in Tacrolimus dose at diagnosis [0.097mg/kg Caucasian vs 0.079mg/kg Asian, p=0.17]. MDRD eGFR was better in Asian patients [54.6 vs 44.5ml/min (p=0.04) at diagnosis]. HbA_{1c} at transplant was similar in both groups [5.4% Caucasian (n=8) vs 5.5% Asian (n=18)]. Random glucose was similar at transplant [5.6mmols Caucasian (n=19) vs 5.5mmols Asian (n=22)] and diagnosis [16mmols Caucasian (n=19) vs 18mmols Asian (n=30)].

Conclusions: Asian patients behave similarly to patients with pre-existing T2DM whereas Caucasian patients have more stable glycaemic control.

SA-PO503

Body Weight Increase and Congestive Heart Failure during Treatment with Avosentan: A Post-Hoc Analysis of the ASCEND Trial Jarno Hoekman,[1] Hiddo Jan Lambers Heerspink,[1] G. Viberti,[2] Damian Green,[4] Johannes F. Mann,[3] Dick de Zeeuw.[1] *[1]Dept Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands; [2]King's College London School of Medicine, London, United Kingdom; [3]Dept of Medicine IV, University of Erlangen, Erlangen, Germany; [4]Quintiles Ltd., Strasbourg, France.*

Background: The ASCEND trial tested the renoprotective effect of the endothelin-1 antagonist avosentan in subjects with type 2 diabetes and nephropathy. Despite the drug's ability to decrease albuminuria, an excess of congestive heart failure (CHF) events occurred in the avosentan treatment arms which was likely due to increased sodium/fluid retention and led to premature termination of the trial. We questioned whether the rise in body weight during the first weeks of avosentan treatment can be used to monitor increased susceptibility to develop CHF during follow-up.

Methods: In a post-hoc analysis of the ASCEND trial (N=761) we assessed change in body weight after 1 month between placebo and combined avosentan treatment arms (25mg and 50mg). Using cox regression we then analysed whether a 1 month change in body weight is associated with increased CHF risk.

Results: The increase in body weight after 1 month was +1.0kg (95%CI 0.8 – 1.2) in the combined avosentan treatment groups, while body weight did not change in the placebo group (0.0kg (95%CI -0.2 - 0.3) P vs. avosentan <0.001). Between 1 month and end of study 28 CHF events occurred in the avosentan group and 9 in the placebo group. Among patients with a CHF event, body weight changed with +1.8kg in the avosentan arm vs. -0.3kg in the placebo arm, whereas patients without a CHF event experienced a body weight change of +0.9kg in the avosentan arm and 0.0kg in the placebo arm. The rise in body weight was associated with increased CHF risk in the avosentan arms (HR=1.15 (95% 1.01 - 1.30) P=0.030) but not in the placebo arm (HR=0.93 (95%CI 0.68 - 1.29) P=0.669). Body weight change in the avosentan arm remained associated with CHF risk after controlling for history of CHF, age, and gender (HR=1.20 (95%CI 1.04 - 1.37) P=0.012).

Conclusions: Short-term change in body weight during treatment with an endothelin-1 antagonist can be used to monitor CHF risk.

Funding: Pharmaceutical Company Support - The Ascend Trial Was Sponsored by Speedel Pharma Ltd.

SA-PO504

Non-Alcoholic Fatty Liver Disease Is Associated with Cardiovascular Risk in Adults with Diabetes Jeffrey C. Sirota,[1] Pamela Mettler,[1] Kim Mcfann,[1] Giovanni Targher,[2] M. Chonchol,[1] Diana I. Jalal.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado, Aurora, CO; [2]Section of Endocrinology and Metabolism, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy.*

Background: While some studies have linked non-alcoholic fatty liver disease (NAFLD) to cardiovascular disease (CVD), it remains uncertain if NAFLD is associated with an increased prevalence of CVD in the US.

Methods: Cross-sectional study examining the relationship between ultrasound-diagnosed NAFLD and CVD in the National Health and Nutrition Examination Survey 1988-1994 (n=11,317). CVD was defined as a composite of angina, myocardial infarction, or stroke. In addition, we tested interactions between NAFLD & chronic kidney disease (CKD) and between NAFLD & diabetes on CVD.

Results: 875 (8%) participants had CVD and 3720 (33%) had NAFLD. Subjects with CVD were older, more likely to have hypertension and diabetes, and had higher waist circumference, triglycerides, & homeostatic model of insulin resistance (HOMA-IR) scores. NAFLD prevalence was 35.6% and 45.3% in patients without and with CVD, respectively (p<0.0001). NAFLD was associated with CVD (OR 1.74, 95% CI 1.41-2.14, p<0.0001) in univariate regression analysis. After adjusting for demographics and other CVD risk factors, the OR for CVD in NAFLD was 1.10 (95% CI 0.88, 1.37, p=0.42). The interaction between NAFLD and CKD on CVD was not significant (p=0.89). The interaction between NAFLD and diabetes on CVD was significant (p=0.049). Stratified analysis showed NAFLD was independently associated with CVD among diabetic subjects.

Table

	with diabetes n=824	no diabetes n=10,493
Unadjusted OR (95% CI)	2.38 (1.37-4.15, p=0.002)	1.45 (1.15-1.83, p=0.002)
Adjusted OR* (95% CI, P)	1.98 (1.05-3.73, p=0.034)	0.99 (0.78-1.28, p=0.98)

* Adjusted for age, sex, race, h/o hypertension, waist circumference, systolic blood pressure, HOMA-IR, HDL-cholesterol, triglycerides

Conclusions: Ultrasound-diagnosed NAFLD is associated with CVD among US adults. This association is attenuated by cardiovascular risk factors. In people with diabetes, however, NAFLD is independently associated with CVD.

SA-PO505

Role of Innate Immunity in Diabetic Nephropathy Can Li,[1] Ying Shun Jin,[1] Hong Bin Zou,[2] Shang Guo Piao,[3] Bi Hu Gao,[4] Chul Woo Yang.[3] *[1]Nephrology, YanBian University Hospital, YanJi, JiLin, China; [2]Nephrology, The First Affiliated Hospital of JiLin University, ChangChun, JiLin, China; [3]Nephrology, The Catholic University of Korea, Seoul, Korea; [4]Nephrology, The Affiliated ZhongShan Hospital of DaLian University.*

Background: There is growing evidence that innate immunity plays a potential role in the pathogenesis of various renal diseases. However, the role of innate immunity in diabetic nephropathy (DN) has yet to be demonstrated. The aim of this study was to investigate the expression of toll-like receptors (TLR) and its ligands in human kidney tissue of DN.

Methods: We studied 12 type 2 DN patients with renal biopsy, and 12 patients with nephrectomy for renal cancer served as controls. Clinical characteristics were recorded, and intrarenal expression of TLRs (TLR2 and TLR4) and its ligands (heat shock protein70, HSP70 and MYD88) was examined by immunohistochemistry.

Results: The intrarenal expression of TLR2 was markedly decreased in glomerulus of the DN group (1.30 ± 0.21%/mm^2 vs. 28.50 ± 3.45%/mm^2, P<0.01), whereas its expression was increased in the tubulointerstitum (16.55 ± 0.75%/mm^2 vs. 8.93 ± 0.62%/mm^2, P<0.05), and this trend was accompanied by MYD88 expression (Glomerulus: 1.76 ± 0.60%/mm^2 vs. 90.92 ± 10.69%/mm^2; tubulointerstitum: 24.48 ± 2.38%/mm^2 vs. 16.15 ± 1.12%/mm^2, P<0.01, respectively). In contrast, TLR4 immunoreactivity was significantly increased in the glomerulus of DN group (45.65 ± 3.08%/mm^2 vs. 31.61 ± 1.32%/mm^2, P<0.01) but not in the tubulointerstitum. HSP70 expression, a TLR ligand, was significantly increased in the DN group compared with the Con group (Glomerulus: 91.40 ± 13.88%/mm^2 vs. 50.91 ± 4.07%/mm^2; tubulointerstitum: 19.27 ± 1.23%/mm^2 vs. 9.25 ± 0.74%/mm^2, P<0.01, respectively). Correlation analysis revealed that TLRs expression was correlated with the proteinuria and the eGFR.

Conclusions: Our findings suggest that an alteration in TLRs and its ligands expression is closely associated with diabetic renal injury, and that innate immunity may be one of important players in type 2 DN.

Funding: Other U.S. Government Support, Government Support - Non-U.S.

SA-PO506

Homeostatic Model Assessment Indices in Evaluation of Insulin Resistance, and Secretion in the End Stage Renal Disease Stanislaw Niemczyk,[1] Katarzyna Szamotulska,[2] Kinga Giers,[1] Mariusz Jasik,[3] Zbigniew Bartoszewicz,[3] Katarzyna Romejko-ciepielewska,[1] Ewa Paklerska,[3] Malgorzata Gomolka,[1] Joanna Matuszkiewicz-Rowinska.[3] *[1]Military Institute of Medicine, Warsaw, Poland; [2]National Research Institute of Mother and Child, Warsaw, Poland; [3]Medical University of Warsaw, Warsaw, Poland.*

Background: There are no conclusive studies on indices of the effectiveness of insulin action in the end stage renal disease (ESRD) pts.

Aim. The purpose of the study was to compare Homeostatic Model Assessment indices (HOMA-%B, HOMA-%S, HOMA-%IR) and Disposition Index (DI) in HD patients without diabetes with healthy control subjects.

Methods: The groups comprised 33 non-diabetic ESRD HD pts and 33 healthy controls matched for age (50-80y.o.), gender (25 M, 8 W) and BMI. Fasting level of glucose and insulin were measured. HOMA-%B, HOMA-%S, HOMA-IR indices and DI were calculated using: HOMA 1 and HOMA 2 as measures of IR. The indices were assessed also in subgroups divided according to BMI (<25; 25-30; ≥30 kg/m^2). Relationships between leptin, LAR, CRP and HOMA were evaluated.

Results: Glucose concentrations were lower in ESRD pts (82.4±10.4 vs. 93.9±11.6, p=0.001). Insulin concentrations were similar (median 6.8 vs 6.0 mU/l, p=0.698). HOMA1-%B were higher in ESRD pts (median 137.1 vs. 81.6, p=0.002). HOMA1-%S (median 75.6 vs. 71.5) and HOMA1-IR (median 1.3 vs. 1.4) were not significantly different (p=0.264 and p=0.189). DI1 levels were higher for HD pts (median 1.16 vs. 0.53, p<0.001). In subgroups analysis all statistically significant differences were restricted to persons with BMI<25kg/m^2. Similar results, as for HOMA 1 model, were obtained for HOMA 2. Among HD patients, significant correlations were found between HOMA-%B, HOMA-%S, HOMA-IR indices and serum leptin, leptin/adiponectin ratio and CRP levels.

Conclusions: The Homeostatic Model Assessment-B (HOMA-%B) and Disposition Index (DI) are strongly correlated with insulin resistance level in the end stage renal disease.

In non-diabetic and end stage renal disease patients undergoing hemodialysis the Homeostatic Model Assessment indices and Disposition Index may be useful and important models in interpretation of glucose metabolism disturbances.

SA-PO507

New Pathologic Classification of Diabetic Nephropathy: Clinical Correlations Loyana Teresa Teofilo Lima Silva, Camila Hitomi Nihei, Raquel Maria Maia, Elerson Costalonga, Michell Alves Oliveira, Denise M.A.C. Malheiros, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, School of Medicine University of Sao Paulo, Sao Paulo, Brazil.*

Background: The aim of this study was to evaluate the new pathological classification of diabetic nephropathy (DN) according to the Research Committee of the Renal Pathology Society in 2010.

Methods: We analyzed 77 diabetic patients submitted to renal biopsy at our center from 1999 to 2010. Thirty-eight patients with biopsy-proven DN meet inclusion criteria of biopsies containing at least 8 glomerulus and follow up longer than 1 year. Biopsies were reviewed and classified according to new pathological classification of DN. Two pathologists scored all biopsies independently.

Results: In these 38 DN patients, none was classified as type I, 1 as type IIa, 6 as type IIb, 29 as type III and 2 as type IV; 16 patients had mild interstitial injury, 12 had moderate interstitial injury, while 9 had severe interstitial injury. Arteriosclerosis was present in 9 biopsies while arteriolar hyalinosis was present in 23. The agreement between the reports of the pathologist was 100%. We couldn't analyze the impact class IIa and IV of DN on outcome because the low number of patients. The clinical features and laboratory findings of patients was classified as type IIb and III are summarized in Table I.

Clinical Features

Class	IIb	III	
N=35	N=6	N=29	
Age(y)	42,3±19,7	51,6±12,1	p=0,15
Male	3(50%)	16(55%)	pNS
Initial eGFR(ml/min)	54,5(±26,5	30±20,5	p=0,01
Final eGFR(ml/min)	41,6±30,3	16,2±13,6	p=0,004
Proteinuria(g/day)	3,8±3,2	5,4±2,9	p=0,2
Glycated hemoglobin(%)	10,9±4	8,9±2,8	p=0,15
Hypertension	6(100%)	28(96,6%)	pNS
ESRD	3(50%)	20(69%)	pNS
Follow up(mo)	46,7±47	25±22,9	pNS
Interstitial Fibrosis			
0 (0%)	0 (0%)	0 (0%)	pNS
1 (<25%)	3 (50%)	14 (48%)	pNS
2 (25-50%)	2 (33%)	9 (31%)	pNS
3 (>50%)	1 (17%)	6 (21%)	pNS

Results are showed as mean ± SD or n(%)

Conclusions: The new pathological classification of DN seems to correlate with baseline renal function and can increase the diagnosis rate and attract more attention to interstitial damage in DN.

Funding: Government Support - Non-U.S.

SA-PO508

Glomerular Filtration and Albumin Excretion Rate Variability in Type 1 Diabetic Patients Are Largely Explainable by Glomerular Lesions Alone Behzad Najafian,[1] Maria Luiza A. Caramori,[2] Michael Mauer.[2] *[1]Pathology, University of Washington, Seattle, WA; [2]Pediatrics and Medicine, University of Minnesota, Minneapolis, MN.*

Background: Structural-functional relationship (SFR) studies are powerful tools to understand the natural history of diabetic nephropathy (DN). We studied SFR in a large cohort of type 1 diabetic (T1D) patients (pts) with a wide spectrum of renal function.

Methods: Renal biopsies from 161 (M/F=60/101), age 35[19-64] years, diabetes duration 22±9 years, albumin excretion rate (AER) 31[2-4630] ug/min, glomerular filtration rate (GFR) 101[31-178] ml/min/1.73 m^2 T1D pts were studied by electron microscopy stereology and correlated with renal function.

Results: Simple regression showed that AER directly correlated with Hb_{A1C} (r=0.21, p=0.007), mean blood pressure (MBP) (r=0.41, p=0.0001), volume fraction of mesangium per glomerulus [Vv(Mes/glom)] (r=0.78, p=0.0001), glomerular basement membrane width (GBMW) (r=0.67, p=0.0001) and inversely with age at the onset of diabetes (r=-0.17, p=0.03) glomerular filtration surface density [Sv(PGBM/glom)] (r=-0.72, p=0.0001). Multiple regression analyses (MRA) showed Vv(Mes/glom) and GBMW explained 68% of AER variability (p=0.0001). Maximum prediction of AER variability (75%) was obtained by piecewise linear regression analyses (PLRA) with these two variables at an AER breakpoint about 40 ug/min. Simple regression showed that 27% of GFR variability can be explained by Vv(Mes/glom). This was increased to 29% when sex and GBMW were added to Vv(Mes/glom) in a MRA model. However, Vv(Mes/glom) alone explained about 70% of GFR variability using PLRA at a GFR breakpoint of 115 ml/min/1.73 m^2.

Conclusions: These studies show that majority of renal functional abnormalities in T1D pts with a wide range of AER and GFR is explainable by the presence of glomerular lesions. If appropriate SFR models are applied, glomerular structural abnormalities can be potentially used as surrogates of GFR decline, especially in future longitudinal studies of early DN.

Funding: NIDDK Support

SA-PO509

Clinical Predictors of Non-Diabetic Kidney Disease in Type II Diabetics Undergoing a Kidney Biopsy: A Retrospective Analysis Gaurav Jain, Gautam K. Bhanushali, Michael Allon. *University of Alabama at Birmingham.*

Background: Diabetic nephropathy (DN) is usually diagnosed in patients with long-standing DM with typical clinical features (slow progression, proteinuria, hypertension). Kidney biopsy is reserved for patients with "atypical" clinical features (acute kidney injury, rapid progression, severe proteinuria, hematuria, or absent retinopathy) that suggest non-diabetic kidney disease (NDKD). We evaluated the association between clinical features and renal pathology in Type II diabetics undergoing kidney biopsy.

Methods: 146 patients with Type II DM underwent native kidney biopsies at our center over 8 years. Two investigators who were blinded to the renal pathology retrospectively extracted clinical information from the electronic medical record. The association between clinical features and kidney pathology was evaluated by Chi square analysis.

Results: Of 146 kidney biopsies, 43 (or 29%) had DN, 80 (or 55%) had NDKD and 23 (16%) had DN+NDKD. The most common NDKD lesions were ATN (20 pts), IgA nephropathy (15 pts), FSGS (12 pts) and pauci-immune glomerulonephritis (11 pts). We combined patients with NDKD and those with DN+NDKD, and compared them to patients with isolated DN. The likelihood of NDKD was increased in patients with AKI, non-nephrotic range proteinuria, absence of diabetic retinopathy, and excellent BP control. In contrast, hematuria, rapid progression of CKD, and good glycemic control did not increase the likelihood of NDKD.

Results

Clinical Features	HR for NDKD	95% CI	p-value
Biopsy due to proteinuria	0.68	0.57-0.82	0.0004
Biopsy due to AKI	1.46	1.22-1.76	0.0004
Hematuria	1.13	0.92-1.39	0.25
Proteinuria <3.5 gm/day	1.56	1.20-2.02	0.0006
No retinopathy	1.57	1.07-2.30	0.009
Rapid progression	1.03	0.79-1.35	0.82
Good glycemic control	1.26	0.97-1.65	0.07
Excellent BP control	1.49	1.24-1.78	0.0004

CI (confidence interval), HR (Hazard Ratio)

Conclusions: NDKD is present in over two-thirds of type II diabetics undergoing a kidney biopsy, and comprises a variety of pathologies. Simple clinical criteria can be used to estimate the likelihood of NDKD, and thereby guide the decision about proceeding with a kidney biopsy.

SA-PO510

Nation-Wide Survey of Body Mass Index in Prevalent Dialysis Patients in Japan Yukitoshi Sakao,[1] Akihiko Kato,[1] Yoshihide Fujigaki,[2] Seiji Hashimoto,[3] Takeshi Hasegawa,[3] Kunitoshi Iseki,[3] Yoshiharu Tsubakihara.[3] *[1]Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [3]Committee of Renal Data Registry, Japanese Society for Dialysis Therapy (JSDT), Bunkyo-ku, Tokyo, Japan.*

Background: The prevalence of overweight (body mass index (BMI) ≥25kg/m^2) is gradually increased in men aged from 20 to 60 years old, while decreased in women aged from 40 to 60 years old in Japanese general population. However, the prevalence of overweight, normal (18.5≤BMI<25 kg/m^2)and lean BMI (<18.5kg/m^2) is still unknown among the dialysis population in Japan.

Methods: A nation-wide data were obtained from Renal Data Registry of JSDT. We calculated BMI by body height and weight at post-dialysis in 165,215 patients (age: 65.2±12.5, time on HD: 7.3±7.2 years, male: 62%). We compared BMI in the general population reported from the Nation Health and Nutrition Survey by Ministry of Health, Labour and Welfare.

Results: Mean BMI were 21.5±3.5 kg/m^2 in male and 20.7±3.9 kg/m^2 in female, lower than those in the general population (M: 23.5 kg/m^2, F: 22.3 kg/m^2). Lean BMI was more prevalent when compared both in men (16.8 vs. 4.3%) and in women (30.1 vs. 10.8%) across all generation. The prevalence of lean BMI in male was 20.2% in chronic glomerulonephritis (CGN), 18.2% in diabetic nephropathy (DN) and 13.7% in benign nephrosclerosis (BN). Lean BMI was more prevalent in DN (33.9%) and CGN (32.9%) when compared to BN (20.4%) in female. The prevalence of overweighed BMI was also lower both in men (12.8 vs. 28.6%) and women (11.7 vs. 20.6%) respectively. The rate of overweighed BMI was almost identical to that in general subjects in patients aged from 20 to 40 years old, while it decreased with ageing over 40 years old. Overweighed BMI was observed more often in DM (M: 17.0%, F: 19.7%) than in CGN (M: 9.9%, F: 8.5%) and in BN patients (M: 11.6%, F: 10.5%).

Conclusions: The prevalence of lean BMI was 3 to 4-fold higher in the dialysis population in Japan. However, the prevalence of overweighed BMI was almost identical to that in general population in young patients aged from 20 to 40 years old.

SA-PO511

Association between Body Composition and Quality of Life in ESRD Patients Somchai Yongsiri,[1] Jiranuch Thammakumpee,[2] Pongtip Unprasert,[1] Ouppatham Supasyndh.[3] *[1]Internal Medicine, Faculty of Medicine Burapha University, Mueng, Chonburi, Thailand; [2]Internal Medicine, Chonburi Hospital, Mueng, Chonburi, Thailand; [3]Internal Medicine, Pharmongkutklao Hospital, Bangkok, Thailand.*

Background: Protein-energy wasting is a significant problem in ESRD patients that compromise the patient's Quality of life (QOL). Multifrequency Bioimpedance Spectroscopy (BIS) is a validated method to assess body composition in dialysis patients. Objective. The study objective is to explore the association between body composition and QOL in ESRD patients who received different treatment modalities.

Methods: Body composition were measured by Body Composition Monitor®, the device provided body composition and quantified hydration status. QOL was measured by WHOQOL-BREF instrument comprises 26 items, which measure the following domains: physical health, psychological health, social relationships, and environment. Pearson's correlation coefficient was used to measure the correlation, $p<0.05$ was considered as statistically significant.

Results: Sixteen predialysis-CKD5, 26 PD and 34 HD patients were included in this study. There were no statistically difference in baseline characteristics including Charlson comobidity index, dietary intake, BMI and blood pressure between groups.Mean QOL score in each group were in the middle range and not significantly difference. PD patients had more over hydration when compare to HD patients (16.18±11.24 vs. 2.36±11.07 %OH/ECW $p<0.0001$).There were inverse correlation between overhydration and physical health in HD patients ($r=-0.372$, $p=0.033$) but not in PD and CKD5 patients. CKD5 patients had more lean tissue index than PD and HD patients (LTI=14.34±3.13, 12.26±3.65, 11.48±3.48 kg/m^2 respectively, $p=0.023$). There were correlation between LTI and overall QOL in CKD5 ($r=0.690$, $p=0.002$) and PD patients ($r=0.498$, $p=0.010$). In HD patients, LTI was associated with better physical health ($r=0.464$, $p=0.007$).

Conclusions: QOL in predialysis-CKD5, PD and HD patients were not statistically different. HD patients had better volume control than PD patients. Higher LTI were associated with better QOL in ESRD patients.

Funding: Government Support - Non-U.S.

SA-PO512

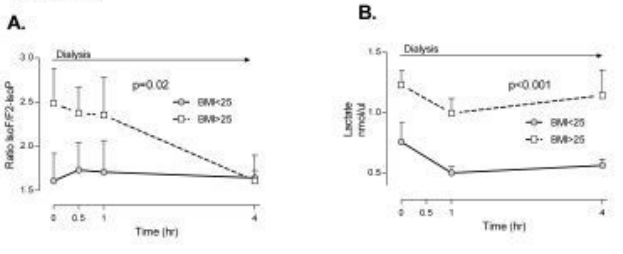
Obesity Influences Oxidative Stress and Mitochondrial Dysfunction in Maintenance Hemodialysis (MHD) Patients Jorge Gamboa,[1] Mias Pretorius,[1] Delia M. Woods,[1] Charles Anthony Dematteo,[1] Jonathan Himmelfarb,[2] T. Alp Ikizler.[1] *[1]Medicine, Vanderbilt University Medical Center; [2]Medicine, University of Washington.*

Background: Patients undergoing MHD have increased cardiovascular morbidity and mortality that cannot be explained by traditional risk factors. Increased oxidative stress burden, usually consequence of increased production of reactive oxygen species (ROS), is associated with increased CV events in MHD patients. The mitochondria is one of the main sources of ROS. In this study we tested the hypothesis that markers of oxidative stress and mitochondrial dysfunction are increased in patients undergoing MHD.

Methods: We evaluated fifteen (15) patients undergoing MHD for at least 6 month. Serial blood sampling was taken during hemodialysis.

Results: We found that F2-Isoprostanes (F2-Iso) and Isofurans (IsoF) were increased at baseline in MHD patients, but did not change during the course of hemodialysis. Interestingly the ratio of IsoF to F2-IsoP was higher than one during hemodialysis, a phenomenon described in mitochondrial dysfunction. Lactate, a marker of mitochondrial dysfunction, was elevated at baseline but did not change significantly during hemodialysis. When patients were categorized by being or not overweight/obese (BMI higher or lower than 25), patients with BMI>25 had higher IsoF to F2-IsoP ratios at the beginning of HD, which resolved by the end of HD (Figure 1A). Higher lactate levels were observed in obese patients at baseline and during hemodialysis (Figure 1B) suggesting a higher extent of mitochondrial dysfunction in these patients.

Figure 1.



Conclusions: MHD patients have increased mitochondrial dysfunction, which is more pronounced in obese individuals. Mitochondrial dysfunction is not affected by hemodialysis procedure. Further studies are required to elucidate the relation between mitochondrial dysfunction, oxidative stress and obesity in MHD patients.

Funding: NIDDK Support, Other NIH Support - NHLBI; NIGMS, Private Foundation Support

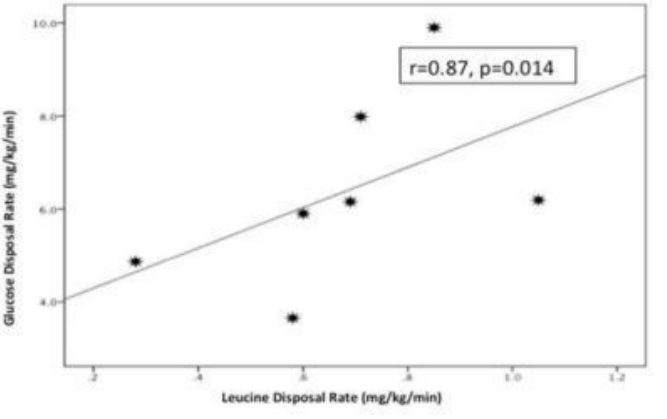
SA-PO513

Insulin Resistance of Carbohydrate and Protein Metabolism Are Correlated in Chronic Hemodialysis Patients Serpil Muge Deger,[2] Adriana Hung,[1,2] Cindy Booker,[1,2] Feng Sha,[2] Phyllis Ann Egbert,[2] Mary B. Sundell,[2] Charles D. Ellis,[2] T. Alp Ikizler.[1,2] *[1]Nephrology, VA THS, Nashville, TN; [2]Nephrology, Vanderbilt University, Nashville, TN.*

Background: Sarcopenia is associated with increased morbidity and mortality in CHD patients. Peripheral insulin resistance has been proposed as a critical player in the development of sarcopenia. Measurement of glucose disposal rate (GDR) by clamp studies is the gold standard of assessing peripheral IR. There are no studies examining the amino acid disposal rate (AADR) in relation to GDR in CHD patients. Here, we investigated the relationship between AADR and GDR using a novel hyperinsulinemic eugylcemic and euaminoacidemic dual clamp technique to assess the effects of IR on amino acid metabolism in prevalent CHD patients.

Methods: Seven male CHD patients underwent hyperinsulinemic euglycemic euaminoacidemic clamp protocol to measure GDR and AADR. Variable infusion of 20 % dextrose and AA solution were infused to maintain the plasma levels at the post-absorptive state. The primary outcome was the correlation between GDR and AADR concentrations in response to insulin administration.

Results: Mean age was 54± 13 years and 29% had previous diagnosis of T2DM. Mean BMI was 29.8±5.5 kg/m^2. During the dual clamp procedure, basal leucine concentrations significantly increased from their basal values [94.5 ±16.2 micromol/L vs. 113.4±17.3 micromol/L ($p=0.018$)]. There was a significant correlation between Leucine disposal rate and GDR(spearman coefficient $r= 0.857$, $p=0.014$).



Conclusions: The results of this study demonstrate that the severity of IR of carbohydrate metabolism is associated with a similar resistance to anabolic actions of insulin in CHD patients. Insulin resistance represents a potential mechanism for development of sarcopenia and can be targeted for treatment of Protein Energy Wasting in CHD patients.

Funding: NIDDK Support, Veterans Administration Support

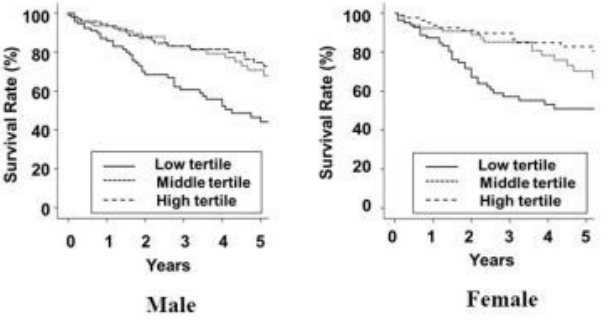
SA-PO514

Limb/Trunk Lean Mass Ratio as a Risk Factor for Mortality in Peritoneal Dialysis Patients Seokhui Kang, Jun-Young Do, Kyu-hyang Cho, Kyung Woo Yoon, Jong-Won Park. *Department of Internal Medicine, Yeungnam University Hospital.*

Background: This study was performed to determine the clinical relevance of limb/trunk lean mass ratio (LTLM) in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: This retrospective cohort study included 534 CAPD patients. Body compositions were measured using a DEXA apparatus.

Results: In males, the sensitivity and specificity for the diagnosis of sarcopenia were 70.3% and 85.9%, respectively. In females, the sensitivity and specificity for the diagnosis of sarcopenia were 62.3% and 83.8%, respectively. The initial low LTLM tertile was associated with mortality in male CAPD patients and in female CAPD patients.



Among patients who maintained CAPD for a year, the maintenance of low LTLM tertile was associated with mortality.

Conclusions: The LTLM is associated with other lean mass indices, nutritional status, and mortality in CAPD patients. Therefore, LTLM may be used as a new marker useful for the prediction of the nutritional status and mortality in patients with CAPD.

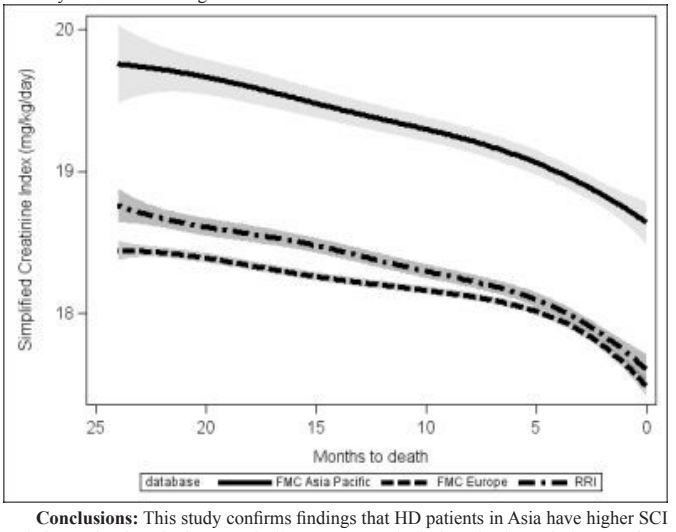
SA-PO515

Muscle Mass Dynamics before Death in Incident Hemodialysis Patients as Represented by a Simplified Creatinine Index: Results of an International Study Bernard J. Canaud,[1] Daniele Marcelli,[1] Len A. Usvyat,[2] Aileen Grassmann,[1] Michael Etter,[3] Yuedong Wang,[4] Adrian Marcos Guinsburg,[5] Cristina Marelli,[5] Adam Tashman,[2] Nathan W. Levin,[2] Peter Kotanko.[2] *[1]FMC, Bad Homburg, Germany; [2]Renal Research Institute, New York, NY; [3]Fresenius Asia Pacific Ltd, Hong Kong; [4]University of California, Santa Barbara, CA; [5]FMC, Buenos Aires, Argentina.*

Background: Muscle wasting is common in hemodialysis (HD) patients and associated with mortality. Previous studies showed HD patients share characteristic trends even weeks before death e.g. deterioration in nutritional, inflammatory and cardiovascular status. This study evaluates the behavior of the new muscle mass indicator SCI (Simplified Creatinine Index) over the 2 years prior to death.

Methods: The MONitoring Dialysis Outcomes (MONDO) consortium encompasses HD databases from Fresenius Medical Care (FMC) clinics in Europe, Asia, Latin America, Canada, RRI clinics in US, Maastricht University in the Netherlands, and KfH clinics in Germany. Databases from RRI, FMC Europe and Asia were queried for $spKt/V_{urea}$, pre-dialysis serum creatinine and demographic characteristics. SCI was calculated using the Canaud formula: SCI (mg/kg/day) = 16.21 + 1.12 x [1 if male; 0 if female] - 0.06 x age (years) - 0.08 x $spKt/V_{urea}$ + 0.009 serum creatinine pre-dialysis (µmol/L). Pre-death SCI dynamics were analyzed by estimating cubic splines.

Results: 13620 HD patients from 22 countries were studied: Europe 16 [N=9883]; Asia 5 [N=800]; USA [N=2937]. Irrespective of region, SCI fell by ~1 mg/kg/day in the 2 years before death. The rate of decline accelerated a few weeks before death and was virtually identical in all regions.



Conclusions: This study confirms findings that HD patients in Asia have higher SCI and shows that lean body mass declines even months before death. Early interventions may change the negative trend.

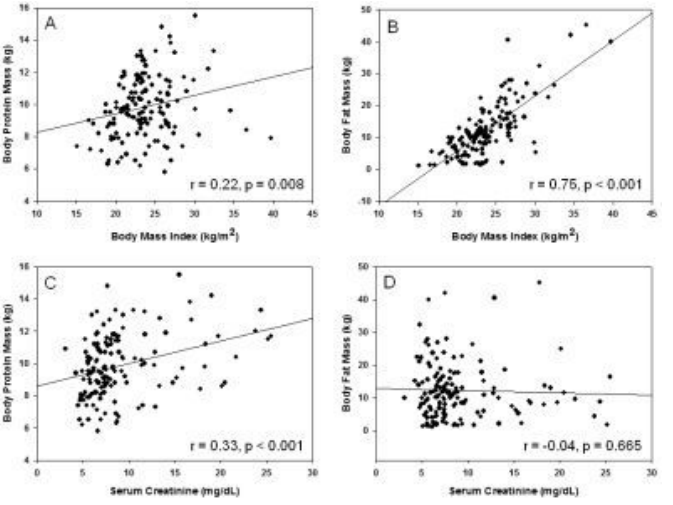
SA-PO516

Body Mass Index and Serum Creatinine: Relation to Body Composition in Incident versus Prevalent Dialysis Patients Jongha Park,[1,2] Kyung Sun Park,[1] Kamyar Kalantar-Zadeh,[2] Miklos Zsolt Molnar,[2] Jong Soo Lee,[1] Hyun Chul Chung.[1] *[1]Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea; [2]Harold Simmons Center for Chronic Disease Research & Epidemiology, LA BioMed at Harbor-UCLA, Torrance, CA.*

Background: Body protein and fat mass may influence clinical outcomes via different pathways in end-stage renal disease. Reliable surrogate measures to discriminate among these body compositions may help classify different types of malnutrition more accurately in individual patients and across populations.

Methods: Relationships of body mass index (BMI) and serum creatinine level (Cr) with body protein and fat mass estimated by bioimpedance method were evaluated in 144 new dialysis (HD 117, PD 27) and 70 prevalent HD patients in South Korea using linear regression models.

Results: Incident dialysis patients were 54.7±13.8 yrs old and included 46.5% women and 56.3% diabetics. BMI was strongly correlated with fat mass ($r = 0.75$, $p < 0.01$), while serum Cr was associated with protein mass ($r = 0.33$, $p < 0.01$).



An increment of 1.0 kg/m² in BMI could independently predict to increase in fat mass by 1.74 kg (95%CI: 1.48-2.00), while a change of 1.0 mg/dl in serum Cr could predict a parallel change of 0.13 kg in protein mass (95%CI: 0.04-0.21) in adjusted models (R^2 = 0.63 and 0.38, respectively). In prevalent HD patients (age, 51.8±13.5 yrs; female 48.6%; diabetes 37.1%) similar but even stronger relationships were observed; BMI to fat mass ($r = 0.78$, $p < 0.01$) and serum Cr to protein mass ($r = 0.48$, $p < 0.01$).

Conclusions: BMI and serum Cr level can be used as clinical surrogates of body fat and protein mass, respectively, in dialysis patients. Since serum Cr is influenced by not only protein mass but also residual renal function, its relation to protein mass seems to be more relevant in long-term maintenance rather than new dialysis patients.

SA-PO517

Sarcopenia as a Predictor of Patient Vulnerability Grahame J. Elder,[1,2] Avalon Moonen,[3] Sjorjina Green,[3] Nathan Hewitt.[3] *[1]Department of Renal Medicine, Westmead Hospital, Sydney, NSW, Australia; [2]Osteoporosis and Bone Biology Program, Garvan Institute of Medical Research, Sydney, NSW, Australia; [3]Clinical School, University of Notre Dame, Sydney, NSW, Australia.*

Background: Patients ≥age 60 on satellite dialysis have ~20% annual mortality. Sarcopenia assessment may identify vulnerable patients in order to target modifiable risks. Sarcopenia has been defined by reduction in the lean tissue index (LTI; mass/height²) adjusted for age and sex, tests of functional ability, muscle strength, relative grip strength and the short physical performance battery (SPPB; balance, repeated chair stands and gait speed). Nutritional assessment; the Subjective Global Analysis (SGA) and laboratory biomarkers are also used to predict increased risk.

Methods: We evaluated 80 stable haemodialysis patients aged ≥60 for sarcopenia using bioimpedance analysis (BIA; Fresenius Medical Care), functional ability; 6 minute walk, timed up-and-go (TUG), repeated chair stands (RCS), grip and knee extension strength and SGA. A further 60 patients were assessed by age and sex adjusted grip strength and SPPB. Blood was tested for laboratory biomarkers, and stored for further analyses.

Results: LTI was below the 10th centile of the reference population in 43%, with no difference in prevalence by age or gender. Values correlated to strength (grip 0.34, p=0.003; knee extension 0.45, p<0.001), distance covered in a 6 minute walk (0.34, p=0.002), inversely to RCS time (-0.42, p<0.001) and TUG (-0.27, p=0.019) but not to SGA, dialysis vintage or laboratory markers. For patients above and below age 70, sarcopenia was present in 75% and 62% respectively, defined by adjusted grip strength, and 54% and 45% defined by SPPB.

Conclusions: While some measures of sarcopenia increase with age, LTI does not, consistent with those patients at lower risk surviving longer. Ongoing assessment will determine which test or combination provides capacity to identify more vulnerable patients.

SA-PO518

Adjusting Dialysis Dose to Metabolic Rate or Body Surface Area Would Deliver Greater Dialysis to Under-Dialysed Groups Enric Vilar,[1,2] Laura Mawer,[1] Ashwini Machado,[1] Ken Farrington.[1,2] *[1]Renal Unit, Lister Hospital, Stevenage, United Kingdom; [2]University of Hertfordshire, Hatfield, United Kingdom.*

Background: In renal failure metabolic waste products accumulate which dialysis must remove. HD dose is adjusted according to body water using the equation Kt/V but this may under-dialyse women and those of low BMI. We studied the relationship between Watson volume, Resting Energy Expenditure(REE), and Body Surface Area(BSA) to determine whether an alternative denominator to V (Kt/REE or Kt/BSA) would provide more dialysis to at-risk groups.

Methods: Survival data were gathered retrospectively in 1016 patients dialysed over a 21 year period, excluding those transferred, transplanted or changing modality. At intervals residual renal function, Kt/V and V were recorded. BSA by the Dubois formula and REE by a novel formula were calculated, allowing Kt/BSA and Kt/REE to be estimated for

each patient. Survival was compared between genders and between those with BMI < or ≥22. Cox models compared survival between those with average Kt/V, Kt/BSA and Kt/REE above or below the median.

Results: Survival was similar between genders(p=0.9). Those with BMI<22 had inferior survival (HR for death 0.78[CI 0.64-0.96]) compared to those whose BMI was ≥22(p=0.03, median survival 3.9 v 4.6 years). REE/V was higher in women than men (p<0.0001) indicating that using Kt/REE instead of Kt/V would increase HD dose in females. BSA/V was higher in women than men (p<0.0001) and in those with BMI<22 (p=0.006), indicating that use of Kt/BSA would increase HD dose in females and slim individuals. Below median Kt/V was associated with a hazard for death of 1.31(CI 1.1-1.6,p<0.001) compared to above median Kt/V. This hazard was greater for Kt/BSA(1.44 [CI 1.2-1.7]) and Kt/REE (1.47 [CI 1.2-1.8] suggesting that Kt/REE and Kt/BSA may predict survival better than Kt/V.

Conclusions: BSA and REE are closely related. In HD worse survival in low BMI patients may be due to higher BSA/V or REE/V ratios. Kt/BSA and Kt/REE appear better survival predictors than Kt/V. A modified HD dose algorithm with these denominators will increase dose to apparently under-dialysed groups. Use of Kt/BSA or Kt/REE should be explored in prospective studies.

Funding: Government Support - Non-U.S.

SA-PO519

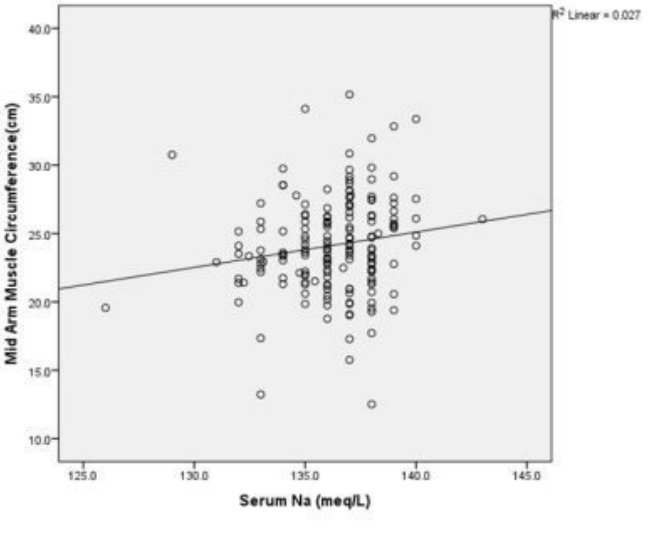
Low Serum Na Is Associated with Decreased Lean Body Mass and Increased Interdialytic Weight Gains Dimitrios J. Poulikakos, Nicholas Jason Lelos, Victoria Marks, Debasish Banerjee. *Renal and Transplantation, St George's Hospital NHS Trust, London, United Kingdom.*

Background: Low serum Na has been associated with increased mortality in hemodialysis patients. Hyponatremia is a recognised complication of inflammation and cytokine induced altered hypothalamic osmoregulation has been postulated. Hypothalamic inflammation is linked with muscle mass wasting in chronic kidney disease. Mid-arm muscle circumference (MAMC) is a good surrogate of muscle mass in haemodialysis patients. We examined the relationship between serum Na and muscle mass in dialysis patients.

Methods: 172 hemodialysis patients were included. Triceps skinfold thicknesses (TSF), mid-arm circumference (MAC), handgrip strength and subjective global assessment (SGA) were assessed using standard techniques. MAMC was calculated with the formula MAMC(cm) =MAC (cm)-3.142X TSF (cm). Annually averaged predialysis sodium and monthly routine laboratory measurements were used.

Results: Age was 66.1 ±14.37, female 62 (36%), diabetes 48 (28.9%), asian 34(19.8%), blacks 56 (32.6%), TSF (cm)1.95±.83, MAMC(cm) 24.1 ±3.52, TSF percentile ≥75th 86 (50%), MAMC percentile ≤10th 66 38%, Handgrip (kgs)25.1±13.1, SGA 5.36±1.14, dialysate Na 138(mEq/L), Serum Na 136.2±2.25, predialysis systolic BP (mmHg) 144.7±34.9, %IDWG 2.51±1.46, dry weight (kgs) 73.62±15.4, albumin 32.5±4.3,CRP 21.98±40.8.

Serum Na correlated positively with MAMC, handgrip and SGA (Pearson correlation r= 0.165, p=0.031, r=0.229 p=0.038 and r= 0.224 p=0.006 respectively) and negatively with %IDWG. Serum albumin correlated positively with lean body mass markers.Patients with serum Na <136.2 meq/L had larger interdialytic weight gains (T-test p=0.016).



Conclusions: This study demonstrates an association between low serum sodium and decreased muscle mass. In this cohort of muscle depleted patients large interdialytic weight gains may be related primarily to thirst.

SA-PO520

Serum Aldosterone Concentrations Relate to Fluid Overload (FO) and Serum Potassium Levels (SK^+) in Hemodialysis (HD) Patients (pts) Jochen G. Raimann,[1] Li Liu,[2] Fansan Zhu,[1] Adam Tashman,[3] Andrew S. Bomback,[4] Philip J. Klemmer,[5] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Renal Research Institute; [2]Peking University First Hospital; [3]University of California, Santa Barbara; [4]Columbia University; [5]UNC Chapel Hill.*

Background: Water and salt homeostasis and SK^+ stimulate aldosterone secretion. In HD pts a right-shifted relation between extracellular volume (ECV) and serum aldosterone was demonstrated (Bomback, 2009). This study aimed to investigate relations between FO [reflected by the ratio of ECV over total body water (TBW)] and SK^+.

Methods: Chronic HD pts underwent gradual reduction of post-HD weight. ECV/TBW [by whole body bioimpedance (DeLorenzo, 1997)], SK^+ (by direct potentiometry) and aldosterone (by ELISA) were measured pre-HD at baseline (BL) and repeatedly during post-HD weight reduction until end of study (End). T-Test, linear regression (LR), linear mixed models (LMM) and Likelihood Ratio Test (LRT) with two degrees of freedom for LMM comparison were employed.

Results: We studied 13 pts (age 55±14 years, 9 females, 8 Blacks, aldosterone 40±24 ng/dL, ECV/TBW 0.43±0.06, SK^+ 4.6±0.6) for 57±40 days. Pre HD weight changed (BL 76.4±14.8, End: 75.0±14.3; P<0.01), but no significant changes in ECV/TBW [0.01 (95% CI -0.012 to 0.04), SK^+ [0.12 (95% CI -0.4 to 0.6) mEq/L] and aldosterone [16.3 (95% CI -80 to 112) ng/dL] were found. LR showed a positive correlation between SK^+ and aldosterone at BL only (R^2=0.48; P=0.02). LRT determined a LMM with random slopes and intercepts (patients and days into the study) as appropriate. LMM showed significant fixed effects of ECV/TBW and SK^+ on aldosterone concentrations (**Table 1**).

Fixed Effects of serum potassium (SK+), ECV/TBW on serum aldosterone in a LMM with random slope and intercepts (pts and days into the study).

	Estimate	Standard error	P-value
Intercept	-2922.52	1020.85	0.008
ECV/TBW	6528.12	2410.02	0.011
SK+	874.30	252.18	0.002
ECV/TBW * SK+	-1733.64	593.36	0.006

Conclusions: Aldosterone concentrations related significantly to SK+ and ECV/TBW in the studied pts during gradual ECV reduction. The response mechanisms of aldosterone secretion to changes in ECV/TBW and SK+ appear intact in HD pts.

SA-PO521

Proton-NMR Metabolomics Reveals Impaired Lactate Metabolism in DM-Derived HD Patients Kazuhisa Takeuchi,[1,2] Itiro Ando,[1] Hiroshi Sekino,[2] Masako Fujiwara.[1] *[1]Graduate School of Pharmaceutical Science, Tohoku University, Sendai, Miyagi, Japan; [2]CKD Center, Koujinkai, Sendai, Miyagi, Japan.*

Background: Lactate has recently been shown as a factor that stimulates production of vascular endothelial growth factor (VEGF). We have tried to analyze the metabolites in HD patients using proton-NMR metabolomics, in which we have identified the signal corresponding to lactate. The aim of this study was to analyze the metabolites including lactate during HD by proton-NMR metabolomics.

Methods: Patients were enrolled after their informed consents had been obtained. The plasma and HD waste fluid were collected at several HD time points. Proton-NMR was measured by 600 MHz NMR spectrometer (JEOL ECA). For quantitation of signals, the flip angle for detection pulse was set to 90^o , and spectra were recorded with the repetition time 37.4 sec which ensure 99% signal recovery. For data analysis, "Alice2 for Metabolome" (JEOL) was used.

Results: The NMR metabolomics clearly demonstrated sharp peak signals of Cr, trimethylamine-*N*-oxide (TMAO), glucose, lactate, pyruvate and some amino acids. The signals of plasma Cr and TMAO were diminished during HD, whereas plasma lactate signals were not decreased but rather significantly increased in CGN-derived HD patients. In HD waste fluid, lactate signal was identified, and during HD the signal was also significantly increased in accordance with that of plasma. In DM-derived HD patients, however, the increase in lactate signals was absent. Pyruvate signal was also identified, and the ratio (Pyr/Lac) was almost half of normal levels.

Conclusions: By proton-NMR metabolomics, a change of metabolite during HD in both plasma and HD waste fluid can be analyzed. Lactate is produced during HD. In DM-derived HD patients, the lactate production is impaired, possibly leading to less VEGF production resulting in their more frequent cardiovascular complications.

SA-PO522

Normalized Protein Catabolic Rate, Not Muscle Mass, Is an Outcome Predictor in Patients Undergoing Maintenance Hemodialysis Ja Seon Kim,[2] Shin Sook Kang,[1] Eun Hee Kang,[1] Jai Won Chang,[2] Jung-Sik Park,[2] Kyung Sun Park.[3] *[1]Dietetics and Nutrition Service, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Division of Nephrology, Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [3]Division of Nephrology, Intenal Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Korea.*

Background: Detection of malnutrition is important because malnutrition is associated with the morbidity and mortality in end-stage renal disease (ESRD) patients on maintenance hemodialysis (MHD). This prospective observational study examined the relationship

among body composition, appetite, malnutrition inflammation score (MIS), nutrition intake, biochemical data, hospital stay and mortality in this population.

Methods: One hundred thirty seven patients (M:F=77:60, age 57.9±12.5 years) were enrolled. Individual patient's recent appetite status was scaled from 1 to 5 (very good, good, fair, poor, very poor). Bioimpedance analysis (BIA) was applied to estimate percentage body fat and muscle mass. We estimated nutrient intake by 1 day dietary recall. The MIS was used to evaluate the malnutrition inflammation complex syndrome. The patients were prospectively followed up for 5 years regarding mortality and hospitalization.

Results: During follow-up period, 25 patients died. Three patients who died from head trauma, traffic accident, and suicide were excluded. Compared with the survival group (n=112), non-survival group (n=22) revealed significant lower level of normalized protein catabolic rate (nPCR, 0.94±0.20 vs. 1.10±0.24, p=0.002), serum creatinine (9.5±2.8 vs. 11.1±2.7, p=0.019) and higher level of MIS (6.6±2.0 vs. 5.5±2.4, p=0.031) and hemoglobin (Hb, 11.3±1.2 vs. 10.7±1.0, p=0.016). Multivariate logistic regression analysis showed that nPCR (p=0.026) and Hb (p<0.01) were significant factors influencing the mortality of MHD patients.

Conclusions: Clinical parameter determining nutritional adequacy, nPCR, would be more important than anthropometric or bioimpedance indices for the survival in MHD patients. On the other hand, relatively high level of hemoglobin could be associated with the mortality in these patients.

SA-PO523

Implications of Dietary Protein Intake on Mortality in Long-Term Hemodialysis Patients

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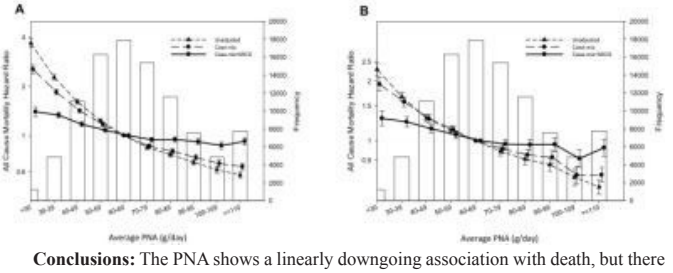
Background: Low and very high dietary protein intake, as measured by protein nitrogen appearance (PNA)(also known as protein catabolic rate [PCR]) have both been shown to influence mortality risk in maintenance hemodialysis patients (MHD). We examined 8-year all-cause mortality and PNA association.

Methods: Using clinical data from large dialysis provider's clinics, we identified 98,489 MHD patients. Mortality risks were estimated using unadjusted and malnutrition–inflammation complex syndrome (MICS) adjusted Cox proportional hazards regressions. PNA was calculated by multiplying nPCR times averaged dry weight.

Results: Patients were 64±15 years old and included 45% women and 59% diabetics. Compared with the reference level (60-69 g/day) low PNA (≤30 g/day, HR:1.40, 95%CI:1.30,1.50) was associated with higher mortality in each level of analysis (Figure A). In all patients, the best survival was associated with a PNA between 100 and 109 g/day. This range was shown to be optimal among hypoalbuminemic (serum albumin <3.5 g/dl) patients as well (Figure B).



Conclusions: The PNA shows a linearly downgoing association with death, but there is no association between high PNA and mortality. Using PNA as a dietary protein intake estimate per each individual was associated with increased risk of death in MHD patients using traditional case mix adjustments. However, after adjusting for MICS only a low daily protein intake is associated with increased risk of death in MHD patients. It is possible that the association between protein intake and survival is mediated by anorexia secondary to protein energy wasting, or additional factors. This possibility should be examined in intervention trials.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

SA-PO524

Normalized Protein Catabolic Rate Is an Independent Predictor of Long-Term Survival in Peritoneal Dialysis Patients

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Background: Normalized protein catabolic rate (nPCR) which reflects daily dietary protein intake in stable dialysis patients (pts) has been reported to be a predictor of mortality in hemodialysis pts. Objective of this study was to investigate the association between nPCR and long term survival in peritoneal (PD) pts.

Methods: Fifty seven PD pts were enrolled in the study from November 2000 to May 2008. On enrollment, demographic, clinical and biochemical data were recorded. Pts were followed through September 2011.

Results: The mean age was 56 years. Fifty-six percent were female and the majority (61%) were African descent. Mean and maximum follow-up were 2.83 and 10.80 years respectively. Mean and median nPCR were 0.944 and 0.910 g/kg/day respectively. Thirty-five per cent of the pts had nPCR below 0.8g/kg/day. Levels of albumin (p=0.049) and prealbumin (p=0.026) were significantly lower in pts with nPCR<0.8 compared to those with nPCR≥0.8g/kg/day. nPCR correlated directly with albumin (r=0.34, p=0.012), prealbumin (r=0.30, p=0.017), magnesium (r=0.48, p<0.0001) and phosphorus (r=0.42, p=0.002). Upon 11 years of observation, cumulative observed survival of pts with enrollment nPCR ≥0.8 was significantly better than those of pts with <0.8 g/kg/day (p=0.048). In multivariate Cox's regression analysis, after adjusting for confounding variables (table footnote), nPCR was an independent predictor of all cause mortality. For each 0.01 unit rise in nPCR, there was a 5.2% decrease in relative risk of mortality.

Multivariate Cox's Regression Analysis

Variable	Relative Risk	p
nPCR (g/kg/day) (x100)	0.948	0.018
Age (years)	1.044	0.03
Albumin (g/dL)	0.077	0.001

Other nonsignificant variables included in the model were body mass index, gender, race, diabetes, hypertension, dialysis vintage at enrollment, creatinine, blood urea nitrogen, hemoglobin and white blood cell count (WBC).

Conclusions: In conclusion, lower nPCR is associated with poorer nutritional status in PD pts. Lower nPCR is independently associated with increased risk of all cause mortality in PD pts followed up to 11 years.

SA-PO525

Improved Outcome by Application of Integrative Clinical Nutrition Dialysis Score (ICNDS) for Assessing of Nutritional Risk in Hemodialysis Patients

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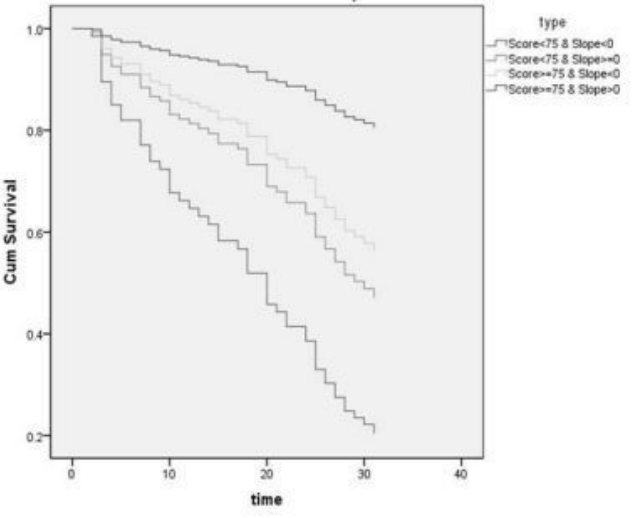
Background: The ***Integrative Clinical Nutrition Dialysis Score (ICNDS)*** is a new quantitative method for identifying nutritional risk in hemodialysis patients.

Methods: The method is based on monthly routine biochemical measures: Albumin, creatinine, urea, cholesterol, Kt/V, CRP, & dry weight change. Each parameter was given a scoring value from 1-5. A score of 5 for a NKF-K/DOQI recommended value & a lower one for sub-optimal values. The Scoring results are between 0-100 given for each patient. High & low Scores reflect a good & poor nutritional status respectively.

Components of ICNDS Score

	Score				
	1	**2**	**3**	**4**	**5**
Albumin	<2.0	2.01 - 2.99	3.0 - 3.49	3.5 - 4.0	>4.0
% Weight change	=>10%	-8% - -9.99%	-6% - -7.99%	-2 % - 5.99%	-2% - 0%
Creatinine	2 - 3.99	4 - 5.99	6 - 7.99	8 - 9.99	=>10
Urea	40 - 59	60 - 79	80 - 99	100 - 149	=>150
Cholesterol	<79.99	80 - 99	100 - 129	130 - 149	=> 150
CRP	>5	3 - 4.99	1.1 - 2.99	0.5 - 1.0	<0.49
Kt/V	<1.0	1.0 - 1.09	1.1 - 1.19	1.2 - 1.39	>1.4

Results: In 59 patients Score results were significantly correlated with SGA score. In 179 patients, baseline *score* was a significant inverse predictor of mortality & hospitalization: each 1-unit increase in score reduced mortality risk by 7.1%, & hospitalization risk by 6.5%. A 1- unit increase of *slope of monthly scores* (31 months) reduced mortality risk by 23.6% & hospitalization risk by 20.1%. A *threshold* Score => 75 reduced mortality by 64.2%. Patients were divided to 4 categories based on baseline *score* (above/below a threshold of 75) & *slope* of monthly scores (smaller/larger to 0).



Worsening nutrition status over time as indicated by both *score* & *slope*, significantly increased death hazard.

Conclusions: The ICNDS is a simple, useful & reliable tool for assessing HD patients' nutrition status which leads to early intervention & improving outcome.

SA-PO526

Determinants of Energy Expended during Physical Activity in Haemodialysis Patients Sivakumar Sridharan,[1] Jocelyn Berdeprado,[1] Enric Vilar,[2] Ken Farrington.[1] *[1]Renal Medicine, Lister Hospital, Stevenage, United Kingdom; [2]Renal Medicine, Basildon Hospital, Basildon, United Kingdom.*

Background: Haemodialysis(HD) patients have reduced levels of physical activity(PA). PA assessment may help to design further interventions in HD patients. Standard methods of measuring PA through accelerometers are not practical for routine clinical use.Self-report activity questionnaires could be a useful alternative.

Methods: Each study subject was administered the Recent Physical Activity Questionnaire(RPAQ) on attendance for a HD session. RPAQ has been validated for ranking individuals according to time spent in vigorous-intensity activity and overall energy expenditure.RPAQ enquires about time spent on activities at home, at work and during various recreational activities in the preceding 4 weeks. We assigned MET(metabolic equivalent of task) values for each activity. Resting energy expenditure(REE) was estimated using a novel predictive equation validated in renal failure. Total energy expenditure(TEE) was estimated from MET and REE. TEE estimation assumed 8 hours sleep and that the remaining unreported hours from RPAQ were spent in light activity.Energy Expenditure during physical activity(PAEE) was calculated as (TEE x 0.9) – REE.

Results: 282 HD patients(105 female, 177 male) completed the RPAQ. Mean age was 64.1(±15.2).There were 201 Whites, 51 Asians and 30 Blacks. Mean REE was 1535 kcal/day(±247), mean TEE 2149 kcal/day(±446) and mean PAEE 399 kcal/day(±214).Male patients, those aged < 65 and those who were employed had significantly higher REE, PAEE and TEE($p<0.001$ for all). There were significant differences in the above among different ethnic groups. TEE and PAEE were significantly less in those with heart disease.In stepwise multiple regression analysis, the independent determinants of log transformed PAEE were age, sex, employment status($p<0.001$ for all) and the presence of heart disease($p=0.01$) with these factors accounting for 57% of the variance in PAEE.

Conclusions: Mean daily MET values of the study subjects indicate a sedentary lifestyle for most HD patients. Age, sex, employment status and heart disease are the principal determinants of energy expended during physical activity in HD patients.

SA-PO527

Prognostic Utility of Plasma S100A12 Levels in Maintenance Hemodialysis Patients: A Novel Score System for Predicting Mortality Yasukiyo Mori,[1] Yayoi Shiotsu,[1] Eiko Matsuoka,[1] Keiichi Tamagaki,[1] Atsushi Kosaki.[2] *[1]Division of Nephrology, Department of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Faculty of Nursing, Setsunan University, Hirakata, Osaka, Japan.*

Background: S100A12 is an endogenous receptor ligand for advanced glycation end products. Here, we assessed plasma S100A12 levels as an independent mortality predictor and examined their utility in clinical settings.

Methods: We have measured plasma S100A12 levels in 550 maintenance hemodialysis (HD) patients to assess the association with the prevalence of cardiovascular disease (CVD) as the cross-sectional study, previously. In this study, we explored the prospective observation to examine the risk of two-year mortality.We also developed an integer scoring system with plasma S100A12 levels to predict mortality.

Results: We found that higher plasma S100A12 levels (≥ 18.79ng/mL) were associated with higher all-cause mortality compared with lower plasma S100A12 levels (< 18.79ng/mL) (P=0.001). Multivariable Cox proportional hazards analysis revealed higher plasma S100A12 levels (hazard ratio [HR], 2.267; 95% confidence interval [CI], 1.195-4.302; P = 0.012), age ≥ 65years (HR, 1.961; 95% CI, 1.017 - 3.781; P = 0.044), serum albumin < 3.5g/dL (HR, 2.198; 95% CI, 1.218 - 3.968; P = 0.012), and prevalence of history of CVD (HR, 2.068; 95% CI, 1.146 - 3.732; P = 0.016) to be the independent predictors of two-year all-cause mortality. The integer score was derived by assigning and totaling the points for these factors and was developed with trends across increasing score values for predictions of all-cause mortality (c-statistic = 0.730 [0.656 – 0.804]). The resulting model demonstrated good discriminative power for distinguishing the validation population from 303 HD patients (c-statistic = 0.721 [0.627 – 0.815]).

Conclusions: We concluded that the plasma S100A12 level is an independent predictor for two-year mortality. We therefore established the simple integer score for predicting mortality based on plasma S100A12 levels.

Funding: Government Support - Non-U.S.

SA-PO528

The Association of the Geriatric Nutritional Risk Index and Total Lymphocyte Count with Mortality in Korean Hemodialysis Patients: A Single Center Study Yeon Soon Jung, Ye Na Kim, Ho Sik Shin, Hark Rim. *Internal Medicine, Kosin University College of Medicine, Busan, Republic of Korea.*

Background: Malnutrition is highly prevalent in maintenance HD patients and is associated with an increased risk of mortality. Recently, Kobayashi reported that GNRI was a significant predictor of mortality in HD patients. Evaluation of TLC was helpful in monitoring the nutritional status and assessing prognosis in PD patients. To date, there have been few longitudinal studies investigating the effect of the relationship between GNRI and TLC on mortality in hemodialysis patients. Thus, we sought to examine these outcomes.

Methods: We enrolled 120 patients receiving stable maintenance HD at Kosin University Gospel Hospital. Blood laboratory tests including TLC were performed once per month, and the data over three months were averaged. The GNRI was calculated by modifying the nutritional risk index for elderly patients, which was reported by Yamada. Survival curves were generated by the life table method. The relative risk of mortality for different parameters was estimated using the Cox proportional hazards model.

Results: Life table analysis revealed that subjects with GNRI < 90 (n = 19) had lower survival rates than did those with GNRI ≥ 90 (n = 101) (Wilcoxon test, P = 0.048), but subjects with TLC < 1500/mm^3 (n = 76) had similar survival rates compared to subjects with TLC ≥ 1500/mm^3 (n = 44) (Wilcoxon test, P = 0.500). TLC was marginally correlated with GNRI and significantly correlated with phosphorus.The association of GNRI ≥ 90 with TLC ≥ 1500/mm^3 excludes the occurrence of complications with moderate reliability.

Table 1. Main patient characteristics and outcomes

Variables	All patients (n = 2211)	Non-cancer patients (n = 1360, 61.5%)	Cancer patients (n = 851, 38.5%)	P-value
Age (years)	61.1 ± 14.1	61.4 ± 15.3	60.7 ± 12.1	0.221
Male gender	1356 (61.3%)	789 (58%)	567 (66.6%)	0.001
DM	833 (37.7%)	687 (50.5%)	146 (17.1%)	0.001
Sepsis	687 (31.1%)	442 (32.5%)	245 (28.7%)	0.001
Hospital mortality	671 (30.3%)	306 (22.5%)	365 (42.8%)	0.001
ICU admission	523 (23.7%)	385 (28.3%)	138 (16.2%)	0.001
ICU mortality	340 (65.0%)	230 (59.7%)	110 (79.9%)	0.001
Inotropics	607 (27.4%)	441 (30.2%)	166 (19.5%)	0.001
Mechanical ventilator	480 (21.7%)	385 (28.3%)	95 (11.2%)	0.001
Chronic hepatitis B	211 (9.5%)	93 (6.8%)	118 (13.8%)	0.001
Chronic hepatitis C	185 (8.4%)	114 (8.4%)	71 (8.3%)	0.512
Cause of death				
cancer	234 (34.9%)	NA	234 (64.2%)	
cerebrovascular disease	58 (8.6%)	51 (16.7%)	7 (1.9%)	0.001
heart disease	49 (7.3%)	45 (14.7%)	4 (1.0%)	0.001
DM	1 (0.2%)	1 (0.3%)	0	0.001
infection	273 (40.8%)	160 (52.3%)	113 (31.0%)	0.001
liver diease	53 (7.7%)	46 (15.0%)	7 (1.9%)	0.001
hypertensive disease	3 (0.5%)	3 (1.0%)	0	0.001
Type of cancer				
Locoregional solid tumor	189 (8.5%)		189 (22.2%)	NA
Metastatic solid tumor	604 (27.4%)		604 (71.0%)	
Hematological malignancy	58 (2.6%)		58 (6.8%)	
Cancer status				
controlled	143 (6.4%)	NA	143 (16.8%)	
uncontrolled/newly diagnosed	81 (3.6%)	NA	81 (9.5%)	
uncontrolled/recurrence progression	627 (28.3%)	NA	627 (73.8%)	

Conclusions: Our results indicate that GNRI is a significant predictor of mortality in HD patients and the use of TLC might improve the evaluation of nutritional risk, but additional studies are needed to confirm these findings.

SA-PO529

Subjective Global Assessment (SGA) and Effects of Aging on Biomarkers in Hemodialysis (HD) Patients (pts) Peter F. Barany,[1] Bengt Lindholm,[1,2] Olof Heimburger,[1] Abdul Rashid Tony Qureshi,[1,2] Peter Stenvinkel,[1] Thiane Gama Axelsson.[1,2] *[1]Renal Medicine, CLINTEC, Karolinska Institutet, Stockholm, Sweden; [2]Baxter Novum, CLINTEC, Karolinska Institutet, Stockholm, Sweden.*

Background: SGA is a simple and valid assessment of protein-energy wasting (PEW). Since the proportion of elderly dialysis patients grow, it is of interest to investigate how biomarkers behave in different age groups.

Methods: 224 non-selected prevalent HD pts were studied during a three month period and survival observed up to 42 months. Nutritional, inflammatory and cardiac biomarkers in plasma were measured before a HD session at baseline and weekly CRP values were obtained during 3 months. The pts were divided into two groups according to age, <65 y (n=110) and >65 y (n=114).

Results: As expected, comorbidity and the analyzed biomarkers (except adiponectin and leptin) differed significantly between the groups. However, the prevalence of poor nutritional status (SGA score >1) did not differ between pts <65 y and pts >65 y (46 vs. 48 %). Inflammatory activity as measured by median CRP over three months was higher in pts>65 y (median 8.2 vs. 4.9 mg/l in pts <65 y.

Spearman rank correlations (rho) to SGA in the two age groups

	<65 y	>65 y
Albumin	-0.47 [c]	-0.24 [a]
Creatinine	-0.41 [c]	-0.37 [c]
IGF-1	-0.30 [b]	-0.19 [a]
Leptin	-0.15	-0.07
Adiponectin	0.15	0.20 [a]
Ghrelin	0.20 [a]	0.02
CRP	0.36 [c]	0.16
IL-6	0.33 [c]	0.26 [b]
NT-proBNP	0.36 [c]	0.27 [b]

[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

In a logistic regression model the odds ratios for having SGA >1 were significant for albumin and creatinine in the <65 y-grp and for creatinine and NTproBNP in the >65 y-grp. SGA strongly predicted survival in both age groups. In Cox regression models including sex, comorbidity and SGA, a lower pre-dialysis s-creatinine was significantly associated with death in pts<65 y whereas in pts >65 y, a lower albumin was associated with a worse outcome.

Conclusions: Ageing, comorbidity and PEW needs to be accounted for when studying biomarkers in ESRD. The differences in the biomarkers in relation to age, SGA and survival suggests that causes of PEW partly may differ between age groups.

Funding: Pharmaceutical Company Support - Amgen

SA-PO530

Prognostic Significance of the Combination of Neutrophil-Lymphocute Ratio and Serum Albumin Levels in Incident Hemodialysis Patient
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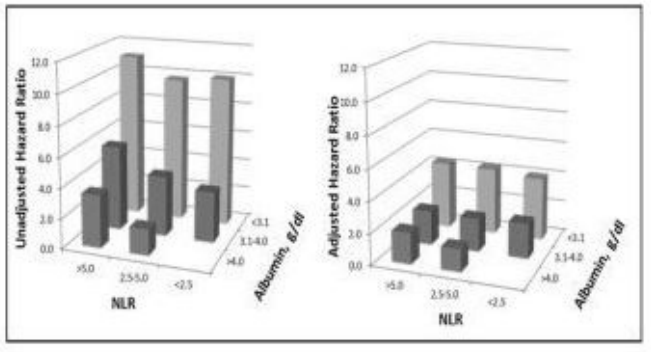
Background: Neutrophil-Lymphocyte ratio (NLR) is a measure of systemic inflammation. The aim of the present study was to evaluate the prognostic significance of the combination of NLR and serum albumin in HD patients.

Methods: Incident HD patients treated in RRI clinics between 1/2000 & 12/2010 were included. In all patients contemporaneous measurements of neutrophil & lymphocyte counts & serum albumin levels were available. The analysis was restricted to first available lab parameters after the start of HD. NLR was calculated as simple ratio of absolute neutrophil & lymphocyte counts. The primary outcome was mortality over 3 years. Cox Model was used to determine the association of trichotomized NLR (< 2.5,2.5-5, >5.0) & albumin levels (<3.1,3.1–4.0,>4.0 g/dL) with mortality. The final model was adjusted for age, gender, race, diabetes, access type, BMI, residual function, body temp, SBP, nPCR, eKt/V, serum creatinine, & (total counts of WBCs, neutrophils, lymphocytes, platelets, platelet:lymphocyte ratio & PNI categories).

Results: 3297 patients were studied (median (IQR) age at start of dialysis 63 (51-73) yrs, 55% male, & 41% Blacks). During a follow-up of 3 yrs, there were 880 (26.7%) deaths. NLR>5.0 vs. <2.5 (unadjusted HR:2.1; P<0.0001) and albumin <3.1 g/dL vs. >4.0 g/dL (unadjusted HR:5.6; P<0.0001) was associated with poor survival. There was significant increase in hazard ratio with each stepwise increase in NLR or decrease in serum albumin levels.



Conclusions: The prognostic tool based on combination of NLR and serum albumin levels may be useful to identify incident HD patients who are at high risk of mortality. Addition of NLR and albumin to existing mortality risk prediction models may further improve their discriminatory ability.

SA-PO531

Correlations between Serum Albumin and Number of Intact Teeth in Relation to Selected Biomarkers (IL-G, FGF-23, PTH) in a Chronic Hemodialysis Randomized Cohort
Judith Beto,[1,2] Miriana Jelebinkov,[2] Ella Trepashko,[2] Kristiyana Kaneva,[1] Louis Scannicchio,[2] Vinod K. Bansal.[1] *[1]Nephrology, Loyola University Healthcare System, Maywood, IL; [2]Nutrition and Biological Sciences, Dominican University, River Forest, IL.*

Background: Hypoalbuminemia in chronic hemodialysis (HD) patients is multifactorial. Purpose: The purpose of this prospective study was to examine correlations between serum albumin (Alb) and number of intact teeth in relation to selected biomarkers in a randomized cohort of 72 chronic HD patients from a single independent dialysis unit.

Methods: Laboratory data collected from single blood draw in Jan 2012. Serum alb, standardized oral exam during prior quarter (Oct-Dec 2011). Analysis: FGF-23, IL-6 by standard ELISA (R&D, Millipore); alb by bromcresol purple. PTH, PO_4, calcium, vit D by assay; oral exam to count number of intact teeth. LOW (<3.6 mg/dl) and HIGH (≥3.6) alb categories calculated. T-test statistics by alb group and gender.

Results: LOW and HIGH alb groups were significantly different (p <0.05) by not by gender. A correlational relationship was seen between alb group and FGF-23, PTH, PO_4. An inverse relationship was seen for CRP-hs, IL-6A, and vitamin D. All patients deficient in Vitamin D. A linear trend relationship was seen for PTH, FGF-23, and PO_4. HIGH alb patients had significantly more intact teeth (p <0.05) compared to LOW alb patients.

TABLE 1:

	Alb g/dl	CRP-hs ug/ml	FGF-23/ PTH pg/ml	Phos mg/dl	IL-6 pg/ml	Vit D ng/ml	% Intact Teeth
Total n=72	3.36(0.3)	1.41(1.0)	2019(1679)/ 638(520)	5.5(1.4)	0.19(0.1)	24.2(13.3)	65(32)
LOW Alb n=52	3.24(0.2)**	1.56(1.1)	1807(1629)/ 609(464)	5.3(1.2)	0.21(0.1)	26.6(15.3)	63(33)**
HIGH Alb n=20	3.70(0.1)**	1.02(0.8)	2503(1728)/ 716(638)	5.9(1.2)	0.15(0.1)	21.7(10.4)	70(30)**
Female n=36	3.35(0.2)	1.38(1.0)	1454(1528)/ 645(508)	5.2(1.3)	0.20(0.1)	23.5(9.7)	62(36)
Male n=36	3.38(0.04)	1.44(0.9)	2546(1654)/ 621(615)	5.8(1.5)	0.18(0.1)	24.4(14.5)	67(29)

Results as mean (SD); **p<0.05 between groups

Conclusions: The percentage of intact teeth may be a simple screening method for albumin risk; no other factors appear to be significantly related in this prospective randomized cohort of HD patients.

SA-PO532

Advance Glycation End Products (AGEs) in Chronic Dialysis Patients
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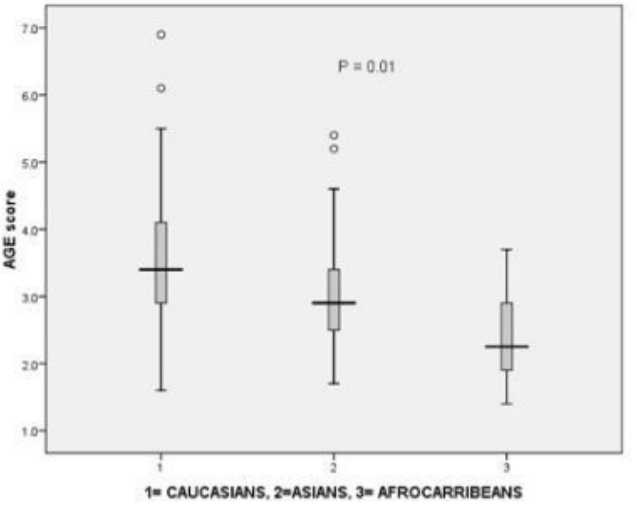
Background: Cardiovascular disease (CVD) is the major cause of death in dialysis patients. There are both traditional and nontraditional risk factors for CVD in dialysis patients, including anemia, hyperphosphatemia, and biomarkers of inflammation. Advanced glycation end products (AGEs) are generated by the Maillard reaction and increased by oxidative stress, and normally metabolized and excreted by kidney, so accumulate in the chronic kidney disease (CKD). Many studies have reported elevated AGEs as cardiovascular risk factors.

Methods: We measured AGEs using a skin autofluorescence (AF) method (DiagnOptics, Groningen, Netherland) in the non-fistula arm of 178 chronic hemodialysis patients.

Results: The patients mean age 63 ± 14.9 years, 58 (32.6%) female, 66 (37%) diabetic and 102 (57.3%) Caucasian, skin AF score for AGEs 3.2 ±9.6 AU, and highest 3.56 ± 0.9 AU in Caucasian population, vs 2.98 ±0.88 AU in Asians and 2.38 ± 0.61 AU in AfroCarribeans (Figure 1), despite diabetes being lowest in Caucasian group.



There was no association between AF-AGEs and age, gender, dialysis vintage, hemodiafiltration, smoking status, history of hypertension, diabetes, peripheral vascular disease, CRP, B2 microglobulin and HbA1C, but AF AGEs were increased in patients with known cardiovascular disease (3.78 ± 0.97 VS 3.01 ± 0.88, p =0.01).

Conclusions: Skin AF AGEs were measured in a multi-ethnic dialysis population and found highest in those with known cardiovascular disease, and may prove to be a simple method for assessing cardiovascular risk.

Funding: Private Foundation Support

SA-PO533

Reliability of Measuring Advanced Glycation End Products by Non Invasive Technique in a Multiethnic Haemodialysis Population
Andrew Davenport, Saurabh Chaudhri. *Department of Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Advanced Glycation end products (AGE) have long been implicated in increasing cardiovascular risk in chronic systemic diseases. Non-invasive methods are now available using its property to fluoresce when exposed to certain wavelength of light. This has led to development of autofluorescence readers (AFR) to measure AGE.

Methods: We measured AGE values using AGE Reader by Diagnoptics Technologies. Mean of 3 values was used to measure AGE concentration in both arms of patients on haemodialysis or haemodiafiltration. Pre and post dialysis readings were also taken in a randomly selected group.

Results: 139 patients were tested (65% males). Data was also collected for ethnicity, gender, access, diabetes, HbA_1C, Beta 2 microglobulin, urine output and RRT modality (HD or HDF).

AGE readings by autofluorescence were found to be to be significantly higher in Caucasians in comparison to Black (mean difference 0.61, p=0.041) and South Asian (mean difference 0.86, p=0.04) population.

In patients with L sided fistula (N= 71) AGE levels were significantly lower in the L arm (3.20 ± 0.79 vs 3.46 ± 0.69, p=0.04) in comparison to the R arm.

AGE levels were also significantly lower in the fistula arm post dialysis in these patients (3.4± 0.54 vs 3.89 ± 0.66, p=0.007).

There was no difference in either arm predialysis (3.23±0.83 in R vs 3.10±0.87 in L) or post dialysis (pre vs post dialysis: p value 0.64 for L arm and 0.85 for R arm). No difference was seen based on variation in gender or modality of RRT.

Conclusions: We have applied this non-invasive technique to a multiethnic group of patients on HD/HDF to show it can be used in a clinical setting.

The results show AGE values to be lower in the (L) fistula arm predialysis and reduce significantly post dialysis.

In patients dialysing with a catheter there was no difference in the AGE reading between the two arms or pre or post dialysis in each arm.

This would suggest measurement of AGE levels in dialysis patients should be done in the non fistula arm and irrespective of the timing (pre or post dialysis). Levels can be done in either arm in patients dialysing with a catheter using this technique.

SA-PO534

Patients in Chronic Hemodialysis Present Increased Indoleamine 2,3-Dioxygenase (IDO) Activity: A Clue for Uremic Immunodeficiency?

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Background: T cell activity has been reported to be depressed in patients in dialysis (HD), in whom regulatory Tcells (Tregs) have been poorly investigated. Since the enzyme indoleamine 2,-dioxygenase (IDO) is known to trigger the suppressive activity of Tregs, we aimed this study at investigating IDO's activity in HD patients looking at the effect of dialysis and correlations with markers of oxidative stress.

Methods: IDO activity was assessed in sera of 52 HD patients (21-88 y.o.) and 20 healthy controls (HC) as change in the ratio between tryptophan (Trp) and its catabolic product kynurenine (Kyn), determined by isocratic RP HPLC with UV detection. Patients were tested before and after HD (26 bicarbonate HD, 26 hemodiafiltration). Markers of oxidative stress (advanced oxidated protein products-AOPP and C reactive protein-CRP) were also tested.

Results: Before HD session Trp levels were significantly lower than in HC (20.44± 7.39 vs 54.37 ± 5.97 µmol/l, P< 0.0001), Kyn levels were significantly higher (4.32± 2.06 vs 2.02± 0.32 µmol/l, P< 0.0001) and the Kyn/Trp ratio, indicating IDO activity, was significantly increased (21.78± 8.06 vs 3.73± 0.55, P< 0.0001). During HD sessions, Trp levels remained unchanged, while Kyn levels significantly decreased (2.49± 1.09 µmol/l, P< 0.0001) producing a significant decrease in Kyn/Trp ratio (11.75± 4.02, P< 0.0001). No correlation was found with signs of oxidative stress (AOPP or CRP levels). No difference was found between patients receiving bicarbonate HD or HDF.

Conclusions: In patients in chronic HD we detected an enhanced activity of IDO, not related with signs of oxidative stress and dialysis modalities, which was partially corrected by the dialysis sessions. The activation of IDO's regulated pathways is expected to depress immune system functions, a feature well known in uremic patients.

SA-PO535

Identifying the Uremic Compounds Specifically Involved in the Pathophysiology of Hemodialysis Patients by Capillary Electrophoresis Mass Spectrometry

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Background: It is suggested that uremic toxins are involved in the pathophysiology of hemodialysis (HD) patients, such as malnutrition-inflammation-atherosclerosis (MIA) syndrome. However, the profile of the uremic compounds in HD patients remains largely unknown.

Methods: We collected serum samples of both pre- and post-HD from 11 HD patients and comprehensively evaluated the concentrations of 119 compounds (53 anions and 66 cations) by capillary electrophoresis mass spectrometry (CE-MS). And the results of pre- and post-HD were compared with those of the CKD patients not undergoing HD we previously reported (Hypertens Res 2010).

Results: Serum concentrations of 33 compounds (19 anions and 14 cations) were significantly higher in pre-HD than those in the patients with CKD stage 5. Among them, 13 anions and 10 cations were removed to the same or below the level of CKD stage 5 by HD. However, serum concentrations of 6 anions (malonate, fumarate, adipate, decanoate, benzoate, N-acetylneuraminate) and 4 cations (Indole-3-acetate, tyrosine, glycerophosphorylcholine and ADMA) were significantly higher in both pre- and post-HD than those of CKD stage 5. On the other hand, 9 compounds (4 anions and 5 cations), which were detected in CKD patients not undergoing HD, were not detected in HD patients.

Conclusions: It was shown that HD patients have the different profile of uremic compounds compared with that of CKD patients not undergoing HD. These 33 compounds whose serum concentrations were higher than those in the patients with CKD stage 5 may be involved with the HD patients-specific pathophysiology.

SA-PO536

Metabolic Profiling Detects Metabolites Associated with Mortality in Hemodialysis Patients

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Background: Circulating biomarkers may identify high-risk hemodiaysis (HD) patients. The ongoing USRDS special study, ACTIVE/ADIPOSE, includes serial collection of blood biomarkers. In an adjunct study, our group is using metabolomics to investigate specific metabolites and metabolic patterns that may be associated with clinical outcomes, including mortality.

Methods: Prevalent patients undergoing HD in Atlanta GA clinics supplied plasma samples at 6-month intervals over an 18-month period. Metabolic profiles of 17 patients who died after study start were compared with profiles of 21 surviving patients who had similar age, race, sex, and HD vintage, using a dual chromatography-LTQ-Velos Orbitrap mass spectrometry (DC-Orbi) method developed at Emory University to generate global metabolic profiles.

Results: The mean (S.D.) ages of deceased and surviving patients, respectively, were 56.7 (13.1) and 58.7 (12.6) at study start, with mean (S.D.) vintage 5.6 (5.0) and 5.5 (4.2) years. All patients were African-American with 57% and 43% male. The DC-Orbi-based metabolomics method detected over 40,000 metabolic features from the 72 plasma samples. Data reduction techniques separated metabolic patterns associated with subsequent mortality (principal component analysis) and identified 81 metabolic features changing significantly between survivors and non-survivors over time (false discovery rate, q=20). Among these features, pathway analysis matched metabolites related to carnitines, biopterins, amino sugars, and amino acids as significantly overrepresented.

Conclusions: Metabolic patterns detected by DC-Orbi indicated metabolic features relating to carnitines (reduced in deceased patients) and amino sugars (increased in deceased patients) which changed over time. Metabolic profiling allows detection of metabolic patterns that may identify HD patients who are at significantly increased risk for mortality and who could potentially benefit from targeted interventions.

Funding: NIDDK Support, Other NIH Support - NCRR, NIA, NIEHS

SA-PO537

Elimination of Proteins by Dialysis and Different Hemodiafiltration Methods

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Background: Various arrangements of hemodiafiltration (HDF) have been introduced to increase the efficacy of hemodialysis (HD). However, also concerns regarding elimination of vital substances emerge.

Methods: The aim of our study was to evaluate elimination of proteins across a wide range of molecular weights (MW) in HD and two HDF methods. A total of 8 dialysis patients were treated in a cross-sectional design with 1) mixed HDF (mHDF, a combination of pre- and postdilution administration of substitution solution), 2) postdilution HDF (pHDF), and 3) HD; all procedures were performed with high-flux polysulfon (Helixon FX1000) dialyzers. Dialysate was collected with the Dialysate Sampling System (Fresenius); the ratio between dialysate sampled and produced was 1:60. A total volume of substitution was 44±4.5 L (pre/post ratio 1.16) in mHDF, and 22±2.3 L in pHDF. Protein concentration in dialysate was measured and proteins resolved by 2D-PAGE and identified by mass spectrometry (MALDI-TOF/TOF). Elimination selectivity of a specific protein was calculated as a ratio of spot volume of mHDF/HD, and pHDF/HD, respectively.

Results: The highest protein elimination was found in mHDF [13.7 g (8.9-17.7), median (interq. range), p<0.05 vs. HD and pHDF], lower in pHDF [6.8 (5.2-10.3, p<0.05 vs. HD] and the lowest in HD [1,9 (1.8-2.2)], ANOVA and Tukey test. In the dialysate 17 proteins with MW from 10 to 80 kDa were identified and matched among the three groups. Elimination selectivity of each protein (ratio of the spot volume in HDF/HD) was 9.6±25.4 (mean±SD) for mHDF and 1.9±4.3 for pHDF. In mHDF the elimination selectivity increased linearly with MW (r=0.59, p=0.015; Spearman correl.), whereas in pHDF it was independent of MW (r=0.26, p=0.3). mHDF seems to remove preferentially larger molecules. Proteins falling into this category were vitamin D binding protein, fetuin A, transthyretin, zinc-α-2-glycoprotein etc.

Conclusions: mHDF removes plasma proteins more effectively than pHDF or HD. Due to high filtration volume mHDF has the potential to remove high MW toxins but at the expense of elimination of useful proteins. Clinical relevance of this ambivalent effect must be tested in long term studies.

Funding: Government Support - Non-U.S.

SA-PO538

Vitamin D and Cognitive Function in Maintenance Hemodialysis Patients

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Background: Vitamin D deficiency and cognitive impairment are both prevalent in US hemodialysis patients. We hypothesized that Vitamin D deficiency may be associated with cognitive impairment due to the vasculo-protective, neuro-protective and immune-modulatory effects of (25 OH) Vitamin D.

Methods: We conducted a cross sectional analysis of 255 patients from the Dialysis and Cognition Study. In linear regression models, Vitamin D was the exposure variable; used first as a continuous variable and then stratified according to the Institute of Medicine guidelines as deficient (<11.9 ng/dl), insufficient (12-19.9 ng/dl) and sufficient (> 20 ng/dl). Principal component analysis was used to obtain the memory and the executive function domains from the individual neuro-cognitive tests. Scores on the individual tests as well as on the memory and executive function domains were used as the outcome variables. Multivariable models were adjusted for age, sex, race, education and other potential confounding variables.

Results: Mean (SD) serum Vitamin D was 17.2 ± 7.4 ng/dl, with 36, 139 and 80 patients in the deficient, insufficient and sufficient groups, respectively. Patients in the deficient group were more likely to be women, African American, diabetic and have longer dialysis vintage. Higher Vitamin D levels were independently associated with better performance on tests assessing executive function, with no association seen with tests assessing memory.

Table: Association between Vitamin D Level and Cognitive Performance

Cognitive Test/Component	Function Tested	Unadjusted		Adjusted	
		Beta	p-value	Beta	p-value
Executive Factor	Composite of 'executive' tests	0.11	0.09	0.14	0.01
Memory Factor	Composite of 'memory tests'	-0.14	0.03	-0.05	0.41
Mini-Mental State Exam	Screen	0.05	0.80	0.22	0.23
Short Delayed Recall	Working memory, recognition, and memory	-0.18	0.33	0.08	0.63
Delayed Recall		-0.30	0.09	-0.05	0.75
Recognition		-0.31	0.11	-0.21	0.28
Block Design	Executive function, processing speed and attention	0.20	0.78	0.24	0.71
Digit Symbol Coding		0.48	0.68	2.02	0.03
Trails A		-4.04	0.15	-5.85	0.04
Trails B		-7.55	0.33	-11.52	0.08

Adjusted for age, sex, race, education, diabetes, smoking, high (May-August) and low (all other months) sunlight months, dialysis vintage and BMI.
Principal component analysis was used to calculate scores on Executive and Memory components, which have a mean of 0 and SD of 1, with positive values representing better performance. Beta coefficients represent a difference in performance on cognitive tests per 1 SD (7.4 ng/dl) higher Vitamin D level. Higher coefficients represent better performance except for Trails A and B where a higher score denotes worse performance.

Conclusions: In conclusion, Vitamin D deficiency in hemodialysis patients is associated with worse cognitive function, particularly in executive domains which are associated with cerebrovascular disease.

Funding: NIDDK Support

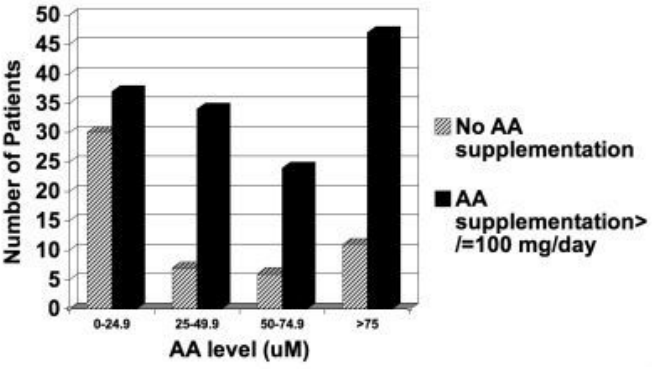
SA-PO539

Relationship of Ascorbic Acid Levels to Vitamin Supplementation in a Prevalent Hemodialysis Population Garry J. Handelman,[2] Nathan W. Levin,[3] Peter Kotanko,[3] Robert L. Benz,[4] Amanda Logan,[1] Jose Albert Avila,[1] Deepali Prasad,[1] Lawrence S. Weisberg,[1] William D. Sirover.[1] *[1]Division of Nephrology, Cooper Medical School of Rowan University, Camden, NJ; [2]Dept of Nutrition, Univ. of Mass at Lowell, Lowell, MA; [3]Renal Research Institute, NY, NY; [4]Division of Nephrology, Lankenau Hospital, Wynnewood, PA.*

Background: Ascorbic acid (AA) is critical for supporting erythropoiesis. AA is cleared by hemodialysis (HD) and patients have the potential to become deficient. AA deficiency is a significant issue as it may require patients to receive higher dosages of erythropoiesis stimulating agents (ESA) in an effort to achieve target hemoglobin level. Some patients take 100 mg/day of supplemental AA that is part of a specially-formulated multivitamin. One cross-sectional study suggests that plasma AA levels <75 μM lead to ESA hyporesponsiveness. We hypothesize that HD patients can have low AA levels even with AA supplementation.

Methods: Pre-HD plasma samples for 196 patients were obtained in the outpatient setting on a Monday or Tuesday after a 72-hour hiatus from HD. Patients' supplemental vitamin intake were reviewed.

Results: One hundred and thirty eight patients (70%), 95 (69%) of whom were taking at least 100 mg of supplemental AA, had plasma AA levels< 75.



Conclusions: These results indicate that the majority of patients will require more than 100 mg of supplemental AA to achieve a plasma AA level >75 μM. Without supplementation, these patients may require higher ESA doses. AA is metabolized to oxalate. Due to decreased urinary oxalate excretion in end stage renal disease, oxalate has the potential to pathologically deposit in organs and cause oxalosis. Further research is needed to determine the risk of oxalosis in HD patients who receive supplemental AA in amounts greater than that in a multivitamin.

Funding: Private Foundation Support

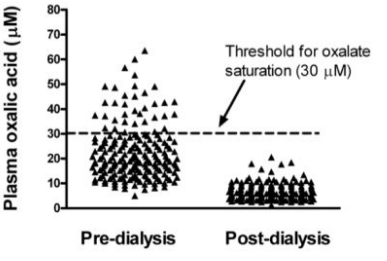
SA-PO540

Plasma Oxalate and Ascorbic Acid Levels in a Prevalent Hemodialysis Population William D. Sirover,[1] Yuguan Liu,[2] Jose Albert Avila,[1] Deepali Prasad,[1] Amanda Logan,[1] Craig B. Langman,[4] Nathan W. Levin,[3] Peter Kotanko,[3] Jeffrey S. Berns,[5] Lawrence S. Weisberg,[1] Garry J. Handelman.[2] *[1]Division of Nephrology, Cooper Medical School of Rowan University, Camden, NJ; [2]Dept of Nutrition, Univ. of MA at Lowell, Lowell, MA; [3]Renal Research Institute, NY, NY; [4]Division of Nephrology, Northwestern University, Chicago, IL; [5]Division of Nephrology, Univ. of PA, Philadelphia, PA.*

Background: Oxalate (Ox) is a metabolite of ascorbic acid (AA) and oxalosis may occur in hemodialysis (HD) patients, as oxalate may pathologically accumulate due to decreased urinary excretion. AA increases iron availability and utilization for erythropoiesis. More information about Ox kinetics is needed before administering AA in a placebo-controlled trial. At plasma Ox levels>30uM, calcium-oxalate supersaturation threshold is reached and oxalosis may occur. Additional evidence in pediatric patients suggests that oxalosis does not develop when pre-HD Ox is >30uM, if post-HD Ox is <30uM. This study tested the hypotheses that pre-HD Ox directly correlates with pre-HD AA and that contemporary HD treatments result in post-HD Ox<30 uM.

Methods: Pre-HD and post-HD plasma samples for 196 patients were obtained in the outpatient setting on a Monday or Tuesday after a 72-hour hiatus from HD.

Results: Pre-HD Ox values ranged from 5.01 to 63.6 uM (mean 22.5 +/- 11.1 uM). Post-HD Ox levels ranged from 1.3 to 20.6 uM (mean 6.4 +/- 3.2 uM). Pre-HD AA ranged from 1.8 to 409.3 uM (mean 65.6 +/- 71.5 uM). Pre-HD Ox correlated directly with pre-HD AA ($p<0.05$).



Conclusions: While oxalate may precipitate temporarily if pre-HD Ox is >30 uM, we demonstrate in adults that oxalate clearance by HD yields post-HD Ox levels that will likely prevent the development of oxalosis. Further research is needed to determine if oxalosis is present if pre-HD Ox is >30 uM.

Funding: Private Foundation Support

SA-PO541

The Impact of Vitamin K_1 Supplementation on Biomarkers of Vitamin K in Hemodialysis Patients: A Randomized Trial Rachel M. Holden,[1] Wilma M. Hopman,[3] Amanda Antczak.[2] *[1]Medicine, Queen's University, Kingston, ON, Canada; [2]Jean Mayer USDA Human Nutrition Research Center of Aging, Tufts University, Boston, MA; [3]Clinical Research Center, Kingston General Hospital, Kingston, ON, Canada.*

Background: Sub-clinical vitamin K deficiency is common in hemodialysis (HD) patients and may be a modifiable risk factor for cardiovascular disease. We aimed to investigate whether short-term vitamin K_1 supplementation improved biomarkers of vitamin K status in HD patients, assessed by circulating vitamin K and uncarboxylated prothrombin (PIVKA-II [protein induced by vitamin K absence]).

Methods: Ths was an interventional randomized placebo-controlled crossover trial. Investigators and patients were blinded to the participant's order of treatment allocation. Twenty (male=12, female=8) prevalent stable HD patients were treated with 1 mg of vitamin K_1 daily versus matching placebo for 2 weeks with a 4 week wash-out period between treatments. Absolute and percent change in plasma levels of vitamin K_1 and PIVKA-II were determined by HPLC and ELISA respectively.

Results: Two subjects were withdrawn from the study due to acute hospitalization. The baseline mean vitamin K_1 level was 0.9±1.0 nmol/L and 0.7±0.6 nmol/L for vitamin K_1 and placebo respectively (p=0.3). The mean increase in vitamin K_1 level in response to supplementation was 6.7 nmol/L (4.0-10.0, P<0.0001) versus 0.6 nmol/L (0.3-1.0, p=.001) in the placebo group (p<0.001). There was a 36.5% reduction in PIVKA-II levels with vitamin K_1 supplementation (1.9 ± 1.3 to1.0 ± 0.5, p=0.002) versus a 10% increase (1.5±1.2 to 1.7±1.4, p=0.07) with placebo (p<0.001). Post-vitamin K_1 supplementation, 94.1% of subjects were in the normal range for PIVKA-II.

Conclusions: This study shows that biomarkers of vitamin K status respond to short-term supplementation with vitamin K_1. Studies evaluating the impact of vitamin K_1 supplementation on cardiovascular outcomes are warranted.

SA-PO542

Phosphate Binders and Thyroid Replacement Therapy in Dialysis Patients: Retrospective Review Yin L. Win, Nnamdi K. Nwaohiri, Farhanah Yousaf, Haroon Rashid, Chaim Charytan, Bruce S. Spinowitz. *Division of Nephrology, New York Hospital Queens, Flushing, NY.*

Background: Prevalence of hypothyroidism among ESRD patients is increased. These patients are frequently prescribed levothyroxine. Many hypothyroid ESRD patients are also taking phosphate binders which could interact with levothyroxine. We explored the prevalence of hypothyroidism and studied the effect of phosphate binders on thyroid profile and thyroid replacement therapy in dialysis patients.

Methods: Medical records of prevalent dialysis patients were reviewed. Patients with prescription order of levothyroxine were grouped according to phosphate binder use; none (G0), sevelamer only (G1), calcium acetate (G2), calcium acetate plus sevelamer (G3), and calcium acetate plus lanthanum carbonate (G4). TSH, T3, T4 as well as monthly doses of levothyroxine and phosphate binders were tabulated.

Results: 342 patients were registered at Trude Dialysis Center between Sep 2008 and Mar 2012. 54 (16%) of 342 patients (27M/27F) had a prescription order for levothyroxine. Fifteen patients were excluded for non-compliance with prescribed phosphate binders or insufficient data.

Table 1: Thyroid Profile

	G0 (n=7)	G1 (n=11)	G2 (n=9)	G3 (n=9)	G4 (n=3)
TSH	2.40	4.81	3.19	5.53	4.15
T3	0.71	0.70	0.76	0.65	0.78
T4	5.31	7.28	8.10	5.83	5.51
Levothyroxine Dose (µg)	80	108	80	100	216*

*p<0.05

Table 2: Relationship of Binder Dose Increase with Levothyroxine Dose Increase

	G1 n=11	G2 n=9	G3 n=9	G4 n=3	Total
Stable Binder Dose	0/5[¶]	0/5	0/4	0/2	0/16
Increase in Binder Dose	5/6	2/4	2/5	1/1	10/16*

*p<0.05

[¶] number of patients with levothyroxine dose increase / total number of patients in group

Conclusions: Our study confirms a high prevalence of hypothyroidism among dialysis patients. TSH was higher in the phosphate binder groups versus no phosphate binder group. Patients taking lanthanum carbonate with calcium acetate may require higher doses of levothyroxine. A statistically significant number of dose increases of levothyroxine were necessary following a dose increase in phosphate binders. Larger longitudinal studies are warranted to define the impact of phosphate binders on thyroid profile and thyroid replacement therapy in dialysis patients.

SA-PO543

Effects of Intradialytic Whey Protein Supplementation on Body Composition in Non-Malnourished Hemodialysis Patients Peter J. Fitschen,[1] Brandon Kistler,[1] Pei-tzu Wu,[1] Hae Ryong Chung,[1] Jin Hee Jeong,[1] Shane Phillips,[2] Ken Wilund.[1] *[1]University of Illinois, Urbana, IL; [2]University of Illinois - Chicago, Chicago, IL.*

Background: Malnutrition is common in maintenance hemodialysis (MH) patients and results in a significantly increased risk of mortality. Moreover, an inverse relationship between body mass index (BMI) and mortality has been shown in MH patients suggesting that an increased BMI may be protective in this population. However, it is not clear if the protective effect of BMI is due to lean or fat mass. Previous nutritional interventions in malnourished MH patients (albumin ≤3.5g/dl) have shown that nutritional supplementation (NS) improves albumin, survival, and bodyweight. However, the effects of NS on body composition in non-malnourished MH patients are not clear.

Methods: The purpose of this study was to examine the effects of intradialytic whey protein supplementation on body composition in non-malnourished MH patients. MH patients (n=23, albumin = 4.0±0.1g/dl) were randomly assigned to intradialytic whey protein (Whey, n=13) or non-nutritive placebo (Con, n=10). All beverages were consumed at the beginning of the dialysis session thrice weekly for 6 months. Body composition was assessed by DXA at baseline and 6 months. Scans were performed 24±3 hrs after dialysis to reduce variations in water balance.

Results: After 6 months, there were no significant changes in BMI. However, change in fat mass was significantly different between groups (p=0.003). A significant increase in fat mass was observed in Con (p=0.042) and a significant decrease in fat mass was observed in Whey (p=0.033). There was a trend for an increase in lean mass in Whey (p=0.051); however, no change in lean mass was observed in Con.

Conclusions: These preliminary results from our ongoing trial suggest that 6 months of intradialytic whey protein supplementation may result in changes in body composition in non-malnourished MH patients. Future studies are needed to determine the effects of interventions that change body composition on outcomes in MH patients.

Funding: NIDDK Support

SA-PO544

Gastrointestinal Factors and Glucometabolic Disturbances in Non-Diabetic Patients with End-Stage Renal Disease Thomas Idorn,[1] Filip K. Knop,[2,3] Morten Jørgensen,[1] Jens Juul Holst,[3] Mads Hornum,[1] Bo Feldt-Rasmussen.[1] *[1]Department of Nephrology, Rigshospitalet, Copenhagen University Hospital, Copenhagen Ø, Denmark; [2]Diabetes Research Division, Department of Internal Medicine, Gentofte Hospital, Hellerup, Denmark; [3]Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Copenhagen N, Denmark.*

Background: Non-diabetic patients with end-stage renal disease (ESRD) have disturbed glucose metabolism. The underlying pathophysiology remains to be elucidated. We evaluated gastrointestinally-mediated glucose disposal (GIGD), incretin effect and secretory responses of glucagon and the two incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (parameters, which are all impaired in diabetic subjects with normal kidney function) in non-diabetic ESRD patients.

Methods: Three groups were investigated: ESRD with normal glucose tolerance (NGT; *N*=10), ESRD with impaired glucose tolerance (IGT; *N*=10) and healthy control subjects (CTRL; *N*=11). Examinations included 75 g-oral glucose tolerance test and isoglycemic intravenous (iv) glucose infusion on separate days. Plasma glucose, insulin, glucagon and total forms of GLP-1 and GIP were measured repeatedly. GIGD, based on glucose amounts utilized, and incretin effect, based on incremental insulin responses, were calculated.

Results: GIGD was significantly reduced in both ESRD groups compared to CTRLs (P<0.016). Incretin effects were 64±5% (CTRL), 54±5% (ESRD+NGT) and 38±8% (ESRD+IGT), with significant difference between CTRLs and ESRD+IGTs (P=0.014). Fasting concentrations of glucagon, GLP-1 and GIP were significantly increased in ESRD patients (P<0.005). Glucagon suppression (% of baseline) was significantly impaired in both ESRD groups compared to CTRLs (P<0.001), while total responses of GLP-1 and GIP (baseline-corrected) were unaltered among groups (P>0.12).

Conclusions: Non-diabetic ESRD patients are characterized by 1) reduced GIGD, 2) diminished incretin effect in those with IGT, and 3) severe fasting hyperglucagonemia that seems irrepressible in response to both oral and iv glucose stimuli. These perturbations may contribute to the disturbed glucose metabolism in ESRD.

Funding: Private Foundation Support

SA-PO545

Glycemic Load(GL) Is Independent Determinant of Oxidative Stress and Inflammation among Prevalent Maintenance Hemodialysis (MHD) Patients Chutatip Limkunakul,[1] Mary B. Sundell,[1] Amy J. Graves,[2] Ayumi Shintani,[2] T. Alp Ikizler.[1] *[1]Nephrology, Vanderbilt University, Nashville, TN; [2]Biostatistics, Vanderbilt University, Nashville, TN.*

Background: High glycemic index (GI) and glycemic load (GL) are associated with increased levels of oxidative stress and inflammation in the general population. MHD patients are known to have excessive oxidative stress burden and inflammation. We examined whether dietary GI or GL is associated with markers of oxidative stress (F2-isoprostanes) and inflammation (C-reactive protein) among MHD patients and, if so, whether body composition or adipokines, i.e. leptin and adiponectin (ADPN) modulate these associations.

Methods: A registered dietitian obtained GI, GL and other dietary data. Two 24-hour diet recalls (a hemodialysis day and a non-hemodialysis day) were analyzed using the Nutrition Data System for Research software. F2-isoprostanes, CRP, leptin, and ADPN were measured in a fasted state. Cross-sectional associations between GI, GL and markers of interest were examined by multiple regressions with adjustment for potential variables.

Results: Fifty-eight MHD patients (mean age 47 ± 12 years, BMI 29.5±6.8 kg/m^2 and DEXA truncal fat 16.4±8.8 kg) were studied. Dietary GI was not associated with F2-isop and log CRP but had significant correlation with truncal fat (r=-0.182, P=0.05). In contrast, GL was significantly associated with F2-isop (P=0.002), both in non-adjusted and adjusted analysis, (P=0.005 after adjustment for age and sex; P=0.004 after including BMI, trunk fat and waist/hip ratio in the model). Addition of leptin or ADPN did not alter the significance of the association. GL also correlated with log CRP (p=0.03) but this association was dependent on BMI and trunk fat. Log CRP correlated with trunk fat and BMI (r=0.05, P=0.01 and r=0.06, P=0.02, respectively).

Conclusions: Dietary GL, but not GI, is significantly associated with markers of oxidative stress and inflammation among prevalent MHD patients, independent of body composition and adipokines. These data may indicate the importance of dietary nutrient intake and its potential role in determining the dysregulated metabolic profile in MHD patients.

SA-PO546

Influence of Hemodialysis on Incretin Axis and Insulin Secretion Anna Masajtis-zagajewska, Ilona Kurnatowska, Malgorzata Jadwiga Wajdlich, Michal P. Nowicki. *Department of Nephrology, Hypertension and Kidney Transplantation, Medical University, Medical University, Lodz, Poland.*

Background: Incretin hormones are secreted in the gut after a meal and stimulate insulin production. Both main incretins, i.e. GLP-1 (glucagon like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide) are eliminated by the kidneys. Little is known about the changes of incretin axis in end-stage renal disease (ESRD). The aim of the study was to assess the influence of elective start hemodialysis therapy on serum GLP-1 and GIP and insulin sensitivity in diabetic and non-diabetic ESRD patients.

Methods: The study comprised 56 patients (23 F, 33 M, mean age 57±14 years) with ESRD in the course of diabetic nephropathy (n=23) and non-diabetic renal diseases (n=34) who were qualified to start chronic HD. Glucose metabolism, including incretin hormones concentration, was assessed before the first HD session and after 6 months of the therapy.

Results: After 6 months of HD a significant increase of GLP-1 concentration was observed in both diabetic and non-diabetic patients (by 2.27 pmol/l (45%) and 1.28 pmol/l (22%); respectively, p=0.0003). Serum GIP increased significantly only in diabetic patients (by 30.9 pg/ml (55%); p=0.008). No significant changes of fasting glucose was found but HOMA-IR and serum insulin decreased significantly in diabetic patients (p=0.01 and p=0.008, respectively). In contrast, HOMA-β was unchanged in both groups of patients.

Conclusions: Our results indicate that hemodialysis therapy helps to restore incretin axis in particular in patients with diabetic kidney disease.

Funding: NIDDK Support

SA-PO547

Effect of Insulin Infusion on Insulin-Like Growth Factor I during Hemodialysis Mark Reinhard,[1] Jan Frystyk,[2] Bente Jespersen,[1] Per R. Ivarsen.[1] *[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus N, Denmark; [2]Department of Endocrinology and Internal Medicine & Medical Research Laboratories, Aarhus University Hospital, Aarhus C, Denmark.*

Background: Hemodialysis (HD) is a catabolic procedure that probably contributes to the high frequency of protein-energy wasting among patients on maintenance HD. In fasting patients undergoing a midweek HD session, a marked reduction in serum levels of bioactive insulin-like growth factor I (IGF-I) has been observed. Accordingly, the aim was to investigate whether it was possible to stimulate bioactive IGF-I by a meal with or without concomitant infusion of glucose±insulin.

Methods: In a randomized cross-over study, 11 non-diabetic HD patients (M/F:8/3, median age 57 years, range 33-79) received a meal after fasting overnight and started either 1) no treatment, 2) glucose infusion (10% glucose, 2.5 mL/kg/h), or 3) glucose-insulin infusion (10% glucose added 30 units of NovoRapid® per liter, 2.5 mL/kg/h) for 2 h before a standardized 4-h HD session. Infusion with either glucose or glucose-insulin was continued throughout the HD session. During infusion blood glucose was maintained at 8.0-10.0 mmol/L by additional glucose infusion. Blood samples were collected at baseline and until 2 h after the HD session.

Results: Data are presented as mean±SD. From baseline to end of HD there was no significant difference in the change in either serum bioactive IGF-I (p=0.99) or total IGF-I (p=0.22) between groups, but there was an overall increase in both bioactive IGF-I (from 0.83±0.27 to 1.01±0.34 µg/L, p<0.001) and in total IGF-I (from 124±43 to 132±52 µg/L, p=0.001). Similarly, from baseline to end of HD there was no significant difference in the change in either serum IGF-binding protein 1 (IGFBP-1) (p=0.43) or IGFBP-2 (p=0.40) between groups. There was an overall decrease in IGFBP-1 (from 267±147 to 143±92 µg/L, p<0.001), whereas IGFBP-2 rose transiently at 120 to 180 min with a maximum of 9% (p<0.001) before returning to baseline levels at the end of HD.

Conclusions: A meal at the beginning of HD increased both total and bioactive IGF-I; there was no further effect of adding either glucose or glucose-insulin infusion.

Funding: Private Foundation Support

SA-PO548

Physical Functionality in Hemodialysis Patients: Body Composition as a Predictor of Functional Capacity M. A. Martinson,[1] T. Alp Ikizler,[3] Y. Zhang,[1] R. Filipowicz,[1] G. Wei,[1] Tom Greene,[1] G. Morrell,[1] S. Beddhu.[1,2] *[1]Univ of Utah; [2]VHASLC, Salt Lake City, UT; [3]Vanderbilt Univ, Nashville, TN.*

Background: Hemodialysis (HD) patients have decreased functional capacity. It is not known if body composition (muscle and/or fat) is a better predictor of physical functioning in this population than body size alone.

Methods: The Protein Intake Cardiovascular Disease and Nutrition in CKD stage V study (PICNIC) is an ongoing longitudinal study of HD patients. In 114 patients, generalized estimating equations (GEE) were used to perform simultaneous cross-sectional regressions relating the 6-min distance walked at baseline, 6 and 18 months to mid-thigh muscle area (MTMA), intra-abdominal fat area (IAFA) measured at the same time points. A similar analysis was used with BMI as the predictor variable. Adjustments were made for age, gender, race, duration of ESRD, vascular access type and study site.

Results: Mean age was 51± 17 years with 59% men, 22% black, 43% with DM, 24% with CAD, and 20% with PVD. Median duration of HD was 33 (IQR 12, 52) months. Average MTMA, IAFA and BMI were 107 ± 28 cm^2, 133 ± 79 cm^2 and 28.5 ± 6.3 kg/m^2, respectively. Average 6-min distance walked was 323 ± 101 m. The regressions of distance walked with body composition and body size indices are summarized in Table 1.

Associations between body composition, body size and 6-min distance walked

	Distance Walked (m) beta [95% CI], p-value
Model 1	
For each SD ↑ MTMA (cm^2)	18 [-3, 38], p=0.09
For each SD ↑ IAFA (cm^2)	-28 [-43, -13], p<0.001
Model 2	
For each SD ↑ BMI (kg/m^2)	-21 [-36, -6], p=0.01

Age, gender, race, duration of ESRD, vascular access type, and study site were adjusted for in both models.

Conclusions: Increased body size and decreased intra-abdominal fat area are associated with decreased physical functionality, whereas higher muscle area may be associated with increased physical functionality. Interventions that target increasing muscle mass and decreasing fat mass may improve physical functioning in the dialysis population.

Funding: NIDDK Support

SA-PO549

Relation between Physical Performance and Daily Physical Activity in Maintenance Hemodialysis Patients Jun Chul Kim,[1] Bryan B. Shapiro,[2] Yinan Li,[2] Min Zhang,[2] Janos Porszasz,[2] Kamyar Kalantar-Zadeh,[2,3] Joel D. Kopple.[2,3] *[1]Division of Nephrology, CHA Gumi Medical Center, Korea; [2]LA BioMedical Research Institute at Harbor-UCLA Medical Center; [3]UCLA Schools of Medicine & Public Health, Los Angeles, CA.*

Background: Maintenance hemodialysis(MHD) patients often display diminished physical performance(PP), and this might influence daily physical activity(DPA). We therefore assessed this relationship in MHD patients and Normal of similar age and gender mix.

Methods: PP and DPA were assessed in 76 patients undergoing MHD and 40 Normals using 3 measures of PP: 6-minute walking distance(6MW), sit-to-stand(STS) and stair climbing. DPA was measured with an Actigraph GT3X+ Activity Monitor®. The daily vector magnitude for DPA was calculated as the square root of the sum of the squares of the three dimensional axes. All comparisons were age and gender adjusted.

Results: Patients were 52±12SD years, 33% female, 40% diabetic; dialysis vintage was 55±45 mos. Normals were 51±13 years and 40% female. The DPA, averaged over 7 consecutive days, was greatly reduced in MHD vs. Normals(p<0.0001). DPA averaged for 7 days correlated with 6MW(r=0.275, p=0.023)(Fig. 1) but not with STS (r= 0.181, p= 0.149) or 22 stair climb(r= 0.157, p=0.201). The 6MW correlated negatively with the % time in sedentary or sleep activity(r=-0.324, p=0.009) and positively with % time in ≥moderate physical activity(r=0.292, p=0.018)(Fig. 2). The STS did not correlate with % time in sedentary or sleep activity(r= -0.142, p= 0.261) nor with time in ≥moderate physical activity(r= 0.175, p= 0.164).

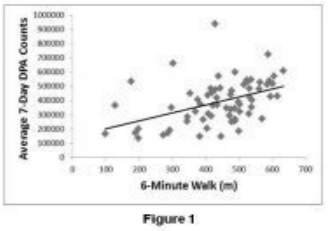


Figure 1

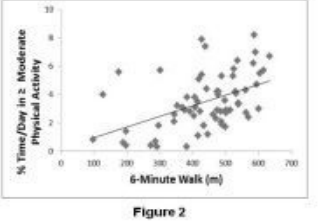


Figure 2

Conclusions: These findings indicate that in MHD patients measures of PP correlated with average DPA; increased PP may be a cause and/or result of greater DPA. Measures that increase 6MW might enhance DPA. 6MW was a more sensitive indicator of DPA than either STS or stair climb measurements.

SA-PO550

Muscle Size and Co-Morbid Conditions Do Not Explain Low Physical Function in Dialysis Patients R. Marcus,[1] Paul Lastayo,[1] R. Filipowicz,[1] G. Wei,[1] Patricia Lynn Painter,[1] G. Morrell,[1] Mary B. Sundell,[2] T. Alp Ikizler,[2] S. Beddhu.[1,3] *[1]Univ of Utah, Salt Lake City, UT; [2]Vanderbilt Univ, Nashville, TN; [3]VHASLC, Salt Lake City, UT.*

Background: Frailty is common in end-stage renal disease (ESRD). We examined whether muscle mass accounts for ESRD associated decreased physical functioning.

Methods: Demographic and co-morbidity baseline data from 3 previous studies (1 HD, 2 non-HD) were utilized. Comorbid conditions included CAD, CHF, PVD, CVD, cancer, diabetes, and lung disease. All subjects underwent baseline testing that included MRI derived mid-thigh muscle area (MTMA) and distance walked in 6 minutes (6MW). Multiple linear regression was used to relate 6 MW to dialysis and non-dialysis groups adjusted for comorbidities and muscle mass.

Results: Clinical characteristics in the HD and non-HD participants are summarized in Table 1.

Characteristics of HD and no-HD participants

	HD, n=122	non-HD, n=109
Age (yrs)	49±16*	74±7
Male (%)	57*	29
White (%)	64*	95
CAD (%)	23	14
CHF (%)	18*	3
PVD (%)	17*	4
CVD (%)	15	17
Lung disease (%)	13	7
Diabetes (%)	42*	17
Alcohol intake	34	45
BMI (kg/m²)	28.6±5.6	28.1±6.5
MTMA (cm²)	110±27	97±24
6MW (m)	316±112*	410±120

*Difference between groups p<0.05

In a multiple linear regression model, 6 MW distance was substantially lower in HD participants compared to non-HD participants (-147.0 m, 95% CI -197 to -97 m, p<0.001) even after adjusting for muscle area and other factors listed in Table 1.

Conclusions: There was a clinically significant difference in physical functioning between non-HD and HD patients, as measured by 6MW. This difference in 6MW was not explained by muscle size or measured co-morbid conditions. HD participants walked substantially shorter distances than non-HD participants despite greater muscle area and younger age. Whether the impaired functional ability of muscle in HD patients is related to other metabolic disturbances such as oxidative stress, inflammation, or other muscle structural abnormalities associated with uremia should be examined in future studies.

Funding: NIDDK Support

SA-PO551

Effects of Exercise and Nandrolone on Frailty among Hemodialysis Patients

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Background: Frailty is common among patients with CKD and has been linked to decreased survival and loss of independence. The extent to which frailty can be reversed, particularly among patients with ESRD, is unclear. The purpose of this study was to evaluate the effect of exercise and anabolic steroids on frailty among HD patients.

Methods: Patients undergoing HD for ≥ 3 mo with evidence of malnutrition or poor quality of life assessed by questionnaire were eligible to participate in the Nandrolone and EXercise Trial (NEXT). Frailty was defined as 3 or more points from: SF-36 Physical Function score of < 75 (2 pts), Vitality score <55 (1pt), and the lowest quintile of weekly energy expenditure PASE questionnaire using normative data (1pt). The intervention consisted of intradialytic resistance exercise training of the lower extremities and/or weekly nandrolone decanoate (ND) by IM injection (2x2 factorial) for 12 weeks. The effect of the interventions on frailty was assessed using general estimating equations (GEE).

Results: 79 patients were enrolled, 66 of whom had measures of frailty before and after the intervention. The majority were male (63%) with a median dialysis vintage of 26 months and a mean KT/V of 1.4 ± 0.3. Hypertension (91%), diabetes (48%) and CAD (42%) were common. At baseline 58% of participants met frailty criteria. Among frail patients assigned to exercise, 36% no longer met criteria for frailty at the follow-up visit. 40% of patients who were not initially frail and did not exercise became frail at f/u (vs. 15% of nonfrail exercisers;p=0.04). There was no statistically significant improvement in frailty among patients who received ND (p=0.77).

Change in Frailty after Exercise

Table 1					
Study Group	Frail-> Frail	Frail -> Not Frail	Not Frai l -> Frail	Not Frail -> Not Frail	Total
Exercise	14	8	2	11	35
No Exercise	13	3	6	9	31
Total	27	11	8	20	66

Conclusions: Almost 40% of frail patients who exercised no longer met frailty criteria after 3 mos, and a similar percentage of nonexercisers became frail. These findings demonstrate that frailty is potentially reversible through exercise and suggest that deterioration is common without intervention.

Funding: NIDDK Support

SA-PO552

Progressive Resistance Exercise Training for Skeletal Muscle Anabolism in Hemodialysis Patients: A Randomized Controlled Trial

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Background: Progressive resistance exercise training (PRET) may reverse muscle wasting but the anabolic response to such exercise in hemodialysis patients remains unclear. This efficacy study determined whether a novel intradialytic PRET technique could reverse atrophy and consequently improve strength and physical function in hemodialysis patients. A second aim was to compare any anabolic response to that of healthy participants completing the same program.

Methods: In a single blind, controlled study 22 hemodialysis patients (mean (SD), age: 53(16) years) and 9 healthy individuals (51(16) years) were randomly allocated to a PRET or an attention control (SHAM) group. PRET completed a high intensity leg extension exercise (3 sets of 10 repetitions at 80% of regularly reassessed maximal strength) using novel equipment. SHAM completed low intensity stretching exercises using ultralight (1.5kg) latex bands. Exercise was completed thrice weekly, during dialysis in hemodialysis patients, over 12 weeks. Outcomes included thigh muscle volume by magnetic resonance imaging, knee extensor strength by isometric dynamometer, lower body tests of physical function and harms. Data were analysed *per protocol* by analysis of variance.

Results: PRET elicited an anabolic response in both hemodialysis patients (PRET +84(123)cm^3; SHAM -109(118)cm^3) and healthy participants (PRET +136(163)cm^3; SHAM -32(54)cm^3) (Omnibus ANOVA: p=0.01). PRET also increased knee extensor strength in both hemodialysis patients and healthy participants. In contrast, PRET only significantly enhanced lower body functional capacity in the healthy participants. Harms possibly related to PRET included one case of muscle soreness and five episodic hypotensive events. All harms were resolved without late effects or sequelae.

Conclusions: Intradialytic PRET safely elicited normal anabolic and strength responses in hemodialysis patients. The lack of a change in functional capacity was surprising and warrants further investigation. *Registered clinical trial NCT01007838.*

Funding: Clinical Revenue Support

SA-PO553

Subclinical Hypothyroidism and Mortality among Dialysis Patients

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Background: Subclinical hypothyroidism is highly prevalent in the general and dialysis populations, but its clinical significance and the benefits of thyroid hormone replacement remain unclear.

Methods: We examined the association between hypothyroidism and all-cause mortality in 2715 prevalent adult dialysis patients with baseline thyrotropin levels measured between January 2004 and April 2011. Mortality was ascertained by the Social Security Death Master Index and local registration systems. In parallel analyses, hypothyroidism overall and subclinical hypothyroidism, defined by thyrotropin levels, and associations with mortality were estimated using Cox proportional hazards models. To reduce risk of observing reverse-causal associations, analyses included 30-day lag periods between thyrotropin measurement and start of at-risk time.

Results: There were 2365 (87.1%) euthyroid controls and 350 (12.9%) hypothyroid subjects (thyrotropin > assay upper limit normal), of whom 238 (68.0%) had subclinical hypothyroidism (upper limit normal < thyrotropin ≤ 10 mIU/L). Hypothyroidism overall and subclinical hypothyroidism were each associated with increased mortality: adjusted HRs (95% CIs) 1.27 (1.06-1.52; p=0.009) and 1.32 (1.07-1.63; p=0.01), respectively. Hypothyroidism treated with thyroid hormone replacement was not associated with increased mortality compared to euthyroidism, whereas untreated hypothyroidism was: HR (95% CI) 1.58 (1.26-1.98; p<0.001); findings were similar when treatment status was considered only in the context of subclinical hypothyroidism. Sensitivity analyses indicated that cardiovascular mechanisms may mediate associations between hypothyroidism overall, subclinical hypothyroidism, and death.

Conclusions: Hypothyroidism overall and subclinical hypothyroidism are associated with increased all-cause mortality in dialysis patients, which may be ameliorated by thyroid hormone replacement.

Funding: NIDDK Support

SA-PO554

Is New Anemia Protocol Resulting in Iron Overload in Hemodialysis Patients?

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Background: Recently several trials reported significant adverse effects associated with Erythropoietin stimulating agents (ESA) therapy. This has led to alterations in protocols used for anemia management in end stage renal disease (ESRD) patients (pts.). The new protocols involve lower hemoglobin (Hb) target and higher threshold for increase ESA dose. Aim of this study is to evaluate the effect of new anemia protocol on Hb and iron storage in ESRD pts.

Methods: Total of 173 were included in this retrospective study from several dialysis units. Data were collected for 6 months before (Pre-protocol) and after (Post-protocol) implementation of new protocol: demographic data, body mass index, dialysis providers, number and days of hospital admission, ESA and iron doses and result of Hb, serum iron, ferritin, total iron binding capacity, and albumin. These variables were used in a logistic regression model for high post-ferritin level (>800 ng/ml).

Results: 107 (62%) pts. were male, average age 53±12 yr, 168 (97%) were African-American.There were 31% reduction in ESA dose (P<0.0001) and 24% increase in serum ferritin level (P<0.0001) with no change in Hb level.

Results before and after change in anemia protocol

Variables	Pre-protocol	Post-protocol	P
Average serum ferritin >800 (ng/ml)	26%	46%	<0.0001
Average serum ferritin >1200 (ng/ml)	8.7%	17.3%	<0.0001
Hb g/dl	10.5±3.6	10.98±1.2	0.1567
Serum iron (mcg/dl)	66.3±24.6	73.1±25.5	0.0002
Serum TSAT (%)	28.9±9.4	32.7±11.5	<0.0001
Iron dose (mg/month)	131±136	103±103	0.0001
Serum Ferritin (ng/ml)	636±423	833±485	<0.0001
Serum albumin (gm/dl)	3.9±0.4	3.9±0.4	0.0218
Erythropoietin dose (IU/session)	4220±6165	2895±4235	<0.0001
Hospital days	5±10	5±11	0.7398

In multivariable model high post-ferritin level (>800 ng/ml) was predicted by low post-serum albumin, low post-iron dose and dialysis provider.The inverse response of serum ferritin to iron dose most likely represent lag effect of iron dosing on serum ferritin.

Conclusions: With the new anemia management protocol a significant reduction in ESA dose was achieved without any change in Hb level at the expense of significant increase in serum ferritin level. This can increase the risk iron overload in pts. with hypoalbuminemia.

SA-PO555

The Comparative Short-Term Effectiveness of Intravenous Iron Dosing on Anemia Parameters among US Hemodialysis Patients Abhijit V. Kshirsagar,[1] Janet K. Freburger,[1] Alan R. Ellis,[1] Lily Wang,[1] Wolfgang C. Winkelmayer,[2] M. Alan Brookhart.[1] *[1]University of North Carolina at Chapel Hill; [2]Stanford University.*

Background: Intravenous (IV) iron is in widespread use in the US hemodialysis population, yet there is limited data on the comparative effectiveness of different dosing strategies. Previous studies have not directly compared doses that are now routinely used in practice, or have had limited sample sizes and follow-up.

Methods: We conducted a retrospective cohort study using data from the clinical database of a large dialysis provider (years 2004-2008) merged with administrative data from the United States Renal Data System. Exposures were 2 sets of dosing patterns assessed over a 1 month period: (1) bolus (consecutive doses of 100 mg with the potential to exceed 600 mg during one month versus maintenance (all other iron dosing), and (2) high (> 200 mg) versus low dose (≤ 200 mg). Outcomes were achieved differences in hemoglobin levels, epoetin alfa (EPO) dose, transferrin saturation (TSAT), and serum ferritin, during a 6 week follow-up period, adjusted for a demographic and medical covariates.

Results: 117,050 patients met study entry criteria. Most individuals received maintenance dosing (47.4 %), 16.3 % received bolus dosing, and 36.3% received no iron. 29% of patients received high dose and 34% received low dose iron. The average dose of iron during the exposure period was 709 mg for the bolus group and 203 mg for maintenance, 558 mg for the high dose group and 140 mg for the low dose. Bolus dosing was associated with higher average hemoglobin (+0.23 g/dl,95% C.I. 0.21-0.26), TSAT (+3.31% 95% C.I. 2.99-3.63), and ferritin (+151 mcg/L, 95% C.I. 134.9- 168.7), and lower average EPO dose (-464 u 95% C.I. -583 to -343) compared to maintenance. High dose of intravenous iron was associated with higher hemoglobin (+0.19 g/dl, 95% C.I. 0.17-0.21), lower EPO dose (-606 u, 95% C.I. -701 to -511), higher TSAT (+3.27%, 95% C.I. 2.99- 3.56) and ferritin (+141 mcg/L, 95% C.I. 133.3-163.6) relative to low dose.

Conclusions: In a large dialysis cohort, both bolus and high dose IV iron led to improved anemia parameters; these benefits should be weighted against any potential harms.

Funding: Other U.S. Government Support

SA-PO556

The Comparative Short-Term Effectiveness of Iron Sucrose versus Ferric Gluconate on Anemia Parameters among US Hemodialysis Patients Abhijit V. Kshirsagar,[1] Janet K. Freburger,[1] Alan R. Ellis,[1] Lily Wang,[1] Wolfgang C. Winkelmayer,[2] M. Alan Brookhart.[1] *[1]University of North Carolina at Chapel Hill; [2]Stanford University.*

Background: Iron sucrose (IS) and ferric gluconate (FG) are the two most commonly used formulations of intravenous (IV) iron among US hemodialysis population. Although they are generally believed to be interchangeable in clinical practice, there is limited data on their comparative effectiveness in anemia management.

Methods: We conducted a retrospective cohort study using data from the clinical database of a large dialysis provider (years 2004-2006) merged with administrative data from the United States Renal Data System. The two exposures were iron sucrose and ferric gluconate delivered in a one-month period. Outcomes were differences in hemoglobin levels, epoetin alfa (EPO) dose, transferrin saturation (TSAT), and serum ferritin, between the formulations measured during a 6 week follow-up period.

Results: 66,207 patients comprised the sample. 39% received iron sucrose 12% received ferric gluconate, and 39% were non-users. The IS and FG users had a comparable baseline distributions of demographic, clinical, and laboratory parameters. The average dose during the exposure period was 325 mg for IS and 329 mg for FG. Iron sucrose was associated with higher average hemoglobin (+0.16 g/dl, 95% C.I. 0.12-0.19) than ferric gluconate during the follow-up period. There were no observed differences in EPO dose, TSAT or serum ferritin.

Conclusions: In a representative population of US hemodialysis patients, these results suggest that iron sucrose is slightly more effective than ferric gluconate at raising hemoglobin.

Funding: Other U.S. Government Support

SA-PO557

Modifying Effects of Iron-Biomarkers on Hemoglobin Changes in Hemodialysis Patients Adam E. Gaweda,[1] Premila Bhat,[2] Gregory A. Maglinte,[3] Chun-lan Chang,[4] Jerrold W. Hill,[4] Grace S. Park,[3] Akhtar Ashfaq,[3] Matthew Gitlin.[3] *[1]U of Louisville; [2]Ridgewood Dialysis Ctr; [3]Amgen Inc; [4]IMS Health.*

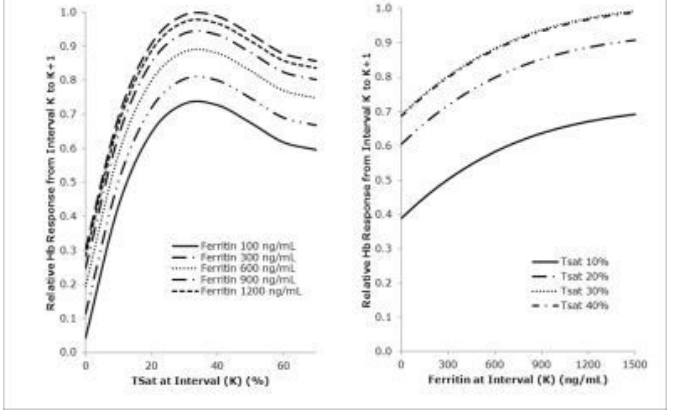
Background: KDOQI guidelines recommends concurrent use of erythropoiesis stimulating agents and iron supplements for anemia in end-stage renal disease patients, targeting levels of transferrin saturation (TSAT) >20% and ferritin >200 ng/mL. The relationship between iron biomarkers, iron and Epoetin alfa (ESA) dosing, and subsequent changes in hemoglobin (Hb) levels were evaluated.

Methods: Electronic medical records of 1,902 hemodialysis patients from 25 hospital-based dialysis clinics and 20 independent dialysis organizations across the US were retrospectively analyzed. From June 1, 2009 to June 30, 2010, patients had ≥1 Hb value during each 4-week interval for 4 consecutive intervals (k-2, k-1, k and k+1, where k is the index interval), ≥1 intravenous (IV) ESA dose during interval k-1 or k, and ≥1 TSAT and ferritin value at interval k. Using a generalized estimating equation approach, the change in Hb (g/dL) from k to k+1 was regressed on the change in ESA (units) and IV iron (mg) from k-1 to k, Hb levels at interval k-2, k-1, and k, TSAT and ferritin at interval k, and interactions of ESA dose change with TSAT and ferritin fit to a polynomial function.

Results: Patients were mean (SD) 62 (15) years of age; 41% Caucasian, 35% African American; 65% had hypertension and 39% diabetes. Polynomial model results indicated TSAT levels had a significant interactive effect ($p<0.05$) on Hb change relative to ESA dose change, but ferritin did not. At an ESA dose change of 1000 units/kg/month, relative Hb response was maximized when TSAT was 35% and ferritin was >600ng/mL (Figure).

Conclusions: Higher TSAT and ferritin values had minimal incremental impact to Hb change. Overall, TSAT had a significant and more pronounced modifying effect on Hb change compared to ferritin.

Figure. Relative Hb response to an Epoetin alfa dose change of 1000 units/kg/month across values of TSAT and ferritin



Funding: Pharmaceutical Company Support - Amgen Inc.

SA-PO558

Therapeutic Effect of Intravenous Ascorbic Acid in Hemodialysis Patients with Normoferritinemic Anemia Byung Chul Shin, Wan Soo Lee. *Internal Medicine, Chosun University Hospital, Gwangju, Republic of Korea.*

Background: Hemodialysis (HD) patients with functional iron deficiency often develop resistance to recombinant human erythropoietin (rhEPO). Recent studies showed that intravenous ascorbic acid (IVAA) administration could override rhEPO resistance in HD patients. This study was undertaken to test the effects of IVAA in HD patients with normoferritinemic functional iron deficiency accompanied with EPO-hyporesponsive anemia.

Methods: Fifty-eight HD patients with normoferritinemic (between 100 and 500 ug/liter) anemia were included and divided into the controls (N = 25) and IVAA (N = 33) groups. IVAA patients received 500 mg of intravenous ascorbic acid with each dialysis session for 3 months and additional 4 month follow-up after the end of the therapy.

Results: Twenty patients has a response to IVAA with significantly increase in their hemoglobin (Hgb > 1.0 g/dL) and reduction of weekly rhEPO dosage compared with control group after three months treatment ($p < 0.05$). Compared with non-responders, transferring saturation (TSAT) was significantly decreased in responders group (26 ± 11 vs. 35 ± 14%, $p < 0.05$) on baseline data. There was a significant rise in serum iron and TSAT (baseline vs. 3 months, serum iron 57 ± 22 vs. 108 ± 22 ug/dL, TSAT 26 ± 11 vs. 52 ± 7%, $p < 0.05$) and a fall in serum ferritin (377 ± 146 vs. 233 ± 145 ng/mL, $p < 0.05$) of responders group (N = 20) but no significant changes in controls and non-responders group (N = 13) at 3 month treatment.

Conclusions: IVAA can be a potent and effective adjuvant therapy for hemodialysis patients with rhEPO-resistant normoferritinemic anemia.Also IVAA can reduce the dosage of rhEPO for anemia correction.

SA-PO559

Diagnostic Biomarkers to Appraise Protein-Energy Wasting and Association of Inflammation in Maintenance Hemodialysis Patients Byung Chul Shin, Wan Soo Lee. *Internal Medicine, Chosun University Hospital, Gwangju, Republic of Korea.*

Background: Protein-energy wasting (PEW) contribute significantly to the increased cardiovascular mortality among dialysis patients. However, there is no isolated marker capable of assessing the nutritional status of patients with chronic kidney disease. We investigated several parameters to appraise PEW and association of inflammation in maintenance hemodialysis patients.

Methods: Sixty patients were enrolled in this cross-sectional study. The nutritional status of the patients were divided three groups according to Subjective Global Assessement (SGA). 1) Severe malnutrition (SGA 1 to 3), 2) Mild to moderate malnutrition (SGA 4 to

5), 3) Well nutrition (SGA 6 to 7). We also simultaneously checked inflammatory markers, nutritional markers and performed an anthropometric measurement.

Results: Of all patients, sixteen patients (26.7%) were malnourished. PEW-positive patients had a difference in body mass index, % usual body weight, % standard body weight, Geriatric nutritional risk index, skinfolds, fat mass, air circumference, cardiovascular disease, albumin, high-sensitivity C-reactive protein (hsCRP), transferrin, ferritin, hemoglobin and hematocrit compared with PEW-negative patients. CRP levels were significantly higher in the malnourished group. Compared with patients without PEW, the presence of PEW was associated with incrementally higher cardiovascular disease ($p<0.05$).

Conclusions: Serum hsCRP is a strong predictor of malnutrition and inflammation in hemodialysis population. The sensitivity can be increased by associating serum albumin with other nutritional and anthropometry markers to correctly evaluate the nutritional status of hemodialysis patients. Also, SGA is a simple and inexpensive method in clinical practice for detection in the patients with PEW.

SA-PO560

Are Greater Cumulative IV Iron Doses in Hemodialysis Patients Associated with Mortality? D. Miskulin,[1,4] Navdeep Tangri,[2,4] Jing Zhou,[3,4] Karen J. Bandeen-roche,[3,4] Wieneke Michels,[3,4] L. Ebony Boulware.[3,4] *[1]Tufts Medical Center; [2]University of Manitoba; [3]Johns Hopkins University; [4]The DECIDE Network Patient Outcomes in ESRD Study Investigators.*

Background: Intravenous (IV) iron use in US hemodialysis (HD) patients has increased and erythropoiesis stimulating agents (ESA) use has declined following studies showing increased mortality with targeting higher hemoglobin (Hb) and recent changes in Medicare payments for ESAs. IV iron impairs phagocytic function, is a mediator of oxidative stress, and deposits in organs, all of which may increase mortality. Toxicity may be greater among patients with higher transferrin saturation (TSat) and ferritin values.

Methods: We studied a cohort of patients initiating HD between 2003-2008 in Dialysis Clinic Inc. dialysis units. We followed patients until death, transplant, loss-to-follow-up or Dec 31, 2008. In a discrete time interval Cox Proportional Hazards model adjusting for age, sex, race, cause ESRD, BMI, eGFR at HD start, and time varying TSat / ferritin, Hb, EPO dose, comorbidity, vascular access, post-HD weight, pre-HD systolic BP, serum albumin, and creatinine, we determined the relationships of cumulative iron dose, defined in 1-, 3-, and 6- month rolling windows, with mortality. We tested for interactions between iron dose and TSat/ferritin level.

Results: Among 12,468 incident patients (median follow up 1.6 years), 3817 (30.6%) died. Median [interquartile range] cumulative iron doses over 1-, 3-, and 6- month rolling windows were 100 [0,300], 500 [150,1000], and 1075 [550, 1600] mg, respectively. Greater cumulative iron doses were not statistically significantly associated with mortality (Table), even among patients with higher TSat/ ferritin values.

1-Month Iron	**None**	**1-100 mg**	**101-200 mg**	**201-300 mg**	**>300 mg**
	Ref	0.99 (0.91,1.08)	0.96(0.88,1.04)	1.00 (0.90,1.12)	1.01(0.94,1.08)
3-Month Iron	**None**	**1-200 mg**	**201-600 mg**	**601-1000 mg**	**>1000 mg**
	Ref	0.99 (0.90, 1.10)	1.00 (0.93, 1.07)	0.94 (0.87,1.02)	1.02 (0.94, 1.10)
6-Month Iron	**None**	**1-700 mg**	**701-1000 mg**	**1001-1800 mg**	**>1800 mg**
	Ref	0.96 (0.88, 1.04)	0.95 (0.86, 1.04)	0.96 (0.89, 1.03)	1.06 (0.98, 1.16)

Conclusions: There was no increase in mortality with greater cumulative 1,3- or 6-month IV iron doses, even among patients with higher TSat/ferritin values.

Funding: Other U.S. Government Support

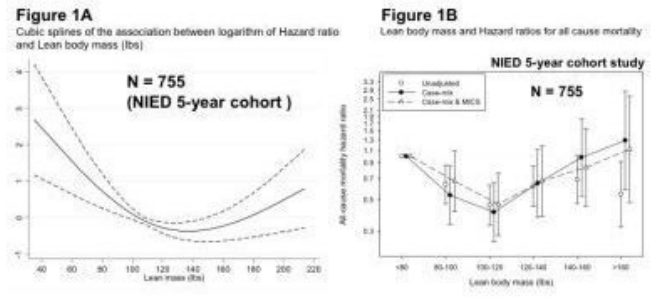
SA-PO561

Association between Lean Body Mass and Mortality in Maintenance Hemodialysis Patients Manoch Rattanasompattikul,[1] Miklos Zsolt Molnar,[1,2] Jongha Park,[1] Rachelle Bross,[3] Jennie Jing,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, LA BioMed at Harbor-UCLA, Torrance, CA; [2]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [3]Harbor-UCLA, Torrance, CA; [4]Nephrology, University of Tennessee, Memphis, TN.*

Background: The link between inflammation and malnutrition resulted in the description of a syndrome called protein-energy wasting (PEW). The mechanisms underlying the associations between PEW and survival are not well established. We hypothesized that lean body mass (LBM) is a predictor of mortality in maintenance hemodialysis patients (MHD).

Methods: In a 5-year prospective cohort study of 755 MHD patients, we examined the impact of LBM on survival. We used near-infrared (NIR) interactance to assess LBM and divided MHD patients into six categories: <80, 80-100, 100-120, 120-140, 140-160, >160 lbs. Cox proportional hazards models estimated death hazard ratios (HRs) (and 95% CIs), and cubic spline models were used to examine associations with mortality over 5 years (2001-2006).

Results: Mean age of patients was 54±15 years, 53% were diabetic and 49% Hispanic. The group with the highest LBM (>160 lbs) had more blacks (60%) and Hispanics (76%). In the unadjusted model, HRs were significantly lower in the LBM 80-100, 100-120, 120-140 and >160 lbs groups (0.64(0.47-0.87), 0.45(0.33-0.63), 0.62(0.45-0.85) and 0.54(0.32-0.92), respectively) compared to the LBM < 80 lbs group. Cubic splines illustrated a J-shape association of LBM with mortality (Figure1A). The associations after case mix and MICS adjustment are shown in Figure 1B.



Conclusions: In MHD patients, the survival advantage of higher LBM appears to be unclear. Many abstruse factors affect mortality in patient with higher LBM. Clinical trials to examine the outcomes of interventions that modify body composition in MHD patients are indicated.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK078012

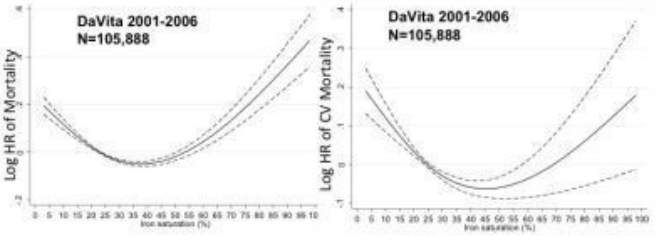
SA-PO562

Mortality-Predictability of Iron Saturation Ratio across Different Racial and Ethnic Groups of Hemodialysis Patients Manoch Rattanasompattikul,[1] Miklos Zsolt Molnar,[1,2] Jongha Park,[1] Joshua Zaritsky,[3] Jennie Jing,[1] Keith C. Norris,[4] Csaba P. Kovesdy,[5] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, LABioMed at Harbor-UCLA, Torrance, CA; [2]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]Charles R. Drew University; [5]University of Tennessee, Memphis, TN.*

Background: Iron stores may be related to outcomes in maintenance hemodialysis (MHD) patients. It is unclear to what extent serum levels of iron saturation (TSAT) are associated with outcomes in this population in different racial and ethnic groups of MHD patients.

Methods: We examined mortality-predictability of iron markers in a contemporary cohort of adult MHD patients, including 36% African Americans (AAs).

Results: We identified 105,888 MHD patients (age, 62±16 years and 55% men) with iron saturation ratio (TSAT) data. In a case mix, malnutrition–inflammation complex syndrome (MICS) adjusted model, TSAT below 20% was associated with all-cause death hazard ratio (HR) [95% confidence interval] of 1.08(1.02-1.15) in AAs, 1.08 (1.03-1.14) in Caucasians and 1.08 (0.98-1.18) in Hispanics. In Hispanic MHD patients, the HR for all-cause of death was increased in the subset with TSAT above 50% (1.13, CI 1.01-1.28, p=0.04). Cubic splines confirmed a reverse J-shaped association of TSAT with all-cause mortality and cardiovascular mortality.



Conclusions: A reverse J-shaped association between TSAT and mortality exists across all races and ethnicities of MHD patients. The reason for significantly different racial/ethnic differences in adjusted mortality risk at lower TSAT levels is unclear and deserves further exploration.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

SA-PO563

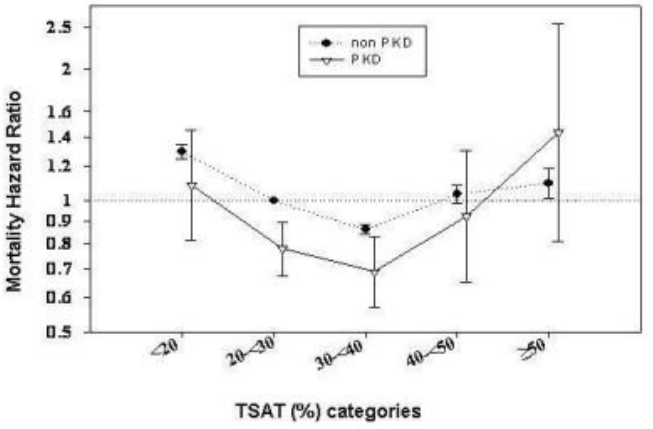
Optimal Level of Transferrin Saturation in Maintenance Hemodialysis Patients with and without Polycystic Kidney Disease Parta Hatamizadeh,[1,2] Joshua Zaritsky,[3] Lilia R. Lukowsky,[1] Miklos Zsolt Molnar,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,3] *[1]Harold Simmons Center, LABioMed at Harbor-UCLA, Torrance, CA; [2]University of Michigan, Ann Arbor, MI; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]University of Tennessee, Memphis, TN.*

Background: Transferrin saturation (TSAT) is one of the indices used to guide management of anemia. Anemia is thought to be less prominent in patients with chronic kidney disease (CKD) due to polycystic kidney disease (PKD) compared to other CKD patients. Therefore, iron metabolism might vary between PKD patients and other CKD patients. Optimal level of TSAT in CKD patients is unclear and is more so in those with PKD.

Methods: We studied 2,969 maintenance hemodialysis (MDH) patients with PKD (57.9±12.9 years old; 46% female) and 128,054 without PKD (62.0±15.4 years old; 45% female). Survival predictability of TSAT was studied using baseline, time-averaged and

time-dependent values with multivariable case-mix and malnutrition inflammation complex syndrome (MICS) adjusted Cox regression models.

Results: In both PKD and non-PKD MHD patients, there was a U-shaped association between TSAT and mortality hazard ratio. A baseline TSAT between 40% and 50% was associated with the best survival in both PKD and non-PKD patients and that of time-averaged TSAT was between 30% and 40% in both groups. A time dependent TSAT between 30% and 40% was associated with the best outcome in PKD patients; however, that of non-PKD patients was a TSAT range of 40% to 50%.



Conclusions: In both PKD and non-PKD patients on MHD, a time-averaged T-SAT between 30% and 40% was associated with the best survival. Additional studies in both PKD and non-PKD dialysis patients are required to determine whether or not therapeutic attempts to keep TSAT levels in that range will improve survival.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK077341

SA-PO564

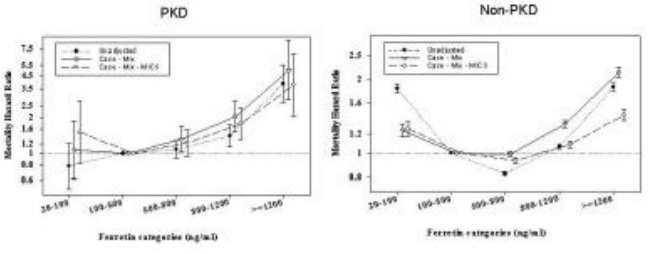
Association between Ferritin Level and Survival in Maintenance Hemodialysis Patients with and without Polycystic Kidney Disease

Parta Hatamizadeh,[1,2] Lilia R. Lukowsky,[1] Joshua Zaritsky,[3] Miklos Zsolt Molnar,[1] Csaba P. Kovesdy,[4] Kamyar Kalantar-Zadeh.[1,3] *[1]LABioMed at Harbor-UCLA, Torrance, CA; [2]University of Michigan, Ann Arbor, MI; [3]David Geffen School of Medicine at UCLA, Los Angeles, CA; [4]University of Tennessee, Memphis, TN.*

Background: Ferritin level is used as a guide for anemia management. Patients with chronic kidney disease (CKD) due to polycystic kidney disease (PKD) have less severe anemia compared to other CKD patients. Therefore, their supplemental iron requirement may also be different. The most favorable level of ferritin in CKD patients, in general, is still unclear and it has not been studied specifically in PKD patients.

Methods: We studied 131,023 maintenance hemodialysis (MDH) patients including 2,969 with PKD. Survival predictability of ferritin (divided into 5 subgroups: 20-<100ng/mL, 100-<500, 500-<800, 800-<1,200, ≥1,200) was studied using time-averaged values via Cox regression models adjusted for case-mix and Malnutrition Inflammation Complex Syndrome (MICS) variables.

Results: In survival analyses of 2,969 PKD (57.9±12.9 years old; 46% female) and 128,054 non-PKD (62.0±15.4 years old; 45% female)patients on MHD using ferritin 100-<500 ng/ml as reference group, a more prominent J-shaped association was observed in non-PKD patients, with the best survival rate in the 500-<800 ng/mL range, whereas for PKD patients ferritin values above 500 ng/ml exhibited a trend towards incrementally higher mortality.



Conclusions: Time-averaged ferritin displays a J-shaped relationship with mortality with best survival at 100-<500 ng/mL for PKD patients and 500-<800ng/mL for non-PKD patients even after adjustment for demographics, comorbidities and surrogates of inflammation and nutrition. Whether iron stores have differential risk implications across PKD vs. non-PKD warrants additional studies.

Funding: Other NIH Support - R01 DK078106, K24 DK091419, R21 DK077341

SA-PO565

Association between Variability of Serum Ferritin Level and Mortality in Maintenance Hemodialysis (MHD) Patients

Takahiro Kuragano, Yoshinaga Otaki, Yukiko Hasuike, Takeshi Nakanishi. *Internal Medicine, Division of Nephrology and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

Background: We have been already reported that ferritin variability is associated with that of Hb in maintenance hemodialysis (MHD) patients. In this study, we evaluated the association between ferritin variability and mortality and morbidity in MHD patients.

Methods: Prospective, observational multi center study. In 1086 MHD patients, we measured serum ferritin, Hb, and TSAT levels every 3 month. Cardiovascular disease (CVD), infectious disease, hospitalization, and all cause death were registered during 24 months. According to fluctuation patterns of serum ferritin, patients were divided into 5 groups: persistently low ferritin (LOW:<100 ng/mL), persistently high ferritin (HIGH : ≥100), decrease form high ferritin to low (H to L), increase from low to high (L to H) and high amplitude (HA). The association between fluctuation patterns of ferritin levels and events were investigated with Cox Proportional Hazards Model for Time-Dependent Variables.

Results: At the start of the study, the mean Hb level was 10.6±1.0g/dL and mean ferritin was 125.4±147.0ng/mL. During observational period (24 months), 79 CVD, 368 infectious diseases, 324 hospitalizations, and 36 deaths were occurred. Patients with HIGH group showed elevated risk for CVD (HR: 95%CI: 2.22, p=0.003), infectious disease (HR: 95%CI: 1.76, p<0.0001) compared with those of LOW group. The risk for hospitalizations in H to L ferritin group was significantly (HR: 95%CI: 1.59, p=0.013) higher than those of LOW group. Moreover, the risk for death of L to H (HR: 95%CI: 6.18, p=0.002) group and HA (HR: 95%CI: 3.75, p=0.029) group were significantly higher than those of LOW group.

Conclusions: MHD patients with ferritin ≥ 100 ng/mL bear an increased risk for CVD and infectious disease. Furthermore, MHD with increased ferritin levels or high amplitude of ferritin fluctuation also increased risk for death.

SA-PO566

Low Hepcidin Level Is Associated with Increased Erythropoiesis in Maintenance Hemodialysis (MHD) Patients with Less Iron Storage

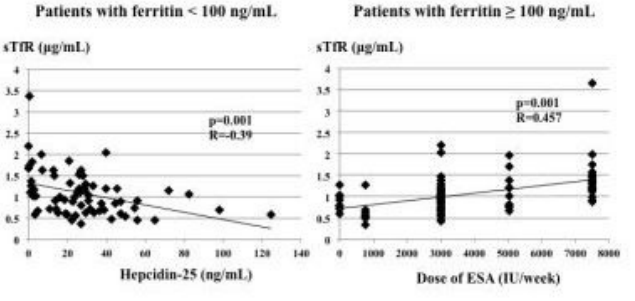
Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Division of Nephrology and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.*

Background: It has been believed that repleted iron storage is essential for erythropoiesis. However, in Japan (J-DOPPS), 40%>MHD patients can maintain hemoglobin(Hb)>11g/dl even in the condition of serum ferritin level<100 ng/ml. For clarifying the determinants of erythropoiesis in MHD patients with low iron storage, we evaluated the regulating factors of serum levels of soluble transferrin receptor (sTfR) in MHD patients.

Methods: 153 MHD patients were recruited in this study and classified into two groups (I: ferritin<100 and II: ferritin≥100) according to iron storages. sTfR, Hb and serum levels of iron, ferritin, albumin, high sensitivity CRP, creatinine, TIBC, intact-parathyroid hormone were measured. Serum levels of hepcidin-25 were also quantified using LC-Ms/MS.

Results: Hepcidin-25, TIBC, and TSAT levels in Group II were significantly higher than Group I. There were no significant differences in Hb, iron, sTfR, and dose of ESA between two groups. In the multivariate analysis, hepcidin-25(p= 0.004, β=-0.3), iron(p= 0.003, β=-0.3), and albumin(p=0.03, β=0.2) were selected as the independent predictors of sTfR in Group I, while iron(p=0.005, β=-0.3), and dose of ESA(p=0.012, β=0.3) were selected in Group II.

Correlation between serum sTfR and hepcidin-25, and dose of ESA



Conclusions: Irrespective of iron storage, serum iron was the significant predictor of sTfR, which could be linked to iron availability for erythroid cells via transferrin. In the patients with low ferritin, the negative correlation between sTfR and hepcidin-25 indicated that iron availability could be paralleled with erythropoiesis, because the release of iron and its transport from reticuloendothelial system and iron absorption from the gut is tightly regulated by hepcidin-25. On the other hand, in the patients with repleted iron storage erythropoiesis was fully dependent on the dose of ESA.

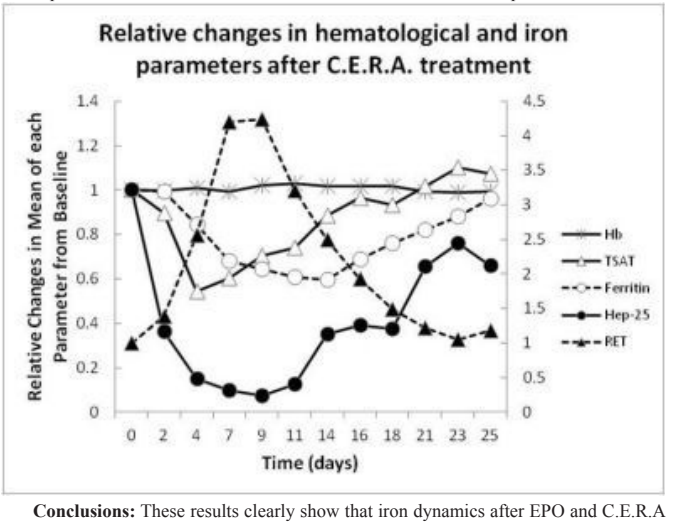
SA-PO567

Iron Dynamics in Hemodialysis Patients Receiving Epoetin-Beta or Continuous Erythropoietin Receptor Activator (C.E.R.A.) Masanobu Horie,[1] Eizou Hasegawa,[1] Ken-ichi Minoshima,[1] Yasushi Shimonaka.[2] *[1]Department of Urology, Daiyukai Daiichi Hospital, Japan; [2]Product Research Department, Chugai Pharmaceutical Co., Ltd., Japan.*

Background: C.E.R.A., methoxy polyethylene glycol-modified epoetin-beta, is a long acting erythropoiesis stimulating agent (ESA), proven to maintain hemoglobin levels with monthly administration. The difference in iron dynamics and erythropoiesis after C.E.R.A. and epoetin-beta (EPO) treatment were investigated.

Methods: Fourteen hemodialysis (HD) patients receiving 100 μg C.E.R.A. Q4W and 8 HD patients receiving 4500 IU/week EPO, who had been stably maintained hemoglobin levels by these ESA, were enrolled. All patients were received anemia treatment with same ESA before registration. Prior to every dialysis during 25 days observation period, blood samples were obtained and hematological and iron parameters including serum hepcidin-25 were measured.

Results: Hemoglobin levels were stably maintained in both groups. Although transferrin saturation (TSAT) declined weekly, no consistent change of other parameters were observed in EPO-treated group. On the contrary, rapid and continuous suppression of hepcidin-25 and reciprocal increase of reticulocytes were observed in C.E.R.A.-treated group. TSAT reduction followed by gradual recovery and gradual reduction of ferritin were also observed. These parameters were recovered toward the end of the observation period.



Conclusions: These results clearly show that iron dynamics after EPO and C.E.R.A treatment are quite different. EPO treatment induces stable iron mobilization. On the other hand, C.E.R.A. induces rapid increase of iron demand triggered by intensive expansion of erythropoiesis, leads to rapid TSAT decline and gradual reduction of ferritin, nevertheless continuous hepcidin-25 suppression. This study demonstrates that C.E.R.A. induces intensive iron mobilization and effective iron usage for erythropoiesis.

SA-PO568

Iron Dynamics and Erythroblast Maturation Kinetics after C.E.R.A. Treatment Yasushi Shimonaka, Yuki Omori, Mariko Noguchi-sasaki, Yusuke Sasaki, Keigo Yorozu. *Product Research Dept., Chugai Pharmaceutical Co., Ltd., Kamakura, Kanagawa, Japan.*

Background: The mechanism of iron mobilization by erythropoiesis stimulating agents (ESA) and the effects of continuous erythropoietin receptor activator (C.E.R.A.), a methoxy polyethylene glycol-modified erythropoietin (EPO), on iron dynamics and erythroblast maturation kinetics were investigated.

Methods: C57BL/6N (B6) mice with total body irradiation (TBI) or TBI followed by bone marrow transplantation (BMT) were intravenously injected EPO or vehicle. EPO or carbamylated EPO (C-EPO) was intravenously injected into B6 mice. These mice were sacrificed and analyzed hematological and iron parameters including serum hepcidin. B6 mice intravenously treated with 2, 10 μg/kg of C.E.R.A. or vehicle were sacrificed and analyzed hematological and iron parameters, as well as the maturation status of bone marrow erythroblast by flow cytometry stained with TER119 and CD71.

Results: Although TBI abolished the reduction of serum hepcidin by EPO injection which was observed in control mice, BMT recovered the reduction of serum hepcidin by EPO. C-EPO, which conserves *in vitro* inhibitory effect of hepcidin production but no erythropoietic activity, did not reduce serum hepcidin in B6 mice. C.E.R.A.-treated mice showed significantly higher hemoglobin than in vehicle treated mice for 14 days after treatment, reciprocally, serum hepcidin was continuously suppressed in C.E.R.A.-treated mice, nevertheless serum iron was markedly decreased at day 5. Rapid increase followed by gradual decrease of TER119(+)CD71(high) immature erythroblast and delayed increase of TER119(+)CD71(low) mature erythroblast were observed in C.E.R.A.-treated mice.

Conclusions: These results indicate that erythropoietic activity of ESA is essential for iron mobilization through serum hepcidin reduction. C.E.R.A. stimulates effective erythropoiesis with marked iron consumption and leads to effective iron recruitment for erythroblasts by remarkable hepcidin reduction. Delayed erythroblast maturation after C.E.R.A. treatment suggests the process might be controlled by iron supply. These unique features of C.E.R.A. might contribute to prevent rapid increase of hemoglobin after C.E.R.A. treatment.

SA-PO569

The Change of Reticulocyte Hemoglobin Equivalent Levels after Administration of Continuous Erythropoietin Receptor Activator in Hemodialysis Patients Midori Kakimoto-Shino,[1] Tetsuya Fujikawa,[1] Tadashi Kuji,[1,2] Nobuhito Hirawa,[1] Yoshiyuki Toya,[1] Satoshi Umemura.[1] *[1]Yokohama City University, Japan; [2]Yokodai Central Clinic, Japan.*

Background: Epoetin beta pegol called CERA (continuous erythropoietin receptor activator), recently approved, has been reported to be well effective in renal anemia control; however, relation between iron status and efficacy of CERA remains unknown. The aim of this study was to investigate iron status and response to erythropoiesis stimulating agents (ESAs) including CERA and epoetin beta (EPO) in hemodialysis patients.

Methods: A total of 106 outpatients received chronic hemodialysis at a satellite clinic were randomized to CERA or EPO. Hb levels and reticulocyte hemoglobin equivalent (RetHe) levels were measured weekly for a month. Baseline serum hepcidin levels as a key regulator of iron homeostasis were measured with mass spectrometry. In the CERA arm, patients who had received 0-750 IU, 750-4499 IU or 4500-9000 IU of EPO per week were given 0 μg, 75 μg or 100 μg of CERA per month, respectively. Target Hb level was 10-11 g/dl. ESAs resistance index (ERI) was defined as weekly weight-adjusted dose of ESAs divided by hemoglobin concentration.

Results: A total of 101 participants (mean age 67.1±11.6 (SD), 31.7% of female, 48.5 % of diabetes) were analyzed. There was no significant difference in mean Hb levels between two groups during the study period (CERA 10.8±0.97 g/dl vs. EPO 10.8±0.60 g/dl, p=0.962). Decline in RetHe level in the first week in CERA group was greater than in EPO group (-4.44±2.57 vs -2.44±1.42, p<0.001). The estimation of ESAs doses to maintain target Hb value was 1μg of CERA corresponded to 286 IU of EPO. Change of RetHe in the first week were negatively correlated with ERI in CERA arm (r = -0.383, p = 0.006), but not in EPO arm. Decline of RetHe in the first week remained significant after adjusting for potential confounders.

Conclusions: Patients with CERA showed greater decline in RetHe value in the first week than those with EPO. Higher hepcidin at baseline was correlated with poor responsiveness to CERA. The efficacy of CERA treatment may be changed by the iron metabolism during the first week.

SA-PO570

Importance of Intensive Iron Supplementation for Dialysis Patients with Continuous Erythropoietin Receptor Activator Toko Hashimoto, Yusuke Tsugawa, Masataka Tsunoda, Ryota Ikee, Naomi Sasaki, Nobuo Hashimoto. *H.N.Medic, Sapporo, Hokkaido, Japan.*

Background: Impact of different iron supplementation protocols for dialysis patients treated with Continuous Erythropoietin Receptor Activator (CERA) is unknown.

Methods: Dialysis patients with stable hemoglobin (Hb) levels for 12 weeks were eligible for this study (N=130). Erythropoietin was switched from short acting agent to CERA and followed up for 13 weeks. Participants were treated with either intensive iron supplementation (intravenous iron administration 120 mg/week for 12 weeks) or conventional treatment (40 mg/week for 4 weeks). The outcomes were change in Hb level at 13 weeks, required CERA dosage, and total costs necessary to control anemia. We constructed multivariable regression models adjusted for the potential confounders.

Results: intensive iron supplementation was associated with 0.9 (95% CI: 0.4 to 1.3) g/dL higher Hb level (p<0.001), 74.7 (95% CI: 141.1 to 8.2) μg lower required dosage of CERA, and US $198.2 (95% CI: 30.0 to 366.4) lower anemia-related mediation costs after 13 weeks.

	Change in Hb level *1 (g/dL) (95% CI)	p-value	Required CERA dosage (μg) (95% CI)	p-value	Related medication costs (US$) (95% CI)	p-value
Intensive iron supplement strategy	0.9 (0.4 to 1.3)	<0.001	-74.7 (-141.1 to -8.2)	0.03	-198.2 (-366.4 to -30.0)	0.02
Conventional iron supplement	Reference		Reference		Reference	
Age	0.0008 (-0.01 to 0.01)	0.9	2.3 (0.4 to 4.3)	0.02	4.9 (-0.006 to 9.9)	0.05
Female	-0.03 (-0.3 to 0.3)	0.8	-3.0 (-46.6 to 40.6)	0.9	-10.5 (-120.9 to 99.8)	0.9
Baseline Hb level	-0.9 (-1.1 to -0.6)	<0.001	-34.1 (-66.8 to -1.4)	0.05	-61.6 (-144.6 to 21.3)	0.1
Serum ferritin concentration	0.00004 (-0.001 to 0.001)	1.0	-0.1 (-0.3 to 0.1)	0.4	-0.1 (-0.7 to 0.4)	0.6
Transferrin saturation*2	-0.008 (-0.02 to 0.005)	0.2	0.8 (-1.2 to 2.9)	0.4	0.4 (-4.9 to 5.6)	0.9
High sensitivity C-reactive protein	-0.3 (-0.006 to -0.003)	<0.001	17.9 (-11.7 to 47.4)	0.2	29.6 (-45.1 to 104.4)	0.4
Average CERA dosage	-0.005 (-0.006 to -0.003)	<0.001				

*1 Defined as the change in hemoglobin level from the baseline at week 13. *2 Calculated by Fe/TIBC*100.

Conclusions: Intensive iron supplementation was associated with higher Hb attainment, lower CERA dosage, and lower costs required for anemia control among dialysis patients treated with CERA.

Funding: Clinical Revenue Support

SA-PO571

Effect of Low-to-Moderate Aluminum Exposure on Iron Status and Bone Markers in Chronic Hemodialysis Patients Paweena Susantitaphong,[1] Khajohn Tiranathanagul,[1] Pisut Katavetin,[1] Kearkiat Praditpornsilpa,[1] Marc E. De Broe,[2] Patrick C. D'Haese,[2] Somchai Eiam-Ong.[1] *[1]Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.*

Background: The incidence of aluminum (Al)-related toxicity has declined in hemodialysis (HD) patients. However, some patients continue to receive Al-based phosphate binders due to costs. This study was conducted to explore the effect of low-to-moderate doses of Al-based phosphate binders and their discontinuation on markers of bone disease and iron status in HD patients.

Methods: Following a first screening of serum Al level in 37 HD patients receiving low-to-moderate doses of Al-based binders, a second screening was performed in 22 of the 37 patients after a 2-year follow-up period during which the use of Al-based phosphate binders was phased out. Desferrioxamine test (DFO; 5mg/kg), iron status and bone marker assessment were performed to explore the association between serum Al level, iron status and bone markers in the second screening.

Results: At first screening, the mean serum Al level was 27.8±10.3 µg/L. Thirteen patients had a serum Al level >30 µg/L, considered a level of possible toxicity. There was a positive correlation between the serum Al level and HD duration (r=0.6; p<0.001) as well as with cumulative dose of Al-based binder. At the second screening, the mean serum aluminum level decreased to 12.5±7.4 µg/L and only one patient had a pre-DFO serum Al level >30 µg/L. The mean serum Al level increased to 26.0±14.7 µg/L post-DFO but in none of the patients did the post-DFO change in serum Al exceed 50 µg/L, a threshold which has previously been associated with Al-induced bone disease. The decrease in serum Al level was associated with a significant increase in iPTH whereas total alkaline phosphatase did not change. At both screenings serum Al and ferritin were inversely associated with each other. Dialysate Al levels were below 2µg/L throughout the study period.

Conclusions: If the use of low-to-moderate doses of Al-based binders is required in HD patients with no available substitution, serum Al level, iron and bone markers should be closely monitored to ensure safe use of these drugs.

SA-PO572

Roles of Hepcidin Isoforms in Hemoglobin Variability and Therapeutic Effects of Recombinant Human Erythropoietin in Maintenance Hemodialysis Patients Takashi Yokoyama,[1] Yasushi Shimonaka.[2] *[1]Dept. of Nephrology and Dialysis, Sapporo Higashi-Tokushukai Hospital, Sapporo, Hokkaido, Japan; [2]Product Research Department, Chugai Pharmaceutical Co., Ltd, Kamakura, Kanagawa, Japan.*

Background: We examined to clarify the roles of hepcidin isoforms in hemoglobin variability and effects of recombinant human erythropoietin (rHuEpo) therapy in patients undergoing maintenance hemodialysis (HD) patients with renal anemia.

Methods: 72 HD patients (M:F=48:24, age=62.63±12.84 years old) were observed in 2011. All patients were treated with intravenous administration of rHuEpo (750~9000units/week). The weekly average values of several clinical markers such as ferritin (FRN), high sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6) and Hb were measured at 2-day intervals just before HD. Serum hepcidin isoforms (hepcidin-20, -22, -25) were measured as reported by Murao, et al (Rapid Common Mass Spectrum 2007;21:4033-8). The Hb variability classified as reported by Ebben, et al was measured throughout the year and erythropoiesis stimulating agents resistance index (ERI) in each patient were also examined.

Results: 1) The mean Hb level and hepcidin isoform levels were as follows: low amplitude low (LAL:n=26, Hb=9.93±0.73 g/dL, hepcidin-20=15.29±11.80 ng/mL, -22=2.37±2.57, -25=34.00±25.07), low amplitude high (LAH:13, 11.43±0.42, 6.31±5.22, 0.98±1.09, 14.58±15.26), high amplitude (HA:21, 11.00±0.38, 13.29±10.58, 1.86±1.26, 28.72±29.91) and target (T:7, 11.00±0.23, 11.46±11.48, 1.98±2.17, 30.82±36.08). Significant differences were found between LAL and LAH (p<0.01), HA(p<0.001) and T(p<0.001) in Hb levels. Marked differences were also detected in hepcidin isoforms (-20:p=0.002, -22:p=0.024, -25:p=0.005) between LAL and LAH. 2) Remarkable differences were detected between LAL and LAH in FRN(LAL:81.97±10.75 ng/mL vs LAH:40.71±7.63, p=0.003) and in IL-6 (16.55±6.00 pg/mL vs 4.55±2.30, p<0.05). 3) In ERI (19.15±8.65 units/kg/g.Hb vs 7.65±5.04, p=0.005), marked differences were detected between LAL and LAH.

Conclusions: These results indicate that three different hepcidin isoforms, togerther with FRN and IL-6, affect strongly the therapeutic effects of rHuEpo in maintenance HD patients.

SA-PO573

Serum Hepcidin-25 Level Is Correlated with Serum Levels of Ferritin and Transferrin Saturation in Hemodialysis Patients Noriko Saito,[1] Kazuhide Saito,[2] Masaaki Shimotori,[1] Kozo Ikarashi,[1] Tetsuo Morioka,[1] Hisaki Shimada,[1] Shigeru Miyazaki.[1] *[1]Nephrology, Shinraku-en Hospital, Niigata, Japan; [2]Urology, Niigata University Graduate School of Medicine, Niigata, Japan.*

Background: Hepcidin-25(HPC) is a crucial player of iron metabolism. Iron-overload and inflammation stimulate HPC production, whereas anemia, iron depletion, growth differentiation factor 15(GDF15) and soluble hemojuvelin(sHJV) inhibit HPC production.

Serum HPC level is elevated in hemodialysis patients, however, the correlation with other iron parameters such as Hb, ferritin, transferrin saturation(TSAT), high sensitive CRP(hs-CRP) and soluble transferrin receptor(sTfR) is not well understood.

Methods: 40 hemodialysis patients(HD) and 20 normal healthy volunteers(N) were studied. Serum HPC level was determined by LS-MS/MS, using HPC-25 as an internal standard. GDF15, sHJV and sTfR levels were measured using ELISA. Standard hematological parameters including hs-CRP were also evaluated.

Results: HPC level (ng/ml) was higher in HD than N (48.2±42.3 vs 11.1±11.9, p<0.01). GDF15 level (ng/ml) and sTfR level (nmol/l) were also higher in HD than N (8.5±3.2 vs 0.6 ± 0.2, p<0.001 and 25.7±16.7 vs 16.3±6.5, p<0.01, respectively).

sHJV level(ng/ml) did not show any differences (85.7 ± 35.8 vs 120.5±73.3, NS).

HPC level of N was correlated to Hb (r=0.662, p<0.005), ferritin (r=0.761, p<0.001), TSAT (r=0.728, p<0.005), hs-CRP(r=0.471, p<0.05) and GDF15 (r=0.539, p<0.05), but not to sHJV or sTfR.

HPC level of HD was correlated to ferritin (r=0.898, p<0.0001), TSAT (r=0.677, p<0.0001) and sTfR (r=-0.454, p<0.005), but not to Hb, hs-CRP, GDF15 or sHJV.

Conclusions: In HD, serum HPC level was strongly correlated to iron metabolism markers and not to inflammation or hypoxia markers. Thus HPC level in HD might be mainly regulated by the state of iron utilization.

SA-PO574

Serum Uric Acid Is Associated with Abdominal Adiposity, Inflammation, and Insulin Resistance in Hemodialysis Patients J. Abraham,[1,2] T. Alp Ikizler,[3] R. Filipowicz,[1] Mary B. Sundell,[3] G. Wei,[1] Y. Zhang,[1] Nestor E. Almeida,[1] Kalani L. Raphael,[1,2] S. Beddhu.[1,2] *[1]University of Utah, Salt Lake City, UT; [2]VA Healthcare System, Salt Lake City, UT; [3]Vanderbilt University, Nashville, TN.*

Background: Uric acid (UA) is thought to play a major role in metabolic syndrome but there is a paucity of data on the association of UA with adiposity, insulin resistance (IR), and inflammation in hemodialysis (HD) patients. Therefore, we investigated these associations in the ongoing Protein Intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) study of chronic HD patients.

Methods: Fasting non-HD plasma was used to measure UA, high sensitivity C reactive protein (hsCRP) and insulin levels. MRI was used to measure intra abdominal fat area (IAFA) at baseline and 6 months follow-up. Separate multiple linear regression analyses were used to relate hsCRP, insulin and IAFA at baseline to plasma UA concentration at baseline (cross-sectional model), and to relate hsCRP, insulin and IAFA at 6 months to baseline serum uric acid (lagged model). Adjustments were made for age, gender, race, vascular access, ESRD duration, and study center.

Results: 119 hemodialysis patients were included in the analysis. The mean age was 52 ±16 yrs, 59% were men, 82% were Caucasians and 47% had diabetes. Mean UA was 5.5 ± 1.8 mg/dL. Plasma UA correlated positively with abdominal adiposity, plasma UA correlated positively with hsCRP and insulin both at baseline and at 6 months.

Association for each standard deviation ↑ in uric acid with log hsCRP, log insulin and intra abdominal fat area (IAFA)

	Baseline	Follow up
	Beta, 95% CI, p-value	Beta, 95% CI, p-value
Log hsCRP (g/dL)	0.27 (0.0004, 0.54), 0.05	0.51 (0.10, 0.92), 0.02
Log insulin (IU/L)	0. 30 (0.08, 0.51), 0.01	0.27 (-0.03, 0.58), 0.08
IAFA (cm²)	17.2 (2.3, 32.1), 0.02	27.1 (5.9, 48.3), 0.01

Adjusted for age, gender, race, duration of hemodialysis, vascular access, and study center.

Conclusions: Serum UA is associated with abdominal adiposity, inflammation and IR in HD patients. Interventions that target UA might decrease inflammation in HD patients.

Funding: NIDDK Support

SA-PO575

Circulating Bacterial-Derived DNA Fragments as a Marker of Systemic Inflammation in Peritoneal Dialysis Cheuk-Chun Szeto, Bonnie Kwan, Kai Ming Chow, Chi-bon Leung, Philip K.T. Li. *Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong.*

Background: Endotoxemia is common in peritoneal dialysis (PD) patients, and circulating lipopolysaccharide (LPS) level is related to the degree of systemic inflammation and atherosclerosis. We hypothesize that circulating bacterial DNA, another microbial component, correlates with the degree of systemic inflammation and predicts the survival of new PD patients.

Methods: We measured plasma bacterial DNA level in the archive blood samples of 300 consecutive new PD patients. The result was compared to serum C-reactive protein (CRP) level, patient survival and peritonitis-free survival.

Results: The average age was 57.8 ± 12.1 years; average plasma bacterial DNA level was 34.3 ± 1.3 cycles; average follow up 37.9 ± 22.2 months. Plasma bacterial DNA level correlated with serum CRP (r = 0.565, p < 0.001) and LPS level (r = 0.224, p = 0.029).

At 36 months, the patient survival was 77.5%, 78.3%, 74.6% and 65.2% for plasma bacterial DNA level quartiles I, II, III and IV, respectively (log rank test, p = 0.034). By multivariate analysis with the Cox proportional hazard model to adjust for confounders, plasma bacterial DNA level had no independent effect. Similarly, peritonitis-free survival was 60.6%, 59.8%, 60.3% and 50.4% for plasma bacterial DNA level quartiles I, II, III and IV, respectively at 36 months (p = 0.020), and the difference was not significant after adjusting for confounding factors.

Conclusions: We found that plasma bacterial DNA level correlated with the degree of systemic inflammatory state in PD patients. Although plasma bacterial DNA level seems to predict patient survival and peritonitis-free survival, the association disappears after adjusting for confounding factors. Further prospective studies are needed to delineate the role of plasma bacterial DNA as a prognostic marker of renal failure patients.

Funding: Pharmaceutical Company Support - Baxter Extramural Grant Program (Baxter Healthcare)

SA-PO576

Tumor Necrosis Factor-α Modifies the Relationship between Obestatin and All-Cause and Cardiovascular Mortality in Maintenance Hemodialysis Patients Ilia Beberashvili,[1] Inna Sinuani,[2] Ada Azar,[3] Hadas Kadoshi,[3] Gregory Shapiro,[1] Leonid Feldman,[1] Zhan Averbukh,[1] Joshua Weissgarten.[1] *[1]Nephrology, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Pathology, Assaf Harofeh Medical Center, Zerifin, Israel; [3]Nutrition, Assaf Harofeh Medical Center, Zerifin, Israel.*

Background: We hypothesized that obestatin, an anorexogenic gut hormone, increase may link to protein-energy wasting in maintenance hemodialysis (HD) patients, predisposing to increased mortality risk.

Methods: We performed a prospective cohort study of 94 prevalent hemodialysis patients (38% women) with a mean age of 64.8±11.2 years. In total, the study period extended 45.8±26.7 months.

Results: Among various nutritional markers only fat mass index (FMI) exhibited statistically significant linear association (r=0.210, p=0.042) with obestatin. Surprisingly, patients with serum obestatin concentrations below the median (<7.12ng/ml) had lower cumulative incidences of survival (log rank χ^2=5.65, p=0.017). The crude all-cause (HR 2.43, 95% CI 1.22-4.86) and cardio-vascular mortality hazard ratios (HR 2.97, 95% CI 1.24-8.77) in these patients persisted to be significant after adjustments for various confounders. We then studied the implications of obestatin levels in combination with TNF-α levels. A significant obestatin x TNF-α interaction was found for daily energy intake: patients with high obestatin and low TNF-a values exhibited the lower daily energy intake. Survival analysis showed the modulating effect of TNF-α on obestatin Across the four obestatin-TNF-α categories, the group with high obestatin and low TNF-α exhibited a worse outcome in both, all-cause (log rankχ^2=10.86, p=0.012**)** and cardio-vascular (log rankχ^2=11.19, p=0.011) mortality.

Conclusions: Low serum obestatin concentration is an independent predictor of mortality in prevalent hemodialysis patients. High obestatin values in HD patents are linked to a markedly decreased mortality risk (especially because of cardio-vascular causes) in co-existence of high TNF-α levels. This interction of obestatin with TNF-α in hemodialysis patients should be further elucidated.

SA-PO577

TAM Ligand and Receptor Expression in Chronic Renal Failure Iris J. Lee,[1] Brendan A. Hilliard,[2] John Madara,[3] Swati Rao,[1] Tapan H. Patel,[1] Jean Lee,[1] Crystal A. Gadegbeku,[1] Eric T. Choi,[4] Philip L. Cohen.[2] *[1]Nephrology, Temple University, Philadelphia, PA; [2]Rheumatology, Temple University, Philadelphia, PA; [3]School of Medicine, Temple University, Philadelphia, PA; [4]Vascular Surgery, Temple University, Philadelphia, PA.*

Background: The vitamin K dependent proteins Gas6 and protein S are ligands for the TAM receptors (tyro3, axl, and mer) and function as crucial regulators of pro-inflammation and mediators of vascular disease in animal models. We examined whether TAM ligands and receptor expression is altered in patients with chronic renal failure.

Methods: After informed consent, blood samples were obtained from 23 controls, 53 hemodialysis patients (HD), and 72 patients with chronic kidney disease (CKD). Expression of TAM ligand/receptor and vitamin K deficiency was evaluated by ELISA flow cytometry (FACS) and western blots.

Results: We observed significant increases in plasma Gas6 in CKD and HD with levels correlating with eGFR (r= -0.28 p=0.02), dialysis vintage (r=0.36, p=.008) and variables linked to chronic inflammation, such as low albumin, (r= -0.34, p=.01). Protein S was also increased in the plasma of CKD. (p<0.001) Further, a marginally-significant association between Gas6 and a history of coronary artery disease was found (r=0.26, p=.058).

We studied whether vitamin K deficiency in HD affected Gas6 levels. By PIVKA-II assay, we found >70% had levels >2ng/ml indicating functional vitamin K deficiency (mean=4.48ng/ml). However we observed no relationship between Gas6 and PIVKA-II (r^2=0.12, p=.11). Furthermore, by western blot, HD sera was able to phosphorylate axl suggesting carboxylation of TAM ligands are not essential for axl signaling. By FACS, increased expression of mer and axl was seen on CD14++ monocytes in HD, a specific subset linked to pro-inflammation and vascular disease. In addition, we found increased turnover of the axl receptor, as soluble axl were markedly increased in the plasma of HD compared to controls. (110.1ng/ml vs. 14.8ng/ml, p<0.0001).

Conclusions: We demonstrate dysregulation of the TAM-ligand receptor pathway in HD. This pathway may be critical in inflammatory-mediated vascular disease in CKD.

Funding: Private Foundation Support

SA-PO578

Patterns of C-Reactive Protein and Mortality in Dialysis Patients Gero D. von Gersdorff,[1] Len A. Usvyat,[2] Mathias Schaller,[1] Inga Bayh,[5] Michael Etter,[6] Aileen Grassmann,[5] Adrian Marcos Guinsburg,[3] Jeroen Kooman,[7] Eric Liu,[6] Daniele Marcelli,[5] Cristina Marelli,[3] Laura Scatizzi,[5] Stephan Thijssen,[2] Edwin B. Toffelmire,[4] Yuedong Wang,[8] Nathan W. Levin,[2] Claudia Barth,[9] Peter Kotanko.[2] *[1]University Hospital Cologne, Germany; [2]Renal Research Institute, New York, NY; [3]Fresenius Medical Care (FMC), Argentina; [4]FMC, Canada; [5]FMC Europe, Germany; [6]FMC Asia Pacific, Hong Kong; [7]University Hospital Maastricht, Netherlands; [8]UC- Santa Barbara; [9]KfH Kuratorium für Dialyse und Nierentransplantation, Germany.*

Background: Monitoring of CRP for risk assessment in hemodialysis (HD) patients (pts) has been questioned. We explore if longitudinal analysis of CRP is useful in HD pts.

Methods: MONDO consortium consists of HD databases from RRI (USA), FMC in Europe, Asia, Canada and Latin America, KfH Germany and from University of Maastricht. We assessed CRP and CRP-variability in HD pts in their 1st year and observed survival in the following year. CRP means and coefficients of variation (CoV) were calculated and pts were grouped into tertiles of baseline CRP and CRP-CoV. A Cox model was constructed for hazard ratios (HR) of survival adjusted for risk factors.

Results: CRP was available in 9918 pts, 3822 from Germany (KfH) and 6096 from 15 other European countries (FMC), including 10 in (South)-Eastern Europe. Results are presented separately. Higher CRP were associated with higher HRs. HRs declined with larger CRP variability when the mean CRP was medium or high.

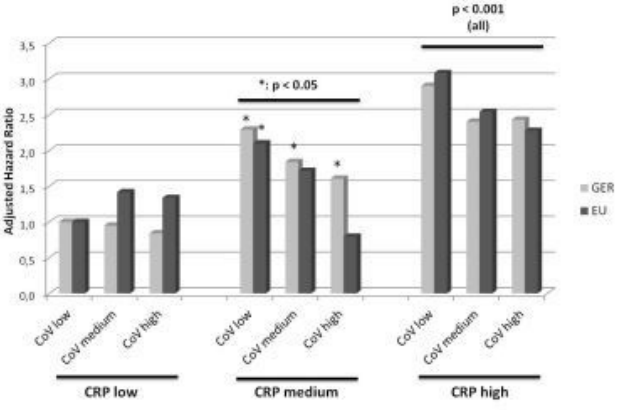


Figure 1: Hazard Ratios of death associated with CRP-elevation and CRP-variability, respectively. Levels of CRP were grouped into low (<7.7 mg/L), medium (7.7 – 18.7 mg/L) and high (>18.7 mg/L), respectively. Similarly, CoV was grouped into low (<0.74), medium (0.74-1.21) and high (>1.21). Hazard ratios were adjusted for age, sex, weight, diabetes, blood pressure, weight gain, and albumin

Conclusions: Not only absolute levels of CRP but also low variability conferred the highest HRs. This suggests that chronic elevations of CRP carry a higher risk than acute CRP increases, such as occur with infections.Longitudinal trends of CRP offer important information for risk prediction in HD pts.

SA-PO579

Circulatory Mitochondrial DNA in Patients on Maintenance Hemodialysis Cao Hongdi, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital of Nanjing Medical University.*

Background: Chronic sterile inflammation plays a crucial role in the development and progression of cardiovascular disease (CVD) in patients with chronic kidney diseases (CKD). Mitochondrial DNA (mtDNA) released into circulation after cell damage can promote chronic inflammation in patients and animal models. However, the role and mechanisms of circulatory mtDNA in chronic sterile inflammation in patients on maintenance hemodialysis (MHD) remains unknown.

Methods: In this study, 74 MHD patients in our dialysis center and 20 health controls were included. The circulatory mtDNA was detected by real-time PCR assay. Plasma C-reactive protein (CRP) and tumor necrosis factor α (TNF α) were quantitated by using ELISA assay.

Results: The results showed that circulatory mtDNA was significantly elevated in MHD patients comparing to those in the health control. The mtDNA level was closely correlated with the levels ofCRP and TNF α in MHD patients. The circulatory mtDNA was also higher in MHD patients with CVD or secondary hyperparathyroidism than those in the others. The effect of different dialysis patterns, including low-flux hemodialysis (LF-HD), high-flux hemodialysis (HF-HD) and online hemodiafiltration (OL-HDF) on the clearance of circulatory mtDNA was evaluated. The results revealed that only HF-HD and OL-HDF but not LF-HD could partially remove plasma mtDNA. In vitro, the human acute monocytic leukemia cell line (THP-1) was treated with purified mtDNA isolated from humanskeletal muscles. The results demonstrated that mtDNA could induce the production and secretion of IL-1β and TNF α in the THP-1 cells, suggesting a direct causal relationship between mtDNA and inflammation.

Conclusions: Collectively, it is concluded that circulatory mtDNA is elevated and its level is closely correlated with chronic inflammation, CVD and secondary hyperparathyroidism in MHD patients. HF-HD and HDF but not LF-HF can partially remove circulatory mtDNA.

Funding: Government Support - Non-U.S.

SA-PO580

Elevated Toll-Like Receptor 4 Expression and Signaling in Muscle from Patients with Chronic Kidney Disease (CKD) Giacomo Garibotto,[1] Alice Bonanni,[1] Irene Mannucci,[1] Antonella Sofia,[1] Stefano Saffioti,[1] Giuliano Brunori,[2] Elena D'Amato,[1] Valeria Cademartori,[1] Daniela Verzola.[1] *[1]Nephrology Division, IRCCS San Martino-IST, Genoa, Italy; [2]Ospedale Santa Chiara, Trento, Italy.*

Background: Toll-like receptors (TLRs) play a pivotal role in pathogen recognition and cytokine synthesis in several tissues, such as immune cells and skeletal muscle. A systemic inflammatory response is common in CKD patients and is associated with a wasting syndrome and a worse prognosis.

Methods: In this study we used both an ex-vivo and an in vitro approach to examine if TLRs can contribute to the development of inflammatory changes in skeletal muscle of CKD patients.

In ex-vivo studies, TRLs (TLR2,TLR3,TLR4),as well as their downward proinflammatory cascade, including IL-6 (RT-PCR and immunohistochemistry), p-p65 and p38 (immunohistochemistry) were evaluated in muscle biopsies (rectus abdominis) of patients with ESRD (21M/5F, age 69±11 yrs) and in 9 controls (7M/2F, age 66±4 yrs).

Results: Muscle from CKD patients showed significantly elevated TLR4 (mRNA and protein + 30-50%, $p<0.05$), while TLR3 and TLR2 were unchanged. Moreover, NFkB signaling (p-p65 and p-ikBα) was ~6-7 fold increased ($p<0.02$- 0.01) and was associated with elevated expression of the NFkB-regulated genes IL-6 and p-p38.

As a next step, to study if circulating factors can cause the observed inflammatory events we studied the effects of uremic serum in a mouse myoblast cell line (C2C12 cells).Uremic serum increased TLR4 mRNA and protein expression (~2-3 folds vs. basal, $p<0.01$), as well as phosphorylated p38 (by 2-3 fold vs. basal, $p<0.01$). Additional studies revealed that p38 and protein kinase C (PKC) inhibition significantly abrogated serum–induced TLR4 upregulation, NF-κB activation and cytokine secretion.

Conclusions: The data suggest that in CKD patients, before the dialytic stage, skeletal muscle recognizes circulating pathogen-associated molecules with specific TLRs to initiate an IL-6 transcriptional response. Collectively, these data suggest that uremic milieu induces TLR4 expression via PKC-α .Abnormal TLR4 expression may play a role in the susceptibility of such patients to protein wasting and altered energy regulation.

Funding: Government Support - Non-U.S.

SA-PO581

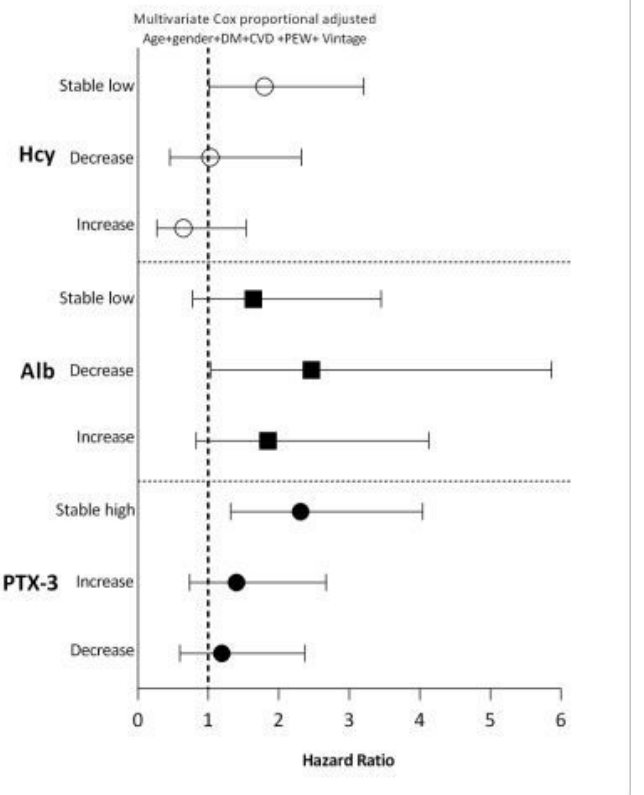
Influence of Variation of Pentraxin 3, Homocysteine and Albumin on the Survival in Hemodialysis Patients Bodil Sjoberg, Abdul Rashid Tony Qureshi, Sunna Snaedal Jonsdottir, Olof Heimburger, Peter F. Barany. *Renal Medicine Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden.*

Background: Persistent micro-inflammation is a strong risk factor for cardiovascular disease and mortality in HD patients. Longitudinal inflammation variation and mortality risk have been sparsely studied. We studied the relation of the variation of three potentially important plasma biomarkers (pentraxin 3 (PTX-3), albumin (Alb) and homocysteine (Hcy)) to mortality.

Methods: In 188 prevalent HD patients (age 63±14 years, 103 males, 26% diabetes, dialysis vintage median (IQR) 28 (13-56) months), PTX-3, Alb and Hcy levels were measured at inclusion and after three months, with median follow-up of 41 (11-50) months. Inflammatory marker variation was categorized according to tertiles at both occasions. The variation of PTX-3, Alb and Hcy was calculated as the intra-class correlation (ICC) from estimates of between-patient and within-patient variance (mixed model). Lower ICC indicates relatively less between-patient variation and higher within-patient variation. Mortality was analyzed by Kaplan-Meier and Cox proportional hazards model, adjusted for age, gender, DM, CVD, PEW and vintage.

Results: ICC for PTX-3 was 0.47 (95% CI: 0.35 to 0.57), ICC for Alb was 0.64 (95% CI: 0.55 to 0.72) and ICC for Hcy was 0.84 (95% CI: 0.79 to 0.88). Within-patient variation of PTX-3 levels was higher than Alb and Hcy variation. During follow-up 88 patients (46%) died. Persistently elevated PTX-3, a decrease in Alb levels and persistently low levels of Hcy were associated with higher mortality (fig).

Conclusions: Persistently high PTX-3 levels were associated with worse outcome. PTX-3 varied more within patients than between patients. Moreover, Low Hcy levels' association with a worse outcome may be linked to protein-energy wasting and hypoalbuminemia.



Funding: Pharmaceutical Company Support - Baxter Healthcare

SA-PO582

Effect of Inflammation on the Relationship of Arteriovenous Fistula Blood Flow Dynamics and Cardiovascular Morbidity among Hemodialysis Patients Rui Zeng,[1] Wenhui Liao,[2] Nan Zhang.[1] *[1]Division of Nephrology, Tongji Hospital, Huazhong University of Science & Technology, Wuhan, Hubei, China; [2]Division of Geriatric, Tongji Hospital, China.*

Background: Cardiovascular disease is the leading cause of death among maintenance hemodialysis patients. Although the presence of an arteriovenous fistula (AVF) has an adverse effect on cardiac function, the exact role of the arteriovenous fistula blood flow in contributing to this morbidity is unclear. The purpose of this study is to investigate how arteriovenous fistula blood flow dynamics(Qa)increase cardiovascular event among maintenance hemodialysis patients.

Methods: Eighty-one maintenance hemodialysis patients bearing an AVF and twelve healthy controls were recruited into the study. Qa and cardiac output (CO) were measured by means of Transonic Hemodialysis Monitor HD02. Predialysis blood samples were taken before Qa mornitoring. HsCRP was measured by immunoturbidimetry(KYOMA). Inflammatory cytokines (IL-2, IL-4, IL-6, IL-10, INF-ϒ and TNF) were measured by Cytometric Bead Array (BD™). Cardiovascular morbidity was monitored prospectively over a nineteen months period.

Results: During the follow-up period, 21% patients (17/81) developed at least one episode of cardiovascular event. Qa, the serum IL-6 and hsCRP levels were significantly higher in patients with CVD than that without(1105±176)vs.(918±215)ml/min, $P<0.05$;6.00(4.40~11.49)vs. 2.60(1.96~4.73)pg/ml, $P<0.01$;8.75(3.83~18.40)vs. 3.80(1.00~7.20)mg/L, $P<0.05$).The risk factors of CVD morbidity was assessed by binary logistic regression analysis. It demonstrated that serum IL-6 was an independent and stronger risk factor for CVD morbidity(HR=1.456, P=0.014, 95%CI (1.078~1.967)) among HD patients. Spearman rank correlation analysis and liner regression analysis showed that Qa was positively correlated with serum IL-6(β=0.429, $P<0.01$). Path analysis suggested that Qa contributed to CVD mortality via the increase of serum IL-6 in HD patients.

Conclusions: IL-6 is an independent risk factor of CVD in maintenance HD patients. AVF blood flow dynamics increases cardiovascular disease morbidity among HD patients via its upregulation of serum inflammatory IL-6.

Funding: Clinical Revenue Support

SA-PO583

Progressive Upregulation of Thrombomodulin, C-Reactive Protein, and D-Dimer in Chronic Kidney Disease versus End Stage Renal Disease Can Be Used to Stratify Risk of Thrombotic Events Korosh Sharain,[1] Debra Hoppensteadt,[1] Vinod K. Bansal,[2] Jawed Fareed.[1] *[1]Pathology, Loyola University Medical Center, Maywood, IL; [2]Nephrology, Loyola University Medical Center, Maywood, IL.*

Background: The progression through the stages of chronic kidney disease (CKD) and into end stage renal diease (ESRD) is unpredictable and thus prognosis and risk are also difficult to predict. Cardiovascular events such as ACS, DVT, and PE are the most common complications in kidney disease. Several studies suggest an elevated inflammatory and thrombotic state in kidney disease, however, the clinical utility of such biomarkers have not been evaluated. This study evaluates the progression of CKD to ESRD through the pro-thrombotic markers Thrombomodulin (TM), C-Reactive Protein (CRP), and D-Dimer.

Methods: 48 patients with CKD, 104 patients with ESRD, and 79 age matched control samples were evaluated. TM, CRP, and D-Dimer were measured simultaneously by Cerebral II biochip immunoassay technology (Randox Evidence Investigator,United Kingdom).

Results: TM levels were 1.24±0.042mg/L (mean±SEM) in controls, 3.44± 0.27mg/L in CKD, and 7.08±0.25mg/L in ESRD, with p<0.0001 by ANOVA. CRP levels were 1.64±0.28mg/L, 4.03±0.55mg/L, and 7.11±0.59mg/L in Controls, CKD, and ESRD respectively, with a p<0.0001 by ANOVA. D-Dimer levels were 89.12±8.61mg/L in controls, 184.76±13.73mg/L in CKD, and 323.64±20.76mg/L in ESRD, with p<0.0001 by ANOVA. Compared to controls, TM levels in CKD and ESRD were increased by 2.76 and 5.68 times respectively, with r^2=0.979. CRP levels were increased 2.45 and 4.33 times in CKD and ESRD respectively, with r^2=0.995 and D-Dimer levels in CKD and ESRD were increased by 2.07 and 3.63 times respectively, with r^2=0.988.

Conclusions: Compared to controls, D-Dimer, TM, and CRP were significantly elevated in the CKD. Compared to the CKD, D-Dimer, TM, and CRP were significantly elevated in the ESRD. When relative concentrations were evaluated, an almost linear progression from control to CKD to ESRD was appreciated. The linear progression of increasing thrombotic state from control to CKD to ESRD can be used as an indicator of cardiovascular disease risk in kidney disease.

SA-PO584

An Evaluation of the Novel Fibrosis Marker Galectin-3 in Hemodialysis Patients Christiane Drechsler,[1] Christoph Wanner,[1] Katja Blouin,[1] Stefan Pilz,[2] Andreas Tomaschitz,[2] Vera Krane,[1] Winfried März,[3] Eberhard Ritz,[4] Pim Van der Harst,[5] Rudolf Allert De Boer.[5] *[1]Div of Nephrology, University of Würzburg; [2]Dept of Internal Medicine, Medical University of Graz; [3]Synlab Center of Laboratory Diagnostics, Heidelberg; [4]Div of Nephrology, University of Heidelberg; [5]Dept of Cardiology, UMC Groningen.*

Background: Galectin-3 is involved in fibrosis and inflammation and has emerged as a biomarker in heart failure. Experimental data suggest that galectin-3 is also linked to renal fibrosis associated with renal failure. Galectin-3 targeted therapy may inhibit this process. We evaluated if plasma galectin-3 concentrations are associated with adverse outcomes in dialysis patients.

Methods: This study investigated the role of galectin-3 in 1168 diabetic hemodialysis patients participating in the German Diabetes and Dialysis Study. Galectin-3 was measured in blood samples taken at baseline by ELISA. We correlated Galectin-3 concentrations with demographic, clinical and biochemical markers. By Cox regression analysis we assessed the association of galectin-3 concentrations with specific cardiovascular endpoints, infectious deaths and mortality during 4 years of follow-up.

Results: Study participants were on average 66±8 years and 54% were male. Mean Galectin-3 concentration was 54±20 ng/µl, being highly elevated as compared to healthy subjects. In multivariable analysis, we identified gender, CRP, HDL-cholesterol, potassium, calcium, NT-pro-BNP, creatinine and thrombocyte count as important factors, which most strongly predicted variation in galectin-3 concentrations. Increased Galectin-3 concentration (log transformed, per standard deviation) independently associated with stroke (HR 1.25 (1.01-1.55)), deaths due to heart failure (1.39 (1.00-1.93)) and infectious deaths (1.25 (1.03-1.52)), thus contributing to a significant rise in all-cause mortality.

Conclusions: Galectin-3 concentrations are highly elevated in dialysis patients and associated with poor outcome including excess stroke, deaths due to heart failure, infectious deaths and all-cause mortality. Further study is needed to assess if interventions targeted at galectin-3 may be useful for patients with kidney disease.

SA-PO585

Changes of Serum Albumin, Body Mass Index, C-Reactive Protein and Interleukin 6 over Time in End Stage Kidney Disease (ESKD) Patients and the Impact of Conventional Risk Factors Claire H. Den Hoedt,[1,2] Michiel Bots,[3] Muriel Grooteman,[4] Neelke C. Van Der Weerd,[4] Erik L. Penne,[4] Pieter M. Ter Wee,[4] Renee Levesque,[5] Menso Jan Nube,[4] Peter J. Blankestijn,[2] Marinus A. Van Den Dorpel.[1] *[1]Maasstad Hospital, Rotterdam; [2]UMCU, Utrecht; [3]UMCU, Utrecht; [4]Centre Hospitalier de l'Université de Montréal, Montréal.*

Background: Inflammation and malnutrition are important features of ESKD patients but data on changes over time are scarce. Therefore we studied changes over time of serum albumin, body mass index (BMI), high sensitivity C-reactive protein (CRP) and interleukin 6 (IL-6). We also evaluated the impact of age, sex, presence of diabetes mellitus (DM), cardiovascular disease, residual kidney function and dialysis vintage on these changes.

Methods: We analyzed data of the CONvective TRAnsport Study (CONTRAST), a randomized controlled trial in 714 chronic ESKD patients (62% men, age 64 ±14, median vintage 2 yrs, 25% DM) comparing online hemodiafiltration with low-flux hemodialysis. Albumin and BMI were measured every 3 months up to six years and predialysis CRP and IL-6 were measured at 6 months and yearly up to three years. The rates of change over time of serum albumin, BMI, CRP and IL-6 were estimated across strata of risk factors with linear mixed effects models.

Results: Albumin concentrations and BMI decreased but CRP and IL-6 increased over time. For every incremental year of baseline age, there was a yearly excess decline in albumin of 0.03 g/L (P<0.001) and an excess decline in BMI of 0.02 kg/m2 (P<0.001). In patients with DM there was a yearly excess decline in albumin of 0.52 g/L (P=0.002) and the decline in BMI doubled as compared to patients without DM (P=0.21). In men, there was an excess decline in albumin of 0.30 g/L per year (P=0.05), and an excess increase in IL6 of 11.5 % (P=0.04) per year as compared to women.

Conclusions: Despite best efforts of care all inflammatory and nutritional parameters worsened over time. In older patients, patients with DM and men the deterioration of these parameters was more pronounced. Special focus on patients at risk by individualizing medical care might improve their prognosis.

Funding: Pharmaceutical Company Support - Fresenius Medical Care (The Netherlands) and Gambro Lundia AB (Sweden), Roche Netherlands; the International Society of Nephrology/Baxter Extramural Grant Program

SA-PO586

On-Line Hemodiafiltration Treatment Using a New Fx CorDiax: Helixone Plus Membranes: Short Term Observation Katarzyna Bladek, Irena Pietrzak. *Fresenius NephroCare, Poznan, Wielkopolska, Poland.*

Background: OL-HDF and capillary haemodiafilters –helixone plus is the most advanced form of extracorporeal renal replacement therapy. This approach gives a higher sieving coefficient for middle molecules and ensures a low albumin loss. The role of OL- HDF with helixone plus in anaemia treatment still is unknown. Anaemia in ESRD pts is commonly associated with inadequate EPO production an its endogenous resistance cause by uremic toxins. Our objective was to compare effectiveness of FX OL- HDF and CorDiax- plus OL-HDF.

Methods: In this study participated 37 uremic pts- treated by OL-Fx HDF for at least 12 mth. Changes in dose of dialysis, hemoglobin level (Hb), Fe, serum albumin, dose-response effect of ESA were determined at the beginning and after 4 mth of CorDiax HDF treatment. Dose of dialysis (eKt/V) was calculated from pre- and post-treatment urea concentrations according to Daugirdas. To evaluate the dose-response effect of ESA we used the erythropoietin resistance index (ERI), calculated as the weekly dose of ESA (µg) divided by the dry body weight (DBW kg) and Hb level (g/dl).

Results: There were no differences between eKt/V at the beginning (1,45±0,11,) and after 4mth (1,44,±0,13,) of observation. Mean levels of Hb (11,02±1,19, 11,26±0,99) and Fe (71,32±27,30, 68,89±21,81) did not changed during observation. ERI decreased significantly (p=0,05) from 0,019±0,021 to 0,012±0,019 and ESA dose decreased from 12,97±12,88, to 8,92±13,29 (p=0,07). There was a trend for serum albumin concentration to increase from 3,28,±0,35, to 3,92, ±0,27.There was found significant correlation (r = - 0,45) between ERI and serum albumin levels after 4 mth of treatment.

Conclusions: 1. OL-HDF with CorDiax helixone plus membrane influences ESA dose and ERI during 4 mth of treatment. It allows considerable anaemia correction and reduces ESA doses.

2. CorDiax membrane with greater clearences of some uremic toxins in comparison to FX gives significant decreased of ERI even stable dose of dialysis in this study.

3. Increasing serum albumin levels may suggest lower loss of it using CorDiax membranes in postdilution OL-HDF as compared with FX-OL-HDF.

4. Further research to confirm these findings is needed.

SA-PO587

The Cardiovascular Effects of Cinacalcet Hydrochloride in Hemodialysis Patients with Secondary Hyperparathyroidism Sun Ryoung Choi,[2] Yu Ah Hong,[1] Ji Hee Lim,[1] Min-Young Kim,[1] Bumsoon Choi,[1] Cheol Whee Park.[1] *[1]Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine,The Catholic University of Korea, Seoul, Korea; [2]Department of Internal Medicine, Hallym University College of Medicine, Seoul, Korea.*

Background: Secondary hyperparathyroidism (SHPT) in hemodialysis patients has an essential role in the endothelial and cardiac dysfunction. We performed this study to evaluate the impact of cinacalcet hydrochloride on cardiovascular system by investigating the clinical cardiovascular parameters, oxidative stress and surrogate markers of endothelial dysfunction in hemodialysis patients with SHPT.

Methods: We studied 12 hemodialysis patients who had high levels of intact PTH(iPTH, >300pg/mL) and corrected calcium (cCa, >9.0mg/dL) with cinacalcet treatment over 20-weeks.Our study consisted of 3 phases;wash-up period for 12 weeks (phase I),medication period for 20 weeks (phase II), follow-up period at 20 weeks after end of treatment (phase III). We performed flow-mediated dilation (FMD), cadio-ankle vascular index (CAVI), and echocardiographic analyses at the end of each phase. Furthermore, we measured serum NOx (nitrate and nitrite), isoprostane, and sICAM-1 to evaluate the degree of oxidative stress and endothelial dysfunction during three phases of this study.

Results: Treatment significantly decreased levels of iPTH, calcium, phosphorus, calcium x phosphorus. But the level of C-reactive protein was not affected by treatment. There were no differences in blood pressure.There was significant improvement in diastolic dysfunction (E/e', P <0.05). There were notable reduction trends in the left ventricular mass index (P=0.06). In contrast, cinacalcet significantly improved FMD (P < 0.01) and enhanced

CAVI (P < 0.05) .The oxidative stress and endothelial dysfunction were improved during the phase II, but returned to the baseline levels at the phase III (NOx, P < 0.05; isoprostane, P < 0.05; sICAM-1, P < 0.05).

Conclusions: Cinacalcet may ameliorate endothelial and diastolic dysfunction by decreasing the oxidative stress as well as increasing the NOx production in hemodialysis patients with secondary hyperparathyroidism.

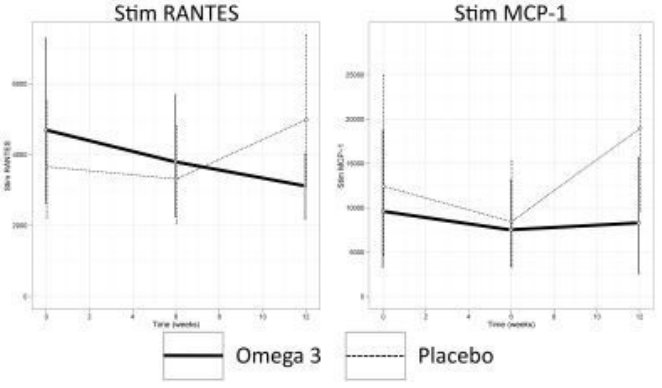
SA-PO588

Omega-3 Fatty Acids Inhibit the Up-Regulation of Endothelial Chemokines MCP-1 and RANTES in CHD Patients Adriana Hung,[1,2] Cindy Booker,[1,2] Edward D. Siew,[1,2] Amy J. Graves,[3] Ayumi Shintani,[3] Charles D. Ellis,[2] Jonathan Himmelfarb,[4] T. Alp Ikizler.[1,2] *[1]CSR&D, Veterans Administration TVHS, Nashville, TN; [2]Nephrology, Vanderbilt University, Nashville, TN; [3]Biostatistics, Vanderbilt University, Nashville, TN; [4]KRI, University of Washington, Seattle, WA.*

Background: One of the early events in inflammation is the upregulation of endothelial chemokines, monocyte chemoattractant protein-1 (MCP-1) and RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted). In this study we tested the hypothesis that Omega-3 fatty acid (O3FA) supplementation will be effective in attenuating chemokine expression.

Methods: This is a double blinded RCT of the supplementation of omega-3 fatty acid in prevalent chronic Hemodialysis patients (CHD). Patients were randomly assigned in a 1:1 fashion to receive 3 gms of eicosapentaenoic acid (EPA) (C20:5 omega 3) plus docosahexaenoic acid (DHA) (C22:6 omega 3) versus placebo for 12 weeks. Blood samples were collected before and after the intervention. MCP-1 and RANTES were measured from LPS stimulated PBMC. Analysis of covariance (ANCOVA) was used to compare percent change from baseline to 12 weeks.

Results: 38 CHD patients were randomized and 31 completed the trial. Mean age was 51 ± 14 years, 73% African American and 79% males. Supplementation of O3FA effectively decreased both lipopolysaccharide (LPS)-induced expression of RANTES (p=0.04) and, with borderline significance, monocyte chemo attractant protein-1 (MCP-1) (p=0.06).



There was no significant effect of the intervention on IL-6 (p=0.08).

Conclusions: The data obtained from this study suggest that omega-3 fatty acids supplementation is beneficial in decreasing the levels of pro-inflammatory chemokines RANTES and MCP-1. Larger studies evaluating the impact of omega 3 supplementation on CV mortality and PEW in ESRD are needed.

Funding: Other NIH Support - NCCAM, Veterans Administration Support

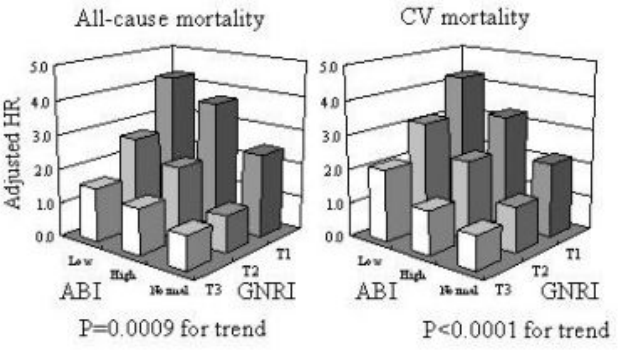
SA-PO589

Association of Ankle Brachial Index and Protein-Energy Wasting with Mortality in Hemodialysis Patients Yasuhiko Ito,[1] Hirotake Kasuga,[2] Masashi Mizuno,[1] Yasuhiro Suzuki,[1] Shoichi Maruyama,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya University, Nagoya, Japan; [2]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan.*

Background: Ankle brachial index (ABI) has been widely recognized as a marker of systemic atherosclerosis. Protein-energy wasting (PEW), currently considered to be due to inflammatory process, is highly prevalent in HD patients, and is associated with increasing risk of mortality. We investigated the association of ABI and PEW with mortality in HD patients.

Methods: A total of 1036 hemodialysis (HD) patients were divided into 3 groups according to ABI levels; normal group: 0.9-1.4 (n=682), high group: >1.4 (n=150) and low group: <0.9 (n=204), and were followed up for 8 years. Patients were also divided into tertiles according to geriatric nutritional risk index (GNRI) levels as a simplified marker of PEW state; tertile 1 (T1): <90.8, T2: 90.8-97.3 and T3: >97.3. GNRI = (14.89 × albumin) + [41.7 × (body weight / body weight at BMI of 22)].

Results: Declined GNRI levels were independently associated with abnormal ABI (odds ratio 0.97, 95%CI 0.96-0.99, p=0.0009). By Kaplan-Meier analysis, 8-year event-free survival rates from mortality were 62.8%, 46.2% and 27.3% among normal, high and low ABI group (p<0.0001), and were 34.3%, 59.7% and 68.0% among T1, T2 and T3 of GNRI, respectively (p<0.0001). In the combined setting of ABI and GNRI, the risk of mortality was 4.26-fold (95%CI 2.63-6.90) higher in the low ABI group with T1 of GNRI and 3.69-fold (95%CI 2.30-5.91) higher in the high ABI group with T1 of GNRI compared to the normal ABI group with T3 of GNRI, respectively (Figure). Similar results were also obtained from cardiovascular mortality.



Conclusions: Abnormal ABI and lower GNRI, might reflect PEW state, were closely linked, and were interactively associated with increasing risk of mortality in HD patients.

SA-PO590

Arterial Stiffening Is Related to Changes in Body Composition in Patients on Regular Hemodialysis Akihiko Kato,[1] Yukitoshi Sakao,[1] Takayuki Tsuji,[2] Naro Ohashi,[2] Hideo Yasuda,[2] Yoshihide Fujigaki.[2] *[1]Blood Purification Unit, University Hospital, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: In the elderly, arterial stiffness is associated with low thigh muscle mass in men but not in women, probably due to reduced testosterone level in men (Atherosclerosis, 2010). We showed that thigh sarcopenia is related to carotid arteriosclerosis in hemodialysis (HD) patients (NDT 2011). However, it remains unclear whether a sex-dependent association may be present in the dialysis population. The purpose of this study was to evaluate the relationship of the mid-thigh muscle cross-sectional area to arterial stiffness in male and female HD patients, respectively.

Methods: The study population consisted of 82 male and 41 female HD patients with a mean age of 61±11 years (time on HD: 11±10 years). We calculated abdominal subcutaneous fat-mass area (ASFA), abdominal visceral fat-mass area (AVFA) and thigh muscle area (TMA) form CT images, and then adjusted these by body mass index (BMI). We also measured brachial-ankle pulse wave velocity (baPWV) automatically using a volume plethysmographic apparatus (VeraSera VS-1000, Fukuda Denshi, Tokyo, Japan).

Results: In both sexes, baPWV was significantly correlated positively with age and systolic blood pressure (SBP) (p<0.01) while negatively with peripheral total lymphocyte count (p<0.05). There was a significant association between TMA/BMI ratio and baPWV in men (r=-0.32, p<0.01). In women, baPWV was negatively correlated with AVFA/BMI ratio (r=-0.38, p=0.02). A multiple regression analysis revealed that TMA/BMI ratio was inversely correlated with baPWV independent of other clinical variables that associated with baPWV in men. In contrast, AVFA/BMI ratio was independently and negatively associated with baPWV in women.

Conclusions: The findings suggest that arterial stiffening is related to skeletal muscle atrophy in men, while with reduced abdominal adiposity in women in HD patients.

SA-PO591

Effect of a Single Hemodialysis Session on Lipid Particle Number and Size Determined by Nuclear Magnetic Resonance Lipoprotein Analysis Anjali Gupta, Frank Modersitzki, Manish P. Ponda. *New York University Langone Medical Center, New York, NY.*

Background: Atherosclerotic vascular disease is the main cause of mortality for patients with end-stage renal disease (ESRD). Mild degrees of renal impairment have been shown to be associated with lipoprotein abnormalities including greater concentrations of small Low-Density Lipoprotein (LDL) and Intermediate-Density Lipoprotein using Nuclear Magnetic Resonance (NMR) lipid analysis. The aim of our study is to evaluate changes in various lipoprotein subclasses during a single hemodialysis (HD) session.

Methods: Thirteen patients on maintenance HD were enrolled into the study. Fasting NMR lipid analysis was obtained before initiation of HD (t1), after two hours of HD (t2), end of HD session (t3) and the next day of dialysis (t4). Measured lipoproteins levels were adjusted for ultrafiltration, based on calculated plasma volume changes from hematocrit values obtained at various time points during the HD session.

Results: Our study shows a decrease in mean concentration of Very Low Density Lipoprotein (VLDL) and chylomicon particles by 67% at t2 and 52% at t3 as compared with t1 (P < 0.05). The reduction was mainly in small and medium VLDL particles, large VLDL particles did not show significant changes. The concentration of triglycerides (TG) decreased by 40% (P < 0.05) during dialysis and was lowest at two hours into dialysis. LDL and high-density lipoprotein (HDL) particle concentrations did not change significantly during a HD session. However, mean concentrations of LDL particles (total, large and small) were 20% higher (p <0.05) on the day after dialysis as compared to t1. Similarly, concentrations of HDL particles were 12% higher (P < 0.05).

Conclusions: Our pilot study shows a significant fluctuation in concentrations of VLDL and chylomicron particles especially, small and medium VLDL particles during a single hemodialysis session. Further investigations are needed to see if these fluctuations contribute to the increased cardiovascular mortality of hemodialysis patients.

SA-PO592

Lipoprotein Associated Phospholipase A2 (LP-PLA2) Activity in CKD Stage 5 Patients Is Associated with Atherosclerosis Christof Ulrich, Felix Kohler, Bogusz Trojanowicz, Antje Spens, Roman Fiedler, Eric Seibert, Matthias Girndt. *Martin-Luther University Halle-Wittenberg, Halle (Saale).*

Background: LP-PLA2, an enzyme mainly derived from macrophages, hydrolyses oxidized phospholipids, thereby producing proatherosclerotic lyso-phosphatidylcholine and free oxidative radicals. We recently demonstrated a significant association between proinflammatory monocytes and atherosclerosis in CKD patients. The aim of the study was to evaluate LP-PLA2 activity and – gene expression in HD patients with and without signs of atherosclerosis and to link these data to inflammatory monocytes.

Methods: 63 HD patients (age 63.2±15.2 years, 40 m/23f, 23 diabetics) were enrolled in the study. Atherosclerosis was diagnosed by ultrasound evaluation of the carotid arteries using a recently established atherosclerosis score system (CJASN 2011; 6:505-511). LP-PLA2 activity was measured in frozen plasma samples. For real time gene expression analysis RNA was isolated from whole blood using the PAX gene system. Monocytes were classified by flow cytometry according to expression of CD14 and CD16.

Results: 21 of 63 patients were scored atherosclerosis positive (At+). These patients were older (70.8±10.9 vs. 59.5±15.7, p=0.04) and had significantly higher LP-PLA2 activity compared to patients without atherosclerosis (At-) (LP-PLA2-activity [nmol/min/ml], At+: 20.1±4.2 vs. At-: 17.6±4.5, p=0.04). Both groups did not differ regarding the soluble inflammatory marker CRP and the frequency of CD14++CD16+ monocytes. Normalized LP-PLA2 gene expression did not correlate with LP-PLA2 activity. However, the frequency of CD14++CD16+ monocytes was significantly associated with both LP-PLA2 gene expression (r=0.26, P=0.03) and CRP (r=0.31, P=0.02).

Conclusions: Our data show that CKD-5 patients with relevant atherosclerosis have higher LP-PLA2 activity compared to patients without severe atherosclerosis. This seems to be independent from CRP. As demonstrated by other studies LP-PLA2 activity and gene expression do not necessarily correlate. The inflammatory monocyte subset CD14++CD16+ is a potential source of LP-PLA2. Further studies will show if differentiation of CD14++CD16+ is linked to higher LP-PLA2 activity levels.

SA-PO593

Is There Association between Electronegative LDL and Bone Mineral Density in Hemodialysis Patients? Julie Lobo,[1] Milena Barcza Stockler-pinto,[2] Viviane Oliveira Leal,[1] Najla Elias Farage,[3] Dulcinéia Saes Parra Abdalla,[4] Denise Mafra.[1] *[1]Federal Univ Fluminense, Brazil; [2]Federal Univ of Rio de Janeiro, Brazil; [3]RenalCor, Brazil; [4]University of São Paulo, Brazil.*

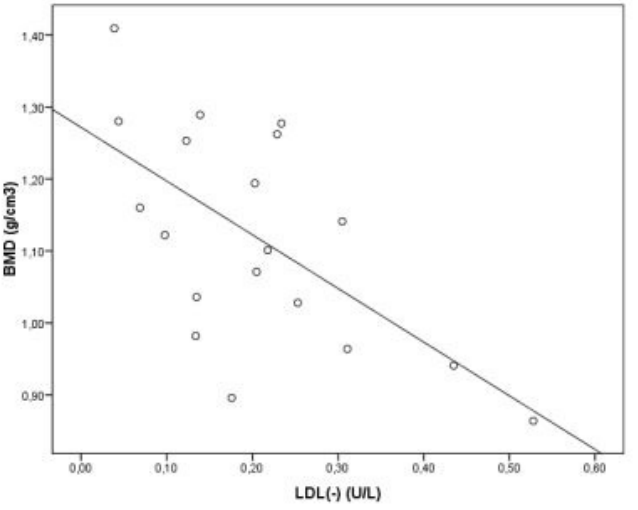
Background: Electronegative LDL [LDL(-)] plays an important role in atherosclerosis and also in bone remodeling. However, there is no information concerning hemodialysis (HD) patients. Our aim was verify the relationship between inflammatory markers, LDL(-) levels and bone mineral density (BMD) in HD patients.

Methods: Twenty HD patients (11M/9F; 52.4±12.8 years) from RenalCor Clinic, Brazil, were studied. LDL(-) and parathyroid hormone (PTH) were measured by ELISA; tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) were measured by a multiplex assay kit. BMD was evaluated by dual energy X-ray absorptiometry (DXA). SPSS for Windows (version 11.0) was used for statistics.

Results: Results are showed in Table 1. Patients presented significantly high levels of LDL(-) and inflammatory markers compared to normal values (Lobo et al., Clin Chim Acta 412:1788-92, 2011). There was a negative correlation between BMD and LDL (-) (r=-0.62, p=0.005) (Figure 1), BMD was not correlated either with PTH or inflammation markers.

Table 1: Biochemical parameters in HD patients

Parameters	HD Patients
Body Mass Index (Kg/m2)	24.1±4.4
Average dialysis time (Months)	69.1±39.7
LDL(-) (U/L)	0.32 ± 0.30
TNF-α (pg/mL)	5.4 ± 1.6
IL-6 (pg/mL)	4.1 ± 1.6
VCAM-1 (ng/mL)	48.5 ± 8.5
ICAM-1 (ng/mL)	20.5 ± 15.9
BMD (g/cm3)	1.11 ± 0.15
PTH (pg/mL)	359 (26-1700)



Conclusions: Our results suggest that LDL(-) might play a role not only in atherosclerosis, but also on bone remodeling, probably contributing to alterations in bone metabolism found in HD patients. More comprehensive studies are needed to better understand this association.

Funding: Government Support - Non-U.S.

SA-PO594

Is Dyslipidemia a Risk Factor for Cardiovascular Disease in Hemodialysis? Milagros Ortiz Librero,[1] Carmen Mon Mon,[1] Juan Carlos Herrero Berron,[1] Olimpia Ortega,[1] Isabel Rodriguez,[1] Paloma Gallar Ruiz,[1] Julie Hinostroza Yanahuaya,[1] Manuel Praga.[2] *[1]Nephrology, Hospital Severo Ochoa, Madrid, Spain; [2]Nephrology, Hospital 12 de Cctubre, Madrid, Spain.*

Background: Cardiovascular(CV)disease is the most common cause of mortality in chronic hemodialysis(HD)patients.Dyslipemia is a cardiovascular risk factor in healthy population.Its treatment has been shown to improve CV prognosis.However,in HD population,this association is less clear.Asses risk factors of mortality in HD,considering:Classical CV risk factors:hypertension,diabetes,lipid abnormalities.Comorbidity,malnutrition,inflammation.

Methods: 64 chronic HD patients followed for 8 years were analyzed(2003-11).71.9% were men with a mean age of 59.97±13.41 years.Follow-up on dialysis was 54.40±38.02 months.65.6% had hypertension,28.1% diabetes and 37.5% had suffered a CV event.In a cross-section at baseline were analyzed:Lipid abnormalities,Charlson comorbidity index (CCI),malnutrition-inflammation parameters(albumin,cholesterol,CRP).Overall and CV mortality were evaluated.

Results: Abnormalities lipid profile:Col>200mg/dl:18.75%.TAG>150mg/dl:43.75%. HDL<40mg/dl:43.75%.LDL>100:32.8%.Atherogenic Index>4.4:31.25%.87.5% had altered lipid profile,48.4% received statins and 42.2% sevelamer.CCIadjusted for age:3.48±1.43. Malnutrition-Inflammation parameters:albumin:4.02±0.3,Cholesterol:166.26±41.01,PCR:12.65±15.21.50% of patients died,65.2% of cardiovascular etiology.Univariated analysis showed age>59 years(p50),cholesterol<137(p25),CCI (>5),CRP>12.7(p75),sevelamer treatment and previous CV disease were associated to overall mortality (OM). In Cox analysis only CCI adjusted for age(Exp:3.82,95%CI:1.63-8.89),elevated CRP(Exp:2,174,95%CI:1.05-4.49) and low levels of cholesterol(Exp:0.29,95%CI: 0.12-0.74)were associated with OM.None of the factors described were correlated with CV mortality.

Conclusions: Despite the high prevalence of abnormal lipid profile,lipids are not predictors of long-term mortality in HD population.Malnutrition-inflammation and comorbidity will be the determinants of mortality in this population.

SA-PO595

Parathyoid Dysfunction in Elderly Hemodialysis Patients with Hemodialysis and Its Related Factors Huiling Wang. *Division of Nephrology, Jiming Hospital, Shanghai, China.*

Background: In this study, we observe the relevant indicators related to calcium and phosphorus metabolism disorders and parathyroid abnormalities for hemodialysis patiens with the age over 65, and analyze its related factors.

Methods: 286 stabile patients with maintenance hemodialysis (MHD), who came from Jimin Hospital of Shanghai from 2010.01 to 2010.12, were divided into two groups according to age: elderly group (≥ 65 years) and non-elderly group (<65 years).All patients underwent conventional HD, among which, most patients took hemodiafiltration(HDF) 1-2 times per month. According to KDIGO guideline, patients with different conditions used erythropoietin, rocaltrol and calcium carbonate when needed. Fasting blood specimen before dialysis were drawn to assay Hb, Scr, BUN, calcium, phosphorus, intact parathyroid hormone (iPTH), high-sensitivity C-reactive protein (Hs-CRP), and albumin (Alb).

Results: Elderly patients accounted for 51.4% of the total people. The major cause was hypertension(35.4%), followed by chronic glomerulonephritis(21.1%) and diabetes(19.7%). While in the non-elderly patients group, the main cause was chronic glomerulonephritis(43.2%), followed by diabetes(23.7%) and hypertension(12.2%). Comparing with those of non-elderly patients, the elderly patients' dialysis ages were shorter, dry weight was lighter, the number of patients who used vitamin D and/or calcium was fewer, systolic blood pressure was higher, diastolic blood pressure was lower, serum BUN, Scr, P, iPTH, Alb((35.3±2.1) vs (37.1±2.7),g/l), standard-protein nitrogen present rate (nPNA) concentration were also lower. The level of Hs-CRP was much higher((7.24±2.41) vs (6.52±2.68),mg/l) (P <0.05).The incidence of emerging lower iPTH (<150pg/L) was much higher(55.8% vs 36.7%)(P <0.05).While the level of Hb, AC-Ca and Kt / V in the two groups showed no differences(P> 0.05).In logistic multiple regression analysis,it showed that the age, plasma phosphorus, Alb and nPNA of patients with MHD were independent factors of secondary hypoparathyroidism.

Conclusions: Most elderly patients with HD have the phenomenon of hypoparathyroidism, its occurrence may be related to age, malnutrition and other potential factors.

Funding: Government Support - Non-U.S.

SA-PO596

Relationship between Body Composition, Change of Body Weight and Prevalence of Cardiovascular Disease, Usefulness of a Home Body Composition Analyzer in Hemodialysis Patients Yukie Kitajima,[1] Yuzuru Sato.[2] *[1]Tokyo Healthcare University, Tokyo, Japan; [2]Sato Junkanki Hospital, Matsuyama, Ehime, Japan.*

Background: The risk factors for cardiovascular disease in hemodialysis patients include age, duration of hemodialysis, diabetes mellitus and hyperphosphatemia. We investigated the relationship among visceral fat area, percentage of body fat with a home body composition analyzer, a multifrequency bioelectrical impedance analysis and anamnesis of cardiovascular complication, between body weight change and body fat mass change.

Methods: Area of visceral fat was measured using CT in 84 non-diabetic patients. Body composition was measured using a home body composition analyzer(OMRON CO., LTD) and a multifrequency bioelectrical impedance analysis(Biospace CO., LTD). Patients were divided into two groups according to visceral fat area; those with visceral fat area < 100cm^2 (Group A) and with > 100cm^2 (Group B). Next, body weight change and body fat mass change were evaluated in 59 stable hemodialysis patients for three years.

Results: Group B showed higher rate of cardiovascular complication, especially ischemic heart disease (p<0.01). Visceral fat area correlated with BMI (R^2=0.523), total body fat (R^2=0.544), percentage of body fat (R^2=0.283). Furthermore, Patients with ischemic heart disease showed higher percentage of body fat (p<0.05). With regard to the relationship between body weight change and body fat mass change over the three-year period, a significant positive correlation was showed between percentage of body weight change and percentage of body fat mass change (male: R^2=0.521, female: R^2=0.786). Percentage of body fat of a home body composition analyzer and a multifrequency bioelectrical impedance analysis showed positive correlation(R^2=0.741).

Conclusions: Higher visceral fat area was found to have the high rate of cardiovascular disease, especially ischemic heart disease in hemodialysis patients. In addition, BMI and percentage of body fat can be measured more easily than CT. Results also showed that body weight change reflect body fat mass change. Those assessments will evade the risk of cardiovascular disease. Use of a home body composition analyzer is effective and inexpensive.

SA-PO597

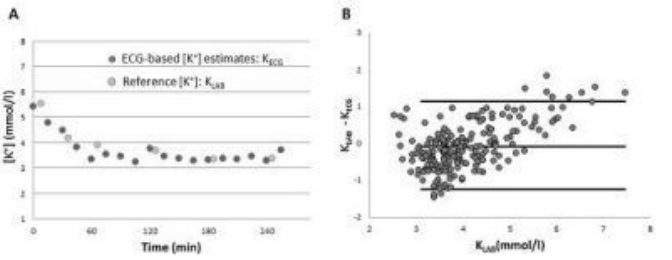
Noninvasive Intradialytic Potassium Estimation in HD Patients Cristiana Corsi,[1] Emanuele Mambelli,[2] Stefano Severi,[1] Antonio Santoro.[2] *[1]Department of Electronics, Computer Science and Systems, University of Bologna, Cesena, Italy; [2]Nephrology, Dialysis and Hypertension, Policlinico S. Orsola-Malpighi, Bologna, Italy.*

Background: The K^+ changes have a strong influence on the T-wave morphology in the ECG signal. Both hypo- and hyperkalemia influence cardiac repolarization and are associated with increased mortality in HD patients. Intradialytic K^+monitoring could be extremely useful to maintain or modify the K^+ balance in patients with high risk of cardiac arrhythmias by means of a sort of closed loop control.

The aims of the study were to develop a new method to estimate serum K^+ from the real-time T-wave analysis and to validate it in HD patients.

Methods: We performed Holter ECG (Mortara H12+) in 13 HD patients (39 sessions, 3 per patient over three weeks). A 2-minute window median value of the ratio of the T-wave slope to amplitude ($T_{S/A}$) was used to estimate K^+. Reference values (K_{LAB}) were obtained from blood samples. The ECG estimate K^+ (K_{ECG}) has been compared to K_{LAB} by correlation and Bland-Altman analyses.

Results: K_{LAB} varied from 5.15±0.88 mM (range: 3.11 – 7.46) at the beginning of HD to 3.28±0.29 (range: 2.63 – 3.95) at the end (-47%±15%).The K_{ECG} estimator gave consistent results in 33/39 sessions. A patient specific calibration of the estimator based on data from the first session allowed a good agreement with the reference measurements (Figure 1A) in all the patients in the two following sessions, with an error of -0.04±0.61 mM (Figure 1B).



Conclusions: The estimate of serum K^+ from the ECG can be realistic. After a more comprehensive validation and assessment of potentially interfering factors (contemporary other electrolytes and/or cardiac electrophysiology abnormalities, etc.) this simple and noninvasive measure could help in preventing K^+ changes-related intradialytic dangerous arrythmias.

SA-PO598

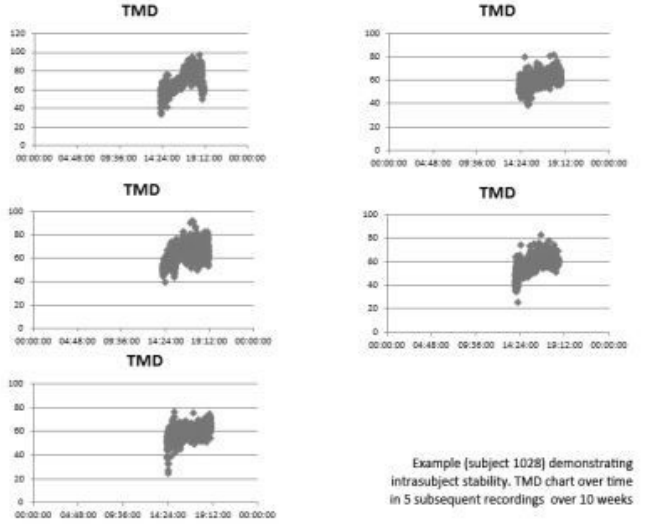
Analysis of T-Wave Morphology for Risk Stratification in Hemodialysis Patients Dimitrios J. Poulikakos, Debasish Banerjee, Marek Malik. *St George's University of London, London, United Kingdom.*

Background: Risk stratification for sudden cardiac in hemodialysis is an urgent clinical need. We hypothetised that dialysis represents a stable cardiovascular challenge for each patient generating a subject-specific repolarisation profile. We prospectively study dynamic repolarisation indices derived from Holter ECG monitoring on dialysis.

Methods: 12-lead Holter ECG recordings are performed during dialysis for five sessions at two weeks intervals. QTC interval, proportion between the principal axes of the T wave loop (CRa), T wave morphology dispersion (TDM), proportion between the dimension and the length of the T wave loop (Loop Ratio), absolute and relative T wave residua (ATWR) and total cosine R to T (TCRT) are calculated automatically and blindly.

Results: 42 out of 49 subjects so far have analysable data (210 recordings). Two sustained cardiac arrest. Mean age is 58.84 ±13, females 17 (39%), diabetic 12(27.9%) and 7 (16.3%) have coronary artery disease. Mean repolarisation values are QTC 437.64±22.9, CRa 0.21±0.09, TDM 34.2±21, Loop Ratio 0.12±0.03,RTWR 0.001±0.001, ATWR= 2124.4±1688, TCRT 0.204±0.61.

QTC, CRA, TMD, Loop Ratio, Absolute TWR, showed intra-subject stability (Repeated measure ANOVA F=0.47, 0.39, 0.87, 0.52, 0.92 and intra-class correlation coefficients ICC 0.9.69, 0.961, 0.967, 0.919, 0.894, respectively) and inter-subject variability (F= 7384, 75.52, 39.8, 199, 34.6 respectively, p <0.001).



Example (subject 1028) demonstrating intrasubject stability. TMD chart over time in 5 subsequent recordings over 10 weeks

Subjects with cardiac arrest had significantly higher mean TDM, relative TWR and absolute TWR (76.38±1.99, 0.0025±.0027, 4784±853 respectively) and lower TCRT (-0.7677±.044). These differences are consistent with risk prediction in cardiac patients.

Conclusions: Repolarisation indices on dialysis can depict subject-specific profiles. Further data is needed to characterise high risk profiles.

SA-PO599

Predicting Mortality in Dialysis Patients: Does a Simple Chest X-Ray Have Prognostic Value? Ethan Bohn, Brent Gali, Navdeep Tangri, Manish M. Sood, Paul Komenda, Claudio Rigatto. *University of Manitoba, Winnipeg, Canada.*

Background: Clinical outcomes of dialysis patients are variable, and improved knowledge of prognosis would inform decisions regarding patient management. We assessed the value of simple, chest x-ray derived measures of cardiac size (cardiothoracic ratio (CTR)) and vascular calcification (Aortic Arch Calcification (AAC)), in predicting death in a prevalent cohort of hemodialysis (HD) patients.

Methods: We used a database of patients starting HD between 2000 and 2010 in Manitoba, Canada. At least one electronically accessible and technically adequate postero-anterior (PA) chest x-ray was available in 791 patients. Two observers measured CTR and AAC independently in all films; disputes were settled by consensus. AAC was graded as 0 to 3 as per Hashimoto et al (Atherosclerosis 2010, 210:137). Cox regression was used for multivariate modelling.

Results: The median age of the study population was 58 years and median dialysis vintage was 1.3 years. 47% of participants were male and 35% were of aboriginal descent. Mean CTR was 0.53±0.07. Of 791 patients, 137 died during follow-up. Age, diabetes, heart failure, and baseline serum creatinine and calcium were the strongest predictors of survival in a base Cox model. Both CTR (HR 1.71 [1.33, 2.20] per 0.1 unit change, AUC= 0.60 [0.55, 0.65]), and AAC>1 (HR 2.48 [1.70, 3.62] AUC=0.62 [0.56, 0.67) were highly associated with death in univariate Cox analysis. CTR remained significant after adjustment for base model variables (adjusted HR 1.36 [1.04, 1.78]), but did not increase the AUC of the base model (0.71 [0.66, 0.76] vs. 0.72 [0.67, 0.77]) and did not improve net reclassification performance (NRI=0). AAC was not associated with mortality after adjustment for age in multivariate Cox models.

Conclusions: Neither CTR nor AAC assessed on routine chest x-ray improved prediction of mortality in this prevalent cohort of HD patients. Our data does not support the utility of simple x-ray derived measures of cardiac size and vascular calcification. More advanced imaging techniques such as cardiac MRI and coronary CT may be needed to improve prognostication in this population.

SA-PO600

Electrocardiographic Early Repolarisation: A Marker for Sudden Cardiac Death: Is Three Times More Common in Patients with Chronic Kidney Disease, and Increases with Disease Severity Reza Hajhosseiny,[1] Frederic Sebag,[2] Ronak Rajani,[2] Kaivan Khavandi,[2] Matthew James Wright,[2] David Goldsmith.[1] *[1]Renal and Transplantation Department, Guy's Hospital, London, United Kingdom; [2]Department of Cardiology, St Thomas' Hospital, London, United Kingdom.*

Background: Although electrocardiographic (ECG) early repolarisation (ER) occurs infrequently (<5%) in general and atherosclerotic populations and is a marker of sudden cardiac death (SCD), the prevalence of ER in patients with chronic kidney disease (CKD), in whom SCD is common, is unknown. We aim to determine the frequency and causes of ER in patients with CKD.

Methods: We retrospectively studied 197 patients with stage 3-5 CKD. Demographic cardiovascular risk data, cardiac history, co-morbidities, and medications were recorded, with bone/mineral biochemistry and C-reactive protein levels. All patients underwent a 12-lead ECG analysed for the presence of ER, bundle branch block, left ventricular hypertrophy and relevant electrical durations (PR, QRS, QTc intervals). ER was defined as an elevation of the QRS-ST junction (J point) of at least 0.1mV from baseline with slurring/notching of the QRS complex. To determine whether the presence of ER was a manifestation of CKD, we also evaluated the ECGs of 39 healthy renal transplant donors.

Results: The patients with CKD had a mean age of 61.5 (±16.1) of whom 57% were male. The prevalence of ER in pre-dialysis patients with CKD stage 4 and 5 (N=106, 54%) was higher than in the transplant donors (26.4% vs. 7.7%, p=0.02). ER frequency increased with CKD stage (stage 3: 8%, stage 4: 30% and pre-dialysis stage 5: 25%), but decreased post-dialysis (13%, p=0.02). On multivariate analysis with ER as the dependent variable, and QRS duration, QTc duration, heart rate, PR interval, dialysis, COPD, anti-aldosterone and age as the covariates, only the QRS duration remained a significant independent predictor of ER (OR 0.97, 95% CI, 0.94-0.99, p=0.01).

Conclusions: ER is more common in patients with pre-dialysis CKD compared to healthy renal transplant donors. Further longitudinal studies are indicated to determine whether this increased prevalence of ER is associated with the increased rate of SCD in this population.

SA-PO601

Non-Compressible Arteries in Hemodialysis Patients: Does a High Ankle Brachial Index Mask the Presence of Peripheral Vascular Disease? Kristopher Joel Swiger,[1] Ronald Walden Milam,[2] Stephen Vance,[2] Alan L. Hinderliter.[2] *[1]Johns Hopkins Department of Internal Medicine, Baltimore, MD; [2]Univeristy of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: The Ankle Brachial Index (ABI) is an effective screening tool for peripheral arterial disease (PAD), but is falsely elevated in some chronic kidney disease (CKD) patients with noncompressible arteries due to medial wall calcification. The Toe Brachial Index (TBI) may be a more reliable indicator of occlusive atherosclerotic disease in these individuals. We hypothesized that a high percentage of patients with CKD and noncompressible arteries, characterized by high ABI levels, would have low TBI values suggesting occlusive PAD.

Methods: To compare ABI and TBI values, we measured both indices in a cohort of patients with stage 4 or 5 CKD undergoing renal transplantation evaluation.

Results: The 88 patients averaged 56±12 years of age; 44% were female; 59% were African-American; 64% had diabetes and 52% had a history of tobacco use. The mean blood pressure was 153±32/83±13 mmHg. The non-HDL cholesterol averaged 121±40 mg/dl. The majority (88%) had end-stage renal disease.

The ABI was low (< 0.9), suggesting occlusive PAD, in 27%; borderline (0.9-1.0) in 24%; normal (1.0-1.3) in 33%; and high (> 1.3), indicating noncompressible arteries, in 16%. Of those with a high ABI, 29% had a TBI < 0.9, suggesting occlusive PAD; additionally, 10% of patients with a normal ABI had a TBI < 0.9.

Conclusions: In summary, in our cohort of patients with stage 4 or 5 CKD, the prevalence of PAD was high, as evidenced by a low or borderline ABI in 51%; and noncompressible arteries, characterized by an ABI > 1.3, were observed in 16%. Low TBI values, suggesting occlusive PAD, were observed in 29% of those with a high ABI, and in an additional 10% who had a normal ABI. These results suggest that a high ABI may mask occlusive PAD in a significant proportion of patients with noncompressible arteries. Our findings support further research to examine the accuracy and prognostic significance of the TBI in patients with advanced CKD, and the incremental value of TBI measurements in patients with noncompressible vessels.

SA-PO602

Penultimate Pulse Wave Velocity, More than Baseline Pulse Wave Velocity, Predicted Mortality in Italian ESRD Cohort Study Macaulay A. Onuigbo,[1,2] Antonio Bellasi,[3] Domenico Russo,[4] Biagio Raffaele Di Iorio.[5] *[1]College of Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System; [3]Nefrology, "S. Anna" Hospital, Como, Italy; [4]Nephrology, "Federico II" University, Naples, Italy; [5]Nephrology, "A. Landolfi" Hospital, Solofra (AV), Italy.*

Background: Cardiac disease, the major cause of death in ESRD is difficult to prognosticate. In 2011, our group for the first time showed that PWV variations determined mortality differences. We examined translational PWV changes vs ESRD mortality.

Methods: PWV was measured by the foot-to-foot method and repeated after six months. Coronary artery calcification (CAC) was measured at 0, 12 and 24 months. Mortality outcomes were analyzed.

Results: In 2007-2010, 466 ESRD patients, 229 M:237F, age 19-97 (65.6) years, were followed up for 28.9 months. 128 patients (74M:54F) died. Causes of death - acute myocardial infarction (AMI) in 47 (37%) (age 70, 26M:21F), sudden death (SD) in 29 (23%) (age 72, 19M:10F). Paired PWV available in 308 surviving patients and 106 patients who died. Baseline PWV was lower in surviving vs dead patients – 8.46 +/- 1.8 vs 9.43 +/- 3.75 (p=0.0005). Repeat PWV values were unchanged in 308 survivors (8.46 +/- 1.8 vs 8.53 +/- 1.85, p=0.5, NS). Repeat PWV increased in the 106 who died from 9.43 +/- 3.75 to 12.11 +/- 4.18 (p<0.0001). Of the 29 dead from SD, death occurred <12 hours after the last dialysis (ATLD) in 7, >24 hours ATLD in 20 and >48 hours ATLD in 17. Of the 47 dead from AMI, 6 died <12 hours ATLD, 35 died >24 hours ATLD and 23 died >48 hours ATLD. CAC values scatter did not allow for analysis but baseline CAC values were higher in the AMI/SD patients vs surviving patients.

Conclusions: This is the first report to show a scalable direct relationship between translational PWV and mortality in ESRD. Penultimate PWV, more than baseline PWV predicted mortality. Higher ESRD deaths from AMI/SD occurred during the long inter-dialytic (weekend) period. We propose that PWV be monitored among all ESRD patients. Patients with high and/or increasing PWV values may require more intense cardiovascular analysis. Further research into new preventative/therapeutic options in this area of ESRD care is warranted.

SA-PO603

Blood Pressure Variability (BPV) and the Risk of Death in Incident Hemodialysis (HD) Patients Tariq Shafi,[1 3] Stephen M. Sozio,[1 3] Wendy L. St. Peter,[1 2] Karen J. Bandeen-roche,[1 3] L. Ebony Boulware.[1 3] *[1]Johns Hopkins University; [2]University of Minnesota; [3]The DEcIDE Network Patient Outcomes in End Stage Renal Disease Study Investigators.*

Background: BPV has been associated with poor outcomes in HD patients, but prior studies did not account for potential confounders including volume status and antihypertensive medications.

Methods: We quantified within-person predialysis systolic BPV among 13,331 incident HD patients treated at Dialysis Clinic, Inc. (DCI), using standard deviation of the residual obtained from mixed-effects linear regression models estimating changes in log SBP over time. This measure reflects the summary of each person's SBP variation from their own mean SBP over time. We estimated the association of BPV with mortality (all-cause and cardiovascular disease [CVD]) overall and after stratifying by quintiles of baseline SBP using discrete time proportional hazards regression adjusted for demographics, comorbidities and treatment factors including interdialytic weight gain, lab tests and antihypertensive medications.

Results: Patients' (mean age 62 yrs; 37% black; 45% female) average baseline SBP was 149±19 mmHg. There were 3,625 deaths (1,773 from CVD) during follow-up (median, 1.9 yrs). Higher BPV was associated with increased risk of mortality and this effect was seen overall and in all SBP quintiles.

Predialysis SBP Variability and the Risk of Death

		Deaths	HR (95% CI) for BPV*	p
All-Cause Mortality				
Overall		3625	1.50 (1.41-1.60)	<0.001
By SBP Quintiles	BP (mmHg)			
Q1	<135	1024	1.57 (1.38-1.78)	<0.001
Q2	135-146	672	1.42 (1.22-1.66)	<0.001
Q3	146-154	569	1.32 (1.13-1.55)	0.001
Q4	154-166	589	1.69 (1.46-1.96)	<0.001
Q5	>166	668	1.40 (1.22-1.61)	<0.001
CVD Mortality				
Overall		1773	1.44 (1.31-1.58)	<0.001
By SBP Quintiles				
Q1	<135	480	1.51 (1.25-1.82)	<0.001
Q2	135-146	298	1.31 (1.04-1.66)	0.02
Q3	146-154	273	1.23 (0.97-1.55)	0.09
Q4	154-166	297	1.61 (1.31-1.98)	<0.001
Q5	>166	358	1.41 (1.17-1.71)	<0.001

*Adjusted HR per 2-SD increase in SBPV

Conclusions: Predialysis SBP variability is an important predictor of both overall and CVD mortality in incident HD patients, even after accounting for BP level and comorbidities. Interventions to reduce BPV are warranted, as potential ways to reduce excess mortality in ESRD.

Funding: NIDDK Support, Other U.S. Government Support

SA-PO604

30-Day Outcomes and Predictors of Mortality in Dialysis Patients Undergoing Abdominal Surgery Arley F. Diaz, Gurmukteshwar Singh, Philip Huh, Mihaela Stefan. *Medicine and Renal Division, Baystate Medical Center/ Tufts University School of Medicine, Springfield, MA.*

Background: Dialysis patients not infrequently undergo abdominal surgery, large studies evaluating and quantifying the post-operative risks and mortality in this population have not been performed.

Methods: We analyzed data from the 2007-08 American College of Surgeons National Surgical Quality Improvement Program to calculate the 30-day mortality risk and post-operative complication rates in chronic dialysis patient who underwent abdominal surgery. We also identified predictors for cardiac (acute MI and cardiac arrest) and respiratory (acute respiratory failure and pneumonia) complications. We used a multiple logistic regression analysis to examine associations between preoperative risk factors and postoperative events, in this model we adjusted for preoperative data, such patient's demographics, co-morbid conditions and laboratory results.

Results: 5178 dialysis patients from 189 hospitals were included in the analysis, 2144 abdominal surgeries were performed; overall 30-day mortality was 18.9%, the rates of cardiac and respiratory complications were 5.4 and 25.6% respectively. Functional status, ASA classification and emergency procedures conferred the highest risk factors for mortality with O.R of 2.7, 2.4, and 1.6 ($P<0.05$) respectively, patients with previous history of coronary disease were 2 times more likely to have cardiac complications (O.R 2.0, 95% C.I 1.2-3.2) and laparoscopic procedures were less likely to have respiratory complications (O.R 0.62, 95% C.I 0.46-0.82). Table 1 shows predictors of mortality.

Conclusions: Dialysis patients are at a very high risk of 30-day post-operative mortality, knowledge of predictive parameters may aid in the identification of patients at risk and help prevent mortality and complications after abdominal surgery.

Predictors of 30-day Mortality in Chronic Dialysis Patients After Abdominal Surgery

	Odds Ratio	P Value
Age	1.41 (1.27-1.57)	<0.001
Functional Status	2.77 (1.9-3.9)	<0.001
ASA Classification	2.46 (1.85-3.27)	<0.001
Emergency Procedure	1.69 (1.24-2.31)	0.001
Sepsis	1.24 (1.12-1.37)	<0.001
Ascites	1.60 (1.11-2.31)	0.012

SA-PO605

Pulmonary Hypertension Predicts Cardiovascular Mortality and Events in Hemodialysis Patients Zhilian Li, Wei Shi, Xinling Liang, Shuangxin Liu, Wenjian Wang, Penghua Hu, Lixia Xu, Ruizhao Li. *Guangdong General Hospital, Guangdong Academy of Medical Sciences.*

Background: Our previous study observed a close link between cardiovascular(CV) event and pulmonary hypertension(PH) in different stages of chronic kidney disease(CKD), particularly in maintenance hemodialysis(MHD) patients. However, whether PH predicts CV mortality and events remains unknown.

Methods: We did a retrospective cohort study to evaluate the predicative value of PH for CV events in 278 MHD patients. 78 patients with PH and 200 patients without PH were followed up for 2 years. Systolic pulmonary artery pressure(SPAP) was evaluated using Doppler echocardiography and calculated using Bernoulli equation, a value of >40mmHg was defined as PH. CV events were evaluated according to records from the computerized hospital database, which included acute heart failure, angina, acute myocardial infarction (AMI), arrhythmia requiring hospitalization, transient ischemic attack, thromboembolic or hemorrhagic stroke, peripheral vascular disease, or sudden cardiac arrest (or sudden death). The time to the first episode within the 2 years was taken for survival analysis.

Results: Among 278 MHD patients, 53(19.1%) died during the follow-up as a result of all causes, 22(28.2%) in the group with PH, and 31(15.5%) in the group without PH. 28(10.1%) died of CV events (52.8% of the death causes), 15(19.2%) in the group with PH, and 13(6.5%) in the group without PH. Survival curve showed the all-cause and CV mortality and new onset CV events of patients with PH were higher than that of patients without PH. In a multivariate Cox proportional hazard model, the adjusted HR for all-cause mortality, CV mortality and CV events (PH vs non-PH) was 2.38 (95%CI: 1.33-4.26), 4.06 (95%CI: 1.86-8.88) and 2.62 (95%CI: 1.66-4.13), respectively.

Conclusions: Our study in the first time showed the impact of PH on predicting CV mortality and events in MHD patients. Evidence of PH in predicting CV events in non-dialysis CKD patients is needed.

Funding: Government Support - Non-U.S.

SA-PO606

Left Ventricular Mass Is Associated with Increased Risk of Sudden Death in End Stage Kidney Disease Patients Irina Mostovaya,[1] Muriel Grooteman,[2,3] Marinus A. Van Den Dorpel,[4] Erik L. Penne,[1,2] Neelke C. Van Der Weerd,[2] Albert H. Mazairac,[1] Claire H. Den Hoedt,[1,4] Renee Levesque,[5] Menso Jan Nube,[2,3] Pieter M. Ter Wee,[2,3] Peter J. Blankestijn.[1] *[1]Nephrology, Univ. Medical Ctr. Utrecht, Utrecht, Netherlands; [2]Nephrology, VU Univ. Medical Ctr., Amsterdam, Netherlands; [3]Institute for Cardiovasc. Research, VU Univ. Medical Ctr., Netherlands; [4]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands; [5]Nephrology, Ctr. Hospitalier de l'Univ. de Montréal, Montreal, Canada.*

Background: Left ventricular mass (LVM) is a known predictor of overall and cardiovascular (CV) mortality in end stage kidney disease (ESKD) patients. The aim of this study is to determine whether LVM is 1) associated with overall and CV mortality in our population of ESKD patients and 2) whether LVM is associated with certain types of CV events.

Methods: Analysis was performed with data of 321 ESKD patients from the COnvective TRansport STudy (CONTRAST), a randomized controlled trial comparing online HDF and HD. The primary endpoint of CONTRAST was all-cause mortality. The main secondary endpoint was a composite of major CV events, including death from CV causes, vascular intervention, cerebrovascular accident (CVA) and amputation. Echocardiography was performed at initiation of the study. LVM was divided into tertiles. Multivariate Cox regression analysis was used to determine whether LVM tertiles were associated with the above described clinical events.

Results: After adjustment for age, sex, dialysis vintage, history of cardiovascular disease, diabetes and smoking, the highest tertile of LVM (>260g) was significantly and independently associated with increased risk of overall mortality (HR:1,82; p=0,015), CV mortality (HR:4,01; p=0,004), and sudden death (HR:14,2; p=0,012). No significant relationship was found with risk of overall CV events (HR:1,54; p=0,097), vascular interventions (HR 0,95; p=0,865), CVA (HR:1,60; p=0,758), and amputations (HR:1,09; p=0,886).

Conclusions: LVM is significantly and independently associated with an increased risk of overall mortality, CV mortality and sudden death. No relationship was found between LVM and risk of nonfatal CV events, vascular intervention, CVA and amputation.

Funding: Pharmaceutical Company Support - Dutch Kidney Foundation, Fresenius Medical Care, Gambro Lundia AB, Dr.E.E. Twiss Fund, Roche Neth., Baxter Extramural Grant Program, Netherlands Organization for Health Research and Development

SA-PO607

Effects of Hemodiafiltration on Change in Left Ventricular Mass over Time in End Stage Renal Disease Patients Irina Mostovaya,[1] Muriel Grooteman,[2,3] Marinus A. Van Den Dorpel,[4] Erik L. Penne,[1,2] Neelke C. Van Der Weerd,[2] Albert H. Mazairac,[1] Claire H. Den Hoedt,[1,4] Renee Levesque,[5] Menso Jan Nube,[2,3] Pieter M. Ter Wee,[2,3] Peter J. Blankestijn.[1] *[1]Nephrology, Univ. Medical Centertrecht, Utrecht, Netherlands; [2]Nephrology, VU Univ. Medical Center, Amsterdam, Netherlands; [3]Institute for Cardiovascular Research, VU Univ. Medical Center, Amsterdam, Netherlands; [4]Internal Medicine, Maasstad Hospital, Rotterdam, Netherlands; [5]Nephrology, Centre Hospitalier de l'Univ. de Montréal, Montreal, QC, Canada.*

Background: Left ventricular mass (LVM) predicts overall and cardiovascular mortality in end stage kidney disease (ESKD) patients. Stabilization or regression of LVM improves the prognosis in patients with ESKD. Recently, a small (n=22) randomized controlled trial showed that hemodiafiltration (HDF) is associated with a decrease in LVM when compared to standard hemodialysis (HD). The aim of this study is to determine the effect of online HDF versus low flux HD on the rate of change in LVM over time in ESKD patients.

Methods: Analysis was performed with data of 321 ESKD patients from the COnvective TRansport STudy (CONTRAST), a randomized controlled trial comparing online HDF and HD. Patient characteristics and dialysis properties were recorded at baseline every 3 months. M-mode echocardiography was performed on an inter-dialysis day at baseline, after 6 months, 12 months and yearly thereafter. LVM was calculated with Devereux formula. Data on echocardiography and patient characteristics from the first 3 years of follow-up was used for analysis. Differences in rate of change in LVM over time were assessed with linear mixed effect models. Treatment effect was evaluated in subgroups of: age, gender, residual renal function, dialysis vintage, history of cardiovascular disease or diabetes, convection volume and dialysis center.

Results: LVM did not change over time (p=0.356). There was no difference in rate change in LVM between treatment groups (p=0.858). No evidence was found for different effect is the subgroups.

Conclusions: Online HDF does not affect change in LVM over time different from HD. We found no change of LVM over time in a period of 3 years in our patient population.

Funding: Pharmaceutical Company Support - Dutch Kidney Foundation, Fresenius Medical Care, Gambro Lundia AB, Dr.E.E.Twiss Fund, Roche Netherlands, Baxter Extramural Grant Program, Netherlands Organization for Health Research and Development

SA-PO608

Severe Left Ventricular Dysfunction Predicts Early Mortality in Incident Asian Dialysis Patients Sabrina Haroon,[1] Nan Luo,[2] Horng Ruey Chua,[1] Boon Wee Teo,[1] Evan J.C. Lee,[1] Titus W. Lau.[1] *[1]Division of Nephrology, National University Health System, Singapore; [2]School of Public Health, National University of Singapore, Singapore.*

Background: Cardiac disease is a leading cause of death amongst dialysis patients. Left ventricular ejection fraction (LVEF) by 2D echocardiography maybe a useful tool in risk stratification of patients starting dialysis. It is however unclear at what level of LVEF (just prior to starting dialysis) predicts early mortality.

Methods: In this retrospective analysis of consecutive patients starting dialysis from Jan 1, 2005 to Dec 31, 2010, we examine 3 different levels of LVEF and its association with early mortality (first 90 days of dialysis). It is part of the protocol in our institution to have a screening echocardiography for all patients just prior to starting dialysis (within a 6 months window). SAS was used for analyses. Logistic regression analysis was used to determine if different LVEF levels in a given model predicts endpoint (90 days mortality).

Results: 870 patients were included in the analysis with 92 deaths (10.6%) occurring within the first 90 days. There were 633 (72.7%) patients with LVEF > 45% (this serve as our reference group of near normal-normal LV function), 173 (19.9%) patients with LVEF 30-45% (this being the group with moderate degree of LV dysfunction) and 64 (7.4%) patients with LVEF < 30% (this being the group with severe LV dysfunction). Multivariate analysis (with inclusion of age, gender, diabetic status, ischemic heart disease status, stroke status, periphery vascular disease status, baseline hemoglobin, albumin and planned start with access into the model) showed amongst different LVEF groups, the group with severe LV dysfunction (LVEF < 30%) was significantly associated with early (90 days into dialysis) mortality (OR 4.27; CI 95% 2.01 - 9.07; p = 0.0002). Interestingly, in this model, presence of ischemic heart disease was not a significant predictor of early mortality and was also not associated with low LVEF.

Conclusions: Severe LV dysfunction (LVEF < 30%) is a strong predictor of early mortality in an incident Asian dialysis cohort but not the group with moderate LV dysfunction and those with known ischemic heart disease.

SA-PO609

Differences in Assessment of Left Ventricular Hypertrophy with Echocardiography Compared to Cardiac Magnetic Resonance Imaging in End Stage Renal Disease: Implications for Clinical Trials Patrick Barry Mark, Rajan K. Patel, Kathryn K. Stevens, Alan G. Jardine. *Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom.*

Background: Left ventricular hypertrophy (LVH) is a risk factor for reduced survival in end stage renal disease (ESRD). Left ventricular (LV) mass is conventionally assessed using echocardiography (echo) although echo may overestimate LV mass compared to cardiac magnetic resonance imaging (CMR). We studied the relationship between LV remodelling and LVH using CMR and echo in ESRD patients.

Methods: 117 haemodialysis patients (63.2% male; median age 54.0, range 24-74) underwent echo and CMR (1.5 Tesla scanner) on the same, non-dialysis day. Echo LV mass was determined using the ASE formula. Characterisation of remodelling (concentric-pressure induced vs. eccentric- volume overload induced) was based on regional wall thickness. CMR LV mass was calculated using analysis software (Siemens Argus). LV mass index (LVMI) was calculated by normalising to body surface area. Power calculations for clinical trials targeting LVH were derived from the data.

Results: 98 patients had suitable images for analysis by both echo and CMR. Mean LVMI was 144.3 g/m^2 by echo and 94.5g/m^2 by CMR. 63.3% of patients had LVH by echo of which 56.5% had eccentric LVH and 43.5% had concentric LVH. 67.5% had LVH by CMR. Echo overestimated LV mass compared to CMR by mean of 48.4g/m^2 (Bland-Altman plot). There was no difference in the degree of overestimation in patients with eccentric compared to concentric LVH (overestimate 46.3 vs. 50.9g/m^2, p=0.77). Based on these data, using CMR in a randomised clinical trial of patients with LVH, 266 patients (133 per group) would be required to have an 80% chance of detecting a reduction in LV mass index of 10g with an intervention, at significance of 5%. A similar trial with echo would require 694 patients.

Conclusions: Overestimation of LV mass with echo compared to CMR in ESRD does not appear to be due to LV remodelling and may reflect other inherent differences between the techniques. CMR does allow for reduction in sample size for clinical trials targeting LVH but not to the dramatic extent previously described.

Funding: Government Support - Non-U.S.

SA-PO610

Left Ventricular Diastolic Dysfunction Is Associated with Coronary and Carotid Atherosclerosis in Dialysis Patients Jwa-kyung Kim, Myung Jin Choi, Min-Gang Kim, Jisuk Han, Sung Gyun Kim. *Department of Internal Medicine, Hallym University Sacred Heart Hospital, Kidney Research Institute, Republic of Korea.*

Background: Left ventricular diastolic dysfunction (LVDD) is common among patients with chronic kidney disease. We evaluated whether LVDD is associated with atherosclerosis in two major vessels, coronary and carotid arteries in incident dialysis patients.

Methods: Myocardial ischemia and the carotid intima-media thickness (cIMT) was evaluated using single photon emission computed tomography and B-mode ultrasonography. The presence of LVDD was considered when the ratio of early peak transmitral inflow velocity (E) to early diastolic mitral annular velocity (E') (E/E') was >15, and carotid atherosclerosis was defined as cIMT >1.0 mm and/or the presence of plaque.

Results: Among 108 patients, LVDD was observed in 27 patients. Those with LVDD had significantly more perfusion defects [relative risk 4.1, 95% CI 1.8-10.2], a higher summed stress score (SSS, p=0.004) and an increased aortic pulse wave velocity (p=0.022) than subjects without LVDD. Similarly, the cIMT was greater in those with LVDD than patients without (1.04 ± 0.15 vs. 0.82 ± 0.17 mm, p<0.001), and the risk of carotid atherosclerosis was 25 times greater for those with LVDD. Multiple logistic analyses showed that old age [odds ratio (OR) 1.10, 95% CI 1.01-1.19], diabetes (OR 1.82, 95% CI 1.11-3.51), 1 mg/L increase of high-sensitivity C-reactive protein (OR 4.48, 95% CI 1.54-13.04), E/E' >15 (OR 17.94, 95% CI 2.12-35.55) and SSS (OR 1.34, 95% CI 1.04-1.73) were significant predictors for carotid atherosclerosis.

Conclusions: Abnormal LVDD is closely associated with coronary ischemia and carotid atherosclerosis in incident dialysis patients. Further studies are warranted to clarify the cause-effect relationship.

SA-PO611

Analysis of Risk Factors for Cardiac Events in End-Stage Renal Disease Patients with Normal Stress Myocardial Perfusion Imaging Jwa-kyung Kim, Jisuk Han, Myung Jin Choi, Min-Gang Kim, Sung Gyun Kim. *Internal Medicine, Hallym University Sacred Heart Hospital, Kidney Research Institute, Republic of Korea.*

Background: Normal myocardial perfusion imaging is closely associated with very low rates of cardiac events and better long-term prognosis in patients with end-stage renal patients (ESRD). However, it is uncertain whether subsets of these patients are at increased risk for serious cardiac events, even in the presence of a normal perfusion scan. The purpose of this work is to determine the characteristics of the patients who had normal perfusion scan but had cardiac events during follow-up.

Methods: A total of 260 consecutive ESRD patients underwent baseline echocardiography and stress/rest single photon emission computed tomography (SPECT) at the start of dialysis. One hundred sixty (61.5%) of patients showed a summed stress score ≤3, which was considered normal.

Results: During the 4-year follow-up period, there were a total of 79 cardiac events (30.4%). Patients with normal SPECT had significantly lower rates of cardiac event than those with abnormal SPECT (HR, 0.54; 95% CI, 0.31-0.94; *P*=0.029). Among normal SPECT patients, 31 (19.3%) of patients had cardiac events within a mean duration of 25 months. Patients with cardiac events were significantly older (p=0.014), had higher prevalence of diabetes (p=0.013) and lower serum hemoglobin (Hb) and albumin level than patients without cardiac events. In addition, baseline left ventricular (LV) ejection fraction was significantly lower and LV mass index was higher in these patients. Cox proportional hazard analysis showed that diabetes mellitus (HR, 2.57; 95% CI, 1.16-5.68), age >60 years (HR, 2.05; 95% CI, 1.05-4.22), hypoalbuminemia (<3.0 g/dL, HR, 4.15; 95% CI, 2.10-8.17) and anemia (Hb <9.0 g/dL, HR, 4.08; 95% CI, 1.76-9.45) were independent predictors of all cardiac events.

Conclusions: The well-known traditional risk factors for cardiac events are also important in patients with normal perfusion scan. Optimal management of these risk factors is necessary in ESRD patients.

SA-PO612

Chronic Fluid Overload (CFO) Is Associated with Increased Left Ventricular Mass Index (LVMI) & LVMI Improves over Time in Euvolemic (noCFO) Pediatric Patients (Ped Pts) Maintained with Chronic Peritoneal Dialysis (CPD) Kimberly A. Burrows, Heather A. Dickerson, Poyyapakkam Srivaths, Eileen D. Brewer. *Pediatric Renal & Cardiology, Baylor College of Medicine, Houston, TX.*

Background: Ped pts with ESRD have increased cardiovascular mortality. Risk factors include CFO, hypertension, no residual renal function (RRF), anemia and inflammation, all of which contribute to increased LVMI as well as abnormal cardiac remodeling and subsequent diastolic dysfunction. Few ped studies have assessed the relationship between CFO & LVMI, & no ped study has assessed prevalence of CFO in ped pts maintained on CPD. **Aim:** To determine prevalence of CFO & increased LVMI in ped CPD pts & whether LVMI changes with time on CPD.

Methods: Prospective monthly follow-up over 1y for ped pts 6-25yo without underlying primary cardiac disease, who were treated with home cycler CPD for >3m. Fluid status was assessed by bioimpedance, weight (wt) & BP at each monthly visit. CFO was defined as >4% over target dry wt for at least 3m in each 6m period or median value >4% for

an entire 6m period. ECHOcardiogram with LVMI was done initially & at end of study. Monthly serum Ca, P, iPTH, 25OHvit D, & Hgb were measured as part of routine care.

Results: 10/15 pts enrolled completed at least 9m of study; 3 were transplanted, 1 changed modality & 1 is still in study. 2/10 (20%) had CFO, & LVMI at the end of study was significantly higher compared to 8/10 noCFO pts (mean LVMI 57.5 vs. 37.5 g/m$^{2.7}$, p=0.006). In CFO pts, LVMI remained elevated throughout the study (initially 61 & 49.7 g/m$^{2.7}$ and at end of study 60.3 & 54.8 g/m$^{2.7}$). In the 8 noCFO pts, LVMI was increased initially, but improved significantly by end of study (median LVMI 48.6 vs. 36.2 g/m$^{2.7}$, p=0.025). There was no difference in total time on CPD or time averaged monthly BP (indexed for gender, age, ht%ile), Hgb, serum Ca, P, iPTH & 25OHvit D between CFO and noCFO pts. 1/2 CFO & 4/8 noCFO pts had no RRF.

Conclusions: In ped CPD pts CFO is associated with increased & persistent LVMI. In noCFO pts, LVMI improves over time with CPD. These data emphasize the importance of maintaining euvolemia in ped CPD pts to reduce future cardiac morbidity.

Funding: Clinical Revenue Support

SA-PO613

Resource Utilization in Patients with Heart Failure Who Develop End Stage Renal Disease Kimberly N. Hong,[1] Sumit Mohan,[2] Mark J. Russo,[3] Deborah D. Ascheim,[1] Jai Radhakrishnan.[2] *[1]Mount Sinai School of Medicine, New York, NY; [2]Columbia University Medicine Center, New York, NY; [3]University of Chicago Medical Center, Chicago, IL.*

Background: While hemodialysis improves volume control, arteriovenous shunting can also impact the progression of heart failure. The purpose of this study was to characterize resource utilization in Medicare beneficiaries with heart failure who were initiated on hemodialysis (HD).

Methods: De-identified inpatient claims data for all Medicare beneficiaries with a Chronic Conditions Warehouse (CCW) heart failure (HF) flag ((N=10,331,062) were obtained from the Centers for Medicare and Medicaid services for the period of 1/1/2001 to 12/31/2005. Medicare beneficiaries with at least 1 year of data prior to converting to an ESRD status code (11, 21) were included (N=68,196). Index hospitalization was defined as the first admission with dialysis that was preceded by at least one admission without dialysis. All outcomes were assessed using paired t-test and included annualized all-cause and HF associated hospitalization rates, LOS and time to rehospitalization before and after HD was started. Principle diagnosis code was used to classify HF hospitalization using the CCW ICD-9 codes definition for HF. Patients were censored following heart/kidney transplant and ventricular assist device use.

Results: Annualized hospitalization rates decreased after the initiation of HD. Specifically, all-cause and HF admissions decreased by 0.48 admissions/yr (7.9±37.5 vs 8.0±12.8, p<0.0067) and 0.62 admissions/yr (1.1±10.2 vs. 0.5±3.0 p<0.0001). Mean LOS, however, was longer following initiation of HD (6.6±9.5 vs 7.4±8.9, p<0.0001).

Conclusions: Patients with HF who were started on HD had lower rates of HF related admissions in the first year of ESRD but tended to have longer hospitalizations suggesting lower utilization among these patients overall but greater utilization among those needing admission. Further analysis is required to identify patients and risk factors that predict continued readmission for HF.

Table - Descriptive Statistics

	Number	%Total/SD
Male (n)	35012	51
White (n)	46378	68
Mean age (yrs)	72	11
Mean follow-up time (days)	984	460

SA-PO614

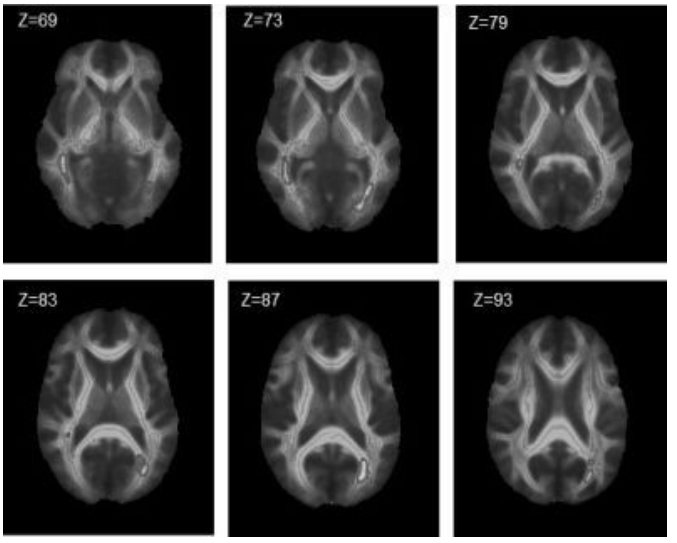
Brain White Matter Microstructure Using Diffusion Tensor Imaging Is Associated with Cognitive Impairment in Haemodialysis Patients Mohamed Tarek Eldehni,[1,2] Aghogho Odudu,[1,2] Chris W. McIntyre.[1,2] *[1]Graduate Entry Medical School, University of Nottingham, United Kingdom; [2]Department of Renal Medicine, Royal Derby Hospital, United Kingdom.*

Background: Structural and functional brain white matter (WM) abnormalities are poorly characterised in Haemodialysis (HD) patients despite the observed deterioration in functional status after initiating HD. We aim to examine the brain WM microstructure using Diffusion Tensor Magnetic Resonance Imaging (DTI) and its correlation with cognitive impairment.

Methods: DTI was done in 71 subjects, 48 incident HD patients and 23 age-matched normal controls (NC). Images were analysed using FSL software. Maps of Fractional anisotropy (FA) (a measure of WM tracts organisation) were computed then a voxelwise statistical analysis of the FA data was done between the groups using TBSS (Tract-Based Spatial Statistic). Cognitive assessment was done using the Montreal Cognitive Assessment (MoCA) and Trail Making Tests (TMT) A & B. Pulse wave velocity (PWV) was done in all subjects.

Results: There were bilateral areas of reduced FA in HD patients compared to NC with statistical significance (P<0.05) indicating distorted WM structure (Figure). MoCA scores were lower in HD patients (25 IQR 22-28 vs 28 IQR 26-30, P=0.004). TMT A & B times were higher in HD patients (TMT A 35s IQR 26-49 vs 22s 21-30, P=0.001) (TMT B 80s IQR 57-121 vs 56s IQR 43-87). PWV did not differ between the groups (P=0.08).

Conclusions: HD patients have significant WM structural abnormalities manifest as cognitive impairment and reduced attention and mental flexibility compared to aged-matched normal controls. The lack of difference in large vessel characteristics could indicate that WM distortion in HD patients is linked to the circulatory and inflammatory stress of HD.



TBSS Projection of all subjects' FA data onto a mean skeleton highlighting voxels with significant FA reduction in multiple levels.

Funding: Government Support - Non-U.S.

SA-PO615

Anatomic Brain Disease in Hemodialysis Patients David A. Drew,[1] Rafeeque Bhadelia,[2] Hocine Tighiouart,[1] Tammy Scott,[1] Saeed K. Shaffi,[1] Daniel E. Weiner,[1] Mark J. Sarnak.[1] *[1]Tufts Medical Center; [2]Beth Israel Deaconess Medical Center.*

Background: Although dialysis patients are at high risk for stroke and have a high burden of cognitive impairment, there are few reports on anatomic brain findings in the hemodialysis (HD) population. Using brain magnetic resonance imaging (MRI), we compared the prevalence of brain abnormalities in hemodialysis patients to a non-end stage renal disease (ESRD) control population.

Methods: We assembled a cross-sectional cohort of 45 maintenance HD patients and 67 non-ESRD controls, both without prior history of stroke. The primary predictor was dialysis status. Covariates included demographics (age, race, sex), vascular risk factors (diabetes and hypertension) and vascular disease (coronary artery disease, congestive heart failure). The primary outcomes were severity of white matter disease and cerebral atrophy (sulcal prominence and ventricular atrophy) as assessed by a semi-quantitative scale (0 to 9), hippocampal size (0 to 3), and prevalence of small and large vessel infarcts. Multivariable linear or logistic regression was used to evaluate the relationship of dialysis status to MRI findings.

Results: The mean (SD) age of HD patients and controls was 55 (17) and 53 (13) years, respectively. HD patients had more severe white matter disease (1.6 v 0.7) and cerebral atrophy (sulcal prominence = 2.3 v 0.6; ventricular enlargement = 2.3 v 0.9; hippocampal size = 1.3 v 1.0) with all p-values <0.001. In multivariable analyses, hemodialysis status was significantly associated with worse white matter and atrophy grades. HD patients had a higher prevalence of small (17.8%) and large (7.8%) vessel infarcts than controls (combined 22% vs 0%, p<0.0001).

Table 1. Association of Hemodialysis Status with MRI findings

MRI finding	Hemodialysis β Coefficient [95% CI]	p	Hemodialysis adjusted† β Coefficient [95% CI]	p
White Matter Grade	0.88 [0.37, 1.38]	0.001	0.69 [0.04, 1.33]	0.04
Sulcal Grade	1.71 [1.23, 2.19]	<0.001	1.36 [0.87, 1.84]	<0.001
Ventricular Grade	1.37 [0.77, 1.97]	<0.001	1.06 [0.39, 1.74]	0.002
Hippocampal Grade	11.82 [2.50, 55.92]*	0.002	13.52 [1.09, 168.19]*	0.04

*presented as OR (odds ratio)
†: adjusted for age, gender, race (African American or other), vascular disease and vascular disease risk factors

Conclusions: Hemodialysis patients have more white matter disease and cerebral atrophy compared to non-ESRD controls. Hemodialysis patients also have a high prevalence of unrecognized infarcts.

Funding: NIDDK Support, Other NIH Support - T32 Training Grant

SA-PO616

A 12 Months Randomized Controlled Trial Evaluating the Effects of a Vitamin E Bonded Dialysis Membrane on Inflammation, Oxidative Stress, Fibrin Clot Parameters, Cardiovascular Events and Mortality

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Background: Inflammation, oxidative stress and alterations in fibrin clot phenotype have all been linked to cardiovascular (CV) disease in hemodialysis (HD) patients. Vitamin E (VE) bonded HD membranes are purported to improve a number of these parameters which we investigated in a 12-months randomized controlled trial.

Methods: All HD patients managed by our unit were screened for the study; principle exclusion criteria were HD<3 months or C-reactive protein (CRP) >50 mg/L. Patients were randomized to HD with either a VE-bonded polysulfone (PS) membrane or a non-VE bonded PS equivalent. Data were collected prospectively on CV events and mortality and blood samples taken at baseline and 12 months for determination of oxidized LDL (ox-LDL), CRP, complement (C3, sC5b9, factor D and properdin) and fibrin clot parameters.

Results: Of the 260 patients enrolled in the study 123 were assigned to VE and 215 completed 12 months. Comparing the change in each parameter after 12 months between groups revealed no significant effects of the VE on the levels of ox-LDL, CRP, C3, sC5b9, factor D or properdin or on fibrin clot parameters (all p>0.30). Similarly there were no statistically significant differences between the VE and control groups in the numbers of deaths (7 vs 8; p=0.96), CV events (27 vs 17; p=0.11) or hospital admissions (98 vs 124; p=0.48). However, there were fewer non-infective dialysis access complications in the VE group (26 vs 54; p=0.04).

Conclusions: In this study, switching prevalent HD patients to a VE-bonded membrane for a period of 12 months was not associated with improvements in the pre-dialysis measures of inflammation, oxidative stress and fibrin clot function tested, the number of CV events or mortality. Larger studies of longer duration are required to fully evaluate the potential of VE membranes to influence CV risk.

Funding: Pharmaceutical Company Support - Asahi Medical Ltd, Japan, Government Support - Non-U.S.

SA-PO617

Increased Fibrin Clot Density Is Associated with Prevalent and Incident Cardiovascular Disease in Hemodialysis Patients

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Background: The formation of denser fibrin clots and prolonged fibrinolysis times are associated with cardiovascular (CV) disease (CVD) in several patient groups including a small study of chronic hemodialysis (HD) patients. We sought to characterize the determinants of fibrin clot parameters and their association with prevalent and incident CVD and CV risk factors in a larger cohort of HD patients.

Methods: Fibrin clot density and fibrinolysis times, in conjunction with clinicopathological data, were available for 219 HD patients who were followed for 12 months.

Results: Fibrin clot density was significantly higher in the 93 patients with a prior history of CVD (0.556[0.529-0.583] vs 0.509[0.487-0.531] absorbance units (au); p=0.007) and in the 28 patients who experienced a new CV event during follow up (0.578[0.526-0.630] vs 0.522[0.503-0.540] au; p=0.03). Fibrinolysis times were not associated with prevalent or incident CVD. Fibrin clot density was significantly correlated with a variety of CV risk factors on univariate analyses. Independent predictors of clot density (identified using backwards stepwise regression) were: fibrinogen, C-reactive protein, triglycerides, weight, diastolic blood pressure and ethnicity which together accounted for 85% of variance. In a logistic regression model, including factors associated with prevalent CVD in univariate analysis at baseline, clot density was independently associated with CVD (odds ratio for increase of 0.1 au: 1.51[1.16-1.96]). This association persisted after adjusting for medications. Age, smoking history, diabetes, malignancy and oxidized low density lipoprotein were also independently associated with CVD in this model. Clot density was not independently associated with incident CVD in a similar logistic regression model, in which smoking and previous history of CVD were the only independent determinants.

Conclusions: This study indicates that increased fibrin clot density is associated with both prevalent and incident CVD in HD patients.

Funding: Pharmaceutical Company Support - Asahi Kasei Ltd., Japan, Government Support - Non-U.S.

SA-PO618

Study of the Optimal Dose of Vitamin K2 in Hemodialysis Patients

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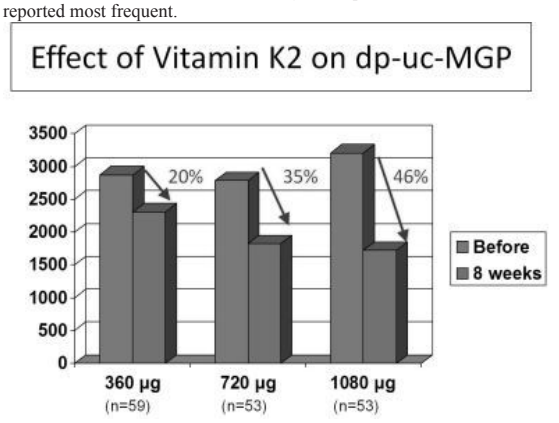
Background: Hemodialysis patients suffer from accelerated vascular calcification. The Vitamin K2 dependent Matrix Gla Protein (MGP) is one of the most powerful inhibitors of vascular calcification. Hemodialysis patients may benefit from pharmacologic doses of Vitamin K2 to improve the biologic activity of MGP. The optimal dose of Vitamin K2 required for this purpose is unknown.

Methods: We randomized 200 chronic hemodialysis patients from 2 tertiary non-university hospital dialysis units to receive uncoated tablets of 360 (group A), 720 (group B) or 1080 µg (group C) of Vitamin K2 thrice weekly for 8 weeks. The inactive form of MGP (dp-uc-MGP) was measured at baseline and after 8 weeks. Patients on coumarin treatment were excluded.

Results: For various reasons (death n=5, study withdrawal n=6, side effects n=15, incompliance n=3, missing samples n=6) 35 patients had no complete analysis. Study groups A (n=59), B (n=53) and C (n=53) had a reduction in dp-uc-MGP levels of 20, 35 and 46% respectively. 13% of patients suffered from gastro-intestinal side effects, unrelated to the dose. Nausea caused by the unpleasant smell of the uncoated tablets was reported most frequent.



Conclusions: Oral Vitamin K2 supplementation in chronic hemodialysis patients effectively and dose-dependently reduces dp-uc-MGP levels. Gastro-intestinal side-effects were frequent but may be overcome by coating the tablets.

Funding: Pharmaceutical Company Support - NattoPharma

SA-PO619

Dental Health and All Cause and Cardiovascular Mortality in Hemodialysis Patients: A Prospective Multinational Cohort Study

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Background: Oral disease may be associated with increased risks of death due to inflammation or as a general indicator of healthcare practices. We evaluated the association between dental status and the risk of all-cause and cardiovascular mortality in adults on hemodialysis.

Methods: ORAL-D is an ongoing multinational prospective cohort study of consecutive adults on hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Italy, Hungary, Poland, Argentina, Portugal, France and Spain. A dental surgeon evaluated dental status by using the DMFT (decayed, missing, filled, permanent teeth) score. Quality of dental health was defined based upon WHO criteria as low, moderate and high DMFT scores of ≤2.6, 2.7-4.4 and >4.4. We assessed survival at 12 months using centralized mortality data. We conducted analyses using Cox regression controlling for age, gender, previous cardiovascular events, income status, clinical performance measures, dialysis prescription and performance indicators, and depressive symptoms.

Results: 4720 hemodialysis patients in the participating clinics received a complete evaluation of their dental status and completed follow up. Median follow up was 19.9 (17.0 to 28.0) months and 516 (11%) died during follow up. The overall DMFT score was 21.67 (9.40). Dental health (DMFT scores) had uncertain associations with all-cause or cardiovascular mortality.

Exposure	Unadjusted risk	p value	Adjusted risk	p value
All-cause mortality				
Low DMFT score	1.36 (0.46-3.96)	0.58	0.63 (0.19-2.08)	0.45
Moderate DMFT score	2.89 (1.14-7.36)	0.03	1.51 (0.58-3.88)	0.40
High DMFT score	5.98 (2.48-14.42)	<0.001	2.07 (0.85-5.07)	0.11
Cardiovascular mortality				
Low DMFT score	1.02 (0.29-3.60)	0.98	0.40 (0.09-1.79)	0.23
Moderate DMFT score	1.76 (0.59-5.19)	0.31	1.02 (0.34-3.05)	0.97
High DMFT score	3.86 (1.44-10.4)	0.007	1.38 (0.50-3.78)	0.54

Conclusions: Dental health has uncertain associations with all-cause and cardiovascular mortality in patients on hemodialysis. ORALD will be completed by end of 2013.

Other steering group: Stroumza P, Frantzen L, Leal M, Torok M, Benarek A, Dulawa J, Celia E, Gelfman R, Wollheim C, Johnson D.

SA-PO620

Periodontal Disease and All Cause and Cardiovascular Mortality in Hemodialysis Patients: A Prospective Multinational Cohort Study
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Background: In the general population, periodontal disease is associated with increased cardiovascular mortality. We have evaluated the association between periodontitis and all-cause and cardiovascular mortality in adults on hemodialysis.

Methods: ORAL-D is an ongoing multinational prospective cohort study of consecutive adults receiving hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Italy, Hungary, Poland, Argentina, Portugal, France and Spain. A dental surgeon evaluated presence of periodontitis with standard methods, defined as a Community Periodontal Index (CPI) score ≥3 during a standardized oral examination. We assessed survival at 12 months using centralized mortality data. We conducted analysis with Cox regression controlling for age, gender, previous cardiovascular event, income status, clinical performance measures, dialysis prescription and performance indicators and depressive symptoms.

Results: 3672 dentate hemodialysis patients in the participating clinics received a complete evaluation for periodontitis and completed follow up. Median follow up was 19.9 (17.0 to 28.0) months. 1516 patients (42%) had periodontitis and 339 (10%) died during follow up. Periodontitis had uncertain associations with risks of all-cause (HR 0.86 [95% CI 0.68-1.10]) or cardiovascular (HR 0.85 [95% CI 0.63-1.15]) mortality.

Conclusions: Contrary to data in the general population, periodontitis has uncertain associations with all-cause or cardiovascular mortality in patients on hemodialysis. ORALD will be completed by end of 2013.

Other steering group: Stroumza P, Frantzen L, Leal M, Torok M, Benarek A, Dulawa J, Celia E, Gelfman R, Wollheim C, Johnson D, Petruzzi M, De Benedittis M.

SA-PO621

Serum Bicarbonate Is Not Associated with Arterial Stiffness in Hemodialysis Patients
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Background: Limited data suggest that metabolic alkalosis may provoke vascular calcification, which increases arterial stiffness. Therefore, we investigated the association between serum bicarbonate with pulse wave velocity (PWV), as a measure of arterial calcification, in the ongoing Protein Intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) study of chronic hemodialysis patients.

Methods: Carotid and femoral pulses were used to measure aortic PWV with a Sphygmacor PVX device. Measurements were conducted at baseline, 6, and 12 months. Linear regression models related PWV at (i) baseline and (ii) 6 and 12 month follow-up values (lag model) with each standard deviation increase in predialysis serum bicarbonate concentration. Adjustments were made for age, gender, race, vascular access, ESRD duration, and study center.

Results: One hundred eighteen hemodialysis patients had non-missing data at baseline and were included in the analysis. The study is ongoing and follow-up PWV data were available in 93 and 74 participants at the 6 and 12 month visits. The mean age was 52 ± 16 years, 59% were men, 81% were Caucasians and 47% had diabetes. Mean serum bicarbonate at baseline was 23.4 ± 5.0 mmol/L and mean (SD) PWV for the three times points were 9.8 (3.4), 10.2 (3.8), and 10.1 (3.4) m/sec, respectively. Each standard deviation increase in predialysis serum bicarbonate concentration was not associated with PWV at baseline or follow-up.

Association of predialysis serum bicarbonate concentration with (i) baseline and (ii) 6 and 12 month (lag) aortic PWV (m/s)

	Coefficient	95% CI	p
Baseline model	-0.36	-0.89 -0.17	0.18
Follow-up (lg) model	0.01	-0.55 0.58	0.96

Conclusions: Higher predialysis serum bicarbonate concentration is not associated with aortic stiffness in hemodialysis patients.

Funding: NIDDK Support

SA-PO622

25-Hydroxyvitamin D Is Associated with Faster Gait Speed, but Not Muscle Area, in Hemodialysis Patients
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Background: Interventions to improve frailty in maintenance hemodialysis (MHD) patients are limited. Therefore, we investigated whether serum 25-hydroxyvitamin D (25-D) is associated with gait speed and mid-thigh muscle area in MHD patients in the ongoing Protein Intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) study.

Methods: Linear regression models were used to relate each SD increase in baseline 25-D as the predictor variable to gait speed, assessed by 6-minute walk (6MW), and mid-thigh muscle area, assessed by MRI as outcome variables at (i) baseline (cross-sectional) and (ii) 6 and 12 month follow-up values (lag). Logistic regression models were used to determine if 25-D predicted completion of the 6MW. Age, gender, race, vascular access, ESRD duration, and study center were included in each model.

Results: The mean age of 93 MHD patients in this analysis was 52±16 years, 53% were men, 75% were white and 43% had diabetes. Mean serum 25-D at baseline was 21.8±11.9 ng/mL. Mean gait speed was 0.85 (±0.32) m/sec and mean muscle area was 103.0 (±24.6) cm^2. Seventy-three, 58, and 55 individuals performed the 6MW at baseline, 6- and 12-months. Baseline 25-D in those that did and did not perform the 6MW were similar and did not predict completion of the 6MW at any time point. Higher 25-D was associated with faster gait speed at baseline and after 6-12 months. Seventy-three and 63 individuals had muscle MRI at baseline and 6-months. 25-D was not associated with muscle area in this cohort of MHD patients.

Table 1. Association of each SD increase in 25-D with gait speed and muscle area at (i) baseline and (ii) with follow-up values (lag).

	Baseline Model	Lag Model
	Coefficient (95% CI)	Coefficient (95% CI)
Model 1: Gait Speed (m/s)	0.09 (0.02–0.16)	0.08 (0.03–0.13)
Model 2: Muscle Area (cm2)	-2.3 (-7.3–2.8)	-3.8 (-8.9–1.4)

Conclusions: In MHD patients, higher 25-D is associated with faster gait speed but not with muscle area. Interventional studies are warranted to determine whether 25-D therapy could improve/slow the decline in gait speed in MHD patients.

Funding: NIDDK Support

SA-PO623

Significant Association of Klotho, FGF-23 and Arterial Stiffness in End Stage Renal Disease Patients on Maintenance Hemodialysis
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Background: Arterial stiffening characterizes the vasculature of end-stage renal disease (ESRD) patients and is a strong predictor of their cardiovascular morbidity and mortality. Pulse wave velocity (PWV) has been widely recognized as a marker of systemic atherosclerosis.Recently emerging evidence suggests the role of Klotho and FGF-23 against endothelial dysfunction that is a determinant of impaired vasoreactivity. We hypothesized that the level of Klotho and FGF-23 may relate with arterial stiffness presented by PWV in ESRD patients.

Methods: We analysed 90 ESRD patients including 51 male and 39 female on hemodialysis. Mean age was 57.8 ± 12.7 years. Patients were tested for plasma level of FGF-23, Klotho, biologic markers of cardiovascular disease and arterial stiffness presented by PWV and simple aortic calcification with plain radiograph of chest. Endothelial function was assessed by flow-mediated dilation(FMD).

Results: Mean concentration of Klotho and FGF-23 were 640.14±256.42 pg/ml and 6432.67±14702.86 pg/ml. The mean aortic PWV was 8.74±2.02m/s at baseline. The aortic PWV and the level of FGF-23 were significantly increased in patients with diabetes compared non-diabetic group. Aortic PWV correlated with age, aortic arch calcification scores and level of log-formed FGF-23 positively. In addition, it correlated with diastolic blood pressure, PTH level and level of Klotho inversely. However, it didn't correlate with serum calcium, phosphorus, 25-vitamin D level and Kt/Vurea.

Conclusions: The level of Klothoand FGF-23 were useful for assessment of arterial stiffness that precedes cardiovascular complicationsin ESRD patients on maintenance hemodialysis.

Funding: Private Foundation Support

SA-PO624

Bone Turnover Markers Associate with Cardiovascular and All-Cause Death in Maintenance Hemodialysis Patients
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Background: Alkaline phosphatase (ALP) is a biomarker which is associated well with cardiovascular (CV) and all-cause mortality in both maintenance hemodialysis (MHD) and predialysis patients. It is, however, inconclusive whether bone turnover markers such as bone-specific ALP (BSAP), serum N-terminal telopeptide of type I collagen (NTX), and tartrate-resistant acid phosphatase 5b (TRAP5b) can predict prognosis of MHD patients.

Methods: In this single-centred prospective observational study, we enrolled 128 MHD patients in May 2006 and followed-up until May 2012. We measured 1-84 PTH, NTX, TRAP5b and BSAP at baseline. We used Cox proportional hazards model employing each of the four parameters (BSAP, NTX, TRAP5b, and PTH) to evaluate the predictors of CV and all-cause death, respectively. Adjustment was performed for age, sex, diabetic status, and dialysis vintage.

Results: The subjects were aged 67+/-13 with a median vintage of 5.0 (IQR 3.0-8.6) years. During a median follow-up of 6.1 (2.5-6.1) years, 40 patients died, 18 of whom died of CV diseases. When the third quartile (Q3) was set to the reference, the hazard ratios (HRs) for all-cause mortality in the quartiles (Q1-Q4) of the four parameters were (2.0, 2.4, 1, 2.9*) for PTH, (1.3, 0.9, 1, 2.2) for NTX, (1.9, 2.6, 1, 3.5*) for TRAP5b, (0.7, 0.7, 1, 0.5) for BSAP, respectively. The HRs for CV death were (3.0, 2.7, 1, 6.1*) for PTH, (1.2, 1.0, 1, 2.5) for NTX, (2.1, 2.4, 1, 5.0*) for TRAP5b, (0.9, 0.2, 1, 0.6) for BSAP, respectively. (* P<0.05).

Conclusions: The associations between all-cause mortality and PTH or bone resorption markers were J-shaped with highest HR in the highest quartiles (Q4). The association with CV death was also J-shaped but the effect sizes of all these biomarkers were higher for CV mortality than all-cause mortality. This may imply the validity of the concept of chronic kidney disease-mineral bone disorders (MBD).

SA-PO625

Association of Geriatric Nutritional Risk Index and C-Reactive Protein with Cardiovascular Morbidity in End-Stage Renal Disease Patients Who Just Began Hemodialysis Therapy

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Background: Protein-energy wasting (PEW), commonly observed in patients with end-stage renal disease (ESRD), is currently considered to be due to not only nutritional disorder but also inflammatory process. Recently, geriatric nutritional risk index (GNRI) has been developed as a simplified screening tool for PEW. We investigated the association of GNRI and C-reactive protein (CRP) with future cardiovascular (CV) morbidity in ESRD patients who just began hemodialysis (HD) therapy.

Methods: A total of 1548 ESRD patients who stably started HD therapy were examined. The GNRI was calculated from medical records at starting of HD, as follows; GNRI = (14.89 × albumin) + [41.7 × (body weight / body weight at BMI of 22)]. Patients were divided into quartiles according to GNRI levels; quartile 1 (Q1): <85.2, Q2: 85.2-91.2, Q3: 91.2-97.0 and Q4: >97.0. They were also divided into quartiles of CRP; Q1: <0.7mg/l, Q2: 0.7-1.8 mg/l, Q3: 1.8-6.1 mg/l and Q4: >6.1 mg/l, and were followed up to 10-year.

Results: Elevated CRP was independently associated with severe PEW defined as Q1 of GNRI (odds ratio 1.04, 95%CI 1.02-1.06, p=0.0001). At the 10-year follow-up, event-free survival rates for CV morbidity were 37.1%, 43.3%, 54.7% and 58.9% in Q1, Q2, Q3 and Q4 of GNRI, and were 63.6%, 55.3%, 47.8% and 32.2% in Q1, Q2, Q3 and Q4 of CRP, respectively (p<0.0001 in both). In the combined setting of GNRI and CRP, the risk of CV morbidity was 8.27-fold (95%CI 3.47-19.7) higher in Q1 of GNRI with Q4 of CRP compared to Q4 of GNRI with Q1 of CRP. Similar results were also obtained for mortality.

Conclusions: Lower GNRI, might reflect PEW state, and elevated CRP were closely linked, and were interactively associated with increasing risk of CV morbidity and mortality in ESRD patients just beginning HD therapy.

SA-PO626

Mortality Rate and Left Ventricular Systolic Function Associated with Interleukin-18 Level among Hemodialysis Patients

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Background: The impact of persistent inflammation on cardiac function is not well illustrated among patients on maintenance hemodialysis. The study explores the prognostic value of interleukin (IL)-18 as an indicator of inflammation status for LV systolic function among asymptomatic ESRD patients.

Methods: Adult ESRD patients undergoing maintenance hemodialysis were enrolled prospectively. The exclusion criteria included LVEF <50%, severe valvular heart disease, chronic atrial fibrillation, acute decompensated heart failure, and acute coronary syndrome. Participants received 2-dimensional (2D) echocardiography with tissue Doppler imaging (TDI) and myocardial deformation analysis (2D strain analysis). Inflammatory cytokines, i.e. high-sensitivity C-reactive protein (hsCRP), IL-6 and IL-18, were measured.

Results: There were 90 patients who were 64.6±9.5 years of age and 40% of them are male. Patients were stratified into 2 groups by the threshold of an IL-8 value of 667 pg/ml, the median of IL-8 values. Compared to the low IL-18 group, the high IL-18 group showed increased hsCRP levels, but not IL-6 concentration. Using the 2D strain analysis, the high IL-18 group had worse LV systolic function: reduced global LV peak systolic longitudinal strain (GLS; high IL-18 group *vs.* low IL-18 group: -16.9 ± 3.6% *vs*. -19.2 ± 3.7%, p=0.01). In a multivariate regression analysis, GLS was the only independent factor of the IL-18 level (odds ratio= 1.25, p =0.03, 95% C.I. 1.02-1.52). Seventeen (24%) patients died during follow-up.Underthe Kaplan-Meier model, the high IL-18 group had higher all-cause mortality (p =0.05).

Conclusions: The study demonstrated LV systolic dysfunction and worse outcome among ESRD patients with increased IL-18 levels. GLS was the only independent correlate of IL-18 level. Thus, subtle LV systolic dysfunction may contribute to poor prognosis of ESRD patients with an elevated IL-18 level.

SA-PO627

Effect of Circulating Soluble Receptor for AGE (sRAGE) and the Proinflammatory RAGE Ligand (S100A12) on Mortality in Chronic Kidney Disease (CKD) Patients Stages 3-5

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Background: Activation of the soluble receptor for AGE (sRAGE) is implicated in the progression of vascular complications. We investigated if increased circulating concentrations of sRAGE, and the RAGE ligand S100A12, predicted mortality in ESRD patients (pts).

Methods: A total of 145 pts (median age 61 y, 61% males; 36 hemodialysis (HD), 55 peritoneal dialysis (PD) and 54 CKD stages 3-5) were studied. All survivors completed 36 months of follow-up. Clinical characteristics were documented, and markers of mineral metabolism including fibroblast growth factor-23 (FGF-23), inflammation (high-sensitivity C-reactive protein, hsCRP; and interleukin-6, IL-6) were analyzed as well as plasma concentrations of S100A12 and sRAGE. The myocardial systolic (peak systolic velocity, PSV) and diastolic (early diastolic velocities, E') velocities were assessed by Tissue Doppler Imaging.

Results: After 36-months follow-up, mortality rate (Kaplan–Meier analysis) was associated with increased plasma levels of S100A12 (χ^2 = 3.58; P <0.05) and sRAGE(χ^2 = 5.95; P = 0.01). S100A12 was positively associated with IL-6 (r= 0.26, p<0.001), FGF-23 (r= 0.18, p<0.001), hsCRP (r= 0.25, p=0.02), TNFα (r=0.16;p=0.03) and OPG (r= 0.44, p<0.0001). sRAGE was negatively associated with E' (r=0.22, p<0.01). In Cox analysis, only sRAGE (hazard ratio, HR=1.91((95% confidence interval, CI) 1.03-1.61) and IL-6 (HR= 1.59(95% CI 1.20-2.15) were independently associated with increased risk of death.

Conclusions: Increased concentrations of sRAGE and S100A12 were associated with increased all-cause mortality in CKD stages 3-5 pts possibly reflecting increased activation and production of RAGE in the context of accelerated vascular disease.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

SA-PO628

The Significance of Interleukin-33 and Soluble ST2 in Coronary Artery Disease Associated with End-Stage Renal Disease

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Background: End-stage renal disease (ESRD) is associated with coronary artery disease (CAD). The IL-33/ST2 pathway is thought to be protective against atherosclerosis due to its important role in Th2 immune responses and its ability to block the differentiation of macrophage-derived foam cells. No studies investigated the role of IL-33 and soluble ST2 (sST2) in CAD associated with ESRD patients.

Methods: To determine whether IL-33 and/or sST2 levels are altered in CAD/ESRD patients, and whether the level of these markers can be used to predict the risk of CAD development. A total of 46 adult ESRD patients, with and without CAD were recruited. The levels of serum IL-33 and sST2 were quantified, using enzyme-linked immunosorbent assays, and compared between CAD and non-CAD patients. The levels were also correlated with triglycerides (TGL), total cholesterol (Tchol), low-density lipids (LDL) and high density lipids (HDL). Statistical analyses was performed using the unpaired two tailed t-test and a P-value of less than 0.05 was considered significant. For correlation analysis, the pearson test was performed.

Results: In ESRD patients, higher levels of IL-33 and sST2 were associated with optimal levels of TGL (< 1.7) and LDL (< 3.3),and the level of IL-33 was negatively correlated with Tchol(P = 0.02). When diabetic and non-diabetic ESRD patients were compared, a negative correlation between IL-33 and Tchol (P = 0.002) and LDL (P = 0.04) was only found in the non-diabetic ESRD group. There was no difference in the level of IL-33 or sST2 between CAD and non-CAD patients, the level of IL-33 was negatively correlated with Tchol (P = 0.025) and positively correlated with sST2 (P = 0.006) in the non-CAD patients only.

Conclusions: IL-33 may act protective against CAD development in ESRD patients; further tests of more patients are currently underway.

Funding: Government Support - Non-U.S.

SA-PO629

Relationship between Baseline Inflammatory Status and Cardiovascular (CV) Events in Hemodialysis (HD) Patients (Pts) on Peginesatide or Epoetin

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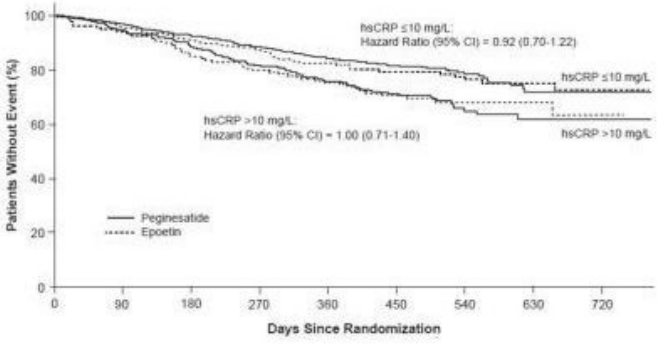
Background: Analysis of a blinded, independently-adjudicated CV composite safety endpoint (CSE) using data pooled from two Phase 3 randomized, active-controlled, open-label trials (EMERALD 1, 2) comparing (2:1) peginesatide once every four weeks vs epoetin 1-3x weekly in HD pts showed similar CSE event rates for peginesatide (22.8%) and epoetin (24.4%; HR for time to first event [95% CI], 0.95 [0.77, 1.17]). High-sensitivity C-reactive protein (hsCRP) is a known predictor of CV outcomes. To evaluate the association between baseline hsCRP and CV events, we performed a prespecified subgroup analysis.

Methods: EMERALD 1 and 2 enrolled HD pts on stable epoetin doses ≥4 weeks. Pooled data from both studies (peginesatide [n=1066]; epoetin [n=542]) were examined for CSEs (all-cause death, stroke, myocardial infarction, and serious adverse events of congestive heart failure, unstable angina, and arrhythmia) by predefined baseline hsCRP level (≤10, >10 mg/L).

Results: Median baseline hsCRP was 5.4 mg/L for peginesatide (n=1051) and 5.8 mg/L for epoetin (n=526). The percent of pts with baseline hsCRP ≤10 and >10 mg/L was similar for peginesatide and epoetin (≤10 mg/L: 68.7% and 67.5%; >10 mg/L: 31.3% and 32.5%, respectively). Compared with the hsCRP ≤10 mg/L subgroup, the CSE event rate in the hsCRP >10 mg/L subgroup was higher for both treatment arms: peginesatide 30.4%, epoetin 29.8% vs peginesatide 19.1%, epoetin 21.1%, respectively. CSE events over time did not differ between treatments in either hsCRP subgroup (Figure).



Conclusions: In HD pts receiving peginesatide or epoetin, baseline hsCRP >10 mg/L was associated with worse CV outcomes. No differences in CV outcomes between treatments were evident in either hsCRP subgroup.

Funding: Pharmaceutical Company Support - Affymax, Inc., and Takeda Pharmaceutical Company Ltd.

SA-PO630

Mortality and Cardiovascular (CV) Events Are Associated with Low Hemoglobin (Hb): Results of Analyses in the MIRCERA Trials Database

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Background: Several randomized controlled trials (RCTs) have raised concerns about potential harm with use of erythropoiesis-stimulating agents (ESAs), especially at high Hb. To explore the relation between Hb and mortality/CV events, we analyzed data from all patients in the MIRCERA RCT program.

Methods: We analyzed the relationship between Hb (last value within 4 weeks before an event; mean over time; deviation from target) and a composite endpoint (fatal adverse event, non-fatal myocardial infarction or stroke) and its components in pooled data from 9 RCTs (3405 CKD patients; 2826 patient exposure years) with both MIRCERA and reference ESAs.

In an analysis of the last value before an event, rate ratios (RRs) and bootstrap 95% BCa confidence intervals (CIs) were calculated from the distribution of all Hb values in comparison to the distribution of Hb values associated with events. Mean Hb over time and deviation from target (baseline ±1 g/dL) were analyzed using a Cox regression model, and hazard ratios (HRs) and 95% CIs were calculated.

Results: Comparison of percentage of Hb values within each Hb category to the percentage of events associated with each category showed the highest RR in the Hb category <10 g/dL and the lowest RR in the category 12 to <13 g/dL (CIs of the estimates excluding unity). Findings for individual components of the composite were similar.

In an analysis of time-adjusted average Hb, HRs >1 with CIs excluding unity were in the lowest Hb categories.

HR >1 with CI excluding unity was found in patients whose Hb fell below the target.

Hb level (g/dL)	< 10	10 to <11	11 to < 12	12 to <13	≥13
RR (95% BCaCI)	**2.44 (1.96-2.98)***	0.96 (0.75-1.19)	0.88 (0.74-1.02)	**0.77 (0.63-0.93)***	0.96 (0.72-1.22)
Hb over time (g/dL)	< 10	10 to <11	11 to < 12	12 to <13	≥13
HR (95% CI)	**4.39 (2.53-7.63)***	**2.10 (1.58-2.78)***	1.00 (ref)	0.85 (0.65-1.13)	1.42 (0.79-2.56)
Deviation from target Hb	Hb decrease by > 1 g/dL		Hb ±1 g/dL of baseline	Hb increase by > 1 g/dL	
HR (95% CI)	**3.00 (2.24-4.01)***		1.00 (ref)	1.43 (0.82-2.52)	

* CIs excluding unity

Conclusions: In multiple analyses from a large program of ESA treatment, risk of mortality and CV events was consistently higher at low Hb and in patients whose Hb fell below target.

Funding: Pharmaceutical Company Support - F. Hoffmann-La Roche Ltd.

SA-PO631

Changes in Key Clinical and Laboratory Parameters Identify Patients at Increased Risk of Future Hospitalization: Results from an International Study

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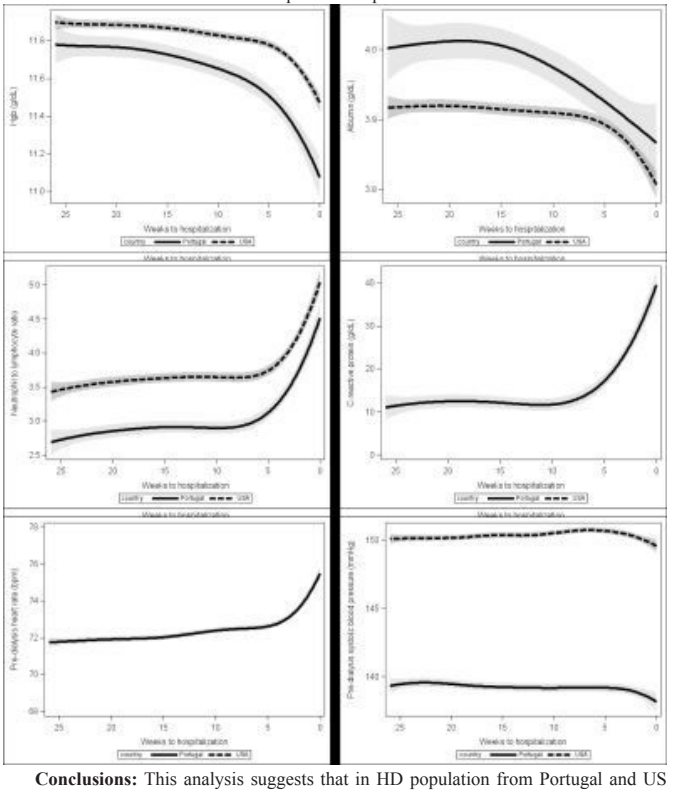
Background: Recent studies in HD indicate a change in several parameters before death. We aimed to understand whether clinical parameters also change before hospitalization in international dialysis patient (pt) population.

Methods: The MONitoring Dialysis Outcomes (MONDO) consortium consists of HD databases from RRI clinics (USA), FMC clinics in Europe, Asia, Latin America and Canada, Maastricht University (Netherlands) and KfH clinics (Germany). Only databases from US and Portugal were queried to obtain data prior to first hospitalization in all HD pts bn 1/2007 and 12/2011. We computed weekly averages for several key parameters up to 26 weeks prior to first hospitalization.

Results: We studied 6458 pts (US, 4789; Portugal, 1669). In both countries hgb and albumin levels started to decline 7 to 12 weeks prior to hospitalization. NLR and CRP started to increase about 7 weeks prior to hospitalization. SBP started to decrease and heart rate started to increase about 5 weeks prior to hospitalization.



Conclusions: This analysis suggests that in HD population from Portugal and US clinical and laboratory indicators change as early as weeks before the hospitalization. Delineating these characteristics may aid the development of alert systems.

SA-PO632

Pre-Procedural Serum Albumin and C-Reactive Protein Levels Predict Long-Term Clinical Outcome after Percutaneous Coronary Intervention in Hemodialysis Patients

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Background: Although percutaneous coronary intervention (PCI) has been widely performed in hemodialysis (HD) patients with coronary artery disease, poor outcome such as higher restenosis or poorer survival after PCI remains clinical problems in such population. On the other hand, lower albumin levels and chronic inflammation state are highly prevalent, and linked to poor cardiovascular outcome in HD patients. We evaluated the possible prognostic values of serum albumin and C-reactive protein (CRP) levels for clinical outcomes after PCI in HD patients.

Methods: A total of 549 HD patients successfully undergoing PCI for stable angina were enrolled. They were followed-up for up to 10 years. Serum albumin and CRP levels were measured prior to PCI. They were divided into the lowest, the middle and the highest tertile (T1, T2 and T3) according to serum albumin and CRP levels, respectively. We analyzed the incidence of major adverse cardiovascular events (MACE) as a composite endpoint of target lesion revascularization (TLR), non-fatal myocardial infarction (MI) and death.

Results: Kaplan-Meier analysis showed that 10-year event-free survival rates from MACE were 14.7%, 21.1%, and 32.1% in T1, T2 and T3 of albumin (p=0.0006), and were 34.2%, 23.9% and 6.5% in T1, T2 and T3 of CRP (p<0.0001), respectively. After adjustment, lower albumin (HR 1.43, 95%CI 1.03-1.99, p=0.045 for T1 vs. T3) and elevated CRP (HR 1.68, 95%CI 1.19-2.36, p=0.011 for T3 vs. T1) were independent predictors for MACE, respectively. In the combined setting of albumin and CRP, the risk of MACE was 3.43-fold (95%CI 1.78-6.59, p=0.0070) higher in the T1 of albumin with T3 of CRP compared to the T3 of albumin with T1 of CRP even after adjustment. Similar results were obtained for TLR and death.

Conclusions: Lower albumin and elevated CRP levels could predict MACE after PCI in HD patients. Furthermore, the combination of these variables is more markedly related to increased MACE than either variable alone.

SA-PO633

The Association of Low Triiodothyronine with Sudden Cardiac Death and Infectious Death in a Cohort of Incident Peritoneal Dialysis Patients

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Background: A direct association between low triodothyronine (T3) and all-cause and cardiovascular mortality have been reported in hemodialysis patients. However, the implication of this syndrome in peritoneal dialysis (PD) patients has not been described even though small studies suggested its association with all-cause mortality. To address this existing gap in knowledge, we examine the association between low T3 and cause-specific and all cause-mortality in a large cohort of incident PD patients.

Methods: After exclusion of 52 patients with thyroidstimulating hormone (TSH) values outside the normal range, 475 clinically and biochemically euthyroid incident PD patients were followed for a median of 46 months. Measurements of thyroid hormones, albumin, CRP, Urea kinetics were performed at baseline. All cause and cause-specific deaths were registered during follow-up. Low T3 was defined as a serum T3 level below the lowest tertile of T3 in the study patients.

Results: Patients with low T3 at baseline were significantly older and have lower BMI, hemoglobin, serum albumin, urine volume and residual renal function (RRF) compared to patients with non-low T3. In multivariable analysis, T3 levels at baseline were independently associated with hemoglobin (p=0.006) and RRF (p=0.03). Patients who died had significantly lower T3 at baseline than those who survived (P<0.001). In Cox analyses, Low T3 at baseline was a significant predictor of all-cause mortality independent of well known risk factors (Adjusted HR 2.05, CI;1.36-3.09) in incident PD patients. Low T3 at baseline was also significantly associated with sudden cardiac death (Adjusted HR 1.97, CI;1.13-3.40) and infectious death (Adjusted HR 3.04, CI;1.49-6.21).

Conclusions: Low T3 at baseline was independent predictor of mortality after adjusting well-known risk factors in incident PD patients. We need to turn attention to low T3 as prognostic marker on mortality, frequently observed in the peritoneal dialysis population.

SA-PO634

Elevated Troponin I Levels and Cardiac-Specific Mortality in Asymptomatic Hemodialysis Patients

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Background: Elevated troponin I (TnI) levels are associated with all-cause mortality in stable hemodialysis patients. However, the relationship to cardiac-specific death has been inconsistent. We hypothesized that elevated TnI levels in chronic stable hemodialysis patients would track best with cardiac-specific mortality.

Methods: Single-centre, cohort study of prevalent hemodialysis patients at a tertiary care hospital. Plasma TnI levels were measured with routine pre-dialysis monthly blood tests in clinically stable patients for 2 consecutive months. Plasma TnI was measured by immunoassay (Beckman Coulter, Inc.) and a value above the laboratory reference range (0.06 µg/L) was considered elevated. The primary outcomes were all-cause and cardiac mortality. Cox proportional hazard models were used to examine the association of TnI with the outcomes of interest.

Results: Of 133 patients (mean age 59.6 ± 27.5; 56% male; 40% diabetic; 40% with prior CAD, 91% hypertensive, and a median dialysis vintage 2.8 years) followed for a median of 1.7 years, there were 38 deaths (58% non-cardiac, 39% cardiac, 3% unknown). A mean TnI >0.06 was found in 27% (N=36) of patients. Elevated TnI was associated with all-cause mortality [HR 2.44 (95% CI 1.16-5.11)] and cardiac death [HR 6.04 (1.77-20.7). After adjusting for age, gender, dialysis vintage, diabetes status, and prior CAD history, only cardiac mortality remained significantly associated with elevated TnI [HR 4.32 (1.16-16.1)].

Conclusions: Elevated TnI was independently associated with cardiac mortality, but not all-cause mortality, in asymptomatic hemodialysis patients. Chronic myocardial injury may explain this increased risk. TnI may reflect recurrent subclinical ischemia-reperfusion injury in otherwise stable patients with underlying atherosclerotic heart disease. Whether patients with an elevated TnI would benefit from targeted investigations and strategies to manage cardiovascular risk remains to be determined.

Funding: Private Foundation Support

SA-PO635

High Sensitivity Troponin as a Predictive Marker for Cardiovascular Events and Mortality in the Stable Dialysis Population

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Background: High Sensitivity Troponin (hsTnT) is a cardiac biomarker used in diagnosing myocardial injury and predicting outcome. Its interpretation and application in the haemodialysis (HD) and peritoneal dialysis (PD) population remains unclear. Our aim is to determine if hsTnT is effective in predicting cardiac events and death in the stable dialysis population.

Methods: A prospective observational study of prevalent nonacute HD and PD patients from July 2011. hsTnT was obtained with routine blood tests and baseline characteristics. Patients were followed up for one year (outcomes; death/new cardiac event). SPSS v.20 used and appropriate tests employed based on data distribution.

Results: 393 patients, 275 HD(70%) and 118 PD(30%) were included. Median hsTnT was 57.5ng/L IQR [36.0,101.1] with no difference between HD and PD patients. There was a statistically significant reduction in hsTnT postHD (p<0.0001). However the amount and rate of change was not clinically significant. Patients who developed a cardiac event had higher hsTnT (102.0ng/L vs 56.0ng/L, p<0.0001) and increased mortality (33.3% vs 6.9%, Likelihood ratio (LHR) 15.8,p<0.0001). This was valid in both HD patients (p=0.003, LHR 8.89 p=0.003) and PD patients (p=0.002, LHR 8.5 p=0.004) for cardiac events and mortality respectively. All cause mortality (but not cardiac mortality) was associated with higher hsTnT (91.0ng/L vs 56.0ng/L p= 0.007). This was only valid in PD patients (p=0.035) and not HD patients (p=0.075). Dividing the study group into quartiles (Q) by hsTnT revealed incremental rise in LHR of developing cardiac events;Q1-Q2 (LHR 10.1,p=0.001), Q1-Q3 (LHR 11.4,p=0.004), Q1-Q4 (LHR 11.5,p=0.001). Mortality similarly increased across quartiles reaching significance between Q1-Q4 (LHR 9.8,p= 0.002).

Conclusions: A higher hsTnT may have a role in predicting cardiac events and death in stable dialysis patients.

SA-PO636

Interaction of B-Type Natriuretic Peptide and Cardiac Troponin T Improves the Prediction of Mortality in Patients on Hemodialysis Kaoru

Kaoru Yasuda,[1] Shoichi Maruyama,[1] Hirotake Kasuga,[2] Yoshinari Yasuda,[1] Tomoki Kosugi,[1] Waichi Sato,[1] Naotake Tsuboi,[1] Yasuhiko Ito,[1] Enyu Imai,[1] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; [2]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Aichi, Japan.*

Background: Patients on hemodialysis (HD) are regarded as the highest risk group for cardiovascular disease (CVD). Thus, the risk stratification for future events is clinically important. Elevated plasma B-type natriuretic peptide (BNP) and serum cardiac troponin T (TnT) levels were highly prevalent in HD patients. We investigated whether combination of BNP and TnT could improve predictive values for mortality in HD patients.

Methods: A total of 516 stable HD patients were enrolled and were followed up for 10 years. They were divided into tertiles according to plasma BNP levels; tertile 1 (T1): <184ng/l, T2: 184-463ng/l and T3: >463ng/l. They were also divided into tertiles of serum TnT levels; T1: <0.05µg/l, T2: 0.05-0.10µg/l and T3: >0.10µg/l, and were followed-up for 10-year.

Results: At the 10-year follow-up, Kaplan-Meier survival rates were 73.8%, 60.6% and 40.1% in T1, T2 and T3 of BNP, and were 82.7%, 54.6% and 36.2% in T1, T2 and T3 of TnT, respectively (p<0.0001 in both). In the combined setting of BNP and TnT, the risk of mortality was 5.04-fold (95%CI 2.61-9.74) higher in T3 of both BNP and TnT compared to T1 of both (p<0.0001). Similar results were also obtained from CVD mortality. C-index also showed that adding both BNP and TnT significantly increased prognostic values for all-cause mortality and CVD mortality.

C-index by combination of BNP and TnT

	All-cause mortality		CVD mortality	
	C-index(95%CI)	P	C-index(95%CI)	P
Basic model	0.745(0.702-0.788)	Ref.	0.692(0.631-0.753)	Ref.
Basic model+BNP	0.785(0.745-0.825)	0.0011	0.712(0.653-0.771)	0.048
Basic model+TnT	0.786(0.746-0.826)	0.0006	0.722(0.664-0.780)	0.028
Basic model+BNP and TnT	0.806(0.767-0.845)	<0.0001	0.733(0.676-0.790)	0.0075

Established risks include male, age, duration of HD, traditional risk factors, BMI, hemoglobin, albumin and CRP.

Conclusions: The combination of BNP and TnT could more accurately predict mortality than either variable alone in C-statistics.

SA-PO637

Prognostic Value of N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) Concentrations in Patients with End Stage Renal Disease Frank Dellanna,[1] Michael Dickenmann,[2] Valeriy Y. Shilo,[3] Werner Kleophas.[1]
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Background: NT-proBNP is a powerful diagnostic tool in chronic kidney disease (CKD) patients not on dialysis receiving erythropoiesis stimulating agents (ESAs). Although the predictive value of NT-proBNP in end stage renal disease (ESRD) patients is not known, a strong association of NT-proBNP levels >10,000 ng/mL with increased mortality has been reported. This analysis explored the prediction of clinical outcomes by NT-proBNP in ESRD patients treated with long-acting continuous erythropoietin receptor activator (C.E.R.A.).

Methods: Data from 10 international multicenter open-label trials including 1597 patients on dialysis who switched to treatment with once-monthly C.E.R.A. were analyzed. Predictive NT-proBNP cut-offs were searched for each parameter leading to an odds ratio (OR) for binary outcomes; pertaining p-values were corrected for optimization bias.

Results: NT-proBNP significantly predicted the safety endpoints cardiac adverse events (AEs; OR 2.9, p<0.0001), vascular disorders (OR 3.6, p=0.022), and hypertensive AEs (OR 3.6, p=0.056). No association to thromboembolic and dialysis-related disorders was observed. Likewise, NT-proBNP predicted the occurrence of serious cardiovascular AEs (OR 2.2, p=0.016). It did not predict ESA maintenance parameters such as Hb stability, Hb fluctuation, or response probability in these 10 trials. However, high NT-proBNP correlated with a higher maintenance dose (OR 1.4, p=0.007), and low dose/Hb effect (OR 1.4, p=0.014).

Conclusions: This pooled analysis confirmed the strong predictive value of baseline NT-proBNP levels for cardiovascular AEs in patients with ESRD. NT-proBNP does not predict thromboembolic effects. Association with Hb maintenance performance parameters in terms of stability was not found, while elevated NT-proBNP was associated with a higher dose requirement and a diminished Hb response.

Funding: Pharmaceutical Company Support - F. Hoffmann-La Roche, Basel, Switzerland

SA-PO638

NT-proBNP in Hemodialyzed Patients: Marker for Overhydration or Cardiovascular Complications? Krzysztof Schwermer, Krzysztof Hoppe, Jolanta Kaczmarek, Peter Sawatiuk, Marcin Czyzewski, Malgorzata Szkudlarek, Bartlomiej Posnik, Dorota Sikorska, Krzysztof Pawlaczyk, Andrzej P. Oko.
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Background: Proper volume control is one of the chief goals in the care for patients undergoing maintenance hemodialysis (HD). An overhydration state (OH) in the inter-dialytic period directly correlates with the risk of cardiovascular complications. NT-proBNP is one of the most popular indicators of heart failure, particularly during overload.

Methods: The study was performed on 136 HD patients (male n=89, female n=47). OH, serum NT-proBNP, cardiac troponin T (cTnT), hemoglobin (Hgb), osteocalcin (Osc), and pre- and post-dialytic blood pressure (BP) were assessed during a 6 month period (at time 0 and 6 months). OH was evaluated using both clinical methods and bioimpedance (BIA - Fresenius Body Composition Monitor). The study group was divided into 3 subgroups according to initial NT-proBNP levels (A: <500pg/ml, n=9; B: 500-5000pg/ml, n=50; C: >5000pg/ml, n=77).

Results: No difference was observed regarding age and gender among subgroups. Most interestingly, at time 0, the clinical determination of OH within subgroups did not show differences compared to the same measurements using BIA (1.13 vs. 2.01 vs. 4.53, p<0.00001), the same relation was observed at 6 months (0.74 vs. 2.08 vs. 4.11, p<0.0001). At time 0 an evident difference was observed in serum Hgb and Osc concentrations (12.1 vs. 11.7 vs. 10.8, p<0.0005; 169.8 vs. 147.7 vs. 211.8, p<0.02 respectively), and after 6 months a continued significance was seen in Osc concentrations (163.2 vs. 171.4 vs. 229.0, p<0.005). cTnT levels differed significantly both at time 0 and after 6 months (0.028 vs. 0.051 vs. 0.115, p<0.00001; 0.037 vs. 0.057 vs. 0.098, p<0.0005). At both time points there were statistical differences in post-HD systolic BP (p<0.05).

Conclusions: The use of NT-proBNP as a measure of intravascular volume is limited due to the intricate relations of pathophysiologic mechanisms. However, according to our results, NT-proBNP seems to be a significant marker of overhydration and heart muscle damage among HD patients.

SA-PO639

Biological Variation of N-Terminal B-Type Natriuretic Peptide (NT-proBNP) in the Stable Dialysis Population Magid Fahim,[1,3] Andrew Hayen,[4] Amanda Coburn,[3] Goce Dimeski,[2,3] Andrea R. Horvath,[4,6,7] David W. Johnson,[1,3] Jonathan C. Craig,[5,6] Scott Campbell,[1,3] Carmel M. Hawley.[1,3]
[1]University of Queensland; [2]Pathology Queensland; [3]Princess Alexandra Hospital; [4]University of New South Wales; [5]Westmead Children's Hospital; [6]University of Sydney; [7]Prince of Wales Hospital, Australia.

Background: Change in NT-proBNP levels correlates with the risk of cardiovascular events on dialysis and may be useful for monitoring cardiac-risk but the biological variation of NT-proBNP in this setting is unknown. The aim of our multi-centre, prospective cohort study was to determine the within- & between-person variation of NT-proBNP in stable dialysis patients.

Methods: 55 prevalent HD and PD patients were assessed 10-times over 5 months, weekly for 5-weeks then monthly for 4-months. Assessments were conducted in a standardised manner between 6-10AM, at the same point in the dialysis cycle. At each visit, subjects underwent clinical review, whole body multifrequency bioimpedance, EKG and NT-proBNP testing. Plasma was frozen within an hour of collection, batched and analysed in duplicate in a single run. Subjects were deemed unstable if they underwent a change in cardiac medication, dialysis prescription, ischaemic symptomatology, extracellular volume > 1L, new arrhythmia or hospital admission between visits. Data from the intervals preceding and following such events were excluded from analysis. Analytical (CV_A), between-person (CV_G) and within-person biological variation (CV_I) were calculated. The difference in serial levels needed to detect a clinically significant change with 90% certainty (RCV) & index of individuality (IOI) were derived.

Results: 41 subjects, 147 weekly & 142 monthly intervals were analysed. Median NT-proBNP= 1974 pg/mL (IQR 711 - 3967 pg/mL). CV_A= 1.9%, CV_G= 189%, weekly CV_I= 27% & monthly CV_I= 35%. The 90% weekly & monthly RCV= -46% – +84% & -54% – +120% respectively. IOI= 0.14.

Conclusions: A large change in serial NT-proBNP levels is needed to confidently exclude change due to analytical & biological variation alone. The best strategy for applying NT-proBNP in dialysis is relative change monitoring rather than comparing results to reference intervals.

Funding: Pharmaceutical Company Support - Fresenius Medical Care, Roche Diagnostics, Private Foundation Support, Government Support - Non-U.S.

SA-PO640

Elevation in Endothelial Progenitor Cells Are Associated with Worse Survival in African-American Hemodialysis Patients Francesca Cardarelli,[1] Qi Long,[3] Monnie Wasse.[1]
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Background: Circulating endothelial progenitor cells (EPC) have been correlated to endothelial function and cardiovascular disease risk. In this study we wanted to evaluate the association between circulating EPC and mortality in patients receiving hemodialysis.

Methods: We measured circulating EPC in 58 African American patients undergoing regular hemodialysis at one of the Emory University-affiliated Davita dialysis centers. We collected all-cause mortality data at a mean follow-up of 1182.53 days (SD=442.3). Cox proportional hazards models were used to analyze the association between EPC and patient survival time.

Results: At follow up 38% of patients died and 62% survived. Higher circulating EPC were associated with increased mortality. After controlling for gender, age, diabetes mellitus, dialysis vintage, and erythropoetin weekly dose, the association remained significant.

Estimated hazard ratio (95% confidence interval) for one unit increase in each EPC type, after controlling for gender, age, diabetes, dialysis vintage, and erythropoetin weekly dose. The outcome variable is the survival time.

EPC type	HR (95% CI)	P value
CD34+	1.74 (1.16,2.59)	0.007
CD34+/CD45 med	1.76 (1.17,2.64)	0.007
CD34+/CD133+	2.19 (1.22,3.94)	0.009
CD34+/CD133+/ CD45 med	2.20 (1.21,4.02)	0.010
CD34+/VEGF2R+/CD45 med	1.00 (0.34,2.97)	0.999
CD34+/CD133+/ VEGF2R+/ CD45 med	0.84 (0.15,4.85)	0.851

Conclusions: In a limited sample of African-American dialysis patients, elevated EPC were associated with increased mortality. Unlike in cardiovascular studies, where elevated EPC are associated with better survival, it is possible that in dialysis patients there is a stimulation of EPC, with subsequent increased circulating level, but the EPC are dysfunctional and are therefore associated with increased mortality. More studies are needed to examine possible correlation between EPC function and outcome.

Funding: Pharmaceutical Company Support - Davita Clinical Research Grant

SA-PO641

Circulating Endothelial Cells Predict Long-Term Cardiovascular Events and Survival in Hemodialysis Patients Rajesh Mohandas,[1] Mehmet Koc,[2] Xuerong Wen,[1] Mark S. Segal.[1] *[1]Division of Nephrology, Hypertension & Transplantation, University of Florida, Gainesville, FL; [2]Division of Nephrology, Marmara University School of Medicine, Istanbul, Turkey.*

Background: Circulating endothelial cells (CEC) are thought to be markers of endothelial injury and dysfunction. We hypothesized that the numbers of CEC may provide a novel means for predicting long-term cardiovascular events and survival in hemodialysis subjects who are known to be at markedly increased risk for cardiovascular disease.

Methods: Twenty-nine hemodialysis patients underwent a single enumeration of their CEC number. Patients were separated into two groups, a low CEC group (≤19 CEC/mL) and high CEC group (>19 CEC/ml). We retrospectively analyzed the survival and incidence of adverse cardiovascular events in these two populations. Survival was compared using Kaplan-Meier curves and the risk of adverse cardiac events using Multivariate Poisson Regression analysis.

Results: Baseline characteristics in both groups were not significantly different. In over 9 years of follow up, only 2 of the patients in the low CEC group had died of cardiovascular causes, as compared with 6 in the high CEC group. When controlled for diabetes and pre-existing CAD each 10 CEC/ml increase was associated with an 18% increased risk of adverse cardiovascular events during follow-up (P= 0.05).

Conclusions: In this hemodialysis population, a single measurement of CEC was an independent predictor of long term future adverse cardiovascular events and survival. CEC number at baseline had better predictive value than diabetes, hyperphosphatemia, low albumin and other known risk factors. CEC may be a novel and relatively inexpensive biomarker to assess endothelial health and cardiovascular risk. Further studies in a larger cohort are needed to investigate the utility of CEC in predicting cardiovascular isk.

SA-PO642

Effect of Membrane Permeability on Cardiovascular Risk Factors and B2m Plasma Levels in Patients on Long Hemodialysis: A Randomized Cross-Over Trial Charles Chazot,[1] Judith Kirchgessner,[2] Tieu Duyen Chung,[2] Cyril Vo-van,[1] Jean-marc Hurot,[1] Christie Lorriaux,[1] Guillaume Jean,[1] Daniele Marcelli.[2] *[1]NephroCare Tassin-Charcot, Sainte Foy Les Lyon, Rhone Alpes, France; [2]Fresenius Medical Care, Bad Homburg, Germany.*

Background: Mortality of HD patients is mainly of cardiovascular origin related to traditional and non traditional risk factors. Also, long-hour dialysis and Beta2-microglobulin (B2m) are related to patient survival. We have evaluated the effects of both session length and membrane flux on CV risk factors and B2m plasma levels.

Methods: One hundred and sixty-eight patients were included in a randomized cross-over trial. Patients were split in 2 groups (5-6 hours - Group 1 - and 7-8 hours -Group 2). The 2 groups were assigned randomly and consequently to a low-flux and a high-flux 9-months period (Period 1 and 2).

Results: 155 patients started the study after the run-in phase. Age, gender, BMI and vintage were not different according to the flux periods. 83 patients ended the whole study. No difference was found regarding Lpa, homocystein, LDL and HDL cholesterol between groups and periods of treatment. B2M was in average 39.0±11.8 and 46.5±10 mg/L respectively in Group 1 and 2 at the end of the low flux period. Independently of treatment time and sequence, B2m was significantly lower at the end of the high flux phase (respectively at 26.9±7.8 and 27.8±4.3 mg/L in Group 1 and 2, p<0.0001). Phosphate and the CaxP product were significantly lower at the end of the high-flux period (respectively p=0.045 and p=0.009), but nPCR decreased under high flux filter (p=0.0025). Triglycerides levels presented a trend to decrease under high flux filter (p=0.067). There was a statistical trend for more deaths during low flux treatment (13 versus 5, p=0.071).

Conclusions: Hence there was no influence of high-flux filters on several traditional CV risk factors in a HD patients treated with extensive treatment time. Treatment time is not enough to reach the targets of B2m and high flux filters are necessary to optimize the middle molecules clearance.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

SA-PO643

Impact of Frequent Hemoglobin Measurement and ESA Dose Titration on Hemoglobin Stability in Dialysis Lynda A. Szczech,[1] William G. Harrison,[2] Alex Yang.[3] *[1]PPD, RTP, NC; [2]ZS Associates, San Francisco, CA; [3]Affymax, Inc., Palo Alto, CA.*

Background: Recent changes in the clinical landscape in ESRD have highlighted a lack of evidence surrounding the frequency of Hb monitoring and ESA dose changes. Using patient (pt) data from a large US dialysis provider, we evaluated the relationship between frequency of Hb measurement, epoetin dose titrations and use, and Hb stability.

Methods: Dialysis facilities were split into cohorts based on 1) Hb measurement frequency (<2, ≥2 to <3, or ≥3 measurements/pt-month), or 2) titration frequency (<0.8, ≥0.8 to <1.2, or ≥1.2 titrations/pt-month). Proportion of dose changes across cohorts (up/down titration or dose hold) was compared.

Results: Across 1630 dialysis facilities (N=141,631 pts) in 2010, 5.8%, 58.5%, and 35.7% averaged <2, 2 to <3, and ≥3 Hb measurements/pt-month, respectively. Demographics, comorbidities, hospital admission/duration, albumin, and ferritin were similar across all groups. Proportion of changes in ESA dose and Hb stability is described.

	Hb Measurements				Titration Frequency		
	<2	≥2 to <3	≥3		<0.8	≥0.8 to <1.2	≥1.2
No. facilities, n	94	954	582		170	1030	430
Mean Hb measurements/pt-month	1.8	2.5	3.6		NA	NA	NA
Mean dose titrations/pt-month[1]	0.8	1.0	1.2		0.6	1.0	1.4
- Up, %	42	44	41		42	41	43
- Down, %	40	39	41		41	41	35
- Holds[2], %	18	17	18		17	18	23
Mean epoetin use, U/pt-month	58K	69K	72K		54K	69K	75K
Hb stability (pts w/Hb 10-12 g/dL), %	59.9	59.9	61.8		61.6	61.0	62.1
Hb variability (mean SD), g/dL	0.72	0.72	0.73		0.73	0.73	0.71

[1] ≥10% change in consecutive doses; [2] ≥3 consecutive sessions with zero dose

Evaluation of a subset of facilities (n=19 facilities, 1461 pts) with the least variability in twice-monthly Hb measurements per pt (avg 2 Hb measurements/pt-month; SD ±0.5) demonstrated a similar trend in association of titration frequency with epoetin use, with no effect on Hb stability or variability.

Conclusions: Across facilities, increased Hb measurement frequency was associated with greater frequency of ESA dose titration, which was, in turn, associated with higher rate of dose holds. Increased frequency of Hb measurements and titrations were both associated with greater epoetin use, but neither was associated with a greater percentage of pts in goal Hb range.

Funding: Pharmaceutical Company Support - Affymax, Inc. and Takeda Pharmaceuticals

SA-PO644

Time-Dependent Modeling of Mortality Benefits of Statins in Dialysis Patients Theresa I. Shireman,[1] Milind A. Phadnis,[1] James B. Wetmore,[1] Sally K. Rigler,[1] Qingjiang Hou,[1] John Spertus,[2] Jonathan D. Mahnken.[1] *[1]University of Kansas Medical Center, Kansas City, KS; [2]Mid-America Heart Institute, Kansas City, MO.*

Background: Cardiovascular disease is a leading cause of death in patients with end-stage renal disease (ESRD). The role of HMG-CoA reductase inhibitors (statins) in the prevention of cardiovascular events in ESRD patients on dialysis is uncertain. Despite the completion of three randomized clinical trials (RCTs) in dialysis patients, the efficacy of statins in reducing mortality or cardiovascular events has not been definitively established. This was unexpected, given two earlier observational studies that had suggested a survival benefit of statins in this population.

Methods: We developed a novel time-dependent model of statin exposure to determine whether they confer survival benefits in dually eligible Medicare-Medicaid ESRD patients. We linked Medicaid pharmacy claims with data from the USRDS core files and Medicare institutional and physician/supplier service claims and selected a cohort of ESRD patients initiating dialysis between 1/1/2000 to 9/30/2005. We followed the cohort until their date of death, loss of eligibility, or 12/31/2005. Medication exposure was defined with three time-dependent covariates: current weekly drug availability (per days supply from claims), cumulative percentage of weeks with a medication available (compliance), and cumulative number of weeks where drug availability switched (available vs not available). Models were adjusted for demographic, functional status, and comorbidity covariates. We used a Cox proportion hazards model with time-dependent covariates including a three-way interaction between the medication exposure measures.

Results: The final sample included 76,372 ESRD patients. Statin compliance and switches were not significant in the final models. The adjusted hazard ratio (AHR) for time-dependent statin use = 0.547 (99% confidence interval, 0.526-0.569).

Conclusions: In this cohort study of new dialysis patients, weekly statin availability was associated with a substantial decline (45.3%) in mortality, suggesting that current statin use is more important than cumulative use.

Funding: NIDDK Support

SA-PO645

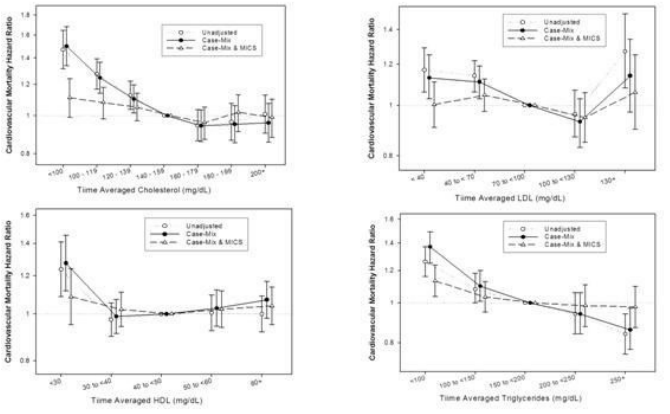
Hypercholesterolemia and Hypertriglyceridemia Are Associated with Better Survival in Hemodialysis Patients Hamid Moradi,[1] Elani Streja,[2] Hamid M. Said,[1] Madeleine V. Pahl,[1] Nosratola D. Vaziri,[1] Kamyar Kalantar-Zadeh.[2,3] *[1]Medicine, University of California, Irvine, Orange, CA; [2]Harold Simmons Center, LA BioMed, Harbor-UCLA, Torrance, CA; [3]Medicine, University of California, Los Angeles, Los Angeles, CA.*

Background: Lipid levels are inversely associated with mortality in maintenance hemodialysis (MHD) patients, a group at high risk for cardiovascular (CV) mortality. We hypothesized that this association would persist despite adjustment for malnutrition-inflammation complex (MIC), body mass index (BMI), serum calcium and phosphorus.

Methods: A 2 year cohort (July 2004 through June 2006) of 32,278 MHD patients was studied in the United States from DaVita dialysis clinics where lipid profiles were measured in at least 50% of all outpatients during a given calendar quarter. Cox proportional hazard models were adjusted for case mix and surrogates of MIC, BMI, calcium and phophorus.

Results: The 32,278 patients were 60 ± 15 years old and included 46% women. Increases in both total cholesterol and triglycerides were associated with an improvement

in survival. LDL level <100 > 130 mg/dL were associated with increased CV mortality therefore indicating a U shaped association. While serum HDL cholesterol <30 mg/dL was associated with an increased risk of death, HDL level greater than 60 mg/dL did not have any clear association with better survival.



Conclusions: The inverse association of total cholesterol and triglyceride levels with mortality in MHD patients persists despite adjustment for MIC, BMI, plasma calcium and phosphorus. While low LDL levels are associated with an increased risk of CV mortality, high LDL levels (>130 mg/dL) also increased risk of death in MHD patients. Furthermore, low HDL levels are associated with an increased risk of death however higher plasma HDL levels (>60 mg/dL) are not associated with improved survival.

Funding: NIDDK Support, Other NIH Support - R01 DK078106; K24 DK091419; F32 DK082130

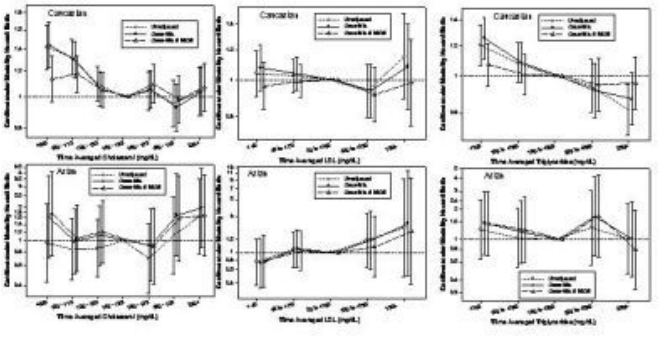
SA-PO646

Impact of Race and Ethnicity on the Association of Lipids with Mortality in Dialysis Patients Hamid Moradi,[1] Elani Streja,[2] Madeleine V. Pahl,[1] Keith C. Norris,[3] Nosratola D. Vaziri,[1] Kamyar Kalantar-Zadeh.[2,3] *[1]Medicine, University of California, Irvine; [2]Harold Simmons Center, Harbor-UCLA; [3]Medicine, University of California, Los Angeles.*

Background: Lipid levels are inversely associated with cardiovascular mortality (CV) in maintenance hemodialysis (MHD) patients. We examined the association of lipids with cardiovascular (CV) mortality in African American (AA), Hispanic and Asian MHD patients.

Methods: A 2 year cohort (July 2004 - June 2006) of 32,278 MHD patients was studied in U.S. DaVita dialysis clinics where lipid profiles were measured in at least 50% of all patients during a given calendar quarter. Cox proportional hazard models were adjusted for case mix and surrogates of malnutrition-inflammation complex.

Results: The 32,278 patients were 60±15 years old with 46% women. Increases in both total cholesterol (TC) and triglyceride levels were associated with improved survival in all groups except Asians in whom TC <140 and >200 mg/dL was associated with increased CV mortality. LDL level <70 and > 130 mg/dL was associated with increased mortality in all groups except Asians in whom CV mortality increased with higher LDL levels. In Caucasian patients, serum HDL cholesterol <30 mg/dL was associated with increased CV mortality, while in AA and Hispanic patients there was no clear association with reduced HDL. In Asians both HDL <30 and > 60 mg/dL were associated with increased CV mortality.



Conclusions: There is an inverse association between total cholesterol and triglyceride levels and CV mortality in all MHD patients except for Asians. Also, elevated LDL cholesterol is associated with increased CV mortality in the Asian MHD population. While low HDL levels are associated with an increased risk of death in the Caucasian and Asian population, higher HDL levels (>60 mg/dL) are not associated with improved survival regardless of race or ethnicity.

Funding: NIDDK Support, Other NIH Support - R01 DK078106; K24 DK091419; F32 DK082130

SA-PO647

Long Chain n-3 Fatty Acids and Risk of Sudden Cardiac Death in Patients Starting Hemodialysis Allon N. Friedman,[1] Zhangsheng Yu,[1] Rebeka Tabbey,[1] Cheryl Denski,[1] Hector Tamez,[2] Julia Beth Wenger,[2] Ravi I. Thadhani,[2] Yong Li,[3] Bruce A. Watkins.[3] *[1]Indiana University School of Medicine, Indianapolis, IN; [2]Massachusetts General Hospital, Boston, MA; [3]University of Connecticutt, Storrs, CT.*

Background: Sudden cardiac death is the single leading cause of mortality in hemodialysis patients and occurs at an exceptionally high rate. No treatment for this problem currently exists. Experimental and clinical evidence suggests that long chain n-3 fatty acids are protective against sudden cardiac death. It is unclear whether such a protective relationship exists in the hemodialysis population, and if so, whether it applies to the early high-risk period after initiating hemodialysis.

Methods: We conducted a nested case-control study using 100 patients who died of sudden cardiac death during the first year of hemodialysis and 300 patients who survived that period. Groups were frequency matched by age, sex, and race. Baseline serum phospholipid fatty acids were measured in stored samples by gas chromatography.

Results: Long chain n-3 fatty acids were inversely related to the risk of sudden cardiac death even after adjusting for relevant co-morbid conditions, biochemical values, and dietary fats (P<0.003). As compared to the lowest quartile of long chain n-3 fatty acids, the 1-year hazard of sudden cardiac death was 50% (95% confidence interval: 0.28 to 0.90%), 70% (0.15 to 0.62%), and 71% (0.13 to 0.69%)) lower for the third, second, and highest quartiles, respectively. Moreover, the inverse relationship between long chain n-3 fatty acids and sudden cardiac death was maintained even during the highest-risk first few months after starting hemodialysis.

Conclusions: Blood long chain n-3 fatty acid levels, which are easily modifiable by dietary supplementation, are strongly associated with a reduced risk of sudden cardiac death in hemodialysis patients throughout the first year after starting hemodialysis.

SA-PO648

Uremic Dyslipidemia and Arterial Stiffness in Hemodialysis Patients R. Filipowicz,[1] Alfred K. Cheung,[1,2] T. Alp Ikizler,[3] Y. Zhang,[1] Tom Greene,[1] T. S. Bjordahl,[1] A. N. Habib,[1] G. Wei,[1] S. Beddhu.[1,2] *[1]U of Utah, Salt Lake City, UT; [2]SLCVHA, Salt Lake City, UT; [3]Vanderbilt Univ, Nashville, TN.*

Background: Uremic dyslipidemia is characterized by ↓ plasma high density-lipoprotein cholesterol (HDL-C) and increased triglycerides (TG) levels, which are potentially atherogenic and increased arterial disease in hemodialysis (HD) patients (pts). We examined the associations of fasting plasma TG, HDL-C, total cholesterol (TC) and low density-lipoprotein cholesterol (LDL-C) levels with arterial stiffness as assessed by aortic pulse wave velocity (PWV) in the ongoing Protein Intake, CVD and Nutrition In CKD stage V (PICNIC) Study.

Methods: Fasting lipids were measured in non-HD day plasma samples. PWV was measured using carotid and femoral pulses with a Sphygmacor PVX. Baseline and 12-month follow-up PWV were related to baseline lipids in separate linear regression models. Average of baseline, 6 and 12 month PWV values were also related to baseline lipids using generalized estimating equations.

Results: 116 participants with baseline lipids collected were included in the analysis. Mean age was 52 ± 16 yrs; 41% were women, 20% were Black, 48% had DM, 27% had CAD, 23% had PVD, and 22% had CHF. Mean baseline TG, HDL-C,TC and LDL-C levels were 144 ± 75, 43 ± 15, 149 ± 35 and 77 ± 30 mg/dL, respectively. Mean PWV were 9.8 ± 3.4, 10.3 ± 3.8 and 10.2 ± 3.4 m/s at baseline, 6 and 12 months, respectively.

Associations * of fasting plasma lipid levels with aortic PWV

	Baseline PWV (m/s)	12 mo PWV (m/s)	Baseline, 6 and 12 mo PWV (m/s)
	β (95%, CI)	β (95%, CI)	β (95%, CI)
Model 1			
For each ↑ SD TC (mg/dL)	-0.10 (-0.65, 0.46)	0.05 (-0.81, 0.91)	-0.13 (-0.56, 0.30)
Model 2			
For each ↑ SD HDL-C (mg/dL)	-0.30 (-1.06, 0.45)	-0.25 (-1.26, -0.76)	-0.17 (-0.72, 0.38)
For each ↑ SD TG (mg/dL)	-0.02 (-0.67, 0.63)	0.03(-0.97, 1.03)	-0.08 (-0.41, 0.57)
For each ↑ SD LDL-C (mg/dL)	-0.03 (-0.60, 0.53)	0.12 (-0.79, 1.03)	- 0.12 (-0.56, 0.31)

*Adjusted for demographics, vascular access, vintage, smoking, DM, BP, serum Ca and P and study site.

Conclusions: In 12 month this study, uremic dyslipidemia was not appear to be associated with arterial stiffness. Further studies are warranted to determine whether Rx of uremic dyslipidemia will impact CV events or mortality in HD pts.

Funding: NIDDK Support

SA-PO649

Regulation of CD36 Expression on Monocytes of Dyslipidemic Hemodialysis Patients Alicja E. Grzegorzewska,[1] Leszek Niepolski,[2] Jan Sikora,[3] Pawel P. Jagodzinski,[4] Dominik Pajzderski.[1] *[1]Dpt. of Nephrology, Poznan University of Medical Sciences (UMP); [2]B.Braun Avitum Dialysis Centre, Nowy Tomysl; [3]Dpt. of Clinical Immunology, UMP; [4]Dpt. of Biochemistry and Molecular Biology, UMP, Poland.*

Background: Role of CD36 receptor in the development of atherosclerosis and atorvastatin effect on CD36 are not clear. Our aim was to check how amelioration of serum lipids with therapeutic lifestyle changes (TLC) or atorvastatin influences monocyte CD36 expression in dyslipidemic hemodialysis (HD) patients.

Methods: HD patients (n=49), dyslipidemic according to KDOQI (2003) and not taking lipid lowering drugs, were enrolled into the prospective study started with 4 week education in low-fat diet and physical activity. In 34 persons, still dyslipidemic after 21 TLC weeks, atorvastatin (10 mg/d) was added for 4 weeks. Patients' evaluation was done after 4, 8, 18, 25, and 29 weeks from the study beginning. CD36 expression was measured as mean fluorescence intensity by flow cytometric analysis, serum salusin-alpha - by RIA.

Results: LDL-Ch decreased (141±56 vs 116±27 mg/dL, p=0.009), HDL-Ch (38.6±9.9 vs 44.2±10.3 mg/dL, p=0.001) and CD36 expression increased (1,387±899 vs 2,064±1,256, p<0.001) during TLC. CD36 correlated with total Ch (r= -0.359, p=0.020), LDL-Ch (r= -0.354, p=0.022), non-HDL-Ch (r= -0.345, p=0.025), anti-atherogenic salusin-alpha (r=0.392, p=0.007), arm circumference (r= -0.312, p=0.044), and triceps skinfold (TSF) thickness (r= -0.477, p=0.001). Patients with CD36 expression >1,000 showed higher chance for total Ch <200 mg/dL and non-HDL-Ch <160 mg/dL (for both OR 10.6, 95% CI 2.51–45.0). Patients with TSF thickness <12 mm had higher probability for CD36 >1,000 (OR 5.20, 95% CI 1.37–19.8). CD36 expression, lower than in controls before atorvastatin (1,152±631 vs 1,650±840, p=0.015), increased with the drug-induced amelioration in serum lipids to 1,365±870, p=0.028.

Conclusions: In dyslipidemic HD patients CD36 expression upregulates in response to changes in serum cholesterols induced by TLC or atorvastatin, and depends on fat depot. Our results support the concept designated monocyte CD36 expression as an anti-atherogenic factor. ClinicalTrials.gov ID NCT01448174.

SA-PO650

Whole-Genome Expression Levels Associated with Cardiovascular Disease in End-Stage Renal Disease Patients Martin Schalling,[1] Karin Luttropp,[1] Bengt Lindholm,[2] Peter Stenvinkel,[2] Olivier Devuyst,[3] Louise Nordfors.[1,2] *[1]Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [2]Renal Medicine and Baxter Novum, Karolinska Institutet, Stockholm, Sweden; [3]Division of Nephrology, UCL Medical School, Brussels, Belgium.*

Background: Patients (pts) with end-stage renal disease (ESRD) are at increased risk of developing cardiovascular disease (CVD). Although the exact mechanisms responsible are unknown, persistent inflammation and oxidative stress may increase the vascular risk. Increased apoptosis in smooth muscle cells (SMC) has been associated with vascular calcification (VC), and the dialysis process itself may induce SMC apoptosis.

Methods: The material consisted of iliac artery (IA) and abdominal subcutaneous fat (ASCF) from 10 pts with ESRD. The pts were divided into matched pairs of 5 pts suffering from CVD (coronary artery calcification, CAC) and 5 pts without known CVD. Pts were matched for age, gender, diabetes, treatment modality, hypertension and previous history of CVD. Samples were collected during renal transplantation. Degree of VC was defined by Agatston score.RNA was prepared using Bio-Rad RNA preparation kit for fibrous tissue. Microarray analysis was performed using Illumina® HumanWG-6 v3.0 arrays. Statistical analysis was performed using limma and samr packages in BioConductor® software.

Results: The statistical analysis resulted in 378 and 252 significant genes for ASCF and IA respectively. Changes in ASCF ranged from 4.6-fold up-regulated, to 2.7-fold down regulated in patients with CVD compared to those without CVD. In IA, fold changes ranged from 2.4-fold upregulated, to 2.4-fold down-regulated in pts with CVD. False discovery ratios (FDR) were below 13 % in ASCF, and below 10 % in IA. The expression of genes involved in pathways regulating apoptosis was up-regulated in ESRD pts with CVD; these candidate genes are now the focus of further studies.

Conclusions: We identified genes whose expression levels differed between ESRD patients with and without VC. These genes may indicate pathways and molecular mechanisms associated with CVD in ESRD. Interestingly, our results indicate that the process of apoptosis is important in the development of VC in ESRD.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corporation, Government Support - Non-U.S.

SA-PO651

The Impact of Different Comorbidity for Survival Analyses in Dialysis Patients: A Long-Term Population-Based Study Wei-Chih Kan,[1] Chih-Chiang Chien,[1] Yi-hua Lu.[1] *[1]Nephrology, Chi-Mei Medical Center, Tainan, Taiwan; [2]Chi-Mei Medical Center; [3]Chi-Mei Medical Center.*

Background: The global prevalence and incidence of end-stage renal disease has been rapid-increasing annually. However, the risk factors for mortality and long-term survival between hemodialysis (HD) and peritoneal dialysis (PD) appear to be inconclusive. This study aims to investigate the risk factors that impact on mortality among dialysis patients.

Methods: We conducted an observational cohort study to investigate the impact of underlying comorbidities on long-term outcome of dialysis patients in Taiwan. Several risk factors possibly affecting mortality were analyzed. Survival rates of different groups were also compared.

Results: In all, 5683 HD patients and 302 PD patients were enrolled for analysis. Patients who selected PD tended to be younger and less likely to have baseline comobidities compared to those on HD. The percentage of diabetes mellitus (DM) is much lower in PD patients (36.8%) than in HD patients (53.1%). The prevalence of hypertension (HTN) was 75.5% in HD patients and 64.2% in PD patients. In HD patients, mortality risk increased with older age, DM, HF, CAD, stroke, peripheral vascular disease (PVD) and liver cirrhosis (LC). In PD patients, mortality risk increased with older age and DM. The cumulative survival rate among HD patients was 95.7% at one year, 66.2% at five years and 44.3% at nine years; that among PD patients was 95.8% at one year, 66% at five years and 50.4% at nine years. There was no statistically difference between HD and PD patients (log-rank: P = 0.398). However, the survival became different after stratification by DM/non-DM. For non-DM patients, the survival rate was higher on PD than HD group; in contrary, higher survival rate was found in HD group among DM patients (log-rank: P<0.001).

Conclusions: Our findings show that each comorbidity had different impact on long-term survival among dialysis patients. Older age and DM were strong predictors for mortality whether in HD or PD patients. There was no statistically difference in long-term survival between HD and PD patients from our study.

SA-PO652

Trade-Offs in Claims-Based Comorbidity Identification with Early Loss of ESRD Patients: Adaptation of the Liu Comorbidity Index James B. Wetmore,[1] Sally K. Rigler,[4] Jonathan D. Mahnken,[2] Qingjiang Hou,[2] Theresa I. Shireman.[3] *[1]Division of Nephrology, University of Kansas Medical Center, Kansas City, KS; [2]Department of Biostatistics, University of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine & Public Health, University of Kansas Medical Center, Kansas City, KS; [4]Medicine, University of Kansas Medical Center, Kansas City, KS.*

Background: Claims-based comorbidity indices are widely used in observational studies. In 2010, Liu et al. published a new comorbidity index for use in USRDS studies which combines information from the baseline Medical Evidence Form (CMS 2728) with Medicare claims incurred during six months of chronic dialysis. Given the high mortality rate in incident dialysis patients, Liu's six month claim capture period, while resulting in high sensitivity for comorbidities, could lead to a survivor bias as well as smaller samples with reduced generalizability.

Methods: We examined potential trade-offs between the original 180-day versus a 90-day claim capture period. We selected persons initiating dialysis with continuous Medicare and Medicaid eligibility during the first 90 days. We computed a Liu original index using 180 days of Medicare claims (starting with 90-day survivors, and continuing on to day 270) and compared it to a modified Liu Index which used only the initial 90 days after initiating dialysis with continuous Medicare coverage.

Results: There were 70,114 persons with complete data. The original Liu Index could be computed for only 52,937, demonstrating a 25% reduction in sample size resulting from the stipulation that patients survive a full 270 days. The Modified Liu score using only the first 90 days of dialysis was 7.3 ± 4.0, but when limited to only those who would have survived the full 270 days required of the original Liu Index, the mean score on the modified index was 6.4 ± 3.6 points. This mean difference of 0.98 ± 2.8 points represents only a 12% reduction from the original score.

Conclusions: This modified approach allows inclusion of 25% more subjects and permits initiation of outcome measure ascertainment from day 91, thereby reducing survivor bias.

Funding: NIDDK Support, Private Foundation Support, Clinical Revenue Support

SA-PO653

The Impact of Advancing Age on Coronary Disease Prevalence and Associated Mortality in End Stage Kidney Disease Liam F. Casserly,[1] Cornelius John Cronin,[1] Ailish Hannigan,[2] Austin G. Stack.[1,2] *[1]Nephrology and Medicine, University Hospital Limerick, Ireland; [2]Graduate Entry Medical School, University of Limerick, Ireland.*

Background: Coronary disease and advancing age are both independent risk factors for death on dialysis. The impact of age on coronary disease prevalence and associated mortality is unclear although it is postulated that the mortality risks increase with advancing age. The purpose of this study was to determine the contribution of age to disease prevalence and associated mortality in national cohort.

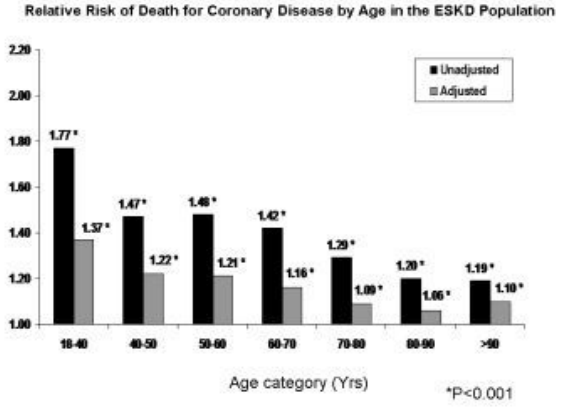
Methods: National incidence data on all new dialysis patients (N=823, 753), between May 1995 and December 2004 and followed until October 2006, were analysed from the U.S. Renal Data System. Age specific prevalence and 1-year mortality rates were determined for each of 7 age groups (18-40, 40-50, 50-60, 60-70, 70-80, 80-90 and > 90) and relative hazard ratios (RR) were calculated for those with and without coronary disease using multivariable Cox regression. All analyses were adjusted for 21 demographic, clinical, socioeconomic and laboratory indicators.

Results:

Prevalence of Coronary Disease by Age Group and Age-specific Death Rates

Age Group Category (Years)	18-40	40-50	50-60	60-70	70-80	80-90	90
Coronary disease (%) *	1.2	4.6	14.2	27.3	35.5	16.3	1.0
Age specific death rates (per 1000 person years)							
Coronary disease (n=222,221) *	158	178	229	338	496	701	1074
No coronary disease (n=601,532) *	89	121	155	237	383	582	893

*P<0.001



The hazard of death for coronary disease was modelled separately in each age category *P<0.001.

Conclusions: The prevalence of coronary disease increased with advancing age up to the seventh decade and declined thereafter. Mortality impact was greatest for younger compared to older patients. Whether this reflects age differences in rates of disease detection and/or severity; patient compliance or use of interventional strategies needs further evaluation.

SA-PO654

Impaired Chronotropic Response Limits Exercise Tolerance in Peritoneal Dialysis and Renal Transplant Patients Maggie Kam Man Ma,[1] David Chung Wah Siu,[1] Wai Kei Lo,[2] Desmond Y.H. Yap,[1] Daniel Tak Mao Chan.[1] *[1]Department of Medicine, Queen Mary Hospital, Hong Kong; [2]Department of Medicine, Tung Wah Hospital, Hong Kong.*

Background: Chronotropic incompetence (CI), defined as blunted increase in heart rate (HR) during exercise, has been accepted as an independent predictor of cardiovascular mortality. Little is known about its prevalence in peritoneal dialysis (PD) and renal transplant (RT) patients. This study aimed to investigate the prevalence of CI and its relationships with exercise intolerance in these patients.

Methods: Ambulatory PD and RT patients were recruited for treadmill stress test using modified Bruce protocol. Exercise duration and HR response was recorded. Maximum age-predicted HR was defined as (220-age). Heart rate reserve was defined as difference between peak heart rate achieved during exercise and resting heart rate. Chronotropic response (CR) to exercise was evaluated by the percentage of heart rate reserve ([peak HR-resting HR/maximal age predicted HR-resting HR]x100%).

Results: 86 patients (mean age 51.72±8.78 years, male 53.5%) were included in this study. RT patients had significantly longer exercise duration than PD patients (10.01±3.59 min Vs 6.26±3.16 min, P=0.000). Both PD and RT patients demonstrated blunted HR response to exercise. PD patients had significantly lower maximum heart rate than RT patients (75.34±13.67% Vs 86.57±23.56% of age-predicted HR respectively, p=0.009). CR was also significantly lower in PD than RT patients (58.64±24.11% Vs 79.73±43.54%, p=0.008). CI (i.e. CR<80%) was noted in 28 (64%) PD and 15 (36%) RT patients respectively.

Exercise Testing Measurements in PD and RT Patients

	RT(n=42)	PD(n=44)	P
Baseline HR	70.90±10.18	71.48±14.41	0.83
Peak HR	146.76±36.73	128.93±23.98	0.009
Rest sBP	137.62±20.38	143.86±20.54	0.163
Rest dBP	85.50±10.15	73.00±11.57	0.000
Treadmill time (min)	10.01±3.59	6.26±3.16	0.000
% maximum age predicted HR	86.57±23.56	75.34±13.67	0.009
HR reserve	75.85±34.34	57.09±26.84	0.007
CR (%)	79.73±43.54	58.64±24.11	0.008
CI(CR<80%), n(%)	15(36)	28(64)	0.01

Conclusions: Chronotropic incompetence was more prevalent in PD than RT patients and such difference might account for the discrepancy in their exercise tolerance.

Funding: Government Support - Non-U.S.

SA-PO655

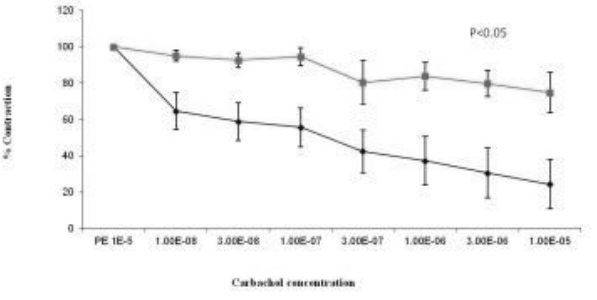
Phosphate Has Deleterious Effects on Vascular Function Kathryn K. Stevens,[1,2] Rajan K. Patel,[1,2] Sarah Kettlewell,[1] Marc J. Clancy,[2] Christian Delles,[1] Godfrey L. Smith,[1] Alan G. Jardine.[1,2] *[1]ICAMS, University of Glasgow, Glasgow, United Kingdom; [2]Renal Unit, Western Infirmary, Glasgow, United Kingdom.*

Background: Elevated serum phosphate is a risk factor for cardiovascular disease. Whether this is a direct effect of phosphate is unknown. We examined the effects of phosphate in human resistance vessels and human umbilical vein endothelial cells (HUVECs).

Methods: Adipose tissue was removed from CKD patients undergoing live donor transplantation and their normal donors. Resistance vessels were dissected and incubated in normal (1.18mM) or high phosphate concentration (2.5mM) solution for 16 hours, then mounted on a myograph. Vasoconstrictor response to phenylepherine (PE) and vasorelaxation responses to carbachol and sodium nitroprusside (SNP) were measured. Concentration-response curves were constructed. HUVECs were grown in normal (0.5mM) and high (3mM) phosphate medium. We measured superoxide production (electron paramagnetic resonance spectroscopy), eNOS and nitrotyrosine expression (Western blot) and intracellular calcium concentration (epifluorescence).

Results: Vessels from patients with and without CKD incubated in high phosphate relax less well to carbachol (p<0.05).

Figure 1: Relaxation of human vessels from patients with CKD to increasing concentrations of carbachol. Vessels incubated in high (pink) and normal (blue) phosphate concentration media.



Vessels from patients without CKD also relax less well to SNP (p<0.05). HUVECs grown in high phosphate produce more superoxide (p<0.05), have reduced expression of eNOS and increased expression of nitrotyrosine. Calcium concentration was similar between HUVECs in high and normal phosphate.

Conclusions: Elevated phosphate decreases endothelium dependent vasodilatation in patients with and without CKD. This may be a marker of endothelial dysfunction, (reduced eNOS and increased nitrotyrosine expression and increased suproxide production in HUVECs). Endothelial independent relaxation is also impaired in patients without CKD. These experiments support the notion that phosphate has direct effects in uremia.

SA-PO656

Directly Toxic Effect of Phosphate on the Vasculature Can Be Reversed by Zaprinast Kathryn K. Stevens,[1,2] Rajan K. Patel,[1,2] Sarah Kettlewell,[1] Christian Delles,[1] Godfrey L. Smith,[1] Alan G. Jardine.[1,2] *[1]ICAMS, University of Glasgow, Glasgow, United Kingdom; [2]Renal Unit, Western Infirmary, Glasgow, United Kingdom.*

Background: Hyperphosphataemia is a risk factor for cardiovascular disease; whether this is a direct effect of phosphate is unknown. This study looks at the effect of phosphate on vessels and smooth muscle cells (SMCs) and endothelial cells (ECs).

Methods: Mesenteric resistance vessels were dissected from WKY rats and incubated in physiological saline solution (PSS) with normal (1.18mM) or high phosphate concentration (2.5mM) for 16 hours. Vessels were mounted on a myograph and vasoconstriction response to phenylepherine (PE) and vasorelaxation response to carbachol and sodium nitroprusside (SNP) were measured ± phosphodiesterase 5 inhibitor (zaprinast). Concentration-response curves were constructed for PE, carbachol and SNP ±zaprinast. Area under the curve was calculated. SMCs and ECs were grown in normal (0.5mM) and high (3mM) phosphate medium. Western blot was performed for eNOS and protein kinase G (PKG) expression. Calcium concentration was measured by epifluorescence.

Results: Vessels incubated in high phosphate relax less well to carbachol and SNP (max relaxation 95.7% vs 61.6% and 85% vs 43%, $p<0.05$). The addition of zaprinast improved relaxation response to carbachol and SNP in both high and normal phosphate PSS. This was most marked in vessels in high phosphate (improvement of 22.9% (carbachol) $p<0.05$) resulting in no difference in relaxation response to carbachol and SNP between vessels in normal and high phosphate. Expression of eNOS was unchanged in SMCs but reduced in ECs grown in high phosphate. PKG expression was lower in SMCs grown in high phosphate. Calcium concentration was not significantly different between either SMCs or ECs grown in high and normal phosphate.

Conclusions: Elevated phosphate impairs endothelium dependent and independent vasorelaxation. The mechanism may relate to reduced available nitric oxide (reduced eNOS) and reduced cGMP production/guanylate cyclase expression (reduced PKG) in vascular SMCs. Zaprinast reduces cGMP breakdown and corrects the impaired vasorelaxation. Elevated phosphate, one aspect of the uraemic environment, has direct effects on vascular cells and function.

SA-PO657

GYY4137 Inhibit the Transdifferentiation of Human Vascular Smooth Muscle Cell Induced by Phosphorus

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Background: Vascular calcification is one of the major pathogenic factors for cardiovascular complication in patients with chronic kidney diseases (CKD). Accumulated evidences suggested that vessel smooth muscle cells (VSMC) to osteoblast-like cells transdifferentiation (VOT) plays a crucial role in promoting vascular calcification. Therefore, Targeting VSMA VOT may ameliorate vascular calcification and reduce the incidence of cardiovascular event in CKD patients. H_2S is now recognized as a gas playing an important role in ameliorating vascular calcification induced by vitamin D_3 plus nicotine in rats. However, the clinical usage of H_2S is limited due to its gas feature. that GYY4137 was recently reported to releases H_2S slowly both *in vitro* and *in vivo,* which may mimic the biological effect of H_2S. Thus, the protective role of GYY4137 on inhibiting VSMC VOT and vascular calcification was investigate in this study.

Methods: The human VSMCs were treated with phosphorus (3.0mM, 4.5mM), bmM, 100mM, 150ma and Cbfa-1, known as marker of VSMCs and osteoblasts respectively, bone morphogenetic protein (BMP)-2 and OC were tested by Western Blotting. mRNA expression of Cbfa-1, BMP-2, osteoponin (OPN) and collagen I were detected by RT-PCR.

Results: 1) Both mRNA and protein expressions of Cbfa-1, BMP-2 and OC were markedly induced in VSMC treated with phosphorus or b-glycerophosphoric acid, while protein level of SM-22a was diminished. 2) GYY4137 inhibited the expression of BMP-2 and OC induced by phosphorus, but exhibited no effect on Cbfa-1 expression. The down-regulation of SM-22a induced by phosphorus could be partially restored by GYY4137.

Conclusions: Our study confirmed that both inorganic phosphorus and donor of phosphorus stimulated VSMC transdifferentiating to osteoblast-like cells, represented by *de novo* synthesis markers of osteoblast and losing markers of VSMC. GYY4137, a donor of H_2S, provided beneficial effects against VSMC phenotypic modulation induced by phosphorus but the precise mechanisms need to be explored in depth.

Funding: Government Support - Non-U.S.

SA-PO658

Magnesium Reduces Vascular Calcification in Uremic Rats

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Background: Phosphate (P) binder containing magnesium (Mg) are used to reduce P absorption in chronic renal disease patients. However, the role of Mg in secondary hyperparathyroidism and vascular calcification (VC) is not completely known.

Methods: To evaluate Mg effects on VC, Wistar rats 5/6 nephrectomized (Nx) were fed with a high P diet (1.2%) and received calcitriol (CTR) i.p. on alternate days. Rats were randomly assigned to groups with different Mg diet content: 0.1%, 0.3%, 0.6% and 0.9%. Control healthy rats were fed normal P diet (0.6%) and 0.1% Mg. Urine was collected on 3 consecutive days to determine the excretion of P (UP/UCr). After 15 days animals were euthanized and plasma levels of ionized Ca (selective electrode), Mg, P, aortic Ca content (spectrophotometry) and PTH (ELISA) were measured.

Results: The results expressed as mean ± standard error are shown in the table.

	Plasma Mg (mg/dL)	Plasma iCa (mM/L)	Plasma P (mg/dL)	PTH (pg/mL)	Aortic Ca (mg/g tissue)	UP/UCr
Control	2.14 ± 0.06	1.17 ± 0.01	6.5 ± 0.29	21.36 ± 6.78	0.42 ± 0.03	0.68 ± 0.06
Nx+CTR+P+Mg 0.1%	2.66 ± 0.23	1.07 ± 0.03 (a)	10.65 ± 1.53 (a)	355.29± 146.42 (a)	33.39 ± 7.57 (a)	8.27 ± 2.38 (a)
Nx+CTR+P+Mg 0.3%	2.97 ± 0.19	1.19 ± 0.02 (a, b)	7.99 ± 0.81 (b)	267.62 ± 129.828 (a)	2.44 ± 0.80 (b)	6.69 ± 0.85 (a)
Nx+CTR+P+Mg 0.6%	3.31 ± 0.36 (a)	1.13 ± 0.03 (a)	7.95 ± 0.92 (b)	79.20 ± 45.65 (b)	1.07 ± 0.41 (b)	2.61 ± 0.66 (b)
Nx+CTR+P+Mg 0.9%	4.43 ± 0.36 (a, b)	1.11 ± 0.03 (a)	7.93 ± 0.55 (b)	22.64 ± 17.32 (b)	0.60 ± 0.17 (b)	1.97 ± 0.30 (b)

a: $P<0.05$ vs. control. b: $P<0.05$ vs. Nx+CTR+P+Mg 0.1%

Increased plasma Mg levels were accompanied by decreased levels of plasma P, PTH, aortic Ca and UP/Ucr.

Conclusions: An increase in diet Mg content decreased P, plasma PTH and also significantly reduced VC in uremic rats.

Funding: Pharmaceutical Company Support - Fresenius Medical Care Deutschland GmbH

SA-PO659

Association of Proton-Pump Inhibitor Use and Serum Magnesium

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Background: Despite the staggering number of U.S. prescriptions for proton-pump inhibitors (PPIs) and their numerous known adverse effects, only case reports that describe the association of hypomagnesemia and PPI use have been published. The FDA released a statement in March 2011 outlining the risk of low magnesium levels for long-term PPI users.

Methods: The MIMIC-II database, consisting of ICU patients from a large, urban academic medical center, was used. Pre-admission medications were determined using a Natural Language Processing (NLP) algorithm on discharge summaries. Sequential multivariate linear and logistic regressions were developed to assess whether acid-suppressive medications were related to serum magnesium.

Results: 2629 (23%) patients were on a PPI prior to admission. PPI use was associated with a 0.012 (±0.004) mg/dL lower serum magnesium concentration compared to users of no acid-suppressive medications ($p=0.003$). Among concurrent diuretic users, PPI use was associated with a 55% increase in the odds of hypomagnesemia (95% CI, 1.21-1.98) and a 0.030 (±0.008) mg/dL lower serum magnesium concentration ($p<0.001$). There was no association between PPI use and magnesium concentrations in those not on diuretics.

Table 1. Association between acid suppression therapy and serum magnesium concentration

	Serum magnesium concentration (mg/dL)				
	Proton-pump inhibitors		H2-receptor antagonists		No acid-suppressive medications
	β-coefficient ± SE	p-value	β-coefficient ± SE	p-value	Reference
Unadjusted model	0.007±0.005	0.12	0.006±0.009	0.38	-
Model I*	-0.012±0.004	0.009	-0.006±0.008	0.44	-
Model II†	-0.012±0.004	0.003	-0.011±0.008	0.17	-
Stratified analysis‡					
Diuretic use (n=2170)	-0.020±0.006	<0.001	-0.017±0.014	0.68	-
No diuretic use (n=6231)	-0.002±0.005	0.68	-0.007±0.010	0.45	-

Abbreviations: SE, standard error.
Reference category is those on no acid-suppressive medications. Beta coefficients ± standard errors and p-values are provided for each variable.

*Model I includes age, gender, ethnicity, and renal function.
†Model II includes all variables in Model I and the addition of systolic blood pressure, heart rate, temperature, serum calcium, serum phosphate, serum glucose, hematocrit, diuretic use, and 38 comorbidities.
‡Given a significant multiplicative interaction term between PPI and diuretic use ($p=0.02$), Model II was stratified by diuretic exposure.

Table 2. Association between acid suppression therapy and hypomagnesemia*

	Proton-pump inhibitors			H2-receptor antagonists			No acid-suppressive medications	
	Cases, n (%)†	Odds ratio (95% CI)	p-value	Cases, n (%)†	Odds ratio (95% CI)	p-value	Cases, n (%)†	Ref.
Study population‡	463 (15.3)	1.09 (0.95-1.25)	0.21	82 (14.82)	0.98 (0.74-1.33)	0.85	1359 (16.6)	-
Diuretic use (n=3173)	168 (15.7)	1.55 (1.21-1.98)	<0.001	12 (5.9)	0.56 (0.28-0.90)	0.02	208(10.7)	-
No diuretic use (n=6231)	243 (15.1)	0.91 (0.77-1.08)	0.29	78 (18.4)	1.18 (0.87-1.57)	0.29	1151(18.4)	-

Abbreviations: Ref, reference category.
Reference category is those on no acid-suppressive medications.

*Hypomagnesemia was defined as a serum magnesium level <1.6 mg/dL.
‡A multiplicative interaction term between PPI and diuretic use was significant ($p<0.001$). A multiplicative interaction term between H2RA and diuretic use was also significant ($p=0.002$).

Conclusions: This is the first study to provide an analysis of PPI use and serum magnesium concentrations in a large population. Magnesium levels on admission were lower among PPI users, especially those concurrently on diuretics. The mechanism of PPI-induced hypomagnesemia may be related to compensatory upregulation of renal tubular magnesium reclamation in response to a negative total body balance. This net loss of magnesium could also explain the increased risk of hip fractures in PPI users described in the literature. Our findings verify initial case reports of hypomagnesemia in long-term PPI users and confirm another adverse effect of PPI use.

SA-PO660

The Relationship between Proton Pump Inhibitor Use and Serum Magnesium Concentration among Hemodialysis Patients

Sharon Nessim,[1] Catalina Hernandez,[3] Mark L. Lipman,[1] Ahsan Alam.[2] *[1]Division of Nephrology, Jewish General Hospital, McGill University, Montreal, QC, Canada; [2]Division of Nephrology, Royal Victoria Hospital, McGill University, Montreal, QC, Canada; [3]McGill University, Montreal, QC, Canada.*

Background: It is known that serum magnesium (Mg) concentration is inversely related to vascular calcification and hyperparathyroidism among patients with end-stage renal disease (ESRD). In recent years, there have been several case reports of hypomagnesemia due to the use of proton-pump inhibitors (PPI), attributed to inappropriate gastrointestinal (GI) Mg loss. We hypothesized that the tendency to GI Mg loss among PPI users is more common than is currently reported. Since patients on dialysis have little to no renal Mg loss to alter Mg balance, they are an interesting population in whom to study the relationship between PPI use and serum Mg levels.

Methods: Using a single-center cross-sectional design, we studied 155 prevalent hemodialysis (HD) patients. Serum Mg concentration for each patient was determined based on the mean of 3 consecutive serum Mg levels drawn at 6 week intervals. PPI use was documented. The relationship between PPI use and serum Mg was determined in unadjusted analyses, as well as after adjustment for age, gender, cause of ESRD, diabetes, time on HD and dialysate Mg concentration.

Results: 55% of patients were on PPIs at the time of the study. The majority of patients (62%) used a dialysate Mg (in mmol/L) of 0.5, and the remainder (38%) used a dialysate Mg of 0.375. Serum Mg levels were significantly lower among PPI users vs. non-users (0.93

vs. 1.02 mmol/L, p<0.0001). In addition, more PPI users vs. non-users had a Mg level < 1 mmol/L (79% vs. 43%) and a Mg level < 0.8 mmol/L (16% vs. 4%). On multivariate analysis, PPI use remained an independent predictor of a lower serum Mg concentration (p<0.0001).

Conclusions: Among HD patients, PPI users have lower serum Mg levels as compared with non-users, supporting the hypothesis that PPI-induced GI Mg loss is more common than is currently recognized. Further research is required to determine whether the magnitude of change in Mg levels among PPI users with ESRD is associated with adverse outcomes.

Funding: Clinical Revenue Support

SA-PO661

Postprandial Mineral Metabolism in Hemodialysis Patients Mark Reinhard,[1] Jan Frystyk,[2] Bente Jespersen,[1] Per R. Ivarsen.[1] *[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus N, Denmark; [2]Department of Endocrinology and Internal Medicine & Medical Research Laboratories, Department of Clinical Medicine, Aarhus University Hospital, Aarhus C, Denmark.*

Background: Abnormal mineral metabolism in hemodialysis (HD) patients is associated with cardiovascular death. The aim was to characterize postprandial mineral ion handling in HD patients compared with healthy volunteers.

Methods: On a non-HD day, 12 HD patients (M/F: 8/4; median age 61 years, range 39-74) and 12 healthy individually matched volunteers consumed a weight-adjusted standardized meal containing between 292-372 mg of calcium and between 441-818 mg of phosphorus. Following an overnight fast, blood samples were collected at baseline and until 9 h after the meal. Urine samples were collected from healthy volunteers to measure fractional excretion of calcium and phosphate.

Results: After the standardized meal, HD patients and healthy volunteers differed significantly in terms of changes in plasma phosphate (p=0.001) and parathyroid hormone (PTH) (p=0.02) levels, but not in ionized calcium levels (p=0.17). Plasma phosphate, ionized calcium, and PTH levels did not significantly deviate from baseline at any time in the healthy volunteers; however, all three parameters showed significant postprandial changes in the HD patients. Phosphate levels decreased at 60 to 240 min (maximum of 9% at 120 min, p<0.001) and then rose slightly by a maximum of 4% before returning to baseline at 540 min. Similarly, ionized calcium decreased slightly after the meal (maximum of 2% at 240 min, p=0.003). PTH levels increased at 240 min to the end of the study (maximum of 14% at 540 min, p<0.001). The healthy volunteers demonstrated an immediate increase in fractional excretion of calcium (maximum of 154% at 120 min, p<0.001) and of phosphate (maximum of 47% at 360 min, p<0.001).

Conclusions: After a dietary calcium and phosphorus load, both plasma phosphate and ionized calcium levels decreased, while PTH levels increased in HD patients. The mechanisms governing the extrarenal phosphate clearance in HD patients are unclear, but may involve both skeletal and extraskeletal soft tissue deposition.

Funding: Private Foundation Support

SA-PO662

A New Equation to Predict 24-Hour Urinary Phosphorus Cassianne Robinson-Cohen,[1] Geoffrey A. Block,[2] Martha S. Persky,[2] Glenn M. Chertow,[3] Bryan R. Kestenbaum.[1] *[1]Kidney Research Institute, University of Washington; [2]Denver Nephrology; [3]Nephrology, Stanford University Medical Center.*

Background: Traditional management of stage III-V chronic kidney disease includes dietary phosphate restriction and/or the use of phosphorus binders. Monitoring dietary phosphorus is key for assessing compliance to and effectiveness of therapeutic interventions. The 24-hour urine collection is the gold standard method for estimating dietary phosphorus; however, it is cumbersome and prone to timing errors. The precision and accuracy of spot urine phosphorus (s-UP) measurement have never been thoroughly investigated.

Methods: We studied 143 participants from the Phosphate Normalization Trial, a randomized trial of phosphorus binders *versus* placebo among patients with eGFR 20-45 mL/min/1.73m^2. We evaluated multiple simultaneous spot and 24-hour urine phosphorus (24h-UP) measurements per participant. We used forward stepwise linear regression to model 24h-UP as a function of s-UP plus the following covariates: spot urine creatinine, treatment assignment, age, sex, height, weight, time of urine collection, and time since last meal. We validated our results using leave-one-out cross-validation.

Results: In addition to s-UP, the following covariates improved the 24hUP model: spot urine creatinine, age, gender and weight. Treatment assignment did not significantly add to the predicted 24hUP concentration. Compared to a model containing only the s-UP to creatinine ratio (r^2=0.12, RMSE=0.45, p<0.001), the novel regression equation correlated more strongly with 24hUP (validation r^2=0.49, RMSE=0.35, p<0.001).

Linear regression coefficients of significant predictors of ln(24-hour urine phosphorus excretion)

Variable	Estimate	Standard error
Intercept	6.39	0.16
ln(Spot Urine Phosphorus, µg/dl)	0.47	0.06
(Spot urine creatinine, µg/dl)^-2	0.02	0.01
sqrt(Spot urine creatinine, µg/dl)	-0.68	0.12
Male gender	0.31	0.05
Age, years	-0.007	0.002
Body weight, kg	0.005	0.001

Conclusions: We describe a new equation that incorporates s-UP and creatinine, age, sex, and weight to predict 24hUP. The equation is more accurate than the s-UP to spot creatinine ratio and provides a simple method for estimating this important characteristic in CKD patients.

Funding: Pharmaceutical Company Support - Funding for the investigator initiated parent study (Phosphate Normalization Trial) was provided by Shire, Inc., Fresenius NA, Genzyme, Inc, Denver Nephrologists, PC, Novartis, Inc. and Davita, Inc. Funding Entities Had No Role in the Design, Conduct, Analysis, Interpretation or Preparation of the Manuscript. Each Funding Entity Was Permitted to Review the Manuscript for Verification That No Proprietary or Confidential Information Was Included

SA-PO663

VS-501: A Novel Phosphate Binder Derived from Natural Plant Polymer J. Ruth Wu-Wong, Yung-wu Chen, Jerry Wessale. *Vidasym, Chicago, IL.*

Background: Inadequate control of serum phosphate in chronic kidney disease (CKD) can lead to pathologies of clinical importance including cardiovascular complications, renal osteodystrophy, and increased mortality. On-market phosphate binders are mainly used in dialysis patients but effectiveness is limited by safety concerns and low compliance due to high pill burden and gastrointestinal (GI) discomfort.

Methods: VS-501 is a chemically-modified, plant-derived natural polymer that absorbs phosphate in the GI tract, and doesn't have many of the shortcomings of the current phosphate binders.

Results: When male Sprague–Dawley rats on normal diet containing 0.7% phosphorus and 1% calcium were treated with 3% (by dry weight) VS-501 or Sevelamer in the food for 6 days, serum phosphate was not significantly altered, but urinary phosphate levels decreased from 135 ± 22 µmol/24 hr (no treatment) to 7.2 ± 0.6 and 5.5 ± 1.4 µmol/24 hr in the VS-501 and Sevelamer treatment groups, respectively. VS-501 had no effect on serum Ca or PTH, while Sevelamer increased serum Ca from 10.0 ± 0.1 mg/dL to 12.3 ± 0.5 mg/dL and reduced serum PTH from 138 ± 15 pg/ml to 35 ± 12 pg/ml. Increasing dietary phosphate (KH_2PO_4 at 0.67% and K_2HPO_4 at 0.33% by dry weight in food) led to an increase in serum phosphate from 3.33 ± 0.11 mmol/L (the pre-dosing level) to 4.67 ± 0.65 mmol/L in the vehicle-treated group, but no significant changes in serum phosphate were observed in rats treated with 1%, 5% or 10% (by dry weight) VS-501 in the food for 5 days. Water and food consumptions were not significantly changed (vs. vehicle alone) in the VS-501 treatment groups. Furthermore, in vitro studies demonstrated that, upon adding 5 ml water to 0.1 g of VS-501 or Sevelamer (dry powder), the volume of Sevelamer increased from 0.1 cm^3 to 2 cm^3 (20-fold) within 20 min at 37°C, while the volume of VS-501 increased from 0.09 cm^3 to 0.25 cm^3 (3-fold) after 6 hours at 37°C.

Conclusions: The results strongly support the conclusion that VS-501 effectively controls serum phosphate levels via absorbing phosphate in GI with minimal swelling volume and without affecting serum Ca and PTH levels, indicating a compelling product profile for clinical development as a prescription drug or medical food.

SA-PO664

Dietary Phosphate Restriction Prevents Renal Anemia in Chronic Kidney Disease Model Rats Mari Nakao, Hironori Yamamoto, Otoki Nakahashi, Mina Kozai, Yutaka Taketani, Eiji Takeda. *Department of Clinical Nutrition, University of Tokushima, Institute of Health Biosciences, Tokushima, Japan.*

Background: The restriction of dietary phosphate (Pi) has been known as a critical nutritional therapy to prevent hyperphosphatemia and development of cardiovascular disease in chronic kidney disease (CKD) patients. However, the effects of dietary Pi restriction on the renal anemia in CKD is still unclear. In order to understand the beneficial effects of multiple associated with improvement of hyperphosphatemia in CKD patients, we examined whether the dietary Pi restriction affects on the development of renal anemia in CKD model rats.

Methods: 8 weeks aged male wister rats were received either a AIN93G based diet containing adenine, or a control diet for about 5 weeks. Then, CKD rats were divided into two groups, the control diet group (CKD-CP; Pi:1.06%) and restricted Pi diet group (CKD-RP; Pi:0.2%). was administered for 7 or 16 days. For histology using paraffin sections of liver tissues, ferric iron was detected by berlin blue staining. Gene expression analysis were performed using western blots and real-time PCR methods.

Results: In blood biochemical data, CKD-CP rats exhibited anemia with hyperphosphatemia, hypocalcemia, high plasma levels of parathyroid hormone (PTH) and FGF-23, low plasma levels of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) when compared with control rats. Interestingly, we observed that dietary Pi restriction for 7 days prevented renal anemia in the levels of hemoglobin, red blood cells, iron and erythropoietin in blood. In the other hand, plasma levels of creatinine, BUN, $1,25(OH)_2D$ and hepcidin had no difference between CKD-CP and CKD-RP. In addition, dietary Pi restriction for 7 days also reduced the CKD-induced hepatic iron accumulation. Moreover, we found dietary Pi restriction also could regulate the expression of iron metabolism related genes including hepcidin, DMT-1 and Cybrd1 in liver and intestine of CKD rats.

Conclusions: These results suggest that the dietary Pi restriction in CKD rats improves the metabolic abnormalities of Pi, calcium, PTH and FGF-23 but also prevent renal anemia through the maintain of plasma erythropoietin levels.

Funding: Government Support - Non-U.S.

SA-PO665

Self-Reported Non-Adherence with Phosphate Binder Prescription Is Associated with High Serum Phosphorus and PTH: Results from the DOPPS

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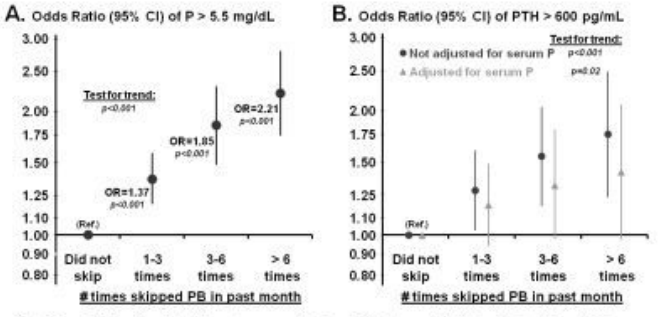
Background: Most patients on maintenance hemodialysis are prescribed phosphate binders (PB). However, practitioners report that patient adherence to PB regimens is less than optimal. The impact of non-adherence on serum phosphorus (P) and PTH levels has not been well reported.

Methods: Self-reported non-adherence to PB prescription (# of times a patient reported skipping PBs in the previous month) was obtained from 5,089 participants in DOPPS phase 4 (2009-2011). Labs, including P and PTH, and PB data were also collected that month. Adjusted logistic regression was used to model high P (> 5.5 mg/dL) and PTH (>600 pg/mL).

Results: PB prescription varied across DOPPS countries (median = 5 PB pills/day, IQR: 3-9). 55% of patients reported taking all prescribed PBs in the past month; 27% skipped 1-3 times; 10% skipped 3-6 times; 8% skipped >6 times. Non-adherence was lowest among patients prescribed 1-2 PB pills/day, but did not vary among patients prescribed more pills (even ≥12/day). Non-adherence showed a strong linear association with elevated serum P and PTH levels (Figure), both with and without adjustment for PB pills/day.

Conclusions: Non-adherence to PB regimens is common and is associated with high P and PTH levels. As high P and PTH may be risk factors for adverse clinical outcomes including cardiovascular events, interventions and patient support activities aimed at improving adherence to PB therapies should be investigated.



Funding: Pharmaceutical Company Support - The DOPPS Is Administered by Arbor Research Collaborative for Health and Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), Abbott (Since 2009), Baxter (Since 2011), and Vifor Fresenius Renal Pharma (Since 2011), without Restrictions on Publications

SA-PO666

Reasons for Phosphate Binder Discontinuation Vary by Binder Type

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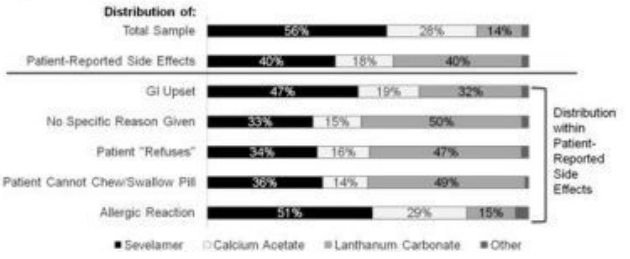
Background: Non-adherence with phosphate binders (PB) is common in ESRD patients (pts) which contributes to elevated phosphorus levels. Pill burden, side-effects, complex regimens, and cost all contribute to the non-adherence seen. With recent reports of PB non-adherence in ESRD pts at 62%, there is an unmet need for new PBs that can overcome these barriers to adherence. We assessed the reasons for PB discontinuation to better understand the drivers of non-adherence for particular PBs.

Methods: PB prescriptions and reasons for discontinuation, either switched or ceased medication entirely, were analyzed for Medicare pts receiving hemodialysis treatment at a large dialysis organization from their electronic medical records. Two independent coders classified each reason for discontinuation from 7/1/2009–6/30/2011 into 6 categories which were subsequently divided into 40 subcategories. The percent of pts on each type of PB was calculated within each category.

Results: 30,933 reasons were classified for this study; 50.1% of records cited the pt discontinued the PB but contained no additional information. "Lab Results", cited for 27.4% of the reasons for discontinuation, and "Patient-Reported Side Effects", cited for 10.8% of the reasons, were the 2nd and 3rd largest categories. While pts on lanthanum carbonate accounted for 14% of the total sample, they comprised 40% of the "Patient-Reported Side Effects" category and were similarly over-represented in 4 of the 5 subcategories.

Figure. Distribution of Binders Within Reasons for Discontinuation



Conclusions: The high percentage of reported side effects (>10%) resulting in discontinuation identifies an unmet need for improved PBs. Although a disproportionate percentage of pts prescribed lanthanum reported side effects, further work is needed to identify the relative tolerability of PBs and potential explanations, such as prescription bias.

Funding: Pharmaceutical Company Support - vifor Pharms

SA-PO667

Fermagate Is a More Potent Binder Compared to Sevelamer HCl in ESRD Patients on Hemodialysis

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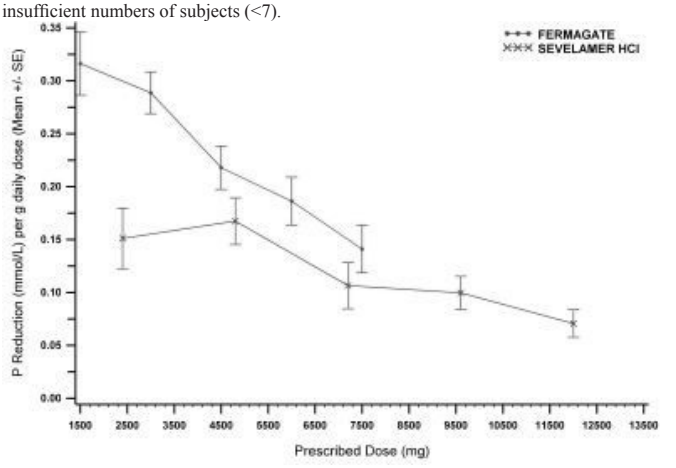
Background: Fermagate (F) is a magnesium iron hydroxy carbonate phosphate (P) binder. In vitro studies have shown that F binds 2.5x more P /g than sevelamer HCl (S). In this phase 3 study, we compared F to S in the management of hyperphosphatemia in hemodialysis (HD) patients.

Methods: After screening, subjects completed a 2-week course of current P binder therapy, followed by a 1- to 4-week washout period, and then were randomized to receive F (1.5 or 3.0 g/d) or S (2.4 or 4.8 g/d). The starting doses were titrated up to 9.0 g/d (F) or 12.8 g/d (S) to reduce serum P to the target range of 0.8-1.78 mM (2.5-5.5 mg/dL) then maintened for up to 5 months. Potency was calculated using each subject's highest dose during titration.

Results: F and S (284 and 138 subjects, respectively) reduced serum P by a mean of 0.71 and 0.61 mM (2.2 and 1.9 mg/dL), respectively (p<0.001). No difference between groups was noted for serum P or Ca x P. Intact parathyroid hormone decreased after 3 months in the F group (p<0.01) and trended downward in the S group. Mean potency of F was greater at all doses and ~2x greater at the lower doses. The potency of both drugs decreased as dose increased. The highest doses in both groups were not plotted because of insufficient numbers of subjects (<7).



Serum magnesium levels increased with F treatment and serum total 25-hydroxyvitamin D levels decreased more with S. The most common related adverse events for F were discoloured faeces (12.7%) and diarrhoea (11.6%) and for S were nausea (3.6%) and dyspepsia (2.2%).

Conclusions: F was generally well-tolerated. Subjects taking F achieved similar serum P reductions compared to S using approximately half the dose.

Funding: Pharmaceutical Company Support - INEOS Healthcare Ltd

SA-PO668

Mechanisms of Hyperphsphaturia in the Osteocyte-Abelated Mice

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Background: Recent studies have shown that alterations in osteocyte metabolism occur in very early stages of chronic renal disease (CKD) and likely mediate altered bone and mineral metabolism in patients even with very mild degree of renal dysfunction. Fibroblast

growth factor 23 (FGF23) is a phosphaturic factor secreted by the osteocytes involved in the control of phosphate homeostasis. FGF23 is elevated from the early stage of CKD. We have established a transgenic mouse model, based on the diphtheria toxin (DT) receptor-mediated cell knockout (TRECK) system, in which inducible and specific ablation of osteocytes is achieved in vivo (Tatsumi S. et al. Cell Metab. 2007). To understand the role of osteocytes in Pi homeostasis, we investigated renal Pi handling in the osteocyte-ablated mice.

Methods: Ten-week old wild-type (WT) and transgenic (Tg) mice were injected i.p. with vehicle or 15-50 μg/kg body weight DT. After 8 days, we investigated Pi metabolism in Tg and WT mice.

Results: Within 48 hours of DT administration, 60–80% osteocytes were killed. The number of ablated osteocytes was dependent on DT concentration. After 8 days, osteocyte-ablated mice exhibited excessive bone resorption, impaired mineralization and adipose tissue proliferation in marrow space. These are hallmarks of the ageing skeleton. Moreover, the osteocyte-ablated mice demonstrated a marked increase of urinary Pi excretion and a significant decrease of renal sodium dependent Pi co-transporters, NaPi-IIa and NaPi-IIc at protein level, whereas no alteration in both plasma Pi and calcium concentration. Further, in this model, plasma FGF23 levels were significantly decreased, while PTH levels were conversely increased. In addition, dietary Pi manipulation was not affected Pi excretion in the osteocyte-ablated mice.

Conclusions: The reduction of osteocyte number appears to be induced urinary Pi excretion through PTH action. On the basis of the present results, we will discuss the role of osteocytes on Pi homeostasis.

Funding: Government Support - Non-U.S.

SA-PO669

Role of Estrogen Receptors ERα and ERβ in Estrogen-Induced Downregulation of NaPi-IIa and Phosphaturia in Ovariectomized Rats

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Background: We have previously demonstrated that estrogen (EST) downregulates NaPi-IIa and causes phosphaturia and hypophosphatemia in ovariectomized (OVX) rats. However, the effects of EST on other renal Pi transporters and the respective roles of estrogen receptor isoforms (ERa and ERb) in this effect remain unknown.

Methods: OVX rats were placed in metabolic cages with access to food and water and treated with EST or estrogen receptors (ER) agonists or their vehicle. The expression of ERα and ERβ was examined by RT-PCR. The expression of phosphate (Pi) transporters was examined with Northern hybridization and immunoblotting and correlated with urinary Pi excretion.

Results: RT-PCR data indicate that both ERα and ERβ are expressed in the proximal tubule (PT) cells of rat kidney. Molecular studies demonstrated that NaPi-IIa mRNA and protein were significantly reduced by EST in a dose-dependent manner, whereas the expression levels of NaPi-IIc and Pit2 were unchanged. Phosphaturia and decreased NaPi-IIa expression were also observed in pelletized slow-release EST experiments, were not hampered by the presence of progesterone and were reproduced by tamoxifen. Using specific agonists of either ERα(PPT) or ERβ (DPN) or both (PPT+DPN), we demonstrated that food intake was decreased in PPT- and PPT+DPN- but not in DPN-treated rats. PPT and DPN alone did not affect NaPi-IIa protein abundance and did not cause phosphaturia despite a decrease in the expression of its mRNA levels. However, PPT+DPN caused a sharp downregulation of NaPi-IIa along with significant urinary Pi wasting.

Conclusions: The phosphaturic effect of EST results from a specific downregulation of NaPi-IIa in the proximal tubule. This effect is not altered by progesterone and is mimicked by tamoxifen, indicating that the latter acts as estrogen agonist in the kidney. The effect of estrogen on NaPi-IIa is mediated via a complex mechanism involving the activation and likely heterodimerization of ERα and ERβ. Lastly, we propose that tamoxifen could be used as a phosphaturic agent to treat hyperphosphatemia.

Funding: NIDDK Support, Clinical Revenue Support

SA-PO670

Klotho and Na+/Pi Cotransporter on High Phosphorous Induced Vascular Calcification and Early Intervention by Sodium Thiosulfate in Remnant Kidney Rat

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Background: To investigate the expression of Klotho and Na+/Pi cotranspoter involved in high phosphorous induced vascular calcification and effect of sodium thiosulfate (STS) on vascular calcification in remnant kidney rats.

Methods: SD rats (n=35) underwent 5/6 nephrectomy (n=21) or sham operation (n=14). Rats were fed with high phosphorus (HP) or normal phosphorus (NP) diet for 16 weeks. They were divided into 5 groups: (1) remnant kidney rats receiving HP diet (NHP, n=7) (2) remnant kidney rats receiving NP diet (NNP, n=7), (3) sham operation rats receiving NP diet (SNP, n=7), (4) sham operation rats receiving HP diet (SHP, n=7). (5) remnant kidney rats receiving HP diet with STS (THP, n=7). The treatment group was given STS intraperitoneally three times a week for 16 weeks. Aortic calcification was confirmed by Von kossa staining. Klotho, Cbfα1 and Pit-1 expression in aorta were determined by immunohistochemistry, while Klotho and NaPi2a mRNA in kidney were determined by Real-time PCR.

Results: After 16 weeks, Scr, P^{3+} and iPTH were higher in NHP than those in SNP group ($P<0.05$). THP group showed significant decrease in Scr, P^{3}+ and iPTH and its pathological changes and aortic calcification alleviated compared with NHP group ($P<0.05$). Von kossa showed significant vascular calcification in NHP, occasional calcification in NNP and SHP, none in SNP group. The aorta expression of Klotho decreased while Pit-1 and Cbfα1 increased in NHP compared with SNP group ($P<0.01$). The aorta expression of Klotho increased and Pit-1 and Cbfα1 decreased ($P<0.01$) in THP compared with NHP group($P<0.01$). The kidney expression of Klotho mRNA decreased, while kidney NaPi2a mRNA increased in NHP compared with SNP group ($P<0.01$). After treatment, the kidney expression of Klotho mRNA increased while NaPi2a mRNA decreased in THP compared with NHP group ($P<0.05$).

Conclusions: The regulation of Klotho and Na+/Pi cotranspoter in both aorta and kidney may contribute to high phosphorous induced vascular calcification in remnant kidney rat. The effect of sodium thiosulfate is encouraging in decreasing serum phosphate and delaying the progression of vascular calcification.

Funding: Government Support - Non-U.S.

SA-PO671

Uromodulin Upregulates TRPV5 by Impairing Caveolin-Mediated Endocytosis

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Background: Uromodulin (UMOD), also known as Tamm Horsfall protein, is synthesized in the thick ascending limb of Henle's loop and secreted into urine as the most abundant protein. Association studies in humans suggest protective effects of UMOD against calcium-containing kidney stones. Mice carrying mutations of Umod found in human uromodulin-associated kidney disease (UAKD) exhibit hypercalciuria, but the potential mechanism for UMOD regulation of urinary Ca^{2+} excretion is incompletely understood. This study examined the hypothesis that UMOD regulates TRPV5 and TRPV6, channels critical for renal transcellular Ca^{2+} reabsorption.

Methods: HEK293 cells were cotransfected with TRPV5 and secreted or transmembrane KL. Whole-cell current was analyzed by patch-clamp recording.

Results: We found that coexpression with UMOD increased whole-cell TRPV5 current density in cultured human embryonic kidney (HEK) cells. Biotinylation studies showed that UMOD increased TRPV5 cell-surface abundance. Extracellular application of purified UMOD or supernatant of media harvested from UMOD-expressing cells upregulated TRPV5 current density and cell-surface abundance within physiological relevant concentration ranges. UMOD exerted a similar effect on TRPV6. It has been reported that TRPV5 undergoes constitutive caveolin-mediated endocytosis. We further showed that UMOD had no effect on TRPV5 in a caveolin-1 deficient cell line and expression of recombinant caveolin-1 in the cells restored the ability of UMOD to upregulate TRPV5. Finally, secretion of UAKD-mutant UMOD was markedly reduced and coexpression of mutant UMOD with TRPV5 failed to increase its current.

Conclusions: Thus, UMOD upregulates TRPV5 by acting from the extracellular side and by decreasing endocytosis of TRPV5. The stimulation of Ca^{2+} reabsorption via TRPV5 by UMOD may contribute to protection against kidney stone formation.

Funding: NIDDK Support, Other NIH Support - K12-HD000850

SA-PO672

Loss of Insulin-Induced Activation of TRPM6 Channels Results in Impaired Glucose Tolerance during Pregnancy

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Background: Gestational Diabetes Mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy. Women with GDM are at a higher risk for pre-eclampsia and as well as developing Diabetes Mellitus type 2 (DM2). Hypomagnesemia affects insulin resistance and is a risk factor for DM2 and GDM. Two single nucleotide polymorphisms in the epithelial magnesium channel TRPM6 were predicted to confer susceptibility for DM2.

Methods: Using HEK293 cells expressing TRPM6, immunoblotting and patch clamp analysis the mechanism of insulin-stimulated channel activity was investigated. Dynamic regulation of TRPM6 at the plasmamembrane was determined by Total Internal Reflection Fluorescent (TIRF) microscopy. A total of 997 pregnant women described as the Berlin Birth Cohort were included in the study.

Results: Using patch clamp analysis and TIRF microscopy, we demonstrated that insulin stimulates TRPM6 activity via a phosphoinositide 3-kinase and Rac1-mediated elevation of cell surface expression of TRPM6. Interestingly, insulin failed to activate the genetic variants TRPM6(V^{1393}I) and TRPM6(K^{1584}E) which is due to the inability of the insulin signaling pathway to phosphorylate TRPM6(T^{1391})and TRPM6(S^{1583}). Moreover, by measuring Total Glycosylated Hemoglobin in 997 pregnant women as a measure of glucose control, we demonstrated that TRPM6(V^{1393}I) and TRPM6(K^{1584}E) are associated with higher TGH and confer a higher likelihood of developing GDM. The impaired response of TRPM6(V^{1393}I) and TRPM6(K^{1584}E) to insulin represents a pathway leading to GDM where the defect is located in TRPM6.

Conclusions: Pregnant women carrying TRPM6(V^{1393}I) and TRPM6(K^{1584}E) possibly lack the physiological regulation of TRPM6 by insulin. These genetic variants of TRPM6 could be used as potential biomarkers to improve diagnosis and identify those at risk for developing GDM/DM2-induced hypomagnesemia.

Funding: Government Support - Non-U.S.

SA-PO673

Osteoblast-Specific Calcium-Sensing Receptor (Casr) Deficient Mice Display Temporary Growth Delay with Decreased Trabecular Bone

Hakan R. Toka,[1,2] Salvatore Dibartolo,[2] Yingben Xue,[3] David B. Mount,[1] Gary C. Curhan,[1] David Goltzman,[3] Edward M. Brown,[4] Martin R. Pollak.[2] *[1]Nephrology, Brigham and Women's Hospital, Boston, MA; [2]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [3]Endocrinology & Metabolism, Royal Victoria Hospital McGill University, Montreal, QC, Canada; [4]Endocrinology, Brigham and Women's Hospital, Boston, MA.*

Background: Casr has an important role in regulating calcium homeostasis within several tissues including bone. *In vivo* and *in vitro* data strongly suggest that Casr regulates osteoblast (Ob) proliferation, differentiation and mineralization, and thereby skeletal growth. Ob Casr-deficient mice under the Osterix (Osx) promoter have been reported to have severe growth retardation and decreased life expectancy.

Methods: We generated a conditional Casr-deficient mouse model targeting exon 3 using the Cre/Lox system. We obtained Cre transgenic mice driven by the Ob-specific Osx promoter and developed Ob Casr-deficient mice (Ob $Casr^{-/-}$).

Results: Immunohistochemistry in Ob $Casr^{-/-}$ shows absence of Casr in Obs, which are born at the expected Mendelian ratio. Ob $Casr^{-/-}$ develop significant growth delay, which is evident between the ages of 2 to 6 weeks compared to control mice (n=10 vs. 10, p=0.0002). DEXA studies show decreased BMC and BMD at age 3 weeks (p=0.002 and p=0.02) that is no longer evident at 3 months of age. Micro CT studies show decreased trabecular bone parameters in experimental mice (BV/TV, p=0.0001; Tb.Th, p=0.03; Tb.N, p=0.0001; Tb.Sp, p=0.0001). Bone histomorphometry displays increased osteoclast (Oc) cell numbers in Ob $Casr^{-/-}$ (N.Oc/BP, p=0.0001). Preliminary qPCR studies show decreased expression of several markers of Ob proliferation (e.g. IGF1R) or differentiation (e.g. Osx).

Conclusions: Our studies show that conditional Ob $Casr^{-/-}$ mice display temporary growth delay. The likely mechanism in our model is a temporary decrease in trabecular bone growth mediated by Ob Casr deficiency until skeletal growth plateaus in the adult mouse. Studies with primary Ob cultures addressing the effects of Casr deficiency on Obs and indirectly Ocs during bone growth are being pursued.

Funding: Other NIH Support - PO1DK070756-05

SA-PO674

Effect of Heparin on Level of Ionized Calcium in Dialysis Patients

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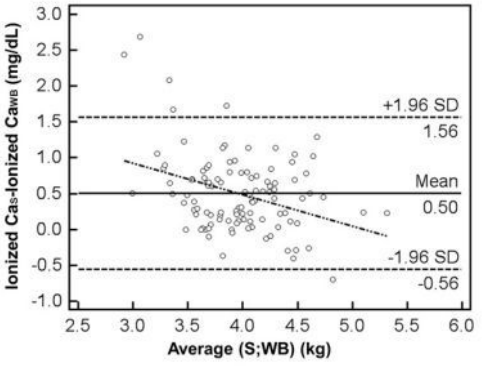
Background: Heparin as anticoagulant is especially associated with lower ionized calcium level in whole blood than that in serum. The aim of this study is to evaluate the bias of ionized calcium between whole blood containing heparin and serum.

Methods: We included 107 patients under maintenance hemodialysis in out hospital hemodialysis (HD) unit. The clinical and laboratory data were collected and were measured simultaneously. Non-heparinized, dry, and vacutainer tube was used to determine serum ionized calcium. Whole blood ionized calcium was obtained using sodium heparin-coated 3-ml syringe.

Results: Total calcium was 8.3 (7.1–11.6) mg/dL in non-corrected and 8.28 (6.93–11.53) in corrected. Ionized calcium was 3.76 (1.70–5.20) mg/dL in whole blood and 4.15 (3.25–5.43) mg/dL in serum. Level of ionized calcium in serum was higher than that in whole blood (P < 0.001). Serum ionized calcium was correlated with whole blood ionized calcium, total calcium, and corrected total calcium (P < 0.001). Bland-Altman analysis showed that a bias for ionized calcium was 0.5027. Most of ionized calcium in serum – ionized calcium in whole blood was within ±1.96 SD of 0.5027.



For bias, non-standardized β was –0.4389 (P < 0.001) and intercept was 2.2418 (P < 0.001). There was a significant difference in distribution of categories according to ionized calcium level between two methods (Kappa, 0.279; P < 0.001). When ionized calcium in whole blood was measured, forty three patients with normocalcemia were miscategorized to hypocalcemia.

Conclusions: This study demonstrate that whole blood ionized calcium is underestimated comparing with serum ionized calcium. Measuring bias will be increased along with the decrement of whole blood ionized calcium. Therefore, significant hypocalcemia in whole blood ionized calcium may be verified by serum ionized calcium.

SA-PO675

Phosphate Is a Key Factor for Chronic Kidney Disease Progression

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Background: Although experimental studies showed that protein (Prot) restriction slows CKD progression, this was not confirmed by clinical studies, suggesting that other factors could offset the benefits of such regimen. Since phosphate (Pi) has been recently recognized as a nontraditional risk factor for CKD progression, we sought to discern the possible deleterious effects of Pi from those of Prot intake. For this purpose, we studied the effect of two different dietary Pi contents in rats subjected to 5/6 nephrectomy (Nx) that received a low-Prot diet.

Methods: Nx rats were divided in 2 groups according to Pi content (0.2 and 0.9%), while all were maintained on a 12% Prot diet. After 2 months, biochemical, histological and imunohistochemical analyses were performed.

Results: Both groups showed low-level interstitial (INT) expansion and glomerulosclerosis (GS), consistent with dietary Prot restriction. Nx animals fed 0.9% Pi exhibited lower hematocrit (Ht) and creatinine clearance (ClCreat), as well as higher fractional excretion of Pi (Fe%Pi) and serum FGF-23, than those receiving 0.2% Pi. Despite Prot restriction, Pi overload was associated with worsening of renal function, and with an increase in the INT area and staining for α-actin (α-ACT). Moreover, FGF-23 correlated with Ht, Fe%Pi and α-ACT (r = -0.6, 0.8 and 0.6, respectively; p< 0.05 for all), consistent with the hypothesis that FGF-23 not only governs Pi homeostasis but may also contribute to the pathogenesis of renal fibrosis.

	0.2Pi	0.9Pi
Ht(%)	44±1	38±1*
Pi(mg/dl)	6±1	7.5±1
Cl_{creat}(ml/min)	0.6±0.04	0.4±0.07*
FePi(%)	0.15±0.07	10±0.7*
FGF-23(pg/ml)	153±16	767±60*
GS%	1.0±0.6	1.15±1
INT%	0.2±0.08	1.2±0.3*
α-ACT%	0.7±0.07	3.7±0.6*

*p<0.05

Conclusions: Pi overload plays a role in CKD progression through an increase in interstitial fibrosis, even in the presence of Prot restriction, suggesting that both Pi and Prot intake should be limited in CKD. These preliminary data may help us to understand the negative findings of some clinical studies of Prot restriction on CKD progression, in which little attention was paid to dietary Pi.

Funding: Government Support - Non-U.S.

SA-PO676

Association of Vitamin D (D25) Deficiency and Low Serum Phosphorus (Phos) with Obesity in a Large Ethnically Diverse Non-CKD Population

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Background: Higher overall dairy intake has been associated with lower obesity rates but serum Phos levels relation to obesity are unknown. Higher dietary Phos and low D25 have been described more in obese populations which suggests the hypothesis that obesity is associated with higher serum Phos particularly in those without CKD. To this end, we sought to determine the risk for obesity in those with D25 deficiency and across differing Phos levels within a large ethnically diverse population of subjects without CKD.

Methods: Subjects age ≥ 18 yrs with measured 25-hydroxyvitamin D (D25), serum Phos, documented eGFR≥60ml/min, and body mass index (BMI) in 1/1/2007-12/31/31. Obesity defined as BMI ≥ 30 and D25 deficiency <30mg/dl. Serum Phos was analyzed as both continuous & categorical quartile variables. Demographic and comorbidity data were extracted from the electronic medical records. Logistic regressions were performed to determine odds ratio (OR) for risk of obesity.

Results: A total of 35,280 subjects met the inclusion criteria for analysis. 44% had BMI ≥ 30 and 65% had D25 deficiency. The lower Phos group (1.9-3.1mg/dl) had the greatest proportion with D25 deficiency (67%). Multivariable logistic regressions obesity OR (95% CI) was 1.84 (1.67-2.03) in those with D25 deficiency after adjusting for age, gender, race, diabetes, hypertension, calcium, and PTH. Conversely, obesity OR were 10% lower for every 1 mg/dl Phos increase [OR = 0.90 (95% CI 0.83-0.97)].

OR for Obesity

Phosphorus	OR	95% CI
1.9-3.1	-	-
3.1-3.5	0.97	0.85-1.11
3.5-3.9	0.96	0.84-1.1
3.9-5.7	0.80	0.70-0.91
D25<30	1.84	1.67-2.03

Conclusions: We found greater risk for obesity with D25 deficiency and lower serum Phos levels. This is consistent with the mechanism of D25 on calcium and Phos balance but contrary to the high Phos diets observed in the obese population. D25 and Phos levels may reflect risk factors for the metabolic syndrome in the non CKD population.

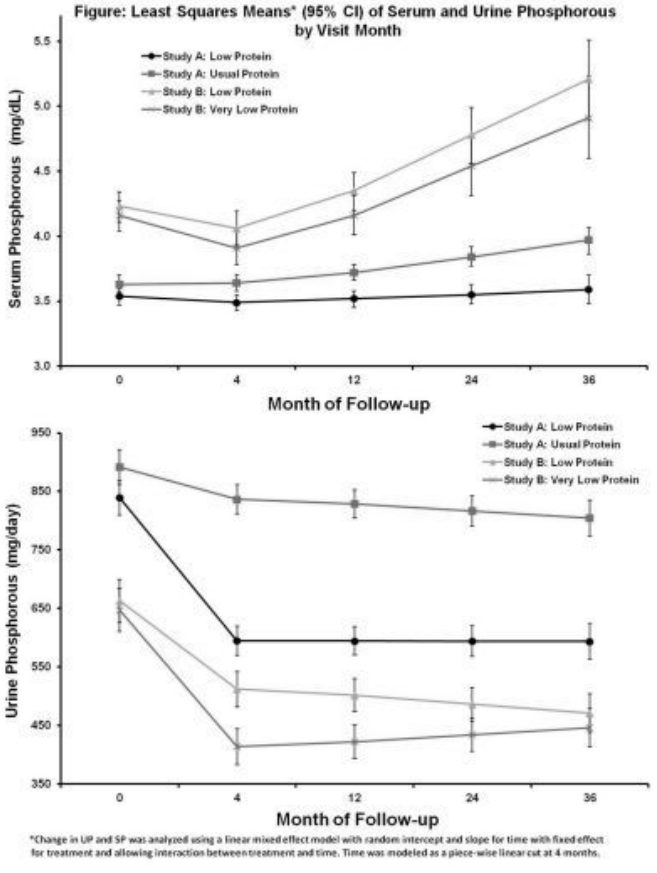
SA-PO677

Diet Modification Effects on Serum and Urine Phosphorous Levels: The Modification of Diet in Renal Disease Study Britt B. Newsome,[1] Joachim H. Ix,[2] Hocine Tighiouart,[3] Mark J. Sarnak,[3] Andrew S. Levey,[3] Gerald J. Beck,[4] Geoffrey A. Block.[1] *[1]Denver Nephrology; [2]UC San Diego; [3]Tufts Medical Center; [4]Cleveland Clinic.*

Background: We examined the relationship between dietary phosphorous (P) and protein modification on serum (SP) and urine (UP) phosphorous levels in chronic kidney disease (CKD).

Methods: Analysis of the Modification of Diet in Renal Disease (MDRD) Study, a randomized trial of dietary protein restriction and blood pressure control on CKD progression. Study A: 585 patients with a glomerular filtration rate (GFR) 25-55ml/min/1.73m^2 assigned to a usual-protein (1.3 g/kg/day) or a low-protein diet (0.58 g/kg/day). Study B: 255 patients with a GFR 13-24ml/min/1.73m^2 assigned to the low-protein or a very-low-protein diet (0.28 g/kg/day) supplemented with ketoacid-amino acids. Dietary P targets (mg/kg/day): usual protein 16-20; low protein 5-10; very low protein 4-9. We used a linear mixed effect model to assess UP and SP change.

Results: All 3 lower protein groups achieved dietary P restriction. Baseline SP was higher for Study B vs. A (Figure). The mean SP (mg/dL) change from 0-4 months was 0.01(Study A, usual protein), -0.05(Study A, low protein), -0.17(Study B, low protein) and -0.25(Study B, very low protein). Follow-up SP was similar among Study B groups while the Study A low protein group remained lower than the usual diet group. Baseline UP was lower for Study B vs. Study A. The mean UP (mg/day) change from 0-4 months was -55(Study A, usual protein), -244(Study A, low protein), -151(Study B, low protein) and -233(Study B, very low protein). UP levels at ≥4 months were different between Study A groups while Study B groups were different only at 4 and 12 months.



Conclusions: Dietary P restriction resulted in significant UP reductions despite minimal SP reductions. Effective SP reduction strategies will require more than diet modification.

Funding: NIDDK Support, Pharmaceutical Company Support - CM&D Pharma, LTD

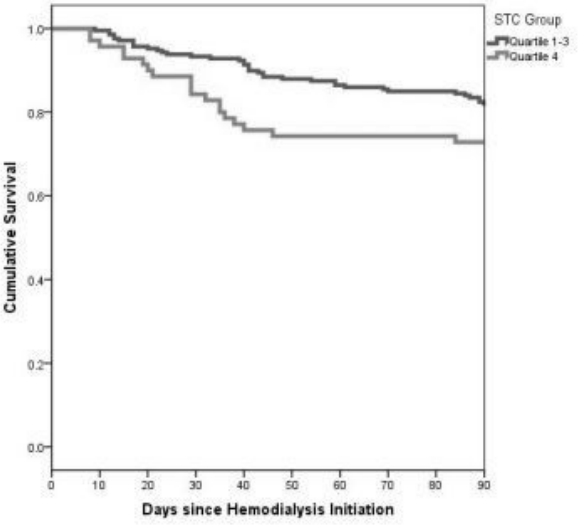
SA-PO678

Serum Stanniocalcin-1 Associates with Mineral Bone Disease, and Mortality in Hemodialysis Sagar U. Nigwekar,[1] Ishir Bhan,[1] Julia Beth Wenger,[1] S. Ananth Karumanchi,[2] Ravi I. Thadhani.[1] *[1]Massachusetts General Hospital; [2]Beth Israel Deaconess Medical Center.*

Background: Disordered mineral metabolism has been implicated in decreased survival among hemodialysis (HD) patients. Stanniocalcin-1, an intracellular and secreted glycoprotein known to regulate calcium and phosphorous balance in bony fish, is also produced in many human tissues. We investigated how stanniocalcin-1 associates with traditional markers of mineral bone disease (MBD) and mortality in HD patients.

Methods: Serum stanniocalcin-1 levels at the time of HD initiation were measured in 280 subjects enrolled prospectively in the Accelerated Mortality on Renal Replacement (ArMORR) cohort. Associations between stanniocalcin-1 levels and traditional serum markers of MBD were assessed. Hazard ratios (HR) and 95% confidence intervals (CI) for 90-day mortality were calculated by comparing quartiles of stanniocalcin-1 using Cox proportional hazard models.

Results: The mean age was 65 years and 53% of subjects were males. Median stanniocalcin-1 level was 11,431 pg/mL (IQR 8,295-14,512). Serum stanniocalcin-1 correlated positively with phosphorous (r= 0.21, p= 0.0004) and negatively with calcium (r= -0.13, p= 0.031). No significant correlations were observed with parathyroid hormone, alkaline phosphatase, and 25-hydroxyvitamin D. In multivariate analyses, patients with serum stanniocalcin-1 levels in the highest quartile had significantly higher 90-day mortality (HR 2.76, 95% CI 1.35-5.67) compared with the remaining subjects.



This relationship was observed across all quartiles of phosphorous and calcium.

Conclusions: Elevated stanniocalcin-1 independently predicts mortality and is associated with higher phosphorous and lower calcium levels in HD. Future studies are needed to identify stanniocalcin-1's mechanism of action and to determine if these associations are causative.

SA-PO679

Non-Invasively Assessed Bone Loss Is a Stronger Predictor than Traditional Risk Factors for Coronary Artery Calcification Progression in Patients with CKD-5D Hartmut H. Malluche,[1] Gustav A. Blomquist,[2] Daniel Davenport,[3] Paul Anaya,[4] Vincent L. Sorrell,[2,4] Marie-Claude M. Faugere.[1] *[1]Div of Nephrology, University of Kentucky; [2]Dept of Radiology, University of Kentucky; [3]Dept of Surgery, University of Kentucky; [4]Div of Cardiology, University of Kentucky, Lexington, KY.*

Background: Coronary artery calcifications (CAC), commonly found in patients with CKD-5, are associated with high morbidity and mortality. Risk factors for elevated CAC in this patient population are not well established. This prospective study evaluated the impact of bone loss, as well as traditional and non-traditional predictors, on CAC.

Methods: At baseline and after one year, bone mineral density was measured at L1-L2 vertebrae and total hip using dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT). CAC was concurrently quantified by Agatston scores using CT.

Results: 148 subjects (age 53±13) with CKD-5D were enrolled. Eighty-four had CAC<400 and 64 had CAC≥400. Those with CAC≥400 were older, had lower QCT scores of the hip and spine and lower DXA scores of the hip (all p<.05). Gender, race, diabetes, dialysis vintage, BMI, hypertension, smoking, exercise, HDL/LDL, serum phosphorus, calcium and iPTH, use of vitamin D or calcium-based phosphate binders did not differ significantly between the CAC groups. In prospective analysis of one year follow-up (at this time n=44), only bone loss at the spine and age correlated with CAC progression.

Predictors of CAC Progression in Patients with CKD-5D

	Spearman's Rho Correlation with CAC Progression after 1 year	P-value
Δ L1-L2 QCT	-.450	.003
Δ L1-L2 DXA	-.300	.048
Age at baseline	.373	.013

Conclusions: The profile of risk factors for CAC in CKD-5D differs from the general population. In cross-sectional analysis the strongest predictors are age followed closely by non-invasively assessed bone mass at both the hip and spine. Serum phosphorus, calcium and iPTH are not predictive. Prospectively, after one year the strongest predictors of CAC progression are bone loss of the spine followed by age. Due to lower bone turnover of the hip, longer observation may be required to identify bone loss at the hip in addition to spine as a predictor.

Funding: NIDDK Support

SA-PO680

Bone Mineral Density 5 Years after Parathyroidectomy in Hemodialysis Patients with Secondary Hyperparathyroidism Masayuki Yamanouchi, Yoshifumi Ubara, Keiichi Sumida, Koki Mise, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Minato-ku, Tokyo, Japan.*

Background: Whether bone mineral density (BMD) improves 5 years after parathyroidectomy (PTx) for secondary hyperparathyroidism (SHPT) remains unknown.

Methods: To investigate BMD after PTx using dual energy X-ray absorptiometry (DXA).BMD was examined at the distal one third of the radius (non-shunt side) and at the lumbar supine (L2-L4, lateral view) before and five years after PTx in 35 hemodialysis patients who underwent surgery from April 1994 to May 2004. Their data were analyzed retrospectively.

Results: Intact PTH decreased significantly from 1100±530 (range, 446 to 2300) pg/mL before PTx to 75±68 (2 to 251) pg/mL at 5 years after PTx (P<0.01). The radial BMD and the lumbar BMD before PTx showed a decrease of -3.3±1.9 SD below and -1.3±2.4 SD below the corresponding normal mean T-score, respectively. The radial BMD (T-score) before PTx was 0.522±0.113 g/cm^2(-3.3±1.9), and increased significantly to 0.545±0.114 g/cm^2 (-2.8±2.0)(*P*=0.01) five years after PTx. While the lumbar BMD (T-score) showed no significant changes by 0.734±0.202 g/cm^2 and 0.746±0.199 g/cm^2 (-1.3±2.4 to -1.1±2.3) between before and five years after PTx. The patients did not suffer any fractures during the follow-up period.

Conclusions: These findings indicate that maintenance of iPTH at a level under 300 pg/mL for five years after PTx can increase radial BMD in patients with SHPT showing a marked preoperative decrease to the osteoporosis range (less than –2.5 SD of the T score) according to the WHO classification, and can be stabilization or no further significant loss of lumbar BMD.

Funding: Private Foundation Support

SA-PO681

Evaluation of Bone Mineral Density, Kidney Function and Arterial Stiffness in 371 Long-Standing Type 1 Diabetic Patients with or without Diabetic Nephropathy Maria Lajer,[1] Simone Theilade,[1] Christel Joergensen,[1] Frederik I. Persson,[1] Lise Tarnow,[1,4] Hans-Henrik Parving,[2,4] Peter S. Lacy,[3] Bryan Williams,[3] Peter Rossing.[1,4] *[1]Steno Diabetes Center, Denmark; [2]Rigshospitalet, Denmark; [3]University of Leicester, United Kingdom; [4]University of Aarhus, Denmark.*

Background: Patients with chronic kidney disease may have lower bone mineral density (BMD).

Methods: Prospective cohort of 141 patients with diabetic nephropathy (DN) (55% men; age [mean±SD] 53±9 years, 42±8 years of diabetes, DN duration 22±6 years) and 230 with normoalbuminuria (50% men, 58±10 years, 40±10 years of diabetes). Femoral and lumbar spine BMD (g/cm^2) was measured by DXA. Osteopenia and osteoporosis were defined by any T-score of -2.5 to -1.0 and <-2.5, respectively. Glomerular filtration rate (GFR) by ^{51}Cr-EDTA was determined in DN and estimated (MDRD) in normoalbuminuric patients. Pulse wave velocity (PWV) was measured by SphygmoCor and 24h blood pressure by BPro watch.

Results: Among patients with DN, 73 (52%) and 36 (26%) had osteopenia or osteoporosis, respectively compared to 124 (54%) and 32 (14%) of normoalbuminuric patients (p=0.013). In males, 34% with DN vs. 14% with normoalbuminuria had osteoporosis (p<0.001). Among women, femoral BMD were different after adjustment for sex and age. Baseline eGFR levels were not associated with declining BMD. Among patients with DN, 24h central PP were higher (p= 0.014) while 24h central systolic dip were lower in osteoporotic compared with osteopenic patients and patients with normal BMD (p<0.001). PWV also tended to be elevated and GFR lower in osteoporotic patients although not significantly (p=0.12 and 0.14). Among normoalbuminuric patients, 24h central BP and PWV were elevated in osteoporotic patients (p=0.005 and 0.028, respectively) while 24h central systolic dip were not significantly different (p>0.05).

Conclusions: The risk of osteoporosis was highest among male type 1 diabetes patients and long-standing DN and femoral BMD was associated with albuminuria status, pulse pressure and dipping. Hence, screening and treatment of osteoporosis in patients with albuminuria or arterial stiffness should be considered.

Funding: Private Foundation Support

SA-PO682

Fibroblast Growth Factor 23, Bone Mineral Density and Risk of Hip Fracture among Older Adults: The Cardiovascular Health Study Anna Jeanette Jovanovich,[1] M. Chonchol,[1] John A. Robbins,[3] Howard Fink,[4] Ian H. de Boer,[2] Bryan R. Kestenbaum,[2] Ronit Katz,[2] Laura Carbone,[5] Gail A. Laughlin,[6] Michael Shlipak,[7] Linda F. Fried,[8] Kenneth J. Mukamal,[9] Joachim H. Ix.[6] *[1]University of Colorado; [2]University of Washington; [3]University of California Davis; [4]VA Minneapolis HCS; [5]VA Memphis HCS; [6]University of California San Diego; [7]University of California San Francisco; [8]VA Pittsburgh HCS; [9]Beth Israel Deaconess Medical Center.*

Background: Fibroblast growth factor 23 (FGF23) regulates renal phosphorus excretion and inhibits calcitriol synthesis. The association of high FGF23 levels with bone loss and fracture in the general population is uncertain.

Methods: We evaluated 3337 ambulatory participants ≥65 years. Total hip (TH) and lumbar spine (LS) bone mineral density (BMD) was measured by dual x-ray absorptiometry in a subset (n=1291). In all participants, hip fracture incidence was assessed prospectively by hospital records review.Associations of plasma FGF23 levels with BMD and incident hip fracture were examined with linear regression and Cox proportional hazard models, respectively. We tested whether associations differ by chronic kidney disease (CKD) status.

Results: Mean age was 78 ± 5 years, 60% were women, and 16% were black. Men had a lower median plasma FGF23 level than women (66 [IQR 52- 92] vs. 75 [56-107] RU/mL; p< 0.001). In models adjusted for important confounders, higher FGF23 levels associated with greater TH BMD and LS BMD in men only (ß per doubling of FGF23: 0.02 [95% CI 0.001, 0.04] g/cm^2 and 0.03 [95% CI 0.006, 0.06] g/cm^2, respectively). During a mean follow-up of 11 years, 328 hip fractures hospitalizations occurred. Higher FGF23 levels did not associate with hip fracture risk in women or men (adjusted HR: 0.95 [95% CI 0.78, 1.15] and 1.09 [95% CI 0.82, 1.46] per doubling of FGF23, respectively). Results were similar irrespective of CKD status (p >0.4 for interaction).

Conclusions: In this large cohort of older adults, higher FGF23 levels were associated with greater BMD in men only, and had no statistically significant association with hip fracture risk in either sex.

Funding: NIDDK Support

SA-PO683

Rapid Cortical Bone Loss in Patients with Chronic Kidney Disease Thomas L. Nickolas,[1] Stephanie Boutroy,[1] Serge Cremers,[1] Donald J. Mcmahon,[1] Mary B. Leonard,[2] Elizabeth Shane.[1] *[1]Columbia University, NY, NY; [2]CHOP, Philadelphia, PA.*

Background: Fracture risk is elevated in chronic kidney disease (CKD) patients. We reported that CKD patients with prevalent fractures have lower areal BMD (aBMD) by DXA at the ultradistal radius (UDR) and lower cortical (Ct) and trabecular (Tb) volumetric BMD (vBMD), thinner cortices and abnormal Tb microarchitecture of the distal radius (RAD) and tibia (TIB) by high resolution peripheral computed tomography (HRpQCT).

Methods: To ascertain if microarchitectural abnormalities associated with CKD are progressive, we enrolled 54 patients in a longitudinal study and measured aBMD by DXA at the lumbar spine (LS), total hip (TH), femoral neck (FN), 1/3 and ultradistal radius (1/3R; UDR), and Ct and Tb vBMD, geometry and microarchitecture of the distal RAD and TIB by HRpQCT (voxel size 82µm). Median (interquartile range) followup was 1.8 (1.3-2.1) years. Serum was archived for batch analysis at study completion. Changes in bone measures were annualized and compared by mixed models adjusted for age, sex, race, dialysis status and baseline bone measures.

Results: At baseline, mean±SD age was 69±10 yrs, 52% were female, 33% had prevalent fractures and 17% were on dialysis. Mean eGFR in nondialyzed patients (37±17 mL/min) did not change (-0.3±7 mL/min, p=1.0). There were significant declines in aBMD at the TH (1.2±2.6%, p<0.01) and UDR (2.4±5.6%, p<0.01) but not the LS, FN or 1/3R. By HRpQCT, there were significant declines in RAD Ct area (2.9±3.8%, p<0.0001), density (1.2±1.6%, p<0.0001) and thickness (1.2±1.6%, p<0.0001). In contrast, Tb vBMD and microarchitecture did not change, excepting a small, significant increase in Tb area (0.4±0.9%, p<0.0001). The pattern and significance of changes were mirrored at the TIB.

Conclusions: CKD is associated with progressive Ct bone loss at the RAD and TIB. Declines in Ct density, thickness and area, coupled with increases in Tb area suggest endocortical cancellization is the primary mechanism of Ct loss. Pharmacologic interventions to decrease Ct loss could represent a strategic therapeutic target to prevent deterioration of bone microarchitecture and lower fracture risk in this vulnerable population.

Funding: NIDDK Support

SA-PO684

Association of Bone Density and Mass with Coronary Artery Calcification in Incident Dialysis Patients Laura H. Mariani, Anne K. Tsampalieros, Mary B. Leonard, Sylvia E. Rosas. *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.*

Background: Vascular calcification has been associated with low bone mineral density (BMD) in the general population and pre-dialysis CKD. In dialysis patients, it has been associated with low trabecular bone volume and low bone turnover. Little is known about the association at the time of dialysis initiation.

Methods: 96 patients were enrolled within 4 months of dialysis initiation and underwent both tibia quantitative CT (QCT) and ECG-triggered multi-slice CT scans. QCT provides measures of trabecular and cortical volumetric BMD and bone mineral content (BMC). Multivariable ordinal logistic regression was used to assess the relationship between coronary artery calcification (CAC) Agatston score and bone density and mass.

Results: Mean (SD) age was 50 (13) yr, BMI was 29 (5.8) kg/m² and dialysis vintage was 66 (31) days. 67% were male, 62% black and 49% diabetic. Total Agatston score was 0 in 36%, >0 to 100 in 25%, >100 to 400 in 17% and >400 in 22%. The mean (SD) z-score for trabecular BMD was -0.28 (1.32), cortical BMD was -0.41 (1.22) and cortical BMC was 0.34 (1.21). There was no statistical difference in bone parameters across the four CAC categories. In univariate models, greater trabecular BMC was associated with a greater odds of elevated CAC. In fully adjusted models, greater cortical BMC was associated with greater CAC (Table 1).

Association of Bone Parameters with CAC using Ordinal Logistic Regression

Bone Parameter	Unadjusted		Adjusted for Age,Sex,Race		Fully Adjusted*	
	OR per SD	p-value	OR per SD	p-value	OR per SD	p-value
Cortical Bone						
BMC(mg)	1.01	0.97	1.30	0.35	1.83	0.05
BMD(mg/cm³)	0.70	0.07	1.00	0.99	1.09	0.71
Trabecular Bone						
BMC(mg)	1.54	0.03	1.45	0.09	1.56	0.08
BMD(mg/cm³)	0.73	0.13	0.80	0.32	1.04	0.88

*Adjusted for age, sex, race, diabetes, smoking and hyperlipidemia

Conclusions: We found that cortical content by QCT was positively associated with CAC after multivariable adjustment in these incident dialysis patients. The relationship between bone and vascular calcification may vary depending on the bone imaging modality used and longitudinal studies are needed to further explore the interplay between bone and vascular calcifcation.

Funding: NIDDK Support

SA-PO685

Changes in DXA Measures of Bone Mineral Density after Pediatric Renal Transplantation Anne K. Tsampalieros,[1] Lindsay M. Griffin,[1] Babette Zemel,[1] Heidi Kalkwarf,[2] Justine Shults,[1] Mary B. Leonard.[1] *[1]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: We recently reported (JASN 2012) that trabecular volumetric bone mineral density (vBMD) decreases, cortical vBMD increases and cortical thickness did not improve following pediatric renal transplantation (Txp), as measured by quantitative CT (QCT). Changes in DXA measures of areal BMD (aBMD) in pediatric Txp have not been well characterized.

Methods: DXA scans were obtained in 56 Txp recipients (age 6 -21 yr) at Txp, and 3, 6, and 12 months later. Sex- and race- specific Z-scores were generated for DXA whole body bone mineral content (WB-BMC-Z) and lumbar spine BMD (LS-BMD-Z) relative to age and adjusted for height-Z based on >900 healthy reference participants. Changes in Z-scores were assessed using quasi-least squared regression models.

Results: At baseline, LS-BMD-Z was significantly elevated in younger Txp recipients only (Table). In all Txp recipients combined, LS-BMD-Z decreased significantly and decreases were independently associated with greater mean glucocorticoid (GC) dose (p=0.001) and greater declines in PTH levels (p=0.002). Although WB-BMC-Z scores on average did not change significantly over the year, greater GC doses (p<0.001) and lesser decreases in PTH levels (p=0.03) were independently associated with declines in WB-BMC-Z scores.

Height-Z Adjusted DXA Z-scores at Txp and 12 Month Follow-up

	0	12	p value of change
LS-BMD <13 yrs	1.36 ± 1.48*	0.68 ± 1.17*	0.006
LS-BMD ≥13 yrs	0.21 ± 0.80	-0.01 ± 0.76	0.06
WB-BMC	-0.33 ± 1.10	-0.38 ± 1.03*	0.43

* p<0.05 compared to reference participants

Conclusions: These data demonstrated that greater GC exposure following Txp was associated with decreases in LS-BMD-Z and WB-BMC-Z scores. The association with declines in WB-BMC-Z may reflect impaired expansion of cortical dimensions with growth, consistent with our prior QCT analysis. The differential effect of changes in PTH is consistent with PTH effects to increase and decrease trabecular and cortical vBMD, respectively. These data suggest that DXA captures GC and PTH effects on trabecular and cortical bone following Txp.

Funding: NIDDK Support, Other NIH Support - NICHD

SA-PO686

Low Fracture Rrates with Normal DXA Scans in Hemodialysis(HD) Patients Scott A. Rasgon,[1] Richard M. Dell,[1] John J. Sim.[1] *[1]Nephrology, Kaiser Permanente, Los Angeles, CA; [2]Nephrology, Kaiser Permanente, Los Angeles, CA; [3]Nephrology, Kaiser Permanente, Los Angeles, CA.*

Background: KDIGO makes the following recommendations for DXA scans in dialysis patients. " **3.2.2 In patients with CKD stages 3-5D with evidence of CKD-MBD, we suggest that BMD testing not be performed routinely, because BMD does not predict fracture risk as it does in the general population, and BMD does not predict the type of renal osteodystrophy (2B)."**This is based on 13 studies with the most subjects in any of the studies was 242. 5 studies showed no correlation with fracture rates and 8 showed some correlation with low BMD and increased fracture rate. Last year we presented data on 1883 patients who had DXA scans. We saw a correlation between a low fracture rate in patients who had a normal DXA. We now have 1954 HD patients with DXA scans and one more year of follow up on our existing patients to identify the predictive value of DXA in HD patients.

Methods: We reviewed the BMD data from DXA scans in a population of HD patients at Kaiser Permanente to see if any correlation exist between BMD testing and hip fracture rates in HD patients greater than 60 years of age. In our 60+ population there was 940,153 DXA done of which 1954 scans were done on HD patients during the time period of 01/01/1998-05/09/12.

Results: We found the following results. 325 HD patients had a normal DXA and only 4 had hip fracture with a 1.2 % rate of fracture.

Hemodialysis DXA and Hip Factures

	No DXA	Normal	Osteopenia	Osteoporosis	Unknown	Total
Hemodialysis Hip Fx	27	4	42	93	0	166
Hemodialysis	954	321	791	689	14	2769
Hemodialysisi Total	981	325	833	782	14	2935

Comparing the risk of hip fracture in a normal DXA vs osteoporosis/osteopenia result showed an unadjusted relative risk of 6.8 with a p=.0001.

Conclusions: Our findings demonstrate that normal DXA scans predict a low fracture risk even in HD patients. This has continued to be supported in our large updated data base with more DXA scans and one more year of observation. We have more subjects in our study than previous studies combined.This data supports the use of DXA scans in HD patients to identify those at highest risk to concentrate on fall prevention and treatment options.

SA-PO687

Mineral Metabolism and Cortical Volumetric Bone Mineral Density in Childhood Chronic Kidney Disease Michelle Denburg,[1] Anne K. Tsampalieros,[1] Ian H. de Boer,[2] Justine Shults,[1] Heidi Kalkwarf,[3] Babette Zemel,[1] Mary B. Leonard.[1] *[1]The Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Washington, Seattle, WA; [3]Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: The objective of this study was to determine the relations among measures of mineral metabolism and cortical volumetric bone mineral density (CortBMD) in children with CKD.

Methods: Cross-sectional study of 172 children (ages 5-21 yr) with CKD stages 2-5D. Serum measures included 25-hydroxyvitamin D (25[OH]D), $1,25(OH)_2D$, $24,25(OH)_2D$, PTH, fibroblast growth factor 23 (FGF23), calcium (Ca, corrected for albumin), and phosphorus (Pi). Free and bioavailable 25(OH)D were calculated using total 25(OH)D, albumin and vitamin D-binding protein levels. Tibia quantitative CT scans were used to measure CortBMD and results expressed as sex-, race- and age-specific Z-scores based on 675 controls. Multivariate linear regression was used to identify independent correlates of CortBMD-Z.

Results: Median CortBMD-Z was lower in CKD stages 4-5D compared with stages 2-3 (-0.47 vs. +0.14; p < 0.01). Median PTH was 182 pg/ml in stages 4-5D and 44.5 pg/ml in stages 2-3 (p <0.0001). In multivariate analysis (Table), Ca and 25(OH)D were positively associated, and PTH and $1,25(OH)_2D$ levels were inversely associated with CortBMD-Z. The positive association between 25(OH)D levels and CortBMD-Z was comparable using total, free or bioavailable levels. CKD severity and Pi were not associated with CortBMD-Z, independent of PTH level. Sex, race, underlying disease, FGF23 level, and vitamin D sterol therapy were not associated with CortBMD-Z.

Multivariate Model of Determinants of CortBMD-Z

Covariate	Change in CortBMD-Z	p
Age (per yr)	-0.048	0.07
PTH (per 10%)	-0.024	0.002
Ca (per 1 mg/dl)	0.37	0.02
25(OH)D (per 10 ng/ml)	0.19	0.03
1,25(OH)2D (per 10%)	-0.075	<0.001

PTH and 1,25(OH)2D log transformed

Conclusions: Greater PTH and $1,25(OH)_2D$ levels and lower calcium and 25(OH)D levels were independently associated with cortical deficits in childhood CKD, as measured by quantitative CT. Bone biopsy studies are needed to determine if these deficits represent increased porosity and/or a mineralization defect.

Funding: NIDDK Support, Other NIH Support - NIH Grants R01-DK060030, R01-HD040714, K24-DK076808, and UL1-RR-024134 and UL1-RR-026314, Pharmaceutical Company Support - National Kidney Foundation / Amgen KDOQI Research Fellowship

SA-PO688

Bisphosphonate Use in Chronic Kidney Disease Meghan J. Elliott,[1] Matthew T. James,[1] Luz Palacios-derflingher,[1] Marcello Tonelli,[2] Braden J. Manns,[1] Robert R. Quinn,[1] Pietro Ravani,[1] Gregory Kline,[1] Brenda Hemmelgarn.[1] *[1]Medicine, University of Calgary, Calgary, AB, Canada; [2]Medicine, University of Alberta, Edmonton, AB, Canada.*

Background: While bisphosphonates are commonly used in the general population, their use in patients with eGFR < 30 mL/min/1.73 m^2 is not recommended due to lack of data regarding safety and efficacy. We aimed to investigate the association between CKD stage and bisphosphonate use in a community-based cohort.

Methods: Participants aged ≥ 66 years were identified from a province-wide lab database, Alberta, Canada between 2002 and 2008. We included patients who had at least 1 outpatient creatinine measurement and did not require renal replacement therapy at baseline. GFR was estimated using the CKD-EPI equation, categorized as ≥ 60, 45-59, 30-44, 15-29 and < 15 mL/min/1.73 m^2 (index date was the date of the first eGFR). Bisphosphonate use was defined as at least 2 prescriptions in the year after their index eGFR. Logistic regression models (adjusted for demographics, comorbidities, steroid use and prior fractures) were used to determine the likelihood of bisphosphonate use by stage of CKD (reference eGFR ≥ 60).

Results: Among the 304,601 participants (median age 74.7 years; 55.7% female), 33% had eGFR < 60. Prevalence of bisphosphonate use declined with decreasing eGFR, however their use remained substantial even among patients with eGFR < 30. Compared to patients with eGFR > 60, patients with eGFR 15-29 were only 30% less likely to use bisphosphonates (OR 0.70; 95% CI 0.65, 0.76) after adjusting for demographics, comorbidities, steroid use and prior fracture.

Prevalence and adjusted likelihood of bisphosphonate use

eGFR (mL/min/1.73 m²)	Crude prevalence of bisphosphonate use (95% CI)	Adjusted* OR (95% CI)
≥ 60	13.7% (13.6, 13.9)	Reference
45-59	13.3% (13.0, 13.6)	0.89 (0.86, 0.91)
30-44	12.0% (11.7, 12.4)	0.80 (0.77, 0.84)
15-29	9.8% (9.2, 10.5)	0.70 (0.65, 0.76)
<15	4.0% (3.0, 5.5)	0.32 (0.23, 0.44)

*Adjusted for age, sex, comorbidities, steroid use and prior fracture

Conclusions: Despite a lack of data on efficacy and safety, the prevalence of bisphosphonate use among patients with severe CKD (eGFR < 30) was only slightly lower than in people with normal kidney function.

Funding: Government Support - Non-U.S.

SA-PO689

Zoledronic Acid Improves Bone Volume but Not Arterial Calcification in CKD-Mineral Bone Disorder Neal X. Chen,[1] Matthew R. Allen,[1] Vincent H. Gattone,[1] Xianming Chen,[1] Drew M. Brown,[1] Alexander J. Carr,[1] Mohammad Walid Aref,[1] Sharon M. Moe.[1,2] *[1]Indiana Univ School of Medicine, Indpls, IN; [2]Roudebush VAMC, Indpls, IN.*

Background: Bisphosphosphonates have not been directly tested in CKD patients. Zoledronic acid (ZOL) is a potent bisphosphonate that could improve bone properties in CKD. It also has anti-VEGF properties and thus may be effective in the vascular disease of CKD-MBD.

Methods: Cy/+ male rats (CKD) or their normal littermates (NL) were treated with a normal (0.6%) phosphorus casein diet, and given a single SQ injection of zoledronic acid (ZOL) or vehicle at 25 wks (~30% NL GFR) of age. Serum biochemistries were followed for 10 wks; blood pressure, heart mass, aorta calcification, and bone were assessed at 35 wks of age (~15% NL GFR).

Results: The CKD rats developed CKD-MBD with a progressive increase in phosph, PTH, FGF23 and BUN. The aorta calcium content in CKD rats was 2x that of NL animals and was correlated with PTH (r=0.65), FGF23 (r=0.73), phosph (r=0.70) and heart wt (r=0.81); all $p < 0.001$. ZOL had no effect on biochemistries of CKD-MBD, arch calcification, or heart wt. CKD animals treated with ZOL had significantly higher proximal tibia trabecular bone volume compared to vehicle ($23.0 \pm 7.5\%$ vs $9.7 \pm 4.4\%$, $p < 0.001$). These effects were similar to those observed in NL animals. ZOL treatment resulted in higher trabecular number, but not thickness in both CKD and NL rats. There was no relationship between arch calcification and bone volume. FGF23, PTH, calcium and phosph did not correlate with trabecular bone volume nor trabecular number, but were strongly correlated with trabecular thickness. The effect of ZOL on bone strength and cortical bone is being evaluated. There were no adverse effects on kidney function.

Conclusions: In a slowly progressive rat model of CKD fed a normal phosphorus diet, there was a substantial positive effect of ZOL on trabecular bone volume. In contrast to studies from other bisphosphonates, ZOL had no effect on aorta calcification, heart size, calcium, phosph, PTH or FGF23. These studies suggest that ZOL may provide an effective therapy for bone preservation in the setting of CKD-MBD.

Funding: Other NIH Support - NIAMS

SA-PO690

Pharmacokinetic Effect of Cinacalcet on Patients with Secondary Hyperparathyroidism (SHPT): Useful Data in Clinical Practice Dolores Arenas,[1] Vanesa De la Fuente,[1] Pablo Delgado,[1] Patricia Gutiérrez,[1] Jorge Ribero,[1] Mariano Rodriguez,[2] Victor Lorenzo,[3] Yolanda Almaden Peña.[2] *[1]H.Perpetuo Socorro, Alicante, Spain; [2]H.Reina Sofía, Córdoba, Spain; [3]H.U.Canary Islands, La Laguna, Spain.*

Background: The pharmacokinetic (PK) effect on patients (pts) controlled and treated chronically with cinacalcet is unknown. Our objective was to describe the PK effect over 48 hours (h) after a single dose of cinacalcet in pts on hemodialysis (HD) with controlled iPTH (100–400 pg/ml).

Methods: Single-center, open, exploratory and uncontrolled PK clinical trial with a single dose of cinacalcet based on clinical practice (30–90 mg) and a 48h follow-up. The treatment the pts received before the start of the study was maintained. iPTH, ionic Ca, P, and calcitonin were determined at baseline (24h after the last cinacalcet dose, trough level in daily dosing regimen), and at 1, 3, 6, 12, 24 and 48h. PTH was determined with the Duo PTH kit (Scantibodies Laboratory,Santee,CA,USA).

Results: We included 10 pts (8 men) with SHPT (median diagnostic age of 4.5 years (y), range 2–31), with a mean age 66 y (39–82), receiving HD for a median of 5 y (2–31). Table 1 shows the mean levels (SD) during the study. Pts received the dose that they were due to receive: 6 pts received 30 mg of cinacalcet, 3 received 60 mg and 1 received 90 mg. They were all treated with hidroferol for 2 months before the study. 25OHD3: 52.6±13.5; 1.25OH2D3: 18.8±14.4.

Table 1. Mean levels (SD) at baseline and after cinacalcet administration, and percentage of patients in range. Repeated measures ANOVA P

Time	Previous 2 months	0 hours	1 hour	3 hours	6 hours	12 hours	24 hours	48 hours	P-Value
Intact PTH (pg/ml)	252.9 (80.5)	140.1 (96.7)	74.6 (42.5)	78.4 (35.1)	131.4 (94.6)	204.2 (147.9)	175.5 (118.2)	250.0 (141.7)	0.007
% pat. with PTH < 100	0	40	70	80	50	30	30	10	-
% pat. with PTH 100-400	100	60	30	20	50	50	70	70	-
% pat. with PTH >400	0	0	0	0	0	20	0	20	-
Ionic calcium (mg/dl)	8.7* (0.6)	4.96 (0.39)	5.05 (0.23)	5.05 (0.25)	5.11 (0.51)	4.87 (0.25)	5.11 (0.30)	5.03 (0.40)	NS
Phosphorus (mg/dl)	4.9 (0.9)	4.51 (0.6)	4.15 (0.52)	4.01 (0.68)	4.02 (0.64)	3.98 (0.76)	4.78 (0.94)	5.32 (1.27)	0.04
Calcitonin (pg/ml)	—	15.0 (14.1)	19.5 (18.2)	33.8 (32.9)	29.5 (32.4)	20.6 (21.3)	14.4 (14.5)	11.0 (10.1)	0.01

*Serum calcium; SD = standard deviation, NS = not significant

Conclusions: In HD pts with SHPT controlled by cinacalcet, a similar PK effect to *de novo* pts is produced, with a predictable response (reduction in PTH and P, and increase in calcitonin in the subsequent 6h with no change in Ca) and a significant increase at 48h in PTH and P and reduction in calcitonin. In pts with excessively suppressed PTH at minimum doses of cinacalcet, it could be suggested that spacing the doses might be useful.

Funding: Pharmaceutical Company Support - Amgen, S.A.

SA-PO691

Effect of Sevelamer Treatment on Fibroblast Growth Factor 23 and Pulse Wave Velocity in CKD Stage 3 Patients Annet Bouma-de Krijger, Frans J. van Ittersum, Pieter M. Ter Wee, Marc G. Vervloet. *Nephrology, VU University Medical Center, Amsterdam, Netherlands.*

Background: FGF23 is independently associated with cardiovascular outcome. In this pilot study we investigated the effect of phosphorus binding therapy on FGF23 and subsequent change in pulse wave velocity.

Methods: 24 normophosphatemic CKD stage 3 patients were treated with a fixed dose of sevelamer-carbonate 2,4 gram twice daily with their meal. Patients remained on their usual diet. Measurement of pulse wave velocity (PWV) was performed and blood samples were obtained 2 weeks prior to baseline, at start of sevelamer treatment, at 8 weeks sevelamer treatment and after a wash-out of 2 weeks. We measured 3 times PWV at each time point with the Sphygmocor® device. Measurements were valid with a SD < 10%. General Estimated Equations (SPSS) was used to evaluate the effect of phosphorus binding on the mean PWV.

Results: Of 24 patients, 2 withdrew because of hypophosphatemia (<0.7 mmol/l) and 4 because of side effects. Analysis was performed in the remaining 18 patients. In these, phosphate levels decreased not significantly during treatment from a mean of 1.12 (± 0.17) to 1.07 mmol/l (± 0.21). During treatment period phosphate excretion decreased from a median of 26,3 to 17,5 mmol/24 h (p 0.02), but not paralleled with a decline in FGF23. FGF23 decreased not significantly from a median of 169 (IQR 123-215) to 155 RU/l (IQR 113-214).

	regression coefficient (B)	p-value	explained variance (model)
PWV (m/s)			0.47
Treatment compared to baseline	-0.27	0.15	
Wash-out compared to treatment	0.27	0.32	
Female compared to male	-1.02	0.02	
Diabetes	0.42	0.34	
Age (years)	0.10	<0.001	
MAP (mmHg)	0.02	0.38	
FGF23 (RU/ml, log transformed values)	0.60	0.04	

Phosphorus binding with sevelamer showed a non-significant trend towards a decline in PWV. In additon to this finding 24 hour phosphate excretion did not influence PWV. lnFGF23 is significantly associated with PWV.

Conclusions: Phosphorus binding therapy by sevelamer leads to a decline in phosphate excretion but not to a decline in FGF23 or improved PWV. For the latter our study was most likely underpowered. However, overall FGF23 was independently associated with increased PWV, suggesting a direct effect on vascular function.

Funding: Pharmaceutical Company Support - Sanofi, the Netherlands

SA-PO692

Effect of Eldecalcitol, a New Active Vitamin D Analog, on Bone Metabolism in Postmenopausal Women Undergoing Maintenance Hemodialysis Naomi Sasaki,[1] Ryota Ikee,[2] Megumi Sato,[3] Masataka Tsunoda,[4] Nobuo Hashimoto.[1] *[1]Department of Nephrology and Dialysis, H. N. Medic, Atsubetsuchuo, Atsubetsu-ku, Sapporo, Japan; [2]H. N. Medic Kitahiroshima; [3]Jinzonaika Megumi Clinic; [4]H. N. Medic Sapporo-Higashi.*

Background: Eldecalcitol (ED) is a new agent that was developed to improve the effects on bone status while maintaining the positive effects of the conventional active vitamin D on Ca metabolism.In cases of primary osteoporosis, ED is known to dose-dependently increases bone density in the lumbar vertebrae while reducing the frequency of new vertebral fractures, and has attracted attention as new therapeutic drug for the

treatment of osteoporosis.However, there has been no report examining the effects of ED treatment on bone metabolism in patients with ESRD to date. We examined the safety and effects on bone metabolism of ED treatment in postmenopausal female patients undergoing maintenance dialysis.

Methods: Forty postmenopausal women who had received maintenance dialysis for at 6 months at our institution were enrolled. Patients with an average Ca(Alb) level of more than 9.5mg/dL prior to ED treatment were excluded. ED (0.5μg/day) was added to the existing treatment regiment, and patients were followed for 6 months after the onset of treatment.

Results: 1) Excluding 4 subjects who changed hospital and 4 subjects removed from the study, 32 subjects were followed-up for 6 months after ED treatment. The reason for removal of the 4 subjects was hypercalcemia (≥11.0), constipation, diarrhea and skin irritation, respectively.

2) A comparison of mean values for the 6 months before treatment and 6 months after ED treatment.

Effects on Bone Metabolism

	Pre-treatment	Post-treatment	
Ca(Alb)	9.0±0.3	9.5±0.5	0.001*
intPTH	108±57	80±56	0.004*
BSAP	133±79	88±45	0.006*
TRACP-5b	659±346	485±266	0.02*
LV-BMD	0.501±0.108	0.525±0.015	0.06
LV-Tscore	66.1±14.3	69.5±16.0	0.04*

LV; Lumber vertebrae, mean±SD

Conclusions: We followed-up postmenopausal woman undergoing maintenance dialysis for 6 months after the onset of ED treatment. ED treatment did not result in severe hypercalcemia and was continued. In addition a significant reduction in a bone metabolism marker and increase in lumbar vertebrae bone density were observed.

SA-PO693

PA21, a Novel Iron-Based Phosphate Binder, Has High Phosphate Binding Capacity and Low Iron Release In Vitro across a Physiological pH Range

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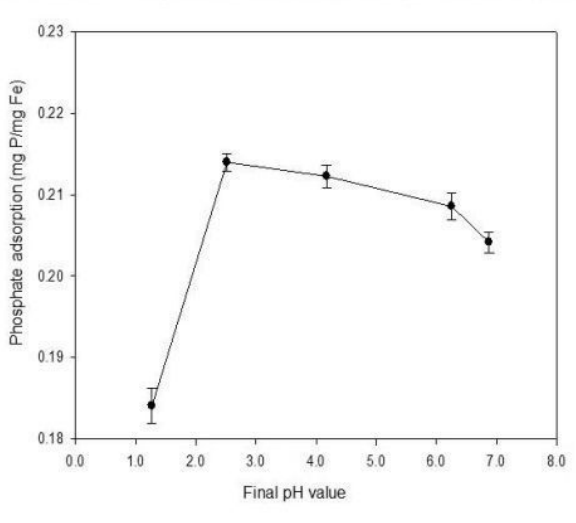
Background: Management of hyperphosphatemia in chronic kidney disease can be challenging due to limitations of current treatments, e.g., high pill burden and poor tolerability. The novel iron-based phosphate binder PA21 is being developed and may provide an alternative treatment for hyperphosphatemia. This *in vitro* study investigated phosphate binding capacity and iron release of PA21 at pH values found in the gastrointestinal (GI) tract.

Methods: PA21 (90mM Fe) was incubated with phosphate buffer (36mM) in an aqueous solution at pH 1.2 for 1 hr. The solution was subsequently adjusted to pH 2.5 for 1 hr, 4.5 for 1.5 hrs, 7.0 for 2 hrs, and 7.5 for 2.5 hrs. After each timepoint, phosphate adsorption (difference between amount of phosphate added and the amount not adsorbed) and final pH of samples were measured. Phosphorus concentration was determined photometrically as molybdenum blue at 740nm. Additionally, iron release was determined by inductively-coupled plasma optical emission spectrometry in samples prepared at pH 1.2, 1.7 and 2.1.

Results: Phosphate adsorption was lowest (0.184 mg P/mg Fe) at pH 1.3, peaked (0.214 mg P/mg Fe) at pH 2.5, then declined slightly with increasing pH (Figure 1). Iron release peaked (6.2%) at a final pH of 1.2 (initial pH: 1.2). Iron release was 1.3% at a final pH of 2.5 (initial pH: 1.7) and was not detectable at a final pH of 5.6 (initial pH: 2.1).

Conclusions: PA21 showed a high phosphate binding capacity even at low pH, and robust phosphate binding capacity at higher pHs. Iron release was negligible except at the lowest pH (1.2), representative of the fasting state of the stomach. These characteristics of PA21, observed *in vitro*, indicate that it could be a potent phosphate binder that minimizes the risk of systemic effects in patients with hyperphosphatemia.

Figure 1: PA21 binding capacity during a simulated passage through the GI tract



Funding: Pharmaceutical Company Support - Vifor (International) Inc.

SA-PO694

Indoxyl Sulfate Induces Oxidative Stress and Apoptosis in Cultured Osteoblast Cells

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Background: Indoxyl sulfate (IS) is an organic anion uremic toxin. IS accumulates in the blood and promotes the progression of renal dysfunction. Recently, a few reports have suggested that IS inhibits osteoblastic cell function, suppresses parathyroid hormone (PTH)-stimulated intracellular cAMP production, and decreases PTH receptor expression. However, whether IS suppresses osteoblastic cell differentiation and induces apoptosis is still unclear. In the present study, we investigated whether IS induces apoptosis and inhibits differentiation in cultured osteoblast cells.

Methods: We examined the cytotoxicity of IS in MC3T3-E1 cell line (newborn mouse calvaria-derived preosteoblast cell line) by MTT assay. To determine whether IS inhibits the differentiation of MC3T3-E1, real time PCR (type I collagen, osteonectin) was performed and alkaline phosphatase (ALP) activity was measured. Generation of reactive oxygen species (ROS) was measured using 5-(and-6)-Carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCF-DA). To investigate IS-induced apoptosis, we performed fluorescence-activated cell sorting (FACS) and measured caspase 3/7 activity.

Results: IS showed cell toxicity in a dose-dependent pattern (0.1, 0.5, 1, 1.5 mM, 72 h) and suppressed ALP activity in dose- and time-dependant patterns in the differentiated osteoblast cells. Real time PCR for collagen I and osteonectin showed that IS suppressed the expression of these genes at doses above 0.5 mM at day 5. The expression of these genes was not supressed at days 1 and 3 in the presence of IS. These findings suggest that IS inhibits the differentiation of osteoblast cells. Further, IS provoked ROS in a dose-dependent pattern as found using DCF-DA. Caspase 3/7 activity peaked at 6 h (IS: 1mM). Antioxidant (n-acetylcysteine) attenuated caspase 3/7 activity that was induced by IS. IS caused apoptosis at doses above 0.1 mM during 24 h (0.1 mM, 63.02%), as observed by FACS.

Conclusions: IS induces ROS and apoptosis in cultured osteoblast cells. In addition, IS inhibits differentiation of osteoblast cells.

SA-PO695

p-Cresyl Sulfate Deteriorates Osteoblast Function through Up-Regulating MAPK Cascade

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Background: Accumulation of uremic toxins (UTx) such as Indoxyl sulfate (IS) is not only a key player in progression of kidney dysfunction, but also a leading cause of various complications in chronic kidney disease (CKD). p-Cresyl sulfate (PCS), the main metabolite of p-cresol, predicts the risk of cardiovascular and all-cause mortality in patients with CKD through mechanism not fully understood yet. In order to prove possible contribution of PCS on bone metabolism in CKD, we analyzed the toxic effects of PCS on osteoblasts in comparison with IS.

Methods: Primary osteoblastic cells from mouse calvariae were exposed with PCS (0 to 0.5mmol/L) for 24hr, and stimulated by 10^{-8}mmol/L parathyroid hormone (PTH) for 10min. We evaluated intracellular cAMP production, reactive oxygen species (ROS) production, cell viability, and amount of DNA fragmentation. MAPK signaling pathways were also determined.

Results: PTH-induced intracellular cAMP production of osteoblasts decreased in the presence of 0.125-0.5mmol/L PCS. Increased DNA fragmentation and decreased cell viability were observed with PCS treatment above 0.125mmol/L. On the other hand, IS did not suppress the PTH response of osteoblasts until at a dose of 2mmol/L and decreased cell viability at the concentration range of 0.5-2mmol/L. IS demonstrated significant increase in ROS production at 0.25mmol/L, while PCS demonstrated that only at 0.5mmol/L. We also observed that PCS induced phosphorylated JNK, a MAP kinase protein.

Conclusions: PCS deteriorated osteoblast function through the activation of MAPK cascade in addition to ROS production. These findings suggest that PCS plays significant roles in abnormal bone metabolism in CKD through mechanisms different from those of IS.

SA-PO696

Falsely Low Parathyroid Hormone Levels in Dialysis Patients Receiving Biotin Supplements

Anuradha Wadhwa, Vinod K. Bansal, Earle W. Holmes. *Loyola University, IL.*

Background: Biotin has been identified as a potential cause of falsely suppressed parathyroid hormone (PTH) levels in samples measured using "classic" Siemens Advia Centaur PTH assay (CLPTH). We compared PTH results measured by CLPTH with the manufacturer's new enhanced PTH assay that is free from biotin interference (ENPTH) and also evaluated the clinical significance of inter-assay differences in PTH results.

Methods: Samples from 128 dialysis subjects taking standard doses of renal vitamin supplements were tested by CLPTH and ENPTH methods. Four patients that showed the largest inter-method differences were further evaluated for potential analytical interference by heterophile antibodies to goat IgG, and by substances that bind to streptavidin (e.g.

biotin). Each patient's PTH results were interpreted relative to KDOQI 2002 Guideline (target: 150-298 pg/mL) and Commentary 2010 (target: 2-9 times ULN of the assay).

Results: 37 of 128 (29%) patients had PTH values that were decreased by > 10% when assayed by CLPTH as compared to ENPTH (median decrease: 20 %; range: 11% -98%). Four samples with largest inter-method differences were free from interference by heterophile antibodies. Pretreatment of these specimens with streptavidin prior to re-assay by the CLPTH method resulted in an increase in the PTH values, suggesting that the interfering substance inhibited the binding of the biotinylated capture antibody to streptavidin. Evaluation of the inter-assay differences relative to the PTH targets in the KDOQI guidelines and the commentary 2010 showed that the negative interference in the CLPTH would have affected the clinical interpretation of results in as many as 8 (6 %) and 7 (5 %) cases, respectively. The prevalence of biotin interference in our patients greatly exceeded the expected 0.5% rate that was reported by the manufacturer.

Conclusions: An interfering substance (presumably biotin) is present in the plasma of dialysis patients taking standard doses of commonly used vitamins at levels sufficient to cause significant negative interference in the CLPTH assay. The resulting false decreases in PTH results had the potential to adversely affect the management of Secondary hyperparathyroidism.

SA-PO697

The Influence of Different Heparins and Sulodexide on Concentration and Gene Expression of OPG/RANK/RANKL Pathway in Human Umbilical Vein Endothelial Cells (HUVECs) Beata Naumnik, Katarzyna Gasowska, Michal Mysliwiec. *Dept. of Nephrology and Transplantation with Dialysis Unit, Medical University of Bialystok, Bialystok, Poland.*

Background: OPG/RANK/RANKL (Osteoprotegerin/Receptor Activator of Nuclear Factor κB/Receptor Activator of Nuclear Factor κB Ligand) system plays an important role in vascular calcification. Heparins and others glycosaminoglycans, interact with OPG, may influence the process of atherogenesis. Von Willebrand Factor (vWF) functionally connected with OPG in Weibel-Palade bodies, presents as well-known endothelial dysfunction marker.

Methods: HUVECs were obtained as described by Jaffa. The cultures were supplemented with unfractionated heparin (UFH), enoxaparin and sulodexide. The concentrations of OPG, sRANKL and vWF were determined (ELISA) in supernatant from the culture before and after drugs' stimulation. To determine the genes expression of all examined factors in endothelial cells, the Real Time-PCR reaction was carried out.

Results: We found 1.5 fold increase in OPG gene expression after 1 hour of incubation ($p=0.027$) with 4 μg/ml of UFH, followed by increase of OPG concentration after 6 hours of incubation. UFH and sulodexide provoked fast increase of sRANKL concentration, but only UFH increased RANKL gene expression after 1 h of incubation ($p=0.042$). vWF level raised accordingly with UFH concentration ($p<10^{-3}$) as well as the duration of incubation ($p=0.006$) and was positively correlated with OPG increase. Sulodexide induced the 2.5 fold increase of vWF gene expression during the second hour of incubation ($p<10^{-3}$).

Conclusions: UFH occurred to be the most potent in releasing OPG, sRANKL and vWF from HUVECs. Similarly, the increase of OPG and RANKL genes expression was observed only with UFH supplementation. The positive correlation between the increase in vWF and OPG after UFH administration reinforces the presumption of the pathophysiological linkage between OPG/RANK/RANKL axis and vascular calcification.

Funding: Government Support - Non-U.S.

SA-PO698

Two Novel Vitamin D Receptor Modulators of Similar Structures Exhibit Distinctly Different Hypercalcemic and Cardiovascular Effects in 5/6 Nephrectomized Uremic Rats J. Ruth Wu-Wong, Megumi Kawai, Yung-wu Chen, Masaki Nakane. *Vidasym, Chicago, IL.*

Background: Vitamin D receptor modulators (VDRMs) such as calcitriol, paricalcitol and doxercalciferol are commonly used to manage secondary hyperparathyroidism in chronic kidney disease (CKD). Clinical observations demonstrate that VDRM therapy may provide cardiovascular benefit in CKD. Current on-market VDRMs have a narrow therapeutic index (TI) at 1-4-fold (estimated from the hypercalcemic toxicity and PTH suppressing efficacy). Hypercalcemia is a serious concern, leading to the need for frequent drug dose titration and serum calcium monitoring. A VDRM with expanded TI and cardiovascular benefit will be desirable. However, it is not well understood why structurally similar VDRMs exhibit different hypercalcemic profile and how hypercalcemia affects VDRM's cardiovascular effect.

Methods: We studied two novel VDRMs with similar structures (one difference in the A-ring) in the 5/6 nephrectomized (NX) male Sprague–Dawley rats with established uremia, elevated parathyroid hormone (PTH), endothelial dysfunction and left ventricular (LV) hypertrophy.

Results: VS-110 and VS-411 at 0.01-1 μg/kg (i.p. 3x/week for two weeks) suppressed serum PTH in the 5/6 NX rats effectively. VS-411 potently raised serum calcium in a dose-dependent manner with 11% increase in serum calcium at 0.01 μg/kg (TI = ~1-fold), while VS-110 didn't raise serum calcium even at 1 μg/kg (TI > 50-fold). When the 5/6 NX uremic rats were treated with VS-110 or VS-411 (0.01- 0.1 μg/kg) for two weeks, VS-110 improved endothelium-dependent aortic relaxation and reduced LV hypertrophy in a dose-dependent manner without affecting serum calcium. VS-411 also exhibited effects on the cardiovascular parameters, but was less potent. VS-110 and VS-411 induced HL-60 differentiation with EC_{50} at 0.8 and 0.3 nM (vs. calcitriol at 13.9 nM), respectively. Both VS-110 and VS-411 inhibited the proliferation of primary human keratinocytes with IC_{50} at 1.40 and 0.04 nM (vs. calcitriol at 10 nM), respectively.

Conclusions: These studies demonstrate that structurally similar VDRMs can exhibit distinctly different hypercalcemic and cardiovascular effects in 5/6 NX uremic rats.

SA-PO699

Fibroblast Growth Factor 23, Parathyroid Hormone and Phosphate Excretion in CKD Stage 3 Maarten W. Taal,[1] Victoria Thurston,[1] Natasha J. Mcintyre,[2] Nigel Lawson,[1] Richard J. Fluck,[1] Chris W. McIntyre.[2] *[1]Department of Nephrology, Royal Derby Hospital, Derby, United Kingdom; [2]School of Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom.*

Background: Fibroblast growth factor (FGF)23 has been identified as an important regulator of phosphaturia that becomes elevated early in CKD to increase urinary fractional excretion of phosphate (FePhos) and maintain phosphate balance. We aimed to investigate FGF23 and PTH levels in relation to GFR and FePhos in persons with predominantly early CKD stage 3.

Methods: Serum intact FGF23 (Kainos ELISA) and PTH (Roche E170) were measured in 1667 persons with estimated GFR 59-30ml/min/1.73m^2 recruited from 32 Primary Care Practices. Detailed medical history and clinical assessment were peformed as well as urine and serum biochemistry tests.

Results: Median values for key variables were: age 74(IQR 67 to 79)y, eGFR 53(46 to 60)ml/min/1.73m^2, PTH 46(34 to 66)pg/ml, FGF23 42(33 to 53)pg/ml, FePhos 23(19 to 29)%. PTH and FGF23 were elevated in similar proportions of participants at all levels of GFR (Table). Multivariable analysis identified GFR, serum calcium, phosphate, logPTH, uric acid concentrations as well as log urine PCR and BMI as independent determinants of serum FGF23 concentration (adjusted R^2=0.22). Both log serum PTH and FGF23 concentration were independent determinants of FePhos, as well as age, gender, diabetes, current smoking status, BMI, eGFR, Hb, HDL cholesterol and log hsCRP (adjusted R^2=0.33).

GFR bands	Both normal	FGF23 ⇑	PTH ⇑	Both ⇑	Totals
20-29ml/min	4 (15%)	9 (35%)	2 (8%)	11 (42%)	26
30-39ml/min	43 (24%)	51 (28%)	32 (18%)	54 (30%)	180
40-49ml/min	198 (46%)	92 (21%)	84 (19%)	57 (13%)	431
50-59ml/min	380 (61%)	113 (18%)	89 (14%)	39 (6%)	621
60-69ml/min	260 (76%)	34 (10%)	38 (11%)	9 (3%)	341
70-79ml/min	49 (79%)	6 (10%)	6 (10%)	1 (2%)	62
80-89ml/min	4 (67%)	1 (17%)	1 (17%)	0	6

Conclusions: FGF23 and PTH become elevated in CKD stage 3 and together mediate an increase in FePhos. Multiple factors in addition to GFR and serum phosphate are independent determinants of FGF23 concentration. Further research is required to identify factors that provoke a rise in FGF23 as well the relative importance of FGF23 and PTH in increasing FePhos.

Funding: Pharmaceutical Company Support - Sanofi; Roche, Private Foundation Support

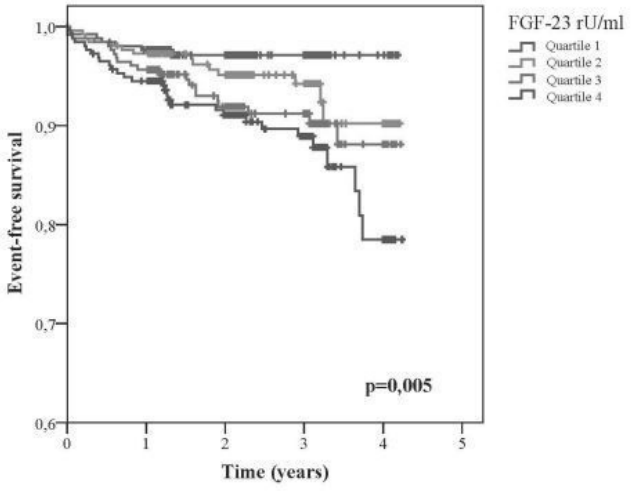
SA-PO700

Increased FGF-23 Levels Are Predictors of Cardiovascular Morbidity and Mortality in Subjects with Intact Renal Function-The HOM SWEET HOMe Study Sarah Seiler,[1] Bodo Cremers,[2] Philipp Ege,[1] Michael Fehrenz,[1] Gunnar Max Große-dunker,[1] Florian Hornof,[1] Niko Rebling,[1] Kyrill S. Rogacev,[1] Bruno Scheller,[2] Michael Böhm,[2] Danilo Fliser,[1] Gunnar H. Heine.[1] *[1]Internal Medicine IV, Saarland University Medical Center, Homburg, Saar, Germany; [2]Internal Medicine III, Saarland University Medical Center, Homburg, Saar, Germany.*

Background: Our HOM SWEET HOMe study aimed to investigate cardiovascular (CV) implications of elevated FGF-23 in subjects with intact renal function. We now report first data on the predictive value of FGF-23 levels on CV morbidity and mortality in HOM SWEET HOMe participants.

Methods: We recruited 1309 subjects admitted at Saarland University Hospital for elective coronary angiography between May 2007 and January 2010. At baseline, we recorded classical cardiovascular risk factors and comorbidity; blood samples were obtained for the measurement of C-terminal FGF-23 levels. Patients with eGFR < 60 ml/min/1.73 m² were excluded from this analysis. We assessed the occurrence of the predefined primary endpoint (acute myocardial infarction, CV death and non-hemorrhagic stroke) and of secondary endpoints (CV events; death from any cause) by yearly follow-up interviews.

Results: 1026 from 1309 patients had an eGFR > 60 ml/min/1.73 m², their mean age was 63 ± 10 years, and 513 patients had coronary artery disease at baseline. During a follow-up period of 2.4 ± 1.0 years, patients in the highest FGF-23 quartile reached the predefined primary ($p = 0.005$) and secondary (CV events $p = 0.041$; death from any cause $p = 0.008$) endpoints more often than patients in lower quartiles.



Conclusions: In line with recent data on a direct cardiotoxic effect of FGF-23, our study found a prognostic impact of elevated FGF-23 levels in subjects with intact renal function. These results suggest that FGF-23 might become a new therapeutical target even in the absence of CKD.

SA-PO701

FGF-23: The New HbA1C of Phosphate Homeostasis? Sarah Seiler, Gaetano Lucisano, Philipp Ege, Matthias Klingele, Anne Lerner-Gräber, Danilo Fliser, Gunnar H. Heine. *Internal Medicine IV, Saarland University Medical Center, Homburg, Saar, Germany.*

Background: It has recently been claimed that the phosphaturic hormone FGF-23 better mirrors long-term systemic phosphate burden than a single phosphate measurement, similar to glycosylated haemoglobin (HbA1c) which reflects average blood glucose levels.

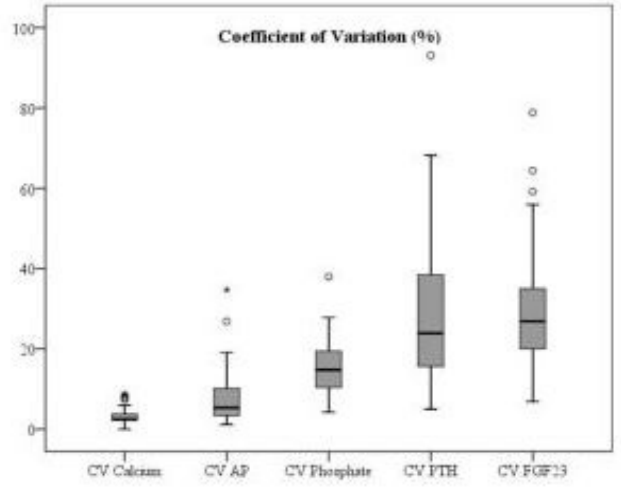
Against this background, we conducted the DIAL HOMe (Dialysis in Homburg Evaluation) study to test the following hypotheses:

1) Plasma FGF-23 values are more strongly associated with average phosphate value over the previous four weeks than with a single random plasma phosphate measurement.

2) Compared to conventional parameters of calcium phosphate metabolism, repeated measurements of plasma FGF-23 have a lower coefficient of variation.

Methods: We included 40 hemodialysis patients into a prospective cohort study. During the study period of four weeks, dialysis dosage, dialysate calcium concentration and co-medication (phosphate binder, calcimimetics, vitamin D, iron) was kept stable. Plasma calcium and phosphate levels were measured at the beginning of each dialysis session, and FGF-23, alkaline phosphatase and parathyroid hormone levels were measured once weekly.

Results: Expectedly FGF-23 correlated with the average phosphate value of the previous four weeks ($r=0.586$; $p<0.01$). However, in contradiction to our hypothesis, this correlation did not significantly differ from the correlation of FGF-23 with a single random phosphate value ($r=0.551$; $p<0.01$). Furthermore, intraindividual stability of FGF-23 was not superior to stability of conventional markers of calcium phosphate metabolism (phosphate, calcium, parathyroid hormone, alkaline phosphatase; [Figure 1]).



Conclusions: In our DIAL HOMe study FGF-23 was neither a specific marker for long term phosphate burden of our patients, nor did it display a higher intraindividual stability than other markers of calcium phosphate metabolism.

SA-PO702

Fibroblast Growth Factor Receptor 1 and Fibroblast Growth Factor Receptor 4 Are the Predominant Receptors for Fibroblast Growth Factor 23 Jyothsna Gattineni,[1] Carlton M. Bates,[2] Michel G. Baum.[1] *[1]Pediatric Nephrology, UT Southwestern Medical Center, Dallas, TX; [2]Pediatric Nephrology, Childrens Hospital of Pittsburgh, Pittsburgh, PA.*

Background: FGF23 is a phosphaturic hormone that is implicated in many hypophosphatemic and hyperphosphatemic disorders. We have previously shown that at baseline individual FGFR1(kidney conditional), FGFR3 and FGFR4 mice had serum phosphorus levels comparable to wild type mice indicating that there is more than one receptor regulating the phosphaturic actions of FGF23. FGFR1 and FGFR4 null mice had ~1.4 times higher serum FGF23 levels suggesting that these receptors are important for the phosphaturic actions of FGF23. Therefore, we studied FGFR1 (kidney conditional) FGFR4 double mutant mice (DKO).

Methods: DKO mice were generating by using the lox-p cre technique. Mice were placed on a phosphate depleted diet for 5 days to study the role of phosphate in the regulation of FGF23.

Results:

Baseline Parameters of Wild Type and DKO Mice

	Wild Type	DKO
Serum FGF23 (pg/ml)	121.3± 6.7	3762 ± 523.5*
Serum Phosphorus (mg/dl)	7.1 ± 0.2	9.2 ± 0.3*
FePhos	6.9 ± 1.5	5.6 ± 0.5
BBM NaPi-2a/β-actin expression	0.6 ± 0.1	0.6 ± 0.2
BBM NaPi-2c/β-actin expression	0.5 ± 0.1	0.7 ± 0.1
Serum PTH (pg/ml)	143.0 ± 26.7	66.4 ± 20.7#
Serum 1,25 Vitamin D (pmol/L)	132.8 ± 9.2	182.5 ± 22.4#

*$p<0.001$; #$p<0.05$

Conclusions: Basal serum phosphorus levels were higher in DKO mice despite a 30 fold higher FGF23 levels in DKO mice compared to wild type mice. Basal BBM NaPi-2a, NaPi-2c expression and FePhos was comparable in DKO and wild type mice showing a resistance to the phosphaturic actions of FGF23. DKO mice have elevated 1,25 vitamin D levels indicating that FGFR1 and R4 also play a role in vitamin D homeostasis. To study the role of serum phosphorus in regulating FGF23 levels, DKO and wild type mice were placed on a LP diet. On an LP diet, serum phosphorus decreased and was comparable in both sets of mice {WT 3.6 ± 0.4 vs 4.0 ± 0.4} but FGF23 levels remained significantly elevated in DKO mice {8.2 ± 3.6 vs 510.1 ± 215.7*, $p<0.05$}. Despite being hypophosphatemic on LP diet, DKO have elevated FGF23 levels. We conclude that FGFR1 and FGFR4 are the principal receptors regulating the phosphaturic actions of FGF23.

Funding: NIDDK Support, Private Foundation Support

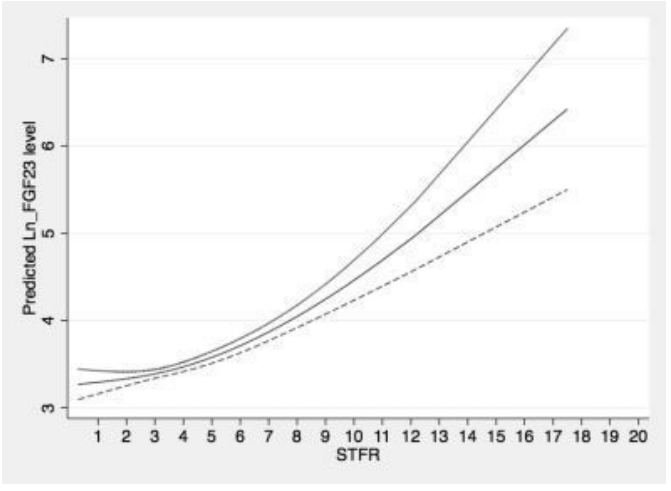
SA-PO703

Serum FGF-23 Levels Are Independently Associated with Markers of Iron Metabolism in Kidney Transplant Recipients Istvan Mucsi,[1,2] Miklos Zsolt Molnar,[2,3] Akos Ujszaszi,[2] Myles S. Wolf.[4] *[1]McGill University Health Centre, Montreal, QC, Canada; [2]Semmelweis University, Budapest, Hungary; [3]Harbor-UCLA Medical Center, Torrance, CA; [4]University of Miami Miller School of Medicine, Miami, FL.*

Background: Recent reports suggested a mechanistic link between iron metabolism and circulating levels of FGF-23. We analyzed the association between markers of iron metabolism versus FGF23 levels in kidney transplant recipients.

Methods: Data from 984 Tx recipients (mean age 51±13 years, 57% males, mean eGFR 51±21 ml/min/1.73m2, median Tx vintage 72 months) were analyzed. Serum FGF-23 was measured using a C-terminal enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). Iron metabolism was characterized by serum iron, transferrin saturation, serum ferritin and also serum soluble transferrin receptor (STFR) levels. eGFR was calculated using the 4-variable MDRD equation.

Results: Age, gender and eGFR adjusted FGF23 levels were weakly and negatively correlated with serum iron and transferrin saturation ($r=-0.079$ and $r=-0.083$, respectively; $p<0.05$). Serum ferritin was negatively ($r=-0.170$, $p<0.001$), STFR positively correlated ($r=0.262$, $p<0.001$) with serum FGF23. In a multivariable linear regression model STFR remained significantly associated with logarithmically transformed serum FGF23 levels (beta=0.200, $p<0.001$), after adjusting for age, gender, eGFR, comorbidity, BMI, Hb, serum albumin, Ca, PO4, PTH, CRP, interleukin 6, TNF-alpha, erythropoietin levels and erythropoietin treatment).



Association between STFR and ln_FGF23 levels (cubic spline model).

Conclusions: Lower levels of iron stores or iron availability are associated with higher serum C-terminal FGF23 levels. These results are compatible with the hypothesis that iron deficiency may modulate FGF23 metabolism in kidney transplant recipients.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO704

Serum FGF23 Levels Are Indepently Associated with Serum Asymmetric Dimethylarginine (ADMA) Levels in Kidney Transplant Recipients

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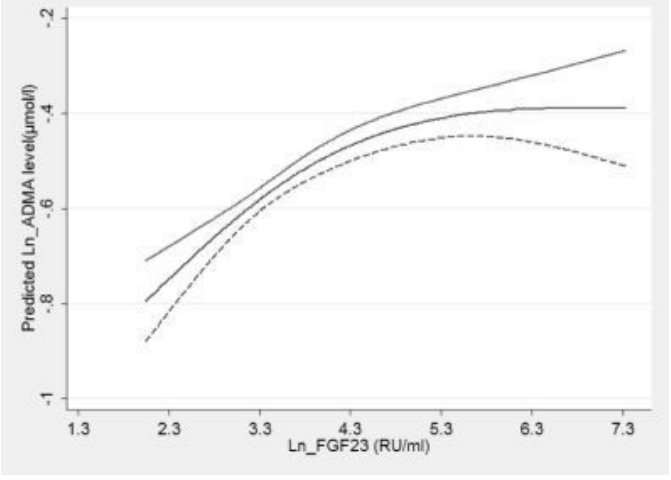
Background: FGF23 may interfere with vascular reactivity induced by the nitric oxide (NO) system. We assessed if serum FGF23 is associated with serum ADMA in stable kidney transplant recipients.

Methods: Data from 258 kidney transplant recipients (age 54+12, mean eGFR 42+21 ml/min/1.73m2) followed at a single transplant center were analyzed. Serum FGF23 was measured using a C-terminal enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). ADMA was determined using liquid chromatography-mass spectrometry. Variables with non-normal distribution were natural log-transformed for the analyses.

Results: Serum FGF23 levels positively correlated with serum ADMA in bivariate Pearson correlation analysis (r=0.385, p<0.001). In a multivariable linear regression model serum FGF23 remained independently associated with serum ADMA levels (beta=0.157, p=0.013) (adjusted for age, gender, serum albumin, eGFR, Charlson Comorbidity Index, systolic blood pressure, serum phosphate, BMI, total cholesterol and serum PO4).



Association between ln_FGF23 levels and ln_ADMA (cubic spline model)

Similar independent association was seen in a model using L-arginin/ADMA as the dependent variable.

Conclusions: Higher serum FGF23 is independently associated with higher serum ADMA levels in stable kidney transplant recipients. These results are compatible with the hypothesis that higher serum FGF23 levels may be associated with endothelial dysfunction in this patient population.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO705

High Serum Phosphate and Vitamin D Deficiency Enhance TACE Activation and TNFα Release in Vascular Smooth Muscle Cells

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Background: High serum phosphate (HP) and vitamin D (VitD) deficiency are associated with a higher risk of cardiovascular (CV) mortality. Increases in serum TNFα, a main cause of systemic inflammation and CV lesions, require enhanced activation of TNFα converting enzyme (TACE). Because parathyroid TACE activity is strongly induced by HP and suppressed by VitD receptor (VDR) activation, we examined whether HP could initiate CV lesions through a distinct up-regulation of TACE activation and TNFαrelease in aortic vascular smooth muscle cells (VSMC) from wild type (wt) and VDR-null mice.

Methods: Changes in TACE expression, subcellular location, and activity were measured in primary cultures of VSMC exposed for 8h to: a) Normal (0.9 mmol/L) or high (3.3 mmol/L) P; b) Lipopolysaccharide (LPS, 100 μg/ml), a known inducer of TACE/TNFα signals, or c) HP with 25-hydroxyvitaminD (10^{-10}M), paricalcitol (10^{-8}M) or both.

Results: In VSMC from wt mice, HP increased cytosolic levels of pro-TACE, inactive TACE, and activated TACE. HP also increased by 2.2±0.2 fold the translocation of active TACE to the cell membrane to release TNFα, as strongly as LPS (2.1±0.19).In VSMC from the VDR-null mice, basal TACE content and activity at the cell surface was 1.8±0.2 fold higher than in wt cells, and was more robustly enhanced by HP and LPS. Accordingly, VDR activation effectively attenuated HP-induced TACE/TNFα signals only in wt cells. Importantly, the 25-hydroxyvitaminD+paricalcitol combination was more potent than either monotherapy.

Conclusions: A cause-effect link between HP and CV damage is that HP enhances TACE/TNFα signals in VSMC as strongly as LPS. Similarly, the CV protective actions of VDR activators could be partially accounted for by their efficacy to attenuate TACE/TNFα signals in VSMC.

** PV is a recepient of an ERA-EDTA fellowship.*

Funding: Government Support - Non-U.S.

SA-PO706

Arterial Klotho Is Regulated via a HSP72-SRF-Dependent Pathway and Is an Obligate Transducer of HSP72 Anti-Calcific Effects

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Background: Klotho is a 130kDa transmembrane protein that is expressed in arterial smooth muscle cells. We previously showed that arterial Klotho deficiency under pro-calcific environments in CKD results in accelerated calcification and causes resistance to anti-calcific effects of the circulating phosphatonin, FGF-23. Heat shock protein (HSP)-72 is an inducible chaperone that can inhibit the development of arterial calcification. Here, we postulate that regulation of vascular health involves cross-talk between Klotho and HSP72.

Methods: *In vitro* model: Human aortic smooth muscle cells (HA-SMCs) +/- Klotho, HSP72 or SRF siRNA. Induction of HSP72 was achieved by heat shock treatment (HST) at 42°C for 30 minutes. Assessment of calcification was achieved by Alizarin red and Arsenazo III method.

Results: We show for the first time that suppression of vascular Klotho under pro-calcific stress (2.7mM CaCl2 and 2mM β-glycerophosphate) can be reversed by induction of HSP72 by HST. These effects were mitigated by HSP72 siRNA. Suppression of vascular Klotho by pro-calcific stress was associated with osteogenic transformation as demonstrated by upregulation of bone phenotypic regulators Runx2 and ALP and dedifferentiation of SMC phenotype as shown by downregulation of serum response factor (SRF), myocardin and α-SMA. Since SRF is a master regulator of SMC contractile genes, we next investigated whether SRF could regulate vascular Klotho expression. SRF suppression by the chemical inhibitor CCG-1423 and SRF siRNA, resulted in loss of Klotho expression. Furthermore, we found HSP72-SRF and SRF-Klotho protein-protein interaction following induction of HSP72 by HST. Finally, induction of HSP72 inhibited the development of HA-SMC calcification and these effects were mitigated by both HSP72 siRNA and Klotho siRNA.

Conclusions: Arterial Klotho is regulated by HSP72 and SRF. Cross-talk between Klotho and HSP72 was mediated by protein-protein interaction with SRF. Arterial Klotho functions as an obligate transducer of HSP72 calcification inhibitory effects.

Funding: Private Foundation Support

SA-PO707

Erythropoietin Enhances 25-Hydroxy-Vitamin D in Rats with Renal Mass Reduction

Tsuneo Takenaka, Tsutomu Inoue, Yusuke Watanabe, Takashi Miyazaki, Hiromichi Suzuki. *Saitama Medical University, Iruma, Saitama, Japan.*

Background: We showed that erythropoietin (EPO) doses correlated to 1,25-dihydroxy-vitamin D (1,25VD) in hemodialysis patients (Int Urol Nephrol 2003;35:407-13). An activation of EPO receptors stimulates JAK/STAT system to induce erythropoiesis. Enzymatic conversion from vitamin D to 25-hydroxy-vitamin D (25VD) occurs in the liver. To date, 6 cytochromes (CYP2J3, 3A2, 27A1, 2C11, 2R1 and 2D25) are known to be involved in conversion to 25VD. In uremia, CYPs including CYP27A1 and CYP2C11 are reduced in liver. Growth hormone transduces its signal through JAK/STAT pathway

to up-regulate hepatic CYP2C11. However, the influences of EPO on 25-hydroxy-vitamin D and CYPs have not been examined.

Methods: In the present study, the influence of human recombinant EPO (300 units/kg/week for 8 weeks) on CYPs and 25VD were assessed in control Wister (n=5) and 5/6 nephrectomized rats treated with (n=5) and without EPO (n=5). Real time RT-PCR was used to evaluate CYPs with GAPDH as a house keeper.

Results: Hematocrit was decreased in uremic rats (48±1 vs 22±1%, p<0.01), and EPO reversed renal anemia (to 30±2%, p<0.05). Furthermore, 25VD (75±2 vs 17±3 ng/ml, p<0.01), 1,25VD (140±34 vs 26±7 pg/ml, p<0.05), hepatic CYP2C11 (0.42 fold, p<0.05) and CYP27A1 (0.43 fold, p<0.05) were lower in rats with reduced renal mass than the control. The administration of EPO reversed the decrements of 25VD (to 29±4 ng/ml, p<0.01) and 1,25VD (to 68±14 pg/ml, p<0.05) in rats with renal insufficiency. In addition, EPO restored CYP2C11 expression to the control level, but CYP27A1 remained reduced. However, EPO altered serum levels of neither parathyroid hormone nor insulin-like growth factor. Although hepatic JAK2 was increased by 2.8 fold (p<0.05) in uremic animals, EPO reduced JAK2 (0.03 fold, p<0.01). Surprisingly, EPO receptors were detectable in uremic liver.

Conclusions: Our data indicate that the administration of EPO increased 25VD in 5/6 nephrectomized rats. In addition, the present results implicate that EPO elicited an induction of CYP2C11 independently of JAK/STAT pathway. Finally, our findings suggest that EPO may have direct action on hepatic cells in uremia.

Funding: Government Support - Non-U.S.

SA-PO708

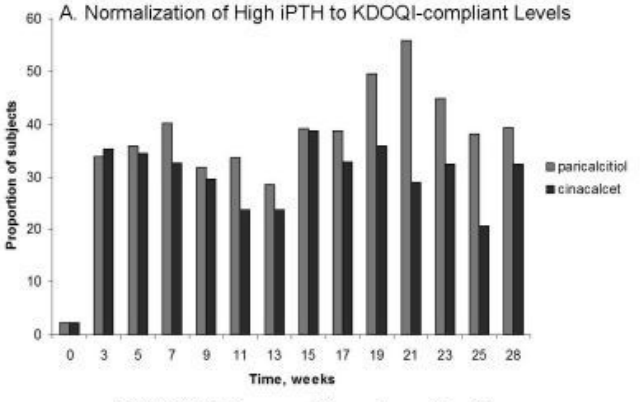
Paricalcitol Compared to Cinacalcet for Treatment of Secondary Hyperparathyroidism (SHPT): Analyses of the IMPACT Study

Markus Ketteler,[1] Kevin J. Martin,[2] Michael Amdahl,[3] Mario Cozzolino,[4] David Goldsmith,[5] Amit Sharma,[6] Samina Khan.[3] *[1]Klinikum Coburg, Coburg, Germany; [2]St. Louis Univ, St. Louis, MO; [3]Abbott, Abbott Park, IL; [4]Univ of Milan, Milan, Italy; [5]Guy's Hospital, London, United Kingdom; [6]Pacific Renal Research Inst, Meridian.*

Background: KDIGO and KDOQI guidelines define target iPTH levels and advise measuring calcium (Ca), phosphorus (P), alkaline phosphatase (AP) and bone-specific AP (BSAP). AP and BSAP are markers of bone turnover; elevated levels are linked to increased mortality. Vitamin D receptor activator (VDRA) therapy is linked to reduced mortality; however, use of non-selective VDRAs leads to an increase serum Ca. Paricalcitol, a selective VDRA, lowers iPTH with minimal impact on Ca and P.

Methods: The IMPACT study compared IV or oral paricalcitol to cinacalcet with low-dose vitamin D. Subjects were initially analyzed by oral and IV randomization strata; for these post-hoc analyses strata were combined to compare overall efficacy of paricalcitol to cinacalcet. iPTH, Ca and P were measured bi-weekly from weeks 3-25 and at week 28.

Results: There were 134 subjects in each treatment group. A greater proportion of paricalcitol subjects had KDOQI-compliant iPTH (upper threshold 300 pg/mL) at every visit except week 3 than cinacalcet (A). Of subjects with baseline iPTH above KDIGO-compliant levels (upper threshold 585 pg/mL), a greater proportion normalized with paricalcitol than cinacalcet at every visit except week 3. Mean iPTH, calcium, and P over time are shown (B). Mean changes from baseline to final were -15.9, +15.2 in AP (IU/L) and -10.3, +9.6 BSAP (U/L) for paricalcitol and cinacalcet, respectively.



Conclusions: Paricalcitol was more effective than cinacalcet at normalizing high iPTH levels, with minimal impact on Ca and P. Paricalcitol treatment reduced, while cinacalcet increased AP and BSAP.

Funding: Pharmaceutical Company Support - Abbott Laboratories

SA-PO709

Fibroblast Growth Factor 23 and Cardiovascular Events in Patients with Chronic Kidney Disease: Findings from the CRIC Study

Julia J. Scialla,[1] Tamara Isakova,[1] Amanda Hyre Anderson,[2] Alan S. Go,[3] Juan E. Grunwald,[2] Takayuki Hamano,[2] Jiang He,[4] John W. Kusek,[5] James P. Lash,[6] Lisa C. Nessel,[2] Akinlolu O. Ojo,[7] Mahboob Rahman,[8] Dominic S. Raj,[9] Huiliang Xie,[1] Wei (Peter) Yang,[2] Xiaoming Zhang,[2] Harold I. Feldman,[2] Myles S. Wolf.[1] *[1]University of Miami; [2]University of Pennsylvania; [3]Kaiser Permanente; [4]Tulane University; [5]NIDDK; [6]University of Illinois; [7]University of Michigan; [8]Case Western Reserve; [9]George Washington University.*

Background: Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone that is elevated in CKD and associates with mortality. FGF23 induces myocyte hypertrophy in animal models. We hypothesized that higher FGF23 is associated with all cardiovascular (CV) events, and particularly congestive heart failure (CHF).

Methods: We measured baseline FGF23 in 3860 CRIC participants with CKD followed up to 6 years for pre-ESRD atherosclerotic cardiovascular disease (ASCVD; myocardial infarction, stroke or peripheral vascular disease procedure) or CHF hospitalizations. We assessed the association between FGF23 and events using Cox models adjusted for demographics, kidney function, traditional/nontraditional CV risk factors, and CV drugs.

Results: Mean eGFR was 44 ± 15 ml/min/1.73m^2. A prior history of ASCVD was present in 1,179 participants (31%), and 373 (10%) had a history of CHF. Median FGF23 was 145 (IQR 96 to 239) RU/ml. Higher log-FGF23 was associated with a linear increase in risk of ASCVD and CHF. Results were similar if death was included in the endpoint or if analyses were not censored at ESRD. For each, results were similar among those without prevalent disease (ASCVD or CHF, respectively) and by level of eGFR (≥45, 30-44, or <30 ml/min/1.73m^2).

	Hazard Ratio (95% CI) per doubling of FGF23	
	ASCVD Outcomes N=287 events 22/1000 person years	CHF Hospitalization N=360 events 27/1000 person years
Unadjusted†	1.37 (1.25-1.50)	1.75 (1.64-1.88)
Fully adjusted†‡	1.23 (1.04-1.45)	1.38 (1.21-1.58)
Event or death‡	1.31 (1.19-1.46)	1.42 (1.31-1.54)

†Censored for death and ESRD
‡Adjusted for demographics, kidney function, proteinuria, comorbidity, traditional cardiovascular risk factors, cardiovascular medications, other mineral metabolites, hemoglobin, C-reactive protein and serum albumin

Conclusions: Higher FGF23 is associated with ASCVD and CHF in patients with CKD, but the association with CHF is stronger.

Funding: NIDDK Support

SA-PO710

Direct In Vivo Effects of Vitamin D Sterol Therapy on Wnt Signaling in End Stage Kidney Disease

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Background: Wnt signaling is suppressed in early CKD resulting in decreased bone turnover, a phenomenon that is overcome by 2^{o}HPT in late CKD (Sabbagh et al JBMR 2012). It has been postulated that PTH stimulates FGF23 expression through increases in Wnt signaling (Rhee et al JBMR 2011). While vitamin D sterols suppress PTH and increase FGF23, the effect of these agents on osteocytic Wnt signaling remains unknown.

Methods: Protein expression of sclerostin (SOST), a known inhibitor of Wnt signaling, and of FGF23 were assessed by immunohistochemistry in the iliac crest of 11 dialysis patients (6M, 5F), age 16 ± 1 years, before and after 8 months of therapy with vitamin D sterols and phosphate binders.

Results: Biochemical values, bone histomorphometry, and bone SOST and FGF23 protein expression are displayed below.

	Pre	Post
Biochemicals		
Calcium (mg/dL)	8.6 ± 0.2	8.8 ± 0.2
Phosphorus (mg/dL)	6.3 ± 0.4	6.0 ± 0.4
Alkaline Phosphatase (IU/L)	298 (146, 488)	175 (124, 414)
PTH (pg/mL)	517 (378, 1048)	559 (375, 922)
FGF23 (RU/mL)	705 (318, 1196)	1696 (565, 3973)
Plasma SOST (pmol/L)	47 ± 9	50 ± 12
Bone Histomorphometry and SOST Expression		
BFR/BS (μm³/mm²/d)	50.0 ± 10.4	30.4 ± 7.5
OV/BV (%)	7.0 ± 1.1	4.4 ± 0.8*
O.Th (μm)	12.7 ± 0.9	10.2 ± 0.6*
OS/BS (%)	41.1 ± 3.9	31.7 ± 3.8*
OMT (d)	15.3 ± 1.4	14.6 ± 2.1
MLT (d)	29 (24, 47)	36 (30, 46)
BV/TV (%)	37.2 ± 3.7	36.1 ± 2.7
Tb.Th (μm)	173 ± 19	159 ± 6
Bone SOST (pixels/mm²)	0 (0, 21)	208 (11, 961)*
Bone FGF23 (pixels/mm²)	216 (136, 465)	469 (328, 1165)*

PTH values did not change with therapy. Bone SOST was expressed almost exclusively in cortical bone while FGF23 was found in both trabecular and cortical bone. At baseline, bone SOST expression was very low and increased with therapy. Bone FGF23 also increased with vitamin D sterol therapy.

Conclusions: Vitamin D sterol therapy may suppress Wnt signaling in cortical bone, independent of any changes in PTH. Increasing bone FGF23, occurring in conjunction with increasing SOST expression, calls into question current theories regarding Wnt-mediated regulation of FGF23 in CKD.

Funding: NIDDK Support, Other NIH Support - UL1 RR-033176

SA-PO711

Fibroblast Growth Factor 23 Increases Rapidly after Acute Kidney Injury

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Background: FGF23 is a bone-derived phosphate-regulating hormone, which acts on the kidney to increase urinary phosphate excretion. Circulating FGF23 levels are elevated in chronic kidney disease but little is known about FGF23 levels in acute kidney injury (AKI).

Methods: We investigated changes in FGF23 levels during AKI using folic acid nephropathy in WT as well as genetically modified mice. We also measured FGF23 levels in 14 patients peri- and post-cardiac surgery and compared levels in patients who developed AKI (n=4) versus those who did not (n=10).

Results: FGF23 levels increase within hours after AKI in mice, similar to NGAL, an established AKI marker. Circulating FGF23 levels also increased more than 10-fold 24 hours after AKI in all genetically modified animals, including mice bearing osteocyte-specific PTH/PTHrP deletion, or PTH deletion, or global VDR deletion, similar to the changes observed in WT animals. In phosphate restricted animals we found that despite reduced baseline serum phosphate levels, circulating FGF23 levels increased similar to animals maintained on a phosphate-replete diet. The increase in circulating FGF23 was associated with (or was induced by) an increase in bone FGF23 production. Finally, FGF23 levels increased in post-surgical patients with AKI within 24 hours post-operatively.

Conclusions: In conclusion, FGF23 is rapidly up-regulated during AKI, both in rodents and humans and it can be used as a novel marker of renal injury. This elevation of serum FGF-23 appears to be independent of serum phosphate, PTH, or $1,25(OH)_2$ vitamin D signaling.

Funding: NIDDK Support

SA-PO712

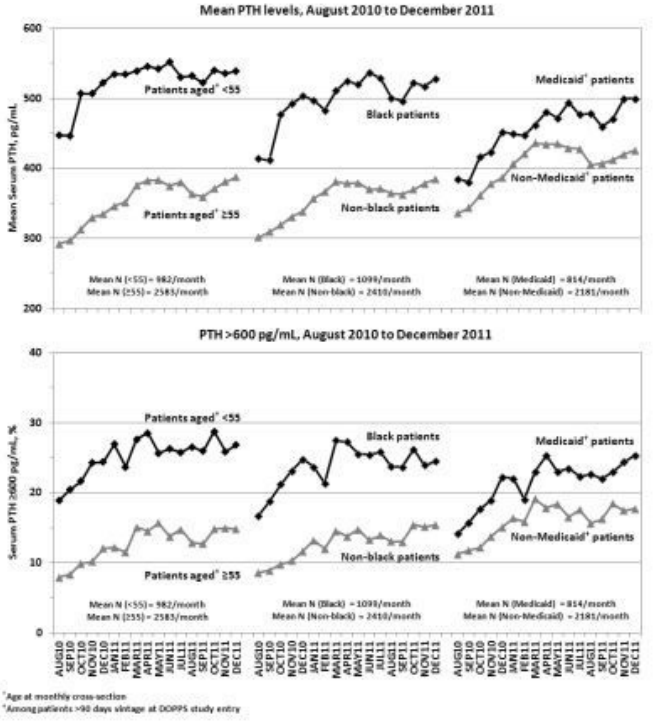
Very High PTH Levels in Hemodialysis (HD) Patients (pts) Who Are Black, on Medicaid, or Aged <55 after Implementation of the Prospective Payment System (PPS): Results from the DOPPS Practice Monitor

Francesca Tentori,[1,2] Douglas S. Fuller,[1] Ronald L. Pisoni,[1] Brenda W. Gillespie,[3] Brian Bieber,[1] Bruce M. Robinson.[1,3] *[1]Arb Res Collab Hlth, Ann Arbor, MI; [2]Vanderbilt Univ Med Ctr, Nashville, TN; [3]Univ of MI, Ann Arbor, MI.*

Background: The 2009 KDIGO guidelines and the 2011 implementation of the PPS may both have contributed to the rise in serum PTH in US HD pts. Due to higher medication use, Blacks, Medicaid pts, and pts aged <55 were identified in a 2010 GAO report as being at higher risk of developing very high PTH levels with the PPS.

Methods: We report trends in mean PTH levels and prevalence of PTH ≥600 pg/mL from Aug 10 to Dec 11 among ~3500 US pts/mo in the nationally representative DOPPS Practice Monitor (DPM; www.dopps.org/dpm). Survey-weighted regressions were used to test group differences.

Results: 48% of US DOPPS medical directors reported higher PTH targets in 2011 vs 2010. Mean PTH levels increased 28% from Aug 10 to Apr 11 and then stabilized. Trends did not differ by race (Black/non-Black, p=0.92), Medicaid status (p=0.16), or age <55 (p=0.05). As at prior time points, the prevalence of PTH ≥600 pg/mL in Dec 11 was higher among Blacks (27%, p<0.001 vs non-Blacks), Medicaid pts (26%, p=0.04 vs non-Medicaid), and pts aged <55 (28%, p<0.001 vs ≥55). Notable changes in secondary hyperparathyroidism therapies were higher oral vitamin D use (3% in Aug 10; 7% in Dec 11) and switch of vitamin D analog type in many facilities (paricalcitol to doxercalciferol). No difference in PTH trend by analog type was seen (p=0.14).



*Age at monthly cross-section
*Among patients >90 days vintage at DOPPS study entry

Conclusions: Following recent guidelines and payment changes, higher PTH targets have been adopted, leading to even higher prevalence of very high PTH levels in certain pt groups. Given the strong association of PTH ≥600 pg/ml with adverse outcomes, clinicians may need to reconsider treatment strategies in these patient groups.

Funding: Pharmaceutical Company Support - The DOPPS Is Administered by Arbor Research Collaborative for Health and is supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), Abbott (Since 2009), Baxter (Since 2011), and Vifor Fresenius Renal Pharma (Since 2011), without Restrictions on Publications

SA-PO713

Association of Vitamin D Status and Secondary Hyperparathyroidism in CKD

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Background: Vitamin D deficiency is prevalent in CKD patients and contributes to secondary hyperparathyroidism. Current guidelines recommend achieving vitamin D levels above 30 ng/ml, but this target is based upon expert opinion rather than quantitative evidence. We asked if PTH falls progressively with higher 25-hydroxyvitamin D (25-D) levels, and specifically if values above 30 ng/ml are associated with additional reduction in parathyroid hormone (PTH) across all stages of CKD.

Methods: We performed a cross-sectional analysis of initial laboratory data collected on 14289 stage 1-5 US CKD patients enrolled in the Litholink CKD program. Serum calcium (Ca), phosphorus (P), 25-D and plasma PTH levels were compared within a given CKD stage and across CKD stages.

Results: Within CKD stages 3-5, ascending 25-D pentiles (<17.8, 17.8-24.9, 25-31, 31.1-39, >39 ng/ml) were associated with progressively lower unadjusted PTH values. Higher serum Ca values associated with higher 25-D pentiles in stages 4 and 5. Results were similar when PTH was adjusted for serum Ca. Serum P values did not differ significantly between 25-D pentiles within CKD stage. Individual scatter plots of unadjusted PTH vs 25-D showed progressively greater negative slope values from stages 1 through 5; slopes were significant in all 5 CKD stages.

Conclusions: Serum PTH values are progressively lower at higher ranges of 25-D in CKD stages 3-5, including 25-D values above 30 ng/ml. Though observational, these findings support dosing to achieve vitamin D levels above 30 to lower PTH.

Funding: Pharmaceutical Company Support - LabCorp

SA-PO714

Fibroblast Growth Factor 23 (FGF23) Plasma Levels Are Elevated with Early Chronic Kidney Disease (CKD) and Positively Associated with Steroid Based (SB) versus Steroid Free (SF) Immunosuppression in Pediatric (ped) Renal Transplant (Tx) Patients (pts) Rachana Srivastava, Poyyapakkam Srivaths, Scott E. Wenderfer, Eileen D. Brewer. *Pediatrics, Renal Section, Baylor College of Medicine, Houston, TX.*

Background: FGF23 is a phosphaturic hormone that also suppresses renal 1α-hydroxylase activity. In adult CKD pts FGF23 increases in early stages. Few studies have focused on FGF23 & CKD in ped Tx pts. **Aim:** Determine plasma FGF23 & evaluate potential associations with renal function & mineral metabolism parameters in ped Tx pts.

Methods: Cross-sectional study of 53 ped Tx pts (33M; median age 15.2y, range 3.9-21.8y; 23 Hispanic/16 white/13 black/1 Asian) with CKD stages 1-3 (mean±SD Schwartz eGFR 102±27 ml/min/1.73m2); median time since Tx 1.18y (range 0.15-11y). Immunosuppression was 43 pts steroid free (SF) & 10 steroid based (SB). Serum Cr, Ca, P, iPTH, 1,25vitD; plasma C-terminal FGF23 by ELISA; & urine Ca, P, Cr were measured. 10/53 pts (19%) were on P supplements for hypophosphatemia for age. 13/53 pts (25%) were prescribed calciferol, & 4/53 pts (7.5%), calcitriol.

Results: 14/53 pts (26%) had plasma FGF23>130 RU/ml (normal±2SD=69±39 in healthy controls in 2 ped studies). Plasma FGF 23 levels were not associated with gender, age, ethnicity, time since Tx, tubular reabsorption P (TRP), or serum P, iPTH or 1,25vitD. Elevation of plasma FGF23 was the only mineral metabolism abnormality associated with lower eGFR to CKD stage 2-3 (Table). Plasma FGF23 was higher in pts receiving SB vs. SF (206±207 RU/mL vs. 95±67 RU/ml, p=0.004).

eGFR Schwartz (ml/min/1.73m2)	Serum Ca (mg/dl)	Serum P (mg/dl)	iPTH (pg/ml)	1,25vitD (pg/ml)	TRP (%)	FGF23 (RU/ml)
eGFR ≥90; n=37	9.8 (9.5-10.2)	4.4 (3.6-4.8)	62 (45-153)	57 (46-82)	85 (81-89)	79 (55-95)
eGFR 30-89; n=16	9.8 (9.5-10.1)	4.4 (3.6-4.7)	45 (19-77)	48 (28-55)	84 (80-88)	115* (85-131)

Values as median (interquartile range); *p=0.013, CKD 1 vs CKD 2-3

Conclusions: In ped Tx pts with CKD stages 1-3: 1) elevated plasma FGF23 is the only mineral metabolism parameter associated with early decrease in GFR; 2) elevated FGF23 is strongly associated with SB immunosuppression, suggesting an effect of steroids on FGF23 metabolism in ped Tx.

Funding: Clinical Revenue Support

SA-PO715

Hypovitaminosis D Is Associated with Elevated Parathyroid Hormone (iPTH) in Pediatric (ped) Renal Transplant (Tx) Patients (pts) with Good Function (fx) Rachana Srivastava, Eileen D. Brewer, Tian Tian, Lorrie J. Moreno, Poyyapakkam Srivaths. *Pediatrics, Renal Section, Baylor College of Medicine, Houston, TX.*

Background: Vitamin D (vitD) deficiency (def) is widespread in adult Tx & CKD pts. Prevalence & risk factors associated with vit D def are not well described for ped Tx pts. **Aim:** Assess prevalence of vitD def assessed by serum 25-OH vit D (25OHD) & associations with other mineral parameters or renal fx in ped Tx pts.

Methods: Cross-sectional study of 54 ped Tx pts (34M; median age 15.2y, range 3.9-21.8y; 23 Hispanic/17 white/13 black/1 Asian) with mild CKD stages 1-3 (mean±SD eGFR Schwartz 102±27 ml/min/1.73m2); median time since Tx 1.19y (range 0.15-11 y). Immunosuppression was 44 pts steroid free (SF), 10 pts steroid based (SB). Serum 25OHD, 1,25vitD, Ca, P, iPTH, Cr & plasma C-terminal fibroblastic growth factor (FGF) 23 by ELISA were measured. 13/54 pts (24%) were prescribed calciferol supplements (sup), & 4/54 pts (7%), calcitriol. Average sun exposure (questionaire) was ≥4h/wk (range 4-14) in 14/54 pts (26%).

Results: 45/54 pts (83%) had hypovitaminosis D (25OHD<30ng/ml); 16/45 pts (36%) had vit D def (<15ng/ml); 29/45 pts (64%) had vit D insufficiency (16-29ng/ml). Serum 25OHD levels were not associated with gender, ethnicity, age, eGFR, time since Tx, SF or SB. Serum iPTH but not Ca, P or 1,25vitD were significantly greater in pts with vit D def (table). Serum 25OHD was positively correlated with average weekly sun exposure (r=0.34, p=0.01), but not prescirbed calciferol sup.

25OHD (ng/ml)	Ca (mg/dl)	P (mg/dl)	iPTH (pg/ml)	1,25vitD (pg/ml)	FGF23 (RU/ml)
vitD def <30; n=45	9.8 (9.5-10.2)	4.4 (3.6-4.8)	62* (45-153)	57 (46-82)	86.4 (56-142)
vitD replete≥30; n=9	9.8 (9.5-10.1)	4.4 (3.6-4.7)	45 (19-77)	48 (28-55)	80.7 (55-104)

Serum/plasma values as median (interquartile range); *p=0.049, vitD def vs replete

Conclusions: In ped Tx pts with good graft fx: 1) vitD def is highly prevalent; 2) low serum 25OHD is associated with elevated iPTH, but not other demographic or biochemical parameters; 3) sun exposure was <4h/wk in most pts (74%) and likely contributed to vitD def. Further studies are in progress to evaluate efficacy of calciferol sup to treat vitD def.

Funding: Clinical Revenue Support

SA-PO716

Spak Regulates FGF23 Formation, Phosphate Homeostasis and Bone Mineralization Michael Föller,[1,2] Ganesh Pathare,[2] Diana Michael,[2] Bernd Pichler,[3] Florian C. Lang.[2] *[1]Campbell Family Institute for Breast Cancer Research, University Health Network, Toronto, ON, Canada; [2]Institute of Physiology, University of Tübingen, Tübingen, Germany; [3]Department of Radiology, University of Tübingen, Tübingen.*

Background: The WNK-dependent STE20/SPS1-related proline/alanine-rich kinase (Spak) regulates the thiazide-sensitive sodium, chloride cotransporter (NCC) and the sodium, potassium, chloride cotransporter (NKCC2) in the distal tubule of the kidney thereby maintaining extracellular fluid volume and blood pressure. NCC is also expressed in osteoblasts where it is implicated in bone mineralization. Osteoblasts control phosphate homeostasis by forming the phosphaturic factor FGF23. Here, we tested *in vivo* whether Spak participates in the regulation of FGF23 formation and of phosphate homeostasis.

Methods: FGF23 serum levels and phosphate homeostasis were analyzed in mice expressing transgenic Spak resistant to WNK-dependent activation ($spak^{tg/tg}$) and in wild type mice ($spak^{wt/wt}$) as well as in wild type mice following NCC inhibition with hydrochlorothiazide (HCT).

Results: As a result, HCT treatment of $spak^{wt/wt}$ mice significantly increased the serum FGF23 level and renal phosphate excretion. Moreover, the serum FGF23 level was significantly higher in $spak^{tg/tg}$ mice than in $spak^{wt/wt}$ mice. Urinary phosphate excretion was significantly larger in $spak^{tg/tg}$ mice compared to $spak^{wt/wt}$ mice despite a lower serum phosphate concentration in $spak^{tg/tg}$ mice. The urinary calcium excretion was significantly reduced in $spak^{tg/tg}$ mice. Serum levels of calcitriol and PTH were not significantly different between the genotypes. Bone density was significantly increased in $spak^{tg/tg}$ mice compared to $spak^{wt/wt}$ mice.

Conclusions: In conclusion, Spak is a strong regulator of FGF23 formation, bone mineralization and phosphate homeostasis.

SA-PO717

High Dose Intravenous Iron and Intact FGF23 in Normal Rats Eva Gravesen,[1] Jacob Hofman-Bang,[1] Ewa Lewin,[1,2] Klaus Olgaard.[1] *[1]Nephrological Department P, Rigshospitalet, Copenhagen, Denmark; [2]Nephrological Department B, Herlev Hospital, Copenhagen, Denmark.*

Background: Administration of intravenous iron has been proposed to induce elevation of fibroblast growth factor 23 (FGF23), hypophosphatemia and osteomalacia. High levels of FGF23 are associated with increased mortality in the CKD population. CKD patients often develop iron deficiency, anemia and the need for intravenous iron therapy. As such it is important to obtain further knowledge on the possible effects of iron on FGF23. Therefore, the effect on FGF23 levels of two different iron preparations, iron (III) isomaltoside 1000 and ferric carboxymaltose was examined in rats.

Methods: A single high dose of 60 mg/kg b.w of iron (III) isomaltoside 1000, or ferric carboxymaltose, or vehicle, was given intravenously and the effects on plasma levels of FGF23, phosphate, Ca^{2+}, PTH, transferrin, ferritin and iron was examined. Normal Wistar rats kept on a standard diet (0.9% calcium and 0,7% phosphorus), weighing 250 g, were used (n=8 in each group). Samples for determination of FGF23 were obtained at time 0, 30, 60, 120, 180 minutes and after 48 hours. FGF23 was measured by the intact FGF23 assay from Kainos lab. Japan.

Results: FGF23 levels at baseline were 91± 9 pg/mL in all 3 groups. In the vehicle group 93±16 pg/mL at 30 minutes, 92±13 pg/mL at 180 minutes, and 148±22 pg/mL at 48 hours. In the iron (III) isomaltoside 1000 group 111±26 at 30 minutes, 124 ±32 pg/mL at 180 minutes and 194±59 pg/mL at 48 hours. In the ferric carboxymaltose group 97±16 at 30 minutes, 86±13 pg/mL at 180 minutes and 156±35 pg/mL at 48 hours. No significant differences in FGF23 levels were observed at any time point between the groups.

Conclusions: A single high dose of either iron (III) isomaltoside 1000 or ferric carboxymaltose had no effect on intact FGF23 levels for up to 48 hours after intravenous injection in normal rats.

Funding: Private Foundation Support

SA-PO718

Ergocalciferol Treatment and Aspects of Mineral Homeostasis in Patients with Chronic Kidney Disease, Stage 4-5 Eva Gravesen,[1] Jacob Hofman-Bang,[1] Ewa Lewin,[2,1] Klaus Olgaard.[1] *[1]Department of Nephrology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [2]Department of Nephrology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark.*

Background: Plasma levels of FGF23 are elevated in patients with CKD. FGF23 is correlated to adverse outcome and cardiovascular mortality in both CKD and non-CKD subjects. Treatment with active vitamin D induces elevated FGF23, while epidemiological studies have found positive effects of active vitamin D on mortality in CKD. In normal subjects an increase in 1,25(OH)2D and FGF23 has been observed after nutritive vitamin D treatment. Focus on non-classical effects and possible less side effects of treatment with nutritional vitamin D, raises the expectation of possible benefits of treating CKD patients with nutritional vitamin D. It is not known if nutritional vitamin D alters FGF23 levels in CKD patients. The objective was to examine the possible effects of treatment with high doses of ergocalciferol on FGF23 levels and other parameters of mineral homeostasis in predialysis CKD patients.

Methods: 43 adult patients with CKD stage 4-5, not receiving vitamin D supplementation, were studied, and allocated by simple randomization to either an intervention (n=26) or a control group (n=17). The intervention group received ergocalciferol, 50.000 IU/week for 6 weeks. Plasma FGF23, creatinine, PTH, phosphate and Ca^{2+} were obtained at baseline and after the six weeks. Data are Mean±SEM.

Results: The intervention group had a significant increase in p-25(OH)D_2 levels from <10 to 90±4 nmol/L, while plasma 1,25(OH)$_2$D (62±6 at baseline and 67±6 at 6 weeks) remained stable. No changes were seen in circulating vitamin D levels in the control group. After the six weeks of treatment no significant changes were seen in plasma FGF23 levels 35±4 to 37±5 control group and 48±6 to 58±11 and the levels of plasma creatinine, phosphate, Ca^{2+} and PTH remained stable in both groups.

Conclusions: No stimulatory effect of treatment with high dose of ergocalciferol was found on FGF23 levels in CKD patients, stage 4-5 and as such no harmful effects of ergocalciferol treatment were observed.

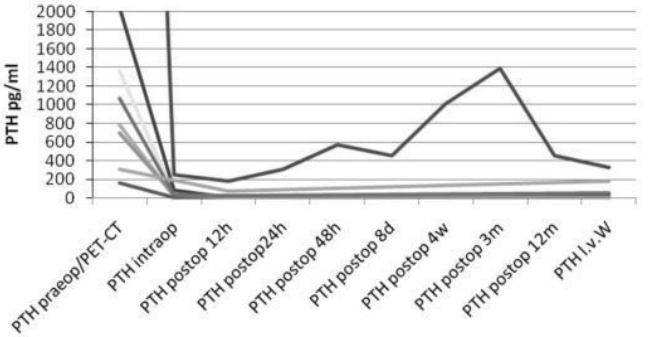
SA-PO719

The Role of Preoperative Positron Emission Tomography/Computed Tomography PET/CT for Localization Diagnostics of Parathyroid Glands Frieder Keller. *Nephrology, Internal Medicine, University Hospital, Ulm, Germany.*

Background: Surgical removal of the parathyroid tissue becomes necessary in many cases. An accurate preoperative localization and identification of the number of epithelial glands is very important, especially in case of recurrent hyperparathyroidism and to identify ectopic epithelial tissue.

Methods: In our retrospective study we investigated the role of positron emission tomography/ computed tomography (PET/CT) as an additional imaging technique. Between January 2001 and March 2011, a total of 12 patients underwent PET/CT because of severe hyperparathyroidism or recurrence after a previous operation. Eight patients underwent ultrasound as well. In nine patients PET/CT was positive and they received surgical treatment. Three patients were not operated because of the negative results of the PET/CT. The analysis was followed by an assessment of the sensitivity, the specificity, the accuracy as well as the positive and negative predictive value of the PET/CT. As the reference the combined scoring was selected based on the last follow-up values for parathyroid hormone, serum calcium and phosphate concentrations.

Results: The sensitivity of PET/CT was superior (87 % vs 66 %) and the specificity was comparable (50 % vs 50 %) to that of standard ultrasound. The positive predictive value in PET/CT and ultrasound was almost equal (77 % vs 80 %) whereas the negative predictive value (66 % vs 33 %) as well as the accuracy (75 % vs 62 %) were more precise in PET/CT than in ultrasound. Two atypical localizations of parathyroid glands were detected by PET/CT none of them by ultrasound. Subnormal, normal and even slightly elevated parathyroid hormone levels were measured after total parathyroidectomy.



Conclusions: Sensitivity, accuracy and negative predictive values for PET/CT are superior to ultrasound. PET/CT may provide valuable topographic information before parathyroidectomy.

SA-PO720

Renal Phosphate Clearance Is an Important Determinant of Serum FGF23 Level in Peritoneal Dialysis Patients Shunsuke Yamada,[1] Hisako Yoshida,[2] Kazuhiko Tsuruya,[2] Masatomo Taniguchi,[1] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [2]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Background: Fibroblast growth factor 23 (FGF23) is a bone-derived phosphaturic hormone that regulates phosphate (P) homeostasis. Recent clinical studies have revealed that serum FGF23 is regulated by various factors including P load, and is closely associated with high morbidity in dialysis patients. However, the clinical characteristics that determine serum FGF23 level remain unclear in peritoneal dialysis (PD) patients.

Methods: The present cross-sectional study was performed to indentify the clinical factors that regulate serum FGF23 level in PD patients. We enrolled 56 prevalent outpatients receiving PD treatment with various dialysis vintages at Kyushu University Hospital on April 1st in 2012. We determined demographic, clinical, and biochemical parameters including serum intact FGF23 (Kainos laboratories Inc., Japan) level, and collected urine and PD fluid for 24-hours. The dependent variable was serum log FGF23 level, and the independent variables were renal P clearance and other clinical parameters. Linear regression analysis and trend analysis were used to determine the effect of renal P clearance on serum FGF23 level.

Results: Patients were 54 ± 13 years old, 64% man, and 42% diabetes. Dialysis vintage was 615 ± 526 days. Serum FGF23 level was 5920 ± 6789 pg/mL. Univariate analysis revealed that dialysis vintage, serum P, renal P clearance, and peritoneal P clearance were significantly associated with serum FGF23 level (p<0.05). In the multivariate analysis, renal P clearance was negatively associated with serum FGF23 level (p<0.05) even after adjusted for age, sex, underlying kidney disease, dialysis vintage, serum P, and peritoneal P clearance.

Conclusions: Renal P clearance is a negative determinant of serum FGF23 level in PD patients. Preserving residual renal function can maintain serum FGF23 level within the appropriate range, leading to the prevention of future cardiovascular events.

SA-PO721

The Type IIa Sodium-Phosphate Cotransporter (NpT2a) Is Transcriptionally Regulated by Parathyroid Hormone (PTH) Rebecca Murray,[1] Nina W. Lesousky,[2] Syed J. Khundmiri,[1,2] Barbara Clark,[3] Eleanor D. Lederer.[1,2,4] *[1]Physiology & Biophysics, University of Louisville, Louisville, KY; [2]Medicine, University of Louisville, Louisville, KY; [3]Biochemistry & Molecular Biology, University of Louisville, Louisville, KY; [4]Robley Rex VAMC, Louisville, KY.*

Background: Primary hyperparathyroidism manifests in patients as high PTH, high serum calcium, and low serum phosphate levels. PTH exerts multiple effects in the proximal tubule, including reduced phosphate reabsorption. While much is known about acute inhibition of NpT2a by PTH, little is known about the mechanisms responsible for chronic hypophosphatemia under conditions of primary hyperparathyroidism. We hypothesize that PTH chronically regulates NpT2a at the transcriptional level either by affecting NpT2a mRNA stability or by decreasing promoter function.

Methods: We used two models to explore this hypothesis: a transgenic mouse that displays a parathyroid-specific Cyclin D1 overexpression (PTH-D1), leading to gradual development of primary hyperparathyroidism; and opossum kidney (OK) WT cells exposed to long-term PTH stimulation.

Results: Chronic PTH exposure (100 nM) suppressed NpT2a mRNA and protein expression in both models. Quantitative RT-PCR revealed that NpT2a mRNA levels were decreased 50% in kidney cortex of PTH-D1 mice versus control. PTH treatment of OK cells decreased NpT2a mRNA levels by 40% within 1 hour, with a maximal 75% decrease within 24 hours. NpT2a mRNA half-life in PTH-treated OK cells was 1.5 hours, in contrast to a 7-hour half-life when transcription was inhibited by actinomycin D (1 µg/mL). When OK cells were treated with PTH in the presence of actinomycin D, the effect of PTH on NpT2a mRNA half-life was blocked.

Conclusions: We conclude that (1) chronic PTH decreases NpT2a mRNA expression, and (2) PTH induces the transcription of an unknown protein that results in enhanced NpT2a mRNA degradation.

Funding: Veterans Administration Support

SA-PO722

Sclerostin and DKK-1 Levels in Pre-Dialyisis CKD Patients Geert J. Behets,[1] Liesbeth Viaene,[2] Bjorn Meijers,[2] Patrick C. D'Haese,[1] Pieter Evenepoel.[2] *[1]Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium; [2]Laboratory of Nephrology, Catholic University Leuven, Leuven, Belgium.*

Background: Sclerostin and Dickkopf-1 (DKK-1) are inhibitors of the canonical Wnt pathway, known to suppress bone formation. In dialysis patients, serum levels of sclerostin are elevated and correlate positively with bone mineral density and bone volume and negatively with bone turnover. Little is known about serum levels of sclerostin and DKK-1 in predialysis CKD patients.

Methods: We performed a cross-sectional observational study in 149 patients (CKD stage 1-5, 81 men, 60 ± 16 years, 22 diabetic). Serum sclerostin and DKK-1 were measured using ELISA kits (Biomedica). Other parameters of mineral metabolism included 1-84 PTH, calcitriol, calcidiol (all DiaSorin), FGF-23 (Kainos) and bone specific alkaline phosphatase (BsAP – electrophoretic method). Glomerular filtration rate was estimated using the 4-variable MDRD method.

Results: Mean eGFR was 43 ± 27 ml/min/1.72 m² (range 4.1 – 139.2). Overall mean serum sclerostin and DKK-1 levels were 68 ± 45 pmol/l and 38 ± 15 pmol/l respectively. Overall, sclerostin correlated positively with 1-84PTH ($r = 0.312$, $p < 0.001$), FGF-23 ($r = 0.320$, $p < 0.001$) and age ($r = 0.474$, $p < 0.001$), and negatively with calcitriol ($r = -0.393$, $p < 0.001$) and eGFR ($r = -0.616$, $p < 0.001$). Multivariate analysis indicated older age, lower eGFR, and gender (male) to be independently associated with higher sclerostin levels. No correlation was found between sclerostin and bone alkaline phosphatase. DKK-1 levels, conversely, showed a positive correlation with eGFR ($r = 0.212$, $p < 0.01$).

Conclusions: Serum sclerostin levels but not DKK-1 levels increase along the progression of renal disease. The finding of positive association between PTH and sclerostin is opposite to what has been observed in other populations (dialysis, primary hyperparathyroidism, general population) and conflicts with experimental data. Whether the increasing sclerostin levels in progressive renal failure protects the bone against the deleterious consequences of high PTH levels or merely reflects decreased clearance of sclerostin remains to be clarified.

Funding: Pharmaceutical Company Support - Diasorin

SA-PO723

25-Hydroxyvitamin D, Parathyroid Hormone, and Coronary Artery Calcification in Pre-Dialysis Chronic Kidney Disease Amrita Kaur Sukhi,[1] Rachel M. Holden,[1] Alexander R. Morton,[1] Robert Louis Nolan,[2] Wilma M. Hopman,[3] Jocelyn S. Garland.[1] *[1]Department of Medicine, Queen's University, Kingston, ON, Canada; [2]Department of Radiology, Queen's University, Kingston, ON, Canada; [3]Clinical Research Centre, Kingston General Hospital, Kingston, ON, Canada.*

Background: The role of Vitamin D in contributing to coronary artery calcification (CAC) in chronic kidney disease (CKD) is challenging to ascertain. Studies evaluating whether 25-Hydroxyvitamin D (25(OH)D) is a risk factor for CAC have mainly considered 25(OH)D as a separate variable. The majority has shown no correlation between 25(OH)D and CAC. However, pre-clinical studies have suggested parathyroid hormone (PTH) may impact on 25(OH)D synthesis, by down-regulating hepatic production of 25(OH)D. In fact, a negative correlation has been demonstrated between 25(OH)D and PTH in uremic animal models. The purpose of our study was (1) to translate the reported negative association between 25(OH)D and PTH *in vivo* in CKD patients, and (2) to evaluate whether 25(OH) D and PTH, when considered as separate variables versus together, are associated with CAC cross-sectionally, and in prospective follow-up.

Methods: 88 stage 3 to 5 pre-dialysis CKD patients underwent multi-slice computed tomography for the evaluation of CAC in 2005 and 2009. 25(OH)D and PTH levels were obtained at the original visit and at four year follow-up.

Results: 25(OH)D and PTH were negatively correlated ($r=-0.32$; $P=0.002$). By multivariable logistic regression adjusted for age, season of blood-work and kidney function, 25(OH)D and PTH as separate co-variates did not reveal an association with CAC. However, when considered as an interaction term, (25(OH)D*logPTH), there was a statistically significant association bewteen 25(OH)D*logPTH with prevalent CAC >100 Agatston units (AU) (OR=0.97; 95% confidence interval (CI) 0.949 to 1.00; P=0.047), and CAC progression >100 AU over 4 years (OR=0.98; 95% CI 0.959 to 1.00; P=0.05).

Conclusions: Similar to the findings in pre-clinical studies, 25(OH)D levels decreased as PTH levels rose. Moreover. the interaction of low 25(OH)D and elevated PTH predicted CAC, suggesting an inter-dependence of 25(OH)D and PTH in contributing to CAC development.

SA-PO724

Proximal Tubular Resistance to the Action of Fibroblast Growth Factor-23 in Sickle Cell Disease Vimal Master Sankar Raj,[1] Dima Hamideh,[2] Wacharee Seeherunvong,[1] Ofelia A. Alvarez,[2] Carolyn L. Abitbol,[1] Jayanthi Chandar,[1] Michael Freundlich,[1] Gaston E. Zilleruelo.[1] *[1]Pediatric Nephrology, University of Miami, Miami, FL; [2]Pediatric Hematology, University of Miami, Miami, FL.*

Background: Proximal tubular supra-normal function in patients with sickle cell (SS) disease has been described with increased proximal tubular re-absorption of phosphorus(P) and secretion of creatinine. Since the mechanisms remain elusive and Fibroblast Growth Factor-23 (FGF23) is an important modulator of P homeostasis, measurements of serum FGF23 and renal P handling were evaluated in SS patients with normal GFR.

Methods: We conducted a cross-sectional study evaluating renal handling of phosphorus in 14 subjects (mean age 16.6± 4.3 yr) with SS disease and normal or elevated GFR.Serum creatinine, cystatin C, mineral biochemical data including 25-(OH)vitamin D and FGF23 were measured. Intact FGF23 was measured by ELISA kit (Millipore, MA; normal range 22-91pg/mL). The tubular re-absorption of phosphorus (TRP) and maximum re-absorption of phosphorus (TMP/GFR) were calculated. GFR was estimated by serum creatinine using modified Schwartz or MDRD per age and by cystatin C.

Results: Of the 14 patients,12 were African Americans (5M,9F).Mean GFR by serum creatinine was 159.5±32.4 and by cystatin C 137.6±34.2 ml/min/1.73m²,respectively. Most had Vitamin D deficiency (13±8ng/ml), normal serum calcium and alkaline phosphatase, but increased serum P for age(5.2±0.7mg/dl). TRP was elevated in most patients for serum P with average 95.6±2.4%(normal >80%) and TMP/GFR at 5.0±0.4mg/dl(normal 2.6-4.4). Intact FGF23 concentrations were elevated at 106.2±28.7pg/ml. Linear regression analysis showed significant correlations of serum FGF23 with TRP ($r=0.6$,$p=0.03$) and urine phosphorus excretion ($r=-0.5$,$p=0.04$).

Conclusions: These preliminary cross-sectional data demonstrate elevated serum FGF23 levels in SS patients with normal GFR. The elevated serum P with concomitant high TRP and TMP/GFR indicates proximal tubular resistance to the phosphaturic action of FGF23. Further studies are needed to better understand the role of FGF23 and its co-factor, Klotho in the proximal tubular function in SS patients.

Funding: Private Foundation Support, Clinical Revenue Support

SA-PO725

Vascular Calcification and Tumoral Calcinosis in a Family with Hyperphosphatemia and an FGF-23 Mutation Anuja P. Shah,[1] Clinton Miller,[2] Cynthia C. Nast,[1] Mark D. Adams,[2] Barbara J. Truitt,[2] John Tayek,[1] Lili Tong,[1] Parag Mehtani,[1] John R. Sedor,[3] Kenneth E. White,[4] Rajnish Mehrotra,[1] Janine A. La Page,[1] Francisco J. Monteon,[5] Patricia Dickson,[1] Sharon G. Adler,[1] Sudha K. Iyengar.[2] *[1]Nephrology, LABiomed Research Institute, Torrance, CA; [2]Genetics, Case Western Reserve University, Cleveland, OH; [3]Nephrology, Case Western Metro Health Center, Cleveland, OH; [4]Medical & Molecular Genetics, University of Indiana, Indianapolis, IN; [5]Nephrology, Instituto Mexicano del Seguro Social, Guadalajara, Mexico.*

Background: We studied a kindred with vascular calcification (VC) requiring limb/digit amputations unlike most cases of tumoral calcinosis. The only metabolic abnormality was hyperphosphatemia (hi P). We conducted genetic/pathobiological assessments to evaluate the cause of their VC.

Methods: High dimensional genetic analysis of 13 family members, and exome analysis of 6 members was performed. To characterize osteogenic features of the patient's vessels in the vicinity of VC, we evaluated amputation tissue with medium to small size vessels from our proband.

Results: Severe VC occurred almost ubiquitously, associated with hi P, but without diabetes, CKD, hypertension, hyperlipidemia, hypercalcemia, hyperparathyroidism, or hypervitaminosis D. There was a novel Q67K FGF-23 mutation and a null (deletion) allele on the other FGF-23 homologue in all 3 affecteds. Affecteds had high plasma C-terminal FGF-23, but little or no circulating intact FGF-23. Endothelial and vascular smooth muscle cells, and few interstitial monocyte/marcophages and spindle cells expressed the osteoblast marker osteonectin, often associated with segmental or, circumferential internal elastic lamina calcification in all size vessels, and focal medial calcification mostly in larger arteries without arteriosclerosis or lipid deposition.

Conclusions: This FGF-23 mutation segregates with hi P and is a plausible causes of the osteogenic changes in intima, media, and capillaries without typical atherosclerosis. This family's novel phenotype differentiates it from reported FGF-23 mutation cases and suggests that FGF-23 fragments may regulate VC, or that other genes, which the affected members share, may modify this process.

Funding: NIDDK Support, Private Foundation Support

SA-PO726

Maxacalcitol Ameliorates Tubulointerstitial Fibrosis in Obstructed Kidneys by Recruiting PPM1A/VDR Complex to pSmad3 Kazunori Inoue,[1] Isao Matsui,[1] Takayuki Hamano,[2] Akihiro Shimomura,[1] Chikako Nakano,[1] Yasuo Kusunoki,[1] Yoshitsugu Obi,[1] Yoshiharu Tsubakihara,[2] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]Comprehensive Kidney Disease Research, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Tubulointerstitial fibrosis (TIF) remains unresolved in nephrology because satisfactory therapies have not been established. One of the candidates protecting the kidney from TIF is active vitamin D (aVD) because aVD negatively regulates renin. We examined if maxacalcitol (22-oxacalcitriol (OCT)) suppressed TIF in unilateral ureteral obstructed (UUO) kidney.

Methods: Six-week-old male Sprague-Dawley rats were randomly divided into four groups; sham + vehicle (S+V), sham + OCT (S+O), UUO + vehicle (U+V), or UUO + OCT (U+O). Vehicle or OCT at a dose of 0.5 μg/kg BW was administrated subcutaneously twice a day. To elucidate the anti-fibrotic mechanism of OCT, *in vitro* experiments were performed using NRK52E cells.

Results: In UUO rats, OCT ameliorated all fibrotic parameters, such as tubular injury index, interstitial volume index, collagen I positive area, and mRNA levels of extracellular matrix genes. We found that TGF-β1 itself induced its expression in a phosphor-Smad3 (pSmad3)-dependent manner, and that OCT ameliorated TIF by abrogating this autoinduction.TGF-β1 increased both the nuclear levels of protein phosphatase Mg^{2+}/Mn^{2+} dependent 1A (PPM1A), a pSmad3 phosphatase, and the interaction levels between the vitamin D receptor (VDR) and PPM1A. Under the presence of OCT, PPM1A/VDR complex was recruited to pSmad3, and pSmad3 was dephosphorylated. Consequently, autoinduction of TGF-β1 was suppressed.

Conclusions: Our findings provide a novel approach to inhibit TGF-β1 signaling pathway in fibrotic diseases.

Funding: Pharmaceutical Company Support - Chugai Pharmaceutical Company

SA-PO727

Mineral and Bone Disorder among Participants in the Saudi Arabia Dialysis Outcomes and Practice Patterns Study (DOPPS): Serum Biomarkers and Therapeutic Regimens

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Background: The DOPPS has recently conducted a pilot study in Saudi Arabia (SA). Abnormalities in markers of mineral and bone disorder (MBD) have been recognized as potential risk factors for cardiovascular disease in the hemodialysis (HD) population and associated with poor clinical outcomes. We report findings related to management of MBD in the SA DOPPS study cohort with comparisons to other DOPPS regions.

Methods: 20 HD facilities treating at least 23 HD pts were randomly selected from a comprehensive roster of dialysis units to be representative of >95% of SA HD pts. Descriptive results for SA, based upon a random sample of 20-25 HD pts in each study facility, are compared to other DOPPS regions. Preliminary results for SA are from 315 HD pts treated at 16 dialysis facilities. Results are presented as weighted estimates, accounting for the sampling fraction in each unit.

Results: Mean s. calcium was similar in the SA DOPPS sample (9.2 mg/dL) compared to other DOPPS regions. Mean s. phosphorus was higher in SA (5.4 mg/dL) compared to Eur/ANZ (5.0), but similar to Japan (5.5) and North America (5.2). Mean PTH in SA (479) was higher than other DOPPS regions(range 168-376), with 32% of SA pts having a serum PTH>600 pg/mL. Mean dialysate calcium in SA (3.1 mEq/L) was also higher than other DOPPS regions(range 2.5-2.8). The % of pts prescribed IV Vit. D, oral Vit. D, a phosphate binder, and cinacalcet were 27, 46, 92, and 24%.

Percent of patients in MBD marker categories and medication use, by DOPPS region

	Saudi Arabia (N=315)	Eur/ANZ (N=4297)	North America (N=4890)	Japan (N=1575)
Serum calcium (mg/dL)*				
< 8.4	21.5	15.2	14.0	12.3
8.4-9.5	45.8	52.0	57.0	55.7
9.6-10.2	20.5	23.5	22.8	23.5
> 10.2	12.3	9.3	6.1	8.6
S. phosphorus (mg/dL)				
< 3.5	17.7	15.3	10.8	4.0
3.5-4.5	15.0	26.6	25.4	19.6
4.6-5.5	22.7	25.6	27.5	31.6
5.6-7.0	27.2	22.4	23.1	32.4
> 7.0	17.5	10.1	13.2	12.5
S. PTH (pg/mL)				
<150	27.2	32.6	20.0	59.1
150-300	15.6	30.5	37.0	27.4
301-600	24.9	25.8	27.6	11.2
>600	32.2	11.2	15.4	2.4
MBD Medications				
IV Vitamin D	27.4	13.5	55.0	27.2
Oral Vitamin D	45.8	50.3	21.7	39.7
Phosphate Binder	91.9	80.2	83.1	81.2
Cinacalcet	23.5	17.3	18.3	10.3

*Albumin corrected

Conclusions: The potential impact of these preliminary practice findings on patient outcomes in SA will be examined in the longitudinal component of the SA DOPPS study.

Funding: Pharmaceutical Company Support - The Initial Cross-Sectional DOPPS Study in Saudi Arabia Was Supported by Amgen without Restrictions on Publications. The International DOPPS Is Supported by Scientific Research Grants from Amgen (Since 1996), Kyowa Hakko Kirin (Since 1999, in Japan), Sanofi Renal (Since 2009), and Abbott Laboratories (Since 2009)

SA-PO728

Acute Changes in Plasma Ca^{2+} and Regulation of Plasma FGF23 Levels

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Background: Fibroblast Growth Factor 23 (FGF23) has an essential physiological role in phosphate and vitamin D homeostasis. Calciotropic hormones are the potent regulators of FGF23. How phosphate and calcium influence FGF23 secretion is less clarified. Dietary phosphate load has been shown to increase FGF23 levels. In parallel, manipulation of plasma calcium levels by dietary means has been shown to regulate FGF23 levels, suppressing FGF23 in hypocalcemia and increasing FGF23 in hypercalcemia. The aim of the present investigation was to study whether plasma Ca^{2+} might participate in the acute regulation of plasma FGF23 and therefore the response of FGF23 to acute changes in plasma Ca^{2+} levels was examined in the rat.

Methods: Acute hypercalcemia was induced by intravenous (iv) infusion of 25 mM calcium for 60 minutes and hypocalcemia by iv infusion of 30 mM EGTA. Control rats had an iv saline infusion. Samples for plasma Ca^{2+}, phosphate, PTH and FGF23 were obtained at time 0, 5, 10, 20, 30, 40, 50 and 60 minutes. FGF23 was measured as intact FGF23 by the Kainos Lab assay.

Results: Significant hypercalcemia was induced with an increase in plasma Ca^{2+} from 1.33±0.03 to 1.65±0.02 mmol/L. The levels of FGF23 remained stable, 90±22 to 86±23 pg/mL. In EGTA infused rats plasma Ca^{2+} decreased from 1.33±0.04 to 1.01±0.04 mmol/L, while FGF23 levels remained stable, 51±6 to 69±12 pg/mL. Control rats has stable Ca^{2+} 1.34±0.01 to 1.30±0.01 mmol/L and again stable FGF23 levels were obtained from 77±9 to 92±10 pg/mL.

Conclusions: Acute induction of hypocalcemia or hypercalcemia did not induce any significant changes in plasma levels of intact FGF23 in normal rats.

SA-PO729

Oxidation Matters when Measuring Parathyroid Hormone in Patients on Dialysis

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Background: Only non-oxidized parathyroid hormone (PTH) has bioactive function. We evaluated the hypothesis that determination of biological active parathyroid hormone (BA-PTH) more accurately predicts mortality in hemodialysis patients.

Methods: PTH was measured by means of the third generation intact-PTH electrochemiluminescence immunoassay system, either directly (iPTH) and after prior removal of oxidized PTH molecules using specific monoclonal antibodies raised against the oxidized human PTH (BA-PTH) in 340 prevalent hemodialysis patients (224 men/116 women; median age, 66 years).

Results: 170 patients (50%) died during a follow up of 5 years. Median BA-PTH levels were higher in survivors (7.2 ng/L; IQR 3.1 to 16.5 ng/L) compared to deceased patients (5.0 ng/L; IQR, 1.9 to 11.1 ng/L; p=0.002). Survival analysis showed increased survival in the highest BA-PTH tertile compared to the lowest BA-PTH tertile (Chi square 14.3; p=0.0008 by log-rank test). Median survival was 1702 days in the highest BA-PTH tertile, whereas it was only 453 days in the lowest BA-PTH tertile. We *a priori* stratified iPTH levels into five categories representing very low (<20ng/L), low (20 to 65ng/L), medium (65 to 150ng/L), target (150 to 300ng/L), and high (>300ng/L). Survival analysis showed that patients with target iPTH levels had longer median survival compared to the other categories. However, the BA-PTH to PTH ratio was significantly different among these five categories, ranging from 9 to 17% (p<0.0001). This finding indicates that the amount of BA-PTH but not iPTH predicted patients' survival.

Conclusions: Biologically active PTH (BA-PTH) is directly associated with all-cause mortality in patients on dialysis. Further larger studies are needed to provide evidence whether current PTH guidelines for target PTH levels (based on currently available assay systems) needs to be revised or BA-PTH measured instead.

Funding: Pharmaceutical Company Support - Immundiagnostik AG

SA-PO730

Renoprotective Effects of Vitamin D Receptor Agonist during Low but Not during High Dietary Sodium in Adriamycin Nephrosis

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Background: Renin-angiotensin-aldosterone system (RAAS) blockade is the standard renoprotective therapy and dietary sodium restriction potentiates its efficacy. Add-on vitamin D receptor agonist (VDRA) may provide additional renoprotection but whether its efficacy depends on sodium intake is unknown. We studied the effect of the VDRA paricalcitol, alone and combined with RAAS blockade during low and high sodium intake in adriamycin (ADR)-induced proteinuric nephropathy.

Methods: Six weeks after ADR injection, male Wistar rats (n=64) were randomized into 8 groups stratified for proteinuria (UP). Four groups received low sodium chow (LS, 0.05% NaCl), with vehicle (LS+V) or lisinopril (75mg/l in drinking water; LS+LIS), paricalcitol (3x/wk 160 ng/kg p.o.; LS+P) or both (LS+LIS+P). The other groups received high sodium chow (HS, 2% NaCl) and the same regimens. Blood pressure (BP) and UP were measured 2-weekly. After 6 wks rats were sacrificed; interstitial macrophage accumulation (mø) and α-smooth muscle actin (α-SMA) protein were determined.

Results: At start of treatment, nephrotic range UP was present (mean±SD 212±196 mg/d) and progressed to 335±214 mg/d at wk 12 in LS+V. UP was reduced in LS+P (160±107, p<0.05) and more prominently in LS+LIS (25±15) and LS+LIS+P (30±46, both p<0.05). Systolic BP was elevated in LS+V (164±23 mmHg) and in LS+P (160±12), with lower BP in LS+LIS (108±18) and LS+LIS+P (112±32, both p<0.05). During LS all treatments reduced renal mø (LS+V 29±6 vs. LS+LIS 14±9, LS+P 17±7, LS+LIS+P 12±9, all p<0.05). α-SMA expression was reduced in LS+P (1.1±0.3%) and LS+LIS+P (1.1±0.4%) compared to LS+V (2.2±1.0%, both p<0.05), whereas in LS+LIS (1.4±0.6%) the decrease was non-significant. None of the treatments reduced UP, α-SMA or mø during HS intake.

Conclusions: VDRA has renoprotective effects independent of BP alone and in combination with RAAS-blockade, reducing UP, renal inflammation and pre-fibrotic changes. The renoprotective effects of VDRA require dietary sodium restriction.

SA-PO731

Stronger Association of Fibroblast Growth Factor 23 (FGF-23) with Neutrophil Gelatinase-Associated Lipocalin (NGAL) than with C-Reactive Protein (CRP) in Mexican Americans with Type 2 Diabetes and Chronic Kidney Disease Shweta Bansal,[1,2] Khaled Khazim,[1,2] Nagarjun Kasaraneni,[1,2] Nedal H. Arar,[1,2] Hanna E. Abboud,[1,2] Paolo Fanti.[1,2] *[1]Division of Nephrology, University of Texas Health Sciences Center at San Antonio, San Antonio, TX; [2]Renal Section, Audie L. Murphy VA Hospital, San Antonio, TX.*

Background: In addition to abnormal phosphorus homeostasis, FGF-23 may be regulated by systemic inflammation in chronic kidney disease (CKD). The aim of this study was to evaluate the association of plasma FGF-23 with NGAL, a novel marker of inflammation, in Mexican Americans with different stages of CKD.

Methods: FGF-23, NGAL, CRP and other parameters were assessed in a cross-sectional analysis of stored plasma samples from 115 Mexican Americans who participated in the Family Investigation of Nephropathy and Diabetes (FIND) study from 2001 to 2004. The enrolled population consisted of type 2 diabetic (T2DM) patients with variable kidney functions and their family members.

Results: Of 115 people, 22% were healthy, 26% had T2DM, 8% had microalbuminuria, 20% had macroalbuminuria and 24% were on hemodialysis. In bivariate correlation analysis, $\log_{10}$ transformed FGF-23 and NGAL levels increased progressively with worsening estimated glomerular filtration rate (eGFR) (r=-0.75, p<0.001), and (r=-0.75, p<0.001), respectively and significantly associated with $\log_{10}$CRP (r=0.4, p<0.001), and (r=0.38, p<0.001), respectively. Serum albumin and phosphate were other parameters associated significantly with $\log_{10}$FGF-23 and $\log_{10}$NGAL. On further analysis, Log_{10}FGF-23 levels associated with plasma $\log_{10}$NGAL (r=0.75, p<0.001) and remained significant after adjustment for age, BMI, eGFR, phosphorus, albumin, and CRP in partial correlation analysis (r=0.32, p=0.004), much more pronounced in advanced CKD (r=0.61, p<0.001). The association between FGF-23 and CRP was lost after adjustment for NGAL (r=0.1, p=0.41).

Conclusions: Higher FGF-23 levels are associated with markers of inflammation in Mexican Americans with different stages of diabetic kidney disease. FGF-23 associates better with NGAL than CRP, suggesting NGAL to be a better marker to evaluate the relationship between FGF-23 and inflammation.

Funding: Veterans Administration Support

SA-PO732

Serum Soluble α-Klotho Is Associated with Coronary Calcification and Insulin Sensitivity Ana L.E. Cancela,[1] Silvia M. Titan,[1] Alexandre Costa Pereira,[2] Luciene M. dos Reis,[1] Fabiana G. Graciolli,[1] Susan Schiavi,[3] Raul D. Santos Filho,[2] Vanda Jorgetti,[1] Rosa M.A. Moyses.[1] *[1]Nephrology, Universidade de São Paulo, Brazil; [2]Heart Institute, Universidade de São Paulo, Brazil; [3]Genzyme Co.*

Background: Klotho is a protein related to mineral metabolism homeostasis. In addition, it seems to be involved in aging, atherosclerosis and insulin sensitivity. However, little is known about serum soluble α-Klotho and its association with CAD or other biochemical markers of mineral metabolism, since these assays have just recently become available. In this study, we have analysed these relationships in patients with normal kidney function and suspected CAD.

Methods: Data on 290 patients (167 males) with suspected CAD and a median GFR of 92 ml/min undergoing elective coronary angiography was collected. Serum soluble α-klotho, FGF-23 and other mineral metabolism biomarkers were ascertained. Patients were genotyped for the KL-VS allele. Coronary obstruction was quantified using the Friesinger score (FS). Coronary calcification was assessed by MSCT and quantified using the Agatston score (AS).

Results: Median serum soluble α-klotho was 489 (361- 665) pg/ml. In the univariate and multivariate linear regression analysis, log serum α-klotho was negatively correlated with total calcium (p = 0.02) and positively related to glycemia (p = 0.04). There was no correlation between serum α-klotho and phosphorus, FGF23 or lipid profile. KL-VS polymorphism was not associated with serum klotho, AS or FS. In calcified patients, a positive association between serum α-klotho and AS was found (p=0.04), even after adjustments for other mineral metabolism variables and cardiovascular risk factors.

Conclusions: In patients with suspected CAD and preserved renal function, serum soluble α-klotho levels correlated positively with coronary calcification and glycemia. Prospective studies are needed to confirm its role as a new marker of calcification and insulin sensitivity.

Funding: Government Support - Non-U.S.

SA-PO733

Is Insulin Resistance Associated with Disrupted Renal Phosphorus Homeostasis in Stage 3-5 Chronic Kidney Disease? Jocelyn S. Garland,[1] Rachel M. Holden,[1] Robert Louis Nolan,[2] Wilma M. Hopman,[3] Alexander R. Morton.[1] *[1]Medicine, Queen's University, Kingston, ON, Canada; [2]Radiology, Queen's University, Kingston, ON, Canada; [3]Clinical Research Center, Kingston General Hosptial, Kingston, ON, Canada.*

Background: Insulin is directly involved in renal phosphorus (PO_4) handling, increasing renal PO_4 re-absorption at the proximal tubule sodium-phosphate co-transporter type II (NaPi-2). In insulin resistance, insulin receptor number remains preserved in the kidney,which we hypothesize could maintain, or increase, renal PO_4 re-absorption. Our primary hypothesis was insulin resistant CKD patients would demonstrate greater disruption in renal PO_4 homeostasis, detected by greater fibroblast growth factor-23 (FGF-23) levels.

Methods: 72 stage 3-5 pre-dialysis CKD patients were enrolled to evaluate associations between insulin resistance (homeostasis model assessment of insulin resistance (HOMA-IR)) and disrupted renal PO_4 homeostasis (FGF-23). Coronary artery calcification (CAC), FGF-23, and mineral metabolism parameters were compared based on median HOMA-IR. Patients with diabetes treated with insulin were excluded.

Results: Patients with HOMA-IR > 2.2 had 30% higher log FGF-23, and 40% higher CAC. FGF-23 correlated with HOMA-IR (r=0.25; P=0.04), waist circumference (r=0.29, P=0.02), and body mass index (r=0.33; P=0.004). Linear regression: insulin resistance was a risk factor for greater log FGF-23 levels, (HOMA-IR, ß = 0.37; 95% CI 0.14 to 0.6; P=0.002), adjusted for age, 1,25 Dihydroxyvitamin D, kidney function, and parathyroid hormone (PTH).

Clinical Characteristics Based on HOMA-IR

Variable	HOMA-IR >2.2	HOMA -IR < 2.2	P
Age	63 ± 13	65 ±1 5	0.64
eGFR	26.8 ± 12.1	24.9 ± 13.5	0.5
iPTH	9.85 (5.8 – 21.4)	8.05 (5.6 – 18.5)	0.7
1,25(OH)2 Vit D	49.9 (33-70)	50.7 (35 – 80)	0.1
phosphorus	1.26 ± 0.21	1.27± 0.27	0.8
calcium	2.35 ± 0.27	2.26 ± 0.2	0.15
BMI	35.1 ± 7.5	25.3 ± 4.3	<0.0001
Log FGF-23	2.32 ±0.02	2.04 ± 0.42	0.01
Log CAC score	2.09 ± 0.87	1.58 ± 1.26	0.05

Conclusions: Insulin resistant CKD patients demonstrated greater disruption in renal PO_4 homeostasis (↑ FGF-23) and CAC, while PO_4 levels remained normal. These findings suggest insulin resistance may modify renal PO_4 handling in CKD, and studies are needed to explore this relationship.

SA-PO734

Serum Soluble α-Klotho Levels in Patients with Chronic Kidney Disease Noriyuki Okada,[1] Kodo Tomida,[2] Akira Suzuki,[2] Terumasa Hayashi,[2] Tatsuya Shoji,[2] Yoshiharu Tsubakihara.[3] *[1]Clinical Labratory, Osaka General Medical Center, Osaka, Japan; [2]Kidney Disease and Hypertension, Osaka General Medical Center, Osaka, Japan; [3]Comprehensive Kidney Disease Reserch, Osaka University Graduate School of Medicine, Suita, Japan.*

Background: Membrane-bound Klotho is known a cofactor for fibroblast growth factor 23(FGF-23) to regulate phosphorus and vitamin D homeostasis. Soluble Klotho in circulation is known to act a humoral factor such as regulating insulin and IGF-1 receptor binding, calcium and phosphorus homeostasis. Klotho deficiency is associated with progression and complications in chronic kidny disease(CKD). A few investigators reported that soluble Klotho level were decreased in patients with CKD from early stage, but other investigator reported no change of soluble Klotho in CKD.

Methods: Fourty eight patients (30 males and 18 females, 34-86 yrs of age) with CKD stage 2-5 were enrolled in this study. We measured serum soluble α-Klotho(sαKl), FGF-23, intact PTH, and 1,25$(OH)_2$D3. SαKl were measured by using human soluble α-Kloth kit(Immuno-Biological Laboratories Co.,Ltd. Gunma, Japan). We calculated eGFR using the Japanese equation for estimating GFR.

Results: 1,25$(OH)_2$D3 levels progressively declined from Stage 2 to Stage 5. Intact PTH and FGF-23 levels begun to rise in Stage 3A, and thereafter drastically elevated. Whereas serum calcium and phosphate levels were within normal range in this group. We couldn't observe significant relationship between eGFR and sαKl level(P=0.78), a few patients revealed low sαKl levels in Stage 4-5. SαKl levels in various CKD stage were as follows (mean±SD) : Stage 2 (n=5) 457.0±68.8pg/ml, Stage 3A (n=9) 540.2±117.5pg/ml, Stage 3B (n=15) 513.3±69.3pg/ml, Stage 4 (n=14) 517.1±144.9pg/ml, Stage 5 (n=5) 438.7±202.8pg/ml. SαKl levels in these stages showed no significant difference each other. SαKl levels had no significant relationship to intact PTH, FGF-23, 1,25$(OH)_2$D3, calcium and phosphate levels, respectively.

Conclusions: In our small group study, we observed progressive decline of 1,25$(OH)_2$D3 levels and elevation of intact PTH and FGF-23 levels with deterioration of renal function, but sαKl levels were not associated with renal function.

SA-PO735

Bone Disease Is an Early Event in Lupus Nephritis Patients Aline Lázara Resende, Cristiane Bitencourt Dias, Luciene M. dos Reis, Vanda Jorgetti, Viktoria Woronik. *University of Sao Paulo, Sao Paulo, Brazil.*

Background: The role of glucocorticoid as the major factor for bone loss in lupus nephritis patients (LN) has been questioned. Our aim was to evaluate bone disease in newly diagnosed LN patients.

Methods: Pre-menopausal female patients with ≤2months of diagnosed LN and GFR>30 ml/min/1.73m^2 were submitted to bone biopsy and histomorphometry analysis. Controls for static parameters were female age-matched healthy subjects (1:2) from a Brazilian database (Dos Reis et al. Bone 1998;23)[1]. Dinamic parameters were compared to female subjects from Melsen et al. (Calcif Tiss Res 1978;26)[2].

Results: Patients presented a mean age of 30.5±9.7years and were on glucocorticoids for 35.3±11.5days. Seven patients presented proliferative LN. Mean proteinuria was 6.0±1.9g/day and estimated GFR was 65.1±34.5ml/min/1.73m^2. All patients presented vitamin D deficiency (25OHD=8.8±2.7ng/ml). Structure parameters of patients and controls were not different. LN patients presented a reduced OV/BV, O.Th, MS/BS and BFR/BS, suggesting an impaired bone formation and mineralization These patients also presented higher ES/BS and Oc.S/BS, revealing an increased bone resorption.

Histomorphometric parameters of LN patients and controls.

	LN Patients (n=8)	Controls (n=16)[1] (n=29)[2]	p
Formation			
OS/BS (%)	2.13 (0.98-12.61)	8.6 (6.6-16.1)	0.06
Ob.S/BS (%)	0.21 (0.09-0.78)	0.75 (0.07-2.37)	0.34
OV/BV (%)	0.11 (0.04-0.71)	1.57 (1.06-2.92)	0.004
O.Th (µm)	2.74±1.17	11.3±2.07	<0.001
Mineralization			
MS/BS (%)	2.18±2.14	12±5.0	0.005
MAR (µm/day)	0.47±0.24	0.64±0.1	0.52
BFR/BS (µm3/ µm2/ day)	0.01±0.01	0.07±0.03	0.002
MLT (days)	26.49±13.06	23.7±2.7	0.84
Resorption			
ES/BS (%)	8.27 (4.71-11.55)	1.9 (1.3-3.1)	<0.001
Oc.S/BS (%)	0.3 (0.19-0.71)	0.01 (0-0.1)	<0.001

OS/BS=osteoid surface;Ob.S/BS=osteoblast surface;OV/BV=osteoid volume;O.Th=osteoid thickness;MS/BS=mineralization surface;MAR=mineral apposition rate;BFR/BS=bone formation rate;MLT=mineralization lag time;ES/BS=eroded surface;Oc.S/BS=osteoclast surface.

Conclusions: Newly diagnosed LN patients presented an impaired bone formation and mineralization associated with an increase in resorption parameters. These findings could not be attributed to glucocorticoids and might be related to vitamin D status and inflammatory factors.

Funding: Government Support - Non-U.S.

SA-PO736

Newly Diagnosed Lupus Nephritis Patients: Bone Disease beyond Glucocorticoid Use and Proteinuria Aline Lázara Resende, Cristiane Bitencourt Dias, Luciene M. dos Reis, Vanda Jorgetti, Viktoria Woronik. *University of Sao Paulo, Sao Paulo, Brazil.*

Background: The etiology of bone loss in lupus nephritis patients (LN) is multifactorial. Our aim was to evaluate bone disease in newly diagnosed LN patients.

Methods: Pre-menopausal female patients with ≤2 months of diagnosed LN and GFR>30 ml/min/1.73m^2 were submitted to bone biopsy and histomorphometry analysis. Controls were primary glomerulopathies (PG) female patients with similar estimated GFR and proteinuria (Dias et al. Kidney Int 2007;71).

Results: Mean period of glucocorticoid use was 35.3±11.5days. LN patients presented significantly lower vitamin D levels, possibly contributing to higher iPTH levels. Structure parameters were not different. LN patients presented a reduced O.Th, suggesting a reduced bone formation, and a higher ES/BS, revealing an increased bone resorption.

Clinical and histomorphometric parameters of LN patients and controls

	Lupus Nephritis (n=8)	Primary Glomerulopahties (n=6)	p
Age (years)	30.5±10.4	33.5±5.3	0.49
24h Proteinuria (g/day)	6.0±2.0	5.6±3.3	0.75
Serum creatinine (mg/dl)	1.32±0.58	0.87±0.16	0.23
Estimated GFR (ml/ min/1.73m2)	65.1±34.5	84.1±22.8	0.35
25(OH)D (ng/ml)	8.75±2.87	18.39±11.10	0.02
iPTH (pg/ml)	72 (22.5-120.8)	18.5 (10-25.5)	0.03
Formation			
OV/BV (%)	0.11 (0.04-0.71)	0.33 (0.13-1.11)	0.34
Ob.S/BS (%)	0.21 (0.09-0.78)	0.47 (0.35-2.58)	0.18
O.Th (µm)	2.74±1.17	7.1±4.25	0.04
OS/BS (%)	2.13 (0.98-12.61)	3.9 (1.68-10.75)	0.75
Mineralization			
MS/BS (%)	2.18±2.14	4.2±3.49	0.10
MAR (µm/day)	0.47±0.24	0.62±0.21	0.39
BFR/BS (µm3/ µm2/ day)	0.01±0.01	0.03±0.03	0.08
MLT (days)	26.49±13.06	17±8.37	0.17
Resorption			
ES/BS (%)	8.27 (4.71-11.55)	2.55 (0.9-3.88)	0.002
Oc.S/BS (%)	0.3 (0.19-0.71)	0.21 (0-0.33)	0.14

OS/BS=osteoid surface;Ob.S/BS=osteoblast surface;OV/BV=osteoid volume;O.Th=osteoid thickness;MS/BS=mineralization surface;MAR=mineral apposition rate;BFR/BS=bone formation rate;MLT=mineralization lag time;ES/BS=eroded surface;Oc.S/BS=osteoclast surface.

Conclusions: Newly diagnosed LN patients presented reduced bone formation and increased bone resorption when compared with patients with similar proteinuria. These findings could not be attributed to glucocorticoid use and might be related to the vitamin D status and inflammatory factors.

Funding: Government Support - Non-U.S.

SA-PO737

FGF-23 Inhibits Hyperphosphatemia-Induced Apoptosis and Stabilizes Tight Junction Protein ZO-1 Expression in Human Brain Microvascular Endothelial Cells in CKD, In Vitro Chih-ping Chung,[1,2] Tzong-Shi Lu,[1] Kenneth Lim,[1,3] Christina Lee,[1] Daniel Zehnder,[3] Li-Li Hsiao.[1] *[1]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Department of Neurology, Taipei Veterans General Hospital, Taipei, Taiwan; [3]Clinical Sciences Research Institute, Warwick Medical School, United Kingdom.*

Background: CKD patients exhibit cerebral small-vessel diseases among other cardiovascular consequences. Fibroblast growth factor 23 (FGF23) levels rise in CKD and functions to counteract hyperphosphatemia. We postulate that hyperphosphatemia is a major contributor of brain microvascular endothelial dysfunction in CKD, whilst rising FGF-23 levels may inhibit this process.

Methods: *In vitro*: Human brain microvascular endothelial cells (HBMECs). CKD conditions: borderline-high (2mM) and high (5mM) phosphate for 72 hours. Cell viability: XTT assay. Apoptosis: caspase-3 fluorometric assay.

Results: We show that treatment of HBMECs with 5mM but not 2mM phosphate decreased cell viability and increased caspase-3 mediated apoptosis. FGF-23 inhibited phosphate-induced caspase-3 mediated apoptosis at the concentrations ranging from 0.5 – 10ng/ml with significance at 5 and 10ng/ml. We next treated HBMECs with recombinant soluble Klotho (0.2 nM) and showed that it also inhibited phosphate-induced caspase-3 mediated apoptosis. Furthermore, we show that 5mM but not 2mM phosphate decreased the tight junction protein, ZO-1 expression in HBMECs and FGF-23 reversed phosphate-induced ZO-1 suppression at the concentration of 5 and 10ng/ml. However, HBMECs treated with soluble Klotho did not modulate phosphate-induced suppression of ZO-1.

Conclusions: Our study is the first to describe hyperphosphatemia as a significant contributor of cerebral microvascular dysfunction. Furthermore, we show that FGF-23 can reverse HBMEC apoptosis and stabilize tight junction protein ZO-1 induced by hyperphosphatemia. We therefore speculate that hyperphosphatemia may be involved in the development of cerebral small vessel disease and propose rising FGF-23 levels as a mediator of a bone-kidney-brain axis in CKD.

SA-PO738

CSF-1 Dependent Defective Kidney Repair Triggers Nephritis in Lupus-Susceptible Mice Yasunori Iwata,[1,3] Elisabeth A. Boström,[3] Julia Menke,[2] Takashi Wada,[1] Vicki R. Kelley.[3] *[1]Kanazawa University; [2]Johannes Gutenberg-University of Mainz; [3]Brigham and Women's Hospital.*

Background: Inflammation is meant to promote healing. Macrophages (Mø) are central to inflammation and require colony stimulating factor (CSF)-1 for survival, proliferation and activation. Our prior studies indicate that CSF-1 is upregulated in tubular epithelial cells and hastens renal tubular repair following transient injury, ischemia/reperfusion (I/R) in normal (B6 and BALB/c) mice. Paradoxically, CSF-1 drives lupus nephritis in genetically-susceptible mice. We hypothesized that CSF-1 dependent Mø-mediated inflammation after I/R leads to non-resolving inflammation that triggers lupus nephritis in lupus-susceptible mice.

Methods: Using MRL-*Fas^{lpr}* mice genetically constructed to express differing levels of CSF-1 in the kidney and circulation: TgC/+ (high), wildtype (moderate), and op/op (none), we compared parameters of kidney repair (tubular pathology, proliferation/apoptosis and KIM expression, magnitude of Mø and fibrosis) and lupus nephritis (glomerular pathology and IgG/C3 deposits, and serum IgG) after I/R. Normal (B6 and BALB/c) mice served as controls.

Results: We detected defective renal tubular repair and non-resolving inflammation (accumulation of Mø) in MRL-*Fas^{lpr}* mice compared to normal mice after I/R. Moreover, I/R triggered early-onset lupus nephritis (glomerulonephritis, IgG/C3 glomerular deposits and elevated serum IgG) in MRL-*Fas^{lpr}* mice, and not in normal mice. Defective renal repair and early-onset lupus nephritis was most pronounced in MRL-*Fas^{lpr}* mice expressing the highest level of CSF-1 in the kidney and circulation. Moreover, we did not detect tubular pathology, an increase in Mø and early-onset lupus nephritis in MRL-*Fas^{lpr}* mice deficient in CSF-1.

Conclusions: CSF-1 released from injury tubules mediates defective Mø mediated renal repair and non-resolving inflammation, that hastens the onset of lupus nephritis in genetically susceptible mice. This suggests that CSF-1 expands Mø in lupus-susceptible individuals that are responsible for triggering lupus nephritis.

Funding: Government Support - Non-U.S.

SA-PO739

TWEAK Transactivates Epidermal Growth Factor Receptor to Modulate Renal Inflammation in the Kidney Sandra Rayego-Mateos,[1] Jose Morgado,[2] Ana Belen Sanz,[2] Daniel Batlle,[3] Janos Pato,[4] Gyorgy Keri,[4] Jesus Egido,[2] Alberto Ortiz,[2] Marta Ruiz-Ortega.[1] *[1]Nephrology, Universidad Autónoma, Madrid, Spain; [2]Nephrology, IIS-Fundacion Jimenez Diaz, Madrid, Spain; [3]Northwestern University Feinberg Medical School, Northwestern University, Chicago, IL; [4]Vichem, Budapest, Hungary.*

Background: The cytokine tumor necrosis factor-like weak inducer of apoptosis (TWEAK), binds to its receptor Fn14 to elicit multiple biological activities, including stimulation of cell growth/apoptosis, angiogenesis and induction of inflammatory cytokines. TNF-α induced transactivation of the EGF receptor (EGFR) in a variety of cells, a process involved in renal disease progression. EGFR transactivation involves the release of EGFR ligands by metalloproteinases of the ADAM family, of which ADAM-17 is the best characterised member. Our aim was to investigate whether TWEAK could transactivate EGFR in the kidney and the involvement of this molecular pathway in TWEAK mediated-renal responses.

Methods: The in vivo effect of Tweak was evaluated by intraperitoneal injection of recombinant human Tweak (0.5 µg/per mouse) into C57BL6 mice and in vitro in tubular epithelial cells.

Results: TWEAK in vivo increased renal EGFR phosphorylation levels, mainly located in tubular epithelial cells. In vitro, TWEAK by binding to Fn14, induced EGFR phosphorylation in tubular epithelial cells. This procces is mediated by ADAM17, as demonstrated by pharmacological inhibition with TAPI-2, or by ADAM17 gene silencing. In Tweak-injected mice treatment of with the EGFR kinase inhibitor erlotinib markedly diminished renal EGFR activation and inhibited TWEAK-induced renal changes observed at 24 hours, including ERK activation (a downstream EGFR signalling), up-regulation of proinflammatory factors and interstitial inflammatory cell infiltration. Moreover, in tubular epithelial cells EGFR transactivation blockade, by TAPI and ADAM17 gene silencing, diminished TWEAK-induced ERK activation and upregulation of proinflammatory factors.

Conclusions: TWEAK transactivates EGFR signalling pathway leading to modulation of downstream mechanisms, such as ERK activation, and cellular responses, including renal inflammatory cell infiltration.

Funding: Government Support - Non-U.S.

SA-PO740

Expression of Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (TWEAK) & TWEAK Receptor, Fibroblast Growth Factor-Inducible 14 (Fn 14) in IgA Nephropathy Jungkook Wi, Hong Joo Lee, Joo Hee Cho, Wha-young Suk, Eun Young Kim, Ju-Young Moon, Sang-Ho Lee, Kyung-hwan Jeong, Tae-won Lee, Chun-Gyoo Ihm. *Department of Nephrology, College of Medicine, Kyung Hee University, Seoul, Republic of Korea.*

Background: Recently,it was reported that TWEAK might play an important role in the pathogenesis of kidney damage. In present study, we investigated the correlation between clinical parameters and urinary TWEAK levels and the degree of TWEAK-Fn14 interaction expressed by immunohistochemistry (IHC) in IgA nephropathy (IgAN).

Methods: We investigated urinary TWEAK and serum TWEAK levels in 21 patients with biopsy proven IgAN and in 15 healthy volunteers. We also examined the renal TWEAK-Fn14 expression by IHC. As a control, we used nonpathological kidney tissues obtained from nephrectomized surgical specimen. The expressions of TWEAK and Fn14 were assessed using Allred scoring system by one pathologist and one nephrologist.

Results: The mean age was 36.1 ± 9.5 years in IgAN group and 36.0±8.3 years in healthy control group. The level of uTWEAK in IgAN was significantly higher compared to healthy controls ($p<0.01$). While the TWEAK and Fn14 interaction was not observed in control human kidney tissues, it was, in contrast, clearly observed in tubular cells of IgAN patients.There was no correlation between the levels of sTWEAK or uTWEAK and score of immunostaining with TWEAK ($p=0.496$) or Fn 14 ($p=0.440$) or other clinical parameters. The score of immunostaining did not correlate with the proteinuria or the degree of histological change. There is, however, the significant correlation between the immunostaining of TWEAK and Fn14 and eGFR of IgAN patients ($P= 0.02$ and 0.03, respectively).

Conclusions: This study shows that the degree of TWEAK & Fn 14 interaction in IgAN has a significant correlation with renal function, suggesting the role in the pathogenesis of IgAN.

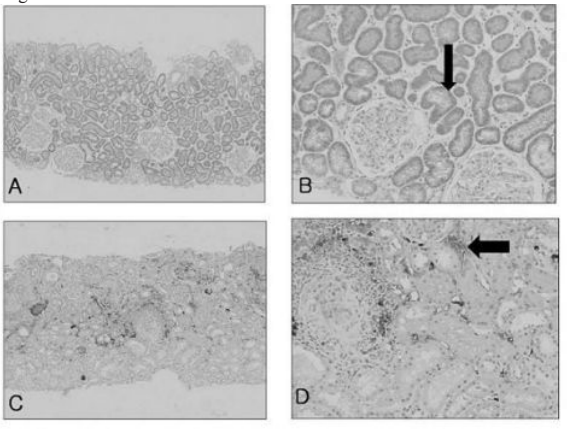


Fig 1. A & B : Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in kidney of IgA nephropathy by IHC. C & D : Expression of fibroblast growth factor-inducible 14 (Fn 14) protein in kidney of IgA nephropathy by IHC.
(A ,C: low power field x100. B,D : high power field x 200)

Funding: Private Foundation Support

SA-PO741

Involvement of Endoplasmic Reticulum Stress in Bovine Serum Albumin Induced NLRP3 Inflammasome Activation Li Fang, Ping Wen, Lei Jiang, Chunsun Dai, Junwei Yang. *Center for Kidney Disease, 2nd Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Albuminuria, an universal feature of chronic kidney disease, contribute to the progression of tubulointerstitial fibrosis. Although it seems that ongoing albuminuria can lead to tubular injury, the molecular machnism remains mostly unknown. Here we show that endoplasmic reticulum (ER) stress is involved in the inflammasome activation of tubular epithelial cells induced by bovine serum albumin.

Methods: Both the renal biopsies samples from patients with albuminuria and the cultured NRK-52E were investigated. The association between the severity of albuminuria or the injury of NRK-52E and the inflammasome activation was estimated. The resting NLRP3 inflammasome was located on ER of kidney epithelial cells according to our results of immunofluorescence staining of renal tissues or cultured cells. To further evaluate the role of ER stress on activation of the NLRP3 inflammasome induced by albumin, NRK-52E were exposed to bovine serum albumin (BSA) with or without the chemical chaperone taurine-conjugated ursodeoxycholic acid (TUDCA) and the inflammasome activation was assessed.

Results: In the biopsies samples, we provide the inflammasome activation characterized by the caspase-1 activation and maturation of IL-1b and IL-18 is associated with the severity of albuminuria. In vitro, BSA can also induce the activation of NLRP3 inflammasome. Because there is a significant overlap of NLRP3 with the ER marker calreticulin, the *ER* stress provoked by BSA seems to play a crucial role in the activation of the NLRP3 inflammasome. For exploration of the functional roles of ER stress, we demonstrated that TUDCA which was proved to be an enhancer for the adaptive capacity of ER could attenuate NLRP3 inflammasome activation induced by albumin not only in vitro but also in diabetic nephropathy.

Conclusions: These findings suggest that ER stress seems to play an important role in BSA-induced NLRP3 inflammasome activation, and elimination of ER stress via TUDCA may hold promise as an novel avenue for preventing NLRP3 inflammasome activation and ameliorating tubular injury induced by albuminuria.

Funding: Government Support - Non-U.S.

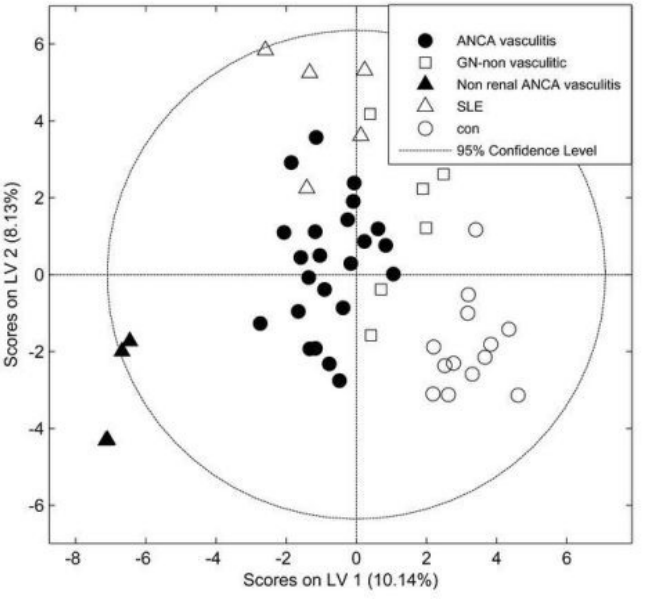
SA-PO742

Urinary Metabolomic Biomarkers in Remitting and Relapsing Vasculitic Glomerulonephritis Bahjat Al-ani,[4] Hamad Al Nuaimi,[4] Martin A. Fitzpatrick,[3] Stuart W. Smith,[4] Caroline O.S. Savage,[4] Yasmeen Ghani,[2] Alan D. Salama,[2] Stephen P. Young,[3] Mark A. Little.[1] *[1]Trinity Health Kidney Centre, TCD, Ireland; [2]UCL Centre for Nephrology Royal Free, UCL, United Kingdom; [3]Dept of Rheumatology, University of Birmingham, United Kingdom; [4]Renal Immunobiology, University of Birmingham, United Kingdom.*

Background: Current vasculitis biomarkers lack predictive value and are insensitive to early vasculitic injury. To address this, we analysed the longitudinal urinary metabolomic profile in a rat model of anti-MPO vasculitis (Experimental Autoimmune Vasculitis, EAV).

Methods: We obtained urine at regular intervals over 6 months after EAV induction after which we induced relapse with MPO and endotoxin. Urine metabolites were assessed using NMR, partial least squares discriminant analysis (PLSDA) and partial least squares regression (PLS-R). To validate findings in humans, we analysed the urine of patients with renal anti-MPO vasculitis (varying disease activity), non-renal vasculitis, non-vasculitic glomerulonephritis (GN) and healthy controls.

Results: In EAV, peak haematuria and anti-MPO titre occurred 8 wks post immunisation and declined steadily thereafter. Four weeks after relapse, histological markers of GN and lung iron staining (lung haemorrhage) increased compared to non-relapsed animals. Rats with acute vasculitis (8 wks) had a significantly different metabolite profile compared to controls; the observed PLSDA clusters dissipated between 8 and 26 weeks and re-emerged after relapse. The most raised metabolites in rats with active vasculitis were betaine, trimethylamine and dimethylglycine, which correlated well with the GN score. In patients with renal vasculitis, we observed similar clustering and some of the same metabolites were elevated in the urine.



Conclusions: Vasculitic GN results in a distinct urinary metabolite profile, forming a basis for novel biomarker development.

SA-PO743

Evidence for a Pro-Inflammatory Role of P2X7 in Anti-Podocyte Nephritis

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Background: The P2X7 receptor is a ligand-gated cation channel that is expressed by a variety of immune cells, including macrophages and lymphocytes. Activation of P2X7 leads to influx of Ca^{2+} ions and the efflux of K^+ ions. Calcium influx is linked with the activation of the pro-inflammatory metalloprotease ADAM17 in T cells. Moreover, P2X7 activation induces the assembly of the NALP3 inflammasome complex resulting in the release of IL-1β. Sustained activation of P2X7 leads to cell death. Increased expression of both P2X7 mRNA and protein was reported in mesangial cells and macrophages infiltrating the glomeruli in animal models of antibody-mediated glomerulonephritis. Cultured podocytes express mRNA of P2X7and react to benzoyl ATP, a selective agonist of P2X7. P2X7 could therefore mediate podocyte injury and be a potential therapeutic target for podocyte disease.

Methods: In this study, we used P2X7-knockout mice in the experimental model of anti-podocyte nephritis. In addition we investigated the role of P2X7 with pharmacological modulation in wild-type mice.

Results: P2X7 deficiency was significantly renoprotective compared with wild-type controls, evidenced by better renal function, a striking reduction in proteinuria, and decreased histologic glomerular and podocyte injury. In addition, the selective blockade of P2X7 attenuated, while the selective activation of P2X7 accelerated and exacerbated the course of anti-podocyte nephritis.

Conclusions: These results support a pro-inflammatory role for P2X7 in podocyte injury and suggest that the P2X7 receptor is a potential therapeutic target in podocyte damage.

SA-PO744

Alternative Pathwayactivation of Complement by Shiga Toxin Induces Podocyte Damage in Experimental HUS

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Background: Shiga toxin (Stx)-producing *E.coli* O157:H7 is the offending agent of diarrhea-associated hemolytic uremic syndrome (HUS), a disorder characterized by microvascular thrombosis and glomerular ischemic changes. We recently found that Stx induced complement (C) alternative pathway-mediated C3b deposition and C3a generation at glomerular level, responsible for endothelial P-selectin expression and thrombus formation in experimental HUS. In search for mechanisms linking C activation and glomerular capillary wall damage, we explored in a murine HUS model whether early glomerular C activation/deposition caused podocyte injury.

Methods: Mice i.p. injected with Stx2 plus LPS were sacrified at 24 and 48h and kidneys used for immunohistochemistry.

Results: In Stx2/LPS mice, exuberant glomerular C3b deposits preceded a substantial decrease in podocyte number (Stx/LPS 24h, 92±10 and 48h, 54±1 vs saline, 138±10podo /glom, $p<0.05$ and $p<0.01$)**.** By contrast, factor B deficient mice challenged with Stx/LPS were protected from C deposition and showed limited podocyte loss (Stx/LPS 24h, 82±6 and 48h, 90±8 vs saline,102±8 podo/glom), indicating that the alternative pathway of C triggers podocyte injury. C3a receptor antagonist given to Stx/LPS mice also lessened podocyte loss to a significant extent as compared to untreated mice (11 vs 44 %, $p<0.05$). Relevance for integrin-linked kinase (ILK) regulation and related proteins on podocyte dysfunction was also addressed. In Stx/LPS mice, increased ILK expression was followed by upregulation of Snail, a transcription factor that promotes podocyte dedifferentiation and motility. In parallel, alpha actinin-4, a podocyte protein associated to F-actin and integrin signals, was markedly reduced (score: Stx/LPS 24h, 2.09±0.1 and 48h, 1.62±0.1 vs saline 2.9±0.1, $p<0.01$). Factor B deficiency limited glomerular changes in ILK, Snail and alphaactinin-4.

Conclusions: These results identify Stx-induced C activation as a key mechanism of C3a-dependent podocyte damage in experimental model of human typical HUS.

Funding: Private Foundation Support

SA-PO745

NF-κB Inhibition Strongly Attenuates Interstitial Inflammation and Renal Functional Loss Induced by Excess Dietary Adenine

Cristiene Okabe, Raquel L. Borges, Denise M.A.C. Malheiros, Camilla Fanelli, Flavia G. Machado, Simone C.A. Arias, Grasiela P. Barlette, Thales de Brito, Niels O.S. Camara, Roberto Zatz, Clarice K. Fujihara. *Univ of Sao Paulo, Brazil.*

Background: Dietary adenine (ADE) excess promotes severe interstitial (INT) inflammation and fibrosis, leading to progressive INT nephritis and renal insufficiency. We showed previously [PONE, 2011;6:e29004] that, on excess ADE, KO mice for TLR-2, -4, MyD88, ASC or Caspase-1 exhibit less renal injury than WT mice, suggesting a pathogenic role for TLR activation and inflammasome assembly in this model. Here we investigated whether a distinct pathway of innate immunity – the NF-κB system – also participates in the chain of reactions triggered by intratubular crystal (Crys) precipitation.

Methods: Forty-two adult male Munich-Wistar rats were divided in 3 groups. **C:** given standard chow; **ADE:** given ADE in chow, 0,75% during 1 week and 0.53% for 2 weeks; **ADE+PDTC**, given ADE as described and the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC), 120 mg/kg/day in drinking water. At the end of this 3-week period, blood pressure (BP, mmHg), serum creatinine (S_{Cr}, mg/dL), glomerular volume (V_G, $10^6\mu m^3$), % cortical interstitium (%INT), the rate of tubulointerstitial (TI) cell proliferation ($PCNA^+$ cells/mm^2), the intensity of Crys deposition/mm^2 and the number of renal granulomas (Gran)/mm^2 were measured.

Results:

	BP	Scr	V_G	%INT	$PCNA^+$	Crys	Gran
C	121±2	0.6±0.1	0.85±0.03	0.2±0.1	35±2	0±0	0±0
ADE	130±4[a]	2.8±0.1[a]	0.58±0.02[a]	20.0±1.1[a]	1187±112[a]	114±7[a]	26±2[a]
ADE+PDTC	132±3[a]	1.5±0.2[ab]	0.68±0.02[a]	9.0±1.2[ab]	725±62[ab]	100±7[a]	4±1[ab]

Mean±SE, [a]$p<0.05$ vs. C, [b]$p<0.05$ vs. ADE+V.

Conclusions: As expected, ADE promoted Crys deposition and Gran formation, in association with slight hypertension, glomerular shrinkage, TI proliferation, severe INT damage and marked creatinine retention. PDTC strongly attenuated ADE-induced renal injury/inflammation/hyperplasia and functional decline, without changing Crys. These findings are consistent with the concept that the NF-κB system is activated by intratubular Crys precipitation and contributes, along with other pathways of innate immunity, to initiate the severe inflammatory response associated with this model. FAPESP/CNPq.

Funding: Government Support - Non-U.S.

SA-PO746

TLR-2/-4 TREM-1 Pathway Is Activated but Dispensable in Inflammatory Macrophages in Sterile Kidney Injury

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Background: Inflammatory macrophages (MΦ) are abundant in kidney disease, stimulating repair, or driving fibrosis by excessive or inappropriate activation. Damage associated molecules (DAMPs), released from injured cells, engage pattern recognition receptors (PRRs) on MΦ, contributing to activation. Triggering Receptor Expressed in Myeloid cells (TREM-1) and Toll-Like Receptors (TLRs) are important activators in MΦ. Understanding mechanisms of MΦ activation during kidney injury would help to prevent fibrosis.

Methods: Kidney MΦ were isolated 5d after Unilateral Ureteral Obstruction (UUO) and Microarray was performed. Crude DAMPS were purified from UUO kidney to stimulate cells *in vitro*. Mice were subjected to UUO and unilateral ischemia and reperfusion injury (IRI), and treated with soluble TREM1-fc and analyzed for responses to injury. TLR2-4-/-, Myd88-/- or Csf1Rcre;Myd88fl/fl mice were subjected to UUO or IRI and analyzed for rsponses to injury.

Results: TREM-1 is highly upregulated in kidney MΦ after injury and is a marker of M1 cells. DAMPs from UUO kidney activated bone marrow MΦ *in vitro* independently of TREM-1, but partially dependent on TLR-2/-4, MyD88 pathway. In UUO and IRI, TREM-1 blockade by TREM1-Fc treatment had no impact on disease progression or MΦ activation *in vivo*. In the IRI model, TLR-2/-4 or MyD88 deficiency was anti-inflammatory. However, deletion of MyD88 only in myeloid cell lineage had no effect on MΦ activation or disease progression. Instead TLR-2/-4 or MyD88 deficiency reduced activation of mesenchymal lineage of cells (pericytes and myofibroblasts) resulting in less fibrosis.

Conclusions: TREM-1 and TLR2/4 pathways are dispensable in MΦ activation in sterile kidney injury but TLRs play a role in mesenchymal cell activation.

Funding: NIDDK Support, Pharmaceutical Company Support - Genzyme GRIP Award

SA-PO747

Integrin Linked Kinase Plays a Key Role in the Regulation of the Renal Inflammatory Response

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Background: Inflammation plays a key role in the progression of renal diseases. Integrin-linked kinase (ILK) is involved in cell-matrix interactions and regulates many processes, including proliferation, migration, and angiogenesis, while its role in the inflammatory process is unknown. We therefore investigated whether ILK-signaling system regulates renal inflammatory response.

Methods: The role of ILK in renal inflammation was studied using the model of systemic infusion of Angiotensin II (AngII) in conditionally ILK deficient mice (cKO). I*n vitro* the blockade of endogenous ILK was done by small interfering RNA in cultured tubular epithelial cells (HK-2).

Results: AngII-infused mice presented an inflammatory response in the kidney, characterized by interstitial infiltration of monocytes/macrophages (F4/80+ cells) and T lymphocytes (CD3+) and upregulation of proinflammatory molecules and adhesion factors (MCP-1, IL-6, RANTES and ICAM-1). In AngII-infused ILK-deficient mice, the number of interstitial inflammatory cells and the renal levels of proinflammatory factors were similar to controls (saline-infused cKO). Moreover, AngII-induced renal Akt and NF-kB activation was abolished in ILK deficient mice, suggesting a downstream mechanism of ILK. To further investigate molecular mechanism in vitro studies were done. In HK2 cells, ILK gene silencing inhibited the activation of Akt/GSK3 induced by AngII, demonstrating that GSK3 and Akt are downstream signals in ILK pathway. The activation of NF-kB pathway is regulated by ILK, as observed by downregulation of NF-kB activation and related gene upregulation by silencing ILK and, its downstream signalling GSK3α/β.

Conclusions: Our data demonstrated a key role of ILK in renal inflammation, by regulation of GSK3α/β/Akt signaling and activation of NF-kB pathway, and suggest a potential therapeutic target for AngII-mediated renal diaseses.

Funding: Government Support - Non-U.S.

SA-PO748

Phenotypic Shift of Activated Glomerular Cells towards Redifferentiation by the Unfolded Protein Response Hisashi Johno, Shotaro Nakajima, Hironori Kato, Jian Yao, Masanori Kitamura. *Department of Molecular Signaling, University of Yamanashi, Chuo, Yamanashi, Japan.*

Background: During recovery from acute glomerulonephritis, cell proliferation, matrix expansion and expression of a-smooth muscle actin (a-SMA; dedifferentiation marker) subside spontaneously. However, molecular mechanisms underlying this recovery process remain elusive. In mesangioproliferative glomerulonephritis, the unfolded protein response (UPR) is induced in activated, dedifferentiated mesangial cells. In the present report, we investigated a role of UPR in mesangial cell deactivation and redifferentiation.

Methods: Mesangioproliferative glomerulonephritis was induced in rats by intravenous administration with anti-Thy 1 antibody. To induce UPR, tunicamycin was injected intraperitoneally at day 3. At day 7, kidneys and isolated glomeruli were subjected to immunohistochemistry and Western/Northern blot analyses. In vitro, activated rat mesangial cells were treated with UPR inducers, and phenotypic shift of mesangial cells was evaluated using Western/Northern blot analyses, reporter assays and assessment of mitogenesis.

Results: We found that, during experimental glomerulonephritis in rats, reinforcement of UPR significantly attenuated mesangial cell proliferation, matrix expansion and expression of a-SMA. Consistent with this in vivo result, induction of UPR suppressed cell proliferation and transcriptional expression of type IV collagen (ColIV) and a-SMA in activated mesangial cells. UPR reduced phosphorylation of Akt selectively, and it was responsible for attenuation of cell proliferation. UPR also preferentially depressed levels of total and phosphorylated Smads without affecting their transcriptional levels. It was responsible for the suppression of ColIV and a-SMA. Translational suppression via the eIF2a pathway, but not proteasome-mediated protein degradation, was responsible for the down-regulation of Smads.

Conclusions: These results suggest the novel potential of UPR to facilitate a phenotypic shift of activated glomerular cells towards deactivation and redifferentiation. UPR may serve as endogenous machinery that supports recovery of glomeruli from acute inflammation.

Funding: Government Support - Non-U.S.

SA-PO749

A Novel Role for the Cytochrome P450 System in Modulating Immune-Associated Inflammation Gabriela E. Garcia,[1] Richard J. Johnson,[1] Luan D. Truong,[2] Lili Feng.[2] *[1]Medicine, University of Colorado Denver, Aurora, CO; [2]Medicine, Baylor College of Medicine, Houston, TX.*

Background: Very little is known about the role of the cytochrome P450 system in modulating glomerular injury and inflammation. Previously, we found that vagus nerve stimulation could attenuate inflammation in anti-glomerular basement membrane (GBM) glomerulonephritis (GN) in association with the induction of cytochrome P4501A1 (CYP1A1) in the kidney. We hypothesized that induction of CYP1A1 might be responsible for the anti-inflammatory effects.

Methods: To test this hypothesis, we used 3-methylcholanthrene (3MC) which is known to increase CYP1A1 expression. Rats received 3MC every other day, starting either before or after the induction of the disease with sacrifice at day 14.

Results: Rats given 3MC showed marked increased expression of kidney CYP1A1 with a 60% and 50% reduction in proteinuria in rats treated before and after the injection of anti-GBM antibody, respectively, compared with control rats. In addition, prominent macrophage (Mø) infiltration was observed in the control group and was reduced significantly by 3MC treatment (44% and 38% in rats treated before and after induction of GN, respectively). Consequently, the severity of glomerular hypercellularity, necrotizing lesion, and crescent formation were significantly reduced in the 3MC treated groups. In addition, sclerotic glomeruli were reduced in the 3MC pretreated group compared to the control group. In control and 3MC treated rats, induction of anti-GBM GN led to a prominent kidney expression of fractalkine/CX_3CL1, MPC-1/CCL2, MIP-1β/CCL4, and MIP-3β/CCL19. The expression of adhesion molecule VCAM-1 was decreased in the 3MC treated groups compared with the control group. Notably, decreased $PCNA^+$ Mø were found in 3MC treated rats compared to control rats.

Conclusions: These findings suggest that vagus nerve stimulation attenuates inflammation in part by CYP1A1-linked inhibition of Mø infiltration through reduction of adhesion molecule expression and Mø proliferation. In addition, these studies demonstrate the novel role for cytochrome P4501A1 as an anti-inflammatory system in GN, and offers insights into novel treatment strategies.

Funding: NIDDK Support

SA-PO750

Renoprotective Effect of Direct Renin Inhibitor in Gentamicin-Induced Kidney Injury in Rats Eun Hui Bae, Joon Seok Choi, Chang Seong Kim, Soo Wan Kim. *Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: Inhibition of the renin-angiotensin system (RAS) is a key therapeutic strategy in slowing progression of kidney injury. Recently, renin inhibition has also emerged as a potential therapy to block the RAS. The protective effect of aliskiren, a direct renin inhibitor, against tubulointerstitial renal injury remains to be defined. This study aimed to examine the protective effects of the aliskiren on gentamicin (GM)-induced kidney injury.

Methods: Male Sprague-Dawley rats (180-200g) were intramuscularly injected with GM (100 mg/kg per day) for 14 days. Aliskiren was infused for two weeks using osmotic minipumps. Human proximal tubular epithelial (HK-2) cells, were cultured with GM in the absence or presence of aliskiren. Inflammatory and profibrotic markers were evaluated *in vivo* and *in vitro.*

Results: GM increased plasma creatinine level, fractional excretion of sodium and urine output, while aliskiren treatment ameliorated these functional changes. Inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ and inducible nitric oxide synthase (iNOS) were increased in the GM-treated kidneys. GM treatment also induced chemokines and adhesion molecules such as monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. These changes were counteracted by aliskiren co-treatment. The accumulation of ED-1 expressing macrophages in GM-treated kidneys was reduced by aliskiren. Masson's trichrome stain demonstrated increased collagen deposition and fibrosis in GM-treated rats, which was ameliorated by aliakiren treatment. Aliskiren also effectively reversed increased expression of TGF-b and α-SMA in GM-treated rat kidney. Along with these changes, aliskiren also attenuated the increased expression of nuclear factor κB and phosphorylated extracellular signal-regulated kinase in HK-2 cells cultured with GM.

Conclusions: These findings suggest that aliskiren prevents GM-induced kidney injury by inhibiting renal inflammation and fibrosis, the mechanism of which was the interruption of NF-κB and/or ERK signaling pathways.

Funding: Private Foundation Support

SA-PO751

A Transcriptional Regulator, Inhibitor of Differentiation 3 (ID3), Is a Novel Risk Factor for Kidney Disease Harini Bagavant,[1] Paromita Dey,[1] Barbara M. Szczerba,[1] Agnieszka M. Szymula,[1] Ani W. Manichaikul,[3] Coleen Mcnamara,[2] Umesh Deshmukh.[1] *[1]Division of Nephrology; [2]Cardiovascular Medicine, Department of Medicine; [3]Department of Public Health Sciences, University of Virginia, Charlottesville, VA.*

Background: The deficiency of a transcriptional regulator, inhibitor of differentiation 3 (*Id3*), in hyperlipidemic Apolipoprotein E deficient mice induces severe glomerulonephritis and proteinuria (Am J Pathol 2011;179:651). ID3 regulates the helix-loop-helix family of transcription factors and a functional deficiency in Id3 is linked to increased atherosclerosis in mice and humans. We postulated that *Id3* deficiency facilitates kidney disease by influencing glomerular cell responses to inflammatory lipids.

Methods: Primary mesangial cell lines were generated from $Id3^{+/+}$and $Id3^{-/-}$ mice and stimulated with oxidized phospholipids (oxPL). Cytokines produced were analyzed by multiplex bead array assays. $Id3^{+/+}$and $Id3^{-/-}$ female mice were fed an atherogenic Western diet (TD.88137; 42% calories from fat) or a chow diet (TD.7012; 17% calories from fat) for 15wks. Mice were evaluated for proteinuria, renal histopathology and inflammatory cell infiltrates. Gene expression analyses were carried out by qPCR.

Results: Compared to $Id3^{+/+}$cells, $Id3^{-/-}$ mesangial cells incubated with oxPL produce higher amounts of CXCL1, an important macrophage and neutrophil chemoattractant. In vivo, both $Id3^{+/+}$ and $Id3^{-/-}$mice fed a Western diet showed increased weight gain and elevated cholesterol levels compared to mice on chow diets. Blood glucose levels were not different between groups. However, only $Id3^{-/-}$ mice fed a Western diet developed severe proteinuria and glomerulonephritis. Glomerulonephritis was accompanied by increased macrophage infiltration and glomerular CXCL1 deposition. A preliminary analysis in humans showed a significant association between *ID3* single nucleotide polymorphisms and albuminuria.

Conclusions: Exacerbated inflammatory responses to lipids in $Id3^{-/-}$ mice facilitate kidney disease in hyperlipidemia. Thus, a functional Id3 regulates chemokine production and is reno-protective. Id3 may provide a critical link between hyperlipidemia, atherosclerosis, and kidney disease.

Funding: NIDDK Support, Private Foundation Support

SA-PO752

Effect of a Serine Protease Inhibitor on the Progression of Renal Injury in Metabolic Syndrome Teruhiko Mizumoto,[1] Kohei Uchimura,[1] Yutaka Kakizoe,[1] Manabu Hayata,[1] Tomoaki Onoue,[1] Miki Ueda,[1] Rika Yamazoe,[1] Jun Morinaga,[1] Sakai Yoshiki,[2] Kimio Tomita,[1] Kenichiro Kitamura.[1] *[1]Department of Nephrology, Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan; [2]Research Headquarters, Ono Pharmaceutical Co., Ltd., Osaka, Japan.*

Background: It is well known that metabolic syndrome (Mets) contributes to the development and progression of CKD. Previously we reported the involvement of serine proteases in the pathogenesis of salt-sensitive hypertension and renal fibrosis. Therefore, we

hypothesized that a synthetic serine protease inhibitor camostat mesilate (CM) attenuates the progression of renal injury induced by metabolic syndrome.

Methods: Thirteen week-old SHR/ND mcr-cp rats, a rat model of Mets, were divided into four groups: 1) normal salt (NS) group, 2) high salt (HS) group, 3) HS+CM group, and 4) HS+hydralazine (hyd) group. NS rats were fed normal diet (0.8% NaCl), and HS rats were fed high salt diet (8% NaCl). The HS+CM rats were subcutaneously implanted with slow-releasing pellets containing CM. Blood pressure (BP) measurements and 24 hr urine collections were made every week, and rats were sacrificed following 4 weeks treatment period.

Results: Severe hypertension and proteinuriawere observed in HS group (proteinuria, NS 54±6.4, HS 345±20.4mg/day), and both were substantially attenuated by CM (28±4.3mg/day). Although reduction in BP levels were comparable between HS+CM and HS+Hyd, the anti-proteinuric effect of CM was much greater than Hyd (110±27.3mg/day), indicating that CM has an ameliorating effect on proteinuria besides its BP lowering effect. Immunohistochemical analysis revealed that CM markedly attenuated the reduced glomerular expression of nephrin induced by HS. Fasting blood sugar and insulin levels as well as insulin resistance index were not affected by CM treatment.

Conclusions: Here we demonstrated that a serine protease inhibitor CM significantly ameliorated the development and the progression of Mets-induced kidney injury by reducing blood pressure and proteinuria. These findings strongly suggest the possibility that CM could be a new class of therapeutic strategy against CKD with Mets.

SA-PO753

Role of Plasmacytoid Dendritic Cells (pDCs) and Interferon-alpha (IFN-α) in the Activation of Tubular Epithelial Cells (RPTEC) in Lupus Nephritis (LN) G. Castellano,[1] Cesira Cafiero,[1] C. Divella,[1] Fabio Sallustio,[2] Margherita Gigante,[3] Loreto Gesualdo.[1] *[1]Renal Dialysis and Transplant Unit, University of Bari, Italy; [2]CARSO Consortium, Valenzano-Casamassima, Italy; [3]Department of Biomedical Science, University of Foggia, Italy.*

Background: PDCs, the major producer of IFN-alpha, play a key role in autoimmune response in SLE. However, the involvement of pDCs in LN is unclear.The aim of this study was to investigate the effects of IFN-alpha in vitro and in biopsies from LN patients.

Methods: Confocal microscopy showed peritubular infiltrating pDC in LN patients, expressing the MXA protein, a specific marker of IFN-alpha local production. We then performed microarray analyses (Illumina), on IFN-alpha activated RPTEC (100U/ml for 48h).

Results: Following stimulation, we found 108 significantly up-regulated genes and 7 down-regulated genes (fold-change >2; false discovery rate, FDR<.05). Gene set enrichment analysis identified 123 processes differentially regulated in IFN-alpha activated RPTEC: the interferon response, the antigen presentation and inflammatory pathways. Among over-expressed genes, we identified the HLA-I, the ubiquitin (FBXO6; DTX3L) and the immunoproteasome subunits LMP7. After validation by RT-PCR, flow citometry experiments on IFN-activated RPTEC confirmed a significant increase of antigen presentation molecules (HLA-I: 75%±2/MnI 7.8±3 basal vs 90%±3/MnI 11±2 IFN-alpha) and inflammatory signalling (LMP7: 49.75%±2 basal vs 72.4%±2 IFN-alpha). Immunohistochemical analysis on renal biopsies of patients with class IV LN showed a significant increase of tubular LMP7 expression (LMP7: 5% ± 2 NL class I vs 16 ± 5% class IV, p<.0001) and MXA expression compared to class I LN (MXA: 0±1.0% class I vs 4.5±1.2% class IV, p<.0001). Confocal analysis in LN patients confirmed the colocalization of IFN-signalling with the immunoproteasome induction (LMP7+/MXA+) and the activation of inflammatory signaling (NF-kB-p65+/MXA+) in renal tubuli.

Conclusions: Our data demonstrate a critical role of pDC-derived INF-alpha in the activation of RPTEC. Therefore we hypothesize that inhibition of IFN-alpha pathway may be a novel therapeutic strategy to reduce renal damage in patients with LN.

SA-PO754

Identification of Urine Biomarkers of Lupus Nephritis: CKD Biomarkers Consortium Jon B. Klein,[1] Michael Merchant,[1] Huijuan Song,[2] Xiaolan Zhang,[2] Alison Marie Mckinley Neal,[2] Brad H. Rovin.[2] *[1]Kidney Disease Program, University of Louisville School of Medicine, Louisville, KY; [2]Internal Medicine/Nephrology, Ohio State University Medical Center, Columbus, OH.*

Background: The current treatment of lupus nephritis is unsatisfactory in terms of both outcome and toxicity. Predictive biomarkers of the course and pathology of lupus nephritis (LN) would lead to less immunosuppression and decrease its associated toxicity. However, no such biomarkers have been discovered with sufficient sensitivity and specificity. We addressed the hypothesis that urine biomarkers would correlate with the presence and extent of inflammation or fibrosis in the tubulointerstitial compartment during LN.

Methods: Urine samples were obtained immediately prior to renal biopsy in patients with LN. Three urine groups (Control, Inflamed, Fibrotic) were defined based on blinded scoring of tubulointerstitial inflammation or fibrosis. Urine samples (n=5 in each group) were depleted of 12 abundant proteins using a ProteomeLab IgY-12 LC-2 column and analyzed using 2D-LC-MS/MS combined with a label-free quantitative strategy to define protein expression differences in urine samples.

Results: Using rigorous standards, 587 proteins across 15 samples were identified. By ANOVA 26 urinary proteins were significantly, differentially abundant (p≤0.05) between C, I, and/or F groups. Of the group of 26 proteins, osteopontin (OPN), hemopexin (HPX) and the endothelial protein c receptor (EPCR) urine levels were examined by ELISA. OPN levels were different in I versus F urine (n= 8 and 18, respectively, p<0.002). HPX levels discriminated between C versus I and F groups (p<0.05). EPCR levels did not differ between groups.

Conclusions: In summary, our results suggest the type and extent of renal tubulointerstitial damage in patients with LN correlates with the abundance of specific urinary proteins. With confirmation, the quantitative assessment of these proteins in individuals may be valuable to guide individualization of lupus treatment. This work was partially supported by the Chronic Kidney Disease Biomarker Consortium funded by NIDDK U01DK085673.

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SA-PO755

Understanding the Molecular Mechanisms of LN Progression and Remission in NZB/W Mice Celine C. Berthier,[1] Ramalingam Bethunaickan,[2] Matthias Kretzler,[1] Anne Davidson.[2] *[1]University of Michigan; [2]Feinstein Institute for Medical Research.*

Background: Systems biology technologies allow integration of current knowledge with large scale experimental data to generate insight into the regulatory events occurring in lupus nephritis (LN). We analyzed the time dynamics of renal molecular changes during LN progression and remission in the NZB/W mouse model.

Methods: Affymetrix based expression profiles at sequential times during LN progression and early or late remission induced by co-stimulatory blockade or BAFF-inhibition respectively, were analyzed for dynamic transcriptional changes using Genomatix Pathway Systems and Ingenuity Pathway Analysis softwares.

Results: Proteinuria onset was characterized by renal inflammatory cell infiltration. STAT-1/-3, EGR1, HIF1A were the top transcription factors (TF) having a binding site (BS) in the regulated gene promoter. Complete remission 3 weeks after co-stimulatory blockade showed a return to baseline expression, reflecting reduction in infiltrating cells. A 654 biomarker dataset (q<0.05) was extracted from the regulated genes overlapping between the proteinuria onset and either age matched non-proteinuric or the remission mice. 17% of these biomarkers had a STAT-1 TF BS in their promoter. STAT-1 was also a major regulated node in human LN kidneys, compared to living donors. LN disease progression to established proteinuria (36 weeks) showed a mitochondrial dysfunction signature, which overlapped significantly with the 403 transcripts that differed between mice in the early and late stages of remission.

Conclusions: NZB/W LN onset is associated with inflammatory cell infiltration of the kidneys but LN disease progression is linked to mitochondrial dysfunction, most probably secondary to hypoxia and tubular damage. A similar mitochondrial dysfunction profile is seen during impending relapse and in human LN. Therapies addressing this intrarenal response to inflammation may synergize with immune modulation in human LN treatment. Many of the inflammatory, metabolic and hypoxia-responsive transcripts appear downstream of STAT-1 dependent regulation, implicating Type I/II IFNs as possible regulators of nephritis onset and remission.

Funding: NIDDK Support

SA-PO756

Laminin α5 Overexpression Accelerates Glomerular Disease Progression in Alport Mice Brooke M. Steenhard, Patricia St. John, Adrian T. Zelenchuk, Larysa Stroganova, Dale R. Abrahamson. *Anatomy and Cell Biology and the Kidney Institute, University of Kansas Medical Center, Kansas City, KS.*

Background: Extracellular matrix expansion in mesangial and glomerular basement membrane (GBM) compartments occurs in many glomerular diseases. Mice on the 129X1/SvJ background harboring a deletion of *Col4a3*, which encodes the collagen α3(IV) chain, are a faithful model of human Alport syndrome. In the absence of collagen α3(IV), a GBM collagen α3α4α5(IV) network fails to form, and ~2 weeks of age, there is upregulation of several laminin isoforms, including laminin α5. These mice develop proteinuria at ~5 weeks and die of renal failure at ~9 weeks. We hypothesized that laminin dysregulation also contributes to the pathogenesis of Alport, and crossed Alport mice with a human (hu) *LAMA5* transgenic line we created that overexpresses hu laminin α5.

Methods: To minimize strain variations known to affect progression of Alport disease in mice, male hu *LAMA5*+/- hemizygotes were mated to female, 129-derived, *Col4a3*+/- heterozygotes. F1 *LAMA5*+/-, *Col4a3*+/- males were backcrossed to 129 *Col4a3* females, and male offspring were analyzed at 2 weeks of age.

Results: Confocal microscope quantification showed that all Alport GBMs (with or without hu laminin α5) contained more mouse laminin α5 than wildtype. Alport mice with hu *LAMA5* expressed 2 fold more hu laminin α5 protein in the GBM than non-Alport, hu *LAMA5* controls, with co-localization of both mouse and human protein to the subepithelial GBM nodules characteristic of Alport. Additionally, an enzyme-linked immunosorbent assay showed that 2 week-old Alport mice with the hu *LAMA5* transgene had urinary albumin levels averaging >300 µg/ml (N=6), which was >11 fold greater than Alport mice without *LAMA5* (N=5). There were no statistical differences in albumin levels between Alport mice without *LAMA5*, non-Alport *LAMA5* mice, or wildtypes.

Conclusions: We conclude that, in the absence of collagen α3α4α5(IV), excess GBM laminin α5 accelerates progression of glomerular structural and functional deficits in Alport *LAMA5* mice. This genetic cross may help uncover mechanisms causing matrix overproduction and proteinuria in glomerular disease.

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SA-PO757

Expression of a Novel Stress-Inducible Protein, Sestrin 2, in Rat Glomerular Parietal Epithelial Cells (PECs) Hiroko Hamatani,[1] Keiju Hiromura,[1] Toru Sakairi,[1] Satoshi Takahashi,[1] Akito Maeshima,[1] Takamoto Ohse,[2] Jeffrey W. Pippin,[3] Stuart J. Shankland,[3] Yoshihisa Nojima.[1] *[1]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Japan; [2]Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan; [3]Division of Nephrology, University of Washington, Seattle, WA.*

Background: Sestrin2, firstly identified as a p53-target hypoxia-inducible gene, accumulates in cells exposed to stress and inhibit mammalian target of rapamycin (mTOR). In this study, we examined the expression of sestrin2 in the kidney.

Methods: Immunohistochemical staining was performed on rat developing and adult kidneys and kidneys with adriamycin-induced (ADR) nephropathy. Immortalized mouse cultured PECs were used for in vitro study.

Results: In embryonic and newborn kidney, immunohistochemical stainings revealed that sestrin2 was moderately expressed in metanephric mesenchyme and comma- and S-shaped body. Increased expression of sestrin2 was observed in PECs of capillary-stage. However, in adult kidney, sestrin2 was selectively expressed in PECs (Figure), similar to claudin-1, the marker of PECs. In ADR nephropathy, sestrin2 expression was markedly decreased in PECs at 8 weeks, when glomerulosclerosis and periglomerular fibrosis were observed. In contrast, increased expression of sestrin2 was detected in some tubular cells (Figure). We then examined the role of sestrin2 using cultured PECs. By western blotting and real time PCR, PECs showed strong sestrin2 expression. Downregulation of sestrin2 by shRNA increased apoptosis of PECs (5.0% vs 2.3%, shRNA vs control, P<0.05), together with increased phosphorylation of p70S6K and 4E-BP1 which are the downstream targets of mTOR.

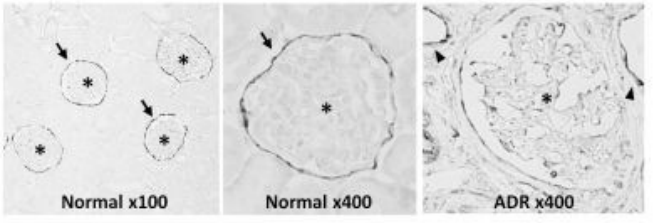

Figure. Sestrin2 expression in rat adult normal kidney and ADR nephropathy

* glomerulus; arrows, sestrin2 in PECs; arrowheads, sestrin2 in tubular cells

Conclusions: These data suggest that increased sestrin2 expression is required for PEC homeostasis.

Funding: Government Support - Non-U.S.

SA-PO758

Cyclin I and p35 Modulate the Subcellular Localization of Cyclin-Dependent Kinase 5 (Cdk5) Yoshinori Taniguchi,[1] Jeffrey W. Pippin,[1] Henning Hagmann,[2] Ronald D. Krofft,[1] Jiong Zhang,[1] Shokichi Naito,[1] Peter J. Nelson,[1] Yoshio Terada,[3] Paul T. Brinkkoetter,[2] Stuart J. Shankland.[1] *[1]University of Washington, Seattle, WA; [2]University of Cologne, Germany; [3]Kochi University, Japan.*

Background: Cdk5 and its activators, cyclin I and non-cyclin p35, have critical survival functions in terminally differentiated podocytes (podo) and neurons.

Methods: To test hypothesis that subcellular localization for Cdk5 is determined by cyclin I and p35, subcellular distribution of Cdk5 was studied in podo and neurons of wild-type ($^{+/+}$), cyclin $I^{-/-}$ ($I^{-/-}$) and $p35^{-/-}$ mice.

Results: In quiescent podo, cyclin I is predominantly nuclear (nucl), whereas p35 cytoplasmic (cyto). In $^{+/+}$ podo and neurons in vivo, Cdk5 stained in nucl and cyto. This was confirmed in cultured $^{+/+}$ podo by western analysis (WB) of subcellular protein fractions, using TATA-box binding protein (TBP) and α-tubulin (tub) as nucl and cyto control proteins for loading. In contrast, $I^{-/-}$ podo and neurons in vivo showed predominantly cyto staining of Cdk5 ($p<0.05$ vs. $^{+/+}$). Transfecting $I^{-/-}$ podo with myc-cyclin I resulted in Cdk5 localizing to both nucl and cyto, similar to $^{+/+}$ cells. In $p35^{-/-}$ podo and neurons in vivo, Cdk5 staining was predominantly nucl ($p<0.05$ vs. $^{+/+}$). When $p35^{-/-}$ podo were transfected with p35-vector, Cdk5 localized to both nucl and cyto, indistinguishable from $^{+/+}$ cells. The subcellular localization for Cdk5 was not altered in either null cells transfected with control GFP-vectors. These were confirmed in null podo using the ratio's of Cdk5/TBP and Cdk5/tub on isolated subcellular fractions ($p<0.05$ vs. $^{+/+}$). To better define the mechanisms of the nucl-cyto localization of Cdk5, the nucl export inhibitor Leptomycin (LM) was used. LM prevented nucl-to-cyto translocation of Cdk5 in $^{+/+}$, $I^{-/-}$ and $p35^{-/-}$ podo.

Conclusions: In summary, these results support that cyclin I anchors Cdk5 to the nucl, while p35 anchors Cdk5 to the cyto, corresponding to primary subcellular localization of each activator. Any movement of Cdk5 from the nucl to cyto is via classical CRM1-dependent mechanism. We conclude that nucl-cyto distribution for Cdk5 is critical for normal survival functions in terminally differentiated podo.

Funding: NIDDK Support, Private Foundation Support

SA-PO759

Cyclin-Dependent Kinase 5 Maintains Podocyte Survival during Experimental Glomerular Disease Yoshinori Taniguchi,[1] Jeffrey W. Pippin,[1] Henning Hagmann,[2] Ronald D. Krofft,[1] Jiong Zhang,[1] Shokichi Naito,[1] Peter J. Nelson,[1] Yoshio Terada,[3] Paul T. Brinkkoetter,[2] Stuart J. Shankland.[1] *[1]University of Washington, Seattle, WA; [2]University of Cologne, Germany; [3]Kochi University, Japan.*

Background: Cdk5 is expressed constitutively in podocytes (podo). Because biological role for Cdk5 in podo health and disease is poorly understood, we generated transgenic mice to selectively and inducibly delete Cdk5 from podo.

Methods: Mice with podocin promoter driven reverse tetracycline transactivator driven cre (NPHS2rtTA/TetO-cre) were crossed with Cdk5 floxed mice (Cdk5-lox/p). When the resultant $TetOCdk5^{-/-}$ mice are given doxycycline (Dox), Dox binds tetracycline receptor present only on podo, resulting in cre recombinase expression (confirmed by immunostaining) which excises the lox/p sites flanking Cdk5, preventing Cdk5 expression in podo (confirmed by immunostaining). Under non-stressed conditions, kidneys from $TetOCdk5^{-/-}$ mice given Dox or control water were normal, as was renal function. To test hypothesis that Cdk5 is required for podo survival during disease, $TetOCdk5^{-/-}$ mice given either Dox or water for 14 days were then injected with anti-podo antibody, which induced podo apoptosis, leading to glomerulosclerosis. Several disease parameters were assessed by immunostaining.

Results: The result of staining showed podo apoptosis was increased compared to diseased control mice (0.3±0.0 vs. 0.1±0.0; $p<0.05$). This resulted in more pronounced decrease in podo number (4.6±0.6 vs. 6.4±1.1; $p<0.05$), proteinuria (Upro/cre 62.4±15.3 vs. 22.1±5.1; $p<0.05$) and glomerulosclerosis (1.7±0.1 vs. 1.4±0.1; $p<0.05$).

Conclusions: In summary, when Cdk5 is reduced, podo are susceptible to increased apoptosis following injury which results in reduced podo number, increased proteinuria and glomerulosclerosis. We conclude that Cdk5 is an important podo survival protein during disease.

Funding: NIDDK Support, Private Foundation Support

SA-PO760

Tissue Transglutaminase: A Novel Enzymatic Pathway Modulating Podocyte Phenotype, Autophagy and Survival Cécile Fligny,[1] Carole Hénique-Gréciet,[1] Marine Milon,[1] Sandra Schordan,[2] Laurent Mesnard,[3] Patrick Bruneval,[1,4] Martin Flamant,[5] Nicole Endlich,[2] Pierre-Louis F. Tharaux.[1] *[1]Paris Cardiovascular Centre - PARCC, INSERM & Université Paris Descartes, Paris, France; [2]Institut für Anatomie und Zellbiologie, Universitätsmedizin Greifswald, Greifswald, Germany; [3]UMR 702, Hôpital Tenon, INSERM & Université Pierre et Marie Curie, Paris, France; [4]Dept of Pathology, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris, France; [5]Service de Physiologie–Explorations Fonctionnelles, Hôpital Bichat, Assistance Publique–Hôpitaux de Paris, Paris, France.*

Background: We found marked podocyte expression of the protein cross-linking enzyme, tissue transglutaminase (TG2) in mice and human kidneys with Rapidly Progressive Glomerulonephritis (RPGN).

Methods: Impact on glomerular phenotype of global and podocyte selective Tgm2 (Tg2) gene invalidation was evaluated. The nephrotoxic serum (NTS) model of RPGN was applied to these strains.

Results: In the NTS-induced RPGN, Tg2 invalidation was associated with marked alleviation of glomerular injury. Albuminuria was 25 fold lower in Tg2 null mice at day 11 ($p<0.001$) that displayed much fewer crescent formation (2 ± 2% of the glomeruli at day 4 and 30 *vs.* 25 ± 6% at day 4 and 45 ± 6% at day 30, $p<0.001$). Accordingly, BUN levels remained within normal ranges at day 30 ($p<0.001$).

Podocin-Cre x Tg2 lox/lox mice also displayed significant protection against the development of RPGN with inhibition of podocyte migration. In addition, TG2 deficiency enhances constitutive autophagy in podocytes. Autophagy is protective in RPGN as selective deficiency in Atg5 in podocyte profoundly altered autophagy and accentuated glomerular damage. Furthermore, we found enhanced anti-oxidant Nrf2 pathway in TG2 deficient podocytes. In fact, TG2 appeared to be essential for normal degradation of p62 by autophagy, a master regulator of Nrf2 transcriptional activity.

Conclusions: Full TG2 activity is essential for severe RPGN, in part through promotion of migration of podocytes and alteration of autophagy. This study unravels a novel enzymatic pathway in podocytes and suggests that targeting TG2 would be clinically beneficial for treatment of severe RPGNs.

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SA-PO761

Molecular Changes in Kidney Cortex Precede Proteinuria in Aging Mice Magali Araujo,[1,2] Marcela Ururahy,[1,3] Nancy L. Koles,[1] Sonia Q. Doi.[1] *[1]Medicine, Uniformed Services University, Bethesda, MD; [2]Nephrology & Hypertension, Georgetown University, Washington DC; [3]Clinical Analysis & Toxicology, Federal University of Rio Grande do Norte, Brazil.*

Background: Aging is associated with renal structural changes and decline of renal function. The progressive decrease in glomerular filtration rate (GFR) and renal blood flow in aging individuals varies widely, and the distinction between age-related loss of GFR and the presence of chronic kidney disease (CKD) in the elderly is yet undefined. This study

was designed to evaluate age-dependent molecular alterations in kidney cortex compared to the onset of albuminuria in mice.

Methods: C57Bl6 male mice were studied at ages 3, 6, 12 and 21 months (m). RNA expression of IL-6, IL-1β, TNF-α, CD68, class A scavenger receptor (SRA), collagen I, fibronectin, TLR2 and TLR4 was measured (normalized to β-actin) in kidney cortex using real time RT-PCR. Urinary albumin and creatinine were measured using commercial kits. Double-staining immunofluorescence (IF) for CD68 (macrophage marker) and Will's Tumor (WT1, podocyte marker) was performed in kidney frozen sections.

Results: Compared to 3m, expression of target genes at older ages showed that IL-6, TNF-α, TLR2 and CD68 increased significantly by 12m, while IL-1β and collagen I increased significantly only at 21m, and TLR4, SRA, collagen IV and fibronectin remained unchanged. Urinary albumin/creatinine ratio increased significantly only at 21m compared to 3m (871±302 vs. 165±40 mg/g creatinine, respectively). IF analysis revealed that CD68 positive signal was predominantly localized in glomeruli overlapping WT1 cytoplasmic staining. Positive staining appeared weakly in approximately 25% of glomeruli at 6m and intensified with age reaching approximately 50% of glomeruli/section.

Conclusions: Our data is consistent with an inflammatory process mediated by TLR2 that begins early at 12m preceding renal fibrosis and proteinuria, and that progresses as the animals age. The co-localization of CD68 with WT1 together with the unchanged SRA expression suggest that the increased renal CD68 in aging mice is due primarily to an aberrant expression by injured podocytes rather than macrophage infiltration.

Funding: Other U.S. Government Support

SA-PO762

Relative Podocyte Depletion in Obese Caucasian American Men without Renal Disease Victor G. Puelles,[1] Rebecca N. Douglas-Denton,[1] Jinhua Li,[1] Wendy E. Hoy,[2] Jens R. Nyengaard,[3] John F. Bertram.[1] *[1]Anatomy and Developmental Biology, Monash University, Melbourne, VIC, Australia; [2]Center for Chronic Diseases, University of Queensland, Brisbane, Queensland, Australia; [3]Stereology and EM Laboratory, Aarhus University Hospital, Aarhus, Denmark.*

Background: Obesity is a worldwide epidemic and a risk factor for CKD. Glomerular hypertrophy and podocyte dysfunction have been described in both obesity and obesity-related glomerulopathy. The aim of this study was to quantify features of podocyte depletion in obese male Caucasian Americans without overt renal pathology.

Methods: Renal tissue obtained at autopsy from Caucasian American men was selected based on Body Mass Index (BMI): 4 obese (BMI ≥30.0 kg/m^2) and 4 lean (BMI ≤24.9 kg/m^2). Individual Glomerular Volume (IGV) was measured using the Cavalieri estimator - 30 glomeruli per subject. A combination of immunohistochemistry, laser confocal microscopy and design-based stereology was used to quantify numbers of podocytes and parietal epithelial cells (PECs) in 6 glomeruli per subject (10th and 90th IGV percentiles). Podocyte number per IGV (Pod/IGV) and percentage of podocytes per glomerulus (podocyte number divided by the total number of tuft cells per glomerulus) were also calculated.

Results: Median IGV was significantly higher (P<0.0001) and IGV variance was three times greater in obese subjects than their lean counterparts. The absolute numbers of podocytes and PECs per glomerulus also varied greatly between subjects in both groups: podocyte number ranged from 263 to 983 (3.7-fold) and PEC number from 124 to 1,279 (10.3-fold). While glomeruli from obese subjects contained more podocytes and more PECs (P<0.01), large glomeruli from obese subjects had lower Pod/IGV (P<0.05) and lower percentage of podocytes per glomerulus (P<0.01) than large glomeruli from lean subjects.

Conclusions: Obese subjects showed evidence of glomerular hypertrophy and relative podocyte depletion. We hypothesize that podocyte depletion predisposes hypertrophied glomeruli to proteinuria and segmental sclerosis.

Funding: NIDDK Support

SA-PO763

Podocyte-Specific Deletion of Signal Transducer and Activator of Transcription 3 Attenuates Nephropathy in a Murine Model of Human Immunodeficiency Virus Associated Nephropathy Leyi Gu,[3] Yan Dai,[2] John C. He,[1] Peter Y. Chuang.[1] *[1]Div of Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Div of Nephrology, Shanghai First Affiliated Hospital, Shanghai, China; [3]Renal Division, Renji Hospital, Shanghai Jiatong University School of Medicine, Shanghai, China.*

Background: Human immunodeficiency virus associated nephropathy (HIVAN) is characterized by collapsing glomerulosclerosis, glomerular epithelial cell proliferation, and reduction of podocyte-specific markers. We previously showed that reduction of signal transducer and activator of transcription (STAT) 3 activity attenuates renal injury in a murine model of HIVAN (Tg26). The role of podocyte STAT3 in the pathogenesis of glomerular pathology of HIVAN has not been confirmed.

Methods: Tg26 mice on the FVBN background with and without podocyte-specific deletion of STAT3 were generated. Podocyte-specific deletion of STAT3 was achieved by crossing the podocin-Cre mice to mice with *loxp* sequences flanking exons 18 to 20 of *STAT3*. Renal function was assessed by serum urea nitrogen. Albuminuria was quantified by ELISA and then normalized to urine creatinine concentration. Glomerulosclerosis and tubular atrophy/dilation was scored using periodic acid-Schiff stained kidney sections. Expression of cell markers were quantified by western blotting, real-time PCR or immunolabeling.

Results: The expression and phosphorylation of STAT3 in the glomeruli of Tg26 mice with podocyte-specific STAT3 deletion (Tg26-STAT3$^-$) were less than that of Tg26 mice without STAT3 deletion (Tg26-STAT3$^+$). Tg26-STAT3$^-$ mice had significantly less proteinuria, serum urea nitrogen, glomerulosclerosis, and tubular dilation/atrophy at 4w and 7w of age compared to Tg26-STAT3$^+$. The expression of podocyte markers (synaptopodin, nephrin, and WT-1) was reduced and the expression of pro-inflammatory STAT3 target genes (MCP-1 and IL-6) was higher in Tg26-STAT3$^+$ mice compared to Tg26-STAT3$^-$ mice.

Conclusions: Activation of podocyte STAT3 is necessary to drive glomerular cell proliferation, loss of podocyte marker expression, loss of kidney function, tubular atrophy/dilation, and glomerulosclerosis in the Tg26 murine model of HIVAN.

Funding: NIDDK Support

SA-PO764

Structure of Endothelial Surface Glycocalyx, Measured In Vivo by Confocal Microscopy, Is Altered in Mesenteric Microvessels of Proteinuric Rats Kai Brodie Betteridge, Kenton P. Arkill, Chris R. Neal, Andy Salmon. *Physiology & Pharmacology, University of Bristol, United Kingdom.*

Background: The luminal surface of blood vessels is coated with a size limiting, specialised, composite endothelial glycocalyx-surface layer (EG-SL) which has a fundamental role in microvessel permeability. Systemic loss of this EG-SL and increased vascular permeability coincides with the onset of albuminuria in diabetic patients (Nieuwdorp *et al.*, 2006), and depletion of the EG-SL in coronary arteries is accompanied by an accelerated development of atherosclerosis in apolipoprotein E deficient mice (Nagy *et al.*, 2010). We hypothesised that albuminuria, independent of diabetes, would result in a reduction of EG-SL in systemic blood vessels, such as mesenteric microvessels. Our aim was to compare measurements of EG-SL depth by Confocal Microscopy (CM) *in vivo* in mesenteric microvessels of albuminuric and control animals.

Methods: Male Sprague Dawley rats were injected with either Puromycin Aminonucleoside (PA) to cause PA induced Nephrosis (PAN) or PBS sham controls. Nine days following injection PAN rats reached maximal proteinuria. Mesenteric microvessels of PAN and sham rats were exposed, cannulated, and perfused with octadecyl rhodamine B chloride (R18: Plasma membrane label) and Fluorescein Isothiocyanate conjugated Wheat Germ Agglutinin (FITC-WGA lectin: EG-SL label) for comparison. EG-SL depth (CM) was estimated based on either: Full Width Half Maximum (FWHM) fluorescence intensity of FITC-WGA; or the anatomical distance between peak fluorescence of FITC-WGA and R18 signals (P-P).

Results: EG-SL depth by the peak to peak method resulted in a value of 242±29nm in sham rats, and this value was significantly reduced to 149±23nm (p=0.0426) in PAN rats. In comparison using the FWHM method resulted in a value of 1557±185nm in the sham rats with no significant change in PAN induced rats (1465±140nm-p=0.7614).

Conclusions: We have shown that EG-SL can be visualised, and depth quantified, *in vivo* using CM, and this depth is significantly reduced in PAN rats compared to control (P-P). Future work will focus on both Electron Microscopy and permeability measurements of EG-SL in the same mesenteric vessels.

Funding: Government Support - Non-U.S.

SA-PO765

HIV-Induced Dedifferentiation in Human Podocytes Is Mediated by Epigenetic Factors through Epithelial Mesenchymal Transition Tejinder Singh, Nirupama Chandel, Partab Rai, Gautam Kishore Valecha, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra Norh Shore LIJ Medical School, Great Neck, NY.*

Background: HIVAN patients have been demonstrated to display proliferation of podocytes and the activation of the renin angiotensin system (RAS). We hypothesize that HIV-induced epigenetic factors stimulate epithelial mesenchymal transition (EMT) in podocytes.

Methods: Immortalized and differentiated human podocytes (IDHPs) were transduced with either empty vector (EV) or HIV (NL4-3) constructs. Both EV/IDHPs and HIV/CIDHPs were de-differentiated at 33°C for 48 hours. Cells were lysed; proteins and RNAs were extracted and probed for VDR, renin, transforming growth factor (TGF)-ß, "-SMA, vimentin, and FSP-1. Ang II contents were measured in cellular lysates of control and experimental cells. EV/IDHP and HIV/IDHPs were assayed for Cytosine-hosphate-Guanosine (CpG) methylatoin and expression of DNA methyltransferase (Dnmt)-1-3. To determine the role of epigenetic factor and the RAS, EV/IDHPs and HIV/IDHPs were incubated in media containing buffer, 5-azacytidine (AZAC, a demethylating agent, 5μM), or losartan (10^{-7}M) for 48 hours. Subsequently, immunoblots were probed for VDR, renin, Snail, "-SMA, vimentin, and and FSP-1. Renal tissues of four weeks old Tg26 (HIV transgenic mice, n=4) were treated with saline or losartan for two weeks and then evaluated for expression (both protein and mRNA) of VDR, renin, Snail, vimentin, and FSP-1.

Results: HIV/IDHPs displayed enhanced (3-fold) expression of Dnmt-3. HIV stimulated CpG methylation of VDR in podocytes. HIV/IDHPs displayed downregulation (2-fold) of VDR and activation of the RAS. HIV/IDHPs displayed enhanced (2-3-folds) expression of TGF-ß, Snail, "-SMA, vimentin, and FSP-1. AZAC inhibited HIV-induced podocyte down regulation of VDR, up regulation of the RAS and expression of podocyte EMT markers. Tg26 mice displayed enhanced renal tissue expression of EMT markers; however, losartan attenuated expression of EMT markers in HIV/IDHPs as well as in renal tissues of Tg26 mice.

Conclusions: These findings suggest that HIV stimulates dedifferentiation of podocyte through the induction of EMT via epigenetic factors.

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SA-PO766

PTP1B Inhibition Protects against Podocyte Injury and Proteinuria
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Background: Protein tyrosine phosphatase 1B (PTP1B) is an ubiquitously expressed nonreceptor protein-tyrosine phosphatase, which regulates various cellular functions including migration. Recent studies suggest that increased migratory phenotype of podocytes may be responsible for foot process effacement *in vivo*. The current study addresses the role of PTP1B in podocyte injury and proteinuria.

Methods: Expression of PTP1B in the kidney was studied by immunohistochemistry. Podocyte-specific deletion of the PTP1B gene was done by crossing $ptp1b^{flox/flox}$ mice with $ptp1b^{flox/+}$; podocin-Cre/+ mice. Podocyte injury was induced by puromycin aminonucleoside (50 mg/kg, i.v.) in rats, adriamycin (12.5 mg/kg, i.v.) in mice or by lipopolysaccharide (LPS, 200 μg each, i.p.) in mice. Urine albumin-to-creatinine ratio (ACR) was determined by enzyme-linked immunosorbent assay. Cell migration was quantified by wound healing assay.

Results: PTP1B was markedly upregulated in the glomerulus, notably in podocytes, in rats with puromycin aminonucleoside nephrosis and in mice treated with adriamycin or LPS. Podocyte-specific PTP1B knockout mice showed normal urine ACR up to 7.5 months of age, while showing increased nephrin tyrosine phosphorylation, as compared with wild-type. LPS-induced albuminuria and foot process effacement were ameliorated in knockout mice (ACR: wild-type: 6255±944 vs knockout: 3274±939 μg/mg, $p<0.05$, n=8-9). Knockout mice also showed ameliorated albuminuria in adriamycin-injected mice. Similarly, PTP1B inhibitor (Calbiochem, #539741, 10mg/kg) reduced LPS-induced albuminuria and foot process effacement in wild-type mice (ACR: LPS alone: 9284±2905 vs LPS + PTP1B inhibitor: 2339±857 μg/mg, $p<0.05$, n=4-5). In cultured mouse podocytes, PTP1B knockdown and the PTP1B inhibitor both reduced LPS-stimulated cell migration and FAK phosphorylation at Y397, which plays a critical role in cell migration.

Conclusions: PTP1B upregulation in podocytes contributes to podocyte injury and proteinuria likely via FAK-mediated migratory response of podocytes. PTP1B may be a promising therapeutic target in proteinuric diseases.

Funding: Government Support - Non-U.S.

SA-PO767

NFKB Protects Human Mesangial Cells against Apoptosis Induced by Changes in Extracellular Matrix Composition: Implication in Renal Physiopathology
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Background: Renal fibrosis leads to chronic renal failure, characterized by a progressive substitution of extracellular matrix (ECM) proteins such as collagen type I (COL I) that may induce significant changes in growth patterns through the interaction with integrins. The aim of this study was to identify the mechanisms responsible for human mesangial cell (HMC) survival, conditioned by changes in extracellular matrix composition.

Methods: HMC where incubated with soluble COL I or collagen type IV, and pretreated with or without selective AKT inhibitor, AKT small interfering RNA (siRNA), PDTC and superrepressor IκBα. HMC apoptosis was determined by Anexin-V and flow cytometric analysis. The NFκB transcriptional activity was analyzed by 3xNFκB-TK-Luc reporter plasmid assay. The protein levels of p65, IκB, P-AKT and Bcl-xL was determined by western blot.

Results: Our results indicate that COL I induce apoptosis in HMC but only after inactivation of nuclear factor NFκB by expression of superrepressor IκBα or PDTC inhibitor. We show that COL I-treated HMC significantly increased NFκB-dependent transcription, IκBα degradation and p65/NFκB translocation to the nucleus. Since AKT activity was increased in this proecess, we wonder the role of AKT and Bcl-xL in the NFκB-mediated apoptosis regulation by COL I. The AKT inhibition by using a specific inhibitor or siRNA blocked the NFκB activity increase leading a sustained cell death by COL I.NFκB mediated the HMC survival by the transcriptional activation of the antiapoptotic Bcl-2 family member Bcl-xL, since the expression of this protein was abrogated by superrepressor IκBα, PDTC or AKT inhibitor.

Conclusions: Our data suggest that HMC exposed to COL I are protected against apoptosis by a mechanism involving AKT-dependent NFκB activation with a consequent Bcl-xL activation and this mechanism may block renal fibrosis.

SA-PO768

Puromycin Aminonucleoside, a Model of Nephrotic Syndrome, Induces the Nuclear Translocation of IQGAP1 Protein in Human Podocytes through the Involvement of ERK Signaling Pathway
Claire Rigothier,[1,2,3] Moin Saleem,[2] Jean Ripoche,[1] Christian Combe,[1,3] Gavin Iain Welsh.[2] *[1]INSERM U1026, Université Bordeaux Segalen, Bordeaux, France; [2]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [3]Service de Néphrologie, CHU Bordeaux, Bordeaux, France.*

Background: IQGAP1, a protein which links the actin cytoskeleton to slit diaphragm proteins, is involved in podocyte motility and permeability. We have treated human podocytes with puromycin aminonucleoside (PAN), inducer of nephrotic syndrome in rat, and studied its effects on IQGAP1 biology and podocyte signaling pathways.

Methods: In human podocytes exposed to PAN, Western blot (WB), immunocytolocalization (IC), quantitative PCR and immunoprecipitation were performed to determine the subcellular expression and localization, the transcription and the interacting partners of IQGAP1. Activation of different signaling pathways, IQGAP2 expression and the degree of IQGAP1 phosphorylation were evaluated.

Results: PAN induced the nuclear translocation of IQGAP1 protein from the cell membrane, a phenomenon observed by IC ($p<0.05$) and confirmed by WB after selective extraction ($p<0.01$). IQGAP2 expression remained cytoplasmic. IQGAP1 nuclear translocation was associated with significant decrease of its interaction with nephrin and podocalyxin and a significant increase of its phosphorylation. Activation of the ERK pathway was observed in PAN treated podocytes with a nuclear translocation of the phosphorylated form of ERK (P-ERK) ($p<0.02$). The ERK signaling pathway was involved in IQGAP1 nuclear translocation: inhibitors of ERK pathway blocked IQGAP1 nuclear translocation ($p<0.02$). IQGAP1 interaction with P-ERK increased upon podocyte exposure to PAN ($p<0.05$) and this was inhibited by inhibitors ($p<0.05$). Chromatin interaction protein assays demonstrated the interaction of IQGAP1 with the chromatin.

Conclusions: PAN induced the translocation of IQGAP1 into the nuclei in human podocytes. IQGAP1 nuclear trafficking required ERK signaling pathway and led to IQGAP1 interaction with the chromatin. Given its implication in podocyte motility, IQGAP1 protein may have a role in the gene regulation of proteins involved in migration or microtubules remodelling.

SA-PO769

Ret Is Critical for Podocyte Survival Following Glomerular Injury In Vivo
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Background: Podocyte injury and loss directly cause proteinuria and progression to glomerulosclerosis. Elucidation of the mechanisms of podocyte survival and recovery from injury is critical for designing strategies to prevent the progression of glomerular diseases. Previously Ret and its ligand, glial cell line-derived neurotrophic factor (GDNF), were demonstrated to be upregulated both in non-immune and immune mediated models of glomerular diseases *in vitro* and *in vivo*. Ret is a receptor tyrosine kinase that is critical for the growth, maintenance, and survival of several populations of neurons in the CNS and PNS. Currently, we are investigating whether Ret is necessary to maintain podocyte function and survival *in vivo*.

Methods: Since deletion of Ret results in perinatal lethality due to renal agenesis, we examined the role of Ret in glomeruli *in vivo* by generating BALB/c mice with a conditional deletion of Ret in podocytes (Nphs2-CRE $Ret^{flox/flox}$). We utilized light microscopy and PAS staining to determine severity of glomerular injury. TUNEL+ cells were imaged by immunofluorescence microsopy. At least 80-100 glomeruli were scored from each animal from a minimum of 4 mice per genotype. Two independent, blinded observers and imagers were used for each experiment.

Results: There were no developmental deficits in $Ret^{-/-}$ podocytes. In contrast to the lack of any developmental and early maintenance phenotype, these Nphs2-CRE $Ret^{f/f}$ mice showed a significantly enhanced susceptibility to adriamycin nephropathy, a rodent model of focal segmental glomerulosclerosis (FSGS). We observed a statistically significant increase in apoptosis between Nphs2-CRE $Ret^{+/+}$ vs Nphs2-CRE $Ret^{f/f}$ mice (10.5±1.05 vs 50.9±4.85; P=0.0002.) We also observed increased glomerular injury between Nphs2-CRE $Ret^{f/f}$ vs Nphs2-CRE $Ret^{+/+}$ mice (1.6±0.22 vs 0.54±0.09; P=0.004.)

Conclusions: Therefore, Ret appears to be critical specifically for podocyte survival and recovery from injury *in vivo*.

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SA-PO770

Albumin Uptake in Podocytes Occurs in a Polarized, Caveolin-Dependent Manner
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Background: Albuminuria is strongly associated with an increased risk of chronic kidney disease progression and albumin-containing endosomes have been found in podocytes from patients with nephrotic syndrome. Albumin uptake into cultured podocytes also occurs but the underlying endocytic mechanisms in podocytes remain to be determined.

Methods: We studied previously characterized human podocytes isolated from urine (Sakairi et al., Am J Physiol Renal, 2010). Uptake kinetics of fluorescein isothiocyanate (FITC)-labeled albumin were measured by spectrofluorometry and Western blotting. Colocalization of albumin and endocytic proteins was studied using confocal microscopy. Total internal reflection fluorescence microscopy (TIRF) examined the polarity of albumin uptake. Intensity correlation coefficient (ICQ) analysis measured the temporal distribution of albumin with components of endocytic pathways.

Results: Albumin uptake by podocytes was minimal at 4 °C, readily observed at 37 °C and plateaued by 1 hour. Although albumin was more freely accessible to the apical cell surface, live-cell imaging showed that albumin was preferentially endocytosed along the basal membrane. FITC-albumin containing endosomes co-localized with megalin and the neonatal Fc receptor, both proteins shown to be involved in albumin uptake in the renal proximal tubule. In contrast to the proximal tubule, albumin endocytosis in podocytes was a caveolin- and not a clathrin-dependent process. FITC-albumin containing endosomes

colocalized with caveolin but not with clathrin and inhibition of caveolin-dependent but not clathrin-mediated endocytosis inhibited albumin uptake. Once in the cell interior, albumin transited through the early endosome and lysosome compartments.

Conclusions: This study demonstrates that podocytes endocytose albumin in a polarized, caveolin-dependent manner. The findings may contribute to the understanding of the pathogenic mechanisms that underlie the chronic kidney disease associated with albuminuria.

Funding: NIDDK Support

SA-PO771

Low Concentration Cadmium Exposure Causes Albuminuria by Inducing Endoplasmic Reticulum Stress and Apoptosis in Podocytes Hsiang-Hao Hsu,[1,2] Kuan-hsing Chen,[1] Cheng-chieh Hung,[1] Hermann Pavenstaedt.[2] *[1]Kidney Research Center, Department of Nephrology, Linkou Chang Gung Memorial Hospital; [2]Medizinische Klinik und Poliklinik D, Universitätsklinikum Münster.*

Background: Proteinuria, especially albuminuria, mainly caused by podocyte injury and ensuing filtration barrier failure, predicts progression and renal outcomes in human glomerular diseases. Cadmium (Cd) is an important industrial and environmental heavy metal pollutant that with chronic, low-level patterns of exposure the kidney is the primary target of toxicity. It is not known if low-level exposure to Cd induces an irreversible dysfunction in glomerular podocytes, which have a significant role in establishing the selective permeability of the kidney filtration barrier.

Methods: In this study, we analyzed the association between blood and urinary Cd levels and kidney abnormalities (serum creatinine level, urine creatinine clearance and albuminuria) in a rodent model. Adult male Sprague-Dawley rats were daily subcutaneous infused with 0.3 mg/kg $CdCl_2$ for up to 12 weeks. In vitro toxic effects and cellular mechanisms of Cd were examined on cultured murine podocytes.

Results: The results showed that significant elevated serum creatinine level and raise of albuminuria were associated with increased serum and urine Cd concentration by continuous infusion in rats. Histological confirmation of podocyte injuries was done by transmission electron microscopy (showing Cd-induced podocyte foot process effacement) and immunohistochemistry exam of augmented cleaved caspase 3 staining. This study further examined in vitro toxic effects and cellular mechanisms of Cd on cultured murine podocytes. We showed that treatment with low concentration $CdCl_2$ (500nM, 48hrs) markedly increases intracellular Cd concentration in cultured podocytes. Accumulation of Cd in podocytes resulted in increased reactive oxygen species production, endoplasmic reticulum stress, inhibition of the survival pathway Akt and Erk, and finally led to cellular apoptosis.

Conclusions: This observation suggests that low concentration Cd exposure causes kidney injuries by inducing endoplasmic reticulum stress and apoptosis in glomerular podocytes.

Funding: Government Support - Non-U.S.

SA-PO772

Fibrates May Protect Glomerular Filtration Barrier by Increasing Synthesis of EETs Ellen T. McCarthy,[1] Virginia J. Savin,[2] Tarak Srivastava,[3] Shuhua Wang,[1] Ram Sharma,[2] Mukut Sharma.[2] *[1]Kidney Institute, University of Kansas Medical Center, Kansas City, KS; [2]Kansas City VA Medical Center, Kansas City, MO; [3]Children's Mercy Hospital, Kansas City, MO.*

Background: We have shown that 20-hydroxyeicosatetraenoic acid (20-HETE) and 8,9-epoxyeicosatrienoic acid (8,9-EET) and the drugs clofibrate and cyclosporine A (CSA) each protect the glomerular protein permeability barrier from injury. We hypothesize that clofibrate and CSA increase synthesis of one or more protective eicosanoids by podocytes.

Methods: Cultured immortalized podocytes were incubated with vehicle, clofibrate 100 μM or CSA 1 μM X 6 hr, clofibrate 100 μM or CSA 1 μM X 6 hr + arachidonic acid (AA) 30 μM X 10 min or AA 30 μM X 10 min. Cells were then incubated with a calcium ionophore to activate phospholipase A_2, and lipids extracted, identified and measured using liquid chromatography-mass spectrometry (LC-MS). In a separate study, mesangial cells were incubated with calcium ionophore and lipids extracted and analyzed.

Results: Both podocytes and mesangial cells can synthesize cytochrome P450 eicosanoids, including 19- and 20-HETE; 5,6-, 8,9-, 11,12- and 14,15-EET; and 5-, 12-, and 15-HETE. Podocytes produced significantly more of each of these eicosanoids that did mesangial cells ($P<0.001$ for each eicosanoid). Clofibrate significantly increased podocyte synthesis of 8,9-, 11,12- and 14,15-EET ($P<0.001$); synthesis of all four EETs was significantly amplified by exogenous AA. Clofibrate did not increase 19- or 20-HETE synthesis even in the presence of exogenous AA. CSA increased synthesis of HETEs and EETs only with the addition of exogenous AA.

Conclusions: We conclude that protection of the glomerular protein permeability barrier by fibrates or CSA does not depend on mesangial cell eicosanoid synthesis or on increased synthesis of 20-HETE. CSA appears to protect the filtration barrier through mechanisms that are independent of eicosanoids. In contrast, fibrate-induced increase in podocyte synthesis of 8,9-EET may protect the glomerular filtration barrier. Studies of fibrates and of anaolgs of 8,9-EET may permit development of novel agents to treat glomerular disease.

Funding: NIDDK Support

SA-PO773

The Uremic Toxin Indoxyl Sulfate Causes Podocyte Injury *In Vivo* and *In Vitro* Osamu Ichii,[1,2] Huiyan Lu,[1] Hideko Takahashi,[3] Patricia M. Zerfas,[4] Jeffrey B. Kopp.[1] *[1]NIDDK, NIH, Besethda, MD; [2]Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan; [3]NEI, NIH, Besethda, MD; [4]Office of the Director, NIH, Besethda, MD.*

Background: Indoxyl sulfate (IS) accumulates in chronic kidney disease (CKD), with serum levels 0.1-1 mM. IS is a ligand of the aryl-hydrocarbon receptor (Ahr), a transcription factor, activating detoxification pathways and other cell functions. Exogenous IS causes tubulo-interstitial injury; we examined whether IS induces podocyte injury.

Results: In FVB/N mouse kidney, Ahr was expressed in podocytes and tubular epithelial cells, most strongly in the former where it was present in the nucleus. In isolated glomeruli from acutely exposed mice (800mg/kg IP, at 2 h), *Cyp1a1,* a sensitive reporter gene for Ahr activation, was induced 470-fold. With chronic exposure (600-800 mg/kg IP daily for 4 wk), renal cortical podocyte mRNA expression (*Nphs1*, *Nphs2*, *Cd2ap, Podxl*, *Synpo*, *Myh9*, *Actn4*, *Wt1*) were reduced to 52-76 % of control mice. Mice undergoing uninephrectomy followed by IS 600-800 mg/kg IP daily for 4 wk manifested microalbuminuria (7-fold over control), while glomeruli showed mild mesangial expansion, capillary loop thickening, and decreased podocyte staining for synaptopodin and non-muscle myosin IIA. Immortalized, differentiated murine podocytes were exposed to 1 mM IS for 30-60 min and manifested Ahr nuclear translocation. At 2 hr cell mRNA expression of *Wt1*, *Podxl*, *Synpo*, *Myh9*, *Cd2ap*, and *Actn4* decreased in a dose-dependent fashion, reaching 33-47 % of baseline. At 24 hr, certain cytokine RNAs increased, in particular *Tnfa* (6-fold), and podocytes showed reduced cell size, loss of actin stress fibers and decreased viability. In normal human kidney, Ahr localized to podocytes and tubular epithelial cells. In immortalized human podocytes, 1 mM IS exposure caused Ahr nuclear translocation and reduced cell viability.

Conclusions: IS caused podocyte injury with altered differentiation markers and cytokine RNA expression. In patients with CKD, accumulation of IS may drive a feed-forward process that contributes to progressive glomerular injury and further deterioration in renal function.

Funding: NIDDK Support

SA-PO774

ApolipoproteinL1-B Splice Variant Is Expressed by Podocytes In Vitro and In Vivo and Shows Both Nuclear and Cytoplasmic Distribution Hidefumi Wakashin, Jeffrey B. Kopp. *NIDDK, NIH, Bethesda, MD.*

Background: Apolipoprotein L1 (ApoL1) is a minor component of HDL particles, although its function there is unknown. ApoL1 promotes lysis of *Trypanosoma brucei brucei,* via enhanced chloride ion channel activity in the parasite lysosome. *APOL1* variants, exclusively present in individuals of recent African descent, are associated with substantially increased risk for glomerular disease. The mechanisms by which *APOL1* variants induce glomerular injury are unknown. ApoL1 has several RNA splice variants. The A isoform consists of 398 amino acid (aa) residues, with a predicted molecular mass (Mw) of 43.9 kDa, and is encoded by exons 1, 3-7. The B isoform consists of 414 aa, having a 16 aa extension at the N-terminus, and is encoded by exon 1-7, with a predicted Mw of 45.9 kDa. The C isoform consists of 381 aa residues, with a predicted Mw of 42.2kD, and is encoded by exons 1, 3, 5-7.

Results: Using the 16 aa sequence unique to ApoL1-B, we raised an rabbit anti-peptide antibody. On western blot, this antibody recognized 4 bands in immortalized human podocyte lysates, at 80, 65, 55, 42 kDa; all 4 bands were eliminated by pre-incubation with the immunizing peptide. The identities of these bands remain to be fully determined. Western blots using normal human kidney tissue showed a single band at 55 kDa. Using immunofluorescence staining, we found that ApoL1-B was expressed by podocytes and proximal tubular epithelial cells in normal human kidney and by cultured human podocytes. Interestingly, ApoL1-B was present in the nuclei of podocytes (in vitro) and tubular epithelial cells (in vivo) in a speckled pattern. In cultured podocytes, ApoL1-B showed partial co-localization with the splicesome marker SC35. ApoL1-B was also present in a filamentous cytoplasmic pattern (in vivo and in vitro), which co-localized with F-actin and myosin heavy chain IIA.

Conclusions: ApoL1-B is expressed in the nucleus and cytoplasm. On-going studies are examining sub-nuclear and sub-cytoplasmic localization, using a GFP-ApoL1-B vector and are seeking to identify nuclear and cytoplasmic binding partners, using flag-tagged ApoL1-B.

Funding: NIDDK Support

SA-PO775

Protein Overload Induced Proteinuria in the Munich Wistar Frömter Rat Does Not Correlate with an Increase in GSC of Surface Glomeruli Measured Using Intravital 2-Photon Microscopy Mark C. Wagner,[1,2] Ruben M. Sandoval,[1] Silvia B. Campos-bilderback,[1] Sarah E. Wean,[1] George Rhodes,[1] Bruce A. Molitoris.[1,2] *[1]Medicine/ Division of Nephrology, IU School of Medicine, Indianapolis, IN; [2]Roudebush VAMC, Indianapolis, IN.*

Background: Injection of large doses of albumin are known to cause nephrotic range proteinuria in rats. Multiple glomerular changes including foot process effacement and cytoplasmic swelling have been documented.

Methods: To ask the fundamental question whether a change in the glomerular sieving coefficient (GSC) occurs during this protein insult event eight Female Munich Wistar Fromter rats were injected IP with either bovine or rat serum albumin at a dose of 1.25g in 5ml of saline on 2 successive days.

Results: Twenty four hour urine collections documented the presence of markedly increased protein which ranged up to 780mg/24 hours. 24 or 48 hrs after the 2nd injection the animals had their GSC measured using intravital 2-photon microscopy using Texas Red-rat albumin as the marker. If the increased in urine protein is simply the result of a glomerular injury than an increase in GSC would be expected. However, if other events i.e. change in albumin uptake or release from tubule segments participate than an altered GSC may not be observed thus implicating non-glomerular processes in the resultant proteinurea. GSC ranged from a low of 0.0073 to a high of 0.0144.

Conclusions: The lack of correlation between an increase in urine protein and GSC supports involvement of non-glomerular processes. We are presently evaluating Texas Red-rat albumin uptake by the tubules and the role of FcRn and will report these results.

Funding: Other NIH Support - P30DK079312

SA-PO776

Podocytes Produce Microparticles in Response to Diabetic Stress Factors: Implications in Diabetic Kidney Disease Dylan Burger, Jean-Francois Thibodeau, Chet E. Holterman, Chris R. Kennedy. *Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada.*

Background: Microparticles (MPs) are submicron (0.1-1.0 µm), anuclear fragments shed from cell membranes and released into the extracellular space. MPs are formed from multiple cell populations in response to stress/injury. However, whether podocytes produce MPs, and whether podocyte MP formation reflects glomerular injury is unknown. We examined MP formation from podocytes in a conditionally immortalized human podocyte cell line (HPOD) and in two mouse models of progressive diabetic kidney disease: streptozotocin (STZ, 50 mg/kg x 5 days) and OVE26 mice.

Methods: HPODs were either exposed to 10% equibiaxial cyclical stretch, high glucose conditions (HG, 25 mM), or treated with angiotensin II (Ang II, 500 nM) or transforming growth factor beta (TGF-β, 5 ng/mL). Additionally, to determine the in vivo significance of podocyte MP formation, we probed whether podocytes release MPs into urine in diabetic mice. MPs were isolated from media/urine by differential centrifugation and quantified by Annexin V (total MPs) or podocalyxin (podocyte MPs) labeling and flow cytometry.

Results: Cyclic stretch was associated with a 3-fold increase in MP release after 5 hours ($P<0.01$). Similarly, HG increased MP release 2-fold after 24 hours ($P<0.05$). Neither Ang II, nor TGF-β had any effect on podocyte MP formation over 24 hours. Both mouse models displayed evidence of glomerular injury (mesangial expansion) and frank proteinuria as evidenced by increased urinary albumin/creatinine levels compared with untreated or wild-type controls (STZ: 1312±224 µg/mg vs 264±130 at 8 weeks, $P<0.001$; OVE26: 391±130 µg/mg vs. 29±6 at 16 weeks, $P<0.05$). STZ-treated mice displayed increased urinary podocyte MPs as compared with untreated mice (17478±8329 MPs/mg Creatinine vs. 7 ±7, $P<0.05$). Similarly, OVE26 mice displayed increased urinary podocyte MPs compared with their wild-type littermates (6956±2386 vs. 9±9, $P<0.01$).

Conclusions: Taken together our results suggest that podocytes produce MPs which are released into urine and may be indicative of glomerular injury. Such processes may be mediated by mechanical stretch and high glucose conditions.

Funding: Government Support - Non-U.S.

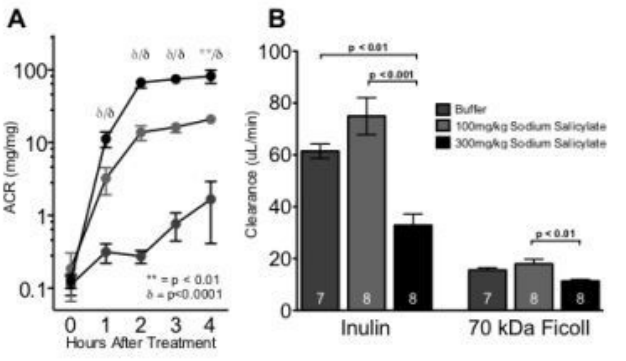
SA-PO777

Rapid and Massive Albuminuria with Undetectable Glomerular Damage Following Sodium Salicylate in Mouse Ryan B. Sanford,[1] John R. Hagaman,[2] Longquan Xu,[2] Jennifer C. Wilder,[2] Mike Kelly Altenburg,[2] J. Charles Jennette,[2] Oliver Smithies.[2] *[1]Division of Nephrology and Hypertension, University of North Carolina Chapel Hill, Chapel Hill, NC; [2]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: The mechanism of renal albumin handling remains debated. Opposed to the dominant theory of a low sieving coefficient with a tight glomerular filter, another model proposes a high sieving coefficient with extensive proximal tubule reclamation. Here we tested these models using salicylate-induced proteinuria.

Methods: We measured urinary albumin, glucose, and gamma-glutamyl transferase (GGT); GFR; ficoll clearance; and renal histology in male CD1 mice after sodium salicylate or buffer IP injections.

Results:



Mice receiving salicylate developed albuminuria peaking between 2-4 hours following injection (A). In addition, glucosuria and increased urinary GGT occurred in treatment groups (data not shown). GFR was decreased compared to buffer in the high dose treatment, but not in the low dose treatment. Clearance of a 70 kDa polydisperse TRITC-Ficoll was unchanged compared to buffer treatment (B). Histology revealed coagulative necrosis of proximal tubular cells; the glomeruli were normal in appearance. By electron microscopy, the glomerular capillary wall was normal in appearance without changes to podocyte architecture.

Conclusions: Within 2 hours of IP sodium salicylate, massive albuminuria occurred accompanied by proximal tubule damage and necrosis. Correspondingly, glomerular damage was undetectable by both structural and functional methods. These findings are consistent with a high glomerular sieving coefficient for albumin with proximal tubular reclamation. Back-leak of albumin from peritubular capillaries into the tubular lumen is also possible.

Funding: NIDDK Support, Other NIH Support - T32 Training Grant

SA-PO778

EphB Receptors Modulate the Nephrintyrosine Phosphorylation: A Potential Mechanism for Adhesion and Repulsion at the Slit Membrane Eva Koenigshausen, Nils Tim Haep, Magdalena Woznowski, Ivo Quack, Lars C. Rump, Lorenz Sellin. *Nephrology, Heinrich Heine University, Duesseldorf, Germany.*

Background: Proteinuria is the strongest predictor for cardiovascular events. Glomerular proteinuria arises from a damaged glomerular filter. The glomerular slit membrane is a specilized cell cell contact with nephrin being its major component. Nephrin is endocytosed after its binding to β-arrestin2, which finally destabelizes the glomerular filter. Eph receptors and their ligands, the ephrins, mediate adhesion and repulsion in specific cell cell contacts. Recently, ephrinb1 was detected at the glomerular slit. The molecular function of the EphB-ephrin family for the podocyte is unknown.

Methods: Podocyte RNA the samples are subjected to qPCR to detect ephrinb1, b2, Eph1 and 2. Furthermore, nephrin, β-arrestin2, Nck1/2 and FAK expressing cells are used. After cell lysis co-immunoprecipitations are done and lysates are subjected to western blot analysis. Serum straved podocytes were stimulated with preclustered ephrinb1-Fc or IgG-Fc for the indicated time. Thereafter nephrin was precipitated from podocyte lysates are analysed by western blotting.

Results: EphB1, B2, ephrinb1 and b2 are expressed in podocytes. EphB1 and EphB2 interact with the extracellular domain of Nephrin and also with podocin, β-arrestin2 and FAK. The expression of EphB1 and EphB2 induces a marked nephrin tyrosine phosphorylation especially at the Nck1/2 binding sites. The stimulation with ephrinb1 decreases tyrosine phosphorylation of nephrin Y1217. EphB1 and B2 expression leads to an enhanced interaction of nephrin with Nck2 and mediates a strong attenuation of the nephrin binding to β-arrestin2. The tyrosine phosphorylation is reproduced in podocytes.

Conclusions: EphB receptors stabelize the glomerular slit membrane thru enhanced nephrin tyrosine phosphorylation at Nck-binding sites. By binding ephrinb1 to EphB receptors a tyrosine desphosphorylation of nephrin is induced. This might cause a destabelization of the slit membrane. The EphB rezeptor mediated cell adhesion and repulsion could contribute to a better understandig of the glomerular pathobiology and open novel therapeutic avenues for glomerular diseases.

SA-PO779

Role of Protein-Tyrosine Phosphatase 1B (PTP1B) in Complement-Mediated Glomerular Injury Lisa Nezvitsky, Michel L. Tremblay, Tomoko Takano, Andrey V. Cybulsky. *Medicine, McGill University, Montreal, QC, Canada.*

Background: Glomerular diseases may be associated with activation of endoplasmic reticulum (ER) stress, notably the unfolded protein response (UPR) and ER-associated degradation (ERAD). PTP1B enhances activation of the inositol requiring-1α (IRE1α) branch of the UPR. IRE1α may regulate ERAD, in part, via induction of ER-associated degradation enhancing α-mannosidase-like protein (EDEM). We propose that PTP1B is an important modulator of ERAD in cells undergoing complement attack.

Methods: Complement was activated in PTP1B wild type (WT) and knockout (KO) mouse embryonic fibroblasts (MEF), and rat glomerular epithelial cells (GEC) by incubation with antibody and normal serum (decomplemented serum in control). To specifically activate the UPR, cells were treated with tunicamycin. Degradation of the T cell receptor subunit, CD3δ, fused to yellow fluorescent protein (CD3δ-YFP) was employed as an ERAD reporter.

Results: Deletion of PTP1B in MEFs impaired tunicamycin-induced activation of c-Jun N-terminal kinase, which is dependent on IRE1α. Complement-mediated cytotoxicity (lactate dehydrogenase release) was reduced in PTP1B KO MEFs, compared to WT, implying that PTP1B enhanced cytotoxicity. After incubation with complement, CD3δ-YFP degradation was decreased in KO MEFs, compared to WT, suggesting that deletion of PTP1B impaired ERAD, possibly via reduced IRE1α-dependent induction of EDEM. In GECs treated with kifunensine (an inhibitor of α1,2-mannosidase and ERAD), degradation of CD3δ-YFP was decreased after exposure to complement, compared to untreated GECs. Similarly to the MEFs, in GECs treated with a chemical inhibitor of PTP1B or transfected with a PTP1B dominant negative cDNA, complement decreased degradation of CD3δ-YFP, compared to control GECs. Surprisingly, in GECs overexpressing PTP1B WT, complement also decreased degradation of CD3δ-YFP, compared to control cells.

Conclusions: After exposure of cells to complement, ERAD and cytotoxicity were reduced upon inhibition of PTP1B. Overexpression of PTP1B also reduced ERAD, suggesting that ERAD is dependent on tight regulation of PTP1B expression. PTP1B is potentially a novel target for modulation of complement-mediated injury.

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SA-PO780

Role of IL-17 Signaling in the Experimental Crescentic Glomerulonephritis Su Mi Lee, Seung Hee Yang, Do Hyoung Kim, Yun Jung Oh, Jung Pyo Lee, Yon Su Kim. *Seoul National University Hospital, Seoul, Republic of Korea.*

Background: T cells play a major role in the pathogenesis of crescentic glomerulonephritis (GN), which is the most severe form of GN and progresses to end-stage renal disease unless treated adequately. Th17 cells have been reported to contribute to renal injury in crescentic GN. In the present study, we examined the notion that the modulation of Th17 response could reduce the renal injury utilizing a murine cresentic GN model.

Methods: IL-17 deficient and STAT-3β deficient mice were used because IL-17 is the major cytokine of Th17 cells and STAT-3 is the essential transcriptional factor of Th17 cell differentiation. Experimental crescentic GN was induced by the injection of anti-glomerular basement membrane (GBM) antibodies into Balb/c, C57BL/6, IL-17 deficient Balb/c, and STAT-3β deficient C57BL/6 mice.

Results: After 7 days of disease induction, the elevated BUN and urine protein/creatinine ratio in wild type mice were significantly reduced in IL-17 deficient (BUN 55 ± 22.7 vs 154 ± 17.5mg/dl, $p<0.01$; U prot/cr 114 ± 10. 6 vs 143 ± 16.6 mg/mg, $p<0.05$) and STAT-3β deficient mice (BUN 116 ± 25.0 vs 156 ± 8.0 mg/dl, $p<0.05$; U prot/cr 53 ± 6.8 vs 99 ± 6.8, $p<0.05$). In addition, the glomerular injury and crescent formation by anti-GBM antibody were attenuated in IL-17 deficient and STAT-3β deficient mice compared to those of wild type mice. Accordingly, intrarenal mRNA expression of IL-1β, TGF-β, MCP-1, IL-12p19 and STAT-3 were elevated in wild type mice, but these changes were attenuated in IL-17 and STAT-3β deficient mice. Treatment of CD3 in co-cultured mesanigal cells and NKT cells induced mRNA expression of IL-17 receptor and secretion of IL-17 and IL-12p70 as well as inflammatory cytokines such as IL-2, TNF-α and IL-6. Blocking of IL-17 receptor reduced these inflammatory reactions.

Conclusions: Taken together, these data suggest that Th17 cells play an important role in the pathogenesis of experimental cresecentic GN. IL-17 and STAT-3 might be a feasible target in protecting renal injury in the disease.

SA-PO781

HIV Related Kidney Disease: A Shift in the Paradigm Sandeep K. Mallipattu,[1] Ruijie Liu,[1] Vivette D. D'Agati,[3] Ali G. Gharavi,[2] Peter Y. Chuang,[1] John C. He.[1] *[1]Nephrology, Mount Sinai School of Medicine, New York, NY; [2]Nephrology, Columbia University, New York, NY; [3]Pathology, Columbia University, New York, NY.*

Background: HIV-associated nephropathy (HIVAN), an aggressive form of focal segmental glomerulosclerosis, has historically been considered the typical presentation of kidney disease in patients with Acquired Immunodeficiency Syndrome. However, with the widespread use of antiretroviral therapy in the United States, the incidence of HIVAN has decreased and the spectrum of HIV-related kidney disease has dramatically changed.

Methods: Although epidemiological studies suggest that HIV infection may accelerate the progression of diabetic nephropathy or age related nephrosclerosis in this population, the mechanism behind this association has yet to be described. To study this, HIV-1 transgenic mice (Tg26) were initially backcrossed to C57BL/6 background; a strain that is typically resistant to classical HIVAN. Next, Tg26 and wild-type mice were treated with low-dose streptozotocin (STZ) to induce diabetes mellitus. Proteinuria was assessed monthly and all mice were sacrificed at 12 months of age.

Results: STZ-treated Tg26 mice exhibited a significant increase in proteinuria, mesangial expansion, glomerular basement membrane thickening, and podocyte effacement compared to untreated age-matched Tg26 mice or STZ-treated wild-type mice. Additionally, Tg26 mice demonstrated a significant increase in proteinuria, podocyte effacement, and GBM thickening at 12 months of age as compared to age-matched wild-type mice. Next, microarray gene expression studies were performed on isolated glomerular extracts from mice revealing a significant upregulation of pro-inflammatory and pro-fibrotic signaling pathways. The upregulation of genes downstream of these pathways was confirmed by real-time PCR and immunostaining.

Conclusions: To date, this is the first study to provide a novel insight into the mechanism of non-HIVAN related glomerular disease progression in the presence of HIV-1 infection and concurrent comorbidities such as diabetes and aging. Finally, we have also confirmed an upregulation of pro-inflammatory and pro-fibrotic pathways.

Funding: NIDDK Support

SA-PO782

1,25-Dihydroxyvitamin D(3) Reduces Proteinurea via Inhibiting uPAR Expression Jianchao Ma, Wei Shi, Shuangxin Liu, Wenjian Wang, Bin Zhang. *Department of Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: Accumulating studies have demonstrated that 1,25-Dihydroxyvitamin D(3) reduces podocytes loss and slows the decline of kidney function in chronic kidney disease. Recent evidences showed that urokinase receptor (uPAR) and its soluble form carry a key role in the pathogenesis of focal segmental glomerulosclerosis. In this study, we hypothesized that vitamin D protects podocytes via modulating uPAR expression.

Methods: 24 C57BL/6 male mice were randomly divided into three groups: control group(Con), LPS group (LPS) and 1,25(OH)2D3 treated group(LPS+1,25(OH)2D3). We injected C57BL/6 mice intraperitoneally with 300 μg LPS. In 1,25(OH)2D3 treatment ,we gavage mice with 1,25(OH)2D3 2.5μg·kg-1·d-1 2 days before LPS injection. We collected the mouse urine for 24 hours after LPS injection. All were executed at the third day. In vitro, immortalized mouse podocytes were cultured and randomized into three groups: control group,LPS ($50mg\cdot L^{-1}$) group, LPS ($50mg\cdot L^{-1}$)+$1,25(OH)_2D_3$ ($1nmol\cdot L^{-1}$) group. The podocytes motility was checked by transwell migration assay .The expression of uPAR in podocytes were detected by immunofluorence method, flow cytometry method and real-time PCR.

Results: The proteinuria were 72.0±35.6mg/gCr,(732.9±233.2)mg/g Cr,(412.3±82.7 mg/g Cr)in control group, LPS group and LPS plus 1,25(OH)2D3 group, respectively. Meanwhile the uPAR expression was increased in LPS group, and was inhibited by 1,25(OH)2D3 in both protein level and mRNA level. The number of podocytes in the transwell migration assay were 8.4±3.8, 31.3±7.9, 19.2±6.8 in the control group, LPS group and LPS+1,25(OH)2D3 group, respectively.

Conclusions: Our findings provided new insights of the renopretection role of 1,25-Dihydroxyvitamin D(3) in podocytes injury, and offer a potential target in reduces proteinurea by inhibiting uPAR expression.

Funding: Government Support - Non-U.S.

SA-PO783

TGF-β Antagonism Is Reno-Protective by Blunting Podocyte Injury in a Novel T2D Model of Diabetic Nephropathy (DN) Mandy M. Smith, Stephen O'Brien, John N. Vassiliadis, Stefan Wawersik, Steven R. Ledbetter, Cynthia M. Arbeeny, Hong Ling. *Sanofi-Genzyme R&D Center, MA.*

Background: Transforming growth factor β (TGF-β) plays a central role in the pathogenesis of DN and TGF-β antagonism has positive effects on renal pathophysiology in diabetic models. However, the impact of treatment on podocyte integrity is less clear and was the goal of this study utilizing 1D11 (a TGF-β antagonist antibody) in a novel T2DN model.

Results: We have reported that female uni-nephrectomized (Unx) KK-A^y mice fed a moderate fat diet develop progressive renal impairment, albuminuria, decline in GFR and pathology characteristic of human DN. Unx-KK-A^y mice were dosed for 12 wk with 1D11 at 2 and 5 mg/kg or 13C4 (control antibody) at 5 mg/kg. The 13C4 group showed glomerular hypertrophy, glomerulosclerosis and tubulointerstitial fibrosis. Serum BUN was increased to >40 mg/dl in the 13C4 group, and the 1D11 group had a 17% reduction at 12 wk. The 13C4 group had marked albuminuria which was ~10 fold above non-diabetic (KK-aa) controls. Albuminuria was attenuated in the 1D11 groups at all timepoints, with a 35% reduction at 12 wk. Urine nephrin proteion (ELISA) increased progressively in the 13C4 group and was correlated with albuminuria; while 1D11 (5mpk) significantly decreased nephrin loss at week 8 post dosing. Nephrin gene expression in kidney homogenates was down-regulated with 13C4, but was upregulated with 1D11, indicating a podocyte protective effect. Western blotting showed that PAI-1 was elevated in the 13C4 group vs non-diabetics and was normalized with 1D11. A similar pattern for activated TGF-β1 was observed. Using immunostaining, PAI-1 was localized in peri-tubular capillaries and glomerular tufts, a pattern similar to TGF-β1 localization observed in other renal disease models. PAI-1 immunostaining was reduced in mice dosed with 1D11 supporting the changes detected by western blotting.

Conclusions: The data indicates that TGF-β neutralization is reno-protective, possibly by maintaining podocyte integrity and reducing endothelial localized PAI-1. It is plausible that a maximal treatment effect requires RAS inhibition and combination studies are planned.

SA-PO784

Investigations Into the Mechanism of Action of the Splice Isoform Vascular Endothelial Growth Factor 165^b on Glomerular Water Permeability In Vivo Megan Stevens, Andy Salmon, David O. Bates, Steve Harper, Sebastian Oltean. *Microvascular Research Laboratories, University of Bristol.*

Background: Several glomerular diseases are associated with increased expression of the pro-permeability, pro-angiogenic vascular endothelial growth factor (VEGF-A, main isoform $VEGF_{165}$). The functionally different splice isoform, $VEGF_{165}b$, has been shown to decrease glomerular water permeability (L_pA/V_i) in kidneys of transgenic mice over-expressing $VEGF_{165}b$ under a nephrin promoter (Qiu et al. JASN 2010;). However,

the molecular mechanisms of this inhibition are unknown. We analyzed the expression and phosphorylation of VEGFR2 in two mouse models.

Methods: Glomeruli isolated from nephrin-$VEGF_{165}$b over-expressing mice and wild-type controls. Western blots used to determine expression and phosphorylation levels of VEGFR2. Isolated glomeruli were incubated with saline or PTK787 (inhibitor of VEGF receptors) and L_pA/V_i measured (Salmon et al. JPhys 2006).Immunofluorescence for expression levels and phosphorylation of VEGFR2 on kidneys from diabetic DBA2J mice treated with i.p. injection of $VEGF_{165}$b, compared to diabetic WT controls.

Results: Western blots show increased phosphorylation of VEGFR2 in the glomeruli of neph-$VEGF_{165}$b over-expressing mice compared to WT littermates (n=3). Western blot for nephrin-$VEGF_{165}$b glomeruli show reduced phosphorylation of VEGFR2 when incubated for 1 hour in 100nM PTK 787 compared to saline (n=1). In WT littermates, phosphorylation of VEGFR2 is blocked by incubation of glomeruli with 100nM PTK 787 (n=3). Incubation of glomeruli from neph-$VEGF_{165}$b mice with PTK leads to a further reduction in L_pA/V_i compared to those incubated with saline ($p<0.05$; mice n=5, glomeruli n=18).In glomeruli from diabetic DBA2J mice treated i.p.with $VEGF_{165}$b (n=3) immunofluorescence revealed increased expression and phosphorylation of VEGFR2.

Conclusions: $VEGF_{165}$b increases phosphorylation of VEGFR2 in glomeruli in both models. However, inhibition of VEGF receptors in neph-$VEGF_{165}$b over-expressing mice further decreases L_pA/V_i, suggesting that although $VEGF_{165}$b is acting through VEGFR2, it is likely that it does not block all VEGFR2 sites, possiblysignaling through other VEGF receptors.

SA-PO785

Systems Analysis of MYH9 Protein-Protein Interactions Reveals Multiple Possible Functions within the Podocyte Thomas Hays,[1] Deborah P. Hyink,[2] Paul E. Klotman.[2] *[1]Medicine, Mount Sinai School of Medicine, New York, NY; [2]Medicine, Baylor College of Medicine, Houston, TX.*

Background: MYH9 encodes the predominant force-generating ATPase in non-muscle cells. Rare nonsynonymous mutations in MYH9 cause a Mendelian disease, which includes a variably penetrant glomerulopathy leading to renal failure. Additionally, work by our group has recently identified reduced MYH9 expression in the setting of HIV-associated nephropathy. MYH9's function in generating tension across the actin cytoskeleton, and in stress fibers has been well characterized in multiple models. However, its precise functions in podocytes remain unknown. Podocytes are highly differentiated cells which maintain the selectively permeable glomerular filtration barrier of the kidney. This function relies upon a mechanically dynamic actin cytoskeleton supporting the podocyte-specific proteins which make up the slit diaphragm apparatus.

Methods: To better understand MYH9's role within the podocyte, we performed an immunoprecipitation mass-spectrometry screen and identified MYH9-interacting proteins within murine and human conditionally immortalized podocytes. Using immunohistochemistry, we characterized the subcellular localization of MYH9 within podocytes in vivo. Finally, we tested the effect of loss of MYH9 expression on podocytes in vitro.

Results: Systems biological analyses revealed that MYH9-interacting proteins belonged to signaling networks known to regulate cytoskeletal actin organization, including the RhoA pathway. Immunohistochemistry revealed MYH9 to be expressed within the podocyte processes overlying glomerular capillary tufts. Knockdown of MYH9 demonstrated that its function was necessary for cytoskeletal organization.

Conclusions: Our results provide the first detailed survey of MYH9 interactions within the podocyte, and demonstrate the important role of this protein in organization the elaborate cytoskeleton of podocytes.

Funding: NIDDK Support, Other NIH Support - NHLBI, NIGMS

SA-PO786

Transgenic Rats Overexpressing a Constitutively Active TGF-ß1 Receptor in Podocytes Develop Podocyte Injury and Albuminuria Sigrid C. Hoffmann, Rebecca Walz, Sabine Neudecker, Carsten Sticht, Norbert Gretz, Wilhelm Kriz. *Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany.*

Background: Glomerular TGF-ß1 is increased early in the course of FSGS and diabetic nephropathy. The podocytes are source and targets of TGF-ß1. This study addressed the hypothesis that increased TGF-ß1 induces podocytes damage leading to glomerular disease.

Methods: Transgenic rats were generated by pronuclear microinjection of a constitutively active TGF-ß1 type 1 receptor [hTßRI$_{(T204-D)}$] cDNA driven by the podocin promoter into fertilized Sprague Dawley oocytes. The transgene expression was verified by Northern blotting, Real-Time RT-PCR, In-situ hybridization and Western blotting. Glomerular expression of 93 genes either specific to the podocyte or implicated in cell survival and TGF-ß signalling, respectively, were examined by Real time RT-PCR, Western blotting and immunohistochemistry. Kidney function was evaluated by urinary albumin excretion in the 24h urine and by creatinine clearance in a monthly interval. Structural changes in the kidney were explored by light and electron microscopy.

Results: hTßRI$_{(T204-D)}$ overexpression in podocytes caused in 83% of male rats a significant slowly progressing albuminuria with at maximum 21mg albumin/24hr excretion in individuals. Morphological alterations correlated with the degree of albuminuria and comprised podocytes injury such as pseudocysts, foot process effacement and decay of podocytes in addition to mesangial expansion, matrix accumulation leading focally to capillary loss and occasionally adhesion of glomerular tuft to Bowman's capsule. Expression profiling revealed that 24 out of 93 tested genes were significantly different regulated resulting in increased glomerular PAI-1/PA and bax/blc-1 ratios, altered collagen composition and down-regulated synaptopodin, podocin and nephrin expression in glomeruli of transgenic rats.

Conclusions: TGF-β regulates the podocyte fate *in vivo* and contributes to the leakage of the glomerular filter. Down-regulation of nephrin, synaptopodin and podocin as well as altered pathways affecting ECM turnover and survival seems to be mechanisms leading to the observed structural and pathophysiological changes.

Funding: Clinical Revenue Support

SA-PO787

Dynamin Activation Rescues PKCε Deficiency Induced Actin Dysregulation in Podocytes Beina Teng,[1] Changkyu Gu,[2] Hermann G. Haller,[1] Sanja Sever,[2] Mario Schiffer.[1] *[1]Division of Nephrology, Department of Medicine, Medical School Hannover, Hannover, Germany; [2]Nephrology Division, Department of Medicine, Massachusetts General Hospital, Charlestown, MA.*

Background: We have previously demonstrated that the novel PKC isoform (PKCε) participated in signalling pathways controlling actin filament dynamics. PKCε deficiency in podocytes resulted in defective actin arrangement which led to a progressive reduction of podocytes and a spontaneous glomerulosclerosis phenotype in PKCε-/- mice *in vivo*.

Methods: PKCε-/- podocytes were infected with adenoviruses expressing wild type and dominant negative PKCε, as well as wild type and different dynamin mutants.

Results: Re-expression of wild type PKCε, but not the enzymatically impaired mutant, resulted in increased formation of stress fibres, focal adhesion complexes, lamellipodia and filopodia in PKCε-/- podocytes. Overexpression of PKCε in PKCε-/- podocytes also resulted in alteration of the signalling pathways, which play a central role in the control of actin-cytoskeletal rearrangement. Loss of PKCε in podocytes dramatically reduced the activity of Rho family small GTPases that are key upstream regulators of actin-cytoskeleton rearrangement, which could be reversed by re-expression of wild type PKCε.

Dynamin is a large GTPase known to regulate actin cytoskeleton through direct dynamin-actin interactions. Overexpression of dynamin mutant with increased affinity for actin (dynE/K) but not the mutants with impaired ability to bind F-actin, significantly enhanced the formation of stress fibres, number of focal adhesions and lamellipodia. All the disordered actin-cytoskeletal phenotypes due to PKCε deficiency could be partially rescued by overexpression of 'gain of function' dynamin.

Conclusions: The ability of dynamin mutant with increased affinity for F-actin to partially rescue organization of the actin cytoskeleton in podocytes lacking PKCε suggests that actin cytoskeleton in podocytes is regulated by two distinct signalling pathways; one regulated the RhoA family small GTPases, and the second one regulated by large GTPase dynamin. In addition, our study demonstrates that activity of dynamin in podocytes is not regulated by PKCε.

SA-PO788

Toll-Like Receptor 9 Signaling Is Activated in Podocytes of Purimycin Aminonucleoside (PAN) Injury Models Yaojun Liang, Chun-xia Zheng, Zhao-hong Chen, Cai-hong Zeng, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.*

Background: Several recent studies have implicated Toll-like receptor 9 (TLR9) signaling in podocyte injury as TLR9 was found upregulated in podocytes of the patients with lupus nephritis and its expression correlated with treatment outcome (Papadimitraki ED, 2009; Machida H, 2010.); in addition, TLR9 upregulation was also found in podocytes of some patients with polyoma nephropathy, haemolytic uraemic syndrome (Batsford S, 2011), and FSGS (our unpublished observation). To determine if TLR9 signaling mediates podocyte injury, we developed in vitro and in vivo experimental models at first.

Methods: Conditionally-immortalized human podocytes and rats were treated with PAN in vitro and in vivo. Expressions of TLR9, the components and downstream targets of TLR9 pathway, were examined by quantitative RT-PCR, western blotting, and immunohistochemistry. Podocyte injury was evaluated by Annexin V flowcytometry and cytoskeletal alteration.

Results: We show TLR9 and two components of the pathway, IRAK1 and TRAF6, were upregulated at both mRNA and protein levels in the podocytes treated with PAN. Immunohistochemical staining revealed significant upregulations of TLR9, IRAK1 and TRAF6 in the podocytes of PAN-treated rats. To determine if TLR9 signaling is activated in the podocytes treated with PAN, we performed qRT-PCR and found the downstream targets of TLR9 signaling, IL-12, IFN-alpha, and MMP9 were upregulated, suggesting a TLR9 signaling activation. Consistent with previous studies, we also observed that PAN induced both apoptosis and cytoskeletal damage of podocytes, two common consequences found in many podocyte injury models.

Conclusions: PAN-treated podocytes and rats may serve as appropriate in vitro and in vivo models for elucidating the role of TLR9 signaling in podocyte injury. We are examining the role of TLR9 signaling in podocyte injury induced by PAN and seeking the possible CpG ligands and their origins.

SA-PO789

Calcium/Wnt/beta-Catenin Signaling Cascade Mediates Angiotensin II-Induced Podocyte Injury and Albuminuria Lei Jiang, Junwei Yang, Chunsun Dai. *Center of Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Angiotensin II (Ang II) plays a pivotal role in promoting podocyte dysfunction and albuminuria, however, the underline mechanisms is not fully delineated yet.

Methods: Four weeks of continuous osmotic pump administration of Ang II and two weeks of administration of exogenous Ang II by vein injection in Balb/c mice were use for in vivo study. cyclosporine A (CsA) or exogenous Dkk1 were administrated in Ang II treated mice.Cultured mouse podocytes(MPC5) were used in vitro.Dkk1 or beta-catenin siRNA were used to block Wnt/beta-catenin signaling activation. CsA or CK59 were used to block calcium signaling.

Results: In this study, we found that Ang II induced Wnt1 expression and b-catenin nuclear translocation in cultured mouse podocytes. Blocking canonical Wnt signaling with *Dickkopf1* (Dkk1) or beta-catenin siRNA attenuated Ang II induced podocyte injury. Ang II could also activate calcium and its two downstream signaling pathways, including calcineurin/NFAT and Camk II, in cultured podocytes. Blockade of the calcium signaling pathways with cyclosporine A (CsA) or CK59 respectively could significantly inhibit Ang II induced Wnt mRNA expression, b-catenin accumulation and podocyte injury. In *in vivo* studies, administration of Ang II promoted glomerular Wnt/b-catenin signaling activation, podocyte damage and albuminuria in mice. CsA could remarkably ameliorate Ang II induced podocyte damage and albuminuria in mice, which is in parallel with the inhibition of glomerular Wnt1 expression. Indeed, administration of exogenous Dkk1 also attenuated Ang II induced podocyte damage and albuminuria in mice.

Conclusions: Taken together, this study demonstrates that Calcium/Wnt/beta-catenin signaling cascade mediates Angiotensin II-induced podocyte injury and albuminuria, targeting this signaling may offer renal protection against the development and progression of proteinuric kidney diseases.

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SA-PO790

Zonulin, a Novel Regulator of Podocyte Function and Mediator of the Intestinal-Renal Syndrome? Britta Sylvia Walter, Alexis J. Sloan, Saurav Singh, Christian Faul. *Division of Nephrology, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL.*

Background: Zonulin is a 47kDa protein that is secreted by enterocytes, and serves as a paracrine regulator of small intestine permeability. While the physiological function of this reversible process is not well understood, it has been shown that in patients with Celiac Disease (CD) zonulin levels are elevated in responses to a gluten-rich diet, leading to abnormal paracellular transport and diarrhea. Mechanistically, zonulin binds to protease-activated receptor (PAR) 2 on enterocytes leading to the phosphorylation of zonula occludens (ZO)-1 and the transient disassembly of tight junctions (TJ). Since zonulin has also been described to be elevated in the serum of patients with CD as well as other autoimmune diseases, zonulin could function as a potential circulating modulator of TJs in other tissues.

Methods: In order to analyze if zonulin can directly affect the podocyte slit diaphragm, a specialized cell-cell junction that contains TJ-proteins, we used the AT1002 peptide, which resembles zonulin's active PAR2 binding domain.

Results: AT1002 treatment of cultured podocytes caused a rearrangement of the actin cytoskeleton accompanied by decreased cell migration, as well as an increase in tyrosine-phosphorylation of ZO-1. Furthermore, in paracellular flux assays, movement of 70kDa dextran particles across podocyte layers was increased in the presence of AT1002. When isolated rat glomeruli where treated with AT1002, albumin permeability was increased. Interestingly, when glomeruli where incubated with serum from patients with FSGS, the elevation of albumin permeability was significantly reduced in the presence of a zonulin blocking antibody or blocking peptide. Finally, when gluten was delivered repetitively by gavage, mice developed mild proteinuria.

Conclusions: Taken together, our preliminary data indicate that zonulin increases paracellular transport across podocytes and modulates the selectivity of glomerular filtration. Future studies aim to determine serum zonulin levels in FSGS patients, and urine albumin levels in patients with CD.

SA-PO791

Mifepristone Is as Effective as Methylprednisolone as Therapy for Experimental Nephrotic Syndrome Danica Petrovic-djergovic, Milan Popovic, Seetharamaiah Chittiprol, Tad Eichler, Phylip Chen, Richard F. Ransom. *Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: Glucocorticoids (GC) are the primary therapy for nephrotic syndrome (NS), and patient response to GC largely defines their prognosis. Experimental NS induced in rats by puromycin aminonucleoside (PAN-NS) is a well-established model of GC-responsive NS. The elegant transplantation experiments of Hoyer *et al*. demonstrated that the kidney is the site of action of PAN, and, by extension, of GC therapy in PAN-NS.

Methods: Injection of 50 mg/kg PAN in Wistar and nude (*Foxn1*$^{rnu/rnu}$) rats was used to induced PAN-NS, daily injections of 5 mg mifepristone (RU486) or 15 mg methylprednisolone (MP)/kg were used for therapy. Protein assay, qRT-PCR, and EM were by standard methods.

Results: Both Wistar and immunodeficient nude rats responded equally to methylprednisolone therapy by a >60% reduction in proteinuria and podocyte foot process effacement. To our surprise, and in contrast to our previous results in cultured podocytes, the GC receptor (GR) antagonist, RU486, and RU486+MP were as effective as MP at reducing PAN-induced proteinuria and podocyte foot process effacement, and were also effective therapy after development of proteinuria. The efficacy of RU486 and MP was associated with modulationi of mRNA expression of specific genes induced by PAN (*Angptl4*, *Trpc6*, *Cxcl10*), but not of typical GC-induced genes (*Dusp1*, *Tsc22d3*).

Conclusions: Our results demonstrated that mature T-cells were unnecessary for either the development of PAN-NS or the therapeutic effect of GC. The common efficacy of RU486, MP, and RU486+MP in PAN-NS strongly support a common mechanism, and highlight the relevance of specific molecules whose expression is altered during nephrosis and modulated by both MP and RU486. Together, our results support the hypothesis that the kidney is a major site of action of GC therapy in NS, and that RU486 is a specific agonist of the GR for the molecular mechanism(s) responsible for the therapeutic effect of GC in NS. Our findings also raise the novel prospect of the use of RU486 as an alternative or supplement to GC in the therapy of NS.

Funding: NIDDK Support

SA-PO792

Secretion of CXCL10 by Podocytes Coordinates Trafficking of Interferon-γ-Activated Macrophages in Puromycin Aminonucleoside Nephrosis in Rats Danica Petrovic-djergovic, Milan Popovic, Seetharamaiah Chittiprol, Santiago Partida-Sanchez, Richard F. Ransom. *The Research Institute at Nationwide Children's Hospital, Columbus, OH.*

Background: The mechanism responsible for trafficking of activated macrophages into kidney tissue in the puromycin aminonucleoside model of nephrotic syndrome in rats (PAN-NS), and the significance of this infiltration, remain largely unknown. Interferon gamma (IFN-γ) inducible protein 10 (IP-10, CXCL10) is a small chemokine secreted in many Th1-type inflammatory diseases, where it plays an important role in trafficking of monocytes and activated T-cells into sites of tissue inflammation. We hypothesized that induction of circulating IFN-γ and glomerular TNF-α during PAN-NS would stimulate release of CXCL10 by glomerular podocytes, leading to infiltration of activated immune cells and greater glomerular injury.

Methods: PAN-NS was by single injection of 50 mg/kg PAN in Wistar and nude (*Foxn1*$^{rnn/rnu}$) rats, macrophage depletion by clodronate liposomes. Cytokine ELISA, protein assay, qRT-PCR, and monocyte (THP-1) migration were by standard methods.

Results: We found that serum INF-γ, glomerular *Cxcl10* mRNA, and intra- and peri-glomerular infiltration of macrophages, but not T-lymphocytes, were strongly induced during the late acute phase of PAN-NS (12 d after injection) in Wistar rats, but not in nude rats lacking functional effector T-lymphocytes. Wistar rats also developed significantly greater proteinuria than nude rats in the late acute phase of PAN-NS, and this greater proteinuria was abolished by macrophage depletion. Stimulation of cultured podocytes with both IFN-γ and TNF-α markedly induced the expression of *Cxcl10* mRNA and the release of a monocyte chemoattractant identified as CXCL10.

Conclusions: Together, these data provide strong support for our hypothesis that increased circulating IFN-γ and glomerular TNF-α synergistically induce the production and secretion of CXCL10 by podocytes, attracting activated macrophages into kidney tissue. The study also suggests that INF-γ, secreted from Th1 lymphocytes, has an important role in priming of pro-inflammatory macrophages whose presence consequently aggravates renal injury.

Funding: NIDDK Support

SA-PO793

Roles of Na^+/H^+ Exchanger Type 1 and Intracellular pH in Angiotensin II-Induced Reactive Oxygen Species Generation and Podocyte Apoptosis Akira Nishiyama, Hiroyuki Kobori. *Department of Pharmacology, Kagawa University Medical School, Japan.*

Background: A growing body of evidence suggests that podocyte apoptosis is a major cause of decreased podocyte number, which leads to albuminuria and glomerular injury. The aim of this study was to clarify the molecular mechanisms of angiotensin II (Ang II)-induced apoptosis in cultured mouse podocytes.

Methods: We examined the effects of Ang II (100 nmol/L) on apoptosis, superoxide anions, and cytosol pH in podocytes. For intracellular pH measurements, image analysis was conducted using confocal laser microscopy after incubation with carboxy-seminaphthorhodafluor-1.

Results: Superoxide anions and intracellular pH were elevated with Ang II treatment. Apoptotic cell numbers, as measured by TUNEL staining and caspase 3 activity, were also augmented in the Ang II-treated group. Pre-treatment with olmesartan (100 nmol/L, an Ang II type 1 receptor blocker), apocynin (50 μmol/L, NADPH oxidase inhibitor), or 5-N,N hexamethylene amiloride (30 μmol/L, Na^+/H^+ exchanger type 1 [NHE-1] inhibitor) abolished Ang II-induced podocyte apoptosis, whereas *NHE-1* mRNA expression was not affected by Ang II treatment.

Conclusions: These results suggest that superoxide production, NHE-1 activation, and intracellular alkalization were early features prior to apoptosisin Ang II-treated mouse podocytes, and may offer new insights into the mechanisms responsible for Ang II-induced podocyte injury.

Funding: Government Support - Non-U.S.

SA-PO794

Cross-Talk between GR, PPARγ and MAPK Signaling Links COX-2 to Injury and Protection in Podocytes Shipra Agrawal,[1] Adam J. Guess,[1] Ruma Pengal,[1] William E. Smoyer.[1,2] *[1]Clinical & Translational Research, The Research Institute at Nationwide Childrens Hospital, Columbus, OH; [2]Department of Pediatrics, The Ohio State University, Columbus, OH.*

Background: γProteinuria is a common feature of glomerular disease as well as a critical determinant of disease progression. Serum albumin (SA) exposure is known to cause podocyte injury both in vitro and in vivo. Moreover, renal injury has been shown to induce COX-2 in podocytes. However, podocyte protection and signaling are now known to be regulated by nuclear receptors [glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor γ (PPARγ)] and MAPK pathways, and cross-talk among them. We thus hypothesized that COX-2 serves as a molecular link between SA-induced podocyte injury and protection, via cross-talk among the GR-, PPARγ- and MAPK-mediated signaling pathways.

Methods: Podocytes treated with SA were analyzed for COX-2 mRNA and protein expression. In addition, the abilities of glucocorticoids (GCs), thiazolidinediones (TZDs), GR and PPARγ antagonists, as well as MAPK and NFκB inhibitors, to regulate SA-induced COX-2 were also analyzed.

Results: SA was endocytosed by podocytes and induced COX-2 mRNA and protein in a dose- and time-dependent manner. SA-induced COX-2 expression was inhibited by both GCs and TZDs. However, pre-treatment with antagonists for GR (RU486 and CORT108297) and PPARγ (GW9662) prevented the GC-mediated inhibition. Surprisingly, some of these antagonists alone enhanced SA-induced COX-2 expression, suggesting a possible role for basal GR and PPARγ activity in podocyte pathophysiology. Additionally, SA activated the kinases ERK1/2, p38 MAPK, MK2, JNK and NFκB, while NFκB inhibition and inhibitors for these kinases (with the exception of JNK) reduced SA-induced COX-2 expression.

Conclusions: Physiologic concentrations of SA induce podocyte COX-2 expression, which can be inhibited by GCs, TZDs, and select MAPK and NFκB inhibitors. Since proteinuria exposes podocytes to increased concentrations of SA, and GCs, TZDs and MAPK inhibitors are all known to directly protect podocytes, our results suggest that COX-2 may serve as a molecular link between injury and protection of podocytes.

SA-PO795

Genetic Alterations in α-Actinins Modulate Effectors of Podocyte Adhesion and Cytoskeletal Dynamics Fangfang He,[1,3] Hui Chen,[1] Philip A. Bondzie,[1] Julie A. Tomolonis,[1] Martin R. Pollak,[2] Joel M. Henderson.[1] *[1]Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA; [2]Division of Nephrology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [3]Department of Nephrology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.*

Background: We previously demonstrated that genetic alterations in α-actinins are associated with changes in traction force generation and cell motility. The aim of this study is to define the mechanisms by which these genetic alterations contribute to podocyte motility.

Methods: α-actinin-1 and α-actinin-4were ablated in cultured murine podocytes using lentiviral RNA interference. Real time PCR was used to detect vinculin mRNA expression, and Western blot and immunohistochemistry were used to characterize vinculin protein expression and distribution in cultured cells. Small GTPase activities were measured by G-LISA RhoA, Rac1 and Cdc42 activity assays.

Results: Ablation of α-actinin-4 resulted in significant increases in vinculin expression at the mRNA and protein levels, larger focal adhesions, decreased RhoA activity and increased Cdc42 activity. No significant changes in vinculin expression, focal adhesions, or GTPase activities were detected in α-actinin-1-ablated cells.

Conclusions: These findings suggest that alterations in podocyte motility in α-actinin-4-ablated cells are associated with changes in focal adhesions and small GTPase activities. Therefore, changes in podocyte adhesion and small GTPase-mediated cytoskeletal dynamics may play a role in glomerular damage and glomerular barrier dysfunction developing secondary to mutations in α-actinin-4.

Funding: NIDDK Support

SA-PO796

Characterization of Signaling Pathways in Cultured Human Mesangial Cells Induced by IgA1-Containing Immune Complexes from Patients with IgA Nephropathy Zhi Qiang Huang, Joshua Anderson, Timothy D. Rohrbach, Stacy D. Hall, Rhubell T. Brown, Bruce A. Julian, Christopher D. Willey, Jan Novak. *University of Alabama at Birmingham, Birmingham, AL.*

Background: In patients with IgA nephropathy (IgAN), galactose-deficient IgA1 (Gd-IgA1) and anti-glycan antibodies form immune complexes (IC) that activate mesangial cells (MC) and induce cellular proliferation. MC activation includes changes in tyrosine kinase (TK) activity, but the specific pathways induced by the Gd-IgA1-containing IC are unknown.

Methods: Cultured human primary kidney MC were stimulated with native IC isolated from sera of IgAN patients or stimulated with *in vi*tro-formed IC using recombinant IgG antibody specific for Gd-IgA1. Cell lysates were preliminarily analyzed by SDS-PAGE/Western blotting using anti-phospho-tyrosine antibody. Specific TK activities in the samples were then determined with a PamStation® 12 high-content peptide substrate microarray. Data were analyzed using BioNavigator to identify peptides significantly altered in phosphorylation, and GeneGo Metacore to identify altered signal-transduction pathways important in IC-mediated activation of MC. MC proliferation was also assessed.

Results: MC proliferation was induced by both native and *in vitro*-formed IgA1-containing ICs. Kinomic profiling showed that phosphorylation of fourteen peptides was significantly altered in the ICs-stimulated MC. Further analysis using a Drug Targeting Network identified ICs-altered TK-inhibitor targetable signaling pathways, including PDGF/PDGFR, c-Kit, and c-Raf pathways. Direct Interaction mapping identified altered signaling in the JAK1, JAK2, FAK, PYK2, PLC-g as well. Enhanced phosphorylation and cell proliferation were blocked by kinase inhibitors targeting some of these pathways.

Conclusions: Gd-IgA1-containing ICs activated multiple signaling pathways in MC and led to cellular proliferation. Inhibition of TK signaling using small molecule inhibitors prevented these changes. Thus, IC-mediated TK pathways in MC represent targets for developing new disease-specific treatment.

Funding: NIDDK Support

SA-PO797

Klotho Is a Target of PPAR-γ and Protective against Cellular Hypertrophy and Apoptosis in High Glucose-Stimulated Glomerular Cells Hye-young Kang,[1] Seong Hun Kim,[1] Dae-Suk Han,[2] Shin-Wook Kang,[1,2] Seung Hyeok Han.[2] *[1]Brain Korea 21, Yonsei University; [2]Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Republic of Korea.*

Background: Klotho is known as an anti-aging hormone that has anti-oxidative and anti-senescence effects. In this study, we investigated whether klotho can play a protective role in high glucose (HG)-stimulated glomerular cells.

Methods: The mRNA and protein expression of klotho and PPAR-γ were examined in mouse mesangial cells and podocytes exposed to 5 mM (NG) and 30 mM glucose (HG) by real-time PCR (RT-PCR) and Western blot, respectively. To explore whether klotho expression was determined by PPAR-γ, we evaluated klotho expression in glomerular cells treated with PPAR-γ agonist (pioglitazone, 50 μM) or antagonist (GW9662, 20 μM), and performed a chromatin-immunoprecipitation (ChiP) assay. The effects of klotho on cell cycle regulation and apoptosis were also elucidated in these cells.

Results: The presence of klotho gene was confirmed in glomerular cells using RT-PCR. Immunofluorescence staining revealed that klotho was mainly expressed in cytoplasm of these cells. The protein expression of klotho was concordant with that of PPAR-γ, which was increased up to 24 hours and decreased at 48 hours in HG-stimulated glomerular cells. In addition, klotho protein expression was increased by PPAR-γ agonist and decreased by PPAR-γ antagonist. The ChiP assay found PPAR-γ responsive element in the 5'-flanking region of the klotho gene. Administration of recombinant klotho (rKL, 1 μg/ml) induced a decrease in G0/G1 phase cells and increases in S and G2 phases cells in HG-stimulated glomerular cells. The expression of $p21^{Clip1}$, $p27^{Kip1}$, cleaved caspase-3, and the ratio of Bax/Bcl-2 was also significantly increased in these cells. rKL significantly attenuated the increased expression of these proteins, whereas klotho siRNA further increased these expression.

Conclusions: Klotho was a target of PPAR-γ in glomerular cells and ameliorated cellular hypertrophy and apoptosis in HG-stimulated glomerular cells, suggesting that klotho may exert a protective effect in glomerular cells under diabetic conditions.

SA-PO798

Inhibition of Notch Pathway Protects the Mice from Human Immunodeficiency Virus Associated Nephropathy Madhulika Sharma,[1] Lynn Magenheimer,[1] Carol G. Carlton,[1] Deborah P. Hyink,[2] Pravin C. Singhal,[3] Paul E. Klotman,[2] Timothy A. Fields,[1] Gregory B. Vanden Heuvel.[1] *[1]University of Kansas Medical Center, Kansas, KS; [2]Baylor College of Medicine, Houston, TX; [3]Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

Background: Notch signaling is activated when Notch receptors (Notch 1-4) bind Jagged and Delta ligands and induce cleavage of Notch intracellular (IC) domain. The Notch IC then translocates to the nucleus, binds to RBP-jk protein and activates transcription of *Hes* and *Hey* genes. Human Immunodeficiency Virus Associated Nephropathy (HIVAN) is characterized by podocyte hyperplasia and glomerular collapse. Here we show that Notch pathway is activated in Tg26 mouse model of HIVAN and Notch inhibition protects these mice from disease progression.

Methods: Notch pathway expression analysis was performed in Tg26 mice. These mice were treated with GSIXX (Notch inhibitor) and disease progression was evaluated. Immortal differentiated podocytes were treated with HIV proteins/ GSIXX and effects on cell proliferation were evaluated.

Results: Notch1 IC, Notch4 IC and Hes1 were all upregulated in the glomeruli. Of these, Notch4 IC expression was most robust and co-localized with the podocyte marker synaptopodin and the proliferation marker (Ki-67). Among ligands, glomeruli showed expression of Jagged2 only. However, tubules showed elevated expression of Jagged1, Jagged2, Delta-like1, and Delta-like4. We treated six week-old Tg26 mice with GSIXX for nine days, and evaluated disease severity. Strikingly, GSIXX treatment resulted in significant improvement in both histologic kidney injury scores and renal function. GSIXX-treated Tg26 mice showed diminished podocyte proliferation and dedifferentiation. Moreover, *in vitro* studies revealed that Notch inhibitors can block podocyte proliferation induced by HIV proteins Nef and Tat.

Conclusions: These studies suggest that Notch signaling, particularly Notch4 IC, may promote collapsing glomerulopathy and HIVAN progression. Thus, Notch inhibition may be a viable treatment strategy for HIVAN.

Funding: Private Foundation Support

SA-PO799

Retinoids Augment the Number of Glomerular Epithelial Transition Cells in Experimental Glomerular Diseases Jiong Zhang,[1] Jeffrey W. Pippin,[1] Ronald D. Krofft,[1] Yoshinori Taniguchi,[1] Paola Romagnani,[2] Peter J. Nelson,[1] Zhi-Hong Liu,[3] Stuart J. Shankland.[1] *[1]University of Washington, Seattle, WA; [2]University of Florence, Italy; [3]Nanjing University School of Medicine, China.*

Background: Reduced podocyte number underlies glomerulosclerosis in membranous nephropathy (MN) and FSGS. Parietal epithelial cells (PEC) that begin to express proteins considered unique to podocytes, called glomerular epithelial transition cells (GETC), might serve as podocyte progenitors. We tested the hypothesis that retinoids improve outcomes in glomerular diseases characterized by reduced podocyte number in part by increasing the number GETC.

Methods: Rats with experimental MN (PHN model) and mice with experimental FSGS (anti-glomerular antibody model) were given all-trans retinoic acid (ATRA) or DMSO (control) following the onset of proteinuria. GETC were defined as cells co-staining for both PAX2 (PEC marker) and WT-1 (podocyte marker).

Results: In MN rats given ATRA (5mg/kg IP, 5x a week until sacrifice), there was a significant increase in the number of GETC lining Bowman's basement membrane (BBM) compared to DMSO at d13 (9.1±0.9/mm of BBM *vs.* 4.0±0.5/mm of BBM, $p<0.01$), d35 (8.7±0.9/mm *vs.* 5.5±0.6/mm, $p<0.01$), and d110 (6.1±0.7/mm *vs.* 0.4±0.1/mm, $p<0.01$). In MN rats, ATRA also increased the number of GETC lining the GBM at d13 (185±36.8/mm^2 of tuft *vs.* 26.4±7.9/mm^2 of tuft, $p<0.01$), and d35 (367±62.8/mm^2 *vs.* 75.2±16.7/mm^2, $p<0.01$). These changes were accompanied by increased podocyte number at d110 in ATRA treated MN rats (1592±84.5/mm^2 *vs.* 962±54.8/mm^2, $p<0.01$ *vs.* DMSO). In FSGS mice, ATRA (16mg/kg IP daily until sacrifice) increased the number of GETC lining Bowman's capsule at d14 (7.5±0.8/mm of BBM *vs.* 1.3±0.5/mm of BBM, $p<0.01$), and along the GBM at d7 (44.1±20.4/mm^2 of tuft *vs.* 11.8±11.8/mm^2 of tuft, $p<0.05$), and d14 (48.8±23.3/mm^2 *vs.* 13.5±13.5/mm^2, $p<0.05$).

Conclusions: In summary, these data show that the normalization of podocyte number by retinoids in experimental MN and FSGS is accompanied by an increase the number GETC. The latter might serve as a podocyte progenitor population to enhance overall glomerular repair in diseases characterized by reduced podocyte number.

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SA-PO800

Podocyte-Specific Constitutive Activation of CaMKII in Mice Results in Reversible Proteinuria and Foot Process Effacement Saurav Singh,[1] Britta Sylvia Walter,[1] Alexis J. Sloan,[1] Ansel P. Amaral,[1] Christian Faul.[2] *[1]Molecular Cell and Developmental Biology, University of Miami Miller School of Medicine, Miami, FL; [2]Department of Medicine, University of Miami Miller School of Medicine, Miami, FL.*

Background: Ca2+ is an important second messenger in podocyte foot processes (FP), and mounting evidence has revealed alterations in Ca2+-mediated signal transduction in podocyte pathophysiology. For example, gain-of-function mutations in TRCP6 calcium channels can cause changes in proper podocyte morphology and glomerular filteration leading to the development of proteinuria. Additionally, elevations of intracellular calcium levels in podocytes can occur under other non-genetic insults such as increases in blood pressure, bacterial infection, and trauma. Calcium/calmodulin dependent kinase II (CaMKII) is key mediator of Ca2+ signaling in various cell types. CaMKII can directly modulate actin fibers, as reported for hippocampal neurons and the regulation of synaptic plasticity, as well as control gene expression via the regulation of histone acetylation as shown in cardiac myocytes. This dual role of CaMKII in the regulation of actin dynamics and epigenetic changes makes it an interesting candidate to function as a key regulator of podocyte morphology and function in health and disease.

Methods: In order to study CaMKII function in vivo, we have generated an inducible transgenic mouse model for the podocyte-specific expression of a constitutive active CaMKII-alpha mutant form.

Results: Podocytes express various isoforms of CaMKII, and the pharmacological inhibition of CaMKII in cultured podocytes induces the degradation of the actin-binding protein synaptopodin, and a loss of actin stress fibers. Transgenic CaMKII mice develop profound proteinuria and podocyte FP effacement, and the proteinuria regresses within 3 months, indicating the activation of compensatory processes.

Conclusions: Our novel data indicate that CaMKII is an important signal mediator in podocytes, and that its inhibition or overactivation can have pathological effects. Future studies aim to determine the precise molecular events underlying CaMKII function in podocytes.

SA-PO801

Active Proteases in Nephrotic Plasma Lead to a Podocin Dependent Phosphorylation of VASP in Podocytes via Protease Activated Receptor-1 Hugh J. McCarthy, Gavin Iain Welsh, Moin Saleem, Jessica Harris. *Academic Renal Unit, University of Bristol, Bristol, United Kingdom.*

Background: Focal Segmental Glomerulosclerosis (FSGS) is known to result in dramatic changes in actin cytoskeleton and injury to the podocyte cell. Post-transplant recurrence of FSGS exemplifies disease due to prescence or absence of a plasma factor(s). There is increasing indication that plasma protease activity may be key.

Methods: Utilising plasma exchange material collected from patients with active post-transplant recurrence of FSGS paired with samples from the same patients in remission the effect on human conditionally immortalized podocytes (ciPods) was analysed.

Results: We show that vasodilator stimulated phosphoprotein (VASP) is phosphorylated in response to relapse plasma from 12 consecutively tested patients, and not in response to paired remission plasma or non-FSGS controls. The phosphorylation signal is absent in human podocytes carrying a pathological podocin mutation. To test for a plasma ligand, inhibition of proteases in relapse plasma leads to the loss of VASP phosphorylation. By the use of siRNA technology we show that proteases in the plasma signal predominantly via protease activated receptor 1 (PAR1) to VASP. Mechanistically FSGS plasma increases podocyte motility, which is dependent on VASP phosphorylation.

Conclusions: These data suggest a specific biomarker for disease activity, as well as revealing a novel and highly specific receptor mediated signaling pathway to the actin cytoskeleton.

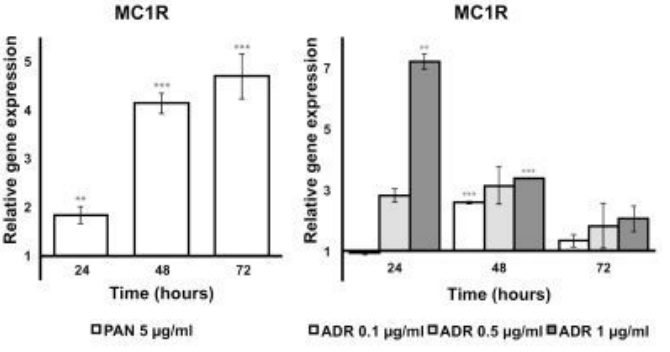
SA-PO802

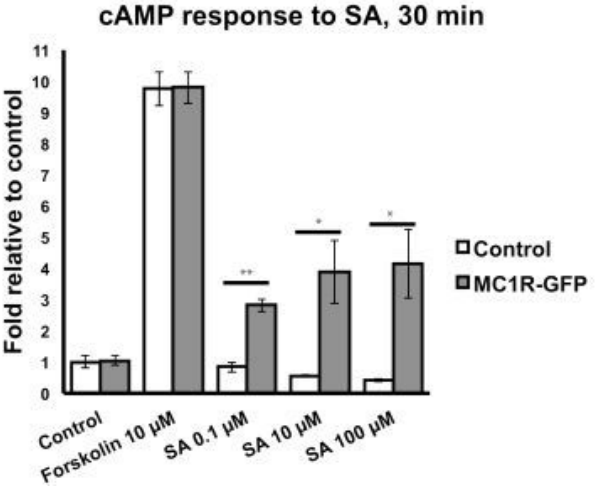
The Melanocortin Receptor 1: A Potential Target for Therapeutical Drugs in Nephrotic Syndrome Johannes Elvin,[1] Annika Lindskog Jonsson,[1] Lisa Maria Buvall,[2] Anna Granqvist,[1] Jenny C. Nystrom,[3] Borje Haraldsson.[1] *[1]Department of Molecular and Clinical Medicine, The Sahlgrenska Academy, University of Gothenburg, Sweden; [2]Department of Nephrology, Massachusetts General Hospital & Harvard Medical School, Boston, MA; [3]Department of Physiology, The Sahlgrenska Academy, University of Gothenburg, Sweden.*

Background: Previously, we have shown that treatment with adrenocorticotropic hormone (ACTH) and agonists targeting the melanocortin receptor 1 (MC1R) reduce proteinuria, improve glomerular morphology and reduce oxidative stress. We propose that MC1R is a potential therapeutic target in renal disease and our aim with this study was to further explore the mechanisms behind MC1R stimulation in vitro.

Methods: In these experiments, we used conditionally immortalized mouse podocytes. The cells were subjected to puromycin aminonucleoside (PAN) or adriamycin (ADR) at different time points. The cDNA was analysed with qPCR and the MC1R gene expression levels were examined. Human MC1R was over-expressed in the podocytes, a novel and Selective Agonist (SA) for MC1R was added and the intracellular cAMP was analysed at different time points in a functional assay.

Results: The native MC1R gene expression is low but stable in podocytes, and we found that both PAN and ADR treatment significantly increased the expression in a dose dependent manner. The effect of the SA against podocytes over-expressing human MC1R was confirmed by a significant increase in intracellular cAMP. MC1R exhibited a high cAMP response to the SA.





Conclusions: The MC1R expression level in mouse podocytes is low, but the up-regulation observed suggests that MC1R is involved in the response to pathological stress. Adding to earlier findings with ACTH, MC1R is proposed to be protective against podocyte-specific renal injury when stimulated with an agonist in a cAMP dependent pathway.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO803

EPLIN (Epithelial Protein Lost in Neoplasm) Interacts with Paxillin and Regulates Mesangial Migration

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Background: Migration of mesangial cells is crucial during glomerular injury or glomerular development. Directional migration requires coordinated actin dynamics and turnover of focal adhesion proteins. The mechanisms how mesangial migration is regulated in mesangial proliferative diseases are still unknown.

The objective was to determine regulatory mechanisms underlying cytoskeletal alteration in migration of mesangial cells.

Methods: During the search for actin regulatory proteins expressed in mesangial cells, we found that EPLIN was highly expre--ssed in rodent and human mesangial cells. We analyzed expression of EPLIN in cultured mesangial cells and in experimental glomerulonephritis. We also investigated its role in mesangial cell proliferation and migration by siRNA-mediated depletion of EPLIN.

Results: By immunoelectron microscopy, EPLIN was localized at the edge of mesangial processes and mesangial angles where they are attached to the GBM. In cultured mesangial cells, EPLIN was colocalized with peripheral actin bundles and paxillin, focal adhesion protein. Immunoprecipiation experiments revealed the interaction between EPLIN and paxillin. By PDGF treatment, localization of EPLIN at focal adhesion was reduced and translocated to peripheral ruffles. Expression of EPLIN was dramatically decreased in Thy-1.1 nephritic rat model. Knockdown of EPLIN facilitated PDGF-induced focal adhesion disassembly and promoted PDGF-induced mesangial cell migration.

Conclusions: We found EPLIN inhibits the migration of mesangial cells possibly by stabilizing focal adhesion complex. These observations shed light on coordinated actin remodeling in the mesangial cells in restorative remodeling processes. Alteration of expression and localization of cytoskeletal regulators underlies phenotypical change of mesangial cells during mesangial injury.

SA-PO804

COL4A3$^{+/-}$/Nphs2$^{+/R140Q}$ Mice: Glomerular Basement Membrane and Slit Diaphragm Interactions with Impact on Podocyte Pathology and Kidney Fibrosis

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Background: Homozygous mutations of the glomerular basement membrane (GBM) collagen IV alpha 3/4/5 (Col4), as well as the slit diaphragm protein podocin have a strong impact on podocyte pathology and renal failure. However, heterozygous patients develop late, if any, changes. Interestingly, patients with double-heterozygous mutations for Col4 and podocin developed early ESRF. Therefore, we hypothesized that an interaction exists between the podocytes, slit diaphragm and GBM-matrix, which is important for maintenance of podocyte structure and function and development of kidney fibrosis.

Methods: COL4A3$^{+/-}$/Nphs2$^{+/R140Q}$ (mutant), respective heterozygous single knockouts (sKO) and wildtype (WT) mice were investigated at different ages using light and electron microscopy, as well as immunohistological and western blot technique.

Results: No difference in lifespan was revealed between mutants, sKO and WT. In mutants, urine analysis showed an age-dependent increased loss of high molecular weight proteins. Profibrotic markers were elevated as well. Light microscopically, laminin-staining did not reveal strong matrix deposition. However, podocyte effacement and moderate tubulointerstitial fibrosis could be detected in mutants using electron microscopy.

Conclusions: We here introduce a new mouse model, to study GBM–podocyte–slit diaphragm-interactions. Compared to the healthy phenotype of heterozygous single knockout mice, the double heterozygous mice showed moderate changes of kidney pathology, like effacement of podocytes, tubulointerstitial fibrosis and proteinuria. We assume a link between GBM and slit diaphragm that might influence podocyte structure. Consequently, patients with heterozygous Col4 mutations who usually present with late changes in kidney function, aggravate earlier due to additional heterozygous podocin mutations. Finding the key players in these interactions could lead to a better understanding of fibrogenesis and new therapeutic targets for common glomerular diseases.

SA-PO805

Small Dense Low-Density Lipoprotein Cholesterol, and Its Clinical Features in Patients with Nephrotic Syndrome

Mayumi Miyamoto,[1] Hidenori Yamazaki,[1] Fumihiro Tomoda,[1] Tsutomu Koike,[1] Satoshi Kagitani,[1] Taizo Nakagawa,[1] Hiroshi Inoue,[1] Masataka Takiwaki,[2] Yoshinori Uji,[2] Isao Kitajima.[2] *[1]The Second Department of Internal Medicine, University of Toyama, Toyama, Japan; [2]The Department of Clinical Laboratory and Molecular Pathology, University of Toyama, Toyama, Japan.*

Background: Nephrotic syndrome (NS) is commonly complicated with hyperlipidemia. Recently, small dense low-density lipoprotein cholesterol (sdLDL-C) has been highlighted as the most harmful lipoprotein on the cardiovascular system. In this study, the associations of sdLDL-C and other lipid parameters to urinary proteinuria and oxidative stress were investigated in patients with NS.

Methods: Fourteen patients with NS (NS group) and 28 age-matched healthy subjects (control group) were enrolled into the study. NS was due to minimal change disease (MCD) in 7 patients and membranous nephropathy (MN) in the remainders. Plasma levels of sdLDL-C (using assay kit supplied by Denka Seiken Co., Ltd., Niigata, Japan), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) were measured. Urinary protein excretion, eGFR and plasma 8-hydroxy-2'-deoxyguanosine (8-OHdG, i.e. a marker for oxidative stress) were also evaluated.

Results: sdLDL-C as well as LDL-C and TG were significantly greater in NS than in control group (69±12, 198±21, 219±37 mg/dL in NS vs 18±1, 63±4, 76±6 mg/dL in control) despite no difference of HDL-C among both groups. In NS group, LDL-C positively correlated with urinary protein excretion ($r= 0.623$, $p< 0.05$), but not with eGFR. Additionally, ratio of sdLDL-C to LDL correlated positively with plasma 8-OHdG ($r= 0.152$, $p< 0.05$), although LDL and sdLDL-C did not associate with it. In contrast, TG and HDL-C did not relate with urinary protein excretion or plasma 8-OHdG in NS group. There was no difference of eGFR, proteinuria and lipid profiles between the patients with MCD and MN.

Conclusions: These results indicated that in NS group, (1) sdLDL-C elevates together with the other lipids and (2) Increased LDL and elevated fraction of sdLDL-C within LDL-C could lead to the aggravations of proteinuria and oxidative stress, respectively.

SA-PO806

Analysis of Gene *SOCS3* and *SOCS5* Sequencing in Children with Steroid-Resistant Nephrotic Syndrome

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Background: The strong evidence of the immunologic factors in the pathogenesis of idiopathic nephrotic syndrome (INS) is known. Glucocorticoids (GCs) are generally applied and highly effective drugs in most cases of INS and steroid responsiveness is the major determinant of prognosis of the disease. Approximately 85-90% of patients with INS respond to glucocorticoid treatment, but 10-15% have partial or no response to GC. Children with SRNS (steroid resistant nephrotic syndrome) progress to end-stage renal failure. There is evidence that either in SSNS or SRNS group of patients, regardless of histological diagnosis, several elements of the Jak/Stat signaling pathway are being excited. After 6-week glucocorticoid treatment expression of all analyzed elements in the SSNS group is back to the level compared to the control group, whereas in the SRNS group there are still high levels of only two elements – *SOCS3* and *SOCS5* transcripts, coding for suppressor proteins of the Jak/Stat pathway. Normal and increased transcription of these genes correlated with absence or presence of proteinuria.

Methods: Sequencing analysis of coding regions of genes *SOCS3* and *SOCS5* was conducted in three groups: *study group 1* counting 40 SRNS patients, *study group 2* counting 30 SSNS patients and *control group* of 30 healthy individuals. All results were establish in terms of Hardy-Weinberg Equilibrium. Odds ratio value (OR) was based on frequencies of alleles and genotypes and SNP (single nucleotide polymorphism) analysis was provided with gene structure based on Haploview software.

Results: We found eight SNP within *SOCS1*, 6 SNP in *SOCS3* and 18 SNP in *SOCS5* (heterozygotic: 2, 1 and 6).

Conclusions: The results showed no significant differences in OR value among the three groups, although *SOCS5* gene structure showed weaker linkage between heterozygous SNPs in SRNS group than in SSNS and control group.

Funding: Government Support - Non-U.S.

SA-PO807

Urinary Proteins Can Be Reliably Measured after Five Years of Frozen Storage

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Background: Little is known about the effects of prolonged frozen storage (>1 year) on the stability of urinary biomarkers. We investigated the recovery of various urinary proteins after prolonged storage at -80°C.

Methods: We used urine samples from a mixed population of 30 adult patients with glomerular diseases or tubulo-interstitial nephritis. All urine samples had a pH >6.0 after premedication with sodium bicarbonate, and bovine serum albumin was added prior to

storage. The fresh measurements were performed on the same day as collection. Analyses of urine biomarkers were performed after a median storage of 5.0 years (range 2.1 - 9.0 years) at -80°C in the same samples.

Results: Median proteinuria in fresh urine samples was 4.5 g/10 mmol creatinine (IQR 2.2 – 9.6 g/10 mmol). Sodium quantification was highly reproducible, indicating that the storage procedures were adequate and not affected by evaporation (Table). There was a strong correlation between repeated measurements. After correction for interassay variation from quality control pools, the large majority of measured protein concentrations were within 99% confidence limits.

Conclusions: The current set of urinary protein concentrations were stable after prolonged storage at -80°C.

Urinary markers at fresh measurement and after frozen storage

	Fresh	After storage at -80°C	Coefficient of determination	Overall (inter)-assay variation (CV%)	Samples with difference within 3 CV (%)
Sodium (mmol/l)	60 (40-74)	60 (41-74)	0.99	0.7	68
IgG (mg/l)	69 (19-121)	67 (20-120)	0.99	4.5	93
Transferrin (mg/l)	72 (24-190)	63 (29-187)	0.98	4.7	93
Alpha 1-microglobulin (mg/l)	37 (16-70)	36 (16-71)	0.98	3.4	83
Beta 2-microglobulin (mg/l)	1.3 (0.3-7.4)	1.5 (0.4-6.2)	0.98	5.8	85

Concentration expressed as median (IQR)

SA-PO808

Higher Precision of Urinary Protein/Creatinine Ratio Compared with 24 Hours Protein Excretion in Kidney Diseases Gianluigi Ardissino, Sara Testa, Francesca Tel, Stefani Rotondo, Luciano Sangaletti, Paolo Vercelloni, Luisa Napolitano, Marieclaire Allaz, Nora Bettinardi. *Center for HUS Control, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Background: Proteinuria, an important biomarker in all nephropathies, is commonly measured as urinary protein excretion on 24-hour timed collection (PE24hrC) a time consuming, burdensome, often unreliable method. In the pediatric setting, the urinary protein-over-creatinine ratio (uPr/uCr) on spot samples is widely employed as surrogate marker of PE24hrC but, to the best of our knowledge, the reliability of uPr/uCr has only been compared with PE24hrC assuming the latter as a gold standard rather then analysing respective precision and accuracy. The present study compares the precision (degree to which repeated measurements under unchanged conditions show the same results) of the two methods of assessing proteinuria (PE24hrC vs uPr/uCr) in a selected group of patients with stable renal disease with the working hypothesis that the most precise method is the one with lowest coefficient of variation (CV).

Methods: Ten patients with longstanding and well documented stable renal disease (hypodisplasia, FSGS and HUS in remission) were provided written instruction on how to perform 4 sets of urine sampling within a short period of time. Each set included a 24hrC and 4 urine samples collected any time during the day in different days across the urine collection (total of 120 determinations). The mean CV of proteinuria was calculated for the PE24hrC and for uPr/uCr as single sample as well as for the mean of 2, 3 and 4 samples assuming that, in patients with stable disease, the CV of proteinuria, with any method, should approach 0, by definition.

Results: The mean CV for PE24hrC was 31.1 while for uPr/uCr it was 29.4, 29.4, 25.5 and 22.7% for 1, 2, 3 and 4 sampling, respectively.

Conclusions: The best method for assessing proteinuria in patients with kidney diseases, as to precision, is uPr/uCr and, even on a single sample it has a higher precision than PE24hrC. However the mean of 4 samples has the lowest CV and therefore the highest precision.

Funding: Private Foundation Support

SA-PO809

The Importance of Screening for Proteinuria in Women of Childbearing Age Jwa-kyung Kim, Jisuk Han, Myung Jin Choi, Sung Gyun Kim. *Internal Medicine, Hallym University Sacred Heart Hospital, Kidney Research Institute, Republic of Korea.*

Background: Detection of protein in the urine, even relatively small amount, can be early sign of kidney damage and often precede a detectable decline in glomerular filtration rate (GFR). Particularly, proteinuria in young-aged women has greater significance because of the possibility for pregnancy. The purpose of this study is to evaluate the prevalence and diagnosis of proteinuria in childbearing-aged women.

Methods: A total of 10,385 Korean women between 20 and 39 years of age, who received health examination from January 2011 to December 2011 were surveyed. Patient were tested for proteinuria with dipstick (-,±,1+,2+,or 3+) and abnormal level of proteinuria was defined as 1+ or greater. Persistent proteinuria was established by confirming proteinuria on subsequent test a week or two after the initial test. In addition, the histopathologic types of kidney diseases in the young-aged women were also evaluated using the renal biopsy data in the ProgressiveREnal disease and Medical Informatics and gEnomics Research (PREMIER) program supported by the Korean Society of Nephrology.

Results: At initial test, abnormal proteinuria was detected in 227 (2.2%) patients: 1+ (184,1.8%), 2+ (40,0.4%) and 3+ (3,0.03%). Among these, 59 (26.0%) patients had repeated testing, and 33 (55.9%) of them showed positive results. Therefore, about 1.2% of patients showed persistent proteinuria. Patients with persistent proteinuria had higher body mass index, lower GFR, and elevated fasting glucose than those with transient proteinuria. However, further diagnostic work-up was performed only about half of the patients with persistent proteinuria (18/33,54.5%). According to the PREMIER data, definite difference in the histopathologic lesions was observed between young-aged women (20–40years) and old-aged women (>40years): the incidence of IgA nephropathy and lupus nephritis was significantly higher, but that of crescentric glomerulonephritis, membranous nephropathy, and diabetic nephropathy was significantly lower.

Conclusions: Approximately 1.2% of women in childbearing age have persistent proteinuria. Specific attention and consultation for further diagnostic work-up is needed.

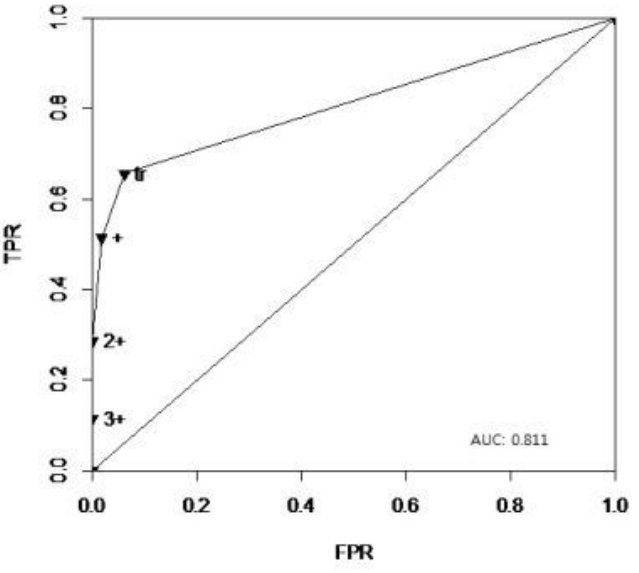
SA-PO810

How to Interpret Trace Result in Urine Dipstick Test for Proteinuria? Beom Kim, Dongyoung Lee, Hye Won Kim, Joong Il Park, Kyoung Hyoub Moon, Soohyun Yang. *Internal Medicine, Veterans Health Service Medical Center, Seoul, Kuwait.*

Background: Urine dipstick test(DIP) is often used for screening for proteinuria owing to simplicity. But it is uncertain we should regard trace result as positive for overt proteinuria. We compared urine dipstick test to spot urine protein to creatinine ratio(PCR) to investigate how to interpret trace result.

Methods: We extracted DIP and PCR results from medical record of Korean Vietnam war veterans who applied for medical check checkup for agent orange poisoning. Regarding PCR as true value, we calculated sensitivity(SN) and specificity(SP) for DIP(cutoff: trace, 1+, 2+). We used KDIGO guideline for PCR result interpretation.

Results: Two hundred sixteen laboratory results were available from as many patients(M: 215). Their median age was 65(61~80). Positive PCR was shown in 6.6%(12/182) of negative DIP, 38.5%(5/13) in trace DIP, 72.7%(8/11) in 1+ DIP and 100%(10/10) in 2+ and 3+ DIP. SN and SP for trace cutoff were 65.7, 93.9%, for 1+ cutoff were 51.4, 98.3%, for 2+ cutoff 28.6, 100%. Area under the curve in ROC curve was 0.811.



Conclusions: To increase SN for proteinuria, it is advisable to regard trace results as positive for proteinuria in mass screening.

SA-PO811

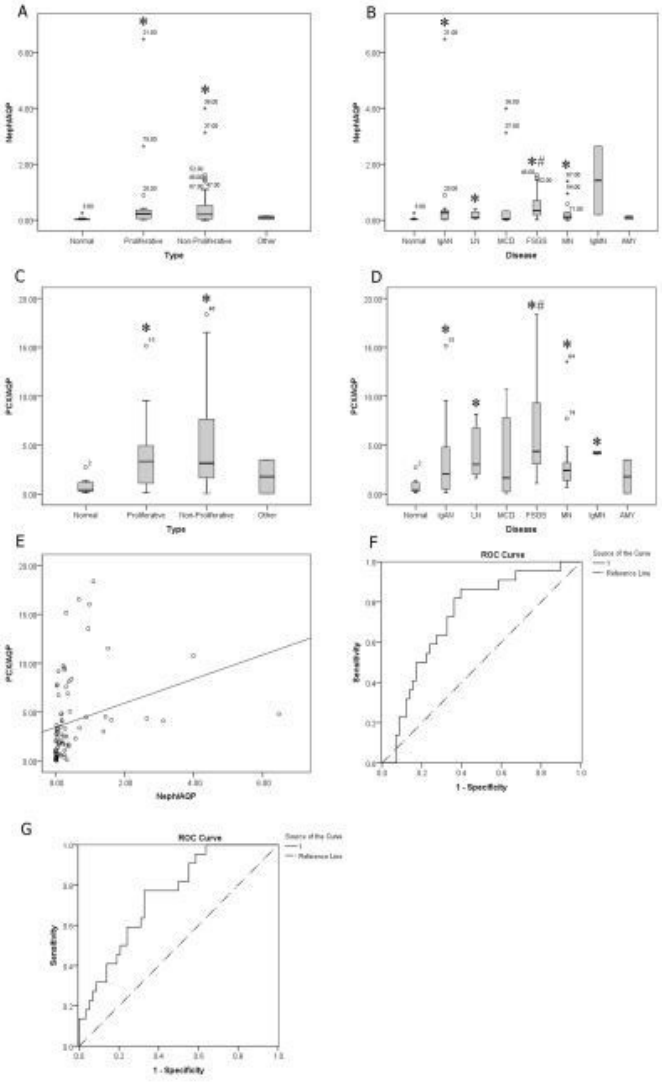
ELISA Analysis of Urinary Nephrin and Podocalyxin Standardized by Aquaporin-2 in the Adult Patients with Nephrotic Syndrome Bin Zhu, Xiaoling Zhu, Yi Lin, Caifeng Zhu, Xiao-xia Cheng, Hongyu Chen, Yongjun Wang. *Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, China.*

Background: To investigate the urinary nephrin and podocalyxin excretion standardized by AQP-2 with ELISA method in the adult nephrotic syndrome patients.

Methods: 71 adult nephrotic syndrome patients including 20 proliferative nephritis patients(13 IgAN, five lupus nephritis, two IgMN), 49 non-proliferative patients(11 MCD, 22 FSGS, 16 MN) and the other two amyloidosis patients. Ten healthy persons were enrolled as the control group. Urinary nephrin, podocalyxin and AQP-2 were measured using the ELISA method. Urinary nephrin and podocalyxin were standardized by AQP-2 (neph/AQP and PCX/AQP).

Results: Urinary neph/AQP correlated positively to PCX/AQP ($r=0.616$, $P<0.001$,Figure 1 E.). Urinary neph/AQP and PCX/AQP were lowest in the normal persons as compared with the nephrotic syndrome patients(Figure 1 A-D). Both proliferative and non-proliferative nephrotic syndrome patients excreted more urinary neph/AQP and PCX/AQP without a significant difference between them ($P>0.05$, Figure 1 A,C). FSGS patients excreted higher neph/AQP and PCX/AQP in the urine than those in the other patients ($P<0.05$,Figure 1 B,D). For the diagnosis of FSGS, a ROC curve analysis indicated that

the sensitivity value is 0.864 while the specificity value is 0.603 when the urinary neph/AQP borderline value was 0.165, and the sensitivity value is 0.773 while the specificity value is 0.672 when the urinary PCX/AQP borderline value was 3.056(Figure 1 F,G).



A.Urinary neph/AQP among proliferative, non-proliferative and renal amyloidosis patients.*: P<0.05, vs. Normal.
B. Urinary neph/AQP among different nephrotic syndrome patients. *: P<0.05, vs. Normal; #: P<0.05, VS MN patients.
C.Urinary PCX/AQP among proliferative, non-proliferative and renal amyloidosis patients.*: P <0.05, vs. Normal.
D. Urinary PCX/AQP among different nephrotic syndrome patients. *: P <0.05, vs. Normal; #: P <0.05, VS MN patients.
E. Urinary neph/AQP correlated positively to urinary PCX/AQP.
F. ROC curve analysis of neph/AQP for the diagnosis of FSGS.
G. ROC curve analysis of PCX/AQP for the diagnosis of FSGS.

Conclusions: Urinary neph/AQP and PCX/AQP by an ELISA method were both significantly increased in the proliferative and the non-proliferative glomerulonephritis patients. FSGS patients excreted higher neph/AQP and PCX/AQP. It is also possible to distinguish FSGS patients from other nephrotic syndrome patients using this method.

Funding: Government Support - Non-U.S.

SA-PO812

Validation of AcSDKP-NH2 as a Non Radioactive Peptidic Marker of Glomerular Filtration Rate in Humans

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[1]Clinical Investigation Center, Assistance Publique - Hôpitaux de Paris, Paris, France; [2]Renal Physiology, Assistance Publique - Hôpitaux de Paris, Paris, France; [3]CEA, CEA Valrho, Bagnols Sur Ceze.

Background: The natural hemoregulatory peptide AcSDKP is degraded by angiotensin I converting enzyme (ACE) and eliminated in the urine by glomerular filtration in humans. We have shown that 1) the amidated form of AcSDKP (AcSDKP-NH_2) is not hydrolysed by murine or human ACE *in vitro* and, 2) measurements of the urinary clearance of AcSDKP-NH_2 and of tritiated inulin in rats give identical results, suggesting that AcSDKP-NH_2 could be used as a new GFR marker (patent N°: WO2004/096292 ;US2008/0199397A1). The objective is to validate AcSDKP-NH_2 as a GFR marker in humans.

Methods: We performed an open label equivalence phase I study where AcSDKP-NH_2 (bolus 0.4 µg/kg, 0.2 µgmin/100 mL eGFR) and inulin (bolus 30 mg/Kg, 15 mg/min/100 mL eGFR) were simultaneously infused during 4h15 to 25 healthy normotensive male subjects (aged 18-35 yrs) with a normal eGFR (MDRD). AcSDKP-NH_2 was measured by an original chromatography/tandem mass spectrometry (LC/MS/MS) assay. Urinary clearance of each marker was calculated. Results are expressed as median [IQR]. The primary end-point is the equivalence between the urinary AcSDKP-NH_2 and inulin clearances.

Results: eGFR (MDRD) was 121 [112 ; 130] ml/min/1,73 m^2. Inulin and AcSDKP-NH_2 urinary clearances were 102 [94 ; 124] and 112 [102 ; 121] ml/min/1,73 m^2, respectively. In the Bland Altman plot, the bias between the two methods was 5 (95%CI: -1; 15) ml/min/1,73 m^2 (p=0.0499). The AcSDKP-NH2 injection was well-tolerated.

Conclusions: This study validate for the first time that AcSDKP-NH_2 is a new and peptidic marker for GFR measurement in humans. Measurements in CKD patients are ongoing.

Funding: Government Support - Non-U.S.

SA-PO813

Diagnostic Cut-Off Values of Creatinine and Cystatin C for Severely Impaired Renal Function in Chinese Children with Kidney Injuries

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Background: There is no data about the normal range of serum creatinine, either measured by the Jaffe method (CrCA) or the enzymatic method (CrE), or cystatin C (CysC) in the Chinese children.

Methods: We determined GFR with plasma clearance of ^{99m}Tc-DTPA in 92 hospitalized children with renal injury and analyze the cutoff value of CrCA, CrE and CysC for diagnosing GFR lower than 60mL/min/1.73m^2, using the Receiver Operating Curve (ROC) analysis. The serum were sampled and divided on morning of the day of GFR measurement, stored at -80 degrees centigrade and measured in the central lab with standard quality control.

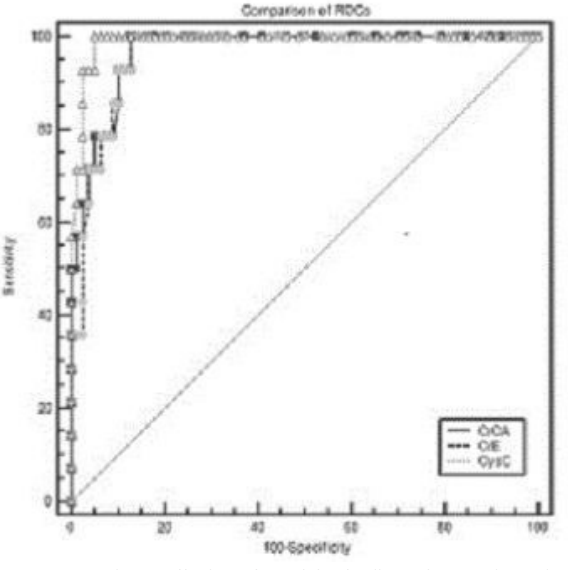
Results: In the 92 children, 49% were male and the mean age was 13 (IQR [10,15]) years old. Their mean GFR was 99.8 (IQR [73.5, 117.7]) mL/min/1.73m^2. The results of ROC analysis, divided by Age=14, are shown in Table 1. The AUC were close to 1 and indicated satisfactory diagnostic power of CrCA, CrE and CysC.

Results of ROC Analysis

	Age	AUC	Cut-Off Value	Se %	Sp %
CrE (µmol/L)	≥14	0.964*	84	100	90
	<14	0.983*	47	100	94
CrCA (µmol/L)	≥14	0.984*	102	100	94
	<14	0.986*	62	100	94
CysC (mg/L)	≥14	0.992*	1.29	100	97
	<14	0.987*	1.46	100	94

*all with p<0.0001; AUC, area under curve; Se, sensitivity; Sp, Specificity; +LR, positive likelihood ratio; -LR, negative likelihood ratio

Paired ROC comparison between CrCA, CrE and CysC showed that in the whole group, not divided by age of 14, the area under the ROC of CysC is significantly greater than the other two (both P<0.05).



Conclusions: The cut-off values of Creatinine for diagnosing GFR lower than 60 mL/min/1.73m^2, measured either by Jaffe method (CrCA) or enzymatic method (CrE), differ a lot between different age groups. The cut-off value of CystatinC, on the other side, remains stable between the two age groups and a CysC level over 1.4mg/L should be highly suspected as an indicator for severely impaired renal function.

Funding: Government Support - Non-U.S.

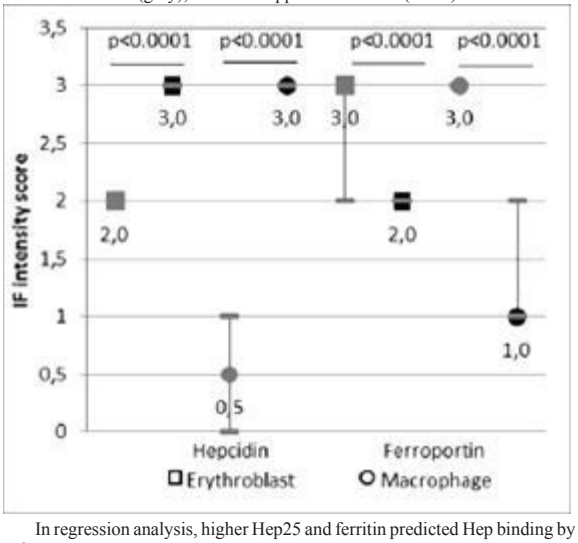
SA-PO814

Bone Marrow Iron Distribution, Hepcidin and Ferroportin Expression in Renal Anemia Liliana Barsan,[2] Simona Stancu,[1] Ana Stanciu,[2] Cristina Capusa,[1] Gabriel Mircescu.[1] *[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; [2]"Dr Carol Davila" Hospital of Nephrology, Bucharest, Romania.*

Background: The hepcidin-ferroportin system is involved in both conditions associated with iron-restricted erythropoiesis in renal anemia: iron deficiency anemia (IDA) and anemia of chronic disorders (ACD). As serum hepcidin could have diagnostic utility, we investigated its relationships with iron distribution, hepcidin (Hep) and ferroportin (Fpn) expression in bone marrow (BM) cells, and peripheral iron indices (ferritin; transferrin saturation) in non-dialysis chronic kidney disease (CKD) patients.

Methods: Prospective observational single center study on 54 CKD patients, epoetin and iron naïve, classified according to BM iron distribution (Perls' stanin) as IDA (n=26), ACD (n=21) or normal iron stores (n=7). Hep and Fpn expression by erythroblast and macrophage was evaluated by immunofluorescence on semiquantitative scales. Serum hepcidin (Hep25) was measured by ELISA (Bachem, UK).

Results: Low Hep and high Fpn expression by erythroblast and macrophage (Figure) were seen in IDA (grey), while the opposites in ACD (black).



In regression analysis, higher Hep25 and ferritin predicted Hep binding by erythroblast (R^2= 0.48; p<0.0001), while lower ferritin and Hep25 predicted erythroblast Fpn expression (R^2=0.29; p=0.003). Inflammation had no contribution. In ROC analysis, Hep25 and ferritin had similar moderate utility in differentiating IDA from ADC (AUC 0.63 95%CI 0.47–0.79 and 0.76 95%CI 0.61-0.90, respectively).

Conclusions: In anemic CKD patients, hepcidin and ferroportin expression by erythroblast and macrophage are correlated and closely related to BM iron distribution. Although the hepcidin-ferroportin system reaction seems to be regulated by the ferritin-driven Hep25, serum hepcidin and peripheral iron indices are of little help in describing bone marrow iron status.

SA-PO815

Hematuria Following Exercise Prem P. Varma,[1] Prashant Sengupta,[1] Ranjith K. Nair.[1] *[1]Department of Nephrology, Army Hospital (Research & Referral) Delhi Cantt-10, New Delhi, Delhi, India; [2]Department of Pathology, Army Hospital (Research & Referral) Delhi Cantt-10, New Delhi, Delhi, India; [3]Department of Nephrology, Army Hospital (Research & Referral) Delhi Cantt-10, New Delhi, Delhi, India.*

Background: Incidence of Exercise induced hematuria has been reported to be between 5-30%. Available literature suggests that it lasts for few hours to a maximum of 3 days. *Aim:* We planned to study incidence and duration of hematuria after a 5 km run in soldiers who are accustomed to 1.5-2 km run 5 days a week.

Methods: All fit and healthy soldiers without any co-morbidities, who volunteered to participate in the study were the subjects. They ran for 5 km at 0630 h. There was no water intake permitted during the run. Timings for the successful completion of run was 25 minutes for those below 30yrs and it was 26.5 and 28 mts for those between the age of 30-40yrs and 40-50 yrs respectively. Urine sediment of soldiers was examined before and after the run. Anyone with an abnormal pre-exercise sediment was excluded from the study. All those who had post exercise hematuria were followed with daily urine examination and those who continued to have hematuria beyond 14 days were thoroughly evaluated and subjected to renal biopsy also.

Results: 491 participants completed the run successfully and fifty nine of them (12%) developed post exercise hematuria. We found that the younger participants (age < 30 yrs) had a significantly higher incidence of hematuria as compared to their older compatriots (p=0.019). Mean duration of hematuria was 1.98 +/- 1.89 days. Hematuria cleared in 81% of the participants within 3 days and in other 19% it persisted. Of these in 12% hematuria lasted between 3-7 days and in another 7% it continued beyond 7 days. Three individuals had persistence of hematuria beyond day 14 and all of them were found to have primary glomerular diseases on kidney biopsy (two had IgA nephropathy and one had FSGS).

Conclusions: Exercise induced hematuria can last upto a fortnight and if it persists beyond a fortnight an underlying cause is likely. We feel hematuria is possibly related to intensity of exercise as in our study younger participants who had lesser time to complete the run had higher incidence of hematuria.

SA-PO816

Hepatitis C Virus P22 Antigen Test: Serological Response, Diagnostic Advantages and Cost Effectiveness in a Tertiary Care Haemodialysis Centre Subash Somalanka, Lennard Lee, David Makanjuola, John Clark. *Epsom & St Helier University Hospitals NHS Trust, London, United Kingdom.*

Background: p22 ELISA stains for a part of the nucleocapsid of Hepatitis C Virus (HCV) genome & has recently become commercially available. The current recommendation is to use ELISA to check for antibodies against HCV. We present a case of acute HCV infection in a tertiary renal unit with subsequent screening, source identification & follow up of all the Haemodialysis [HD] patients & staff who might have had contact with the index case. Serological response, alanine transaminase[ALT], HCV antibody[Ab], HCV p22 antigen[Ag], HCV RNA PCR test, advantages of p22 Ag test & financial implications are discussed.

Methods: Blood samples were obtained from the index patient. ALT, p22 Ag, HCV Ab & RNA PCR were performed retrospectively. 165 patients were investigated. p22 HCV Ag test was used as a screening test which being less sensitive than PCR required a week of additional screening.

Serological tests of the index case

Time[Weeks]	ALT[5-40 IU/L]	HCV Ab	p22 Ag[<3 fmol/L]	RNA PCR[<50 IU/ml]
0	26	-	-	-
2	22	-	3474	140,000
8	394	-	>20,000	2,100,000
11	474	+	2399	106,000
16	181	+	6573	558000

Results: Following screening, 1 patient previously known to have HCV was found to have the same HCV genotype as the index case. Gene sequencing showed only 2 different base pairs, suggesting that this individual was the source of transmission. Additional cost implications to the renal unit were minimised by the use of p22 Ag test in comparison to RNA PCR (£12 Vs £40 per test). p22 Ag test was run on an existing automated serological testing platform [ARCHITECT i2000 SR]. It was cheaper, easy to run & had a rapid result turnover.

Conclusions: p22 Ag ELISA is a robust, cheap & reliable test that allows for earlier detection of HCV viraemia. This would be of crucial importance in patient subgroups that need early treatment or where early detection is required to reduce risk of cross-transmission. Even though the HCV PCR was more specific & sensitive, p22 Ag ELISA can be used as a cost effective way of early serological diagnosis. It would also be useful in patients who fail to mount an adequate Ab response.

SA-PO817

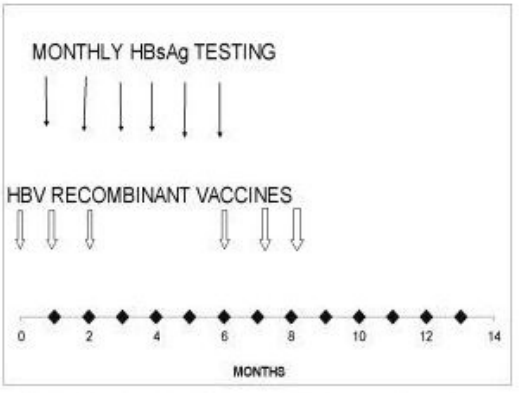
Transient Post-Vaccination Recombinant HBsAgenemia among ESRD Patients: How Common Is This Phenomenon? What Implications for Dialysis Care? Macaulay A. Onuigbo,[1] Nnonyelum T. Onuigbo.[2] *[1]College of Medicine, Mayo Clinic; [2]Information Technology, NTEC Solutions.*

Background: Hepatitis B Virus (HBV) infection affects millions worldwide, and constitutes a serious public health scourge; complications including heptaocellular carcinoma, liver failure and death. Hemodialysis (HD) exposes ESRD patients to significantly higher risks of de novo HBV infection. Therefore, current US CDC guidelines call for monthly HBsAg testing for all HD patients, perpetuated indefinitely. Furthermore, US CDC guidelines stipulate 100% coverage of all ESRD patients with Recombinant HB vaccinations, usually administered in a series of 3-4 scheduled booster doses. The phenomenon of transient post-vaccination Recombinant HBsAgenemia soon following HB vaccinations, which could be easily mistaken for de novo HBV infection, with dire consequences to the patient, has been rarely reported. The incidence in a routine HD Unit over 10 years has rarely bee studied.

Methods: We retrospectively analyzed our Mayo Clinic Renal Database for all patients seen in Northwestern Wisconsin, July 2000-July 2010, to ascertain the frequency of such phenomenon.

Results: The electronic databases and paper records of 965 HD patients were analyzed. One case of falsely positive HBsAg was detected. We described this rare phenomenon in an 81-year old HD patient in 2010, two days after the first of three planned Engerix B vaccines.

Conclusions: This scenario of indefinitely repeated monthly HBsAg screening tests among all HD patients, superimposed on often repeated series of Recombinant HB vaccination booster doses, lends itself to the potential of errors of false positive HBsAg test from vaccine-related Recombinant HBsAgenemia (Fig 1). It appears rare in routine ESRD care. We however re-echo previous recommendations that HD patients must not be screened for HBsAg for at least 4 weeks following a Recombinant HB vaccination.



The scenario of increasing potential for falsely positive post-vaccination Recombinant HBsAgenemia resulting from repeated monthly HBsAg testing among ESRD patients who receive multiple doses of HB Recombinant vaccinations in series together with sometimes the need for repeated booster HB vaccine doses.

SA-PO818

Imbalance of CCN2/CCN3 as a Marker for Detecting Glomerular Matrix Expansion Long Chen, Min Zheng, Dan Liu, Linli Lv, Bi-Cheng Liu, Jiandong Zhang. *Institute of Nephrology, Southest University, Nanjing, China.*

Background: Accumulating evidence suggests that members of CCN family proteins such as connective tissue growth factor (CCN2) and nephroblastoma overexpressed (CCN3) play important role in remodeling extracellular matrix.

Methods: We initially examined CCN2, CCN3 expression in a dynamic period of anti-Thy1 glomerulonephritis. Next, we asked whether the imbalance of CCN2/CCN3 seen in our animal experiments has its clinical implication as a biomarker in detecting glomerular histological changes. Our previous study suggested that urinary mRNA measurement is a powerful tool to monitor renal damages in patients with chronic kidney diseases (CKD). We investigated whether urinary mRNA of CCN2 and CCN3, can provide some clinical insight into the management of patients with CKD with a total of 35 patients with CKD and 12 healthy volunteers.

Results: Our results indicated that both mRNA expression of CCN2 and CCN3 was increased in nephritic glomeruli isolated from rats 4 days after injection of OX-7 Ab (16.85±1.36 au; 4.4±0.52 au,), as well as their protein level (1.14±0.1 vs. 0.68±0.04au, $p<0.01$; 0.6±0.05 vs. 0.33±0.04au,$p<0.01$). Intriguingly, enhanced mRNA expression of CCN3 was shown to decline by 40.3% in d6 nephritic glomeruli (3.03±0.18 au), by contrasting to steadily exaggerated expression of CCN2 (29.3±6.88 au). Of note, this imbalance of CCN2/CCN3 in glomeruli was in coincidence with massive glomerular matrix accumulation as indicated by PAS score (65.2±3.1vs. 53.2±3.2, $p<0.05$).In CKD patients compared to healthy controls the results indicated that urinary mRNA of both CCN2 and CCN3 were distinctively greater. Urinary mRNA of CCN3 was shown to inversely correlate to the degree of glomerular histological changes(r=-0.615, p=0.009). Furthermore, the ratio of urinary mRNA of CCN2 to CCN3 had an even stronger correlation efficient with glomerulosclerosis(r=0.647, p=0.005).

Conclusions: Our study demonstrates that the ratio of CCN2/CCN3 urinary mRNA correlates to the degree of glomerular histological changes in CKD patients, suggesting that imbalance of CCN2/3 may be a useful marker for evaluating CKD.

Funding: Government Support - Non-U.S.

SA-PO819

Gas Chromatography: A New Opportunity of Renal Diseases Diagnostics P. Miarka,[1] B. Grabowska-Polanowska,[2] J. Faber,[2] M. Skowron,[2] A. Pietrzycka,[3] P. Zagrodzki,[2] I. Sliwka,[2] Wladyslaw Sulowicz.[1] *[1]Dept. of Nephrology, UJ-CM, Poland; [2]Institute of Nuclear Physics of Polish Academy of Sciences, Poland; [3]Faculty of Pharmacy, UJ-CM, Poland.*

Background: During last decade, number of patients suffering from chronic kidney disease (CKD) has significantly increased. The physicians' efforts are focused on early CKD diagnosis and reduction of the end-stage renal diseases incidence. The breath test seems to be promising diagnostic device offering early noninvasive diseases detection. Trimethylamine (TMA) ismentioned in literature as potential marker of chronic kidney diseases. The aim of presented study was the determination of breath composition in persons suffering from CKD.

Methods: Exhaled air samples were enriched using solid phase microextraction (SPME) and analyzed by gas chromatography equipped with mass spectrometer (GC–MS).

Results: Breath samples were collected from 14 patients with CKD and 7 healthy volunteers. In the whole group of CKD patients the mean creatinine values were 171.2±74.2umol/l, urea 11.9±4.0mmol/l and eGFR 37.4±18.9ml/min/1/73m2. Trimethylamine was detected in 11 CKD patients and in none of control group. TMA mean values in exhaled air were 21.49±19.68ppb in CKD. Among breath components were also detected sulfur compounds such as dimethyl sulfide (DMS), carbon disulfide (CS_2) and also potential markers of oxidative stress. The presence of DMS in breath was confirmed in all participants. In CKD patients DMS concentrations ranged from 0.01 to 1.02nmol /l in control group from 0.05 to 0.39nmol/l $CS_2$was detected in all patients, and its concentration ranged from 0.009 to 0.15nmol/l. In the control group, in 4 out of 7 cases we detected CS_2 in the concentration range from 0.01 to 0.02nmol/l.TMA significantly correlated with eGFR ($r=-0.18$ $p<0.05$) and creatinine ($p<0.05$). Interestingly a significant correlation between DMS and eGFR ($r=-0.44$ $p<0.05$) was found. In addition, CS_2 correlated with creatinine ($r=0.44$ $p<0.05$) and uric acid concentration ($r=0.73$ $p<0.05$).

Conclusions: In the future, current diagnostic procedures (blood and urine tests, kidney biopsy) may be supplemented by detection of TMA concentration in human breath in early stages of chronic kidney diseases.

SA-PO820

Immunochemical Determination of Monomer, Homodimer and Total Neutrophil Gelatinase-Associated Lipocalin Kristian Bangert, Niels Rosenkilde, Jakob Ploug Jørgensen, Alexandra Baer, Lars O. Uttenthal. *BioPorto Diagnostics A/S.*

Background: NGAL (neutrophil gelatinase-associated lipocalin) released from the kidney in early acute kidney injury is reported to be chiefly in the monomer form, whereas NGAL released from neutrophils in inflammation contains a substantial amount of homodimer in addition to monomer. Immunochemical assays that can specifically measure monomer and homodimer forms of NGAL without the need for molecular size separation are therefore of interest for the rapid measurement of NGAL in different pathological conditions, as well as a total NGAL assay that measures monomer and homodimer forms identically.

Methods: By pairwise testing of a large series of mouse monoclonal antibodies raised against recombinant human NGAL in sandwich ELISA, we have developed immunochemical assays specific for monomer and homodimer NGAL, as well as an assay for total NGAL. The specificities of the assays were verified by means of recombinant human NGAL monomer and homodimer standards and peaks of native NGAL obtained by molecular exclusion chromatography of urine and plasma.

Results: The monomer NGAL assay cross-reacted <1% with homodimer NGAL and the homodimer NGAL assay cross-reacted <0.1% with monomer NGAL, while the total NGAL assay reacted equally with NGAL monomer and homodimer on a mass basis.

Conclusions: These assays are more convenient for the rapid analysis of different molecular forms of NGAL than methods involving molecular size separation and may aid in distinguishing between NGAL responses due to different pathological processes and of different cellular origin.

Funding: Pharmaceutical Company Support - BioPorto Diagnostics A/S

SA-PO821

Plasma Basigin/CD147 as a Biomarker of the Kidney Diseases Mayuko Maeda, Tomoki Kosugi, Waichi Sato, Tomohiro Masuda, Hiroshi Nagaya, Kayaho Maeda, Yuka Sato, Hiroshi Kojima, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: Basigin (Bsg)/CD147 is a transmembrane glycoprotein, and contributes to cell survival, invasion and metastasis. Recently, we documented in the experimental studies that Bsg is involved in the pathogenesis of renal inflammation caused by ischemia and renal fibrosis. Regardless of the primary disease process, these are the key determinant of diseased kidney and the prognostic predictors of renal function. We therefore investigated whether Bsg could serve as a novel biomarker for a differential diagnosis and prognosis in kidney diseases.

Methods: Plasma and spot urine samples were collected from the 261 patients, who underwent renal biopsy in our affiliated hospitals between 2008 and 2011. They included minor glomerular abnormality (MGA) as a pathological control (n=13), lupus nephritis (LN; n=64), minimal change nephrotic syndrome (MCNS; n=45), diabetic nephropathy (DM; n=24), IgA nephropathy (IgAN; n=43), focal segmental glomerulosclerosis (FSGS; n=32), and membranous nephropathy (MN; n=40). Plasma and urine Bsg values were measured by ELISA. Bsg expression in kidneys was examined by immunohistochemistry staining. Particularly in lupus nephritis, we verified the benefit of Bsg, using histopathological index.

Results: In normal kidney, Bsg is mainly distributed in tubular epithelial cells, but not glomeruli. Its expression was extremely lower in atrophic tubules. In kidney diseases with active injury, Bsg was more detected in macrophages and fibroblasts around damaged tubules and blood vessels. Positive but weak staining for Bsg was observed in injured glomeruli. Indeed, there was the strong relationship between plasma Bsg and serum creatinine. Bsg values in plasma and urine were significantly higher in LN, MCNS, DM, FSGS and MN patients compared with MGA, but not IgAN. Particularly, Bsg values with LN showed strong correlation with histological index. These data indicate that Bsg may be one of reliable biomarkers for renal injuries.

Conclusions: Plasma Bsg may be a prime candidate for developing a new procedure for the evaluation of renal injury and the prognostic predictor.

SA-PO822

Urine Exosomal Ceruloplasmin: A Potential Biomarker of Early Kidney Disease Krishnamurthy P. Gudehithlu,[1] Amit J. Joshi,[1] Mark A. Kraus,[1] Peter D. Hart,[1] Jose A.L. Arruda,[2,3] George Dunea,[1,3] Ashok K. Singh.[1,2,3] *[1]Division of Nephrology, John H. Stroger, Jr., Hospital of Cook County, Chicago, IL; [2]Section of Nephrology, University of Illinois at Chicago and VAMC, West Side Division, Chicago, IL; [3]The Hektoen Institute of Medicine, Chicago, IL.*

Background: Urine exosomal proteins could act as fingerprints of glomerular and tubular cells and as such may offer new biomarkers for diagnosing early kidney disease. We investigated this by identifying in the urinary exosomes of renal disease patients abundant proteins that were present in low concentrations in control urine.

Methods: We isolated urine exosomes from biopsy-proven patients of membranous nephropathy (n=9), IgA nephropathy (n=7), lupus nephritis (n=10), focal segmental glomerulosclerosis (n=10) and normal controls (n=5) by differential centrifugation. Proteins were extracted from the exosomes and subjected to SDS-PAGE fractionation followed by liquid chromatography / tandem mass spectrometry (LC-MS/MS) analysis. A total of 88 proteins (probability score of 95% or more) were identified that were abundant in urinary exosomes of patients and present at low concentrations in controls. Among them, we selected three proteins, ceruloplasmin, nephrilysin and alpha-1-antitrypsin, for further investigation. The three selected proteins were quantified in the urine exosomal extracts by ELISA.

Results: Quantitative results showed that of the three proteins ceruloplasmin was the most promising; it was 30-60 fold higher in the exosomal extracts of renal disease patients (expressed as ug ceruloplasmin/mg exosomal protein) and approximately 300 fold higher (expressed as ug exosomal ceruloplasmin/mg of urinary creatinine) than in controls. Increased ceruloplasmin levels correlated poorly with the degree of proteinuria in these patients suggesting that increased urine exosomal ceruloplasmin was specific to the underlying kidney disease rather than to increased leakage of systemic proteins.

Conclusions: We conclude that urinary exosomal ceruloplasmin is up-regulated in renal disease patients and may be a biomarker of early kidney disease.

Funding: Private Foundation Support

SA-PO823

Urinary Exosomes: A Novel Means to Non-Invasively Assess Changes in Renal Gene Expression Anja Susanne Muhlfeld,[1] Silvia Spanu,[2] Claudia R.C. van Roeyen,[1] Bernd Denecke,[3] Jürgen Floege.[1] *[1]Department of Nephrology and Clinical Immunologie, RWTH Aachen University, Aachen, Germany; [2]Department of Nephrology, University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]IZKF, RWTH Aachen University, Aachen, Germany.*

Background: In clinical practice there is a lack of markers for the non-invasive diagnosis and follow up of kidney disease. This study was designed as a proof-of-concept-study to evaluate urinary exosomes as diagnostic markers for kidney disease. Exosomes are membrane vesicles, which are secreted from their cells of origin into surrounding body fluids and contain proteins and mRNA which are protected from digesting enzymes by a cell membrane.

Methods: Toxic podocyte damage was induced by puromycin aminonucleoside in rats (PAN). Urinary exosomes were isolated by ultracentrifugation at different time points during the disease (day 0: prior to disease induction, day 5: at the beginning of proteinuria, day 10: at maximal kidney damage). Exosomal mRNA was isolated, amplified, and the mRNA species were globally assessed by gene array analysis. Tissue specific gene and protein expression was assessed by RT-qPCR-analysis and immunohistochemistry.

Results: Gene array analysis of mRNA isolated from urinary exosomes revealed cystatin C mRNA as one of the most highly regulated genes during the course of PAN. Its gene expression in urinary exosomes increased 7-fold by day 5 and remained high at a 2-fold increase until day 10. This was paralleled by a 2-fold increase in cystatin C-mRNA expression in the renal cortex. Protein expression in the kidneys also dramatically increased with *de novo* expression of cystatin C in glomerular podocytes, in parts of the proximal tubule and the renal medulla. Urinary excretion of cystatin C increased about 2-fold after the induction of podocyte damage.

Conclusions: In this proof-of-concept-study we could demonstrate that changes in urinary exosomal cystatin c mRNA expression are representative of changes in renal mRNA and protein expression during the course of PAN. Because cells lining the urinary tract produce urinary exosomal Cystatin C mRNA, it might be a more specific marker of renal damage than glomerular filtered free cystatin C.

Funding: Private Foundation Support

SA-PO824

Divergent Time-Dependent Prediction of the Severity of Mouse Folic Acid-Induced Kidney Fibrosis by Urinary Exosomal microRNA-27b Taro Horino,[1] Xuzhen Hu,[1] Takayuki Tsuji,[1,2] Alejandro Alvarez-Prats,[1] Ana Carolina Souza,[1] Robert A. Star,[1] Peter S.T. Yuen.[1] *[1]NIDDK, NIH, Bethesda, MD; [2]1st Department of Medicine, Hamamatsu University, Hamamatsu, Japan.*

Background: Epidemiological studies indicate that renal fibrosis is a predictor of rapidly declining kidney function during CKD, but more accessible biomarkers are needed. Urinary exosomes contain proteins, mRNAs, and miRNAs that can serve as biomarkers of renal injury. We tested how well urinary exosomal miRNAs, collected at different times post-injury, correlate with the degree of kidney fibrosis.

Methods: Outbred CD-1 mice (n = 7) were administered folic acid (FA) (250 mg/kg, i.p.) then urine was collected for 24 h in metabolic cages ending on days 2, 6, 10, and 14. Urinary exosomal fraction was isolated by 2-step centrifugation (17,000 x g for 15 min, and 200,000 x g for 1 hr). We measured 4 kidney-enriched miRNAs (miR21, miR27b, miR192 and miR200b) by RT-qPCR (Taqman). We scored the degree of kidney fibrosis at 14 days and compared it with urine concentrations of exosomal miRNAs.

Results: All 4 urine exosomal miRNA levels were elevated on day 2 post-FA; miR27b and miR200b were nearly maximum on day 2. Urinary miRNAs displayed a time-dependent correlation with fibrosis score. At day 6, all 4 urinary exosomal miRNAs were negatively correlated to fibrosis score; miR27b had the strongest negative correlation (r^2=0.793). At day 10, miRNAs were not correlated with fibrosis. At day 14, urine exosomal miR27b was again correlated to fibrosis score, but this time positively correlated (r^2=0.854).

Conclusions: We found that urinary exosomal miR27b predicted kidney fibrosis in the FA mouse model. Interestingly, the association was time-dependent: the correlation was negative early on (day 6) but changed to a positive correlation later (day 14). This switching could be explained by multiple roles for miR27b in renal tubule cells. This time dependence complicates the use of miR27b as a potential biomarker or therapeutic target for renal fibrosis, as time-dependent staging would be required to determine whether to inhibit or stimulate miR27b in order to treat the progression of kidney disease.

Funding: NIDDK Support

SA-PO825

Endogenous Human Inhibitor Interferes with Detecting microRNA-27b in Urine and Non-Exosomal Urine Fractions Taro Horino, Robert A. Star, Peter S.T. Yuen. *NIDDK, NIH, Bethesda, MD.*

Background: Urinary exosomal and non-exosomal miRNAs are potential biomarkers for acute and chronic kidney diseases, but we found that human urine contains an inhibitor of miRNA purification/detection. Therefore, we developed a more sensitive purification/assay system, then measured and validated the distribution of miR27b in different fractions of normal urine.

Methods: We separated healthy human or rat urine into 4 subfractions: 17K pellet, 17K supernatant, 200K supernatant, and 200K pellet (Exosomes). We compared 3 methods to purify miRNA [TRIzol, miRNeasy, or TRIzol/miRNeasy]. We measured [RNA] by NanoDrop, and [miR27b] by Taqman (RT-PCR) using synthetic, pure, mature miRNA standards. We validated assays by the Standard Addition Method (SAM; parallel addition of 4 known increasing amounts of pure miRNA to an unknown sample, then extrapolate to 0 addition).

Results: TRIzol/miRNeasy had the highest recovery of miR-27b. miR-27b was undetectable in supernatant fractions from 2 ml of human urine. Pure miR-27b recovery was inhibited by <5 µl of urine, with as much as 63% inhibition by 50 µl of urine. Inhibitory activity was not in mouse or rat urine, but was in human supernatant fractions. The urine inhibitor prevented miR-27b from binding to the purification column. We could reduce inhibitory activity by capturing the inhibitor on a first column then purifying the the flow-through on a second column. However, a simpler solution was to use 0.1 ml of urine, as all fractions were almost completely unaffected by inhibitory activity, which we validated by SAM. We found that in human urine 60% of mi-R27b was found in the 200K sup, 27% in the 17K pellet, and 13% in the 200K (exosomal) pellet.

Conclusions: We validated a sensitive assay for urinary miRNAs from 0.1 ml of urine, using a combined method (TRIzol/miRNeasy). In larger volumes of human urine or supernatant fractions, miR-27b could not be accurately assayed due to endogenous inhibitory activity. We postulate that inhibitor(s) could compete with miRNA binding to the purification column, lowering assay results (yield). Now that technical issues have been resolved, we can test miRNAs from each urine fraction as potential biomarkers for renal diseases.

Funding: NIDDK Support

SA-PO826

Urinary Expression of miR-21, miR-29 Family, miR-93 and TGF-β_1/SMAD Signaling Pathway Associated Molecules in Patients with IgA Nephropathy Gang Wang,[1] Yongcheng He,[2] Bc Kwan,[1] Kai-ming Chow,[1] Fm Lai,[1] Philip K.T. Li,[1] Cheuk-Chun Szeto.[1] *[1]Medicine & Therapeutics, The Chinese University of Hong Kong, Hong Kong, China; [2]Nephrology, Shenzhen University, Shenzhen, China.*

Background: MicroRNAs play important roles in the development and progression of renal fibrosis. We studied the expression of miR-21, miR-29 family, miR-93, miR-377 and miR-216a, together with messenger RNA of TGF-β_1, SMAD2, SMAD3, SMAD4 and SMAD7 in the urinary sediment of patients with IgA nephropathy.

Methods: We studied 43 patients with biopsy-proven IgAN. Urinary expression of miRNAs and mRNAs was determined and compared to that from 13 healthy controls.

Results: The urinary expression levels of miR-29b and miR-29c were significantly lower and the urinary expression levels of miR-93 was higher in patients with IgA nephropathy than normal controls. Proteinuria significantly correlated with urinary expression levels of miR-29b (r = -0.388, P = 0.003) and miR-29c (r = -0.409, P = 0.002). GFR significantly correlated with urinary expression levels of miR-21 (r = 0.338, P = 0.028), miR-29b (r = 0.333, P = 0.031) and miR-29c(r = 0.304, P = 0.050). Urinary expression level of miR-93 significantly correlated with glomerular scarring (r = -0.392, P = 0.010). There was significant correlation between the levels of urinary SMAD3 and miR-21 (r = 0.624, P < 0.001), miR-29b (r = 0.566, P < 0.001), miR-29c (r = 0.619, P < 0.001) and miR-93 (r = 0.332, P = 0.032). The level of urinary SMAD4 negatively correlated with the level of urinary miR-29c (r = - 0.325, P = 0.036).

Conclusions: We find the urinary expression of miR-29b and miR-29c is downregulated and miR-93 is upregulated in patients with IgA nephropathy. The expression of miR-29b and miR-29c significantly correlates with proteinuria and renal function and the expression of miR-93 significantly correlates with glomerular scarring. Our results suggest these miRNAs might play an important role in the pathophysiology of IgA nephropathy and the levels of urinary miR-29b, miR-29c and miR-93 have the potential to be developed as non-invasive markers for IgA nephropathy.

SA-PO827

Evaluation of Disease State of Various Glomerulonephritis by the Levels of Claudin1 and CD68 mRNA in Urine Kojiro Yamamoto,[1] Takashi Oda,[1] Takahiro Uchida,[1] Atsushi Watanabe,[1] Hanako Takechi,[1] Naoki Oshima,[1] Yutaka Sakurai,[2] Hiroo Kumagai.[1] *[1]Nephrology, National Defense Medical Collage, Tokorozawa, Saitama, Japan; [2]Preventive Medicine and Public Health, National Defense Medical Collage, Tokorozawa, Saitama, Japan.*

Background: Urine test is non-invasive and can reflect more general condition of the whole kidney than findings of renal biopsy. We reported at the last ASN that the immunostaining for claudin1 (CLDN1) and CD68 of urinary sediment was useful for the evaluation of the activity of glomerular diseases. In order to confirm the finding at the mRNA level, we measured CLDN1 and CD68 mRNA using real-time RT-PCR.

Methods: We collected morning urine samples from 196 patients who had been hospitalized for kidney biopsy from '08 to '11. Total RNA isolated from sediments of collected urines was subjected to real-time RT-PCR. We used TaqMan Probes for CLDN1, CD68, and Aquaporin2 (AQP2) (all from Applied Biosystems). AQP2 was used as a kidney-derived reference gene. The relationship between relative levels of target mRNAs divided by AQP2 mRNA levels and the histological changes such as existence of crescent formation (CF) (with or without) or global sclerosis rate (under 30% global sclerosis is low and over 30% global sclerosis is high) was evaluated. We also evaluated the relationship of mRNA results with the state of glomerular crescents (cellular crescent (CC), fibro-cellular crescent (FCC), fibrous crescent (FC), and no crescent (NC)).

Results: The relative levels of the CLDN1 and CD68 mRNA were significantly increased in those patients with CF ($p<0.01$ and $p<0.01$, respectively). On the other hand, no significant differences in the relative levels of the CLDN1 and CD68 mRNA were found in relation to the global sclerosis rate. Interestingly, the relative levels of the CLDN1 mRNA was significantly increased in the patients with FC but was unchanged in those with CC or FCC. The relative levels of the CD68 mRNA was significantly increased in patients with CC but was unchanged in those with FC or FCC.

Conclusions: These data suggest that the quantification of the CLDN1 and CD68 mRNA (adjusted by AQP2 mRNA) of urinary sediment is useful for the evaluation of the state of the glomerular disease.

SA-PO828

Next-Generation Sequencing of Urinary microRNA in Human Lupus Nephritis Beatrice Goilav,[1] Iddo Z. Ben-Dov,[2] Irene Blanco,[3] Olivier Loudig,[4] Dawn Wahezi,[5] Chaim Putterman.[3] *[1]Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]Laboratory of RNA Molecular Biology, Rockefeller University, New York, NY; [3]Rheumatology, Albert Einstein College of Medicine, Bronx, NY; [4]Epidemiology, Albert Einstein College of Medicine, Bronx, NY; [5]Rheumatology, CHAM, Bronx, NY.*

Background: Lupus nephritis (LN) is associated with significant morbidity and mortality. microRNAs (miRs) are small non-coding RNAs that regulate translation. Previous studies report changes in miR expression in kidney tissue, urine and PBMC that correlate with LN disease activity. However, use of RNA deep-sequencing methods has not been previously described. We aimed at identifying miR expression patterns in LN by RNA sequencing.

Methods: Cell-free urine from adult (n=9) and pediatric (n=4) female patients with LN were obtained at time of active disease and in remission. Total RNA was used to prepare small RNA cDNA libraries for sequencing. Multiplexing through sample-specific 3' adapters was applied to limit batch effects and cost. Sequence reads were mapped to the human genome and small RNA databases. miRs were quantified by relative read abundance. qRT-PCR was used for quantitative validation.

Results: We obtained reproducible profiles of miRNA from cell-free urine. In a paired-sample analysis comparing miR abundance in urine of active vs inactive LN, we found significant changes in 19 miRs, including upregulation of miR-185, -328, and -423 by 400-1,000x during active disease. Differential read abundance was confirmed by qRT-PCR.

Conclusions: We detected miRs (most of which had not been previously linked to LN) with significantly higher presence in the urine during active LN. These miRs may be biomarkers of disease activity or indicators of specific histology. Several of the miRs we detected in the urine were previously found to be differentially expressed in PBMCs during active disease. This may imply that their presence in urine originates from infiltrating rather than kidney cells. Finally, upregulated miRs during active LN could point towards "protective" target genes that are repressed in relapse and identifying the latter may reveal new therapies.

Funding: Clinical Revenue Support

SA-PO829

Clinical Investigation of Galectin-1 in SLE Patients Yinghong Liu, Wenzhen Xie, Guochun Chen, Fuyou Liu. *Department of Nephrology, The Second Xiangya Hospital, Changsha, Hunan Province, China.*

Background: Galectin-1(Gal-1) is an endogenous glycan-binding protein which controls a diversity of immune cell processes and involved in immunoregulation. **Objectives** To analyze the expression of Gal-1 in the serum, urine and kidney tissues that derived from SLE patients with the aim of establishing a correlation between these parameters and the clinical profile.

Methods: 50 SLE patients were selected. Serum and urine were collected and the expression of Gal-1 was examined by ELISA .Kidney tissues were obtained from 36 LN patients. The expression of Gal-1 in the kidney tissues were analyzed by immunofluorescent or immunohistochemical staining.

Results: We found significantly enhanced level of Gal-1 in urine from SLE patients compared to controls. The urine levels of Gal-1 were significantly higher in SLE patients with active disease than inactive individuals.But no difference were observed between the urine Gal-1 level of inactive group and that of control group. We detected a significant positive association between the urine Gal-1 level and SLEDAI scores. Similarly, a positive correlation were observed between urine Gal-1 level and anti ds-DNA antibody titers, ESR, urine protein. And a negative correlation between the urine Gal-1 level and the serum total protein,seralbumin,complement C 3 were observed.Galectin-1 immunoreactivity was found in renal of LN cases, while control glomeruli were negative. The expression of Gal-1 in renal whose AI(acute indexes) were above 4 were significantly higher compare to that were less than 4.And a positive correlations were observed between the expression of Gal-1 and the AI,AI+CI(chronic indexes) and SLEDAI scores .However, no significant differences were found between the serum Gal-1 level of control group and that of SLE patients.

Conclusions: Our data suggest that urine Gal-1 could serve as a novel biomarker to predict the disease activity of SLE and involvement of kidney. As Gal-1 participates in modulating the immune system, we suggest that the presence of Gal-1 in the kidney may contribute to the immune deregulation observed in LN.

SA-PO830

Severe Lupus Nephritis: The Predicitve Value of a ≥50% Reduction in Proteinuria at 6 Months Stephen M. Korbet, Edmund J. Lewis, The Collaborative Study Group. *Nephrology, Rush University Medical Center, Chicago, IL.*

Background: In severe lupus nephritis (SLN), attainment of a complete remission (CR) in proteinuria (UPro) with treatment is associated with a favorable outcome. While the initial course of therapy can be up to 6 mo, the time to remission in the majority of patients is >6 mo and many don't attain a CR until after 12 mo. We assess the value of a ≥50% reduction in UPro at 6 months in predicting outcome in SLN pts.

Methods: We studied 86 adult pts in the prospective, controlled trial of plasmapheresis (PP) in SLN (NEJM 1992). All pts had ISN/RPS class IV ± class V (25 pts) lesions. All pts received pred and oral CYC and 40 pts received PP. Pts were categorized based their status at 6 months: (a) CR ≤6 mo, (b) ≥50% reduction in UPro, (c) <50% reduction in UPro, (d) ESRD/ death. The outcomes were compared between pts with ≥50% or <50% reduction in UPro at 6 mo. A CR was defined by a SCr of ≤ 1.4 mg/dL and UPro of ≤0.33 g/day and partial remission (PR) by a ≤25% increase in baseline SCr and ≥50% reduction in UPro to ≤1.5 g/d.

Results: The 86 pts were 32±12 yrs old, 84% were female, 63% white and 24% were AA. At 6 mo. 13 pts (15%) had died or reached ESRD and 12 pts (14%) had attained a CR while 34 pts (40%) had a ≥50% reduction and 27 pts (31%) a <50% reduction in UPro. Comparing pts with ≥50% and <50% reduction in UPro at 6 mo there was no difference in baseline age (31±12 v. 29±12 yrs), gender (76% v. 85% female), race (68% v. 63% white), SCr (1.8±0.9 v. 1.9±0.9), histology or treatment. However, the baseline UPro was higher in pts with ≥50% reduction in UPro at 6 mo (7.1±3.6 v. 4.6±3.2, P 0.002). At follow-up (120±65 mo), pts that had a ≥50% reduction in UPro at 6 mo attained a CR in 56% and PR in 35% of cases compared to pts that had a <50% reduction in UPro at 6 mo who had a CR in 22% (P 0.009) and PR in 33% (P- NS). The 15-yr renal survival (71% v. 25%, P 0.003) and 15-yr pt survival without ESRD (66% v. 18%, P 0.002) was greatest in pts with a ≥50% reduction in UPro at 6 mo compared to pts with <50% reduction and similar to that of pts with CR at ≤6 mo (82% and 82%).

Conclusions: A ≥50% reduction in UPro at 6 mo is predictive of a favorable outcome in pts with SLN.

SA-PO831

Does H Ficolin Contribute to the Pathogenesis of Lupus Nephritis? Zofia I. Niemir,[1] Anna Swierzko,[2] Maciej Cedzynski,[2] Anna Maciejewska,[3] Jolanta Lukasiewicz,[3] Magdalena Polcyn-adamczak.[1] *[1]Department of Nephrology, University of Medical Sciences, Poznan, Poland; [2]Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland; [3]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.*

Background: Recently, increased levels of H-ficolin in sera of patients with systemic lupus erythematosus (SLE) have been reported. Given the potential importance of this protein in autoimmunity, we compared its serum levels in patients with different morphological forms of primary glomerulonephritides (PGN), active and inactive lupus nephritis (LN) and healthy controls (C).

Methods: The study involved 120 patients with PGN (43 with IgA-mesangial proliferative GN, 36 with non-IgA-mesangial proliferative GN, 6 with membranoproliferative GN, 19 with idiopathic membranous GN, and 16 with focal-segmental glomerulosclerosis), 55 with LN (40 with active and 15 with inactive phase of the disease) and 65 C. The activity of LN was assessed by scoring the symptoms of SLE according to the systemic lupus erythematosus disease activity index. The serum level of H-ficolin (s-H-ficolin) was determined using the NUNC Maxisorp plates coated with *H. alvei* 1200 LPS, monoclonal anti-H-ficolin antibodies and HRP-labeled anti-mouse IgG.

Results: Median s-H-ficolin concentration was 23.9 mg/ml (range 1.6 to 59) in patients with LN, 16.8 mg/ml (range 2.6 to 53.5) in those with PGN and 18.9 mg/ml (range 6.4 to 35.9) in C (LN vs. PGN and C, $p<0.0001$). We compared the number of subjects that could be allocated to the fourth quartile obtained in C. This resulted in 24.6% of those from the C group, 29.1% of patients with PGN and 63.6% of patients with LN (LN vs. PGN and C; $p<0.0001$) In LN, s-H-ficolin levels tended to be higher in patients with active lupus flare than in those with inactive disease, but the difference did not reach the statistical significance. No statistically significant differences could be found between patients with various morphological forms of PGN.

Conclusions: Our results indicate that H-ficolin may be involved in the pathogenesis of LN. Further studies are required to resolve this issue.

Funding: Government Support - Non-U.S.

SA-PO832

Development of a Novel High Throughput Assay of Hydroxychloroquine in Whole Blood Samples Enables Identification of Medication Non-Adherence in Patients with Lupus Nephritis

Gary Chusney, Tom Cairns, Janet Lee, Liz Lightstone. *Leslie Brent Laboratory, Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Use of hydroxychloroquine (HCQ) in lupus patients is associated with improved long term outcomes. It has a long half life & recent data suggest low levels identify non adherence with therapy – a major cause of poor outcomes in lupus. Our aim was to develop & validate a liquid chromatography with tandem mass-spectrometric detection (LCTMS) method for the routine monitoring of HCQ in whole blood from patients treated for lupus.

Methods: The LCTMS method used a sample of 25µL whole blood with a simple protein crash treatment using deuterated (d4)-HCQ as the internal standard. The compounds were detected in the column effluent by monitoring specific m/z transitions of HCQ (336.1>247.1) & d4-HCQ (340.1>251.1). Throughput was 15 samples per hour. Calibrators (range 0 – 3.80mg/L) & quality controls, at 3 concentrations, were prepared in-house.

Results: The LCTMS assay was linear up to a concentration of at least 4.00mg/L. The LOQ was 25µg/L. The intra-assay imprecision was 8.7% @ 0.23mg/L, 4.8% @ 0.82mg/L & 6.8% @ 1.82mg/L & total imprecision was 8.7% @ 0.23mg/L, 6.9% @ 0.82mg/L and 6.8% @ 1.82mg/L (n=36). Absolute recovery was (mean±SD, n=5) 70.0±5.3% @ 0.5mg/L & - 73.7±3.6% @ 1.0mg/L.

In an initial cohort of 52 patients taking 200mg bd, 44 had levels >0.2mg/L. In these patients the median HCQ concentration was 0.87mg/L (range 0.23-1.89mg/L) – slightly lower than the suggested optimum of 1mg/L. Importantly, these preliminary data suggest 15% were not taking their prescribed medication.

Conclusions: The LCTMS method described is simple, precise, requires small sample volumes and therefore effective for routine therapeutic monitoring of HCQ. Identifying non adherent patients may well improve future adherence and therefore outcomes. To our knowledge it is the first LCTMS method described for measuring this drug in a clinical environment.

SA-PO833

Metabolomics Profiles in Different Stages of Chronic Kidney Disease (CKD)

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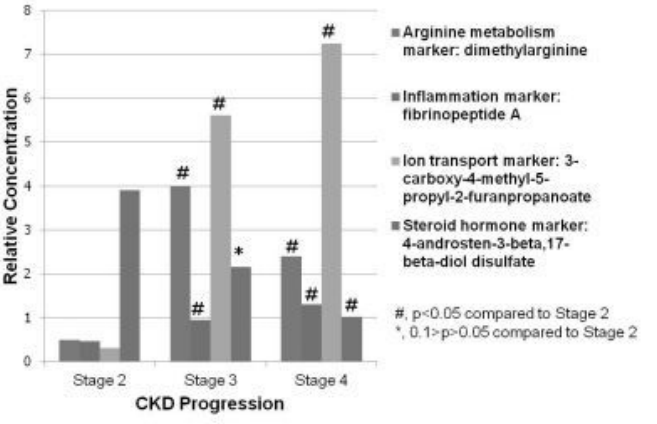
Background: CKD is a common public health problem. Identifying biomarkers adds prognostic / diagnostic value by contributing to an understanding of CKD at the molecular level possibly defining new drug targets. Metabolomics provides a snapshot of biochemical events at a particular time in the progression of CKD. In this pilot study we ascertained whether metabolite profiles are significantly different in CKD stages 2, 3 and 4.

Methods: An analysis of plasma metabolites, using GC/MS and LC/MS platforms, was conducted on thirty participants comprised of non-diabetic males ages 40-52 with ten each in stage CKD-2, CKD-3 and CKD-4 based on eGFR.

Results: Comparison of CKD-3/CKD-2 identified 62 metabolites that differed ($p\leq0.05$), with 39 higher and 23 lower in CKD-3 compared to CKD-2; comparison of CKD-4/CKD-2 identified 111 metabolites, with 66 higher and 45 lower; and comparison of CKD-4/CKD-3 identified 11 metabolites, with 7 higher and 4 lower. Major changes in metabolic profiles with increasing stage of CKD were observed, including altered arginine metabolism, elevated coagulation/ inflammation, impaired carboxylate anion transport, and decreased adrenal steroid hormone production.



Conclusions: Global metabolic profiling uncovered potential biomarkers of stages of CKD offering insight into pathophysiological processes. This pilot study suggests metabolomics can be used to formulate testable hypotheses of the usefulness of measuring specific metabolite patterns as indicators of kidney disease in diverse populations.

Funding: NIDDK Support, Other NIH Support - NCRR Now NIGMS, Local NCAT Supported CTSC

SA-PO834

Elevated Plasma Levels of Vasohibin-1 Predict Renal Dysfunction and Renal Levels of Vasohibinb-1 Are Associated with Histological Alterations in CKD Patients

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Background: Vasohibin-1 serves as a negative feedback regulator of angiogenesis and we reported the therapeutic efficacy of Vasohibin-1 in mouse diabetic nephropathy models. The aim of the current study is to examine the plasma, urinary and renal levels of Vasohibin-1 in patients with renal disorders and to evaluate its clinical usefulness.

Methods: We studied 78 Japanese CKD patients with (n=54) or without renal biopsies and 6 control subjects. The levels of plasma, urinary Vasohibin-1 were determined by ELISA and immunohistochemistry for Vasohibin-1 was conducted. Correlation among clinical, histological parameters, the plasma, urinary levels of Vasohibin-1 and the number of renal Vasohibin-1^+ cells were examined.

Results: The plasma levels of Vasohibin-1 inversely correlated with age and systolic blood pressure and positively correlated with the number of renal Vasohibin-1^+ cells. Vasohibin-1 was observed in renal endothelia of normal subjects and additionally in mesangial cells, crescent and interstitial inflammatory cells in CKD patients. Double immunostaining showed partial co-localization of Vasohibin-1 and αSMA/CD31 in glomeruli. Significant positive correlation was observed between [1)]crescent formation and the number of Vasohibin-1^+ cells in glomerulus or cortex, and [2)]interstitial cell infiltration and the number of Vasohibin-1^+ cells in cortex. The plasma levels of Vasohibin-1 and the number of glomerular Vasohibin-1^+ cells correlated with glomerular VEGFR-2^+ area. Baseline plasma levels of Vasohibin-1 inversely correlated with the annual alteration of eGFR (r=-0.36, *P=0.03).*

Conclusions: Elevated plasma levels of vasohibin-1 predicts renal dysfunction and renal expression of vasihibin-1 is associated with histological alterations in CKD patients.

SA-PO835

The Association of Complement 3 (C3) and Proteinuria in Non-Nephrotic Chronic Kidney Disease

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Background: Obesity and hypertriglyceridemia (hTG) are risk factors for progression of chronic kidney disease (CKD). Adipocytokine such as Complement 3 (C3) is associated with metabolic abnormality and vascular damage. However, the roles of C3 on the association with the metabolic marker, renal arteriolopathy, and proteinuria are not clear. The clinical significance has not been investigated among CKD patients.

Methods: In the present study, we examined 145 non-nephrotic (serum albumin ≥3 g/dl) CKD patients excluding lupus, who had undergone renal biopsy (54% male). Arteriolar-wall thickening was assessed semi-quantitatively. We further investigated the variables based on the median level of serum C3.

Results: The mean (SD) age was 42 (17) years, urine protein (UP) 1.2 (1.2) g/gCr, and eGFR 80 (25) ml/min/1.73 m^2, C3 level 80 (26) mg/dl. Although the mean age, eGFR and blood pressure was comparable between higher and lower C3 groups, UP was significantly higher in the higher C3 group than those in the lower C3 group. Patients with higher C3

level were significantly larger in body mass index, grade of arteriolar-wall thickening and higher prevalence of diabetes mellitus (DM) and hTG. Logistic analysis adjusted for age showed higher C3 was significantly associated with higher risk for the presence of above the mean value of UP (higher UP) as well as hypertension, DM and hTG. Higher C3 level was a significant factor for higher UP after adjustment with age, sex and other significant factors. The adjusted odds ratios (95% CI) of higher C3 level was 2.6 (1.2 to 5.8). Coexistence of higher C3 level and hTG was significantly associated with higher UP and the odds ratio (95% CI) was 3.4 (1.2 to 9.9) compared to the lower C3 level and hTG(-).

Conclusions: Increased level of C3 was related to metabolic abnormalities and associated with higher UP in non-nephrotic CKD patients, especially when complicated with hTG. The present study suggests the clinical significance of C3 among patients with CKD. However, the causal role of C3 on proteinuria remained to be studied.

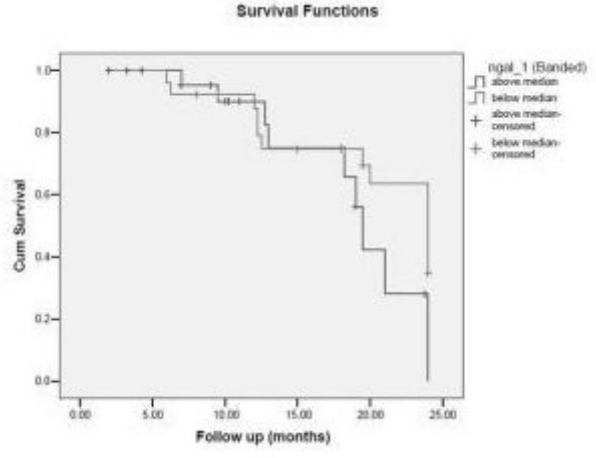
SA-PO836

Plasma NGAL Does Not Predict ADPKD Progression Grazia Maria Virzì,[1,2] Dinna N. Cruz,[1,2] Fiorella Gastaldon,[1] Valentina Corradi,[1,2] Maurizio Clementi,[3] Claudio Ronco.[1,2] *[1]Nephrology, St Bortolo Hosp, Italy; [2]International Renal Research Institute Vicenza; [3]Clinical Genetics, Univ of Padua, Italy.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease with variable rate of progression. Neutrophil gelatinase-associated lipocalin (NGAL) has been implicated in pathological conditions and it is proposed as a biomarker for CKD progression in kidney diseases. We hypothesized that NGAL could also be a good marker for progression of ADPKD. Our aim was to evaluate if NGAL could predict loss of function in ADPKD patients.

Methods: ADPKD patients with confirmed mutations (PKD1, n=33; PKD2, n=17) were enrolled and followed prospectively. Creatinine (sCr) and NGAL values were measured at baseline and on follow-up (median 18 mos, IQR 11-28). Plasma NGAL was measured by Triage point of care test. CKD progression was defined as 15% decrease in eGFR from baseline to follow-up. Patients were divided into 2 groups based on median baseline NGAL and compared by the Kaplan-Meier curve.

Results: We enrolled 50 ADPKD pts (60%M age 41yrs); mean sCr 1.3±0.7mg/dl and median eGFR was 62 mL/min/1.73m². NGAL values are inversely correlated with baseline eGFR (r=-0.64, p<0.001). There was weak correlation between baseline NGAL and subsequent change in eGFR (r=0.28, p=0.05) 9/50 patients had NGAL below limits of detection (60pg/ml); median NGAL level was 79pg/ml. On follow-up, 12 patients (24%) had progression as defined. No statistically significant relationship between higher NGAL and ADPKD progression was observed.



Conclusions: We did not observe a relationship between NGAL and CKD progression in patients with genetically confirmed ADPKD; however 18% of patients had undetectable plasma NGAL levels. This raises doubt about the utility of the current NGAL assay as a biomarker for CKD progression in this population. The new extended range NGAL assay may prove more useful.

SA-PO837

Urinary Vitamin D Binding Protein: A Potential Novel Marker of Interstitial Inflammation and Fibrosis Katarina Mirkovic,[1] Carolina R.C. Doorenbos,[1] Wendy Dam,[1] Hiddo Jan Lambers Heerspink,[2] Ferdau L. Nauta,[1] Maartje C.J. Slagman,[1] Andrea B. Kramer,[1] Ron T. Gansevoort,[1] Jacob van den Born,[1] Gerjan Navis,[1] Martin H. De Borst.[1] *[1]Nephrology; [2]Clinical Pharmacology, University Medical Center Groningen, University of Groningen, Netherlands.*

Background: The severity of interstitial fibrosis strongly predicts progression of renal function loss. We explored the role of urinary vitamin D binding protein (uVDBP), a low-molecular-weight protein reabsorbed by receptor-mediated uptake in tubular cells, as a marker of tubulointerstitial inflammation and fibrosis in rats and tested if uVDBP responds to therapy intensification in humans.

Methods: Wistar rats were sacrificed at 6, 12, 18, 24, and 30 wks after unilateral adriamycin proteinuric (UAP) nephropathy (n=60). Saline-treated rats (n=30) served as controls. uVDBP was measured by ELISA and related to interstitial α-smooth muscle actin (α-SMA) and collagen III staining and macrophage accumulation. uVDBP was also measured in subjects with normoalbuminuria or microalbuminuria (both n=100), and in 47 stage I-III non-diabetic CKD patients after two 6-wk treatment periods: ACE inhibitor (ACEi) under normal sodium (NS) or ACEi+ARB under low sodium (LS) diet (intensified therapy).

Results: In rats, uVDBP was increased at 6 wks after UAP induction (523 [236-1235] ug/d vs controls 6 [3-13], p<0.01) and increased over time (30 wks: 940 [656-2107] vs 168 [105-342] ug/d, p<0.01). uVDBP was associated with interstitial α-SMA (standardized β 0.43, p=0.01), collagen III (st.β 0.44, p=0.02) and macrophage accumulation (st.β 0.47, p=0.01) independently of albuminuria.

In humans, uVDPB was increased in microalbuminurics (15 [8-38] ug/d) and in CKD patients (ACEi+NS: 4446 [712-14214] ug/d) compared to normoalbuminurics (7 [3-18] ug/d, p<0.001). uVDBP responded to therapy intensification in CKD patients (ACEi+ARB+LS: 987 [200-3498], p<0.001 vs ACEi+NS), but remained >100-fold higher than in normoalbuminurics (p<0.001).

Conclusions: uVDBP may be a novel urinary biomarker of tubulointerstitial inflammation and fibrosis. Persistently elevated uVDBP suggests ongoing interstitial damage despite optimal renoprotective therapy in CKD patients.

SA-PO838

The Atlas Database of Kidney Tubular Proteins Is Refined with the Gene Expression Information for New Urinary Biomarkers Discovery Hidehiko Fujinaka,[1] Tadashi Yamamoto.[2] *[1]Institute for Clinical Research, Niigata National Hospital, Kashiwazaki, Niigata, Japan; [2]Structural Pathology, Institute of Nephrology, Niigata University, Niigata, Japan.*

Background: Recently, several proteins have been proposed as new urinary biomarkers of kidney injury. But they do not always identify the injured sites of kidney nephron segments, especially in case the biomarker may be also derived from plasma. An ideal biomarker must originate from injured cells. Discovery of new urinary biomarkers originated from the specific nephron segments or cells may be difficult in patients with massive proteinuria since most of proteins arederived from plasma. We compared the immunohistochemistry atlas database of kidney tubular proteins (http://www.proteinatlas.org/) with our microarray database of kidney genes expression for new biomarkers discovery.

Methods: Kidney tubule-predominant proteins were defined that the antibody staining intensity level in tubular cells was strong (weak or negative in glomerular cells) or moderate (negative in glomerular cells). The microarray analysis was done by preparation of Cy5-labeled cRNA from human glomeruli and Cy3-labeled cRNA from human cortices. The genes of positive Cy3/Cy5 ratio were defined as kidney tubule-predominant genes.

Results: From the atlas database of immunohisotochemistry, 468 proteins were determined as kidney tubule-predominant proteins. Among genes of these 468 proteins, 268-gene-derived proteins were confirmed kidney tubule-predominant genes, some of which were established tubule-predominant proteins such as SLC12A1 and FABP1. Among the 268 kidney tubule-predominant proteins, 62 proteins were listed in the urinary proteome database (http://141.61.102.16/urine/). In the listed 62 proteins, the expressions of CALB1 (in distal tubule cells) and ALDOB (in proximal tubule cells) were reduced in kidney sections with tubulointerstitial injury, and urinary CALB1 and ALDOB were shown reduced in some patients with tubulointerstitial injury by Western blotting.

Conclusions: The atlas database of kidney tubular proteins can be refined by combination with the gene expression information, which may be a good tool for new urinary biomarkers discovery.

Funding: Government Support - Non-U.S.

SA-PO839

Renal miRNA-mRNA Expression Signatures in Progressive Chronic Kidney Disease Michael Rudnicki,[1] Paul Perco,[2] Barbara D'Haene,[3] Judith Sunzenauer,[1] Pieter Mestdagh,[3] Jo Vandesompele,[3] Bernd Mayer,[2] Gert J. Mayer.[1] *[1]Dept. of Internal Medicine IV - Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria; [2]Emergentec Biodevelopment GmbH, Vienna, Austria; [3]Biogazelle, Zwijnaarde, Belgium.*

Background: MicroRNAs (miRNAs) significantly contribute to the pathophysiology of chronic kidney disease (CKD) progression but their association with clinical outcome remains poorly understood.

Methods: Large scale miRNA and mRNA expression profiling was performed on 45 renal biopsies from patients with proteinuric kidney diseases. Patients were defined as progressive (doubling of serum-creatinine or end-stage renal disease during follow up) or stable. Median follow-up time was 44 months. Differentially expressed miRNAs between stable and progressive patients were determined, and inversely correlated to global mRNA expression. These miRNA-mRNA pairs were further characterized.

Results: In progressive subjects 12 miRNAs (miR-140-3p, miR-148a, miR-190, miR-192, miR-192*, miR-194, miR-204, miR-206, miR-216b, miR-30d, miR-30e*, miR-532-3p) were downregulated, which correlated with upregulated mRNAs involved in inflammatory response, cell-cell-interaction, metabolism, apoptosis, and intracellular signalling. While we also observed upregulation of 9 miRNAs (miR-142-3p, miR-143-5p, miR-150, miR-223, miR-223*, miR-31, miR-31*, miR-511, miR-629*) in progressive cases, these did not correlate to a significant extent with downregulated mRNAs. Eight of the 12 downregulated miRNAs were associated with creatinine at biopsy and creatinine at follow-up, proteinuria at follow-up, and the degree of pathohistological injury. Bioinformatics analysis of predicted targets of these 12 miRNAs revealed 2 groups of upregulated genes (CCR7, CCL19, CXCL1, S1PR4, PLCB2, and RASGRP2, RASGRP1, ITPR3), which positively correlated with arterial hyalinosis, tubular atrophy/interstitial fibrosis and histological diagnosis.

Conclusions: We identified renal miRNA- and mRNA-profiles being differentially expressed in progressive chronic kidney disease. These miRNAs and mRNAs were mainly associated with inflammatory pathways, and the degree of expression correlated with renal disease severity.

Funding: Government Support - Non-U.S.

SA-PO840

Mass Spectrometry- and Antibody-Based Proteomics of Human Kidney Tissues for Clinical Application Tadashi Yamamoto, Hidehiko Fujinaka. *Department of Structural Pathology, Institute of Nephrology, Niigata University, Niigata, Japan.*

Background: The glomerulus and other compartments of the human kidney are aimed to analyze by proteomics using mass spectrometry (MS) and antibody (Ab)-based immunohistochemistry (IHC).

Methods: Proximal tubules, distal tubules and collecting ducts were differentiated in formalin-fixed paraffin-embedded (FFPE) human kidney sections by IHC using anti-AQP1, calbindin and AQP2 antibodies, respectively and a laser-capture microdissection (LCMD) method. To apply proteomics to clinic, a new method, On-Site Direct Digestion (OSDD) method was developed to prepare peptides efficiently from FFPE tissues since conventional methods were not satisfactory for MS sample preparation from tiny FFPE tissue samples such as 1 mm^2 area-tissue section, which corresponds roughly to 50 human glomerular sections. To elucidate difference in glomerular proteome, 50 glomerular sections were collected from each FFPE kidney biopsy specimens and peptides were prepared by the OSDD method.

Results: Approximately 500-3000 proteins were identified in each FFPE 1 mm^2 area-section of the kidney compartments. The numbers of identified proteins were significantly higher (about ten-times) than the conventional methods. The results were summarized into proteome databases, which provide MS-based information and protein localization in the kidney. Proteomes of the glomerulus were compared between normal controls and glomerular sclerosis or other glomerular diseases, which confirmed glomerular expression of already-known proteins but also elucidated not-known disease-unique proteins. These studies also proposed protein enrichment and interactions in glomerular disease conditions.

Conclusions: Human kidney compartments, such as the glomerulus and other nephron segments were analyzed by MS and Ab-based proteomics. The OSDD method was successfully developed for analysis of proteomes of small compartments in FFPE human biopsy samples, which provided new significant information for understanding physiology and pathology.

Funding: Government Support - Non-U.S.

SA-PO841

Massively Parallel Sequencing of Human Urinary Exosome/Microvesicle Nucleic Acids Reveals a Predominance of Non-Coding RNA Leileata M. Russo,[1] Kevin Miranda,[2] Anna Scott.[1] *[1]Exosome Diagnostics, Inc., New York, NY; [2]Program in Membrane Biology, Massachusetts General Hospital, Boston, MA.*

Background: The recent demonstration that exosomes/microvesicles (collectively referred to as microvesicles) contain RNA and DNA has sparked much interest in their use as nucleic-acid based biomarkers for the non-invasive analysis of disease. We have previously shown that high integrity RNA can be isolated from micorvesicles and here we determine the complete nucleic acid content of urinary microvesicles using massively parallel sequencing.

Methods: Extraneous nucleic acids were digested using RNase and DNase treatment and the microvesicle inner nucleic acid cargo was analyzed without DNase digestion (to examine both DNA and RNA based sequences) and with DNase to examine only RNA sequences using the Illumina Genome Analyzer.

Results: Approximately 18 million reads were found to map to the human genome. A substantial proportion (~88%) of reads aligned to ribosomal RNA sequences. Of the remaining sequences, ~35% aligned to protein coding genes and splice sites encompassing approximately 16,000 of the known ~23,000 protein coding genes of the human genome. Analysis of protein coding genes specific to the renal and genitourinary tract revealed that complete segments of the renal nephron and collecting duct as well as genes indicative of the bladder and prostate could be identified. Surprisingly, the remaining ~65% of sequences aligned to non-coding and repeat sequences which were shown to be RNA based (ncRNA).

Conclusions: This data supports the use of deep sequencing of microvesicles to analyze both coding and non-coding RNA which is thought to play an emerging role in cell regulation broadening the potential of microvesicles as a unique source of non-invasive biomarkers for disease monitoring without the need for invasive biopsy. Deep sequencing may be used to identify novel ncRNA sequences (beyond miRNA) that may be useful in the identification of much needed renal disease biomarkers.

Funding: Private Foundation Support

SA-PO842

Distribution of Nucleic Acids in Urinary Exosomes/Microvesicles Sub-Fractionated via Percoll Gradient Leileata M. Russo, Guillermo Salazar, Anna Scott, Kendall Bate. *Exosome Diagnostics, Inc., New York, NY.*

Background: Urinary exosomes/microvesicles (collectively referred to as microvesicles) may be used to non-invasively examine the transcriptional profile of the kidney without the need for biopsy. However, before this powerful technology can be utilized, it is important to understand the distribution of mRNA, rRNA, small RNA (including miRNA) and DNA within microvesicles. Here we examine the nucleic acid content of urinary microvesicles fractionated on a Percoll gradient to better understand their utility as a potential non-invasive source of nucleic acid biomarkers for acquired renal disease diagnosis and monitoring.

Methods: Microvesicles were isolated via differential centrifugation to remove whole cells and cellular debris and the microvesicle pellet was then fractionated on a Percoll gradient and the nucleic acid composition of each fraction determined. RT-qPCR was used to examine transcript expression in each Percoll fraction.

Results: Analysis of various renal mRNA transcripts including H+/Cl- exchange transporter 5 (CLCN5), cubilin (CUBN), megalin (LRP2) as well as bladder markers including uroplakin (UPK2) were shown to have a strong correlation with the presence of rRNA detected via RT-qPCR of 18S rRNA and via the correlation of 18S / 28S rRNA peaks analyzed via the Agilent Bioanalyzer. Small RNA and high integrity RNA (with 18S / 28S rRNA peaks) was present in similar fractions. Genomic DNA was not contained within microvesicles and could be removed via DNase digestion of the microvesicles prior to their lysis.

Conclusions: Protein coding transcripts detected at the mRNA level correlated strongly with the amount of rRNA (R^2=0.78 to 0.94) suggesting that rRNA is packaged together with mRNA and that the two are not present in separate microvesicle populations. Genomic DNA may co-isolate with microvesicles thus it is important that DNA is removed or mRNA specific primers and probes are used for transcriptional analysis in microvesicles. Overall, these findings support the use of rRNA integrity as a quality control of general RNA integrity which is critical when using microvesicles as a source of non-invasive transcriptional biomarkers for renal disease diagnosis.

Funding: Pharmaceutical Company Support - Exosome Diagnostics, Inc.

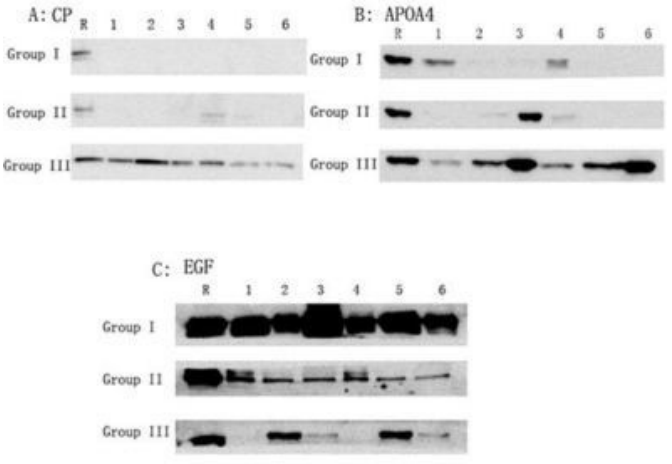
SA-PO843

Establishment of Urinary Proteome and Identification of Protein Biomarkers of Metabolic Syndrome with Early Renal Injury Xuejiao Liu,[1] Mingxi Li,[1] Bixia Gao,[1] Jianfang Cai,[1] Youhe Gao,[2] Xuemei Li,[1] Xuewang Li.[1] *[1]Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Physiology and Pathophysiology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, Beijing, China.*

Background: To identify potential urinary biomarkers of metabolic syndrome (MetS) with early renal injury by spectral counting and iTRAQ quantitative proteomic approaches.

Methods: 1. Participants were divided into three groups: group I health control, group II MetS patients (IDF 2005 criteria) without MAU and group III MetS patients with early renal injury (20μg/min≤UAE<200μg/min and eGFR≥60mL/min.1.73m2). 2. Overnight urine were collected. After intra-group mixture, proteins were digested by trypsin. 3. Peptides of intra control, group I, II and III were labeled by iTRAQ reagents 114, 115, 116 and 117, and were mixed together. 4. Proteins of three groups were analyzed by LC-MS/MS for spectral counting, and the mixture of labeled proteins were identified by LC-MS/MS for iTRAQ. 5. Differential proteins were determined by ANOVA by both quantitative approaches. 6. Western Blot was applied to validate differentially expressed proteins.

Results: 1. Total of 807, 630 and 456 proteins were identified respectively in group I, II and III, and 566 proteins were identified in iTRAQ labeled sample. 2. Totally, 47 proteinsbetween group III and II, 35 proteins between group III and II were identified differentially by two quantitative approaches. 3. CP and APOA4 in group III was higher expressed than that in group I and II by western blot, and EGF in group II and III was lower than that in group I.



Conclusions: 1. The urinary proteome of MetS with early renal injury is established. 2 Differential proteins between MetS and MetS with early renal injury are identified 3. CP and APOA4 may be biomarkers of MetS with early renal injury, and EGF is a potential biomarker of MetS.

Funding: Government Support - Non-U.S.

SA-PO844

A Novel Approach for Discovering Chronic Kidney Disease Associated Human Podocyte-Enriched Genes Wenjun Ju,[1] Casey Greene,[2] Felix H. Eichinger,[1] Viji Nair,[1] Jeffrey B. Hodgin,[1] Markus Bitzer,[1] Young-suk Lee,[2] Qian Zhu,[2] Min Li,[3] Masami Ikehata,[3] Maria Pia Rastaldi,[3] Clemens D. Cohen,[4] Olga Troyanskaya,[2] Matthias Kretzler.[1] *[1]Internal Medicine, University of Michigan, Ann Arbor, MI; [2]Department of Computer Science, Princeton University, Princeton, NJ; [3]Renal Research Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy; [4]Institute of Physiology, University of Zurich, Zurich, Switzerland.*

Background: Cell-lineage-specific transcripts are essential for differentiated tissue function in metazoan organisms. They are frequently found to be the cause of hereditary disease and mediate end organ specific damage in acquired chronic diseases.

Methods: We developed a genome-scale method to identify genes with novel cell-lineage-specific expression, referred to as "in silico nano-dissection", leveraging high-throughput functional genomics data from tissue homogenates using a machine learning framework. This approach was tested in chronic kidney disease (CKD) to predict proteins enriched in renal visceral epithelial cells (podocytes), whose dysfunction is responsible for hereditary proteinuric syndromes and acquired glomerular diseases.

Results: In silico nano-dissection identified transcripts with cell-lineage enrichment for podocytes. Podocyte-enriched expression was validated using the human protein atlas and compared to expression profiles derived from fluorescence tagged podocytes in murine models. Our result indicates that the prediction accuracy exceeds predictions from transcriptional profiles of the murine models. The relevance for human monogenetic glomerular disease was demonstrated by prediction of the recently reported association of MYO1E with autosomal-recessive glucocorticoid resistant nephrotic syndrome. In acquired human glomerular disease, the podocyte gene set correlated significantly and positively with the level of renal impairment in 139 biopsies of CKD patients.

Conclusions: In summary, nano-dissection can define cell-lineage-enriched transcriptome that can serve as highly specific markers of disease stage and provide a starting point for the development of targeted, organ specific therapeutics.

Funding: NIDDK Support

SA-PO845

Urinary Proteomics for Diagnosis and Prognosis of Chronic Kidney Disease Marius A. Øvrehus, Petra Zürbig, Knut Aasarod, Bjorn Egil Vikse, Stein I. Hallan. *St Olav University Hospital, Norway.*

Background: CKD markers beyond eGFR and proteinuria are needed, and urine proteomic analyses have shown promising promising results in some primary kidney diseases. However, few data from unselected CKD patient series have been published.

Methods: 19 consecutive out-patients with CKD stage 4-5 and 17 healthy volunteers were included in a single-blinded proof-of-concept study. Mid-stream morning urinary samples were stored at –80°C. Specific patterns of 273 urinary biomarker peptides (from a total of >5000 peptides measured with CE-MS analysis from Mosaiques Diagnostics) were used to predict classification scores (previously validated cut-off value ≥0.343 indicating CKD). Annual eGFR decline over 2-11 years, proteinuria and other relevant data for characterizing phenotype were recorded.

Results: The CE-MS method diagnosed CKD with a sensitivity/specificity of 0.89/1.00, and ROC analysis estimated an AUC value of 0.972 (95% CI 0.924-1.020). Corresponding data for proteinuria were 0.74/0.82 and 0.70 (95% CI 0.494-0.905). Regression analyses showed that the CE-MS score were strongly associated with both proteinuria and loss of GFR (p<0.001 and p=0.002, respectively) independent of age, sex, BP, and other relevant risk factors. Table 1 exemplifies that patients with a wide range of diagnosis, including nephrosclerosis, were correctly classified.

Exemplary participants illustrating subject characteristics and urine proteomics results

Subject characteristics					Urine Proteomic	
Age (y)	Cause of CKD	GFR (ml/min/1.73m2)	Proteinuria	GFR decline/y	Score	CKD
48	Glomerulonephritis	23	+	-23	0.942	Y
70	Nephrosclerosis	26	+++	-15	0.672	Y
19	Alport syndrome	13	+++	-12	0.468	Y
81	Nephrosclerosis	20	0	-11	0.933	Y
77	Nephrosclerosis, DM	30	++	-11	0.934	Y
47	Glomerulonephritis	9	+++	-10	0.782	Y
77	Nephrosclerosis	30	+	-7	0.451	Y
61	Healthy volunteer	78	0	-1	-0.100	N
33	Healthy volunteer	>90	0	-1	-0.725	N

Conclusions: Although based on small numbers and patients with advanced disease, our data show that urine proteomics have a high diagnostic accuracy for CKD over a wide range of kidney diseases. Strong associations with the rate of eGFR decline and degree of proteinuria indicate a potential for CKD risk staging as well.

Funding: Government Support - Non-U.S.

SA-PO846

Integrated Epigenetic Analysis of Chronic Kidney Disease Yi-An Ko,[1] Davoud Mohtat,[3] Ae Seo Deok Park,[1] Maria Concepción Izquierdo,[3] James M. Pullman,[4] Thomas H. Hostetter,[3] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA; [2]Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; [3]Department of Pathology, Montefiore Medical Center, Bronx, NY; [4]Department of Medicine/Nephrology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Epidemiological data indicate the adverse intrauterine and postnatal environment have long lasting roles in chronic kidney disease (CKD) development, epigenetic information can be a plausible carrier for mediating the effects. However, as of yet epigenetic differences have not been shown between control and CKD kidneys, we performed genome wide analysis for cytosine methylation (CM) and histone modification (HM) in normal and CKD human kidney tissue samples.

Methods: The DNA methylation profiles in micro-dissected human kidney tubule samples of patients with moderately advanced CKD are used for a genome-wide restriction enzyme based CM assay. In addition, the ChIP-seq data were generated with a panel of histone marks (H3K4me1, H3K3me3, H3K9ac, H3K36me3, H3K27me3) using human kidney lysate. The chromatin annotation with CM data and gene expression data are integrated in a genome wide manner.

Results: We observed differences both in CM and HM when comparing CKD to healthy human kidney tissues. Most differentially methylated regions (DMR) (51%) localized to gene body and were relatively rare on promoters. The difference in CM level was modest (<20%) and more loci showed loss of CM in CKD samples. Gene ontology showed enrichment for regions/genes involved in cell adhesion, development and immune processes (key pathways in CKD progression) influenced both by CM and the gene expression level. The DMRs showed the most overlap with H3K4me1 modification and computationally these regions were annotated as enhancers.

Conclusions: Our report is the first to demonstrate epigenetic differences in tubule epithelial cells obtained from patients with CKD, raising the possibility that epigenetic deregulation could play a critical role in CKD development via influencing core profibrotic pathways.

Funding: Other NIH Support - This Work Is Supported by the NIH Roadmap Epigenomics Program, 5R01DK087635-02 Susztak and Greally

SA-PO847

Propensity-Matched Mortality Comparison of Peritoneal Dialysis Patients with Technique Failure Jinn-Yang Chen. *Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: Comparisons of mortality in peritoneal dialysis patients with technique failure and matched hemodialysis patients are lacking. We aimed to compare survival of peritoneal dialysis patients with technique failure and hemodialysis by intention-to-treat analysis in a matched-pair cohort.

Methods: We estimated the propensity score for peritoneal dialysis prescription at the start of follow-up with a logistic regression model that included all listed covariates as predictors. We constructed matched pairs with a greedy matching 5→1 algorithm by randomly selecting for each peritoneal dialysis patient with score p a corresponding hemodialysis patient with score between p + 0.1 and p - 0.1. The degree of matching was checked by standard difference. The hazzard of survival was estimated by Cox model robust standard errors.

Results: During this period, 973 patients experienced technique failure. We matched 952 patient pairs from a retrospective cohort of who initiated dialysis from 2005~2008 in Taiwan. In the primary intention-to-treat analysis of survival from day 0, mortality hazzard was higher for peritoneal dialysis patients with technique faioure than for hemodialysis patients (PD vs. HD, HR= 2.95, 95% CI: 2.54~3.41, p<0.001).

The hazzard was especially high at the second year. When stratified by age group (>= or< 65 years), with/without diabetes and with/without cardiovascular disease, PD patients all have higher hazzard compared to matched HD patients.

Conclusions: Peritoneal dialysis patients with technique failure have excess mortality risk comared to their matched hemodialysis patients.

Funding: Government Support - Non-U.S.

SA-PO848

The Detrimental Effects of Comorbidity Burden and Baseline Characteristics on Peritoneal Dialysis Technique Failure in Taiwan: A National Cohort Study 2005~2009 Jinn-Yang Chen. *Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: The comorbidity burdens were different between hemodialysis (HD) and peritoneal dialysis (PD) patients. This study aimed to compare comorbidity burdens and to identify baseline and time-dependent risk factors for PD technique failure.

Methods: Those who have survived for the first 90 days among patients initiated dialysis between July 1, 2005 and December 31, 2008 were analyzed. A novel comorbidity index developed for Taiwan dialysis patients was used to quantify comorbidity burden. PD technique failure was defined as shifting to HD for more than 30 days or death. The outcome was censored by transplantation, recovery of renal function and loss of follow-up. An extended Cox's proportional hazard model was used to estimate hazard ratios.

Results: HD patients have higher comorbidity score than that of PD patients (median score, HD vs. PD= 6 vs. 4). Among 3308 PD patients, 439 patients (13.3 %) died, 534 patients (16.2%) were transferred to haemodialysis, 105 patients (3.2%) received renal transplantation and 126 patients (3.8 %) loss follow up. Age, male gender (HR=1.24, 95% CI: 1.09~ 1.42, p <0.001), and peritonitis-free survival as time-dependent variable (HR=2.15, 95% CI: 1.75~ 2.65, p=0.006) affected PD technique survival independently. Icodextrin use before the attack of peritonitis did not improve PD technique survival. Diabetic patients had poorer PD technique survival (HR=1.91, 95% CI: 1.65~2.20, p<0.001). SLE (HR=1.68, 95% CI: 1.11~2.54, p=0.013) affect PD survival, while polycystic kidney disease (HR=0.88, 95% CI: 0.53~1.44, p=0.603) did not affect PD technique survival. The chance of PD technique survival for those who had comorbidity score > 10 were only 25% after 3 years.

Conclusions: High comorbidity burden affected the technique survival of PD patients in Taiwan.

Funding: Government Support - Non-U.S.

SA-PO849

A Retrospective Analysis of Patient Outcomes on Peritoneal Dialysis: Technique and Patient Survival and the Impact of Switching to Haemodialysis Shiv Bhutani, Jernej Pajek, Angela M. Summers, Helen Hurst, Paul E. Brenchley, Anand Vardhan, Alastair J. Hutchison. *Manchester Institute of Nephrology & Transplantation, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: PD remains an integral form of renal replacement therapy and has witnessed changes in the last decade including increase in automated PD, assisted PD, use of icodextrin and biocompatible solutions. To establish the impact of prevailing PD practice at our centre we carried out a retrospective outcome analysis.

Methods: Medical records of all patients commencing PD over a 7 year period between 2004 and 2010 to the end of the observation period in March 2011 were reviewed. Data collected included demographic, clinical, PET and adequacy. Outcome was analysed in terms of patient and technique survival using Cox's proportional hazards model.

Results: 286 patients commenced PD in the seven year period. Mean age was 55.2y (SD 17.6), 39% were female and median follow-up was 24 months (Range 0.8-84). 76 patients (27%) were transplanted and 102 (36%) died in the observation period. Patient survival probabilities at 3 and 5 years were 0.69 and 0.53, technique survival 0.67 and 0.58, transplantation 0.23 and 0.32 respectively. Peritonitis was the main cause for technique failure (42%) while ultrafiltration failure 6.3%. High Davies comorbidity grade, creatinine and BMI were independent predictors of technique failure. Sudden cardiovascular events were the predominant mode of death (31%), followed by debilitation (19%) and peritonitis (13%). PD failure was an independent predictor of death. When successful switch to HD was analysed, haemodialysis via catheter presented an elevated death hazard compared to staying on PD or HD through fistula.

Conclusions: Technique and patient survival of our cohort is comparable to published reports. However, the incidence of ultrafiltration failure and predictive value of residual renal function in technique survival are less so. Cardiovascular mortality and morbidity from peritonitis are predominant adverse events despite achieving recommended peritonitis targets. Nature of vascular access has serious impact on outcome when switching from PD to HD.

Funding: Government Support - Non-U.S.

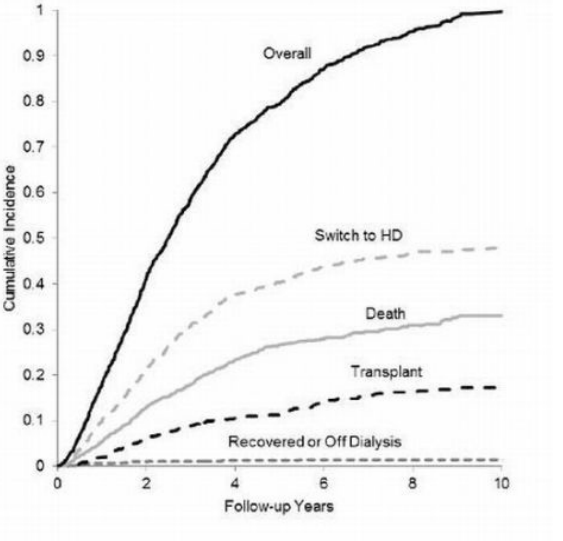
SA-PO850

Technique Survival for Incident Peritoneal Dialysis (PD) Patients: The Kaiser Permanente Experience Wan-ting Yang,[1] Margo A. Sidell,[2] Jason Jones,[2] Victoria A. Kumar.[1] *[1]Nephrology, Kaiser Permanente, Los Angeles, CA; [2]Research and Evaluation, Kaiser Permanente, Pasadena, CA.*

Background: Few large studies have focused on outcomes for peritoneal dialysis (PD) patients in the United States (US). Our aim was to examine technique survival among patients who initiated PD at our institution over the past decade.

Methods: We used an internal ESRD registry to identify all patients who initiated PD between January 1, 2001 and December 31, 2010. Inclusion criteria included age > 18 years and 1 year of prior KP membership at initiation of PD. Patients were censored from the technique survival analyses at the earliest of any of the following: transplantation, recovered GFR, study end, loss of KP coverage or death. Patients who transitioned to HD temporarily were allowed up to 120 days off PD, after which they were deemed technique failures.

Results: A total of 1,710 patients initiated PD during the study period and 1,378 patients met inclusion criteria. Mean patient age was 56.5±14.0 years. Approximately 54% of patients were male and 63% were diabetic. The median size of individual PD programs was 42 patients/year (range 15-115). One and two year technique survival was 83% and 63%, respectively. Technique failure by competing risks is plotted below.



Two year technique survival for patients with ESRD due to cystic kidney disease, diabetes mellitus, glomerulonephritis, hypertension and other was 50%, 59%, 65%, 68% and 73% respectively, p=.004. For every ten year increase in age at initiation of PD, the RR of technique failure was 1.06, p=0.02. Vintage, sex and race were not associated with technique survival in our study.

Conclusions: Cause of ESRD and baseline age were associated with technique survival in our patients, while baseline vintage, sex and race were not. Technique survival in our study may reflect center size and experience.

Funding: Pharmaceutical Company Support - Baxter Healthcare Incorporated

SA-PO851

Combination Therapy with Peritoneal Dialysis and Hemodialysis from the Initiation of Dialysis Preserve the Residual Renal Function and Serum Albumin-Retrospective Cohort Study for 30 Months- Atsushi Ueda,[1,3] Aki Hirayama,[2] Chie Saito,[3] Kunihiro Yamagata.[3] *[1]Kidney & Dialysis Center, Namegata District General Hospital, Namegata, Ibaraki, Japan; [2]Tsukuba University of Technology, Tsukuba, Ibaraki, Japan; [3]University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: In ASN 2011, we reported advantages of the combination of peritoneal and hemodialysis (PD+HD) from the initiation of dialysis therapy on the patients' survival rate compared to PD or HD groups. In this study, we investigated the impact of PD+HD on the residual renal function (RRF), and the relation between RRF and oxidative stress.

Methods: We employed 86 end-stage renal disease patients received an adequate explanation about the three modalities and selected by our criteria (HD=52, PD=21 and PD+HD=21). Daily urine volume as a marker of RRF, blood analysis data, peritoneal permeability and serum beta-2-microglobulin (β_2MG) from the start of dialysis until 30 month in every 6 month were measured. Pentosidine were measured in the 35 patients at the start of dialysis and 12 month later.

Results: Urine volume at the start in HD group was 991±500mL/day, and decreased rapidly from 6 to 30 months (136±260 mL/day). Urine volume in PD group gradually declined from 6 month and maintained until 30 month (1077±813 mL/day). However, that in the PD+HD group was kept during 30 months (1234±684 mL/day). The peritoneal permeability was not different between PD and PD+HD group. Albumin level in PD group was significantly lower than that of the other groups from 6 to 30 months. β_2MG levels in PD+HD group in 0 and 6 months were lower, but there were no significant differences among the three groups in 12, 18 and 24 months. There was no correlation between pentosidine level and urine volume at start of dialysis, however, there was a negative correlation in 12 month.

Conclusions: It is considered that RRF is the most important factor, and serum albumin level is also meaningful for dialysis patients' prognosis. Our study demonstrated that the PD+HD therapy showed both advantages to preserve RRF and serum albumin. Moreover, the patients with preserved RRF indicated lower oxidative stress. Therefore, these beneficial effects may bring an excellent prognosis.

SA-PO852

Is Peritoneal Dialysis Better than Hemodaialysis for ESRD Patients with End-Stage Liver Disease? Chih-Chiang Chien. *Chi-Mei Medical Center.*

Background: Patients with end-stage renal disease (ESRD) are at a higher risk for chronic hepatitis, liver cirrhosis (LC) and death than the general population. Optimal renal replacement therapy for ESRD patients concomitant end-stage liver disease remains controversial. We investigated the long-term outcome among dialysis patients in an endemic area for chronic liver disease.

Methods: We did a longitudinal cohort study and selected all adult ESRD patients (≥18 years old) on maintenance dialysis who began renal replacement therapy between January 1st, 1999, and December 31st, 2000 (n = 12902). For 11293 incident hemodialysis (HD) and 761 peritoneal dialysis (PD) patients, follow-up was from the start of dialysis until the date of death or the end of database (December 31, 2008). A Cox proportional hazards model identified the risk factors for all-cause mortality.

Results: Patients on PD were predominantly younger than those on HD. There were many more HD than PD patients who had DM and cardiovascular diseases. However, there were no significant differences between these 2 groups based on HTN, PAD, LC, cancer, dementia, and hemiplegia or paraplegia. The percentage of LC was 6.2% in HD patients and 5.3% in PD patients. After adjustment, male patients had a higher mortality rate than female patients. Other than well-known risk factors, LC (hazard ratio [HR] 1.473, 95% CI: 1.329-1.634) and dementia (HR 1.376, 95% CI: 1.083-1.750) were also independent predictors of mortality. With all-cause mortality as the outcome, there were only 3 significant factors that interacted with dialysis mortality: (1) DM, (2) chronic lung disease, and (3) dementia.

Conclusions: In conclusion, 3 baseline comorbidities—DM, chronic lung disease, and dementia—had different effects on long-term outcomes in HD and PD patients. This nationally representative study of incident dialysis patients in an endemic area for chronic hepatitis shows that LC was an important predictor of mortality; however, the effect on mortality was not different between HD and PD patients.

SA-PO853

N-Terminal Pro-Brain Natriuretic Peptide as Useful Predictor for Decline of Residual Kidney Function in Peritoneal Dialysis Patients Takeshi Yokoyama,[1] Katsuomi Matsui,[2] Tsutomu Sakurada,[2] Yusuke Konno,[1] Kenjiro Kimura.[2] *[1]Nephrology and Hypertension, Kawasaki Municipal Tama Hospital, Kawasaki, Kanagawa, Japan; [2]Nephrology and Hypertension, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.*

Background: Preservation of residual kidney function (RKF) has been recognized as an independent predictor of survival in peritoneal dialysis (PD) patients. Although heart failure is known to associate with decline of RKF, little is known which neurohumoral factorsare helpful to predict decline of RKF. The aim of present study is to determine which neurohumoral factors are helpful to predict decline of RKF.

Methods: Neurohumoral factors such as N-terminal pro-brain natriuretic peptide (NT-pro-BNP), BNP, atrial natriuretic peptide (ANP) were measured in 28 PD patients at the initiation of PD. We calculated RKF as the half of the sum of weekly urea and creatinine clearances normalized to 1.73m^2 BSA, and defined the rate of decline of RKF as [(latest RKF - intial RKF) / duration of PD]. We examined the correlations between the rate of decline of RKF and the neurohumoral factors. Furthermore, the patients were divided into 2 groups based on NT-pro-BNP value and we compared the initial parameters between 2 groups.

Results: Average RKF at the initiation of PD was 40.7 24.5 L/week/1.73m^2 and average rate of decline of RKF was -1.10 0.97 L/week/1.73m^2/month. Rate of decline of RKF was significant correlated with NT-pro-BNP (r = -0.43, p > 0.05), but not correlated with BNP and ANP. In high NT-pro-BNP group, proportion of diabetic nephropathy and daily consumption of peritoneal dialysate were singificantly higher than low NT-pro-BNP group. BNP and ANP were also significantly higher in high NT-pro-BNP group.

Conclusions: Among the neurohumoral factors, NT-pro-BNP was the most useful predictor for decline of RKF in PD patients.

SA-PO854

Prospective Multicenter Cohort Study of the Effect of Residual Renal Function on Clinical Parameters in Patients with Peritoneal Dialysis: PDR-CS Hitoshi Sugiyama,[1] Yasuhiko Ito,[2] Kazuhiko Tsuruya,[3] Hisako Yoshida,[3] Hiroki Maruyama,[4] Shin Goto,[4] Masaaki Nakayama,[5] Hidetomo Nakamoto,[6] Hiroshi Morinaga,[1] Seiichi Matsuo,[2] Hirofumi Makino.[1] *[1]Okayama University, Japan; [2]Nagoya University, Japan; [3]Kyushu University, Japan; [4]Niigata University, Japan; [5]Fukushima Medical University, Japan; [6]Saitama Medical University, Japan.*

Background: Previous literatures and the guidelines for PD in the 2009 Japanese Society for Dialysis Therapy document the importance of the residual renal function (RRF) in PD patients.

Methods: A prospective multicenter observational study, known as the Peritoneal Dialysis Registry and Cohort Study (PDR-CS), began enrolling patients in December 2009 (UMIN000003659). This investigation analyzed the clinical parameters significantly associated with RRF as evaluated based on the daily urine volume (UV).

Results: Four national university hospitals participated in the PDR-CS and 178 patients were enrolled as of December 2010 (mean age, 58.8 years; male, 68.0%; diabetic nephropathy (DM), 30.3%). The analysis showed that 86.4% and 68.5% of the maintenance PD patients (n=140) exhibited a daily UV of at least 100 ml and at least 400 ml, respectively. The group with a daily UV of 100 ml or over (n=121) showed significant differences in the duration of PD and serum beta 2-microglobulin (B2M, p<0.01) in comparison to the group with a daily UV under 100 ml (n=19). Dividing the patients based on a daily UV of 400 ml or over (n=87) or under 400 ml (n=40) revealed dramatic differences in the 8 clinical parameters, including PD duration (30 vs. 42 months, p<0.01), PD drain volume (391 vs. 1000 mL, p<0.01), cause of ESRD (DM, 21.8 vs. 42.5%, p<0.05), PD Kt/V (1.10 vs. 1.53, p<0.01), residual renal Kt/V (0.74 vs. 0.06, p<0.01), total Kt/V (1.83 vs. 1.65, p<0.01), serum phosphate (5.00 vs. 5.53 mg/dL, p<0.05), and B2M (21.1 vs. 35.4 mg/L, p<0.01).

Conclusions: The current study suggested that PD patients with a daily UV of 400 mL or over exhibited better demographic and clinical data. Further studies are necessary to clarify the daily UV of 400 mL or over as being an adequate marker of RRF and to elucidate its association with the patient prognosis in PD.

Funding: Private Foundation Support

SA-PO855

The Glomerular Filtration Rate Estimated from Serum Creatinine Predicts Residual Renal Function in Peritoneal Dialysis Patients Seokhui Kang, Kyu-hyang Cho, Kyung Woo Yoon, Jong-Won Park, Jun-Young Do. *Department of Internal Medicine, Yeungnam University Hospital.*

Background: The aim of this study was to evaluate the clinical relevance of estimated GFR (eGFR) equations in predicting residual renal function (RRF) in peritoneal dialysis (PD) patients.

Methods: We reviewed the medical records to identify all adults who underwent PD between April 2001 and March 2012. We identified 515 patients whose creatinine clearances were measured by both eGFR and 24-hr urine, dialysate collections one or more times, for a total of 3141 pairs of measurements. The eGFRs were calculated using Cockcroft-Gault's formula (CG), four variable MDRD formula (MDRD4), six variable MDRD formula (MDRD6), and CKD-EPI formula.

Results: In anuric patients, there was no significant correlation between the eGFRs and peritoneal clearance. In non-anuria patients, significant correlations were noted between the eGFR and residual renal function, total creatinine clearance, but not peritoneal clearance. (male, r = 0.7830 for CG, r = 0.7804 for MDRD4, r = 0.8097 for MDRD6, r = 0.7782 for CKD-EPI; female, r = 0.6717 for CG, r = 0.6610 for MDRD4, r = 0.6584 for MDRD6, r = 0.6624 for CKD-EPI; $P < 0.0001$ for all variables). In males, simple linear regression analyses using eGFRs for the prediction of residual renal function showed that non-standardized-βs of eGFR were 0.783 for CG, 0.780 for MDRD4, 0.810 for MDRD6, and 0.778 for CKD-EPI ($P < 0.0001$ for all variables). In females, non-standardized-βs of eGFR were similar to those in males (0.672 for CG, 0.661 for MDRD4, 0.658 for MDRD6, and 0.662 for CKD-EPI).

Conclusions: The present study demonstrates that residual renal function is associated with eGFR using serum creatinine. The eGFR using serum creatinine may be an alternative method for predicting residual renal function without 24-hr urine collection.

SA-PO856

The Role of Residual Renal Function in Plasma and Dialysate Clearances of Icodextrin in APD Patients Talerngsak Kanjanabuch, Somchai Eiam-Ong. *Chulalongkorn University.*

Background: To investigate the role of residual renal function (RRF) over pharmacokinetics (PK) of icodextrin and its metabolites in APD patients.

Methods: A prospective non-randomized PK study was conducted. Each patients was administered 1.5 L of solution containing 7.5% icodextrin for 12 hours (8:00-20:00). Afterwards the solution was drained and the patients resumed NIPD (1.8-2L/cycle x 5cycles during 20:00-8:00) using glucose-based solution. Icodextrin and its metabolites (DP2-DP7) were measured in blood, urine, and dialysate by HPAE-PAD with Carbopac PA-1 detector and enzymatic methods. After 24 hours, patients were requested to return on days 3,7,14, and 28 for collections of blood, dialysate, and urine samples. The total carbohydrate (CHO) levels were assessed by enzymatic amyloglucosidase method.

Results: Twelve patients (7 with RRF, 5 without RRF) on APD were included. All patients had been used icodextrin during the 12-hour long dwell for at least 28 days. Approximately two thirds of both groups had diabetes. At baseline both groups were similar in baseline demographics, laboratory measurements, and dialysis parameters besides urine volume and renal clearance. Plasma AUC as well as peak and trough levels of icodextrin were significant higher in the no-RRF group. However, differences between the peak and trough concentrations of icodextrin in both groups were <20%. Peak plasma levels of icodextrin metabolites (DP2-DP7) were observed at 12 and 14 hours after icodextrin administration in the group with RRF and without, respectively. There were trends of lower individual and total plasma DP2-7 levels in the group without RRF. However, the levels seemed to be stable during 1-month period of observation. Of interest, the total CHO concentration faster disappeared from the dwelt dialysate (t0-t12) in the group with RRF but seemingly not differed between groups with or without DM.

Conclusions: RRF plays a significant role in clearance of icodextrin from the plasma and dialysate. Hence administration of icodextrin solution in patients without RRF should warrant for accumulation effects in the long-term usage.

SA-PO857

Skin Autofluorescence, a Measure of Cumulative Advanced Glycation End Products, Is a Strong Predictor of Cardiovascular Events in Patients with Peritoneal Dialysis

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Background: Advanced glycation end products (AGEs) are biomarkers of metabolic stress and are thought to play a role in the pathogenesis of chronic complications of chronic kidney disease. Tissue autofluorescence (AF) is related to the accumulation of AGEs. Evaluation of accumulative AGEs by skin AF predicts cardiovascular outcomes in patients with predialysis CKD and hemodialysis. However, little is known whether skin AF is associated with cardiovascular outcomes in patients with peritoneal dialysis (PD).

Methods: This multicenter, prospective cohort study enrolled 105 PD patients, aged 20-80 years, between April 2007 and March 2009. Skin AF and clinical parameters concerning residual kidney function were measured at baseline and study months 6, 12, and 24. The primary outcome was the occurrence of major cardiovascular events.

Results: There was a progressive and significant increment in skin AF levels from 2.89 ± 0.97 A.U. at baseline to 3.89 ± 1.13 A.U. at months 24. Changes in skin AF levels were significantly associated with changes in serum beta-2 microglobulin (< 0.01) and renal Kt/V (< 0.05). During the follow-up periods (3.46 ± 1.2 years), 32 patients had a major cardiovascular event, of which 7 died from cardiovascular causes. Cox proportional hazards analysis showed that skin AF was a significant predictor of cardiovascular events (hazard ratio, 0.28; 95% CI, 0.12 to 0.66), independently of serum beta-2 microglobulin (hazard ratio, 0.21; 95% CI, 0.12 to 0.67), and renal Kt/V (hazard ratio, 0.19; 95% CI, 0.11 to 0.58).

Conclusions: Skin AF level is associated with residual kidney function, and a strong and independent predictor of cardiovascular events in PD patients.

SA-PO858

The Effect of Residual Renal Function and Other Patient Factors on Gram Positive Peritonitis Outcomes

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Background: Gram positive organisms are the most common cause of peritonitis in patients treated with peritoneal dialysis. Pharmacokinetic studies have indicated that clearance of antibiotics is higher with Continuous Cyclic Peritoneal Dialysis (CCPD) than Continuous Ambulatory Peritoneal Dialysis (CAPD), and that patients who are non-anuric have lower cefazolin concentrations compared to patients who are anuric. Few studies have examined how these and other factors affect peritonitis treatment outcomes.

Methods: Between 2003 and 2010, all gram positive peritonitis episodes treated with cefazolin at a large tertiary care hospital were included. A Cox proportional hazards model was used to examine the relationship between the primary outcome, time to resolution of the intraperitoneal (IP) white blood cell (WBC) count, and the following factors: PD modality, renal creatinine clearance (CrCL), PD vintage, hospitalization during peritonitis treatment, age, change in antibiotic during peritonitis treatment, and cefazolin dose per kilogram of body weight. Polymicrobial infections were excluded.

Results: There were 119 patients with 177 peritonitis episodes in this study. Lower CrCLwas associated with a greater likelihood of resolution of the IP WBC count (p=0.0002). Shorter duration of PD was associated with a greater likelihood of resolution (p=0.005). Interestingly, age was also statistically significant (p=0.03) with older age associated with greater resolution.

Conclusions: Longer PD vintage may be associated with changes to the peritoneal membrane that leads to decreased resolution. The association between greater renal function and non-resolution suggests renal cefazolin clearance contributing to lower cefazolin concentrations and treatment failure. The unexpected association of younger age with non-resolution warrants further investigation.

SA-PO859

Factors Affecting Treatment Outcomes of Peritonitis in Peritoneal Dialysis Patients

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Background: Peritonitis associated with peritoneal dialysis is usually treated successfully. However, failed treatment can lead to relapse, catheter removal, change to hemodialysis, and even death. Few studies have examined factors that affect outcomes of peritonitis treatment.

Methods: Data from all peritonitis episodes at a large tertiary care hospital was collected prospectively from 2003-2010. A Cox proportional hazards model was used to examine the relationship between the primary outcome, time to resolution of the intraperitoneal (IP) white blood cell (WBC) count, and the following factors: PD modality, renal creatinine clearance (CrCL), PD vintage, hospitalization during peritonitis treatment, age, and change to an alternative antibiotic during peritonitis treatment. CrCL was analyzed in three groups: 0 mL/min (group 0), >0 to 5 mL/min (group 1), and >5 mL/min (group 2). Polymicrobial, fungal, and mycobacterial infections were excluded.

Results: There were 215 patients with 393 episodes of peritonitis in the study. Lower CrCL was associated with a greater likelihood of resolution of the IP WBC count (HR 1.52, p=0.0076 for group 1 vs. 2). Treatment at home was associated with a greater likelihood of resolution of the IP WBC count compared to hospitalization (HR 1.59, p=0.0005).

Conclusions: Greater residual kidney function and hospitalization during peritonitis treatment were associated with a decreased likelihood of resolution of peritonitis. PD modality, PD vintage, age, and change to an alternative antibiotic during peritonitis treatment did not significantly affect the primary outcome. Hospitalization was likely a surrogate for more severe peritonitis. However, the surprising association of better renal function with non-resolution suggests that current antibiotic regimens may lead to underdosing and may not account for renal antibiotic clearance.

SA-PO860

Peritoneal Dialysis as Rescue Therapy for Patients with No Vascular Access: A Prospective Study

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Background: The choice between peritoneal dialysis (PD) and hemodialysis (HD) is usually based on clinical criteria and on patient preference. However, some patients are referred to PD because of difficulties in obtaining vascular access for HD and are commonly considered "negative selection" (NEG) patients, who will probably have a worse prognosis than will those who are incident dialysis patients and choose to start PD (POS patients). However, there have been few prospective studies comparing outcomes in such patients.

Methods: We compared POS patients (n=72) and NEG patients (n=14) referred to our PD clinic between Jan. 2009 and Sep. 2011, following them until May 2012.

Results: NEG patients tended to be younger (56 ± 12 vs. 64 ± 16 yrs; p = 0.09), had been on HD for 32 months and had a median of 5 previous vascular access failures. No differences were seen regarding gender, race or prevalence of diabetes (55.6 vs. 35.7%; ns). After 475 ± 240 days of follow-up, 41 episodes of peritonitis had occurred in 29 patients, although there was no difference between POS and NEG patients (33 vs. 36%; ns). All deaths (n = 14) occurred among POS patients. A composite analysis of survival and return to HD showed no significant differences between POS and NEG patients. Analysis of patients that present a failure in PD modality identified peritonitis as a positive risk factor (RR=3.94; p<0.0001), independent of status (POS or NEG).

Conclusions: Our data show that patients referred to PD as a last recourse for dialysis do not present a worse prognosis than do those choosing PD as a first option. However, the occurrence of peritonitis can lead to modality failure, HD no longer being an alternative for some. Therefore, such patients should be closely monitored to avoid this common complication of PD.

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SA-PO861

Peritoneal Dialysis-Associated Peritonitis in Austria: Epidemiology, Risk Factors, and the Role of Oral Active Vitamin D

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Background: Peritonitis is a major complication of peritoneal dialysis being associated with hospitalization, technique failure, and increased mortality. Data on incidence and risk factors for peritonitis are scarce.

Methods: Patients who performed PD between January 2000 and December 2009 in seven Austrian PD-units were included (n=720). Date and culture result for peritonitis, concomitant medication and comorbidities were identified from the patients records.

Results: We collected data from 720 patients representing 1703 patient years and 44.3 % of the PD population treated in Austria in this time period. The median peritonitis incidence rate was 0.34 episodes/patient–year (interquartile range: 0.32-0.39). In a multivariate analysis the risk for peritonitis was decreased by 57 % in patients treated with oral active vitamin D (HR 0.43; 95 % CI 0.28-0.64). Furthermore, renal diagnosis and decreased albumin levels were associated with an increased risk for peritonitis. Detailed analysis revealed that vitamin D especially reduced the risk for gram-positive and -negative episodes of peritonitis, whereas in sterile episodes no significant association could be shown. Beside decreased levels of albumin, higher age, and cardiomyopathy which increased the risk for all-cause mortality, treatment with oral active vitamin D was also associated with a significantly lower risk of death (HR 0.46; 95% CI 0.27-0.81).

Conclusions: In this nationwide retrospective cohort study we identified several factors related to increased risk for peritonitis. Oral active vitamin D was independently associated with decreased risk for peritonitis and all-cause mortality in PD-patients.

SA-PO862

Pets-Related Peritonitis and Its Prevention in Peritoneal Dialysis: A Case Study Mekdess Abebe,[1] Cheryl Laveglia,[2] Nand K. Wadhwa.[1] *[1]Division of Nephrology, Stony Brook University, Stony Brook, NY; [2]Dialysis Clinic, Inc, East Setuaket, NY.*

Background: Many peritoneal dialysis (PD) patients keep animals/pets as life time companion for their social support. However, the interaction with pets can increase the risk for peritonitis. We encountered a patient who kept pets and had recurrent peritonitis due to Pasteurella multocida. This prompted us to evaluate the prevalence of animals/pets in PD patients and to incorporate a training session about pets when the patient initiates PD. Thus we evaluated the prevalence of pets and associated peritonitis before and after the training.

Methods: All PD patients in our dialysis unit were included. A training session was instituted for PD patients who have kept domestic animals as pets. The training included interventions including patient education, counseling on hygiene and placement of barriers limiting the pets' access to the dialysis equipment. Questionnaires related to the interactions of the patients with the pets during treatment and/or set up were distributed to all PD patients before and after the training. The prevalence of pets and peritonitis rates were analyzed.

Results: The patient population included 17 males and 11 females with a mean age of 55 years. The PD patients who kept companion animals were 21% (6/28) compared with 23% (6/26) two years ago. Only 50% (3/6) of patients allowed pets in the room during set up and/or treatment as compared to 83% (5/6) before our intervention. Only one patient reported that pets are allowed in the room during set up and treatment. Two patients don't allow pets in the room during set up but occasionally pets enter the room during treatment. Three patients never allowed their pets to interact during setup, exit site care or treatment. Subsequently we have no episode of peritonitis related to pets in our unit. Our patient with recurrent peritonitis due to Pasteurella multocida has been free of peritonitis for three years after the training on handling of pets during PD treatment.

Conclusions: We recommend a periodic questionnaire and an educational session for all PD patients who have or intend to have pets for their social support to prevent pets-related peritonitis.

SA-PO863

Low Serum Cultured Adipose-Derived Mesenchymal Stromal Cells Ameliorate Rat Model with Zymosan Induced Severe Peritonitis Hangsoo Kim, Masashi Mizuno, Kazuhiro Furuhashi, Takayuki Katsuno, Takenori Ozaki, Kaoru Yasuda, Waichi Sato, Naotake Tsuboi, Yasuhiko Ito, Enyu Imai, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: In peritoneal dialysis,fungal peritonitis is a major problem as the fibrosis can progress rapidly and it may evolve into encapsulating peritoneal sclerosis even after the peritoneal dialysis catheter has been removed.

In this study, we used rat peritonitis model induced by zymosan administration after peritoneal scrape accompanied complement activation and attempted to treat the model by low serum cultured adipose-derived mesenchymal stromal cell(LASC).

Methods: The fungal peritonitis was induced in the rat model by administrating zymosan daily for 5 days after scraping the peritoneum mechanically. The rats were divided into two groups; LASC (L group) or vehicle (V group) administration intraperitoneally with PD solution every day. On day 5, rats were euthanized and the peritoneums were harvested, then the thickness of the peritoneum, the infiltration of inflammatory cells, and the deposition of the complement were compared between the groups.To trace LASCs,CFSE labeled LASCs were administered, then assessed by immunofluorescent staining.For the in vitro study, we obsereved the interaction between mesothelial cells(MCs) and LASCs.

Results: On day 5, microscopic findings in L group had less plaques and less edematous. Histologically, the thickness of the peritoneum, the infiltration of inflammatory cells and the deposition of complements in L group was significantly more reduced than those in V group. In addition, the mesothelial cell layer on the peritoneal surface in L group recovered more quickly compared with that in V group. Also, the layer in L group showed complement regulatory factors clearly. In the tracing study, LASCs were laid mainly on the surface of peritoneum along with the recovered MCs.In vitro study, the supernatant of LASCs stimulated the growth of MCs.

Conclusions: The results suggest that LASCs have the multiple effects on peritoneal damage. In the future, LASC therapy may be useful for treating peritoneal injury during fungal infection of the peritoneum.

SA-PO864

Probiotics Decrease the Incidence of Gram Negative Peritonitis in Patients on Peritoneal Dialysis An-Bang Wu, Ming-cheng Wang, Yu-tzu Chang, Chin Chung Tseng. *Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan.*

Background: Peritonitis may cause catheter removal in uremia patients. Gram negative bacteria (GNB), most coming from intestinal tract, may cause worse result. The probiotics may decrease pathogens in intestinal tract. So we use probiotics to prevent peritonitis, and improve the gastrointestinal function.

Methods: This was a randomized double blind study. The inclusion criteria were 1. Uremia patients on peritoneal dialysis (PD) more than 3 months. 2. Age between 16 and 75 years. The exclusion criteria were 1. Patients had advanced malignance. 2. Patients had more than two episodes of G(+) peritonitis within one year. 3. Patients had expected life less than one year. 4. Patients had drug abuse. 5. Poor compliant patients. 6. Patients had active infection. 7. Patients had uncontrolled autoimmune disease. Fifty patients received probiotics one pack per day (Lactobacillus acidophilus, Bifidobacterium, Enterococcus faecium, Bifidobacterium longum and prebiotics (Fructo Oligo Saccharides)) and the other fifty patients received placebo (prebiotics only). The primary end point was time to the first episode of peritonitis. The secondary outcome was SGA score and serum albumin change.

Results: The first patient was recruited on March 16, 2010 and the last on April 26, 2010. Total 100 patients were included. The baseline characteristics were similar.Eight patients drop out. Two received renal transplantation, two expired, and four shifted to hemodialysis (due to refractory peritonitis, colon perforation, tunnel infection and patient's request). There were 12 episodes of peritonitis including 3 GNB peritonitis. No difference was found in peritonitis incidence between two groups. However, no GNB peritonitis developed since four months of study in two groups. There was no significant difference in the nutrition marker.

Conclusions: There was no significant difference in peritonitis incidence between the two groups. But no GNB peritonitis developed since four months of the study. Both probiotics and prebiotics may prevent the GNB peritonitis in patients receiving PD.

SA-PO865

The Improvement of Peritoneal Dialysis (PD) Device and Biocompatible Dialysate Might Decrease the Frequency of PD-Associated Peritonitis, and Result in Reducing the Risk for Encapsulating Peritoneal Sclerosis Masatsugu Nakao, Keitaro Yokoyama, Izumi Yamamoto, Yudo Tanno, Ichiro Ohkido, Hiroshi Hayakawa, Masato Ikeda, Hiroyasu Yamamoto, Tatsuo Hosoya. *Division of Nephrology & Hypertension Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.*

Background: Peritoneal dialysis (PD)-associated peritonitis is a serious complication in patients undergoing PD that results in discontinuation of PD and encapsulating peritoneal sclerosis (EPS). It is therefore important to elucidate the prevalence of PD-associated peritonitis and the causative pathogens. Particularly in Japan, changes to PD devices and fluid pH, from acidic to neutral, are expected to affect the prevalence of PD-associated peritonitis and associated pathogens, and thus the prevalence EPS.

Methods: This study assessed patients who had undergone PD at our hospital between January 1990 and May 2012. For each PD device, patients were divided according to the following: single-bag and acidic PD fluid (S+A group); twin-bag and acidic PD fluid (T+A group); and twin-bag and neutral PD fluid (T+N group). The prevalence of PD-associated peritonitis, pathogens, and the incidence of EPS were then compared between groups.

Results: The T+A group had fewer cases of peritonitis (76.0±11.7 patient-months vs. 114.8±61.3 patient-months P=0.001) and a lower percentage of *Staphylococcus sp.* (43.5% vs. 26.1%, P=0.001) and *Pseudomonas aeruginosa* (25.8% vs. 5.6%, P=0.0003) than the S+A group. Although the number of peritonitis cases was similar in the T+A and T+N groups (114.8±61.3 patient-months vs. 122.3±60.7 patient- months, NS), the T+N group had a higher percentage of *Streptococcus sp.* (4.9% vs. 27.4%, P=0.001) and Gram-negative rods, excluding *Pseudomonas aeruginosa*, (4.9% vs. 13.9%, P=0.03) than the T+A group. The rate of EPS was 25.2% (24/95 patients) in the S+A group, 6.3% (10/158 patients) in the T+A group, and 0% in the T+N group, showing that the incidence was extremely low or zero with the use of twin bags (P<0.0001).

Conclusions: Changes in PD devices and fluids resulted in a different prevalence of peritonitis, type of pathogen, and incidence of EPS.

SA-PO866

Lack of Patient Free Choice of Technique Predicts Peritonitis in Peritoneal Dialysis Clara Santos, Joao Carlos Fernandes, Ana Marta Gomes, Joaquim Seabra. *Nephrology, CHVNG/E, Portugal.*

Background: Peritonitis is a major complication of peritoneal dialysis (PD) and its occurrence is associated with significant morbidity and mortality. Determining factors that are associated with PD peritonitis might have an important role in its prevention.

Methods: Retrospective longitudinal cohort study of all patients on PD program between 1998 and 2011. Demographic and clinical variables were evaluated and compared between patients who had at least one episode of peritonitis and patients who never had peritonitis. In statistical analysis, we used the chi-square, Student's t test and Mann-Whitney test. In univariate and multivariate longitudinal analysis we used log-rank test and Cox regression model, respectively. A p-value less than 0.05 was considered statistically significant.

Results: We studied 122 patients with mean age 53±15years, 61%male, 16%diabetic, with an average length of stay in PD for 23 months. 140 episodes of peritonitis were identified in 58 patients, yielding a total rate of 1 episode per 20.6 months. 35% of first peritonitis episodes were observed during the first 6 months of treatment. The occurrence of peritonitis was not correlated with gender, age, diabetes, first renal replacement therapy, PD modality, loss residual renal function and D/P creatinine at the time of PD start. In multivariate analysis, obligatory inclusion on PD, compared to patient free choice, was the only predictive factor of the development of peritonitis (HR = 2.05; 95% CI 1.10 to 3.84; p=0.025).

Conclusions: The principal finding of the study was that obligatory inclusion on PD, usually for lack of vascular access for hemodialysis, was the only significant predictive factor of the development of peritonitis. These patients may benefit from a more intense and closely supervised PD training. The fact that 35% of first peritonitis episodes occur during the first 6 months of technique also stresses the importance of more intensive training and attention during the initial period of treatment. The occurrence of peritonitis is higher in patients who start PD without other choice than those who freely choose this technique. Those patients may benefit from more intense training programs and more attention.

SA-PO867

The Long Term Observation of Conservative Therapy for Encapsulating Peritoneal Sclerosis (EPS) Kyoko Kishida,[1] Keitaro Yokoyama,[1] Nanae Matsuo,[1] Masatsugu Nakao,[1] Yudo Tanno,[1] Ichiro Ohkido,[1] Hiroshi Hayakawa,[1] Kenji Kasai,[2] Hiroyasu Yamamoto,[1] Tatsuo Hosoya.[1] *[1]Division of Kidney, Hypertension, The Jikei University School of Medicine, Tokyo, Japan; [2]Division of Internal Medicine, Fuji City General Hospital, Shizuoka, Japan.*

Background: Encapsulating peritoneal sclerosis (EPS) is a severe complication of peritoneal dialysis (PD). For reason that surgical treatment for EPS is technically difficult; in patients who have relieved symptoms of the intestinal obstruction without surgery, the operation for EPS is hesitated even if the risk for the recurrence of intestinal obstruction is not necessarily low. In fact some patients with EPS died due to the recurrence of intestinal obstruction. So it is required to evaluate the validity of this conservative treatment.

Methods: In order to investigate long-termoutcome in patients with EPS, in this historical cohort study, we recruited 50 EPS cases admitted to our Hospital from 1989 November to 2011 May. Patients were divided into two groups, the conservative therapy group and the surgical treatment group, and their outcomes were compared.

Results: Twenty nine of the 50 EPS cases have relieved symptoms of the intestinal obstruction conservatively such as IVH, NPO and so on, but 7 of the 29 cases underwent emergency surgery within a few years because of bowel perforation, and 3 of them died after surgery. For death cases of the conservative therapy group, 8 of them were EPS related-death. The survival rate was higher in the surgical treatment group.

Conclusions: During long-term observation, the prognosis of EPS patients with conservative therapy was poor depend on developing bowel perforation. Accordingly, surgical treatment for EPS should not be hesitated for the reason why it is technically difficult. The indications and timing of surgery for EPS should be reconsidered based on the results of this study.

SA-PO868

Laparoscopic Approach for Evaluation of Encapsulating Peritoneal Sclerosis in Peritoneal Dialysis Patients Yudo Tanno, Masatsugu Nakao, Izumi Yamamoto, Nanae Matsuo, Ichiro Ohkido, Hiroyasu Yamamoto, Keitaro Yokoyama, Tatsuo Hosoya. *Division of Kidney and Hypertension, Jikei University School of Medicine, Tokyo, Japan.*

Background: Encapsulating peritoneal sclerosis (EPS) is a serious complication of peritoneal dialysis (PD). Therefore, assessment of peritoneal injury in patients undergoing PD is important in preventing EPS. Clinical significance of assessing peritoneal permeability with peritoneal equilibration test (PET) and of measuring biomarkers in PD effluent is not established. Furthermore, since examination of peritoneal tissue samples only assesses pathology of parietal peritoneum around the catheter insertion site, it is difficult to predict any existing visceral peritoneal injury from this result. We reported that it was possible to detect early changes observed in the development of EPS by using laparoscopy (Kidney International 2012 in press). Therefore, we studied laparoscopic findings in patients who had undergone PD for more than 4 years.

Methods: 14 patients underwent laparoscopy at the time of PD catheter removal. Duration of PD in these patients was 7.8±4.8 years. Clinically, none of these patients had developed EPS by the time of the investigation. The findings of both parietal and visceral peritoneal tissues were categorized according to color changes, presence of neovascularizations and adhesions.

Results: It was found that longer the duration of PD, the worse the peritoneal injury. Changes in the parietal and visceral peritoneum had heterogeneous distributions. Moreover, with regard to adhesions in the visceral peritoneum, they were dominant in the right lower abdomen. There was discrepancy in the findings between the parietal and visceral tissues, as shown in the table below.

Percentages of macroscopic changes observed in the peritoneum

	color changes	neovascularizations	adhesions
parietal peritoneum	85%	64%	42%
visceral peritoneum	39%	35%	28%

Conclusions: These findings suggest that EPS can occur focally in isolation therefore, blind biopsies of the peritoneum may not be sufficient to detect EPS. For this reason, evaluation of the entire peritoneum by laparoscopy provides more accurate and extensive information about the peritoneal injury.

SA-PO869

Influence of Peritoneal Membrane Phosphate Transport Characteristics on Choice of Peritoneal Dialysis Modality Asmaa Y. Mohammed Al- Chidadi, Andrew Davenport. *UCL Center for Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Hyperphosphatemia is associated with an increased risk of mortality in dialysis populations. There is little data on peritoneal phosphate clearance (PPiCl) in PD patients, and we set out to review its determining factors.

Methods: In a cross sectional observational prospective study, measurements of D/P Pi and D/P Cr were made during a standard PET, and PPiCl with 24 hour PD effluent in 327 consecutive adult PD patients, 161 female (49.2%). Patients were stratified according to their D/PCr and D/PPi and modality (CAPD or APD).

Results: D/PPi correlated with D/PCr together with peritoneal Cr Clearance (PCrCl) and PPiCl. Among anuric patients with Pi≥1.7 there was no significant difference between total PiCl compared to those with Pi<1.7 (47.9 ±37.5 vs 60 ±27.9 l/wk). Similar results were found in those with RRF (44.8 ±42 vs 43 ±28 l/wk). There was no significant difference in dietary protein intake (nPNA) according to Pi level (0.94 ±0.3 vs 0.96 ±0.2). In both anuric patients and those with RRF, high Pi transporters had significantly lower serum Pi, indicating D/PPi as an independent factor predicting hyperphosphatemia (0.86 ±0.3 vs 1.2 ±0.3, p<0.05). There was a significant difference between D/P Pi groups in regard to PPiCl and PCrCl (p<0.05) but not in peritoneal KT/V (p<0.09). PCrCl and PPiCl were significantly higher in CAPD vs APD (36 ±24 vs 24.9 ±27, p<0.001 and 31.7 ±20.7 vs 22 ±24.6, respectively p<0.001). D/PCr was significantly higher in CAPD patients(0.7 ±0.1 vs 0.7 ±0.1, p=0.04). D/PPi did not achieve significance in CAPD.

Among fast average and slow average Pi transporters, PCrCl and PPiCl were significantly higher in CAPD patients (36 ±24 vs 24.9± 27, p< 0.001 in fast average transporters and 32.6 ±17.9 vs 15.9 ±18.9, P 0.03 in slow average transporters and 31.7 ±20.8 vs 22 ±24, P 0.001 in fast average transporters and 33 ±17 vs 12.9 ±16.5 in slow average transporters respectively).

Conclusions: Peritoneal Pi transport characteristics should be considered when optimizing PD modality in hyperphosphatemic patients. Increasing APD dwell times and switching to CAPD can improve PPiCl in patients with inadequate phosphate control on APD.

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SA-PO870

Influence of Dialysis Modality on Chronic Kidney Disease-Mineral Bone Disorder: Hemodialysis x Peritoneal Dialysis Rodrigo Azevedo de Oliveira,[1] Fellype Carvalho Barreto,[1,2] Zita Maria Leme Britto,[3] Viviane Felis Pinheiro,[3] Luciene M. dos Reis,[1] Rosa M.A. Moyses,[1] Vanda Jorgetti.[1] *[1]Universidade de São Paulo, São Paulo, SP, Brazil; [2]Uninove, São Paulo, SP, Brazil; [3]Cetene, São Paulo, SP, Brazil.*

Background: Few studies have evaluated Chronic Kidney Disease - Mineral Bone disorder (CKD-MBD) with bone biopsy in peritoneal dialysis patients. They suggest a higher prevalence of low turnover bone disease in comparison to hemodialysis population. However, there is not any study with homogeneous compared groups. The aim of our research was to compare CKD-MBD profile (laboratorial abnormalities and renal osteodysthrophy) between peritoneal dialysis (PD) and hemodialysis (HD) patients matched to age, sex and length on dialysis.

Methods: Thirty-two HD patients (68.7% male; 48.8± 12.3 years old; length on HD: 23.0 ± 15.3 months) were comparedto 32 PD patients (50% male; 49.0 ± 10.4 years old; length on PD: 18.8 ± 17.5 months). Both groups were submitted to laboratorial evaluation and iliac crest bone biopsy. Bone tissue was evaluated according to TMV classification (Turnover, Mineralization and Volume).

Results: Concerning the laboratorial evaluation, in general, HD patients presented higher levels of biochemical markers of mineral metabolism than PD patients: alkaline phosphatase: 229.6 ± 93 x 107.7 ± 36 U/L; p <0.00i; iPTH: 207.5 (88.7 – 656.0) x 359.0 (242.0 – 483.5); p = 0.04; FGF-23: 11826 (3369 – 28763) x 497 (411 – 1186) pg/ml; p < 0.001; 25(OH)vitaminD: 35.9 ± 15.6 vs 12.1±7.0; p<0.001; and albumin: 3.8 ± 0.2 vs 3.5 ± 0.5; p<0.001. According to TMV classification, peritoneal dialysis was associated to low bone turnover (p=0.04), low bone mineralization (p=0.04) and to a normal/high trabecular bone volume (p = 0.005) compared to HD patients.

Conclusions: Our bone-biopsy based study confirmed that PD is associated to low bone turnover in comparison to HD treatment, probably due to lower PTH and vitamin D levels. Interestingly, PD treatment seems to preserve trabecular bone volume.

Funding: Government Support - Non-U.S.

SA-PO871

Oral Sodium Bicarbonate May Ameliorate Hyperphosphatemia in Peritoneal Dialysis Patients with Metabolic Acidosis Subir K. Paul, Narasimha R. Boorgu, Rajesh Boorgu, Shejuti Paul. *Shoals Kidney & Hypertension Center, Florence, AL.*

Background: Despite achievement of adequate solute clearance with peritoneal dialysis (PD), metabolic acidosis (MA) remains a frequent problem in PD patients and is associated with adverse outcome. Similarly, despite implementation of current therapeutic approaches, hyperphosphatemia (HP) management remains sub-optimal in many PD patients. HP and high calcium phosphorus product (CaPP) are known causes of vascular, cardiac, and soft tissue calcifications. We have found a strong inverse correlation (r=-0.56) between serum bicarbonate concentration and phosphate level in patients on chronic PD. This study tests the hypothesis that correction of MA in chronic PD patients with oral $NaHCO_3$ will be beneficial for control of HP.

Methods: 13 patients on chronic PD with MA (serum HCO_3 < 22 meq/L) with mean age of 52.5 were included. 9 were male, and 4 were female. Serum HCO_3, phosphate, and Ca levels were measured in DaVita laboratory 4 weeks prior to initiation of therapy, at baseline, and 2-4 weeks following start of therapy. $NaHCO_3$ was given orally with total dose of 3900 mg daily for one month. No change was made in dietary phosphate restriction, phosphate binders, or in vitamin D dosages. Data was analyzed by student's paired t-test and linear regression.

Results: During pre-treatment phase, serum HCO_3 worsened from 21.4 meq/L to 19 meq/L in 4 weeks (p <0.0001). With oral $NaHCO_3$ therapy, serum HCO_3 improved from 19 meq/L to 24.9 meq/L (p < 0.0001). In pretreatment phase serum phosphate increased from 5.9 mg/dl to 7.3 mg/dl. (p<0.0001) With treatment, serum phosphate decreased from 7.3 mg/dl to 5.4 mg/dl (p <0.0001). In pretreatment phase CaPP increased from 54.4 to 63.7 (p<0.0001) but decreased from 67.7 to 50.4 (p<0.0001) following treatment. 69% of treated patients met K/DOQI guidelines of phosphate <5.5 and 77% met recommended value of CaPP < 55.

Conclusions: We conclude that oral sodium bicarbonate therapy corrects metabolic acidosis in chronic PD patients and may be therapeutically beneficial in the management of HP. Double blind, large, randomized, placebo controlled studies are warranted.

SA-PO872

Effects of Paricalcitol on Peritoneal Membrane in a Rat Peritoneal Dialysis Model Arzu Velioglu,[1] Zeynep Bozkurt,[1] Halil Tugtepe,[2] Deniz Filinte,[3] Ebru Asicioglu,[1] Izzet Hakki Arikan,[1] Serhan Tuglular,[1] Ishak Cetin Ozener.[1] *[1]Nephrology; [2]Pediatric Surgery; [3]Pathology, Marmara University, School of Medicine, Istanbul, Turkey.*

Background: Experimental studies showed activation of vitamin D receptor reduced the fibrotic lesions in kidney and heart. Effects of vitamin D and its analogs on peritoneal function and morphology in peritoneal dialysis (PD) were not suggested previously. In this study, we aim to examine the effects of paricalcitol on peritoneal membrane.

Methods: We use 30 rat to 4 groups: Group N, Sham operated rats as control group (n=6); Group U, Ureamic rats (5/6 nephrectomy, 2weeks) (n=8); Group PD, Ureamic rats (2 weeks) + PD (4 weeks) (n=8); Group PD-P, Ureamic rats (2 weeks) + [PD + paricalcitol (0.24 mcg/kg/day, IP)] (4 weeks) (n=8). For PD, a silicone catheter was introduced into the abdominal cavity after development uremia. At the end of study, 24-hour urine samples were collected and 1-h peritoneal equilibration test was performed. Blood pressure (BP) was measured using tail-cuff system. Peritoneal thickness was measured, and inflammation, fibrosis, and vascularization were evaluated semi-quantitatively score.

Results: PDF instillation, resulted in obvious alterations in peritoneal transport (increased D/P urea, decreased D1/D0 glucose) and peritoneal morphology (increased neovascularization, inflammation and fibrosis) compared to control and ureamic groups. Paricalcitol administration significantly improved UF capacity of peritoneum decreasing peritoneal thickness, neovascularization and inflammation compared to the group PD (42,5±16,6 vs. 25,6±4,9 µm, p=0,038; 72,7±19,1 vs. 14,6±5,4, p=0,02; 2,63±0,51 vs. 1,38±0.51, p<0,0001;respectively). 24-h urine output was not decreased in group PD-P. Increased BP was observed in uremic rats (group U, PD, PD-P). Paricalcitol administration was decreased BP levels in PD-P group compared to PD group (173,2±3,2 mmHg vs. 163,7±4,6 mmHg, p=0,005).

Conclusions: Paricalcitol ameliorated peritoneal function and morphology in subtotally nephrectomized rats who were receiving peritoneal dialysis.

Funding: Pharmaceutical Company Support - Abbott

SA-PO873

Prevalence of Chronic Kidney Disease: Mineral and Bone Disorder in Incident Peritoneal Dialysis Patients and Its Impact on Short Term Morbidity and Mortality Shen Hui Chuang,[1,2] Hung Chew Wong,[3] Vathsala Anantharaman,[4] Priscilla P. How.[1,4] *[1]Pharmacy, National University of Singapore (NUS), Singapore; [2]Pharmacy, Tan Tock Seng Hospital, Singapore; [3]Biostatistics Unit, Yong Loo Lin School of Medicine, NUS, Singapore; [4]Medicine (Nephrology), National University Hospital, Singapore.*

Background: A complex relationship exists between chronic kidney disease-mineral and bone disorder (CKD-MBD) and adverse clinical outcomes in incident peritoneal dialysis (PD) patients. The objectives of this study were to report the prevalence of CKD-MBD and examine the impact of attaining target CKD-MBD parameters on one-year morbidity and mortality in incident PD patients in Singapore.

Methods: In this retrospective cohort study, patients electively initiated on PD from June 2006 to August 2010 were followed up for one year from date of PD initiation. CKD-MBD parameters were collected and the prevalence of CKD–MBD at 4 to 6 months after PD initiation was determined based on KDOQI and KDIGO guidelines. Linear regression and Cox proportional hazards model were used to evaluate the effects of attaining target CKD-MBD parameters at 4 to 6 months post-PD initiation on hospitalization, peritonitis or exit-site infections and mortality at one year.

Results: Eighty-six patients were included in this study. The prevalence of CKD-MBD was 86.0% (KDOQI) and 54.7% (KDIGO). Duration of hospitalization was similar in patients who met CKD-MBD goals and those who did not. In the adjusted analyses, patients who failed to meet all of KDIGO's CKD-MBD goals [regression coefficient (RC): 1.24, p= 0.01] or calcium (Ca) targets [RC: 1.18, p= 0.02] had a significantly higher incidence of peritonitis or exit-site infections. Additionally, there was a trend towards shorter time to death in patients who failed to meet KDIGO's phosphorus (P) targets [adjusted hazard ratio: 9.54, 95% CI: 0.98 - 92.86, p=0.05].

Conclusions: Prevalence of CKD-MBD in our patient population was moderate (KDIGO) to high (KDOQI). Achievement of all of KDIGO's CKD-MBD goals or individual Ca targets was associated with reduced peritonitis and exit-site infections. There was a trend towards improved survival in patients who met KDIGO's P goals.

SA-PO874

Short Term Impact of the Peritoneal Dialysate Switch from Low Calcium to Standard Calcium Concentration Minoru Ito. *Division of Nephrology, Yabuki Hospital, Yamagata, Japan.*

Background: In patients on peritoneal dialysis (PD) therapy, calcium (Ca) concentration of peritoneal dialysate is an important factor influencing serum Ca concentration and parathyroid hormone (PTH) levels. In Japan, low Ca (2.5 mEq/L) dialysate and standard Ca (3.25 - 4.0 mEq/L) dialysate are available. Actually, low Ca dialysate is commonly used compared with standard Ca dialysate in Japan. However, there is a no guideline about the choice of Ca concentration. The adequate Ca concentration of PD solution is still unclear. The aims of this study are to assess the influence of the change from low Ca solution to standard Ca solution and to consider the optimal Ca concentration of peritoneal dialysate.

Methods: 9 patients (male 4, female 5) on PD with hyperparathyroidism were included in this study. A mean age was 70.4 ± 16.9 years and mean PD duration was 26.6 ± 7.8months. They were treated with low calcium dialysate from initiation to the time of this study. We analyzed their clinical data before and after the switch of peritoneal dialysate from low Ca to standard Ca.

Results: Three months after the switch of dialysate, the average PTH level was decreased significantly, from 527.1 ± 526.8 to 189.3 ± 92.6 pg/ml ($p < 0.01$). However, effects to serum Ca and phosphate (P) level were not found. The prescribed doses of vitamin D analogs and P binders had to be reduced in some patients.

Conclusions: Low Ca dialysate is commonly used, since favorable effects were expected in terms of good control of serum Ca, P and PTH levels with high dose of vitamin D analogs and Ca containing P binders. However, low Ca calcium dialysate could not maintain PTH levels within the target range in this study. And we could control secondary parathyroidism better after the change to the standard Ca dialysate without hypercalcemia. In this study, we couldn't estimate the calcification effect of the Ca concentration of peritoneal dyalaysate. Therefore, Further investigations must be needed to consider the optimal Ca concentration of peritoneal dialysate. At this point, the Ca concentration of dialysate should be determined on an individual basis with a deliberate consideration.

SA-PO875

Total Alkaline Phosphatase Is Related to Parathyroid Hormone Increases in Patients with Peritoneal Dialysis Juan Carlos Ramirez-Sandoval, Jorge Jesus-silva, Olynka Vega, Luis E. Morales-Buenrostro, Ricardo Correa-Rotter. *Nephrology, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico DF, Mexico.*

Background: Total alkaline phosphatase (tAP) in conjunction with intact parathyroid hormone (iPTH) can be useful as a surrogate of bone turnover. However, there is no consensus about the interpretation of tAP's concentration in patient follow-up as most studies are cross-sectional. The aim of this study was to determine the ability of routine measurement of tAP to mirror a change in serial measurements of iPTH concentration of patients treated with peritoneal dialysis.

Methods: We examined 179 collected data from 54 stable peritoneal dialysis patients in a 3-year cohort. We included those with more than three serial routine measurements of tAP and iPTH taken simultaneously, all negative for hepatitis B and hepatitis C virus and with normal LFTs. The value of each tAP and iPTH were assessed by comparing changes in each measurement to obtain a ratio of recent tAP/previous tAP (rtAP/ptAP) and recent iPTH/previous iPTH (riPTH/piPTH-) for every patient. Indeed, patients were assigned to one of two groups according to iPTH behavior: (group A, n=13) patients with initial stable iPTH who subsequently showed an increase of at least nine times the upper normal value, and (group B n=41) patients with stable iPTH who did not show a meaningful increase. Furthermore, we compared tAP´s increase between both groups.

Results: The Spearman correlation coefficient between rtAP/ptAP and riPTH/pPTH was 0.67 (P: 0.001).Correlation between tAP and iPTH was 0.47 (P: 0.001). Group A had significantly different tAP respect group B (median tAP group A: 214.9 IU/L, 94-503; group B: 107.6 IU/L, 22-473, P <0.0001). The rtAP/ptAP was different between both groups aswell (median rtAP/ptAP group A: 1.87, 1.30-4.04, and group B: 1.01, 0.38-1.60, P=0.0004).

Conclusions: In this study we found that a 60% or greater tAP rise is a good marker of a clinically important iPTH increase. tAP may be an alternative surrogate of mineral metabolism status, applicable in the routine monitoring of dialysis patients in low income countries when iPTH is not available or limited due financial constraints.

Funding: Government Support - Non-U.S.

SA-PO876

Pleiotropic Effects of Sevelamer in Peritoneal Dialysis Patients with Type II Diabetes Mellitus Sudha P. Chennasamudram, Tetyana L. Vasylyeva. *Pediatrics, Texas Tech University Health Sciences Center, Amarillo, Tx.*

Background: In chronic kidney disease (CKD) patients, hyperphosphatemia promotes vascular calcification and is a strong risk factor for cardiovascular mortality. Sevelamer, a phosphate binder used to treat hyperphosphatemia, may act as an anti-inflammatory agent in hemodialysis patients. The pleiotropic properties of sevelamer have not been studied in peritoneal dialysis (PD) patients with diabetes. This study compared the effects of sevelamer and calcium carbonate on endothelial function (EF) and inflammation in patients with type II diabetes mellitus (T2DM) on PD.

Methods: This open-labeled, randomized, cross-over study with 15 subjects (average age: 54±9 years) whose serum phosphate levels were ≥ 5.5 mg/dL was approved by TTUHSC Institution Review Board. After consent, subjects discontinued all phosphate binders to undergo a 2-week washout period. Subjects were then randomly assigned to receive either sevelamer or calcium carbonate for 8 weeks. Subjects underwent a second 2-week washout period to cross-over to either the alternate treatment for another 8 weeks. At the beginning and end of each treatment phase, EF was evaluated by reactive hyperemia index (RHI). Serum levels of EF biomarkers (endothelin-1 [ET-1], plasminogen activator inhibitor-1 [PAI-1], soluble vascular adhesion molecule [sVCAM] and soluble intercellular adhesion molecule [sICAM]) and pro-inflammatory cytokines (interleukin 6 [IL6], interleukin 1 [IL1], tumor necrosis factor alpha [TNF-α] and C reactive protein [CRP]) were measured. Serum calcium, phosphate, and lipids were also measured.

Results: As expected, both phosphate binders decreased phosphorus levels, and when treated with calcium carbonate serum calcium levels were elevated. There was a significant improvement in lipid profile with sevelamer treatment compared to calcium carbonate

treatment. There was no difference in RHI with both the treatments, but serum levels of ET-1, PAI-1, CRP and IL6 were significantly lower with sevelamer treatment compared to calcium carbonate treatment.

Conclusions: Treatment with sevelamer has beneficial effects compared to calcium carbonate in decreasing inflammation and improving EF in patients with T2DM on PD.

Funding: Pharmaceutical Company Support - Sanofi

SA-PO877

The Relationship between Apelin and Cardiac Parameters in Patients on Peritoneal Dialysis: Is There a New Cardiac Marker? Serhat Karadag,[1] Savas Ozturk,[1] Meltem Gursu,[1] Zeki Aydin,[1] Sami Uzun,[1] Abdullah Sumnu,[1] Huseyin Oflaz,[3] Rumeyza Kazancioglu,[2] Egemen Cebeci.[1] *[1]Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey; [2]Nephrology, Bezmialem Vakif University, Medical Faculty, Istanbul, Turkey; [3]Cardiology, Istanbul University Istanbul Medical Faculty, Istanbul, Turkey.*

Background: Many markers have been proposed for CVD risk assessment in dialysis population. Apelin is a peptide that has roles in cardiovascular functions and volume regulation namely vasodilation, decreased blood pressure(BP), positive inotropic effect and inhibition of antidiuretic hormone release. The aim of this study was to examine relationship of apelin levels with echocardiographic findings and laboratory parameters related with cardiovascular function and bone mineral metabolism among peritoneal dialysis(PD) patients.

Methods: This is a cross-sectional study in which chronic PD patients aged between 18 and 80 without active cardiac, infectious or malignant diseases and hypervolemia have been included. Apelin levels and echocardiographic findings were recorded as well as clinical and laboratory data.

Results: Of the 53 patients, the mean age and female/male ratio was 52.8±15.3years and 30/23, respectively. Mean apelin level was 1.45±0.37ng/ml. Gender, drugs (renin-angiotensin-aldosteron inhibitors, statins), presence of left ventricular hypertrophy, diabetes mellitus, hypertension, hyperlipidemia and residual renal function did not affect apelin levels. Apelin was correlated negatively with age and left atrium diameter; and positively with diastolic BP, ejection fraction (EF), total cholesterol, LDL-cholesterol, HDL-cholesterol, parathyroid hormone and alkaline phosphatase levels. Diastolic BP, LDL-cholesterol, ALP and EF were found to be the independent determinants of apelin levels with linear regression analysis. In patients with hypertension; diastolic BP, LDL-cholesterol and EF remained as the determinants of apelin level.

Conclusions: Apelinergic system has important roles in volume regulation, cardiovascular functions, lipid metabolism and bone mineral disorders in PD patients. It may be a treatment alternative for these disorders in the future.

SA-PO878

Cholecalciferol Therapy: LongTermEffectin Peritoneal Dialysis Patients Francesca K. Martino,[1,2] Elisa Scalzotto,[1,2] Manish Kaushik,[1,2] Jeong Chul Kim,[1,2] Maria Pia Rodighiero,[1,2] Claudio Ronco.[1,2] *[1]Department of Nephrology, Dialysis & Transplantation, San Bortolo Hospital, Vicenza, Italy; [2]International Renal Research Institute, Italy.*

Background: The deficiency of vitamin D is a widespread problem in general population and in patients with chronic kidney disease (CKD).Moreover, in such patients vitamin D plays a central role in the pathogenesis and treatment of mineral bone disorder (MBD). A recent metanalysis showed that Cholecalciferol may improve MBD in CKD patients. However, there is no evidence on its long term effect. The purpose of the study is to evaluate the long term effect of Cholecalciferol in PD patients.

Methods: We studied on 65 patients on peritoneal dialysis treated with Cholecalciferol for at least 6 months. We measured calcium before and after the treatment with vitamin D. The following characteristics of patients were evaluated: age, primary renal disease, duration of RRT, history of CVD, other drug therapy with Calcitriol and calcium based binders. Finally, we evaluated every 3 months Albumin, phosphorus and PTH and every 6 weeks calcium levels. Continuous non-normally distributed variables were presented as median values and interquartile range (IQR). Friedman's test was used to evaluate the differences before and after the treatment with Cholecalciferol. Multivariable logistic regression models were used to evaluate the variable related with hypercalcemia.

Results: The median period of follow-up was 10 month (IQR 8-12). 56.9% were male. 58.5% in the period of observation received therapy with Calcitriol with maximal dosage 0.5 mcg, while 29.2% received calcium carbonate with maximal dosage 2g. Finally, the dosage of chelecalciferol was between 625 and 8750 U per week. During the follow-up, 35.6% had at least one hypercalcemia episode. There was no difference in calcium values from baseline (median 9.4 mg/dl IQR 9.2-10.7) to study end (median 9.8 IQR 9.3-10.2) with p=0.25. In multivariable analyses, we found that only additionalCalcitriol therapy was independent predictor of hypercalcemia episode.

Conclusions: In this analysis, we conclude that hypercalemia episode in PD patients is not related with Cholecalciferol.

SA-PO879

Clinical and Laboratory Features in Uremic Patients on Continuous Ambulatory Peritoneal Dialysis before and after Parathyroidectomy Ying-tang Wang, Yu-Juei Hsu, Sung-Sen Yang, Shih-Hua P. Lin. *Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.*

Background: Although parathyroidectomy (PTX) in indicated for severe hyperparathyroidism (HPT) refractory to medical control, a detailed pre- and post-PTX assessment in uremic patients on continuous ambulatory peritoneal dialysis (CAPD) is rarely reported.

Methods: Uremic patients on CAPD who received PTX for severe HPT defined as serum PTH greater than 800 pg/ml were enrolled for analysis. Pre-PTX clinical and biochemical findings, and post-PTX predictors for "hungry bone syndrome" (HBS) and management were evaluated.

Results: There were 22 patients (M:F = 8:14, age 49.1 ± 10.3 years) with serum PTH concentrations 1619.6 ± 473.1 pg/ml. The most common clinical manifestations associated with severe HPT were pruritus (59.1%), fatigue (40.9%), bone pain (40.9%) and multiple uremic tumoral calcinosis (UTC) (22.7%). Despite calcium and vitamin D supplements, thirteen patients developed HBS (serum total $[Ca^{2+}] < 8.5$ mg/dl with neuromuscular symptoms). These patients had significantly higher pre-PTX serum i-PTH and alkaline phosphotase (ALP), and lower post-PTX serum calcium at 4th hour than 9 patients without HBS. Six patients with more severe HBS needed the addition of intraperitoneal calcium supplement (360~1080 mg elemental calcium) for 1 to 4 months to correct hypocalcemia and symptoms. Of note, UTC completely resolved over 4 to 8 months in 4 patients with severe HBS.

Conclusions: UTC is also not uncommon in CAPD patients with severe HPT and has a favorable response to PTX. A higher pre-PTX serum ALP, iPTH and lower post-PTX calcium are the indicators of post-PTX HBS. Intraperitoneal calcium supplementation is an alternative effective treatment for severe HBS in CAPD patients after PTX.

Funding: Clinical Revenue Support

SA-PO880

Serum Alkaline Phosphatase Is an Independent Predictor of Mortality in Peritoneal Dialysis Patients Paul A. Fein, Oluwatoyin Akinrinade, Jyotiprakas Chattopadhyay, Morrell M. Avram. *Avram Division of Nephrology, S.U.N.Y. Downstate Medical Center UHB at Long Island College Hospital, Brooklyn, NY.*

Background: Elevated levels of serum alkaline phosphatase (AlkP) have been reported to be associated with increased mortality risk in hemodialysis (HD) patients (pts). We have examined the association of serum AlkP with all-cause mortality in our PD pts.

Methods: Ninety PD pts were enrolled in this study beginning in 1995. On enrollment, demographics, clinical and biochemical data were recorded. Pts were followed up to September 2011.

Results: The mean age was 62 years. Sixty-one percent were female and majority (81%) were African descent. Mean and median AlkP were 135 U/L and 113 U/L, respectively. Fifty-six percent of the pts had elevated levels (greater than 104 U/l) of AlkP. Mean and maximum follow-up were 2.61 and 16.2 years, respectively. As expected, AlkP correlated directly with serum intact parathyroid hormone (PTH) (r=0.36 p=0.003). In Cox's multivariate regression analysis, adjusting for confounding variables (Table 1 footnotes), AlkP remained a significant independent predictor of mortality (Relative Risk=1.016, p=0.004). For each unit increase in enrollment AlkP, there was a 1.6% increase in relative risk of death. Albumin corrected calcium (Relative Risk: 2.20, p=0.035) and PTH (Relative Risk: 0.998, p=0.02) were also significant predictors of mortality in this model (Table 1).

Table 1. Multivariate Cox's Regression Analysis

Variables	Relative risk	p
AlkP (U/L)	1.016	0.004
PTH (pg/ml)	0.998	0.02
Albumin corrected calcium (mg/dl)	2.20	0.035

Other variables included in the model, but were not significant: age, race, gender, diabetes, hypertension, dialysis vintage at enrollment, albumin, creatinine, blood urea nitrogen, glutamic oxaloacetic transaminase (SGOT), hemoglobin, iron, and white blood cell (WBC) count.

Conclusions: Higher levels of serum AlkP is a strong independent predictor of increased risk of mortality in our PD pts followed up to 16 years. Further studies are needed to elucidate the mechanism underlying the association between AlkP and mortality in PD pts.

SA-PO881

Differences in Coagulation Protein Concentrations between Dialysis Modalities Daniel E. Carl,[1] Erika Martin,[2] Todd W. Gehr,[1] Donald F. Brophy.[2] *[1]Internal Medicine, Nephrology, Virginia Commonwealth University, Richmond, VA; [2]Pharmacotherapy, Virginia Commonwealth University, Richmond, VA.*

Background: It is widely accepted that ESRD patients are predisposed to increased cardiac and thrombotic events; and that differences likely exist between hemodialysis (HD) and peritoneal dialysis (PD). We evaluated coagulation protein concentrations in HD and PD patients, including tissue factor (TF), tissue factor pathway inhibitor (TFPI), prothrombin fragment 1+2 (F1+2), von Willebrand's factor (vWf), and fibrinogen. The goal was to determine whether significant differences in these protein concentrations exist between dialysis modalities.

Methods: 47 patients were enrolled: 20 HD, 20 PD, and 7 healthy volunteers. Patients on anti-platelets or chronic anticoagulation were excluded. In the HD patients, labs were drawn pre-treatment and heparinase was added to counteract any potential heparin effect.

Results: Both HD and PD groups had significantly greater coagulation protein concentrations compared to healthy controls. PD patients had significantly higher plasma concentrations of fibrinogen, TF, and TFPI compared to HD and controls. HD and PD patients had similar levels of vWf and F1+2.

	Normal values	Controls	HD	PD	P value
vWF	0.52-1.54 U/mL	0.8±0.3	2.3±1.5	2.2±1.2	<0.05
TF	23-130 pg/mL	102.0±22.6	375.0±124.8	521.6±153.7	<0.0001
TFPI	75-120 ng/mL	45.7±11.8	86.1±21.8	114.5±34.3	<0.0001
F1+2	66-229 pmol/L	136.9±55.8	379.6±152.6	348.5±162.4	0.002
Fibrinogen	200-400 mg/dL	297.1±87.1	445.2±132.4	560.0±142.9	<0.0001

Conclusions: ESRD patients have elevated coagulation protein concentrations compared to healthy controls, which likely contributes to the increased prevalence of thrombotic disease in ESRD. While fibrinogen is also an inflammatory marker, it further contributes to hypercoagulability. PD patients appear to have higher plasma levels of coagulation proteins relative to HD. The relationship between increased coagulation protein levels and thrombotic events in these patients needs to be addressed.

SA-PO882

Peritoneal Dialysis Patients Exhibit Pro-Thrombotic Platelet Function and Blood Viscoelasticity Compared to Hemodialysis Patients

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Background: ESRD patients have pro-inflammatory disease states that pre-dispose them to increased cardiac and thrombotic events. The goal of this study was to assess for differences in platelet function and viscoelasticity between patients receiving hemodialysis (HD) and peritoneal dialysis (PD).

Methods: 47 patients were enrolled: 20 HD, 20 PD, and 7 controls. Patients on anti-platelets or chronic anticoagulation were excluded. HD patient labs were drawn pre-treatment with added heparinase. The time to clot onset and was measured by Force Onset Time (FOT) and the Reaction time (R). K represents the time from clot initiation until final clot firmness. Platelet contractile force (PCF) measured the force produced by platelets during clot retraction, while clot rigidity was measured as clot elastic modulus (CEM) and maximum amplitude (MA). FOT, CEM and PCF were measured by Hemodyne; R, K and MA were measured by TEG.

Results: PD and HD patients had high fibrinogen levels, with PD patients being statistically greater than HD. The PCF, CEM and FOT in the HD group was similar to the controls, while the PD group was significantly greater than both groups. PD patients also had shorter K times and made firmer clots (MA) compared to HD and controls.

	Normal Range	Controls	HD	PD	P value
PCF	4.0-9.5 Kdyne	7.51±0.97	8.46±3.94	11.88±4.26	<0.01
CEM	14.0-35.0 kdyn/cm2	26.0±4.7	30.0±14.0	40.7±16.0	0.02
FOT	3.0-8.0 min	5.3±1.6	7.1±2.4	5.6±1.8	NS
R	3.0-8.0 min	7.2±2.0	7.9±2.0	6.9±1.9	NS
K	1.0-3.0 min	2.1±0.6	1.6±0.3	1.3±0.3	<0.001
MA	51-69 mm	65.4±3.6	65.8±6.0	72.7±5.5	<0.001
Fibrinogen	200-400 mg/dL	297.1±87.1	445.2±132.4	560.0±142.9	<0.0001

Conclusions: This study suggests PD patients exhibit a more pro-thrombotic profile compared to HD. PD PCF,CEM and MA values were markedly greater than the HD and control patients. Time to clot onset (FOT and R) however was not different compared to controls. These data may help explain the observational findings of greater thrombotic events in PD patients compared to HD. Further study is needed to study the links between coagulation proteins, platelet function and clinical outcomes.

SA-PO883

Coronary Artery Calcification Score as a Predictor of All-Cause Mortality and Cardiovascular Morbidity in Peritoneal Dialysis Patients

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Background: This study aimed to examine whether coronary artery calcification is associated with all-cause mortality and cardiovascular events in peritoneal dialysis (PD) patients.

Methods: Adult PD patients who were clinically stable for at least 2 months were recruited to this prospective observational cohort study. Coronary artery calcification was assessed using multislice spiral computed tomography (MSCT) and recorded according to Agatston score (CaCS). The primary endpoint was all-cause mortality and secondary endpoint was cardiovascular events including acute coronary syndrome (ACS), acute ischemic stroke, acute cerebral hemorrhage and peripheral vascular occlusion. The clinical data including general information, adequacy of PD and laboratory data were collected. Multivariate Cox regression was used to identify predictor of all-cause mortality and cardiovascular events in these patients.

Results: 179 PD patients (86 men) with a median age of 67 years were recruited to this study. CaCS ranging 0 to 5257 was stratified as follow: low (CaCS = 0, n = 54, age 52.2±15.8), moderate (0 < CaCS < 400, n = 72, age 65.8±11.7), high (CaCS ≥ 400, n = 53, age 71.9±9.6). The follow-up duration was 30.6±16.2 (24 to 63) months. Univariate analysis revealed that serum Ca, P, iPTH, cholesterol, triglyceride, LDL and dialysis duration were not significantly different, while age ($p<0.001$), diabetes mellitus (DM, $p=0.012$), BMI ($p=0.02$), renal GFR ($p=0.037$), serum albumin ($p=0.003$) and HDL ($p=0.024$) were significantly different among the 3 groups. Multivariate analysis (Cox regression) revealed that CaCS had a positive correlation with all-cause mortality ($p = 0.001$) and cardiovascular events($p = 0.011$) in these PD patients after adjustment for age, sex, DM, albumin, HDL, BMI, residual renal GFR and dialysis duration.

Conclusions: CaCS is an independent predictor of all-cause mortality and cardiovascular morbidity in PD patients.

SA-PO884

Comparison of Indices of Insulin Resistance in Peritoneal Dialysis Patients

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Background: Insulin resistance (IR) is common in chronic dialysis patients and is associated with excess mortality. Previous studies using indirect measures show that IR is prevalent in those receiving peritoneal dialysis (PD). There are limited studies using the gold standard hyperinsulinemic euglycemic glucose clamp (HEGC) in this patient population. Also, the comparison of indirect and practical indices of IR to HEGC has not been adequately performed in PD patients.

Methods: This is a cross-sectional, single center study performed in 7 prevalent PD patients (median age 50 years (interquartile range [IQR] 42.0, 55.5), 43% men, median body mass index (BMI) of 26.6 kg/m2 (IQR 25.1, 36.2) who were studied three consecutive times. IR was assessed by HEGC (glucose disposal rate [GDR]), homeostatic model assessment of IR (HOMA-IR), HOMA-IR corrected by adiponectin (HOMA-AD), leptin adiponectin ratio (LAR), QUICKI, McAuley's index, and oral glucose tolerance Test (OGTT) at each time point. Fasting state was ensured by withholding PD for 8 hours prior to and during study times.

Results: Seventy-one percent of the subjects displayed abnormal glucose tolerance by HGEC (GDR median 6.42; IQR 6.1, 7.3); 1 of which was overtly insulin resistant (GDR 3.5 mg/kg/min) and not previously known to be diabetic. HOMA-AD and McAuley's index were correlated with IR measured by HGEC ($p = 0.038$ and $p < 0.001$, respectively). HOMA-IR, LAR, QUICKI were not correlated. All 7 patients had at least 1 abnormal OGTT demonstrating impaired glucose tolerance.

Conclusions: Insulin resistance is highly prevalent in chronic peritoneal dialysis patients, which might be related to continuous, hypertonic glucose exposure. McAuley's index performed well in PD patients despite performing poorly in chronic hemodialysis patients. This potentially could be explained by PD patients showing more pronounced hypertriglyceridemia than prior reports in the HD population. Other indirect indices of IR such HOMA-AD also perform well in PD patients and may be used in large epidemiological outcome studies.

Funding: NIDDK Support, Other NIH Support - NCRR, Pharmaceutical Company Support - Baxter Renal Care

SA-PO885

Usefulness of the Brachial-Ankle Pulse Wave Velocity in Assessment of Earlier Atherosclerosis Risks in Peritoneal Dialysis Patients

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Background: Cardiovascular disease is a major cause of morbidity and mortality in patients with end-stage renal diseases. Management of risk factors for the atherosclerosis is therefore crucial to improve the prognosis. This study aimed to define the clinical factors that accelerate earlier atherosclerotic changes in peritoneal dialysis (PD) patients.

Methods: A total 42 PD patients (average age 55.4, male/female ratio 1.2, follow-up 26 months) were studied. We evaluated the vascular stiffening by measuring brachial-ankle pulse wave velocity (baPWV, n=41), which relies not only on pathologically visible sclerosis but also on functional resistance in peripheral muscular arteries of lower limbs. Carotid intima-media thickness (cIMT), which reflects the stiffening in central elastic arteries, was also examined (n=26).

Results: In multivariate models, cIMT correlated only with reduced diastolic pressure ($\beta=-0.45$, $p<0.02$), likely resulting from the stiffened central arteries. In contrast, baPWV, was independently associated with urinary protein excretion ($\beta=0.58$, $p<0.001$), elevated systolic blood pressure ($\beta=0.28$, $p<0.001$) and hemoglobin A1c ($\beta=0.27$, $p<0.002$). Both cIMT and baPWV did not significantly correlate with other parameters including serum calcium, phosphorus, lipids, residual renal functions, C-reactive protein and dialysis duration.

Conclusions: Our observations suggest that (1) baPWV could predict the ongoing endothelial injuries and/or stress with much higher sensitivity than cIMT, thereby represents a useful, non-invasive modality to grasp earlier treatable atherosclerotic risks and (2) blood pressure and glycemic levels should be rigorously controlled in PD patients who are prone to hyperglycemia and/or hypervolemia.

SA-PO886

Adipokines and Coronary Artery Calcification in Uremic Patients Undergoing Peritoneal Dialysis Kuo-hsiung Shu, Chi-Hung Cheng, Ming-Ju Wu, Cheng-Hsu Chen, Tung-min Yu, Ya-Wen Chuang. *Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan.*

Background: Uremic patients are associated with various cardiovascular complications. Coronary artery calcification (CAC) may serve as a surrogate marker of atherosclerotic coronary artery disease. Adipokines have been linked to atherosclerotic diseases. The aim of our study is to investigate the prevalence of CAC and alteration of adipokines in uremic patients undergoing peritoneal dialysis (PD).

Methods: Newly diagnosed uremic patients undergoing PD as their mode of renal replacement therapy at our hospital were recruited for the study. A multislice detector computer tomography (MDCT) heart scan were performed at the initiation of PD. Fasting venous blood samples were collected for routine biochemistry, blood routine and adipokines, including adiponectin (APN), leptin, visfatin and resistin. Univariate and multivariate logistic regression were used to identify factors that were associated with CAC and serum levels of adipokines.

Results: A total of 21 PD patients were recruited for the study. The mean age was 52.3±12.3 years. Six (26.1%) had diabetes mellitus (DM) as their underlying chronic kidney diseases. CAC was found in 14 (66.7%) patients, of which 3 (14.3%) had severe calcification (calcification score >1000). No significant risk factor for CAC was identified in this study. A high serum APN level (51.5±35.8 mg/ml) and leptin level (17.6±18.8 mg/ml) were found. Univariate logistic regression revealed female was associated with a higher APN level while DM, fasting blood sugar and serum uric acid level were negatively correlated with APN level. Total serum protein and albumin were positively correlated with leptin level, while serum uric acid was negatively correlated with visfatin level. On multivariate study, only female gender and DM were associated with APN level.

Conclusions: The current study revealed a high prevalence of CAC and alteration of adipokines in PD patients and provided potential treatment targets for the prevention of cardiovascular diseases.

SA-PO887

Prevalence and Risk Factors of Vascular Calcification in Coninuous Ambulatory Peritoneal Dialysis Patients Dhavee Sirivongs,[1] Jinvibha Anusri,[1] Sajja Tatiyanupangwong.[2] *[1]Medicine/Nephrology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [2]Medicine/Nephrology, Northeast Kidney Physician Network of Thailand, Khon Kaen, Thailand.*

Background: Cardiovascular disease is a major cause of death in chronic dialysis patients, resulting from their mineral abnormalities. Vascular calcification (VC) is associated with many cardiac events, currently used as an index of cardiac mortality. This study aimed to determine the prevalence and risk factors of VC in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: The multicenter cross-sectional study of CAPD patients under Thai PD first policy from 7 hospitals was conducted during January-December 2011. Plain radiographs of lumbar spine and pelvis were performed to detect the calcification of aorta, iliac and femoral arteries, read by a radiologist. VC score was assessed by diagnostic criteria of Bellasi and NKF.

Results: Total 557 CAPD patients were enrolled, female in 50.09% and DM in 33.57%. Mean age was 52±13.74 years. Average of CaxP level and dialysis vintage were 36.58±14.45 mg/dL and 21.64±12.53 months, respectively. There were 144 patients (25.85%) had aortic calcification, male in 43.75%, and DM in 53.47%. Regarding the VC group, average age was 58±11.90 years, mean VC score>0 was 6.43±5.47, rates of iliac and femoral VC were 14.13% and 18.29% respectively. Mean CaxP, PTH and calcium-based phosphate binder dosage were 36.55±14.41 mg/dL, 225.17±333.75ng/ml and 1,164.84±795.61 mg/day, respectively. CaxP > 55 was seen in 10.53% of VC group. Mean dialysis vintage was 22.02±12.96 months. By multivariate analysis, only diabetes (OR =2.82, p=0.000) and age>30 years (OR =5.93, p=0.015) were risk factors for VC.

Conclusions: Prevalence of VC in CAPD patients of our series is quite low compared to that of hemodialysis patients, may be due to shorter in dialysis vintage.Age>30 years and DM are risk factors for VC, so every patient who has one of them may need to be monitored for VC and should be treated properly to prevent cardiac mortality.

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SA-PO888

Risk Factors Associated with Development of Peripheral Artery Occlusive Disease in Patients on Maintenance Peritoneal Dialysis Chin Chung Tseng, An-Bang Wu, Yu-tzu Chang, Meng-Fu Cheng, Ming-cheng Wang. *Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan.*

Background: The prevalence and risk factors for peripheral artery occlusive diseases (PAD) have been widely studied in patients with end-stage renal disease. However, the risk factors for development of PAD after starting of dialysis therapy in peritoneal dialysis (PD) patients have rarely been evaluated.

Methods: Between 1 October 2006 and 31 December 2009, our PD unit provided chronic renal replacement therapy for 194 patients. Among these patients, 167 patients, from whom informed consent was obtained, were included for study. Diagnosis of PAD was made by measurement of ankle–brachial blood pressure index (ABI) and clinical manifestations. PAD was defined in the patients with an ABI value less than 0.90 in either extremity or having the typical signs and symptoms of intermittent claudication. ABI was performed and clinical manifiestations of PAD were re-evaluated every year for each patient during the study course. Baseline characteristics, comorbid conditions, and history of dialysis were obtained. Patients' clinical and laboratory data after initiation of PD were collected.

Results: 141 patients were initially negative in PAD. Among these patients, 22 % (31/141) developed PAD during the follow-up course. Preexisting diabetes mellitus (DM) was the patient characteristic associated with development of PAD. Longer PD duration, lower residual renal function (urine amount, kidney Kt/V urea and weekly CrCl) were associated with development of PAD. Higher ALK-P, history of parathyroidectomy, and lower serum potassium levels were significant risks, but serum triglyceride and cholesterol levels were not. Among these factors, DM, Lower residual renal function, and higher serum ALK-P level were independently associated with PAD.

Conclusions: DM, PD duration, lower residual kidney function, history of parathyroidectomy, higher serum ALK-P level, and lower serum potassium level were associated with new development of PAD. Among these factors, DM, Lower residual renal function, and higher serum ALK-P level were independent risk factors.

SA-PO889

The Relationship of Plasma ADMA Levels with Cardiac Functions and Metabolic Parameters in Peritoneal Dialysis Patients Savas Ozturk,[1] Serhat Karadag,[1] Meltem Gursu,[1] Zeki Aydin,[1] Sami Uzun,[1] Abdullah Sumnu,[1] Egemen Cebeci,[1] Rumeyza Kazancioglu.[2] *[1]Department of Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey; [2]Department of Nephrology, Bezmialem Vakif University, Medical Faculty, Istanbul, Turkey; [3]Cardiology, Istanbul University Istanbul Medical Faculty, Istanbul, Turkey.*

Background: Asymmetric dimethylarginine(ADMA) is accepted as a risk factor for coronary artery disease by causing endothelial dysfunction and vasospasm. We aimed in the present study to investigate the relationship between ADMA levels and echocardiographic parameters in PD patients; and to determine its utility as a cardiovascular risk determinant in this population.

Methods: This is a cross-sectional study in which PD patients aged 18-80; with at least three month duration of dialysis and without active cardiac, infectious or malignant diseases, and clinically evident hypervolemia were included. ADMA levels and echocardiographic parameters were recorded.

Results: Of the 55 patients included, the mean age was 53±15years. Mean ADMA level was 81.9±48.0µmol/L. The variables found to be positively correlated with ADMA levels were weight, body surface area, BMI, glucose, uric acid, sodium, ultrafiltration volume, left atrium diameter, intraventricular end-systolic diameter and intraventricular end-diastolic diameter. PTH, Kt/V and EF were found to be negatively correlated with ADMA levels. Male gender, use of acetyl salicylic acid and the presence of hypertension were found to increase plasma ADMA levels; while presence of ischemic heart disease, DM, hyperlipidemia, and use of statins, ACEi, ARB, beta blockers, erythropoiesis stimulating agents, vitamin D and nitrate did not change plasma ADMA levels. Although ADMA levels in patients with IHD had higher levels of ADMA; the difference was not statistically significance. ADMA levels were similar in patients with or without significant daily urine volume. With multivariate analysis; gender, BMI and use of ASA were found to be the independent variables determining ADMA levels.

Conclusions: The correlation of ADMA with BMI, gender and hypertension leads to the idea that ADMA may be used as a CVD risk determinant in PD patients.

SA-PO890

Increased Peritoneal Permeability at Peritoneal Dialysis Initiation Is a Possible Cardiovascular Risk in Patients Using Biocompatible Peritoneal Dialysis Solution Yoshifumi Hamasaki,[1] Yoshitaka Ishibashi,[2] Kent Doi,[1,3] Masaomi Nangaku,[1] Eisei Noiri.[1] *[1]Nephrology and Endocrinology, University of Tokyo, Japan; [2]Nephrology, Japanese Red Cross Medical Center, Japan; [3]Emergency and Critical Care Medicine, University of Tokyo, Japan.*

Background: Peritoneal dialysis (PD) patients suffer from cardiovascular disease (CVD) partly because of malnutrition-inflammation-atherosclerosis syndrome. It is still unclear what factors increase CVD risk even when patients are treated using biocompatible PD solution (BPDS). We investigated the relationship between clinical parameters at PD initiation and CVD occurred during PD therapy using BPDS.

Methods: The data from patients who started PD from 2001 to 2009 at University of Tokyo Hospital were collected retrospectively. To identify contributing factors for CVD events (cerebrovascular disease, ischemic heart disease or heart failure, and peripheral arterial disease) occurred until March 2012, we analyzed data including clinical parameters measured within 6 months after starting PD using BPDS.

Results: 116 patients were included; 98 patients were started PD as the first renal replacement therapy (RRT) and 18 patients were switched from HD to PD. New CVD events occurred in 31 patients. Age (66.2 vs 57.6 y.o.), history of CVD before starting PD (50 vs 17%), systolic BP (139 vs 127 mmHg), CRP (0.73 vs 0.38 mg/dl), and D/P(4h) creatinine values of peritoneal equilibration test (D/Pcre) (0.64 vs 0.57) were significantly higher in the new onset CVD group than the non-CVD group ($p < 0.05$ in each). Serum albumin (Alb) was significantly lower in the new onset CVD group than the non-CVD group (3.3 vs 3.6 g/dl, $p < 0.01$). In patients starting PD as the first RRT, D/Pcre was significantly correlated with Alb and serum iP ($r = -0.37$ and -0.24). When patients were split down the middle based on the D/Pcre, CVD morbidity was higher and duration of PD monotherapy was shorter in the high D/Pcre subgroup than the low D/Pcre subgroup.

Conclusions: Peritoneal permeability at PD initiation was associated with new onset of CVD events. Increased peritoneal permeability at PD initiation may contribute to progress atherosclerosis through malnutrition even in PD patients using BPDS.

SA-PO891

Hepatocyte Growth Factor (HGF) as a Long Term Predictor Factor for Total and Cardiovascular Mortality in Peritoneal Dialysis (PD) Patients

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Background: The aim of the study was to access the influence of increased HGF levels on total and cardiovascular mortality (CV) in PD patients on the basis of a 6 year observation period.

Methods: The study group consisted of 67 patients (31 W, 36 M) with a mean age of 53 +/- 13 years, treated with PD for a median period of 24 months. We assessed the following parameters: HGF, IL-6, hsTNF-α, hsCRP, albumin concentration, WBC count and ejection fraction (EF) %. Calcium scoring (CaSc) was measured using multirow spiral computed tomography (MSCT).

Results: During the 6 year observation period, 22 patients died, where 17 patients died due to CV causes. Survival was 1-72 months (median 20 months). Significant influence of HGF and proinflammatory cytokines on total and CV mortality in dialysis patients were shown using univariate Cox regression:

Total mortality:

Albumin (HR-0.89; p=0.026); hsTNF-α (HR-1.57; p=0.006); hsCRP (HR-1.06; p=0.0002); IL-6 (HR-1.05; p=0.0003); HGF (HR-2.39; p=0.004); EF% (HR-0.96; p=0.033); CaSc (HR-1.0003; p=0.024)

CV mortality:

Albumin (HR-0.96; p=0.5); hsTNF-α (HR-1.7; p=0.004); hsCRP (HR-1.06; p=0.0005); IL-6 (HR-1.05; p=0.001); HGF (HR-2.51; p=0.007); EF% (HR-0.98; p=0.3); CaSc (HR-1.0005; p=0.003)

Multiple regression analysis model was shown in table 1.

Table 1. Multiple Cox regression models to predict total and CV mortality.

independent variables	total mortality	total mortality	CV mortality	CV mortality
	HR (95% CI)	p	HR (95% CI)	p
HGF	3.64 (1.74-7.60)	**0.0006**	3.84 (1.70-8.66)	**0.001**
PD duration	1.00 (0.98-1.02)	0.8	0.99 (0.96-1.01)	0.2
Age	0.95 (0.90-0.99)	**0.03**	0.94 (0.89-1.00)	0.06
EF%	0.97 (0.93-1.01)	0.2	0.98 (0.93-1.04)	0.5
CaSc	1.0007 (1.0004-1.001)	**0.0001**	1.0008 (1.0004-1.001)	**0.0001**
Albumin	0.87 (0.78-0.97)	**0.01**	0.89 (0.78-1.02)	0.1

Conclusions: HGF is an independent risk factor for total, as well as for CV mortality in the PD patients.

Funding: Clinical Revenue Support

SA-PO892

Multiple Factors Affect Sleep Quality in Peritoneal Dialysis Patients

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Background: Studies have shown that sleep disorders are prevalent in dialysis patients, and poor sleep quality impacts the life quality of those patients. However, few studies investigate the influence factors of sleep quality in dialysis patients. The objectives of the present study were to evaluate influence factors of sleep quality in peritoneal dialysis patients.

Methods: 77 patients (61 male, 16 female) were included in the study, who were treated with CAPD from 3 months to 63 months (mean 10months).The mean age was 47 years(from 16 to 82 years). Pittsburg Sleep Quality Index(PSQI) was used to assess sleep quality. Quality of life parameters were assessed by Kidney Disease Quality of Life Questionnaire(KDQOL- SF™1.3).We also evaluated depressive state with Beck Depression Inventory (BDI) as well as anxiety with Self-rating Anxiety Scale(SAS)in those patients.

Results: The average of overall PSQI scores was 7.9±3.8. Positive correlation was found among PSQI scores and age (R = 0.41), BDI scores (R = 0.31), and SAS scores(R = 0.35). However, negative correlation was found between PSQI scores and serum albumin (R = - 0.36). Subjective sleep quality was positively related to general health scores (R = 0.34), social function scores (R = 0.39), physical fitness scores (R = 0.36), physical symptoms scores (R = 0.38) as well as disease burden scores (R = 0.39) of KDQOL-SF ™-1.3. Multivariate regression analysis showed somatic symptom was the independent risk factor of PSQI scores *(P=0.0002) and subjective sleep quality (P=0.007).*

Conclusions: Our study shows that many factors come together to induce poor sleep quality, especially somatic symptom. Medical staffs should attach importance to comprehensive evaluation of each patient, and then develop the individual therapy and nursing intervention.

SA-PO893

The Perspectives of Adults Living with Peritoneal Dialysis: Systematic Review and Thematic Synthesis of Qualitative Studies

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Background: Most patients with end-stage kidney disease require dialysis to survive, unable to access kidney transplantation. Peritoneal dialysis (PD) is generally recommended as the first choice of dialysis modality for adults without significant comorbidities or those with residual kidney function. This study aims to synthesize qualitative studies on patients' experiences and beliefs about PD.

Methods: We conducted a systematic review and thematic synthesis of qualitative studies on adult perspectives of living with PD. MEDLINE, Embase, PsycINFO, CINAHL, theses databases and reference lists were searched to November 2011.

Results: Thirty-nine studies involving 387 participants were included. We identified seven themes: resilience and confidence (determination, overcoming vicissitudes); support structures (strong family relationship, peer support, professional dedication, social abandonment, desire for holistic care); overwhelming responsibility (disruptive intrusion, family burden, onerous treatment regimen); control (gaining bodily awareness, achieving independence and self-efficacy, information seeking); freedom (flexibility and autonomy, retaining social functioning, ability to travel); sick identity (damage to self-esteem, invisible suffering); and disablement (physical incapacitation, social loss and devaluation).

Conclusions: PD can offer patients a sense of control, independence, self-efficacy and freedom. However, holistic and multidisciplinary care is needed to mitigate the risks of impaired self-esteem, physical incapacitation, reduced social functioning, and poor sense of self-worth. Strategies that aim to strengthen social support, and promote resilience and confidence in patients are integral to achieving positive adjustment, improved psychosocial outcomes and treatment satisfaction.

SA-PO894

Sexual Dysfunction in Men and Women in Peritoneal Dialysis: Differential Link with Metabolic Factors and Quality of Life Perception

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Background: Peritoneal dialysis (PD) patients face a demanding challenge in all aspects of their lives: physical, psychological and social. Although several conditions affect the sexual function of patients on PD, there are few studies that have been dedicated to these issues in PD. This cross-sectional study aims to evaluate sexual dysfunction (SD) and the determinants among PD patients.

Methods: All chronic PD patients in one Center were asked to complete the International Index of Erectile Function (IIEF) for men and the Index of Female Sexual Function (IFSF) for women. Both groups answered the Hospital Anxiety and Depression Scale (HADS) and the Euroqol (EQ5D) to measure the quality of life (QoL).

Results: We evaluated 57 PD patients (50,9% males), aged 53,9±15,7 years, 27,6% diabetics.

SD was diagnosed in 54,4% of the patients. These were older (P=0,029), had lower albumin (P=0,042), lower PTH (P=0.015)and lower EQ5D scores (P=0,012) without significant differences in HADS score.

In male patients, metabolic factors such as diabetes (P=0,005), hypercalcemia (P=0,038), lower PTH levels (P=0,014) and lower testosterone (P=0,002), were associated with SD. EQ5D score was not significantly corrrelated with reported IIEF score in this group. In female patients, a worse SD score was associated with lower QoL index (P=0,003) as well as with worse nutricional parameters, such as albumin (P=0,007), nPCR (P=0,008) and phosphorous (P=0,0014). Time in peritoneal dialysis, previous hospitalization and peritonitis episodes were not associated with erectile or female sexual dysfunction.

Conclusions: Sexual dysfunction is highly prevalent in PD patients, with impact on quality of life. Differences in male and female were highlighted, pointing to distinct modifiable risk factors for this complication. A comprehensive PD approach should address these issues for a better patient rehabilitation.

SA-PO895

Economic Burden of Incident Unplanned Starts on Peritoneal Dialysis in a High Specialty Health Care Facility in Mexico City

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Background: Few studies have examined unplanned initiation of peritoneal dialysis (PD). This study used detailed 2010 data from a Health Care Facility in Mexico to examine patient demographics, hospitalization costs, and outcomes associated with unplanned PD and tested differences for those requiring urgent hemodialysis (HD) while initiating PD.

Methods: Highly detailed data were collected to characterize patients. Descriptive analyses were conducted with a focus on costs and differences for patients requiring urgent HD were measured and tested using Stata 11.

Results: Of 195 hospitalized patients in 2010 for PD placement, 51 incident patients met criteria for unplanned PD initiation. Among them, 25 required urgent HD prior to PD initiation and 26 did not. Overall, for a Mexican population with kidney disease, this was a relatively young population with relatively low cardiovascular comorbidities, similar diabetes presence. 92% of the patients received a 90% or greater government subsidy for hospital costs. Average total costs associated with hospitalization for unplanned start of dialysis were 72,123.23 MXN ($5,233.91 US) with most of the costs associated with hospital bed rates and procedures. Costs were 88,078.03 MXN ($6,391.73) per patient for those requiring urgent HD and 56,782.16 MXN ($4,120.62) for those not requiring urgent HD, a difference (P<0.05) of over 30,000 MXN ($2,200). These results are from a single site and may not generalize, although they may apply to Public Health institutions providing acute renal replacement therapy to uninsured ESRD patients with low socio-economic status.

Conclusions: The results highlight the potential cost savings to payers for developing a well structured PD program in order to improve the handling of resources related to PD starts, and should help inform policy regarding oversight and coverage of low income patients at risk of dialysis.

Funding: Pharmaceutical Company Support - Baxter Healtcare Corporation

SA-PO896

Survival of Patients with Heart and Lung Transplants on Peritoneal Dialysis and Hemodialysis Pramod Kumar Guru, Heena S. Sheth, Rachita Prakash, Renee A. Burr, Filitsa H. Bender, Beth M. Piraino. *Renal-Electrolyte Division, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: ESRD, mostly due to calcineurin inhibitor toxicity, impacts negatively on survival of those with heart and lung transplants. Outcomes in relation to dialysis modalities, PD vs HD, are sparsely reported. The purpose of the study is to compare the survival of patients with heart and lung transplants on PD and HD.

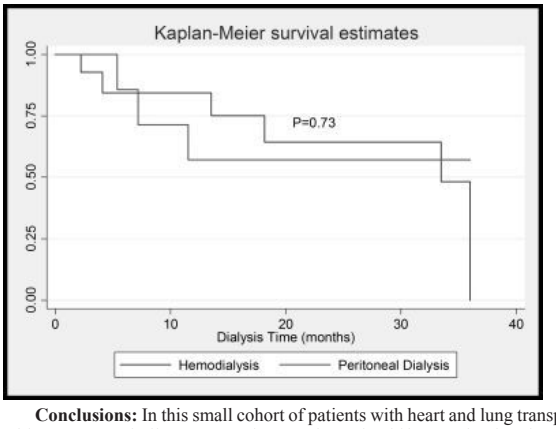
Methods: A single-center, retrospective review of prospective IRB approved registry was performed including patients with heart, lung and both transplants between 1992-2011 on dialysis at our outpatient dialysis center. Patients with kidney and abdominal organ transplants were excluded as were 2 heart/lung transplant patients who switched modalities.

Results: Cohort consists of 26 patients: heart (17), lung (8), heart & lung (1); 10 on PD and 16 on HD. Demographics are shown below.

Table 1

Demographics	PD	HD	P value
Women (%)	2 (20)	3 (19)	0.93
African American (%)	0	5 (31)	0.05
Mean age, years(SD)	55.9 (12.7)	54.0 (13.0)	0.67
Diabetes mellitus (%)	3 (30)	8 (50)	0.31
Charlson Comorbidity Index (median)	5	5	0.8
Initial serum albumin (median) gm/dl	3.8	3.05	0.03
Transplant (%)			0.43
Lung	2 (20)	6 (37.5)	
Heart	8 (80)	9 (56.2)	
Both	0	1 (6.2)	

There were 3 deaths in PD (30%) and 8 on HD (50%). From start of outpatient dialysis, survival at 6 month was 90% & 87.5%, and at 1 year was 80% & 81.2% for PD and HD patients respectively. Multivariate analysis controlling for initial albumin showed no difference in survival on PD and HD (HR 1.37, P=0.7). Kaplan-Meier survival curves are shown below.



Conclusions: In this small cohort of patients with heart and lung transplants, survival with ESRD was similar on HD and PD. Patients should be permitted to choose the modality best suited to their life-style.

SA-PO897

Ultrafiltration, Free Water and Solute Transport in Cirrhotic and Non Cirrhotic High Transport Patients on Peritoneal Dialysis Javier De Arteaga, Fabian Ledesma, Walter Guillermo Douthat, Carlos R. Chiurchiu. *Nephrology, Hospital Privado, Postgraduate Nephrology, Catholic University Cordoba, Cordoba, Argentina.*

Background: Besides being mostly high transporters (HT), cirrhotic pts have an unusually high UF volume on PD. Objective: to compare fluid and solute transport parameters between cirrhotic and non cirrhotic HT pts. Pts: We evaluated all of our HT pts (n= 17) since 2001 5 pts are cirrhotic.

Methods: Modified (4.25%) PET tests are done yearly since 2001.Minipets are done since 2006 for free water transport and NA removal data. Peritoneal ultrafiltration is measured manually hourly. HT is defined as a D/P creatinine ≥ 0.80 at 4 hs. We have not corrected NA sieving for NA difussion from the circulation for any pt from both groups. table 1 shows the comparative results between both HT groups: cirrhotic (Gr 1) vs non cirrhotic (Gr2).

Results:

	Gr1	G2	P
N	5	12	-
Age	58,2 ± 9,23±	46,8 ± 15,74	0,1
KTV(wk)	2,15 ± 0,89	1,9 ± 0,46	0,71
Prot (gr)	6,12 ± 2,87	9,8 ± 3,34	0,04
Time on PD (ms)	43,75 ± 23,37	25 ± 20,60	0,14
Time to Pet (ms)	19,00 ± 23,44	9,25 ± 17,27	0,35
D/P Creat 4 hs	0,80 ± 0,03	0,84 ± 0,03	0,03
DNa T 0 (meq/lt)	132,80 ± 4,76	129,54 ± 2,23	0,12
DNa 60 (meq/lt)	129,78 ± 2,99	124,54 ± 3,5	0,02
Plasma Na (meq/lt)	141,2 ± 2,86	137,61 ± 3,81	0,03
DIP Na	-3,0 ± 3,8	-5 ± 2,49	0,14
NAR final (meq/lt)	132,45 ± 32,95	87,96 ± 17,90	0,02
% FWT	15,60 ± 6,50	35,67 ± 21,24	0.01
UF 1 h (ml)	636 ± 132,21	373,33 ± 172,56	0,01
UF final (ml)	950 ± 265,59	665 ± 139,62	0,08

Conclusions: Cirrhotic pts have a higher drainage volume (UF) and peritoneal sodium removal (P=0.02). As expected for a HT state, NA sieving is blunted in both groups (Na difussion correction not done for neither) and this reflects a lower free water transport mostly for cirrhotic pts (p=0.01). The fact that peritoneal drainage volume is significantly higher in cirrhotics despite a HT state is probably due to the ascitic fluid generated" per se" more than true osmotic ultrafiltration and this might explain a lower free water transport in this group.(p<001) Daily loss of protein is slightly higher in non cirrhotics (p=0.04). HT cirrhotic pts have peritoneal physiologic parameters that allow to differentiate them from non cirrhotic HT pts.

SA-PO898

Randomized Cross-Over Study to Analize the Degree of Hydratation, Mesured by BCM with the Use of Conventional and Biocompatible Peritoneal Dialysis Solutions Jesus Montenegro, José Ignacio Cornago, Maria Isabel Gallardo, Paula García, Ainhoa Hernando, Rosa Ines Munoz. *Nephrology, Hospital Galdakao-Usansolo, Bilbao, Spain.*

Background: The Ultrafiltration(UF) is less with biocompatible Peritoneal Dialysis Solutions (PDS) than conventional PDS, but those preserved better the residual renal function (RRF), although a few studies don´t find these differences.

Methods: We designed a randomized cross over study to compare the hydration in stable CAPD patients treated with biocompatible and conventional PDS, measuring the RRF and the UF. The degree of hydration was measured by bioimpedance with the equipment Body Composition Monitor (BCM, Fresenius Medical Care). Patients with some RRF were randomized into two groups. Group 1: the patients started with a pure bicarbonate PDS (bicaVera®, FMC) bicaVera-standard-bicaVera. Group 2: standard-bicaVera-standard. The patients changed the type of PDS every month. In the last day of each period of study the RRF was calculated and a peritoneal equilibrium test (PET) with the solution in study was done.

Results: Results of BCM of 16 patients in each arm are shown in the table.

Degree of hydratation in liters with both PDS

Results BCM	1 month	2 months	3 months
Group 1:Liters	1.22±1.5	0.84±1.2	0.77±1.1
Group 2: Liters	1.16±1.6	1.19±1.6	0.85±1.8
p	0.93	0.57	0.81

A tendency of increase in diuresis and GFR is observed when dialyzed with bicaVera®, however, the daily UF as the UF of the PET increases significantly when dialyzed with a standard PDS (p< 0.05) but the daily loss of liquids (Diuresis+UF) was similar with both PDS. According to the measurement with BCM, there were no significant differences between the degree of hydration of the patients when dialyzed with biocompatible PDS and the degree of hydration in patients dialyzed with conventional PDS (p>0.05).

Conclusions: This decrease of the UF is statistically significant, but clinically isn´t important due to the fact that a lower UF is compensated by a greater diuresis. Measuring hydration with BCM is recommended in clinical practice and the BCM helps to know the degree of overhydration more precisely. The study will need to be continued with more patients to reach major statistic strength.

SA-PO899

Effluent Cancer Antigen 125: A Predictor of Technique Survival in Peritoneal Dialysis Deirisa Lopes Barreto,[1] Nynke Halbesma,[2] Martijn Leegte,[3] Raymond T. Krediet.[1] *[1]Internal Medicine, Division of Nephrology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; [2]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; [3]Hans Mak Instituut, Naarden, Netherlands.*

Background: Cancer antigen (CA) 125 is the best known effluent marker in peritoneal dialysis (PD). It reflects the mesothelial cell mass of the peritoneal membrane. A decline is observed with duration on PD. In this study we aimed to investigate effluent CA125 as a predictor of technique survival.

Methods: Adult patients with a PD duration of at least 24 months were eligible for this study. Technique failure was defined as switch from PD to hemodialysis (HD). To determine the predictive value of CA125 a Kaplan-Meier analysis was performed, where the difference between low (<6.5 kU/L) versus high (>6.5 kU/L) CA125 levels was determined by means of a log-rank test. Censored observations consisted of death, transplantation, lost to follow-up and end of study period.

Results: One hundred and six incident PD patients were included in this prospective study with a maximum follow-up of 60 months after commencing PD. The Kaplan-Meier curve showed a borderline significance, where technique failure was more often observed in patients with low CA125 levels as compared to patients with high CA125 levels. After a longer follow-up this difference became more pronounced. Technique survival rates were 83% in patients with high levels of CA125 and 38% for those with low CA125 levels. The two CA125 groups were comparable according to their characteristics. Only the group with low levels of CA125 had a longer PD duration and utilized more automated PD.

Conclusions: Patients with high CA125 levels tend to have a better technique survival as compared to patients with low CA125 levels. These results support the importance of membrane failure as cause of drop-out in long term PD.

SA-PO900

Root Cause Analysis of Low KT/V in Peritoneal Dialysis Farsad Afshinnia, Ziad S. Zaky, Jonathan H. Segal. *University of Michigan.*

Background: A target total weekly KT/V of 1.7 may not be achieved in all peritoneal dialysis (PD) patients. This study identifies risk factors for a KT/V < 1.7 and assesses the response to intervention in a single center out-patient dialysis unit.

Methods: Clinical information including demographic, duration of PD, details on prescription, prior abdominal surgery, catheter dysfunction, hernia, leak, and peritonitis were collected. Primary endpoint is defined as the first KT/V below 1.7 after initiation of PD. Time to event analysis was performed with Cox regression analysis. McNemar test was used to test the change in KT/V category after intervention.

Results: 13 of 35 patients (37.1%) were found to have at least one KT/V of < 1.7 during the study period. Mean (SD) age was 49.2 (15) years. Mean age, weight and fill volume were not statistically different between the low and acceptable KT/V groups, but total volume was 14.3 (2.8) vs 11.6 (3.8) L (p=0.033), and residual KT/V was 0.2 (0.4) vs 0.6 (0.5) (p = 0.041), respectively. History of prior surgery was 76.9% (10) vs 72.7% (16), catheter dysfunction 46.2% (6) vs 27.3% (6), hernia 38.5 (5) vs 22.7% (5), leak 23.1% (3) vs 9.1% (2) and peritonitis was 36.4% (8) vs 46.2% (6) in patients with low and acceptable KT/V, respectively. Relative risk of low KT/V was 3.6 times higher (95% CI: 1.5-8.7) with each additional risk factor. Incorporation of time-varying covariates showed that every 1 kg increase in weight without a change in PD prescription was associated with a 5% (95%CI: 1%-10%) increased risk of low a KT/V. After appropriate interventions including adjusting total volume in PD prescription 11 patients (84.6%) with low KT/V increased to acceptable range (p = 0.001).

Conclusions: Number of risk factors and loss of residual renal function are significant predictors of poor clearance. Correcting modifiable risk factors and increasing total therapy volume may improve Kt/V and can be used as targeting intervention for quality improvement.

SA-PO901

Intercalated Cells Maintain Sterility of the Urinary Tract John David Spencer, Andrew L. Schwaderer, David S. Hains. *Pediatrics, Nationwide Children's Hospital, Columbus, OH.*

Background: Although urine is sterile, little is known how the kidney maintains sterility. We have shown that the renal collecting duct produces several antimicrobial peptides (AMP) that help maintain sterility. Intercalated cells (IC) produce one of the most potent human AMPs – Ribonuclease 7 (RNase7). When RNase7 is neutralized in the urinary tract, bacterial growth increases. This study was designed to further characterize the biological relevance of RNase7 and Ribonuclease Inhibitor (RI) in the human kidney during infection.

Methods: *Gene expression*: RNA from non-infected and pyelonephritic human kidney tissue was used to quantify RNASE7 and RI using real-time PCR. *Protein expression*: IF localized RNase7 and RI production. Western blot (WB) and ELISA quantified RNase7 and RI production in non-infected/infected kidney and urine samples. RNase7 function: Live/Dead kill assays assessed the antimicrobial function of RNase7 on uropathogens in the presence and absence of RI.

Results: *Gene expression:* With pyelonephritis, RNASE7 mRNA expression increased from 1028 ng to 2927 ng/10ng total RNA (p<0.04) while RI mRNA expression decreased from 806 ng to 549 ng/10ng total RNA (p<0.05). Protein expression:IF localized RNase7 production to the apical surfaces of IC and RI to the basolateral surfaces of IC. Extracellular staining showed that IC secrete RNase7 into the urinary space. ELISA showed kidney and urinary RNase7 peptide production increase with infection. WB showed concurrent decreases in kidney RI production with pyelonephritis. RI was not detected in sterile urine. Degraded RI was identified in infected urine. RNase7 function: Recombinant RNase7 rapidly kills Gram-positive/negative uropathogens at micromolar concentrations (0.1-2.5μM). In the presence of equal concentrations of RI, the antimicrobial effects of RNase7 significantly decreased.

Conclusions: ICs are important in maintaining sterility of the urinary tract as they produce potent AMPs like RNase7. IC also produce regulatory proteins like RI, which abrogates the antimicrobial effects of RNase7. Further elucidation of the IC factors that regulate production and function of AMPs like RNase7 may lend insight into the pathogenesis of UTIs.

Funding: NIDDK Support

SA-PO902

The Crucial Role of Th2 Lymphocytes in Renal Fibrosis Lili Liu,[1] Qiao Zeng,[1] Sheng Chen,[2] Gang Xu.[1] *[1]Tongji Hospital, Wuhan, Hubei, China; [2]The Cancer Center, Union Hospital, Wuhan, Hubei, China.*

Background: Renal tubulointerstitial fibrosis is the final common stage of renal failure. $CD4^+$ T lymphocytes recruitment and activation after injury could be the very important early event that mediates the onset of renal fibrogenesis. But the role of $CD4^+$ T lymphocytes in renal fibrosis is controversial and its cellular mechanism needs to be further investigated.

Methods: Biopsy specimens were from patients with minimal change or IgA nephropathy. Mouse renal fibrosis was induced by unilateral ureteral obstruction. $CD4^+$ T lymphocytes of wild BALB/c mice were deleted with anti-CD4 monoclonal antibody. Flow cytometry analyzed the ratios of subtypes of $CD4^+$ T lymphocytes. $CD4^+$ T lymphocytes were isolated by magnetic-activated cell sorting (MACS). BALB/c Nu/Nu mice were reconstituted with polarized Th1 or Th2 cells by tail vein injection which were labeled and tracking with DiI. Kidney sections were examined for HE, Masson's trichrome's staining and immnohistochemistry of α-SMA and vimentin. ELISA quantified the levels of cytokines. RT-PCR detected the mRNA expression of collagen I, fibronectin and TGF-β1 and specific transcriptional factors.

Results: Our study demonstrated that the depletion of $CD4^+$ T lymphocytes inhibited the process of UUO-induced mouse renal fibrosis.In the process of UUO-induced renal fibrosis, Th1 cells were predominant on day 3 after UUO, the ratios of Th2/Th1 increased with time gone and Th2 cells were predominant on day 14 after UUO. Results also have shown that Th2-reconstituted mice developed renal fibrosis easier than Th1-reconstituted mice manifested by interstitial expansion and collagen deposition, higher expression of α-SMA and Vimentin and increased expression of fibronectin, TGF-β and collagen I. We also found that $CD4^+$ T cells from Th1-reconstitued mice tended to secret IL-4 and IL-13 Th2-like cytokines.

Conclusions: In conclusion, our study demonstrated the importance of $CD4^+$ T lymphocytes in renal fibrosis and gave the first direct evidence that Th2 cells play the pivotal role in UUO-induced renal fibrosis. Deletion of Th2 cells or inhibition of $CD4^+$ T lymphocytes differentiation to Th2 would be a potential therapeutic intervention to prevent renal fibrosis.

SA-PO903

The Uremic Toxicity Affects the Oxidative Burst and Antigen Presentation on Monocyte-Derived Macrophages: Potential Link between Uremia and Adaptive Immune Dysfunction Andrea Novais Moreno-Amaral,[1] Marina Luise Viola de Azevedo,[1] Natalia Borges Bonan,[1] Bruna Bortolini,[1] Elis Fernanda Demenech,[1] Wesley M. Souza,[2] Andréa Marques Stinghen,[2] Roberto Pecoits-Filho.[1] *[1]Pontifícia Universidade Católica do Paraná, Curitiba, Parana, Brazil; [2]Federal University of Parana, Curitiba, Parana, Brazil.*

Background: The immune system of CKD is characterized by disorders on innate immune activation that leads to a deficiency in the adaptive immune response, established by an increased susceptibility to infections and poor response to vaccination. To understand the mechanisms behind these events, we investigated the role of uremic serum (US) and isolated uremic toxins on burst oxidative production and antigen presentation markers expression on U937 monocyte derived-macrophage (MDM).

Methods: MDM was incubated with p-cresol (pC), p-cresyl sulphate (pCS) or US obtained from CKD patients in stage II/III and superoxide anion production was evaluated through NBT assay. Cell surface markers expression related to antigen presentation were evaluated using mAb anti-HLA-ABC-FITC, -HLA-DR-PE, and to co-stimulatory molecules using -CD80-FITC and -CD86-PE and analyzed by flow cytometry.

Results: Our results showed that both uremic toxins and US were able to induce an increase in oxidative stress in MDM, assessed by superoxide anion production, and were able to increase HLA-ABC and CD80 expression in the same level when compared to the LPS. Interestingly, we observed that US was not able to modulate HLA-DR expression and the isolated toxins showed slight effect on this expression. Equally results were observed for CD86, where basal expression was expressed after MDM incubation with US and with both toxins pC and pCS.

Conclusions: It is know that CD86 has higher affinity to CD28 and this binding induce T cell proliferation while CD80 is the preferred CTLA-4 ligand, which promote the cell-cycle arrest. Our results suggest that the uremic toxins can induce macrophage dysfunction causing increase oxidative stress and also an important factor for the increased incidence of infections in uremia, since no modulation on HLA-DR and CD86 expression, suggesting a possible negative signal to T cells during antigen presentation.

SA-PO904

Receptor-Mediated Extravasation of IgG Auto-Antibodies: A Novel Role of FcRn in Autoimmune Pathology Florina Olaru,[1] Wentian Luo,[1] Linna Ge,[1] Yoshikazu Sado,[2] Dorin-Bogdan Borza.[1] *[1]Vanderbilt University School of Medicine, Nashville, TN; [2]Shigei Medical Research Institute, Okayama, Japan.*

Background: In most capillary beds, endothelial cells restrict the passage of macromolecules from plasma to the interstitial space. IgG autoAbs targeting extravascular autoAgs, such as α3(IV) collagen, can only induce inflammation and tissue injury after crossing this barrier. How this occurs is not known. We hypothesized that IgG extravasation is mediated by FcRn, a key regulator of IgG homeostasis expressed in endothelial cells.

Methods: We determined how genetic and pharmacologic targeting of FcRn affects in vivo binding of IgG autoAbs to mouse tissues at 18-24h after passive immunization with IgG mAbs to α3/α5(IV) collagen NC1 domains (a model for Goodpasture autoAbs). The duration of the experiments was chosen to minimize the impact of accelerated IgG catabolism in the absence of FcRn.

Results: In vivo binding of IgG autoAbs to α3/α5(IV) collagen in the basement membranes (BMs) of alveoli, skin, and neuromuscular junction (NMJ) was strongly inhibited by genetic ablation of FcRn. Binding of IgG autoAbs to extra-renal BMs was also reduced by treatment with high dose intravenous immunoglobulin (IVIG) and effectively prevented by treatment with a blocking anti-FcRn mAb. Ablation of FcRn function did not affect the binding of IgG autoAbs to the GBM and tubular BMs, and only slightly reduced the titers of circulating IgG autoAbs.

Conclusions: The mechanisms of IgG extravasation are tissue-specific. Our results indicate that FcRn mediates this process in microvascular beds with continuous but not fenestrated endothelia (e.g. in lungs, skin and skeletal muscle but not kidneys). In these tissues, FcRn acts as a "Trojan horse" that transports IgG autoAbs for binding to autoAgs in the extracellular matrix (e.g. collagen IV), thereby promoting autoimmune pathology. Competitive inhibition of FcRn-mediated IgG extravasation may be a novel mechanism for the beneficial activity of high-dose IVIG in autoimmune diseases. Targeted FcRn inhibitors more specifically and effectively blocking this process may provide a novel approach for treating IgG-mediated diseases affecting lungs, skin, NMJ and other sites.

Funding: NIDDK Support, Private Foundation Support

SA-PO905

C-Reactive Protein Exacerbates Renal Ischemia Reperfusion Injury Melissa A. Pegues, Mark Mccrory, Alexander J. Szalai. *Medicine, University of Alabama at Birmingham, Birmingham, AL.*

Background: Acute kidney injury (AKI) is a serious complication of hypertensive crisis, cardiovascular surgery, and ischemia reperfusion injury (IRI). Mortality from AKI is as high as 80% due to incomplete knowledge of the mechanisms leading to AKI. It is known that a systemic inflammatory response always accompanies AKI and that increasing levels of the acute phase protein C-reactive protein (CRP) associate with worsening of AKI. These studies were undertaken to determine if CRP is actively involved in AKI-and not just marking its occurrence.

Methods: Wild-type, human CRP transgenic (CRPtg), and mouse CRP deficient ($CRP^{-/-}$) mice were subjected to bilateral renal IRI for 30 minutes and outcomes measured after 24 hours of reperfusion.

Results: Serum levels of human CRP increased in CRPtg mice subjected to IRI (14.81±3.00 μg/ml at baseline, 59.76±7.86 μg/ml 24 hours post-surgery), but did not increase in CRPtg mice subjected to sham surgery without IRI (23.54±1.16 μg/ml at baseline, 14.51±5.06 μg/ml 24 hours post-surgery). Mouse CRP was minimally elevated by IRI (2.41±0.42 μg/ml at baseline, 12.77±4.71 μg/ml 24 hours post-surgery). Compared to their wild-type and $CRP^{-/-}$ counterparts, CRPtg mice subjected to IRI had increased serum creatinine and urinary albumin and more histological evidence of kidney damage (increased necrosis, brush border loss, and cast formation). $CRP^{-/-}$ mice showed improvement in all measured markers of renal damage compared to wild-type mice. RT-PCR analysis revealed decreased expression of FcγRIIb mRNA in injured kidneys of CRPtg mice compared to wild-type mice subjected to IRI. Also, kidneys of $CRP^{-/-}$ mice subjected to IRI had increased expression of markers of alternatively activated macrophages (arginase 1 and mannose receptor) compared to controls.

Conclusions: Taken together these data indicate that CRP plays an active role in renal IRI induced AKI, perhaps by depressing expression of the inhibitory FcγRIIb receptor and promoting classically activated macrophage polarization.A better understanding of this damaging sequence in AKI will allow for the development of interventions to shorten the course of AKI, prevent the need for hemodialysis, and improve survival.

Funding: NIDDK Support

SA-PO906

In Fabry Disease the Storage of Globosides but Not of Isoglobosides Impacts the Development of Invariant Natural Killer T Cells and Their Interaction with Dendritic Cells Hermann-Josef Groene, Stefan Porubsky, Mahnaz Bonrouhi. *Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany.*

Background: Invariant natural killer T cells (iNKT) are important players at the crossroad between innate and adaptive immunity with implications also in kidney diseases such as FSGS or lupus erythematodes. Recognition of endogenous lipid antigen(s) on CD1d molecules is required for iNKT cell development. Isoglobotrihexosylceramide (iGb3) has been implicated as this endogenous selecting ligand and recently suggested to control over-stimulation of iNKT cells in α-galactosidase A-deficient mice ($\alpha GalA^{-/-}$, human Fabry disease). A higher incidence of autoantibodies in Fabry patients has been also reported. In addition, iGb3 bears a terminal Galα3Gal epitope which is recognized by xeno-antibodies mediating hyperacute rejection in pig-to-human transplantation. However the presence and function of iGb3 in murine tissues remained controversial.

Methods: Here we generate a Gb3-synthase deficient mouse ($Gb3S^{-/-}$).

Results: We show that in $\alpha GalA^{-/-}/Gb3S^{-/-}$ double knockout mice, which store isoglobosides but no globosides, minute amounts of iGb3 (1619±380 molecules of iGb3 per thymocyte (mean±SEM, n=3)) can be detected by high performance liquid chromatography. Analyzing multiple gene targeted mouse strains, we demonstrate that globoside, rather than iGb3, storage is the major cause for the 50% reduced iNKT cell frequencies and the 80% less efficient antigen presentation in $\alpha GalA^{-/-}$ mice. Finally, we show that correction of globoside storage in $\alpha GalA^{-/-}$ mice by crossing them with $Gb3S^{-/-}$ normalizes iNKT cell frequencies and DC function.

Conclusions: We conclude that - although detectable in murine thymus in $\alpha GalA^{-/-}/Gb3S^{-/-}$ mice - iGb3 does not influence either the development of iNKT cells or their interaction with peripheral DCs. Moreover in $\alpha GalA^{-/-}$ mice it is the Gb3-storage that is responsible for the decreased iNKT cell numbers and impeded antigen presentation on DCs.

Funding: Government Support - Non-U.S.

SA-PO907

Abstract Withdrawn

SA-PO908

Paricalcitol and Mesenchymal Stem Cells Additively Suppress Primary T-Helper 17 Differentiation via Distinct Mechanisms Michelle M. Duffy, Bairbre A. McNicholas, Matthew D. Griffin. *Regenerative Medicine Institute, College of Medicine, National University of Ireland, Galway, Ireland.*

Background: T-helper 17 (Th17) cells play a pathogenic role in acute kidney injury (AKI), glomerulonephritis and transplant rejection. We previously reported that mesenchymal stem cells (MSCs) suppress primary Th17 induction via PGE2 production induced by direct contact with activated $CD4^+$ T-cells. Vitamin D receptor agonists (VDRa) also have direct and indirect suppressive effects on T-helper differentiation. The current study aimed to investigate the combined effects of MSCs and the VDRa paricalcitol on Th17-mediated responses in vitro and in unilateral ureteral obstruction (UUO).

Methods: Th17 differentiation was induced by 3-day activation of total $CD4^+$ T-cells or FACS-purified naive and memory $CD4^+$ T-cells with anti-CD3 + APCs in the presence of IL-6, TGFβ1, anti-IFNγ and anti-IL-4. Paricalcitol or vehicle was added in graded concentrations (10^{-12}-10^{-7}). MSCs were added at 1:400 MSC:T-cell ratio. Analyses included ELISA, surface and intracellular flow cytometry and immunoblotting. UUO was carried out for 72 hours in adult mice. Kidney cells suspensions were enriched for $CD45^+$ cells by magnetic separation and stimulated with anti-CD3ε followed by IL-17A ELISA in the presence or absence of paricalcitol and/or MSCs.

Results: Paricalcitol suppressed IL-17A production in dose-dependent fashion in Th17 cells *in vitro*. At its optimal concentration (10^{-8}M) paricalcitol mildly inhibited T-cell proliferation but strongly reduced the frequency of $IL\text{-}17A^+$ cells. Combined MSCs and paricalcitol further inhibited Th17 induction from both naive and memory responders to a greater degree than either intervention alone. MSCs reduced expression of the Th17-associated transcription factors RORγt, RORα, Runx1 and IRF4. MSC-mediated Th17 suppression was reversed by selective COX 2 inhibition while paricalcitol-mediated suppression was not. MSCs and paricalcitol also suppressed IL-17A secretion by effector memory Th17 cells from obstruced kidney.

Conclusions: MSCs and paricalcitol additively suppress primary Th17 differentiation via distinct mechanisms and inhibit re-activation of Th17 cells from obstructed kidney.

Funding: Pharmaceutical Company Support - Abbott Laboratories, Government Support - Non-U.S.

SA-PO909

microRNA-155 Deficiency in Peripheral Lymphocyte Is Related to IgA Nephropathy Ping Fu, Wei Qin. *Division of Nephrology, Department of Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: MicroRNA-155 (miR-155) involves in lymphocyte homeostasis and adjusts multiple immune genes. Lymphocytes dysfunctions and impaired homeostasis are key pathogenesis of IgA nephropathy (IgAN). miR-155 level in peripheral lymphocytes of IgAN patient was studied to clarify potential pathogenic relationship.

Methods: Forty biopsy-proven IgAN patients and 15 unrelated healthy controls were included. Expression of miRNAs in peripheral lymphocyte was first determined using Exiqon microRNA microarray. Realtime RT-PCR of miR-155 was performed. The expression level of Foxp3, a regulator of miR-155, was also measured. Correlation between miR-155 and Foxp3 expression level as well as clinical indexes, such as proteinuria, hematuria, renal function and albumin level was analyzed.

Results: Microarray results indicated that in the 1035 microRNAs successfully analyzed, 533 are upregulated and 499 are downregulated compared with normal controls. The expression level of miR-155 in IgAN patients was dramatically lower than that in normal control (fold change: -0.61), which was further confirmed by realtime RT-PCR (IgAN 0.197±0.07 vs Control 0.796±0.13, $p<0.01$). Further study showed that baseline proteinuria (24 hour quantification) and hematuria (RBC per high power view) level was significantly correlated to the miR-155 expression level (proteinuria: $r=-0.594$, $p<0.001$, hematuria: $r=-0.590$, $p<0.001$). However, no apparent correlation was observed in baseline serum creatinine level, serum albumin level, serum albumin concentration. Significantly

correlation between miR-155 and Foxp3 expression level was also noticed(R=0.681, p<0.001). Moreover, it is noticed that the peripheral lymphocytes miR-155 level is higher in patients with mild pathological changes (Lee's grade) as well as IgA1 glycosylation level.

Conclusions: Remarkable lower expression of miR-155 in peripheral lymphocytes was observed in IgA nephropathy patients, which was correlated with proteinuria, hematuria, kidney lesion scores and IgA glycosylation level. These results suggested that miR-155 might play important roles in the pathogenesis of IgAN and might be a potential biomarker of the disease.

Funding: Government Support - Non-U.S.

SA-PO910

Differential Effect of High-Density Lipoprotein from Healthy Subjects and from Chronic Kidney Disease Patients on Polymorphonuclear Leukocytes

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Background: Cardiovascular disease (CVD) and infections of chronic kidney disease (CKD) patients are related to disturbed functions of immune cells such as polymorphonuclear leukocytes (PMNLs). Gradual changes in the protein composition of high-density lipoprotein (HDL) with decreasing renal function may lead to a loss of its anti-atherosclerotic properties. While the effect of unmodified HDL and HDL modified by the uremic milieu on monocytes has already been investigated, no such experiments with PMNLs have been conducted so far.

Methods: HDL was isolated from plasma of healthy subjects (HS-HDL; n=7), patients with CKD stage 3 (CKD3-HDL; n=8) and hemodialysis patients (HD-HDL; n=8). Final HDL concentrations were 10 and 100 µg/ml. PMNLs were isolated from HS. The oxidative burst was stimulated with phorbol-12-myristate-13-acetate (PMA) or E. coli and measured via cytochrome c reduction. The surface expression of CD14, a pattern recognition receptor, was quantified by flow cytometry. Apoptosis was assessed by evaluating morphological features under the fluorescence microscope and by measuring the DNA content by flow cytometry.

Results: HS-HDL, CKD3-HDL and HD-HDL significantly increased the basal surface expression of CD14 at a final concentration of 100µg/ml. Only HD-HDL showed this effect already at 10µg/ml. The formyl-methyl-leucyl-phenylalanine stimulated CD14 expression was increased by all isolates tested. CKD3-HDL and HD-HDL strongly reduced the PMA stimulation of the oxidative burst already at 10µg/ml whereas HS-HDL had a much lesser effect at 100µg/ml. The activation of the oxidative burst by E. coli was diminished only by HD-HDL. While HS-HDL and CKD3-HDL had no significant effect on PMNL apoptosis, HD-HDL significantly decreased PMNL apoptosis, an effect characteristic for pro-inflammatory mediators.

Conclusions: The different effect of HDL modified by the uremic milieu on PMNLs may contribute to the deranged immune response in CKD3 and HD patients.

Funding: Private Foundation Support

SA-PO911

CD4+CD25+Nrp1+ Regulatory T Cells Induce the Renal Protect Effect of Ischemic Preconditioning

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Background: Ischemic reperfusion is one of important causes of acute kidney injury. T cell mediated inflammatory is the major mechanism of kidney ischemic reperfusion injury (IRI). A short-time preconditioning ischemia(PI) spurs kidney tolerance to following ischemic dysfunction. And the underlying mechanisms of IP are thought to involve adaptive changes within the injured tissue. Because regulatory T cells play a key role in inflammatory suppressing and tolerance maintenance, we hypothesized that CD4+CD25+Nrp1+ regulatory T cells induce the protect effect of ischemic preconditioning in kidney.

Methods: C57BL/6 mice underwent 25 min of bilateral renal clamping or sham operation. CD4+CD25+Nrp1+ T cells were analyzed by flow cytometry. At 5 days after IP (or sham operation), purified leukocytes from spleen were adoptively transferred into T cell-deficient (nu/nu) mice. After 1 week, these mice underwent 35 min of renal IRI, the renal functioan and pathology were detected. We used anti-CD25 monoclonal antibody to deplete regulatory T cells, and evaluated the renal protect effect of ischemic preconditioning. And we transfered CD4+CD25+Nrp1+ T cells isolated from PI mouse to assess whether thus can reverse the renal protection.

Results: 7 days after operation(bilateral renal clamping or sham operation), the proportion of CD4+CD25+Nrp1+ regulatory T cells was 4-folder higher in IP group than in sham operation group. In vitro analysis the CD4+CD25+Nrp1+ T cells isolated from PI mice, the Foxp3 expressing rate was 96.7%±1.3%. The nu/nu mice receiving leukocytes from ischemic wild-type mice had significantly reduced renal injury and inflammatory compared with nu/nu mice receiving leukocytes from sham-operated, wild-type mice. Depletion of regulatory T cells deprived the adoptive protect effect of PI. Adoptive transfer the CD4+CD25+Nrp1+ T cells protect the kidney from subsequent ischemic reperfusion injury and inflammation.

Conclusions: These results demonstrated that immune cells primed after renal IRI with an increasing proportion of CD4+CD25+Nrp1+ regulatory T cells and thereby suppressed the inflammatory and injury during a second episode of IRI.

SA-PO912

HLA-DQB1*05:02 Allele and DPB1*05:01-DQB1*03:02 Haplotype Are Associated with Susceptibility to Chronic Glomerulonephritis (CGN)

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Background: Association of HLA loci with specific diseases has been widely studied. However, such an association is not well documented about Chronic Glomerulonephritis (CGN). The objective of this study was to examine the association between the susceptibility to conventional CGN with HLA-C,-DPB1,-DQB1 loci in Zhejiang Han population.

Methods: Genotyping for HLA-C, -DPB1 and -DQB1 loci was performed in 112 patients with CGN and 115 control individuals. Frequencies of the allele and haplotype were analyzed to clarity the association of HLA-C,-DPB1,-DQB1 loci with CGN.

Results: The results were showed that the frequency of DQB1*05:02 allele (2.23%) was significantly lower in patients than that in controls (10.0%). Linkage disequilibrium were confirmed for C-DPB1,C-DQB1,DPB1-DQB1 and C-DPB1-DQB1 haplotypes in patients and controls. The frequency of DPB1*05:01-DQB1*05:02 haplotype in patients (0%) was significantly lower than that in controls (5.50%).

Conclusions: It was concluded that the decreased expression of DQB1*05:02 allele and DPB1*05:01-DQB1*03:02 haplotype are strong predisposing factor for conventional CGN in Chinese Han population.

Funding: Clinical Revenue Support

SA-PO913

Highly Significant Association of HLA Serotypes with Autoimmune End-Stage Renal Diseases

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Background: Autoimmune kidney diseases may cause end stage renal disease (ESRD). The UNOS Kidney Registry routinely collects HLA serotypes to help match registrants with kidney donors. We mined this large dataset and evaluated association of HLA serotypes with primary diagnoses of ESRD.

Methods: A total of 346,317 subjects registered on the UNOS Kidney Registry waitlist (1987-2011) were available with complete gender, primary diagnosis and HLA serotyping data. We performed association analyses with 107 HLA A, B, DR serotypes for 43 primary renal diagnoses in four ethnic groups (N=316,369). The control group consisted of 29,948 patients registered with a diagnosis of polycystic kidney disease (PKD), which has established genetic risk variants outside of the MHC region. A step-wise logistic regression was applied in the ethnicity-matched case-control groups after adjusting for sex. We excluded diagnoses with small sample size (N < 200). After correction for multiple testing, a significance threshold of $1.1x10^{-5}$ was applied.

Results: We found significant associations with HLA serotypes for 23 primary diagnoses of European (EA), African (AA), Hispanic (HSP) and Asian (ASN) ancestry. Novel associations were found for membranous nephropathy (MN) with DR5 (EA: OR=1.47, $P=2.66x10^{-21}$; AA: OR=1.57, $P=6.61x10^{-14}$) and with DR6 (EA: OR=1.28, $P=7.28x10^{-10}$). For the first time, we discovered significant HLA associations in AA for systemic lupus erythematosus (SLE) with DR2 (OR= 1.76, $P=4.81x10^{-33}$) and B8 (OR=1.84 $P=1.32x10^{-12}$) and chronic glomerulonephritis A68 (OR=0.57, $P= 6.67x10^{-14}$). We also observed significant association for IgA nephropathy with DR2 (EA: OR=0.71, $P=1.87x10^{-32}$; AA: OR=0.68, $P=1.39x10^{-05}$) and Wegeners granulomatosis for DR4 (EA: OR=1.4 $P=1.14x10^{-12}$) and DR6 (EA: OR=0.62,$P=2.59x10^{-11}$), which have not been reported. We confirmed known associations for Type 1 diabetes with DR3, DR4, DR2, B39, Goodpasture syndrome with DR2, SLE with DR2 and DR3 and MN with DR3.

Conclusions: This data mining approach revealed several novel HLA serotypes primarily from the MHC II region (HLA-DR) that are significantly associated with autoimmune kidney diseases.

Funding: Other NIH Support - R01-DK090029 Role of PLA2R and Anti-PLA2R in Idiopathic Membranous Nephropathy

SA-PO914

The Role of γδ T Cells in Pyelonephritis

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Background: Cells of the innate immune system are the first responders to, and are responsible for, the clearance of bacterial infections. IL-17 has been established as one of the key cytokines in pathways of Polymorphonuclear neutrophilic granulocyte (PMN) recruitment and activation. Recent studies also point to a role for this cytokine in various forms of glomerulonephritis. In addition to the well-known Th17 cells, another major producer of IL-17 are gamma delta (γδ) T cells. Here we investigate the role played by γδ T cells in pyelonephritis.

Methods: The clinical uropathogenic *E. coli* isolate 536 (UPEC 536) was instilled into the bladder of anaesthetised C57Bl/6 mice using a catheter. Mice were infected again three hours later to induce an ascending renal infection. Kidneys were excised enzymatically digested and the inflammatory infiltrate analysed by flow cytometry. The level of kidney infection was determined by plating dilutions of the renal digest onto CPS ID plates.

Results: Renal infection with *E. coli* was maximal 3 hours after the second infection, with the numbers of *E. coli* present dropping 1000 fold over the following 72 hours. Infiltration of the renal tissue by PMNs peaked at day 2 post-infection and decreased sharply as the bacteria were cleared. However, PMN numbers rose again if the bacteria were not cleared and a chronic infection was established. Notably although γδ T cells were extremely rare in the normal kidney, their numbers increased significantly, from 4 fold at

72 hours post-infection up to 12 fold at day 7 post-infection. When the cytokine profile of the γδ T cells was investigated they produced IL-17 rather than IFNγ (27.3% versus 2.4% respectively). Additionally γδ T cells predominately produced IL-17A alone (17.6%) rather than a combination of IL-17A and IL-17F (3.9%) or IL-17F alone (4.4%).

Conclusions: Pyelonephritis induces the infiltration of γδ T cells into the renal parenchyma where they express IL-17A. The expression of IL-17 by γδ T cells later in renal infection may be responsible for amplification of the innate immune response, enabling optimal recruitment and activation of PMNs and clearance of persistent renal infections.

Funding: Government Support - Non-U.S.

SA-PO915

Role of CCR2 and CX_3CR1 in Homeostatic and Inflammatory Recruitment of Kidney Mononuclear Phagocytes Daniel Engel,[1] Torsten Urzynicok,[1] A. Richard Kitching,[2] Christian Kurts.[1] *[1]Institute for Experimental Immunology, Bonn, Germany; [2]Medicine, Monash University Clayton, Melbourne, Australia.*

Background: The kidney harbors a dense network of mononuclear cells, including macrophages and dendritic cells (DC), which directly contribute to both innate and adaptive immunity and can originate from blood monocytes. Nevertheless, the mechanisms that regulate migration of macrophage and DC- progenitors in homeostasis and inflammation are currently unclear.

Methods: The abundance of macrophages (CX3CR1+, F4/80+, CD11c-) and DCs (CX3CR1+, CD11c+) within the healthy and the inflamed kidney of $CCR2^{-/-}$x $CX3CR1^{GFP/+}$, $CCR2^{+/+}$x $CX3CR1^{GFP/+}$ and $CCR2^{+/+}$x $CX3CR1^{GFP/GFP}$ mice were analysed by flow cytometry. Renal inflammation was induced by unilateral ureteral obstruction or by instillation of uropathogenic *E.coli* into the urinary bladder. Injected BM-derived monocyte-progenitors (BMMP) were isolated out of $CCR2^{-/-}$x $CX3CR1^{GFP/+}$, $CCR2^{+/+}$x $CX3CR1^{GFP/+}$ and $CCR2^{+/+}$x $CX3CR1^{GFP/GFP}$ mice by sorting or by negative MACS enrichment. The number of CX3CR1-expressing donor macrophages and DCs within the kidney was defined as mentioned above.

Results: We found that homeostatic migration of macrophages and DCs into the kidney depends on the chemokine receptor CCR2, but that renal DCs additionally depend on CX3CR1. In Homeostasis, BMMP did not efficiently migrate into the kidney. In contrast, migration of donor BMMP into the kidney was significantly increased by inflammation, for example by unilateral ureteral obstruction and bacterial pyelonephritis. Of note, such BMMP that infiltrated into the kidney were able to differentiate into both renal macrophages and DCs. Consistent with the findings under homeostatic conditions, migration of BMMP and the subsequent generation of macrophages within the inflamed kidney was dependent on CCR2, whereas generation of DCs mainly required CX3CR1.

Conclusions: These findings suggest a non-redundant role for CCR2 and CX3CR1 in the migration of macrophages and DC-progenitors into the kidney, with DCs having a greater dependence on CX3CR1. This differential dependency might allow to therapeutically modulating renal inflammatory processes in which either DCs or macrophages are involved.

SA-PO916

Immune Complexes Enhance Dendritic Cell Migration to Lymph Nodes Menna R. Clatworthy,[1,2] Caren Petrie-aronin,[2] Rebeccah J. Mathews,[1] Ken Smith,[1] Ronald N. Germain.[2] *[1]CIMR/Department of Medicine, University of Cambridge, Cambridge, United Kingdom; [2]LSB, NIAID, NIH, Bethesda, MD.*

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by immune complex (IC) deposition in tissues, including the kidneys, causing inflammation and disease. Many deleterious effects of IC are mediated via Fcγ receptors (FcγR), which are found on most immune cells, including dendritic cells (DCs). DCs are important antigen presenting cells. Following antigen acquisition in tissues, DCs migrate to lymph nodes and activate T cells. We sought to determine how FcγR cross-linking with IgG IC might affect DC migration.

Methods: Bone marrow DCs (BMDCs) were generated from wild type (WT) & FcγRIIB-/- mice & stimulated with IC. *In vivo* migration assay: BMDCs were injected subcutaneously (S/C), lymph nodes harvested and DCs enumerated histologically and by flow cytometry. *In vitro*, 3D chemotaxis assay: Murine BMDCs & human monocyte-derived DCs were placed in a CCL19 gradient in a collagen matrix and time-lapse confocal imaging performed.

Intravital two-photon microscopy: Dermal DCs from WT & FcγRIIB-/- CD11cEYFP mice were imaged following stimulation with IC or lupus serum.

Results: FcγR-crosslinking with ICs increased BMDC migration from peripheral tissues to draining lymph nodes; In a 3D collagen matrix, IC-stimulated murine and human DCs showed enhanced directional migration in a CCL19 gradient;· FcγR-crosslinking with IgG ICs increased CCR7 expression on BMDC and on human monocyte-derived DCs; Intravital, two-photon imaging demonstrated that S/C administration of IC resulted in dermal DC mobilisation *in vivo*. S/C application of autoantibody-containing serum from mice (NZB/WF1) and humans with SLE increased dermal DC mobilisation *in vivo.*

Conclusions: Our study demonstrates a novel effect of IC in increasing DC migration to lymph nodes. Furthermore, we show that DC mobilisation *in vivo* is increased following S/C administration of IC or autoantibody-containing lupus serum. Together, these data suggest an additional mechanism by which ICs might drive autoimmunity in SLE via the inappropriate localisation of autoantigen-bearing DC.

SA-PO917

Expression of Toll-Like Receptors and Cathelicidin in Hemodialysis Patients with Vitamin D Deficiency Caren Cristina Grabulosa,[1] Edgar Ferreira Cruz,[1] Silvia Regina Manfredi,[1] Silvia Regina Moreira,[1] Lilian Cuppari,[1] Beata Marie Redublo Quinto,[1] Marcelo Costa Batista,[1,2,3] Miguel Cendoroglo-Neto,[1,2,3] Maria Dalboni.[1] *[1]Nephrology, Universidade Federal de São Paulo, Sao Paulo, Brazil; [2]Nephrology, Hospital Israelita Albert Einstein, Sao Paulo, Brazil; [3]Nephrology, Tufts-New England Medical Center, Boston, MA.*

Background: Patients in hemodialysis (HD) may have vitamin D (vit D) deficiency which is associated with inflammation, impaired immunity and increased susceptibility to infections. Besides, vit D regulates toll-like receptors (TLR) and antimicrobial peptides, such cathelicidin. However, data on expression of TLR and cathelicidin in HD patients with deficiency of vit D are limited.

Objective: To evaluate the expression of TLR-2 and TLR-4 on PMN and MN and serum levels of cathelicidin from HD patients with deficiency of vit D.

Methods: Blood samples from 38 HD patients and 31 age-and gender-matched controls were analyzed for TLR2 and TLR4 expression on PMN (CD66+) and MN (CD14+) by flow cytometry. We measured serum levels of cathelicidin, CRP and vit D.

Results: The HD patients had low vit D ($p = 0.008$), high CRP ($p = 0.04$), high cathelicidin ($p = 0.01$) and exhibited significant upregulation of CD66TLR2 (313±168 vs. 249±56; $p = 0.04$), CD66TLR4 (212±157 vs. 121±35; $p = 0.02$) and CD14TLR2 (369±210 vs. 269±144; $p = 0.03$) expression. Vit D correlated negatively with age ($r = - 0.31$; $p = 0.01$) and CRP correlated positively with cathelicidin ($r = 0.50$; $p < 0.0001$). We did no observe any correlations between vit D and TLR2, TLR4 and cathelicidin expressions.

Conclusions: CD66TLR2, TLR4 and CD14 TLR2 and cathelicidin expressions are increased in hemodialysis patients. This suggests that these results are associated with a priming inflammatory state in this population. Besides, it is possible that there are other molecules enrolled in vit D regulation of TLR and antimicrobial peptide in HD patients. Futures studies are necessary to investigate the mechanisms between vitamin D and impaired immunity associated with increased susceptibility to infections in hemodialysis patients.

Funding: Government Support - Non-U.S.

SA-PO918

Identification of Candidate Target Antigens for Membranous Lupus Nephritis Dawn J. Caster,[1] Erik Korte,[1] Michael Merchant,[1] Brad H. Rovin,[2] John Barker Harley,[3] Bahram Namjou,[3] Jon B. Klein,[1] Kenneth R. McLeish,[1] David W. Powell.[1] *[1]Medicine, University of Louisville School of Medicine, Louisville, KY; [2]Medicine, Ohio State University College of Medicine, Columbus, OH; [3]Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Membranous lupus nephritis (MLN) is described in 15%-40% of LN renal biopsies, but the mechanism of immune complex deposition is unknown. An autoantibody to the ~ 145kDa M-type phospholipase A2 receptor (PLA2R) on podocytes was shown to be present in 70%-80% of patients with idiopathic membranous nephropathy (iMN). However, this antibody is rarely present in patients with MLN. This study tested the hypothesis that patients with MLN develop unique autoantibodies against a protein expressed on podocyte plasma membranes.

Methods: Human podocyte membrane and cytosol proteins were separated by SDS-PAGE and immunoblotted with 1:200 dilution of pooled human sera from 5 patients with biopsy confirmed MLN, 10 patients with Class III/IV LN, 4 SLE patients with no kidney involvement, or 4 normal individuals. Proteins in the MW region corresponding to a band reactive only with MLN sera were characterized by LC-MS/MS.

Results: Sera from patients with SLE with or without class III/IV or class V LN had multiple reactive bands against cytosol proteins. MLN sera specifically reacted with one prominent ~ 50kDa band in membrane proteins that was not present in cytosol proteins. Several promising candidate proteins were identified in the ~ 50kDa region including a recognized lupus-associated auto-antigen (Sjögren Syndrome auto-antigen, lupus La protein), proteins implicated in LN (Annexin A2 and the PDGF receptor), antigens associated with other autoimmune diseases (SLC9A3R1, snRNP70, LEPREL4), and auto-immune signaling proteins (TRAF2, TRIP6).

Conclusions: Our findings support a hypothesis that MLN patients express unique auto-antibodies against a ~ 50kDa endogenous podocyte plasma membrane protein. These findings support the hypothesis that iMN and MLN have a similar pathophysiology, in that they result from auto-antibodies binding to podocyte membrane proteins.

SA-PO919

Regulation of Glomerular DAF by Heme: Role of HO-1 Maria Detsika,[1] Pu Duann,[2] Elias A. Lianos.[1] *[1]Medicine, University of Athens, Greece; [2]Nephrology, UMDNJ, NJ.*

Background: In systemic hemolysis and in hematuric forms of glomerulopathy, glomeruli are exposed to high heme concentrations. Although cytotoxic, heme can have cytoprotective effects by inducing heme oxygenase (HO). It also attenuates complement (C)-mediated cell injury by up regulating endothelial cell Decay-accelerating factor (DAF). Rat glomeruli express DAF exclusively in glomerular epithelial cells (GEC). The effect of heme on glomerular DAF expression is unknown and highly significant in C-mediated forms of injury.

Methods: Glomeruli were isolated from wild type (WT) and *hmox1*+/-, Sprague-Dawley rats. The latter were obtained by Zinc Finger Nuclease (ZFN) technology designed to target a specific exon 3 HO-1 sequence. Glomeruli were treated with Heme (Hemin) or

Cobalt Protoporphyrin (CoPP) (HO inducers) or Zinc (ZnPP) or Tin (SnPP) Protoporphyrins (HO enzyme activity inhibitors) for 18 h. HO-1 and DAF expression was assessed by real-time PCR and western blot analysis.

Results: Constitutive HO-1 and DAF expression was observed in WT and *hmox1*+/- glomeruli. In the latter, there was 60-70% HO-1 protein depletion while DAF protein decreased by 3-fold. Heme and CoPP increased both HO-1 and DAF expression. However, their effect on HO-1 synthesis was biphasic: a dose-dependent increase at low concentrations (6-200 μM) followed by reduction to constitutive or sub-constitutive HO-1 levels at high concentrations (400 and 800 μM, respectively). In contrast to HO-1, high heme or CoPP concentrations (≥400 μM) failed to reduce DAF expression; it further increased by 2-3 fold in both WT and *hmox1*+/- glomeruli. ZnPP and SnPP (12.5, 25, 50 and 100 μM) also increased DAF protein levels dose-dependently. This was independent of changes in HO-1 synthesis, which was increased by ZnPP but reduced by SnPP. In the presence of hemin (200 μM and 400 μM), ZnPP and SnPP (50 μM) had an additive effect on DAF induction.

Conclusions: HO-1 regulates glomerular DAF expression. Heme and non-Fe metalloporhyrins upregulate DAF independently of effects on HO-1 expression/activity and may thus have a dual protective effect on C-dependent glomerular injury.

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SA-PO920

VEGF Splicing in the Normal Kidney and in Diabetic Nephropathy Rosanne Jane Turner, Jan A. Bruijn, Hans J. Baelde. *Pathology, Leiden University Medical Center, Leiden, Netherlands.*

Background: The cytokine vascular endothelial growth factor (VEGF) is a key regulator in the development and maintenance of the glomerular filtration barrier. VEGF plays an important role in the development of diabetic nephropathy. There are seven proangiogenic VEGF splicing variants. Important differences between these isoforms are the in- or exclusion of exon 6 and 7 of the VEGF gene, on which the neurophilin- and heparin binding sites are encoded. Longer isoforms containing these binding sites, such as the common variants VEGF 165 and 189, have a prolonged and more effective mode of action in comparison to shorter isoforms, such as the common variant VEGF 121. In rodents the dominant isoform in the kidney and glomeruli is the 165 variant. In this study, we set out to investigate the splicing pattern of VEGF in isolated glomeruli and whole tissue in the normal kidney and in the kidneys of patients with diabetic nephropathy.

Methods: The relative expression of each individual splice variant was measured in samples of glomeruli isolated by laser captured microdissection and whole kidneys from 23 controls and 28 patients with diabetic nephropathy with the use of RT-PCR followed by quantitative fragment analysis with capillary electrophoresis.

Results: VEGF 121 was the dominant isoform with a relative expression of 69% and 74% in the glomeruli and kidneys respectively. The expression of VEGF 165 was 23% and 24% and the expression of VEGF 189 was 2.6% and 5.6% in the glomeruli and kidneys. In a few samples the VEGF 181 isoform was detected with a relative expression of less than 1%. Overall, splicing patterns in diabetic patients were similar to those in controls.

Conclusions: In conclusion, this is the first report to show VEGF 121 to be the dominant isoform in both the human kidney and the glomerulus. This is in contrast with findings in rodents, in which the 165 variant is the most common. VEGF splicing patterns were the same in patients with diabetic nephropathy and controls.

SA-PO921

VEGF-A Regulates Glomerular Endothelial Cell Expression of Protective Complement Regulators Lindsay S. Keir,[1] Gavin Iain Welsh,[1] Richard Coward,[1] Simon C. Satchell,[1] Anna Richards,[2] Moin Saleem.[1] *[1]Academic Renal Unit, University of Bristol, Bristol, United Kingdom; [2]Queens Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom.*

Background: Glomerular thrombotic microangiopathy (TMA) is the pathological feature of hemolytic uremic syndrome (HUS). The rare atypical HUS is predominantly caused by mutations in regulatory proteins of the alternative complement pathway. While Shiga toxin (Stx)-HUS is more common, it has a poorly defined pathogenesis. Recently, Stx-treated human podocytes were found to secrete less VEGF-A. A proportion of patients treated with VEGF-inhibitor drugs and adult mice with a specific podocyte VEGF-A knock down also develop TMA. We hypothesised that reduced podocyte VEGF-A decreases glomerular endothelial cell (GEnC) expression of complement regulators making them vulnerable to complement injury and development of TMA.

Methods: VEGF-A treated conditionally immortalised human GEnC were analysed for expression of complement regulators using immunofluorescence, western blot and qPCR. GEnC viability, C3d and C4d deposition was assessed using a complement-mediated cytotoxicity assay with or without preceding VEGF-A treatment.

Results: VEGF-A increased GEnC surface expression of CD46, CD59 and factor H. CD55 expression was not altered by VEGF-A. Factor H was also secreted by GEnC but complement factor H related protein 5 (CFHR5) and factor I were not. Functionally, VEGF-A treated GEnC had significantly improved viability and showed 30% less C3d and C4d compared to untreated cells. Kidneys from adult mice with a podocyte specific VEGF-A knock down and TMA are now being examined to explore this in vivo.

Conclusions: This work identifies a link between podocyte VEGF-A and the up-regulation of protective complement regulator expression on glomerular endothelium which in vitro, protects GEnC from complement activation. Preliminary in vivo work supports this but further analysis is required.

Funding: Government Support - Non-U.S.

SA-PO922

Analysis of Germline VH Sequences of IgG Autoantibodies Specific for Galactose-Deficient IgA1 in Patient with IgA Nephropathy Zhi Qiang Huang,[1] Milan Raska,[1] Zhixin (Jason) Zhang,[2] Hitoshi Suzuki,[3] Stacy D. Hall,[1] Robert J. Wyatt,[4] Bruce A. Julian,[1] Ali G. Gharavi,[5] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]University of Nebraska Medical Center, Omaha, NE; [3]Juntendo University, Tokyo, Japan; [4]University of Tennessee, Memphis, TN; [5]Columbia University, New York, NY.*

Background: In IgA nephropathy (IgAN), glomerular deposits of immune complexes (IC) with galactose-deficient IgA1 (Gd-IgA1) likely originate from circulating IC wherein Gd-IgA1 (autoantigen) is bound by anti-glycan autoantibodies of IgG or IgA1 isotype. Variable region of the heavy chains (VH) of IgG autoantibodies from IgAN patients have a unique sequence at the N-terminal part of CDR3, YCSR/K, whereas antibodies from healthy controls have a YCAR/K sequence. However, it is unknown whether the unique VH sequences are of germline origin, *i.e.*, rare VH alleles, or from somatic mutations or other processes.

Methods: Using genomic DNA of three IgAN patients and two healthy controls, we PCR-amplified, cloned, and sequenced VH CDR3 coding regions with primers designed according to the corresponding VH gene of each IgG specific for Gd-IgA1 (J. Clin. Invest. 119, 1668-1677, 2009).

Results: Analyses of VH genomic DNA revealed that the unique CDR3 of IgG anti-Gd-IgA1 antibodies in IgAN patients did not originate from rare alleles of germline VH sequences. Using recombinant IgG antibodies and site-directed mutagenesis, we showed that the S residue in CDR3 is required for binding to Gd-IgA1. These data indicate that a complex process of abnormal immune responses is involved in production of pathogenic antibodies specific for Gd-IgA1. Our recent GWAS data associated three loci on chromosome 6 in the HLA region with IgAN, whichis consistent with this hypothesis.

Conclusions: Unique VH CDR3 regions of Gd-IgA1-specific antibodies in IgAN patients are not encoded by rare alleles of germline VH sequences but may be generated through somatic mutation upon antigenic stimulation. Removal or blockade of Gd-IgA1 antigen or anti-Gd-IgA1 antibodies would prevent IC formation and such strategies may lead to future disease-specific treatment of IgAN.

Funding: NIDDK Support, Other NIH Support - GM098539

SA-PO923

Aberrant *O*-Glycosylation of IgA1 in IgA Nephropathy (IgAN) and the Role of Initiating Enzymes Tyler J. Stewart, Kazuo Takahashi, Koshi Yamada, Milan Raska, Milada Stuchlova-Horynova, Matthew B. Renfrow, Jan Novak. *University of Alabama Birmingham, Birmingham, AL.*

Background: IgAN is associated with aberrant glycosylation of IgA1 hinge-region (HR). Circulatory IgA1 has 3 to 6 of the 9 potential HR sites *O*-glycosylated and in patients with IgAN some of these sites are galactose deficient, *i.e.,* contain terminal or sialylated *N*-acetylgalactosamine (GalNAc). HR glycopeptides with 5 or 6 *O*-glycansare more abundant on IgA1 from IgAN patients, whereas those with 3 or 4 *O*-glycans predominate on IgA1 from healthy controls. These data suggest that the postulated primary initiating enzyme, GalNAc-transferase 2 (GalNAc-T2), or other GalNAc-Ts contribute to the glycosylation abnormality in IgAN.

Methods: *In vitro* time-course reactions of purified recombinant GalNAc-T2 and -T14 with IgA1 HR synthetic peptides were characterized using high-resolution mass spectrometry. Homology models of GalNAc-T14 to crystal structure of GalNAc-T2 were created and aligned with HR peptides. GalNAc-T2 co-crystalized with a mucin peptide was compared to IgA1 HR peptide models.

Results: We showed that GalNAc-T2 is expressed in IgA1-secreting cells from IgAN patients and controls, but GalNAc-T14 was the only initiating enzyme that was overexpressed in the cells from IgAN patients. To understand better the biochemical mechanisms of IgA1 *O*-glycosylation, we performed comparative structural analysis and modeling of GalNAc-T2 and -T14. The first and second preferred glycosylation site of IgA1 HR, as observed in *in vitro* reactions, contained the amino-acid motif TPSP where the first threonine is glycosylated. The first glycosylated site of IgA1 HR localized to the GalNAc-T2 catalytic site. Modeled GalNAc-T14 varied from GalNAc-T2 crystal structure in the lectin domain and a variable loop involved in the catalytic site of GalNAc-Ts, thus underscoring differences between the two enzymes.

Conclusions: These results suggest a structure function relationship between GalNAc-Ts that could help to explain how upregulation of GalNAc-T14 in IgAN patients could lead to aberrant *O*-glycosylation of IgA1 HR. We postulate that the lectin domain and the variable loop in GalNAc-Ts affect *O*-glycosylation.

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SA-PO924

Molecular Characterization of IgA1 Secreted by IgA1-Producing Cell Lines from Patients with IgA Nephropathy Kazuo Takahashi,[2,1] Hitoshi Suzuki,[3,1] Koshi Yamada,[1] Stacy D. Hall,[1] Zina Moldoveanu,[1] Knud Poulsen,[4] Jiri F. Mestecky,[1] Bruce A. Julian,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Fujita Health University School of Medicine, Toyoake, Japan; [3]Juntendo University Faculty of Medicine, Tokyo, Japan; [4]Aarhus University, Aarhus, Denmark.*

Background: IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans plays a key role in the pathogenesis of IgA nephropathy (IgAN). We established IgA1-producing cells derived from the circulation of IgAN patients and healthy controls (HC). IgA1 secreted by cells from IgAN patients has more Gal-deficient *O*-glycans compared to IgA1 from HC cells. To characterize IgA1 involved with IgAN, *O*-glycan microheterogeneity and attachment sites should be analyzed, as each HR has nine potential sites for *O*-glycosylation.

Methods: In this study, IgA1 secreted by cells from IgAN patients (IgAN-IgA1; n=6) and HC (HC-IgA1; n=7) was individually analyzed to define the HR glycosylation patterns and glycosylation sites using high-resolution mass spectrometry (MS).

Results: High-resolution MS analyses revealed that the total content of *N*-acetylgalactosamine (GalNAc) in IgA1 from IgAN-IgA1 was higher that in HC-IgA1 (4.73 *vs*. 4.37 mol/HR peptide, *P*=0.003). Moreover, the amount of Gal-deficient *O*-glycans in IgAN-IgA1 was greater than that in IgA1 from HC-IgA1 (0.28 *vs*. 0.21 mol GalNAc/ HR peptide, *P*=0.004). HR glycopeptides with 5 or 6 *O*-glycan chains were more abundant in IgAN-IgA1, whereas those with 3 or 4 *O*-glycan chains predominated on HC-IgA1. Four HR glycoforms, including three glycoforms with Gal-deficient *O*-glycans, predominated in IgAN-IgA1. Tandem MS revealed that the sites with Gal-deficient *O*-glycans or nonglycosylated sites included mainly S230, T233 and T236, whereas T225, T228 and S232 were glycosylated predominantly by GalNAc-Gal disaccharide.

Conclusions: This study shows first definitive identification of HR *O*-glycosylation microheterogeneity on IgA1 from IgAN-IgA1 and HC-IgA1. The glycoforms predominating in IgAN-IgA1 may be candidates for biomarkers specific for IgAN.

Funding: NIDDK Support, Other NIH Support - DK078244, DK082753, DK083663, DK075868, and GM098539

SA-PO925

Synthesis of Galactose-Deficient IgA1 *O*-Glycans by GalNAc-Transferases Expressed in IgA1-Producing Cells: Implications for the Pathogenesis of IgA Nephropathy Kazuo Takahashi,[2,1] Milan Raska,[3,1] Milada Stuchlova-Horynova,[3,1] Alena Kasperova,[3,1] Stacy D. Hall,[1] Yoshiyuki Hiki,[2] Yukio Yuzawa,[2] Bruce A. Julian,[1] Zina Moldoveanu,[1] Matthew B. Renfrow,[1] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Fujita Health University, Toyoake, Japan; [3]Palacky University in Olomouc, Olomouc, Czech Republic.*

Background: IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans (Gd-IgA1) plays a pivotal role in IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential *O*-glycosylation sites occupied; some Gal-deficient glycans consist of terminal *N*-acetylgalactosamine (GalNAc). IgA1 HR *O*-glycosylation was reported to be initiated by GalNAc-T2. However, the expression of GalNAc-T2 did not differ between cells from patients and those from healthy controls (HC). In contrast, expression of GalNAc-T14, the enzyme with highest similarity to GalNAc-T2, was 5-fold greater in IgA1-producing cells derived from IgAN patients than in those from HC. Here, we analyzed kinetics and site-specificities of GalNAc-T2 and -T14 on HR using high-resolution mass spectrometry (MS).

Methods: We produced recombinant soluble GalNAc-T2 and -T14 enzymes. A synthetic HR (sHR) and a panel of synthetic HR glycopeptides (sGP) with a single GalNAc residue at different sites were used as enzyme acceptors.

Results: GalNAc-T2 showed higher activity *i.e.*, faster rate of glycosylation of sHR, than GalNAc-T14. GalNAc-T14 can add GalNAc to up to 5 sites in HR of IgA1. Distinct sHR *O*-glycoforms generated by GalNAc-T2 and -T14 were subjected to tandem MS to localize glycosylated sites. The sites of glycosylation on sHR catalyzed by GalNAc-T2 and -T14 were the same and appeared in an ordered fashion: GalNAc was attached to T7 first and then to T15, followed by S11 and T4. GalNAc-T14 ineffectively glycosylated the sGP with a GalNAc at S9, the site that corresponds to S230 on IgA1 HR, which is the dominant site with terminal GalNAc in Gd-IgA1 proteins.

Conclusions: GalNAc-T2 and -T14 have similar site-specificity on IgA1 HR, but differ in kinetics and how they are affected by preexisting glycosylation. Elevated expression of a specific GalNAc-T is a possible mechanism for production of Gd-IgA1 in IgAN.

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SA-PO926

MAPK/ERK Signaling Pathway Activation in Mesangial Cells Is Predictive of Renin Angiotensin System Blockers Efficiency in IgA Nephropathy Jonathan M. Chemouny,[1,2,3] Houda Tamouza,[1,2] Leona Raskova-Kafkova,[4] Laureline Berthelot,[1,2] Martin Flamant,[1,2,5] Meetu Kaushik Tiwari,[1,2] Marie Demion,[1,2] Laurent Mesnard,[6,7,8] Bruce A. Julian,[4,9] Emilie Tissandie,[1,2] Marc Benhamou,[1,2] Niels O.S. Camara,[10] Jan Novak,[4] Francois Vrtovsnik,[1,2,3] Renato C. Monteiro,[1,2] Ivan Cruz Moura.[1,2] *[1]INSERM U699, Paris, France; [2]UPDiderot, Paris, France; [3]Néphrologie, Bichat, Paris, France; [4]Microbiology, UAB, Birmingham, AL; [5]Physiologie–Explorations Fonctionnelles, Bichat, Paris, France; [6]Urgences Néphrologiques et Transplantation Rénale, Tenon, Paris, France; [7]INSERM U702, Paris, France; [8]UPMC, Paris, France; [9]Medicine, UAB, Birmingham, AL; [10]Laboratory of Transplantation Immunobiology, Department of Immunology, IBS IV, USP, São Paulo, Brazil.*

Background: IgA nephropathy (IgAN) has a significant morbidity as 20-40% of patients progress to ESRD within 20 years after disease onset. We aimed to gain insight into the molecular mechanisms involved in IgAN progression.

Methods: We evaluated renal biopsy specimens from IgAN patients (n=31) for the activation of MAPK/ERK signaling pathway. We tested the association between p-ERK1/2 staining and clinical and histological data.

Results: MAPK/ERK signaling pathway was activated in the mesangium of patients presenting with over 1 g/day proteinuria and elevated blood pressure, but absent in biopsy specimens of patients with IgAN and modest proteinuria (1 g/day). In human mesangial cells in vitro, MAPK/ERK activation through mesangial IgA1 receptor (CD71) controlled cytokines secretion and was induced by large-molecular-mass IgA1-containing circulating immune complexes purified from patient sera. Moreover, IgA1-dependent ERK activation required renin–angiotensin system (RAS) as its blockade was efficient in reducing proteinuria in those patients exhibiting substantial mesangial activation of ERK.

Conclusions: These results suggest a major role of MAPK/ERK activation in mesangial cells in glomerular injury in IgAN. We hypothesize a new mechanism sustaining anti-proteinuric properties of RAS blockers. ERK1/2 phosphorylation in mesangial cells on diagnostic biopsy may have a pronostic value concerning RAS blockers efficiency in decreasing proteinuria.

Funding: Government Support - Non-U.S.

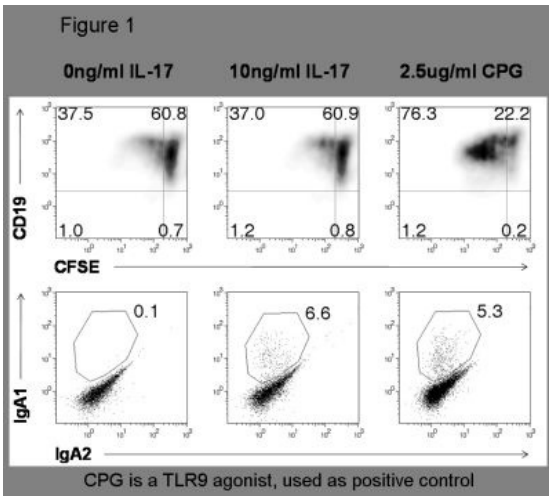
SA-PO927

IL-17-Producing CD4 T-Cells Induce B Cells Secreting IgA1 in IgA Nephropathy Patients Wei Chen, Xumin Chen, Mengjun Liang, Wang Zhang, Xueqing Tang, Xueqing Yu. *Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.*

Background: Immune complexes containing aberrantly glycosylated IgA1 are the features of IgA Nephropathy (IgAN). IL-17-Producing CD4 T-Cell cells can induce B cells proliferation, and trigger antibody production. We aimed to elucidate the role of Th17 in the pathogenesis of IgAN through promoting B cell response.

Methods: Expression of cell surface markers and intracellular cytokines was assessed by flow-cytometer, data were analyzed using FlowJo software. In vitro study, circulating B cells were enriched from healthy donors and cultured with 10ug/ml anti-IgM and 100ng/ml sCD40L, with or without 10ng/ml IL-17. B cell proliferation was monitored by measuring CFSE dilution by FACS.

Results: $IgA1^+$ B cell was found to be increased in IgAN patients as compared with healthy controls (4.97%±2.58% vs. 2.76%±1.96%, P =0.009). No significant difference in the total B cells between two groups (P =0.98). IgAN patients had more Th17 (1.88%±0.95% vs. 1.08%±0.46%, P =0.003) and Th2 cells (2.67%±1.12% vs. 1.59%±0.84%, P =0.005) compared with controls. Th17 was found to be significantly positive correlation with Th2 cells (r=0.555, P<0.001),and no significant association was observed between the frequency of Th2 cells and B cells in IgAN patients (r=0.049, P =0.760). Moreover, in vitro, induced $IgA1^+$B cells were significantly increased with IL-17 (6.6% vs. 0.1%, P =0.025), there was no change of proliferation rate of total B cells (37.5% vs. 37.0%, P=0.513).



Conclusions: Our data support the hypothesis that Th17 and Th2 participate in inducing IgA1 secreting B cells in IgAN, and IL-17 may contribute to the pathogenesis of IgAN by promoting IgA1 production.

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SA-PO928

DNA Vaccination with Plasmids Encoding Different Human Glycosyltransferases Induces High Titers of Specific Serum Antibodies in Mice Milada Stuchlova-Horynova,[1,2] Kazuo Takahashi,[2,3] Katerina Zachova,[1] Alena Kasperova,[1,2] Jiri F. Mestecky,[2] Jan Novak,[2] Milan Raska.[1,2] *[1]Palacky University, Olomouc, Czech Republic; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Fujita Health University School of Medicine, Toyoake, Japan.*

Background: Galactose (Gal)-deficient *O*-glycans on IgA1 molecules play a key role in pathogenesis of IgA nephropathy (IgAN). *O*-glycosylation is initiated by attachment of *N*-acetylgalactosamine (GalNAc) to Ser/Thr residues of the hinge region by *N*-acetylgalactosaminyltransferases (GalNAc-T2 and GalNAc-T14) followed by addition of Gal to the GalNAc catalyzed by β1,3-galactosyltransferase 1 (C1GalT1). This structure can be modified by attachment of sialic acid to the Gal residues in a reaction catalyzed by a α2,3-sialyltransferase (ST3Gal) and/or to the GalNAc residues catalyzed by α2,6-sialyltransferase II (ST6GalNAcII). We explored DNA vaccination approach for the generation of hyperimmune sera for detection of individual glycosyltransferases.

Methods: The cDNAs coding for human GalNAc-T2, -T14, C1GalT1, ST6GalNAc II, and ST6GalNAcI were cloned into mammalian expression plasmids hydrodynamically administered to BALB/c mice. In parallel, the secretory versions of above glycosyltransferases were expressed in HEK 293T cells for Western blotting. Hyperimmune sera as well as commercially available monoclonal antibodies specific to cis- and trans-Golgi markers were used for colocalization of target glycosyltransferases by fluorescence microscopy.

Results: Purified recombinant glycosyltransferases were identified by MALDI-TOF mass spectrometry and used for Western blot evaluation of specific serum antibodies elicited in mice by DNA immunization. Hyperimmune sera allowed localization of respective glycosyltransferases into cis- or trans-Golgi subcompartments of tested B cell lines.

Conclusions: We used hydrodynamic DNA vaccination for successful preparation of hyperimmune mouse sera specific for glycosyltransferases involved in generation of *O*-glycans on IgA1 molecules as a tool for detection of potential abnormalities in glycosyltransferases localization, a mechanism potentially contributing to IgA pathogenesis.

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SA-PO929

Increased Levels of Indoleamine 2,3-Dioxygenase (IDO) Activity and Regulatory T-Cells in Henoch Schoenlein Purpura Roberta Camilla,[1] Elisa Loiacono,[1] Maria Elena Donadio,[1] Margherita Conrieri,[2] Manuela Bianciotto,[2] Francesca Maria Bosetti,[2] Licia Peruzzi,[1] Davide Defedele,[3] Maria Paola Puccinelli,[3] Giovanni Conti,[4] Laura Morando,[1] Alessandro Amore,[1] Rosanna Coppo.[1] *[1]Nephrology, Dialysis, Transplantation, R. Margherita Hospital, Turin, Italy; [2]Pediatric Emergency, R. Margherita Hospital, Turin, Italy; [3]Diagnostic Department, R. Margherita Hospital, Turin, Italy; [4]Pediatric Nephrology, G. Martino Hospital, Messina, Italy.*

Background: Indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan (Trp) producing kynurenine (Kyn). IDO-expressing dendritic cells possess T cell regulatory properties that block T responses to antigenic stimulation leaded by interleukin 17 producing T cells (Th17). We aimed at investigating IDO metabolites and Th-17 cells activity in children with Henoch-Schoenlein purpura (HSP) presenting with acute onset followed by complete resolution.

Methods: 23 children (3-14 y.o) with HSP - with or without renal involvement - were studied in different phases of systemic vasculitic activity with multiple sampling. IDO activity was assessed in sera as change in Trp and Kyn determined using isocratic RP HPLC with UV detection. The Kyn/Trp ratio was also calculated. In mononuclear cells real time PRC (Taqman) detected mRNA levels of TFG-β1, which modulates the differentiation of Th17.

Results: Children with HSP had increased Kyn levels (2.31±0.54 vs 2.02±0.32 μmol/l; P= 0.048), while Trp levels were similar to controls. TGFβ1mRNA transcriptional levels were significantly depressed (0.89±0.57 vs 1.43±0.51; P<0.0001). There was a significant inverse correlation between Kyn levels and TGFβ1mRNA values (P= 0.023). In phases of clinically active purpuric rash with or without renal involvement, children presented with a Kyn/Trp ratio - indicating IDO activity - similar to controls, while it increased during healing phase and inactive disease (3.82±0.76 vs 4.58±0.77, P<0.05).

Conclusions: We report for the first time a modulation of IDO in children with HSP: the activation of this pathway, known to regulate tolerogenic immune mechanisms, is associated with remission of signs of systemic vasculitis. IDO may play a role in regression/progression of disease activity in HSP.

SA-PO930

Chemokines Usage by Tubular Epithelial Cells in IgA Nephropathy: Role of TNF-α and Angiotensin II Ai Ing Lim, Sydney C.W. Tang, Loretta Y.Y. Chan, Kar Neng Lai, Joseph C.K. Leung. *Department of Medicine, University of Hong Kong, Hong Kong.*

Background: Tubulointerstitial infiltration of immunocompetent cells is associated with a rapid progression in IgA nephropathy (IgAN). We examined the role of tumor necrosis factor-α (TNF-α) and angiotensin II (AngII) in tubular chemokines usage in IgAN.

Methods: Using a transwell co-culture system, we studied the chemokines usage and chemotactic responses of peripheral blood mononuclear cells (PBMC) towards proximal tubular epithelial cells (PTEC) following activation by conditioned medium prepared from mesangial cells cultured with IgA from IgAN patients (IgA-medium; n=45) and healthy subjects (n=30).

Results: Increased expression of chemokines, including CXCL1, CXCL2, CXCL8, CCL2, CCL5 and CCL11, was observed in PTEC cultured with IgA-medium. In response to IgA-medium, chemotaxis of CD45+ PBMC, and in particular, the activated GMP-17+, CD3+ lymphocytes and CD14+ monocytes towards PTEC were increased. The transmigrated subsets exhibited differential up-regulated expression of CXCR2, CCR2 and CCR5. Chemotactic response of activated CD3+ cells was attenuated by anti-CCL5, followed by anti-CCL2 but not by anti-CXCL1 or anti-CXCL2. Transmigrated activated CD14+ cells was reduced by anti-CCL2, anti-CCL5 and anti-CXCL2. Transmigrated activated GMP-17+ was inhibited by anti-CCL5. Under all circumstances, anti-CCL11 have no effect on the cellular transmigration. PTEC activated by IgA-medium had elevated expression of AngII, transforming growth factor-β (TGF-β) and TNF-α. The release of CXCL1, CXCL2, CXCL8, CCL2, CCL5 and CCL11, was increased by TNF-α, but not by TGF-β. AngII priming further increase all chemokines examined. Synthesis of chemokines and transmigration after activation of PTEC with IgA-medium was blocked by anti-TNF-α or losartan, but not by anti-TGF-β or PD123319 (AT2R blocker). Interestingly, TGF-β blockage enhanced the apoptosis of transmigrated GMP17+ subset.

Conclusions: Our data show the differential chemotactic responses of PBMC subsets towards PTEC in IgAN. These events utilize different chemokine-chemokine receptor axes and are tightly regulated through the interaction of IgA-medium induced TNF-α and AngII.

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SA-PO931

Cytokine-Mediated Dysregulation of Specific Glycosyltransferases Contributes to Aberrant Glycosylation of IgA1 in Patients with IgA Nephropathy Hitoshi Suzuki,[1,2] Milan Raska,[3] Koshi Yamada,[1] Zina Moldoveanu,[1] Bruce A. Julian,[1] Robert J. Wyatt,[4] Yasuhiko Tomino,[2] Ali G. Gharavi,[5] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University Faculty of Medicine, Tokyo, Japan; [3]Palacky University in Olomouc, Olomouc, Czech Republic; [4]University of Tennessee Health Science Center, Memphis, TN; [5]Columbia University, New York, NY.*

Background: IgA1-producing cell lines from patients with IgAN (IgAN cells), but not those from healthy controls (HC cells), secrete galactose (Gal)-deficient IgA1 (Gd-IgA1), with *O*-glycans with terminal or sialylated N-acetylgalactosamine (GalNAc). The aberrant glycans constitute the neoantigen that leads to formation of nephritogenic immune complexes. This aberrancy has been associated with decreased activity of β1,3-galactosyltransferase (C1GalT1) and elevated activity of α2,6-GalNAc-sialyltransferase II (ST6GalNAcII) in IgAN cells, but the regulation of these enzymes is not well understood.

Methods: To confirm the roles of these glycosyltransferases in the production of Gd-IgA1, we used knocked-down of *C1GalT1* and *ST6GalNAcII* genes by siRNA using Amaxa electroporation.

Results: Expression of *C1GalT1* decreased by 70% after siRNA knock-down in all cells,resulting in the increase of Gd-IgA1 by 50% in HC cells ($P<0.01$) and 20% in IgAN cells ($P<0.05$). Conversely, galactosylation of IgA1 *O*-glycans in IgAN cells increased after knock-down of *ST6GalNAcII*($P<0.01$). Expression of these glycosyltransferases was regulated by several cytokines, including IL-6 that decreased enzyme activity of C1GalT1

and elevated activity of ST6GalNAcII in IgAN cells. Moreover, serum levels of IL-6 were elevated in patients with IgAN compared to those in healthy control ($P<0.05$).

Conclusions: Our results confirmed the roles of C1GalT1and ST6GalNAcII in the synthesis of Gd-IgA1. Furthermore, increased levels of IL-6 contributed to the dysregulation of these enzymes. These cell lines are a valuable tool for studies of pathways leading to production of Gd-IgA1 and development of new therapeutic strategies for IgAN.

Funding: NIDDK Support

SA-PO932

Dietary Zn Condition May Determine the Susceptibility of IgA Nephropathy

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Background: Many etiologic factors including mucosal infections, genetic predisposition, diet and environmental agents are associated with the onset of IgA nephropathy (IgAN). Some experimental data have shown the relationship between IgAN and innate-immunity particularly via toll-like receptors (TLR). It has recently been revealed that Zn may play an important role in various immune-related diseases. Zn homeostasis is thus critically involved in dendritic cell (DC) maturation and affects the magnitude of immune-responses via TLR, such as TLR4. It is recently discussed that chronic Zn insufficiency by excess intake of instant food may influence the susceptibility of atopic or autoimmune diseases.

Methods: IgAN-prone mice which we recently established (Okazaki K, JASN 2012) were divided into three groups given with low, normal and high Zn diets. To assess exogenous pathogen-mediated immune responses, lipopolysaccharide (LPS) was nasally administered to each group. The activity of IgAN was biochemically and pathologically evaluated during the disease course. To approach detailed mechanisms, we cultured whole spleen cells or B cells with T cells or DCs under various Zn conditions with/without LPS, and then measured IgA production.

Results: Levels of urinary albumin and serum IgA-IgG immune-complex were increased in low Zn group. In high Zn group, serum IgA concentration and mesangial IgA depositions were significantly lower than those in other groups. IgA production by spleen cells was increased in low Zn condition and decreased in high Zn condition. In normal and low Zn condition, IgA production tended to be higher in presence of LPS. However, only in high Zn condition, IgA concentration remained at a low level despite of LPS stimulation. Furthermore, these influences of Zn concentration on IgA production by B cells were enhanced in the presence of DCs.

Conclusions: Dietary Zn may affect the amplitude of immune-responses to exogenous antigens including nephritogenic IgA production and thus play important roles in the susceptibility of IgAN.

SA-PO933

Effects of Long-Acting Erythropoiesis Stimulating Agents on Glomerulosclerosis in Acute or Chronic Model with Mesangial Proliferative Nephritis

Michinori Hirata, Ken Aizawa, Yoshihito Tashiro, Satoshi Takeda, Koichi Endo. *Product Research Department, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan.*

Background: It has been reported that darbepoetin-α (DA) prevents proteinuria in a reversible model, anti-Thy1 antibody-induced glomerulonephritis (Thy-1-GN) rats. However, it has not been elucidated whether long-acting erythropoiesis stimulating agents (ESAs) can also protect against glomerulosclerosis in chronic state. This study aimed to assess whether continuous erythropoietin receptor activator (C.E.R.A.), a newly developed long-acting ESA, could exert renoprotective effects 1) in Thy-1-GN rats (acute model) and 2) in chronic Thy-1-GN (an irreversible chronic model) rats.

Methods: Thy-1-GN rats (F344, male, 6 weeks old) were produced by the injection of anti-Thy1.1 monoclonal antibody (OX-7). At the induction of nephritis (day 0) C.E.R.A. or DA (25 mcg/kg, n=10, respectively) was intravenously injected. Chronic Thy-1-GN rats (F344, male, 7 weeks old) were produced by the injection of OX-7 and uninephrectomy. C.E.R.A. was intravenously injected 4 hour before or 24 hour after of the injection of OX-7.

Results: Both of them significantly inhibited proteinuria and cystatin C at day 6 and day 8 but not at day 4 compared to vehicle-treated Thy-1-GN rats (n=10). In addition, C.E.R.A. significantly suppressed glomerulosclerosis as well as DA at day 8. Simultaneously, the significant inhibition of C.E.R.A. on α-SMA and type I collagen expressions were observed by immunostaining method. In separated glomeruli, C.E.R.A. significantly prevented mRNA levels of fibronectin and type I collagen at day 6 and day 8 but not at day 4. In irreversible chronic model rats, both pre- and post-treatment of C.E.R.A. (25 mcg/kg, n=12) significantly inhibited proteinuria at day14. Furthermore, the effect was maintained until day 28 ($p<0.01$).

Conclusions: C.E.R.A. significantly inhibited mesangial proliferative glomerulonephritis partly due to preventing the production of matrix proteins in glomeruli. This beneficial effect of C.E.R.A. was exerted both in acute and chronic state. The renoprotective effect of post-treatment of C.E.R.A. could be of advantage in clinical conditions.

SA-PO934

A Survey of US Transplant Center Practices on Immunosuppression after an Allograft Fails

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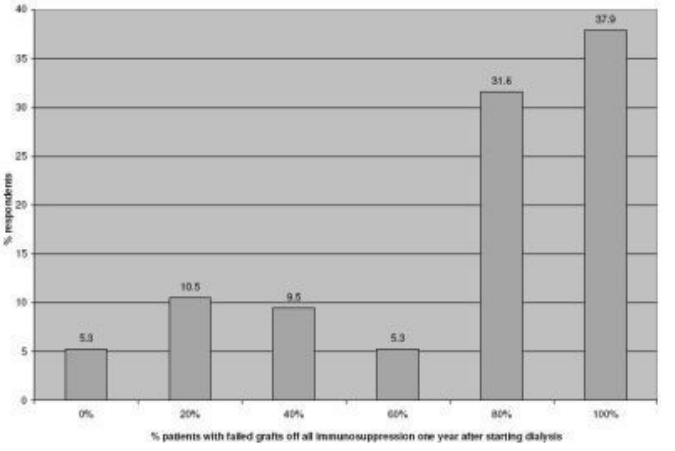
Background: The decision to continue or stop immunosuppression medication in the patient who has returned to dialysis after failure of a transplanted kidney is complex. Continuing immunosuppression increases infection risk. Stopping immunosuppression may increase risk of sensitization, reducing the chance of retransplant, or of rejection of a subsequent renal transplant. Drug cessation also increases risk of acute rejection from a failed transplant, possibly necessitating allograft nephrectomy. There is no clear standard of care. We surveyed US transplant centers to define current practices.

Methods: Using the REDCap survey program, we emailed a 19-question survey to medical and surgical transplant directors as identified by the United Network for Organ Sharing.

Results: Emails were sent to 231 programs; 97 (42%) responded. 25% of respondents reported adjusting immunosuppression according to a standard protocol; 73% said practices are physician-dependent. The majority said that 80% or 100% of patients are off all immunosuppression 1 year after returning to dialysis.



Only 5% of respondents have no patients off immunosuppression at 1 year, and 1% continued full immunosuppression without a taper. The most important factors cited in deciding whether to stop immunosuppression were plans to retransplant (38%) and signs and symptoms of rejection (36%). Respondents most commonly said they performed graft nephrectomy only if there are signs and symptoms of rejection (45%) or if signs and symptoms of rejection fail to respond to steroids (33%).

Conclusions: We lack good data to guide decisions on immunosuppression in patients with failed allografts. More study is needed to determine which policies lead to the best outcomes in terms of infection rates, success of retransplantation and overall survival.

Funding: Clinical Revenue Support

SA-PO935

Immunosuppression and Outcomes in African-American (AA) Kidney Transplant Recipients: A 4-Year Analysis from the Mycophenolic Acid Observational Renal Transplant (MORE) Registry

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Background: AA kidney transplant patients are at increased immunological risk. Tacrolimus absorption is reduced in AAs, and they may have a higher risk of rejection following mycophenolic acid (MPA) dose reductions vs non-AAs. Data on outcomes using modern immunosuppressive regimens in AAs, or comparing enteric-coated mycophenolate sodium (EC-MPS) vs mycophenolate mofetil (MMF), are lacking.

Methods: The MORE registry is a prospective, observational study of *de novo* adult kidney transplant patients receiving MPA at 40 US centers. 4-year data from tacrolimus-treated patients were analyzed.

Results: 218 AA (149 EC-MPS, 69 MMF) and 686 non-AA (467 EC-MPS, 219 MMF) patients were analyzed. The groups were similar other than living donors (24% AAs, 48% non-AAs; $p<0.01$). Mean tacrolimus trough level was similar in AAs and non-AAs at all time points. More AA vs non-AA patients received steroids, but mean steroid dose was similar in steroid-treated AA and non-AA patients. Within the AA group, more patients received the full recommended MPA dose (1.44g EC-MPS, 2.0g MMF) with EC-MPS vs MMF at month 6 (56% vs 36%, $p=0.02$) and month 36 (47% vs 17%, $p=0.03$). Graft survival at 4 years was 89% in AAs vs 96% in non-AAs (log rank $p<0.01$, Kaplan Meier); patient survival was 96% and 94% ($p=0.99$). Biopsy-proven acute rejection (BPAR) occurred in 19% AAs and 11% non-AAs ($p<0.01$). Efficacy endpoints were similar with EC-MPS or MMF in AAs and in non-AAs. Adverse events were similar in AAs vs non-AAs other than diabetes (17% vs 11%, $p=0.02$), cardiovascular events (11% vs 6%, $p=0.03$) and bone-related events (8% vs 14%, $p=0.02$).

Conclusions: BPAR and graft loss were more frequent in AAs vs non-AAs despite similar tacrolimus exposure and more frequent steroid therapy. In AAs, full MPA dose was maintained more frequently with EC-MPS than MMF at months 6 and 36, but immunosuppression may still have been inadequate.

Funding: Pharmaceutical Company Support - Novartis

SA-PO936

Effect of HLA DR Matching on Renal Transplant Outcomes Using Contemporary Immunosuppression

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Background: Does HLA DR matching has an impact on graft outcomes using contemporary immunosuppression (IS).

Methods: Retrospective analysis of 555 patients receiving a kidney transplant at MUSC from 1/2005-6/2008 was performed. Patients received an IL-2 receptor antagonist(IL-2A) as induction unless they were a re-transplant, had PRA>20%, or if the kidney had a cold ischemia time >24 hours, in these cases they received Thymoglobulin(Thymo). Maintenance IS consisted of a calcineurin inhibitor, mycophenolate, and prednisone. Kaplan–Meier analyses were used to examine associations between graft failure and variables using a log rank test to determine differences. Cox regression model was used to determine graft survival by HLA DR matching after adjusting for demographic & clinical characteristics.

Results: 62% were male, mean age 49+/-15 yrs, median f/u 1324 days, 54% were African American(AA), 68% received IL-2A induction and 32% received Thymo. 85.6% subjects had either 1 or 2 DR mismatch(DRMM). AA had an increased number of at least 1 DRMM compared to Caucasians. Patients with increasing numbers of DRMM had higher numbers of HLA A and B MM(p<.001 for both). DRMM were also associated with an increased incidence of acute rejection in the first 6 months; 0 DRMM 5%, 1 DRMM 14%, 2DRMM 19%(p=.015). Cox regression model that examined DRMM and graft survival revealed that AA had a 2.5 fold higher risk of graft failure relative to Caucasians(p=.006). Subjects with 1 DRMM had a 6.6 fold increased hazard of graft failure (p=.010) and 2 DRMM had an 8.2 fold increased hazard(p=.006) when compared to 0 DRMM. If the data is examined based on induction therapy type, the IL-2A group had a decrease in graft survival in 2 DRMM compared to O DRMM(p=.016). Thymo group did not show a decrease in survival in 2 DRMM compared to 0 DRMM.

Conclusions: DRMM is associated with increased early acute rejection even with use of contemporary immunosuppression. DRMM increases the risk of graft failure. Small subset of patients with DRMM with Thymoglobulin induction did not show increased incidence of graft failure.

SA-PO937

Early Corticosteroid Withdrawal after Kidney Transplantation: Paradoxical Effects on the Central and Peripheral Skeleton

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Background: In the first year after kidney transplantation (KTx), patients on corticosteroid-based immunosuppression experience significant loss of areal bone mineral density (aBMD) and high fracture rates. In some studies, early corticosteroid withdrawal (ECSW) is associated with lower rates of bone loss; its effects on bone quality are unclear.

Methods: We enrolled 48 adult KTx recipients, mean age 50±14 yrs, in a prospective study to quantify effects of ECSW on bone quality. We measured aBMD by dual energy X-ray absorptiometry (DXA) of the lumbar spine (LS), total hip (TH), femoral neck (FN), 1/3 radius (1/3R) and ultradistal radius (UDR) at baseline, 3, 6 and 12 months (m) after KTx. We used high resolution peripheral quantitative computed tomography (HRpQCT) to assess volumetric BMD (vBMD), bone geometry and microstructure at the radius and tibia. Comparisons were made using mixed models adjusted for age, BMI, sex, diabetes, race and pretransplant dialysis and diabetes; results are expressed as Means±SD.

Results: 39, 40 and 41 patients completed 3-, 6- and 12m follow-up, respectively. At KTx, BMI was 28±6 Kg/m^2; 29% were women and 71% were white. Over 12m, TH and FN aBMD declined at 3m (-1.2±4.1%, p<0.01 and -1.3±5.9%, p<0.05, respectively), but recovered to levels not significantly different from baseline by 12m. In contrast, 1/3R and UDR aBMD declined linearly over 12m (-1.9±6.3%, p<0.001 and -2.9±8.7%, p<0.01, respectively). LS aBMD did not change. By HRpQCT at the radius, cortical (Ct) and trabecular (Tb) vBMD declined over 12m (-2.1±4.9% and -4.7±7.1%, p<0.001, respectively). Tb microstructure did not change. These changes were mirrored at the tibia, though the amount of loss was smaller. Older age and female gender were associated with Ct bone loss. Older age was associated with Tb bone gain.

Conclusions: Over the first year of KTx, ECSW is associated with bone loss at the peripheral but not the central skeleton. Future studies will elucidate biochemical mechanisms of these bone changes and their effects on bone strength and fracture risk.

Funding: NIDDK Support, Private Foundation Support

SA-PO938

Prolonged-Release Tacrolimus (QD) Is Non-Inferior to Immediate-Release (BD) Tacrolimus Using Different Measures of Clinical Efficacy: A Combined Analysis

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Background: Non-inferiority of tacrolimus QD vs BD was assessed in two large prospective studies in *de novo* kidney transplantation. Study 12-03 was a double-blind, double-dummy, randomized trial; OSAKA was an open-label, parallel-group, randomized trial. Efficacy was assessed at Week 24 by biopsy-proven acute rejection (BPAR) in Study 12-03, and a composite endpoint based on EMA guidelines (graft loss, BPAR, renal dysfunction (eGFR; MDRD4) at the end-of-study visit) in OSAKA. These combined analyses investigate a number of different efficacy parameters.

Methods: The analyses (n=1278) included 633 patients receiving tacrolimus QD (12-03: 331 patients; OSAKA: 302) and 645 receiving tacrolimus BD (12-03: 336; OSAKA: 309). Only patients receiving a starting dose of tacrolimus 0.2mg/kg/day (QD or BD) with MMF and low-dose corticosteroids are included in these analyses. Data were collected up to the end-of-study/follow-up visit for BPAR, the US FDA composite endpoint (graft loss, BPAR, loss to follow-up), and the EMA composite endpoint. Renal dysfunction was defined as eGFR <40mL/min/1.73m^2. 95% CIs were calculated using a 10% margin for non-inferiority.

Results: Baseline demographics were balanced between groups. In both groups, 49% of kidneys were from deceased male donors. BPAR incidence was comparable between groups (13.9% vs 14.1% tacrolimus QD vs BD, respectively) (table). Of the patients who received a kidney from a deceased donor, 9.6% vs 9.9% experienced BPAR, respectively. The US FDA composite endpoint was reached by 21.5% of tacrolimus QD- and 19.8% of BD-treated patients, and the EMA composite endpoint was reached by 40.3% vs 38.3%, respectively (table). The incidence of graft dysfunction was comparable between groups.

	BPAR	FDA composite EP	EMA composite EP
Tacrolimus QD	13.9%	21.5%	40.3%
Tacrolimus BD	14.1%	19.8%	38.3%
Difference (QD–BD)	–0.2%	+1.7%	+2.0%
95% CI	–4.0%, 3.6%	–2.7%, 6.1%	–3.4%, 7.4%

Conclusions: Non-inferiority of tacrolimus QD vs tacrolimus BD, measured by standard efficacy endpoints (BPAR, US FDA and EMA composite endpoints), was demonstrated in a large combined database of kidney transplant recipients.

Funding: Pharmaceutical Company Support - Astellas Pharma Europe

SA-PO939

Efficacy and Safety of Anti-Thythymocyte (ATG) Induction for Highly Sensitized Kidney Recipients with Hepatitis C Virus (HCV) Infection

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Background: ATG induction has been reported to improve graft outcomes in highly sensitized (panel reactive antibody (PRA) >80%) kidney recipients, but its efficacy and safety is still uncertain in HCV + patients.

Methods: From 2003 to 2009, 509 deceased donor kidney recipients who received ATG induction, followed by tacrolimus, mycophenolate mofetil and steroid maintenance were retrospectively studied and divided into 6 groups based on PRA and HCV status. HCV progression as reflected by Alanine Aminotransferase (ALT), aspartate aminotransferase (AST) and HCV titers was monitored. Infection, malignancy, rejection, graft and patient survival rates were compared among these groups.

Results: Acute humeral rejection (AHR) rate was higher in highly sensitized groups, but no difference between the HCV+ and HCV-groups. Acute cellular rejection (ACR) at 1 year and late rejection at 3 and 5 year were similar among all groups. Compared to the other groups, HCV+/PRA>80% group had equivalent patient and graft survival rates at 1, 3 and 5 year. There was no increased incidence of infection and malignancy in HCV+/PRA>80% group. AST, ALT and HCV titers were also found stable among HCV+ groups.

Results

	HCV+/PRA>80	HCV-/PRA>80	HCV+/PRA 79-21	HCV-/PRA 79-21	HCV+/PRA<20	HCV-/PRA<20	P
N	23	164	14	91	40	177	
AHR 1 yr	8.7%†	8.5%†	0	4.4%	0	1%	0.0002 (0.99†)
ACR 1yr	21.7%	10.4%	6.7%	7.7%	7.5%	8.5%	NS
Rejection 5yr	0	4%	0	3%	0	4.4%	NS
Patient survival 1yr	91.7%	97%	100%	98.9%	90%	96%	NS
Patient survival 5yr	82.5%	82.7%	100%	91.9%	70.4%	84.5%	NS
Graft survival 1yr	83.3%	90.2%	100%	94.5%	84%	93.2%	NS
Graft survival 5yr	64.8%	72.7%	93.3%	79.8%	60%	74.6%	NS
Infection 1yr	47.8%	37.8%	29%	48.4%	50%	39.5%	NS
Infection 5yr	16.7%	27.3%	14.3%	17.5%	15%	32.4%	NS
Malignacy	4.5%	6.6%	0	2.2%	0	2.3%	NS

Conclusions: By using ATG induction, HCV+ highly sensitized kidney recipients did not have higher rates of rejection. They could have comparable short and long-term graft and patient survival outcomes. ATG induction was well tolerated by HCV+ patients. Our data suggested ATG induction can be efficaciously and safely used in highly sensitized patients with HCV infection.

SA-PO940

Low Dose Schemes of Thymoglobulin as Induction Therapy in High Risk Kidney Transplant Recipients Daniel Perez-Vega, Jorge Andrade-Sierra, Francisco Molina-Ruiz. *Nephrology and Transplant Uit, Hospital de Especialidades CMNO, IMSS, Guadalajara, Jalisco, Mexico.*

Background: The use of Thymoglobulin (rATG) as induction therapy in high risk kidney trasplant recipients(HKR) to prevent acute rejection (AR) is a common practice, however the evidence is scarce respecting low dose schemes. Aim: To show the AR incidence and renal function 1 year after transplantation in HKR according to two different low dose schemes.

Methods: KR were classified as high risk when: they had a previous trasplant or a cadaveric donor, and if they had a crossmatch test or PRA>10%. 93 HKR were prospectively analyzed according to total rATG dose (4mg/kg vs 4.1-6mg/kg). The primary endpoint was the rate of AR and graft failure at 1 year. The secondary endpoints were serum creatinine, hematological adverse effects and infections. All patients received inmunosupression with a calineurine inhibitor, mycophenolate mophetil and steroids.

Results: In group 1 the mean accumulated dose was 3.7 mg/kg vs 5.4mg/kg in group 2. There were no significant differences in AR(biopsy proved) rates at 1 year as shown in Table 1. Infection was significantly more frequent in the higher dose group (62.1 vs 33.9%; p=0.045).

Table 1. One year outcomes according to total rATG dose

Variable	**All (93)**	**≤4mg/kg(56)**	**>4mg/kg(37)**
Acute rejection n(%)	10 (10.7%)	4 (7.1%)	6(16.2%)
Graft failure n(%)	1 (1.1%)	1 (1.7%)	0 (0%)
Infections n(%)	42(45.1%)	19(33.9%)	23(62.1%)**
Urinary Tract Infections n(%)	40(43%)	19(33.9%)	21(56.7%)
Cytomegalovirus n(%)	1(1.1%)	1(1.8%)	0 (0%)
BK virus n(%)	1 (1.1%)	1(1.8%)	0 (0%)
Death n(%)	5 (5.3%)	3 (5.2%)	2 (5.4%)
Serum Creatinine (mg/dl)	1.13 ±0.47	1.18±0.45	1.07±0.28
Creatinine Clearence (ml/min)	81.1 ±16.7	78.7±16.2	84.6±17.1
Lymphocyte count(cels/mm3)	1005±426	1026±423	975±435
Platlet count (1000 cels/mm3)	205±58	203±62	209±52
Hemoglobin (g/dl)	13.9 ±2.1	13.8±2.1	14.2±2.0

* *p= <0.05

Conclusions: Lower dose of rATG was not associated to more AR or graft failure and it was associated to lower infection frequency compared to those with higher dose in HKR.

SA-PO941

Systematic Review and Meta-Analysis of Alemtuzumab versus Anti-Thymocyte Globulin in Induction Therapy for Kidney Transplants Kevin C. Roe, Ridhmi P. Rajapakse, Nasrollah Ghahramani. *Nephrology, Penn State College of Medicine - Hershey Medical Center, Hershey, PA.*

Background: In renal transplant recipients, induction therapy with lymphocyte depleting agents is effective at decreasing incidence of acute rejection (AR). This review compares reported outcomes of several randomized controlled trials (RCTs) using anti-thymocyte globulin (ATG) versus alemtuzumab.

Methods: We conducted a systematic review and meta-analysis to compare outcomes from induction therapy using ATG and alemtuzumab in renal transplant. Two of the authors independently conducted literature searches and data extraction from the PUBMED and Cochrane Central databases to identify RCTs comparing outcomes in adult kidney-alone transplants using alemtuzumab versus ATG induction therapy.

Results: We identified 6 RCTs (447 patients). The studies were published from 2007-2011. Four studies (n= 244) reported on AR at 1 year. For these studies, the odds ratio (OR) of AR with alemtuzumab compared with ATG induction was 0.80 (95% CI: 0.38 to 1.70; p=0.57). Among the three studies (n= 379) that reported on Cytomegalovirus (CMV) disease, the OR of CMV disease with alemtuzumab compared with ATG induction was 0.71 (95% CI: 0.37 to 1.36; p=0.30). Among the three studies (n= 379) that reported on polyoma BK-virus nephropathy(BKVN), the OR of BKVN with alemtuzumab compared with ATG induction was 0.58 (95% CI: 0.23 to 1.47; p=0.25). Four studies (n=405) reported on delayed graft function (DGF). For these studies, the OR of DGF with alemtuzumab compared with ATG induction was 0.84 (95% CI: 0.47 to 1.52; p=0.57).

Conclusions: Meta-analysis of results of RCTs of the two induction regimens showed no significant difference in the incidence of: a) AR at 1 year; b) DGF; c) BKVN; d) and CMV. Therefore, although there are multiple analyses and some conflicting data on outcomes from the use of alemtuzumab versus ATG for induction therapy, our review does not confirm a difference in the incidence of the outcomes studied.

SA-PO942

Influence of a Long-Term Immunosuppression with Everolimus on the Aortic Pulse Wave Velocity in Renal Transplant Recipients Joerg Seckinger, Anna K. Schlenker-Bø, Lars Kihm, Claudia Sommerer, Martin G. Zeier, Vedat Schwenger. *Department of Nephrology, University Hospital Heidelberg, Heidelberg, Germany.*

Background: In renal allograft recipients cardiovascular disease is the leading cause of death. The aortic pulse wave velocity (PWV) is an established cardiovascular risk factor and easy to assess in a daily clinical routine. While the therapy with calcineurin inhibitors (CNI) is suspected to promote aortic stiffness the effect of a long-term therapy with mTOR-inhibitors remains unclear.

Methods: In a clinical trial we examined the effect of a long-term immunosuppressive therapy with everolimus (EVR) compared to the standard immunosuppression with CNI on the aortic PWV in renal transplant recipients.

Results: A total of 24 renal allograft recipients were investigated (EVR: n=12, CNI: n=12). The demographic data of both patient groups (EVR vs. CNI) was comparable (age: 44.5±15.2 vs. 47.1±11.4 years, p=0.39; time on dialysis: 55.4±32.6 vs. 60.5±39.2 months, p=0.33; 75% males in both groups). The therapy switch from CNI to EVR occurred at an average of 7.5 months post transplant. While the PWV in the two patient groups was similar at the time of the therapy switch (9.74 vs. 9.68 m/s, p=0.42) a significant difference was noted at the time of the follow-up approximately 5 years (59.1±14.4 vs. 60.3±11.1 months, p=0.44) after the transplantation: 10.9±2.45 vs. 12.3±2.28 m/s (p=0.04).

Conclusions: In renal transplant recipients a long-term immunosuppressive therapy with everolimus was associated with less aortic stiffening compared to the standard treatment with calcineurin-inhibitors. The routine assessment of pulse wave velocity may aid the clinician in the decision process to switch patients at an increased risk for cardiovascular disease to an mTOR based regimen.

SA-PO943

Influence of a Switch from a Cyclosporine A to an Everolimus Based Immunosuppression on the Diabetic Status in Renal Transplant Recipients Joerg Seckinger, Florian Kälble, Christian Morath, Matthias Schaier, Vedat Schwenger, Martin G. Zeier, Claudia Sommerer. *Department of Nephrology, University Hospital Heidelberg, Heidelberg, Germany.*

Background: The choice of the primary immunosuppressive drug has a significant influence on the glucose metabolism in transplant recipients. While a calcineurin inhibitor or steroid therapy is associated with an increased risk for the development of an impaired glucose tolerance or a new onset diabetes mellitus after transplantation (NODAT) the role of mTOR-inhibitors remains unclear to date.

Methods: In a case-control study we examined renal transplant recipients that were switched from a cyclosporine A (CyA) and mycophenolate based immunosuppression to an everolimus (EVR) based therapy within the first year after transplantation with regard to the development of impaired glucose tolerance (fasting blood glucose values 110-125 mg/dl) and NODAT (>=126 mg/dl). Follow-up data was collected at 12 and 24 months after the therapy switch.

Results: A total of 146 patients (54 female, 92 male) at the age of 45.8±14.8 years were enrolled in the study (BMI 25.3±4.1 kg/m²). The demographic data in both patient groups was comparable. The switch to EVR (n=73) occurred at a median of 3.4 months post transplant. The average trough levels 12 and 24 months after the therapy switch were 6.0±2.2 mg/l and 6.4±2.9 mg/l for EVR, and 148±65 µg/l and 134±47 µg/l for CyA, respectively. The steroid doses in both groups (EVR versus CyA) at the time of the therapy switch (8.7±6.7 mg vs. 7.1±4.3 mg, p=0.78) and during the follow-up at 12 months (4.2±2.5 mg vs. 4.3±2.8 mg, p=0.15) and 24 months (3.6±0.9 mg vs. 3.8±0.9 mg, p=0.22) were comparable. The cumulative incidence of impaired glucose tolerance and NODAT 12 and 24 months after the switch of the primary immunosuppression was significantly lower for patients in the EVR group: 8.2% vs. 24.7% (p=0.007) and 1.4% vs. 12.3% (p=0.009), respectively.

Conclusions: In this retrospective study a therapy switch from a cyclosporine A to an everolimus based immunosuppression was associated with a significantly lower rate of impaired glucose tolerance and NODAT.

SA-PO944

Variation of Tacrolimus Blood Levels and Transition Readiness in Adolescent Renal Transplant Recipients Edward Iglesia,[1] Maria E. Ferris.[2] *[1]Robert Wood Johnson Medical School; [2]UNC Kidney Center.*

Background: Medication nonadherence is a major cause of rejection and graft failure in adolescents. The transition from pediatric to adult services is a particularly vulnerable period. We examined the relationship of the variation of tacrolimus blood levels with transition readiness.

Methods: We performed a retrospective study of renal transplant recipients ≥12 years old followed at the UNC Kidney Center pediatric nephrology clinic who are on tacrolimus and had a transition readiness assessment (UNC TR$_x$ANSITION Scale). Using tacrolimus (TAC) trough levels, we calculated medication adherence based on standard deviation (SD) and percent coefficient of variation (%CV). The TR$_x$ANSITION Scale was used to assess self-reported adherence (higher score = greater adherence) and overall transition readiness (higher score = greater adherence). TAC SD and %CV were calculated for 52 weeks prior to the transition readiness assessment. Spearman's correlation was performed to examine the relationships among TAC SD, %CV, self-reported adherence, and overall transition readiness. Patients with a SD>2 or a %CV>30 were considered nonadherent.

Results: Of 14 patients, 36% were female and 57.1% white. Mean age at transition assessment was 15.5±2.4 years and mean number of weeks post-transplantation was 221.6±135 (range 68-485). Mean number of TAC trough levels was 9.3±3.5 (range 3-14). Mean TAC SD was 1.7±.80, mean %CV was 25±9, mean TR$_x$ANSITION adherence subscale score was .86 ±.19 (max 1.0), and mean overall TRANSITION Scale score was 6.3±2.0 (max 10). Twenty-nine percent were considered nonadherent by TAC SD>2 and 36% by %CV>30. TR$_x$ANSITION adherence subscale score had a significant negative correlation with %CV (rho= -.62, p=.02) and trended towards significance with TAC SD (rho= -.52, p=.06). Overall TR$_x$ANSITION score (rho= -.56, p=.03) was negatively correlated with %CV (rho= -.58, p=.03).

Conclusions: Adherence based on %CV of tacrolimus blood levels significantly correlated with self-reported adherence and overall transition readiness. This supports that the acquisition of transition-related knowledge and skills may play an important role in decreasing morbidity related to medication adherence.

Funding: Private Foundation Support

SA-PO945

The Relationship between Hemorheological Factors and Vascular Endothelial Dysfunction in Cyclosporine A or Tacrolimus Treated Renal Transplant Recipients

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Background: Calcineurin inhibitors, mainly cyclosporine A (CsA), are associated with endothelial dysfunction in renal transplant recipients (RTR). Hemorheological disturbances including decreased erythrocyte deformability (ED), increased plasma viscosity and erythrocyte aggregation (EA) have also been reported in CsA treated RTR. We aimed to investigate the relationship between hemorheological factors and endothelial dysfunction in CsA and tacrolimus (Tc) treated RTR.

Methods: Thirty one RTR and sixteen healthy subjects were recruited. The RTR group included patients receiving CsA (n=16) or Tc (n=15). Endothelial function was evaluated by flow-mediated dilation (FMD) of the brachial artery.ED and EA were measured with laser-assisted optical rotational cell analyzer and plasma viscosity by a cone-plate viscometer. Transthoracic echocardiography was performed to assess left ventricular mass index (LVMI).

Results: FMD of CsA group was significantly lower than the controls (6.3±5.1vs 11.9±5.6 %, P=0.024), whereas, there was no significant difference between Tc group (8.8±5.4%) and controls. Both CsA and Tc groups had significantly higher LVMI than the controls (104.1±31.8 and 90.5±17.1 vs 72.0±16.7 g/m^2, P=0.003). At shear stresses ranging between 0.95 and 30 Pa, ED of CsA group were significantly lower compared with the controls. In Tc group, the decrease in ED was significant at shear stresses ranging between 0.53 and 5.33 Pa. ED indices did not correlate with FMD or LVMI in any of the groups.

Conclusions: The degree of endothelial dysfunction and reduction in ED were significant in patients on CsA therapy. Hemorheological factors were not likely to be associated with endothelial dysfunction in RTR.

SA-PO946

Novel Substitution of Basiliximab for FK506 in Renal Transplant Patients

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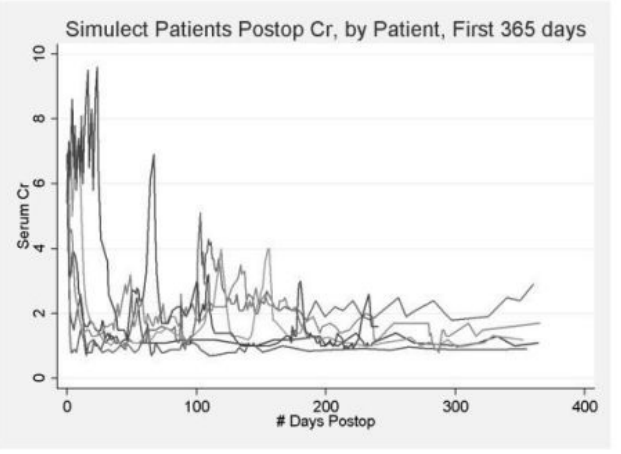
Background: Kidney transplant recipients with calcineurin inhibitor (CNI) toxicity and contraindication to sirolimus have limited maintenance immunosuppressant options. Replacement of CNI/sirolimus with Basiliximab (anti-CD25) provides a novel substitution for long-term maintenance therapy. We present 6 such cases at our institution between 2007 and 2011.

Methods: Four patients received living donor transplants (LDKT) and two received deceased donor transplants (DDKT). All LDKT recipients were highly sensitized; 3 had a positive flow crossmatch (XM+) with their donors before desensitization and one was ABO incompatible (XM+/ABOi). Besides delayed graft function experienced by DDKT recipients, all transplants were uncomplicated. Patients were discharged on MMF, Prednisone, and FK506 (n=5) or sirolimus (n=1). At mean time of 0.9 year (range 0.2-4.1 years), five patients switched to Basiliximab due to CNI toxicity and 1 patient due to non-healing wounds on serolimus. A monthly dose of 20 mg IV was administered in our infusion center. Mean follow-up time was 2.3 years (range: 0.7-5.3 years).

Results: One death was reported 13 months after DDKT due to sepsis and one graft was lost 2 years after transplantation due to recurrent hemolytic uremic syndrome. Figure 1 shows the allograft function in the survived patients. Three patients were hospitalized with diarrhea due to C. difficile colitis or CMV; one of them had urinary tract infection (UTI) as well. One patient developed BK viremia and 1 had UTI and CMV viremia but did not require hospitalization. One case of acute cell-mediated rejection was noted (Banff 2A) that resolved with therapy.



Conclusions: Basiliximab provides a novel substitution for CNI/sirolimus as maintenance immunosuppression therapy. In spite of the small size of our study, patient and allograft survival seemed comparable with our overall kidney transplant recipients.

SA-PO947

Concomitant Use of Proton Pump Inhibitors and Mycophenolate Mofetil Is Associated with an Increased Risk of Rejection in Kidney Transplant Recipients

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Background: Recent pharmacokinetic studies have demonstrated that proton pump inhibitors (PPI) reduce exposure of mycophenolic acid (MPA); however the clinical significance of this drug-drug interaction on transplantation outcomes has not been determined.

Methods: This was a retrospective cohort study to evaluate the impact of PPI use on the one-year rates of biopsy-proven acute rejection (BPAR) in kidney transplant recipients who were prescribed rabbit anti-thymocyte globulin (rATG), calcineurin inhibitor, mycophenolate mofetil (MMF) and steroids.

Results: 225 patients that were prescribed PPI were compared to 390 patients who were on standard acid-suppressive therapy with ranitidine. BPAR occurred more frequently in the PPI group (20.9% vs. 16.4%, RR=1.5, p=0.03). More black patients had BPAR (28.5% vs. 11.7%, RR=2.1, p<0.001) but were less likely to receive PPIs (46.2% vs. 53.7%, p=0.03). Age, gender, HLA mismatch, cold-ischemic time, donor age, CMV status, rATG dose and MMF dose at discharge were similar between groups. Although the PPI group had more patients with prior transplantation (21.7% vs. 13.8%) and with PRA >20% (28.8% vs. 16.7%), neither variable was associated with BPAR (both p>0.35). At one year, BPAR type, BPAR grade, patient and graft survival, graft function and time to BPAR were unaffected by PPI exposure.

Conclusions: In this retrospective study, PPI use in the first transplant year was associated with an increased risk for BPAR. It is possible that a reduction in MPA exposure contributed to the increased risk. In kidney transplant recipients on MMF, PPIs should be judiciously prescribed.

SA-PO948

Influence of the Type of Calcineurin Inhibitor and Severity of Metabolic Alterations Following Fructose Consumption in Renal Transplant Recipients

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Background: Metabolic disturbances are common in renal transplant recipients (RTR). Alterations in lipid and uric acid (UA) metabolism that have been linked to adverse graft outcome may be a consequence of impaired graft function, a choice of immunosuppressive therapy and other factors including excessive fructose consumption. The aim of the study was to examine influence of short-term fructose load on serum UA, plasma lipids and blood pressure (BP) among RTR treated with cyclosporine-based or tacrolimus-based standard triple immunosuppressive therapy.

Methods: 55 cadaveric RTR were enrolled. 26 RTR were treated with cyclosporine A (CsA), age 42.8±12.9 years, GFR$_{C\text{-}G}$ 54.4±13.8 mL/min/1.73 m^2 and 29 with tacrolimus (Tac), age 46.1±10.8 years, GFR$_{C\text{-}G}$ 55.3±14.2 mL/min/1.73 m^2. An oral fructose tolerance test (OFTT) was based on consumption of 70 g of fructose. Blood samples for UA, total cholesterol (TC), triglycerides (TG), LDL and HDL cholesterol were obtained at baseline and 60, 120, 180, and 240 min. thereafter.

Results: There was a significant increase of serum UA during OFTT in both groups from 416.6 ± 84.6 to 452.2±89.4 µmol/L after 240 min. in CsA group and from 420.4±102.4 to 449.9±101.9 µmol/L in Tac group; p <.001 in both groups. The peak increase of UA to 459.0±89.8 µmol/L and 464.1±102.8µmol/L, respectively was noticed 120 min. after fructose intake. Serum TG increased during OFTT from 2.31±1.74 mmol/L at baseline to

2.59±1.68 mmol/l after 240 min. in CsA (p <.001), and from 1.89±1.28 to 2.05±1.34 mmol/L in Tac-treated patients (p <.01). In both groups TC, LDL and HDL cholesterol significantly decreased during OFTT. No significant changes in BP were noticed.

Conclusions: RTR treated with CsA when compared to Tac patients, show similar predisposition to purine metabolic disturbances following fructose consumption. Use of immunosuppressive protocols including CsA is associated with less favorable, as compared to Tac profile of lipid changes induced by fructose.

SA-PO949

High Intrapatient Tacrolimus Variability and Early Sub-Therapeutic Tacrolimus Levels Predict Worse Renal Transplant Function at 1 Year

Henry Whalen, Julie Glen, Marc J. Clancy. *Transplant Unit, Western Infirmary, Glasgow, United Kingdom.*

Background: High intrapatient tacrolimus variability (ITV) is associated with worse clinical outcomes post-renal transplant. High tacrolimus levels cause toxicity, whilst sub-therapeutic tacrolimus (STT) levels predispose to acute rejection (AR) and graft loss. We investigated the effects of ITV and STT on renal transplant outcomes at 1 year.

Methods: Data was collected from the prospectively-compiled electronic patient record for 239 adult kidney transplants performed between 01/01/07 and 01/03/09. Patients received Basiliximab induction and triple therapy immunosuppression with prednisolone and mycophenolate. Target tacrolimus trough levels were 5-8ng/dL.

Median ITV 6–12 months post transplant was calculated, with high variability (HV) defined as variability > observed median and low variability (LV) as </= observed median.

For STT analysis, the % of STT levels in the post transplant year were calculated. Patients > observed median were considered High Frequency (HF) and those </= the median were low frequency (LF).

Groups were compared for AR, graft survival and eGFR at 1 year.

Results: Median ITV was 16%. AR in HV patients was 21% (25/119), compared with 8% (9/120) for LV patients. (p=0.0049). eGFR at 1 year was worse in the HV group (mean 54 µMol/L, SD 21.2) compared to the LV group. (mean 64 µMol/L, SD 20.0) p=0.0008.

Median STT was 14%. AR in HF patients was 21.7% (25/115) compared with 7.3% (9/124) for LF patients. (p= 0.0015). eGFR at 1 year in the HF group (mean 52.5 µMol/L, SD 22.4) was lower compared to the LF group (mean 65.0 µMol/L, SD 17.8). p=0.0001.

Conclusions: Our findings support the hypothesis that high levels of ITV and STT are associated with worse clinical outcomes. This may represent covariation with patient's compliance but the importance of achieving target tacrolimus levels is confirmed.

Our follow up may be insufficient to show an association between shorter graft survival and high ITV or STT levels. However, we find a reduced eGFR in both HV and HF groups that may predict shorter graft survival in the long term.

SA-PO950

Steroid Resumption after Early Withdrawal Predicts New Onset Diabetes and Morbid Obesity after Kidney Transplantation

Christopher A. Carlos, Donald E. Hricik, Joshua J. Augustine. *Medicine, University Hospitals Case Medical Center, Cleveland, OH.*

Background: New onset diabetes (NODAT) and morbid obesity (BMI ≥ 35 kg/m^2) are associated with poor outcomes after kidney transplantation. Randomized studies have shown modest improvements in weight gain and diabetic severity with steroid avoidance. We hypothesized that resumption of steroids after early withdrawal may lead to an increased risk of NODAT and morbid obesity and thus attenuate the metabolic benefits of steroid withdrawal.

Methods: We analyzed outcomes in 228 consecutive patients from 1/2006 to 12/2010 who all underwent steroid withdrawal at 4 days post-transplantation. NODAT was defined by outpatient fasting glucose levels of ≥ 126 mg/dl, a random glucose ≥ 200 mg/dl, or a HbA1c of ≥ 6.5% in 157 patients with no pretransplant diabetes. BMI values were calculated at 24 months in 189 patients and compared to baseline and 12 month values.

Results: Within a year of transplantation, 49 (21%) patients resumed steroid therapy (SR). There were no differences in recipient age, gender, ethnicity, or donor source between steroid-free (SF) and SR patients. SR patients had less HLA matching at transplantation, more rejection, and a lower GFR at one year. NODAT was subsequently diagnosed in 29% of the SR patients vs. 15% of the SF patients (p=0.05). At the time of transplantation, there were no differences in the rates of morbid obesity between SR and SF groups (14% vs. 10%, p=ns). However, by 24 months post-transplantation, the rates were 28% in the SR group vs. 15% in the SF group (p=0.05). Between 12 and 24 months post-transplantation, SR patients had an increase in BMI of 1.3±3.1 kg/m^2, vs. 0.5±1.5 kg/m^2 in SF patients (p=0.02). SR remained a predictor of weight gain during this time period after controlling for baseline BMI, age and ethnicity (p=0.02).

Conclusions: Resumption of corticosteroid therapy in the first year following early withdrawal in kidney transplant recipients was associated with a near doubling of NODAT and a near doubling in the rate of morbid obesity at two years post-transplantation. Steroid resumption was an independent predictor of weight gain after the first year of transplantation.

SA-PO951

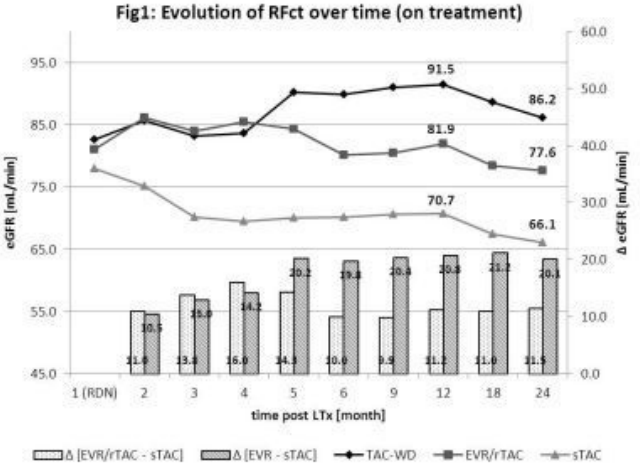
Evolution of Renal Function after Liver Transplantation: Do Patients Benefit from mTOR Treatment? 2-Year Follow-Up of 719 De Novo LTx Recipients from Study H2304

G. Junge, Patricia M. Lopez, Sven Kohler, Heike Schwende, J. Fung. *H2304 Study Group.*

Background: mTOR inhibitor facilitated CNI reduction and/or elimination may reduce CNI-associated nephrotoxicity in de novo liver transplant recipients (LTxR). Little is known about long-term treatment with everolimus(EVR) in LTx LTxR. Maintained efficacy, development and amount of proteinuria as well as evolution of renal function represent open questions to be addressed in this abstract.

Methods: H2304 (NCT00622869) is a 24-month(M) multinational RCT that randomized n=719 de novo LTxR 1:1:1 into (1) EVR (3-8ng/mL) + reduced tacrolimus (3-5ng/mL; EVR+rTAC; N=245), (2) EVR (6-10ng/mL) + TAC withdrawal (TAC-WD; N=231) at M4, and (3) standard TAC (6-10ng/mL; TAC-C; N=243). Main endpoints at M12 and 24M include composite efficacy failure rate of treated BPAR, graft loss or death and evolution of renal function(RF) from randomization to M12 and M24.

Results: BPAR rates at M12/24 were 4.1/6.1% for EVR+rTAC vs 10.7/13.3% with TAC-C demonstrating significantly lower rates with EVR/rTAC at M12 p= 0.0052 and M24 p=0.010. Enrollment in TAC-WD arm was stopped early due to higher AR rate. ITT and on-treatment analysis of evolution of renal function showed significantly better results for EVR/rTAC over TAC-C (Fig1). Urinary protein excretion was highest at M6 (290 mg/g) and further decreased in the EVR/rTAC arm from 245 mg/g at M12 to 194 mg/g at M24, clearly below the proteinuria threshold (300 mg/g).



Conclusions: H2304 24-month data confirm numerically better efficacy with significant fewer BPAR events and superior renal function with EVR/rTAC over TAC-Control. The overall safety profile was not distinct from previous experiences with everolimus and expectations in a *de novo* liver transplant population.

SA-PO952

Hepatitis C Related Renal Disease: Do HCV Patients Benefit from mTOR Treatment after LTx?

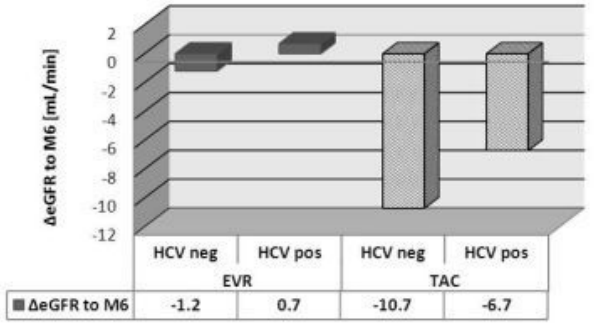
G. Junge, Patricia M. Lopez, Sven Kohler, Heike Schwende, J. Fung. *H2304 Study Group.*

Background: Tx recipients treated with CNI are at high risk of developing renal injury. CNI nephrotoxicity is manifested as acute azotemia often responsive to dose reduction, or as progressive CKD which is usually irreversible. In addition to symptomatic CKD, clinically silent glomerular disease has been described in HCV patients, primarily in those who underwent LTx due to chronic HCV. Here, the impact of an everolimus(EVR) facilitated CNI-sparing regimen on the evolution of renal function(RFct) in HCV patients post LTx is presented.

Methods: H2304 (NCT00622869) is a 24-month(M) international RCT. 719 de novo LTx recipients were 1:1:1 randomized to EVR(3-8ng/mL)+Reduced TAC(3-5ng/mL; EVR+rTAC, N=245) or EVR(6-10ng/mL)+TAC withdrawal (TAC-WD; N=231) at M4 or TAC-C(6-10ng/mL; N=243). Composite efficacy failure(tBPAR, graft loss or death) and RFct(eGFR) were assessed in the ITT and HCV population.

Results: Comparison of EVR+rTAC vs TAC-C is presented. Enrollment into TAC-WD arm was prematurely stopped due to higher tBPAR (16.5%). Patients were stratified for HCV viral status and eGFR leading to homogenous distribution of HCV patients (31.8/31.3%) and RFct at RDN. In the ITT population, composite efficacy failure at M12: 6.7 vs 9.7% (p=0.230) and BPAR: 4.1 vs 10.7% (p=0.005) for EVR+rTAC vs TAC-C. In HCV patients, efficacy failure was comparable for both arms, but fewer EVR patients had fibrosis progression. Mean eGFR in HCV patients at M6: 78.8 vs 69.9 mL/min and change in eGFR to M6: +0.7 vs -6.7 mL/min for EVR+rTAC vs TAC-C (Fig1). Complete M12/24 data will be presented.

Fig1: ΔeGFR to M6 in HCV patients post LTx



Conclusions: Early EVR-facilitated TAC reduction demonstrated comparable efficacy and superior renal function in HCV patients indicating that patients with HCV related nephropathy significantly benefit from treatment with mTOR inhibition, also suggesting lower rates of HCV related fibrosis compared to standard CNI treatment.

SA-PO953

Current Reduction in the Toxicity of Calcineurin Inhibitor on Protocol Allograft Biopsy during the Period of Two Decades (1990-2010) Ken Sakai, Yasushi Ohashi, Kentaro Tanaka, Sonoo Mizuiri, Atsushi Aikawa. *Nephrology, Toho University, Tokyo, Japan.*

Background: Calcineurin inhibitors (CNIs); the cornerstone of most immunosuppressive regimens in the past 20 years, have undesirable chronic effects. According to the improvement of therapeutic drug monitoring, we re-examined the frequency of CNI toxicity.

Methods: 1hour, 1year, 3year and 5year protocol biopsy was performed after kidney transplant (KTx) for all recipients in this institution. From 1990 to 1995, a total of 30 recipients underwent protocol biopsies up to 5years after KTx (1990's group). From 2000 to 2003, another 56 recipients underwent protocol biopsies up to 5 years after KTx (2000's group). Both of two groups took protocol biopsy completely and analyzed in this retrospective study. Anti CD25 antibody was used as an initial immunosuppressant only for 2000's group. Biopsy tissue was evaluated by Banff classification 1997. Newly appearance of Ah (arteriolar hyalinosis) score is defined as CNI toxicity.

Results: In 1990's group (13% for ABO incompatible KTx, 13% for secondary KTx, mean age 30.4±14.1yrs, donor 47.8±14.1yrs at KTx), Cv (intimal thickening) increased sequentially (0.22±0.44, 0.85±0.69, 0.83±0.75, 1.25±0.5 at 1hour, 1y, 3y, 5y respectively). Ah also increased sequentially (0, 0, 0.71±0.95, 1.0±0.81). In 2000's group (21% for ABO incompatible KTx, 7% for secondary KTx, mean age 32.8±18.4yrs, donor 52.1±12.6yrs), Cv remained constant (0.25±0.59, 0.22±0.48, 0.09±0.39, 0.16±0.25 at 1hour, 1y, 3y, 5y respectively) and Ah also remained constant (0, 0.08±0.28, 0.31±0.54, 0.17±0.31 at 1hour, 1y, 3y, 5y respectively). Serum creatinine (mg/dl) elevated more in 1990's group (1.15±0.35 to 1.31±0.62, P=0.02) during 5 years than in 2000's group (1.04±0.33 to 1.18±0.52, P=NS). Ah score was significantly higher at 5yrs after KTx in 1990's group than in 2000's group (P=0.03).

Conclusions: Contemporary use of CNI with CD25 antibody significantly reduced the CNI toxicity and improved graft function in this era.

SA-PO954

Cytomegalovirus Infection Rates Post Renal Transplant: 5 Years Single Centre Experience in United Kingdom Riaz Bavakunji, Jeetendra Ramesh Rathod, Linda Evans, Amanda J. Knight, Catherine Byrne. *Renal Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, Nottinghamshire, United Kingdom.*

Background: To evaluate the overall incidence, severity and outcome of CMV infection after 100 days of prophylactic Valganciclovir. We also estimated cost and clinical effectiveness of 200 days prophylaxis.

Methods: Retrospective data of all incident lone-kidney transplant recipients and infections between 1.1.2006 to 30.6.2011.

Results: 290 lone-kidney transplants were performed in 285 recipients. Fifty six (19%) recipients were high risk CMV Donor+/Recipient–. All appropriate recipients received prophylaxis. Forty six recipients (16%) had CMV infection, regardless of sero-status. The mean age was 46 years. Nineteen of the 46 (41.3%) had late onset (>200days) CMV infection. Of these 11 (23.17%) recipients in this group were high risk. In the late onset high risk group (11 recipients) there were two episodes of biopsy proven acute rejection, both preceding CMV infection by several months. One patient had gastrointestinal haemorrhage. All except two, patients were treated with Valganciclovir. The average dose of valganciclovir was 900mg/day. The median treatment duration 51days. The average out-patient treatment cost with Valganciclovir was £2200 ($3451) In our high risk late onset there were no proven tissue invasive disease. There was one graft failure unrelated to CMV infection or no CMV related deaths. Renal function remained unchanged after treatment up to 3 yrs. If we were to extend our prophylaxis by a further 100 days for the 56 high risk recipients our estimated additional costs would be £ 121,072($189968) when the actual treatment cost was £32620 ($51182).

Treatment Cost Analysis

	Outpatient days	Inpatient days	Cost of treatment
Valganciclovir £43.24 ($66)	417	-	£18030 ($29289)
IV Ganciclovir £35.71 ($ 56)	107	20	£4535 ($7115)
IV Foscarnet £41.39 ($65)	-	40	£1655 ($2596)
Average Bed Tariff £210 ($329)	-	60	£8400 ($13180)
Total	549	35	£32620 ($51182)

Conclusions: Pre-emptive therapy and prompt immunosuppression reduction, as opposed to longer prophylaxis, is cost effective in our population and does not seem to cause any significant mortality or rejection.

SA-PO955

Outcome of Hepatitis B and C Virus Associated Hepatocellular Carcinomas Occurring after Renal Transplantation Nada Kanaan,[1] Claudia Raggi,[1] Eric Goffin,[1] Ziad Hassoun.[2] *[1]Nephrology, Cliniques Universitaires Saint Luc, Brussels, Belgium; [2]Gastroenterology, Cliniques Universitaires Saint Luc, Brussels, Belgium.*

Background: Chronic hepatitis B (HBV) and C (HCV) virus infections are causes of morbidity and mortality in kidney transplant recipients (KTR). Immunosuppressive agents enhance HBV and HCV replication, leading to decreased patient survival due to progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC). The aim of this study was to assess the incidence and outcome of HCC in KTR.

Methods: We performed a case-control study in patients with chronic HBV and/or HCV infection who underwent kidney transplantation (KT) between 1976 and 2011 and subsequently developed HCC. Patients' characteristics and outcomes were compared to a control group of HBV and/or HCV positive patients with HCC matched for age and gender who did not have KT.

Results: Among 2944 KTR, 330 had hepatitis B and/or C. Fourteen developed HCC, an incidence of 4.24%. All patients were Caucasian, and 86% were male. Mean age at HCC diagnosis was 52.6 ± 2 years (53.2 ± 1.5 in controls, p = ns). Mean time between transplantation and HCC diagnosis was 16.7 ± 2.7 years. Six HCCs were related to HBV, 6 to HCV, and 2 to co-infection with HBV and HCV. Immunosuppressive therapy was comparable in HBV, HCV and HBV+HCV patients. All patients had corticosteroids. Sixty-four percent of patients received induction treatment and were on triple therapy. At diagnosis, 71% of patients met Milan criteria (65% in the control group, p = ns). Tumour characteristics and treatment modalities including surgical resection, chemoembolization, radiofrequency ablation, or liver transplantation were comparable between the two groups. Patient survival 2 years after HCC diagnosis was 43% in KTR, compared to 76% in the control group (p=0.03). There was no significant difference in overall survival between HBV- and HCV-infected KTR with HCC.

Conclusions: HCC occurs with an incidence of 4.24% in HBV and/or HCV infected patients after KT. Survival after HCC diagnosis is significantly worse compared to a control group of non-transplanted patients with HBV and/or HCV, matched for age and gender, and with similar tumour characteristics.

SA-PO956

Renal Allograft Outcomes in Recipients with BKVN Diagnosis Using Combination IVIG and Leflunomide Therapy Jun B. Lee,[1] Surya Seshan,[3] Lisa Walters,[1] Choli Hartono,[1] Thangamani Muthukumar,[2] David Serur,[1] Manikkam Suthanthiran,[2] Darshana Dadhania.[2] *[1]Nephrology, Rogosin Institute, New York, NY; [2]Nephrology, Weill Medical College of Cornell University, New York, NY; [3]Pathology, Weill Medical College of Cornell University, New York, NY.*

Background: BKV Nephropathy (BKVN) is associated with greater than 50% risk of graft loss. The cornerstone of management is reduction in immunosuppression. In 2005, we initiated a standard treatment protocol using IVIG and leflunomide with discontinuation of MMF and reduction of tacrolimus to troughs of 3-5ng/dL (ILTR). Here we evaluated the impact of this therapy on one of the largest cohort with biopsy proven BKVN diagnosis.

Methods: We performed a retrospective review of individuals with biopsy proven BKVN transplanted at our center between 5/1998 and 5/2010 and evaluated the impact of ILTR therapy and other risk factors on graft outcomes.

Results:

Table 1

	No ILTR Rx (N=16)	ILTR Rx (N=31)	P value
Age (mean±SD)	46 ±14	55 ±14	0.05
Gender (% Female)	8/16 (50.0%)	6/31 (19.4%)	0.03
Post Tx Month (mean±SD)	17 ±14	14 ±14	0.8
Living Donor Tx (%)	6/16 (37.5%)	14/31 (45.2%)	0.6
ATG Induction (%)	4/16 (25.0%)	27/31 (87.1%)	<0.001
Steroids Maintenance (%)	12/16 (75.0%)	14/31 (45.2%)	0.05
Baseline Creatinine (mean±SD, mg/dL)	1.5 ±0.5	1.6 ±0.5	0.5
Creatinine at Time of BKVN (mean±SD, mg/dL)	2.5 ± 0.8	2.5 ±1.1	0.4
Steroids Rx (%)	4/16 (25.0%)	4/31 (12.9%)	0.3
IVIG Rx (%)	7/16 (43.8%)	31/31 (100%)	n/a
Leflunomide Rx (%)	3/16 (18.8%)	31/31 (100%)	n/a
Graft Loss (%)	10/16 (62.5%)	11/31 (35.5%)	0.08
Median Time to Graft Loss (months)	33	37	0.5

We found a trend towards lower graft loss in those treated with ILTR therapy. A multivariate logistic regression model with stepwise backward elimination found treatment with ILTR therapy (P=0.08) and lower serum creatinine at time of BKVN diagnosis to be protective against graft loss (P=0.03) independent of age, gender, induction and steroid therapy. However, time to graft loss was not different between the ILTR group and the No ILTR group.

Conclusions: Combination therapy with IVIG and leflunomide may be associated with decreased risk of graft loss. Prospective randomized study using this combination therapy and stratification by serum creatinine level at time of biopsy is warranted.

SA-PO957

Assessment of Anti-HLA Antibody Production in Pediatric Renal Transplant and Dialysis Patients with the H1N1/Influenza A Combination Vaccine Vaishali Bansilal,[1] Gail Prado,[1] Morris J. Schoeneman,[1] Anil K. Mongia,[1] Bandana Paudyal,[1] Valeriya M. Feygina,[1] David Hochman,[2] Allen J. Norin,[2] Hanan K. Tawadrous.[1] *[1]Div. of Pediatric Nephrology, Dept. of Pediatrics, SUNY Downstate Medical Center, Brooklyn, NY; [2]Transplant Immunology and Immunogenetics, Dept. of Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Pediatric patients (pts) with ESRD who are on dialysis and awaiting for transplant, and transplanted kidney pts are immunosuppressed. It is recommended that such pts receive yearly vaccinations with the influenza A/H1N1 preparation. The immunogenicity, safety and tolerability among pediatric transplant pts for influenza A vaccines have been previously reported. Similar data is not available for dialysis pts, especially those who have been lost their renal allograft.

Methods: The aim of this study is to investigate the immunogenicity of influenza A/H1N1 given to pediatric pts with ESRD on dialysis (D) and with kidney transplant (T), and whether there is an increase in anti-HLA antibodies secondary to vaccination. 21 pediatric pts were enrolled. Mean age was 15.5 years old, 29% female, 71% male, 57% African American, 29% Hispanic. They were divided into 2 groups;12 pts with ESRD on dialysis, 4/12 with previous history of kidney transplant (D), and 9 kidney transplant recipients with good graft function (T). All subjects were tested for anti-HLA antibody 1 month prior to vaccination, and 1 month and 6 months afterwards.

Results: Among the T group, no pt tested positive for either anti-HLA class I or class II before or after Flu A/ H1N1 vaccine. In the D group, 4 pts with a previous history of graft failure were mildly sensitized before immunization; 2 showed no change in class I & II, one patient had mild increase in class I after vaccination, and one patient had an increase in class I by 24%.

Conclusions: None of the the T pts had either cell mediated or humoral rejection secondary to Flu A/H1N1 vaccine. In the D group, no pt had evidence of anti–HLA antibody following vaccination. Our study suggests that influenza A/H1N1 vaccination may be immunogenic, safe and tolerable in pediatric dialysis pts with or without a failed kidney allograft.

SA-PO958

Use of Tenofovir Did Not Negatively Impact Long-Term Graft Function in HIV (+) Kidney Transplant Recipients Gregory Malat, Dwight M. Matthew, Karthik M. Ranganna, Alden Michael Doyle. *Department of Nephrology, Drexel Medicine, Philadelphia, PA.*

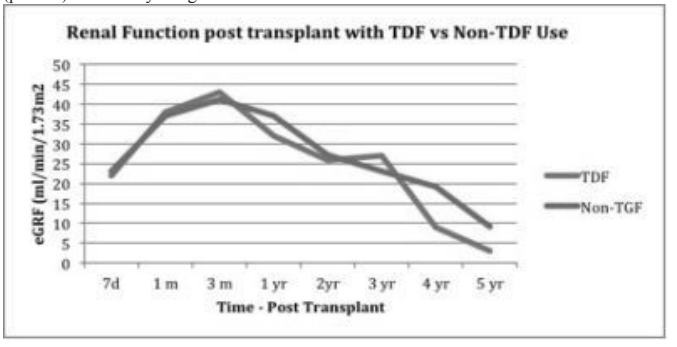
Background: Dosing calcineurin inhibitors in HIV(+) kidney transplant recipients (KTR) is difficult secondary to the drug interactions noted with concurrent HAART medications. Tenofovir (TDF) is not metabolized through the same CYP3A enzyme, but has been reported to cause nephrotoxicity in non-transplant patients. Whether TDF would have similar deleterious effects on kidney allograft function is not known. Herein, we report the first long-term experience with HAART regimens including TDF in HIV(+) KTR.

Methods: Drexel has performed 98 HIV(+) KTR since 2001. We divided the patients into 2 groups, HAART +TDF (TDF) vs. HAART without TDF (nonTDF), and analyzed long-term kidney function (~5 years).

Results: The demographics were generally comparable in both groups.

Demographic	TDF (n=21)	nonTDF (n=77)	p-value
Recipient			
Male	81%	87%	0.49
African American	62%	92%	0.00
Age, years	48 (28-62)	48 (22-71)	0.23
Donor			
Male	62%	58%	0.81
African American	19%	23%	0.78
Age, years	27 (7-60)	42 (2-76)	0.05
CVA	10%	45%	0.00
Hypertension	14%	33%	0.11
Deceased Donor	90%	91%	1.0
Terminal creatinine>1.5mg/dL	33%	32%	1.0
Cold Ischemia Time	15 (0-29)	15 (0-33)	0.59
Post-operative Characteristics			
Delayed Function	62%	60%	1.0
ACR (cumulative)	48%	77%	0.02
ACR (< 1 year)	42%	62%	0.20

The incidence of delayed function was similar in both groups. The nonTDF group had a statistically higher incidence of cellular rejection (ACR). This result was cumulative, and was not seen early post-transplant (< 1 year). Both groups had comparable graft survival (p=0.41) and kidney allograft function.



Conclusions: When HIV(+) KTR who received TDF as part of their HAART were compared with those who did not, we found that both groups had similar long-term kidney survival/function. We therefore suggest that use of TDF in HIV(+) KTR is safe when dosed appropriately.

SA-PO959

Characterization of EBV Infections in Children and Adolescents Post-Renal Transplant Nilka deJesus-Gonzalez, Isa F. Ashoor, Deborah R. Stein, Michael J. Somers. *Nephrology, Boston Children's Hospital and Harvard Medical School, Boston, MA.*

Background: EBV infections(infx) are common after pediatric renal transplants(Tx). Whether the impact and complications are different in asymptomatic vs. symptomatic EBV infx is uncertain.

Methods: We characterized the spectrum of EBV infx in pts <18yo who underwent Tx at our center over a 9-yr period. Post-Tx EBV infx was defined as >25K EBV DNA copies/ml by PCR. We ascertained post-Tx primary EBV infx or EBV reactivation incidences, episodes of acute rejection(AR) and PTLD, modifications in immunosuppression and eGFR changes during EBV viremia.

Results: In 158 pts evaluated, median age was 11yo(IQR,4-16yo), 52% boys, 48% LD recipient. 41% of recipients were EBV seropositive(+) at Tx while 92% of donors were EBV+. 97% of EBV+ recipients received an EBV+ donor graft. 57/158 pts had an EBV infx at a median of 8mo after Tx(4-19mo); 42/57 manifested a primary EBV infx with 44% having clinical symptoms. Median peak EBV copies was 178,500/ml(55,500-500K). 45/57 pts who developed EBV post-Tx were on antiviral prophylaxis. Immunosuppression was reduced in 21/57 pts who developed EBV infx. In 52/57 pts, EBV amplification turned negative in 28days(11-87days). In the 5 pts with persistent EBV viremia, median EBV copies of 80K/ml (14K-244,500 copies/ml) were found at last f/u of median 52days of EBV+ (8–648days). Children <5yo had a lower EBV+ at the time of Tx and higher incidence of EBV infx postTx compared to older children. During EBV viremia, mean eGFR was 83ml/min/1.73m2 (±43) and decreased to 66ml/min/1.73m2 (±45) on their last

f/u(p<0.0001). There were also 6 cases of AR during EBV viremia compared to 0 cases in EBV- pts(p=0.007). 10 of 57 EBV viremic pts developed PTLD vs. 1 of 101 EBV-pts(p=0.009). PTLD developed in 0-7.5mo post-infx with median EBV copies of 55,200/ml at diagnosis(40,500-292Kcopies/ml).

Conclusions: 1. 1/3 of pts developed EBV infx, despite the majority being on prophylaxis. 2.Post-tx EBV infx was more likely in younger pts, most of them having a primary infx rather than EBV reactivation. 3.Immunosuppression reduction led to EBV infx remission in most pts. 4.Pts with post-Tx EBV infx had higher incidence of PTLD and AR.

SA-PO960

Risk Factors and Outcomes of Acute Graft Pyelonephritis Prem P. Varma, Ashok Kumar Hooda, Sonia Badwal. *Department of Nephrology, Army Hospital (Research & Referral) Delhi Cantt-10, New Delhi, Delhi, India.*

Background: Acute graft pyelonephritis (AGPN) is a common post transplant complication which can result in acute graft dysfunction. Since the diagnosis is largely clinical, there is conflicting data on short and long term graft outcomes. Aim: To prospectively follow biopsy proven cases of AGPN for graft outcome.

Methods: All patients transplanted between 2002 and 2007 at our centre were followed for graft dysfunction. Anyone with graft dysfunction underwent an allograft biopsy, even if the diagnosis was apparent clinically. Diagnosis of AGPN was made by presence of neutrophilic infiltrate in the interstitium/ and/or neutrophilic casts in the tubules. AGPN was classified as "Early" if it occurred within 6 months post transplant and "late" if it occurred after that. Risk factors for AGPN were analysed. Each episode of AGPN was treated with 4-6 weeks of antibiotics. Individuals with concomitant acute rejection were given anti rejection therapy with steroids after 3-7 days of antibiotics if the graft dysfunction persisted. All patients with AGPN were given prophylactic antibiotic cover for the next 6 months and were followed for graft outcomes. SPSS 11 was used for statistical analysis.

Results: Over a mean follow up period of 30.9 months, 110 of 265 patients developed graft dysfunction. 26 (10%) had evidence of graft pyelonephritis; 9 "early" and 19 "late" AGPN. Seven had evidence of concomitant acute rejection. HCV infection was the only significant risk factor for AGPN (p= 0.03), while immunosuppression, rejection, stent placement etc did not show any significance. After a median follow up 14.6 months both early and late AGPN had poor outcomes with significantly higher Creatinine values (p=0.02 for early and p<0.01 for late AGPN) as compared to the immediate post treatment value. Those with concomitant acute rejection did not fare any better despite anti rejection therapy. 75% of patients relapsed requiring repeat courses of antibiotics.

Conclusions: AGPN is an important cause of graft dysfunction, which frequently precipitates acute rejection. The presence of HCV infection was found to be a risk factor for development of AGPN. Outcome of both early and late AGPN was observed to be poor.

SA-PO961

Long-Term Outcome of HCV-Associated *Versus* Non-HCV-Associated Glomerulonephritis after Renal Transplantation Laura Garcia-puente Suarez, Esther Gonzalez Monte, Natalia I. Polanco Fernandez, Manuel Praga, Amado Andres, J. Morales. *Nephrology Department, Hospital 12 de Octubre, Madrid, Spain.*

Background: Many studies have described the worse progression over time of hepatitis C virus (HCV) associated nephropathies, however there isn't much information available on the evolution of HCV-associated glomerulopathies in the renal transplant on subjects with active HCV infection. We collected a series of membranous and membranoproliferative glomerulopathies (MGN and MPGN respectively) which either recurred or appeared *de novo* in our transplant population and tried to define whether HCV influenced progression towards end-stage renal failure.

Methods: Single-center, observational study of subjects with biopsy proven MPGN or MGN which either recurred or appeared *de novo* after renal transplantation. Two groups were designed according to the coexistence of HCV infection.

Results: 44 patients were collected with the following demographic characteristics: 9 female *vs* 35 male, mean age 50.2 ± 13.8 years, 45.4% presenting MGN and 54.5% MPGN, and 50% being HCV positive. We analysed the evolution of renal function over time measured as serum creatinine in mg/dl (sCr), the amount of 24-hour proteinuria developed over time (24-h Prot), the long-term allograft survival, censored graft survival and patient survival.

Characteristics of study group

	HCV positive	HCV negative
Number of patients	22	22
Mean age (years)	48.09 ± 11.1	52.32 ± 16
Gender (M/F)	18/4	17/5
Mean basal sCr (mg/dL)	1.45 ± 0.43	1.47 ± 0.37
Mean final sCr (mg/dL)	4.18 ± 2.09	3.9 ± 2.55
Median basal 24-h proteinuria (g/L)	0.4 (0-1.8)	0.52 (0-2.5)
Median final 24-h proteinuria (g/dL)	2.9 (0.10-17)	3.9(0.1-12)
Mean allograft survival (months)	39.11 (3-125)	42.04 (3-144)

p<0.05 for all demographic characteristics analysed

There were no differences between the mean final sCr or the median final proteinuria of both groups. Kaplan-Meier overall survival analysis was the same for both groups.

Conclusions: In our group of patients the diagnosis of MGN or MPGN in the kidney allograft led to rapid worsening of the real function, and end-stage renal disease in most subjects although the presence of HCV infection did not result in a worse evolution over time.

SA-PO962

Urinary Tract Infections in Renal Transplant Recipients: Does Prophylaxis Prevent? Fatima Khalid, Syed Hassan, Hasan Zahid, Sarim Rashid, Tamim Hamdi, Waqas Qureshi. *Henry Ford Hospital.*

Background: Symptomatic urinary tract infections(UTI)are common in renal transplant(RT)patients. Scarce data is available about the risk factors and outcomes of symptomatic UTI in patients on antibiotic prophylaxis. The aim of this study is to analyze these features in renal transplant patients during first year post–transplant.

Methods: Consecutive patients(n=490)that underwent RT at our institute from 2008-2011 were evaluated retrospectively.227 patients were placed on prophylactic antibiotics which were included in the final analysis. Symptomatic Urinary tract infection(UTI) was defined as:presence of signs and symptoms of infection with≥10^5CFU/ml of bacteria. Urinary frequency,urgency,dysuria, suprapubic pain,flank pain,fever,chills and pain over the graft site were considered symptoms. Primary outcomes were graft failure and death within 1 year. Results were computed using univariate and multiple logisitic regression analysis.

Results: Out of 227-RT patients on antibiotic prophylaxis,102 patients developed asymptomatic and 27 developed symptomatic UTIs in the first year. Symptomatic UTI was associated with ESBL positive bacteria in 14 patients(51.8%) and E. coli in24(88.8%). Polymicrobial infections were frequently associated with symptoms17(62.9%). Symptomatic UTI was associated with diabetes,female gender,deceased kidney donors,CMV infection,longer cold ischemia time(16.7±6.8hours)and early graft loss(OR1.7;95% CI 1.1-2.9,p=0.02)but not with acute rejection or death. Previous hospitalization within 30 days with UTI was not a predictor of symptomatic UTI(p=0.319).Most common symptoms were fever in19(70.3%)patients,dysuria in16(59.2%),frequency and urgency in6(22.2%), while only 2(7%)had graft tenderness. Most of the patients had imaging characteristics of pyelonephritis25(92.5%).

Conclusions: In patients on antibiotic prophylaxis, it seems that there is still a significant incidence of symptomatic UTI and prophylactic antibiotics do not improve outcomes regarding graft loss. A recent UTI is not a predictor of recurrent UTI in these patients so there is less role of nosocomial infections in this group of patients but more of community acquired infections.

SA-PO963

Dapsone Induced Methemoglobinemia: Increased Prevalence in a Cohort of Renal Transplant Recipients Nicos Mitsides, Darren Green, Rachel Middleton, David I. New, Elizabeth H. Lamerton, Jude Allen, Jane Redshaw, Paul Chadwick, Chinari Pradeep Kumar Subudhi, Grahame Wood. *Salford Royal Hospital, United Kingdom.*

Background: Dapsone is the commonest cause of drug-induced methemoglobinemia (MHb). The prevalence of MHb in patients on dapsone is reported to be up to 20%. Following an outbreak of pneumocystis pneumonia (PJP) in our tertiary nephrology unit, dapsone 50-100mg daily was used as second line chemoprophylactic agent. Because dapsone is renally excreted, we hypothesized that the rate of MHb would be even higher in this CKD population. We also aimed to describe the demographics, risk factors and presenting features of MHb in these patients.

Methods: This was a case series of 26 consecutive transplant recipients commenced on dapsone for chemoprophylaxis against PJP from February to September 2011. All patients had negative Glucose-6-phosphate dehydrogenase levels prior to treatment. Measurement of MHb level was triggered either by symptoms or drop in serum hemoglobin (Hb) and cases were diagnosed based on elevated levels (>1.5%). MHb patient characteristics were compared against the rest of the cohort to determine potential risk factors.

Results: Of the 26 patients, 46% developed MHb (MHb levels 6.4±4.1%). 50% of cases were symptomatic on presentation, with breathlessness most common (33.3%). Cases had a mean drop in Hb of 19±7.0%. Hb drop was associated with low serum albumin (correlation coefficient [cc] -0.805, p=0.002) and baseline Hb (cc -0.715, p<0.001). MHb led to 5 admissions (median length of stay, 5 days, range 1-10 days) with MHb level showing a strong correlation with length of stay (cc 0.762, p=0.002). 17% of cases required blood transfusion, 17% had computed tomography of the Chest and 8% had bronchoscopy. Following the high number of adverse reactions, the use of dapsone as chemoprophylaxis was stopped.

Conclusions: This is the highest reported prevalence of MHb in patients receiving dapsone and its use led to significant morbidity in this renal transplant population. This study raises concerns to its use as chemoprophylaxis in this setting. As half the cases were asymptomatic on diagnosis, we recommend MHb levels be measured routinely in all patients.

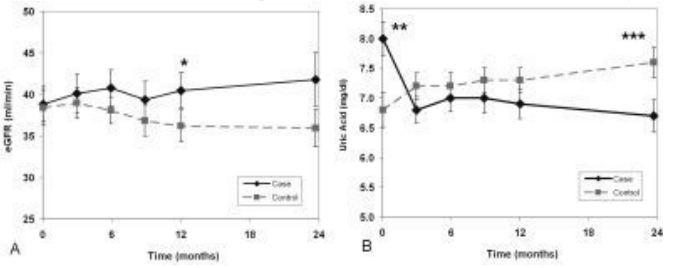
SA-PO965

Effect of Allopurinol in Slowing Allograft Functional Decline in Kidney Transplant Recipients Bhargavi Tangirala, Liliana Osadchuk, Muhammad H. Bashir, Richard J. Marcus, Sabiha M. Hussain, Khaled Nashar, Kalathil K. Sureshkumar. *Division of Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: There is some evidence that hyperuricemia may increase risk for allograft functional decline and the development of chronic allograft nephropathy in kidney transplant recipients (KTR). We aimed to explore any potential beneficial effects of allopurinol in KTR.

Methods: A retrospective chart review of KTR at our institution from 2003-2008 was performed to identify patients on allopurinol (cases), who were then matched with controls based on the time of transplant (±3 months) and estimated GFR (eGFR; ±10 ml/min). Serum uric acid (UA) levels, eGFR, blood pressures (BP), dipstick proteinuria (grade 0-4) and graft loss were compared between the groups.

Results: Out of 428 KTR,54 cases and 54 controls were identified. At the start of allopurinol, mean serum UA level (mg/dl) was 8.0±2.0 vs. 6.8±2.1 (p=0.001) and eGFR was 38.8±15.5 vs. 38.4±15.5 (p=0.64) for cases vs. control groups. Serum UA level was significantly lower at 2 years (6.7±1.7 vs. 7.6±1.6, p=0.02) and eGFR significantly higher at 1 year (40.5±15.2 vs. 36.2±13.4, p=0.04) for cases vs. controls as observed in the figure.



*p=0.04,**0.001,***0.02.

Proportion of patients on cyclosporine A (CsA), BP, proteinuria at 1 year, graft loss at 3 years were similar in both groups as shown in the table.

outcome	Case(n=54)	Control(n=54)	p-value
Graft loss(n)	3	8	0.11
% on CsA	39	42	0.51
Proteinuria(0-4)	0.23±0.77	0.23±0.60	1.00
SBP(mm Hg)	136±20	134±16	0.76
DBP(mm Hg)	75±11	73±10	0.67

Conclusions: Our study showed a potential beneficial effect of allopurinol in preserving eGFR in KTR. This could be related to allopurinol's ability to ameliorate the harmful effects of hyperuricemia such as oxidative stress, endothelial dysfunction, proinflammatory and proliferative actions.

SA-PO966

Osteoporosis after Renal Transplantation Siren Sezer,[1] Emre Tutal Tutal,[1] Mehtap Erkmen Uyar,[1] Zeynep Bal,[1] Bahar Gurlekdemirci,[1] Firdevs Tugba Bozkurt.[2] *[1]Nephrology, Baskent University, Ankara, Turkey; [2]Internal Medicine, Baskent University, Ankara, Turkey.*

Background: By the improvement of long-term patient and graft survival after renal transplantation,post transplantation complications gained importance. The restoration of vitamin D synthesis, clearance of phosphate, and reduction of parathyroid hormone (PTH) levels decreases bone loss but on the other hand, osteoporosis still remains as an important problem due to steroid use, persistent hyperparathyroidism in some cases and other disturbances. In this present study, we investigated the effects of renal function and antropometric measurements on bone mineral density in renal transplant recipients.

Methods: 103 renal transplant recipients (34 female, age, 35.9 ± 9.3 years) with bone mineral densitometer (BMD) analysis at the end of post-transplant 1 year were included in the study. BMD (femoral neck) were measured by dual-energy X-ray absorptiometry (DEXA).Body compositions were analyzed by using the Body Composition Analyzer (Tanita BC-420MA). Laboratory tests including creatinine, GFR, albumin, CRP, calcium, phosphorus, PTH, hemoglobin levels at the end of first post transplantation year were retrospectively collected. Patients were grouped according to femoral neck t scores as osteoporotic (n: 34, t score < -2) and normal (n: 69) BMD patients.

Results: Female gender was more common in osteoporosis group (%52 vs %23, p: 0.003). Glomerular filtration rate of osteoporotic patients were significantly lower (65.4 ± 28.2 vs 79.4 ± 22.8 ml/min, p: 0.03). We found that osteoporotic patients had lower waist-hip ratios, sagittal abdominal diameters, body mass index, fat mass, visceral fat ratio, muscle mass and degree of obesity (p: 0.0001). A correlation analysis revealed that femur t score was positively correlated with glomerular filtration rate (r:401, p: 0.002), body mass index (r:.427, p: 0.001), fat mass and visceral fat ratio (r:.361, 446 respectively, p: 0.001), and negatively correlated with PTH level (r: -.370, p: 0.008).

Conclusions: Our results indicate that better graft function improves bone mineral disease and prevents post transplantation osteoporosis despite of corticosteroid usage in renal recipients.

SA-PO967

Delays as an Indicator of Dysfunctional Systems and Processes in Kidney Transplantation Daniela Ladner, Olivia A. Ross, Pamela H. Sharaf, Kathleen R. Hoke, Krutika Lakhoo, Anton I. Skaro, Elisa J. Gordon, Amna Daud, Donna Woods, Michael Abecassis, Jane Holl. *NU Transplant Outcomes Research Collaborative, Northwestern University, Chicago, IL.*

Background: Kidney transplantation is a complex procedure involving multidisciplinary teams, which can unveil OR system and process failures. To assess safety vulnerabilities, a proactive Failure Modes Effects Analysis (FMEA) was performed.

Methods: FMEA participants included transplant nurses, anesthesiologists, surgeons, technicians, and students. A process map was created by observation and FMEA. System and process failures and causes were identified. Frequencies and potential consequences were assigned. Failure causes were coded applying Joint Commission root cause categories.

Results: 10 FMEA sessions (17 participants) and 12 observations identified 82 steps, beginning with OR set up and ending with patient transfer from OR. 155 system and process failures were identified, with 203 total potential consequences grouped into 7 types (Table 1).

Failure Mode Consequences

Consequence Type	n (%)	Examples
Delay	49%	Waiting for lab results, OR supplies not available
Wasted resources	19%	Incorrect supplies opened, Contamination of central line kit
Injury to patient	14%	Incorrect positioning leading to neuropraxia, Omitted antibiotics leading to infection
Poor teamwork	8%	Telephones/pagers interrupting care, Unprofessional behavior leading to frustration
Risk to personnel	4%	Uncovered cords leading to falls, Inappropriate disposal of sharps leading to injury
Suboptimal care	4%	Oversedation, Critical information missed during timeout
Incomplete/Inaccurate Documentation	2%	EMR not available, incomplete recording of urine output

Nearly half of process failures result in delay. Types of causes leading to delays were: Human Factors (57%), Leadership (20%), Physical Environment (18%), and Communication (5%).

Conclusions: Despite their frequency, delays are often ignored as an accepted part of patient care. Causes of delays offer insight into existing systems and process vulnerabilities. FMEA findings can support proactive systems and process re-designs to reduce human error and improve and standardize communication, care planning, and leadership.

Funding: Private Foundation Support

SA-PO968

Effect of a Multifactorial Intervention with the Aid of Nursepractitioners on Cardiovascular Outcome in Kidney Transplant Recipients Arjan D. Van Zuilen,[1] Michiel Bots,[2] Peter J. Blankestijn,[1] Jack F. Wetzels.[3] *[1]Nephrology, UMC Utrecht, Netherlands; [2]Julius Center, UMC Utrecht, Netherlands; [3]Nephrology, Radboud University Medical Center Nijmegen, Netherlands.*

Background: The MASTERPLAN study evaluated if implementation of current guidelines with aid of a nurse practitioner improved cardiovascular outcome in patients with chronic kidney disease(CKD). Despite small but significant improvements of blood pressure(BP), LDL-cholesterol(LDLc) and proteinuria in the intervention group(IG), no differences in the primary outcome were observed. We performed a post hoc analysis in prevalent transplant recipients that participated in the study.

Methods: MASTERPLAN is a randomized controlled clinical trial, performed in nine Dutch hospitals. 788 patients with CKD (eGFR 20-70 ml/min/1.73m2) were randomised to either receive care by the nephrologist (CG) or intensified care with nurse practitioner support(IG). At baseline stratification on transplantstatus was performed. Both groups were subject to identical guidelines. Patients were followed for a median of 4.8 yrs. The primary endpoint was a composite of myocardial infarction, stroke and cardiovascular death. Also changes in quality of care of cardiovascular risk factors were evaluated.

Results: We analysed 110 transplant recipients (66M), age 51 (12) yrs, eGFR 40(13) ml/min/1.73m^2. Mean follow-up after transplantation 7.5 (5.4) years. The intervention resulted in significantly lower diastolic BP 77 vs 81 mmHg (p=0.006), no significant differences were noted for systolic BP, LDLc, proteinuria, smoking cessation, body weight, physical activity or sodium excretion. The primary outcome occurred in 33.1/1000py in IG and in 25.2/1000py in CG (HR 1.33; 95%CI 0.46 to 3.84). Incidence of ESRD was 39.1 vs 38.6/1000py (HR 0.99; 95%CI 0.39 to 2.49). There were no adverse consequences associated with the intervention.

Conclusions: In this subgroup of 110 patients only diastolic BP is significantly better in IG than in CG. For all other factors direction of difference was similar to the main study, however no statistical significant changes were found. The intensified treatment did not significantly reduce the rate of the composite endpoint or the rate of ESRD.

Funding: Pharmaceutical Company Support - Genzyme, Sanofi, Amgen, Pfizer, Private Foundation Support

SA-PO969

Vitamin D Deficiency in Renal Transplantation Is Not Associated with Graft Fibrosis Kristin Vibeke Veighey,[1] David C. Wheeler,[1] Anne B. Dawnay,[2] Francis Lam,[2] Martyn Egerton,[3] Alexander J. Howie,[1] Mark Harber,[1] Aliyye Karasu,[1] Cono A. Ariti,[4] John Cunningham.[1] *[1]UCL Centre for Nephrology; [2]UCLH; [3]Epsom General Hospital; [4]LSHTM.*

Background: Vitamin D deficiency is endemic in CKD patients undergoing kidney transplantation and may contribute to increased ischaemia-reperfusion injury and graft fibrosis. We postulated that patients deficient in 25-hydroxyvitamin D (25OHD) and/or 1,25-dihydroxyvitamin D (1,25OH_2D) would therefore have increased fibrosis on subsequent protocol biopsies.

Methods: 103 patients (53% White, 22% Black, 15% Asian, 3% Mixed, 7% other) transplanted between 2008-11 had protocol biopsies at 6 (t6) and 52 weeks (t52) and had stored pretransplant serum available. None received supplemental vitamin D at transplantation, 47% were supplemented thereafter. Serum 25OH D2/D3 and total 1,25OH_2D levels were quantified by mass spectrometry and enzyme immunoassay respectively. Fibrosis was quantified using a validated index of chronic damage (ICD) measurement (%). Linear regression was used to assess associations between 25OHD/1,25OH_2D and: 1). ICD at t6 and t52, 2). MDRD eGFR at 1 year.

Results: At transplantation, 80% were 25OH vitamin D deficient (<75nmol/l), including 60% of Asian patients. In 94%, 25OHD2 was below the assay range. 1,25OH_2D levels were subnormal in 60% (80% Asians) and normal (40-150pmol/l) in the remainder. 25OHD levels did not predict 1,25OH_2D levels (r =-0.04 p=0.689). 68% of those replete in the former were deficient in the latter. 1,25OH_2D was not significantly higher in those receiving 1alphacalcidol (45 vs 38pmol/l). At t52 sera were available from 37 patients. Of those

supplemented, (cholecalciferol 20,000iU/wk) 92% became vitamin D replete (>75nmol/l). Despite adjustment for cold ischemic time, donor age, and recipient diabetes, there was no association between vitamin D metabolites and ICD at either t6/t52, or eGFR at 1 year.

Conclusions: Only 20% of CKD patients were vitamin D replete at transplantation. 25OHD2 status had little bearing on overall D status. Oral supplementation effectively repleted 25OHD. There was no association between vitamin D status and chronic damage on protocol biopsies or eGFR.

Funding: Pharmaceutical Company Support - Abbott

SA-PO970

Assessment and Management of Chronic Pain InRenal Transplant Patients
Rosa Sanchez, Juan Pablo Leiva, María José Fernández-reyes, Manuel M. Heras, Alvaro Molina Ordas, Maria Astrid Rodriguez Gomez, Fernando Alvarez-Ude, Carmen Centeno. *Nephrology, General Hospital Segovia, Segovia, Spain.*

Background: Little is known about chronic pain and long-term impact on renal transplantation (RT). We studied the prevalence of pain, clinical and psychological risk factors associated, treatment and analgesic response in recipients of RT.

Methods: Prospective, transversal in 101 TR. Variables were analyzed clinical, laboratory and radiological weapons. We used the following scales: Brief Pain Inventory-Short Form (BPI-sf), Hospital Anxiety and Depression Scale (HADS), Short Portable Mental Status Questionnaire (SPMSQ) and Palliative Performance Scale (PPS). Statistical analysis SPSS 18.0.

Results: Mean age of 59.3 years. 65.3% men and 46% (N = 47) of the population had pain. Of these, 74% (N = 35) noted pain in more than two locations, the average of the highest intensity of pain is 7/10 points, the lowest average intensity 4/10. 25% (N=12) had previously prescribed analgesia and reported relief an average only 30%. Anxiety was found 14% (N= 7) and 8% depression (N= 4). 88% of patients had between 90-100 points in the PPS. 96% of the population had no cognitive impairment. Pain patients show a higher incidence of bone loss (BL), digestive disease and greater use of steroids. In the study univarientes confirm a correlation between pain and: use of steroids and calcineurin inhibitors ($p<0.0001$ and $p=0.027$ respectively), BL ($p=0.000$) gastrointestinal disease ($p=0.032$). In multivariate analysis with correction for age, sex, and years of transplantation the only factors that independently correlated with pain were quantified steroids (ExpB: 9.171, CI: 2.602 to 32.328, $p=0.001$) and osteoporosis (ExpB: 6.527, CI: 2.049 to 20.788, $p=0.002$). Importantly, pain occurred in patients regardless of stage of disease. No relationship was found between pain and laboratory parameters examined, duration of hemodialysis years, and transplant years evolution.

Conclusions: About half of patients with RT have chronic pain, mainly associated with steroids and BL. The transplant patient's functional capacity is excellent despite the pain. Analgesic treatment in this population is underutilized.

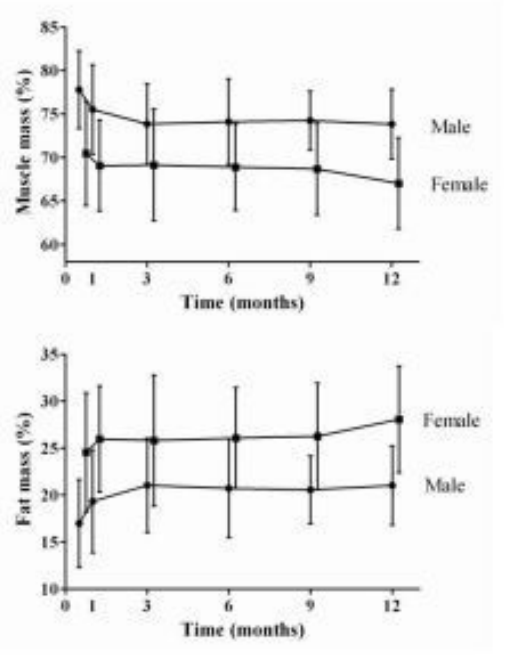
SA-PO971

Change in Body Compositions of Asian Recipients after Kidney Transplantation
Seung Seok Han, Curie Ahn, Yon Su Kim. *Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.*

Background: Kidney transplantation and accompanying medical conditions may result in changes in body composition. Such changes have been evaluated in Caucasian recipients, but not in Asian recipients. Herein, we conducted a study on Asian recipients because Asians have a different body composition from Caucasians.

Methods: A total of 50 Asian recipients (M : F, 24 : 26; body mass index, 21.8 ± 2.6 kg/m^2) were enrolled as a prospective cohort. Using bioelectrical impedance analysis, body composition (muscle and fat mass) was assessed after 2 weeks (baseline), and at 1, 3, 6, 9, and 12 months following kidney transplantation. To find predictors related to changes, the data were analyzed by multivariate analysis using forward selection.

Results: All of the patients had good graft function during the study period (last serum creatinine, 1.16 ± 0.28 mg/dL). Patients gained approximately 3 kg within 1 year of kidney transplantation. The proportion of muscle mass significantly decreased (P trend = 0.001) and the proportion of fat mass significantly increased over time (P trend = 0.002).



The multivariate results revealed that male recipients, deceased donor type, and low protein intake were associated with an increase in fat mass and a decrease in muscle mass.

Conclusions: The results from this study may help to investigate differences in body composition changes between races, as well as the factors related to these changes.

SA-PO972

Cholecalciferol Supplementation Reduces Proteinuria and Parathormone in Kidney Transplant Patients
Ines Aires, Manuel A. Ferreira, Fernando Barbosa Nolasco. *Nephrology and Transplantation, HCC, Lisbon, Portugal.*

Background: Native vitamin D (25-OH vitD) levels are frequently reduced in chronic renal failure patients (pts). Less is known in the kidney transplant (KTx) population. Chronic kidney disease-mineral and bone disorder (CKD-MBD) is common in KTx pts but frequently underlooked.

Methods: We prospectively evaluated the effects of 1 year cholecalciferol supplementation, in 133 KTx pts, 84 men, mean age 54.4±13 years, 34 diabetic, mean post KTx follow up 80.6±64.9 months. All pts were naïve to 25-OH vitD therapy. Immunosuppression was based in calcineurin inhibitors in 79% and sirolimus in 21% pts.

Pts were supplemented accordingly to basal (T0) calcidiol serum levels (SerL): deficiency (<15), insufficiency (15 to 30) and normal (>30 ng/mL). Paired t and Anova tests were used.

Results: At T0, 25-OH vitD levels were 15.4±8.1 ng/mL. 54.9% pts were deficient and 38.4% insufficient. Mean PTH levels were 134.8±90.9 (pcg/mL). 25-OH vitD deficient pts showed higher PTH levels (153±107 vs 97±45 in calcidiol sufficient pts).

All pts completed 6 months (T6), cholecalciferol supplementation (median dose 2664 UI) and 78 pts completed 1year (T12). At T6 mean calcidiol SerL increased to 29,7±11 and at T12 to 30,8±10,2. At T6 4,5% pts remained in deficiency, and 45,8% achieved normal SerL (vs 6.7% T0).At T12, 55% pts attained normal calcidiol levels.

Proteinuria was significantly reduced from 0.99±1.6g/day to 0.64±0.9 g/day (T6), and to 0.56±1 g/day ($p<0.001$) (T12). Pts under sirolimus had the higher proteinuria at T0 and in his group the reduction was higher. PTH levels were significantly reduced to 113±92 pg/mL (T6) and to 107±79,4 ($p<0.001$) at T12. No differences were observed in calcium, phosphorous and creatinine SerL. 25-OH vit.D supplementation was well tolerated, and no adverse events were reported.

Conclusions: Our results shows that vitamin D deficiency is highly prevalent in KTx pts. Oral supplementation with cholecalciferol is efficient, cheap and safe in the correction of calcidiol SerL, leading to significant reduction in proteinuria and improvement in CKD-MBD.

Larger and longer randomized controlled studies are needed to confirm these results in this particular population.

SA-PO973

Vitamin K Intake and Vascular Vitamin K Status in Kidney Transplant Recipients Paul Boxma,[1] Else van den Berg,[1] Johanna M. Geleijnse,[2] Gozewijn Dirk Laverman,[3] Leon J. Schurgers,[4] Cees Vermeer,[5] Gerjan Navis,[1] Stephan J.L. Bakker,[1] Martin H. De Borst.[1] *[1]Dept of Nephrology, University Medical Center Groningen; [2]Dept of Nutrition, Wageningen University; [3]Dept of Medicine, ZGT Almelo, Netherlands; [4]Dept of Biochemistry, Maastricht University, Netherlands; [5]VitaK BV, Maastricht.*

Background: Vitamin K insufficiency is associated with an increased risk of cardiovascular morbidity and mortality. Vitamin K is essential for activation of vascular calcification inhibitor proteins such as matrix Gla-protein (MGP). Insufficient vitamin K intake results in accumulation of uncarboxylated MGP, contributing to an increased risk of vascular calcification. In kidney transplant recipients, cardiovascular risk is high but vitamin K intake and status have not been defined.

Methods: We investigated dietary vitamin K intake, vascular vitamin K status and its determinants in a cohort of kidney transplant recipients (n=60) with stable renal function (creatinine clearance 61 [42-77] (median [interquartile range]) ml/min), 75 [35-188] months after transplantation). Vitamin K intake was estimated by 3-day food records and food frequency questionnaires, and vascular vitamin K status was measured as plasma desphospho-uncarboxylated MGP (dp-ucMGP) by ELISA.

Results: Total vitamin K intake was below recommendations (<120 mg/d for men or <90 mg/d for women) in 50% of patients, probably due to less consumption of green vegetables. Patients below the median of vitamin K intake had higher dp-ucMGP levels (621 (481-927) vs 753 (543-1091), p<0.05). Accordingly, the majority of patients (80%) had elevated dp-ucMGP levels (>500 pmol/L), suggesting vitamin K insufficiency. Multivariate regression revealed CrCl (standardized β -0.51, p<0.001), coumarin use (st. β 0.31, p=0.005), body mass index (st. β 0.35, p=0.001) and sodium excretion (st. β 0.22, p=0.05) as independent determinants of vascular vitamin K status.

Conclusions: A considerable part of the kidney transplant population has increased plasma dp-ucMGP levels, which may contribute to arterial calcification. Improving vitamin K status may have health benefits for kidney transplant recipients.

Funding: Government Support - Non-U.S.

SA-PO974

Elevated Circulating Desphospho-Uncarboxylated Matrix Gla Protein Is an Independent Risk Factor for Mortality and Graft Failure after Kidney Transplantation Paul Boxma,[1] Elke Magdeleyns,[2] Cees Vermeer,[2] Gerjan Navis,[1] Stephan J.L. Bakker,[1] Martin H. De Borst.[1] *[1]Department of Nephrology, University Medical Center Groningen; [2]VitaK BV, Maastricht, Netherlands.*

Background: An increased circulating level of desphospho-uncarboxylated matrix Gla-protein (dp-ucMGP) species, indicating vitamin K insufficiency, has been associated with cardiovascular morbidity and mortality and with progression of chronic kidney disease. Its role in allograft and patient survival after kidney transplantation is unknown. We hypothesized that plasma dp-ucMGP is an independent risk factor of mortality and graft failure in kidney transplant recipients (KTR).

Methods: Baseline plasma dp-ucMGP levels were measured by a conformation-specific sandwich ELISA in a cohort of stable outpatient KTR (n=528) at 7.8 ± 6.4 (mean ± SD) years after transplantation. Associations between dp-ucMGP levels and the composite outcome of all-cause mortality and graft failure were analyzed by multivariable Cox regression analysis adjusting for age, gender, eGFR (CKD-EPI), proteinuria, hemoglobin, use of ACEi/ARB, waist circumference, smoking, diabetes, total cholesterol, HDL cholesterol and systolic blood pressure. Sensitivity analyses were performed in a subgroup with eGFR >30 and <90 mL/min.

Results: At baseline, patients (55% male, age [mean±SD] 52±12 yrs, eGFR 62±23 ml/min) had plasma dp-ucMGP levels of median [interquartile range] 1039 [733-1542] pmol/L; 91% of the population was above the upper-normal limit of 500 pmol/L, indicating vitamin K insufficiency. After 6.4±1.7 years of follow-up, 133 (22%) patients had died and 53 (9%) had developed graft failure. Plasma dp-ucMGP levels were associated with an increased risk of all-cause mortality or graft failure (full model hazard ratio 1.86 per 1 SD increase [95% CI 1.23-2.82], p=0.004). Analyses per tertile of dp-ucMGP and sensitivity analyses provided similar results.

Conclusions: Elevated plasma dp-ucMGP, indicating vitamin K deficiency, is an independent risk factor for mortality and graft failure after kidney transplantation. Future studies should address whether vitamin K supplementation improves outcome after kidney transplantation.

Funding: Pharmaceutical Company Support - VitaK BV, Maastricht, The Netherlands, Government Support - Non-U.S.

SA-PO975

Long-Term Management of Severe Recurrent Focal Segmental Glomerulosclerosis by Soluble Urokinase Receptor Modification Changli Wei,[1] Jochen Reiser,[1] Martin G. Zeier,[2] Christian Morath.[2] *[1]University of Miami; [2]University of Heidelberg, Germany.*

Background: Soluble urokinase receptor (suPAR) is a cause of native and recurrent focal segmental glomerulosclerosis (FSGS) through pathological activation of podocyte β3 integrin. Lowering of suPAR to ranges whereby podocyte β3 integrin is turned off, can lead to clinical remission.

Methods: We report the effects of suPAR modification in long-term management of severe recurrent FSGS accompanied by renal failure.

Results: A 59-year old female patient with primary FSGS had elevated pretransplantation serum suPAR level (5,782 pg/mL), suggesting high risk for FSGS recurrence, which was later confirmed by graft biopsies and by proteinuria of 9.7 g/d. As serum suPAR remained high post-transplantation, intensive plasmapheresis (PP) treatments were initiated with significant improvement of proteinruia. Attempts to wean the patient from PP failed due to severe oliguria following suPAR rebound, suggesting dependence of improved podocyte foot process structure and hydraulic permeability on sufficient circulating suPAR reduction. Since PP was not well tolerated, the patient was instead treated with immunoadsorption (IA), which showed equal efficacy in producing a 42% suPAR reduction rate per session. Overall, the serum suPAR was maintained at a median level of 3,878 pg/mL through PP or IA treatments. Using cellular podocyte integrin activation assay, we found that PP reduced podocyte β3integrin activation gradually, which was associated with a parallel decrease in proteinuria. Reducing PP to twice a week however led to a reversible increase in podocyte β3 integrin activation and rising proteinuria to 10.8 g/d. Both proteinuria and β3 integrin activity was lowered significantly again with IA. More than 15 months after transplantation, the patient is now successfully managed with one IA treatment on alternate weeks with a serum creatinine of 1.5 mg/dL and proteinuria of 2.6 g/d. Electron microscopy shows partial recovery of podocyte foot process pathology on postoperative day 437.

Conclusions: This study suggests that circulating suPAR reduction is critical for renal output, foot process recovery and management of severe recurrent FSGS.

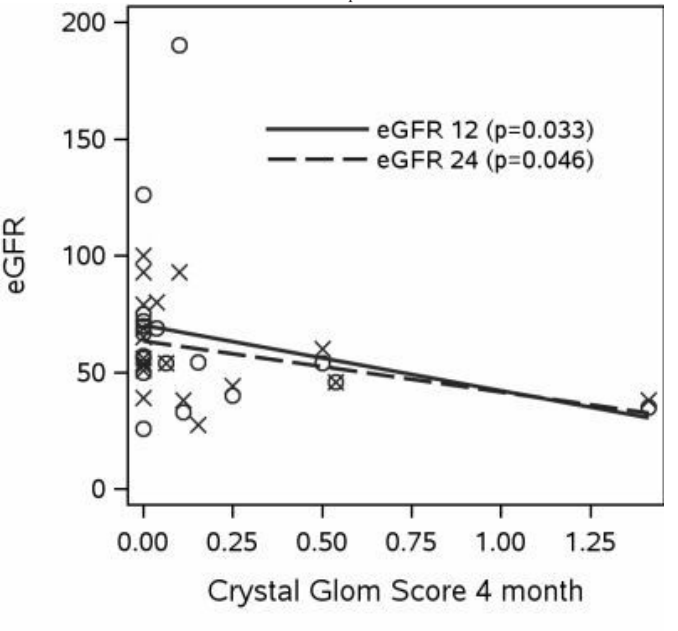
SA-PO976

Post Transplant Recurrence of Oxalosis in Primary Hyperoxaluria Type 1: A Study of Protocol Biopsies Nageswara Pamidi,[1] Lynn D. Cornell,[2] Eric J. Bergstralh,[3] Ramila A. Mehta,[3] Julie Heimbach,[4] Dawn S. Milliner.[1] *[1]Nephrology, Mayo Clinic; [2]Pathology, Mayo Clinic; [3]Biostatistics, Mayo Clinic; [4]Transplantation Surgery, Mayo Clinic.*

Background: Primary hyperoxaluria (PH) accounts for 1% of pediatric ESRD in US and European registries. After transplant (Tp) recurrent oxalosis in the allograft remains a problem. Protocol biopsies were studied to assess the frequency of recurrence and its effect on renal allograft function.

Methods: Of 330 patients enrolled in Rare Kidney Stone Consortium PH Registry 26 patients who received 27 transplants[8 kidney alone (K),19 simultaneous liver and kidney(L/K)] and had at least one protocol biopsy(4, 12, 24 or 60 months)were included. Median age at Tp was 33 years.Crystal score in kidney tissue was estimated as number of crystals /total glomeruli.Spearman's correlation was used to test associations.

Results: 73 protocol biopsies were done in 27 allografts.Calcium oxalate(CaOx)crystals were found in atleast one biopsy in 55%(15/27)of allografts. No associations were found between CaOx crystals and donor age, time on dialysis before Tp & Tp type (K or L/K). Crystals were more often seen in 4 month biopsy (45%) than at 12 and 24 months (15% & 31% respectively).15% of implantation biopsies showed crystals 20 min after graft placement.The crystal/glomerulus score (mean 0.08, range:0-1.41) correlated with 24 hour urine oxalate (r=0.41,p=.0007),calcium(r=-0.42,p=.003),magnesium(r=-0.48,p=.006)and with plasma oxalate at time of biopsy(r=0.44,p=.0008).Crystal score at 4 months(n=19) correlated with eGFR at 12 and 24 mos after Tp



After 24 months 87.5% grafts survived with mean eGFR of 63.2 ml/min/1.73m².2 grafts were lost to oxalosis & 1 to rejection.

Conclusions: Recurrence of Caox crystals was seen in 55% of allografts.Intensity of crystal deposition correlates with concurrent urine analytes and has a negative impact on 2yr graft function.

SA-PO977

Desensitization Protocols for Highly Sensitized Patients on the Waiting List for Deceased Donor Kidney Transplant Hyuk Yong Kwon,[1] Yoonjung Kim,[1] Myung-gyu Kim,[1] Tai Yeon Koo,[1] Curie Ahn,[1,2] Jaeseok Yang.[1] *[1]Transplantation Center, Seoul National University Hospital, Seoul, Korea; [2]Department of Nephrology, Seoul National University Hospital, Seoul, Korea.*

Background: Along with decreased organ availability, HLA sensitization is the most important barrier to deceased donor kidney transplantation (DDKT). Here, we present our experiences of desensitization (DST) for wait-listed patients for DDKT.

Methods: Highly sensitized adult Korean patients with peak panel reactive antibody (PRA) level above 50% that had waited for DDKT for longer than 4 years from 2010 to 2011 in Seoul National University Hospital were enrolled. High dose intravenous immunoglobulin (IVIg, 2 g/kg) and rituximab (375mg/m^2) were administered at day 0. Another dose of IVIg (2 g/kg) and a cycle of bortezomib (1.3mg/m^2) were administered at day 30.

Results: A total of 8 patients were enrolled. Mean age was 53.0±7.6 years, and 6 patients were male. Initial peak PRA level was 98.1±2.2% and sum of mean fluorescent intensity (sMFI) of class I and II antibodies was 26549.8±9036.7. Six cases were retransplants and 2 patients had a history of multiple transfusion. A mean waiting time was 7.8±4.2 years. The mean follow up PRAs were 88.8±19.4% (sMFI 23984.1± 14849.5), 83.5±23.8% (sMFI 20814.6±11488.0), and 85.0±15.6% (sMFI 22554.1±13624.6) at 3, 6, and 12 months after DST, respectively.

4 among 8 patients (50%) had succeeded in getting KT at 2.5±1.4 months after DST with mean reduction of PRA by 17% (sMFI by 30%) just before KT. Donor sepcific antibody (DSA) was converted to negative or titer of DSA decreased at time of KT compared with that before DST. Patient and graft survival were 100%, with 3 patients having at least 12 months of follow up. Their mean serum creatinine at 12months after KT was 1.23±0.31 mg/dl. Acute rejection or infection did not occur.

The other 4 patients are still on the waiting list. Their mean PRAs were 97.3±2.2% (sMFI 28529.8 ±6450.8) before DST and 93.3±5.6% (sMFI 32311.8.0±5623.5) 1 year after DST with a tendency of less reduction compared with the KT group.

Conclusions: DST using high dose IVIg with rituximab/bortezomib could be a promising strategy for the highly sensitized, long-waiting patients for DDKT.

SA-PO978

Incidental Finding of IgA Deposits in Deceased Donor Kidneys in Zero-Hour Biopsies and Impact on Allograft Function Rachelle V. Dyquiangco,[1] Chalit Wanthakawikran,[1] Lillian W. Gaber,[2] Venkataraman Ramanathan.[1] *[1]Nephrology, Baylor College of Medicine, Houston, TX; [2]Pathology, The Methodist Hospital, Houston, TX.*

Background: Given that IgA nephropathy is the most common primary glomerulonephritis worldwide, it is not uncommon to see incidental IgA deposits in kidney biopsies performed in donors without overt renal disease. We studied the prevalence of such deposits among our deceased donors and the impact on allograft function.

Methods: During the 2-year study period, 186 patients underwent deceased donor (DD) kidney transplantation at The Methodist Hospital, Houston, and zero hour biopsy was performed in 156 DD kidneys. We identified biopsies with significant IgA deposits and compared renal outcomes in recipients of those kidneys with matched controls. Microscopic exam of urine, random protein-creatinine ratio (expressed in gm/gm of creatinine) and serum creatinine were obtained at 12 and 18 months post-transplant for both groups. Microscopic hematuria was defined as >3 RBC/HPF. Student t-test or Fisher Exact test was used as appropriate.

Results: IgA mesangial deposits were seen in 22 donor kidney biopsies (14%) and we compared the recipients of these kidneys with controls (n=22). Donor (age, gender, race, cold ischemia time, ECD kidney, and history of HTN) and recipient characteristics (race, etiology of ESRD, number of kidney transplants and high PRA, defined as >20%) were similar in both groups. As shown in table, patients with IgA deposits had similar allograft function, hematuria incidence and proteinuria at 12 and 18 months, compared to patients without these deposits.

	IgA deposits in Donor kidneys (n=22)	Controls (n=22)
Serum creatinine @ 12 months (mg/dL)	1.6±1.5	1.3±0.4 *
Serum creatinine @ 18 months	1.4±0.5	1.5±0.6 *
Microscopic Hematuria at 12 months	1 (5%)	1 (5%) *
Microscopic Hematuria at 18 months	0	1 (5%) *
Proteinuria at 12 months	0.25±0.63	0.17±0.28 *
Proteinuria at 18 months	0.06±0.16	0.18±0.2 *

Conclusions: IgA deposits noted during zero-hour biopsy do not have significant impact on allograft function during short-term follow up. Incidental finding of these deposits should not preclude donation.

SA-PO979

Renal Transplantation in Patients with Atypical Hemolytic Uremic Syndrome Jacobien Verhave,[1] Henk W. Van Hamersvelt,[1] Maria J. Van Helden,[1] Nicole Van De Kar,[2] Jack F. Wetzels.[1] *[1]Nephrology, University Medical Center, Nijmegen, Netherlands; [2]Pediatric Nephrology, University Medical Center, Nijmegen, Netherlands.*

Background: Atypical hemolytic uremic syndrome (aHUS) is mostly caused by complement dysregulation. After renal transplantation, 50% of patients with aHUS develop disease recurrence. Risk factors of recurrence include use of calcineurin inhibitors, rejection, cold ischemia, recurrence of aHUS in a previous graft. It is proposed that all patients should receive prophylactic therapy with plasma exchange or eculizumab.

Methods: We have used an adapted transplantation protocol in patients with aHUS who received a living donor kidney.

Results: Four patients were transplanted. Two patients had lost a previous graft due to recurrent aHUS (patient 3 and 4). In three patients genetic abnormalities in complement regulation were found (Table). Immunosuppression consisted of basiliximab induction and low doses of tacrolimus (0.03 mg/kg BID), prednisone and mycophenolate mofetil (AUC 40-60 mg*h/ml) with the possibility of eculizumab rescue treatment. All patients received a statin, and were targeted to a low blood pressure (CCB, ACEi) to prevent endothelial damage. Follow-up is now 6 – 11 months. No patient developed a rejection or needed eculizumab for recurrent aHUS. Outcome is given in table.

Conclusions: Our data indicate that renal transplantation in aHUS patients, using a protocol that minimizes endothelial damage, seems to have a high success rate without plasma exchange or eculizumab prophylactic therapy. Although long term outcome is unknown, our data suggest that in adult patients controlled trials are needed to demonstrate cost efficacy of prophylactic therapy compared to rescue treatment with eculizumab. Our data are not (yet) applicable to recipients of cadaveric donor grafts.

Patient and outcome data

Patient	Sex	Age	Compl	Follow up (months)	Creatinine (umol/l)/ Uprot	LDH	Thrombocytes
1	F	35	Lower C3	11	115/ neg	177	274
2	F	54	polyCFH	9	113/ neg	233	285
3	F	29	CFH	9	73/ neg	214	317
4	M	46	C3	6	118/ neg	169	170

Tk = number of transplantation; Compl = abnormalities in complement regulation; CFH = factor H; poly = polymorphisms

Funding: Clinical Revenue Support

SA-PO980

Reversibility of Calcineurin Inhibitor Related Thrombotic Microangiopathy with Short Course Eculizumab Miriam R. Berry, Andrew Butler, Meryl Griffiths, Nicholas Torpey. *Addenbrooke's Hospital, Cambridge, United Kingdom.*

Background: Thrombotic microangiopathy (TMA) affects up to 10% of patients with renal dysfunction following solid organ and hematopoietic stem cell transplantation. The etiology is multi-factorial, but calcineurin inhibitors (CNIs) are likely to play a major role. Eculizumab, a monoclonal antibody against the complement component C5, has been used in a number of clinical settings including paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. It may have a potential role in the treatment of post-transplant TMA.

Methods: We report 4 patients (aged 28-51 yrs) treated with eculizumab for de novo post-transplant TMA; 2 following multi-visceral transplantation, one renal and one stem cell allograft. All 3 solid organ recipients had previously been treated for acute rejection but none had detectable donor specific antibodies nor evidence of humoral rejection. No complement mutations were detected.

Results: TMA was diagnosed at a mean of 20.5 (range 4-48) weeks post transplant; 2 patients had biopsy proven renal TMA and 2 had diagnostic peripheral blood films and acute kidney injury. The mean peak creatinine was 425 (range 180-600) µmol/l and one patient was hemodialysis dependent. All were taking CNIs which were discontinued at the time of diagnosis. 3 patients were treated with plasma exchange without improvement in renal parameters. All patients received 900 mg eculizumab weekly for 4 weeks, and one patient received maintenance therapy for 4 months. No major adverse events were observed, and in particular there were no infective episodes attributable to this treatment. Mean creatinine improved to 200 (range 70-400) µmol/l (p=0.025) and no patients required ongoing hemodialysis. In 3 patients there was no evidence of ongoing or recurrent TMA following completion of eculizumab therapy with mean follow up time of 20 (range 4-36) weeks.

Conclusions: Short course eculizumab therapy is a safe and effective therapy which completely or partially reversed TMA and significantly improved renal function in 4 patients with refractory post-transplant TMA.

SA-PO981

Renal Transplantation and Alports's Syndrome in Ireland: A National Retrospective Review of Outcomes Kai Ching Peter Leung, Carol A. Traynor, Frank J. O'Brien, Catherine M. Brown, Peter J. Conlon. *Department of Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: Alport's syndrome is a progressive hereditary nephritis caused by abnormal alpha IV collagen chains of the basement membrane. Patients with renal failure from Alport's syndrome account for 1.6% of all transplants in Ireland. We present one of the largest series of transplanted patients with Alport's syndrome.

Methods: Data was collected from patients who underwent renal transplantation in Ireland between 1980 and 2011. Transplanted Alport's syndrome patients were identified based on biopsy and compared to a matched non- Alport's transplanted population which comprised of non diabetic living and deceased donor transplants with recipients greater than 16 years of age. Incidence of anti-GBM antibody disease post- transplant was also recorded.

Results: 51 renal transplants were identified in 42 Alport's syndrome patients. The mean age at transplant was 32.7 years (SD 11.2 years) and 85.7% were male. There was a known family history in 23 patients. There were 47 cadaveric transplants and 4 living related transplants. 2 patients had preemptive transplants. Of the 42 patients transplanted the 1, 5 and 10 years patient survival rate was 97.4%, 97.4% and 72.9% respectively compared to 95.8%, 86.6% and 72.9% for non Alport's (p=0.021). Overall graft survival was 94.1% at 1 year, 80.6% at 5 years and 60.8% at 10 years. This is compared to 88.3%, 73.2% and 53.8% graft survival in the non- Alport's population (p=0.131). Median graft survival was 15.6 years, compared to 11.3 years in the control group. 20 patients failed their first graft and 9 of these patients went on to receive a second transplant Graft loss was due to chronic allograft nephropathy in 57.9% of cases and acute rejection occurred in 38.8% of transplants. There was no evidence of anti-GBM antibody disease recorded in the Alport patient cohort.

Conclusions: Renal transplantation was successful in patients with Alport's syndrome. Chronic allograft nephropathy accounted for almost half of graft loss. Acute rejection was high in our cohort. Second transplant is effective and safe. In our series, there were no cases of post-transplant anti-GBM antibody disease.

SA-PO982

The Decline in GFR in Children Following Renal Transplant Khurrum Siddique,[1,2] Leisa Borders,[2] Mouin Seikaly.[1,2] *[1]Pediatric Nephrology, UT Southwestern Medical Center, Dallas, TX; [2]Pediatric Nephrology, Children's Medical Center, Dallas, TX.*

Background: Measured Glomerular Filtration Rate (mGFR) remains the gold standard for evaluating renal function. Our primary end point is to: 1) evaluate the mGFR at 1, 2 and 3 years post renal transplant in our pediatric patients, 2) estimate an annual rate of decline of mGFR following kidney transplant, and 3) evaluate factors that may affect mGFR over time.

Methods: Plasma disappearance of ^{125}I-iothalamate (C_{IO}) was used as a marker to mGFR. C_{IO} was performed on patients who received renal transplant between June 1987 – Sept. 2011. The average follow-up time post renal transplant was 4.9 years (range 0.8-16.2 years). Mixed linear regression analysis of log mGFR after renal transplant was performed to evaluate 220 C_{IO} studies, and mGFR was estimated at 1, 2 and 3 years post-transplant. Variables tested for association with decline in renal function were: age at the time of transplant, sex, ethnicity, primary renal disease as glomerular (G) versus non-glomerular (NG), source of allograft (deceased versus living donor), and length of hospital stay (LOS) following renal transplant.

Results: A total of 220 C_{IO} studies were performed on 123 renal post-transplant patients. Mean age at transplant was 9.7±4.7 years (age range: 1.1-18.8 years). 48% of our cohort had more than one C_{IO} test performed. mGFR at 1, 2 and 3 years post-renal transplant was 68.2, 62.4 and 57.2 ml/min/1.73m^2 respectively. The overall rate of mGFR decline following transplant was 8.8%/yr. Patient's age, sex, ethnicity, primary renal disease, source of allograft, and LOS were not associated with the rate of decline of renal function. Higher mGFR at baseline was associated with younger age at transplant (2.7% higher per year of age below the mean) and G disease (29% higher than NG disease).

Conclusions: In our cohort of transplanted children the rate of decline of mGFR following renal transplant was 8.8% per year. Younger age at transplant and glomerular diseases were associated with higher mGFR at baseline but were not associated with variation in the rate of decline of mGFR.

SA-PO983

Efficacy and Safety of Enzyme Replacement Therapy in a Cohort of Kidney Transplant Recipients with Fabry Disease Markus Cybulla,[1] Sandro Feriozzi,[5] Michael L. West,[2] Joan Torras,[6] Kathleen M. Nicholls,[3] Gere Sunder-plassmann.[4] *[1]Nephrology and Rheumatology, FGM, Center of Internal Medicine, Muellheim, Germany; [2]Nephrology, Dalhousie University, Halifax, Canada; [3]Nephrology, Royal Melbourne Hospital, Melbourne, Australia; [4]Nephrology, Medical University, Vienna, Austria; [5]Belcolle Hospital, Viterbo, Italy; [6]Hospital of Bellvitge IDIBELL, Barcelona, Spain.*

Background: Enzyme replacement treatment (ERT) was introduced in 2001 as a new treatment option for patients with Fabry disease. The Fabry Outcome Survey (FOS, sponsored by Shire HGT) is a worldwide outcome database which was established to monitor the long-term efficacy and safety of ERT with agalsidase alfa. The study presents the clinical data of a follow up analysis in kidney transplant recipients (KTRs) with Fabry disease.

Methods: The effects of long term ERT with agalsidase alfa (Replagal®; Shire HGT) on renal outcome were analyzed.

Results: Renal function was analysed in 77 patients (male 70 (90.9%), female 7 (9.1%), 62 (80.5%) received ERT at any time, mean time since transplantation to start of ERT 25,7 (77.3) months, mean time on ERT until last visit, 3.7 (2.9) years; mean time on dialysis before transplantation 4.2 (5.4) years). During the observation time (start of ERT and last visit) the average slope per year in eGFR was -0.03 (-1.66, 1.61; 95%CI) mL/min/1.73 m2 in men and -0.25 (-7.08, 6.59) mL/min/1.73 m2 in women. Patients with proteinuria had poorer graft function at follow-up compared with patients without proteinuria. Arterial hypertension (RR > 120/80 mmHg) has no negative impact on values of proteinuria and renal function respectively. Additionally dialysis treatment before transplantion has also no negative impact on the later graft function. Only minor side effects or infusion related reactions are reported.

Conclusions: This study showed, that graft function in KTR Fabry patients remains stable under enzyme replacement therapy and that the treatment seems to be well tolerated.

Funding: Pharmaceutical Company Support - Shire HGT

SA-PO984

Role of Monocyte in the Development of Chronic Kidney Disease in Kidney Transplant Recipient Elena Guillen-gomez,[1] Lluis Guirado Penit,[2] Carme Facundo,[2] Jose Ballarin,[2] Montserrat M. Diaz Encarnacion.[2] *[1]Laboratory Medicine, Fundación Puigvert, Barcelona, Spain; [2]Nephrology, Fundación Puigvert, Barcelona, Spain.*

Background: Chronic allograft tubular atrophy/interstitial fibrosis (TA/IF) occurs early after transplantation. Interstitial fibroblasts seem to be one of the principal sources of kidney fibrosis. There are several studies that show a close association between macrophage infiltrate and excessive extracellular matrix protein accumulation. In addition, the number of infiltrating macrophages has been shown to correlate with the number of myofibroblasts. The aim of this study is to determine the role of the activated circulating monocytes in kidney allograft recipients pre-and post-transplant, as an initial response to tissue damage and a chronic inflammatory stimulus that could be related to early onset of chronic kidney allograft dysfunction.

Methods: Whole blood samples from 24 kidney recipients and 17 living donors were collected at different time points. Monocytes were analyzed by flow cytometry and levels of IL-10 and soluble CD163 were determined by ELISA. Clinical dates were get it from clinical database.

Results: Classical monocytes (CD14+/CD16-) difference between month 4 and baseline correlated positively with creatinine levels (p=0.0018) and negatively with MDRD (p=0.006) at month 12, and with creatinine at month 18-24 (p=0.02). As described, at 24 hours there is a pick of surface CD163 (p=0.0001), after one week post-transplant surface CD163 and IL-10 levels remain higher than baseline (p=0.0003 and p=0.0463, respectively), while the concentration of soluble CD163 decreased (p=0.0107). Monocytes CD163+ difference between 1/2 weeks and baseline correlated positively with creatinine levels (p=0.04) at 4 month.

Conclusions: These results of CD163 could be initially associated with an early onset of healing, but the persistence of higher levels than baseline could be associated with mechanisms that have been related to fibrosis.

Increasing total amount of classical monocytes (CD14+/CD16-) correlation with serum creatinine and MDRD confers on these cells a new and promising prognostic value on the development of chronic kidney disease in renal transplant patients.

Funding: Private Foundation Support

SA-PO985

FOXP3 Expression in Peripheral Blood Lymphocytes of Kidney Transplant Recipients under Different Induction Antibody Therapies Mark L. Lipman,[1] Yan Zhang,[1] Minh-tri Nguyen,[2,3] Michel Marcil,[1] Jean Tchervenkov,[3] Steven Paraskevas.[2,3] *[1]Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada; [2]Human Islet Transplantation Laboratory, McGill University Health Centre, Montreal, QC, Canada; [3]Department of Surgery, McGill University, Montreal, QC, Canada.*

Background: Regulatory T cells (Treg) have been recognized as a mechanism to promote tolerance in organ transplantation. Forkhead box P3 (Foxp3) is an important transcription factor for the development and function of Treg. Here, we describe FOXP3 expression in peripheral blood lymphocytes of 35 renal transplant recipients treated with either one of the three common induction therapies: rabbit antithymocyte globulin (rATG) or alemtuzumab (ALTZ), which are both lymphocyte-depleting agents (LDA), and basiliximab (BSLX), a nondepleting agent.

Methods: FOXP3 was determined by qPCR on Day 0 prior to transplantation and then on Day 1, 7, 14, 30, and 180 post-transplantation.

Results: Both rATG and ALTZ resulted in immediate and dramatic suppression of FOXP3 expression to less than 10% of baseline at Day 1 and 7 post-transplantation. Subsequently, with LDA, FOXP3 levels recovered modestly to 40% of baseline by Day 14 and to 50% and 70% at 1 and 6-months post-transplantation, respectively. Early results, based on a limited number of cases treated to date with BSLX, demonstrated a much more modest reduction in FOXP3 expression to only 40% at Day 1 and 7 and a full recovery to baseline by Day 14.

Conclusions: It appears that FOXP3 expression varies considerably between lymphocyte-depleting rATG/ALTZ treatment vs. non-depleting BSLX treatment with a more profound and prolonged suppression of FOXP3 expression with the former. These findings do not support a determining role for absolute levels of FOXP3 transcripts in the lower rejection rates reported with LDA compared to BSLX. However, since the lower FOXP3 levels with rATG/ALTZ are likely a function of the lymphopenia, it does not rule out the possibility of a relative shift in T cell subsets (within an overall reduced T cell population), in favor of a higher Treg/Teffector ratio with rATG/ALTZ, as mechanistically implicated in the lower rejection rates described with LDA.

Funding: Private Foundation Support

SA-PO986

Metallothioneins as Functionally Relevant Markers for Biological Organ Age in Preimplantation Kidney Biopsies Johannes Leierer,[1] Michael Rudnicki,[1] Paul Perco,[2] Irmgard Muehlberger,[2] Christian Koppelstaetter,[1] Wolfgang Steurer,[4] Robert Öllinger,[3] Gert J. Mayer.[1] *[1]Dept. of Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria; [2]Emergentec Biodevelopment GmbH, Vienna, Austria; [3]Dept. of General and Transplant Surgery, Medical University Innsbruck, Austria; [4]Dept. of Surgery, Robert-Bosch-Krankenhaus, Stuttgart, Germany.*

Background: Renal transplantation is the treatment of choice for patients with end stage renal failure. Despite overall inferior outcome kidneys from elderly donors are used more frequently due to organ shortage. However chronological donor age considerably lacks sensitivity and specificity for prediction of individual short and long term outcome and hence biomarkers for biological age are an urgent clinical need.

Methods: Age-regulated gene expression changes in 77 zero hour donor kidney biopsies were determined using microarray technology followed by ANOVA and SAM analysis. Expression changes of selected genes were confirmed by quantitative real-time PCR. *In situ* hybridization was used to localize mRNA expression in zero hour biopsies. Functional aspects were examined comparing cell lines (HK-2, htert-RPTEC) grown in normoxic and hypoxic conditions.

Results: Donors were classified into 3 age groups (<40, 40-59, >60 years). 349 genes with altered expression associated with age were identified. These genes were mostly related to Gene Ontology classes of immunity, apoptosis, cell structure and motility and stress response. Three predominant patterns of gene expression were: (1) increasing expression across the age groups, (2) decreasing with age and (3) increasing with age but reduced expression in donors >70 years. Group 3 was dominated by genes encoding for metallothionein (MT) isoforms. *In situ* hybridization demonstrated localization of MT mRNA in renal proximal tubular cells. Cell lines overexpressing MT2A were less sensitive towards hypoxia-induced apoptosis.

Conclusions: Metallothionein expression might serve as a marker for biological age in zero hour biopsies from elderly donors. Furthermore our data support the idea that a reduced expression of MT isoforms predisposes to a reduced anti stress response capacity.

Funding: Government Support - Non-U.S.

SA-PO987

Level of Virus-Specific T Cells (Tvis) as an Indicator of Overimmunosuppression after Pediatric Kidney Transplantation Thurid Ahlenstiel,[1,3] Urban Sester,[2] Lars Pape.[1,3] *[1]Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Nephrology, University Saarland, Homburg, Germany; [3]IFBTx, Hannover, Germany.*

Background: After transplantation (Tx) immunosuppression leads to impaired cellular immune defense resulting in increased risk of viral complications. Post-Tx follow-up of virus-specific T cells (Tvis) may serve as an indicator of viral diseases and overimmunosuppression.

Methods: Within a prospective longitudinal study we monitored Cytomegalovirus (CMV)- and Adenovirus (ADV)-Tvis in 37 children (1-17 years, median 13 years) during the first year after kidney Tx. Based on specific cellular activation and induction of intracellular cytokine production, CMV- and ADV-CD4+ and CD8+Tvis were determined by flow cytometry.

Results: CMV- and ADV-CD4+Tvis were permanently detectable and fluctuated depending on the grade of immunosuppression: Under the intensified immunosuppression during the initial post-Tx period we found temporary decrease of CD4+Tvis. When immunosuppression was reduced, Tvis were increasing. In the presence of sufficient numbers of ADV- or CMV-CD4+Tvis (>2 cells/µl) we did not detect any relevant viral infections or reactivations. Patients with low CD4+Tvis were susceptible for various symptomatic viral infections (e.g. CMV, EBV): The absence of CMV- and ADV-CD4+Tvis (<2 cells/µl) was correlated with a high risk of EBV-infections/-reactivations with persistence of EBV-DNA (Spearman r= -0.68 and -0.49, p<0.0001). In case of high EBV-DNA load (>2500 cop/ml) CMV- and ADV-CD4+Tvis were significantly lower than without relevant EBV-DNA-detection (CMV-CD4+Tvis: 1.6±1.3/µl versus 18.8±13.3/µl; p<0.0001). In contrast to CD4+Tvis, CD8+Tvis were only temporarily detectable.

Conclusions: After kidney Tx CMV- and ADV-CD4+Tvis represent not only virus-specific, but also general cellular immune defense: Sufficient levels of CD4+Tvis (>2 cells/µl) prevent from symptomatic viral infections whereas a decrease is associated with an elevated risk of viral complications. Serving as an indicator of overimmunosuppression, monitoring of CD4+Tvis may improve post-Tx management and optimize individual timing of antiviral therapy and dosing of immunosuppression (effect-related drug-monitoring).

SA-PO988

Minimal Peritubular C4d Staining Is Associated with Clinical and Molecular Features of Antibody-Mediated Rejection Nicole A. Hayde,[1] James M. Pullman,[2] Enver Akalin.[3] *[1]Pediatric Nephrology; [2]Department of Pathology; [3]Adult Nephrology, Transplant Center, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.*

Background: Diffuse (>50%) and focal (11-50%) peritubular (PTC) C4d positive staining is accepted as a footprint of antibody-mediated rejection (AMR) and is strongly associated with donor-specific antibodies (DSA). The significance of minimal (1-10%) PTC C4d staining by immunoperoxidase is unclear. We investigated the clinical and molecular significance of minimal PTC C4d staining in kidney transplant biopsies.

Methods: We analyzed 255 clinically indicated transplant biopsies. C4d staining was performed on paraffin sections using a polyclonal rabbit anti-C4d antibody. Gene expression profiles in a subset of patients were studied using Affymetrix HuGene 1.0ST arrays.

Results: Of the 255 biopsies, 130 (51%) were C4d negative, 78 (31%) had isolated glomerular C4d, 38 (15%) had focal/diffuse PTC C4d and 9 (3%) had minimal PTC C4d+ staining. Minimal and focal/diffuse PTC C4d+ staining were associated with a higher frequency of donor-specific anti-HLA antibodies (DSA) (67% vs. 82% vs. 25%), histopathologic findings of acute or chronic AMR (66% vs. 89% vs. 19%), glomerulitis (0.88 vs. 0.65 vs. 0.25, p=0.003), interstitial inflammation (1.25 vs. 1.41 vs. 0.79; p=0.003) and peritubular capillaritis scores (1.5 vs. 1.5 vs. 0.34; p < 0.001), compared to the C4d negative group, respectively. Using gene ontology, both minimal and focal/diffuse C4d+ biopsies showed increased expression of genes related to activation of the immune response when compared to native pre-implantation biopsies. There was no significant differential gene expression profile between minimal and focal/diffuse PTC C4d+ biopsies. Using the Pathogenesis Based Transcripts, both groups showed enrichment of the gamma-IFN and rejection induced (GRIT), quantitative cytotoxic T cell-associated (QCAT) and quantitative constitutive macrophage-associated transcripts (QMAT) compared to C4d negative controls.

Conclusions: Minimal PTC C4d positive staining is associated with AMR and DSA and demonstrates similar clinical and molecular features compared to diffuse/focal PTC C4d+ biopsies.

SA-PO989

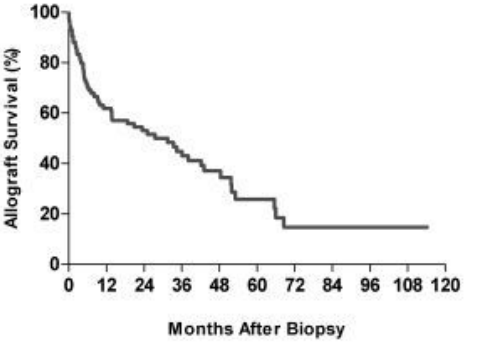
Independent Risk Factors for Allograft Failure Following the Diagnosis of Transplant Glomerulopathy Pallavi Patri, Thangamani Muthukumar, Choli Hartono, Darshana Dadhania, Manikkam Suthanthiran, Surya Seshan. *Weill Medical College of Cornell University.*

Background: Transplant glomerulopathy (TG) is a pattern of glomerular injury characterized by duplication of glomerular basement membrane. Chronic antibody-mediated injury is a major cause for its development. While TG portends a poor prognosis, risk factors of allograft failure following TG have not been well defined.

Methods: We reviewed 1523 consecutive for-cause kidney allograft biopsies at our center (1/2000-12/2010) and identified 84 recipients with TG. We performed Cox proportional hazard regression to identify independent risk factors for graft failure.

Results: TG was diagnosed 41 mo (median) after transplant. eGFR-23ml/min; proteinuria >1g/day-56 (67%); glomerulitis-57 (69%); peritubular capillary inflammation-51 (61%); tubulitis/interstitial inflammation-40 (48%); severe interstitial fibrosis (IF/TA)-19 (23%), severe vascular fibrous intimal thickening-16 (19%) and positive C4d staining-33 (40%). Donor specific antibody: available-28 (34%); positive-22 (79%). Forty-two (51%) patients received combination of pulse steroids, intravenous immunoglobulin, plasmapharesis, rituximab (Ritux) or antithymocyte globulin (Thymo). Median follow up after biopsy-46 mo. There were 31 allograft failures within 1 year of biopsy. One-year post-biopsy allograft survival-63% (Panel-A). By Cox regression, eGFR, proteinuria, IF/TA and additional therapy with Ritux or Thymo were independent predictors of allograft failure (Panel-B).

A) Allograft Survival Following the Diagnosis of TG (N=84)



B) Independent Risk Factors of Allograft Failure Following the Diagnosis of TG (N=84)

Variable	Reference	Hazard Ratio	95%CI	P Value
Time to biopsy	months	0.99	0.99-1.00	0.21
eGFR at biopsy	**10 ml/min**	**0.53**	**0.41-0.69**	**0.000**
Proteinuria >1 g/day	**<1 g/day**	**3.92**	**1.59-9.68**	**0.003**
Glomerulitis, yes	no	0.83	0.39-1.77	0.64
Peritubular capillary inflammation, yes	no	0.50	0.23-1.04	0.07
Tubulitis/interstitial inflammation, yes	no	1.49	0.75-2.94	0.24
Interstitial fibrosis, severe	**nil / mild-moderate**	**2.65**	**1.36-5.16**	**0.004**
Vascular fibrosis intimal thickening, severe	nil / mild-moderate	0.83	0.40-1.71	0.62
Peritubular capillary C4d, positive	negative	1.07	0.54-2.13	0.83
Treatment with pulse steroids, IVIG or PP	no treatment	0.56	0.25-1.24	0.15
Additional therapy with Ritux or Thymo	no additional therapy	2.77	1.03-7.46	0.04

Conclusions: In our single-center analysis of 1523 renal allograft biopsies, we identified 84 transplant recipients with TG. One-year post-biopsy allograft survival was 63%. Level of graft function and proteinuria at the time of biopsy as well as IF/TA were independent risk factors of allograft failure. Unexpectedly, additional therapy with Ritux or Thymo was associated with allograft failure.

SA-PO990

Natural Killer Cells Were Involved in Human Renal Allograft Rejection

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Background: Natural killer (NK) cells play an important role in xenograft rejection by way of "natural" antibody mediated acute humoral xenograft rejection. Expression of NK cells in human renal allograft rejection has not been described. We have evaluated for presence of NK cells by screening CD56 expression, a major receptor of NK cells, in human renal allograft biopsies (Study 1) and explant specimens (Study 2) from patients with cellular and humoral rejection.

Methods: In Study 1, we compared the cytoplasmic CD56 expression in 3 groups of renal transplant biopsies including 1) acute tubular injury as controls, 2) acute cellular rejection (ACR), and 3) antibody mediated rejection (AMR, also called humoral rejection) with or without ACR. In Study 2, we evaluated CD56 expression in 1) control kidney sections (removed for renal tumors), 2) renal allograft explants with ACR only, and 3) renal allograft explants with ACR and AMR. All kidney sections were stained for CD56 (monoclonal antibody from Dako) and their presence in cellular infiltration (CI) area of ACR and peritubular capillaries (PTC) were counted per high power filed (x400) for comparison using ANOVA.

Results: Control cases stained entirely negative for CD56. In rejection cases, CD56 positive NK cells represented less than 5% of inflammatory cells in cellular infiltration. Their presence in peritubular capillaries was also scattered in general (see table below, *p< 0.05 vs control; #p< 0.05 vs ACR only). There were no differences in the presence of CD56 positive NK cells between ACR and AMR in two clinical settings evaluated.

		Biopsy	Biopsy		Explant	Explant
	n	CI	PTC	n	CI	PTC
Controls	12	0.00±0.00	0.00±0.00	10	0.00±0.00	0.00±0.00
ACR only	13	3.54±1.36*	0.31±0.18	6	7.33±3.61*	6.17±5.03*
AMR	11	0.64±0.31#	1.28±0.69	12	5.25±1.81*	3.92±0.76

Conclusions: According to our knowledge, it is the first human study to show that CD56 positive NK cells were present in both ACR and AMR, although their amount in both cellular infiltration areas and peritubular capillaries were relatively small. Further study is needed to determine the significance of NK cells involved in human allograft rejection.

Funding: Clinical Revenue Support

SA-PO991

Integrin β3 Expression May Contribute to Kidney Allograft Failure

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Background: Late allograft failure is a considerable problem in kidney transplantation. Expression of integrin $\alpha v\beta_3$ ($ITG\beta_3$) has been detected in rejecting kidney allografts. However, the role of $ITG\beta_3$ in the late kidney allograft failure is unknown. We hypothesize that $ITG\beta_3$ mediates interstitial fibrosis (IF) and tubular atrophy (TA), the hallmarks of late kidney graft failure.

Methods: Expression of $ITG\beta_3$ and its ligand vitronectin (VN) were evaluated in a well-characterized mouse kidney allograft model of IF/TA, in serum, urine and kidney allografts from human recipients, and in mouse collecting duct epithelial cells (mIMCD) by real-time PCR, immunofluorescence staining, Elisa, and western blot.

Results: Compared to isografts, gene expression of $ITG\beta_3$ was substantially elevated in mouse kidney allografts at 1w and 2w (6.6 and 5.3-fold, p<0.05) prior to development of IF/TA and remained elevated at 6w and 12w after IF/TA was established (1.6 and 2.5-fold; p<0.05). Moreover, $ITG\beta_3$ staining was localized in the renal collecting ducts and increased in allografts compared to isografts. To begin to explore the relationship between this expression and allograft failure, mIMCD cells were exposed to 5ng/ml $TGF\beta_1$ for 24h. Compared to vehicle, $TGF\beta_1$ markedly induced $ITG\beta_3$ mRNA expression (5.9-fold) and was associated with up-regulation of col1A1 (8.6-fold) and fibronectin1 (3.2-fold) (p<0.05). Finally, we evaluated the expression of $ITG\beta_3$ in human kidney transplant recipients. Compared to stable function recipients, $ITG\beta_3$ was elevated by 5.2-fold in graft biopsies with IF/TA (p=0.02). Accompanying these changes were marked elevations in ITGβ3 ligand VN in recipient urine with IF/TA (6.85 ± 1.66 ng/mg creat) compared to recipients with stable allograft function (1.89 ± 0.89 ng/mg creat; p<0.05). Moreover, the extent of elevation was associated with the severity of IF/TA.

Conclusions: Increased expression of $ITG\beta_3$ in kidney allografts within renal collecting duct cells and the ligand VN are associated with the development of IF/TA. Further investigation into this pathway may provide new insights into the development of allograft fibrosis in kidney transplants.

SA-PO992

Obesity Impairs Foxp3+ Treg Cell Function in Clinical Allograft Recipients

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Background: Being overweight or obese (ow/ob) increases risk of hypertension, diabetes and other illnesses, including in transplant recipients, but has been little studied with regard to its immune effects. We have undertaken clinical, translational and basic studies of this link.

Methods: We assessed if CNI or rapamycin (RPM) affected metabolic events in children (n = 62) with stable, long-term kidney or liver allografts (32 M, 30 F), and if being overweight or obese (Ow/Ob) was associated with impaired Treg phenotype or function.

Results: Most patients received CNI (39) or RPM (9) monotherapy, though 12 had CNI (8) or Rapa (4) plus MMF, Aza or steroids. 45 children were normal weight, 7 were overweight and 10 were obese. The CNI-group had more Ow/Ob patients (p=0.045) and higher BMI-for age percentile than RPM-group (61.7 vs. 42.9, p=0.047). CNI dose correlated with BMI (0.521, p=0.013), while RPM use negatively correlated with BMI (-0.888, p=0.044). Ow/Ob patients on CNI therapy had reduced Treg suppressive function compared to patients with normal weight (p=0.045). Hence, CNI use is associated with ow/ob post-Tx, and both CNI and ow/ob are independently associated with impaired Treg function and phenotype, favoring a pro-inflammatory phenotype. In mice, administration of a high fat diet for 5 weeks markedly impaired Treg function, and we are now assessing the effects of leptin on both inflammation and Treg function. Lastly, we are currently testing whether the decreased Treg function caused by a high fat diet can be reversed by HDAC inhibitor therapy in vivo (experimental mice) or in vitro (clinical transplant recipients).

Conclusions: Obesity has typically not been considered when assessing risk factors negatively impacting to long-term graft outcomes. However, the current studies suggest that obesity, like CNI therapy, has a negative effect on Treg function. Efforts to understand these interactions and to find ways to reverse these effects may have important clinical applications for all transplant recipients, as well as for an increasing proportion of the US population who are ow/ob.

Funding: NIDDK Support, Other NIH Support - NIAID

SA-PO993

Sirolimus (SRL) Induces $ILT3^{high}ILT4^{high}$Dendritic Cells (DC) Promoting a New Immunoregolatory Pathway

Giovanni Stallone,[1] Paola Pontrelli,[3] Barbara Infante,[1] Margherita Gigante,[2] Giuseppe Stefano Netti,[2] E. Ranieri,[2] Loreto Gesualdo,[3] Giuseppe Grandaliano.[1] *[1]Dept of Medical and Surgical Sciences-Nephrology Unit, University of Foggia, Italy; [2]Dept of Medical and Surgical Sciences-Clinical Pathology Unit, University of Foggia, Italy; [3]Dept. of Emergency and Organ Transplant, University of Bari, Italy.*

Background: $ILT3^{high}ILT4^{high}$DCs may cause anergy in $CD4^+$ effector T cells converting them into regulatory-type T cells (Treg). The aim of the present study was to investigate in renal transplant recipients whether chronic exposure to SRL may modulate this immunoregulatory pathway.

Methods: Fortyrenal transplant recipients on calcineurin-inhibitor (CNI)-based therapy with biopsy-proven chronic allograft nephropathy were randomly assigned to either CNI dose reduction or CNI withdrawal/SRL introduction. At conversion and 2 years thereafter, we evaluated the SRL effects on circulating DCs (BDCA1+/BDCA2+ and ILT3/ILT4 expression), $CD4^+/CD25^{hi}/Foxp3^+$Tregs and $CD8^+/CD28^-$T cells as well as on ILT3/ILT4 expression and the Th1/Th2 balance in graft biopsies.

Results: In SRL-treated patients, peripheral $BDCA2^+$ cells were significantly increased (p=.0001) along with $ILT3/ILT4^+$DC (p=.0002). In the SRL group we observed an increase in the number of circulating $CD4^+/CD25^{high}/Foxp3^+/CTLA4^+$Tregs (p=.0001) and $CD8^+CD28^-$T cells (p=.002). $ILT3/ILT4^+BDCA2^+$DCs number was directly and significantly correlated with circulating Tregs (z=3.392; p=.0007) and $CD8^+CD28^-$T cells (z=2.016; p=.04). Finally, ILT3/ILT4 expression was increased in kidney biopsies at the end of the study period (p=.001) along with a significant bias toward a Th2 response (evaluated by GATA3 protein expression) within the graft only in the SRL-treated patients (p=.0001).

Conclusions: In conclusion, our data demonstrate that SRL induces the up-regulation of ILT3and ILT4on DC surface and this effect is associated with an increase in the number of Tregs and with the expansion of the $CD8^+CD28^-$ T cell population. This observation would suggest that mTOR inhibition may promote a novel immunoregulatory pathway.

Funding: Government Support - Non-U.S.

SA-PO994

Immunologic Monitoring of T-Lymphocyte Subsets and HLA-DR Positive Monocyte in Kidney Transplant Recipients

Jang-Hee Cho,[1,2] Jun-Seop Kim,[1,2] Yunjeong Kang,[1,2] Ga-young Park,[1,2] Owen Kwon,[1,2] Ji-Young Choi,[1,2] Sun-Hee Park,[1,2] Yong-Lim Kim,[1,2] Chan-Duck Kim.[1,2] *[1]Kyungpook National University Hospital, Republic of Korea; [2]CRC for ESRD in Korea.*

Background: T-lymphocyte and HLA-DR positive monocyte play immunomodulatory roles in kidney transplantation. However, the clinical significance of circulating T-lymphocyte subsets and HLA-DR positive monocyte in the peripheral blood remains unclear. We examined the efficacy of the measurement of these cells for immunologic monitoring in kidney transplant recipients (KTR).

Methods: Blood samples were obtained before transplantation, 2 weeks after transplantation, at the diagnosis of biopsy-proven acute rejection, and at the diagnosis of cytomegalovirus infection. $HLA\text{-}DR^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD25^+$ T lymphocytes and HLA-DR positive monocyte in the peripheral blood were analyzed by flow cytometry.

Results: Seventy-seven specimens were included in this study (64 before and after transplantation, 8 acute cellular rejection, 5 cytomegalovirus infection). The frequencies of $CD4^+CD25^+$ T cells, $CD8^+CD25^+$ T cells, and HLA-DR positive monocyte were significantly decreased at 2 weeks after transplantation compared with those before transplantation (8.75±4.19% vs. 4.34±3.14%, 14.14±5.99% vs. 7.45±5.96%, and 97.96±3.36% vs. 96.85±3.18%, respectively; all P < 0.005). When comparing the frequency at 2 week after transplantation, the frequency of $CD4^+CD25^+$ T cell was significantly increased in KTR with acute rejection (7.45±5.96% vs. 11.79±6.53%; p = 0.019). However, no significant differences were found between stable KTR and KTR with cytomegalovirus infection.

Analysis involving the receiver-operating-characteristic curve demonstrated that acute rejection could be predicted with a sensitivity of 87.5% and a specificity of 68.7% using cutoff value of 7.7% frequency of the $CD4^+CD25^+$ T cells.

Conclusions: The measurement of circulating $CD4^+CD25^+$ T cells might be used as a useful noninvasive immunologic monitoring tool by which acute rejection can be identified after transplantation.

Funding: Government Support - Non-U.S.

SA-PO995

In Situ Genetic Analysis of Cellular Chimerism in Gender Independent Kidney Transplantation Maristela L. Onozato, Ladan Fazlollahi, Rex Neal Smith, A. Bernard Collins, A. John Iafrate. *Department of Pathology, Massachusetts General Hospital, Boston, MA.*

Background: Intragraft chimerism has generated much interest for its immunological implications and to elucidate the origin of the regenerating cells. Recently our laboratory developed highly specific fluorescence *in situ* hybridization (FISH) probes termed polymorphic deletion probes (PDP) that target a highly polymorphic subclass copy number variation loci that result in true deletions of genomic content. The deletion loci on chromosomes 2p, 4q and 8p were identified from single nucleotide polymorphism null genotype database or by array comparative genomic hybridization. Individuals can have homozygous, heterozygous or null genotype at each of these loci and population frequency data predicts that PDP analysis for these 3 loci can distinguishing two individuals at nuclear level *in situ* 95% of times. Importantly these probes are not on the sex chromosomes so can be used in same-sex transplants.

Methods: PDP-FISH combined with immunofluorescence (IF) for common leukocyte antigen (CD45) and the endothelial marker, CD34 were used to identify the frequency and distribution of chimeric cells in 14 renal explant cases. Only cells with two copies of a non-polymorphic control probe were scored.

Results: We performed CD45-IF and FISH with the 3 PDP probes separately in order to differentiate the genotype of transplanted tissue (from the donor) from genotype of CD45 stained leukocytes within the tissue (mainly from the recipient). All cases were informative with at least one of the three applied probes different between donor and recipient. Next, we combined CD34-IF with PDP-FISH to observe the genotype of the endothelial cells. Chimerism was observed in tubular epithelial, transitional cell and endothelial cells. In order to confirm findings with PDP-FISH, XY-FISH was also done on 3 sex mismatched cases.

Conclusions: We found that chimerism is a common event in transplanted kidneys using a highly specific method of PDF-FISH. Epithelial lineage cells tended to present higher frequency of chimerism than vascular endothelial cells raising the question of the role of non-blood stream seeded origin of these cells.

Funding: Pharmaceutical Company Support - Roche Organ Transplantation Research Foundation Grant

SA-PO996

Tacrolimus Can Not Increase Serum Cystatin C Concentration of Adult Recipients in the Early Postoperative Stage of Renal Transplant Fei Liu. *Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.*

Background: Assesment of renal function is of vital importance for patients with renal transplants. The glomerular filtration rate (GFR) is generally considered the best measure of renal function. Previous reports have suggested benefits of serum cystatin C measurements in patients with renal transplants. However, increased levels of serum cystatin C in adult renal transplant recipients associated with administration of tacrolimus compared with administration of cyclosporine A has been reported recently. The purpose of the present study was to evaluate possible different levels of serum cystatin C between tacrolimus and cyclosporine A-treated renal transplant recipientsunder steady-state in the early postoperative stage.

Methods: One hundred and nine patients with renal transplants under steady-state in the early postoperative stage were included in the study. Steady-state was defined as lack of acute rejection and estimated GFR >60 ml/min/1.73m^2 calculated by the MDRD formulas. The early postoperative stage was defined as between 2 and 6 weeks after renal transplantation. The patients were divided into two groups according to their different Immunosuppression. Serum cystatin C was measured using latex particle enhanced turbidimetric immunoassay(PETIA).

Results: Thirty seven renal transplant recipients treated with cyclosporine A and seventy two recipients treated withtacrolimus under steady-state in the early postoperative stage were included in the study in our hospital between March 2010 and October 2011. Serum cystatin C concentration (reference: 0.51–1.09 mg/L) was 1.41±0.15mg/L in cyclosporine A group and 1.44±0.27mg/L (reference 0.51–1.09 mg/L) in tacrolimus group. There was no statistically significant difference between two groups for serum cystatin C concentration (P=0.71). No correlation was obtained between serum cystatin C and cyclosporine A or tacrolimus blood concentration .

Conclusions: We concluded that there is no significant difference for serum cystatin C between two groups. Tacrolimus can not increase serum cystatin C concentration of adult recipients in the early postoperative stage of renal transplant.

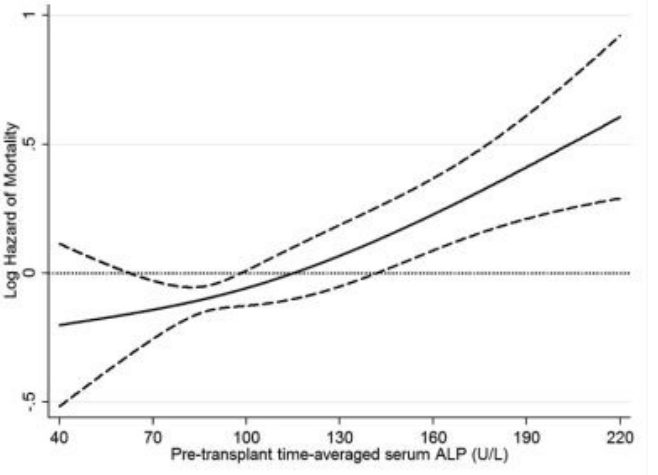
SA-PO997

Impact of Pre-Kidney-Transplant Serum Alkaline Phosphatase Level on Post-Transplant Outcomes Csaba P. Kovesdy,[1] Miklos Zsolt Molnar,[2] Istvan Mucsi,[3] Isidro B. Salusky,[4] Kamyar Kalantar-Zadeh.[1,4] *[1]University of Tennessee; [2]Harold Simmons Center, LABioMed at Harbor-UCLA; [3]McGill University Health Centre; [4]David Geffen School of Medicine at UCLA.*

Background: Mineral-and-bone disorders (MBD) are common in long-term dialysis patients and are risk factors for unfavorable outcomes. The associations between pre-transplant levels of alkaline phosphatase (ALP) and outcomes after kidney transplantation are not well defined.

Methods: Data of the Scientific Registry of Transplant Recipients (SRTR) up to 6/2007 were linked to the 5-year (7/2001-6/2006) cohort of a large dialysis organization in the United States. We identified all dialysis patients who received a kidney transplant during this period and divided them into groups according to increments of pre-transplant ALP. Unadjusted and multivariate adjusted predictors of transplant outcomes were examined.

Results: The 11,776 patients were 47±14 years old and included 39% women. There was linear increasing association between serum ALP level and risk of mortality. Compared to recipients with pre-transplant time-averaged serum ALP 80-<120 U/L, recipients with pre-transplant serum ALP 120<-160 and ≥160 U/L had 49% (HR: 1.49, 95% CI: 1.14-1.93) and 64% (HR:1.64, 95% CI:1.21-2.23) higher graft failure censored all-cause mortality in fully adjusted models.



Compared to recipients with pre-transplant serum ALP 80-<120 U/L, recipients with pre-transplant time-averaged serum ALP of 120<-160 and ≥160 U/L had 111% (HR:2.11, 95% CI:1.26-3.52) and 100% (HR:2.00, 95% CI:1.10-3.65) higher graft failure censored cardiovascular mortality. There was no significant association between time-averaged serum ALP categories and risk of death censored graft failure, delayed graft function or acute rejection.

Conclusions: Hemodialysis patients with pre-transplant serum alkaline phosphatase >120 U/L have unfavorable post-transplant mortality.

Funding: Other NIH Support - R01 DK078106, K24 DK091419

SA-PO998

Long-Term Improvement of Oxidative Stress via Kidney Transplantation Ameliorates Serum Sulfatide Levels, a Novel Candidate Inhibitory Factor of Cardiovascular Diseases Yuji Kamijo, Makoto Harada, Taro Kanno, Yasufumi Takahashi, Koji Hashimoto, Makoto Higuchi. *Department of Nephrology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan.*

Background: Oxidative stress is a strong risk factor for cardiovascular diseases (CVD). The incidence of CVD is lower among kidney transplant recipients than hemodialysis patients, and the reduction in oxidative stress may be one reason for this difference. Recently, serum sulfatides were recognized as a candidate inhibitory factor of CVD affected by oxidative stress. However, the long-term changes in oxidative stress and serum sulfatide levels in kidney transplant recipients are unknown.

Methods: We investigated the long-term changes in a serum oxidative stress marker, malondialdehyde (MDA), and the serum sulfatide levels in 17 kidney transplant recipients. Multiple regression analysis was used to analyze the factors correlated with serum sulfatide levels.

Results: The high serum levels of MDA in the kidney transplant recipients decreased dramatically but were still high 1 year after kidney transplant surgery. MDA levels decreased further and reached near-normal levels more than 3 years after the surgery. Similarly, over the same 3 years, the low serum sulfatide levels increased to near-normal levels, reaching saturation. Multiple regression analysis showed that the most significant factors influencing serum sulfatide levels were MDA and total cholesterol content.

Conclusions: The current results show that over the long term, the internal improvement brought about by successful kidney transplantation can normalize oxidative stress. Oxidative normalization was significantly correlated with the restoration of serum sulfatide levels, which were also influenced by lipoprotein metabolism. The amelioration of serum sulfatide levels might contribute to the low incidence of CVD in kidney transplant recipients.

SA-PO999

GDF-15 Is Related to Anemia and Hepcidin in Kidney Allograft Recipients Jolanta Malyszko,[1] Jacek S. Malyszko,[1] Ewa Koc-Zorawska,[1] Iain C. Macdougall,[2] Michal Mysliwiec.[1] *[1]Nephrology, Medical University, Bialystok, Poland; [2]Renal Unit, King's College of London, United Kingdom.*

Background: Anemia is more prevalent in transplant recipients than in GFR matched CKD-patients, as a series of additional reasons come into play in the transplant situation. Hepcidin is a small defensin-like peptide whose production by hepatocytes is modulated in response to anemia, hypoxia or inflammation. Growth differentiation factor 15 (GDF-15) was identified as hepcidin-suppression factor that is expressed at high levels in patients with ineffective erythropoiesis.

The aim of the study was to assess GDF-15 levels and its correlations with iron parameters, including hepcidin in 60 stable kidney allograft recipients maintained on triple immunosuppressive therapy. Healthy volunteers were studied to obtain the normal ranges for the studied parameters.

Methods: Complete blood count, urea, serum lipids, fasting glucose, creatinine, iron status, were studied by standard laboratory method in the hospital central laboratory. We assessed GDF-15, hepcidin, sTfR, hemojuvelin, IL-6 and NGAL with commercially available assays.

Results: The mean GDF-15, NGAL, hepcidin and hemojuvelin were significantly higher in kidney allograft recipients when compared to the control group ($p<0.001$). GDF 15 was significantly higher in patients with anemia according to WHO definition when compared to non-anemic counterparts ($p<0,05$). GDF 15 levels were not dependent on the type of immunosuppressive therapy (MMF, azathioprine, CNI). In univariate analysis GDF-15 was related to the kidney function (creatinine $r=0.39$, $p<0.01$, eGFR by MDRD $r=-0.37$, $p<0.01$), urea ($r=0.39$, $p<0.01$), uric acid ($r=0.42$, $p<0.01$), hepcidin ($r=-0.32$, $p<0,01$), IL-6 ($r=0.28$, $p<0.,05$), hemoglobin ($r=-0.32$, $p<0.05$), hematocrit ($r=-0.31$, $p<0.05$), erythrocyte count ($r=-0.38$, $p<0.01$), NGAL ($r=-0.35$, $p<0.01$). GDF-15 was not related to serum iron, ferritin, sTfR. In multivariate analysis predictor of GDF-15 was hepcidin (beta value 0.32, $p=0.03$). explaining 38% of the variation of the hepcidin levels.

Conclusions: GDF 15 by affecting hepcidin expression might be involved on the pathogenesis of anemia in kidney allograft recipients.

Funding: Government Support - Non-U.S.

SA-PO1000

Renal Allograft Calcification: Prevalence, Associated Clinical Conditions and Implications for the Long-Term Transplant Outcome Wilfried Gwinner,[1] Irina Scheffner,[1] Johan M. Lorenzen,[1] Verena Broecker,[3] Bernhard Vaske,[1] Michael Mengel,[2] Hermann G. Haller,[1] Anke Schwarz.[1] *[1]Hannover Medical School, Germany; [2]University of Alberta, Canada; [3]University of Cambridge, United Kingdom.*

Background: We have previously reported that renal allograft calcification is frequent in the first months after transplantation (Tx) and is associated with a deterioration of the graft function at one year (Am J Transplant 5:1934,2005). In this study, a large cohort of renal Tx recipients is examined for allograft calcification, with emphasis on factors associated with the calcifications and the long-term graft outcome.

Methods: 420 patients with protocol biosies (6 weeks, 3 & 6 months) were included. Clinical and lab data were recorded at the biopsy time points and then yearly. Allograft outcome was assessed over a period of up to ten years post-Tx.

Results: Of 420 patients, 106 (25%) had calcifications in the tubular lumen and interstitium in one or repeated protocol biopsies. In univariate analyses, pre-Tx hyperparathyroidism, duration of dialysis treatment, post-Tx serum levels of calcium and parathormone and acute tubular injury were related to the calcifications. Calcifications were not related to rejection episodes, calcineurin inhibitor toxicity, or tubulointerstitial fibrosis and tubular atrophy. By regression analysis, serum levels of calcium and parathormone were confirmed as significant factors. Long-term outcome over a period of up to 10 years was assessed by patient and graft survival and renal function. Patient and graft survival was similar in patients with and without allograft calcification. A progressive decline in allograft function was observed in patients with severe or repeated findings of calcification in the biopsies, compared to patients without calcification (eGFR -4.3 vs. -2.3 ml/min*year; $p=0.006$). Parathyroidectomy was associated with a stabilization of allograft function in these patients.

Conclusions: Renal allograft calcification is a relevant complication after renal Tx leading to an inferior long-term function. Decision for parathyroidectomy and its timing or use of potential medical alternatives like calcimimetics need further study.

Funding: Government Support - Non-U.S.

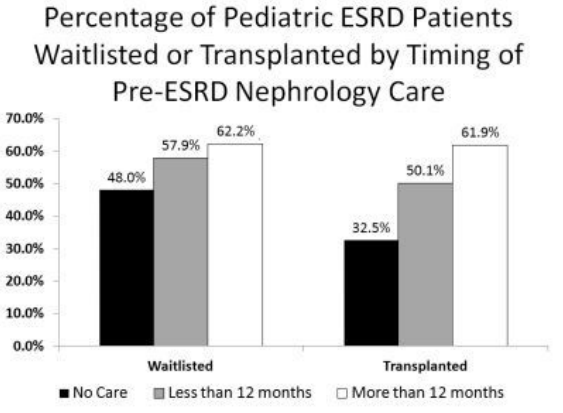
SA-PO1001

Access to Pre-ESRD Nephrology Care Is Associated with Improved Transplant Access for Children with ESRD Rachel E. Patzer,[1,2] Nancy G. Kutner,[3] William M. McClellan,[2,4] Sandra Amaral.[5] *[1]Emory Transplant Center, Emory University, Atlanta, GA; [2]Department of Epidemiology, Rollins School of Public Health, Atlanta, GA; [3]USRDS Rehabilitation/QoL Special Studies Center, Emory University, Atlanta, GA; [4]Division of Nephrology, Emory University, Atlanta, GA; [5]Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: In 2005, the United States Renal Data System (USRDS) began collecting information about receipt of nephrologist care prior to ESRD. We examined whether pre-ESRD nephrology care was associated with transplant access for children with ESRD.

Methods: We examined all pediatric (< 21 yrs) ESRD patients in USRDS from 2005-2009 who had complete data on pre-ESRD nephrology care. Multivariable Cox models were used to examine the impact of pre-ESRD nephrology care on waitlisting and transplantation.

Results: Among 5,776 children with incident ESRD between 2005-2009, the median age was 15 years, 40.4% were white, 27% Hispanic, and 24% black. 66.2% of patients received nephrology care prior to ESRD. Public or no health insurance, African American race, cystic/hereditary renal disease, and younger age were associated with lower likelihood of receiving any nephrology care prior to ESRD (all $p<0.0001$). A greater proportion of patients who had < 12 months and ≥ 12 months vs. no pre-ESRD nephrology care were waitlisted (57.9% and 62.2% vs. 48%, $p<0.0001$) and transplanted (50.1% and 61.9% vs. 32.5%, $p<0.0001$), respectively.



In multivariable analyses, pre-ESRD nephrology care was associated with an increased rate of waitlisting (HR=1.68; 95% CI: 1.54-1.82) and transplant (HR=1.85; 95% CI: 1.69-2.04) compared with no preceding specialty care.

Conclusions: Access to nephrology care prior to ESRD is associated with a higher rate of waitlisting and transplantation among children with ESRD.

SA-PO1002

Clinical Features of Tuberculosis in Kidney Transplant Recipients in Brazil Igor Marques,[1] Bernadete M.C. Ferreira,[2] Luiz Sergio Azevedo,[1] Ligia C. Pierrotti,[3] Victor Sato,[2] Renato Antunes Caires,[2] Elias David-Neto.[1] *[1]Renal Transplant Service, Hospital das Clínicas, University of Sao Paulo School of Medicine, São Paulo, SP, Brazil; [2]Nephrology Division, Hospital das Clínicas, University of Sao Paulo School of Medicine, São Paulo, SP, Brazil; [3]Department of Infectious Disease, Hospital das Clínicas, University of Sao Paulo School of Medicine, São Paulo, SP, Brazil.*

Background: Most studies about post-transplant tuberculosis (TB) describes small patient samples, not comparing kidney transplant recipients (KTRs) with and without TB. The aim of this study was to identify risk factors and to describe the incidence and clinical features of *Mycobacterium tuberculosis* infection after kidney transplantation in Brazil.

Methods: A retrospective case-control single center study with total of 47 cases (2.9%) in Brazil (2000 to 2010) comparing it with 94 KTRs without TB.

Results: The incidence was 945 cases/100,000 patients/year, much higher than the general population of Brazil. Ten-year death-censored graft survival and patient survival were similar between KTRs with TB and those without (62% vs. 72%; p=0.33 and 65% vs. 72%; p= 0.73, respectively). The groups were compared regarding age, gender, re-transplants, cause of end-stage renal disease, renal replacement therapy prior to the transplantation, hepatitis C virus infection, diabetes, type of donor, cytomegalovirus infection and immunosuppressive protocol, but no statistical difference was found between them. Among the cases, 19 (40,4%) had prior acute rejection against 34 (36%) controls ($p=45$). Of the KTRs with TB, 81% were diagnosed within the first 2 years post-transplant and 83% had pulmonary TB, 11% had previous history. The commonest symptoms were fever (81%) and cough (32%). The median time for the diagnosis was 32 days.Mortality during treatment was 13% (6 cases) and 5 deaths were attributed to TB.

Conclusions: This study didn't identify risk factors for TB post-transplant, but there is the necessity of more studies. It is imperative to know and understand the behavior and presentation of TB in KTRs and determinate the possible risk factors for its occurrence to delineate possible interventions to decrease the incidence of TB post-transplant.

SA-PO1003

Inflammatory Infiltrates and Renal Outcome in BKVAN and Acute Renal Allograft Rejection Ünsal Yapici,[1] Jesper Kers,[1] Frederike J. Bemelman,[2] Joris J. Roelofs,[1] Nike Claessen,[1] Ineke Ten Berge,[2] Sandrine Florquin.[1] *[1]Pathology, Academic Medical Center, Amsterdam, Netherlands; [2]Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

Background: Cellular infiltrates in renal biopsies are the hallmark of acute rejection and viral infections, particularly BK-virus nephropathy (BKVAN). Characterization of the heterogeneous interstitial infiltrate might have the potential to discriminate between specific/harmful and non-specific/harmless infiltrates and perhaps between viral infections and acute rejection.

Methods: Using immunohistochemistry, we quantified T- and B-lymphocytes, plasma cells and macrophages in two cohorts comprising of 50 patients with an episode of acute rejection and 30 patients with BKVAN. In addition, the expression of MHC class II, C4d and C3c in tubular epithelial cells was investigated in both cohorts. All biopsies were scored according to Banff 2007. The BKVAN cohort was also scored for viral load (immunohistochemistry) and viral stage (histology). Late renal allograft dysfunction was defined as an eGFR < 30 mL/min/1,73m² or return to dialysis.

Results: No differences in the number of T-lymphocytes, B-lymphocytes and macrophages were observed between the two cohorts. In both cohorts variable expression of MHC class II was found, without significant differences between the groups. We could not detect C4D or C3C staining along the tubular basement membrane. In BKVAN, a significantly higher number of plasma cells was found ($P = 0.03$) and interstitial fibrosis and tubular atrophy was more pronounced ($P < 0.01$). There were no significant differences in outcome between acute rejection and BKVAN. In the BKVAN cohort, the viral load score was not associated with a detrimental outcome. However, stage B was associated with worse outcome, even compared to stage C.

Conclusions: These findings indicate that the composition of the inflammatory infiltrate is comparable in acute rejection and BKVAN, except for higher amount of plasma cells in BKVAN. There is no difference in tubular expression of HLA class II, C4d or C3c and both groups show a comparable renal outcome. However, in the BKVAN cohort a stage B at histological examination is related to late renal allograft dysfunction.

Funding: Private Foundation Support

SA-PO1004

Increased Renal Tubular Cell Apoptosis in Donation after Cardiac Death (DCD) Kidneys Compared with Standard Criteria Donor (SCD) Kidneys Swati Jain, Danica Galesic Ljubanovic, Charles L. Edelstein, Alkesh Jani. *UC Denver, Aurora, CO.*

Background: DCD kidneys suffer increased DGF due to warm and cold ischemia (CI). The effect of pulsatile perfusion (PP) vs. static preservation (SP) on apoptotic pathways is unknown. In a porcine model of DCD, we hypothesized that DCD kidneys have increased apoptosis and caspase-3, and reduced X-linked inhibitor of apoptosis (XIAP) protein compared with SCD kidneys. We also sought to determine whether PP protected DCD kidneys by reducing apoptosis and increasing XIAP expression.

Methods: Male Yorkshire pigs subjected to cardiac death were perfused with cold UW solution and subjected to SP or PP for 24 hrs. Kidney biopsies were obtained at 0 and 24 hrs of CI. Apoptotic renal tubular cells (RTE) were quantitated by a renal pathologist. SCD kidneys not subjected to cardiac death were used as controls. Immunoblot was used to assess active caspase-3 (17kDa) and XIAP(53kDa).

Results: Apoptotic RTEs and caspase-3 expression were significantly increased in DCD vs SCD kidneys whereas XIAP and BCL-XL expression were not detected in DCD kidneys.

Table 1

	SCD		DCD	
CI Time (hrs.)	0	24	0	24
Apoptotic RTEs/ hpf	1.3±0.6	2.2±0.5	0.2±0.1	9.8±2.3*
Caspase-3 expression	ND	ND	++	+++
XIAP expression	+++	+++	ND	ND
BCL-XL expression	+++	+++	ND	ND

n=4; *P < 0.005 vs SCD 0 and 24 hr CI

Next we hypothesized that DCD kidneys subjected to PP would have less apoptosis and increased XIAP expression vs. DCD kidneys stored by SP. PP significantly reduced RTE apoptosis in DCD kidneys but was not associated with increased XIAP expression. Table 2.

	SP		PP	
CI Time (hrs.)	**0**	**24**	**0**	**24**
Apoptotic RTE/hpf	**0.2± 0.1**	**9.8± 2.3***	**0.1± 0.1**	**2.5± 1.4****
Caspase 3/7 activity nmol/min/mg	**84.3 ± 20.5**	**453.3 ±307.7**	**88.5 ±33.8**	**466.3± 234.1**
XIAP protein expression	**ND**	**ND**	**ND**	**ND**

n=4; *P < 0.005 vs. SP 0 hr CI; ** P<0.005 vs. SP 0 and 24 hr CI; ND = not detected

Conclusions: These findings suggest that DCD kidneys experience more RTE apoptosis vs. SCD kidneys associated with loss of anti-apoptotic XIAP and BCL-XL protein expression. DCD kidneys subjected to PP had significantly decreased apoptosis that was independent of caspase-3 and XIAP protein expression.

Funding: NIDDK Support

SA-PO1005

Assessment of Beta Cell Function Following Renal Transplantation David Langsford,[1] Varuni Obeyesekere,[2] Robyn G. Langham,[1] Glenn Ward,[2] Karen M. Dwyer.[1] *[1]Department of Nephrology, St Vincent's Hospital, Melbourne, Australia; [2]Department of Endocrinology, St Vincent's Hospital, Melbourne, Australia.*

Background: Up to fifty percent of non-diabetic patients on the renal transplant waiting list have unrecognised hyperglycemia and are at risk for new onset diabetes after transplantation (NODAT). We aimed to assess the risk of developing NODAT by measuring peri-transplant β cell function in patients with end stage renal failure (ESRF) and following transplantation.

Methods: The recruited patients all had ESRF and had received a transplanted kidney from a living donor. All patients received induction therapy with basiliximab and standard triple therapy with a calcineurin inhibitor, mycophenolate mofetil and steroids. A modified oral glucose tolerance test (oGTT: 0, 30, 60 and 120 minutes) was performed pre-, 3 and 12 months post transplantation. A shortened intravenous glucose tolerance test (IV GTT) was performed pre-, day 7, 3 and 12 months post transplantation. The oral disposition (DIo) and IV disposition indices (DIiv) were calculated. Baseline, post-transplant metabolic and renal specific data were collected.

Results: Thirty percent (3/9) of patients with ESRF had unrecognised hyperglycemia pre-transplant. Fifty percent (5/10) patients developed hyperglycemia post transplant within three months of transplantation, which resolved in three patients within one year. Changes in DIo and DIiv were detected as early as day seven post transplant, however the changes in DIo and DIiv are not necessarily concordant.

Conclusions: Unrecognised hyperglycemia is common in patients with ESRF. β cell function is dynamic and changes rapidly following renal transplantation. Such changes can be measured by calculation of the DI and reflect the impact of surgery and high dose immunosuppression and changes in pre-existing insulin resistance. DIo and DIiv do not necessarily change in parallel, which together with transient hyperglycemia may reflect inhibition of the incretin effect in the setting of the high dose steroids given in the early post transplantation period.

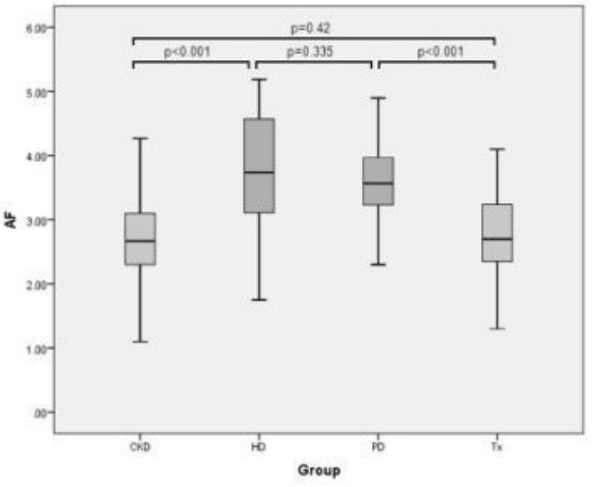
SA-PO1006

Transplantation Is Associated with Reduced Tissue-Advanced Glycation End Product Deposition Lisa E. Crowley,[1] Catherine Johnson,[1] Richard J. Fluck,[1] Maarten W. Taal,[1] Chris W. McIntyre,[1,2] Janson Leung.[1] *[1]Department of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]School of Graduate Entry Medicine and Health, University of Nottingham, Nottingham, United Kingdom.*

Background: Tissue-Advanced glycation end products (AGEs) are a measure of cumulative metabolic stress. Measurement of tissue AGEs by skin autoflourescence (SAF) correlates well with cardiovascular outcomes in diabetic and dialysis patients and is independently associated with cardiovascular risk factors in chronic kidney disease stage 3. This study aimed to evaluate tissue AGEs in a population of renal transplant patients.

Methods: We studied 66 transplant patients (31 female, 35 male). Tissue AGEs were measured in transplant recipients using a SAF reader (AGE reader, DiagnOptics). Values were compared with a matched cohort of CKD 3 patients (on a 2:1 basis) and 115 dialysis patients (62 HD, 53 PD).

Results: Mean value of measured SAF in the transplant patients was 2.79±0.63. SAF correlated most strongly with age (r=0.31) but there was no correlation between SAF and eGFR, transplant age or renal replacement therapy vintage. There was a significant difference between values in dialysis patients and those found in transplant recipients but no difference between transplant and CKD stage 3. In the small number of patients with results recorded both on dialysis and following transplantation SAF decreased by around 25% (mean post transplant time of 20 months).



Conclusions: Tissue AGE values in transplant patients are elevated compared to non CKD control values. However they were similar to patients with established CKD 3 and significantly lower than patients still receiving dialysis. Our data suggest that transplantation may be associated with a reduction in accumulated tissue AGE products and this might be an important component of the observed reduction in cardiovascular risk compared to dialysis patients.

SA-PO1007

Dietary Omega-3 Fatty Acids Intake Is Independently Associated with Lower Serum FGF23 in Renal Transplant Recipients Leandro Cunha Baia,[1] Else Van den Berg,[1] Marc G. Vervloet,[2] Ita Pfeferman Heilberg,[3] Gerjan Navis,[1] Stephan J.L. Bakker,[1] Martin H. De Borst.[1] *[1]Nephrology, University of Groningen (UMCG), Groningen, Netherlands; [2]Nephrology, VU-MC, Amsterdam, Netherlands; [3]Nephrology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil.*

Background: Fibroblast growth factor 23 (FGF23) has been identified as an independent risk factor for cardiovascular morbidity and mortality in patients with chronic kidney disease and renal transplant recipients (RTR). Little is known about determinants of FGF23. We aimed to identify dietary factors as independent modifiable determinants of FGF23 after kidney transplantation.

Methods: In this cross-sectional study, the diet of 707 RTR was assessed with a food frequency questionnaire, inquiring about intake of macro- and micronutrients. Serum intact FGF23 levels were determined by ELISA and values were log-transformed for analyses. We assessed known determinants of FGF23 including intake of protein and phosphate, BMI, serum hsCRP, phosphate and PTH, eGFR and proteinuria as potential confounders. Urinary calcium and phosphate excretions were measured in 24h urine samples. We applied uni- and multivariate regression analyses to identify independent determinants of FGF23 levels.

Results: Mean age was 53±12.8 years; 57% of patients were male. Median time after transplantation was 5.4 [1.9-12.2] years and mean eGFR was 52.3 ± 20.2 ml/min. Multivariate regression analyses revealed significant independent associations of eGFR (standardized $\beta = -0.49$ $p<0.001$), serum phosphate (st. $\beta = 0.21$, $p<0.001$) and BMI (st. $\beta = 0.14$, $p=0.004$) with FGF23. Of the dietary factors, the omega-3 fatty acids docosahexaenoic acid (DHA) (st. $\beta = -0.14$, $p=0.004$) and eicosapentaenoic (EPA) (st. $\beta= -0.15$, $p=0.001$) were inversely associated with FGF23, independent of eGFR, serum phosphate and BMI. No association was found between protein or phosphate intake and FGF23.

Conclusions: Dietary intake of DHA and EPA emerged as novel factors inversely associated with serum FGF23, independent of known determinants of FGF23 in RTR. Accordingly, an increase in dietary DHA and EPA intake might reduce serum FGF23 levels and help lowering cardiovascular morbidity and mortality in RTR.

Funding: Government Support - Non-U.S.

SA-PO1008

Altered Gene Expression in Transplant Kidneys in the Early Stage of New Onset Diabetes after Transplantation Zoltan G. Laszik,[1] Sindhu Chandran,[2] F. Vincenti.[2] *[1]Pathology, UCSF, San Francisco, CA; [2]Transplant Service, UCSF, San Francisco, CA.*

Background: New-onset diabetes after transplantation (NODAT) is an independent risk factor for graft loss of unclear etiology. The aim of our study was to determine global gene expression differences in transplant kidney surveillance biopsies of patients with NODAT at 6 months post transplantation.

Methods: Renal transplant recipients with NODAT who underwent a 6-month surveillance biopsy at UCSF between July 1, 2009 and October 31, 2010 were identified. Controls included 6-months surveillance biopsies with no NODAT and no pre-existing diabetes mellitus. Only biopsies with normal morphology and with at least 3 months duration of diabetes in the NODAT group were included in the study. Agilent whole human genome 4x44K ink-jet arrays were used to determine global gene expression in 5 biopsies with and in 16 biopsies without NODAT. A one-way ANOVA linear model was fit to the comparison to estimate the mean M values, B statistic, false discovery rate (FDR) and p-value for each gene for the comparison of interest.

Results: With a stringent FDR of < 0.01 there were 25 differentially expressed transcripts identified in NODAT versus control kidneys. The average difference in gene expression level was 1.51-fold with 19 genes being up-regulated and 6 genes being down-regulated. Some of the differentially expressed genes code for structural proteins such as elastin while others play a role in mediating growth and repair (GDF7), apoptosis (SLC39A4), acquired podocyte diseases (CMIP), inflammation (chemokine receptor CCR1), metabolism (NADH dehydrogenase), protein trafficking (RAB2A), vascular homeostasis (HSPG2) and signal transduction (PRR5, GDF7). Most of these genes have no known function in the kidney. Possible therapeutic targets in the dataset include CCR1 and HSPG2.

Conclusions: Defining altered molecular phenotype in transplanted kidneys with recent onset NODAT may help to understand the pathogenetic pathways during the early preclinical stages of progressive renal injury. This approach may also help to identify therapeutic targets for further analysis aimed to prevent disease progression.

Funding: Pharmaceutical Company Support - Novartis

SA-PO1009

Urinary Polyomavirus-Haufen Shedding Marks Polyomavirus Nephropathy: A Proof-of-Concept Study for a Novel Diagnostic Non-Invasive Urine Biomarker Volker Nickeleit, Bruna Brylawski, Harsharan Singh. *Pathology, Division of Nephropathology, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Polyomavirus Nephropathy (PVN) is the most significant viral renal allograft infection with 4% incidence. Currently a definitive diagnosis is only established by renal biopsy. Recently we described a novel, non-invasive urinary assay to diagnose PVN, i.e. the "polyomavirus (PV)-Haufen-test", with positive and negative predictive values of > 95%. This test has great diagnostic potential. Hypothesis: PV-aggregation and Haufen formation depend on a high concentration of Tamm-Horsfall protein (THP) that is only present in injured renal tubules and not in voided urine. Thus the presence of PV-Haufen in voided urines is specific for intrarenal disease.

Methods: Part-A- association of PV-Haufen and THP: A1) Immunohistochemistry of PVN cases (n=3) with double labeling to detect PV and THP in renal tubules. A2) Immunogold electron microscopy of urinary PV-Haufen to detect Haufen-bound THP (n=3). A3) Immunoprecipitation studies on urinary PV-Haufen (n=6). A4) Dilution curves altering THP concentrations in fluids mimicking primary and secondary urines and evaluating for PV-aggregation and Haufen formation (n=5). Part-B – THP concentrations in voided urine samples (with concurrent kidney biopsies; n=20).

Results: Part-A. A1) In PVN intratubular PV aggregates show abundant THP. A2) Urinary PV-Haufen are intimately admixed with THP. A3) Immunoprecipitation of urinary PV-Haufen shows abundant coprecipitation of PV and THP. A4) PV aggregation and PV-Haufen formation is THP dose dependent and only occurs with high THP concentrations of > 1mg THP/ml fluid mimicking primary urine in injured tubules. Part-B. THP concentrations in voided urine samples are low, median THP 4.5 microgram/ml urine, range: 0.7-19.5.

Conclusions: PV-Haufen are closely associated with THP. PV-Haufen formation occurs in the setting of very high THP concentrations found in injured renal tubules exceeding voided urine THP concentrations 50 fold. The genesis of PV-Haufen is similar to the formation of intratubular red- or white blood cell casts. Urinary PV-Haufen are novel biomarkers for intrarenal PVN.

SA-PO1010

Prevanlence of Donor Specific HLA-Antibodies (DSA) after Pediatric Kidney Transplantation and Follow Up after Therapeutic Intervention Lars Pape, Nele K. Kanzelmeyer. *Pediatric Nephrology, MHH, Hannover, Germany.*

Background: DSA can lead to deterioration of graft function, associated with chronic humeral rejection after ped. KTx. Until now, no data about prevalence and treatment protocols for children with early detection of DSA are available.

Methods: We retrospectively analyzed 256 routine-measurements of HLA ab-class I and II on a yearly basis in 88 children after KTx (mean age 15 ± 4 years, 30 girls) from 2008-2012 with Luminex xMAP technology. In case of positive HLA ab, donor-specificity was evaluated. The clinical course, therapeutic interventions and kidney biopsy results were analyzed.

Results: In 48% of patients no DSA were found. 52 % patients showed HLA antibodies, donor specifity was confirmed in 12,5% of patients. 4/11 patients (mean DSA MFI 7964) received no treatment because of normal renal function. Transplant function was stable and DSA-MFI increased to 9105. 3/11 were treated with Rituximab because of an increase in s-creatinine > 10% and one child was treated with immuneglobulins. In these children kidney function stayed stable, mean DSA MFI was stable 6820 to 7717). In 3 patients with an increase of s-creatinine > 25%, kidney biopsies showed chronic humeral rejection, in 2 cases associated with transplant glomerulopathy. One patient was treated only with Prednisolon-pulses and Rituximab. No further increase in s-creatinine could be detected and mean DSA MFI increased from 8997 to 17180. In the other three children DSA (mean MFI 7231) were associated with the most severe increase in s-creatinine. They were treated with Prednisolone-pulses, plasmapheresis, rituximab and immuneglobulins. Kidney function and DSA-MFI stayed stable (mean MFI 7300).

Conclusions: In an unselected population after ped KTx, the prevalence of DSA is approximately 12,5 %. It remains unclear, if treatment with Rituximab will help to avoid later humoral rejection in children with DSA who experience no graft dysfunction. Intensive therapy with Prednisolon-pulses, Rituximab and immuneglobulins ± Plasmapheresis seems to be a good option for treatment of DSA-associated chronic rejection with deterioration of graft function. Effect of treatment can be seen on graft function but not on DSA-MFI values.

SA-PO1011

Cytokines and Chemokines as Markers of Tolerance and Rejection after Kidney Transplantation Cornelia Anneliese Blume,[1] Hermann G. Haller,[1] Christine S. Falk.[2] *[1]Nephrology and Hypertensiology, Medical School Hannover, Hannover, Germany; [2]Institute for Transplant Immunology, Medical School Hannover, Hannover, Germany.*

Background: Defining biomarkers suitable for prediction of operational tolerance or rejection is of central interest in future transplantation medicine in order be able to classify patients for individualized immunsuppression.

Methods: Using multiplex protein arrays with 60 different parameters (cytokines. chemokines and growth factors), we analyzed the microenvironment in 32 renal biopsies of 131 renal transplant recipients with histological definition of transplant condition. The patient group comprised 30 patients with T cell mediated rejection (TCMR) according to borderline changes (group 1). 5 of 15 patients with TCMR (group 2). 5 of 18 patients with chronic humoral rejection (group 3) and 16 of 68 patients with unsuspicious biopsies and stable transplant function (tolerant patients, group 0). Levels of three biopsy compartments (capsule, subcapsule and cortex region, re-evaluated by pathological analysis) were compared to plasma levels of these soluble factors (T-test).

Results: 1. We found characteristic gradients for SCF as well as HGF, M-CSF and CXCL9 in the biopsy of group 0 that were mostly abrogated in case of rejection. 2. The plasma level of CXCL9 was indicative for chronic humoral rejection (group 3) and the plasma level of CCL2 for acute rejection (group 2) (table 1). 3. The cortex levels of SCF, CXCL9, the chemokine CCL5 (Rantes), M-CSF, HGF and CCL2 were generally elevated in rejection (Table 2-6) as compared to unsuspicious biopsies (group 0).

Conclusions: SCF should be tested as a marker of tolerance. CXCL9–already described as a urine marker of rejection – may also serve as a plasma marker of rejection. During rejection, HGF may reflect tissue repair, M-CSF may indicate a shift of macrophages towards the graft. With this protein-based technique, novel biomarkers in kidney biopsies and plasma may be verified by ROC- analysis in order to classify patients by the microenvironment in addition to the pathological staging which may substantially improve the reliability of the classification.

Funding: Clinical Revenue Support

SA-PO1012

Utility of ImmuKnow Assay in BK Infection after Kidney Transplantation Nashat Burhan Imran,[1] Vikyath Prakash,[1] Rachelle V. Dyquiangco,[1] Medha Airy,[1] Charles G. Minard,[1] Richard J. Knight,[2] Venkataraman Ramanathan.[1] *[1]Nephrology, Baylor College of Medicine, Houston, TX; [2]Methodist Hospital, Houston, TX.*

Background: By quantifying cellular ATP production, ImmuKnow® assay provides an estimate of immune function. We studied the utility of this assay in kidney transplant (KT) patients with BK infection.

Methods: We identified KT recipients who had BK viremia (BKV) during 2-year study period. ATP level and blood BK PCR was obtained at 1 month post-transplant and then quarterly for a year. BKV was defined as PCR >1000 copies and first detection was defined as incident viremia. We compared ATP values in BK negative patients to patients with varying levels of BKV (1-10K, 10-100K and >100K). ATP values were subdivided into recommended "low" (≤225), "moderate" (226-524) and "strong" immune response categories. A general linear mixed model with an unstructured residual covariance matrix was used to model ATP values as a function of BK infection status and time. Bonferroni correction was used.

Results: We had 261 paired ATP/BK PCR observations and 72 patients developed BKV during the study period. After adjusting for time, BK infection was significantly associated with low ATP (P=0.01). Mean ATP levels were in "moderate" immune response category in all groups. But ATP was significantly lower in patients in BK >100k compared with the BK negative (359vs.468, P=0.03) and BK 1k-10k groups (359vs.493, P=0.007).

	No BK (n=124)	1K-10K (n=60)	10K-100K (n=43)	>100K (n=34)
ATP	468±21	493±26	420±30	359±35
ATP≥525	51(41%)	25(42%)	7(16%)	5(15%)
ATP≤225	13(10%)	10(17%)	10(23%)	15(44%)

When subdivided into immune response categories, high viral load patients had "low" immune response (Fisher Exact test P=0.0001). Compared to their baseline ATP values, incident viremia patients had 32% decline in ATP values (541±272 vs. 366±204, p 0.0001) at time of first BK positivity. Even though, every 100 value drop in ATP had a 6.6% greater hazard for BK infection, it was not statistically significant.

Conclusions: Even though ATP level is significantly low when BK PCR >100K copies, most BK patients are in "moderate" immune category. Incident viremia patients have a significant decline in ATP value compared to baseline values.

Funding: Private Foundation Support

SA-PO1013

Renal and Urinary Levels of EPCR Correlate with Acute Renal Allograft Rejection Lionel Lattenist,[1] Jesper Kers,[1] Nike Claessen,[1] Ineke Ten Berge,[2] Frederike J. Bemelman,[2] Sandrine Florquin,[1] Joris J. Roelofs.[1] *[1]Pathology, Academic Medical Center, Amsterdam, Netherlands; [2]Nephrology, Academic Medical Center, Amsterdam, Netherlands.*

Background: The Endothelial protein C receptor (EPCR) is expressed on endothelial cells of large blood vessels and to a lesser extent on capillaries. Through cleavage by a metalloprotease soluble EPCR (sEPCR) is formed. EPCR increases activation of proteinC. Active protein C has anticoagulant, anti-inflammatory and cytoprotective effects.

Acute rejection of kidney allograft can be divided in acute T-cell and antibody-mediated rejection (respectively TCMR and ABMR). Especially ABMR is characterized by activation of coagulation. Currently no reliable non-invasive biomarkers are available to monitor rejection.

In this study we investigate the EPCR expression pattern in kidney transplants and correlate plasma and urine sEPCR levels to acute renal allograft rejection.

Methods: Renal biopsies were available from 87 renal transplant patients (39 without rejection, 29 TCMR and 19 ABMR), we had access to matched plasma and urine samples for a portion of this cohort. Renal EPCR expression was assessed by RT-PCR and immunostaining. Plasma and urine sEPCR levels were measured by ELISA. Staining intensity was scored semiquantitatively on a scale from 0 to 3.

Results: ABMR patients showed higher levels of EPCR mRNA than TCMR patients.

EPCR expression in venules did not differ whereas EPCR expression on glomeruli and peritubular capillaries was significantly elevated in ABMR patients than in TCMR or control patients. EPCR expression was higher in tubules and arteries of rejection patients than in control patients.

Plasma sEPCR levels did not differ. Urine sEPCR levels were elevated in the ABMR group than in patients with TCMR or without rejection. ROC analysis demonstrated that urinary sEPCR is appropriate to discriminate between ABMR patients and TCMR or control patients.

Conclusions: We conclude that urinary sEPCR could be a novel non-invasive biomarker of antibody mediated rejection in renal transplantation.

Funding: Private Foundation Support

SA-PO1014

Angiopoietin-2: A Prognostic Marker in Kidney Transplantation Welmoet H. Westendorp,[1] Harry Van Goor,[2] Lyan G. Koudstaal,[1] Jeffrey Damman,[1] Hans Burgerhof,[3] Rutger J. Ploeg,[5] Marc Seelen,[4] Henri G.D. Leuvenink.[1] *[1]Surgery, University Medical Center Groningen, Groningen, Netherlands; [2]Pathology, University Medical Center Groningen, Groningen, Netherlands; [3]Epidemiology, University Medical Center Groningen, Groningen, Netherlands; [4]Nephrology, University Medical Center Groningen, Groningen, Netherlands; [5]Nuffield Dept. of Surgical Sciences, University of Oxford, Oxford, United Kingdom.*

Background: Organs derived from deceased brain dead (DBD) donors show worse organ function and more acute rejection episodes than organs derived from living donors. Human studies have provided evidence that DBD donors suffer from bacterial translocation and higher endotoxin load. A link between endotoxemia and Angiopoietin 2 (Ang2) is established. In humans, LPS triggers Ang2 release, which binds to the Tie2 receptor. Use of Ang2 as biomarker of endothelial integrity has gained much attention since Ang2 reflects the immunogenic state of an organ and could therefore be used as a predictor of organ quality and survival.

Methods: We measured serum Ang2 by ELISA in 100 DBD and 220 living donors (LD). Serum was obtained immediately after the declaration of brain death (T0) and just before organ retrieval (T1). Serum from living kidney donors retrieved after start of the operation (T0) and just prior to organ retrieval (T1) was used as control.

Results: Serum Ang2 levels in DBD donors are higher at T0 and T1 (T0: 1925 ± 198.9 pg/ml and T1: 2418 ± 305.2 pg/ml) compared to living donors (T0: 690.7 ± 47.60 pg/ml and T1: 1384 ± 102.9 pg/ml). In LD, Ang2 levels increased at T1 compared to T0 ($p<0.05$). In LD, T1 Ang2 levels are associated with glomerular filtration rate (GFR) at 12 months after transplantation (Spearman's ρ -0.193 p=0.019). In DBD donors, T0 Ang2 levels are associated with serum creatinin 14 days after transplantation (Spearman's ρ -0.333 p=0.017).

Conclusions: These results show elevated Ang2 levels in DBD compared to LD, which is illustrative of an inflammatory response. A clinical validated Ang2 test and therapeutic interventions that modulate the Ang2 response in the DBD donor might be novel tools to improve organ quality.

Funding: Government Support - Non-U.S.

SA-PO1015

Reduced Urinary NOx-Excretion Is Associated with Increased Cardiovascular Risk Factors in Renal Transplant Recipients

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Background: Cardiovascular (CV) risk is high in renal transplant recipients (RTR) and a leading cause for graft loss and mortality. Since endothelial dysfunction is common in RTR, reduced NO production might relate to increased CV risk in RTR. We studied 1) NO production in RTR vs controls; 2) its determinants in RTR; 3) its association with CV risk factors in RTR.

Methods: We included 707 outpatient RTR (age 53±13 yrs, 57% male, ≥1yr post-transplantation (Rtx), time since Rtx 5.4 [1.9-12.2] yrs) and 107 healthy controls (53±10 yrs, 47% male). Systemic NO production was assessed by measurement of 24h urinary excretion of its stable end products NO_2/NO_3 (UNO_x). Detailed data on diet (questionnaires) and drugs were obtained to assess exogenous NO-sources (mainly vegetables). CV risk factors (blood pressure, pulse pressure, heart rate, plasma sodium, Nt-pro-BNP, hs-CRP, HbA1c, proteinuria) were measured.

Results: UNO_x was markedly decreased in RTR (588 [400-812] vs. 1020 [796-1285] nmol/24h in controls, p<0.001), while vegetable intake was similar (92±57 vs. 93±58 g/d, ns) and approximately half of the WHO-recommended intake. Gender, vegetable intake and renal function were independent determinants of UNO_x in RTR. SBP and DBP were 136±18 and 83±11 mmHg resp; creatinine clearance was 66±26 ml/min. On linear regression analyses, UNO_x was inversely associated with SBP (β=-0.29; p=0.005), pulse pressure (β=-0.16; p=0.02) heart rate (β=-0.19 p=0.008), plasma sodium (β=-0.03 p=0.04), hs-CRP (β=-0.02 p=0.008), HbA1c (β=-0.01 p=0.05) and positively with CrCl (β=0.51, p=0.002), independent of age, sex, BSA, drug use and vegetable intake. No association was observed with proteinuria.

Conclusions: The pronounced reduction in UNO_x in RTR compared to controls, and its robust, independent association with CRP and functional characteristics of CV risk points to a role for endogenous NO in CV risk in RTR.

SA-PO1016

Urinary Excretion of Sulfate and Thiosulfate in Renal Transplant Recipients

Else van den Berg,[1,3] Andreas Pasch,[2] Gerjan Navis,[3] Elizabeth Brink,[1] Reinold O.B. Gans,[3] Harry Van Goor,[3] Stephan J.L. Bakker.[3] *[1]TI Food&Nutrition, Wageningen; [2]Dept of Nephrology and Hypertension, University Hospital and University of Bern; [3]University Medical Center Groningen (UMCG), the Netherlands.*

Background: Sulfuric acid is the main degradation endproduct of the sulfur-containing amino acids (SAA) methionine and cysteine. This sulfur metabolism is allegedly adverse by its contribution to metabolic acid load, particularly in renal patients. However, sulfur-containing compounds may have protective effects by intermediated conversion to hydrogen sulfide (H_2S). The major metabolite of SAA excreted in urine is sulfate (SO_4^{2-}). A smaller portion is excreted as thiosulfate ($S_2O_3^{2-}$), reflecting endogenous H_2S-production.

Methods: Urinary SO_4^{2-} and $S_2O_3^{2-}$ excretion was studied in 707 renal transplant recipients (RTR) (age 53±13y, 57% male, time since Rtx 5.4y) and 107 healthy controls (53±10y, 47% male). Diet was assessed for SAA content. Blood pressure, pulse pressure, blood gases, metabolic acid load (NAE), hs-CRP, NT-pro-BNP and renal function were measured and related to urinary SO_4^{2-} and $S_2O_3^{2-}$ excretion.

Results: Urinary $S_2O_3^{2-}$was higher in RTR (7.0 [3.9-11.9] vs 2.1 [0.3-10.1] µmol/24h in controls, p<0.001), whereas $SO_4^{2-}$was similar (both 18±6 mmol/24h; p=0.3). Correlations with intake of SAA were 0.10; p=0.01 for $S_2O_3^{2-}$and 0.32; p<0.001 for SO_4^{2-}. $S_2O_3^{2-}$correlated with renal function (r=0.28), serum HCO3- (r=0.20), serum pH (r=0.20), NAE (r=0.14) and was inversely related with hs-CRP (r=-0.18) and NT-Pro-BNP (r=-0.28), all p<0.001. SO_4^{2-}correlated positively with renal function (r=016; p<0.001), NAE (r=0.51; P<0.001) and serum pH (r=0.08; p=0.05) and inversely with hs-CRP (r=-0.15; p=0.003) and Nt-Pro-BNP (r=-0.24; p<0.001).

Conclusions: In line with the classic view, urinary SO_4^{2-} excretion is associated with NAE, but is not adversely associated with acidosis. The associations of urinary SO_4^{2-} and $S_2O_3^{2-}$ with a favorable risk profile suggests a larger role for H_2S-production as an intermediate pathway than appreciable from the relatively small amount of $S_2O_3^{2-}$ excretion compared to SO_4^{2-}. Exogenous sulfur might serve as therapeutic agent in RTR.

SA-PO1017

Increased Urinary CCL2: Cr Ratio at 6 Months Is Associated with Late Renal Allograft Loss

Julie Ho,[1] Chris J. Wiebe,[1] David N. Rush,[1] Claudio Rigatto,[1] Leroy J. Storsley,[1] Martin Karpinski,[1] Ang Gao,[1] Ian W. Gibson,[2] Peter W. Nickerson.[1] *[1]Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Pathology, University of Manitoba, Winnipeg, MB, Canada.*

Background: Early non-invasive markers that identify patients at risk of renal allograft loss may stratify patients for more intensive monitoring or therapy. CCL2 is a CCR2 receptor chemokine that is a chemoattractant protein for monocytes/macrophages, T cells and natural killer cells. We have previously demonstrated in a multi-centre cohort that urinary CCL2 at six months is an independent predictor for the development of IFTA at 24 months. The goal of this study was to determine if early urinary CCL2 is a predictor of graft loss in an independent patient cohort.

Methods: A prospective, observational cohort study was conducted in the Transplant Manitoba Adult Kidney Program (n=231 patients) from 1997-2008. Six-month urinary CCL2 was measured by ELISA, corrected for urinary creatinine and correlated with long-term graft outcomes.

Results: Urine CCL2: Cr at six months was significantly associated with death-censored graft loss [OR 1.01, 95% CI 1.003-1.020, AUC 0.78, p=0.004]. On multivariate analysis, urinary CCL2: Cr at six months remained an independent predictor of death-censored graft loss [OR 1.01, 95% CI 1.005-1.021, p=0.0015] after adjustment for donor specific antibody and delayed graft function. For each 10ng CCL2/mmol Cr incremental increase in 6-month urinary CCL2 there was a 1.13-fold increased risk of graft loss. The combined model of CCL2, donor specific antibody and delayed graft function yielded an AUC 0.87 for prediction of death-censored graft loss.

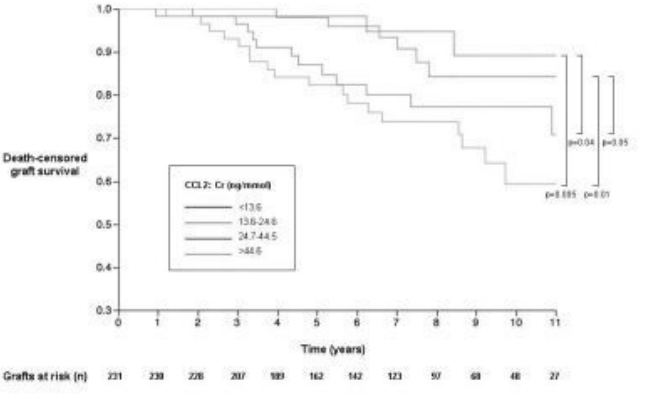

Figure 1. Death-censored graft survival by 6-month urinary CCL2: Cr quartiles, p=0.006

Conclusions: This study confirms in an independent prospective cohort that early urinary CCL2 at six months is a non-invasive independent predictor for late renal allograft loss.

Funding: Private Foundation Support

SA-PO1018

Three Month Protocol Biopsies Show Clinically Significant Diseases and Correlate with Graft Dysfunction at One Year: Genomics of Chronic Allograft Rejection Cohort

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Background: Protocol biopsies provide the opportunity to determine graft histologic status and to identify potential subclinical events. The Genomics of Chronic Allograft Rejection (GoCAR) study is an ongoing, prospective multicenter study using differential gene expression and pathology to predict development of chronic rejection using 0,3,12 and 24-month protocol biopsies. We report pathologic features in 3mo biopsies and the correlates with dysfunction at 12 mos.

Methods: All 3mo protocol biopsies with microarray studies presently available (n=100) were scored by 2 pathologists for 55 variables including Banff categories and analyzed for correlations with 3 and 12mo eGFR.

Results: 12% of biopsies had pathology diagnoses with clinical implications: 8 had evidence of rejection, 2 had polyoma virus infection and 2 had C4d+ with no evidence of rejection. 3 lost their graft during the 1st year (2 due to polyoma, 1 death with function). Histologic variables differing most between patients with 3mo eGFR<50mL/min (n=36) vs >50mL/min (n=64) were % tubular atrophy (p<0.025) and Banff scores for interstitial fibrosis (ci, p<0.02) and tubulitis (t, p<0.05, 2-tailed t test). Features that correlated to the greatest degree with function at 12 mos (eGFR/graft loss) were % fibrosis (p<0.05), % tubular atrophy (p<0.001) and their sum (p<0.001), and Banff ci (p<0.02) and t (p<0.05) scores, and scores for tubular atrophy (ct, p<0.05), and total inflammation (ti, p<0.025). The Chronic Allograft Damage Index (p<0.02) and 3mo eGFR (p<0.001) were both associated with 12mo function.

Conclusions: A substantial fraction of 3mo protocol biopsies reveal diseases that warrant a change in therapy. Extent of tubular atrophy and fibrosis in 3mo protocol biopsies, and active lesions like tubulitis and cortical inflammation, indicate graft status relative to eGFR in this cohort and correlate with later function.

SA-PO1019

A Novel Approach for Detection of Renal Allograft Rejection Mark J. Lerman,[1] Sandra Hinton,[1] Judson M. Hunt,[1] Brandt Moore,[2] Cindy L. Corpier,[1] Richard Dickerman,[1] Tina Worley,[1] Karen Roush,[3] Afzal Nikaein.[2] *[1]Transplant Services, Medical City Hospital, Dallas, TX; [2]Texas Medical Speciality, Medical City Hospital, Dallas, TX; [3]Transplant Immunology, Methodist Medical Center, Dallas, TX.*

Background: Diagnosis of renal transplant (tx) rejection is based on serum creatinine (Cr) and invasive biopsies. However, quality of biopsies are not always adequate to show graft injuries. Urine produced and excreted by the kidney, has the potential to reflect injuries to the renal allograft. We have investigated the presence of biomarkers in urine of patients with or without rejection. The biomarkers included osteoprotegerin (OPG), monokine induced by interferon- γ (MIG) and interferon-γ induced protein of kDa (IP-10). These three biomarkers were increased in 100% of patients with cellular and >80% with humoral rejection.

Methods: 52 renal tx recipients with possible rejection were included. Biopsies were evaluated for rejection, tubular injury, glomerulonephritis, BK virus, and C4d staining. Patient's sera and urine were obtained at the time of biopsies for presence of HLA antibodies and biomarker respectively. Tests were performed by solid phase immunoassays.

Results: According to the following table, all patients with cellular rejection had high level of biomarkers in urine. Only one had lower level of MIG, but close to cut-off value. In addition, 4/6 patients with humoral rejection had high level of all 3 markers. One had lower IP-10 and 2 had lower MIG level, but still elevated. In contrast, only 6/30 (20%) of patients with no rejection had high level of all 3 markers. One patient had BK virus.

# patients	Sr. Cr.	IP-10 > 25pg/ml	MIG>25 pg/ml	OPG >75 pg/ml
Cellular Rejection (12)	1.1-9.9	12 (100%)	11 (91.6%)	12 (100%)
Humoral Rejection (6)	1.2-4.5	5 (83%)	4 (66.6%)	6 (100%)
Glomerulonephritis (3)	1.8-2.8	1 (33%)	1 (33%)	1 (33%)
No Rejection (30)	1.03-8.9	6 (20%)	6 (20%)	7 (23%)

Conclusions: This noninvasive test has potential to be used for diagnosis of rejection and in some cases would eliminate an invasive test which may cause complications with bleeding and infection.

SA-PO1020

Identification of miRNA Pathways Discriminating Acute Cellular and Humoral Renal Allograft Rejection by Using a Newly Developed miRNA Pathway Tool Sara Ivcevic,[1,2] Andreas Heinzel,[1,2] Julia Wilflingseder,[1,3] Rainer Oberbauer.[1,3] *[1]Nephrology, KH der Elisabethinen, Linz, Austria; [2]Bioinformatics, University of Applied Sciences Upper Austria, Austria; [3]Nephrology, Medical University of Vienna, Austria.*

Background: Aim of this project was to develop a miRNA pathway tool for providing fast and reliable information about miRNAs and their affected pathways. We elucidated the test characteristics of our algorithm in miRNAs discriminating acute vascular, interstitial and anti-body mediated rejection after renal transplantation.

Methods: The core of this tool is a relational database for systematic collection and management of information on miRNA target prediction and pathways. Ten algorithms have been selected to predict target genes of miRNAs in-silico. To reduce the high false-positive rate of these predictions we focused on genes predicted as targets by more than one method as well as information on experimentally verified miRNA targets. The obtained targets were used to find pathways which are regulated by those genes to get a global miRNA pathway landscape. For this purpose several statistical methods were applied and the calculation performed for each miRNA as well as for the whole set of miRNAs combined. The tool is available at www.meduniwien.ac.at/nephrogene.

Results: Results from miRNA profiling experiments covering 15 cases with acute vascular rejection, 15 cases with acute interstitial rejection, 11 cases showing antibody-mediated rejection and 10 protocol biopsies (control group) were used for verification of the tool. miRNAs discriminating the different forms of acute renal allograft rejection have been linked to several pathways. A detailed analysis of the identified pathways and involved target genes will be presented at the meeting.

Conclusions: The miRNA pathway tool produces fast and biologically relevant results. The application on acute rejection provides novel insights in the molecular regulation of this complication after renal transplantation and paves the way for new diagnostic and therapeutic targets.

Funding: Government Support - Non-U.S.

SA-PO1021

The Banff Fibrosis Trial of Visual Assessment of Interstitial Fibrosis in Kidney Biopsies Michael Mengel,[3] Robert B. Colvin,[2] Alton B. Farris.[1] *[1]Emory; [2]MGH; [3]UAlberta.*

Background: Interstitial fibrosis (IF) in renal biopsies is a valuable correlate for organ function, provides prognostic information and is a potential end point in trials.

Methods: In a multi-centre trial using 15 allograft and 15 native kidney biopsies with a full range of IF, sections were stained with trichrome, PAS, Collagen III, and virtual microscopy scans (Aperio) were prepared for scoring. Slides were assessed by 27 pathologists following two different definitions of IF shown in figure 1. Reproducibility was assessed by kappa statistics. Results were correlated with collagen III IHC slides after computer based image analysis (reference standard) and GFR at time of biopsy.

Results: Using the trichrome stain better correlations with image analysis were obtained compared to the PAS stain. Approach B, i.e. estimating the % abnormal cortex, was superior to approach A (Table 1). A stronger inverse association between IF and GFR was seen with the trichrome stain. Tubular atrophy and the Banff total i-score correlated less well with the collagen III stain and GFR. Independent of the approach and the stain, reproducibility between observers for assessing IF was weak (kappa <0.25). We then ask the participants to score the Collagen III stained slides. This revealed similar correlations as observed with the trichrome stain: approach A r=0.66 with image analysis and r= -0.37 with GFR; approach B r=0.68 with image analysis and r= -0.4 with GFR.

Figure 1

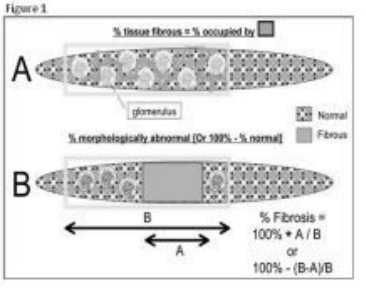


Table 1: Spearman correlations: mean r-values derived from the 27 participant scores are given for the listed associations between the two stains and the two scoring approaches with Collagen IHC and GFR.

	Approach A PAS Stain	Approach A Trichrome Stain	Approach B PAS Stain	Approach B Trichrome Stain	Tubular Atrophy PAS stain	Total I-Score	Collagen III immunohistochemistry
Collagen III immunohistochemistry	0.50	0.61	0.57	0.66	0.57	0.18	
GFR-Cockroft - Gault	-0.36	-0.42	-0.39	-0.43	-0.40	-0.22	-0.44

Conclusions: Trichrome and collagen III stains are similar but superior to the PAS stain in assessing IF. Pathologists are better capable of assessing the extent of abnormal cortex than the extent of interstitial space occupied by fibrous tissue. Visual assessment of IF correlates with organ function as well as computer-based image analysis. However, significant variability between observers argues for more precise consensus criteria for measuring IF visually.

Funding: Pharmaceutical Company Support - Astellas Pharma Canada Inc

SA-PO1022

Pre-Transplant Plasma Levels of PRA and Soluble CD30 in Kidney Graft Recipients as Predictors of Acute Allograft Rejection: Single Centre Experience Lilija Supranaviciene,[1] Tadas Daugela,[2] Marius Miglinas.[1] *[1]Nephrology Department, Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania; [2]Vilnius University Faculty of Medicine, Vilnius, Lithuania.*

Background: Our aim is to evaluate the influence of panel reactive antibodies (PRA) and plasma pre-transplant levels of soluble CD30 (sCD30) on the incidence of acute rejection episodes (ARE) following 2 years after renal transplantation.

Methods: We retrospectively analyzed medical records from renal transplant recipients >17 years old who received kidney graft between November 2008 and December 2011. Patients have been followed for 2 years and the incidence of the first ARE was verified both on histological and clinical findings. Patients had their plasma sCD30 levels measured on the day of the transplantation. By this finding they were divided into high and low risk groups: sCD30 levels >115 U/ml were considered as high risk group and <115 U/ml – low risk group. Maximum percentage of historical PRA was considered positive when > 5%. Other analysed variables were: age, gender, re-transplants and serum creatinine levels at 1, 3, 6, 12 and 24 months after transplantation.

Results: Among 70 transplants, 35 (50%) had ARE episode. Univariate analysis showed, that positive PRA was more frequent in the ARE patients (p = 0,015). In the low sCD30 group there were 53 (75.7%) patients and 25 of them experienced an ARE episode vs high group – 17 (24.3%) patients with 10 rejection episodes (p = 0.41). Kaplan - Meier estimate showed that in the high risk group (with sCD30 > 115 U/ml) there were more ARE after the first year, but results were not significant (p = 0,776). There was no significant correlation between sCD30 levels and recipients creatinine levels measured at 1, 3, 6, 12 and 24 months after transplantation (p > 0.05).

Conclusions: In this single centre study, the positive pre-transplant PRA was a predictive factor of developing ARE. Pre-transplant sCD30 levels did not show any significant difference between rejection and non rejection group in our transplant population and had no influence on graft function after transplantation.

Funding: Private Foundation Support

SA-PO1023

Kidney Allograft SHROOM3 Predicts Fibrosis Madhav C. Menon,[1] Peter Y. Chuang,[1] Yi Luan,[1] Zhengzhe Li,[1] Weijia Zhang,[1] Philip O'Connell,[4] Lorenzo G. Gallon,[2] Robert B. Colvin,[3] Bernd Schroppel,[1] John C. He,[1] Barbara T. Murphy.[1] *[1]Nephrology, Mount Sinai Hospital, New York, NY; [2]Nephrology, Northwestern University, Chicago, IL; [3]Pathology, Harvard Medical School, Boston, MA; [4]Nephrology, University of Sydney, Sydney, Australia.*

Background: The Genomics of Chronic Allograft Rejection (GoCAR) is a multicenter study investigating the ability of differential gene expression to predict the development of Chronic allograft nephropathy.

Methods: 589 patients have been enrolled. Allograft biopsies were obtained were obtained at 0, 3, 12, and 24 months post-transplant with Chronic allograft dysfunction index score (CADI) reported from a core lab. Gene expression microarray analysis was performed on 3 month biopsies (Affymetrix: human exon-1chip) and correlation to 12-month CADI and eGFR analysed (n=160). Overexpression studies were performed on human primary tubular cells (RPTE).

Results: Expression of the SHROOM3 gene was found to correlate linearly with fibrosis and negatively with eGFR at 1 year (n=101; $p<0.05$). This was confirmed by RTPCR independently (n=32) No correlation was seen between SHROOM3 expression and 3 month CADI (n=100). A SNP in SHROOM3 has been linked to CKD in genome-wide association studies. We found that homozygosity for this risk allele (A/A) in donors was associated with higher intragraft SHROOM3 expression at 3 mths (n=134;p=0.03). The risk allele was more prevalent in white vs. non-white donors. While in white donors SHROOM3 expression was predictive of CADI at 12 mths, this was not true for non-whites. In vitro, overexpression of SHROOM3 in human primary tubular cells (RPTE) significantly increased type-1 collagen and fibronectin-1 transcripts, and accentuated TGF-β mediated production of mesenchymal markers (Collagen-1, matrix metalloproteinase-2, SNAIL) and phosphorylation of Smad-3.

Conclusions: These novel findings demonstrate that SHROOM3 expression in kidney allografts is predictive of the development of fibrosis. Initial mechanistic studies suggest that it potentiates the effects of TGF-b. These data support the further investigation of SHROOM3 as a potential therapeutic target for the prevention of renal fibrosis.

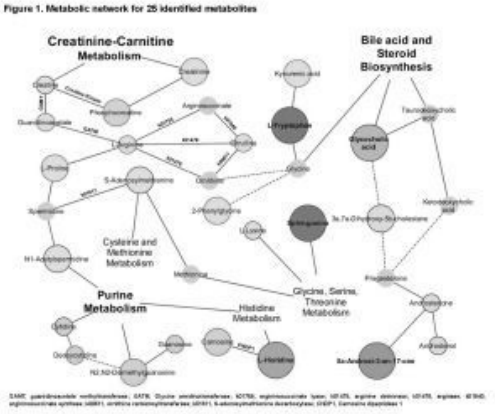
Funding: Other NIH Support - NIAID

SA-PO1024

Impact of Urinary Metablolic Profiles in Predicting Graft Function after Renal Transplantation Jung-Ju Seo,[1] Prasad B. Phapale,[2] Sun-Hee Park,[1] Chan-Duck Kim,[1] Young-ran Yoon,[2] Yong-Lim Kim.[1] *[1]Division of Nephrology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea; [2]Clinical Trial Center and Department of Molecular Medicine, Kyungpook National University School of Medicine and Hospital, Daegu, Republic of Korea.*

Background: Metabolomics can yield markers with improved precision and accuracy for monitoring clinical outcomes. We evaluated clinical applicability of metabolomics to predict GFR after kidney transplantation(KT) using liquid chromatography–mass spectrometry (LC/MS)-based global metabolic profiling along with integrative data analysis and metabolic network analysis.

Methods: In this prospective and exploratory study, LC/MS-based analysis was performed to analyze global metabolic profiles of urine collected from 12 recipients before(-2 days) and after(5, 30, and 90 days) KT. We applied partial least squares(PLS) model and variable influence on projection(VIP) to find metabolite correlated with GFR(VIP>1.3) and identified metabolites by matching MS/MS spectrum to metabolite database, such as HMDB. After metabolic network analysis, we selected most significant metabolites to predict GFR using integrative approach and drew new prediction equation for GFR.



Results: We selected five representative metabolites based on statistical and biological significance in predicting GFR; 5a-androst-3-en-17-one(AS), glycocholic acid(GC), sphingosine(SG), tryptophan(TR), and histidine(HT). We built clinically applicable prediction equation using these metabolites; $GFR_{Predicted}$=0.45HT+0.46AS+0.39TR+0.54SG+0.38GC. The predicted GFR showed strong correlation with observed GFR(R^2=0.71) and could predict long-term GFR at 2 years after KT(R^2=0.50).

Conclusions: In conclusion, we confirmed that urine metabolic phenotype would be more comprehensive biomarker for predicting early and long-term GFR after KT up to 2 years.

Funding: Government Support - Non-U.S.

SA-PO1025

Surveillance Biopsies: The Imapact on Immunosuppression Management in Kidney Transplant Recipients Nadia N. Chaudhri, Emilio Ramos, Joseph M. Nogueira, David K. Klassen, Matthew R. Weir, Abdolreza Haririan. *Medicine, University of Maryland Medical Center, Baltimore, MD.*

Background: The utility of surveillance biopsies in management of immunosuppression(IS) in kidney transplant recipients in the modern era remains unclear. The purpose of our study was to assess whether histological findings on surveillance biopsies help guide changes IS regimen.

Methods: We examined 320 protocol biopsies: 205 at 3.7±0.97 and 115 at 13.1±1.7 mos, scored according to Banff 97 criteria. Tacrolimus, MMF/MPA and early steroid withdrawal were used for maintenance IS. For cause biopsies were excluded.

Results: Patient characteristics are summarized below.

Baseline Characteristics

Age(mean±SD)	50.1±22.9yrs
African American(%)	122 (51.9)
Male(%)	141 (60)
DM(%)	101 (43)
HTN(%)	228 (97)
Hemodialysis (%)	143 (64.7)
DDRT(%)	183 (78.1)
Alemtuzumab induction(%)	138 (59.5)
Basiliximab(%)	70 (30.2)
Thymoglobulin(%)	23 (9.9)
HLA mismatch	4.8±1.5

Mean cr at 3 and 12 mo was 1.62±0.57 and 1.68±0.64 mg/dl, and the incidence of subclinical acute cellular rejection(ACR) was 8.8% (n=18) and 15.7 % (n=16), respectively. In pts with and without ACR, the mean cr at 3 and 12mos was not statistically different (1.78 and 1.62, p=0.16; 1.83 and 1.60, p=0.2, respectively). Progression of IF/TA from 3 to 12mo was seen in 29.2% of ps. A change in IS was made in 31.5% of pts at 3mos and 49.3% of pts at 12 mos. 4.2% and 5.6% of pts were treated for ACR and 1.4 % and 1.4% for antibody mediated rejection at 3 and 12 mos, respectively. Maintenace IS was modified in 26.4% of pts at 3 mos and 40.6% at 12 mos. These changes included increasing or decreasing CNI/MPA/MMF, adding steroids, or changing to mTORi. Progression of IF/TA was observed in 31.7% with no IS adjustment versus in 14.3% with treatment adjustment (p=0.16).

Conclusions: Surveillance biopsies reveal significant subclinical histological changes that could guide adjustment in IS regimen. The biopsy guided IS modification may decrease risk of progression of IF/TA post transplant. Larger prospective studies are needed to examine the impact of surveillance biopsies on long term renal allograft survival.

SA-PO1026

Systems Biology Analyses of Renal Allograft Injury Julia Wilflingseder,[1,2] Andreas Heinzel,[3] Alexander Kainz,[1,2] Bernd Mayer,[4] Rainer Oberbauer.[1,2] *[1]Nephrology, Medical University of Vienna, Austria; [2]KH der Elisabethinen, Linz, Austria; [3]Bioinformatics, University of Applied Science Upper Austria; [4]Emergentec Biodevelopment, Vienna, Austria.*

Background: Large scale molecular characterization of renal allograft pathology is essentially based on transcriptomics data. However, the test characteristics of diagnostic or predictive markers derived on this basis proved as being rather low.

Methods: Thus we set out to study this enigma using a considerable wider approach by incorporating a broad range of publicly available omics data (genetic background, protein coding as well as non-coding transcripts, as well as proteomics and metabolomics signatures) into the analysis of the prediction of DGF, ABMR, TCMR and early IFTA. We complemented this molecular feature set with our transcriptomics data (mRNA and miRNA) derived from a clinical biopsy repository holding tissue and clinical information of renal allograft recipients at baseline, together with management and indication biopsies (n=170).

We use a hybrid molecular interaction network covering about 15,000 molecular features from the human protein coding gene set, and holding about 800,000 molecular relations for integrating the given cross-omics data sets. This renal allograft-specific network is then segmented into distinct molecular processes relevant in the pathology, in their totality providing us with a molecular model of the different clinical phenotypes.

Results: The workflow for generating the molecular model and characteristics of the identified molecular processes will be presented at the meeting. Preliminary results suggest molecular units being specific for the distinct clinical entities. Such molecular units serve as basis for selection of biomarkers being specific for the individual clinical presentations, and their functional analysis may further serve as basis for better understanding of relevant molecular pathophysiology, in turn being the prerequisite for improving therapeutic measures.

Conclusions: We will present the first results of this cross-omics, Systems Biology-motivated approach in identifying prognostic and diagnostic as well as potential therapeutic molecular candidates for clinico-pathological entities.

Funding: Government Support - Non-U.S.

SA-PO1027

Age as an Effect Modifier for Renal Transplantation in Canada's Aboriginal Peoples Manish M. Sood,[1] Brenda Hemmelgarn,[2] Claudio Rigatto,[1] Paul Komenda,[1] Karen E. Yeates,[3] Julie Mojica,[1] Navdeep Tangri.[1] *[1]Medicine, Section of Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Medicine, Section of Nephrology, University of Calgary, Calgary, AB, Canada; [3]Medicine, Section of Nephrology, Queen's University, Kingston, ON, Canada.*

Background: Previous studies have demonstrated Aboriginals are less likely to receive a renal transplant in comparison to Caucasians however whether this applies to the entire population or specific subsets remains unclear. We examined age as an effect modifier on the outcome of transplantation in Aboriginals.

Methods: Data on 30,688 dialysis (Aboriginal 2,361, Caucasian 28, 327) patients obtained between Jan. 2000 and Dec. 2009 were included in the final analysis. Racial status was self-reported. Cox proportional hazards and the Fine and Grey sub-distribution method to account for competing events were used to determine the association between race, age and transplantation.

Results: In comparison to Caucasians, Aboriginals were less likely to receive a renal transplant (Adjusted HR 0.66 95% CI 0.57-0.77, P<0.0001) however after stratification by age and treating death as a competing outcome, the effect was more predominant in younger Aboriginals (Age 18-40: 20.6% aboriginals vs. 48.3% Caucasians transplanted; aHR 0.50(0.39-0.61), p<0.0001, Age 41-50: 10.2% aboriginals vs. 33.9% Caucasians transplanted; aHR 0.46(0.32-0.64), p=0.005, Age 51-60: 8.2% aboriginals vs. 19.5% Caucasians transplanted; aHR0.65(0.49-0.88), p=0.01, Age >60: 2.7% aboriginals vs. 2.6% Caucasians transplanted; aHR 1.21(0.76-1.91), P=0.4, Age X race interaction p<0.0001). Both living and deceased donor transplants were lower in Aboriginals under the age of 60 compared to Caucasians.

Conclusions: Younger Aboriginals are less likely to receive a renal transplant compared to their Caucasian counterparts, even after adjustment for comorbidity. Determination of the reasons behind these discrepancies and targeted interventions are urgently required.

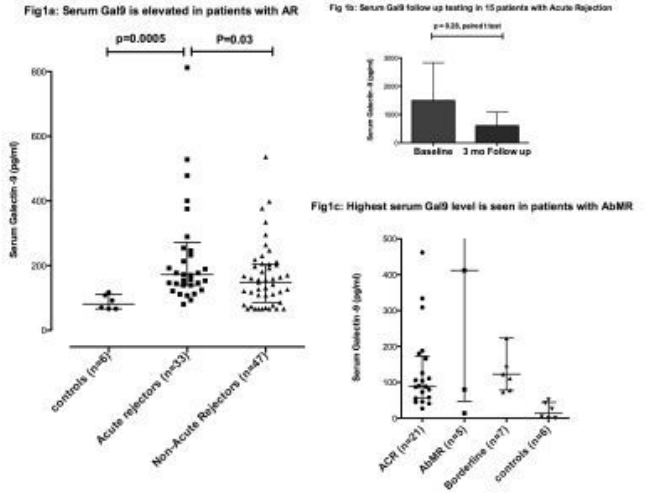
SA-PO1028

Galectin-9: A Novel Serum Biomarker of Acute Rejection in Renal Transplant Recipients with Allograft Dysfunction Isa F. Ashoor,[1] Usaila Ahmad,[1] Sacha A. De Serres,[1] Monica Grafals,[2] Toshiro Niki,[3] Nader Najafian.[1] *[1]Nephrology, Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Boston, MA; [2]Nephrology, Lahey Clinic, Burlington, MA; [3]Kagawa University, Kagawa, Japan.*

Background: Acute renal allograft rejection remains a significant problem. A non-invasive test to predict rejection would be greatly beneficial. Galectin-9 (Gal9) is a lectin that binds to TIM3+ effector Th1/Th17 cells inducing apoptosis. We previously demonstrated accelerated rejection in a murine vascularized cardiac transplant model with Gal9 blockade suggesting a protective role for Gal9. We hypothesize that human serum Gal9 (sGal9) is elevated as a compensatory response and may serve as a biomarker of acute rejection.

Methods: Blood was collected from 6 healthy controls, and 80 renal transplant recipients admitted for allograft biopsy due to an acute rise in serum Cr. Pts were classified by histology as acute rejectors (AR) or non-acute rejectors (NAR). sGal9 was measured using a sandwich ELISA assay.

Results: 33 pts were classified as AR (21 ACR, 5 AbMR, 7 borderline cellular rejection) and 47 as NAR (32 chronic changes,10 GN, 5 ATN). There were no significant differences in terms of age, gender, donor type or immunosuppressive regimen. Median sGal9 levels in AR was 106 pg/ml (IQR 73-205) significantly higher than HCs (15 pg/ml (IQR 0-44), p=0.0005) and NAR (82 pg/ml (IQR 20-138), p=0.03). In 15 AR pts who underwent f/u testing, sGal9 decreased at 3 mo. Within AR, sGal9 was highest in AbMR group.



Conclusions: 1) sGal9 is a novel biomarker elevated in acute rejection at the time of biopsy 2) This elevation is transient suggesting an acute involvement of the TIM-3:Gal9 pathway as a compensatory response 3) Moderate elevation of sGal9 with chronic allograft changes likely reflects an attempt to curtail an ongoing low-grade alloimmune injury.

Funding: Other NIH Support - Clinical Trials in Organ Transplantation Cooperative Research Program (NIAID)

SA-PO1029

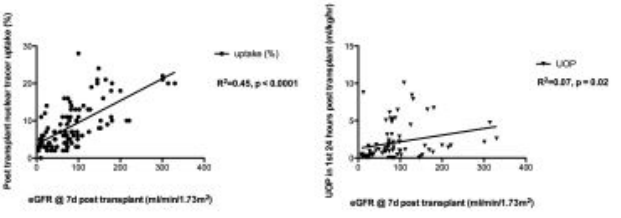
Immediate Post-Renal Transplant Nuclear Scans in Children Correlate with Transplant Function at One Week Isa F. Ashoor, Nilka deJesus-Gonzalez, Michael J. Somers. *Nephrology, Boston Children's Hospital and Harvard Medical School, Boston, MA.*

Background: Delayed graft function (DGF) impacts long term renal graft survival. We assessed if immediate post transplant (tx) Mag3 nuclear tracer uptake correlates with 1 week graft function. This could allow early DGF risk assessment with targeted intervention to improve outcome.

Methods: We reviewed 100 consecutive renal tx pts ≤ 21 yo. Ischemia times, urine output in 1st 24hr post tx (UOP) and POD7 eGFR were calculated. Mag3 was performed by protocol within hrs of tx. DGF was defined as dialysis by POD7.

Results: 47 children were LD recipients (55% boys, median age 8 yo (IQR 2-15), cold ischemia time (CIT) 0.95hr (0.78-1.13), warm ischemia time (WIT) 28min (22-36), UOP 2.1 ml/kg/hr (0.9-5.3), POD7 eGFR 88 ml/min/1.73m^2 (63-145); 2 developed DGF. 53 were DD recipients (60% boys, median age 10 yo (3-16), CIT 9.4hr (7-12.9), WIT 31min (24.5-38.5), UOP 0.9 ml/kg/hr (0.3-1.5), POD7 eGFR 64 (22-92)); 5 developed DGF. DGF pts had lower Mag3 tracer uptake (4% vs 7%,p=0.048), longer WIT (38 vs 29min,p=0.02), and lower POD7 eGFR (10 vs 78,p<0.0001). Age, CIT and UOP were not different. Using POD7 eGFR >60 ml/min/1.73m^2 as surrogate for good early graft function, those pts had both higher UOP (1.7 vs 0.4 ml/kg/hr) and Mag3 tracer uptake (10% vs. 4%,p<0.0001). Times to peak Mag3 activity and 20 min residual were lower in the good eGFR group. POD7 eGFR correlated with Mag3 tracer uptake (R^2=0.45, p<0.0001) but not with UOP (R^2=0.07, p=0.02).

Fig 1:
Post transplant nuclear tracer uptake correlates well with POD7 eGFR while UOP in the 1st 24 hours does not



Conclusions: 1) Patients who develop DGF show reduced Mag3 tracer uptake within hrs of tx.

2) Early Mag3 tracer uptake correlates with POD7 eGFR.

3) Early Mag3 tracer uptake predicts DGF and POD7 eGFR better than early UOP.

4) Mag3 parameters (tracer uptake, time to peak, residual) can be used to develop predictive models for DGF/POD7 tx function, allowing early intervention to improve graft outcomes.

Funding: Clinical Revenue Support

SA-PO1030

Efficacy of Rituximab Compare to Conventional Treatment for Recurrent Idiopathic Membranous Nephropathy after Kidney Transplantation Damien Ambrosetti,[1] Laetitia Albano,[2] Elisabeth Cassuto.[1,2] *[1]Anatomopathologie, CHU de Nice, Nice, France; [2]Transplantation Rénale, CHU de Nice, Nice, France.*

Background: Recurrence rate of membranous nephropathy (MN) after kidney transplantation (KT) varies between 7 to 42% depending on whether biopsies are performed according to protocol or based on symptoms The possibility that AntiPLA2R1 antibody could be a biomarker for IMN recurrence after KT is still debated. The therapy for IMN is challenging. Rituximab has been shown to reduce proteinuria and prevent MN progression.

Methods: We report two male kidney recipients developing recurrent IMN with nephrotic syndrome after KT, treated with or without rituximab, and we analyze clinical, histological and immunological outcome. Patient #1 was treated with high-dose cyclosporin, pulse corticosteroid therapy and ACE inhibitors. Proteinuria decreased drastically and persisted at 0.2 g/dL. Thirteen years later, while serum creatinine was moderately increased, an allograft biopsy was performed. The histologic analysis found a recurrent stage 3 IMN associated with interstitial fibrosis and tubular atrophy. Anti PLA2R1 Ab in serum of patient #1 were detected before KT, at the initial recurrence of IMN and at the time of the last biopsy.Patient #2 was treated and cured with 4 weekly rituximab infusions. Currently, 5 years later, the patient is still asymptomatic. In view of the case of patient #1, a kidney graft biopsy was performed showing no recurrence of MN, but a moderate interstitial fibrosis and tubular atrophy. Anti PLA2R1 Ab were detected in the serum of the patient #2 before KT and at the time of recurrence but were undetectable at the time of the last biopsy.

Conclusions: So, both patients have a similar history of early recurrent IMN after KT, with favorable long-term clinical outcome after treatment. It's remarkable that the presence of histological recurrence is associated with AntiPLA2R1 Ab in the serum of the patient treated without rituximab. In conclusion, no proteinuria doesn't mean no recurrence of IMN after KT. The presence of serum antiPLA2R1 Ab could incite to look at a histological recurrence. In our case, rituximab seems more efficient than cycloporin to avoid IMN recurrence.

SA-PO1031

Reemergence of Adrenocorticotropin Hormone as Novel Therapy for Focal Segmental Glomerulosclerosis Sireesha Koppula, Barry R. Gorlitsky, Amy Nicole Sussman. *Nephrology, University of Arizona, Tucson, AZ.*

Background: Focal segmental glomerulosclerosis (FSGS) accounts for nearly one-third of all nephrotic syndrome (NS) cases in the adult population. Unresponsive patients progress to ESRD 50% of the time. Patients who experience partial or complete remission have up to 90% 10-year kidney survival rates. Many patients remain refractory to standard treatments. Adrenocorticotropin hormone has been used as primary and secondary therapy for nephrotic syndrome but there is no published experience for its use in the treatment of FSGS.

Methods: We report a case of a 28-year-old Filipina female with history of NS, biopsy proven in 2004 consistent with minimal change disease. After multiple treatment failures utilizing cyclosporine and prednisone, a repeat biopsy in 2008 showed FSGS (>50% podocyte effacement in both affected and unaffected capillary loops). Most recently she relapsed with NS, characterized by hypoalbuminemia (1.5g/dl), volume overload, dyslipidemia, and >10 grams of proteinuria quantified by spot protein to creatinine ratio. She was initiated on prednisone 1mg/kg daily; however, by week 8 she continued to have high-grade proteinuria and persistent NS. She subsequently underwent treatment with repository corticotropin injection (H.P. Acthar® Gel, repository corticotropin injection; Questcor), a long-acting formulation of the full sequence ACTH [1-39] that includes other pro-opiomelanocortin [POMC] peptides) at a dose of 80IU twice weekly SC for 6 months. By month 2, she demonstrated >50% reduction of proteinuria and by month 6 she demonstrated a near complete remission and normalization of her serum albumin and lipid panel.

Conclusions: In FSGS, ACTH may work through its melanotropic effect apart from steroidogenesis. There is considerable debate on the ideal treatment regimens for FSGS, particularly in poorly responsive cases. ACTH can be an effective treatment in NS due to FSGS and should be considered in patients who are recalcitrant to standard therapy. Further studies are ongoing to determine efficacy of ACTH as treatment for NS due to FSGS.

SA-PO1032

Hepatitis C-Associated Thrombotic Microangiopathy (TMA) without Cryoglobulinemia Reem Daloul, Francis Dumler, Gampala Harish Reddy, Ping L. Zhang. *Division of Nephrology, William Beaumont Hospital, Royal Oak, MI.*

Background: We report a case of hepatitis C associated TMA in a patient who presented with nephrotic syndrome and progressive renal failure.

Methods: 57 y/o male with a history of hepatitis C and hypertension was admitted with progressive shortness of breath, edema, and easy bruising. Hemoglobin was 8.2 g/dL and platelets 60,000 bil/L. Baseline BUN and serum creatinine where 6 and 0.4 mg/dL. Urinalysis: 1+ blood, 3+ protein, 5 rbc/hpf, 3 wbc/hpf, and no casts. Urine protein creatinine ratio was 5.03. Schistocytes were present in the peripheral blood and haptoglobin was undetectable, Cryoglobulins were negative on several occasions. ADAMTS 13 activity was 86% and ADAMTS13 inhibitor was not detected. Combs ANA, ANCA, Rheumatoid factor, C3, C4, and total complement were normal. Renal biopsy showed thickened glomerular capillary loops, a membranoproliferative pattern but no diffuse proliferation, necrosis or crescent formation. Several small arteries exhibited thrombi formation. Mild interstitial fibrosis and tubular atrophy were also present. Stains for IgM and fibrinogen were positive but others were negative. On electron microscopy glomerular basement membranes were thickened, and mesangial interposition and scattered subendothelial lucent areas were seen. Fibrin material was identified in the subendothelial areas mixed with lucent ischemic changes. No immune complex deposits were identified in the mesangial, subendothelial, or subepithelial areas. No paired tubular structures consistent with cryoglobulins were found. Blood counts stabilized after five cycles of apheresis but renal function continued to deteriorate. Hemodialysis was initiated six weeks later. The patient continues to be dialysis dependent at present.

Conclusions: TTP is known to occur in hepatitis C patients with cryoglobulinemia and/or treatment with pegylated- interferon alpha 2a, in the presence of ADAMTS13 inhibitor and/or a low ADAMTS13 activity. Our patient developed TTP and renal TMA lesions in the absence of these risk factors suggesting that other pathogenetic factors may be involved in the development of TMA in hepatitis C patients.

SA-PO1033

IgA Nephropathy in Sarcoidosis: A Consequence or Just a Coincidence? Nripesh Pradhan, Rochak Varma, Roger F. Carbajal Mendoza, Ashok P. Chaudhari, Donald I. Baumstein. *Nephrology, New York Medical College/Metropolitan Hospital Center, New York, NY.*

Background: Renal involvement in sarcoidosis is largely due to hypercalcemia. Granulomas in interstitium are found in 40% cases. Glomerular diseases due to sarcoidosis are very rare and Ig A nephropathy has been reported in very limited case reports. The association may be more than just a coincidence. We describe a case of sarcoidosis with renal impairment and Ig A nephropathy on kidney biopsy.

Methods: A 38 year old Hispanic male presented with a generalized nodular skin eruption for 2 months. He had a past medical history of kidney stones two years ago. Physical examination showed multiple erythrematous non-tender nodules in arms, flanks and thighs. Routine laboratory test revealed elevated creatinine of 2.6 mg/dl and calcium of 10.7 mg/dl. Mild proteinuria (Spot urine protein creatinine ratio- 0.4) was noted on urine analysis with no cells and bilateral hilar enlargement on chest roentogram. Further work up was noteworthy for elevated 1, 25 OH Vitamin D, suppressed Parathyroid hormone, elevated serum Angiotensin converting enzyme level of 294 (9-67 U/l) ,24 hour urine calcium of 733 mg while serological markers were all negative. Diagnosis of sarcoidosis was confirmed with skin biopsy of the nodular lesions. Renal ultrasound was normal. A Renal biopsy to confirm renal sarcoidosis surprisingly revealed mesangial proliferative glomerulonephritis with immunoflurorescence staining consistent with Ig A nephropathy. Patient was discharged on oral prednisone with close outpatient follow up.

Conclusions: Murray FE et al and Chung Park M et al have in past described simultaneous occurrence of sarcoidosis and Ig A nephropathy in 1987 and 1990 respectively. Sarcoidosis is a state of heightened immune response manifested by polyclonal increase in immunoglobulins. Whether such state is responsible for Ig A deposition in mesangium of glomerulus is not clear. Besides this, familial clustering has also been reported in both Ig A nephropathy and sarcoidosis. So in our case, we think Ig A nephropathy is more likely to be a consequence of sarcoidosis rather than just a coincidence.

SA-PO1034

Proliferative Glomerulonephritis with Monoclonal IgG Deposits Associated with Immune Disorder by Parvovirus B19 or Hepatitis C Virus Infection Emiko Fujita,[1] Akiko Mii,[1] Yukinari Masuda,[2] Megumi Fukui,[1] Shinya Nagasaka,[2] Akira Shimizu.[2] *[1]Department of Internal Medicine, Nippon Medical School, Bunkyo-ku, Tokyo, Japan; [2]Department of Analytic Human Pathology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Background: Proliferative glomerulonephritis (PGN) with monoclonal immunoglobulin G (IgG) deposits (PGNMID) is a recently described disease entity, characterized by non-organized electron-dense deposits in glomeruli and immunofluorescence findings indicating monoclonal IgG deposits. The pathogenesis of many cases of PGNMID remains unknown. We herein report three cases of monoclonal IgG deposition disease associated with viral infection.

Methods: Two cases are with human parvovirus B19 (HPV) who developed nephritic syndrome with hypocomplementemia (case 1) or persistent proteinuria and congestive heart failure (case 2). Other one case with hepatitis C virus (HCV) infection showed severe nephrotic syndrome (case 3). All 3 cases, however, neither patients had detectable levels of serum and urine monoclonal IgG. Renal biopsy in both case1 and case2 showed diffuse endocapillary PGN with monoclonal IgG3-κappa deposits, whereas membranous nephropathy in case 3 with monoclonal IgG1-lamda deposits. Deposits have no organization by electron microscopy in all cases. Clinical symptoms, abnormal laboratory findings, and urinary abnormalities recovered spontaneously in both case 1 and case 2 within 4 weeks. In case3, combined therapy with steroid and an immunosuppressive agent are needed to recover.

Conclusions: Our cases may be the first reported PGNMID patients possibly associated with viral infection. Immune disorders including cryoglobulinemia and monoclonal gammopathies can result from HPV and HCV that may be associated with PGNMID in our cases. Viral infection-associated immune disorders could be implicated in the pathogenesis of PGNMID.

SA-PO1035

Anti-Glomerular Basement Membrane Disease Superimposed on IgA Nephropathy Lin Liu, Lijun Mou, Yubing Wen, Wenling Ye, Jianling Tao, Mingxi Li, Yang Yu, Xuemei Li, Xuewang Li, Limeng Chen. *Department of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: Anti-GBM disease with concurrent IgA nephropathy is rare. We try to observe the clinicalpathologic features of 3 such cases with crescents and to identify the other 21 anti-GBM disease patients without IgA deposition.

Results: 3 cases, including 1 male and 2 female, were identified as anti-GBM disease superimposed on IgA nephropathy in PUMCH, during the past 11 years. Two of them had recent history of infection or fever. Hematuria, oliguria, hemoptysis was not seen in any of them. No one was hemodialysis dependent. Lab results revealed a mean serum creatinine (SCr) level of 283±221μmol/L at diagnosis, anti-GBM antibody maximum titer of 260±35 EU/ml, and negative ANCA. In kidney biopsy, glomerular crescents were observed in all 3 cases with a cellular/fibro-cellular proportion of 72±49%. With treatment of pulse methylprednisolone and cyclophosphamide in all and plasmapheresis in 2 of them, they showed improvement in renal function with SCr decreasing to 120±60μmol/L. Further, compared with data of 21 IgA-free anti-GBM disease patients with crescents, the 3 cases showed lower SCr than those without IgA nephropathy at diagnosis (819±606μmol/L, p=0.020), with better outcome that 100% had their SCr≤300μmol/L after similar treatment (vs. 29% of those without IgA nephropathy, p=0.042). But no significant differences showed in age, sex, and other clinicalpathological features.

Conclusions: The 3 cases with anti-GBM disease superimposed on IgA nephropathy showed milder kidney damage and better prognosis. The more which may indicate some different mechanism involved requires further research.

Funding: Government Support - Non-U.S.

SA-PO1036

Crescentic Glomerulonephritis (GN) in a Child with X-Linked Agammaglobulinemia (XLA) Sabahat Afshan,[1] Theodore Matthew Eison,[1] Margaret Colleen Hastings,[1,2] Christie Michael,[1] Karen Maltby,[1] Patrick D. Walker,[3] Bettina H. Ault.[1] *[1]Pediatrics, University of Tennessee Health Science Center, Memphis, TN; [2]Medicine, University of Tennessee Health Science Center, Memphis, TN; [3]Nephropath, Little Rock, AR.*

Background: XLA is caused by a mutation in Bruton's tyrosine kinase gene resulting in absent or marked reduction in antibody synthesis.

Methods: A five year-old Caucasian male with XLA received gammaglobulin from age three months. Two weeks prior to presentation, he had sore throat and fever; four days prior to presentation he developed cola-colored urine. On presentation blood pressure was 152/112 mm Hg and he had periorbital edema and ascites. Laboratory studies revealed blood urea nitrogen 24 mg/dL, creatinine 0.98 mg/dL, albumin 2.4 g/L, C3 16.6 mg/dL and C4 3.3 mg/dL. Urinalysis showed 3+ blood, 3+ albumin, 316 RBC, 112 WBC, and 1-2 RBC casts/hpf. Plasma IgG (316 mg/dL, normal 470-1400) and IgM (28.1 mg/dL, normal 37-117) were obtained one day after a gammaglobulin dose. Blood culture grew *Streptococcus pneumoniae*. Kidney biopsy showed crescentic GN with diffuse mesangial expansion of intact glomeruli on light microscopy (LM); 3 of 9 glomeruli were globally sclerotic. Plasmacytoid cells were noted in the interstitium. Immunofluorescence showed 3+ IgG, trace IgA, 3+ C3, 3+ kappa and 3+ lambda. Electron microscopy showed subendothelial and mesangial immune complex type electron dense deposits and rare intramembranous deposits. Six doses of 30 mg/kg intravenous methylprednisolone (MP) and then oral prednisone 2 mg/kg/day was given. Serum creatinine fell to 0.58 mg/dl after the fourth dose of MP.

Conclusions: Two cases of GN in XLA are reported, one with membranous and one with membranoproliferative GN. Presence of immune complexes would seem unusual in XLA. Measurable plasma IgM and interstitial plasmacytoid cells on LM and trace IgA on IF suggest that the antibody in the immune complexes could be endogenous. Immune dysregulation resulting from patient's immunodeficiency likely contributed to his severe immune complex GN.

SA-PO1037

Successful of Humanized Anti-Interleukin-6 Receptor Antibody (Tocilizumab) Therapy for a Case of AA Amyloidosis Complicated by Familial Mediterranean Fever Satoshi Hamanoue, Yoshifumi Ubara, Tatsuya Suwabe, Koki Mise, Keiichi Sumida, Masayuki Yamanouchi, Junichi Hoshino, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: Familial Mediterranean fever (FMF) is a cause of AA amyloidosis and is the most frequent type of familial amyloidosis. Colchicine has proved to be an effective treatment for FMF. However, treatment for patients who are refractory to colchicine remains controversial.

Methods: We encountered 4 patients who were diagnosed as having FMF by detection of the specific gene for MEFV. Although 3 patients were negative for AA-amyloidosis, one was shown to have AA-amyloidosis by biopsy of the kidney and stomach. Treatment for this disease was investigated.

Results: Three patients were successfully treated by conservative therapy including colchicine. Colchicine was also administered first to a 51-year-old man with AA-amyloidosis. However, his arthralgia and nephropathy showed progression. Therefore, a humanized anti–interleukin-6 receptor antibody (tocilizumab) was administered at a dose of 8 mg/kg monthly. After 2 years, his serum creatinine decreased from 2.3 mg/dL to 1.5 mg/dL, CRP decreased from 2.5 mg/dL to 0.0 mg/dL, and proteinuria improved from 2.5 g daily to 0.2 g daily. His arthralgia also subsided. Repeat biopsy of the stomach showed the disappearance of AA-amyloidosis.

Conclusions: This case suggests that treatment with tocilizumab can achieve significant improvement and represents a new therapeutic option for patients with FMF and AA amyloidosis if colchicine is not effective.

SA-PO1038

Unusual Presentation of Anti-Glomerular Basement Disease with Linear IgA Staining Gaurav Agarwal,[1] Olatokunbo O. Shobande,[1] Jose R. Torrealba,[2] Weixiong Zhong,[2] Micah R. Chan.[1] *[1]Department of Medicine, Division of Nephrology, University of Wisconsin School of Medicine and Public Health, Madison, WI; [2]Department of Pathology, University of Wisconsin School of Medicine and Public Health, Madison, WI.*

Background: Anti-GBM disease is an aggressive form of glomerulonephritis usually mediated by circulating IgG autoantibodies to the non-collagenous (NC1) domain of alpha3 subunit of Type IV collagen. There have been few case reports about an IgA variant of anti-GBM disease.

Methods: A 72-year-old white male presented with complaints of one episode of gross hematuria. He was found to have acute kidney injury (AKI) with a creatinine of 2.7 mg/dL, which rapidly increased to 5.6 mg/dL over 3 days. His baseline creatinine was 1.0 mg/dL.

His urinalysis was significant for 1+ protein and 2+hematuria. Complement levels were normal. Serologic studies were negative for anti-nuclear antibodies, anti-neutrophil cytoplasm antibodies, cryoglobulins, hepatitis B and C panels, anti-GBM ab.

Due to concern for a rapidly progressive glomerulonephritis, a renal biopsy was performed.

Renal biopsy yielded 3 glomeruli with cellular crescents and fibrin deposits. Immunofluorescence revealed +3 capillary wall linear staining for IgA and segmental +3 staining for fibrin. Electron microscopy reveals mild increase in mesangial matrix and normal cellularity. Glomerular basement membrane is of normal thickness with segmental, severe ischemic wrinkling. No electron-dense immune-like deposits.

The patient was treated with methylprednisolone 1 g IV daily for 3 days, then transitioned to oral prednisone (1 mg/kg/day), oral cyclophosphamide (1.5 mg/kg/day), and received a 6-week course of plasmapheresis. His renal function improved gradually from 5.6 mg/dL to 3.2 mg/dL by 3 weeks of treatment.

Conclusions: We report a case of IgA anti-GBM disease suggesting a possibility of a novel GBM antigen. The antigenic heterogeneity of anti-GBM IgA antibody disease demonstrates the necessity of renal biopsy for reliable diagnosis of this atypical presentation, as serological assays are likely to give false negative results. Further studies are needed to identify these novel antigen targets.

SA-PO1039

Eculizumab Therapy in an Atypical Haemolytic Uraemic Syndrome (aHUS) Patient (pt) with Long-Term Renal Failure and Posterior Reversible Encephalopathy Syndrome (PRES) Hannah Povey, Rahul Vundru, Mahdi Jibani. *Ysbyty Gwynedd, Bangor, United Kingdom.*

Background: aHUS is a rare, life-threatening, genetic disease of chronic, uncontrolled complement activation. Eculizumab, a monoclonal antibody that selectively inhibits terminal complement activation, is approved in all pts with aHUS.

Methods: 21-year-old female with a late diagnosis of aHUS (STEC-HUS diagnosed at age 3, kidney impairment at age 14, iron deficiency and anaemia). Medical records are summarized.

Results: The pt presented with malaise, headache and easy bruising. Thrombotic microangiopathy (TMA) was shown by decreasing platelets (90×10^9U/L), increasing creatinine (166µmol/L), elevated lactate dehydrogenase (LDH; 1175U/L) and presence of schistocytes. ADAMTS13 activity, levels of C3 and C4 were normal (77%, 0.91g/L and 0.28g/L respectively). No complement mutations were identified (factor H, I and MCP activity was normal; CFH auto-antibodies were negative). Despite intensive plasma exchange (PE) she became anuric and required haemodialysis (HD). During the 8th week on HD, the pt lost vision in both eyes, followed by 3 grand mal seizures. A cerebral MRI showed parietaloccipital lobe edema consistent with PRES. The pt received labetalol infusions and eyesight was regained in 3 days. Support with HD and PE continued (36 sessions over 10 weeks total), however, thrombocytopenia continued and LDH remained elevated (Table). Eculizumab administration (900mg/wk for 4 wk, 1200mg wk 5 and 1200mg/2wk thereafter) improved platelet count, reduced LDH and decreased creatinine (Table). After the second eculizumab dose, renal function recovered and HD was discontinued (2.5 months on HD). The patient is currently on chronic eculizumab treatment.

Conclusions: Eculizumab, in contrast to intensive PE, inhibited complement-mediated TMA, improved renal function and eliminated the need for HD in a pt with life-threatening aHUS.

Day	Creatinine (µmol/L)	LDH (U/L)	Platelets (x10^9 U/L)	Management Since Previous Measure
1	166	1172	91	
7	328	585	28	PE x5, HD x2
14	436	646	77	PE x5, HD x5
20	420	437	128	PE x4, HD x2
28	369	917	75	PE x4, HD x5
35	435	532	124	PE x7, HD x4
42	453	509	53	PE x6, HD x4
49	470	571	120	PE x5, HD x4
58	514	622	172	HD x5
68	356	483	85	PE x2, HD x4, Ecu x1
78	327	490	73	HD x1, Ecu x1
88	177	412	96	Ecu x2
99	168	634	41	Ecu x1
110	223	406	129	Ecu x1
123	201	299	177	Ecu x1
137	144	348	209	Ecu x1
151	139	339	154	Ecu x1
165	145		202	Ecu x1

Ecu=eculizumab.

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SA-PO1040

Eculizumab in Genetic Form of Atypical Hemolytic Uremic Syndrome Associated with Cocaine Consumption Carola Arcal Cunillera, Josep Miquel Blasco Pelicano, Nestor Yesid Rodriguez, Luis F. Quintana, Federico Oppenheimer, Francisco Maduell, Josep Maria Campistol Plana. *Nephrology and Kidney Transplant Division, Hospital Clínic, Barcelona, Spain.*

Background: We present the case of two brothers with Atypical Hemolytic Uremic syndrome (aHUS) associated to factor H (FH) gene mutation and triggered by cocaine, with completely different renal outcome related to treatment.

Methods: A 27-year-old man presented with acute kidney failure, non-immune hemolytic anemia and thrombocytopenia. Complementary blood test showed raised lactate dehydrogenase, presence of schistocytes and low haptoglobin and C3 levels. A renal biopsy was performed showing thrombotic microangiopathy. After ruling out other causes, he was diagnosed with aHUS. Despite plasma exchange (PE) was administered every-other-day for 6 weeks (20 sessions), mild signs of hemolysis persisted (20 red-cell bags were

transfused) with no improvement on kidney function, hence the patient started chronic hemodyalisis. Genetic analysis revealed a mutation on complement FH gene (c.3514G>T; Glu1172Stop) with low concentration levels of protein 14.7 mg/dl (normal 12-56 mg/dl). Cocaine consumption was documented, considering it a potential trigger.

Six years later his younger brother (30 years old at that time) presented with severe kidney failure (creatinine 12 mg/dl), non-immune hemolytic anemia and thrombocytopenia. ADAMTS 13 activity was normal (85%) and test for cocaine was positive again. With the suspicion of aHUS and considering the poor outcome of PE on his brother, early treatment with eculizumab was started on day 4 (900 mg weekly during four weeks, then mantainance therapy with 1200 mg every two weeks). The hemolysis was reverted in a few days and kidney function progressively improved, currently mantaining a serum creatinine of 1.5 mg/dl after 11 months of treatment. Genetic analysis showed the same mutation on FH as his brother.

Conclusions: The opposite evolution of the brothers according to the treatment performed suggests that early treatment with eculizumab may prevent progression to end stage renal disease. These cases also suggest that cocaine could be acting as an enviromental trigger over a genetical succeptibility.

SA-PO1041

Efficacy of Rituximab in Adult Patients with Multirelapsing, Steroid-Dependent and Steroid-Resistant Nephrotic Syndrome: Report of 4 Cases and a Meta-Analysis Andreas Kronbichler,[1] Julia Kerschbaum,[1] Martin Busch,[2] Gert J. Mayer,[1] Michael Rudnicki.[1] *[1]Department of Internal Medicine IV - Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria; [2]Department of Internal Medicine III – Nephrology, Endocrinology and Rheumatology, Medical University Jena, Jena, Germany.*

Background: Rituximab (RTX) has shown efficacy in adult patients with nephrotic syndrome due to minimal-change disease (MCD) and focal segmental glomerulosclerosis (FSGS). Here we report 4 cases, and we analyzed the efficacy of RTX in multirelapsing (MR), steroid-dependent (SD) and steroid-resistant (SR) nephrotic syndrome from published reports treated with RTX, thereby identifying factors predicting a favorable therapeutic response.

Methods: Studies were identified through Pubmed. Studies on children were excluded. Four patients with MCD and FSGS treated with RTX in our centers were included. Clinical characteristics, laboratory data, treatment modalities, and data on outcome parameters were collected. Primary outcome parameters were complete/partial remission.

Results: We analyzed data from 30 patients from the literature, and we included 5 patients from our cohort (22 SD patients, 13 SR patients). In total, 26 patients received complete or partial remission, while 9 patients were non-responsive to RTX treatment. All seventeen patients with MCD were responsive to RTX therapy, whereas only half of the patients with FSGS received complete or partial remission ($p=0.001$). SR patients were non-responsive in 8 out of 13 cases, whereas SD patients received remission in 21 out of 22 patients ($p<0.001$). In univariate binary regression steroid-dependency as compared to steroid-resistance was associated with a 21-fold increased chance of remission. Furthermore, serum albumin at time of referral (per unit increase) and a low sum score of immunosuppressive agents before treatment with RTX were associated with a favorable outcome ($p=0.007$ and $p=.017$, respectively).

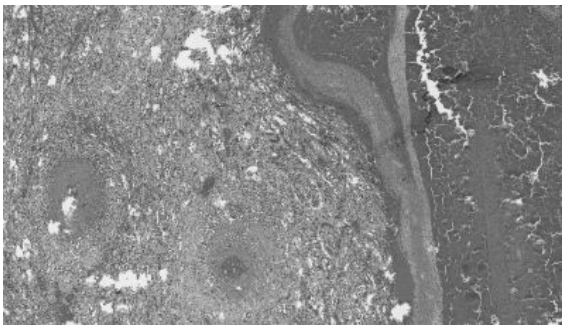
Conclusions: In multirelapsing, steroid-dependent and steroid-resistant nephrotic syndrome due to MCD or FSGS in adult patients we identified several factors associated with treatment response to RTX. These results need to be validated in a prospective study.

SA-PO1042

Acute Peri-Renal Hemorrhage as a Complication of Necrotizing Granulomatous Vasculitis Kiran B. Mandya Chikkalingaiah,[1] Hieu Q. Vo,[1] Arif Showkat,[1] Cuong Tan Nguyen,[1] Barry M. Wall.[1,2] *[1]UTHSC, Memphis, TN; [2]VAMC.*

Background: Multifocal necrotizing gramulomatous arteritis with no pulmonary involvement.

Methods: 66-yr old Asian female with hypertension and osteoarthritis on meloxicam 15 mg/d with serum creatinine (SCr) of 0.8 mg/dL was admitted for non-oliguric acute kidney injury. She had recently received Ciprofloxacin for presumed urinary tract infection. She denied fever or respiratory symptoms. Physical examination was unremarkable with normal vital signs and no skin lesions. Urinalysis: 25 RBC/hpf, 2-3 WBC/hpf, and granular casts, no eosinophiluria. Urine protein:creatinine ratio was 2.0, BUN 44 mg/dL, SCr 5.5 mg/dL, serum albumin 2.7 g/dL, WBC 17,200/m, hemoglobin 8.5 g/dL, platelet 442. Serologies for HIV, Hepatitis B and C, ANA, cANCA and pANCA were negative. C3,C4, CPK, LDH, CXR, and renal ultrasound were normal. Urine and blood cultures were negative. Ciprofloxacin was discontinued as a possible source for allergic interstitial nephritis, but SCr continued to rise, peaking at 7.3 mg/dL on day 4 and 6.8 mg/dl on day 6. Kidney biopsy was planned, but on day 6 she reported lower abdominal and back pain. She collapsed 6 hr later with hypotension and subsequently expired. Hemoglobin had dropped from 11 g/dL to 3.2 g/dL. Autopsy revealed multifocal necrotizing granulomatous vasculitis involving small arteries of kidneys (no glomerular crescents or proliferative changes (figure 1), esophagus, bladder, and ovaries. There were bilateral sub capsular renal hematomas and massive retroperitoneal hemorrhage with normal lung pathology.



Conclusions: This case illustrates several unusual features of granulomatous polyangiitis: ANCA negativity, non-pulmonary involvement and catastrophic spontaneous peri-renal hemorrhage. Vasculitides require tissue diagnosis such that a high index of suspicion should lead to prompt biopsy of involved organs, including the kidney.

SA-PO1043

Favorable Response to Rituximab in Diffuse Sclerosing Variant of Fibrillary Glomerulonephritis Asad J. Chaudhary,[1] Geeta G. Gyamlani,[1,2] Barry M. Wall.[1,2] *[1]University of Tennessee Health Science Center, Memphis, TN; [2]VAMC.*

Background: Fibrillary GN with sclerosing features has been associated with rapidly deteriorating renal function. We present 2 cases with favorable responses to Rituximab with 1 yr follow up.

Methods: Case 1: 62 yr old Caucasian male with type II diabetes mellitus developed rapidly worsening nephrotic range proteinuria despite lisinopril (80mg/day) and losartan (50 mg/day). HIV, hepatitis B and C serologies, serum and urine IFE, and ANA were negative. Renal biopsy showed diabetic nodular glomerulosclerosis. Congo red negative. Immunofluoresence (IF) positive for IgG,IgM, IgA, C3,C4, κ and λ light chains. Electron microscopy (EM) showed frequent intramembranous nonbranching, randomly arranged fibrils segmentally extending into subepithelial areas forming feather-like spikes. Biopsy results were consistent with Fibrillary Glomerulonephritis with extensive scarring and diabetic changes. He received 4 weekly doses (700 mg) of rituximab. Nephrotic range proteinuria has persisted, likely due to underlying diabetic nephropathy, however, SCr remains 1.4 mg/dl after 12 mos of follow-up.

Case 2. 55 year old Caucasian male presented with proteinuria and microscopic hematuria with SCr of 1.5 mg/dl and spot protein creatinine ratio (PCR) 8. C3 and C4, hepatitis B,C,and HIV serologies, and ANCA panel were normal or negative. Renal biopsy showed global and focal glomerulosclerosis. Congo red negative. IF positive for IgG, C3, C4. κ and λ light chains. EM revealed markedly thickened basement membranes. The mesangium was expanded with presence of randomly oriented fibrils which extend into the basement membranes. SCr had increased to 2.15 mg/dl with random PCR of 12. He received rituximab, 2 doses of 750mg, one week apart. Over the course of one year follow up his most recent spot PCR is 1 gram and SCr is 2.5 mg/dl.

Conclusions: Both patients with idiopathic sclerosing forms of fibrillary GN treated with rituximab demonstrated stable renal function with stable or declining proteinuria, suggesting that rituximab may be effective therapy for this variant of fibrillary GN, which typically progressees rapidly to end stage renal disease.

SA-PO1044

Combined Kidney and Auxiliary Liver Transplantation in a Patient with Primary Hyperoxaluria Type I: A Case Report Bojana Maksimovic,[1] Branislav Kocman,[2] Karlo M. Mihovilovic,[1] Mladen Knotek.[1] *[1]Renal Division, University Hospital Merkur, Zagreb, Croatia; [2]Surgery Division, University Hospital Merkur, Zagreb, Croatia.*

Background: Primary hyperoxalurias (PH) are rare inborn errors characterized by the overproduction of oxalate, which is deposited as calcium oxalate in various organs. PH type 1 is due to the defects in the gene that encodes the hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT). Kidney is the prime target of oxalate deposition. Combined liver and kidney transplantation is treatment of choice in patients with ESKD due to PH1. Kidney transplantation alone results in early graft loss, due to recurrent disease.

Methods: Case Description: Here we present the case of a 21 year old Caucasian male who was diagnosed with PH1, and who developed ESKD subsequent to recalcitrant renal stone formation. In February 2012 the patient received simultaneous deceased-donor auxiliary liver and kidney transplantation. The patient underwent left hepatectomy with cholecystectomy, which was followed by the orthotopic transplantation of the second and third liver segment and a kidney transplantation. Immediate function of both grafts was established with substantial drop in serum creatinine and liver enzymes level. However, in the early post transplantation period dialysis was continued five days for the enhanced oxalate removal. Renal biopsy performed one month post transplantation showed normal renal parenchyma and 5-10% of renal tubules containing oxalate crystals. This is similar to 1-month protocol biopsy findings in our two previous patients who received combined whole liver and kidney transplant for PH1. Three months following transplant, the patient maintains good liver and kidney function.

Conclusions: Discussion: Combined auxiliary liver and kidney transplantation is a good treatment option for patients with PH1 and ESKD. It is also a safer treatment option, because whole liver transplantation requires removal of native liver which is functional in all other aspects, but AGT enzyme defect. With this approach life-threatening risk of potential liver graft failure is avoided, with maintenance of sufficient AGT enzyme level to prevent recurrent kidney disease.

SA-PO1045

Acute Kidney Injury Post Transplant: Broadening the Differential Diagnosis Lindsay Kruska, Jason M. Kidd, Karin A. True. *UNC Kidney Center, Chapel Hill, NC.*

Background: Annually, approximately 18,000 patients undergo renal transplantation. Often the etiology of their native disease is unknown. This case illustrates the importance of keeping a broad differential diagnosis for graft dysfunction.

Methods: A 74 yo Caucasian man underwent living unrelated donor renal transplant 9 months after reaching ESRD. His underlying renal insufficiency was thought to be related to acute tubular necrosis related to a prolonged hospital stay several years before, but had never had a diagnostic biopsy. Immunosuppression consisted of alemtuzimab 30mg IV, methylprednisolone 500mg IV followed by mychophenolate mofetil, tacrolimus and steroid taper. Renal function improved rapidly in first 5 days after transplant, but in the setting of Pseudomonas bacteremia he developed acute kidney injury requiring dialysis. Urine sediment showed monomorphic RBC and granular casts. Biopsy was performed and showed pauci-immune necrotizing and crescentric glomerulonephritis. He had a positive ANCA with MPO titer of 115 u/mL. Pretransplant banked serum sample was checked for ANCA and showed MPO titer of 137.6 u/mL. An unexpected diagnosis of recurrent ANCA vasculitis was made and the patient was treated with methylprednisolone 500mg IV daily for 3 days followed by prednisone 60mg daily and plasmapheresis. He has recovered enough renal function to be dialysis independent.

Conclusions: ANCA associated vasculitis (AAV) is the most common cause of rapidly progressive glomerulonephritis, though still rare with an estimated incidence of 20 cases per million. Approximately 30% of those diagnosed with progress to ESRD. Transplantation is a good option with 5 year graft survival rates comparable to all transplant recipients. The risk of relapse has been estimated between 11 – 50%, though in more recent series estimates range 9 – 17% with some relapses extrarenal. Though mean time to relapse is 31 months, reports of relapse range 5 days to 13 years. This case illustrates the importance in establishing an etiology of ESRD prior to transplant, but if impossible, maintaining a broad differential diagnosis for graft dysfunction.

SA-PO1046

Biopsy Proven Polyomavirus JC-Associated Nephropathy, Eleven Years after a Kidney Transplantation Marie-christine Guilbert,[1] Mathieu Latour,[1] Marie-chantal Fortin,[2] Harsharan Singh,[3] Volker Nickeleit,[3] Marie-Josee Hebert,[2] Edith A. Renoult.[2] *[1]Pathology Department, Centre Hospitalier de l'Université de Montréal, Canada; [2]Internal Medecine, Nephrology Service, Centre Hospitalier de l'Université de Montréal, Canada; [3]Nephropathology Laboratory, University of North Carolina at Chapel Hill.*

Background: Polyomavirus-associated nephropathy (PVAN) is a severe complication in renal transplant recipients. It is attributed to BK virus (BKV) and, more rarely, to JC virus (JCV), typically associated with progressive multifocal leukoencephalopathy. We describe a case of PVAN caused by JCV, in a 59-year-old male presenting with progressive kidney failure, 11 years after kidney transplantation.

Methods: This patient underwent cadaveric renal transplantation in 2001. Over the last 4 years, he was regularly lost to follow-up and serum creatinine rose from 139 to 241µmol/l, prompting a transplant biopsy. The biopsy specimen showed interstitial inflammation, slight tubulitis with viral inclusions and atypical enlarged nuclei, in a background of interstitial fibrosis and tubular atrophy, as well as severe arteriolar hyalinosis, consistent with calcineurin inhibitor toxicity. Immunohistochemical staining for SV40 large T antigen was positive. Decoy cells were present in the urine and cast-like polyomavirus aggregates (Haufen) were also detected by negative electron microscopy. Quantitative PCR in blood was negative for BKV and positive for JCV (7.38×10^4 copies/ml). Finally, JCV specific large T antigen was detected in the atypical tubular nuclei seen in the biopsy specimen. In order to improve JCV-specific immunity, immunosuppressive treatment has been reduced. Neurological evaluation is ongoing.

Conclusions: In contrast to the accumulating data of PVAN due to BKV, there are limited data reported on PVAN due to JCV. These two infectious nephropathies share some clinical and pathological signs but they may have different pathophysiological mechanisms. It is important that clinicians are aware that JCV as well as BKV should be considered a possible cause of PVAN. With wider clinical recognition and experience with this feature, we will better understand its prognostic significance.

SA-PO1047

Oxalosis after Kidney Transplantation in Bariatric Surgery Patients: A Retrospective Analysis Bhavna Chopra, Hatem Amer, Emilio Rodrigo, John C. Lieske. *Division of Nephrology, Mayo Clinic, Rochester, MN.*

Background: Obesity is a growing epidemic. We increasingly encounter candidates for transplantation who have had bariatric surgery. Roux en Y gastric bypass (RYGB) has been associated with enteric hyperoxaluria and renal failure from oxalosis, raising concern for early graft loss after kidney transplant (KTx). The objective of this study was to review our KTx experience in patients after RYGB.

Methods: All KTx recipients at our center with greater than 1 year follow-up who had undergone previous RYGB for obesity were retrospectively reviewed. Results were expressed mean±SD [range].

Results: Nine patients, all female and primarily Caucasian (7/9) were identified. Majority (8/9) were on dialysis prior to KTx, for 2.8±2.6yr. Causes of renal failure were diabetes (4), nephrectomy (2), oxalate nephropathy (1), ADPKD (1), and unknown (1). Age at transplant was 61.5±7.0 yrs. Time from RYGB to KTx was 6.9±4.4 yrs and 7/9 received a living KTx. All grafts were functioning during a study follow-up of 3.2±1.8 yrs [1.4-6.6] yrs. BMI at RYGB was 51.7±7.7 kg/m^2 and 32.4±8 kg/m^2 at KTx. Length of small intestinal common channel was 141.6±17.6 cm. Kidney stones pre-KTx occured in 4/9 pts. The peak plasma oxalate (PlOx) pre-KTx was 29.1±27.4 [5.2-88.7] µmol/L (normal < 3; with risk of oxalosis at > 25-30). Therapy for enteric hyperoxaluria (low oxalate diet + calcium binders) lowered immediate pre-KTx PlOx to 18.4±9.5 [6.6-28.0] µmol/L. PlOx at 3 wk post KTx was 3.7±1.7, and then 9.1±6.7µmol/L at last follow up. The 24 hr urine oxalate at last follow-up post KTx was elevated at 0.64±0.24 mmol/24hr (nl < 0.45). eGFR (MDRD) at 1, 3, 6, 12 months was 46.2, 48.3, 51.5, 46.5 ml/min/1.73 m^2, respectively. Two patients had oxalate crystals in biopsy at 8 months and 2 yrs post-KTx respectively.

Conclusions: This experience suggests that short term KTx graft survival is reasonable after RYGB. Patients in our group were treated to reduce PlOx to an accepted limit for systemic CaOx supersaturation (highest PlOx 28µmol/L). Long term graft outcomes need to be determined. Urine and plasma oxalate should be measured and promptly treated as indicated. A standardized approach to this high risk group is advisable.

SA-PO1048

Two Cases of Passenger Lymphocyte Syndrome Following Renal Transplantation Luxme Nadarajah,[1] Neil Ashman,[1] Raj Thuraisingham,[1] Colin Barber,[2] Shubha Allard,[2] Laura Green.[2] *[1]Nephrology Department, Barts Health NHS Trust, London, United Kingdom; [2]NHS Blood and Transplant, Barts Health NHS Trust, London, United Kingdom.*

Background: Passenger lymphocyte syndrome (PLS) is an immune-mediated red blood cell (RBC) haemolysis which occurs following ABO-mismatch solid organ and/or bone marrow transplantation. Passive transfer of viable donor B-lymphocytes occurs during transplantation and when these are stimulated, they cause the production of antibodies which are capable of reacting towards recipient RBC antigens, leading thus to haemolysis.

Methods: We report 2 cases of PLS occurring after renal transplantation. Both recipients were young males who had biopsy proven end-stage renal failure secondary to hypertension. They received live-related kidney transplants; one from his mother (case-1) and the other from his brother (case-2). The direction of blood group transfer, from donor to recipient, was O Rh + to A Rh + in both cases. Approximately 12 days after transplantation both recipients showed a rapid fall in their haemoglobin levels with no identifiable bleeding source. Their haemolytic screens were consistent with DAT positive haemolysis and anti-A antibody was detected in their sera, thus confirming the PLS-induced haemolysis. Treatment with blood transfusion and alteration of immunosuppressive therapy led to the recovery of haemoglobin and subsequent renal graft function.

Conclusions: In the literature we have identified 82 PLS cases related to renal transplants. Previous ABO sensitisation, group O donor to group A recipient transplant, cyclosporine treatment and patient secretor status, have all been identified as risk factors. Clinical outcomes in general are good; nonetheless, cases of graft failure and deaths have been reported. Therefore, it is imperative that clinicians are made aware of this condition so as to enable early diagnosis and appropriate treatment. It should also be highlighted that good communication between haematologists and nephrologists is key to managing PLS-induced haemolysis following ABO-mismatched renal transplants.

SA-PO1049

Aeromonas Caviae Infection in a Renal Transplant Recipient: More than Just an Innocent Bystander Amy J. Zwettler, Kevin C. Abbott, Robert Nee. *Nephrology Department, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Aeromonas species are gram-negative anaerobic rods that are ubiquitous in water and soil. Aeromonas has been considered a colonizer in asymptomatic hosts or a contaminant in cultures; however, more recent data suggests that certain strains are emerging enteropathogens. Transplant patients may be at increased risk of infection given their immunosuppressed state.

Methods: A 54-year old male with history of end-stage renal disease secondary to hypertension, underwent a living unrelated donor transplant. Transplantation was from a 35-year old male donor, with 5 antigen mismatches, and Thymoglobulin was used for induction. The donor was cytomegalovirus (CMV)-negative and the recipient positive. His postoperative course was unremarkable until day 14 when he developed abdominal pain and diarrhea. Stool studies were negative for CMV and Clostridium difficile PCRs. However, stool culture grew Aeromonas caviae for which the patient received a 5-day course of levofloxacin, followed by resolution of his symptoms.

Conclusions: Aeromonas is becoming an increasingly recognized cause of diarrhea. While patients with immunosuppression may be at increased risk, this is to our knowledge the first reported case in a renal transplant patient. Interestingly, this patient was a prior undersea medical officer, and therefore may have had occupational exposure and colonization to Aeromonas that was then allowed to cause diarrheal illness in the setting of immunosuppresion. A growing body of evidence supports the pathogenicity of Aeromonas, and our case suggests that Aeromonas infection should be considered in kidney transplant patients with diarrhea, as treatment may lead to relief of symptoms and prevention of

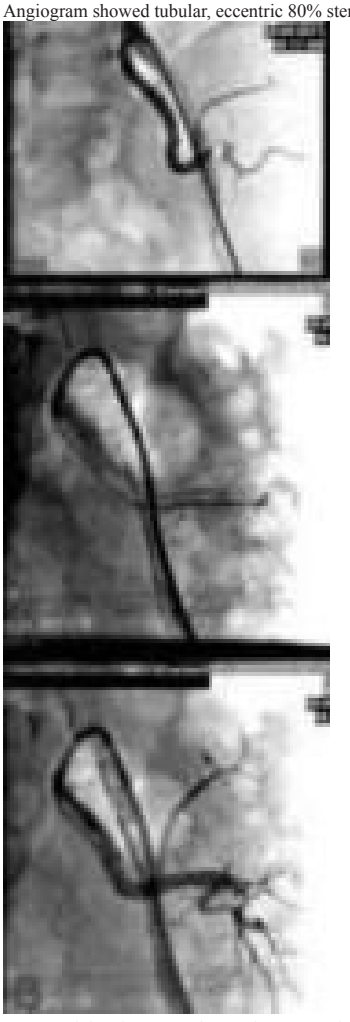
poor outcomes. "The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, the department of the Navy, the Department of Defense, or the United States government."

SA-PO1050

Distal Stenosis of the Transplanted Renal Artery Masquerading as Acute Rejection: Improvement in Renal Function after Percutaneous Transluminal Angioplasty Brian Michael I. Cabral, Ma Czarlota M. Acelajado-valdenor. *Center for Renal Diseases, St. Luke's Medical Center, Global City, Philippines.*

Background: A case of renal failure in a kidney transplant recipient. History was suspicious for acute rejection. Workup showed Doppler findings of distal TRAS. Renal function improved after angioplasty.

Methods: A 33 y/o male cadaveric kidney recipient sought 2nd opinion for azotemia. 2mos post-transplant, crea increased (2.8 from baseline 2mg/dL). Biopsy showed CNI toxicity. Immunosuppressants were adjusted with temporary improvement. However, azotemia recurred, accompanied by proteinuria, HPN & anemia. Upon presentation, crea was 6.8mg/dL, BP was high despite 5 anti-hypertensive drugs. Biopsy showed borderline changes suspicious for acute rejection: mild tubulitis,focal c4d, interstitial fibrosis & tubular atrophy. Duplex scan: increased peak systolic velocity in the distal segment of the allograft artery with RIR>3.5, suggestive of stenosis. Methylprednisolone 1500 mg was given. Angiogram showed tubular, eccentric 80% stenosis of the distal segment.



A peripheral stent was inserted. Post-stenting angiogram showed a patent, well apposed distal stent. Renal function & BP improved post-angioplasty. After a month, crea stabilized at 2mg/dL, BP controlled w/ 2 drugs.

Conclusions: We present a case of transplant renal artery stenosis arising early post-transplant in a cadaveric allograft recipient, accompanied by HPN, graft failure, biopsy findings consistent with borderline rejection, &stenosis occurring at an unusual location distal to anastomotic site. Here we see the value of conducting a thorough workup in patients with allograft dysfunction: while a rejection episode might have adequately explained the azotemia, the biopsy showed borderline rejection not consistent with the increase in crea nor the presence of refractory HPN prompting workup for vascular complications.

Funding: Clinical Revenue Support

SA-PO1051

Mycoplasma Hominis Infection in Three Pediatric En-Bloc Kidney Transplant Patients: Is the Recipient the Source? Janet Y. Young, Deborah B. Adey. *Internal Medicine, Univ. of Calif, Davis, Sacramento, CA.*

Background: M. hominis is an organism that colonizes GU tract, posterior pharynx, and lower GI tract. It is of low pathogenicity but can cause severe infections in immunosuppressed patients. We report three cases of M.hominis infection in pedi-en-bloc kidney transplant recipients.

Methods: Case 1: 41yo female with ESRD from IgA nephropathy received pedi-en-bloc kidney transplant from 4mo male. On POD 7, she developed a retroperitoneal hematoma. Subsequent fever and leukocytosis triggered empiric antibiotics and surgical evacuation on POD 14. Peritoneal fluid cultures were initially negative and later grew M.hominis. She was switched to doxycycline/azithromycin and later to ciprofloxacin. She received 14 weeks of antibiotics.

Case 2: 28yo male with ESRD from reflux nephropathy received pedi-en-bloc kidney transplant from 2mo female. Readmitted POD 11 for fever and leukocytosis. Abdominal CT showed 4cm perinephric fluid collection with air. The incision appeared erythematous and the wound was surgically explored. Initial cultures were negative but grew M.hominis at 10 days. He received 6 weeks of doxycycline/ciprofloxacin.

Case 3: 28yo female with ESRD from unknown etiology received pedi-en-bloc kidney transplant from 1mo male. On POD 8, she had fever and leukocytosis. CT abdomen showed 4.4cm peritoneal abscess and 2.7cm perinephric hematoma. Abscess was drained, cultures grew M.hominis at 10 days. She received 6 weeks of ciprofloxacin/doxycycline.

Conclusions: M.hominis is undetectable by gram stain and grows slowly. Risk factors for infection are immunosuppression, tissue trauma, GU manipulation. Optimal treatment regimen and duration is unclear. Antibiotics effective against M.hominis include tetracyclines, clindamycin, fluoroquinolones, though resistance is emerging.

In kidney transplantation, it has led to perinephric abscesses/hematomas, perihepatitis, peritonitis, graft loss, death. For the 3 patients above, source of infection is likely the recipient given donor age. M.hominis must be considered when transplant patients present early with signs of infection. Early suspicion for the organism will hasten diagnosis and treatment, given difficulty in organism isolation.

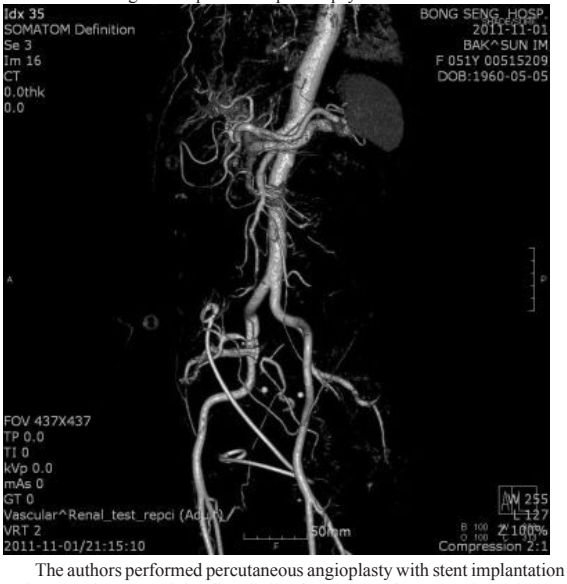
Funding: Clinical Revenue Support

SA-PO1052

A Case of Acute Renal Failure in a Renal Allograft Recipient Caused by a Post-Biopsy Renal Arteriovenous Fistula Superimposed on the Onset of Renal Artery Stenosis Seong Min Kim, Joon Seok Oh, Joong Kyung Kim. *Internal Medicine, Bong Seng Memorial Hospital, Busan, Korea.*

Background: Renal biopsy of the transplanted kidney is an essential diagnostic tool of acute and chronic rejection as well as recurrent and de novo nephropathies in renal allograft recipients. Arteriovenous fistula(AVF) is an uncommon but well-known complication of percutaneous renal biopsy. Most postbiopsy AVFs are asymptomatic and regress spontaneously ; however, some AVFs result in hypertension, hematuria and renal insufficiency. Whether postbiopsy AVF superimposed on transplant renal artery stenosis(TRAS) also regress spontaneously is unknown.

Methods: The authors present a case of acute renal insufficiency in a 51-year-old female renal allograft recipient with postbiopsy AVF and TRAS.



The authors performed percutaneous angioplasty with stent implantation for the TRAS and transcatheter arterial coil embolization therapy for AVF. The patient's renal function returned to baseline level and is being followed up for 6 months.

Conclusions: What is noteworthy from our case is that before renal biopsy, our patient had arterial hypertension and showed mild elevated serum creatinine. This frasgile equilibrium was broken by postbiopsy arteriovenous fistula superimposed with arterial

stenosis. This patient showd abnormal lab finding on post-trasnplantation 3 months. This combination caused acute renal hypoperfusion, and indeed oliguria and pre-renal azotemia. After embolization of the AVF and stent implantation on TRAS, renal function parameters returned to basal levels. Of course, the findings of allograft biopsy was normal.

Funding: Private Foundation Support

SA-PO1053

Two Cases of Successful Renal Transplantation in MYH9-Related Disorder

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Background: MYH9-related disorders (MYH9-RD) is a group of autosomal dominantly inherited disorders caused by mutations of the MYH9 gene, which encodes the non-muscle myosin heavy chain IIA. Renal transplantation (RT) has been rarely reported in patients with MYH9-RD.

Methods: A 17-year-old-girl was admitted for living-related RT. She was diagnosed at 2 years old as having idiopathic thrombocytopenic purpura (ITP) which proved refractory to medications. At age 9, she developed hematuria and proteinuria and two years later, she was diagnosed of mesangial proliferative glomerulonephritis. Subsequently, her renal function progressed to end-stage renal disease (ESRD) at age of 17 years. On admission for RT, her platelet count was 44,000/mm^3. To prevent and control bleeding, she received IVIG and platelets perioperatively. The operation was performed without excessive bleeding and the platelet count was 126,000/mm3. However, large hematoma developed and after removing hematoma, her graft function was improved. She performed genetic study and a mutation was detected in MYH9 exon 16 (p.Arg702Cys), compatible with Fechtner syndrome. This mutation was a de novo mutation. An audiogram revealed bilateral high-frequency hearing deficit and she needs a hearing aid. The peripheral blood smear showed giant platelets, but no apparent inclusion bodies. On ophthalmic examination, cataracts were found in both eyes. An one year follow-up of the patient showed a favorable clinical course.

Second case is of 23 year-old-man diagnosed as ITP. Proteinuria was found on his school screening test and he was diagnosed with mesangial proliferative GN. He underwent genetic study which showed a mutation in MYH9 gene motor domain exon 1 suggesting MYH9-RD. This mutation was also a de novo mutation. He also performed renal transplantation successfully and his graft function is well maintained.

Conclusions: Our case suggests that renal transplantation is also the treatment of choice for ESRD in patients with MYH9-RD and the macrothrombocytopenia is not a contraindiation to aggressive management of ESRD.

SA-PO1054

Retroperitoneal Fibrosis in Context of Diffuse Large B Cell Lymphoma Presenting as Pseudoobstructive Uropathy

Buthayna Dinary, Khaldoon Shaheen, Ismail Hader, Keyvan Ravakhah. *Internal Medicine, St. Vincent Medical Center, Cleveland, OH.*

Background: Retroperitoneal fibrosis (RPF) encompasses a range of diseases characterized by the presence of a fibro-inflammatory tissue, which usually surrounds the abdominal aorta and the iliac arteries and extends into the retroperitoneal to envelop neighboring structures—eg, ureters. RF is generally idiopathic, but can also be secondary to certain drugs, malignancy, infections, and surgery. Here we describe a case of sudden onset of acute renal failure (ARF), hydronephrosis, in association with diffuse large B cell lymphoma and RPF.

Methods: A 71 year-old previously healthy man was admitted with two days history of fever, dull abdominal pain, decreased urine output, left leg and scrotal swellings. Examination was significant for scrotal and left leg pitting edema. Laboratory studies showed potassium (6.4mmol/l), creatinine (3.1 mg/dl), BUN (70 mg/dl), Hg (10.9 gm/dl), LDH 1240 u/l and proteinuria. US showed bilateral hydronephrosis. CT abdomen revealed retroperitoneal LAP and a solid retroperitoneal mass consistent with RPF. Duplex US of renal veins revealed left RVT. Retrograde pyelogram showed patency of the calyceal system. Lymph node biopsy confirmed B Cell Lymphoma. He was hydrated, anticoagulated and treated with IV dexamethasone. Patient creatinine improved to 2.5 mg/dl. He was discharged on prednisone, Lovenox, and Rituximab with plan to start CHOP.

Conclusions: Retroperitoneal Fibrosis should be suspected in any patient presenting with unexplained ARF and enlarged kidneys, especially in the setting of widespread lymphoma. It remains a mystery whether B cell lymphoma triggered the symptoms of a latent RPF or whether the two diseases merely coincided. Prompt diagnosis of RF improves chances of preserving renal function. Steroids are typically used to treat RPF, although other options—eg, tamoxifen-available. The outlook is usually good, but, if not appropriately diagnosed or treated, the disease can cause severe complications, such as ESRD.

Funding: Private Foundation Support

SA-PO1055

Metastatic Breast Cancer Causing Type B Lactic Acidosis

Hala Yamout, Cybele Ghossein. *Nephrology, Northwestern University Feinberg School of Medicine, Chicago, IL.*

Background: To report an unusual case of type B lactic acidosis caused by metastic breast cancer.

Methods: A 24 year old woman with breast cancer complicated by metastasis to the bone, liver, and lung, presented with new right facial nerve palsy. Work up showed new brain metastasis and treatment included dexamethasone and radiation. Labs showed an anion gap metabolic acidosis with elevated lactate levels despite hemodynamic stability.

Date	pH	CO2 (mmHg)	HCO3 (mEq/L)	Lactate (mMol/L)	Metabolic Acid
1/12: 10:49	7.02	44	11	>20	18
1/12: 18:45	7.11	22	7	>20	33
1/13: 1:00	7.38	33	20	>20	34
1/14: 5:00am	7.34	30	9	>20	
1/17: 4:00	7.20	27	11	>20	42

Metabolic acidosis was treated with a bicarbonate drip. Lactate levels remained elevated. The patient's family eventually withdrew care.

Conclusions: There are different types of lactic acidosis: type A caused by tissue hypoperfusion; type B caused by to malignancy, medications, or hereditary disorders; or type D found in short bowel syndrome or a history of jejuno-ileal bypass surgery. Type B lactic acidosis is mainly described with hematological malignancies. The mechanism of disease is partially secondary to liver and kidney dysfunction with impaired clearance of lactate. Tumor overproduction of lactate likely contributes through overexpression of glycolytic enzymes, mitochondrial dysfunction, and use of anaerobic metabolism.

Treatment of lactic acidosis depends on treating the underlying cause, with no randomized controlled trials comparing the treatments of type B lactic acidosis. Chemotherapy is effective in a small group by reducing lactate production. Bicarbonate use is controversial as it may increase mortality and cause intracellular acidosis by CO2 generation. The role of thiamine remains unclear as it may either shunt away production of lactate by pyruvate yet may be a cofactor in tumor DNA production. Dialysis has been used to medically stabilize the patients and possibly clear excess lactate.

We present an unusual case of metabolic acidosis caused by type B lactic acidosis from a solid malignancy. Type B lactic acidosis should be considered in the differential diagnosis of patients with both solid and hematological malignancies who present with an anion gap acidosis.

SA-PO1056

Severe and Prolonged Hypocalcemia Following a Single Subcutaneous Dose of Denosumab in a Patient with Metastatic Prostate Cancer

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Background: This case describes the onset of life-threatening hypocalcemia following a standard dose of denosumab (AMG-162), a full human monoclonal antibody with high affinity for the receptor activator of NF-kappaB (RANK) ligand. While prior studies involving denosumab have made mention of hypocalcemia as a mostly transient and asymptomatic adverse effect, this is the first reported case of clinically significant hypocalcemia, requiring a prolonged course of an intravenous calcium drip.

Methods: A 73 year old male with a history of prostate cancer with diffuse osseous metastatic disease, presented to the emergency department for evaluation of weight loss and low back pain. His prostate cancer had been diagnosed several days prior, after a biopsy was performed. He was admitted to the hospital and received sporadic treatment with androgen deprivation therapy. Admission serum calcium was 9.0 mg/dL. On hospital day five, he received one dose of 120mg of denosumab subcutaneously. In the days that followed, the serum calcium decreased to a range of 6.5 to 7.1 mg/dL and within one week, the level reached a nadir of 4.6 mg/dL. This prompted the initiation of an intravenous calcium drip. The patient required this calcium infusion of 148grams of elemental calcium over a total of eighty consecutive days, as well as aggressive oral supplementation with 375grams of elemental calcium, in order to sustain acceptable serum calcium levels.

Conclusions: Denosumab has proven to be effective at preventing bone loss due to several medical conditions, including postmenopausal osteoporosis and metastatic disease, with a relatively innocuous safety profile. The causation of hypocalcemia has previously been looked at as an occasionally expected but temporary and slight occurrence, manageable with oral supplementation. This case has brought to light the potential for severe hypocalcemia; one that is more difficult to manage and is of longer duration than first thought. As the use of denosumab becomes more widespread, clinicians in all fields need to be aware of potential severe, refractory hypocalcemia.

SA-PO1057

56 Year Old African American Female with Stage IV Metastatic Colon Cancer Was Given Oxaliplatin Causing Immune-Related Intravascular Hemolysis and Acute Tubular Necrosis

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Background: Oxaliplatin (OXP) is a third-generation diaminocyclohexane platinum analogue. OXP compared to cisplatinis characterized by low-plasma accumulation and renal eliminationvia simple glomerular filtration without tubular metabolism.OXP is used for extended periods in advanced colorectal cancer causing unexpected nephrotoxicities; acute interstitial nephritis, thrombotic microangiopathy, and immune-related intravascular hemolysis causing ATN.Reported cases of OXP-induced ATN are rare.

Methods: A 56 y/o African American female with a history of type II diabetes, hypertension, Stage II Colon Cancer s/p resection on 11/04. The patient relapsed to Stage IV with mets to the lung on 07/05. The patient received chemotherapy with OXP/5-fluorouracil/leucovorin with bevacizumab. Oxaliplatin was restarted due to progression of cancer. During the second cycle, patient developed chest tightness/rash/itchiness/fever and chills immediately after OXP infusion. The patient's symptoms improved after the infusion was stopped. Two hours later patient's urine turned dark brown. Patient's physical exam was unremarkable. Urine analysis was strongly dipstick positive for blood with no RBCs/HPF. Hemoglobin decreased from a baseline of 10.3 to 8.2 g/dl. The serum increased to Cr 2.6 mg/dl from baseline of 0.7 mg/dl. The BUN was 42 mg/dl, LDH 396 U/dl, and haptoglobin 62 L. The patient received IV fluids and OXP was held. The serum Cr returned to baseline.

Conclusions: ARF occurs rarely during OXP treatment. OXP most likely causes the formation of a drug-antibody immune complex that binds via Fab to a 110 kDa protein on the RBC membrane leading to acute hemolysis. The latent period of several cycles before the development of overt hemolytic anemia is probably secondary to a gradual buildup of the immune response to OXP. After OXPtreatment, hematological abnormalities may occurduring ARFand mimic TMA. Nephrologists should be aware of thispossibility,since in addition to the supportive therapy ofARF, the treatmentof acute episodes of TMA requires plasma exchanges.

SA-PO1058

Mycophenolate Mofetil as a Steroid Sparing Agent in Renal Sarcoidosis
Anita A. Zaidi, Jordan L. Rosenstock, Maria V. DeVita. *Department of Nephrology, Lenox Hill Hospital, New York, NY.*

Background: Steroids are the mainstay of treatment for renal sarcoidosis. Many patients with sarcoidosis are chronically dependent on steroids and there is limited data on the use of steroid sparing agents. This is a case of a patient that has remained in remission using mycophenolate mofetil (MMF).

Methods: This is a 56 year old female with a history of sarcoidosis who developed acute renal failure when weaned from steroids. Her baseline creatinine ranged from 1.5-2.0 mg/dl on chronic prednisone and plaquenil. The patient had 3 relapses in 2 years while being weaned from steroids. Her most recent relapse occurred when the prednisone dose was lowered to 7.5 mg/day. On exam, she was an African American woman in no distress. Blood pressure was 110/80 with pitting edema in the lower extremities. Labs revealed a serum creatinine of 3.5 mg/dl (prior 2.0), ACE level was 147 (prior 38) and calcium 10.6. It was decided not to perform a kidney biopsy due to her being a Jehovah's Witness, but it was felt she had interstitial nephritis related to sarcoidosis. The patient was started on prednisone 40mg with improvement in creatinine to 2. Due to possible lifelong need for prednisone, MMF was started as a steroid sparing treatment. MMF was started and titrated to 1gm BID which she tolerated and the prednisone was decreased to 10mg daily. The serum creatinine improved to 1.6. Prednisone was decreased to 5mg and than tapered off after 3 months. The patient's most recent creatinine was 1.7 after being off prednisone for 14 months and on MMF for 22 months.

Conclusions: There have been only a few case reports about the use of MMF as a steroid sparing agent in renal sarcoidosis in which patients could be successfully weaned off steroids. The cases included a 56 year old patient who remained in remission on MMF after 18 months. Another case was of a 15 year old male that remained in remission for one year and a third case mentions a 57 year old female that has remained in remission after 28 months. Although renal failure from sarcoidosis is treatable by steroids, MMF should be studied further as a potential steroid sparing agent in the treatment of renal sarcoidosis.

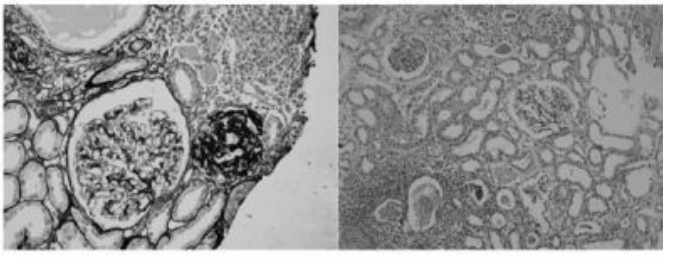
SA-PO1059

14-Year Follow-Up of a Chinese Boy with Lowe' Syndrome
Li Lin, Hong Ren, Jingyuan Xie, Nan Chen. *Nephrology Department, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China.*

Background: Oculocerebrorenal (Lowe) syndrome is a rare X-linked recessive disorder characterized by congenital cataracts, mental retardation, and renal tubular dysfunction.

Methods: An eight-month-boy first admitted to our hospital 14 years ago because of proteinuria. We followed up him for 14 years, all laboratory tests were recorded and renal biopsy was performed.

Results: The patient had congenital cataract and mental retardation. He was admitted to our hospital for mild to moderate proteinuria at 14-month-old. The patient was given glucocorticoid treatment (prednisone 2.5mg/kg/d at initiation then decreased) for 2 years but proteinuria persisted. At the age of 14, he came to our hospital for hypermicrosoma and obvious proteinuria (2-3 g/d). Physical examination showed facial edema, mild X-type legs and short-waisted(Height:120cm,Weight:30kg). Blood test showed creatinine was 59umol/L, phosphorus was 1.14mmol/L and HCO_3 was 22mmol/L. The increase of urine PH to 7.2 as well as over secretion of all kinds of amino acid and calcium in urine indicated proximal tubular injury(5.14mmol/24h).Urinary glucose and UA were normal (1.08 mmol / 24 h and 2.43mmol/l).Renal biopsy was conducted consequently, which showed interstitial infiltration of inflammatory cells and deposit of both IgM and C3 in mesangial region. Electron microscope was normal.The pathogenesis of proteinuria in Lowe syndrome remains unknown and further study needs to be done.The patient was given cyclosporine (125mg/d) and prednisone(15mg/d) therapy but the proteinuria persisted after 4 months.



Conclusions: Lowe syndrome not only presents defects in eyes, brain and renal tubule but also in glomeruli. This case indicated lack of response to immunosuppressive therapy in patients with Lowe syndrome.

SA-PO1060

Variation in Presentation and Presence of DNA Adducts and p53 Mutations in a Patients with Endemic Nephropathy: An Environmental Form of the Aristolochic Acid Nephropathy
Sandra Karanovic,[1] Ivana Vukovic-Lela,[1] Bojan Jelakovic,[1] Kathleen G. Dickman,[2] Anamarija Kovac Peic,[3] Damir Dittrich,[3] Andrea Fernandes.[2] *[1]Department of Nephrology, Arterial Hypertension and Dialysis, UHC Zagreb, School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Departments of Pharmacological Sciences & Medicine/Nephrology, State University of New York at Stony Brook; [3]General Hospital "Dr. Josip Bencevic", Slavonski Brod, Croatia.*

Background: Endemic nephropathy (EN) and associated upper urothelial cancers (UUC) are a form of aristolochic acid nephropathy (AAN), in which ingestion of bread contaminated with Aristolochia leads to chronic dietary intoxication.

Methods: This abstract presents the cases of 3 members of the same family who showed different EN clinical courses and pathological spectra. Although they shared the same household and familiar environment for 18 years and were therefore similarly exposed to aristolochic acid (AA), they exhibited different clinical courses. EN can present as renal insufficiency or UUC alone, or in combination, regardless of which develops first. Moreover, UUC commonly affects both the right and the left sides, and our study clearly showed this. The patient in Case 1 had UUC with typical EN histopathological signs, whereas that in Case 2 had right UUC without EN followed by left UUC with typical EN histopathological signs. In contrast, the patient in Case 3 initially showed renal insufficiency, complicated afterwards by right UUC, and later on by left UUC with histopathological end-stage chronic changes but without typical EN changes. AA-DNA adducts and specific p53 mutational spectra (A:T→ T:A transversion) were found in tissues of cases 1 and 2. For Case 3, the appropriate tissue sample to detect adducts couldn't have been obtained. This patient possibly had specific p53 mutations in exons that were not examined.

Conclusions: The diverse clinical courses reported herein could be attributed to differences in metabolic activation or detoxification of AA and/or DNA repair resulting from different genetic polymorphisms.

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SA-PO1061

Tubulointerstitial Nephritis in Behcet's Disease Sumi Sukumaran Nair, Shuchi Anand, Neiha Arora. *Stanford University.*

Background: Behcet's Disease[BD] is a rare disease with multisystem involvement, with a prevalence of about 1 in 300,000 persons per year in the US. Renal involvement is known to occur with BD, with AA amyloidosis being the most common manifestation. We report a case of tubulo-interstitial nephritis in a patient with BD.

Methods: A 26-year-old Brazilian man with a history of treated *Helicobacter pylori* gastritis and recurrent oral ulcers presented with severe abdominal pain. He also reported fatigue, weight loss and night sweats that began 1-month prior. His initial labs were significant for a SCr of 2.7mg/dl and ESR 136mm/hr. Abdominal imaging showed thrombus in the inferior vena cava, with extension into the bilateral renal veins. Urine analysis was bland without any proteinuria. An extensive workup for coagulopathy (including ANA, antiphospholipid antibody, factor V leiden, Prothrombin A mutation, JAK 2 mutation, ß2 glycoprotein), malignancy (SPEP/UPEP, colonoscopy, testicular ultrasound, PET scan, biopsy of the IVC lesion and lymph node), infection and autoimmune disease was unrevealing. His renal function rapidly improved with hydration (SCr decreased from 2.7 to 1.2 mg/dL), supporting a prerenal etiology. Given the history of recurrent oral ulcers, two previous episodes of painless red-eye, and severe thrombosis with a negative workup, he was given a presumptive diagnosis of BD.

He was started on azathioprine and prednisone therapy. Roughly 7 months after the initial episode, he noted recurrent oral ulcers while tapering the prednisone; at this time, he was found to have an increase in SCr (from a baseline of 1.2 mg/dL up to 1.6 mg/dL), with significant proteinuria (spot urine protein/creatinine 3.2 g/g). Renal biopsy showed tubulo-interstitial nephritis (TIN). He was continued on prednisone and azathioprine and lisinopril was added, with subsequent improvement in his SCr (1.2mg/dl) and proteinuria (1.2g/g).

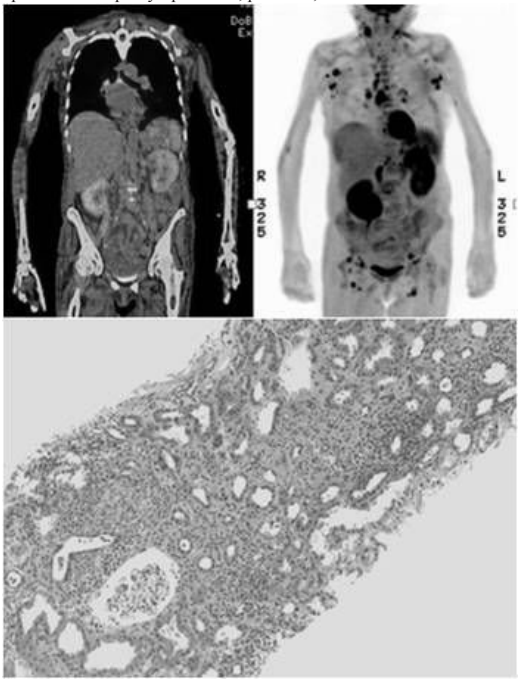
Conclusions: Although renal manifestations of BD including AA amyloidosis, glomerulonephritis, and renal vascular disease have been well described, BD- associated TIN has not been widely reported- a review of the literature showed only 6 reported cases to date. This case adds to our understanding of the spectrum of renal pathology in patients with BD.

SA-PO1062

DRESS due to Vancomycin Mandana Rastegar, Mark A. Perazella. *Section of Nephrology, Yale School of Medicine, New Haven, CT.*

Background: Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) syndrome is a severe, multi-system drug reaction characterized by three of the following: fever, skin rash, peripheral eosinophilia, atypical circulating lymphocytes, lymphadenopathy, and hepatitis. These features are common to many systemic disorders, often delaying prompt diagnosis.

Methods: A 54-year old female with history of chronic pancreatitis complicated by pancreatic pseudocyst and duodenal artery pseudoaneurysm required coil embolization 3 months prior to presentation. Two months later, she developed fever, vomiting and abdominal pain, found to have *Staphylococcus* bacteremia and candidemia. IV vancomycin and fluconazole were administered for 14 days. Two weeks later, she presented with similar complaints. Vancomycin, fluconazole, ciprofloxacin and flagyl were administered, but discontinued as cultures remained negative. Two days later, patient developed fever, hypotension, cervical lymphadenopathy, hepatomegaly, and worsening morbilliform rash. Serum creatinine was 2.6mg/dL (baseline 0.6 mg/dL) and WBC was 37.4K/mm^3 with 31% eosinophils. CT Scan showed diffuse lymphadenopathy. PET scan demonstrated extensive uptake in multiple lymph nodes, pancreas, and renal cortices.



Kidney biopsy revealed granulomatous AIN with numerous eosinophils. This patient had classic features of DRESS syndrome secondary to vancomycin. She received IV steroids followed by oral prednisone taper with complete resolution of the systemic illness and AKI.

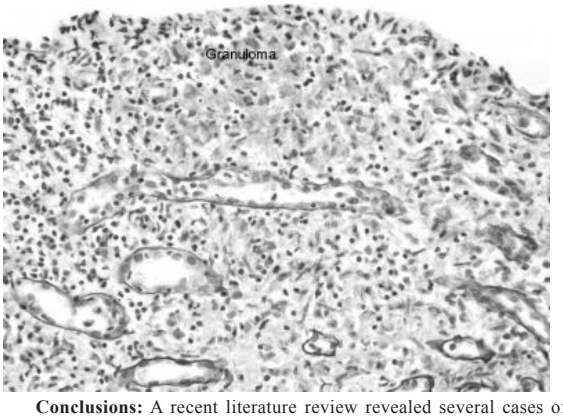
Conclusions: Early recognition and prompt removal of causative agents are critical in managing DRESS syndrome. PET-CT is used to detect tumors and inflammatory processes; it has potential as a non-invasive diagnostic tool for AIN. The etiology of AKI often rests between ATN or AIN. When biopsy is risky, PET scan offers a diagnostic alternative to distinguish the cause of AKI. This test may provide same day results for timely treatment.

SA-PO1063

Etanercept Induced Acute Granulomatous Interstitial Nephritis in a Rheumatoid Arthritis Patient Case Report Amr Yafi,[1] Laurie Matt,[1] Nairmeen Haller,[1] Natthavat Tanphaichitr.[2] *[1]Internal Medicine, Akron General Medical Center, Akron, OH; [2]Nephrology, Akron Nephrology Associates, INC, Akron, OH.*

Background: Acute interstitial nephritis (AIN) is a common cause of acute kidney injury in adults. The majority of cases are associated with drug-induced AIN. Tumor necrosis factor (TNF)-α inhibitor is a frequently used medication in advanced autoimmune diseases. Granuloma formation has been associated with AIN, however it is a rare occurrence.

Methods: This is a 55 year old female patient with a several-year history of rheumatoid arthritis (RA). She presented to the hospital with deterioration in renal function and hypertension. Approximately seven years prior to her presentation, etanercept therapy was started and her symptoms have been under control since. The initial laboratory tests were consistent with acute kidney injury, with no active urine sediments. A kidney biopsy revealed acute granulomatous interstitial nephritis. Her acute renal injury improved following withdrawal of etanercept and initiation of steroid therapy.



Conclusions: A recent literature review revealed several cases of sarcoid-like reaction secondary to initiation of TNF-α inhibitors, but none of these cases showed granulomatous interstitial nephritis formation. To our knowledge, this is the first case of etanercept-induced acute granulomatous interstitial nephritis in an adult RA patient. This case underscores the importance of having a high index of suspicion for AIN with the use of TNF-α inhibitor therapy.

SA-PO1064

Hemodialysis Improved CO2 Narcosis in Mixed Respiratory Acidosis and Metabolic Alkalosis Shamik Bhadra, Ziauddin Ahmed. *Nephrology, Drexel University College of Medicine, Philadelphia, PA.*

Background: Acute on chronic respiratory acidosis with metabolic alkalosis commonly cause CO2 narcosis in patients with a combination of COPD, CHF, and OSA .Since ventilator dependency can be a huge problem in such patients, any innovative treatment of Metabolic Alkalosis can be extremely helpful. We present a case where we used Hemodialysis as rescue therapy.

Methods: A 49 y/o AAM with h/o DM2, CKD stage 2, with baseline creatinine b/w 1.13-1.3 with nephrotic syndrome, diastolic heart failure and OSA who presented to the hospital with complaints of dyspnea of exertion and orthopnea of one month duration. Clinical exam showed pulmonary edema and hypercapnic respiratory failure. He was started on a bumex drip at 2 mg/hr. P/E revealed, an obese male, drowsy with BP of 140/90, bilateral +3 pedal edema, with O2 sat of 94% on BIPAP. His serum bicarb was 35, chloride 101, and Scr -1.33. ABG of 7.25/86/73/37/92%. Patient diuresed well with urine output of 1.4 L in the first 24 hrs and 6 L on the 2nd day. His PCO2 rose from 86 to 94, and subsequently to 108 but his pH remained at 7.25. He became obtunded but was not intubated because of concern for difficulty weaning. Serum creatinine increased from 1.33 at admission to 1.41 and serum bicarb rose from 35 to 43, with chloride decreasing to 94. Potassium remained 4.2 after 4 days of diuresis and PCO2 rose to 95. The diuretic was discontinued and patient was dialyzed once with a HCO3 bath of 25 and 3k potassium bath for 3 hours and given 500 ml of NSS. At 2 hours post HD his ABG was 7.28/78/131/36.7/98% and bicarb improved to 31. Ten hours post HD the ABG was 7.32/75/115/38/98% with HCO3 31, chloride 101 and potassium 4.8. His urine pH was 5.0. Patient clinically improved and was taken off the BIPAP. Two days after HD he was discharged with ABG of 7.40/60/84/36/96%, HCO3 of 31, Scr of 1.14 and with normal mental and respiratory status.

Conclusions: Hemodialysis with low bicarbonate bath may have a role in improving the metabolic alkalosis component of the CO2 narcosis and avoid ventilator dependence.

SA-PO1065

Demyelinating Neuropathy and Rhabdomyolysis Induced by Simvastatin in a CAPD Patients Somchai Yongsiri,[2] Jiranuch Thammakumpee.[1] [1]*Internal Medicine, Chonburi Hospital, Mueng, Chonburi, Thailand;* [2]*Internal Medicine, Burapha University, Mueng, Chonburi, Thailand.*

Background: Statin is a well-known drug causing rhabdomyolysis. Demyelinating neuropathy is another serious side effect that reported less frequently. This current case illustrate a CAPD patients who suffered from both rhabdomyolysis and demyelinating neuropathy induced by simvastatin.

Methods: Case description. A 37 year-old female had been on CAPD for 10 months because of hypertensive renal disease presented at the outpatient clinic with generalized muscle weakness and muscle pain for 2 days. Medical history showed an episode of peritonitis 4 months after started CAPD. Her medications included simvastatin, amlodipine, losartan and furosemide. On physical examination, she had muscle tenderness, motor power grade 1 all extremities with reduced deep tendon reflex and decreased pinprick sensation all extremities. Cranial nerves were intact. Serum creatinine phosphokinase level was elevated at 1208 U/L (normal 24-170 U/L). MRI of C-spine found no spinal cord or nerve root lesion. Lumbar puncture showed clear, colorless fluid without cell, normal glucose and protein level. Electromyography and nerve conduction velocity studied revealed sub-acute demyelinating neuropathy. Diagnosis. Demyelinating neuropathy and rhabdomyolysis secondary to simvastatin. Clinical course. She had been treated conservatively with temporary hemodialysis and physical rehabilitation. Her conditions were progressively improved over 3 months after simvastatin withdrawal and regain muscle power to grade 4/5.

Conclusions: Discussion. The incidence of peripheral neuropathy and rhabdomyolysis associated with the use of statins are about 1 in 14,000 and 1 in 10,000 person-years respectively. It can occur many months or years after starting the drug. A possible mechanism of neuropathy and rhabdomyolysis from statin is the inhibition of mitochondrial respiratory chain and an intracellular deficiency of ubiquinone. Risk factors include statin characteristic and higher dose, pre-existing neuromuscular disease, concurrent drug use, and renal dysfunction. Nephrologist should be aware of these serious side effects in ESRD patients taking statins.

SA-PO1066

Peritoneal Drainage of Chronic Ascites Secondary to Right Ventricular Failure and Endstage Renal Disease Siddiq Anwar, Aman Gupta, Merajul Haq Khan, Sohail Ahmad. *Renal Unit, East Kent University Hospitals NHS Foundation Trust, Canterbury, United Kingdom.*

Background: We report two cases of haemodialysis (HD) patients where severe right ventricular failure and resultant chronic ascites was successfully managed using peritoneal drainage.

Methods: The first case is a 63 year old female on HD via arteriovenous fistula (AVF) secondary to polycystic kidneys and congestive cardiac failure (CCF). She had a tenchkoff catheter inserted for drainage of chronic ascites. Her pulmonary artery pressure (PAP) was 50mmHg + right atrial pressure (RAP) and had an ejection fraction of 10-15%. Ultrafiltration (UF) on HD proved futile due to severe symptomatic hypotension. Her dialysis adequacy could not be met via peritoneal dialysis alone due to large polycystic kidneys. We simulated UF by removing fluid thrice per week via the tenchkoff catheter before HD sessions. This enabled effective dialysis and fluid balance without compromising hemodynamic stability. Over two months her exercise tolerance improved (NYHA IV to NYHA II) and serum albumin increased by 73% (11 to 19 g/l). This might have been related to alleviating the compressive effect of chronic ascites. Our second case is a 72 year old male also on HD via AVF. He has a background of chronic glomerulonephritis and CCF. His PAP was 44mmHg + RAP. Like the previous case, cardiovascular fragility made UF difficult on HD. His ascites was drained just before his HD session thrice per week via tenchkoff catheter. It was his choice to stay on HD. His albumin improved 48% (23 to 34 g/l) and exercise tolerance (NYHA IV to NYHA II) over a 3 month period.

Conclusions: Peritoneal Dialysis alone has been described in patients with ESRF and severe CCF. These cases are unique and demonstrate that intermittent peritoneal drainage via tenchkoff catheter can be used to successfully drain chronic ascites in patients with heart failure. We feel that intermittent peritoneal drainage via tenchkoff is an underused therapeutic measure. We believe that this has a role in patients with severe right heart failure with chronic fluid overload. This can be a palliative measure, as in our case or in patients waiting for a heart transplant even without severe renal impairment.

SA-PO1067

Recurrent Catastrophic Anti-Phospholipid Antibody Syndrome Triggered by Peritoneal Dialysis Peritonitis Ahsan Ahmed Syed, Vinod Mathrani, Mangalakumar Veerasamy. *Renal, Hull Royal Infirmary, Hull, United Kingdom.*

Background: Catastrophic antiphospholipid syndrome (CAPS)is a rare and life threatening variant of antiphospholipid syndrome. It is characterized by microvascular thrombosis leading to involvement of 3 or more organs, developing over short period of time (less than one week), and the presence of antiphospholipid antibodies (aPL) in high tires. Infection acts as a trigger to increase aPL by molecular mimicry.

Methods: We present a patient who reached ESRD due to CAPS developed recurrence of CAPS following PD peritonitis. PD peritonitis was treated appropriately along with consideration of PD catheter change. Due to poor venous access, temporary replacement of warfarin to unfractionated heparin (UH) was challenging, therefore commenced on low molecular weight heparin at reduced dose. Over the next 10 days she developed pyrexia of unknown origin and a full septic screen was negative. She rapidly developed respiratory insufficiency with pulmonary infiltrates, acute coronary event, abdominal pain and lactic acidosis, suggesting rapidly evolving microvascular thrombosis affecting above organs. This was associated with high aPL titres and low anti-Xa activity. In spite of optimising the anti-coagulation, she developed generalised seizure & CT brain showed bilateral subdural haemorrhages. All above features suggested recurrence of CAPS. She was treated with plasma exchange, UH along with multi-organ support. Since she had prolonged activated prothrombin time (aPTT) at the baseline we needed to monitor anti-Xa activity whilst receiving UH which is not the standard practice. She made a good recovery and discharged on warfarin.

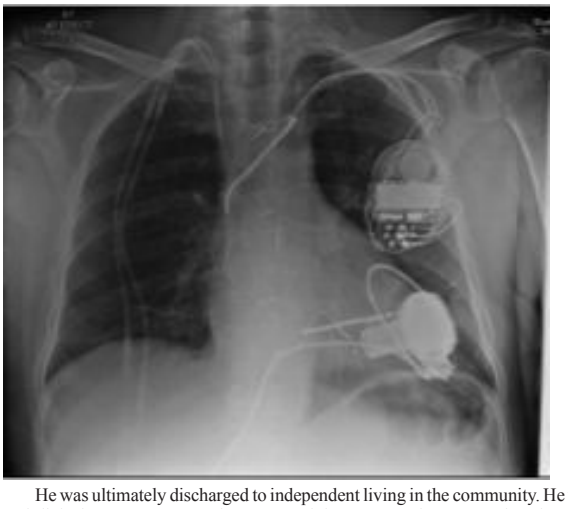
Conclusions: Patients with CAPS can develop thrombotic events due to increase in aPL titre after infection. Since infection is a common occurrence in ESRD patients with indwelling catheters, close monitoring and optimisation of anti-coagulation is essential to prevent thrombotic events during episodes of infection. Since these patients could have prolonged aPTT due to the presence of lupus anti-coagulant, aPTT measurement alone might result in under-anticoagulation hence we suggest factor Xa activity rather than aPTT monitoring while on UH.

SA-PO1068

Successful Long-Term Intermittent Haemodialysis in a Patient with Left Ventricular Assist Device Haridian Sosa Barrios,[1] Andrew Palmer,[1] Nick Banner,[2] Neill D. Duncan.[1] [1]*Renal Medicine, Hammersmith Hospital, London, United Kingdom;* [2]*Cardiac Surgery, Harefield Hospital, London, United Kingdom.*

Background: Left ventricular assist devices (LVAD) in patients with refractory heart failure are bridging treatment to cardiac transplantation. With coexistent end-stage renal failure (ESRF) continuous veno venous haemofiltration (CVVHF) is employed, but ESRF is an ineligibility criterion for cardiac transplantation and the LVAD becomes the destination treatment. CVVHF cannot be provided in the long-term or outside of an intensive care. Uncommonly LVAD-supported patients are treated with intermittent haemodialysis (HD), we present a 48yr male treated in this way.

Methods: Despite cardiac resynchronisation and a pacemaker defibrillator his cardiac function worsened, a Heartware LVAD was inserted. Following CVVHF, intermittent HD four times a week treatment was delivered via a cuffed central venous catheter.



He was ultimately discharged to independent living in the community. He was pulseless and dialysis nurses measured mean arterial pressure using a Doppler ultrasound device. In the event of circulatory collapse fluid replacement was the advised but not required. He had three access-related bacteraemias treated with line salvage - 2.26 episodes per year. Other medical complications were two episodes of epistaxis resulting from nasal septal perforation and obligatory anticoagulation for the LVAD and a gastrointestinal bleed from a duodenal ulcer. He describes good quality of life 17 months after starting HD.

Conclusions: 14% of patients required renal replacement therapy in a recent study of patients who received a LVAD as bridging treatment, and ESRF was associated with 80% mortality. Our novel report of outpatient HD in a LVAD recipient demonstrates this should be considered and can provide long-term quality of life in those without prospects for cardiac transplant.

Funding: Government Support - Non-U.S.

SA-PO1069

Successful Pregnancy after Intracytoplasmic Sperm Injection in a 36 Year Old Hemodialysis Patient Daniel Zickler, Achim Joerres, Ralf Schindler, Andreas Kahl. *Nephrology and Intensive Care, Universitatsmedizin Berlin, Berlin, Germany.*

Background: Fertility is markedly reduced in the dialysis patient population. Causes are multifactorial and include hormonal imbalances. Here we report a case of successful intracytoplasmic sperm injection (ICSI) in a 36 year old hemodialysis patient.

Methods: The patient was diagnosed with end stage renal disease at age 28 caused by interstitial nephritis secondary to sulfasalazine intake for ulcerative colitis. After 4 years of regular sexual intercourse without contraception and a complete infertility workup

of the patient's spouse the diagnosis of infertility was made. The patient and treating gynecologist decided to move to ICSI. After two implantation failures a third embryo could be successfully implanted. During pregnancy hemodialysis was intensified (6 times/week 5 hours). Adequate substitution of erythropoeitin, vitamines, trace elements and iron was given on the basis of monthly measurements.

Results: During pregnancy an exarcerbation of the ulcerative colitis was successfully treated with steroids. Furthermore hypertension had to be treated with alpha-methyl-Dopa and metoprolol. Moreover, an impairment of regurgitation of the mitral valve occurred. Despite these problems the pregnancy proceeded without further complications. At week 34 a healthy male infant (1750g) was delivered spontaneously. 5 weeks after delivery the resection of a small segment of the child´s colon was necessary due to necrotizing enterocolitis, requiring neonatology care for two months. At present, the physical and mental development of the 18 month old boy is normal. Shortly after delivery the mother showed signs of progressive cardiac insufficiency which resolved spontaneously. It remains unclear if the cardiac impairment was associated with pregnancy or fluid overload. One year after the delivery the mother underwent successful kidney transplantation.

Conclusions: To our knowledge this is the first reported case of a successful pregnancy induced by ICSI in a patient on longterm dialysis treatment (>7 years). Successful outcome was achieved by close monitoring of mother and child during and after the pregnancy, as well as intensified hemodialysis treatment.

SA-PO1070

Intraperitoneal Oxacillin in Methicillin Sensitive Staph Aureus (MSSA) Infective Endocarditis: A Case Report Priyanka Singh, Paul A. Fein, Morrell M. Avram. *Avram Division of Nephrology, SUNY Downstate University Hospital at LICH, Brooklyn, NY.*

Background: The treatment of local infection (peritonitis) in patients maintained on continuous ambulatory peritoneal dialysis (CAPD) typically includes repeated administration of intraperitoneal (IP) antibiotics, which are systemically absorbed to varying degrees, but sufficient to reach measurable serum levels. Factors influencing systemic absorption include molecule size, dialysate drug concentration, dwell period, protein binding, distribution volume, and presence or absence of peritonitis. There is limited data available, however, regarding the use of IP antibiotics for systemic infections.

Methods: Case Report: A 42 y/o African-American woman with aortic valve replacement and mitral valve repair receiving hemodialysis via left upper arm arteriovenous (AV) graft developed MSSA endocarditis with aortic root abscess. She was initially treated with intravenous (IV) nafcillin using a Hickman catheter. Cardiac surgery was deferred due to severe anemia (6.8Gm/dL) and religious transfusion objection. The clinical course was complicated by vascular access failure and catheter-related infections, so she was switched to CAPD. Nafcillin-related liver enzyme elevations necessitated a switch to oxacillin. Due to line/catheter infection, we switched IV to IP oxacillin added to each PD exchange using the following conversion: 2Gm IV every 4 hours to 3Gm IP every 6 hours (each totaling 12Gm/day). The serum oxacillin level after 8 PD exchanges was 50 mcg/ml (reference range: peak serum level after 500mg IV oxacillin is 43 mcg/ml). We continued this regimen while the patient was optimized for cardiac surgery using high dose epoetin and iron sucrose, and she remained afebrile, non-toxic, and non-bacteremic for the following month.

Conclusions: Intraperitoneal antibiotics can be considered for systemic infection as long as therapeutic drug levels are achieved in serum. Given the very limited data available, varied antibiotic kinetics and peritoneal absorption rates, therapy must be individualized, and modified as clinically warranted.

SA-PO1071

Ischemic Monomelic Neuropathy Associated with HeRO Vascular Access Device: A Case Report Jagannath H. Saikumar, Sandeep S. Soman, Lalathaksha Murthy Kumbar. *Nephrology, Henry Ford Hospital, Detroit, MI.*

Background: Complications arising from vascular access for hemodialysis are not uncommon. Ischemic Monomelic Neuropathy (IMN) is one the most serious non-vascular complication resulting in irreversible neuronal damage. Here we present an interesting case of a patient who underwent a HeRO (Hemodialysis Reliable Outflow)™ graft implantation resulting in the onset of symptoms suggestive of IMN requiring emergent ligation of the vascular access.

Methods: A 51 y.o woman with ESRD presented to our hospital emergency room with onset of altered mental status shortly after hemodialysis at an outside facility. An acute cerebro-vascular event was ruled out. Her mental status improved with supportive care over two days. She then complained of numbness in her left hand and inability to extend her left middle 3 fingers and elbow. Her husband informed us that she had a left upper extremity HeRO™ graft implantation 3 days earlier at another facility as she was found to have bilateral subclavian vein occlusion. Adequate radial pulsation was noted on Doppler study in the left arm. Due to the possibility of IMN, the patient underwent an emergent graft ligation and placement of a temporary tunneled cuffed catheter. Electromyogram performed post-ligation showed decreased nerve conduction velocity in the left median nerve and in sensory and motor nerve amplitudes in the left arm and forearm suggestive of an acute pan-brachial plexopathy involving primarily the lower trunk. No significant improvement was seen in symptoms and motor function in her left hand at the time of discharge,3 days post ligation. She was lost to follow-up.

Conclusions: IMN is a serious but uncommon complication of hemodialysis access that can result in permanent neurological sequelae.Clinical manifestations of IMN can be misinterpreted as post surgical changes.Previous experience has shown that even with timely intervention, the axonal damage done may be permanent with residual loss of function as in our patient.This case underlines the importance of a high index of suspicion for IMN in the appropriate setting.To the best of our knowledge,this is the first report of IMN in a patient who received a HeRO™ graft.

SA-PO1072

Successful Treatment of Diltiazem Sustained Release (SR) Overdose with Charcoal Hemoperfusion Sreedhar R. Adapa, Tarun Chugh, Rajan Kapoor, Savneek S. Chugh, Maureen E. Brogan. *Department of Nephrology, New York Medical College, Valhalla, NY.*

Background: Hemoperfusion is an extracorporeal technique that works on the principle of adsorption and is used to remove protein-bound and lipid-soluble drugs more efficiently than hemodialysis.

Methods: 50 yo male with PMH of HTN, schizophrenia admitted after being found unconscious in prison cell. Vitals signs: HR of 40/min, BP of 70/40 mm Hg, and RR of 12/min. Physical examination revealed unconscious patient with bradycardia and hypotension. EKG showed sinus arrest with a ventricular escape rhythm at 45 bpm. Significant labs: BUN 31 mg/dl, creatinine 2.2 mg/dl, bicarbonate 16 mEq/L and blood glucose 39 mg/dl. The patient was intubated in the ER. Initial treatment comprised of gastric lavage, IV calcium chloride, IV atropine, insulin and dextrose drip and glucagon for presumptive diltiazem overdose (as few tablets of diltiazem SR were found with the patient). Patient required maximal doses of three pressors, transvenous pacing for symptomatic bradycardia and sustained anuric acute kidney injury (AKI). With no improvement in his hemodynamic status and refractory diltiazem overdose, he got charcoal hemoperfusion for three hours. After a few hours, his cardiorespiratory status improved, transvenous pacer and pressor support was weaned off. Patient required three treatments of intermittent hemodialysis for his AKI. Diltiazem levels were subsequently confirmed to be 5900ng/ml (reference range 50-200 ng/ml) on admission, and 990ng/mlafter hemoperfusion. Transient thrombocytopenia and hypocalcemia were seen with hemoperfusion that were self-limiting.

Conclusions: Hemoperfusion was used with success in standard formulation diltiazem overdose, which didn't respond to intense medical management in few case reports previously. Diltiazem SR formulations cause prolonged toxicity and potentially life threatening. The efficacy of hemoperfusion in treating diltiazem SR overdose was debated recently. We successfully treated diltiazem SR overdose with one of the highest serum concentrations reported in a patient who survived, with hemoperfusion which was refractory to intense medical management.

SA-PO1073

A Surviving Patient with Record High Creatinine Naing L. Htike,[1] Andrew C. Storm,[3] David A. Cohen,[2] Robert L. Benz.[1] *[1]Internal Medicine, Nephrology, Lankenau Medical Center and Lanenau Institute of Medical Research, Wynnewood, PA; [2]Internal Medicine, Lankenau Medical Center, Wynnewood, PA; [3]Internal Medicine, The Johns Hopkins Hospital, Baltimore, MD.*

Background: Creatinine is a product of muscle protein breakdown cleared by the kidneys at a constant rate. The glomerular filtration rate is estimated based on serum creatinine. Higher serum creatinine concentration is associated with either a reduced renal excretion of creatinine or increased production by the body during myositis or rhabdomyolysis. There is no definitive level of serum creatinine which is itself incompatible with human survival. We present the highest serum creatinine level associated with survival based on a thorough review of the literature.

Methods: We report a 34 year old male patient with baseline serum creatinine 1.2 mg/dl who presented to Lankenau Medical Center's emergency department with a six week history of new onset of uremic symptoms including nausea, vomiting, ankle swelling and 30 lb weight loss. His past medical history was unremarkable and was on no prescribed medication. On exam, he had no acute distress. His BMI was 28. His exam was significant only for an elevated blood pressure of 184/93 and asterixis. His oxygen saturation was 100% on room air. His peak serum creatinine of 53.9 mg/dl with an estimated GFR of 1ml/min. There was no hydronephrosis on renal ultrasound. The patient subsequently required maintenance hemodialysis and later changed to long term peritoneal dialysis.

Conclusions: To our knowledge, based on a thorough review of the literature using PubMed, Cochrane Data Base and USRDS, this is the highest level of serum creatinine ever reported. The USRDS records show that over the past 15 years, less than 1.3% of all new ESRD patients have a reported initial creatinine over 20 mg/dl. The USRDS data-base also includes no value over 30 mg/dl. We conclude that serum creatinine itself is non-lethal. It is more likely that other electrolyte and retained metabolic product abnormalities of renal failure frequently cause symptoms or death before lethal toxicity, if such a level exists, of creatinine has been reached.

SA-PO1074

The Treatment Dilemma of Hypodipsic Hypernatremia Ghassan Bandak,[1] John Manllo,[1] Javier A. Neyra,[1] Jerry Yee.[2] *[1]Internal Medicine, Henry Ford Hospital; [2]Nephrology, Henry Ford Hospital.*

Background: Serum osmolality, serum sodium and water homeostasis are hypothalamically regulated via thirst sensation of AVP secretion in response to tonicity and ECF volume changes. Hypothalamic pathology may alter this homeostatic balance, producing disorders of serum sodium.

Methods: A 50 y.o. previously healthy male presented with acutely altered mental status and severe hypernatremia of 188 mEq/L. Laboratory evaluation revealed acute kidney injury with SCr 2.4 mg/dL, Sosm 394 mOsm/kg, UNa 16 mEq/L, Uosm 483

mOsm/kg, measured after IV fluid administration, and inappropriately low serum AVP levels. A brain MRI was consistent with diffuse encephalomyelitis. The patient received DDAVP and isotonic IV fluids with improvements of kidney function, hypernatremia and mental status that approached his baseline by time of discharge. However, symptomatic hypernatremia recurred and required repeated hospitalization. Inpatient evaluation demonstrated increased Uosm to 924 mOsm/kg and reduced 24-h urine volume of 900 mL during water restriction. Urine volume increased to 1750 mL at Uosm of 609 mOsm/kg after isotonic fluid infusion. On a fixed water intake of 2 L per day, the patient currently maintains a SNa at approximately 144 mEq/L.

Conclusions: Hypodipsic hypernatremia represents an infrequently reported, rare, hypothalamic disorder of impaired osmoregulation. In this case, cerebral inflammation impaired thirst function with consequent symptomatic hypernatremia, but the urinary concentration mechanism remained intact, characteristic of patients with hypodipsic hypernatremia. A therapeutic regimen of scheduled water intake is challenging, but was salutary in this case. Lastly, the role of desmopression as thirst stimulant in this uncommon problem is controversial.

Funding: Clinical Revenue Support

SA-PO1075

A Simple Screening Test for the Detection of Anti-Erythropoietin Antibodies

Soma Meran, Alexa Wonnacott, Mat Davies, Aled O. Phillips. *Institute of Nephrology, Cardiff University, Cardiff, United Kingdom.*

Background: Antibody mediated Pure Red Cell Aplasia is a rare complication of erythropoietin (EPO) therapy. Identification and demonstration of functional activity of EPO antibodies required to diagnose this condition is difficult and only performed in select laboratories worldwide. **Objectives:** We report three recent cases of of antibody mediated PRCA associated with subcutaneous Epoetin Alfa (EPREX) use and describe the use of a simple inhibitor assay that can be used to diagnose PRCA in local laboratories.

Methods: Assay Description: A sample of patient serum is incubated for 1h at 37 degrees with an equal volume of serum of known concentration (e.g 50 mIU/ml). By measuring the EPO in the patient sample in conjunction with the level in the incubated mixture, the percentage recovery can be calculated. If the recovery is found to be less than 80% of the expected value, the presence of EPO inhibitor activity is suspected. The assay has been validated by running the test for 6 patients with known EPO antibodies, and compared to 10 CKD patients without PRCA.

3 patients with CKD 4, all on subcutaneous EPREX, developed a sudden onset profound anaemia after 17, 7 and 21 months on EPO therapy. All patients had absolute reticulocyte counts below 7x109/mL. 2 patients had diabetic nephropathy and 1 patient had CKD of unknown cause. The in-house inhibitor assay was performed for all three patients and demonstrated EPO recovery of 0, 11 and 62 percent. Subsequently frozen serum samples were sent to USA for investigation and found to contain neutralising EPO antibodies at titres of 1/1000, 1/10000 and 1/100 respectively. EPO therapy was discontinued for all the patients. Immunosuppressive therapy was discounted for all the patients and all patients are now on unit Haemodialysis and remain transfusion dependent.

Conclusions: These 3 recent cases identifies that the risk with subcutaneous EPO still requires careful observation, and diagnosis and monitoring of new cases of EPO-mediated PRCA remains mandatory. We propose that our novel and simple in-house assay could have widespread use in local laboratories to guide clinical suspicion in suspected cases.

SA-PO1076

Plasmapheresis in a Triad of Hypertriglyceridemia, Acute Pancreatitis, and Diabetic Ketoacidosis

Yin L. Win, Farhanah Yousaf, Sheng F. Kuo, Chaim Charytan, Bruce S. Spinowitz, Marilyn Galler. *Division of Nephrology, New York Hospital Queens, Flushing, NY.*

Background: Severe hypertriglyceridemia induced acute pancreatitis has been well described. This condition may be debilitating and fatal. Currently accepted therapeutic options are limited. We report a case of acute pancreatitis associated with hypertriglyceridemia that was safely and effectively managed with plasma exchange.

Methods: A 38-year-old female with history of gestational diabetes presented with acute onset of nausea and abdominal pain. She denied alcohol use and was on oral contraceptive pills. On physical exam, she was afebrile, normotensive, and tachycardic with mild abdominal distension and diffuse tenderness. Diagnostic tests revealed a serum glucose of 414 mg/dL, triglycerides >816 ng/dL, amylase of 106 U/L, lipase of 272 U/L, anion gap of 23, BUN of 13 mg/dL, creatinine of 0.3 mg/dL, sodium of 120 mmol/L, HCO3 of 14 mmol/L, and positive urine ketones. Computed tomography of the abdomen showed severe acute pancreatitis without evidence of pseudocyst, abscess formation, or cholelithiasis.

The patient was aggressively hydrated and treated with gemfibrozil, insulin, heparin, and morphine on Day 1. Due to persistence of symptoms, plasma exchange using NxStage system with plasmaflo OP-5W dialyzer was performed on Day 2 and 3, which resulted in significant reduction of the triglyceride level and resolution of abdominal pain. Patient was discharged home with gemfibrozil and glyburide as maintenance therapy.

Conclusions: The exact mechanism of hypertriglyceridemia-induced pancreatitis is not clear. It has been postulated that hyperviscosity of blood due to lipid particles cause ischemia in the pancreas, releasing inflammatory mediators and leading to pancreatic necrosis and inflammation. The advantage of plasma exchange over conservative management is the removal of lipid particles in a relatively short period of time and clearance of triglyceridemia associated pro-inflammatory agents. To our knowledge, this is the first adult case of hypertriglyceridemia-induced acute pancreatitis with diabetic ketoacidosis that was successfully treated with plasma exchange.

SA-PO1077

Severe Hyperkalemia Caused by a Commonly-Used Antibiotic in the Outpatient Setting

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Background: Six patients with severe symptomatic hyperkalemia arrived to the emergency department of our institution. The severity of hyperkalemia was out of proportion to the degree of renal dysfunction. All patients were recently prescribed Trimethoprim/Sulfamethoxazole (TMP/SMX) in the outpatient clinics. Trimethoprim reduces renal potassium excretion through the competitive inhibition of epithelial sodium channels in the distal nephron principal cells. We retrospectively identified several risk factors for the development of hyperkalemia in these patients. Failure to recognize them before considering use of TMP/SMX might result in a life-threatening complication.

Methods:

Summary of clinical characteristics

Patient #	1	2	3	4	5	6
Age (yrs)	71	83	68	80	78	72
Base Creat (mg/dl)	1.9	4.5	N/A	N/A	2.4	1.7
Peak Creat (mg/dl)	5.9	8.8	5.0	4.5	4.7	2.6
Peak [K+] (meq/l)	7.5	8.3	7.7	7.3	7.6	8.8
Hx of Diabetes	Yes	N/A	Yes	Yes	Yes	Yes
ACEI use	Yes	Yes	No	No	Yes	Yes
ARB use	No	No	No	Yes	No	Yes
Beta-blocker	Yes	No	No	No	No	Yes
TMP160/SMX800 (# tabs bid)	2	1	N/A	2	1	1

All patients had an acute increase in serum creatinine levels. Five out of the six patients had changes on ECG consistent with hyperkalemia. Of these, three required acute dialysis. Cardiac pacing was necessary in two patients and one required mechanical ventilation. Most patients were on drugs that block the Renin-Angiotensin-Aldosterone System (RAAS). The patients were discharged home without the need of chronic dialysis.

Conclusions: The liberal use of TMP/SMX in the primary care setting could lead to a rise in the incidence of severe hyperkalemia in patients with chronic kidney disease (CKD), particularly those taking medications that impair renal potassium excretion. Although the previously reported incidence of serum potassium level greater than 5.5 meq/l in healthy subjects treated with TMP/SMX was 6%, our report suggests that the incidence is much higher in patients with CKD with RAAS inhibition. This complication can be avoided with the careful identification of clinical risk factors, proper antibiotic dose adjustment, and close monitoring of serum electrolytes.

Funding: Veterans Administration Support

SA-PO1078

Mind the Gap, but Do Not Be Fooled by Its Absence

Hanni Menn-Josephy, Jasvinder S. Bhatia. *Nephrology, Boston University Medical Center, Boston, MA.*

Background: Chronic ingestion of ethylene glycol is a challenging diagnosis that can present as AKI with subtle physical findings and without the classical metabolic derangements.

Methods: 41 year old male with a past history of hypertension and depression was admitted for 5 days of abdominal pain, nausea and vomiting. He was found to have oliguric AKI with creatinine up to 7.4 mg/dL from a baseline of 0.9. On presentation he was hemodynamically stable with an unremarkable physical exam. Lab tests were notable for mild anion gap metabolic acidosis, but no other electrolyte abnormalities. Urine sediment showed non pigmented granular casts, but no cellular casts or crystals. Despite aggressive fluids and resolution of his anion gap within 48 hours, his Cr continued to rise to a peak of 15.5. Radiologic evaluation with abdominal MRA/CT and renal US were unremarkable. Serologies for hepatitis B, C, HIV, ANA, ANCA and immunofixation were unrevealing. A kidney biopsy revealed extensive oxalate crystal deposition in the tubules with associated signs of acute tubular injury and mild focal interstitial inflammation. The patient had no evidence of primary hyperoxaluria or enteric hyperoxaluria and he repeatedly denied ethylene glycol ingestion. His kidney function gradually improved with supportive care and recovered 3 months post admission. Seven months later, he represented with altered mental status post suicidal attempt and was found to have acute kidney injury, severe metabolic acidosis with an anion gap of 25 and osmolar gap of 43. His ethylene glycol level was 94mg/dl, and he required urgent dialysis. The patient later confessed to ingesting small amounts of ethylene glycol over the past year. He had mild improvement of kidney function but Cr remained elevated at 4.9 on discharge.

Conclusions: Ethylene glycol metabolizes to oxalic acid within 24-72 hours, with the resolution of both anion and osmolar gap. This resolution may lead to a delay in diagnosis leading to CKD. This case illustrates the need to consider chronic ingestion despite the lack of exposure history or presence of a significant anion gap or urinary crystals, particularly in high risk patients with a history of depression and an unexplained AKI.

SA-PO1079

Inversion Posture of Standing Head Down Precipitating Acute Kidney Injury: Report of Two Cases

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Background: Although reports of acute kidney injury (AKI) occurring in the setting of physical exercises are numerous in publications none has addressed the impact of change in body posture on kidney function. Therefore, we report 2 cases of young nigerian men

who developed AKI after engaging in physical exercise involving inversion posture of standing head down. This is very unusual, in view of the acclaimed benefits of this posture to practitioners of yoga.

Methods: Two young men, aged 27 and 29 years respectively, were referred to our centre with strikingly similar presentations; they developed severe kidney dysfunction within one week of undergoing military training in a camp. Both had no prior history predisposing to kidney disease and data regarding their pre-recruitment records confirmed their medical fitness. Their camp daily routines included participation in physical exercises entailing inversion posture of standing head down for several minutes.

At presentation, both patients had pressure ulcers on their scalps consistent with prior assumption of this unusual posture. They were hypertensive (150/90, 144/100 mm Hg), had markedly impaired kidney function (urea 73 mmol, and 90.3 mmol, creatinine 1890umol and 2305 umol). Daily urine outputs were normal which showed bland sediments with no pigments or proteinuria. These abnormalities reversed completely with conservative management in one (urea 2.9 mmol, creatinine 102 umol) and hemodialysis in the other (urea 3.8 and creatinine 122 umol). Computerized angiography revealed diffuse bilateral renal ischaemia despite normal renal arteries.

Conclusions: Inversion posture of standing head down may trigger kidney injury. Predisposing factors for this unusual cause of AKI in persons with seemingly intact renal arteries remain to be ascertained.

Since man assumed the erect posture billions of years ago, the position of both native and transplant kidneys, below the heart, offers peculiar advantage to glomerular hemodynamics. Reversal of this position places the heart above the kidneys and diverts greater than usual cardiac output to organs and tissues above the heart in the inverted posture, thus creating a 'steal' syndrome in the kidneys.

SA-PO1080

Hyperparathyroid Lung Disease in a Chronic Hemodialysis Patient Adepeju A. Jinadu, Niama Huda, Sayed Husain, Sandeep S. Soman. *Nephrology, Henry Ford Hospital.*

Background: Hyper parathyroid lung disease is usually a subclinical entity diagnosed at autopsy. Only a few cases have been reported in which it lead to significant clinical symptoms. We report a case leading to respiratory symptoms.

Methods: The patient is a 70 years old male with history of end stage renal disease on hemodialysis for 7 years. He presented with a complaint of dyspnea. His Chest X-ray revealed a right lower lobe infiltrate he was treated with antibiotics for pneumonia and discharged.

He presented a month later with persistent dyspnea. Repeat Chest X-ray showed persistent right sided infiltrate, CT scan was done which showed a mass like airspace opacity in the right lower lobe with adjacent small airspace nodularity and non-specific visceral pleural thickening. Differential diagnosis included a neoplastic or Fibrotic process.

He underwent a CT guided trans-thoracic lung biopsy which revealed Metastatic calcification in the airspace with foreign body giant cell response. His Calcium phosphorus product was 48, iPTH level; 595. 25OH-vitamin D level was 8. Sevelamer was continued and calcitriol 0.5mcg daily was initiated.

Conclusions: Hyperparathyroid lung disease is usually an indolent disease, it is found on autopsy in 60 to 70 % of Hemodialysis patient. Clinical manifestations are minimal but can lead to dyspnea and respiratory failure. Chest radiography is insensitive for the diagnosis, and may show only diffuse airspace abnormality or nodular infiltrate. CT scan may show infiltrate, ground-glass infiltrate, consolidation, calcified nodules, HRCT Scan and Technetium scans are sensitive for diagnosis of this condition. The progression and management of the disease is poorly understood, with aim of treatment including correction of elevated calcium phosphorus product and treatment of hyperparathyroidism. Our patients, symptoms and radiographs were initially misinterpreted, and only after a lung biopsy was diagnosis made. We present this case history to heighten awareness of this condition, which should be kept in mind when dialysis patients develop unexplained radiographic changes or pulmonary symptoms, to avoid patient undergoing an invasive procedure like lung biopsy which is not required for diagnosis.

SA-PO1081

A Case Report: Renal Failure in a Cutaneous T-Cell Lymphoma/Sezary Syndrome Patient Sujay Dutta Paudel, Hui Peng, Yuanqing Li, Meirong Zhong, Tan-qi Lou. *Division of Nephrology, Department of Medicine, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: Sezary syndrome (SS) is a rare form of cutaneous T-cell lymphoma and the renal failure due to sezary cells infiltration is even rare. Till date only one similar case has been reported in the literature. Here we report a case of SS and its involvement in renal failure (RF).

Methods: A 39 years old Chinese male was presented to our hospital with high fever, diffuse skin rash and renal failure with a family history of congenital ichthyosis. He gives history of ingestion of prescribed antibiotics (streptomycin), and therefore the skin rash due to drug reaction was presumed.His urinary output did decrease. During admission, he had fever (38^0C), peripheral edema, lymphadenopathy of supraclavicular, axillary and inguinal region. Scaly, erythematous and desquamated skin rash and murmur on auscultation. Initial laboratory parameters revealed; Serum creatinine-907µmol/L, BUN- 23.92mmol/L, Hb-91gm/L, Albumin-29gm/L. Ultrasonography abdomen showed both kidneys enlarged and enlargement of hilar lymph nodes. Cervical lymph node biopsy revealed peripheral T-cell lymphoma and skin biopsy revealed the presence of sezary cells. Hemodialysis started and patient kept on oral steroids, antibiotics. Fever gradually subsided and there was improvement in his skin rash. But on the 9th day of admission, patient complained of sudden severe pain in the right inguinal region. His HGB dropped to 29gm/L and CT-scan showed right retroperitoneal bleeding. He was then referred to intervention radiology for embolization and patient stabilized. Next day his Serum amylase increased to 428U/L and therefore was further managed in the line of acute pancreatitis. He was discharged on request from his family members citing financial problem. Few weeks after the discharge, the patient died.

Conclusions: Literature addressing SS and renal failure are very few. In this patient, the bigger size of the kidney, persistent proteinuria, no oliguria and the presence of sezary cells in skin biopsy indicate that RF is a result of leukemic infiltration of the sezary cells.

SA-PO1082

IgG4 Related Tubulointerstitial Nephritis Associated with Chronic Lymphocytic Leukemia Andrew F. Malone,[1] David Howell,[2] John Paul Middleton,[1] Stephen R. Smith,[1] Ruediger W. Lehrich.[1] *[1]Division of Nephrology, Duke University Medical Center, Durham, NC; [2]Department of Pathology, Duke University Medical Center, Durham, NC.*

Background: IgG4 related systemic diseases are characterized by plasma cell infiltrates positive for IgG4 staining. The extent of involvement of the kidney is unknown. Here we describe a case of IgG4 related tubulointerstitial nephritis (IgG4-TIN) associated with chronic lymphocytic leukemia (CLL).

Methods: A 72yo African American man was referred with proteinuria and recently normal renal function. Patient was easily fatigued and had frothy urine. He had no family history of kidney disease. BP was 135/80 mmHg. The rest of his exam was normal. On admission his creatinine was 6.8 mg/dl, dipstick trace positive for blood, 1 rbc/hpf and 10 wbc/hpf and no casts. He had 0.9 g/day of proteinuria. The rest of his renal failure work up was normal except ANA of 1:40. Hemoglobin was 8.9 g/dl; white cell count 18,000/ml with 79% lymphocytes. Marrow aspirate showed abnormal monoclonal B cells. Total serum IgG was 3150mg/dl with normal IgG4. Kidneys were 12.9cm bilaterally. The patient did not require dialysis. Renal histology revealed an interstitial plasma cell infiltrate with scattered eosinophils and extensive fibrosis and tubular atrophy. Plasma cells stained heavily for IgG4 by immunohistochemistry. There was mesangial matrix expansion. Therapy with prednisone 60mg daily was started and creatinine improved to 2.7mg/dl.

Conclusions: To our knowledge this is the first reported case of IgG4-TIN and CLL. Obtaining a kidney biopsy and specifically staining for IgG4 was crucial in obtaining a diagnosis. Diagnosis and treatment was guided by a recent report by Raisson et al (J Am Soc Nephrol, 22: 1343-52, 2011). The fact that CLL was diagnosed simultanously with IgG4-TIN suggests that there is a causal relationship. IgG4-TIN needs to be considered in a patient with CLL and abnormal renal function. Our case demonstrates the importance of making a diagnosis of IgG4-TIN because it responds readily to corticosteroids.

SA-PO1083

Treating One Disease Causes Another: Renal Sarcoidosis Anila P. Sankar,[1] Alex J. Hernandez,[1] Shalini Barlapudi.[1] *[1]Department of Nephrology, The Ochsner Clinic Foundation, New Orleans, LA.*

Background: Renal sarcodosis (RS) is a multisystem granulomatous disorder of unknown etiology characterized pathologically by nonceasating granulomas. Common renal manifestations are nephrocalcinosis and nephrolithiasis. We present a rare case of Etanercept (tumor necrosis factor inhibitor) induced RS.

Methods: Case: 42 year old female with psoriasis treated with subcutaneous injections of etanercept 50 mcg twice weekly for three years presents to renal clinic with weakness and acute kidney injury. Current medications were prednisone10 mg, etanercept ,and multivitamin. Recent gastric biopsy for reflux gastropathy revealed multiple nonceasating granulomas. No pertinent family history or use of herbal medications ,NSAIDS, or IV drugs. Physical examination: vitals :BP 100/72, HR 89,100% O2,with multiple healing erythematous plaques on joints. Laboratory data creatinine of 3.8 mg/dL (10/2011) increased from 1.3 mg/dL,BUN 29 mg/dl,Hb 9.3 gm/dl, calcium 8.6 ,ace level 53 U/L and no eosinophilia. Urine analysis negative. Serological evaluationand renal ultrasound were normal. With worsening creatinine of 6.2 mg/dL a renal biopsy was done which reported subacute and chronic granulomatous interstitial nephritis. Etanercept was discontinued and high dose steroid treatment was started. Creatinine has improved to 2.4 mg/dl with steroid taper.

Conclusions: Discussion: The possibility of Etanercept induced sarcoid was considered given previous cases reported by Gifre et el(J ClinRheumato April 2011). With this in mind we attributed etanercept to cause RS. This case highlights importance for monitoring renal function and discontinuation of drug if suspicious for RS.

SA-PO1084

Pregnancy and Kidney Disease in a Patient with Sjogren's Syndrome and Tubulointerstitial Nephritis Abhishek Joshi,[1] Keelin O'Donoghue,[2] Uzma Mahmood,[2] Debasish Banerjee,[3] Anita Banerjee.[1] *[1]Directorate of Medicine, Princess Royal University Hospital, Orpington, Kent, United Kingdom; [2]Department of Obstetrics and Gynaecology, Cork University Maternity Hospital, University College Cork, Cork, Ireland; [3]Cardiovascular Research Centre, St. George's Hospital, London, United Kingdom.*

Background: Maternal and fetal outcome in pregnancy with renal failure is unpredictable, where each condition can adversely affect the other. We present a case of steroid sensitive Sjogren's nephritis worsened by pregnancy, demonstrated over the course of multiple pregnancies and investigated the aetiology.

Methods: A 28-year-old nullipara with a diagnosis of primary Sjogren's syndrome (SS) presented with a deterioration of renal function. A diagnosis of secondary tubulo-interstitial nephritis was made on renal biopsy.

Her first pregnancy ended in the second trimester with a decision to deliver a female infant at 27 weeks due to worsening maternal renal function. Renal function improved immediately. A second pregnancy ended in a 1st trimester miscarriage. The 3rd and 4th pregnancies delivered male infants at 35 and 34 weeks, with worsening renal function in each pregnancy, reaching end-stage. Repeat biopsy showed extensive glomerulosclerosis and male cells were identified.

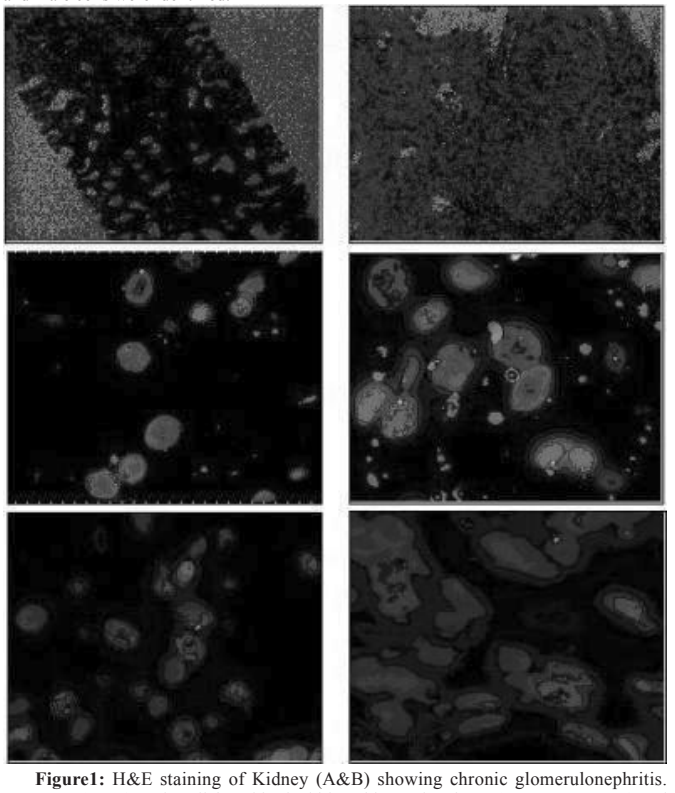


Figure1: H&E staining of Kidney (A&B) showing chronic glomerulonephritis. XY-FISH (C&D) Male cell found in the kidney biopsy, bearing X(SpectrumOrange) and Y(SpectrumGreen) chromosomes. Adjacent are female cells with (XX) genotype. Y-FISH (E&F) confirms presence of male cells.

Conclusions: This case of SS with renal disease demonstrated the increased risk of fetal and maternal adverse pregnancy outcomes. Renal function worsened in each pregnancy and progressed to end-stage renal disease. Fetal microchimerism offers an interesting mechanism for our patient's renal failure and its apparent relationship to her pregnancies.

SA-PO1085

Bacterial Peritonitis Following Esophagogastroduodenoscopy in a Patient on Peritoneal Dialysis Manmeet Brar, Tibor Fulop. *Nephrology, University of Mississippi, Jackson, MS.*

Background: We report the case of a 54 year old African American female with end stage renal disease on CAPD presenting with generalized abdominal pain along with nausea and vomiting. Two days earlier, she has undergone an esophagogastroduodenoscopy (EGD) with biopsy. Surgical exploration revealed murky peritoneal fluid and stain showed mixed flora (both Gram negative and Gram positive rods); blood and peritoneal fluid culture grew only *Streptococcus pneumoniae*. An occult perforation, not obvious to naked eye and without contrast extravasation, can occur after EGD and may lead to peritonitis, especially in the high risk setting of a patient with end stage renal disease on PD. To our knowledge, this is the first reported case of mixed peritonitis attributable to suspected microperforation after EGD. It is important to recognize the importance of antibiotic prophylaxis to decrease the occurrence of infectious complications in PD patients undergoing upper gastrointestinal procedures. In addition, prompt recognition of possible mixed bacterial infection is essential in this setting.

SA-PO1086

Role of Dialysis in a Patient with Intracranial Hemorrhage due to Dabigatran *Pradaxa*®) Muhammad W. Khattak, Hasan Arif, Ami Patel. *Nephrology, Drexel School of Medicine, Philadelphia, PA.*

Background: Dabigatran is a new direct thrombin inhibitor which is mainly used to prevent stroke in patients with atrial fibrillation. There is no antidote for this medication. The primary clearance of dabigatran is through kidneys with the half-life around 12-18 hours. This drug is 30% protein bound and its clearance with dialysis is around 60 % in 2-3 hours. There is very limited data available regarding the role of dialysis treatment in case of toxicity related to dabigatran. We report a case of using dialysis in reversing the anticoagulation effects from dabigatran in the setting of intracranial bleed.

Methods: We report a case of an 83 year old white male with a history of CAD with cardiac bypass surgery and chronic atrial fibrillation who was discovered to have a spontaneous subdural hematoma after presenting with altered mental status. His home medications included asprin 81 mg, dabigatran 150 mg twice daily. The last dose of dabigatran was at the morning of admission. His vitals were stable and had right sided weakness with aphasia. His admission creatinine was 0.93 mg/dl, INR was 1.17, and partial thromboplastin time (PTT) was 47.9 seconds. On the second day of admission he had worsening intra cerebral bleed with midline shift and the repeat PTT was 48.1 sec. The thrombin time (TT) was not available. As a last effort in reversing coagulopathy, the patient underwent 4 hours of HD using Optiflux® F160 dialyzer with blood flow of 350 ml/min. The PTT normalized after dialysis and since the thrombin time remained elevated on the third admission day, our patient underwent a second hemodialysis treatment to possibly aid in the clearance of dabigatran. TT improved to 27.7 sec. Repeat imaging of the brain showed stability of the bleeding. Although his coagulopathy was reversed, the patient expired the following day due to respiratory complications.

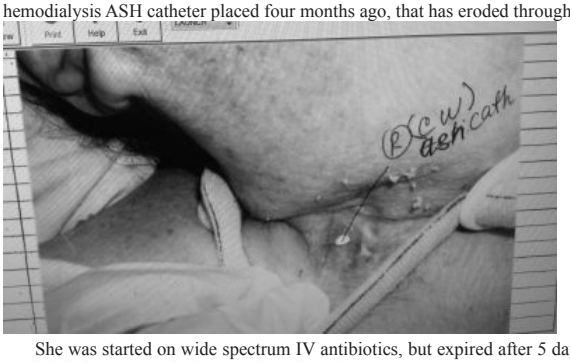
Conclusions: This case illustrates the possible role of hemodialysis in reversing the coagulopathy due to dabigatran-related bleeding in which there is no clear antidote available. Although, it is difficult to determine the degree of renal clearance of the drug in this patient, we believe that hemodialysis enhanced it.

SA-PO1087

Erosion of the Skin by the Hemodialysis Catheter: An Infrequent Complication Chadi Saifan, Rabih Nasr, Elie El-Charabaty, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: The use of chronic hemodialysis tunneled catheters has been increasing. The two major complications seen are infections and thrombosis. We report a case of an elderly woman who presented from nursing home secondary to altered mental status and found to have an erosion of the skin by an ASH catheter.

Methods: A 74 year old female was transferred from the nursing home because of hypotension and worsening confusion. She is known to have ESRD secondary to hypertension, metabolic encephalopathy, CVA, chronic respiratory failure vent dependent. On admission her vitals showed a BP of 104/70 mmHg, temperature of 97. Her physical exam was remarkable for decreased breath sounds bilaterally, decubitus ulcer on her back and sacrum, and lower extremities edema. She was noted by the nurse as well to have on the upper right chest wall, a 1 cm white silicone catheter exposed to the air. It was the tunneled hemodialysis ASH catheter placed four months ago, that has eroded through the skin.



She was started on wide spectrum IV antibiotics, but expired after 5 days secondary to Klebsiella pneumonia bacteremia and sepsis.

Conclusions: Chronic tunneled dialysis catheters have been increasingly used for patients with comorbidities and with a life span less than one year. They can be a useful bridge for patients waiting on the transplant list and those refusing a permanent access such as graft or fistula. They are the preferred vascular access for about 10% of patients on dialysis. The main disadvantages of the tunneled catheters are infections and thrombosis. While erosion of the catheter through the skin is not a very frequent complication, it requires a prompt intervention and removal to avoid subsequent infections and blood stream bacteremia.

SA-PO1088

Arteriovenous Graft VS Tunneled-Cuffed Hemodialysis Catheter: A Hidden Focus of a Hemodialysis Access-Related MSSA Bacteremia Ekamol Tantisattamo, Royden S. Young, Chuong Dinh. *Medicine, University of Hawaii John A. Burns School of Medicine, Honolulu, HI.*

Background: Vascular access-related blood stream infection is a common complication leading to high morbidity and mortality. Arteriovenous graft (AVG) thrombosis is a common complication which not only indicates a possible infection, but also needs invasive therapeutic procedures. We report a case of ESRD presenting with bacteremia and AVG thrombosis. Signs of infection were persistent even after tunneled-cuffed hemodialysis catheter (TCHDC) removal. AVG removal revealed pus around the graft.

Methods: A 56 year-old ESRD woman started hemodialysis via right internal jugular TCHDC 1 month ago when the left arm AVG was placed. The last two hemodialysis treatments werepreformed via the AVG. One day after the last hemodialysis, she developed fever and chills. There was no discharge from the exit site or tenderness along the TCHDC area. On the AVG, there was no thrill, bruit, or tenderness. CBC revealed WBC of 17,100/mm^3. Blood culture grew methicillin-sensitive *Staphylococcus aureus* (MSSA).

Transesophageal echocardiography showed mitral valve endocarditis. Nafcillin was started, and the TCHDC was removed. The catheter tip culture was negative. Repeated blood cultures showed no growth. However, she still had persistent leukocytosis and developed bandemia 2 days later when the AVG thrombolectomy was scheduled. Intraoperatively, copious pus was found surrounding the AVG. The graft was removed, and culture grew MSSA. Nafcillin was continued for 4 weeks with resolution of her symptoms.

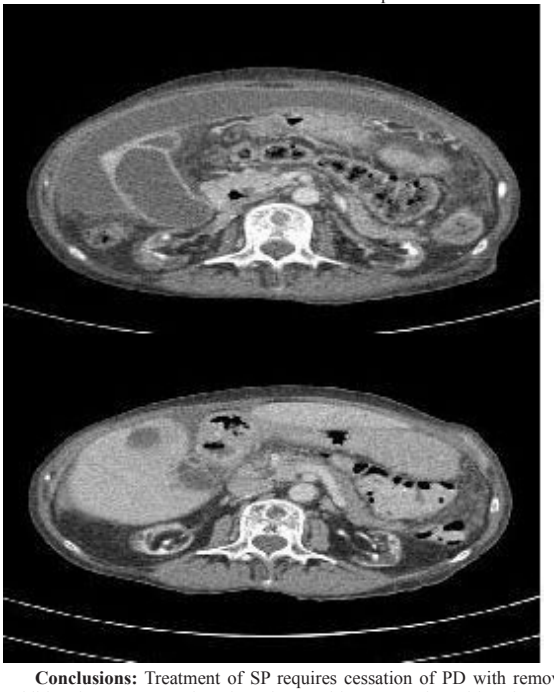
Conclusions: Our patient with TCHDC and AVG presented with MSSA bacteremia. Statically, the most likely cause of vascular access dialysis is from TCHDC. However, given AVG thrombosis and bacteremia, our patient's AVG appears to have been the source of sepsis despite a clinically benign appearance externally. Our case should serve as a reminder to consider all intravascular foreign material as a potential source of infection. Moreover, AVG thrombosis with bacteremia should raise a suspicion for the graft infection and prompt surgical removal should be highly considered.

SA-PO1089

Steroid Responsive Sclerosing Peritonitis: A Case Report and Review of Literature Srinivasa Iskapalli,[1] Deepthi Panjam,[2] Kavitha Kesari,[2] Raviprasenna K. Parasuraman.[3] *[1]Internal Medicine, Beaumont Health System, Royal Oak, MI; [2]Internal Medicine, McLaren Regional Medical Center; [3]Nephrology and Transplantation, Beaumonth Health System.*

Background: Sclerosing Peritonitis (SP) is a rare complication seen in ESRD patients on long-term peritoneal dialysis (PD) and/or in patients with recurrent bacterial peritonitis. SP is associated with high morbidity and mortality and a mortality rate of 56% was reported by Rigby et al.

Methods: A 72y-old female on PD for 16 years presented with vomiting and abdominal pain of 1 week duration. Two months prior to this presentation, she was treated for staphylococcus aureus peritonitis with antibiotics and Tenckhoff catheter removal. Patient evaluation showed distended tender abdomen with significant ascites and leukocytosis (WBC, 15,000/mL). CT of the abdomen showed significant peritoneal enhancement with large loculated ascites and mass effect on bowel loops. Ascitic fluid showed lymphocytic pleocytosis and was negative for bacterial infection. A diagnosis of SP was made based on duration of PD, recent peritonitis and CT findings and was initiated on low dose prednisone 15mg/day immunosuppression. Patient responded well with significant resolution of symptoms and loculated ascitic fluid on follow-up CT scan. Below is the picture showing CT scans before and after treatment over 6months period.



Conclusions: Treatment of SP requires cessation of PD with removal of catheter. Additional treatment such as bowel rest with parenteral nutrition, immunosuppression, tamoxifen and surgical viscerolysis depends on severity and duration. Kawanishi et al. reported 38.5% recovery for those receiving steroids. Commonly oral prednisone is initiated at 0.5-1mg/kg/day and tapered over 6 months depending up on the response and side effects. Early diagnosis and treatment is important to reduce SP related morbidities as demonstrated in this case.

SA-PO1090

Acute Bleeding and Dabigatran: Is Hemodialysis an Option? Tripti Singh,[1] Thin Maw,[1] Mark L. Unruh,[1] Kenneth R. Hallows,[1] Thomas D. Nolin.[2] *[1]Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, PA; [2]Department of Pharmacy and Therapeutics, University of PIttsburgh, Pittsburgh, PA.*

Background: Dabigatran (DB) is an oral, reversible-direct thrombin inhibitor for anticoagulation. It is FDA-approved for prevention of stroke in patients with atrial fibrillation. DB has predictable pharmacodynamics and is 80% renally excreted, so it does not require monitoring routinely. The half-life of the drug is prolonged in CKD as well as AKI. The rate of major bleeding in patients on DB is similar to the rate of major bleeding associated with warfarin. However, no specific antidote is currently available for reversal of the anticoagulant effect of DB. We hypothesized that hemodialysis (HD) could be an effective measure to eliminate DB and thus potentially life-saving in cases of acute bleeding with renal dysfunction.

Methods: Four patients on DB 150 mg po bid were admitted for acute bleeding in setting of renal dysfunction causing high DB levels. Factor VII was given to all patients to reverse the anticoagulant effect of DB. HD was performed to remove dabigatran.

Results: HD was effective in removing 55-65% of DB from blood with one HD session. However, there was a substantial rebound in the blood levels of DB >6 h after completion of HD.

Age (yrs)	Serum creatinine (mg/dl)	Reason for admission	HD Duration	Pre-HD DB Conc (ng/mL)	Post-HD DB Conc (ng/mL)	% Δ in DB Conc	Rebound DB Conc (ng/mL) / Time post-HD
77	1.2	Acute Abdomen	3 h	875	382	56% ↓	538/48 h
86	3.1	*SAH	4 h	318	115	64% ↓	437/12 h
65	1.4	GI Bleeding	2 h	1200	420	65% ↓	626/6 h
81	1.2	*SAH	4 h	269	118	56% ↓	44/24 h

* Sub Arachnoid Hemorrhage

Conclusions: DB is clinically dialyzable and HD can be effective in accelerating plasma clearance of DB, especially in patients with renal impairment. However, the benefits of HD should be carefully weighed against the risk of bleeding with HD catheter insertion in these patients with severely deranged coagulation profile. Further work is needed to better characterize the role of renal replacement therapy in the treatment of acute bleeding in the setting of DB use.

SA-PO1091

Impact of Diagnosing and Treating Primary Hyperparathyroidism in Chronic Kidney Disease Surju Patel,[1] Samer S. Nasser.[2] *[1]The Kidney Center, Johnston, PA; [2]Internal Medicine, Conemaugh Memorial Hospital, Johnstown, PA.*

Background: Intact parathyroid hormone (iPTH) levels are checked once every 3-6 months in stage 3 and 4 chronic kidney disease (CKD) patients. Vitamin D analogues (VDA) are used to treat renal osteodystrophy by targeting certain PTH level depending on the CKD stage. Few CKD patients present with hyperparathyroidism with mild hypercalcemia. Assumption of secondary hyperparathyroidism in patients with mild hypercalcemia or high normal calcium levels may preclude the use of VDA to prevent worsening of the hypercalcemia. There is limited data on primary hyperparathyroidism in the setting of CKD.

Methods: Two CKD patients present with hyperparathyroidism and hypercalcemia that worsened overtime. All VDA were discontinued. During the hypercalcemia work-up, a Sestamibi scan revealed solitary parathyroid adenomas in both patients. Parathyroidectomies on the 2 patients led to lowering of the iPTH levels with correction of the hypercalcemia. At least 6 months later, the 2 patients had iPTH levels that were off target with normal or low calcium, and were treated with VDA without ensuing hypercalcemia. One patient had improvement in the kidney function, after the correction of the hypercalcemia.

PTH level, Calcium levels, and Glomerular Filtration rate (GFR) pre- and post- parathyroidectomy

	Patient 1			Patient 2		
	iPTH (pg/ml)	iCa (mg/dL)	GFR (ml/min)	iPTH	Ca	GFR
preparathyroidectomy	961	12.3	25	193	11.3	22
postparathyroidectomy	171	8.8	28	152	9.1	36
3 months follow up after adding Vitamin D analogue	117	8.8	29	129	8.8	45

Conclusions: Some patients with hyperparathyroidism and hypercalcemia may remain misdiagnosed as secondary hyperparathyroidism especially if they are started on VDA and then develop hypercalcemia. Secondary Hyperparathyroidism may coincide with primary hyperparathyroidism and manifest later after the parathyroidectomy. After the correction of the hypercalcemia with parathyroidectomy, it is safe to target iPTH levels with VDA. Further guidelines for work-up and management may be warranted especially with the advent of new medical therapy for primary hyperparathyroidism.

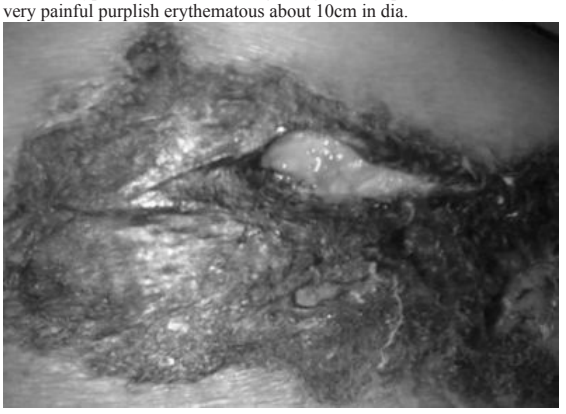
SA-PO1092

Calciphylaxis in a Patient on Hemodialysis, Pathological Clinical Study Carmen H. Sckell de Duarte. *Department of Nephrology, Facultad de Ciencias Médicas - UNA, Asuncion, Central, Paraguay.*

Background: Calciphylaxis is a condition that is increasing with the onset of CRF and HD in diabetics.

Methods: This clinical and pathological case that was studied, diagnosed and certified by skin biopsy.

Results: 47 years old white female patient with a with a HX of T2DM for 18 years at TX with crystalline insulin and obesity (BMI> 40 kg/m2), deadly CKD, regular HD periodic 2 years, presented thrombosis of the superior vena cava a year before, coagulated later with warfarin. Secondary anemia TX with erythropoietin.Secondary hyperparathyroidism TX with CaCO3 until a month ago. Previously hypertensive patient once the HD Tx, is prone to severe hypotension. 5 months beginning with lump in abdomen (umbilicus), indurated, very painful purplish erythematous about 10cm in dia.



The lesion grew covering the entire lower abdomen, becoming a crusty ulcer, necrotic, much more painful,not calmed by painkillers, including opioids. 2 months ago another similar lesion appeared in right breast. Ultrasound of soft parts: thrombosis in this region. Lesion interpreted as calciphylaxis. ECHO: mitral annular calcification, aortic valve opening and slightly calcified with preserved motility. Pelvis RX: shows little air density radiopaque calcium in soft tissues. CXR: mild calcification of the aortic knob. Lesion biopsy is performed, the results: calcific uremic arteriopathy (calciphylaxis) disseminated thrombophlebitis with perivascular inflammatory changes (panniculitis associated with vasculitis of medium caliber of the hypodermis). Hyaline arteriosclerosis.

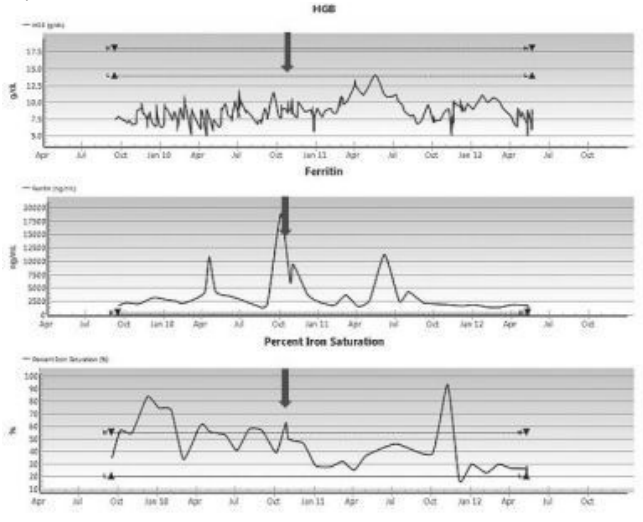
Conclusions: Through this study we characterized the group of sensitive patients to develop calciphylaxis and identify potential risk factors for the emergence of this disease, and thus facilitate prevention.

SA-PO1093

A Case of a 72 Year Old Man with ESRD and MDS with Anemia Which Improved with Desferrioxamine Therapy Ankita B. Patel, Ahmad Waseef, Subodh J. Saggi. *Nephrology, SUNY Downstate, Brooklyn, NY.*

Background: Management of anemia in patients with ESRD and MDS is a unique challenge as EPO requirements are huge.Also, they require multiple PRBC transfusions causing iron overload.

Methods: A 72 year old man with history of ESRD and MDS on HD since Sept '09 was severely anemic requiring massive doses of Epo in excess of 90,000 units/week.Fe sat was consistently >60%; ferritin was >2500. Desferrioxamine (DFO) 125 mg IV/wk was begun in Nov '10. His PRBC transfusion and Epo requirements declined after that. He had 33 ER visits for PRBC transfusions (1-3 transfusions/visit) from Sept '09 to Nov '10 (avg:2.35/mo) which decreased to 18 visits for in 20 months after getting DFO (avg:0.9/mo).His Hb and iron indices are as shown.



Conclusions: Iron excess increases oxidative stress:Excess non-tranferrin bound labile iron is a source of reactive oxygen species. It also causes a decrease in reduced glutathione(antioxidant) and increases membrane lipid peroxidation. Furthermore, iron overload itself has an *in vitro* suppressive effect on erythroid progenitors and seems to increase transfusion requirement.

Epo is regulated by hypoxia via HIF-α. At normal o2 tension the HIF-α undergoes post-translational modification by proline hydroxylation causing it to rapidly degrade. At low o2 tension, HIF-α escapes degradation and translocates into the nucleus stimulating Epo production.DFO has been shown to activate HIF-α in vitro, with kinetics similar to those associated with hypoxia, and to increase expression of HIF-1 target genes, including EPO.

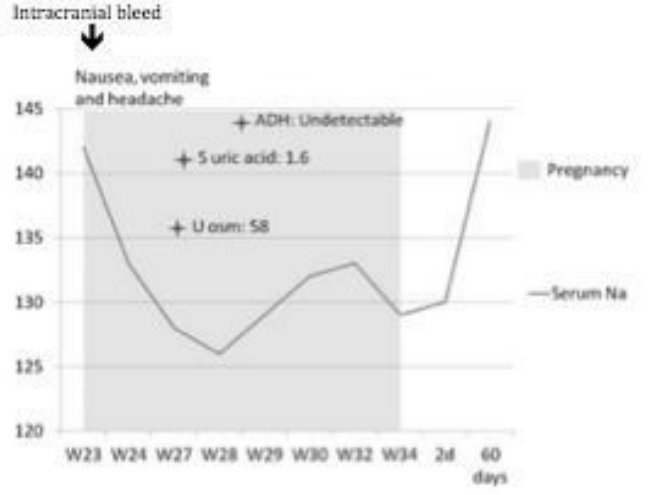
DFO promotes iron release from storage sites and seems to upregulate Epo response. It improves anemia and decreases transfusion requirements. Larger studies are required to validate it as a treatment option.

SA-PO1094

Hyponatremia: Does Intracerebral Bleeding Cause Thirst? Ashish Kataria,[1] Hitesh Patni,[1] Deepa Malieckal,[2] Kenar D. Jhaveri.[1] *[1]Division of Nephrology, Hoffstra North Shore LIJ School of Medicine, Great Neck, NY; [2]Department of Internal Medicine, Hoffstra North Shore LIJ School of Medicine, Manhasset, NY.*

Background: Hyponatremia is a common electrolyte derangement during pregnancy,but hyponatremia secondary to intense thirst after intracranial bleeding from arteriovenous malformation(AVM) has not been described.

Methods: We report a previously healthy,25-year-old woman who had sudden onset severe headache and nausea, 23 weeks into her first pregnancy. A MRI scan showed an AVM with intracranial bleeding in the lateral, third and fourth ventricles with mild hydrocephalus. She developed intense thirst and started drinking 8-10 liters of water each day. She was in no acute distress, euvolemic and her psychiatric evaluation was unremarkable. She developed persistent hyponatremia, with serum sodium ranging between 122-126 mmol/L



Her urine sodium and chloride were low and urine osmolarity ranged between 50-60 mosm/L. Upon water restriction, she retained the urinary concentrating ability and serum sodium increased, suggesting the etiology to be water intoxication. Her ADH level was undetectable (<0.6 pg/ml). She was managed with fluid restriction, and had a caesarean section at 34 weeks of pregnancy. Thirteen weeks after the bleeding in the brain, her serum sodium levels normalized with improvement in her thirst impulses.

Conclusions: Although hyponatremia has been reported with intracerebral bleeding, the etiology implicated mostly is anti diuretic hormone excess. We report a case of hyponatremia after intracerebral bleeding with suppressed ADH hormone, with intense thirst leading to excessive water ingestion. Our patient had a midline brain AVM, which could have contributed to the thirst. However, since the symptoms resolved with conservative management, it suggests the contribution of intracerebral bleed or possibly pregnancy related changes in AVM.

SA-PO1095

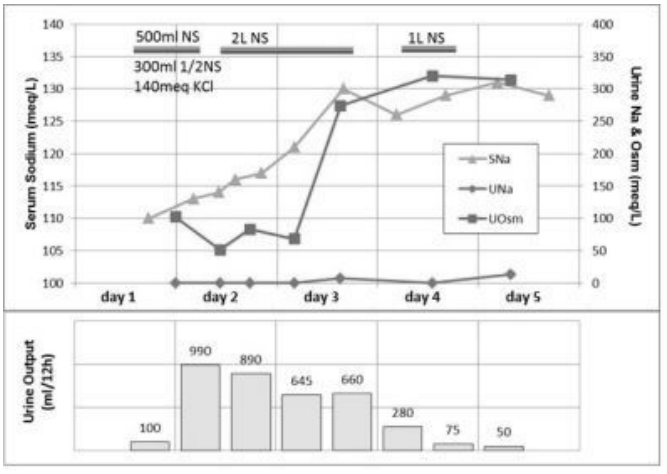
Hyponatremia: Don't Forget the Gut Matthew J. Volk. *Nephrology, University of North Carolina.*

Background: Evaluation of urinary indices is needed for analysis of most electrolyte derangements. In a nephrologist's enthusiasm to marshal related equations, stool and gut function can be missed as contributing factors.

Methods: A 29-year-old female with ulcerative colitis status post total colectomy and ileostomy was admitted with a sodium of 110 meq/L. Since surgery she had suffered high ostomy output and partial bowel obstruction. She had had poor intake aside from clear fluid and recently had developed oliguria and dizziness. On exam she had a pressure of 99/70 and a pulse of 118. She was alert with flat neck veins, clear lungs, and no peripheral edema. Labs appear below. Urine sodium was <5, so saline was infused. Ostomy output increased to over 2L per day of 89 meq Na/L solution, nearly equal to infusion sodium content. Though serum sodium corrected, urine sodium remained <5. Urine briefly became more dilute and plentiful with sodium exposure. It then became much more concentrated as the hyponatremic stimulus resolved. Urine sodium and output did not normalize until 3 weeks later, after completion of ileoanal anastomosis and initiation of TPN. At that point urine sodium was 146 meq/L.

Serum Electrolytes

	Admission	3 Weeks	6 Months
Na meq/L	110	131	141
K meq/L	2.0	4.5	4.3
BUN mg/dl	<2	5	7
Cr mg/dl	0.32	0.37	0.62



Conclusions: Three gut-related mechanisms contributed to the hypotonic hyponatremia. Sodium losses from the ileostomy resulted in volume depletion and low urine sodium, a complication discussed in the surgical literature. Partial obstruction limited the patient's protein intake over a long period resulting in low obligate solute excretion, consistently just 160 mosm/day (1.5L/day free water excretion). This effect has been reported with beer potomania and ovolactovegetarianism. Finally, the low free-water content of this patient's stool (424ml/day), also typical of end-ileostomies, limited the protective effect against hyponatremia typically conferred by diarrhea.

SA-PO1096

Clinical Features of Patients with Stress-Induced Cardiomyopathy Associated with Renal Dysfunction: 7 Cases Series Min Ji Shin, Eun Young Seong, Harin Rhee, Byeong Yun Yang, Sang Heon Song, Ihm Soo Kwak, Soo Bong Lee, Il Young Kim. *Department of Internal Medicine, Pusan National University School of Medicine, Republic of Korea.*

Background: Stress-induced cardiomyopathy(sCMP) is characterized by transient wall-motion abnormalities involving the left ventricular apex,precipitated by emotional or physical stress.As the heart and kidneys influence each other's function by bidirectional pathways,sCMP can induce renal dysfunction or be induced by renal dysfunction.These case series examined the clinical characteristics and outcomes of patients with confirmed sCMP associated with renal dysfunction.

Methods: Patients who met the following criteria of sCMP were included:(1) development of takotsubo on transthoracic echocardiography(TTE) after emotional or physical stress;(2)no evidence of obstructive coronary artery disease on coronary angiography(CAG);for those patients who could not undergo CAG,follow-up TTE showed improvement of LV function were used;(3)new ECG abnormalities or elevation in troponin.Acute kidney injury(AKI) was diagnosed on the RIFLE classification.There were 30 patients with sCMP From 2010 to 2012,and 7 patients were associated with renal dysfunction.3 patients were under prevalent hemodialysis(HD) and 4 patients had AKI(injury in 1,failure in 2 and ESRD in 1).3 of the 7 patients were women.The triggering events were pneumonia in 4 patients,infectious colitis in 2 patients,and RPGN in 1 patient. The mean ejection fraction(EF) in initial TTE was 36%,and in follow-up TTE,mean EF was 56%.Pericardial effusion was detected in all HD patients in initial TTE and 2 of them had uremic encephalopathy,and these patients were treated with intensive HD as suspected under-dialysis status.In AKI patients,mean of maximum level of serum creatinine(SCr) was 4.17 mg/dL and 2 patients were treated with CRRT.SCr restored to normal at 7 and 14 days,respectively in 2 patients.1 patient was required maintenance HD and 1 patient died because of recurrent ventricular tachycardia.

Conclusions: sCMP can be occurred in patient with renal dysfunction including ESRD or AKI.Additionally,as sCMP can induce renal dysfunction,renal function must be closely monitored.

PUB001

Epicatechin Prevents Renal Injury by Targeting Mitochondria in Mouse Cisplatin Nephropathy Katsuyuki Tanabe, Yoshifuru Tamura, Miguel A. Lanaspa, Richard J. Johnson, Takahiko Nakagawa. *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.*

Background: Cisplatin nephropathy is one of the common causes of hospital-acquired acute kidney injury, and it can be regarded as a mitochondrial disease. However, intervention to prevent such deleterious injury has not been established. Recently, epicatechin, a derivative from cocoa, emerges as a novel compound to protect mitochondria in cardiovascular and other organ systems. Here, we examined if epicatechin could prevent the kidney injury by protecting mitochondria in the mouse cisplatin nephropathy.

Methods: Kidney Injury was induced by intraperitoneal single injection of 30mg/kg cisplatin to 8-week old C57BL/6 mice, and epicatechin injection at dosage of 1mg/kg twice a day intraperitoneally was started 8 hours after cisplatin injection. Blood samples were collected and kidneys were harvested on day 3. Immortalized mouse proximal tubular cells (TKPTS) were stimulated by 100μM cisplatin for 1 hour, followed by treatment with or without 10^{-7}-10^{-6}M epicatechin for 4-24 hours.

Results: Cisplatin caused increase in serum creatinine while such elevation was significantly prevented by epicatechin (3.85±1.00 vs 1.86±0.86). This compound also ameliorated renal tubular injury (ATN score 3.7±0.7 vs 2.3±0.8) in association with a decrease in number of apoptotic cells. An increase in oxidative stress in cisplatin group was demonstrated by higher levels of nitrotyrosine in the renal cortex as well as isolated mitochondria while such oxidative stress was significantly reduced by epicatechin. Likewise, levels of the mitochondrial DNA amount and the mitochondrial proteins were also reduced by cisplatin yet epicatechin blocked such alterations. In in vitro study using mouse proximal tubular cells, epicatechin was shown to prevent the cisplatin-induced mitochondrial injury (dysfunction, decreased COX-IV level and fragmentation). Importantly, we confirmed that epicatechin did not disturb anticancer effect of cisplatin in the HeLa cells.

Conclusions: These results suggest that epicatechin prevents the progression of kidney injury in mouse cisplatin nephropathy. A mechanism is likely its mitochondrial protection.

Funding: Pharmaceutical Company Support - Cardero Therapeutics Inc.

PUB002

Alteration of the Gut Microbiome by Vancomycin: Impact on Renal Ischemia Tolerance Kieran Mccafferty, Conor J. Byrne, Julius Edward Kieswich, Magdi Yaqoob. *William Harvey Research Institute, Queen Mary University London, London, United Kingdom.*

Background: The importance of the intestinal microbiome in the pathogenesis of human disease is becoming increasingly recognized. Alteration of intestinal microbiota is associated with multiple disease states including obesity, diabetes and cardiovascular disease. A recent study[1] in rats demonstrated that alteration of gut microbiota using oral vancomycin, increased myocardial ischemia tolerance, which was mediated by a reduction in serum leptin. The role of leptin in ischemia reperfusion injury is controversial: leptin has been shown by different groups to both increase and reduce myocardial ischemia tolerance. The effects of vancomycin induced alteration in gut microbiota on renal ischemia reperfusion injury is unknown.

Methods: 24 male Wistar Rats were split into 2 groups. Group 1 were treated with Vancomycin 0.5mg/ml added to their drinking water for 7 days, Group 2 were given water with no additive. After 7 days both groups underwent a right nephrectomy followed by 45 minutes of left renal artery occlusion. The rats were then left to recover for 48 hours before being sacrificed with blood taken for markers of renal dysfunction and serum leptin.

Results:

	Control	Vancomycin	p
Creatinine (umol/l)	327 (203)	395 (200)	NS
Urea (mmol/l)	48 (28)	55 (21)	NS
Potassium (mmol/l)	4.7 (2.3)	4.7 (1.3)	NS
Sodium (mmol/l)	142 (6)	145 (5)	NS
Phosphate (mmol/l)	4.4 (1.9)	5.1 (2.1)	NS
AST (U/l)	82 (32)	67 (19)	NS
Leptin (ng/ml)	13.0 (6.4)	8.1 (3.2)	0.04

Values diaplayed as Mean (SD), with p value results of 2 tailed t test

Conclusions: In this study we confirm that treatment with vancomycin leads to a reduction in serum leptin levels. However, in contrast to the heart, this does not result in improved renal ischemia tolerance. 1. Lam V et al. Intestinal microbiota determine severity of myocardial infarction in rats. *FASEB J*. 2012;26:1727-1735.

Funding: Other NIH Support - National Institute of Health Research UK

PUB003

The Effect of Dietary Supplementation with Alpha Lipoic Acid in Young Rats on Reduced Glutathione Levels in Mitochondria from Kidney Cortex and Medulla Marianna J. Zamlauski-Tucker, Halah Albar, Robert J. Faulkner, Bingwei Ye. *Physiology & Health Science, Ball State University, Muncie, IN.*

Background: A previous study reported that mitochondrial reduced glutathione (GSH) levels in kidneys from young rats did not increase when the rats were given dietary antioxidant supplementation. GSH is the principal antioxidant inside cells and its level is maintained to protect cells against oxidative injury. The present study investigated whether dietary supplementation with alpha lipoic acid affects mitochondrial GSH levels in kidney cortex and medulla from young rats.

Methods: Young (i.e., 3 months of age) female Lewis rats were given D,L-alpha lipoic acid (100 mg/Kg body wt) via intraperitoneal injection for one week. Age-matched control rats were not given any exogenous supplementation. The kidneys were harvested at the end of the treatment period and separated into cortical and medullary sections. The sections were further separated into cytosolic and mitochondrial fractions by differential centrifugation. GSH levels in the fractions were measured using a spectrophotometric assay. Statistical comparisons were done using a Student's t test.

Results: There were significant increases in both mitochondrial and cytosolic GSH levels in kidney cortex and medulla from young rats receiving dietary supplementation with alpha lipoic acid.

Kidney GSH Levels

		Control (n = 8)	Experimental (n = 6)
GSH - Cytosol	Cortex	5.5 ± 0.4	9.9 ± 0.2 *
μmol/g kid wet wt	Medulla	3.07 ± 0.2	6.3 ± 0.3 *
GSH - Mitochondria	Cortex	100 ± 6	157 ± 11 *
nmol/g kid wet wt	Medulla	92 ± 7	174 ± 8 *

All data expressed as X ± SEM. * Significantly different ($p < .05$) from Control

Conclusions: Dietary antioxidant supplementation in young rats is effective in increasing both mitochondrial and cytosolic GSH levels in kidney cortex and medulla.

PUB004

Shortening of Primary Cilium in Cisplatin-Induced Tubular Cell Apoptosis Shixuan Wang, Zheng Dong. *Cellular Biology and Anatomy, Georgia Health Sciences University and Charlie Norwood VA Medical Center.*

Background: Cilium is an organelle protruding from the apical surface of the cell. Most of the cells in human body have cilia. In the kidney, each tubular epithelial cell contains only one cilium, termed primary cilium or mono-cilium. It has been proposed that primary cilia of kidney tubular cells are mechanosensors to fluid flow. Interestingly, ciliary dysfunction in polycystic models sensitizes animals to acute kidney injury (AKI) induced by renal ischemia-reperfusion, although the underlying mechanism is unknown.

Methods: This study examined the change of primary cilium and is role in tubular cell apoptosis induced by cisplatin, a chemotherapy agent with notable nephrotoxicity.

Results: In cultured human proximal tubular epithelial cells (HK-2), cilium became shorter during cisplatin treatment, followed by apoptosis. To determine if ciliary shortening affects apoptosis, HK-2 cells were transfected with shRNAs targeting KIF3A and TG737, respectively. Ciliary length and frequency were reduced in the knockdown cells. Cisplatin induced higher apoptosis rate and caspase activity in KIF3A/TG737 knockdown cells than that in the non-target sequence transfected cells. We further subcloned HK-2 cells and derived cell clones with long or short cilia. HK-2 clones with shorter cilia were more sensitive to cisplatin-induced apoptosis.

Conclusions: Taken together, the results suggest a role of ciliary regulation in tubular apoptosis during AKI.

Funding: NIDDK Support, Veterans Administration Support

PUB005

EPO Intervention on Controlling of Acute Kidney Injury Repair by Bone Marrow Mesenchymal Stem Cells Transplantation for Mice and Its Possible Mechanism Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: To construct Acute Kidney Injury (AKI) mouse model which is induced by ischemia/reperfusion (I/R). Bone marrow mesenchymal stem cells (BM-MSCs) transplantation has been taken through tail vein injection, observing controlling of exogenous EPO on AKI repair by BM-MSCs transplantation, the role of SDF-1/CXCR4 axle in the controlling effect has also been discussed.

Methods: Altogether 100 C57BL/6 mice had been randomly divided into normal group, model group (AKI group), BM-MSCs transplantation group, EPO intervention group, EPO intervention + BM-MSCs transplantation group, transplanted BM-MSCs were marked by BrdU. Some mice had been sacrificed after the model been constructed 1d, 3d, 7d, and 14d respectively; arterial blood was reserved for testing blood BUN and Scr level; pathological changes for nephridial tissue have been observed and the injury degree for renal tubular has been scored; BM-MSCs distributed in nephridial tissue for recipient mice were observed with immunohistochemistry method and SDF-1 level in injured nephridial tissue was tested by Western Blot.

Results: Contrasted with BM-MSCs transplantation group and EPO intervention group, in EPO internvention + BM-MSCs transplantation group, BUN, Scr and ATN scores were declined sharply; on the 14th day after the transplantation, BUN, Scr degrees for the mice were even close to that of normal group. IHC shown that $BrdU^+$ cells distribution could be detected in the mice kidneys for BM-MSCs transplantation group, and the value reached to the peak on the 7th day after the surgery [(11.32±1.38)%]; EPO intervention combined with BM-MSCs transplantation could increase the number of $BrdU^+$ positive cells in nephridial tissue. SDF-1 level was raised for nephridial tissue after AKI model been constructed, which went to the peak on the 7th day after the model been constructed, and EPO intervention could obviously increase SDF-1 expression.

Conclusions: EPO intervention could increase the number of transplanted cells after BM-MSCs transplantation, enhance AKI repair effect. The high level of SDF-1 expression, which could promote stem cells homing to kidneys, may be one of possible mechanisms.

Funding: Government Support - Non-U.S.

PUB007

JNK Signalling Is Required for LPS-Induced Albuminuria in Mice

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Background: Activation of c-Jun amino terminal kinase (JNK) signalling has been shown to promote leukocyte-dependent proteinuria in rat models of nephrotoxic serum nephritis. However, recent studies have reported that blockade of JNK signalling exacerbates podocyte injury and albuminuria in models of diabetic nephropathy. We sought to examine the role of JNK signalling in other models of proteinuria kidney injury. Systemic administration of lipopolysaccharide (LPS) to mice induces podocyte damage and transient induction of albuminuria. *In vitro* studies have shown that LPS-induced signalling via its receptor (TLR4) operates, in part, via JNK. Therefore, the aim of this study was to determine whether JNK signalling is required for LPS-induced albuminuria *in vivo*.

Methods: Male C57Bl/6J mice were given an intraperitoneal injection of 5mg/kg LPS and 24hr later a 6hr urine collection was performed. Mice were treated with the selective JNK inhibitor CC-930 (60mg/kg/BID) or vehicle, beginning 1hr before LPS and then killed at 4hr (n=2) or 30hr (n=10).

Results: At 4hr after LPS administration, vehicle treated mice showed a dramatic activation of JNK signalling (immunostaining for phospho-c-Jun Ser63) in glomerular cells, including podocytes, and in tubular epithelial cells. This was associated with a 300-fold and 30-fold increase in NOS2 and TNF-α mRNA levels in the kidney, respectively. At 30hr, vehicle treated mice exhibited a 10-fold increase in the urine albumin to creatinine ratio (ACR) and a 67% reduction in nephrin mRNA levels. CC-930 treatment prevented JNK activation and abrogated the up-regulation of NOS2 and TNF-α mRNA levels at 4hr after LPS administration. Furthermore, CC-930 treatment reduced urine ACR by 75% compared to vehicle controls and prevented the down-regulation of nephrin mRNA levels at 30hr after LPS administration.

Conclusions: LPS-induced podocyte damage and albuminuria is dependent upon JNK signalling. These data suggest that blockade of JNK signalling may be a novel therapeutic strategy for sepsis-induced acute renal failure.

Funding: Government Support - Non-U.S.

PUB008

Oxidative and Carbonyl Stress: Hemoglobin Modification in Snakebite Mediated Acute Kidney Injury: A Pathogenesis Link

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Background: The aims of the study were to evaluate structural modification of hemoglobin, oxidative and carbonyl stress in snakebite mediated acute kidney injury (SAKI) patients and established a pathogenesis link.

Methods: Demographical, clinical and biochemical data was recorded in 175 SAKI patient prospectively.Various biophysical characterization of purified Hb including tryptophan quenching, surface hydrophobicity, synchronous fluorimetric study were carried out with the blood sample of SAKI patients. Non-heme iron (NHI) and heme degradation product (HDP) were measured from hemolysate. Advance oxidation protein product (AOPP), Pentosidine, Dityrosine, Thioberbituric acid reactive substance and methyl glyoxate (MG) measured as oxidative and carbonyl stress marker.All data were analyzed with appropriate statistical methods.

Results: Hb degradation and modification increases in SAKI patients. Elevated HDP, PFA and NHI level indicate denaturation and oxidative damage of heme moiety. Increased intrinsic fluorescence of Hb, elevated hydrophobicity of the globin chain and FRET changes indicate a alteration in the secondary and tertiary structure of Hb in SAKI patients. Synchronous studies indicate alteration in tryptophan and tyrosine micro environment of Hb as well as unstable quarternary structure in SAKI patients. These alterations may leads to increased ROS generation. AOPP and MG was also increased linearly suggesting oxidative stress.This is the initiator of inflammatory cascade leading to acute kidney injury.

Conclusions: Structural modification of Hb in SAKI associated with increased non-heme iron and heme degradation product which may lead to functional alteration and profound oxidative and carbonyl stress leading to kidney injury.

PUB009

A Porcine Model of Acute Kidney Injury to Investigate Putative Renoprotective Factors

Mark A.J. Devonald,[1,2] Simon J. Welham,[2] Louise J. Lloyd,[2] Thomas A. Mcculloch,[1] Zsolt Hodi,[1] David S. Gardner.[2] *[1]Nottingham University Hospitals NHS Trust, United Kingdom; [2]University of Nottingham, United Kingdom.*

Background: Ischemia-Reperfusion Injury (IRI) has been studied extensively in rodents but the pig is a better model for human disease. We developed a porcine IRI model that produces mild histological acute tubular injury (ATI). We characterised blood and urine biochemistry and investigated 2 putative renoprotective interventions: erythropoietin (EPO) and remote ischemic preconditioning (RIPC). Primary outcomes were plasma creatinine and histological scoring of ATI. Secondary outcomes included urine electrolytes, plasma and urine biomarkers (NGAL, KIM-1), apoptosis and histology of distant organs (lung, liver, brain).

Methods: 30 pigs were anaesthetised and both renal arteries clamped (40min) or sham operated via abdominal incision. Reperfusion followed for 48h with blood and urine sampled at intervals. Design was 2 (sham/IR) × 3 (saline/EPO/RIPC) factorial, n=5 pigs/group (30 total) with 90% power to observe moderate AKI in 4/5 untreated IR pigs and 0/5 sham control pigs. EPO (1000 iu/kg) was given as i.v. bolus 30min before sham/IR. RIPC was conducted 30min before sham/IR and comprised 3×cycles of 5min inflation/deflation (200mmHg) of cuff around hind-leg followed by 3×cycles of 5min deflation.

Results: IRI elicited a significant (P<.001) increase in kidney mass. This was accompanied by early (<2h) pronounced (from baseline 140 to 250 umol/L at 8-24h after IR) increments in plasma creatinine. Plasma alanine aminotransferase increased significantly in controls but more so after IR, suggesting further liver injury. Urine albumin:creatinine ratio peaked +2h after IR, declining to baseline by 24h. Plasma osmolality did not deviate from baseline but urine osmolality declined significantly. From plasma and urine biochemistry, EPO or RIPC appeared to offer no significant renoprotection.

Conclusions: We have established a large animal model of AKI and have conducted preliminary analyses of plasma and urinary biochemistry to characterise moderate renal and distant organ injury. Histological assessment of injury in kidney at +24 and 48h and in liver and lung at +48h are continuing and will be presented.

Funding: Private Foundation Support

PUB010

Renal Treg Recruitment by DMS Is Not due to De Novo Conversion

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Background: Regulatory T cells (Tregs) have become an immunotherapeutic agent for treating inflammatory processes. Previously, we reported that pretreatment of mice with a sphingosine derivative N, N-dimethylsphingosine (DMS) increases both tissue infiltrating T effectors (Teff) and Tregs in the early phase of renal ischemia/reperfusion injury (IRI), and protects mice from IRI. This Treg recruitment plays a key role in DMS-induced renoprotection because depleting or disabling Treg abolishes renal protection. The peripheral Tregs can be induced by homing and/or de novo conversion. In this study, we used adoptive transfer to determine if DMS causes homing or de novo conversion using $Foxp3^{EGFP}$ mice as a donor, which express EGFP/Foxp3 fusion protein via an endogenous Foxp3 promoter.

Methods: We isolated CD4+EGFP- (Teff) and CD4+EGFP+ (Treg) cells respectively from splenocytes which were first subjected to a negative selection to enrich for CD4+ cell then the flow cytometry sorting to separate EGFP+ cells from EGFP- cells. The EGFP- cells (10^6 cells, > 98% purity) were transfused to wild type BALB/c mice via tail vein injection. On day 4, the recipients were treated with either vehicle or DMS (0.43 mg/kg) for 1 h then kidney, spleen, thymus, bone marrow, and peripheral blood were obtained. Cell suspensions were subjected to CD4 staining and flow cytometry analysis to quantify the CD4+EGFP+ cells. If DMS induces de novo conversion, we expect to see the EGFP+ cells in organs of mice that received EGFP- cells.

Results: We found no EGFP+ cells in any of the organs we tested in either group, suggesting that de novo conversion is not the mechanism of DMS-induced Treg recruitment. In addition, DMS was found to up-regulate the renal expression of chemokines CXCL9 and CXCL10, which are ligands for CXCR3, a key receptor for Treg migration signaling.

Conclusions: Collectively, our findings suggest that DMS may induce renal expression of chemokines, which attract Tregs into the inflamed site and offer the protection against IRI.

Funding: NIDDK Support, Private Foundation Support

PUB011

The Effects of Extracorporeal Shock Wave for Renal Ischemia Reperfusion Injury in Rats

Mai Yoshida,[1] Takashi Nakamichi,[1] Takefumi Mori,[1] Kenta Ito,[2] Hiroaki Shimokawa,[2] Sadayoshi Ito.[1] *[1]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan.*

Background: Extracorporeal shock wave (ESW) is a novel potential and safe treatment for ischemic disorders such as angina pectoris and peripheral artery diseases by mediating angiogenesis. We designed this study to investigate whether ESW ameliorated ischemic AKI.

Methods: We created renal ischemia reperfusion (I/R) models on male 8-week-old Sprague-Dawley rats. Under general anesthesia, the left renal pedicles were clamped for 45 min, and then declamped. The operated rats were divided into ESW group (ESW, n=8) and control group (CON, n=8). Both kidneys of only ESW group were treated with ESW (200 shocks/day for each kidney, 0.09 mJ/mm^2) just after I/R operation, and 24, 48 hours after the operation. Immediately after the third ESW treatment, the rats of both groups were sacrificed to analyze kidney weights, renal histological changes, and renal function.

Results: Among CON group, the left, I/R operated, kidney weight was significantly increased compared with the right kidney (Lt 0.52 ± 0.02 vs. Rt 0.39 ± 0.01 g/100 g body weight). The left kidney weight of ESW group was light compared with that of CON group (ESW 0.45 ± 0.02 vs. CON 0.52 ± 0.02 g/100 g body weight). Plasma creatinine of ESW group was significantly lower than CON group (ESW 0.32 ± 0.03 vs. CON 0.43 ± 0.03 mg/dl). Elastica-Masson stain revealed that tubular injury scores in the outer medulla of ESW group were significantly lower than CON group (ESW 1.6 ± 0.2 vs. CON 2.0 ± 0.1).

Conclusions: ESW ameliorates tubular injury and recovers renal function in a rodent I/R model. ESW may be an effective, safe, and noninvasive therapy for ischemic AKI, although a further investigation is required to solve the underlying mechanisms.

PUB012

Egr-1 Mediates Hypoxia-Induced Epithelial-Mesenchymal Transition via PKC/ERK Pathway in Tubular Epithelial Cells Shiren Sun,[1] Lijie He,[2] Hanmin Wang.[3] *[1]Xijing Hospital, FMMU; [2]Xijing Hospital, FMMU; [3]Xijing Hospital, FMMU.*

Background: Tubular epithelial cells under hypoxia can undergo Epithelial-mesenchymal transition (EMT), and finally lead to renal fibrosis. Egr-1, as a transcription factor, is proven to be important in promoting EMT and be induced by hypoxia in different cell lines. However, the role and underlying mechanisms of Egr-1 in hypoxia-induced renal tubular cells EMT are not well known.

Methods: Western blot (WB)and quantitative reverse transcriptase PCR was performed to detect the protein and mRNA level of Egr-1 under low oxygen. Tubular epithelial cells (HKC and HK-2 cells) were treated Calphostin C or PD98059 under low oxygen to explore the underlying mechanisms of hypoxia-induced Egr-1. Sense and siRNA vector of Egr-1 was transfected in HK-2 and HKC cells to investigate the role Egr-1in hypoxia-induced EMT. SiRNA against snail was done to elucidate the downstream pathway of Egr-1. The expressions of HIF-1α, Egr-1 and Snail in kidney tissues from 5/6- nephrectomized rat model and CKD patients were detected by immunohistochemistry and WB.

Results: Hypoxia enhanced the expression of Egr-1 in tubular epithelial cells, which started at 0.5h and reached to peak at 4h and persists to 72h in HK-2cells. Overexpression of Egr-1 in both HKC and HK-2 cells promoted a mesenchymal phenotype accompanied by reduced expression of the epithelial marker E-cadherin and increased expression of the mesenchymal markers FSP-1, while Egr-1 knockdown by siRNA effectively reversed hypoxia-induced EMT. Calphostin C or PD98059, specific inhibitors to PKC or MAPK/ERK could abolish hypoxia-induced Egr-1, indicating hypoxia induced Egr-1 through PKC/ERK pathway. Egr-1 promoted the expression of snail under hypoxic conditions through transactivation of the snail promoter, which in turn upregulated FSP-1 and downregulated E-cadherin. In 5/6-nephrectomized rats and CKD patients' kidney tissues, Egr-1 and Snail were overexpressed in tubular epithelial cells showing EMT.

Conclusions: Our results indicate that hypoxia induced Egr-1 expression through PKC/ERK pathway and Egr-1 mediates hypoxia-induced renal tubular epithelial cells EMT and renal fibrosis through the activation of the snail pathway.

PUB013

Gender Differences Control Susceptibility to ER-Stress Induced Acute Kidney Injury Rawad Hodeify,[1] Judit Megyesi,[1] Adel Tarcsafalvi,[1] Nang San Hti Lar Seng,[1] Peter M. Price.[1,2] *[1]UAMS; [2]VA Med Ctr.*

Background: ER stress contributes to acute kidney injury induced by several causes.

Methods: Mice were injected intraperitoneally (1 mg/kg body weight) with tunicamycin, an agent known to cause ER stress. Renal function and histology were assessed 3 or 5 days after injection. In addition, a group of female mice was treated with testosterone (5 mg pellet) before tunicamycin.

Results: We observed differences in the severity of kidney injury between male and female mice in response to tunicamycin. Treated male mice showed a severe decline in kidney function (BUN and creatinine), and extensive damage in the outer cortex of the kidney (S1 and S2 segments). Kidney function in female mice did not decline, although their kidneys showed damage of the inner cortex (S3 segment). Protein markers of ER stress were also more elevated in male mice compared with females. Specifically, levels of glucose-regulated protein (GRP-78/Bip), and CHOP/GADD153 were significantly higher in tunicamycin-treated male mice, and was localized primarily to nuclei of proximal tubule cells that showed signs of injury. RT-PCR analysis showed that the splicing of XBP1 (X-box binding protein 1) to its active form (XBP1S), was higher in male than female mice after tunicamycin. Furthermore, staining for active XBP1 was significantly increased in proximal tubules in the deep cortex in male mice, while female mice showed very few active-XBP1-stained nuclei, that mainly localized to the outer cortex. Apoptosis induction, (Bax and caspase-3 activation), was also higher in tunicamycin-treated male mice. Testosterone pretreatment of female mice before tunicamycin exposure resulted in a phenotype similar to male mice with a comparable decline in renal function, and similar kidney damage and induction of ER stress markers.

Conclusions: Male mice are much more susceptible to ER stress-induced AKI than females, and this sexual dimorphism is due to testosterone. ER stress and gender could play a major role in promoting kidney injury. The effect of this sexual dimorphism could provide further insight to understand the relationship between segment-specific damage and kidney function.

Funding: NIDDK Support, Veterans Administration Support

PUB014

Pretreatment with Paricalcitol Attenuates Inflammation from Ischemia-Reperfusion Injury in Mice Hyeonseok Hwang,[1] Yoon-Kyung Chang,[1] Cheol Whee Park,[2] Suk Young Kim,[1] Sangju Lee,[1] Chul Woo Yang.[2] *[1]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, Daejeon, Korea; [2]Division of Nephrology, Department of Internal Medicine, The Catholic University of Korea, Seoul, Seoul, Korea.*

Background: The effect of paricalcitol on renal ischemia–reperfusion injury (IRI) has not been investigated. We examined whether paricalcitol is effective in preventing inflammation in a mouse model of IRI, and evaluated the prostaglandin E2 (PGE2) pathway as a potential protective mechanism of paricalcitol.

Methods: Paricalcitol (0.3 mg/Kg) was administered to male C57BL/6 mice 24 h before IRI. Bilateral kidneys were subjected to 23 min of ischemia, and mice were killed 72 h after IRI. The effects of paricalcitol on renal IRI were evaluated in terms of renal function, tubular necrosis, apoptotic cell death inflammatory cell infiltration and inflammatory cytokines. The effects of paricalcitol on PGE2, its synthetic enzymes and receptors were also investigated.

Results: Paricalcitol pretreatment improved renal function (decreased blood urea nitrogen and serum creatinine levels), and renal pathology (decreased tubular necrosis and apoptotic cell death) in IRI-mice kidneys. The infiltration of inflammatory cells (T cells and macrophages), and production of proinflammatory cytokine (RANTES, tumor necrosis factor-a, interleukin-1b and interferon-g) were reduced in paricalcitol-treated mice with IRI. Paricalcitol upregulated cyclooxygenase-2 expression and PGE2 synthesis in postischemic renal tissue. EP4 mRNA expression increased in paricalcitol-treated mice with IRI, whereas it did not affect EP2 mRNA expression.

Conclusions: Our study demonstrates that paricalcitol pretreatment prevents renal IRI via inhibition of renal inflammation, and we propose upregulation of PGE2 and EP4 as protective mechanisms of paricalcitol in IRI.

PUB015

Renoprotection Derived from Regulatory Changes of OAT1/3 in Ischemic Acute Kidney Injury (iAKI) Marcus Meusel,[1] Michael Kersten,[1] Christopher Held,[1] Boris Betz,[1] Kerstin Möller-Ehrlich,[2] Christoph Wanner,[1] Reinhard Schneider.[1] *[1]Dept. Nephrology, University Hospital Wuerzburg; [2]Center of Experimental Molecular Medicine (ZEMM), Wuerzburg; [3]Clinic of Anaesthesiology, University Hospital Halle, Germany.*

Background: Prostaglandin E_2 (PGE_2) diminishes expression organic anion transporters (OAT1, OAT3) in proximal tubules (PT). In iAKI, OAT down regulation is induced by the COX inhibitor (indomethacin [INDO]) in low doses, and correlates with improvment of renal function. INDO accumulates in proximal tubular cells (PCTs) when internalized by OAT1/3. Thus, we applied a competetive inhibitor of OAT (probenecid [PBC]) to block INDO uptake into PTs with the aim to abolish the effect on OAT1/3 and renal function.

Methods: iAKI was induced in rats by bilateral clamping of renal arteries for 45 min. INDO (1mg/kg) +/- PBC (50mg/kg) was applied i.p. before reperfusion. 24h after ischemic injury the clearance of inulin (C_{IN}, resp. GFR) and para-aminohippuric acid (C_{PAH}, resp. RPF) were measured. PAH net secretion (NS_{PAH}) was determined to elucidate transporter capacities and respective expressions (OAT1/3). Protein- and mRNA-expression were analyzed in renal cortical, and ED1+ infiltration was depicted by immunohistology.

Results: In iAKI INDO restored OAT1/3 expression as well as NS_{PAH} net secretion and PGE_2 clearance. Additionally, INDO substantially improved renal function as measured by C_{IN} and C_{PAH} clearance. Besides, renal cortical apoptosis and iNOS expression was diminished. Notably, low-dose INDO did not affect inflammatory parameters in the renal cortex (e.g. MCP-1, ED1+-cells). In addition, PBC blocked the restoration of OAT1/3 expression induced by INDO. Actually, PBC moreover abrogated all the beneficial effects of INDO on renal outcome described above.

Conclusions: (I) Beneficial effect of low dose INDO is not due to its anti-inflammatory potency, but (II) due to its effects on regulation of OAT expression. PBC competitively inhibits INDO uptake into the PCT, thereby (III) restoring PGE_2 induced down regulation of OAT after iAKI and re-establishing renal damage. This is (IV) evidence for a mechanistic role of OAT1/3 in renoprotection in iAKI.

Funding: Government Support - Non-U.S.

PUB016

Aging Enhances Renal Tubular Cell Apoptosis Following Renal Ischemia-Reperfusion Injury Zygimantas C. Alsauskas, Madhavi J. Rane, Kenneth R. McLeish. *Kidney Disease Program, University of Louisville, Louisville, KY.*

Background: Aging is associated with an increased risk of acute kidney injury following renal ischemia-reperfusion (IRI). Apoptosis of renal tubular cells contributes significantly to renal dysfunction in IRI. The effect of age on development of tubular cell apoptosis following IRI is unclear. This study tested the hypothesis that advanced age enhances tubular cell apoptosis and renal dysfunction in mice with IRI.

Methods: Young (7-20 weeks, n=6) and old (84 weeks, n=3) male C57B6 mice underwent renal ischemia-reperfusion via bilateral renal pedicle clamping for 20 minutes at 37C, followed by sacrifice after 24 hours of reperfusion. Plasma creatinine was measured at sacrifice, and TUNEL+ cells in sections of fixed kidney were counted in outer medulla (at least 10 HPF in at least 2 sections).

Results: Creatinine was routinely less than 0.5 mg/dL after 24 hr in mice following sham-surgery. Creatinine elevation after 24 hr did not differ between younger mice (2.22±0.35 mg/dL) and older mice (2.13±0.18 mg/dL) with IRI, p=0.86. Older mice

exhibited lethargy and hypothermia at 24 hours that was not observed in younger mice. The mean number of TUNEL+ cells per HPF was significantly increased in older mice (23.2±2 cells/HPF) compared to younger mice (11.9±1.1 cells/HPF), p=0.01.

Conclusions: Renal tubular epithelial cells in older mice are more susceptible to apoptotic cell death following ischemia-reperfusion, which may explain increased susceptibility to ischemic kidney injury observed in aged animals. Despite an increase in apoptosis, no differences in the early loss of renal function were observed.

PUB017

Role of Estrogen Receptor α and β in Ischemia/Reperfusion-Induced Acute Kidney Injury Rodrigo Maranon,[1] Arnaldo F. Lopez-Ruiz,[2] Andrea P. Soljancic,[2] Kiran B. Chandrashekar,[2] Ruisheng Liu,[1] Luis A. Juncos.[1,2] *[1]Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS; [2]Medicine/ Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: Premenopausal females are less susceptible to I/R-AKI than males. We previously found that this protective effect is eliminated by blocking the ERα with fulvestrant (FVT). In the present study, we examined whether ovariectomizing female rats (OVX) mimics the effects of FVT, and then examined the role of each of the two estrogen receptors (ERα and ERβ) and their interactions with the cytoprotectant HO in mediating the protective effects of estrogens on I/R-AKI.

Methods: We induced I/R-AKI (40min of bilateral clamping) in intact and OVX female Sprague-Dawley rats. In addition, subsets of OVX rats received either PPT (an ERα agonist) or DPN (ERβ agonist). Renal function (plasma creatinine), injury (KIM-1), inflammation (TNFα) and HO-1 expression were determined 24 hours after ischemia.

Results: OVX and FVT-treated rats had more severe I/R-AKI than intact rats. Administration of either PPT or DPN to OVX rats ameliorated I/R-AKI.

	PL. Creat	TNFα	HO-1	Urine KIM-1
Sham-intact	0.4 ±0.03	0.3±0.04	0.2±0.07	9351±3
I/R-AKI	1.7±0.2	1±0.1	1.2±0.1	148980±1
I/R-AKI + FVT	2.4±0.4	2±0.2	0.79±0.1	183532±1
Sham-Ovx	0.7±0.3	0.3±0.02	0.73±0.3	51409±2
I/R-AKI-Ovx	3±0.1	0.6±0.1	1.6±0.1	676554±4
I/R-AKI -Ovx +PPT	1.8±0.1	0.3±0.02#	2.3±0.1	411578±3
I/R-AKI -Ovx +DPN	2±0.1	0.3±0.01#	2.05±0.1	479708±2

Data: Mean±SEM: *p<0.05vs Sham; # p<0.05vs AKI

Conclusions: The decreased susceptibility to I/R-AKI in females appears to be due to activation of both estrogen receptors; OVX exacerbated injury, whereas administering agonists of either ER ameliorated injury. Activation of the estrogen receptors facilitates AKI-mediated induction of HO-1, which may be responsible for their protective effects against I/R-AKI.

PUB018

Increased Endogenous Hydrogen Sulfide Formation Exacerbates Renal Damage Induced by Cisplatin Injection Heloisa Francescato, Cleonice G. Da Silva, Terezila Machado Coimbra. *Physiology, University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazil.*

Background: Cisplatin (CP)-induced renal damage is associated with an inflammatory process. Hydrogen sulfide (H_2S) is an important signaling molecule involved in inflammation. The inflammatory process is more intense five days after CP injection.This study evaluated the effect of *Lawesson* reagent (LR), a donor for endogenous H_2S formation, on the renal damage induced by CP in rats.

Methods: Twelve rats were injected with CP (5 mg/kg, i.p.), 6 of them received LR (30 µmol/kg/once a day, s.c.), for four days, starting 1 hour before CP injection. Six control rats were injected with 0.15 M NaCl. Blood and urine samples were collected 5 days after saline or CP injections to evaluate plasma urea levels, and sodium and potassium fractional excretions. The kidneys were removed for determination of renal H_2S formation and histological analysis. Immunohistochemistry for vimentin expression in renal tissue, a marker of recent tubular cell lesion, was also performed.

Results: CP-treated rats presented increases in plasma urea levels and in sodium and potassium fractional excretions. Treatment with LR exacerbated all these functional alterations. Plasma ureia levels [mg/dl; expressed as median and interquartile range (25;75%)] were 173.8 (140.0;227.1) in CP-injected rats, 287.1 (83.0;317.4) in the rats treated with LR+CP and 43.4 (34.1;48.8) in control rats (p<0.05). The score for tubulointerstitial lesions (tubular necrosis, interstitial inflammatory infiltrate, tubular lumen dilation) were increased in the renal outer medulla in both groups injected with CP. A greater immunostaining for vimentin on outer medulla tubular cells, evaluated by scores, was observed on LR+CP-group [2.2 (1.8;2.6)], compared to CP [1.4 (1.2;1.8)] and control [0.2 (0.1;0.2)] groups. These alterations were associated with increased H_2S formation in LR+CP group (79.5±10.5 µg/mg prot/h), compared to CP (60.3±5.8) and control (32.4±4.2) animals (p<0.05).

Conclusions: In conclusion, treatment with a donor for H_2S formation aggravates the renal lesion induced by CP. Research Support: CNPq.

Funding: Government Support - Non-U.S.

PUB019

Protein Kinase C-α Regulates Mitochondrial Function and Cell Survival by Associating with Heat Shock Proteins Grazyna Nowak, Sridharan Soundararajan. *Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: Primary cultures of renal proximal tubular cells (RPTC) respond to stress by synthesizing different heat shock proteins (HSP), including HSP70, which prevents cell death following ATP depletion. HSP70 interacts with PKC-α to protect ventricular myocytes against ischemia. The goal of this study was to determine whether PKC-α associates with HSP70 in RPTC mitochondria and if this complex plays a role in mitochondrial and cellular repair following oxidant injury.

Methods: Wild-type PKC-α (wtPKC-α) and inactive PKC-α (dnPKC-α) were overexpressed in primary cultures of RPTC and mitochondrial PKC-α and iHSP70 levels, mitochondrial function, ATP content, and monolayer regeneration were assessed following exposure to the model oxidant, *tert*-butylhydroperoxide (TBHP).

Results: TBHP exposure decreased active PKC-α levels in mitochondria, mitochondrial function, and ATP content, and induced 40% cell loss. Overexpressing wtPKC-α promoted, whereas overexpressing dnPKC-α blocked, recovery of mitochondrial function, ATP content, proliferation, and cell survival. Co-immunoprecipitation and proteomic analysis revealed an association between the inducible HSP70 (iHSP70) and the inactive PKC-α in RPTC mitochondria. Mitochondrial iHSP70 levels increased as the levels of active PKC-α decreased after TBHP exposure. Overexpressing wtPKC-α abrogated whereas overexpressing dnPKC-α augmented TBHP-induced increases in mitochondrial iHSP70. Overexpressing iHSP70: 1) maintained active PKC-α levels in mitochondria, 2) improved recovery of state 3 respiration and ATP content, 3) decreased RPTC death, and 4) accelerated proliferation following oxidant injury. In contrast, iHSP70 inhibition decreased mitochondrial levels of PKC-α, blocked recovery of ATP content, and exacerbated cell death after injury.

Conclusions: We conclude that inactive PKC-α and iHSP70 form nonfunctional complex in mitochondria, which inhibits iHSP70 and blocks recovery of mitochondrial function and ATP content, and regeneration of oxidant-injured RPTC. Active PKC-α maintains iHSP70 functional and improves mitochondrial functions, ATP content, and RPTC regeneration following oxidant injury.

Funding: NIDDK Support

PUB020

Effect of Erythropoietin on Short-Term I/R Injury in Rats Ciro Esposito,[1] Massimo Torreggiani,[1] Francesca Castoldi,[2] Clara Migotto,[2] Nicoletta Serpieri,[2] Fabrizio Grosjean,[1] Alessandra Manini,[2] Enrico Pertile,[2] Antonino Dal Canton.[2] *[1]Nephrology, Fondazione IRCCS S. Maugeri, University of Pavia, Pavia, Italy; [2]Nephrology, Fondazione San Matteo, University of Pavia, Pavia, Italy.*

Background: Oxidative stress, apoptosis, necrosis and inflammation contribute to the pathogenesis of ischemia/reperfusion (I/R) injury. Bone marrow progenitor cells (BMC) are thought to participate in tissue repairing. We tested the efficacy of erythropoietin (EPO) to recruit BMC and ameliorate renal damage at 48 hours in a I/R rat model.

Methods: Female Sprague-Dawley rats were irradiated with a single dose of ^{137}Cs (10 Cy) and transplanted with 10^7 BMC from male donors of the same strain. After 4 weeks, animals were anesthetized and, through a bilateral subcostal incision, renal arteries were clamped for 45 minutes. Reperfusion of the kidneys was visually confirmed. 8 rats were randomized to receive EPO (5000UI/Kg of body weight, i.p.) 30 minutes before I/R while 8 rats received an equal volume of saline. Animals were sacrificed 48 hours later.

Results: There was no difference between the two groups in serum creatinine (3.2±0.8 vs. 2.4±0.8mg/dl), creatinine clearance (0.44±0.20 vs. 0.45±0.16ml/min), diuresis (24.75±7.90 vs. 16.00±3.29ml/24h), proteinuria (0.59±0.25 vs. 0.45±0.18g/24h) and hemoglobin (9.39±0.67 vs. 11.63±0.34g/dl). The FISH technique did not show any difference in the number of Y+ cells in the kidney, mostly of inflammatory origin. Histologically, we observed foci of tubular necrosis, a mild inflammatory infiltrate and leucocyte margination along peritubular capillaries in both groups. However, EPO-treated rats had less apoptotic cells than controls, as shown by TUNEL (7.27±1.73 vs. 19.61±4.56%, p<0.05) and less oxidative stress, as demonstrated by the extent of protein oxidation.

Conclusions: EPO does not ameliorate renal function through BMC recruitment, confirming the results from our previous study that prolonged the observation at 4 weeks after injury. However, EPO seems to act blunting the oxidative burden and preserving cells from apoptosis. The short follow-up time could have masked a faster renal function recovery in the EPO group, as suggested by their higher diuresis.

Funding: Government Support - Non-U.S.

PUB022

Early Postoperative Serum Uric Acid May Be Associated with Early Acute Kidney Injury A. Ahsan Ejaz,[1] Noel I. Ejaz,[2] Ganesh Kambhampati,[1] Bhagwan Dass,[1] Vijay Lapsia,[3] Michiko Shimada,[4] Rajesh Mohandas,[1] Negiin Pourafshar,[1] Richard J. Johnson.[5] *[1]Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL; [2]University of Chicago; [3]Mount Sinai School of Medicine; [4]Hirosaki University; [5]University of Colorado Health Sciences Center.*

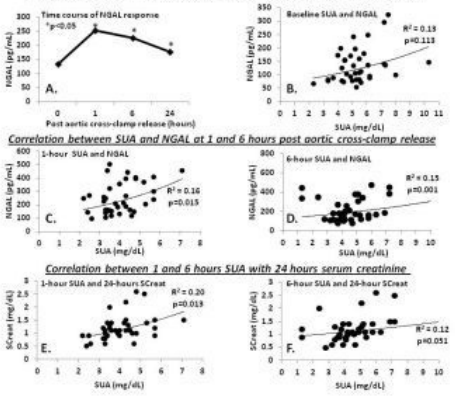
Background: We have reported that pre- and postoperative serum uric acid (SUA) is associated with an increased risk factor for acute kidney (AKI) as measured by serum creatinine (SCr) criteria (AJM, J Nephrol 2012). Based on our previous studies, we

hypothesized that SUA measured in the early, post aortic cross-clamp (ACC) release period may be associated with renal parenchymal damage as measured by serum neutrophilgelatinase-associated lipocalin (NGAL), a reliable predictor of early kidney injury.

Methods: Samples for SUAandNGAL were prospectively collected at 0, 1, 6 and 24 hours post ACC release and stored at -70C in a previously reported study (JTCVS). The relationship of SUA, NGAL and SCr were investigated. We only compared absolute values for each variable in the time period specified to minimize the effects of dilution.

Results: 37 patients were included for analyses. As expected, NGAL peaked between 1 and 6 hours following ACC release (Fig. 1A). There were no significant correlation between SUA and NGAL measured at baseline (Figure 1B). However, there were significant correlation between SUA and NGAL measured at 1 hour (Fig. 1C) and at 6 hours post ACC release (Fig. 1D). Similarly, there were significant correlation between SUA measured at 1 hour (Fig. 1E) and and at 6 hours post ACC release with SCreat measured at 24 hours (Fig. 1F).

Figure 1. Association of postoperative SUA with early and late markers of AKI



Conclusions: The data suggests that early postoperative SUA may be associated with early renal parenchymal injury as evidenced by elevated NGAL and subsequent 24-hour SCr concentrations.

PUB023

Exploring Factors Determine Prognosis of Patients Need Continuous Renal Replacement Therapy (CRRT) due to Acute Kidney Injury (AKI) after Major Cardiovascular Surgery Hiromichi Suzuki, Tsutomu Inoue, Hirokazu Okada, Tsuneo Takenaka. *Department of Nephrology, Saitama Medical University, Iruma Gun, Saitama, Japan.*

Background: Acute kidney injury is a common and major complication of cardiovascular surgery with a high mortality rate. Although multiple recent clinical trials have prospectively evaluated the impact of dose and modality of RRT, the literature on examining the factors to start CRRT in AKI is far less robust.

Methods: In a retrospective observational cohort study from April 2009 to March 2012, we linked electronically stored intra and perioperative clinical data with prognosis of patients who needed CRRT due to AKI after major cardiac surgery. We excluded patients presenting with pre-operative end-stage kidney disease requiring dialysis, AKI preceding surgery and patients having received contrast media within 3 days pre-operatively. All patients were cared for by the same team of cardiologist, surgeons, anesthetists and nephrologists.

Results: One hundred sixty eight patients were recruited. Thirty one patients (18%) did not survive to hospital discharge. There were no significant differences in age, gender and serum levels of creatinine between the survival and non survival groups. However, co-morbidity diseases such as diabetes, hypertension and dyslipidemia were more frequently recorded in non survival group. Among 137 patients survived to hospital discharge, twent-four patients needed CRRT. The pre-operative levels of serum creatinine in patients free from RRT at discharge were lower than those of patients needed RRT at discharge, however, no significant differences in urinve bolume between the two groups at the start of CRRT. Receiver operation curve of preoperative eGFR for prediction of RRT at discharge in AKI patients after cardiac surgery revealed 27ml/min/1.73m^2.

Conclusions: Prognosis of severely ill patients suffering AKI after cardiac surgery who needed CRRT depends on pre-operative levels of eGFR and co-morbid diseases.

PUB024

Urinary RANTES May Predict Long-Term Renal Recovery among Acute Kidney Injury Survivors Osun Kwon, William Brian Reeves. *Medicine/Nephrology, Penn State College of Medicine, Hershey, PA.*

Background: Acute Kidney Injury (AKI) is a common disorder associated with a high morbidity and mortality. Noninvasive parameters which reflect the ongoing pathology in the kidney and predict outcomes are needed to facilitate improved management and decrease morbidity and mortality. AKI is often multifactorial and its pathogenetic mechanism involves inflammation and regeneration. Recently we reported that urinary IP-10, IL-8, VEGF, TGF-α and EGF may predict renal functional outcome within the first week of ischemic AKI and urinary IL-8, MCP-1, IP-10, RANTES, EGF and VEGF may predict 3 month mortality. We questioned whether the urinary markers have predictability for long-term renal outcome and mortality in those patients.

Methods: The subjects were 82 adult patients with AKI referred for renal consultation who had a known baseline serum creatinine concentration (b-s-Cr). Urinary cytokine levels were measured on the day of consultation by microsphere based immunofluorescence multiplex assay and their ability to predict renal functional and patient survival outcome was evaluated at 3 year follow-up.

Results: Among the 82 subjects, 27 (33%) had b-s-Cr > 1.3 mg/dl, suggesting baseline CKD and 48 (59%) had died by 3 year follow-up. Among 9 survivors in the CKD group, 6 recovered renal function (67%), whereas among 25 survivors in normal b-s-Cr group, 21 showed recovery of renal function (84%). A receiver operating characteristic curve was used to determine the cutoff point of each urinary cytokine level with respect to distinguishing subjects with different outcomes at 3 years. Among cytokines examined (IL-1β, IL-6, IL-8, MCP-1, IP-10, RANTES, EGF, TGF-α, and VEGF), no urinary cytokine predicted mortality or survival at 3 years. Interestingly, among survivors at 3 years, high urinary RANTES levels tended to predict renal recovery; AUC 0.735, cutoff value 9.71 pg/mg urine creatinine (U-Cr), sensitivity 0.741, specificity 0.714, P=0.058, especially in patients with normal b-s-Cr; AUC 0.810, cutoff value 9.71 pg/mg (U-Cr), sensitivity 0.762, specificity 0.750, P=0.054.

Conclusions: This finding suggests that high urinary RANTES might predict renal recovery within 3 years among survivors of AKI.

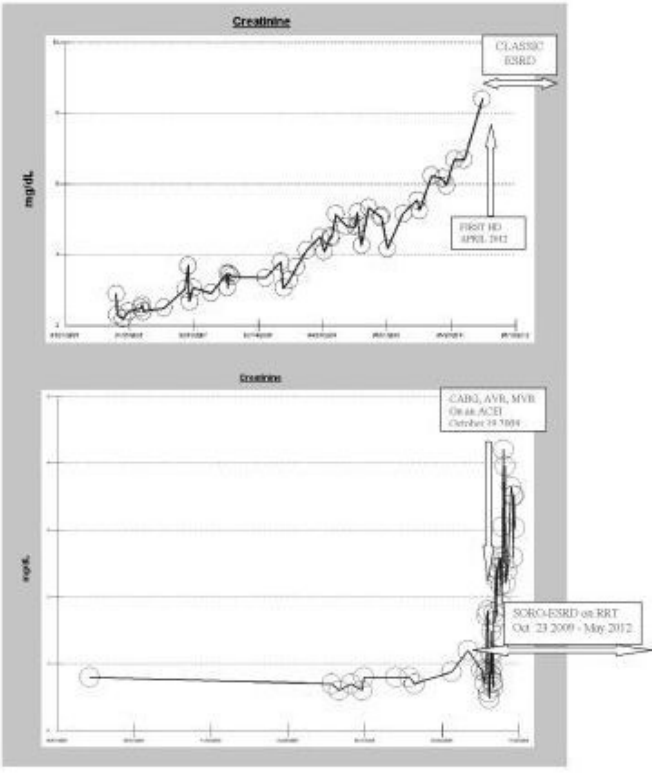
Funding: Clinical Revenue Support

PUB025

Syndrome of Rapid Onset ESRD versus Classic ESRD: A Tale of Two Different Cities: A New Unrecognized Syndrome and Implications for Improved Dialysis Care Macaulay A. Onuigbo. *Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: Classic CKD-ESRD progression is a predictable, linear, progressive and time-dependent decline in renal function, leading inexorably to ESRD and need for RRT (Fig 1). The syndrome of rapid onset end-stage renal disease (SORO-ESRD), which we first described in 2010, is irreversible ESRD rapidly following AKI superimposed on apriori stable CKD (Fig 1). SORO-ESRD demands study.

Methods: In June 2011, we retrospectively analyzed serum creatinine trajectories of the last 100 ESRD patients on RRT for >90 days. Any patient with an eGFR of >/= 30 ml/min/1.73 sq m BSA on or before 90 days antedating the first RRT was categorized as SORO-ESRD.



Results: Excluding 9 patients (incomplete data), of 91 ESRD patients, 57M:34F, age 39-93 years, 31 (34%) including two RTR had SORO-ESRD – 18M:13F, age 72 (50-92) years. AKI precipitating SORO-ESRD followed pneumonia (8), ADHF (7), pyelonephritis (4), post-operative (5), general sepsis (3), contrast-induced nephropathy (2), and others (2). Time between AKI and initiation of RRT was less than one week following cardiac surgery. Incidentally, 7 of 31 (23%) SORO-ESRD patients were concurrently on RAAS blockade at the time of initiation of first hemodialysis.

Conclusions: SORO-ESRD, after the fact, on maintenance RRT, appears similar to ESRD. However, SORO-ESRD and Classic ESRD are so very different – a tale of two different cities. With the former, there is no time for patient education, AVF planning and counseling. Furthermore, there are often huge communication and information gaps between

the nephrologist, the nurses, social workers and the patients/families as to the expectations of renal recovery or otherwise, at the outset, before irreversible ESRD is confirmed, so many months later. This new syndrome must be further studied to unravel these significant unanswered questions.

PUB026

Sodium Bicarbonate versus Ascorbic Acid in the Prevention of Contrast-Induced Nephropathy Bernardo Fargier,[1] Dulce M. Winterdaal,[1] Aristarco Diaz,[1] Abdel J. Fuenmayor.[2] *[1]Unidad de Nefrologia, University Hospital of the Andes, Merida, Venezuela; [2]Instituto Investigaciones Cardiovasculares, Universidad de Los Andes, Mérida, Venezuela.*

Background: Renal injury caused by contrast media is the third leading cause of in -hospital acute renal failure (ARF). Special attention is currently being directed at developing strategies for the prevention of contrast media nephropathy (CMN). The aim of this study is to determine the effectiveness of ascorbic acid vs. sodium bicarbonate in the prevention of CMN.

Methods: This is a prospective, experimental, single-blind, randomized study that was conducted between June 2010 and April 2011. We included 31 patients randomly assigned to 4 groups as follows: Group 1 (n = 8) received 0.9% saline at a rate of 1ml/kgr/hr during twelve hours before the beginning of the study and twelve hours after its completion; Group 2 (n = 8) was administered sodium bicarbonate 5% at a dose of 3ml/Kg before the beginning of the study followed by a dose of 1ml/Kg 6 hours after completion of the study; Group 3 (n = 8) was administered 3 grams of ascorbic acid endovenously before the beginning of the study and 2 grams every 12 hours in 3 additional doses upon completion of the study; Group 4 (n = 7) was given sodium bicarbonate, ascorbic acid at the same doses as those administered to the three previous groups. Groups 2, 3 and 4 received 0.9% saline at the same dose as that given to Group 1. The end point of follow-up was a relative increase ≥ 25% or an absolute increase of 0.5 mgrs / dl of serum creatinine, above baseline, 24 or 48 hours after exposure to a contrast medium. The results were expressed as absolute values , standard deviation and percentages. The ANOVA test was applied to assess between-group differences with a p value < 0,05.

Results: No significant differences were found among the groups. The serum creatinine and glomerular filtration rate 24 and 48 hours following the administration of contrast media did not increase in relation to the baseline levels.

Conclusions: Saline hydration is the ideal protective measure to prevent the occurrence of CMN, and the association of bicarbonate, ascorbic acid or both does not increase its protective effect.

PUB027

Is Intravesicular Pressure a Better Tool to Predict Renal Failure in Critically Ill Patients Compared with Routine Hemodynamic Parameters? Elie El-Charabaty, Chadi Saifan, Morton J. Kleiner, Suzanne E. El Sayegh. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: Acute renal failure is a frequent complication in patients admitted to the ICU. Several studies have tried to find a correlation between hemodynamic parameters and worsening of renal function in acute CHF and were unsuccesful. The aim of the study is to find if there is a linear relationship between worsening of renal function, bladder pressure (bp), hemodynamics (CVP) and blood values.

Methods: We analyzed patients admitted to the ICU with evidence of acute CHF. bp was measured using the transvesical method. Parameters measured were: weight, MAP, urine output, Cr, the use of a diuretic or pressor.

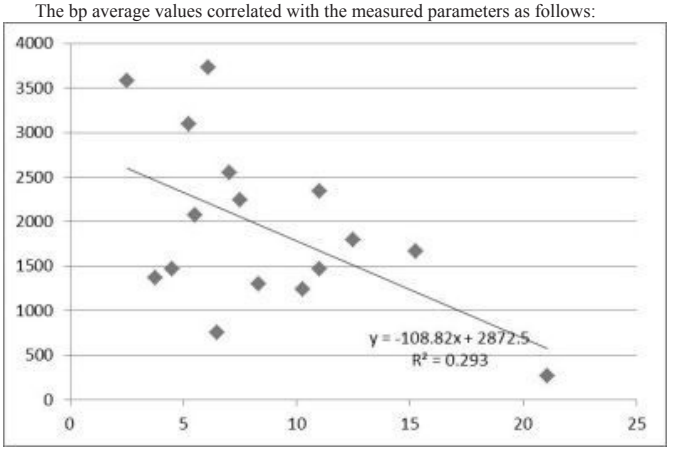
Results: 16 patients were included (5 M, 11 F) with a mean age of 72.9 y, and a mean EF of 37.14 %, 50% were on pressors and 75 % on diuretics. The mean bp measured on admission and within the hospital stay was 9.7 and 7.45 mmHg respectively. The averages of the bp, weight, creatinine level, MAP and urine output were calculated.

Patients characteristics

	Average	SD
Weight admission, Kg	80.44	18.79
Weight later, Kg	80.35	20.32
Cr admission	1.71	1.1
Cr later	1.63	1.03
MAP admission	75.99	8.25
MAP later	83.16	15.85
UO admission	2011	954
UO later	1856	982
bp admission	9.7	5.75
bp later	7.45	1.07

UO: urine output

The bp average values correlated with the measured parameters as follows:



1) For the urine output: y= -108.82 x + 2872.5; r= 0.54
2) For the MAP: y= -0.6555 x + 85.232; r=0.27
3) For the creatinine: y= 0.0246 x + 1.4613; r= 0.11
4) For the weight: y= 1.8277 x+ 64.634; r= 0.43.

Conclusions: Our study showed that bladder pressure measurements correlated better with urine output and weight change than with serum creatinine or mean arterial pressure. It is considered an easy non invasive method to monitor patients volume status and should be used more often during hospitalizations.

PUB028

Association of Left Ventricular End Diastolic Pressure with Acute Kidney Injury in Patients Undergoing Cardiac Catheterization Rebekah Adia Neal, K. Edmund Tse. *Huntington Hospital, Pasadena, CA.*

Background: The use of contrast for computed tomography scans, cardiac catheterizations, and other procedures is a well-known risk factor for the development of acute kidney injury (AKI). Proposed mechanisms include renal ischemia due to reduction of blood flow or increase in oxygen demand and, possibly, by direct toxicity to the renal tubular epithelial cells. It is unclear if volume status at the time of cardiac catheterization, as indicated by the subject's left ventricular end diastolic pressure (LVEDP), has any significant impact on the development of AKI. The primary purpose of the study is to assess for a correlation between LVEDP and the risk for AKI after cardiac catheterization. The secondary purpose of the study is to assess for other risk factors which may contribute to the development of AKI.

Methods: This is a retrospective review of 200 subjects who underwent cardiac catheterization at Huntington Memorial Hospital, Pasadena, California from 2010-2012. Inclusion criteria were documented LVEDP and pre- and post-procedural creatinine levels. AKI definition was based on the critieria from the Acute Kidney Injury Network. LVEDP was assessed in relationship to AKI. Other variables, which included patient age, gender, creatinine levels, amount of contrast, amount of peri-procedural intravenous fluids, ejection fraction (EF), and the use of bicarbonate, N-acetylcysteine, and RAAS inhibitors, were also assessed. Variables were analyzed using the student's t test and ANOVA.

Results: Of the 200 subjects, 39 were found to meet the criteria of AKI. Elevated LVEDP was associated with increased risk of AKI (27mmHg vs. 31 mmHg, p-=0.0313). Only one of variable, EF < 40%, showed a statistically significantly increased risk for the development of AKI in patients with elevated LVEDP > 19 mmHg compared to LVEDP <19 mmHg (p=0.0299).

Conclusions: Elevated LVEDP in subjects with reduced left ventricular ejection fraction is a predictor of AKI in subjects who have undergone cardiac catheterization. Caution should be made to avoid excessive amount of fluid to be given to patients with LVEDP > 19mmHg and EF < 40%.

Funding: Clinical Revenue Support

PUB029

Overload of Either Monoclonal or Polyclonal Light Chains in Proximal Tubules Is Associated with Renal Dysfunction Ping L. Zhang. *Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, MI.*

Background: Free light chains are small molecules passing through glomerular barriers freely and reabsorbed via their receptors in proximal tubules under normal circumstances, but overload of free light chains, either monoclonal or polyclonal, to proximal tubules may cause acute tubular injury. This study was to investigate expression of monoclonal and polyclonal light chains in proximal tubules in human renal biopsies, and to correlat light chain expression with both renal functional index and tubular injury index in renal biopsies.

Methods: A sensitive immunohistochemical staining (IHC) method (polymer technique) was used to stain unremarkable renal parenchyma from tumor associated nephrectomy (control group 1, n = 39), renal biopsies with history of renal failure and proteinuria (polyclonal group 2, n = 33) and renal biopsies with diagnosis of variants of monoclonal light chain associated nephropathy (monoclonal group 3, n = 36). Diminished brushed borders of proximal tubules on PAS stained sections were scored 0 to 3+ for indicating acute tubular injury. The highest light chain stain from each case was scored from 0 to 3+. Serum creatinine levels were obtained from each patient based on the clinical record.

Results: Both polyclonal and monoclonal groups had significantly stronger staining scores for light chains in proximal tubules (2.33 ± 0.12 and 2.79 ± 0.08 arbitrary units [AU], higher serum creatinine levels (2.56 ± 0.21 and 4.47 ± 0.56 mg/dL) and higher tubular injury scores (PAS scores 1.52 ± 0.16 and 2.06 ± 0.16 AU), when compared to those in control group (0.97 ± 0.10 AU, 1.00 ± 0.05 mg/dL and 0.39 ± 0.12 AU, respectively) (ANOVA, $p<0.05$). There was a significantly linear correlation with between light chain scores or tubular injury scores and serum creatinine levels when three groups were taken together for analysis, although the correlation with polyclonal group alone was not as strong as that with the monoclonal group alone.

Conclusions: Our current data suggest that the overload of free light chains, being either monoclonal or polyclonal type, in the proximal tubules can all be associated with the acute tubular injury thus renal dysfunction.

Funding: Clinical Revenue Support

PUB030

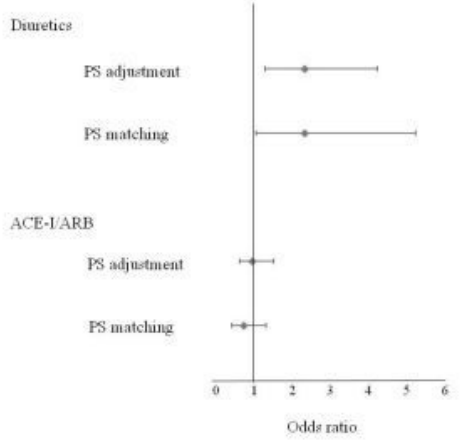
Study of Which Antihypertensives Are Predictors of Acute Kidney Injury in What Kind of Patients after Non-Cardiac Surgery Using Propensity Score

Miho Tagawa,[1] Takayuki Hamano.[2] *[1]Kyoto Katsura Hospital; [2]Osaka University.*

Background: Most of the studies on predictors of postoperative acute kidney injury (AKI) have been performed in cardiac surgery. No study has evaluated which kind of preoperative prescription of antihypertensives are risk factors of AKI after non-cardiac surgery.

Methods: This is a retrospective observational study. Inclusion criteria were adult (age ≥ 18) patients who underwent non-cardiac surgery under general anesthesia from 2007-2009 at our institute. Exclusion criteria were urological surgery, those without creatinine (Cre) values, and who had underwent dialysis preoperatively. Exposure of interests were preoperative use of diuretics and angiotensin converting enzyme inhibitors (ACE-I)/ angiotensin receptor blockers (ARB). Outcome variable was postoperative AKI defined by AKI network (increase in Cre ≥ 0.3 mg/dL or 150 %, or urine output < 0.5 ml/kg/hour for > 6 hours). A propensity score (PS) of receiving diuretics or ACE-I/ARB therapy preoperatively was estimated and adjusting for PS quintiles in multiple logistic regression analyses and matching on log(PS) were performed (user: non-user = 1:2).

Results: There were 137 AKI (5.0 %) among 2,725 subjects. Diuretics but not ACE-I/ ARB were significant predictors of postoperative AKI.



In the patients with the lower quintiles of PS, the greater effect sizes of diuretics were observed.

Odds ratio of AKI according to diuretics PS quintile comparing users with non-users

	Odds Ratio	95% CI	p
Q1+Q2	7.99	1.69-37.82	0.009
Q3	6.92	1.39-34.37	0.018
Q4	1.30	0.16-10.31	0.804
Q5	1.87	0.92-3.82	0.086

Conclusions: Diuretics but not ACE-I/ARB were significant predictors of postoperative AKI. Diuretics were predictors of postoperative AKI in patients to whom physicians were not likely to prescribe diuretics.

PUB031

Neutrophil Gelatinase Associated Lipocalin (NGAL) and Renal Impairment after Non-Cardiac Surgery

John F. Mooney,[1,2] Clara K. Chow,[1,2] Siddharth Trivedi,[3] Federica Barzi,[1] Jagnoor Jagnoor,[1] Stephanie Wilson,[1] Stephen C.H. Li,[4] Michael Clough,[4] Graham Hillis,[1,5] Richard Halliwell,[6] Vincent W.S. Lee.[3] *[1]The George Institute for Global Health, Sydney, NSW, Australia; [2]Dept of Cardiology, Westmead Hospital, Sydney, NSW, Australia; [3]Centre for Transplant and Renal Research, Westmead Millenium Institute, University of Sydney, Sydney, NSW, Australia; [4]Institute for Clinical Pathology and Medical Research, Westmead Hospital, Sydney, NSW, Australia; [5]Dept. of Cardiology, Concord Repatriation and General Hospital, Sydney, NSW, Australia; [6]Dept. of Anaesthesia, Westmead Hospital, Sydney, NSW, Australia.*

Background: Acute kidney injury (AKI) is a known complication of surgery. Neutrophil gelatinase associated lipocalin (NGAL) is a potentially novel means of detecting AKI early.

Methods: A representative sample of patients ≥45 years undergoing elective major non-cardiac surgery were followed with daily creatinine measurement. Patients with dialysis dependence or undergoing nephrological procedures were excluded. Urinary NGAL was collected 4-6 hours after commencement of surgery (Abbott diagnostics). We conducted an interim analysis of the first 101 patients enrolled. We used a linear regression model to assess relationship of NGAL to change in peak creatinine measured in the first 3 days after surgery.

Results: Patients underwent vascular, gastrointestinal, neurosurgical, orthopaedic, urological and gynaecological procedures. Mean age was 65, 52% were female, 51% had hypertension and 14.3% diabetes, 8.3% had baseline serum creatinine > 105 mmol/L and 26% had baseline proteinuria detected on urinalysis. Six patients had AKI determined by the AKIN criteria. Three of these patients had a NGAL > 100 mmol/L, compared with 7 of 95 patients without AKI. NGAL remained an independent predictor of post operative creatinine change after adjusting for age, gender, hypertension, diabetes, higher risk surgery and pre-operative creatinine levels (coefficient 3.17; CI 0.65-5.67, *p > 0.01).*

Conclusions: Urinary NGAL is associated with postoperative creatinine independent of some known predictors of AKI and potentially may have utility in early identification of patients that develop AKI after non-cardiac surgery.

Funding: Pharmaceutical Company Support - Abbott Diagnostics

PUB032

Is Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers Associated with Acute Kidney Injury Post Coronary Artery Bypass Grafting in a Mixed Ethnicity Population in a Community-Based Hospital in Hawaii?

Ekamol Tantisattamo, Ma Clarisse M. Toledo, Dorothy M. Shigaki, Roland C.K. Ng. *Medicine, University of Hawaii.*

Background: Acute kidney injury (AKI) after coronary artery bypass grafting (CABG) is common, and preoperative risk factors are well-established. Angiotensin-converting enzyme inhibitors/Angiotensin receptor blockers (ACEI/ARB) therapy have shown cardio-protective effects in coronary artery disease patients; however, there were concerns that preoperative use of ACEI/ARB poses patients at risk for the development of AKI post-surgery. We aim to determine whether preoperative use of ACEI/ARB is associated with AKI post CABG in a mixed ethnicity population in Hawaii.

Methods: This was a retrospective study of 101 adult patients undergoing CABG between January 2009 and December 2011 in one community hospital in Hawaii. Baseline data collection included patients' characteristics, perioperative variables, and postoperative complications. The incidence of AKI was likewise obtained.

Results: Of the 101 patients, AKI defined by AKIN criteria occurred in 35 patients (35%). The incidence of stage 1 and 2 AKI was 94% and 6%, respectively. There was no incidence of stage 3 AKI. From all of the variables, only advanced age (≥65 years old) was significantly associated with an increased incidence of AKI ($p = 0.022$); otherwise, there were no significantly increased incidence of AKI with the following variables: gender ($p = 0.308$), insulin-dependent diabetes mellitus ($p = 0.716$), hypertension ($p = 0.251$), hyperlipidemia ($p = 1$), COPD ($p = 0.608$), preoperative use of ACEI/ARB ($p = 0.527$) / furosemide (0.072), postoperative use of aspirin ($p = 0.269$), and postoperative atrial fibrillation ($p = 0.593$). With a mixed ethnicity in our population, Japanese was account for the main population (51%) followed by Filipino (18%), mixed ethnicity (8%), and others (23%).

Conclusions: Incidence of AKI post CABG in Hawaii is not different from that of available data. In addition to cardiovascular benefit, ACEI/ARB therapy is not associated with an increased risk of AKI post CABG. Since the outcome of CABG is correlated with AKI, preoperative discontinuation of ACEI/ARB may not be warranted.

PUB033

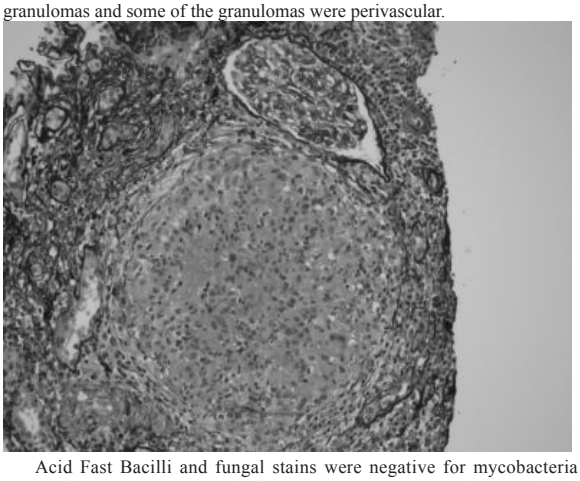
A Rare Case of Drug Induced Interstitial Nephritis with Granuloma Formation in Kidney Biopsy

Rohan Garje,[1] Arash Rashidi.[2] *[1]Internal Medicine, Fairview Hospital; [2]Nephrology, Fairview Hospital, A Cleveland Clinic Hospital.*

Background: Interstitial nephritis (IN) is associated with multiple etiologies but granulomatous IN is a rare finding. Here we report a 48 years old white female with granulomatous IN secondary to Allopurinol.

Methods: A 48 y/o Caucasian female was evaluated for weakness, fatigue and nocturia. She had history of hypertension controlled with Valsartan and was recently diagnosed with gout for which she was started on Colchicine, Allopurinol and Ibuprofen about two

weeks prior to the presentation. She did not have any significant drug allergies. Physical examination was normal and vitals were stable. Renal function testing revealed a serum creatinine of 3.24 mg/dl from the baseline which was 1.0 mg/dl. Urinalysis was negative for red blood cells and eosinophils. Spot urine protein to Cr ratio did not show significant proteinuria. Complete blood count did not reveal eosinophilia. Serology tests including hepatitis B and C, Anti-Nuclear antibody, cytoplasmic-ANCA and peripheral-ANCA were negative and C3 and C4 were normal. Kidney biopsy revealed tubulointerstitial inflammation composed of lymphocytes, eosinophils, epithelioid histiocytes and giant cells forming granulomas and some of the granulomas were perivascular.



Acid Fast Bacilli and fungal stains were negative for mycobacteria and fungi. Immunofluorescence was negative for IgG, IgA, IgM, C3c, C1q, kappa and lambda. Electron microscopy revealed thickened glomerular basement membranes and extensive epithelial foot process effacement. Patient was treated with steroids over a course of 2 months and Allopurinol was stopped. Her renal function significantly improved and her present serum creatinine level is 1.38mg/dl.

Conclusions: Granulomatous IN is an uncommon form of drug induced IN. Our patient had AKI because of granulomatous IN, most likely secondary to Allopurinol.

PUB034

Incidence and Mortality in Critical Units Patients Associated to Acute Kidney Injury: 30-Days and One-Year of Evolution Andres Boltansky,[1] Gabriel Cavada,[2] Guillermo Eugenio Villamizar,[1] Carlos E. Irarrazabal,[2] Antonio Vukusich.[1] *[1]Nefrología, Clinica Davila, Santiago, Chile; [2]Medicina, Universidad de los Andes.*

Background: The aim of this work was determine incidence and risk of mortality in Intensive Care Unit (ICU), Coronary Care Unit (CCU) and Intermediate Critical Care Unit (ICCU) patients associated to Acute kidney injury (AKI), considering intra hospital and 1-year evolution since discharge.

Methods: This is a historic cohort analysis of clinical data from patients admitted in the Critical Units (CU): ICU, CCU and ICCU, which belong to a tertiary health center in Santiago-Chile. Serum creatinine (SCr) variations in 48 hours were used for AKI classification (AKIN criteria). Information from each patient included demographics (age, sex), SCr measurements (estimated baseline and observed creatinine), and 30-days and one-year mortality. We enrolled 2703 patients discharged in 2010. A group of patients (969) were excluded, leaving 1734 patients for analysis (ICU: 366; CU: 347; IU: 981).

Results: The median age was 60.88 ± 19.65 (15 - 99) years, 53.23% were male. The incidence of AKI was 27.69% in CU (30.05; 33.72 and 27.69 % for ICU; CU; and ICCU, respectively). The mortality at before 30 days for no AKI patients was 6.38% (8.76; 2.63 and 3.52 % for ICU; CU; and IU, respectively) and increased for AKI patients: AKI-I 10.00% (Odds ratio [OR]: 1.63; Confidence interval [CI]: 1.02-2.58); AKI-II 15.38% (OR: 2.669, CI: 1.38-5.15); and AKI-III: 28.18% (OR: 5.762; CI: 3.57-9.27). The relationship between intra-hospital mortality and AKI III in the distinct CU was: ICU 35.29%, CCU 21.43% and, ICCU 16.33%. The mortality at before 1 year for no AKI patients was 16.05% and increased for AKI patients: AKI-I: 21.85% (OR: 1.42, CI: 1.02-1.96); AKI-II: 35.06% (OR: 2.73, CI: 1.67-4.47); and AKI-III: 43.60% (OR:3.918; CI: 2.60-5.88).

Conclusions: This data shows that an episode of AKI during a stay in critical units was associated to an increased general mortality before 30-days and one-year of evolution. Also, it shows that the general mortality was elevated in patients with higher AKI stages.

PUB035

Perfusion Pressure during Cardiac Surgery and Its Relation to Acute Kidney Injury Kristian Kandler,[1] Jens C. Nilsson,[2] Daniel Steinbrüchel.[1] *[1]Cardiothoracic Surgery, Rigshospitalet, Copenhagen, Denmark; [2]Cardiothoracic Anaesthesia, Rigshospitalet, Copenhagen, Denmark.*

Background: The aim was to investigate if a higher-than-standard mean arterial pressure (MAP) during cardiopulmonary bypass (CPB) could lower the incidence of acute kidney injury (AKI) after cardiac surgery.

Methods: Preliminary data from the first 16 patients (5 women and 11 men) in a randomized controlled trial was collected. Inclusion criteria are high preoperative risk of AKI (age > 70 years, coronary artery bypass plus valve surgery). The patients were randomized to a MAP > 60 mmHg (HG) or a control group (CG). Norepinephrine infusion was used to maintain a higher MAP. The outcome was measured by glomerular filtration rate (GFR) using Cr-EDTA clearance and urine neutrophil gelatinase associated lipocalin (NGAL) levels. The incidence of AKI was assessed using the AKIN criteria. The study is planned to include 100 patients.

Results: MAP during CPB was 63.4 ± 3.0 mmHg and 48.8 ± 1.7 mmHg ($p<0.0001$) in the HG and CG respectively. Comparing baseline GFR with 5 days postoperative measurements no differences were found between the groups, -4 vs. -15 ml/min ($p=0.49$) in the HG and CG. Urine NGAL levels increased by 33.5 [2.5–1386] ng/ml in the HG and by 163 [-73–1128] ng/ml in the CG ($p = 0.86$), comparing baseline values with postoperative values just prior to admission to the ICU. Five patients in the HG and 7 patients in the CG developed AKI ($p=0.57$).

Conclusions: No significant advantage were found in regard to the incidence of AKI using a higher-than-standard MAP during cardiac surgery.

Funding: Government Support - Non-U.S.

PUB036

Tacrolimus-Induced Thrombotic Microangiopathy Post Allogeneic Stem Cell Transplant Laith F. Al-rabadi,[1] Rawan T. Al-odat,[2] Christopher D. Blosser.[1] *[1]University of Iowa; [2]Jordan Hospital.*

Background: Calcineurin inhibitors (CNI), Tacrolimus and Cyclosporine, are key immunosuppression medications in this era of transplant. Graft versus host disease (GVHD) remains a major complication after allogeneic stem cell transplant (allo-SCT) with incidence of 5-20 %. Immunosuppression therapy is the standard response to GVHD, and Tacrolimus is preferred immunoprophylaxis for patients at high risk of GvHD. However, Tacrolimus has known systemic and nephrotoxic clinical and histopathologic features, including the rare, but clinically devastating thrombotic microangioathy (TMA). Renal injury is one of the most serious consequences of TMA, and hence nephrologists play an integral role in diagnosis and treatment.

Methods: Two Case reports and literature review.

Results: We describe two cases of Tacrolimus-induced TMA associated with renal injury. Both patients had high FK levels on presentation and experienced renal recovery after discontinuation of Tacrolimus. Interestingly, despite reintroduction of a CNI, both patients did not experience recurrent clinical or pathologic disease.

Conclusions: Members of the bone marrow transplant community posit that transplantation-associated TMA (TA-TMA) is distinct from de novo thrombotic thrombocytopenic purpura (TTP), and is associated with GVHD, infections, and medications. Others believe TMA is a form of TTP, and tacrolimus as one medication associated with the disorder. High FK levels have been associated with greater systemic disease, including kidney injury. Nasesen et al. suggested that the local effects are more essential than systemic factors in determining the toxicity of Tacrolimus, thereby arguing against the importance of dose-dependent toxicity. The treatment of Tacrolimus-induced TMA differs from idiopathic TTP, and portends a poor prognosis (mortality 60-90%). Medication discontinuation is the mainstay of treatment, as plasmapheresis is not indicated in Tacrolimus-induced TMA. Recent introduction of Rituxmab, and Daclizumab show promise in inducing remission. Here we discuss the potential mechanisms of the disease, compare and contrast dose related effect and local effect-hypotheses and modalities of treatment for Tacrolimus-induced TMA.

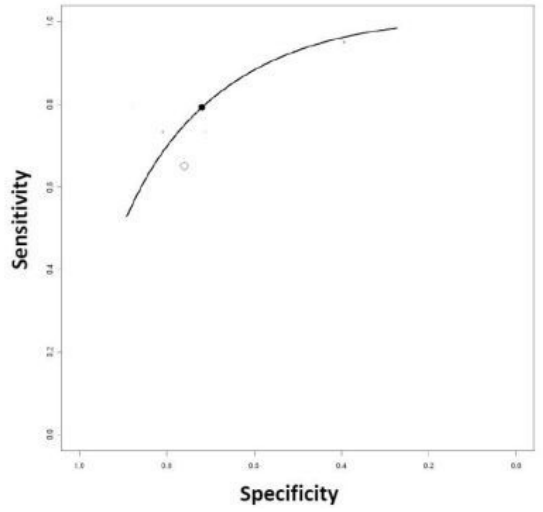
PUB037

Urinary Liver-Type Fatty Acid Binding Protein (L-FABP) for Detection and Prognostication of Acute Kidney Injury (AKI): A Systematic Review Paweena Susantitaphong,[1,2] Kent Doi,[3] Eisei Noiri,[3] Norma Terrin,[4] Bertrand L. Jaber.[1] *[1]Medicine, St. Elizabeth's Medical Center, Boston, MA; [2]Medicine, Chulalongkorn University, Bangkok, Thailand; [3]Department of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan; [4]Institute for Clinical Research and Health Policy Studies, Tufts University, Boston, MA.*

Background: Urinary L-FABP is a proximal tubular epithelial cell biomarker for early detection of AKI, with variable performance characteristics according to clinical settings. We performed a meta-analysis of all observational studies that have tested this biomarker in AKI.

Methods: We performed a literature search using MEDLINE, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, American Society of Nephrology scientific abstracts, and bibliographies of retrieved articles. Human studies investigating the performance characteristics of urinary L-FABP for diagnosis and prognosis of AKI were included.

Results: 13 prospective cohort studies and 2 case-control studies were identified. Only 5 cohort studies could be meta-analyzed. The estimated sensitivity of urinary L-FABP for the diagnosis of AKI was 78.6% (95% CI 63.1, 88.7) and the specificity 70.7% (95% CI 53.8, 83.3). The hierarchical summary receiver-operator-characteristic (HSROC) curve is displayed in figure 1. The estimated sensitivity of urinary L-FABP for predicting dialysis requirement was 69.1% (95% CI 34.6, 90.5) and the specificity 42.7% (95% CI 3.1, 94.5), and for predicting in-hospital mortality, 93.2% (95% CI 66.2, 99.0) and 78.8% (95% CI 27.0, 97.4), respectively.



Conclusions: Urinary L-FABP is a promising biomarker for diagnosing AKI and predicting adverse outcomes. However, the potential value of this biomarker in AKI needs to be further validated in large cohort studies and across a broader spectrum of clinical settings.

PUB038

Acute Kidney Injury (AKI) by Following Pulmonary Thromboendarterectomy (PTE) in Patients with Chronic Thromboembolic Pulmonary Hypertension (CTEPH) Jang Won Seo,[1] Soo Young Yoon,[1] Kim Kerr,[2] William Auger,[2] Michael M. Madani,[3] Stuart Jamieson,[3] Ravindra L. Mehta.[1] *[1]Department of Medicine, UCSD, San Diego, CA; [2]Division of Pulmonary and Critical Care Medicine, UCSD, San Diego, CA; [3]Division of Cardiothoracic Surgery, UCSD, San Diego, CA.*

Background: PTE is an established technique to improve pulmonary and cardiovascular (CV) function in CTEPH, however its effect on renal function is not known. Since PTE surgery requires complete circulatory arrest, we hypothesized that it would be associated with development of AKI. In addition, we postulated that the enhancement in CV performance would result in an improvement in renal function at hospital discharge.

Methods: We reviewed data from 123 adult pts. with CTEPH undergoing PTE enrolled in a prospective trial of a lung protective ventilation strategy to prevent acute lung injury. AKI was determined by the AKIN/KDIGO AKI criteria [sCr change ≥0.3 mg/dl or ≥50% within 48 hrs or urine output (UO) <0.5 ml/kg/hr >6 hrs]. Outcomes included duration of mechanical ventilation, ICU and hospital stay, and renal function at discharge.

Results: Fifty-one (41%) pts. developed AKI following PTE surgery; 25 (20%) met the sCr and 26 (21%) the UO criteria. Underlying CKD (CKD-EPI GFR < 60 ml/min per 1.73 m^2) was present in 10% and was more frequent in pts. with AKI (AKI 19.6% vs no-AKI 2.8%, p=0.003). The mean time to development and duration of AKI were 2.7 and 2.3 days, respectively. AKI and no-AKI pts. had a similar duration of ventilator requirement (median of 1.0 day, p=0.77) and significantly longer median lengths of stay in the ICU (AKI 4.0 vs no-AKI 3.0 days, p=0.02) and hospital (AKI 13 vs no-AKI 11 days, p=0.05). At discharge, renal function had improved in 40.6%, was unchanged in 56.1%, and had worsened in 3.3%. A similar trend was seen in pts. with pre-existing CKD (n=13) with over 76% showing improvement to an eGFR >60 ml/min.

Conclusions: We observed a high incidence of AKI following PTE which was associated with worse outcomes. Renal function improved following PTE and was similar in AKI and no-AKI pts. These findings highlight the importance of measuring renal function changes in CTEPH pts. undergoing PTE.

PUB039

Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) as a Biomarker for Early Identifying Acute Kidney Injury in Critically Ill Patients Maria De Fatima Vattimo,[1] Gabriela Fulan-silva,[2] Cassiane Dezoti Fonseca,[1] Mirian Watanabe.[1] *[1]School of Nursing, University of Sao Paulo, Sao Paulo, SP, Brazil; [2]Heart Institute, University of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Acute kidney injury (AKI) in intensive care unit (ICU) is frequent and is associated with increased mortality. Levels of uNGAL are correlated with the degree of tubular injury and it has been confirmed as the novel biomarker for early identification of AKI, renal replacement therapy (RRT) and death in critically ill patients. The aim of this study was to evaluate the utility of uNGAL compared with other renal parameters for the early detection of AKI.

Methods: Eighty three (n=83) critically ill patients were enrolled in this study. Urinary output, serum creatinine and uNGAL were evaluated. Urine and serum samples were collected 24 and 48 hours after intensive care unit (ICU) admission. AKI patients were stratified based on AKIN (stages I, II and III). The study was approved by Ethical Committee.

Results: Prevalence of AKI was 78.3%; being 44.6% patients admitted with AKI and 33.7% acquired ICU AKI. Among AKI patients, 50.8% were AKIN I, 20.0% AKIN II and 29.2% AKIN III. Highest levels of uNGAL were observed in the AKIN III group ($p<0.05$). uNGAL in the 24 and 48 hours were significantly higher in ICU AKI patients ($p<0.05$), with an area under the curve (AUC) 0.70. Non survivors and RRT patients, at every time point, presented higher uNGAL levels, while serum creatinine and urinary output did not show such tendency.

Conclusions: Our study concludes that uNGAL is a useful biomarker of AKI and is an early good predictor of the onset of ICU AKI.

Funding: Government Support - Non-U.S.

PUB040

Relationship between Urinary Fractional Excretion of Sodium and Life Prognosis in Liver Cirrhosis Patients Naro Ohashi,[1] Yukitoshi Sakao,[2] Tomoyuki Fujikura,[1] Hideo Yasuda,[1] Akihiko Kato,[2] Yoshihide Fujigaki.[1] *[1]First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: Severe renal vasoconstriction in the setting of generalized vasodilatation with blood pooling in areas of vasodilatation and consequent reduction of effective arterial volume is the pathophysiologic basis in liver cirrhosis patients. Low levels of fractional excretion of sodium (FENa) are one of the effective markers to determine hypoperfusion of renal artery. However, the relationship between FENa and life prognosis in liver cirrhosis patients has not been elucidated yet.

Methods: We examined 19 liver cirrhosis patients (14 men and 5 women; mean age: 66.0±11.1 years; underlying liver cirrhosis: type B hepatitis in 2 patients, type C hepatitis in 11, alcoholic hepatitis in 5 and autoimmune hepatitis in 1) with renal damage (estimated glomerular filtration rate (eGFR) < 60 ml/min) that admitted to our hospital to treat complications of liver cirrhosis. Some parameters were compared between patients who survived and those who died during hospitalization.

Results: Eight of 19 patients were died because of uremia in 3 patients, liver failure in 3, and gastrointestinal bleeding in 2, respectively, and 11 patients had survived. Diuretics were administered to all of the patients. There were no differences in patient background, and the levels of serum creatinine and eGFR. However, in addition to total-bilirubin (T-Bil), glutamic oxaloacetate transaminase (GOT), prothrombin time (PT) (%), and Child-Pugh score, dead patients had significant difference about FENa (dead patients: 0.44±0.51% and alive patients: 1.08±0.45%, respectively, p=0.010). FENa levels were significantly and inversely correlated with T-Bil, GOT, and Child-Pugh score, and were significantly and positively correlated with PT (%). Moreover, the sensitivity (75%) and the specificity (100%) of FENa<0.5% about the death were extremely high.

Conclusions: In conclusion, it is possible that FENa in liver cirrhosis patients reflects the severity of disease condition and that it is associated with life prognosis.

Funding: Government Support - Non-U.S.

PUB041

Urinary NGAL, L-FABP, and KIM-1 Trends in Pediatric Patients after Living-Donor Kidney Transplantation Akihiko Shirasu,[1] Akira Ashida,[1] Hyogo Nkakura,[1] Hideki Matsumura,[2] Motoshi Hattori,[3] Hiroshi Tamai.[1] *[1]Pediatrics, Osaka Medical College, Takatsuki, Osaka, Japan; [2]Pediatrics, Hirakata City Hospital, Hirakata, Osaka, Japan; [3]Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Neutrophil gelatinase-associated lipocalin (NGAL), L-type fatty acid binding protein (L-FABP), and kidney injury molecule 1 (KIM-1) have emerged as early marker proteins that are predictive of acute kidney injury (AKI) in various clinical settings, including transplantation. However, the patterns of these marker proteins in pediatric patients after renal transplantation have not been studied in depth. Therefore, we examined the levels of these proteins in pediatric patients undergoing living-donor kidney transplantation. Additionally, we examined the relationship between these marker proteins and eGFR at discharge, in order to clarify whether they would be predictive of the graft function.

Methods: NGAL, L-FABP and KIM-1 in urine samples from seven pediatric patients on days 0, 1, 2, and 7 after transplantation, and at discharge, were examined. Urinary NGAL, L-FABP and KIM-1 were measured using an ELISA kit. eGFR was estimated using the Schwartz formula at discharge.

Results: Seven patients (5 males and 2 females, age 11.6 ± 4.3 years, range 5 to 17 years) were enrolled. All underwent living-donor kidney transplantation without any delay in graft function. The levels of NGAL and L-FABP were highest on day 0, and decreased rapidly to the normal range by day 2. The level of KIM-1 was increased on day 2 and then decreased gradually to the normal range. The eGFR at discharge ranged from 82.8 to 102.3 ml/min/1.73 m^2, and showed a tendency to be negatively correlated with the level of NGAL at day 0 (p=0.06).

Conclusions: In pediatric recipients, the levels of urinary NGAL and L-FABP decreased rapidly and normalized within 2 days after transplantation. This trend is similar to the adult. The level of NGAL on day 0 after transplantation appeared to be potential predictor of renal graft function.

PUB042

Renal Recovery Induced by Eculizumab in a Peritoneal Dialysis Dependent Child with Atypical HUS Omar Nihad Al Masri, Zubaida Al-Ismaili, Eslam Tawfik. *Pediatric Nephrology, Sheikh Khalifa Medical City Managed by Cleveland Clinic, Abu Dhabi, United Arab Emirates.*

Background: Atypical hemolytic uremic syndrome is an ultra-rare disease resulting from defective regulation of the alternative complement pathway. Kidney involvement can be severe resulting in renal failure or death despite therapy with plasma exchange or plasma infusion. Eculizumab (Soliris, Alexion), a monoclonal antibody directed against the complement protein C5, has shown success in inducing and maintaining remission in patients with atypical HUS. Only few cases of dialysis dependent patients that benefited from eculizumab have been reported.

Methods: We report a 2 y/o male who presented 3 months earlier to another hospital with severe acute renal failure associated with hemolytic anemia, thrombocytopenia, low C3 level and biopsy evidence of thrombotic microangiopathy. There was no history of diarrhea and ADAMTS 13 activity was normal .He remained on peritoneal dialysis since presentation despite treatment with daily plasma infusion for 1 week then thrice weekly for 3 months. He was transferred to our hospital where repeat kidney biopsy confirmed the presence of thrombotic microangiopathy with ischemic looking glomeruli and less than 50% chronic interstitial fibrosis. Eculizumab was initiated at dose 600mg then 1 week later 300 mg. Urine output slowly improved and plasma creatinine level continues to fall. 12 days after first dose of Eculizumab infusion the patient is currently dialysis free with creatinine level of 161µmol/L.

Conclusions: This case supports the role of Eculizumab in atypical HUS even if a patient has been dialysis dependent and oliguric for several months. The role of the repeat kidney biopsy prior to commencing Eculizumab was important in both confirming the diagnosis and degree of chronic damage thereby justifying its use.

PUB043

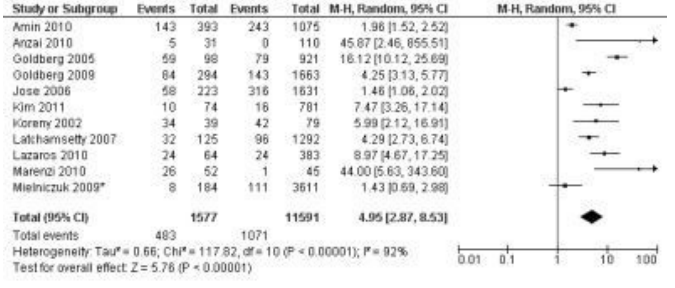
Cardio-Renal Syndrome Typre 1 in Acute Coronary Syndrome: A Systematic Review Dinna N. Cruz,[1] Manish Kaushik,[1] Claudio Ronco,[1] Sean M. Bagshaw.[2] *[1]St Bortolo Hospital, Italy; [2]University of Alberta, Canada.*

Background: Acute kidney injury (AKI) or worsening renal function (WRF) is an important complication of acute cardiac conditions, including acute heart failure and acute coronary syndrome (ACS). This was recently classified as Cardio-Renal Syndrome Type 1 (CRST1). The aim of this systematic review was to establish the incidence of CRST1 in patients hospitalized with ACS and describe its impact on mortality.

Methods: We searched Medline, EMBASE and CENTRAL for studies with original data on AKI and its associated mortality in ACS. Literature search, study selection and data extraction were performed in duplicate. Primary outcome was all-cause mortality. Pooled analysis was performed using a random-effects model. Adjusted odds/hazard ratios were extracted when available, and used in a secondary analysis.

Results: We identified 15 studies from 10 countries (n=249,482 patients). Sample size ranged from 76 to 147,007; follow-up ranged from duration of hospitalization to 48 mos; 2 large studies had 10-yr follow-up. AKI was variably defined using change in serum creatinine (ΔsCr) only (n=11 studies), ΔeGFR, either ΔsCr and/or ΔeGFR, either ΔsCr and/or urine output, or by Δblood urea nitrogen only (n=1 for all). CRST1 occurred in 67,978 (27.2%) subjects. CRST1 was associated with significantly higher odds for death (Fig.1, Odds Ratio 4.95; 95% CI 2.87-8.53, p<0.001; 11 studies, n=11,591). There was statistical heterogeneity across studies (I^2 92%, p<0.001). Using adjusted-OR (n=10 studies, n=10,522), CRST1 remained associated with a significantly higher odds of death (OR 1.99; 95% CI, 1.76-2.25, p<0.001; I^2 87%, p<0.001). The 2 largest studies reported mortality by AKI severity; in both, worse AKI was significantly associated with higher mortality.

Conclusions: In patients presenting with ACS, 1 in 4 develop clinically important CRS Type 1 that portends a higher risk of death.



PUB044

Weight Loss by Bariatric Surgery May Permit Patients with Significant Residual Renal Function to Come Off Dialysis Jack Rubin. *Medicine, Los Alamitos Medical Center, Los Alamitos, CA.*

Background: Many years ago a wise nephrologist was heard to say; "Patients shrink to the amount of dialysis provided them." But what if patients were shrunk to the amount of residual kidney function to support their body mass? Could they then be removed from dialysis? A BMI > 35 is present in almost 14% of Network 18 patients and of these 14% almost 22% have Diabetes. The average GFR of patients starting dialysis approximates 10 ml/min.

We report two patients who underwent bariatric surgery and after substantial weight loss came off dialysis. Patient 1, age 60, had chronic interstitial nephritis from repeated urinary tract infections and recurrent stone disease as well as type 2 diabetes as the major etiologies of renal failure. She was on dialysis 1208 days of which 508 days occurred after banding and gastric bypass surgery. She weighed 312 pounds at the start of dialysis and 142 at the end. Her residual renal function was 6 ml/min at the start and 13 ml/min at the end. She has been off dialysis over 2 years. Her residual renal function remains 13 ml/min. Patient 2, age 55, had type 2 diabetes and acute renal failure culminating in dialysis (possible cortical necrosis) as the cause of renal failure. He was on dialysis 828 days of which 469 were after gastric banding. His residual renal function at the start of dialysis was 15 ml/min and 20 ml/min when removed from dialysis. He weighed 292 pounds at the start of dialysis and 200 pounds when removed from dialysis. He has been of dialysis 3 months and his residual renal function remains 20 ml/min.

It seems sensible to consider offering bariatric surgery to patients with adequate residual renal function, especially since it may convert a dialysis patient to a non dialysis office patient with "just" chronic renal failure. Removing these potentially many selected patients from dialysis by bariatric surgery seems a better and perhaps less expensive option then a long wait on dialysis for an expensive transplant that may never to come because they are too big.

PUB045

Acute Renal Failure due to Anaplasmosis or Thrombotic Thrombocytopenic Purpura? Manish K. Saha,[1] Tarek Hamieh,[1] Vishal Sagar.[1] *[1]Internal Medicine, Health Partners; [2]Internal Medicine, Health Partners; [3]Nephrology, Health Partners.*

Background: Patients who present with fever, confusion, in the setting of acute renal failure and thrombocytopenia almost always begin with a presumptive diagnosis of TTP/HUS. We present a case series of 3 patients who had similar presentations. Peripheral blood smear (PBS) did not reveal schistocytes, but rather, anaplasmosis/ehrlichiosis.

Results: All patients presented in the summer months, from Midwest, with fever, confusion, and diarrhea. Relevant clinical and laboratory data as noted in the table.

Table 1

Case	Creatinine(mg/dl)	Post Rx creatinine	FENA	HGB(g/dl)	Platelet(K/UL)	ALT	CPK
1	4	1.4	<1	13	27	40	451
2	11	1.5	3	12	22	252	3648
3	6.5	1.6	2	12	44	40	235

Urinalysis showed active sediments with minimal proteinuria.

Conclusions: The combination of fever, confusion, renal failure, and thrombocytopenia in our patients initially directed us to entertain TTP/HUS as the main differential diagnosis. However, there was no evidence of hemolysis on PBS. Given the prevalence of tick-borne illnesses in the summer, a PBS of Buffy coat was done. All patients were found to have anaplasmosis/ehrlichiosis. Two patients initially required hemodialysis on presentation. Treatment with doxycylcine resulted in rapid improvements in renal function. Anaplasmosis-Ehrlichosis are tick born bacterial illnesses most prevalent in Midwestern and Northeastern region. Our case series highlights the need to consider anaplasmosis in the work up of patients presenting with TTP/HUS like picture. A high index of suspicion leads to early diagnosis and initiation of therapy. Although one case report had suggested rhabdomyolysis causing renal failure with anaplasmosis, our patients had minimally elevated creatine kinase. The presence of active sediment on urinalysis and improvement in renal function with doxycycline suggest infectious interstitial nephritis as a possible mechanism of renal failure. Acute tubular necrosis or hypovolemia due to diarrhea can also play a role. Renal biopsy may assist in understanding the pathogenesis; however, given the profound thrombocytopenia in our patients and rapid improvement with initiation of antibiotics, biopsy was not preformed.

PUB046

Urine Sediment Contains Acute Kidney Injury-Specific Proteins Nithin Karakala, Benjamin Neely, Alison Bland, Joseph Alge, Michael G. Janech, Juan Carlos Q. Velez, John M. Arthur. *Department of Medicine, Nephrology, Medical University of South Carolina, Charleston, SC.*

Background: Urine sediment(SED) is an important source of information in the diagnosis of acute kidney injury (AKI). While urine supernatant (SUP) has been investigated for identifying diagnostic and prognostic biomarkers of AKI, no studies have attempted to identify candidate markers in SED. We hypothesized that urine SED may contain proteins of diagnostic value for AKI.

Methods: Urine was collected from 4 patients in the medical ICU without AKI and 4 with AKIN stage 3 AKI. Liquid chromatography/tandem mass spectrometry was used to identify proteins that are different between SUP and SED.

Results: We identified 666 proteins (327 SUP no AKI, 459 SED no AKI, 413 SUP AKI and 420 SED AKI). 16 and 36 proteins were unique to the SED AKI and SED no AKI groups respectively. 26 proteins were statistically (p<0.5) more abundant and 15 less abundant in SED AKI compared to the other groups. Analysis of Gene Ontology (GO) terms showed that SED AKI is enriched in proteins derived from mitochondria. These differences could be related to mitochondrial injury or to biogenesis. No other differences in GO term frequencies were seen between groups. Tsukushin and Xaa-Pro dipeptidase were present in 3 out of 4 AKI SED samples and none of the other 12 samples. These proteins are predominantly present in the cytoplasm of renal tubular cells. Tsukushin is a

small leucine-rich repeat proteoglycan that forms a coat on the fibril surface that impedes access to collagenolytic proteinases. It could be playing a role in tubular cell regeneration after AKI. Xaa-Pro dipeptidase splits dipeptides with a prolyl or hydroxyprolyl residue in the C-terminal position. It plays a role in collagen metabolism and may be important in the regulation of basement membrane deposition or degradation.

Unique Proteins

	Supernatant	Sediment
AKI 3	49	15
Without AKI	16	36

Conclusions: Urine sediment contains proteins present in different abundances than the supernatant in AKI. Analysis of the sediment could be a gateway to identifying novel biomarkers and understanding mechanisms of injury and repair.

Funding: NIDDK Support

PUB047

Predictors of Acute Kidney Injury Following on Pump Cardiac Surgery Keren Grynberg,[1,3] Kevan Polkinghorne,[1,2] Jonathan Barrett,[3] Shaun A. Summers.[1,2] *[1]Department of Nephrology Monash Medical Centre, Southern Health, Melbourne, Victoria, Australia; [2]Department of Medicine, Monash University, Melbourne, Victoria, Australia; [3]Intensive Care Unit, Cabrini Health, Melbourne, Victoria, Australia.*

Background: Acute kidney Injury (AKI) post cardiac surgery is a poor prognostic indicator, which independently predicts mortality. Newer published biomarkers are expensive and are not routinely available in clinical practice. We aimed to define the incidence of AKI post cardiac surgery, to identify pre-morbid and operative risk factors for developing AKI, and to accurately predict patients at risk of AKI using routine biochemical tests.

Methods: We prospectively studied 93 consecutive patients undergoing elective (on pump) cardiac surgery. Baseline patient characteristics, including presence of medical co-morbidities, proteinuria, procedural data and kidney function (serum creatinine (sCr) were collected. Internationally standardised criteria for AKI was used, (sCr >1.5 times baseline, elevation in sCr >26.4 µmmol/L (0.3mg/dl), or urine output< 0.5ml/kg/hr for >6 hours within 48 hours). Measurements were collected pre-operatively, within 2 hours of surgical completion and daily for two days. Logistic regression was used to assess predictive factors for AKI including immediate post operative (OPsCr). Model discrimination was assessed using ROC AUC curves.

Results: 18 (19%) patients developed AKI in the post operative period. Inotrope use (OR 3.90, p<0.026), preoperative eGFR (OR 0.96, p=0.013), but not proteinuria (OR 2.58, p=0.11) were associated with AKI in univariate analysis. A multivariate logistic model with preoperative and surgical factors (age, gender, eGFR, proteinuria, cardiac bypass time & inotropes) demonstrated moderate discrimination for AKI (ROC AUC 0.76). The addition of OPsCr improved model discrimination for AKI (AUC 0.83) independently associated with AKI (OR 1.39/µmol increase, 95% CI 1.05-1.82, p=0.018).

Conclusions: 1 in 5 patients developed AKI post cardiac surgery. The development of AKI post cardiac surgery is strongly linked to patient morbidity and mortality. OPsCr accurately predicts the development of AKI, providing a cheap readily available prognostic marker.

PUB048

Comparison of Interstitial Inflammatory Pattern of Acute Interstitial Nephritis with Various Etiologies Cui Li, Tao Su, Rong Chu, Xiaomei Li, Li Yang. *Renal Division, Peking University First Hospital, Beijing, China.*

Background: Acute interstitial nephritis (AIN) accounts for 10% of biopsied acute kidney injury, and is pathologically characterized by dramatic interstitial inflammation with intact glomeruli. Drug hypersensitivity and autoimmune diseases are the major causes of AIN. This study aimed to investigate the difference of inflammatory infiltrate in AIN patients with various etiologies.

Methods: One hundred patients, 21 men and 79 women, aged 47±13y, were clinical-pathologically diagnosed as AIN from 2001 to 2011 and enrolled in the study. More than one year follow-up was performed to ascertain the etiology of AIN. Inflammatory cells were determined on the renal biopsy specimens through immunofluorescence staining of anti-CD3 (T lymphocyte), CD20 (B lymphocyte), CD68 (Monocyte/Macrophage), CD38 (Plasma cell) and neutrophil elastase (Neutrophil). Eosinophil was detected with H&E staining. The number of positive cells was counted and the difference among the AIN cases with various etiologies was tested.

Results: Sixty-three patients had drug hypersensitivity AIN (DAIN), 22 developed Tubulointerstitial Nephritis and Uveitis syndrome (TINU, 13 had later onset uveitis) and 15 were of Sjogren's syndrome (SS). Patients of DAIN and TINU exhibited comparable amount of interstitial infiltrates (402±120 vs 436±122 /400xHP) with similar inflammatory cell proportion, T lymphocyte (46±8% vs 48±8%), Monocyte/Macrophage (26±7% vs 24±6%), B lymphocyte (12±6% vs 13±3%) and Plasma cell (11±5% vs 10±4%). Neutrophil and Eosinohil were common in both groups. Patients with SS had fewer infiltrates (323±107, p=0.02), more plasma cells (30±14%, p=0.000) and less percentage of the rest kinds of inflammatory cells (p<0.001). Neutrophil or eosinohil was rarely seen in SS kidneys (p=0.000).

Conclusions: DAIN and TINU exhibit the same pattern of renal inflammatory infiltration, making the differential diagnosis rather difficult in those who develop uveitis later on. Cellular immunity is the major immune response while the pathogenic role of the 20% humoral immune infiltrates needs further investigation. The SS related AIN lacks acute inflammatory response, and the humoral immunity plays a key role.

Funding: Government Support - Non-U.S.

PUB049

Kidney Injury Molecule-1 Is More Sensitive Biomarker on Contrast-Induced Nephropathy in Children Min Hyun Cho, Youngju Hwang. *Pediatrics, Kyungpook National University Hospital, Korea.*

Background: Contrast-induced nephropathy (CIN) is an important cause of acute kidney injury (AKI) in hospitalized patients. The aim of this study was to discover the reliable and sensitive biomarkers on AKI caused by the contrast material used for cardiac catheterization in pediatric patients with congenital heart disease.

Methods: A variety of clinical findings were analyzed in 26 children that received cardiac catheterization for congenital heart disease; including the amount of contrast agent used and the level of serum creatinine before and after the catheterization. No patient had a prior history of renal disease. The level of urine KIM-1, NGAL, IL-18, L-FABP as candidate biomarkers for AKI were evaluated before and 6, 24, 48 hours later after catheterization by ELISA and compared with each other.

Results: The mean age of the patients was 7.1 years and the male: female ratio was 12:14. Although only one patient had cyanosis caused by pulmonary atresia (oxygen saturation 89%), the others had no cyanosis or congestive heart failure. Ultravist®, a low osmolar dye, was used as the contrast media in all 26 cases; the amount of contrast agent used was on average 31.2±16.7 mL (2.5±2.8 mL/kg of weight). The levels of serum creatinine were checked before, 6h, and 24h after catheterization and showed little change within the normal range as: 0.4±0.19, 0.41±0.19, 0.41±0.22 mg/dL, respectively. The level of urine IL-18, NGAL and L-FABP also showed no significant change to the infusion of contrast. However, the average levels of the urine KIM-1 evaluated before, at 6h, 24h and 48h (N=11) after catheterization were: 70.94±80.83, 78.33±52.5, 107.98±94.2 and 133.21±136.86 pg/mL respectively, showing a progressive increase after catheterization. There was significant difference between the level of KIM-1 before and at 24h after catheterization.

Conclusions: KIM-1 is more sensitive biomarker on contrast-induced AKI in children than other biomarkers including NGAL, IL-18 and L-FABP. Further prospective research is needed.

PUB050

Three Demensions of Hospital-Acquired Acute Kidney Injury ImpactLong-Term Outcome Jinyoung Yoo, Jiyeon Lee, Jin Seok Jeon, Hyunjin Noh, Dong-Cheol Han, Soon Hyo Kwon. *Internal Medicine, Soon Chun Hyang University Hospital, Seoul, Korea.*

Background: Acute kidney injury (AKI) has 3 dimensions; severity, reversibility and duration. We investigated whether these dimensions of AKI impact long-term survivals in hospital-acquired AKI patients.

Methods: We monitored serum creatinine everyday for all patients admitted in Soon Chun Hyang Hospital using a hospital data survey system from Sep. 2007 to August 2008. One hundred twenty three hospital-acquired AKI patients were followed up until 2011. Duration of AKI was categorized as AKI for 1 to 2, 3 to 6, at least 7 days and persistent AKI on the basis of renal recovery back to baseline at the time of discharge. And the severity of AKI was classified by AKIN staging criteria. Kaplan-Meier techiniques were used to conduct the survival analysis.

Results: Duration of AKI: 1 to 2 days (32; 26%), 3 to 6 days (23; 18.7%), at least 7 days (27; 22%), persistent AKI (41; 33.3%) were categorized. AKI stage was classified as stage 1, 28.5%, stage 2, 30.9%, and stage 3, 40.7%. The median follow-up duration was 240 days (53 - 1428 days). Our data showed that persistent AKI and AKIN stage 3 were associated with high mortality (p<0.001, p=0.023). There was no difference in long-term outcome between each duration group (p=0.365).

Conclusions: Severity and reversibility of AKI impact mortality in hospitalized patients. Even short duration of AKI has same effects in long-term outcome compared to long duration group.

PUB051

Carpediem: Cardio-Renal Pediatric Dialysis Emergency Machine: A New Hope for Infant Francesco Garzotto,[1] Anna Clementi,[1] Jeong Chul Kim,[1] Roberto Cena,[2] Monica Zanella,[1] Claudio Ronco.[1] *[1]Dep of Nephrology and IRRIV, St. Bortolo, Vicenza, Italy; [2]Bellco, Mirandola, Italy.*

Background: Although acute kidney injury is a well known independent risk factor for increased morbidity and mortality in critically ill paediatric patients, a safe and specific renal replacement therapy does not still exist.The Cardio-Renal Pediatric Dialysis Emergency Machine (CARPEDIEM) was designed for newborns and infants.The aim of our study was to evaluate their performance respect to some of the design constraints.

Methods: Arterial and venous line pressures were evaluated with a 5FR (3L,11cm) and a 5FR(2L,13cm) catheters, using bovine blood (hct 35%) at increasing values of blood flows.The filtration fraction was kept constant at 20%.The UF vs TMP characteristics was assessed using the 0.147m^2 dialyzer and a 4 FR catheter(2L, 5cm) varying UF from 0 to 4 ml/min at 4 different Qb(10–30mL/min) recording the corresponding stabilized TMPs. The m-hemolysis was evaluated by measuring the normalized (by hematocrit) index of hemolysis (NIH).We tested, with 1L of bovine blood hct 45% circulated for 10 hours,3 different kits with 3 tests each:1st with the 0.075m^2 dialyzer and a Qb of 40 ml/min,2nd with the 0.245m^2 and a Qb of 50mL/min,3rd without the dialyzer.

Results: As shown in Figure 1, arterial and venous blood pressures,varied within a safe range up to 30mL/min of Qb. Figure2 reports the UF vs TMP relationships which are non linear at low Qb (10-15) with a plateau at 80 mmHg,while are linear at higher Qb in the range of allowed UF.As expected,an increase of Qb was associated to a decrease of TMP. Figure3 reports the mean of NIH.The µ-hemolysis index which is always lower than 10,7x10^-4,no difference were observed.

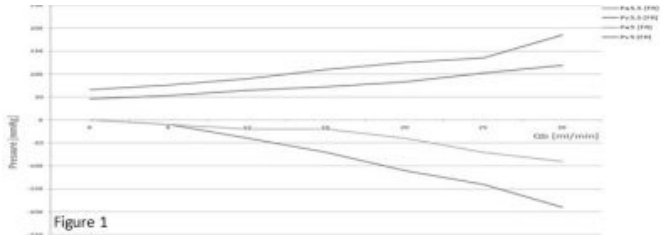


Figure 1

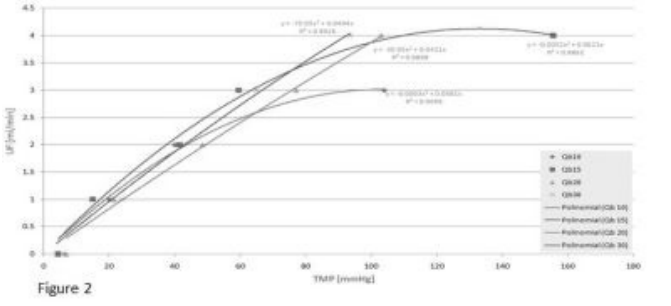


Figure 2

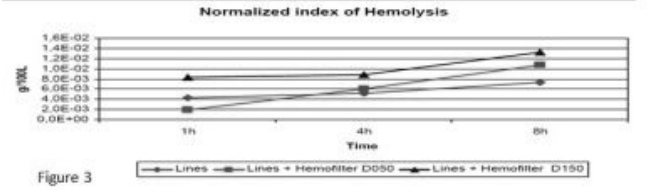


Figure 3

Conclusions: The CARPEDIEM performance connected to different small catheters seems to be adequate to treat paediatric patients safely.Pressure values were stable during all the phases of the test.

PUB052

Caucasian Race as Risk Factor for Vancomycin Nephrotoxicity Srujana Polsani,[1] Jolina Pamela C. Santos,[1] Kisra Anis,[1] Andi Qipo,[1] Belinda Bun Jim.[1] *[1]Jacobi Medical Center; [2]Albert Einstein College of Medicine, Bronx, NY.*

Background: The incidence of vancomycin nephrotoxicity ranges from 14.6 to 29.6%. Risk factors include high trough levels, duration of therapy, concomitant nephrotoxins, obesity and advanced age. Here we confirm well known risk factors, as well as describe novel features of vancomycin nephrotoxicity from an inner city hospital that serves predominantly a minority population.

Methods: We report on 7 cases of vancomycin nephrotoxicity encountered between July 2011 and June 2012. Clinical data such as age, sex, race and ethnicity, comorbidities, dose and duration of vancomycin therapy, vancomycin levels, BUN and serum creatinine were collected.

Results: We found that the median (interquartile range) age was 87 years (63, 90), vancomycin level 40.7 (30.1, 50.8), and peak BUN-to-serum creatinine ratio 11.5 (10.6, 16.9) for all cases. Administration of vancomycin ranged from 4 to 14 days, all via intermittent dosing. There was a predominance of females (71%) vs males (29%) affected. All of the patients were on piperacillin-tazobactam (100%). Forty-two percent of patients had BMI of less than 18 kg/m^2, while 58% had BMI of greater than 20 kg/m^2. Unexpectedly, there was a high percentage of Caucasians affected (43%) vs. African Americans (29%), Hispanics (14%) and Asian Americans (14%). Given that racial mixture of our hospital consists of only 6.5% Caucasians, with African American making up 31.5% and Hispanics 48.5%, this suggests the Caucasian race is an additional risk factor. We also note that the BUN-to-serum creatinine ratio is relatively low (<20), helping to discriminate from a pre-renal state.

Conclusions: We conclude that though our series of patients exhibited traditional risk factors, we also discovered unique features such as the Caucasian race as a new risk factor, a stronger than usual association with piperacillin-tazobactam, and a notable "low" BUN-to-serum creatinine ratio. Given that novel risk factors still being discerned, we recommend to initially follow vancomycin levels until proper dosing can be established. These findings are being confirmed in a larger cohort.

PUB053

The Risk Stratification for the Development of Acute Kidney Injury after Orthopedic Surgery: Preliminary Report Tae Hee Kim,[1] Mi Seon Kang,[2] Sunwoo Kang,[1] Yang Wook Kim.[3] *[1]Internal Medicine, Busan Paik Hospital, University of InJe, Busan, Republic of Korea; [2]Pathology, Busan Paik Hospital, University of InJe, Busan, Republic of Korea; [3]Internal Medicine, Heaundae Paik Hospital, University of InJe, Busan.*

Background: Acute kidney injury (AKI) following operation is associated with increased morbidity and mortality. Specific risk stratification and prognostic scores for the development of AKI has been reported well after cardiac surgery. Recently orthopedic surgery was increased due to aging, trauma, and accident. But, there is little known about postoperative AKI risk after orthopedic surgery. We could easily and accurately identify high-risk patients for the development of AKI following orthopedic surgery.

Methods: From January 2007 to November 2011, 488 patients undergoing orthopedic surgery at Busan Paik Hospital, University of InJe were evaluated. Inclusion criteria were the following: male and female: above 18 years of age: using general anesthesia: femur surgery. Univariate and multivariate analyses were used to evaluate pre-, intra-, and postoperative parameters associated with AKI (underlying disease, serum creatinine, albumin, hemoglobin, bicarbonate, transfusion or not, and pre-op cardiac function). The definition of AKI used an increase of 50% above baseline serum creatinine.

Results: The incidence of AKI was 2.7% (n=13), there was no one who required dialysis.

58 patients had already chronic kidney disease or AKI. 96.6% (n=56) among 58 patients after surgery improved or didn't change their baseline serum creatinine. We suggest that preoperative hydration, short operation time, and postoperative intensive care.

The limitation of this preliminary report is that the incidence of AKI is very low (2.7%), and the reliability of statistical analyses is not confident.

Conclusions: We extended and reevaluated patients including knee orthopedic surgery.

PUB054

Acute Kidney Injury with Normal Urinalysis Alon Antebi, Daniel Kushnir, Victor Frajewicki. *Department of Nephrology and Hypertension, Carmel Medical Center, Haifa, Israel.*

Background: Acute kidney injury (AKI) is associated with increased risk for development of CKD or death. Urine examination is the oldest and one of the most commonly used tests for differential diagnosis of AKI.

Methods: We reviewed complete electronic records for all patients who were admitted to the our service due to AKI, during a 3 year period (2004-2007). Patients with normal urinalysis were selected.The cause of AKI was recorded from the final diagnosis. Urinary ultrasound was performed in all patients before admission to the Nephrology ward.

Results: Of 227 patients (266 admissions) who met inclusion criteria of AKI, 19 cases (7%) were found with normal urinalysis, aged 71±14 years.

Cause of AKI

Clinical Diagnosis	Incidence (%)
Prerenal Azotemia	68.0
Cardio-renal Syndrome	10.5
Obstruction	5.2
Sepsis	5.2
CKD	5.2
Unknown	5.2

In 6 cases AKI was considered drug related, mostly ACEI. Serum creatinine at admission was 3.2 ± 1.5 mg/dl (range 1.5-6.5) with a concomitant urea level of 162 ±93 mg/dl (range 33-380). Admission blood pressure was 127±26/69±16 mmHg. No kidney biopsy was performed in these patients. Two (10%) needed temporary dialysis treatment, but no patient required permanent dialysis. All patients were discharged alive with improvement of their kidney function. Hospitalization length was 5.9±2.4 days.

Conclusions: AKI with normal urine examination is related to prerenal azotemia in most cases if urinary tree obstruction is rouled out before admission. Drug related renal dysfunction should be considered in the differential diagnosis. Short term prognosis was excellent, which allowed to discharge 100% of patients.

PUB055

Path Batch Hemodialysis (PBH): A Safe and Efficient Method for Acute Kidney Injury (AKI) Cancer Patients in the Intensive Care Unit (ICU) Veronica T. Costa e Silva,[1] Ana Paula Leandro Oliveira,[1] Elerson Costalonga,[1] Henrique Palomba,[1] Ludhmila Abrahão Hajjar,[2] James Hung,[1] Cilene Muniz Soares,[1] Juliana Silva Bezerra,[1] Luciane Oikawa,[1] Luis Yu,[1] Emmanuel A. Burdmann.[1] *[1]Nephrology Division, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Intensive Care Unit Department, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Background: The adequacy and safety of PBH have not been evaluated in AKI ICU cancer patients (pcts).

Methods: We prospectively analyzed all PBH performed in AKI adult cancer pcts in the Sao Paulo State Cancer Institute ICU from June 2010 to February 2012.

Results: A total of 373 PBH were performed in 116 AKI cancer pcts. Their characteristics were age 61 14 y, 62.1% male, 18.5% on vasopressors and 10.2% on mechanical ventilation. Most pcts (81%) had solid cancer (urinary tract 41.4%,

gastrointestinal tract 13.8% and gynecologic system 12.1%). The most important AKI etiologies were sepsis (55.2%), obstructive uropathy (27.6%) and surgeries (25.0%). Hospital mortality was 59.5%. Venous access was temporary catheter in 97.9% (58% femoral and 44% internal jugular veins), high-flux polysulphone membrane filters (FS80 in 67%) were utilized and median blood and dialysate flows were 250 (200 300) mL/min. They received 2.97 1.1 hemodialysis (HD) during 4.9 +/- 2.1 days of therapy. Median received Kt/V per session was 0.95 (0.80 – 1.10) and an adequate metabolic control was achieved (post dialysis tests results): Cr 2.70 (1.96 – 3.59) mg/dL; urea 71 (49 – 102) mg/dL; bic 24.2 (22.5 – 25.5) mEq/l; K 3.8 (3.5 – 4.1) mEq/l. The prescribed ultrafiltration was 1500ml (500 2000) which was obtained in 66% of the procedures. The prescribed dialysis time was 240 (240 360) min which was attained in 75% of HDs. The main complications were: hypotension (mean blood pressure <70 mmHg) in 24.9% of HDs, but only 6.3% required dialysis interruption; prescribed blood flow reduction in 6.7%; lines reversion in 19.8% and lines clotting with dialysis interruption in 16.9%.

Conclusions: PBH was as a safe and adequate alternative for HD in this group of AKI ICU cancer pcts.

PUB056

Acute Kidney Injury in a London District General Hospital: Retrospective Audit Data from the North Central London Acute Kidney Injury Network
Arunraj Navaratnarajah,[1] Peter Simon West,[2] Chris Laing,[3] Shyama Dakshina Jayasena.[2] *[1]Kings College London Hospital, London; [2]North Middlesex University Hospital, London; [3]Royal Free Hospital, London.*

Background: The NCEPOD audit 'Adding Insult to Injury' highlighted deficiencies of care in AKI management. The North Central London Acute Kidney Injury Network was established to address these deficiencies at a local level. We present baseline audit data from a London District General Hospital prior to network implementation.

Methods: Patients aged >16 admitted with AKI to North Middlesex University Hospital, London, between March 1st 2009 and March 1st 2010, were reviewed. Cases were included if criteria for AKI stage 3 (AKIN classification) were fulfilled.

Results: 40 patients with AKI3 were audited. No risk assessment was documented in any patient, though co-morbidity scores were high on review. Risk factors included dehydration (65%), sepsis (55%) and ACE-i / ARB / NSAID therapy (53%). 98% of patients had appropriate admission observations. 63% of patients had timely consultant review. 93% received appropriate fluid resuscitation. Cessation of potential nephrotoxins occurred in 83%. 50% were imaged either by US (40%) or CT (10%) within 12 hours. Of septic patients (63%), 84% were given timely administration of antibiotics. 50% of patients were referred to nephrology. 1 patient required transfer to the local tertiary centre for renal replacement therapy (RRT). 5 patients (13%) were admitted to ITU, 2 for RRT alone. In 35%, renal function returned to baseline pre-discharge. Of those that did not (40%, excluding deaths), 38% had nephrology follow-up, 31% primary care follow-up and 25% no follow-up. 20% died during admission due to renal failure. Subsequent follow-up revealed a further 13% mortality.

Conclusions: Problems were highlighted at individual, local and regional level. Local factors include i) delays in access to imaging ii) use of critical care beds due to lack of specialist renal beds iii) limitations of coding in identifying serious AKI. Regional issues include lack of co-ordination of care between district general and tertiary centres for specialist renal care. We hope the network initiative will improve all aspects of AKI care.

PUB057

Factors Associated with Acute Kidney Injury in Patients with Post Hepatic Resection
Mary Muoneke, Bruce E. Berger. *[1]Case Western Reserve University/ St Vincent Charity Medical Center; [2]Nephrology, University Hospitals Case Medical Center.*

Background: We looked at the development of Acute Kidney Injury(AKI) following hepatic resection. We defined AKI as an absolute increase in the serum creatinine level of 0.3mg/dl or above from baseline within 48hours. In this study, we explored the relationship between AKI post resection & co-morbidities like Diabetes, Hypertension, Chronic Kidney Disease (CKD)& use of medications such as Angiotensin converting enzyme inhibitors(ACEI) or non steroidal anti-inflammatory drugs(NSAIDS).

Methods: To understand the distribution of important variables in the study, a univariate analysis was conducted. Between Dec, 2010 & Dec 2011, 69 consecutive patients underwent hepatic resection. 31 for primary malignancy& 38 for metastatic malignancy.8 patients developed AKI post hepatic resection& were compared to 8 randomly selected patients from a pool of 61 patients who had hepatic resection but no AKI.

Results: 75% of the participants in both groups were males. Mean age in the AKI group was 60yrs & 62yrs in non AKI group.

The odds of Hypertension among the individuals with AKI was 80% higher compared to the odds among those without AKI.

The odds of Diabetes among individuals with AKI was 57% lower compared to the odds among those without AKI

The odds of CKD among those with post hepatic resection AKI was similar to the odds among those without AKI.(OR=1.00)

Prior use of NSAIDS and/ or ACEI showed a remarkable association with AKI post resection.There was a 21 fold increase in the odds of regular use of NSAIDS and/or ACEI among those with AKI compared to those without AKI.

There was no observed changes in liver function tests among those with AKI compared to those without AKI.

Conclusions: AKI post hepatic resection was found to be significantly associated with prior use of NSAIDS and/or ACEI & hypertension and inversely associated with diabetes. There may be a need to investigate these variables as collective factors in AKI as well as replicate this study in a larger population.

Limitation:This study has a relatively small sample size although the findings may help direct future investigations into the cause of AKI in post resection patients.

PUB058

Factors Related to Coagulation in Non Continuous Hemodialysis (NCHD) for Acute Kidney Injury (AKI) Cancer Patients in the Intensive Care Unit (ICU)
Veronica T. Costa e Silva,[1] Cilene Muniz Soares,[1] Henrique Palomba,[1] Elerson Costalonga,[1] Ludhmila Abrahão Hajjar,[2] James Hung,[1] Ana Paula Leandro Oliveira,[1] Luciane Oikawa,[1] Juliana Silva Bezerra,[1] Luis Yu,[1] Emmanuel A. Burdmann.[1] *[1]Nephrology Division, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Intensive Care Unite Department, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Background: Factors which could lead to dialysis inefficiency or interruption have not been well studied in AKI ICU cancer patients (pcts).

Methods: We prospectively analyzed all NCHD performed in AKI adult cancer pcts in the Sao Paulo State Cancer Institute ICU from June 2010 to February 2012.

Results: A total of 373 NCHD sessions were performed in 116 AKI pcts. Pcts' characteristics were age 61 +/- 14 y, 62.1% male, 18.5% on vasopressors. Most (81%) pcts had solid cancer, 35.7% with metastatic disease (MD) and 16.4% with previous chemotherapy (CT). Sepsis was the most important AKI etiology factor (55.2%). Hospital mortality was 59.5%. Venous access was temporary catheter (11 Fr) in 97.9% (58% femoral and 44% internal jugular veins). Catheter malfunctioning (CM), defined as prescribed blood flow reduction or lines reversion was observed in 6.7% and 19.8% of hemodialysis (HD), respectively. High-flux polysulphone membrane filters were utilized (FS80 in 67%). Median blood and dialysate flows were 250 (200 300) mL/min, achieved ultrafiltration was 1000 (500 2000) ml and dialysis time was 240 (180 300) min (18.5% of HD > 6 h). Heparin was not used in 66% of the procedures due to contra-indications. Lines clotting causing dialysis interruption was observed in 16.9% of the sessions and was related to CM (OR: 3.01/CI:1.72 - 5.22) and heparin use (OR: 0.33/0.79 – 0.93). The following factors were not related to clotting: catheter site (P: 0.105 and 0.100, for femoral and jugular sites, respectively); HD > 6 h (P: 0.209); MD (P: 0.332); previous CT (P: 0.293); thrombocytopenia (P: 0.265); coagulopathy (P: 0.090) and hypotension (P: 0.103).

Conclusions: Factors related to clotting in NCHD AKI ICU cancer pcts were mostly related to CM and heparin use.

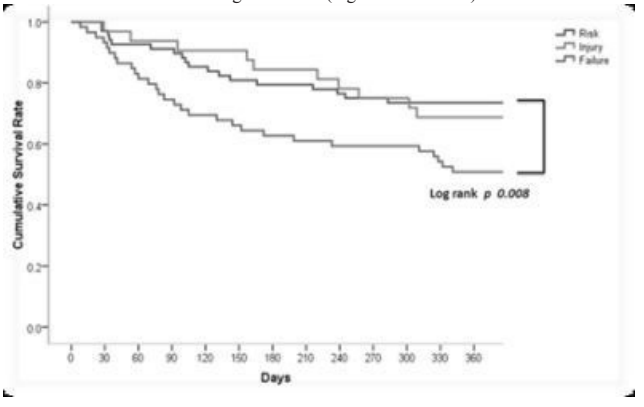
PUB059

One Year Clinical Outcome of Acute Kidney Injury Admitted to Medicine Service: Single Tertiary Care Center Experience
Nuttasith Larpparisuth, Nongnuch Lawattanatrakul, Thawee Chanchairujira, Somkiat Vasuvattakul. *Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.*

Background: Acute kidney injury (AKI) is the considerable problem which associated with higher in-hospital mortality and subsequent development of chronic kidney disease (CKD). Study of long-term outcome of AKI patients admitted to medicine service which have more complex entities is sparse.

Methods: We retrospectively reviewed all consecutive 5,570 patients admitted to medicine service, including ICU, in a single tertiary hospital between 1 January and 30 June 2010. AKI occurred in 870 patients (15.6%) with in-hospital mortality rate of 43% (374 patients). The retrieved data included demographic data,severity of AKI stratified by RIFLE criteria and mode of renal replacement therapy (RRT). 496 AKI patients who survived at discharge had been followed up for 1 year. The crucial factors for mortality and development of CKD stage V were analyzed by the Kaplan-Meier method.

Results: One-year mortality rate of surviving AKI patients was 33.9% (168 patients). Pre-existing CKD stage III and IV, lack of renal recovery at discharge, sepsis, cirrhosis and malignancy were independent determinants of one year survival. In aspect of change in renal function, the lowest one year survival was observed in patients with prior CKD who suffered from 'Failure' degree of AKI (log rank P = 0.008).



All patients with CKD stage 5 required RRT at one year follow-up (3.4%) had baseline eGFR < 60 ml/min. Pre-existing CKD was the most important risk factor for development of CKD stage 4 and 5 after AKI event.

Conclusions: AKI not only affects on short-term but also on long-term outcome in both mortality and renal survival especially in patients with CKD. Surviving AKI patients should be closely monitored in the months for potential development or progression of CKD and death.

Funding: Government Support - Non-U.S.

PUB060

Testing of a Multi-Platform IT Medication Inquiry System (MIS) in Chronic Kidney Disease (CKD) Clarissa Jonas Diamantidis,[1] Marni Zuckerman,[1] Jeffrey C. Fink.[1] *[1]Department of Medicine, University of Maryland, Baltimore, MD; [2]Tech Moksha Consulting, Herndon, VA; [3]Novel Health Strategies, Columbia, MD.*

Background: Patients with CKD are at high risk for medication errors. Establishing a simple health information technology (IT) application designed to guide the safe use of medications in CKD, and making it available on various technology platforms may reduce medication errors in patients with CKD who have a range in familiarity and comfort with IT tools.

Methods: Participants with CKD underwent formal usability testing of a medication inquiry search system (MIS) on three technology platforms: 1) mobile device with SMS text, 2) Personal Digital Assistant (PDA; e.g., iPod Touch), and 3) Internet website. A single interviewer (and 2nd recording observer) conducted all usability test with participants randomized to a predetermined testing sequence. The interviewer provided each participant with a list of 19 tasks on the mobile device and website, and 22 tasks on the PDA. Example tasks include entering a medication name in the MIS, and interpreting the safety response. Tasks were rated as either "completed", "non-critical error", or "critical error" (user cannot complete a task without significant interviewer intervention).

Results: 20 participants representative of the CKD population completed formal usability testing.

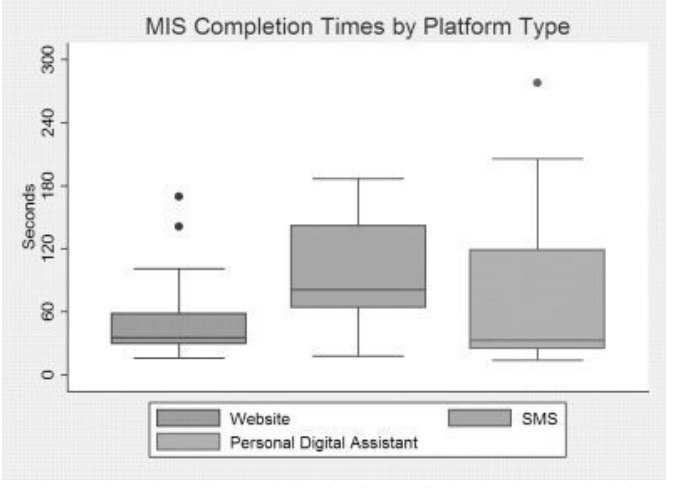


Figure 1 shows the median completion time for a medication inquiry on each platform. Total number of critical errors in the SMS, PDA, and Website group were 28 (7.36%), 23 (5.22%), and 28 (7.36%), respectively. After usability testing, 50% preferred to use the website-based MIS application, 30% favored the PDA, and 20% preferred the SMS application.

Conclusions: Understanding patient preference in technology use and adapting applications to a variety of technology portals will ensure most effective use of targeted interventions to improve patient safety in CKD.

Funding: Private Foundation Support

PUB061

Assessing Computer Literacy in Chronic Kidney Disease (CKD): Results from Safe Kidney Care Clarissa Jonas Diamantidis, Marni Zuckerman, Wanda Fink, Jeffrey C. Fink. *Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Background: Several reports have shown that health outcomes of patients with chronic disease can improve when they are empowered with information technology (IT) tools, however there are few such applications employed in CKD and it is not well-known how often CKD patients' use such technologies. As part of Safe Kidney Care (SKC), a study of patient safety in CKD which employs an educational Web site, we report here on computer and Internet usage characteristics of participants.

Methods: The SKC study (R01DK084017) is conducting two sequentially enrolled cohort studies in pre-dialysis CKD to assess patient safety among individuals with Stage III or greater CKD. The first cohort evaluates an alert system using a medical alert bracelet (or necklace) linked to an informational Web site to evaluate the effect of increased recognition of CKD on unsafe medical practices and errors. As part of baseline visits, SKC participants were asked about their computer access, and Internet usage and preferences. The results from Phase 1 baseline visits are presented here.

Results: Of 108 participants completing baseline visits, 85% (n=92) reported available computer access. 67% (n=72) reported using the Internet, with 55% of participants (n=59) reporting weekly to daily Internet use. Of those using the Internet, devices used to access the Internet were computer (97%, n=70), mobile device (21%, n=15), and entertainment device (6%, n=4). The majority of Internet users claimed use of the Internet without assistance (85%, n=61). 51 Internet users (71%) reported using the Internet to search for health information. Factors associated with regular Internet use are shown below.

Table 1. Adjusted odds of regular internet use among CKD patients in SKC

Baseline Characteristic*	Odds Ratio (OR)	P-Value
> High school diploma (ref. ≤ high school)	3.70	0.01
Adequate health literacy (ref. inadequate)	1.66	0.03

*age, gender, marital status, race, or GFR were not significant

Conclusions: Patients with CKD have variable access to a computer and the Internet. Distribution of health information in CKD should target a wide range of IT ability.

Funding: NIDDK Support

PUB062

Effects of Flow Configurations on Flow Dynamics and Solute Transport in a Helmofilter during Continuous Renal Replacement Therapy Jeong Chul Kim,[1,2] Francesco Garzotto,[1,2] Manish Kaushik,[1,2] Dinna N. Cruz,[1,2] Federico Nalesso,[1,2] Alessandra Brendolan,[1,2] Claudio Ronco.[1,2] *[1]Department of Nephrology, Dialysis & Transplantation, San Bortolo Hospital, Vicenza, Italy; [2]International Renal Research Institute, Vicenza, Italy.*

Background: In hemodialysis for CKD patients flow configurations between blood and dialysate are countercurrent to facilitate maximum diffusion of solutes. In contrast, in CRRT dialysate flow is less than 35 ml/min and flow configuration and blood flow direction are random depending on nurses' preference or machine design. Therefore, for a best practice of CRRT in ICU, it is necessary to evaluate the effects of flow configurations (cocurrent vs. countercurrent) and blood flow direction (upward vs. downward) on solute transport and flow dynamics in a hemofilter.

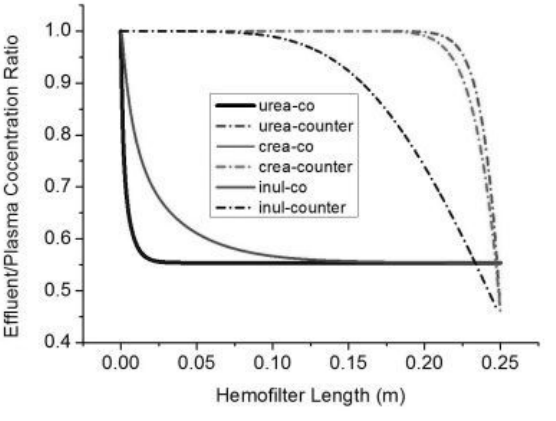
Methods: We analyzed flow dynamics and solute transport (urea, creatinine and inulin) in a hemofilter using computational fluid dynamic modeling according to 4 types of extracorporeal circuit setup during CVVH with blood flow rate of 200 ml/min and dialysate of 2 liters/h.

Results: Pressure profiles in a hemofilter are different depending on flow configuration and blood flow direction.

Pressure profiles in a hemofilter according to flow configuration and blood flow direction

Configuration	Cocurrent Flow		Countercurrent Flow	
Blood Flow Direction	Up	Down	Up	Down
ΔPB (mmHg)	29	5	23	1
ΔPD (mmHg)	24	10	8	20

For downward blood flow, pressure drops in the blood compartment were lower than upward because blood flow was assisted by gravity. Solute extraction ratio in countercurrent configuration is higher than that of cocurrent by 9.8% for urea. As molecular weight increased, the extraction ratio difference increased (9.9% for creatinine and 10.5% for inulin).



Conclusions: Countercurrent flow configuration and downward blood flow direction setting of CRRT could enhance solute removal in a stable flow dynamic condition.

PUB063

NEFRORED Developing a Registry of Patients with Lupus Nephritis and Primary Glomerulonephritis in the Caribbean Region of Colombia Gustavo Jose Aroca Martinez, Andres A. Cadena. *Nephrology, Clinica de la Costa, Barranquilla, Atlantico, Colombia.*

Background: In Colombia there is no reliable information about the local incidence, prevalence and outcomes of patients with lupus nephritis and other glomerular diseases. The Colombian healthcare system is limited in coverage and resources, the patients have

barriers to reach appropriate evaluations and there is insufficient availability of nephrologists for the diagnosis and management of glomerular diseases.

The Objective of NEFRORED is to establish a local network of early detection and attention of patients with lupus nephropathy and other glomerular diseases using a web-based platform.

Methods: NEFRORED was developed in 2008 under a web based protocol (MySQL), the project is shared by all the nephrologist of the northern region of Colombia (Caribbean) and has provided information on the epidemiology of the disease, outcomes, incidence and prevalence of cases. Figure 1

There are two groups of patients: children and adults, and the categories are lupus nephritis and all the other glomerular diseases are grouped in a second category. The university Simon Bolivar (http://www.unisimon.edu.co) has provided assistance with software development and also provides the hosting for the webpage www.nefrored.com.co, under all the standards of information safety and retrieval.

Results: As of 2012 NEFRORED includes data entry for 7 different states in the north of Colombia: Total of Lupus Nephritis 175 cases

LN in adults 166 cases LN in children 9 cases. Other Glomerular diseases 287 Total 486 cases reported.

Conclusions: NEFRORED is the first organized attempt of a group of nephrologists in Colombia to establish a local network for the identification, treatment, prevention and follow up evaluation of patients with lupus nephritis and other glomerular diseases in the Caribbean region of Colombia under a very simple and intuitive web base platform.

Funding: Clinical Revenue Support

PUB064

VEGF Production by Intracellular Prostatglandin-E_2: Role of Retinoic Acid Response Element (Rare) and EGF Receptor (EGFR) Javier Lucio, Ana Belen Fernandez. *Physiology, Alcala University, Alcala de Henares, Madrid, Spain.*

Background: In HK2 cells PGE_2 increases the production of renal protector VEGF through up-regulation of transcription factor retinoic acid receptor-ß (RARß) by intracellular PGE_2. Our aim here was to study the regulation of RARß expression and VEGF production by PGE_2.

Methods: Cells: HK2 (human proximal tubular) Treatment: 16,16-dymethyl-PGE_2 (analogue of PGE_2). Pre-incubations: transcriptional inhibitor actinomycin D (AMD), antagonists of EP receptors (EPR) AH6809/GW627368X, inhibitor of prostaglandin transporter bromocresol green (BG) or inhibitor of EGFR activation AG1478. Transfections: luciferase reporter construct containing RARE (RARE-Luc) from the RARß promoter. Quantifications: mRNA, Q-RT-PCR protein, WB, IF or ELISA. Statistical Analysis: nonparametric Bonferroni test.

Results: i) PGE_2 increased RARß mRNA and protein, the latter being prevented by AMD ii) AH6809, GW627368X or BG prevented PGE_2-induced RARß mRNA up-regulation. Therefore, PGE_2-induced RARß up-regulation is likely due to increased RARß mRNA transcription upon activation of intracellular EPR by intracellular PGE_2. iii) PGE_2 determined an AH6809-, GW627368X- and BG-sensitive increase in RARE-Luc activity (RARE sequences in the RARß promoter are the key for RARß mRNA up-regulation), iv) PGE_2 increased phosphorylated EGFR (a potential consequence of intracellular EPR activation) and VEGF, these effects being inhibited by AH6809, GW627368X or BG. v) AG1478 prevented the activation of RARE, the increase in RARß mRNA/protein expression and in VEGF production induced by PGE_2.

Conclusions: PGE_2-induced VEGF production is due to the sequential activation of EPR and EGFR by intracellular PGE_2, which results in activation of RARE in the RARß promoter leading to transcriptional up-regulation of RARß expression.

Funding: Government Support - Non-U.S.

PUB065

Identification of Translocated Promoter Region and α-Actinin-4 as Two Novel Ste20-Like Kinase, SLK-Interacting Proteins Aala Jaberi, Erika Hooker, Julie Guillemette, Joan Papillon, Andrey V. Cybulsky. *Medicine, McGill University, Montreal, QC, Canada.*

Background: Expression and activity of the Ste20-like kinase, SLK, are increased during kidney development and recovery from ischemic injury. SLK mediates apoptosis in renal tubular and glomerular epithelial cells (GEC). SLK can also regulate cell cycle progression and cytoskeletal remodeling. In cells, SLK is present as a high molecular mass complex, suggesting that SLK is a dimer/oligomer, or is in tight association with other proteins. To better understand the regulation, localization and function of SLK, we sought to identify proteins in this high molecular mass complex.

Methods: Proteins eluted from a SLK affinity column were analyzed by mass spectrometry. Two putative interacting proteins were then tested for interactions with SLK by co-immunoprecipitation and pull-down techniques. Interactions were further validated by immunofluorescence microscopy in GEC, COS-1 and HeLa cells.

Results: Analysis by mass spectrometry identified the nucleoporin, Tpr (translocated promoter region), and the cytoskeletal protein, α-actinin-4, as potential SLK-interacting proteins. The interaction of SLK with Tpr and α-actinin-4 was confirmed by co-immunoprecipitation with full-length SLK, and by pull-down with the 350 amino acid C-terminal, coiled-coil domain of SLK. The region of α-actinin-4 that interacted with SLK was within amino acids 1-300. Tpr and a portion of SLK colocalized in the perinuclear regions of cells, and there was cytoplasmic colocalization of SLK and α-actinin-4. Co-expression of SLK and Tpr in COS-1 cells resulted in reduced autophosphorylation of serine-189 in SLK, in keeping with reduced kinase activation; however, serine-189 phosphorylation was not affected by α-actinin-4.

Conclusions: Interactions of SLK with Tpr and α-actinin-4 are at least in part responsible for the high molecular mass complex of SLK that is present in cells. These protein-protein interactions may control the subcellular localization of SLK. Moreover, the interaction between SLK and Tpr reduces SLK activity. The interacting proteins may impact on the functions of SLK, including regulation of apoptosis, cell cycle, or cytoskeleton.

Funding: Government Support - Non-U.S.

PUB066

Role of Human ß Defensins in Patients with Chronic Kidney Disease Youn-su Park,[1] Min-jee Han,[1] Jung-ho Shin,[1] Su Hyun Kim,[1] Hye Ryoun Kim,[2] Suk-hee Yu.[1] *[1]Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea; [2]Laboratory Medicine, Chung-Ang University College of Medicine, Seoul, Korea.*

Background: DN was known as the main cause of chronic kidney disease (CKD), which was associated with alterations in the two major branches of the immune system, innate and adaptive immunity. Taken together, our aim was to determine whether HBD, pro-inflammatory cytokines including interleukin (IL)-6 and tumor necrotic factor (TNF)-α could be relevant factors of the development and aggravation of patients with CKD, especially from DN.

Methods: Serum samples were obtained from 338 patients who visited the department of nephrologic clinic between February and November 2011 and 88 sex, age-matched healthy controls (61.1±14.3 vs. 57.9±5.96 yrs, p>0.05). The concentration of HBD-1 were assayed using radio immunoassay kit. The concentration of HBD-2 ,IL-6 and TNF-α were assayed using enzyme-linked immunosorbent assay kit. And we reviewed the medical records of the patients.

Results: Serum concentrations of HBD-1, HBD-2, IL-6 and TNF-α were higher in CKD patients over healthy controls (2282.8±792.6 vs. 1605.7±331.7, p=0.000, 1535.9±420.1 vs. 1349.4±775.3, p=0.003, 3.91±3.14 vs. 2.49±2.45, p=0.000, 1.62±2.52 vs. 1.00±0.62, p=0.000, respectively). Serum concentration of HBD-1 was negatively correlated with hemoglobin (Hb)(β=-0.264, p=0.000), estimated GFR (β=-0.586, p=0.000) and positively correlated with fasting blood glucose level (β=0.226, p=0.000) in patients with CKD. Serum concentration of HBD-2 was correlated with WBC count (β=0.158, p=0.003) in patients with CKD and duration of hemodialysis period (β=-0.258, p=0.003). Serum concentration of HBD-1, IL-6 and TNF-αin patients with CKD from DN was increased compared to patients with CKD from non-DN and healthy controls. In contrast, there was no significant statistical difference of serum HBD-2 concentration among above groups.

Conclusions: These data suggest that HBD-1 could affect on both kidney function and Hb level in patients with CKD. In addition, increase of HBD-1, IL-6 and TNF-α may be causative factors of reduction of renal function in DN.

PUB067

c-Abl Mediates Angiotensin II-Induced Apoptosis in Podocytes Xinghua Chen,[1] Tean Ma,[1] Wei Liang,[1] Dongqing Zha,[1] Zhilong Ren,[1] Yipeng Liu,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Our previous studies have demonstrated that angiotensin II(Ang II) induces podocyte apoptosis. c-Abl, the nonreceptor tyrosine kinase has been implicated in the regulation of cell proliferation, survival, cell adhesion and migration. A recent study has demonstrated c-Abl expression in rat kidney. The present study investigates whether c-Abl is involved in Ang II-induced podocyte apoptosis.

Methods: (1) 24 male Sprague-Dawley rats (Group C, D, E and F) were assigned to receive Ang II (400 kg-1min-1) by osmotic minipump and of which 12 rats (Group D and

F) were assigned to receive Telmisartan (3 mg·kg-1d-1) together, 6 rats received normal saline (Group B), and 6 rats used as normal contro1 (Group A). Expression of podocyte c-Abl was examined by immunohistochemistry, and podocyte apoptosis was examined by TUNEL staining. (2) Conditionally immortalized mouse podocytes were used in vitro. The cultured podocytes were treated with Ang II10-9M to 10-6M for various periods with or without c-Abl inhibitor Src-I1, or specific c-Abl siRNA, c-Abl plasmid alone. Expression of podocyte c-Abl, c-Abl-phospho Y245 and Y412 were examined by Real-time PCR, Western blot and immunofluorescence staining. c-Abl and p53 in the nucleus were examined by co-immunoprecipitation and western blot. Podocyte apoptosis was examined by flow cytometry and Hoechst-33342 staining.

Results: Ang II induced podocyte apoptosis in vivo and in vitro, and podocyte c-Abl expression was increased by Ang II both in vivo and in vitro. Y245 and Y412 phosphorylation of c-Abl was also upregulated. Ang II induced an increase in nuclear p53 protein and nuclear c-Abl-p53 complex in podocytes. Down-regulated c-Abl expression by c-Abl inhibitor (Src-I1) or specific siRNA inhibited Ang II-induced podocyte apoptosis. Up-regulated c-Abl expression by plasmid transfection increased Ang II-induced podocyte apoptosis.

Conclusions: c-Abl mediates Ang II-induced podocyte apoptosis, and inhibition of c-Abl could protect podocyte injury induced by Ang II.

PUB068

Role of Smad2/3 Acetylation in Angiotensin II-Induced Extracellular Matrix Synthesis Chen Yu,[1] Lunjun Fu,[1] Ying Yu,[1] Limin Lu.[2] *[1]Division of Nephrology, Tongji Hospital, School of Medicine, Tongji University, Shanghai, China; [2]Department of Physiology and Pathophysiology, Fudan University, Shanghai Medical College, Shanghai, China.*

Background: Angiotensin II (AngII) has long been recognized in stimulating extracellular matrix (ECM) synthesis and plays a critical role in renal fibrosis. The phosphorylation and nucleus translocation of Smad proteins have been demonstrated to be involved in AngII- induced ECM synthesis. Acetylation was recently noticed as another way of Smads modification. The aim of this study is to investigate the role of acetylaiton of Smad2/3 on AngII-induced ECM synthesis and the related mechanism.

Methods: Cultured rat renal interstitial fibroblast cells (NRK-49F) were used in the experiment. The collagenIV and fibronectin protein levels, phosphorylation of Smad2/3 were measured by Western blot. Acetylation of Smad2/3 was measured by co-immunoprecipitation. The siRNA interfering was used to knockdown p300 or CBP expression.

Results: 1) AngII increased the expressions of ECM proteins, including collagenIV and fibronectin, in a concentration dependent manner. 2) AngII stimulated both the acetylation and phosphorylation of Smad2/3. 3) Transfection of siRNA-p300, but not siRNA-CBP, blocked AngII-induced acetylation of Smad2/3, and reduced the expressions of collagenIV and fibronectin.4) The inhibitor of p38 (SB203580) reversed AngII-induced phosphorylation of smad2/3 as well as the expressions of collagenIV and fibronectin. 5) SB203580 abrogated AngII-induced increase in Smad2/3 acetylation; however, neither C646 (p300 inhibitor) nor siRNA-p300 had obvious effects on the increase of Smad2/3 phosphorylation, which was induced by AngII.

Conclusions: AngII stimulates Smad2/3 acetylation and phosphorylation in cultured NRK-49F. Both Smad2/3 phosphorylation and acetylation participate in AngII-induced ECM synthesis. P300 is the key acetyltransferase which responsible for the AngII-induced Smad2/3 acetylation, while p38 plays the crucial role in Ang II-induced Smad2/3 phosphorylation. The phosphorylation of Smad2/3 seems prior and nessecary for the acetylation.

Funding: Government Support - Non-U.S.

PUB069

Dyslipidemia Activates Intracellular Renin-Angiotensin System and Accelerates the Progression of Renal Tubular Interstitial Fibrosis Jie Ni,[1] Kun Ling Ma,[1] Chang Xian Wang,[2] Yang Zhang,[1] Bi-Cheng Liu.[1] *[1]Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiang Su Province, China; [2]Infection Management Department, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiang Su Province, China.*

Background: Dyslipidemia and activation of the intracellular renin-angiotensin system (RAS) are risk factors in the progression of tubular interstitial fibrosis (TIF). This study was to investigate potential synergistic effects of dyslipidemia with RAS using human tubular epithelial cell line (HK-2) and explore its underlying mechanisms.

Methods: HK-2 cells were cultured and divided into Control group (serum free medium) and cholesterol loading group (serum free medium containing 30 μg/ml cholesterol plus 1μg/ml 25-hydroxycholesterol). Lipid accumulation in cells was evaluated by Oil red O staining. The gene and protein expressions of molecules involved in Epithelial-mesenchymal transition (EMT), RAS components and mammalian target of Rapamycin (mTOR) pathway were examined by real-time PCR and Western blot.

Results: Cholesterol loading increased intracellular lipid accumulation. Further analysis demonstrated that there were decreased mRNA and protein expression of E-cadherin and increased mRNA and protein expression of α-smooth muscle actin (α-SMA) in cholesterol loading group compared to the control, suggesting that lipid accumulation contributes to EMT. These were closely associated with increased mRNA and protein expression of angiotensinogen, angiotensin II, rennin, angiotensin-converting enzyme, agiotensin II type 1 and type 2 receptors in HK-2 cells. Interestingly, lipid accumulation increased mRNA and protein expression, and protein phosphorylation level of mTOR, eukaryotic translation initiation factor 4E binding protein 1(4EBP1), ribosomal protein S6 kinase 1(S6K1), which was closely correlated with intracellular RAS activation.

Conclusions: Dyslipidemia contributed to the progression of TIF through intracellular RAS activation. It suggests a possible synergistic effect existed in the pathogenesis of TIF. These effects mediated by intracellular RAS activation could be correlated with the up-regulation of mTOR pathway.

Funding: Government Support - Non-U.S.

PUB070

Identification of Two Direct Carbonylation Sites in the Na/K-ATPase α1 Subunit Stimulated by Ouabain and Glucose Oxidase in LLC-PK1 Cells Yanling Yan,[1,3] Anna P. Shapiro,[1] Vinai Kumar Katragadda,[1] Deepak K. Malhotra,[1] Zi-jian Xie,[2,1] Joseph I. Shapiro,[1,2,3] Jiang Liu.[1,3] *[1]Medicine, University of Toledo College of Medicine, Toledo, OH; [2]Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, OH; [3]Pharmacology and Physiology, Marshall University JCE School of Medicine, Huntington, WV.*

Background: We recently found that protein carbonylation regulated renal proximal tubular Na/K-ATPase/c-Src signaling and sodium reabsorption. We reported here that both ouabain and glucose oxidase stimulated direct carbonylation of two amino acid residues in the Na/K-ATPase α1 subunit.

Methods: Immunoprecipitation, LC-MS/MS.

Results: LLC-PK1 cells were treated with ouabain (100nM) or GO (3mU/ml) for 1 hr. The Na/K-ATPase α1 subunit was immunoprecipitated with anti-α1 antibody and separated with SDS-PAGE. The α1 bands were processed for in-gel trypsin digestion and LC-MS/MS analysis. Direct carbonylation modification of two amino acid residues, Pro^{234} (mass 113.0477 with mass diff 15.9949) and Thr^{237} (mass 99.0320 with mass diff -2.0156), in peptide ^{226}SPDFTNENPLETR^{238} (*UniProtKB/Swiss-Prot No P05024 (AT1A1_PIG)*) were present in control, ouabain- and GO- treated cells. Furthermore, direct carbonylation of both Pro^{222} and Thr^{224} in peptide ^{211}VDNSSLTGESEPQTR^{225} were shown only in response to ouabain and GO treatments. Peptide ^{211}VDNSSLTGESEPQTR^{225} is highly conserved in Na/K-ATPase α1/α2/α3/α4 isoforms. These two peptides are linked and located in the actuator (A) domain of the α1 subunit, and Pro^{222}/Thr^{224} locate on the surface facing the nucleotide binding (N) domain. It was indicated that the conformation changes favor high affinity ouabain binding and affect binding of signaling molecules such as c-Src, PI3K and IP3R (Yatime, L., et al., J Struct Biol. **174**(2): p. 296-306), which are critical in the Na/K-ATPase signaling. The highly conserved ^{217}TGES^{220} motif has been shown to affect conformation change and stabilize the Na/K-ATPase in E2P:Ouabain state.

Conclusions: Ouabain and glucose oxidase stimulated direct carbonylation of two amino acid residues (Pro and Thr) in the A domain of the Na/K-ATPase α1 subunit.

Funding: Other NIH Support - HL-109015 GM-78565, Clinical Revenue Support

PUB071

Calcitriol Decreased TNF-α Induced TGF-β1 Production in Aortic Smooth Muscle Cells via Rac1/Syk Pathway Su-Kil Park, Won Seok Yang. *Internal Medicine, Asan Medical Center University of Ulsan, Seoul, Republic of Korea.*

Background: Interestingly, TGF-β1 is known to have interwoven atherogenic and atheroprotective properties. The various studies depending on animal studies or human studies show conflicting results as well as dependent on damaging insults. Recently, the interest in vitamin D is increasing because it is known to have cardiovascular protective effects. Therefore, we investigated the effect of calcitriol on the production of TGF-β1 and its intracellular signaling pathways after stimulation of cultured aortic smooth muscle cells by TNF-α.

Methods: We performed western blot, EMSA and Rac1 pull down assay. Intracellular reactive oxygen species(ROS) production was evaluated with the probe 5-(and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate by confocal microscopy.

Results: TNF-α (10ng/ml) increased TGF-β1 expression after 8hr, which was decreased by calcitriol (120nM, 240nM) in western blot assay. Calcitriol (120nM) decreased intracellular ROS production after 30min stimulation of TNF-α, which was evaluated by confocal microscopy. TNF-α increased p-Syk after 30min stimulation, which was decreased by calcitriol, however there was no change in TNF-α induced p38 activation, p65 nuclear translocation or IkBα degradation by calcitriol in western blot assay. In EMSA, TNF-α-increased DNA-binding activities of AP-1 and NF-κB, whereas calctriol (120nM) downregulated TNF-α-induced DNA-binding activities of both AP-1 and NF-κB. In Rac1 pull-down assay, active Rac1 increased by TNF-α in 15 min incubation, which was decreased by calcitriol.

Conclusions: In conclusion, calcitriol could decrease TNF-α induced Rac1/ROS/Syk/TGF-β1 production which means that calcitriol might have antioxidant activity by itself. This pathway may explain the mechanism of cardiovascular protective activity of calcitriol.

Funding: Private Foundation Support

PUB072

Mechanisms of Renoprotective Effects of Cordyceps Sinensis in 5/6 Nephrectomy Rats Shanmai Guo, Fang Zhong, Qiao Zhou, Ying Lu, Xu Hao, Weiming Wang, Nan Chen. *Ruijin Hospital, Shanghai Jiao Tong University.*

Background: To explore the mechanism of C. sinensis on chronic renal insufficiency and renal fibrosis.

Methods: The CKD rat model was established by performing 5/6 subtotal nephrectomy. Male SD rats were divided into three groups: sham-operated group (SO group); untreated nephrectomized group(OP group); and administration of C.sinensis-treated nephrectomized group (CS group). Kidneys, sera and urine were collected for analysis.

Results: Scr, BUN and UACR were significantly increased in OP groups than in SO groups ($p<0.05$), and the Alb significantly decreased ($p<0.05$). Obvious pathological changes were detected in OP groups, which including focal or diffused glomerulosclerosis, tubular interstitial changes such as infiltration of inflammatory cells, more deposition of ECM and thickening of arterial wall. The lesion was more serious at 8 weeks after operation. Number of CD68, FSP1 positive cells and the expression area of FN, Col-I, Col-III, and α-SMA in the OP groups were increased compared with SO groups($p<0.05$). However, these changes were partly reversed after C. sinensis treatment. Pathological changes were improved and the mitochondrial damage was alleviated in CS groups. Glomerulosclerosis and interstitial fibrosis were also significantly relieved. Besides Alb were significantly increased, and UACR were significantly decreased in CS groups ($p<0.05$). The number of CD68 and FSP1 positive cells declined ($p<0.05$), and the protein and mRNA level of FN, Col-I, Col-III and α-SMA were obviously decreased($p<0.05$) in CS groups. Compared with SO groups, the protein levels of GRP78, p-eIF2α, ATF4, CHOP, caspase12 and c-caspase 3 in OP groups were significantly increased ($p <0.05$). But they were significantly reduced after treatment with C. sinensis preparations ($p <0.05$). Expression of oxidative stress indicators, such as SOD and MDA were disturbed in OP groups, and they were significantly improved by C. sinensis.

Conclusions: C. sinensis could improve renal dysfunction, alleviate glomerulosclerosis, inflammation and tubular interstitial fibrosis through inhibiting the ERS, alleviating oxidative stress damage, renal tubular cell apoptosis and mitochondrial damage.

PUB073

Nicotine-Induced ERK1/2 Phosphorylation in Rat Mesangial Cells: Role of PKC, PKA, EGFR and CaMKII Ping Hua,[1] Wenguang Feng,[1] Gabriel Rezonzew,[1] Phillip H. Chumley,[1] Edgar A. Jaimes.[1,2] *[1]University of Alabama at Birmingham; [2]Birmingham VA Medical Center, Birmingham, AL.*

Background: Tobacco smoking is associated with accelerated progression of chronic kidney disease of different etiologies including diabetes and hypertension. The mechanisms involved are however not well understood. We have previously reported that nicotine, a biologically active compound present in high concentrations in tobacco, induces cell proliferation and fibronectin production in human mesangial cells (MC) which are prevented by ERK1/2 inhibition (AJP'05). In these studies we determined whether rat MC express nicotine receptors and characterize the signaling pathways that lead to ERK1/2 phosphorylation in response to nicotine.

Methods: Rat MC were grown in DMEM and several nicotinic Ach receptor (nAChR) subunits (α1-7 and β1-4) assessed by western blot (WB). In separate experiments MC were first pre-treated with inhibitors for PKC, PKA, EGFR and CaMKII for 30 min and then the cells were treated with nicotine (10^{-7}M). In other experiments MC were pre-treated with the calcium channel blocker Verapamil (10^{-9} M) before nicotine. Cells were then harvested and ERK1/2 phosphorylation detected by WB.

Results: We first demonstrated that MC are endowed with several nAChR subunits (α2-7 and β1-4). Treatment with nicotine at 10^{-7}M caused a time-dependent ERK1/2 phosphorylation which peaked after 10 min of stimulation (N=3). The calcium/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN93 (10^{-7}M) decreased the ERK1/2 phosphorylation level by ~57% as compared to nicotine. The PKC inhibitor Go6983 at 10^{-9}M, the PKA inhibitor H89 (10^{-8} M) and the EGFR inhibitor AG 1478 (10^{-7} M) also inhibited ERK1/2 phosphorylation by 60%, 48% and 68% respectively as compared to nicotine. Treatment of MC with Verapamil resulted in 33% inhibition of ERK1/2 phosphorylation as compared to nicotine alone.

Conclusions: In these studies we have demonstrated that rat MC are endowed with several nAChR subunits and that ERK1/2 phosphorylation in response to nicotine requires CaMK II, PKA, PKC and EGFR. In addition we have shown that these effects require Ca^{2+} consistent with the role of the nAChRs as agonist-regulated Ca^{2+} channels in MC.

Funding: Other NIH Support - NIEHS

PUB074

Differential Ubiquitylation of the Mineralocorticoid Receptor Is Regulated by Phosphorylation Nourdine Faresse, Olivier Staub. *Pharmacology &Toxicology, University of Lausanne, Lausanne, Switzerland.*

Background: Aldosterone stimulation of the mineralocorticoid receptor (MR) is involved in numerous physiological responses, including Na^+ homeostasis, blood pressure control, as well as heart failure. Aldosterone binding to MR promotes different post-translational modifications (PTMs) that regulate MR nuclear translocation, gene expression and finally receptor degradation.

Methods: Here we studied by different biochemical experiments, the effects of aldosterone stimulation (from 0.1 to 10 nM) on MR PTMs in renal epithelial cells.

Results: We found that aldosterone stimulates rapid phosphorylation of MR via ERK1/2 in a dose dependent manner in renal epithelial cells. This phosphorylation induces an increase of MR apparent molecular weight, with a maximal upward shift of 30 kDa. Strikingly, these modifications are critical for the regulation of the MR ubiquitylation state. Indeed, we find that MR is monoubiquitylated in its basal state and this status is sustained by the tumor suppressor gene 101 (Tsg101). Phosphorylation leads to disruption of MR/Tsg101 association, and monoubiquitin removal. These events prompt polyubiquitin-dependent destabilization of MR and degradation. Preventing MR phosphorylation by ERK1/2 inhibition or mutation of target serines affects the sequential mechanisms of MR ubiquitylation and inhibits the aldosterone mediated degradation.

Conclusions: Our data provide a novel model of negative feedback of aldosterone signaling, involving sequential phosphorylation, monoubiquitin removal and subsequent polyubiquitylation/degradation of MR.

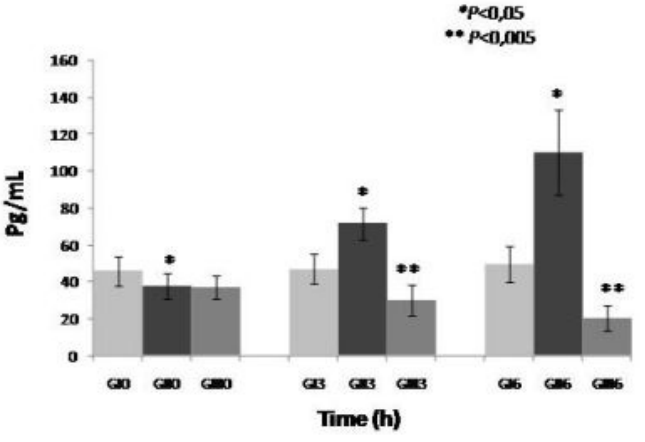
PUB075

Advanced Glycation End Products and MCP-1 Expression by Endothelial Cells through PKC-β Pathway Lisienny Campoli Tono Rempel,[1] Rayana Ariane Pereira Maciel,[1] Bruna Bosquetti,[1] Andrea Novais Moreno-Amaral,[2] Rodrigo B. Oliveira,[3] Roberto Pecoits-Filho,[2] Andréa Marques Stinghen.[1] *[1]Basic Pathology Department, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; [2]Center for Health and Biological Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Paraná, Brazil; [3]Nephrology Department, Universidade de São Paulo, São Paulo, Brazil.*

Background: Studies have demonstrated the role of uremic toxins in the development of cardiovascular disease, the first cause of mortality in CKD patients. However, its mechanisms are not fully understood. We investigated the expression of MCP-1 triggered by advanced glycation end products (AGES), targeting PKC-β pathway in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were cultured in MEM-199, FBS and pen/strepto. AGES were prepared with BSA and glucose. The cells were treated according to the following groups: GI-control (HUVECs + BSA), GII (HUVECs + AGES), and GIII (HUVECs + AGES + PKC-β inhibitor, Gö-6983). All groups were incubated in a kinetics of 0 (baseline), 3 and 6h, and the supernatants were collected to evaluate MCP-1 levels (ELISA).

Results: HUVECs exposed to AGES (GII) showed an important increase of MCP-1 levels after 3 and 6h respectively (72 ± 9; 110 ± 25 pg/mL) in comparison to baseline (38 ± 7 pg/mL), $P<0.05$. In the opposite, in the control group (GI), the MCP-1 expression in 0, 3 and 6h was respectively 46 ± 8; 47 ± 8 and 50 ± 10 pg/mL. HUVECs exposed to AGES + PKC-β inhibitor (GIII) demonstrated a significant decrease in MCP-1 expression after 3 and 6h respectively (30 ± 8; 20 ± 7 pg/mL), $P<0.005$, when compared to AGES (GII).



Conclusions: We demonstrated an increase in MCP-1 production by HUVECs when exposed to AGEs in a time-dependent way. In addition, PKC-β blockade reduced MCP-1 expression, suggesting a potential antiinflammatory action by the inhibition of this pathway.

Funding: Government Support - Non-U.S.

PUB076

Advanced Glycation End-Products Reduced Podocyte Adhesion via Upregulation Integrin Linked Kinase Expression and Cytoskeleton Reorganization Cailian Cheng,[1] Zhenda Zheng,[1] Xun Liu,[1] Ying Tang,[2] Tan-qi Lou.[1] *[1]Department of Nephrology, The 3rd Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [2]Department of Nephrology, The 2rd Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: Recent studies showed podocyte detached from the glomerular basement membrane played an important role in the pathogenesis ofpodocytopenia in diabetic kidney disease. Advanced glycation end-products (AGEs) increase at an accelerated rate in diabetes and associate with the severity of diabetic complications. Little is known about the effects of AGEs on podocyte adhesion and the roles of integrin linked kinase (ILK) and actin cytoskeleton in the process.

Methods: Podocyte adhesion was measured by cytometry and hexosaminidase assay. The levels of receptor for AGEs (RAGE), ILK, α-actinin-4, and synaptopodin were evaluated by western blotting and the distribution of F-actin, α-actinin-4, and synaptopodin were observed by immunofluorescence and confocal fluorescence microscope.

Results: AGEs caused an increase in the expression of RAGE and ILK by 70% and 100%, respectively ($P<0.05$). The expression levels of α-actinin-4 and synaptopodin decreased by 50% and 60%, respectively ($P<0.05$). And AGEs also caused rearrangement of F-actin, redistribution of α-actinin-4 from the membrane to the cytoplasm. AGEs (80 μg/ml) decreased podocyte adhesion by 60% ($P<0.05$). However, pretreatment with anti-RAGE antibody (50μg/ml) inhibited the effects induced by AGEs.

Conclusions: AGEs reduced podocyte adhesion via upregulation of both ILK and RAGE, downregulation of both α-actinin-4 and synaptopodin and reorganization of actin cytoskeleton.

Funding: Government Support - Non-U.S.

PUB077

Advanced Glycation End-Products Activate the Renin-Angiotensin System through the RAGE-PI3-K /Akt Signaling Pathway in Podocytes

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Background: Renin-angiotensin system (RAS) play an important role in the pathogenesis of chronic kidney disease. Advanced glycation end-products (AGEs) were involved in the progression of diabetic kidney disease. However, little is known about the effects of AGEs on RAS in podocytes and the mechanism of these effects.

Methods: Immortalized mouse podocytes were exposed to various concentrations of AGEs (0, 20, 40, 80, 160 μg/ml) for different time intervals(12h, 24h, 48h. The expression levels of angiotensinogen (AGT), renin, and angiotensin II (Ang II) type 1 and 2 receptors (AT1R and AT2R), receptor for AGEs (RAGE) and both Akt and phosphorylated Akt were examined by western blotting. Ang II levels were assayed by ELISA, and angiotensin converting enzyme (ACE) activity was evaluated by ultra violet spectrophotometry.

Results: Treatment with AGEs resulted in significant increase in the expression of AGT (62%, P<0.05) and AT1R (59%, P<0.05). Moreover, Ang II levels increased significantly in both cell lysates and conditioned media (70% and 65%, P<0.05); ACE activity was also significantly higher in cell lysates and conditioned media (68% and 65%, P<0.05). However, there were no changes in renin or AT2R expression (P>0.05). AGEs did increase the expression of RAGE by 50% (P<0.05) and the phosphorylation of Akt by 100% (P<0.05). When podocytes were pretreated with anti-RAGE antibody (50 μg/ml) or the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (10 μM), the AGEs-induced increase in AGT and AT1R expression was reduced significantly. Likewise, Ang II levels and ACE activity decreased significantly.

Conclusions: AGEs activated the RAS in podocytes through the RAGE-PI3K/Akt-dependent pathway, which may provide a new therapeutic for diabetic kidney disease.

Funding: Government Support - Non-U.S.

PUB078

Acidosis Attenuates Leucine Induced Signal Transduction and Protein Synthesis in Rat Skeletal Muscle

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Background: Metabolic acidosis is a common cause of skeletal muscle wasting which occurs mainly due to increased proteolysis, though protein synthesis (PS) may also be impaired. Since leucine (LEU) comprises ~20% of daily essential amino acid requirements and is a known stimulator of PS, we set out to evaluate the impact of acidosis on LEU induced anabolic signaling and PS. LEU apart from serving as a substrate, stimulates PS largely by directly activating the nutrient sensitive mTOR signaling complex, with downstream phosphorylation of eukaryotic 4E-binding protein1 (4E-BP1) and S6 kinase-1 (S6K1).

Methods: Acidosis was induced in rats by gavaging with NH4Cl daily while pairfed controls were given saline. After 2 days rats from both groups were gavaged once with LEU or saline and sacrificed 45 mins later. PS was assayed in isolated extensor digitorum longus muscles of saline gavaged acidotic and control rats, incubated at PH 7.15 or 7.4 respectively, with or without LEU present. ^{14}C-phenylanine incorporation into protein was determined.

Results: PS in the absence of LEU was unaffected by PH. Addition of LEU increased PS significantly by 44±12 % (p<0.05) in pairfed control rat muscle, but was without significance in acidotic rat muscle. Signal transduction was assayed in plantaris muscle collected at sacrifice by Western immunoblotting. Phosphorylation of mTOR and 4E-BP1 increased significantly post-LEU gavage to a similar extent in acidotic and control rats. However LEU stimulated S6K1 phosphorylation was significantly attenuated in acidotic rats vs pairfed controls, increasing to 174±14 vs 309±25% above the basal control value, p<0.001.

Conclusions: In conclusion we have established that sustained acidosis attenuates LEU stimulated activation of signal transduction via the mTOR pathway at the level of S6K-1, a regulator of translation initiation and protein synthesis. This abnormality presumably contributes to the suppression of LEU induced protein synthesis in skeletal muscle and likely contributes to the muscle wasting of acidosis, common in kidney failure.

Funding: Veterans Administration Support

PUB079

Advanced Glycation End-Products Reduces Podocyte Adhesion via Activation of the Renin-Angiotensin System in Podocytes

Cailian Cheng,[1] Zhenda Zheng,[1] Xun Liu,[1] Ying Tang,[2] Tan-qi Lou.[1] *[1]Department of Nephrology, The 3rd Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China; [2]Department of Nephrology, The 2nd Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.*

Background: Renin-angiotensin system and podocytopenia were involved in the pathogenesis of diabetic kidney disease and recent studies showed podocyte detach from the glomerular basement membrane played a key role in the process of podocytopenia. Advanced glycation end-products (AGEs) were involved in the progression of diabetic kidney disease. However, the effects of AGEs on podocyte adhesion and its possible mechanisms is poor known.

Methods: Immortalized mouse podocytes were exposed to various concentrations of AGEs (0, 20, 40, 80, 160 μg/ml) for different periods (6h, 12h, 24h, 48h), podocyte adhesive capacity was evaluated by cytometry and hexosaminidase assay. The expression levels of angiotensinogen (AGT), renin, and angiotensin II type 1 and 2 receptors (AT1R and AT2R) were examined by western blotting. Ang II levels were assayed by ELISA, and angiotensin converting enzyme (ACE) activity was evaluated by ultra violet spectrophotometry.

Results: Treatment with AGEs (80μg/ml) resulted in significant decrease in podocyte adhesion by 60%. AGT and AT1R expression, ACE activity, and Ang II levels increased significantly in podocyte lysates and conditioned culture media exposure to AGEs (80μg/ml) (P <0.05). Pretreatment with losartan and captopril partly improved the reduced adhesive capacity of podocytes compared with cells exposed to AGEs (85%±19% vs. 40%±20%, *P*<0.05), but it was still lower than that podocytes exposed to BSA.

Conclusions: Our findings indicate that AGEs activates the renin-angiotensin system in podocytes, which is partly involved in the down regulation of adhesive capacity induced by AGEs.

Funding: Government Support - Non-U.S.

PUB080

Effects of Leukemia Inhibitory Factor and Oncostatin M on IgA1-Producing Cells from Patients with IgA Nephropathy

Koshi Yamada,[1,2] Zhi Qiang Huang,[1] Milan Raska,[1,3] Zina Moldoveanu,[1] Hitoshi Suzuki,[2] Yusuke Suzuki,[2] Krzysztof Kiryluk,[4] Robert J. Wyatt,[5] Yasuhiko Tomino,[2] Bruce A. Julian,[1] Ali G. Gharavi,[4] Jan Novak.[1] *[1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University, Tokyo, Japan; [3]Palacky University, Olomouc, Czech Republic; [4]Columbia University, New York, NY; [5]University of Tennessee, Memphis, TN.*

Background: Galactose-deficient IgA1 (Gd-IgA1) is a key pathogenetic factor in IgA nephropathy (IgAN). Our recent GWAS associated a locus on chromosome 22q12.2 with elevated serum levels IgA in patients with IgAN. This locus contains genes encoding leukemia inhibitory factor (LIF) and oncostatin M (OSM) cytokines that play a role in mucosal immunity and inflammation. However, their effect on IgA1-producing cells is not known.

Methods: EBV-immortalized IgA1-secreting cells derived from the circulation or tonsils of IgAN patients and healthy controls (HC) were stimulated with LIF and OSM, and serum IgA1 and Gd-IgA1 levels were determined by ELISA. STAT3 and STAT1 phosphorylation was analyzed using SDS-PAGE and Western blotting and confirmed by using specific inhibitors of signaling.

Results: LIF and OSM decreased production of IgA1 in IgAN and HC cells. In contrast, production of Gd-IgA1 was increased by 15% after LIF and by 14% after OSM stimulation, but only in IgAN cells. LIF and OSM signaling was mediated through STAT1 but not STAT3 phosphorylation.

Conclusions: LIF and OSM enhanced production of Gd-IgA1 in the cells from IgAN patients but not in those from HC. This process was mediated by STAT1. This signaling pathway may represent a possible therapeutic target in IgAN.

Funding: NIDDK Support, Other NIH Support - GM098539

PUB081

Differential Expression of Uric Acid Transporters in the Vessels of the Normal Rat Leads to Distinct Responses to Uric Acid

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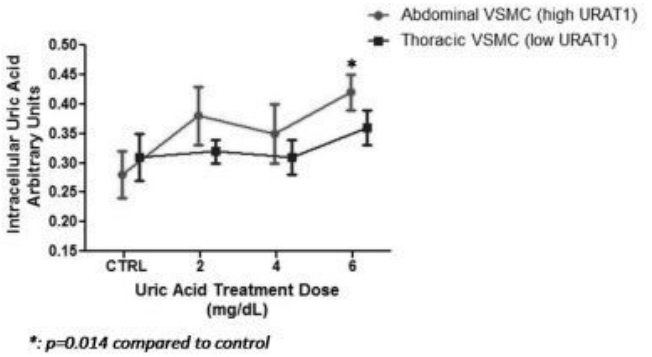
Background: URAT1 (SLC22A12) & GLUT9 (SLC 2A9) are involved in uric acid transport. We have previously shown URAT1 is highly expressed in the abdominal aorta whereas GLUT9 is highly expressed in the thoracic aorta of the normal rat. We hypothesized that different spatial expression of these transporters leads to distinct responses to uric acid by vascular smooth muscle cells (VSMC).

Methods: VSMCs were isolated from the thoracic and abdominal aorta of male Sprague Dawley rats, then plated in DMEM with 10% FBS and Penicillin/ Streptomycin. The cells were treated with uric acid at 0, 2, 4, and 6 mg/dL. Intracellular uric acid was measured via QuantiChrom Uric Acid Assay Kit on cell lysates. MCP-1 in cell culture media was measured by ELISA. To determine if cell proliferation differed in response to uric acid between both cell lines, BrdU incorporation assay was performed at 0 and 6 mg/dL of uric acid.

Results: Intracellular uric acid increased in the abdominal VSMCs with higher uric acid dose; this was significant at 6 mg/dL (Figure 1). MCP-1 doubled in cell culture media of abdominal VSMC treated with 6 mg/dL of uric acid (p=0.002) but was unchanged in the media of the thoracic VSMCs. The percentage of BrdU positive cells increased with 6 mg/dL of uric acid in both cell lines but was significantly higher in the abdominal vs thoracic VSMCs (p=0.04).

Conclusions: Intracellular uric acid increases in response to uric acid in VSMCs expressing URAT1. This is associated with increased MCP-1 and greater cell proliferation. Further studies are needed to better understand the role of URAT1 in uric acid transport in VSMCs.



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PUB083

Aquaporin-2 Is Regulated by Integrin-Linked Kinase

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Background: Progressive interstitial fibrosis produces structural changes in the kidney, where abnormal extracellular matrix (ECM) may lead to functional changes in the tubular reabsortion. In the collecting tubules, the vasopressin-regulated Aquaporin isoform AQP2 is the main regulating water absorption channel. The AQP2 expression is mainly regulated by both transcriptionally and post-translationally pathways which includes its intracellular trafficking to the apical membrane of the tubule that facilitate the water absorption. However the molecular mechanisms implicated in both types of regulation are still under study. Since the traffic of the AQP2 depends in the phosphorilation of its structure, several kinases such those dependent of cAMP or cGMP have being studied. Very few studies have related the changes present in the ECM and their receptors, the integrins, in the AQP2 expression and trafficking regulation. We studied for the first time the implication of the kinase linked to integrins (ILK) in the transcriptional regulation of AQP2 and its intracellular location.

Methods: We used conditional ILK-deleted mice (ILK-cKO) compared with controls (WT). We analyzed urine volumes and its osmolality. Cortical and medullar expression of AQP2 and its intracellular location was determined by western blot, quantitative PCR and immunofluorescence.

Results: We observed a quite significant poliuria, with increased osmolality in the ILK-cKO mice urine compared with WT. In order to study the role of AQP2 regulation pathways we determined its mRNA and protein levels, both significantly decreased in ILK-cKO mice. We observed also a decreased presence of AQP2 in the apical membrane of the tubules in the animals where ILK was depleted.

Conclusions: We propose ILK as a major player in the tubular physiology and pathology since the lack of ILK decreases the AQP2-dependent water absorption, because its expression and presence in the tubular membrane is decreased.

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PUB084

Participation of Endoplasmic Reticulum Stress in the Pathogenesis of Spontaneous Glomerulosclerosis: Role of Intra-Renal Angiotensin System

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Background: Endoplasmic reticulum (ER) is the site of synthesis, folding, assembly, and degradation of proteins. Disruption of ER function leads to ER stress, which is marked by accumulation of unfolded proteins in the ER lumen. Detection of unfolded proteins by the ER membrane receptors triggers the ''unfolded protein response (UPR)'' designed to restore ER function via activation of the adaptive/cytoprotective responses. Failure of UPR or persistent stress triggers activation of ER stress-mediated apoptotic pathway. Several in vivo and in vitro studies have demonstrated the association of ER stress with glomerular diseases. Imai rats develop progressive glomerulosclerosis (GS), which is associated with oxidative stress, inflammation and activation of intra-renal angiotensin system, and can be prevented by AT-1 receptor blockade (ARB). Since persistent oxidative and inflammatory stresses trigger ER stress-induced apoptosis and tissue injury, we hypothesized that kidneys in the Imai rats may exhibit failure of the adaptive and activation of the apoptotic ER stress responses, which could be prevented by ARB.

Methods: To this end 10-week old Imai rats were randomized to untreated and ARBtreated groups and observed for 24 weeks.

Results: At age 34 weeks, untreated rats showed heavy proteinuria, azotemia, advanced GS, impaired ER stress adaptive/cytoprotective responses (depletion of UPR-mediating proteins), and activation of ER stress apoptotic responses. ARB treatment attenuated GS, suppressed intra-renal oxidative stress, restored ER-associated adaptive/cytoprotective system, and prevented the ER stress mediated apoptotic response in this model.

Conclusions: Thus, progressive GS in Imai rats is accompanied by activation of ER stress-associated apoptosis, which can be prevented by ARB.

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PUB085

Human Serum Reduces Shigatoxin 2-Induced Unfolded Protein and Ribotoxic Stress Response and Apoptosis in Human Podocytes

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Background: Shigatoxin 2, the active toxin in hemolytic uremic syndrome (HUS) activates the unfolded protein response (UPR), because of accumulation of unfolded proteins and the ribotoxic stress response through binding to ribosomes. In endothelial cells both pathways contribute to apoptosis, which leads to microangiopathy. Glomerular proteinuria is one early symptom in HUS-patients and persistent proteinuria is associated with a poorer renal outcome. Thus podocytes may be one target for Stx2.

Methods: Human podocytes were incubated with Stx2. Incubation medium was supplemented with fetal calf serum (FCS), serum from healthy human controls (normal human serum = NHS) and serum from pediatric HUS-patients. Lysates were tested for UPR and ribotoxic stress marker, caspase 3 (casp3), a key enzyme in apoptosis and Bcl-2, a prosurvival protein.

Results: Upon Stx2-incubation human podocytes showed an increase in IRE1 ("sensing" levels of unfolded proteins) and an increase in p-EIF2α (transcription factor, inhibiting further protein translation). When normal human serum (NHS) was given to the cells instead of fetal calf serum (FCS) the increases in both proteins were diminished. P38MAPK-α was activated upon Stx2 incubation, while p38MAPK-β (most likely anti-apoptotic) was not activated. NHS suppressed the activation of p38MAPK-α, but activated p38MAPK-β instead. Casp3 activity was increased after Stx2 after 48hrs. This peak was not found with NHS ($p<0.05$) but Bcl-2 was upregulated with NHS compared to FCS supplementation. Sera from HUS-patients did not diminish the activation of p38MAPK-α and casp3 to the same extent as NHS.

Conclusions: Stx2 induces unfolded protein and ribotoxic stress response, leading to apoptosis in human podocytes. Podocytes might be an additional target of Stx2-associated HUS. Activation and apoptosis can be blocked by NHS, but not completely by sera from HUS patients. These data suggest a potential circulating protecting factor in healthy humans, which might be consumed or blocked in patients with HUS.

PUB086

Expression of Genes Associated with Telomere Maintenance in Patients after Kidney Transplantation

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Background: Chronic administration of immunosuppressants has long-term consequences including higher risk of neoplasms development. The processes regulating telomere function have major influence on human cancer biology. The study aimed to assess the impact of immunosuppressive therapy on expression of genes associated with telomere maintenance and protection in patients after renal transplantation.

Methods: Expression profiles of 129 transcripts, which represented 70 genes, were assessed in peripheral mononuclear blood cells (PBMCs) with oligonucleotide microarray technique (HG–U133A Affymetrix) in 8 euglycemic transplant recipients (5M and 2F; aged 46.5±8.6 years) on a three drug immunosuppressive regimen including: cyclosporine, prednisone and mycophenolate mofetil or sodium and 4 healthy controls (2M and 2F; aged 59±4.5years). Statistical analysis was performed with use of Agilent GeneSpring GX 11.0 (unpaired t -test with asymptotic p-value corrected with Benjamini-Hochberg multiple test).

Results: Among the analyzed transcripts there were 4 with significantly different expression between the studied groups of subjects (Fold Change > 2.0, p-value < 0.05) shown in Table 1.

Gene symbol	p-value	Fold Change	Expression regulation in transplant recipients vs. controls	Association with telomere maintenance regulation
ACD	0.006	2.11	down	one of six core proteins (TPP1) in the telosome/ shelterin, mediates the access of telomerase to the telomere
TGFBR2	0.02	2.42	up	regulation of a telomerase transcription
MAP3K1	< 0.001	4.02	up	regulation of a telomerase transcription
YWHAB	0.002	2.97	up	nuclear transport of a telomerase

Conclusions: Our results show detrimental changes in telomere maintenance facilitating cell immortalization and carcinogenesis on a gene expression level in kidney transplant recipients. The outcomes may not only contribute to the knowledge of post-transplant cancer pathogenesis, but influence the strategy of immunosuppressive therapy in the future, as well.

PUB087

High Glucose Induces Autophagy in Podocytes Tean Ma,[1] Xinghua Chen,[1] Dongqing Zha,[1] Pravin C. Singhal,[2] Guohua Ding.[1] *[1]Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.*

Background: Autophagy is a cellular pathway involved in protein and organelle degradation. It is relevant to many types of cellular homeostasis and human disease. High glucose can induce podocyte injury, but it is still unknown about the relationship between high glucose and autophagy in podocytes. This study investigates the effects of high glucose on autophagy in podocytes.

Methods: (1) Conditionally immortalized mouse podocytes were cultured in vitro. The cultured podocytes were treated with either high glucose or normal concentration of glucose in the presence or absence of rapamycin and 3-methyladenine. Autophagy was evaluated by electron microscopy and detection of GFP-LC3 overexpression by fluorescence microscopy. Western blots examined LC3-2 and beclin-1. (2) Conditionally immortalized mouse podocytes were treated with either high glucose or normal glucose in the presence or absence of NAC. ROS generation was detected, and MnSOD and catalase was examined by Western blots.

Results: High glucose promoted autophagy in podocytes. Rapamycin further enhanced this effect which could be inhibited by 3-MA. The proautophagic effect of high glucose was associated with podocyte expression of LC3-2 and beclin-1, and antioxidants such as NAC inhibited high glucose-induced autophagy. High glucose induced the generation of ROS by podocytes in a time-dependent manner. High glucose also enhanced podocyte expression of MnSOD and catalase.

Conclusions: High glucose promotes autophagy in podocytes. High glucose-induced podocyte autophagy is mediated through the generation of ROS.

PUB088

The Activity of GSK-3β and Podocyte Injury in db/db Mutant Diabetic-Nephropathy Mice Liu Zhangsuo,[1] Guo Jia,[2] Wan Jia.[3] *[1]Department of Nephrology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan, China; [2]Department of Nephrology, The First Affiliated Hospital, Zhengzhou University; [3]Department of Nephrology, The First Affiliated Hospital, Zhengzhou University.*

Background: To investigate the activity of GSK-3β and podocyte injury in db/db mutant diabetic- nephropathy mice.

Methods: The db/+m mice (n=15) were served as control. And the db/db diabetic-nephropathy mice(n=30) were randomly divided into intervention group(n=15, subcutaneously injected with BIO, 320 μg/kg per day, since the mice were 90 days old.) and non-intervention group(n=15).When the mice were 90,110 (injection of BIO for 20 days),130 (injection of BIO for 40 days) day-old respectively, 5 mice of each group were sacrificed.Then the tissue of kidneys were used to observe the structure of glomerular by electron microscope or light microscope. The localization or expression of proteins was determined by immunofluorescence analysis, fluorescence quantitative PCR or Western blot.The glycogen synthase kinase-3β (GSK-3β) inhibitor 6-Bromoindirubin-3'-oxime (BIO) was used to inhibit the activity of GSK-3β which could be assayed by TRAPEZE enzyme detection kit.

Results: Compared with control, the glomerular basement membrane(GBM)of non-intervention group become more thick and the messegium was increased. And the expression of nephrin was less (P<0.05) combined with the increasing expression ofα-SMA, which mean the podocyte injury in db/db mutant diabetic- nephropathy mice. Although the protein expression of total GSK-3β had no obvious change, the activities of GSK-3β was enhanced in a time dependent manner in the non-intervention group. After BIO injection for 20 days, the activity of GSK-3β was inhibited but the expression of total GSK-3β had no obvious change. When BIO injection for 40 days but not 20 days, the thickness of GBM, the increasing of messegium and the expression of nephrin were alleviated compared with non-intervention group(P<0.05). But compared with db/+m mice, the pathological changes still existed.

Conclusions: The activity changes of GSK-3β may lead to the podocyte injury in db/db mutant diabetic- nephropathy mice. And BIO could partly ameliorate the expression of nephrin.

Funding: Government Support - Non-U.S.

PUB089

High Glucose Promotes Cellular Senescence in the Kidney by Inducing a Mild Hyperosmolar Stress Maria Del Nogal, Nuria Troyano, Jose Luis Cano-peñalver, Alicia Cortes, Gemma Olmos, Sergio De Frutos Garcia, Manuel Rodriguez-Puyol, Maria P. Ruiz-torres. *Department of Physiology, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain.*

Background: Senescence is a stress response that withdraws the cell cycle and leads to morphological and functional cellular changes. Senescence appears in aged and diabetic kidney suggesting an important role on kidney dysfunction. Our aim was to analyze the role of the extracellular osmolarity induced by high glucose in human mesangial and rat kidney cellular senescence and the mechanism involved.

Methods: In order to increase extracellular osmolarity, Human mesangial cells (HMC) or Ex vivo rat kidney slices were treated with high glucose (HG) or the control osmolyte mannitol during different periods of time. Male Wistar rats were treated with mannitol, with streptozotocin to induce experimental diabetes or with vehicle. Cellular senescence was measured as β-galactosidase activity using a fluorescent substrate. p53, p21, p16 and Ras expression were measured by immunoblotting and immunohistochemistry. Ras activation was analyzed by pull down assay. Reactive oxygen species (ROS) production was evaluated by flow cytometry.

Results: Both HG and mannitol mildly increased extracellular osmolarity in HMC promoting cellular senescence by increasing p53, p21 and p16 protein expression. Both HG and Mannitol promoted an increase in reactive oxygen (ROS) production and the constitutive activation of Ras, however the inhibition of ROS and Ras prevented cellular senescence. Both streptozotocin and mannitol rats shown serum osmolarity and p53 and p16 protein expression increase compared with controls. Both HG- or mannitol-treated rat kidney slices shown increased p53 and p16 expression.

Conclusions: HG induced cellular senescence in HMC and rat kidney cortex by inducing extracellular osmolarity increase, since the control osmolyte mannitol reproduced the same results. This effect was ROS and Ras dependent.

Funding: Government Support - Non-U.S.

PUB090

Impact of Erythropoietin on Mononuclear Cells Signaling in Chronic Kidney Disease (CKD) Patients Stage 4 and 5 Mariusz Kowalczyk, Piotr Bartnicki, Jacek Rysz. *Department of Nephrology, Hypertension and Family Medicine, Lodz Medical University, Poland.*

Background: The main objective of the research was to study the influence of erythropoietin (Epo) on membrane cells phospholipids metabolism (myoinositol incorporation) which play a key role in a synthesis of secondary messengers such as inositol triphosphate and diacylglycerol.

Methods: 15 patients (7 women, 8 men) with CKD stage 4-5 and 20 healthy subjects (14 women, 6 men) were enrolled. Blood was sampled prior and after Epo treatment. Triplicate cultures of peripheral blood mononuclear cell (PBMC) were suspended in liquid nutrient with or without myo-[2-^{3}H]-inositol (INOZ) and phytohaemagglutinin (PHA) as shown in the table below. After incubation INOZ incorporation was estimated by a scintillation counter. Spontaneous activation (SpAct) and phytohemagglutinin-inducible activation (PHAAct) were calculated by the following equations: SpAct = [(PBMC + INOZ) - PBMC]; PHAAct = [(PBMC + PHA + INOZ) - (PBMC + PHA)].

Results:
Mean value results in study groups

	Healthy Subjects	CKD prior treatment	CKD after treatment
PBMC♦	42.7	43	42.4
PBMC+PHA♦	39.5	43.1	47
PBMC+INOZ♦	233.6	168	368.8
PBMC+INOZ+PHA♦	152.7	158.7	^227.1*
Sp Act♦	163 (82-260)	134 (45-198)	208 (120-451)
PHA Act♦	112 (73-142)	103 (42-179)	^182 (132-223)*
Age years	56.6±9	71.9±11	-
HGB g/dl	13.8	9.6*	^11.4*

♦dpm; *p<0.05 vs healthy subjects; ^p<0.05 vs CKD prior treatment

No statistical differences of INOZ incorporation in SpAct and PHAAct cultures were found in the group of healthy subjects and CKD patients prior treatment. INOZ incorporation in the group of patients after Epo treatment was significantly increased in PHAAct and insignificantly increased in SpAct cultures in comparison to the healthy subjects. INOZ incorporation in the group of patients after Epo treatment was significantly increased in PHAAct and SpAct cultures (p<0.05) in comparison to the patients group before treatment.

Conclusions: This study, despite its limitations, disclosed that INOZ incorporation after Epo treatment was significantly higher than before treatment. Therefore, Epo may be partially responsible for the synthesis of the secondary messengers and improvement of the cells signaling in CKD patients.

Funding: Government Support - Non-U.S.

PUB091

Effect of the F11R Receptor Antagonist P4D on Human Umbilical Vein Endothelial Cell (HUVECs) Proliferation Moro O. Salifu, Arye Kremer, Anna Babinska. *Division of Nephrology, SUNY Downsate Medical Center.*

Background: The F11R/JAMA receptor is expressed on the surface of platelets, endothelial cells (EC) and smooth muscle cells and has been implicated in plaque formation and thrombosis and could be a potential therapeutic target in intimal hyperplasia. Here we examined the possibility that blockade of F11R /JAM using the specific F11R inhibitor peptide 4D (P4D) on human vein endothelial cells (HUVECs) could inhibit the proliferation of endothelial cells.

Methods: Cultured HUVEC were treated with increasing concentrations of P4D (0, 100, 250 or 500μM) alone (non-TREATED) or increasing concentration of P4D with TNFa and INFg (TREATED) and mitochondrial activity (Formazan production) was measured at different time points. Comparison of formazan production was made between the two groups at 24 hours and within each group.

Results: In non-TREATED HUVECs, there was no significant difference in formazan production at the three different concentrations of P4D compared to control. In TREATED HUVECs, we observed significantly higher inhibition of formazan production with increasing concentrations of P4D in a dose dependent manner (9% inhibition at 100μM, p=0.14, 18% inhibition at 250μM, p<0.002 and 28% inhibition at 500μM, p<0.005). There was statistically significant inhibition of formazan production between TREATED and non-TREATED groups at 250μM P4D (18% vs. 0.5%, p <0.005) and at 500μM (28% vs. 1.3%, p <0.005).

Conclusions: We observed a dose dependent inhibitory effect of P4D on mitochondrial activity of HUVECs only under inflammatory conditions but no effect on intact HUVEC. Further studies are needed to determine the role of P4D in intimal hyperplasia, characteristic of dialysis vascular access stenosis.

PUB092

Effect of the F11R Receptor Antagonist P4D on Human Smooth Muscle Cell (HSMCs) Proliferation Moro O. Salifu, Arye Kremer, Anna Babinska. *Division of Nephrology, SUNY Downstate Medical Center.*

Background: The F11R/JAMA receptor is expressed on the surface of platelets, endothelial cells and smooth muscle cells and has been implicated in plaque formation and thrombosis and could be a potential therapeutic target in intimal hyperplasia. Here we examined the possibility that blockade of F11R /JAM using the specific F11R inhibitor peptide 4D (P4D) could inhibit the proliferation of human smooth muscle cells (HSMC) in vitro.

Methods: Cultured HSMC were treated with increasing concentrations of P4D (0, 100, 250 or 500 μM) alone (non-TREATED) or increasing concentration of P4D with TNFa IL-1b and INFg (TREATED) and mitochondrial activity (Formazan production) was measured. Comparison of formazan production was made between the two groups at 24 hours and within each group.

Results: In non-TREATED HSMCs, there was significantly higher dose dependent P4D inhibition of formazan production (5.2%@100μM, 20.2%@250μM and 24.8%@500μM) compared to control ($p<0.05$). In TREATED HSMCs, there was also significantly higher dose dependent P4D inhibition of formazan production (8.5%@100μM, 16.6%@250μM and 25.6%@500μM) compared to control ($p<0.05$). There was no significant inhibition of formazan production between TREATED and non-TREATED groups at 250μM P4D (16.6% vs. 20.2%) and at 500μM (25.6% vs. 24.8%).

Conclusions: We observed a dose dependent inhibitory effect of P4D on mitochondrial activity of HSMCs under inflammatory and non-inflammatory conditions. Further studies are needed to determine the role of P4D in intimal hyperplasia, characteristic of dialysis vascular access stenosis.

PUB093

Nicotine Induces Apoptosis in Human Proximal Tubular Cells Joon Seok Choi, Chang Seong Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Korea.*

Background: Cigarette smoking is considered as independent risk factor for progression of chronic kidney disease and nicotine is the major compound of cigarette. The present study was aimed to investigate the effect of nicotine on renal tubular epithelial cell apoptosis in vitro and explore the potential mechanisms involved.

Methods: Human proximal tubular epithelial (HK-2) cells were treated with 200 μM of nicotine. Cell viability was examined using MTT assay. The DCF-DA staining was used to measure intracellular levels of reactive oxygen species (ROS). The protein expression of extracellular signal-regulated kinase (ERK), P38 and c-Jun N-terminal kinase (JNK), NF-κB, Bax and Bcl-2 was determined by semiquantitative immunoblotting.

Results: Nicotine treatment resulted in dose-dependent decreases of cell viability and increases of ROS. Nicotine increased the expression of p-ERK, p-JNK and p-P38. Nicotine induced expression of p-ERK and p-JNK in HK-2 cells, which was attenuated by pretreatement of N-acetyl-cysteine. The expression of NF-κB started to increase 1 hour after nicotine exposure. Increased nuclear NF-κB activation was counteracted by inhibitors of ERK (PD98059) or JNK (SP600125). Nicotine decreased the expression of Bcl-2, while increased that of Bax.

Conclusions: These results suggest that nicotine induce apoptosis by increased ROS generation through activation of NF-κB and mitogen-activated protein kinase signaling pathways in HK-2 cells.

PUB094

High Glucose Induce Apoptosis through the PERK-eIF2α Endoplasmic Reticulum Stress Pathway in Peritoneal Mesothelial Cells Junichi Nakamata,[1] Hiroyuki Morimoto,[2] Ryoko Baba,[2] Nana Ishimatsu,[1] Tetsu Miyamoto,[1] Kaori Kanegae,[3] Ryota Serino,[1] Narutoshi Kabashima,[3] Yutaka Otsuji,[1] Yoshiaki Doi,[2] Masahito Tamura.[3] *[1]The 2nd Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan; [2]The Department of Anatomy, University of Occupational and Environmental Health, Kitakyushu, Japan; [3]The Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Japan.*

Background: It is important to maintain an adequate peritoneal function and structure for long-term PD therapy. Endoplasmic reticulum (ER) stress is induced by an accumulation of unfolded or misfolded protein in the ER, and it is associated with the progression of diabete mellitus. We investigated whether ER stress-induced apoptosis is involved in the glucose-induced peritoneal damage in cultured rat peritoneal mesothelial cells (PMCs).

Methods: The primary PMCs obtained from the parietal peritoneum of Wistar rats were treated with a high glucose culture medium. The cell viability of the PMCs was measured by a WST-1 assay. The induction of apoptosis was examined by a DNA ladder formation assay. The activation of the PERK-eIF2α pathway was determined by Western blotting. The subcellular localization of CHOP was analyzed by immunocytochemistry.

Results: The treatment of PMCs with glucose higher than 3% suppressed cell viability and induced DNA fragmentation. High glucose increased the phosphorylation of PERK and its downstream signal, eIF2α. High glucose also enhanced CHOP expression, which is a downstream element of eIF2α, and altered the subcellular localization of CHOP from cytosol to nucleus. Sal 003, an eIF2α inhibitor, partially attenuated the high glucose-induced suppression of cell viability, thus suggesting that other ER stress pathways such as ATF6 or IRE1, might also be involved in glucose-induced PMC apoptosis.

Conclusions: We demonstrated that high glucose induced ER stress-mediated apoptosis mainly via the PERK-eIF2α pathway in PMCs. These findings suggest that the ER stress-mediated apoptosis might be involved in glucose-induced peritoneal damage, thus indicating a potentially useful therapeutic approach by manipulating eIF2α in patients on PD therapy.

PUB095

The Role of GSK-3β in Epithelial-Mesenchymal Transition and Apoptosis of Podocytes with High Glucose Liu Zhangsuo,[1] Guo Jia,[1] Su Chenhao.[1] *[1]Department of Nephrology, The First Affiliated Hospital, Zhengzhou University; [2]Department of Nephrology, The First Affiliated Hospital, Zhengzhou University; [3]The First Affiliated Hospital, Zhengzhou University.*

Background: To investigate the role of GSK-3β (glycogen synthase kinase 3 beta) in epithelial-mesenchymal transition (EMT) and apoptosis of podocytes with high glucose.

Methods: The matured podocytes were cultured in RPMI 1640 medium with different concentration of glucose (5.6mmol/L, 12.5mmol/L, 30 mmol/L, 50 mmol/L for 48h) or different time (30mmol/L for 24h, 36h, 48h). Western blot or reverse transcription-PCR was used to detected the protein or mRNA expression. The viability or apoptosis of podocytes with different treatments were detected by MTT assay or flow cytometry. The activity of GSK-3β in different conditions was detected by using the GSK-3β activity assay kit. The leakage of bovine serum albumin in transwell cultured plates was analysed to study the functional loss of podocytes caused by high glucose.

Results: Both the protein and mRNA expression of nephrin were decreased while the expression of α-SMA was up-regulated by high glucose treatment in a dose- and time-dependent manner ($P<0.05$). And the leakage of bovine serum albumin was increased when the concentration of glucose increasing, which mean the injury and functional loss of podocytes. Companied with the increasing concentration of glucose, the cell viability was decreased and the Bcl-2 mRNA was decreased while the Bax mRNA expression increasing. This indicated the high glucose could lead the podocytes to apoptosis.

Although the total GSK-3β was not changed significantly ($P>0.05$), the activity of GSK-3β in podocytes was enhanced ($P<0.05$) and the expression of p-Ser9-GSK-3β was increased when the glucose increased.

Conclusions: High glucose could lead the EMT, loss of function and apoptosis of podocytes. The enhanced activity of GSK-3β caused by high glucose may be one of the mechanisms of podocyte injury.

Funding: Government Support - Non-U.S.

PUB096

Polymorphonuclear (PMN) Cells Apoptosis in Chronic Kidney Disease (CKD) Patients Treated with Methoxy Polyethylene Glycol: Epoetin beta (Mircera) Piotr Bartnicki, Jacek Rysz. *Department of Nephrology, Hypertension and Family Medicine, Lodz Medical University, Poland.*

Background: In patients with CKD is observed increased PMN cells apoptosis quantified by flow cytometry using annexin V and propidium iodide staining. Erythropoietin (Epo) in these patients, beyond anemia correction seem to have renoprotective effect and slow progression of CKD. One of possible renoprotective mechanism of Epo is antiapoptotic effect. In this study we aimed to determine the effect of methoxy polyethylene glycol – epoetin beta (Mircera) on PMN cells apoptosis in predialysis patients with CKD.

Methods: 28 patients in predialysis and anemia, treated with Mircera, were enrolled. The healthy control group included 15 volunteers. Data about hemoglobin level (Hg) and estimated glomerular filtration rate (eGFR) are shown in the table below. Apoptosis of PMN cells was quantified by flow cytometry using annexin V and propidium iodide staining and changes of FAS, FAS Ligand, CD16 and CD11b activity were assessed by flow cytometry too using respective monoclonal antibody.

Results:
The study results.

	CKD before treatment	CKD after treatment	Control group
Hg [g/dl]	9.6 (9.1 – 10.1) *	11.6 (11.1 – 12.1) *, #	14.4 (13.3 – 14.9)
eGFR [ml/min]	16.4 (13.9 – 21.8) *	16.6 (14 .0 – 23.3) *	65 (63 – 74)
FAS [MFI]	29.4 (24.8 – 36.2) *	25.2 (20.1 – 29.4) #	23.7 (21.8 – 26.6)
FAS Ligand [MFI]	47.7 (41.4 – 54.0) *	42.9 (30.4 – 54.8)	36.5 (28.9 – 49)
CD16 [MFI]	242 (174 – 309) *	283 (232 – 359)	328 (260 – 458)
CD11b [MFI]	235 (122 -344)	273 (175 – 381)	166 (123 – 280)
ANXLL [%]	94.7 (89.1 – 96.2)	94.5 (92 – 96.4)	95.5 (92.6 – 96.8)
ANXLR [%]	2 (1.5 – 2.5) *	1.5 (0.7 – 1.9) #	1.1 (0.4 – 1.4)
ANXUR [%]	1.5 (1.0 – 2.6) *	1.0 (0.6 – 1.7)	1.0 (0.6 – 1.6)
ANXLR+ANXUR [%]	3.5 (2.4 – 5.1) *	2.6 (1.9 – 3.2) #	2.1 (1.3 – 3.2)
PI [%]	2.6 (1.3 – 5.0) *	2.4 (1.6 – 3.0) *	1.7 (1.5 – 2.1)

* $p < 0.05$ to control group, # $p < 0.05$ to CKD before treatment

We found significant ($p < 0.05$) correlation between Hg and ANXLR + ANXUR measured as Kendall's tau coefficient values.

Conclusions: These data suggest that methoxy polyethylene glycol – epoetin beta (Mircera) used in correction of anemia in patients with CKD might have antiapoptotic effect.

Funding: Clinical Revenue Support

PUB097

Suramin Alleviates Glomerular Injury and Inflammation in the Remnant Kidney Shougang Zhuang.[1,2] [1]*Department of Medicine, Rhode Island Hospital, Alpert Medical School, Brown University, Providence, Providence, RI;* [2]*Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China.*

Background: Although suramin, a compound that inhibits the interaction of multiple cytokines/growth factors with their receptors, can attenuate the development of renal interstitial fibrosis in the murine model of unilateral ureteral obstruction (UUO), it remains unclear whether suramin can alleviate glomerular and vascular lesions. Here we tested the efficacy of suramin in the remnant kidney after 5/6 nephrectomy, a model characterized by the slow development of glomerulosclerosis, vascular sclerosis, tubulointerstitial fibrosis and renal inflammation.

Methods: 5/6 of normal renal mass was surgically ablated in male rats. On the second week after surgery,rats were randomly divided into suramin treatment and non-treatment groups. Suramin was given at 10 mg/kg once per week for two weeks.

Results: In the remnant kidneyof mice receiving suramin, glomerulosclerosis and vascular sclerosis as well as inflammationwere ameliorated. Suramin also attenuated tubular expression of two chemokines, monocyte chemoattractant protein-1 and regulated upon expression normal T cell expressed and secreted (RANTES). After renal mass ablation, several intracellular molecules associated with renal fibrosis, including NF-kappaB p65, Smad-3,signal transducer and activator of transcription-3 and extracellular regulated kinase 1/2, are phosphorylated; suramin treatment inhibited their phosphorylation. Futhermore, suramin abolished renal ablation-induced phosphorylation of epidermal growth factor receptor and platelet derived growth factor receptor, two receptors that mediate renal fibrosis.

Conclusions: These findings suggest that suramin attenuates glomerular and vascular injury and reduces inflammatory responses bysuppression of multiple growth factor receptor-mediated profibrotic signaling pathways. Therefore, suramin may be a useful drug in preventing the fibrosis and sclerosis that characterizes progression of chronic kidney disease.

Funding: NIDDK Support

PUB098

Transforming Growth Factor-β Attenuates Prostaglandin E_2-Mediated Responses in Mouse Proximal Tubule Cells Rania Nasrallah, Fadey F. Farid, Andrew J. Karam, Richard L. Hebert. *Cellular and Molecular Medicine, Kidney Research Centre, University of Ottawa, Ottawa, ON, Canada.*

Background: The proximal tubule is the primary tubular segment affected in diabetic kidneys. Transforming growth factor (TGF)-β is increased by high glucose in the proximal tubule, serving as an important pathophysiological factor in the renal complications of diabetes. Prostaglandin (PG) E_2 regulates diabetic growth, cell fate, fibrosis and electrolyte balance under a number of conditions in different renal cells but the role of PGE_2 in this segment has not been established in the diabetic kidney.

Methods: In the mouse MCT proximal tubule cell line, we observed a 2.5 fold increase in TGF-β protein levels by Western blotting in response to high glucose. We hypothesized that PGE_2 can modify TGF-β dependent injurious responses in MCT cells. MCT cells were treated for 24 hours with 2ng/ml TGF-β in the presence or absence of 1 μM PGE_2.

Results: First TGF-β caused an 8-fold increase in fibronectin protein, which was augmented to 15-fold in the presence of PGE_2. PGE_2 alone however, resulted in a 2-fold increase in fibronectin. In contrast, TGF-β caused a 60% reduction in p27 levels but increased p21 by 5.4-fold, while PGE_2 increased p27 by 2.5 fold and reduced p21 by 70%. TGF-β co-treatment abolished p27 stimulation by PGE_2, but PGE_2 had no effect on TGF-β mediated induction of p21. PGE_2 also increased NaK-ATPase levels by 2-fold, and TGF-β attenuated this response by 50%. While neither TGF-β nor PGE_2 alone altered sgk1 levels (a known activator of NaK-ATPase), co-treatment resulted in a 33% reduction in sgk1 that may account for some of the observed TGF-β mediated attenuation of NaK-ATPase levels in co-treated cells.

Conclusions: Altogether our data indicate that though PGE_2 does not antagonize TGF-β mediated responses in MCT cells, TGF-β does attenuate the effects of PGE_2 with the exception of fibronectin. Future studies will examine the mechanisms associated with this interaction.

Funding: Government Support - Non-U.S.

PUB099

The Correlation of the Anti-Plasminogen Antibodies with Disease Activity in ANCA-Associated Vasculitis Min Chen, Jian Hao, Ming Hui Zhao. *Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.*

Background: The patients with PR3-specific ANCA had antibodies against a protein coded by the antisense strand of the PR3cDNA ($cPR3^{105\text{-}201}$), which participated in the autoantigen complementarity as a potential mechanism of ANCA-associated vasculitis (AAV). Plasminogen was identified as a target of anti-$cPR3^{(105–201)}$. The purpose of the current study was to investigate whether anti-plasminogen antibodies can be used as a biomarker for assessing disease activity of AAV.

Methods: One hundred and four Chinese patients with AAV were recruited in the current study. Anti-plasminogen antibodies were detected in sequential serum samples at initial onset and remission of the disease. Association of anti-plasminogen antibodies with clinico-pathological paramenters was analyzed.

Results: The prevalence of anti-plasminogen antibodies was significantly higher in AAV patients 19/104 (18.3%), as compared with four 0/50 healthy control subjects (19/104 vs. 0/50, χ^2=8.8, *P*=0.003). The prevalence of anti-plasminogen antibodies was significantly higher in AAV patients in active phase of initial onset than in remission (19/104 vs. 1/48, χ^2=7.5, *P*=0.013). The level of anti-plasminogen antibodies (expressed as the percentage of positive control) correlated with the level of ESR (r=0.207, *P*=0.042), Scr (r=0.302, P=0.002), D-dimer (r=0.273, *P*=0.009) and the percentage of glomeruli with crescents (r=0.393, *P*=0.004). The level of BVAS, the prevalence of arthralgia, gastrointestinal involvement and the percentage of glomeruli with crescents with anti-plasminogen antibodies patients was significantly higher than that without anti-plasminogen antibodies patients (22.5±5.63 vs. 19.4±4.66, *P*=0.015, 63.2% vs.25.8%, >*P*=0.002, 57.9% vs. 21.1%, *P*=0.001 and 54.71% vs. 21.36%, *P*=0.002), respectively.

Conclusions: The level of anti-plasminogen antibodies in AAV patients was correlated with reduced renal function and the percentage of glomeruli with crescents. Anti-plasminogen antibodies might be a good biomarker for assessing disease activity of AAV.

Funding: Government Support - Non-U.S.

PUB100

Gene Polymorphisms of Interleukines-17 and Interleukin-17 Receptor Are Associated with End-Stage Kidney Disease Eun Young Kim, Wha-young Suk, Joo Hee Cho, Jungkook Wi, Hong Joo Lee, Young Wook Choi, Ju-Young Moon, Kyung-hwan Jeong, Tae-won Lee, Chun-Gyoo Ihm, Sang-Ho Lee. *Division of Nephrology Department of Internal Medicine, Kyung Hee University, Seoul, Korea.*

Background: Inflammation could be a causal factor in progression of chronic kidney disease. To date, there is convincing experimental and clinical evidences to support the notion that IL-17-producing T cells contribute to kidney injury in renal diseases. But it has never been studied about genetic relationship between ESRD and Th17 pathway.

In this study, we hypothesized that polymorphisms of interleukin 17 or their receptors may be associated with ESRD.

Methods: A total of 290 non-diabetic ESRD patients and 289 normal controls were included. We analyzed fourteen single-nucleotide polymorphisms (SNPs) located within the 4 genes of *IL17A, IL17E, IL17R* and *IL17RB.*

Results: The ESRD patients had a significantly higher allele frequency compared to control subjects for the *IL17E rs10137082*C* and *rs1124053*G* and *IL17R rs4819554*A* alleles. Genotyping analysis demonstrated that 3 SNPs (21.4%) among 14 were significantly associated with ESRD after adjusting for age and sex, which were showed by *IL17E rs10137082* (OR=1.50 dominant, OR=1.47 log-additive), *IL17E rs1124053* (OR=2.15 codominant2, OR=1.46 dominant, OR=1.94 recessive, OR=1.41 log-additive) and *IL17R rs4819554* (OR=1.52 codominant1, OR=1.86 codominant2, OR=1.62 dominant, OR=1.39 log-additive).

Conclusions: Three polymorphisms within the *IL17E* and *IL17R* genes are associated with ESRD independent of age and sex. This is first finding to suggest that polymorphisms of *IL17* genes affect the risk of development of ESRD.

Funding: Private Foundation Support

PUB101

Role of Glycoprotein Nonmetastatic Melanoma Protein B in Regulation of Inflammatory Responses of Macrophages in Hyperglycemia Satyesh K. Sinha,[1] Susanne B. Nicholas.[1,2] [1]*Research, Charles R Drew University of Medicine and Science, Los Angeles, CA;* [2]*David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA.*

Background: Several studies have demonstrated that diverse inflammatory molecules play a significant role in the setting of diabetic nephropathy (DN), one of the profound consequences of diabetic mellitus (DM). The process of inflammation requires the selective expression of genes in cells of the macrophage lineage. Glycoprotein nonmetastatic melanoma protein b (GPNMB), a growth factor, has been identified as a regulator of the inflammatory and phagocytic activities of macrophages. However, little is known about its role in the inflammatory responses of macrophages in hyperglycemia. Therefore, in the present study, we tested our hypothesis that high glucose suppresses the expression of GPNMB in activated macrophages, leading to aberrant cytokine release.

Methods: Murine macrophage cells (J774A.1) were cultured in RPMI 1640. Following serum starvation, the cells were treated with D-glucose (5, 25 and 40mM). The expression levels of GPNMB, CD36 and TGF-β were determined by immunoblotting. Concentrations of IL-6 and TNF-α in culture supernatants were assessed by enzyme-linked immunosorbent assay.

Results: High glucose treated macrophages exhibited significant reduction in the expression of GPNMB (30%) and an increase in the expression of CD36 (13%) and TGF-β compared to normal glucose. Relative to physiological level, under high glucose, secretion of IL-6 was 258% higher (12pg/ml vs. 43pg/ml) and TNF-α was 34% higher (70pg/ml vs. 94pg/ml).

Conclusions: These results suggest that GPNMB may play an important role in the regulation of inflammatory responses of macrophages in DN.

Funding: Other NIH Support - NIH/NIMHD Accelerating Excellence in Translational Science U54M007598 (Formerly U54RR026138) at Charles R. Drew University

PUB102

Acoustic Radiation Force Impulse (ARFI)-Based Elastometry Possibly Represents Diminution of Blood Flow Rather than Tissue Fibrosis of the Kidneys in CKD Patients Ai Ogata,[1] Kenichiro Asano,[1] Yoko Ide,[1] Akiko Sankoda,[1] Keiko Tanaka,[1] Mana Nishikawa,[1] Masaru Kinomura,[1] Noriaki Shimada,[1] Masaki Fukushima.[2] *[1]Nephrology, Kurashiki Central Hospital, Kurashiki, Japan; [2]Internal Medicine, Shigei Research Institute Hospital, Okayama, Japan.*

Background: ARFI is a novel ultrasound method for quantitating tissue elasticity. Velocity of shear wave (Vs) which represents the stiffness of the organ is measured by a virtual touch tissue quantification technique. A higher Vs value implicates progression of fibrosis for chronic liver disease. In this study, the Vs value was measured in the kidneys of CKD patients at all stages and was compared with other clinical parameters.

Methods: Renal imaging and quantification were performed by using an Acuson S2000 ultrasound system (Siemens AG) in 319 CKD patients (M: 198 F: 121, age: 62.0±15.7). Mean Vs was determined after several measurements in each renal parenchyma. The main causes of chronic kidney injury were classified by histology and clinical course (glomerulonephritis: 129, diabetic nephropathy: 107, nephrosclerosis: 83). Estimated GFR was calculated by serum creatinine concentration and age. Brachial-ankle pulse wave velocity (baPWV) was measured within a year from Vs determination in 183 patients. Normal control values were measured in 14 healthy volunteers.

Results: In healthy controls, mean Vs was higher in the renal medulla than in the cortex. The Vs value measured in the right and left kidneys of a patient correlated well though the variation between individuals was considerably large. In CKD patients, Vs and eGFR showed a positive correlation. The correlation was weak in glomerulonephritis patients and Vs was the lowest in diabetic patients with low eGFRs. A negative correlation was obtained between Vs and baPWV. All these results suggest that progression of CKD and arteriosclerosis diminishes blood flow and reduces tension of the kidney which may be reflected by a low Vs value.

Conclusions: Vs possibly represents diminution of blood flow which succeeds arteriosclerosis rather than fibrosis of the kidneys in CKD patients. However, the variation of Vs between subjects is too large for the method to be applicable as is for clinical use.

PUB103

The Relationship among Depression and Treatment Compliance in Hemodialysis Patients Jin-mei Yin,[1] Kang-lai Li,[2] Nian-chang Chen,[1] Tan-qi Lou.[1] *[1]Department of Nephrology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China; [2]Department of Special Care, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: To investigate the relationship among depression and treatment compliance in hemodialysis patients.

Methods: 79 hemodialysis patients were recruited and investigated with Beck Depression Rating Scale (BDI) for depression survey. According to the score of depression, patinets were divided into two groups (non-severe group and severe group). Assessmeng of treatment compliance including dialysis dose, water and salt control, rational drug use, reasonable exercise of compliance were analyzed.

Results: 62% patients were in the severe group. Compared to the non-severe group, the severe group were with poorer treatment compliance ($P < 0.05$). It shows that depression have a significant impact on treatment adherence.

Conclusions: Deprssion were common in hemodialysis patients. Depression had significant effect on treatment compliance, reducing the treatment effect, reducing the patient's quality of life.

PUB104

Single Centre Review of Pregnant Patients Reviewed in a Joint Renal/ Obstetrics Clinic Maharajan Raman, Arvind Ponnusamy, Daniel J. Hall, Hayley L. Mcmanus, Philip A. Kalra, Teresa Kelly, David I. New. *Renal, Salford Royal NHS Trust Foundation, United Kingdom.*

Background: Patient with chronic kidney disease have less ability to cope with renal changes needed for a healthy pregnancy. Pregnancy in a CKD patient carries a risk to both mother and fetus.

Methods: We retrospectively reviewed data patients from Joint Renal/Obstetrics clinic. A total of 30 patients were reviewed between the period of 2005 till 2011. We excluded patients receiving dialysis and transplant patients.

Results: Mean age of pregnant patients were 28.1 years. At the time conception, there were 5 patients on ACE inhibitor and these were stopped immediately. There were no reported foetal abnormalities. Only eight patients had proteinuria at the time booking. Mean creatinine was 79 mircromol/l± 42. Three patients had creatinine over 130 umol/l. Average urine protein creatinine ratio in the first trimester was 149 g/mol, 2nd trimester was 151 and 3rd trimester was 238 g/mol Only nine patients were some form of anti-hypertensive. At 3 months, patient with pre-pregnancy creatinine more 130 umol/l had loss GFR and an increase in proteinuria of over 20%. 26 patients had successful delivery with 4 had miscarriage during the pregnancy. Average gestational birth weight was 2635 gms. Complication during pregnancy included pre-elampsia (2 patients), placenta previa, chorioamnionitis and increase of proteinuria. Mean serum creatinine and urinary PCR at 3 months post pregnancy was 89 umol/l and 140 gms.

Conclusions: In our group of patients there appears to be good maternal and fetul outcome. This is a result from a multidisciplinary team involving comprising nephrologists, obstetricians, midwives, dietician in the joint clinic.The baseline creatinine and proteinuria is associated with worsening in renal function post pregnancy in our cohort of patients as reported in the literature.

PUB105

Assessment of Cellular Mass Index (BCMI) for Bioimpedance Vector (BIVA) as a Marker of Muscle Mass in 212 Patients with CKD Guillermina Barril, Ángel Nogueira Pérez, Maria Bernardita Puchulu, Fernando F. Hadad, Secundino Cigarran, Sara Jericó, Jose-Antonio Sanchez-Tomero. *[1]Nephrology, Hospital U. de la Princesa, Madrid, Spain; [2]Nephrology, Hodpital U. de la Princesa, Madrid, Spain; [3]Nephrology, Hospital U. de la Princesa, Madrid, Spain; [4]Nephrology, Hospital Clinico, Madrid, Spain; [5]Nephrology, Hospital Da Costa, Burela, Spain; [6]Nephrology, Hospital U. de la Princesa, Madrid, Spain; [7]Nephrology, Hospital U de la Princesa, Madrid, Spain.*

Background: To evaluate in 212 patients from ACKD Unit the utility the BCMI by BIA Vctor(celular/talla2 mass) as a marker of muscle mass and its relationship to body composition, Kcorporal total (cell mass x 108.6), visceral proteins, inflammation, age, sex and ERCA fuerza of patients.

Methods: In the nutrition care BIVA with ERCA is performed in 212 patients HD01 monitor ERCA. Xedad men with a 59.4 overall.., Most frequent etiology Diabetes Mellitus 32.6% and compared the results between men and women according to whether BCMI <8 (Group 1) or => 8 (Group 2).

Results: We found 75 patients G2, 55% men and 33.4% are women and G1 with 135 patients, 66.7% men and 33.3% women, total body .xde significant higher in group 2. Results attached table. We found no significant differences. between groups:% total body water,% triceps fold,% arm muscle circumference and arm circumference. NP NA. HB. Total lymphocytes, Ccr and MDRD. If significant direct correlation between dynamometry BCMI and p 0.009, and inversely related to extracellular water. We found differences in both groups of men and women with BMI no significant differences in each group between men and women. We have divided the patients according BCMI<7,9 and =>8 showing significant differences with higher values in the goup with higher BCMI for Phase angle, Intracelular water, % len body mass, % mascle massm albumin nd prealbumin values. Patients with BCMI =>8 showing less PCR than lower than 7,9.

Conclusions: The correspondence BCMI and cell mass can be seen as a key parameter in the study of body composition with BIVA, interrelated with visceral proteins, inflammation AIC and parameters of muscle mass and muscle strength.

PUB106

Dilemmas in Diagnosing Active TB in CKD Population of India Dilip A. Kirpalani, Shrinivas Nalloor, Hardik Shah, Ashok L. Kirplani. *Nephrology, Bombay Hospital Institute of Medical Sciences, Mumbai, Maharashtra, India.*

Background: In India the incidence of TB in CKD is higher than that in the general population. The overlap of clinical and laboratory features of TB and azotemia makes that diagnosis of TB difficult. This study highlights the diagnostic dilemma due to TB in CKD & their solutions.

Methods: 330 consecutive new patients of CKD between stage I and V (excluding those already on anti TB therapy) were investigated for active TB.

Results: Incidence of TB overall – 34/330 (10.3%; CKD stage-wise I-3%, II-5%, III-4%, IV-6.5%, V-9.43%, VD -28%. Sites of TB: (N=43 sites/34 patients) :Pulmonary 12, Pleural 11, Lymph nodular 8, Skeletal 4, Peritoneal 2, Genito urinary 2 & Miliary, Thoracic empyema, Cerebral, Vocal cord 1 each. Method of confirming TB – Bacteriological 7/34(20.6%), Histopathological 13/34 (38.2%), Clinical 14/34 (41.2%) àexudative fluid with lymphocytic predominance 13/14, milary on imaging 1/14. Pitfalls in fluid analysis (N=32 samples/17patients) - Ascitic 6/32, pleural 26/32, using protein content criteria exudates 18/32, transudate 14/32. Cellular predominance in exudative fluid : neutrophillic 5/18, lymphocytic 13/18. ADA levels in exudative fluids higher than transudative fluids ($p<0.05$) but no exudative sample revealed abnormally high ADA.

Conclusions: 1. The incidence of TB is 15 times greater in CKD & it increases with severity of CKD

2. Cardinal symptoms of TB such as fever, cough, weight loss & anorexia may not be present.

3. Usual markers of TB such as high ESR, anemia, +ve MT have poor value in CKD.

4. A history of TB contact & the stage of CKD have high predictive value.

5. Extra pulmonary TB is more common in CKD.

6. The positive predictive value of chest x ray in thoracic TB is limited to 65% & a CT chest may be needed for confirmation.

7. Pleural & ascitic fluid protein estimation, ADA levels and cellular characteristics are frequently confounding.

8. Yield of AFB & histopathological confirmation from TB sites is limited to 60% & the remaining 40% cases are diagnosed by supportive laboratory, clinical judgment & circumstantial evidence. A high index of suspicion is the best way to avoid missing this dangerous comorbidity of CKD.

PUB107

Protecting against Obesity-Induced Chronic Kidney Disease Using Pro-Resolving Lipids Emma Borgeson,[1] Debra F. Higgins,[1] Paula Maderna,[1] Helen M. Roche,[2] Catherine Godson.[1] *[1]Diabetes Research Centre, The Conway Institute, University College Dublin, Dublin, Ireland; [2]Nutrigenomics Research Group, The Conway Institute, University College Dublin, Dublin, Ireland.*

Background: There is a growing appreciation that obesity and its associated complications reflect chronic low-grade inflammation. Recent evidence highlights that the resolution of inflammation is actively regulated by endogenously generated counter regulatory and pro-resolving lipid mediators, such as lipoxins (LXs) and resolvins. We have investigated the potential of LXs to attenuate adipose inflammation, which is linked to pathologies such as chronic kidney disease.

Methods: Adipose tissue explants from perigonadal depots of aging female C57BL/6J mice were used as model of age-associated adipose inflammation. The explants were incubated with vehicle or LXA_4, after which cytokine secretion and expression of insulin sensitizing signaling pathways were assessed. To investigate specific regulatory pathways we also explored the effect of LXA_4 on macrophage-adipocyte crosstalk *in vitro*.

Results: We report that LXA_4 (1 nM) attenuates adipose inflammation, decreasing IL-6 and increasing IL-10 expression ($p<0.05$). The altered cytokine milieu correlated with increased GLUT-4 and IRS-1 expression, suggesting improved insulin sensitivity. Further investigations revealed the ability of LXA_4 to rescue macrophage-induced desensitization of insulin-stimulated signaling and glucose uptake in cultured adipocytes. This was associated with preservation of Akt activation and reduced secretion of pro-inflammatory cytokines, including TNF-α.

Conclusions: We propose that LXA_4 may represent a potentially useful and novel therapeutic strategy to subvert adipose inflammation and insulin resistance and thereby protect against obesity related pathologies including chronic kidney disease.

Funding: Government Support - Non-U.S.

PUB108

Comparison of Urine Biomarker Profiles between Kidney Donors with and without Nephrosclerosis on Biopsy and Diabetic Nephropathy Patients Michael P. Linnes, John C. Lieske, Samuel Edeh, Jennifer R. Geske, S. Jeson Sangaralingham, John C. Burnett, Andrew D. Rule. *Mayo Clinic, Rochester, MN.*

Background: Nephrosclerosis on renal biopsy is strongly correlated with age, but is inadequately detected by traditional kidney disease markers. This pilot study compared potential biomarkers relating to fibrosis, inflammation, and tubular injury in order to identify those that may be predictive of nephrosclerosis.

Methods: Sectioned and stained core-needle biopsies of living kidney donors at the Mayo Clinic from 2000 to 2008 were reviewed to identify those with nephrosclerosis (glomerulosclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis) and banked urine. Donors without nephrosclerosis were matched to donors with nephrosclerosis on age and sex. Patients with diabetic nephropathy served as positive controls. Biomarkers were measured via ELISA and compared between the 3 groups.

Results: There were 46 kidney donors with nephrosclerosis (mean 12% of glomeruli sclerosed, 61% had interstitial fibrosis >5%, 92% had tubular atrophy, and 84% had arteriosclerosis), 46 matched donors without nephrosclerosis (mean 1.9% of glomeruli sclerosed, 0% had interstitial fibrosis, 2% had tubular atrophy, and 8% had arteriosclerosis) and 81 diabetic nephropathy patients. Biomarker levels differed in diabetics compared to donors (see table). Col-IV was increased in the donors with nephrosclerosis compared to donors without (median 3.2 vs 1.1 ug/L, p=0.008). A1M was higher in donors with >5% interstitial fibrosis compared to donor controls (5.5 vs. 2.5 ug/mL, p=0.028).

Urine biomarker levels

Biomarker (ng/mL)	Donors (n=92)	Diabetics (n=81)
Kim-1	0.50	1.13*
NGAL	14.2	32.6
LFABP	2.0	59.3*
THP (ug/mL)	18.0	7.6*
IL-18	.04	.07
Col IV	2.8	8.2*
MCP-1	187	437
aGST	0.7	2.6
π-GST	17	40
A1M (ug/mL)	5	25

*p<0.05 adjusted for Age, Sex, BMI, and UACR

Conclusions: There were notable differences in several urine biomarkers between diabetics and donors independent of traditional kidney disease markers. There were fewer differences in these biomarker between donors with and without nephrosclerosis. Imprecision of the renal biopsy pathology due to sampling variation may limit detection of biomarker associations.

Funding: NIDDK Support

PUB109

Construction of Gene Engineering *L.bulgaria* that Can Express Uricase and Creatininase and Its Function Research Yunsheng Jiang, Fang Liu, Yafen Jiang, Lin Sun, Fuyou Liu. *Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China.*

Background: Recent studies that artificial cell therapy such as therapeutic delivery of live bacteria may become the new strategies for the chronic renal failure. Lactic acid bacteria is generally regarded as safe bacteria. We recently do some research on this field.

Methods: We construct a combination of uricase gene and creatininase gene creatinine, and clone it to Lactobacillus bulgaricus(L.B), so that it could express uricase and creatininase, and then explore the ability of decomposing the urotoxin of creatinine and uric acid in the serum of patients with end-stage renal failure, genetic stability of the recombinant plasmid, as well as the activity of crude enzyme of gene engineering Lactobacillus bulgaricus(L.B-UC). On the other hand, we use the 5/6 nephrectomy rat as an animal model to explore the capacity of creatinine and uric acid removal of the L.B-UC *in vivo*, the improvement of serum lipid balance and its function in the development of chronic renal failure.

Results: From in vitro experiments we can confirm that L.B-UC has a strong decomposition to creatinine and uric acid, a good genetic stability, high activity of uricase andcreatininase; The in vivo studies indicated that the L.B-UC can decompose the Cr and UA, improve the dyslipidemia, and alleviate renal interstitial injury and fibrosis.

Conclusions: we sucessfully constructed the gene engineering Lactobacillus bulgaricus, which can decompose the Cr and UA, have good genetic stability, as well as improve the dyslipidemia, alleviate renal interstitial injury and fibrosis.

Funding: Government Support - Non-U.S.

PUB110

Patient-Reported Complaints of Pruritus Are Common in Dialysis Patients but Infrequently Reflected in Physician-Reported ICD-9 Coding T. Christopher Bond, Gilbert Marlowe, Helen M. Wilfehrt, Amy Young, Mahesh Krishnan, Tracy Jack Mayne. *DaVita Clinical Research, Minneapolis, MN.*

Background: The Kidney Disease Quality of Life 36 (KDQOL) questionnaire—administered to all dialysis patients yearly at a large dialysis organization—quantifies itchiness. We hypothesized that patient-reported itchiness would not correlate with physician-coded pruritus given the relative priority placed on such diagnoses in medical coding.

Methods: We analyzed health records of 70,499 dialysis patients with available itchy skin data on KDQOLs (Jan 2009-May 2012) and compared these to associated physician-provided ICD-9 codes (698 or 698.x) for pruritus. The latter were collected predominantly by treating nephrologists as part of the medical justification for various dialysis-related labs and treatments.

Results: Sixty percent of patients report itchy skin on the KDQOL. The percentage of patients with ICD-9 codes of pruritus was low, and rose slightly with increasing patient-reported levels. The pruritus diagnostic code in these data has low sensitivity—with only 8.52% of patients who report being extremely bothered by itchy skin receiving a diagnostic code of pruritus.

Table. Physician diagnoses of pruritus by categories of patient-reported itchiness

During the past 4 weeks, to what extent were you bothered by itchy skin?	Patient-reported itchiness – n (%)	Physician diagnosis of pruritus – n (%)
Not bothered at all	28,070 (39.82%)	846 (3.01%)
Somewhat bothered	21,318 (30.24%)	859 (4.03%)
Moderately bothered	10,872 (15.42%)	509 (4.68%)
Very much bothered	6,520 (9.25%)	408 (6.26%)
Extremely bothered	3,719 (5.28%)	317 (8.52%)
Total	70,499 (100.00%)	2,939 (4.17%)

Conclusions: Data from a large dialysis organization suggest that physician-reported coding of pruritus does not typically reflect patient-reported itchiness. It is possible that patients do not report itchiness to physicians, physicians choose not to code pruritus, or perhaps physicians believe that itch is a symptom captured within the larger ESRD syndrome. The lack of options to adequately treat pruritus may complicate this situation.

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Funding: Pharmaceutical Company Support - Mitsubishi Tanabe Pharma Corporation

PUB111

Effectiveness and Safety of MIRCERA® in Patients with Anemia Associated with Chronic Kidney Disease: The MINERVA Study Aleix Cases,[1] Jose Portoles,[2] J. Calls,[3] Alberto M. Martinez-Castelao,[4] Domingo Sanchez-guisande,[5] Alfonso Segarra.[6] *[1]H.Clinic Barcelona, Spain; [2]H.U.Puerta de Hierro, Spain; [3]H.Manacor, Spain; [4]H.Bellvitge, Spain; [5]H.Barbanza, Spain; [6]H. Vall d'Hebron, Spain.*

Background: MIRCERA® is the first continuous erythropoietin receptor activator approved for treatment of anemia associated with chronic kidney disease (CKD). We assessed the effectiveness and safety of MIRCERA® in patients with CKD anemia in clinical practice.

Methods: Multicenter prospective observational study carried out in stage 3-5 CKD patients treated with MIRCERA® for a year.

Results: 227 patients were evaluated (mean age 71.2±14.7years; men 55.5%; main CKD etiology: diabetic nephropathy 27.8%): 85 (37.4%) on hemodialysis (HD; correction n=2, maintenance n=83) and 142 (62.6%) CKD not on dialysis (CKDND; correction n=31, maintenance n=111). Hb levels, MIRCERA® doses and dose adjustments are shown in the table.

	CKDND correction	CKDND maintenance	HD maintenance
Hb ranges, n(%)			
Baseline:			
<11g/dl	19(61.3)	24(21.8)	25(30.1)
11-13g/dl	12(38.7)	70(63.6)	49(59.0)
>13g/dl	0(0.0)	16(14.5)	9(10.8)
Month12:			
<11g/dl	7(33.3)	20(30.8)	15(28.8)
11-13g/dl	12(57.1)	42(64.6)	33(63.5)
>13g/dl	2(9.5)	3(4.6)	4(7.7)
Hb levels (g/dl), mean±SD			
Baseline	9.8±0.8	11.7±1.2	11.5±1.4
Month6	11.4±1.2	11.7±1.2	11.5±1.3
Month12	11.4±1.4	11.4±1.0	11.6±1.2
MIRCERA® doses (µg), mean±SD	89.8±45.3	98.1±66.5	171.0±105.7
No 1-year dose adjustments, mean±SD	0.3±0.5	0.7±1.1	2.1±1.9

No MIRCERA®-related adverse events were reported. Mean conversion doses of MIRCERA® were 90.8±48.8µg, 150.1±64.8µg and 230.6±115.8µg in patients previously receiving darbepoetin<40µg/epoetin<8000UI, darbepoetin40-80µg/epoetin8000-16000UI and darbepoetin>40µg/epoetin>16000UI,respectively. After 7.0±5.4months, they were 104.8±58.5µg p=0.006, 157.3±66.5µg p=0.269 and 188.9±148.0µg p=0.195,respectively. Mean 1-year dose adjustments were 0.3±0.5, 0.7±1.1 and 2.1±1.9 in CKDND correction, CKDND maintenance and HD maintenance patients,respectively.

Conclusions: Once-monthly MIRCERA® was effective and safe for CKD anemia management in clinical practice. Conversion doses of MIRCERA® were lower than those described in the SPC and there were a low number of dose adjustments.

Funding: Pharmaceutical Company Support - The Spanish Society of Nephrology ("Sociedad Española de Nefrología") Is the Promotor and Roche Provided Financial Support

PUB112

Functional Iron Deficiency, Is a Limiting Factor of IV Iron Use in Non Hemodialysis Renal Insufficiency Patrick Fievet. *Nephrologie Hemdoialyse, Groupe Hospitalier du Sud de l'Oise, Creil, France.*

Background: Iron deficiency is an important cause of anaemia in non dialysis chronic kidney disease (ND-CKD). The new forms of IV iron (IV Fe) allow use of high doses to treat it. This is interesting to protect venous capital and to reduce the number of administrations. Nevertheless The risk of iron overload has not been studied even though these patients are more exposed than haemodialysis patients since lack of regular blood loss.

Methods: We have studied the efficacy of high doses of low molecular weight iron dextran (LMWD) to correct iron deficiency ant to treat anaemia in ND-CKD. Total doses have been prescribed, according to Ghanzoni formula, taking account hemoglobin concentration (Hb) and patient's weight. 40 doses from 500 to 1600 mg (mean = 760 ± 291 mg) have been administered to 29 ND-CKD patients (DFG = 23.7 ±12.6 ml/min/1.73 m²) with iron deficiency defined by transferrin saturation (TSAT) lower than 20% and/or ferritin lower than 100 mg/L and treated by erythropoiesis stimulating agent (ESA) in 22 cases on 40. ESA doses have not been modified. Patients have been controlled 1 month later. Results of iron markers have been confronted to target values defined by KDOQI and ERBP.

Results: Hb has significantly increased (11,4 ± 1.4 vs 10.3 ± 1.3 g/dL, p < 0,001) as TSAT (22.6 ±8.5 vs 14.0 ±3.9%, p < 0,001) and ferritin (320 ±265 vs 97 ± 61 µg/L, p < 0,001). In 9 cases, ferritin reaches higher value than 500 mg/L after treatment exposing to risk of iron overload. Iron profile in these patients was compatible with functional iron deficency (ferritin values before treatment higher than others patients (169 ± 65 vs 76 ±42 µg/L, p = 0,03) whereas TSAT were identical (13,8 ± 3,7% vs 14.1 ±4%, NS)). In patients with ferritin upper 500 µg/L after treatment, Hb has not increased whereas it has in the others.

Conclusions: Administration of high doses of LMWD is efficient to correct iron deficiency and anemia in ND-CKD. High doses of IV Fe exposes to risk of iron overloadwithout efficacy on erythropoïesis, in patients with functional iron deficiency profile (TAST < 20% and ferritin > 150 µg/L). So it seems to be reserved for patients with ferritin lower or equal to 150 µg/L.

Funding: Other NIH Support - Groupe Hospitalier du Sud de l'Oise

PUB113

Understanding the Molecular Basis of Ventricular Arrhythmias in Uremic Patients Elie El-Charabaty,[1] Georges Khoueiry,[1] Bertrand Mukete,[2] Estelle Torbey,[2] Suchita J. Mehta,[1] Roger Hajjar,[2] Suzanne E. El Sayegh.[1] *[1]Medicine, Staten Island University Hospital; [2]Mount Sinai School of Medicine.*

Background: Uremic cardiomyopathy characterized by LVH and interstitial fibrosis plays a vital role in fatal arrhythmias and sudden cardiac death (SCD) in CKD patients. The molecular basis of uremic cardiomyopathy is not completely understood. Few animal studies, showed a decrease in cardiac sarcoplasmic reticulum (SERCA2a) gene expression with CKD. Other studies showed that SERCA2a underexpression leads to action potential alternans, which is linked to SCD. We hypothesize that patients with stage 5 CKD have lower levels of SERCA2a protein compared to those with normal kidney function.

Methods: Brachial venous tissue was collected from 6 patients with stage 5 CKD at time of A-V fistula creation. Saphenous vein tissue from 6 patients with normal creatinine were collected at time of CABG to serve as the control group. RNA was isolated from these tissues using Trizol reagent. The RNA purity/concentration was assessed using A260/A280 ratio by spectrophotometry. DNA was prepared by using a high capacity cDNA reverse transcription kit. Serca2a gene was assessed using SYBR Green Fast Mix in reverse transcription-PCR.

Results: SERCA2a gene expression in the CKD versus the control group was calculated using PCR fold change method. Our analysis showed a 66% decrease in SERCA2a gene expression with uremic subjects versus control.

Conclusions: This is the first evidence that patients with CKD have an altered gene expression of SERCA 2a, a protein that plays a key role in cardiac cell calcium homeostasis during excitation-contraction coupling. Action potential alternans, a predictor of ventricular arrhythmias, was associated to SERCA2a deficiency in guineapig model. Our findings might suggest a molecular cause for the susceptibility of this population to arrhythmias and SCD. This will be the first vital step that will allow us in the future to link SERCA2a levels to action potential alternans, microvolt T-wave alternans, or ventricular arrhythmias on cardiac telemetry as electrophysiological evidence of increased susceptibility to lethal ventricular tachyarrythmias in this population.

Funding: Other U.S. Government Support

PUB114

Subendocardial Viability Ratio, Albuminuria and Pulse Wave Velocity in Chronic Kidney Disease Patients Robert Ekart,[1] Nina Hojs,[2] Sebastjan Bevc,[2] Radovan Hojs.[2] *[1]Clinic for Internal Medicine, Department of Dialysis, University Clinical Centre Maribor, Maribor, Slovenia; [2]Clinic for Internal Medicine, Department of Nephrology, University Clinical Centre Maribor, Maribor, Slovenia.*

Background: Applanation tonometry can be used for pulse wave analysis (PWA) and pulse wave velocity (PWV) measurements. PWA provides information on subendocardial viability ratio (SEVR) or the Buckberg index, which is a non-invasive estimate of subendocardial perfusion. Albuminuria and chronic kidney disease (CKD) are associated with increased risk of cardiovascular disease. The purpose of our study was to evaluate the relationship between SEVR and albuminuria in CKD patients.

Methods: PWA and carotid-femoral PWV were performed (SphygmoCor, AtCor Medical, Australia) to determine SEVR and aortic stiffness in 74 patients with CKD (52 men, 22 women; age 60.7±12.6 years, range 22-88 years). Eighteen (24.3%) patients were diabetics. Albuminuria was determined with the urinary albumin/creatinine ratio (UACR), values were log-transformed. Standard laboratory analyses (cystatin C, cholesterol, triglycerides, hs-CRP) and 24-hour ambulatory blood pressure measurements (ABPM) (Schiller BR-102 plus, Switzerland) were performed.

Results: SEVR was 152.1±33.3 % (82-235 %), PWV was 11.7±3.5 m/s (5.95-23.03 m/s), UACR was 823.1±1264.4 mg/g and mean 24-hour systolic/diastolic ABPM was 137/77 mmHg. SEVR correlated with UACR (r=-0.331; p<0.004) and PWV (r=-0.349; p<0.002), but not with cystatin C, cholesterol, triglycerides, hs-CRP and 24-hour systolic/diastolic ABPM. Multiple regression analysis showed statistically significant association of SEVR with UACR (p<0.03) and PWV (p<0.002).

Conclusions: According to our data SEVR was associated with albuminuria and carotid-femoral PWV in CKD patients.

PUB115

Chronic Kidney Disease Is Associated with Poor Outcomes in Veterans with Peripheral Vascular Disease Jose Jesus Perez, Charles G. Minard, Ojas A. Naik, Venkataraman Ramanathan. *Division of Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Peripheral vascular disease (PVD) and chronic kidney disease (CKD) coexist and share common risk factors including diabetes and hypertension. We studied the risk factors for PVD and the independent effect of CKD on patient outcomes and further vascular evaluation.

Methods: We retrospectively evaluated veterans who were referred for vascular study for symptomatic PVD. PVD was defined as: abnormal ankle-brachial index (0.7≥ABI≥1.3), abnormal digital pressure, or qualitative waveforms. CKD EPI calculation was used for GFR estimation. Veterans with a history of PVD were excluded. Primary end points included abnormal vascular study, further vascular evaluation after abnormal doppler, and a composite endpoint of ulcer, death, gangrene, rehospitalization, amputation, or osteomyelitis during one year of follow up. Logistic regression analysis was used to identify risk factors.

Results: 593 veterans had vascular evaluation during one year study period. Risk factors associated with abnormal vascular study include age >65 years (OR 2.1; 95%CI 1.4-3.2), smoking (OR 3.1; 95% CI 2.0-4.6), and African-American ethnicity (OR 1.7; 95% CI 1.1-2.6). In veterans with abnormal vascular studies, patients >65 years (OR 0.5, 95%CI 0.3-0.8, p 0.004) were less likely to have further studies, whereas smokers (OR 1.9, 95%CI 1.1-3.2, p 0.02) were more likely to get further work up. Other variables including history of diabetes, hypertension, hyperlipidemia, or ischemic heart disease did not influence the physician's decision for further tests. CKD did not have an independent effect on abnormal vascular study or further vascular evaluation after abnormal doppler exam. However, eGFR<60 was the only independent risk factor for reaching composite endpoint during follow up (OR 2.2, 95%CI 1.2-3.8, p 0.006).

Conclusions: In symptomatic veterans, CKD is neither an independent risk factor for abnormal vascular study nor does it influence referral pattern for further evaluation after abnormal doppler exam. There is no diagnostic or therapeutic nihilism towards CKD veterans. However, eGFR <60 ml/min is associated with poor patient outcomes during one year follow up.

PUB116

Ratings of Executive Functioning in Children and Young Adults with Moderate to Severe CKD Stephen R. Hooper,[1] Jerilynn Radcliffe,[2] Divya Moodalbail,[2] Kathryn Reiser,[2] Abbas F. Jawad,[2] Susan L. Furth.[2] *[1]UNC School of Medicine; [2]CHOP.*

Background: Cognitive deficits in individuals with moderate to severe CKD have been well documented. To date, however, few studies have examined ratings of executive functioning in children and young adults with CKD. Such measurement approaches may provide a cost effective strategy for nephrologists working with this population.

Methods: Cross sectional observational study comprising 18 children and young adults with CKD (eGFR below 60 ml/min/1.73m2), ages 8 to 25 years, followed by nephrology, transplant, and dialysis clinics. Instruments included the Behavior Rating Inventory for Executive Functioning (BRIEF) completed by either the parent (ages 8 to 18) or the older adolescent/young adult (ages 19 to 25). Scores across the two versions of the BRIEF were combined for comparison purposes. Initial data analyses included descriptive findings of the CKD sample, with elevated ratings of at least one standard deviation above the normative mean reflecting impairment.

Results: Median age was 15 yrs (Interquartile Range = 14-17.5 yrs.) and median eGFR was 32 ml/min/1.73m2. The sample was 76% male and 81% Caucasian. About 29% had transplant, and 5% were on dialysis after a failed transplant. Results showed that none of the Behavior Regulation scales were significantly elevated when compared to normative standards, with the Behavioral Regulation Index (BRI) falling within the average range (BRI T-Score = 54.22). In contrast, the Metacognitive Index (MI) was significantly elevated (MI T-Score = 62.5), with specific elevations on the Working Memory, Plan/Organize, and Organization scales. The Global Executive Composite (GEC) also was significantly elevated (GEC T-Score = 60.34), reflecting significant concerns for the presence of overall executive dysfunction in individuals with moderate to severe CKD.

Conclusions: These findings indicated the presence of mild executive dysfunction, with weaknesses noted in the areas of planning, organization, and working memory. It will be important for nephrologists to be aware of the executive capabilities of their patients, especially with respect to their impact on adherence to medical care and ongoing medical management.

Funding: Government Support - Non-U.S.

PUB117

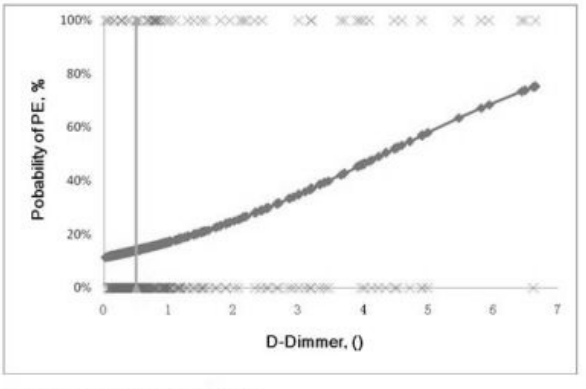
Risk Factors of Pulmonary Embolism in Patients with Nephrotic Syndrome, Results from a Large Retrospective Study Fude Zhou, Yihe Yang, Jicheng Lv, Min Chen, Ming Hui Zhao, Haiyan Wang. *Renal Division, Peking University First Hospital, Institute of Nephrology Peking University, Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China.*

Background: Nephrotic syndrome (NS) is associated with an increased risk for thromboembolic complications .Until now most studies focused on the renal vein thrombosis, few studies have ever evaluated risk of pulmonary thrombosis (PE) in nephrotic syndrome. In this study we assessed the risk of PE in a large, single-center, retrospective study and attempted to identify risk factors in these patients.

Methods: Patients with nephrotic syndrome who had received lung ventilation-perfusion scan between 2001 and 2011 from Peking University First Hospital were collected and analyzed. Multivariate Logistic regression was used to detect the independent risk factors of pulmonary embolism. Logistic regression was used to identify the threshold value of d-dimer.

Results: Among the 312 patients, 65 patients (20.8%) had pulmonary embolism. Elevated level of serum d-dimmer was an independent risk factor of pulmonary embolism on multivariate analysis. (OR= 1.543, 95%CI: 1.269-1.876). The cumulative probability of pulmonary embolism was in a linear association with the serum D-Dimmer level even within the normal range.

Figure 1:Logistic curve ofrisk of pulmonary embolism in nephrotic syndrome patients



Blue line: Logistic curve of probability of PE

Red cross: PE. 0% means the patient did suffered PE, 100% means the patient had suffered PE.

The Logistic curve was almost linear. The yielding points was not obvious.

Green Line: D-Dimmer =0.5.

While proteinuria at presentation and serum albumin which had been reported with deep vein thrombosis were not associated with pulmonary embosis (P>0.05).

Conclusions: In adult patients with nephrotic syndrome, high level of serum d-dimmer was closely associated with pulmonary embolism, while proteinuria or serum albumin was not.

PUB118

Prevalence of Anemia and Ferropenia in Patients with CKD Stages 3, 4 and 5 Not on Dialysis in Nephrology Units in Catalonia (The MICENAS I Study) Aleix Cases,[1] Alberto M. Martinez-Castelao,[2] Joan Fort,[3] Jordi Bonal,[4] Xavier Fulladosa,[2] J.M. Galceran,[5] J. V. Torregrossa,[1] Elisabeth Coll.[6] *[1]H. Clínic Barcelona, Spain; [2]H.Bellvitge, Spain; [3]H. Vall Hebron, Spain; [4]H. Germans Trias i Pujol, Spain; [5]Fundació Althaia, Spain; [6]Fundació Puigvert, Spain.*

Background: Anemia and ferropenia are common among CKD patients not on dialysis, but their prevalence and management in Catalonia are unknown.

Methods: Cross-sectional study conducted in nephrology units in Catalonia. Patients aged≥18 years, with CKD stages 3, 4 and 5 not on dialysis, with an Hb measurement in the previous 2 months who gave informed consent were recruited. Analysis criteria: Anemia Hb<13.5g/dL in men, Hb<12.0g/dL in women or patients receiving ESA therapy; Ferropenia:ferritin<100 ng/mL or TSAT<20%.

Results: 531 patients were recruited by 22 investigators, 504 were valid for analysis. 56.4% were men, mean age 7.8±15.5 years and mean time from CKD diagnosis 6.6±7.7 years. Regarding CKD Stage, 61.5% were in stage 3 (3a:23.0%, 3b:38.5%), 30.2% stage 4 and 8.3% stage 5. Mean Hb was 12.6±1.6g/dL, statistically significant differences were observed depending on CKD stage (Hb progressively decreased from 13.6±1.6g/dL in stage 3a to 11.4±1.2g/dL in stage 5,p<0.0001). Anemia prevalence was 58.5%, increasing in parallel with advancing CKD stages (3a:35.3%, 3b:52.1%, 4:73.7%, 5:97.6%). 53.5% of anemic patients were receiving treatment for it; 26.4% ESA and iron (in stage 5, 51.2% treated with both ESA and iron). 14.9% of the patients had Hb<11g/dL of whom 32.0% did not receive any treatment for anemia. 41.3% had Hb≥13g/dL, of whom 12.5% were receiving treatment for anemia (8.2% ESA). Ferropenia was found in 36.3% of anemic patients, but non statistically significant differences were observed among CKD stages. Among ferropenic patients, 36.4% did not receive any treatment for anemia, 35.5% received both iron and ESA and 17.8% only iron.

Conclusions: Anemia and ferropenia were frequent among CKD patients in stages 3, 4 and 5 not on dialysis attended in Catalonia nephrology units; the significant percentage of undertreated anemic patients found, suggests room for improvement in anemia management in this population.

Funding: Pharmaceutical Company Support - Roche Farma, S.A.

PUB119

Angiotensin Type1 Receptor Blockers Reduce Accumulation of Methylglyoxal in Chronic Kidney Disease Patients Takehiro Suzuki,[1] Daisuke Saigusa,[2] Yasutoshi Akiyama,[1] Eikan Mishima,[1] Yoichi Takeuchi,[1] Sadayoshi Ito,[1] Takaaki Abe.[1] *[1]Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan.*

Background: Methylglyoxal (MG) is a spontaneously formed metabolite of carbohydrates and the pathological roles of MG have been postulated in diabetes and chronic kidney disease (CKD). The plasma MG level is also associated with increased risk of cardiovascular disease (CVD). Hence, a method(s) for reducing methylglyoxal is recommended. Ameliorative effects of Angiotensin type1 receptor blocker (ARB) for CKD and CVD were reported. Renoprotective mechanisms beyond renin-angiotensin system (RAS) blockade by ARB were not fully elucidated. We estimated the renoprotective effects of ARB by assessment of MG changes in the ARB treatment on patients with CKD.

Methods: 33 patients with CKD stage 2 to 5 received ARBs (olmesartan 5-40 mg/day, telmisartan 40-80 mg/day, losartan 25-50 mg/day, irbesartan 50-150 mg/day). Serum creatinine (S-Cr), serum lipid profiles, fasting blood glucose (FBG), HbA1c, blood count and office blood pressure were measured after 6 months from ARB started. The plasma MG was measured by ElectroSpray Ionization Liquid Chromatography/Mass Spectrometry (ESI/LC/MS).

Results: S-Cr decreased and estimated GFR (eGFR) increased significantly after ARB treatment. Both systolic and diastolic blood pressures were significantly lowered after ARB treatment. T-chol, LDL-chol, HDL-chol, tryglyceride, FBG, HbA1c, red blood cell counts and Hb were not significantly changed. ESI/LC/MS revealed that MG was significantly decreased. The correlation between MG and eGFR was evaluated by Spearman's correlation coefficients. However, the decrease of MG was not correlated significantly with eGFR or Cr (r =0.37532).

Conclusions: After the ARB treatment, eGFR improved and BP was decreased. The plasma MG level was also significantly decreased. These results suggest that treatment of ARBs could be a novel pharmacologic strategy in CKD patients.

Funding: Government Support - Non-U.S.

PUB120

Association of the Metabolic Syndrome with Death, Cardiovascular Events or Progression to Dialysis Initiation in Non-Diabetic Chronic Kidney Disease
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Background: Metabolic syndrome (MetS) is an important risk factor for death, cardiovascular events (CVE) and chronic kidney disease (CKD) in the general population. However, the relationship between MetS with death, CVE and kidney disease progression in CKD patients is unclear.

Methods: We performed an analysis on 495 non-diabetic patients with severe kidney disease, not yet on dialysis, who participated in the Homocysteine in Kidney and End Stage Renal Disease study. MetS was defined according to recent guidelines from the National Cholesterol Education Program. The primary outcomes were all-cause death, CVE (defined as composite event of myocardial infarction, stroke or lower extremity amputation) and progression to dialysis initiation. Cox proportional hazard models were used to evaluate the association between MetS with death, CVE, and dialysis initiation adjusted for important confounders.

Results: Mean (SD) age was 69 ± 11 years, 25% were black and mean (SD) eGFR was 18 ± 7 ml/min/1.73m^2. A total of 292 (59%) patients in the cohort met criteria for MetS. Patients with MetS were more likely than those without MetS to have a history of cardiovascular disease (CVD) (33% vs. 18%, p=0.006). Over a mean follow-up of 3 years, there were 169 (34%) deaths, 70 (14%) CVE and 246 patients (50%) initiated chronic dialysis. After adjustment for demographics, smoking status, alcohol use, body mass index, history of CVD and hypertension, systolic blood pressure, eGFR, albumin, and 25-hydroxyvitamin D level, presence of MetS was not associated with an increased risk of death (HR 0.80, 95% CI 0.58 to 1.11), CVE (HR 1.08, 95% CI 0.64 to 1.84) or progression to dialysis initiation (HR 0.91, 95% CI 0.69 to 1.18).

Conclusions: The presence of MetS was not independently associated with an increased risk of death, CVE or progression to dialysis initiation in non-diabetic patients with severe CKD not yet on dialysis.

Funding: NIDDK Support

PUB121

Renal Lipodystrophy in Mild Chronic Kidney Disease Was Associated with Insulin Resistance and Ectopic Fat Accumulation
Hitoshi Minakuchi, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio University, Tokyo, Shinanomachi35, Shinjuku, Japan.*

Background: Chronic kidney disease (CKD) is complicated with metabolic disorder including insulin resistance and dyslipidemia even at the early stages. These clinical conditions suggested abrogation of insulin effects on insulin-target tissues. This study was undertaken to delineate the mechanism for metabolic dysregulation and the role of ADMA in mild CKD.

Methods: 5-week-old SD rats were rendered CKD by hemi-nephrectomy or five-sixth nephrectomy. Twenty weeks after the operation, biochemical parameters in insulin target tissues were examined. Both serum and tissue levels of ADMA were measured by ELISA system. Tissue oxidative stress and fatty acid levels were examined by TBARS and Folch methods, respectively. In in vitro analysis, cultured 3T3L1-fibroblasts were differentiated into mature adipocyte with or without ADMA to examine its effects on adipocyte differentiation.

Results: Serum levels of creatinine, fasting glucose and free fatty acid and urinary protein excretion were increased in CKD rats. Oral glucose tolerance test and insulin tolerance test revealed that impaired glucose tolerance and insulin resistance were evident in CKD rats. Insulin stimulation failed to activate the Akt in adipose tissues. Both the average size of adipocytes and the expression levels of differentiation markers for adipose tissue including PPARγ were decreased in CKD rats. Tissue levels of ADMA and oxidative stress levels were increased in adipose tissue of CKD rats. Expression of DDAH2 and DDAH1 were decreased in adipose tisseu of CKD rats. Finally, ectopic tissue fatty acid accumulation in liver and muscle were increased in CKD rats. In vitro analysis revealed that ADMA inhibited adipocyte differentiation, triglyceride accumulation and insulin signaling as assessed by the phosphorylation levels of Akt .

Conclusions: In mild CKD, increased tissue levels of oxidative stress and ADMA in adipose tissue blocked insulin signaling and adipocyte differentiation. The disordered renal maturation indicated lipodystrophy, which were associated with systemic insulin resistance, dyslipidemia and ectopic fat accumulation in mild CKD.

Funding: Government Support - Non-U.S.

PUB122

5-Aminolevulinic Acid Improves Erythropoietin Production in CKD
Sadamitsu Ichijo, Yasutoshi Akiyama, Takehiro Suzuki, Yoichi Takeuchi, Eikan Mishima, Sadayoshi Ito, Takaaki Abe. *Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University, Sendai, Miyagi, Japan.*

Background: In patients with chronic kidney disease (CKD), anemia is a clinical issue and the involvement of chronic inflammation has been postulated.

Recently this chronic inflammation, as well as uremic toxins, have been known to involve in reducing erythropoietin (Epo) production.

Moreover, it is reported that up-regulation of mitochondrial function may be one of the clues to restore Epo production.

5-aminolevulinic acid (5-ALA) is the first intermediate in the heme biosynthetic pathway and may have a potential to restore mitochondrial function. Here we examined the effect of 5-ALA on Epo production under inflammatory or uremic condition.

Methods: Human Epo-producing Hep3B cells were obtained from ATCC and cultured. Cells were cultured in inflammatory cytokineTNF-α (220U/ml) or a representative uremic toxin, indoxyl sulfate (IS, 0.3mM) with 5-ALA at the various concentrations under hypoxic condition (1% O2) for 24hr. Epo concentration in the medium was measured using ELISA kit. Epo and heme oxygenase-1 (HO-1) mRNA was measured by QT-PCR. Total heme (free heme and heme protein) was measured by fluorometric assay. The amount of HO-1 protein was also determined by Western blot.

Results: The Epo concentration in the culture medium was reduced by TNF-α and IS. Under the condition, 0.3μM and 1μM 5-ALA were significantly restored the reduction of Epo concentration. Although this effect of 5-ALA was also observed by 5-ALA alone, the simultaneous addition of ferrous fumarate (equal concentration) further potentiated the ALA effect. Under the condition, HO-1 protein was increased, suggesting the increase of free heme pool, although total amount of heme was not changed. Because 5-ALA have a potential to increase cytochrome C oxidase and may restore aerobic respiration, these data suggest that 5-ALA may involve the Epo production through the induction of certain heme proteins.

Conclusions: These data suggest that 5-ALA may have a potential to improve renal anemia by increasing Epo production under CKD condition. Further experiments should be necessary to elucidate the precise mechanism of 5-ALA.

PUB123

Hepcidin Is a Bioactive Marker for Iron Status in Non-Dialysis Chronic Kidney Disease Patients
Aya Eguchi, Ken Tsuchiya, Kosaku Nitta. *Tokyo Women's Medical University, Shinjyuku-ku, Tokyo, Japan.*

Background: Hepcidin (Hp), whose expression is stimulated by inflammation and by iron loading, is also the key mediator of renal anemia. However, since the Hp expression is affected by these different factors, its significance as iron status in CKD has been controversial issue.

Methods: Reliable measurement of serum Hp have been made possible by the ProteinChip system, we therefore investigated the iron status and Hp levels of non-dialysis CKD patients (stage IV, V) who had not received frequent erythropoiesis stimulating agent (ESA) or iron therapy.

In addition to the usual iron parameters, serum Hp levels were measured by SELDI-TOF MS-analysis, and IL-6was measured by enzyme immunoassay.

Results: The mean serum Hp level of the CKD patients (n=33) was 46.1±34.2ng/ml. Their Hp levels were significantly and positively correlated with their ferritin (P<0.001) and TSAT levels (P=0.01), and the low Hgb tended to be correlated with low Hp level (P=0.08). There were two distinct Hp levels in the CKD patients, low Hp levels group (mean Hp 19.7±13.4 ng/ml) and high Hp levels group (mean Hp 71.0±28.5 ng/ml), respectively. Serum ferritin was 68.7±45.3 ng/ml and TSAT was 25.8±10.1% in low Hp group, in contrast, 186.2±151.4 ng/ml and 36.4±11.6% in high Hp group. There was no difference of Hgb level between both groups, the dose of ESA administered was lower in low Hp group (P=0.03) and also low amount of ESA was necessarily to maintain the Hgb levels during the observational three months.

Conclusions: Since there has been no golden standard for iron status in advanced CKD patients, measurement of Hp level is one of the useful indicators of assessment of iron status to choose either ESA or iron regimen for maintaining the targeted Hgb levels. Especially, to manage an appropriate ferritin level with keeping Hp low, it is possible to reduce the prescribing dosage of the ESA.

PUB124

Effects of a Low Salt Diet on Glomeruli from Diabetic Apolipoprotein E Null Mice
Chris Tikellis,[1] Mark E. Cooper,[1] Merlin C. Thomas.[1] *[1]Diabetic Complications, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia.*

Background: It is recommended that individuals with diabetes restrict their dietary sodium intake to <65mmol/day. But while salt intake is correlated with blood pressure, it also partly determines the activation state of renin-angiotensin-system (RAS), a key mediator of diabetes-associated glomerulosclerosis.

Methods: *ApolipoproteinE* KO (*apoE* KO) mice were allocated for the induction of diabetes with streptozotocin or citrate-buffer (controls) and further randomized to isocaloric diets containing, 0.05% (low salt), 0.3% (normal salt), or 2.5% (high salt) sodium with or without the ACE inhibitor, perindopril. After 6 weeks of study, glomeruli were isolated and markers/mediators of renal fibrosis assessed using RT-PCR and immunohistochemistry.

Results: A 0.05% sodium (low salt) diet was associated with significantly increased glomerular fibrogenesis in diabetic *apoE* KO mice, and increased glomerular expression of fibronectin, TGFb, CTGF, MMP-2, collagen I, III, and IV. This was associated with activation of the systemic RAS and increased expression of the angiotensin II type 1 receptor (AT1R) in diabetic glomeruli of mice on a low salt diet. A similar induction of fibrogenic markers was observed in diabetic *ACE2/apoE* KO mice that had a constitutively active RAS. In contrast, a diet containing 2.5% sodium (high salt) suppressed glomerular fibrogenesis associated with suppression of the RAS, with an efficacy comparable to ACE inhibition.

Conclusions: Whilst blood pressure lowering is an important goal for the management of diabetes, off-target actions of a low sodium diet, such as activation of the RAS, may contribute to less favourable outcomes in diabetic glomeruli.

PUB125

Daily Activity Levels Are Associated with Fractures in Stage 3-5 Chronic Kidney Disease Sarah L. West,[1,2] Scott G. Thomas,[2] Sophie Jamal,[1,2] Charmaine E. Lok.[2,3] *[1]Women's College Hospital, Toronto, ON, Canada; [2]University of Toronto, Toronto, ON, Canada; [3]Toronto General Hospital, Toronto, ON, Canada.*

Background: Fractures are common in patients with chronic kidney disease (CKD). We determined if daily activity levels could discriminate fracture status in men and women with stages 3-5 CKD.

Methods: We measured daily activity by triaxial accelerometry (StayHealthy RT3) worn for 7 consecutive days on the hip in men and women with stages 3-5 CKD. Each minute of activity was categorized as sedentary (vector magnitude <100), light (vector magnitude between 100-1771), or moderate/vigorous (vector magnitude ≥1772). Logistic regression and area under receiver operating characteristic curves (AUROC) determined whether daily activity could discriminate among those with and without self-reported low trauma fractures since the age of 40/prevalent vertebral fractures. Analyses were performed using STATA and were adjusted for stage of CKD.

Results: Subjects (n=45; n=25 men) were primarily Caucasian (69%) with a mean age of 61±18 years and weight of 76±18 kg. The most common cause of CKD was diabetes (n=15, 33%), most subjects had stage 3 CKD (n=21, 47%), and 14 subjects (31%) had prevalent fractures. Subjects participated in a mean of 1117±113 min/day of sedentary activity, 315±107 min/day of light activity, and 8±11 min/day of moderate/vigorous activity. Compared to those without fracture, those with fracture participated in more sedentary activity (1094±119 vs. 1167±82 min/day, p=0.04) and fewer minutes of light activity (337±113 vs. 267±79 min/day, p=0.04). Increased amounts of sedentary activity and participating in less light activity were associated with fracture (Odds Ratio (OR):1.01, 95% Confidence Interval (CI):1.00-1.02; OR:0.99, 95% CI:0.98-0.99, respectively). As well, both sedentary (AUROC:0.72, 95% CI:0.56-0.87) and light activity (AUROC:0.71, 95% CI:0.55-0.87) were able to discriminate among fracture status.

Conclusions: In conclusion, in our study, patients with stage 3-5 CKD and prevalent fractures participate in less activity compared to those without fractures. Larger, longitudinal studies are needed to confirm these findings.

Funding: Government Support - Non-U.S.

PUB126

Daily Activity Levels Are Associated with Tests of Neuromuscular Function in Stage 3-5 Chronic Kidney Disease Sarah L. West,[1] Maryum Chaudhry,[2] Tanya D. Dahonick,[2] Charmaine E. Lok.[1,2] *[1]University of Toronto, Toronto, ON, Canada; [2]Toronto General Hospital, Toronto, ON, Canada.*

Background: Patients with chronic kidney disease (CKD) report low energy with declines in kidney function which may affect their functional ability; however, the association between energy expenditure and functional tests in patients with stage 3-5 CKD has not been objectively measured.

Methods: In patients ≥18 years with stages 3-5 CKD (using NKF criteria by MDRD), we measured tests of neuromuscular function (NMT: timed up and go [TUG] & 6 minute walk [6MW]) and daily energy expenditure by triaxial accelerometry (StayHealthy RT3) worn for 7 consecutive days on the hip, and categorized activity as sedentary, light, or moderate/vigorous. Statistics were performed using SAS.

Results: Overall, 59 men and 44 women completed NMT/accelerometry assessments, with a mean age of 64 years. The primary cause of CKD was diabetes (25%) and the mean eGFR was 26±15 ml/min/1.73m^2. On average, subjects completed the TUG in 12±4 seconds, and walked 331±111 meters in 6 minutes. Subjects expended 397±263 kcal/day performing daily activities and were sedentary:1145±114 min/day were spent sedentary, while 288±114 and 7±10 min/day were spent completing light and moderate/vigorous activity, respectively. The TUG was negatively correlated with the number of kcal expended/day (r=-0.385, p<0.0001), the amount of light & moderate/vigorous activity/day (r=-0.471, p<0.0001;r=-0.331, p=0.0006), and was positively correlated with the amount of sedentary activity/day (r=0.504, p<0.0001). The 6MW was positively correlated with the number of kcal expended/day (r=0.293, p=0.004), the amount of light & moderate/vigorous activity/day (r=0.342, p=0.0007;r=0.372, p=0.0002) and was negatively correlated with sedentary activity (r=-0.392, p<0.0001).

Conclusions: Low daily energy expenditure (i.e. sedentary activity and reduced light and moderate/vigorous activity) is associated with impaired performance on the TUG/6MW. Since poor performance on NMT is associated with adverse clinical outcomes, future studies should evaluate whether improvements in daily activity energy expenditure translates into improved NMT and outcomes in patients with stage 3-5 CKD.

PUB127

Fetuin A: A Relationships with Inflammation and Secondary Hyperparathyroidism in Patients with End Stage Renal Disease Marek Kuzniewski,[1] Danuta Fedak,[2] Pawlica Dorota,[2] Maria Kapusta,[2] Wladyslaw Sulowicz.[1] *[1]Department of Nephrology, Jagiellonian University, Krakow, Poland; [2]Department of Clinical Biochemistry, Jagiellonian University, Krakow, Poland.*

Background: Fetuin A is a negative acute phase protein, which is produced constitutive by liver and negatively regulated by proinflammatory cytokines. Epidemiological studies have shown that fetuin A can act as systemic inhibitor of vascular calcifications in hemodialysed (HD) patients. Due to the fact that Chronic Kidney Disease (CKD) is characterized by Mineral and Bone Disorder syndrome, the aim of this study was to evaluate relationship between fetuin A concentrations in blood and calcification scores (CaSc) in the context of altered bone metabolism and diminished bone mineral content (BMD) caused by secondary hyperparathyroidism in patients with CKD.

Methods: We enrolled 71 hemodialysed patients, 31 women, 40 men, mean age 60 ± 12 year. C-reactive protein was determined nephelometrically, iPTH using Nichols method, Fetuin A measured by ELISA. CaSc was assessed by MSCT and BMD by DEXA.

Results: We observed statistically significant correlations between serum fetuin A concentrations and imaging techniques: log CaSc (r=-0.29; p=0.03), BMD femoral neck (r=0.26; p=0.04) and biochemical indices: log hsCRP (r=-0.31; p=0.02) and log iPTH (r=-0.31; p=0.02). After extracting the four groups: with iPTH ≤ 300 pg/mL, iPTH > 300 pg/ml, hsCRP ≤ 10 mg/L and hsCRP > 10 mg/L we have observed diminished fetuin A concentration in high PTH group in comparison do group with low PTH levels (p=0.03), and also in high hsCRP compared with low hsCRP group (p=0.01). The lowest fetuin A levels have the patients with high iPTH and high hsCRP levels.

Conclusions: Our results confirm that fetuin A may be influenced both by inflammatory state and by secondary hyperparathyroidism in hemodialysed patients. In those patients group low fetuin A levels may play a role in renal osteodystrophy, or renal osteodystrophy due to secondary hyperparathyroidism, may accelerate vascular calcifications via fetuin A bioavailability reduction.

Funding: Clinical Revenue Support

PUB128

Calciphylaxis: A Multimodal Care Concept Including Sodium Thiosulfate Improving Outcome Gernot Schilcher,[1] Werner Ribitsch,[1] Alexander R. Rosenkranz,[1] Kathrin Popodi,[1] Barbara Binder,[2] Joerg H. Horina.[1] *[1]Division of Nephrology, Medical University of Graz, Austria; [2]Department of Dermatology, Medical University of Graz, Austria.*

Background: Calciphylaxis, predominantly observed in patients with chronic kidney disease (CKD), is a rare but life threatening disease characterized by painful ischemic skin ulceration. Despite increasing incidence and high mortality rates of up to 80% no definite therapeutic regimen is well established. Aim of our present retrospective analysis was to evaluate a therapeutic approach based on a multimodal care concept including (a) medical interventions (sodium thiosulfate, cessation of coumarins, high dose vitamin K, cinacalcet, cessation of vitamin D and calcium containing phosphate binders, prophylactic antibiotics, probiotics, opioids, laxatives), (b) extended nursing (pain control, patient bedding, no subcutaneous injections in any suspicious area), (c) dermatological wound management, (d) dietary modifications (low phophorus and low calcium diet), (e) surgical interventions (parathyreoidectomy in case of primary hyperparathyreoidism) and, optionally, (d) modification of dialysis modality in CKD 5D patients.

Methods: We report on a case series of 4 adult patiens (2 with CKD stage 3 and CKD stage 5D, respectively). Retrospectively collected clinical, laboratory and medication data from patients treated between June 2009 and January 2012 were analyzed. We compared our single center experience and mortality rates before and after implementation of the multimodal care concept and furthermore to previously published outcome data.

Results: All 4 patients (100%) treated according to the multimodal concept showed a complete remission compared to a survival rate of only 50% before implementation of the new approach.

Conclusions: A multimodal therapeutic approach aimed at disease reversal might improve outcome in patients with calciphylaxis. Additional individualized therapies such as parathyreoidectomy in patients with primary hyperparathyreoidism should be considered.

PUB130

Treatment of Hyperuricemia in Chronic Kidney Disease (CKD) Patients May Improve Renal Function Subir K. Paul, Narasimha R. Boorgu, Rajesh Boorgu, Shejuti Paul. *Shoals Kidney & Hypertension Center, Florence, AL.*

Background: Recent studies have demonstrated an association between hyperuricemia and CKD progression, hypertension and cardiovascular disease. In CKD patients with mild hyperuricemia reduction of serum uric acid level (UA) has been shown to be modestly beneficial to preserve estimated glomerular filtration rate (eGFR). We found an inverse correlation between UA and eGFR. (r=-0.37) and a positive correlation between UA and serum Creatinine (CR) (r=0.42). This study was undertaken to evaluate the effects of allopurinol on renal function in CKD patients with moderate to severe hyperuricemia.

Methods: 26 patients with mean age of 61.7 were studied. 19 were Caucasians. 7 were African-Americans. 18 were female. 8 were male. Serum UA, CR, and eGFR were obtained 6 to 12 weeks prior to initiation of therapy, at baseline, at three months and at six months subsequent to start of therapy. Allopurinol was given with dose of 100 to 200 mg daily. No lifestyle modification was initiated and no medications were changed. Data was evaluated by student's paired t-test and linear regression.

Results: During pre-treatment phase, serum UA increased from 9.18 mg/dL to 11 mg/dL (p<0.0001). With allopurinol therapy, Serium UA decreased to 6.9 mg/dL (p<0.0001) at 3 months and to 6.85 mg/dL (p<0.0001) at 6 months. In pretreatment phase serum CR increased from 1.82 mg/dL to 2.12 mg/dL (p<0.0001). With allopurinol therapy, serum CR decreased from 2.12mg/dL to 1.85 mg/dL (p<0.0001) at 3 months and to 1.78 mg/dL at 6 months (p<0.0001). In pretreatment phase estimated eGFR decreased from 40.50 mL/min/1.73 m^2 to 33.69 mL/min/1.73 m^2 (p<0.0001). With treatment eGFR improved from 33.69 mL/min/1.73m^2 to 39.12 mL/min/1.73 m^2 (p<0.0003) at 3 months and to 41.5 mL/min/1.73 m^2 (p<0.0001) at 6 months. All patients tolerated therapy well.

Conclusions: We conclude that in CKD patients correction with allopurinol therapy of moderate to severe hyperuricemia may improve serum CR and eGFR. Large, randomized, double blind, placebo-controlled studies are warranted.

PUB131

Uremic Disruption of Macrophage Cholesterol Transporter ATP-Binding Cassette A1 (ABCA1) Ryohei Kaseda,[1] T. Alp Ikizler,[2] Valentina Kon.[1] *[1]Pediatric Nephrology, Vanderbilt University, Nashville, TN; [2]Nephrology, Vanderbilt University, Nashville, TN.*

Background: Macrophage foam cell is central in atherogenesis. We previously showed that foam cell development in patients with end stage renal disease on hemodialysis (ESRD-HD) reflects reduced cellular cholesterol efflux due to impaired cholesterol acceptor functions of high density lipoprotein (HDL). In uninephrectomized mice, we linked the reduced cholesterol efflux to downregulation of macro-phage ABCA1. We now examine if uremia affects cellular cholesterol transporters.

Methods: Peripheral blood monocytes were isolated from patients with ESRD-HD (n=11) before and after dialysis and Control subjects with normal renal function (n=3). ABCA1, ABCG1 and scavenger receptor type I (SRBI) gene expression was measured by RT-PCR. Since the HDL particles normally stabilize ABCA1, we also examined the effect of HDL isolated from ESRD-HD and Control on gene and protein expression of this transporter in human macrophage-like THP-1 cells exposed to HDL of ESRD-HD or Control by RT-PCR and western blotting.

Results: Compared with Control, monocytes of ESRD-HD had reduced expression of ABCA1 (normalized mRNA, 1.27±0.23 vs 0.77±0.10, $p<0.05$) and SRBI (1.11±0.36 vs 0.45±0.03, $p<0.05$) but not ABCG1 (1.28±0.06 vs 1.00±0.18). This expression pattern was not affected by dialysis treatment. HDL isolated from ESRD-HD increased ABCA1 mRNA in THP-1 cells (HDL of Control: 1.01±0.02 vs HDL of ESRD-HD: 1.30±0.08, n=8 in each group, $p<0.05$), a response not paralleled by enhanced cellular protein levels which rather decreased by upon exposure to the uremic HDL (HDL of Control: 1.11±0.05 vs HDL of ESRD-HD: 0.97±0.08, n=13 and 11, respectively).

Conclusions: The results suggest that hemodialysis patients have monocytes with reduced cholesterol transporters reflecting divergent effects of uremic HDL on transcription and protein degradation of ABCA1 together an effect of the uremic milieu. The data suggest cholesterol transporters as a novel target to lessen CVD in this population.

Funding: NIDDK Support

PUB132

HEMOX: *In-Vitro* and *In-Vivo* Assessement of a Non-Invasive Optical Sensor for Monitoring Blood Volume Variations during Dialysis Elena Mancini,[3] Claudia Perazzini,[1] Andrea Visotti,[1] Irene Selle,[2] Stefano Severi,[1] Antonio Santoro.[3] *[1]HST-CIRI, University of Bologna, Italy; [2]Bellco Srl, Mirandola, Italy; [3]Nephrology, Dialysis and Hypertension, "S. Orsola-Malpighi" Hospital, Bologna, Italy.*

Background: Blood volume (BV) decrease is an accepted marker of cardiovascular refilling of patients undergoing dialysis. The aim of the present study was to evaluate HEMOX sensor's (Bellco s.r.l, Italy) performance in assessing hematocrit and RBV variations comparing it with the gold standard sensor Crit-Line (HemaMetrics,USA) both in *in-vitro* and *in-vivo*.

Methods: *In-vitro* tests were conducted in different operating conditions by changing blood pump flow, dialysis solution conductivity, initial hematocrit and ultrafiltration rate. 22 standard bicarbonate dialysis were carried out using 5 L of bovine blood, a Formula Therapy monitor (Bellco) and data was recorded simultaneously by HEMOX and Crit-Line. In addition, volume decrease observed in the scaled beaker containing the blood was considered. *In-vivo* study included 7 patients enrolled in Nephrology, Dialysis and Hypertension unit, S.Orsola-Malpighi Hospital Bologna. Every patient was monitored during 3 standard bicarbonate dialysis sessions. HEMOX and Crit-Line were used simultaneously and initial and final hematocrit were also measured by CBC in the hospital analysis Laboratory. All data were analyzed in MATLAB (MathWorks, Inc.).

Results: Comparison between HEMOX and Crit-Line was summarized by correlation coefficient (r_{RBV}, r_{Hct}), mean error and standard deviation for both RBV and hematocrit (e_{RBV}, e_{Hct}). *In-vitro*: $r_{RBV}=0.88$, $r_{Hct}=0.93$, $e_{RBV}=2.97\pm2.19$ ($p<0.05$), $e_{Hct}=1.77\pm1.02$ ($p<0.05$). *In-vivo*: $r_{RBV}=0.92$, $r_{Hct}=0.86$, $e_{RBV}=1.56\pm1.71$ ($p< 0.05$), $e_{Hct}=-1.18\pm1.64$ ($p< 0.05$). Comparison of HEMOX and Crit-Line with hematocrit Laboratory measures: $r_{LAB\text{-}HEMOX}=0.85$, $e_{LAB\text{-}HEMOX}=-1.99\pm1.79$ ($p<0.05$); $r_{LAB\text{-}CL}=0.85$, $e_{LAB\text{-}CL}=-1.76\pm1.89$ ($p<0.05$).

Conclusions: *In-vitro* and *in-vivo* studies and comparison with Laboratory results demonstrated that HEMOX and Crit-Line can be considered comparable. A good linearity between the sensors was found, together with amoderate variance of both sensors with respect to laboratory data and a systematic HEMOX underestimation.

PUB133

What Prompts Initiation of Renal Replacement Therapy in Older Patients? Helen Alston, Aine Burns. *Centre for Nephrology at University College London, Royal Free Hospital, London, United Kingdom.*

Background: Many patients start renal replacement therapy (RRT) to alleviate uremia. Symptoms often do not correlate with eGFR, and previous studies found the elderly and those with comorbidities have more uremic symptoms. Symptoms due to other chronic disease may be indistinguishable from uremia, leading to early RT starts but no resolution of symptoms. We conducted a baseline study to determine what proportion of patients start RRT solely to alleviate uremic symptoms, and to identify differences between older and younger populations.

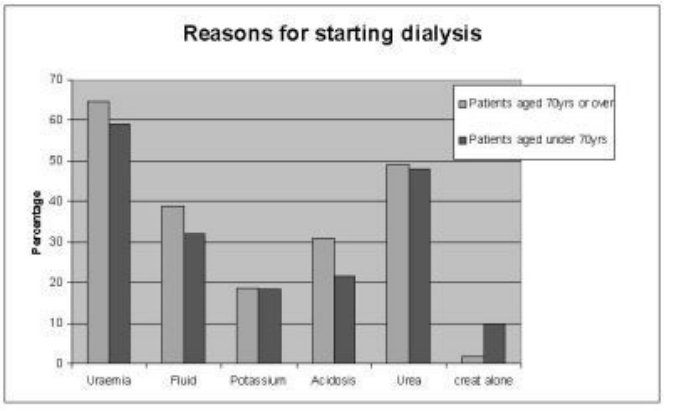
Methods: We reviewed the electronic patient record and blood results for all patients starting RRT in our unit Jan2009-Sept 2011 (n=254). We excluded failing transplants. We recorded age, gender, haemoglobin, eGFR, and Charlson comorbidity index. We coded the reasons for starting RRT. If multiple reasons were given, all were recorded. We noted unexpected RRT starts after acute deterioration in renal function (ie intercurrent illness).

Results: Our sample is characterised below:

Characteristics of our sample

	≥70yrs	<70yrs
Male	65.6%	65.1%
Mean Charlson comorbidity index	7.82	4.8
Diabetes	60.7%	52.6%
Ischaemic heart disease	49%	27.6%
Mean eGFR at initiation RRT	8.83	7.66
Mean Haemoglobin	10.26	9.91

Reasons for initiation of RRT are shown below:



There were also more unplanned starts in the older group (18.6% vs 13.8%). This was a higher incidence than expected.

Conclusions: Older dialysis patients form a distinct group, with higher comorbidities. They do not tolerate low eGFRs as well as younger patients, developing symptoms earlier. They are more likely to have diabetes and heart disease, and to have an unplanned start on RRT. It is vital to plan for deterioration in renal function even if renal function is currently stable. More work is needed to establish whether symptoms do in fact improve in this patients group following initiation of RRT.

PUB134

Immunogenicity of Investigational HEPLISAV Compared with Licensed Hepatitis B Vaccine (Engerix-B) in Patients with Chronic Kidney Disease (CKD) Robert Janssen, Sophia Rahman, Hamid Namini, William Heyward, Tyler Martin. *Dynavax Technologies Corporation.*

Background: Hemodialysis patients are at increased risk of hepatitis B virus infection. Current recommendations for CKD patients require 4 double-doses (2x20 mcg) of Engerix-B® (EB).

Methods: A multicenter, observer-blind, phase 3 study was conducted among 521 patients 18-75 years of age with CKD (GFR ≤ 45 mL/min/1.73 m^2) comparing 3 doses of HEPLISAV™ (H: 20 mcg rHBsAg+3000 mcg 1018 ISS Adjuvant, a toll-like receptor 9 agonist) given at 0, 1, and 6 months to 4 double-doses of EB (2x20 mcg rHBsAg+500 mcg alum) given at 0, 1, 2, and 6 months. Safety was evaluated for one year after the first study injection. Subjects were recruited at 69 sites in the US, Canada, and Germany and randomized 1:1 to receive H or EB. This represents the final immunogenicity and safety analyses.

Results: Among 507 subjects in the modified intent-to-treat population (H: 247; EB: 260), the mean age and gender distribution was similar in both groups. At Week 12, the seroprotection rate (SPR = anti-HBs ≥ 10 mIU/mL) in the H group (65.1%) was significantly higher than the SPR in the EB group (50.8%) with a difference in SPRs (H minus EB) of 14.3% (95% CI, 5.5%, 22.8%). At the primary endpoint at Week 28, the SPR in the H group (89.9%) was significantly higher than the SPR in the EB group (81.8%) with a difference in SPRs of 8.0% (95% CI, 1.7%, 14.3%). At Week 52, 28 weeks after the last study treatment, the SPR in the H group (84.3%) remained higher than the SPR in the EB group (77.3%) with a difference in SPRs of 7.1%. At Week 52, the percentage of subjects with anti-HBs ≥ 100 mIU/mL in the H group (66.8%) was significantly higher than in the EB group (49.1%) with a difference in the percentage of subjects with high antibody levels of 17.7%. At Week 52, the GMC in the H group (170.8 mIU/mL) was significantly higher than the GMC in the EB group (51.0 mIU/mL) with a ratio of GMCs of 3.40 (95% CI, 1.98, 5.84). The incidence of post-injection reactions and adverse events were similar in both groups.

Conclusions: In CKD patients, HEPLISAV induced significantly higher, earlier, and more durable seroprotection than Engerix-B and had a similar safety profile.

Funding: Pharmaceutical Company Support - Dynavax Technologies Corporation

PUB135

Restless Legs Syndrome in Children with Chronic Kidney Disease Sandeep K. Riar,[1] Roberta Leu,[2] Donald L. Bliwise,[3] Laurence A. Greenbaum.[1] *[1]Pediatrics, Division of Nephrology, Emory University and Children's Healthcare of Atlanta, Atlanta, GA; [2]Pediatrics, Division of Pulmonology, Allergy, Cystic Fibrosis and Sleep Medicine, Emory University and Children's Healthcare of Atlanta, Atlanta, GA; [3]Neurology, Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA.*

Background: Restless leg syndrome (RLS) is increased in adults with chronic kidney (CKD) and it is associated with increased morbidity and mortality. There is limited information on RLS in children with CKD.

Methods: This was a prospective, questionnaire-based, cross-sectional study that included patients 8-18 years old with CKD from a tertiary pediatric nephrology center. Healthy children 8-18 years old from two community pediatric practices were included as controls. The primary outcome of interest was Restless Legs Syndrome (RLS), which was assessed via a questionnaire, with exclusion of mimics. Patients were also assessed for sleep quality, use of sleep medications, daytime sleepiness and health related quality of life.

Results: RLS prevalence was more prevalent in children with CKD (n= 124) than in 85 normal children (15.3% versus 5.9%, p-value 0.0355). Among children with CKD, there was no significant association between RLS and gender, race, body mass index, estimated GFR, duration of CKD, etiology of CKD, percentage of life with CKD, stage of CKD, or current treatment modality (dialysis vs. transplant vs. non-dialysis, non-transplant). Children with RLS were older (14.8 years vs. 13.1 years; p=0.03). Children with RLS were more likely to rate their sleep quality as fairly bad or very bad; 41.2% vs. 8.8%; p=0.003) and were reported to be more likely to use sleep medications (42.1% vs. 14.7%; p=0.01). RLS was also associated with lower health related quality of life by parent report (p=0.027). 5/19 patients with CKD and RLS had discussed RLS symptoms with a healthcare provider and only 1 patient had been diagnosed with RLS prior to this study.

Conclusions: RLS is increased in children with CKD compared to normal controls and appears to be underdiagnosed. Systematic screening for RLS and sleep problems appears warranted in children with CKD.

Funding: Private Foundation Support

PUB136

Analysis of the Basal Timepoint in the Cohort of the Multicenter Prospective and Observational Study of Atheromatosis in CKD in Spain (NEFRONA Study): Data on Intima-Media Thickness Angels Betriu, Montserrat Martinez-alonso, Jose M. Valdivielso, Elvira Fernandez. *IRBLleida.*

Background: There are no previous prospective studies assessing the impact of CKD on atheromatosis development, stratified by gender and age. The NEFRONA study is a multicenter prospective observational study monitoring, in patients in CKD 3 to 5D, the changes in atherosclerosis, and their relationship with biochemical and genetic biomarkers.

In the present abstract we report the data on median (mm) intima-media thickness (IMT) in the baseline time point of the NEFRONA cohort (2455 patients without previous CV event, at different stages of CKD), compared with a control population of 559 individuals without CKD.

Methods: Data on IMT was obtained with carotid ultrasound by the same team and assessed by one reader in a blinded fashion. Images from 6 different carotid territories were obtained (bulb, common and internal carotid in both sides). In case the territory presented a plaque, the IMT value was truncated to 1.5mm. We analyze the trend according to degree of CKD stratified by age and sex using logistic regression analysis for the values of IMT. Moreover we also analyze the effect of sex stratified by degree of CKD and age group.

Results: 2455 patients were enrolled of which 61% are male, 25% diabetics and a population of 559 controls of which 53% are male and 11% diabetics. 98% Caucasian in both groups.

Age (y)	No CKD (n=559)		CKD3 (n=944)		CKD4-5 (n=821)		CKD 5D (n=690)		Trend p value along CKD stage	
	M	W	M	W	M	W	M	W	M	W
25-35	.600	.563	.580	.548	.538	.520	.565	.543	.176	.270
35-45	.648	.580	.640	.638	.583	.598	.660	.615	.592	.191
45-55	.795	.680	.745	.620	.670	.660	.735	.670	.018	1.00
55-65	.818	.733	.840	.740	.848	.740	1.06	.788	.028	.361
65-75	.895	.805	1.00	.880	1.12	.830	1.13	.970	.002	.186

* p<0.01 men vs women

Conclusions: IMT increases along with age in all stages and in both sexes, strongly supporting the reliability of the measurements. IMT is lower in women than in men at all stages of CKD and age. The median IMT only shows a significant tendency for progression through the stages of CKD in men older than 55 years. Prospective analysis of the study will identify the risk and protective factors for progression of atherosclerotic disease, distinguished by gender, age and stage.

Funding: Government Support - Non-U.S.

PUB137

Patients with Diabetic Kidney Disease Exhibit Endothelial Dysfunction Independent of CKD Stage Ulf Gunnar Bronas,[1] Marc L. Weber,[2] Mark E. Rosenberg,[3] Daniel Duprez.[2] *[1]Nursing, Univ of Minnesota, MN; [2]Medicine, Univ of Minnesota, MN; [3]Medicine, Univ of Minnesota, Mpls VAMC, MN.*

Background: Cardiovascular disease (CVD) is the leading cause of mortality in patients with chronic kidney disease due to type 2 diabetes (DKD). One of the initial mechanisms of the pathophysiology of atherosclerotic CVD involves endothelial dysfunction. The purpose of this study was to determine the relationship between CKD stage and indices of arterial function and structure in patients with stage 2-4 DKD.

Methods: Participants (n=91) with stage 2-4 DKD (62 male, age 64.1±9.2) underwent assessment of arterial function and structure. Endothelial function was studied by brachial artery flow mediated vasodilatation (FMD) using ultrasonography; endothelial- independent vasodilatation was assessed by sublingual nitroglycerine administration (NTG). Arterial stiffness was assessed by pulse wave velocity (PWV) and carotid incremental elastic modulus (cIEM). Arterial structure was examined by measuring the common carotid intima-media thickness (cIMT).

Results: There were no significant differences in demographics, medications, or medical variables between groups based on CKD stage. All groups exhibited impaired FMD (mean FMD=3.4%) compared to historical controls (FMD=8.9-10.5%). ANOVA did not indicate any significant differences between CKD stage in any of the indices of arterial function or structure. There were no significant associations observed between stage of CKD and indices of arterial function or structure.

Results

CKD Stage	FMD(%)	NTG(%)	cIEM(mm Hg)	PWV(m/s)	cIMT(mm)
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)
2 (n=24)	3.6(2.9)	9.7(5.0)	3287(1313)	8.1(1.6)	0.6(0.2)
3 (n=48)	3.4(3.3)	9.5(6.6)	3224(1186)	8.2(2.2)	0.7(0.3)
4 (n=19)	3.6(3.1)	10.2(3.9)	3572(1608)	8.5(1.6)	0.6(0.2)

Conclusions: Endothelial dysfunction occurs early in DKD, independent of CKD stage, and may contribute to the high incidence of CVD mortality in this population. Vascular smooth muscle function appears to be less affected in early DKD. This finding supports the need to initiate therapies to improve endothelial function in early stage DKD and to investigate interventions that target vascular dysfunction in this population.

Funding: NIDDK Support

PUB138

Role of Unphosphorylated STAT3 and HO-1 in Chronic Nicotine (NIC)-Induced Exacerbation of Renal Fibrosis Istvan Arany,[1] Dustin Reed,[1] Robert Kampen,[1,3] Christine Maric-Bilkan,[2] Luis A. Juncos.[2,3] *[1]Pediatrics, University of Mississippi Medical Center, Jackson, MS; [2]Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS; [3]Medicine/Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: There is a strong epidemiological association between smoking (via NIC) and accelerated progression of chronic kidney disease (CKD). Smoking increases plasma levels of TGFβ1 (a known mediator of renal fibrosis), hence NIC-induced alterations in TGF β1 signaling may augment tubulointerstitial fibrosis and contribute to progression of CKD. Because un-phosphorylated STAT3 (U-STAT3) and heme-Oxygenase-1 (HO-1) are important factors regulating pro-fibrotic signaling. In this study, we tested if these two factors play a role in the adverse effects induced by chronic NIC on renal fibrosis.

Methods: *In vivo*: A mouse model of unilateral ureteral obstruction (UUO) was employed to assess effect of chronic NIC exposure on interstitial fibrosis as well as expression of HO-1 and U-STAT3 in the kidney exposed to chronic NIC.

In vitro: NRK-49F cells were used to study the impact of chronic NIC on TGFβ1-mediated activation of renal interstitial fibroblasts.

Results: Chronic NIC augmented tubulointerstitial fibrosis in the obstructed kidney as evidenced by expression of αSMA and Masson trichrome staining. This exacerbation of UUO-induced tubulointerstitial fibrosis was associated with increased expression of U-STAT3 and decreased expression of HO-1. In vitro, chronic NIC increased TGFβ1-mediated activity of the αSMA promoter in a U-STAT3-dependent manner while attenuating activation of the HO-1 promoter. In addition, activation of endogenous HO-1 by CoPP greatly diminished the promoter activity of αSMA.

Conclusions: Our results suggest that chronic NIC augments pro-fibrotic signaling by enhancing promoter activity of the αSMA gene via increased expression of U-STAT3 and down-regulation of HO-1. Manipulation of these pathways may present therapeutic means to ameliorate the adverse effects of chronic NIC/smoking.

Funding: Other NIH Support - 5RO1DK073401-05S1 to Luis A. Juncos

PUB139

Effects of Tenofovir on CKD Progression Srujana Polsani,[1] Rakesh Malhotra,[1] Erian Shehu,[1] Anjali Acharya.[1] *[1]Jacobi Medical Center; [2]Albert Einstein College of Medicine, Bronx, NY.*

Background: Tenofovir is an effective first line agent in the treatment of human immunodeficiency virus infection. Tenofovir is known to cause proximal tubular injury and Faconi syndrome and is thought to have a low overall toxicity profile. Information on its effect on proteinuria decline in renal function and risk for chronic kidney disease (CKD) is limited. The aim of the study is to evaluate if tenofovir use is associated with increased risk of CKD, proteinuria, hypophosphatemia or faster decline in renal function in a inner city HIV population.

Methods: This is a retrospective chart review of patients with HIV followed in our institution. Information was collected from electronic medical records and/or personal interview with enrolled patients on demographic data, date of HIV diagnosis, HAART regimen, comorbidities, urinalysis, eGFR, serum phosphorus and rate renal function decline. CKD was defined as GFR <60 ml/min for > 3 months. Decline in renal function was defined as greater than 3ml/min in a year for 2 consecutive years. Hypophosphatemia was defined as serum phosphorus less than 3 mg/dl.

Results: Of the 200 patients enrolled 40 patients were excluded had insufficient laboratory data. 80.6% of patients were on tenofovir and 19.3% patients were on non tenofovir based regimen.

Table 1

	Tenofovir n=129 (80.6%)	Nontenofovir n=31(19.4%)
CKD3 and higher 3.2%(n=1)	6.9%(n=9)	3.2%(n=1)
Proteinuria	24%(n=31)	19.3%(n=6)
Hypophosphatemia	27.1%(n=35)	12.9%(n=4)
Renal function decline	7.5%(n=12)	0%(n=0)

Conclusions: Incidence of CKD in our patient population is similar to that shown in other studies.80% of patients were on Tenofovir. Tenofovir use was associated with a higher incidence of CKD, proteinuria and hypophosphatemia and faster rate of renal decline. These findings are being confirmed in a larger cohort. Limitation of the study is that only 20% of patients are on non tenofovir based regimen which may mask the difference between the groups. We recommend that patients on Tenofovir need to have estimations of GFR, UA, Urine albumin, protein, creatinine, urine and serum phosphorus on a regular basis.

PUB140

Hemoglobin Variation of Hospitalized Jehovah's Witness Patients with Chronic Kidney Disease Muhammad W. Khattak, Chioma O. Onyekwelu, Sandeep Aggarwal, Ziauddin Ahmed. *Department of Nephrology, Drexel School of Medicine, Philadelphia, PA.*

Background: Management of anemia and acute blood loss is challenging in Jehovah's Witness patients with different stages of CKD during hospitalization.. Primary prevention of anemia is important goal by using blood less protocol, and keeping adequate HG level by appropriate ESA and iron therapy. It is important to know the Hemoglobin (Hg) variation in clinical practice to formulate a standard policy to manage them during the hospitalization.

Methods: We reviewed the charts of 55 Jehovah's Witness patients with different stages of CKD admitted to our hospital for last two years. Mean age was 55 years, 35.7 % were Males, 64.3% females, 78.56 % African Americans and16.07 % were Caucasians and 5.36% hispanics. Thirty eight were CKD stages 5 on HD and 17 with different stages of CKD and not on HD.

The patients were covered under the blood less policy of the hospital where judicial blood drawn using small tubes was undertaken. The pre admission and discharge Hg were noted.

Paired t test was used to analyzed the variation of HG on admission and discharge.

Results: The mean Hg of patients with CKD stage 5 on HD on admission were 11.54 ±2.84 and on Discharge 11.36 ± 1.5 with p value of 0.026. Five out of 29 patients were within the goal or 10 gm and above on discharge. Ten patients or 26.3% were less than 10 gm of Hg on discharge.

The mean Hg of patient's with other stages of CKD who were not on HD was 10.2 ± 2.17. The discharge Hg was 9.35 ± 2.03 with p value of 0.21. Five out of 9 patients had Hg less than 10 or 55.5% on discharge.

Conclusions: The baseline Hg of CKD stage 5 were higher than the other stages of CKD may be due to routine use of ESA use in HD population. Despite use of blood less policy of the hospital the patients with CKD stage 5 on HD had significant decrease of Hg during hospitalization. This group needs a clear policy for primary prevention by maintenance of threshold HG level and evidence based aggressive treatment protocol with frequent ESA and Iron therapy for acute anemia during hospital course.

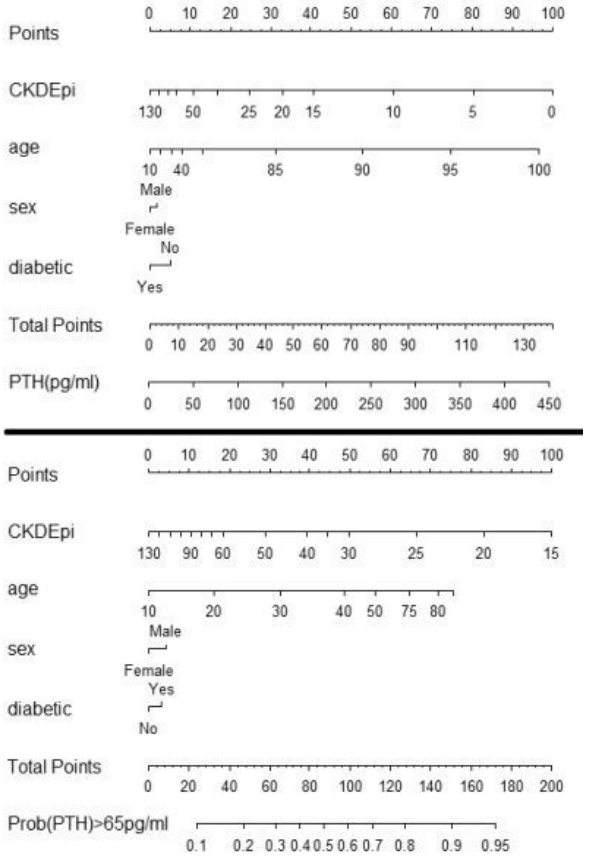
PUB141

An Equation to Estimate PTH Levels in CKD Patients Not Receiving VitD Receptor Activating Agents (ePTH) Kostas I. Sombolos,[1] John Boletis,[3] Dimitrios V. Vlahakos,[4] Kostas C. Siamopoulos,[5] Vassilios A. Vargemezis,[6] Pavlos Nikolaidis,[7] Christos E. Iatrou,[11] Eugene Daphnis,[10] Christos Argyropoulos,[9] Kostas Xynos.[8] *[1]Papanikolaou Hosp.; [2]Laiko Hosp.; [3]Attiko Hosp.; [4]Ioannina University Hosp.; [5]Alexandroupoli University Hosp.; [6]AHEPA Hosp., Thessaloniki, Greece; [7]Abbott Labs Hellas; [8]Abbott International; [9]Herakleion, University Hosp.; [10]Nikea Hospital, Athens.*

Background: Chronic Kidney Disease (CKD) complications are directly related to the level of renal function. Despite the availability of equations for eGFR, no tools exist for the prediction of CKD complications, e.g. Secondary Hyperparathyroidism(SHPT). We develop nomograms for estimating Parathyroid Hormone (ePTH) in CKD.

Methods: Age, sex, iPTH, diabetes(DM) and eGFR (CKD-Epi), from a cross-sectional epidemiology study in Greece, were used to develop spline models for PTH (as continuous and as> 65pg/ml i.e. the upper normal limit for the assay used). Model's discriminatory abilitity was assessed with R^2 (iPTH) & ROC (probability PTH>65 pg/ml) using the bootstrap(bst,2000 samples).

Results: A total of 516 pts who have never received therapy with vitamin D analogs were examined: 55%(women), 26%(diabetics); median(IQR) of: PTH 87.5(87.85), eGFR 34.5(25.6), distribution of patients in CKD stages: 1(6%),2(11%),3(43%),4(32%),5(9%) . Model performance for iPTH R^2: 0.22 & for PTH>65pg/ml ROC: 0.74. Corresponding nomograms are shown below:



Conclusions: It is possible to estimate PTH values (ePTH) based on demographics and eGFR. Nomograms and/or implementation of ePTH in smartphone applications, may reduce cost of CKD care by prioritize pts for SHPT screening (measured PTH) based on their likelihood of having an elevated ePTH. Addition of Ca and P values may improve model performance, but validation in other populations is required.

Funding: Pharmaceutical Company Support - Abbott Laboratories Hellas

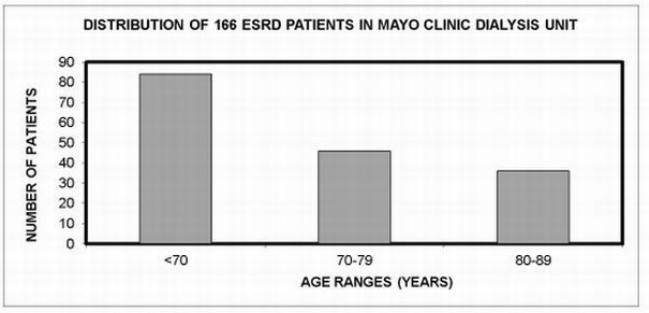
PUB142

Nondialytic Therapy (NDT) Is a Neglected Paradigm in US Nephrology Practice: It's Time for a Robust Reappraisal: This Is Not Rationing! Macaulay A. Onuigbo.[1,2] *[1]Medicine, Mayo Clinic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: Non-dialytic therapy (NDT) is a growing modality of treatment for ESRD for a certain group of CKD patients, in the UK and in Europe, but not in the US. Over 500,000 US ESRD patients are on RRT. In 20 years, the rate of ESRD patients >75 years starting dialysis has almost tripled from 550 to 1550 per million. Elderly CKD patients >70 years with multiple co-morbidities and who slowly progress to ESRD, when managed by multidisciplinary teams (MDT), showed median overall survival (life expectancy) of 1.95 years; 65% one-year survival, not that different with dialytic therapy. NDT led to possibly better quality of life with less hospitalizations. In a recent UK study, over 3 years, 60% of NDT patients, aged >70 years, had no admissions at all. We studied NDT or lack thereof, in a Mayo Clinic HD Unit.

Methods: The census of a NW Wisconsin Mayo Clinic dialysis population was analyzed on May 15, 2012. The second objective was to document any recent NDT activity.

Results: Of a total current ESRD population of 166, 82 (49%) patients were aged 70 years and over, with 46 aged 70-79 years and 36 aged 80-89 years (Fig). NDT was virtually nonexistent. These older ESRD patients experienced frequent hospitalizations and it is unclear if their quality of life would be better than with NDT in a majority of them who concurrently have major multiple co-morbidities.



Conclusions: Virtually one half of the ESRD population in this NW Wisconsin Mayo Clinic HD unit were >70 years. NDT is a neglected paradigm in this practice, as in most US nephrology practices. The place of NDT modality of care for US ESRD patients demands urgent attention and reappraisal. It should be seen for what it is – another alternative to dialytic therapy in a specific group of older ESRD patients. Furthermore, it is not tantamount to rationing!

PUB143

Estimation of GFR: A Comparison of Methods in a Large Caucasian Population Ludwig F. Merker,[1] Baptist Gallwitz,[2] Katja Schoene,[3] Barbara Waldeck.[3] *[1]Diabetes- und Nierenzentrum Dormagen, Dormagen, Germany; [2]Medizinische Klinik IV, Universität Tübingen, Tübingen, Germany; [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany.*

Background: There is only few data available from systematic comparisons between different methods of estimating GFR, so as Cockcroft-Gault (CG) vs MDRD vs CKD-EPI, in Caucasian people with type 2 diabetes (T2DM).

Methods: We recruited 2541 consecutive patients with T2DM from 245 primary care physicians in a prospective, randomized, cross-sectional epidemiological study according to GCP standard; pts. with CKD 5 were excluded. The following data was collected by questionnaire: antidiabetic and antihypertensive medication, renal status, hypertension and comorbidities. On site urinalysis was carried out by dipstick, the same for mircoalbuminuria (MAU). Central lab included HbA_{1c}, serum creatinine, albumin-creatinine-ratio (ACR) and lipid profile; eGFR (ml/min/1,73m^2) was estimated using MDRD formula, CKD-EPI formula and CG caculation. To ensure data quality 10 % of the study sites were monitored. The study was performed from 01-10/2011.

Results: Using the CG formula, 41.1 % had CKD 1, 35.9 % had CKD 2, 21.7 % had CKD 3 and 1.3 % had CKD 4. In contrast, estimating GFR by MDRD formula, 16.1 % had CKD 1, 54.7 % had CKD 2, 27.7 % had CKD 3 and 1.4 % had CKD 4. The correlation between MDRD estimation and CG formula overall was R=0.77, between CG and CKD-EPI slightly better with R=0.81 and MDRD and CKD-EPI showed quite similar results with an overall correlation of R=0.97. Regarding the BMI, the majority of patients with CKD 3 had a BMI of 20-35 kg/m^2. There was a positive correlation between the higher stadium of CKD and diabetes duration as well as the age of the study population and a negative correlation for BMI, independently of the gender and the methodology of eGFR calculation.

Conclusions: In Caucasian Germans and as previously shown by Levey et al., using the CG formula likely leads to overestimation of GFR, as MDRD formula seems to underestimate GFR: Using either CKD-EPI formula or MDRD gets to quite similar results. The correlation of renal impairment is positive for age and diabetes duration and negative for BMI for both genders.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany

PUB144

Thoracic Electrical Bioimpedance (TEB) in Cardiorenal Syndrome (CRS) Management: Relationship with Vectorial Bioimpedance (VBIA) in Chronic Kidney Disease (CKD) Patients Stage 2-5 Secundino Cigarran,[1] Juan Villa Rincon,[1] Emilio E. Gonzalez-parra,[2] Jesus Calvino.[3] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Fundación Jimenez Díaz, Madrid, Spain; [3]Nephrology, Hospital Lucus Augusti, Lugo, Spain.*

Background: CRS may occur by renal and/or cardiac dysfunction. CKD take part of CRS type 2 and 4. When both coexist overhydration is the rule. TEB is a non-invasive, validate and valuable method of evaluating cardiac haemodynamic. VBIA is used to assess body composition in CKD. The aim of this cross-sectional study is to assess the agreement between TBE&VBIA.

Methods: 92 pts. 33.7% female, 47.8% diabetic, 26.2% LVEF < 55%. Mean age 70.89 ± 10.06 yr. Mean GFR-EPI 38.3 ±18.4 ml/min. Heart evaluation was assessed by Echo &TEB (Modular Hotman System. Hemo sapiens INC, Bucarest,Romania) determinating Cardiac Index CI, Lef Systolic Work index,LSWI, Inotropic State index ISI, Systemic Vascular Resistance Index SVRI, Thoracic Fluid conductivity TFC & Eyection Phase Contractility Index EPCI). VBIA used was EFG (Akern Firenza Ita). Hydration status (ECW,ICW TBW), nutritional status (BCM%, muscle, lean body mass & basal metabolism) were determinated. Data were processed with SPSS18 (SPSS INC, Chicago IL, USA). A test P < 0.05 was considered significant.

Results: TFC positively corelated with Na-K exchange, TBW, and negatively with BCM%, reactance and resistance. EPCI positively with TBW and resistance and negatively with BCM%.

Bivariate correlation TEB & VBIA parameters

Variable	Na-K exchange	BCM (%)	TBW (%)	Reactance (Ω	Resistance Ω
Systolic Volume (ml/m^2	106 (NS)	-147 (NS)	300 (p<0.001)	147(NS)	188 (p<0.05)
SVR (F.Ω/m^2)	-162NS)	262 (p<0.05)	-164 (NS)	-039(NS)	-245(p<0.05)
LSWI (g.m/m^2)	064 (NS)	-062(pp<0.05)	325 (p<0.001)	147 (NS)	188 (NS)
EPCI (l/min)	138 (NS)	-244 (p<0.05)	230 (p<0.05)	104 (NS)	294 (p<0.05)
TFC (1/Ω	433 (p<0.001)	-259 (p<0.05)	580 (p<0.001)	-264 (p<0.05)	-235 (p<0.05)
LVFE (%)	-367 (p<0.001)	300 (p<0.05)	-266 (NS)	140(NS)	-063 (NS)

NS= No significance. Person (significance)

Conclusions: TBE parameters correlated with VBIA parametres.TBE &VBIA are valuable tools in CKD patients to assess fluid overload and hemodynamic heart function Futher studies are required to develop and confirm this findings.

Funding: Government Support - Non-U.S.

PUB145

Equations Based on Enzymatically-Measured Serum Creatinine Significantly Overestimate GFR in Chinese Children with Kidney Injuries Mengchun Gong,[1] Xuemei Li,[1] Ke Zheng,[1] Yan Qin,[1] Hongmei Song.[2] *[1]Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Pediatrics, Peking Union Medical College Hospital, Beijing, China.*

Background: There is no data about glomerular filtration rate (GFR), measured through plasma or renal clearance of the exogenous markers, in the Chinese children.

Methods: We determined GFR with plasma clearance of ^{99m}Tc-DTPA in 92 hospitalized children with renal injury and compared the performance of ten different equations, biochemically based on sCr (4), sCysC (3) or their combination (3), in estimating GFR, with statistical parameters of linear correlation and regression, precision and accuracy.

Results: The Schwartz1976, Schwartz2009 and CKiD equations, the most widely accepted tools for GFR estimation in children, significantly overestimate GFR (bias 29.2, 29.1, 22.1, respectively, all p<0.0001 in the paired t-test between eGFR and nGFR). Compared with the other eight equations, the Counahan equation and the Filler equation produced eGFR with better correlation with nGFR, stronger explanation capacity of variance in nGFR, smaller bias, higher intraclass correlation coefficients, higher ratio of eGFR within nGFR±10% and eGFR within nGFR±30% and higher ratio of correct CKD staging.

Estimation Performance of the Eleven Equations

Equations	Mean mL/min/1.73m2	Bias mL/min/1.73m2	γ¶	R-square†	ICC‡	Ratio within 10%	Right Staging
Schwartz1976	126.2	29.2*	0.733	0.54	0.70	15	67
Schwartz2009	128.8	29.1*	0.766	0.59	0.73	17	69
Leger	90.8	-6.2	0.588	0.35	0.59	17	53
Counahan	96.1	-3.2	0.748	0.56	0.73	25	70
Filler	99.6	2.8	0.796	0.63	0.78	35	69
Zappitelli-CysC	88.8	-14.3*	0.796	0.63	0.78	25	60
Grubb	110.6	13.0*	0.795	0.63	0.68	17	67
CKiD	119.3	22.1*	0.808	0.64	0.80	19	72
Bouvet	75.6	-21.5*	0.588	0.35	0.62	25	48
Zappitelli-combi	150.1	53*	0.807	0.65	0.68	12	71

* significant bias between eGFR and nGFR in paird t-test;¶ linear correlation factor; † linear regression factor; ‡ ICC: intraclass correlation factor.

Conclusions: No equation based on enzymatically measured sCr is validated to evaluate renal function in the Chinese CKD children, indicating an urgent need for the development of a new GFR estimating equation based on data from this population.

Funding: Government Support - Non-U.S.

PUB146

Pregnancy Outcomes in Chronic Kidney Disease Zichun Feng,[1] Charles G. Minard,[2] Rajeev Raghavan.[1,3] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Biostatistics, Baylor College of Medicine, Houston, TX; [3]Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Approximately 1 in 150 pregnant women have Chronic Kidney Disease (CKD). These women have a higher incidence of pre-eclampsia, preterm delivery, and adverse maternal / fetal outcomes. In pregnant women with severe CKD (eGFR<30ml/min), pregnancy outcomes and optimal time to initiate dialysis are unknown.

Methods: Ten pregnant women with severe CKD (not on dialysis) and 36 pregnant women with moderate CKD (eGFR 30-100 ml/min, proteinuria) were identified from chart review. Inclusion criteria were >20 weeks of gestation, baseline and interim serum creatinine values. All patients had follow-up and delivery at the same urban public hospital between 2000 and 2012. Comparison was also made to published data of pregnant women on hemodialysis. Statistical analysis was done with Fisher's exact test and nonparametric Kolmogorov-Smirnov test.

Results: Compared to women with moderate CKD, women with severe CKD have higher incidence of preterm delivery and reduction in eGFR during pregnancy (p<0.05).

Pregnancy Outcomes in CKD

Outcomes	Severe CKD	Moderate CKD	p-value
Preterm Delivery	90%	41%	0.01
Reduction in eGFR During Pregnancy	26.1%	0%	0.04
Preeclampsia	70%	63%	1.0
Intrauterine Growth Retardation	20%	6.3%	0.24

Both groups had similar rate of caesarean section and perinatal death (p=1.0). Adverse fetal outcomes in pregnant women with CKD are associated with: maternal hypertension, severe CKD, maternal proteinuria and pre-eclampsia (Odds Ratios= 1.33, 17.83, 1.73, 1.66, respectively). Diabetic nephropathy as the cause of CKD correlates with fewer adverse fetal outcomes, compared to other causes of CKD (Odds Ratio=0.22, 0.05-0.9). Finally, compared to published data in pregnant women on hemodialysis. This cohort of women with severe CKD (not on dialysis) have higher incidences of pre-eclampsia / eclampsia (12.5% vs 70%) and premature delivery.

Conclusions: In pregnancy, the severity of CKD correlates with a higher incidence of pre-term delivery, small for gestational age, and decline of maternal renal function. Pregnant women with severe CKD (eGFR < 30 ml/min) may benefit from pre-emptive initiation of dialysis.

PUB147

Serum Free Light Chains as Markers of Progression in Chronic Kidney Disease Hyun Chul Whang, Eun Sil Koh, Yu Ah Hong, Sungjin Chung, Cheol Whee Park, Yong-Soo Kim, Yoon-Sik Chang. *Department of Internal Medicine, The Catholic University of Korea College of Medicine, Seoul, Korea.*

Background: In patients with chronic kidney disease (CKD), as the glomerular filtration rate (GFR) reduces, the renal clearance of free light chains (FLC) decreases. The aim of this study was to investigate FLC as a marker for kidney damage and as predictors for progression of CKD.

Methods: Serum and urine FLC were assayed in 318 CKD patients and the control. Clinical and biochemical parameters including estimated GFR (CKD-EPI) were evaluated.

Results: There were no significant differences in age, gender, hs-CRP and LDH between the control and CKD group, however, serum kappa and lambda FLC levels were higher in the CKD group than in the control (p <0.01). In the univariate regression analysis, serum and urine kappa and lambda FLC, age and LDH level were associated with estimated GFR. In multivariate linear regression analysis, only serum kappa and lambda FLC were associated with eGFR (β=-1.435, p <0.01 for kappa and β=-1.227, p <0.01 for lambda, respectively). Furthermore, urine lambda was significantly associated with urine albumin after adjusting factors (β=1.63, p <0.01).

Conclusions: These data suggest that serum kappa and lambda FLC may be used as predictors for progressive renal dysfunction in patients with CKD, and urine lambda FLC might be a marker for the detection of kidney damage.

PUB148

Prevalence of Renal Dysfunction in HIV-Positive Patients in Rio de Janeiro Patricia Santiago,[1] Cynthia Cunha,[1] Sandra Wagner Cardoso,[1] Ruth Friedman,[1] Valdilea Veloso,[1] Beatriz Grinsztejn,[1] Jose H. Suassuna.[2] *[1]IPEC, FIOCRUZ, Rio de Janeiro, Brazil; [2]Nefrologia, UERJ, Rio de Janeiro, Brazil.*

Background: Widespread use of highly active antiretroviral therapy (HAART) has altered the course of HIV infection from a lethal disease to chronic medical condition. HIV-associated kidney disease changed likewise. We investigated the prevalence of kidney dysfunction and associated factors in people living with HIV in a country with free access to HAART.

Methods: This was a retrospective cross-sectional study of 2354 outpatients under follow-up in the calendar year 2008. Renal dysfunction was defined as a CKD-EPI eGFR of ≤ 60 ml/min. We collected demographic, clinical and laboratorial data.

Results: The cohort was mostly male (64%), white (67%) and young (80% under 50 yo). Median current CD4 count was 460 mm³ and median nadir CD4 was 189 mm³. Forty-two percent of the cohort had AIDS (CDC-1993) and 81% had a CD4 nadir < 350 cells/mm³. Nevertheless, 70% had undetectable viral load and 68% had ≥ 350 CD4+ cells/mm³ by the time of renal function assessment. Hypertension was the most common comorbidity (26%) followed by diabetes (9%). Six percent of the cohort was HCV+ and 3% had Hepatitis B. Only 17% of the cohort did not meet treatment criteria and were HAART-naïf. Fifty-two percent of patients started atazanavir or lopinavir and 33% still used one of them. Indinavir was previously used by 15% of the patients and 41% were exposed to tenofovir, of which 92% were current users. Median eGFR was 111.4 ml/min and 3.8% of the cohort had an eGFR ≤ 60 ml/min. Seven patients (0.4%) reached CKD stage V. Associated fators were age ≥ 50 years, ≤ 350 TCD4+ cells/mm3, diabetes, hypertension, past tenofovir use, past indinavir use, and current use of atazanavir or lopinavir.

Conclusions: Our cohort had advanced HIV infection but also free universal access to HAART. Age, comorbid degenerative diseases and advanced infection were detrimental to kidney health. Tenofovir, atazanavir and lopinavir showed potential nephrotoxicity. However the risk seems acceptable as, contrary to earlier series of untreated or under-treated patients, the point prevalence of kidney dysfunction was remarkably low.

Funding: Government Support - Non-U.S.

PUB149

Prevalence of Chronic Kidney Disease in High Risk Patients in a Primary Health-Care Petra Martínez, Laura Cortes-sanabria, Héctor R. Martínez Ramírez, Alfonso M. Cueto-Manzano. *Unidad de Investigación Médica en Enfermedades Renales, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

Background: End-stage renal disease has remarkably increased. The magnitude of chronic kidney disease (CKD) in Mexico, particularly at early stage, is not completely known.

Methods: Aim. To determine the prevalence of CKD in patients with type 2 diabetes mellitus (DM2) of a primary health-care setting. A CKD screening in DM2 patients was performed in 3 Family Medicine Units; subjects ³18 years without previous diagnosis of renal disease or transitory cause of proteinuria were included. All patients were subjected to a questionnaire, blood pressure measurement and anthropometry. Serum creatinine was measured, and glomerular filtration rate (eGFR,Simplified MDRD/formula) estimated. A dipstick urinalysis and albuminuria-specific dipstick were performed in a random urine sample, and confirmed.

Results: One thousand eight patients were included: 760 had normal results and 248 had CKD (classified according with K/DOQI). 93 (37%) patients had stage 1, 87 (35%) stage 2, 54 (22%) stage 3, 12 (5%) stage 4 and 2 (1%) stage 5. For analysis, patients of stage 4 and 5 were considered together. The characteristics of patients with CKD are shown.

Prevalence of CKD for Stages

Variable	Stage 1,n93	Stage 2,n87	Stage 3,n54	Stage 4/5,n14
Age (yrs)	58±12	66±9	70±8	66±13
Duration of DM2 (yrs)	12(7-16)	14(9-20)	19(13-22)	20(15-15)
Hypertension,n(%)	47(50)	65(75)	43(80)	11(77)
Systolic blood pressure (mmHg)	126±19	136±17	132±20	137±22
Diastolic blood pressure (mmHg)	78±10	82±10	78±10	85±7
Triglycerides (mg/dl)	165(118-260)	181(221-235)	202(137-274)	147(132-177)
LDL-Cholesterol (mg/dl)	117±31	111±31	115±35	118±32
Hb1Ac (%)	9.3±2.4	8.9±2.3	8.4±2.0	7.5±1.5
eGFR (ml/ min/1.73m2)	110(103-126)	75(68-80)	47(41-57)	24(18-27)
Albuminuria/ Creatinuria (mg/g)	121(53- 291)	120(57-260)	180(52-436)	894(195-2847)

Conclusions: The 25% had CKD. From the latter subjects, 72% had early (stages 1 and 2), 22% intermediate (stage 3) and 6% had advanced CKD (stages 4 and 5). Patients displayed poor metabolic control. It is important to evaluate CKD in high risk populations (such as diabetics) in order to implement early nephroprotective measures.

PUB150

Genetic Impact on Renal Function Decay in Radical and Partial Nephrectomy Maria Teresa Sciarrone Alibrandi,[1] Francesco Trevisani,[1] Simona Delli Carpini,[1] Marco Simonini,[1] Tommaso Camerota,[2] Lino Merlino,[1] Laura Zagato Villa,[1] Elena Brioni,[1] Roberto Bertini,[2] Francesco Montorsi,[2] Patrizio Rigatti,[2] Paolo Manunta.[1] *[1]OU Nephrology, San Raffaele Scientific Institute, Milan, Italy; [2]OU Urology, San Raffaele Scientific Institute, Milan, Italy.*

Background: Historically, renal cancer has been treated with radical nephrectomy (RN). Consequently, many patients developed renal failure and several health problems associated with it, such arterial Hypertension and cardiovascular diseases. Partial nephrectomy (PN) is increasingly being used to treat patients with solid renal lesions. Preservation of renal function is also a relevant clinical consideration. PN is associated with more favorable postoperative renal function outcomes relative to RN in the setting of small renal masses. On the other hand onset of hypertension and chronic renal failure after nephron loss is quite variable among single patients who undergo renal surgery. Aim was to compare RN and PN in renal function outcome according to several genetic polymorphisms involved in blood pressure control.

Methods: 134 patients younger than 80 years with renal cell carcinoma in absence of metastates, treated by RN (n = 65) or PN (n = 69) at Urology Unit of our Hospital between 2005-2012. ΔGFR has been evaluated in the follow up (mean 23.5 months). Statistical analysis has been performed by GLM adjusted for sex, age, blood pressure, comorbid conditions, therapy and previous renal function.

Results: At follow up patients who underwent RN present a significant loss of DGFR vs the ones treated with PN (-23.9 ml/min vs -5.7 ml/min P<0.05). According to genetic polymorphisms a strong correlation has been observed between SIK1 gene (salt inducible kinase 1) and SLC12A1 gene (Na/K/Cl transporters) and the outcome of renal function decay.

Conclusions: Both genes seem to play a role in worsening renal after renal surgery independently from RN or PN used.

PUB151

Is the Chronic Kidney Disease Epidemiology Equation Useful for the Estimation of Glomerular Filtration Rate in Chinese Patients with Chronic Kidney Disease? Xun Liu,[1] Chenggang Shi,[1] Linsheng Lv,[2] Hua Tang,[1] Cailian Cheng,[1] Tan-qi Lou.[1] *[1]Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China; [2]Operating Room, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China.*

Background: The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is most accurate for estimating glomerular filtration rate (GFR) in U.S. population but requires more evaluations in other racial as well as ethnic minorities.

Methods: From January 2005 through December 2010, 1180 Chinese chronic kidney disease (CKD) patients were enrolled. SC was measured by the enzymatic method. Mean sGFR measured by ^{99m}Tc-DTPA GFR estimation was 46.5±27.1 (2.73-137.6) ml/min/1.73 m^2. The patients' GFRs were estimated by Cockcroft-Gault-equation (CG), 6-variable MDRD equation (MDRD1), 4-variable MDRD equation (MDRD4) and CKD-EPI equation. eGFR measured by the CKD-EPI equation was chosen as the reference GFR.

Results: The medians of difference of the Cockcroft-Gault-equation and the 4-variable MDRD equation were significantly less than the CKD-EPI equation. The median percents of the absolute difference of the Cockcroft-Gault-equation, the 6-variable MDRD equation and the 4-variable MDRD equation were significantly less than the CKD-EPI equation. The 15 to 50% accuracy of the Cockcroft-Gault-equation were all significantly higher than the CKD-EPI equation. Detailed performances are listed in Table 1.

Overall performance of difference and accuracy between eGFR

	Median of difference	Median % Absolute difference	15% Accuracy	30% Accuracy	50% Accuracy
CG	-1.34*	27.33*	29.4*	54.1*	78.3*
MDRD1	-2.11	30.90*	26.5	48.4	73.1
MDRD4	-1.40*	30.87*	26.4	48.3	71.9
CKD-EPI	-1.72	31.23	25.5	48.8	71.6

*:P<0.05 compared with CKD-EPI-GFR.

Conclusions: When SC was measured by the enzymatic method, the performances of the CKD-EPI equation was disappointing. The Cockcroft-Gault equation may be more suitable in Chinese patients with CKD.

Funding: Government Support - Non-U.S.

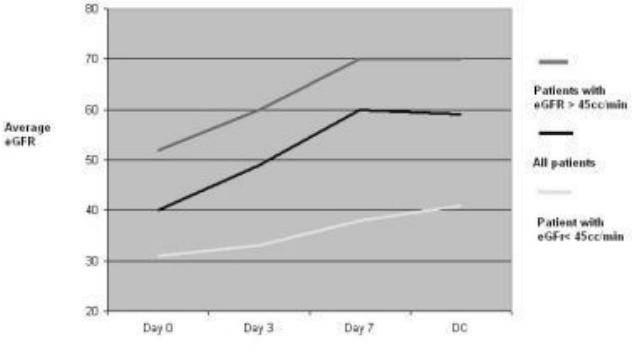
PUB152

Can Critically Ill CKD Patients Augment Their GFR? Krunal M. Patel, George N. Coritsidis, Marie France R. DeLeon, Nechama Diamond, Kasun I. Navarathna. *Elmhurst Hospital Center, New York, NY.*

Background: Critically ill patients, primarily young and with trauma, have demonstrated the ability to augment their GFR. Augmentation is dependent on various mechanisms with renal function being paramount. Heightened GFR may play an important role in pharmacodynamics and especially in antibiotic dosage.

Methods: We reviewed the records of 212 neurocritically ill patients admitted to the surgical/trauma ICU at Elmhurst Medical Center in New York City between 2009 and 2011. Patients with CKD-EPI GFR (eGFR) >60cc/min/1.75m^2; those that developed AKI or had initial AKI with recovery later in the course were excluded. Charts were reviewed for APACHE II, eGFR and hospital length of stay (LOS) were calculated.

Results: In the remaining 36 patients with CKD, eGFR increased by 36% (42±2 to 57±5c/min, p=.0483, ANOVA). Age was 71±3, 50% were trauma and LOS was 28±6 days. In patients presenting with eGFR between 45 and 60cc/min, a 35% increase in GFR was seen (52±1 to70±5, p=.0098, ANOVA). No significant increase in eGFR was seen in patients who presented with eGFR ≤45 (n=17, p=.446). There were no differences in LOS between the eGFR subgroups.



Conclusions: Critically ill CKD patients were able to significantly increase their eGFR if admission eGFR was at least 45cc/min. No increase was seen in those patients with admission eGFR≤ 45 cc/min.

PUB153

Gait Speed as a Predictor of Dialysis and Mortality among Elderly with Stage 5 Chronic Kidney Disease Sameena Z. Iqbal, Leora Birnbaum, Sidi Salami, Ahsan Alam, Murray L. Vasilevsky, Elham Rahme, Gary Inglis, Paul E. Barre, Chi-lan Tran. *Medicine, McGill University Health Centre, Montreal, QC, Canada.*

Background: Gait speed is a validated tool to measure function in both the elderly and among those with chronic kidney disease. It has been proposed to consider a timed gait test of 10 feet or 3 meters back and forth to assess frailty among the elderly. The objective was to assess the association of gait speed in older subjects with Stage 5 CKD with the composite outcome of the progression to dialysis or all-cause mortality.

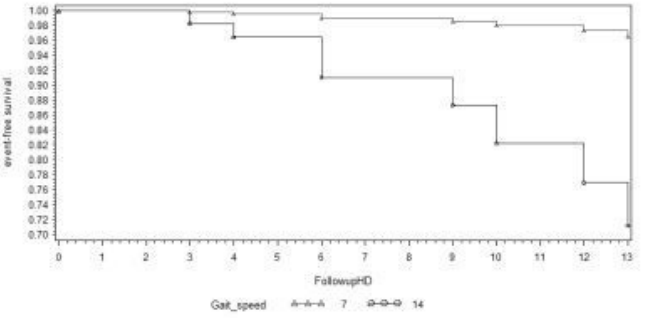
Methods: Between June 2010 and June 2012, we performed a prospective cohort study among individuals over the age of 65 with Stage 5 CKD at two tertiary care hospitals.

Results: In the study, 23 individuals were enrolled, 52% with diabetes type 2, 61% male and 13% African-American. The median age was 79 (65-87) years, median Charlson Comorbidity Score (CCS) was 5 (3-10), the baseline median eGFR was 13 (9-15) ml/min/1.73 m^2, and baseline median serum albumin was 37 (20-44) g/l. Upon enrollment, the median gait speed was 10.4 (6.0-21.6) seconds. During the two year observation period, there were 3 (13%) deaths and 9 (39%) dialysis starts. A Cox proportional hazard model was used to assess the association of gait speed with the composite outcome. The unadjusted and adjusted HRs are shown in table 1.

Cox Hazard model for composite outcome of dialysis start and mortality

Variable	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Gait speed (sec)	1.2 (1.01-1.4)	1.6 (1.04-2.4)
Serum Albumin (g/L)	0.9 (0.8-1.1)	0.8 (0.6-1.1)
baseline eGFR (ml/min/1.73m^2)	0.5 (0.4-0.8)	0.5 (0.3-0.8)
CCS	1.2 (0.9-1.8)	2.7 (1.1-6.6)

Event-free curves for gait speeds of 7 seconds compared to 14 seconds are shown in figure 1.



Conclusions: Gait speed may be a useful predictor of dialysis start and mortality in the elderly with stage 5 CKD, but a large prospective study is warranted.

PUB154

Laboratory Predictors of Kidney Disease in Pediatric Patients with SLE Sangeeta D. Sule,[1] Divya Moodalbail,[2] Julia R. Maisel,[2] Barbara A. Fivush,[1] Susan L. Furth.[2] *[1]Pediatrics, Johns Hopkins University, Baltimore, MD; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: Studies of adult patients with systemic lupus erythematosus (SLE) have shown an association between changes in complement and dsDNA levels with flares of overall disease. Renal specific outcomes have not consistently correlated with changes in these laboratory markers. Our goal was to determine if changes in C3, C4, and dsDNA levels were associated with renal disease in a longitudinal cohort of pediatric patients with SLE.

Methods: Longitudinal logistic regression models using generalized estimating equations to account for repeated measures were used to determine the association of C3, C4, and dsDNA titers with renal disease defined as proteinuria >3+ on urine dipstick or active urinary sediment defined as >5 rbc/hpf, >5 wbc/hpf or cellular casts. Patients were included in this analysis if they had at least 2 clinic visits within 6 months of each other. Patients were followed from time of entry into the cohort through Jan 31, 2012, the last date of follow-up.

Results: 47 children with SLE were followed in a retrospective cohort study. All patients had at least 2 longitudinal visits within 6 months. Patients were 90% female, 55% African American with a median age of diagnosis of 14 years. The median serum creatinine at initial presentation was 0.6 mg/dL, and 59% of these pts had dipstick proteinuria >1+. The median C3 level at presentation was 32 mg/dl; C4 was 12 mg/dl, both below normal values. A 1 mg/dl decrease in serum C3 between visits was associated with an increased risk of cellular casts (OR 1.5, p=0.003) and proteinuria (OR 1.1, p=0.02). There was no association with changes in C4 or dsDNA. Diffuse proliferative GN was associated with an increased dsDNA titer between visits, although not statistically significant (OR 2.2, p=0.07).

Conclusions: In this analysis, pediatric patients with SLE were more likely to have abnormal urinalysis if there were a decrease in serum C3 levels between visits. There was a trend towards increased dsDNA titers associated with diffuse proliferative GN. Abnormalities in SLE-specific labs over time can be predictive of renal disease.

Funding: Other NIH Support - NIAMS

PUB155

Distribution of Albuminuria and Low Glomerular Filtration Rate in a Surburb General Population in Beijng Yan Qin, Ke Zheng, Xiaohong Fan, Jianfang Cai, Xuemei Li. *Kidney Division, Peking Union Medical College Hospital, Beijing, China.*

Background: Chronic kidney disease (CKD) is becoming a major public health problem worldwide. The aim of this study was to screen for CKD among the general population of the surburb area of Beijing in China.

Methods: In an epidemiology study of the Pinggu District of Beijing, a total of 6287 participants aged over 18 years old were selected by systematic randomized cluster sampling. Participants were characterized by eGFR category as G1(>90), G2(60-89), G3a(45-59), G3b(30-44), G4 (29-15) and G5(<15 mL/)min/1.73 m^2. Albuminuria were characterized by ACR category as A1(ACR ≤30mg/g), A2(ACR 30-299mg/g), and A3(ACR≥300mg/g). The CKD-EPI eGFR equation was used for estimation of GFR.

Results: Albuminuria was present in 10.58% of the participants, but GFR less than 60 mL/min/1.73m^2 was found only in 0.76% of the study population. The prevalences are not higher than for other chronic diseases leading to an increased risk for cardiovascular disease mortality, such as hypertension (45.1%), diabetes (8.96%), metabolism syndrome (39.0%) and central obesity(52.7%) in the same population. When distributed to the CKD "heat map" generated by a composite ranking system of relative risks with the combinations of eGFR and ACR as the definition and classification of chronic kidney disease in KDIGO 2009 conference, the G1A1, G1A2 and G2A1stage was constituted by 81.53%, 8.45% and 7.37% of the population, respectively. While the participants in the higher risk stage for mortality or ESRD are summed up to only 0.27%. In multiple regression anlysis for this general population, ACR levels affected by total cholesterol level, serum uric acid level and intracellulerfluid which measured by Bioelectrical impedance analysis.

Conclusions: The combination of eGFR and ACR make clear the composite of CKD in general population and be helpful to make risk prediction. In this surburb general population, the higher prevalence of alubuminuria than low eGFR relatives with the high prevalence of metabolism disease.

Funding: Government Support - Non-U.S.

PUB156

Outcome of Patients Diagnosed with Granulomatous Interstitial Nephritis Ben Anthony Oliveira, Sapna Shah. *King's College Hospital, United Kingdom.*

Methods: This retrospective study reports on all patients presenting with a histological diagnosis of granulomatous interstitial nephritis (GIN) between 2000 and 2012 at our unit.

Results: 21 patients were identified. 38% were of Black ethnicity, 57% were male and the mean age was 59 years. 8 cases were associated with sarcoidosis with evidence of extra-renal disease and 5 with renal-limited sarcoid. 5 patients had tuberculosis (TB) infection, 1 case was related to medication and 2 were idiopathic. The mean duration of follow-up was 46 months. Those with sarcoidosis presented with a mean eGFR of 23 mls/min, minimal proteinuria (0.4g/day) and serum angiotensin-converting-enzyme (ACE) of 106 mg/L. All were treated with prednisolone (mean dose 32 mg/day). Mean duration of treatment was 47 months. At 1 year, renal function and proteinuria had improved to 43 mls/min and 0.3g/24h respectively with a reduction in serum ACE to 32 mg/L. At the final clinic visit, renal function remained stable (mean eGFR 43 mls/min). Mean eGFR was 27 mls/min and proteinuria was 0.7g/day at presentation for those with TB infection. Treatment of TB infection in 3 patients was delayed by a mean of 29 months as TB cultures were negative. Development of extra-renal TB infection led to the initiation of treatment. However, response was disappointing as 2 patients developed end stage kidney disease and the remaining 3 patients had a mean eGFR of 24 mls/min at 1 year and 30 mls/min at last clinic visit. The idiopathic and medication related cases of GIN, presented with a mean eGFR of 46 mls/min and all commenced steroid therapy. During the first year, 1 patient died following an unexpected cardio-respiratory arrest. The mean eGFR at the last follow-up was 34 mls/min. In all groups, the degree of fibrosis on the renal biopsy did not correlate with outcome.

Conclusions: This study represents the largest cohort of patients with GIN in the UK and supports previous findings that patients with sarcoid have a favourable outcome with steroid treatment. Those with TB have an inferior prognosis perhaps due to delayed diagnosis. Efforts to expedite diagnosis along with a consideration of a trial of anti-TB therapy may be warranted in order to preserve renal function.

PUB157

Influence of High Blood Pressure on the Prevalence of Chronic Kidney Disease in Patients with Type 2 Diabetes Mellitus and Essential Hypertension Karla Gabriela Meza-torres, Laura Cortes-sanabria, Victor Omar Frías-navarro, Héctor R. Martínez Ramírez, Miguel Tapia Alanis, Erika Fabiola Gómez-garcía, Blanca Liliana Maldonado-ruiz, Alfonso M. Cueto-Manzano. *Unidad de Investigación Medica en Enfermedades Renales, IMSS, Guadalajara, Jalisco, Mexico.*

Background: Type 2 diabetes mellitus (DM2) and hypertension (HT) are leading causes of chronic kidney disease (CKD). Coincidence of metabolic and hemodynamic abnormalities results in higher frequency of cardiovascular and renal damage. However, there are scarce data about the prevalence of CKD in DM2 and essential HT.

Aim: To compare the prevalence of CKD of patients with DM2 (with and without HT) *vs* those with essential HT (without DM2).

Methods: Patients of 3 primary health-care units were randomly selected. Glomerular filtration rate was estimated (eGFR) from the simplified MDRD formula. Albumin/creatinine ratio was performed in a first morning urine sample. CKD was classified according to K/DOQI guidelines.

Results: 1480 patients were included. Main results are shown in the table.

Variable	DM2 (N 519)	DM2+HT (N 615)	HT (N 346)
Age (yrs)	57 ± 12	63 ± 10*	60 ± 11* †
Duration of DM2 (yrs)	8 (3-13)	10 (4-15)	-
Duration of HT (yrs)	-	7 (3-13)	8 (3-13)
Systolic BP (mmHg)	124 ± 18	137 ± 20*	135 ± 19*
Diastolic BP (mmHg)	74 ± 10	80 ± 11*	80 ± 10*
Glucose (mg/dL)	180 (122-229)	164 (113-202)*	100 (91-107)* †
Urine albumin/creatinine (mg/g)	61 (6-13)	86 (8-31)	9 (6-8)* †
Microalbuminuria, N (%)	66 (13)	110 (18)*	12 (3)* †
Macroalbuminuria, N (%)	17 (3)	44 (7)*	0* †
eGFR (mL/min/1.73m2)	95 (78-116)	84 (66-105)*	77 (67-91)* †
Presence of CKD, N (%)	113 (22)	208 (34)*	64 (18)†
Normal function	405 (78)	405 (66)*	281 (81) †
Stage 1	46 (9)	55 (9)	2 (1)* †
2	25 (5)	51 (8)*	7 (2)* †
3	33 (6)	72 (12)*	42 (12)*
4	8 (2)	29 (5)*	14 (4)*
5	-	1 (0.2)	-

*p<0.05 vs DM2; †p<0.05 vs DM2+HT

Conclusions: Prevalence of CKD was significantly higher in patients with DM2 with HT. Patients with HT (both diabetic or non-diabetic) had higher prevalence of intermediate-advanced CKD than non-hypertensive ones. It is important to perform CKD screening activities in DM, but it is also imperative to evaluate patients with HT, who are frequently relegated in this regard.

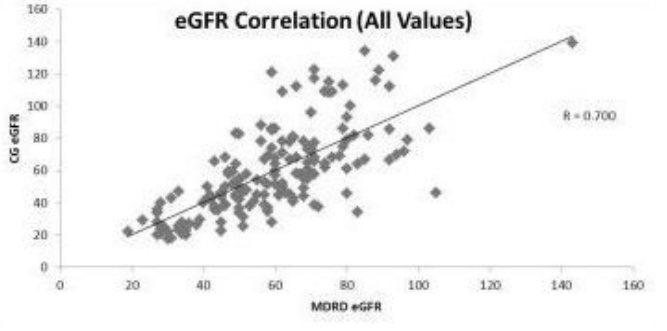
PUB158

Is There Sufficient Correlation or Difference in Using CG and MDRD Formulae to Calculate eGFR in ACS Patients Muhammad Ali Abdool, Hsu Pheen Chong, Mahvash Zaman, Matthew George Parry, Andrew Kuk, Bhavna Pandya. *Aintree University Hospital, Liverpool, United Kingdom.*

Background: In the UK and worldwide, there has been adoption of the 4 variable MDRD in reporting of eGFR and hence CKD staging. However, the cardiologists use CG in the calculation of CRUSADE bleeding risk scores. This can lead to potential confusion in using different formulae for GFR estimations, as well as clinical implications such as renal protection for peri-contrast procedures, ie. angiography and CT scans. Underestimation of eGFR is not as significant as overestimation as renal protection does little harm but can significantly minimise risk of Contrast Induced Nephropathy.

Methods: 166 patients admitted to Aintree University Hospital, Liverpool over a 5 month period (April - August 2012) with a diagnosis of NSTEMI had their notes analysed and eGFR calculated with the 2 formulae.

Results:



The number of patients with CKD 3 and above was 95 (57%) with the CG formula and 83 (50%) with the MDRD formula. There was adequate correlation between the calculated values for eGFR but when the data was subdivided into CKD stages, there was adequate correlation in only 86 (52%) patients.

When the 2 formulae are compared with each other with respect to moderate CKD, CG overestimates the eGFR in 3(4.2%) cases whereas MDRD overestimates in 17(23.3%) cases. This trend is similar in mild CKD with CG overestimating in 12(23.5%) cases compared to MDRD in 24(32.9%) cases. However, The trend is reversed for eGFR>90.

Conclusions: · About half of the population presenting with NSTEMI has moderate CKD or worse as calculated by both formulae

· There is good correlation between CG and MDRD absolute values (R=0.700, p<0.005) but this does not hold true when the data is categorised by CKD staging.

· With mild and moderate CKD, MDRD (43.2%) tends to overestimate compared to CG (18.1%)

· With eGFR>90 CG tends to overestimate compared to MDRD (85% vs 70%).

PUB159

Impacts of Serum Cystatin C and Creatinine on the Efficacy of Mild Renal Dysfunction in Patients with CKD Ke Zheng, Yan Qin, Xuemei Li. *Department of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: To investigate the impacts of serum creatinine(sCr) (including by Jaffe and enzymatic methods) and serum cystatin C(sCysC) within CKD patients in mild renal dysfunction, compared with dual-plasma-sample method (DPSM) to measure the glomerular filtration(GFR) with (99m)Tc Diethylene Triamine Pentaacetic Acid ((99m) Tc-DTPA) plasma clearance rate, to find the better predictor in the earlier stage of CKD.

Methods: nGFR of 300 patients with CKD were determined by dual-plasma-sample method (DPSM) to measure the glomerular filtration(GFR) with (99m)Tc Diethylene Triamine Pentaacetic Acid ((99m)Tc-DTPA) plasma clearance rate. Serum creatinine was detected with Jaffe and enzymatic methods. Serum cystatin C was detected by immunoturbidimetry simultaneously. To find out the optimal diagnostic value of sCr and sCysC at nGFR≤90ml/min/1.73m^2, Receiver Operating Curve(ROC) was used.

Results: The optimal cut off value of sCr(Jaffe)was 96 μmol/L, the optimal cut off value of sCr(enzymatic) was 88 μmol/L, the optimal cut off value of sCysC was 1.2mg/L. The value of area under curve(AUC) of sCr(Jaffe)was 0.892, the AUC value of sCr(enzymatic) was 0.878, the AUC value of sCysC was 0.924. There was significant difference of AUC value between sCysC and sCr(Jaffe and enzymatic)($p<0.05$).

Conclusions: Serum cystatin C is a better indicator than serum creatinine for predicting mild renal dysfunction in CKD patients.

Funding: Government Support - Non-U.S.

PUB160

Early Nephropathy Detection According to a New Classification of Chronic Kidney Disease Miguel Tapia Alanis, Victor Omar Frías-navarro, Erika Fabiola Gómez-garcía, Blanca Liliana Maldonado-ruiz, Laura Cortes-sanabria, Héctor R. Martínez Ramírez, Karla Gabriela Meza-torres, Alfonso M. Cueto-Manzano. *Unidad de Investigacion Medica en Enfermedades Renales, Instituo Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico.*

Background: A new classification of chronic kidney disease (CKD) was proposed by the Kidney Disease: Improving Global Outcomes (KDIGO) in 2011. The major point of revision of this classification was the introduction of a two-dimensional staging of the CKD according to the level of albuminuria in addition to the GFR level. Furthermore, the previous CKD stage 3 was subdivided into two stages (G3a and G3b). We estimated the prevalence of CKD in patients with type 2 diabetes mellitus and/or essential hypertension (HT) based on the new classification.

Methods: Three out of 24 primary health-care units were randomly selected; from these units, patients with DM2 or essential HT were randomly selected. Subjects with previous diagnosis of renal damage and secondary HT were excluded. Serum creatinine was measured and glomerular filtration rate (eGFR) estimated from the simplified MDRD formula. Albumin/creatinina ratio was performed in a first morning urine sample. CKD was classified according to KDIGO, 2011.

Results: One trousand four hundred eighty patiens were studied, (DM2 519; DM2+HT 615; HT 346) with mean age of 62 ±11 years, 936 (63%) women, median duration of DM 8 (3-15) and HT 7 (3-13) years respectively. The prevalence de CKD is shown in the table.

			Albuminuria stages, description and range (mg/g)				
			A1		A2	A3	
			Optimal and high-normal		High	Very high and nephrotic	
STAGE			<10%	10 - 29%	30 - 299%	300 - 1999%	>2000%
G1	HIGH	>105	18.2	5.1	3.1	0.8	0
	OPTIMAL	90 - 104	14	1.3	2.3	0.6	0.1
G2	MILD	75 - 89	16.6	3	2.3	0.5	0
		60 - 74	14.2	1.9	1.9	0.5	0
G3a	Mild moderate	45 - 59	5.1	1.1	2	0.8	0.1
G3b	Moderate severe	30 - 44	1.6	0.7	1	0.2	0.1
G4	Severe	15 - 29	0.2	0.1	0.2	0.3	0.1
G5	Kidney failure	<15	0	0	0.1	0	0

Conclusions: The prevalence global of CKD was 26%. Higher prevalence was observed on Stage G1 - A2, 6.5% of subjects had more risk of complications (G1-A3, G2-A3, G3a-A3, G3b-A2, G4-A1, G5-A1 stage).

PUB161

Ketoanalogue Supplementation Fails to Slow Decline of Renal Function in Chronic Kidney Disease Patients Li Ping Tan,[1] Anis Farhanah Abdul Rahim,[1] Chew Ming Wong,[1] Soo Kun Lim,[1] Yip-Boon Chong,[1] Kok Peng Ng,[1] Tee Chau Keng,[1] Wai Yew Kong,[1] Wan Ahmad Hafiz Wan Md Adnan,[1] Mun Hoe Wong,[1] Abdul Hafidz Muhammad Iqbal,[2] Li Han Lim,[1] Maisarah Jalalonmuhali.[1] *[1]Medicine, University of Malaya Medical Center, Kuala Lumpur, Wilayah Persekutuan, Malaysia; [2]Medicine, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.*

Background: Ketoanalogues have been shown to reduce patient morbidity, relieve uremic symptoms and improve nutritional status in chronic kidney disease (CKD). We aim to study whether ketoanalogue supplementation in combination with a low protein diet managed to slow CKD progression.

Methods: This was a retrospective review. CKD patients using ketoanalogues within the time period of 2009 to 2010 under follow up in our center were identified. Control patients were randomly selected from the same clinic. All patients were counselled by renal dieticians regarding the adoption of a low protein diet. Blood pressure, serum creatinine, serum potassium, serum albumin, serum calcium and serum phosphate levels were obtained at baseline and at every 3 months for a year. statistical analysis was done using SPSS (version 19.0).

Results: 62 patients were recruited. 31 patients in the ketoanalogue group and 31 in the control group. Mean age was 68.5±9.3 years. Majority were male (n=37, 59.7%). The predominant cause of CKD was diabetic nephropathy (n=33, 53.2%). Mean blood pressures were lower at baseline for the ketoanalogue group (139/74±21/13mmHg) compared to control (151/77±23/15mmHg). Baseline renal function was worse in the ketoanalogue group with mean serum creatinine at 291.9±85.6 μmol/L (eGFR 18.4±7.7 ml/min/1.73m2) compared to 213.4±62.7 μmol/L (eGFR 26.8±10.2ml/min/1.73m2)($p<0.05$). At the end of 12 months, serum creatinine in the ketoanalogue group rose to 308.9±105.1 μmol/L, p=0.116 (eGFR 17.7±8.1 ml/min/1.73m2, p=0.42) compared to the control group (serum creatinine 257.2±115.7 μmol/L, p=0.06; eGFR 24.3±13.2 ml/min/1.73m2, p=0.09).

Conclusions: We were not able to demonstrate that the addition of ketoanalogues in combination with a low protein diet was able to significantly slow the decline of renal function in a mixed cohort of patients with CKD.

PUB162

Quality of Reporting of Randomization Methodology in Nephrology Trials Steven Fishbane,[1] Azzour Hazzan,[1] Shayan Shirazian,[2] Ezra Israel,[1] Giovanni F.M. Strippoli.[3] *[1]Division of Nephrology, Hofstra North Shore-LIJ School of Medicine; [2]Division of Nephrology, Winthrop-University Hospital; [3]Consorzio Mario Negri Sud, Italy.*

Background: Inadequate randomization procedures and reporting detract from a trial's value. The purpose of this analysis was to determine the quality of reporting on randomization in nephrology trials.

Methods: Randomized Controlled Trials (RCT) from 7/1/2010 - 6/30/2011 identified from general medical and nephrology journals by Medline search and Table of Contents reviews were abstracted for four aspects of randomization reporting, 1) sequence generation, 2) type of randomization, 3) allocation concealment, and 4) randomization implementation. For two critical criteria, randomization type and method of concealment allocation, predictors of acceptable reporting and related outcome effect size were studied.

Results: Only 5/74 (6.8%) articles reported sufficiently on all 4 criteria. There was no reporting on: 1) sequence generation in 59.5%, 2) randomization type (39.2%), 3) method of allocation concealment (58.1%) and randomization methodology (86.5%) of publications. For two key criteria (randomization type and allocation concealment), reporting was adequate in 32.4% of articles. Significant predictors of acceptable reporting were multicenter vs. single center studies and primary endpoints of time to event or binary vs. continuous. When reporting on these criteria was inadequate, studies had significantly increased effect size for the primary outcome (0.34±0.16 vs. 0.16±0.09, $P<0.001$).

Conclusions: Nephrology studies often have inadequate reporting of randomization methodology. Authors and journal editors should work to adhere to reporting standards.

Randomization Characteristics

Sequence Generation:	n (%)
None Reported	44 (59.5)
Computerized	26 (36.5)
Other	4 (5.3)
Randomization Type:	
None Reported	29 (39.2)
Simple	9 (12.2)
Stratified	16 (21.6)
Permuted Block	8 (10.8)
Block and Stratified	12 (16.2)
Allocation Concealment:	
None Reported	43 (58.1)
Central	19 (25.7)
Pharmacy	4 (5.4)
Sequentially Numbered Opaque Sealed Envelopes (SNOSE)	8 (10.8)
Randomization Implementation:	
Not Reported	64 (86.5)
Yes	10 (13.5)

Funding: Clinical Revenue Support

PUB163

Summary of Chinese Literature Review on Iron Use for IDA in CKD Patients

Bao Liu,[1] Rong Hao,[2] Zhong Li,[3] Xiaoyu Nie,[4] Tang Mi,[1] Yue Gao,[3] Hongyu Yang,[1] Shanlian Hu.[1] *[1]School of Public Health, Fudan University, Shanghai, China; [2]Vifor Pharma, Glattbrugg, Switzerland; [3]Shanghai Centennial Scientific, Shanghai, China; [4]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.*

Background: Iron deficiency anemia (IDA) is very common in patients with chronic kidney disease (CKD). Intravenous (IV) and oral iron are used to treat IDA in CKD patients.

Methods: A comprehensive literature search was conducted in China for 2006-2010. The sources of publications were China Academic Journals Full-text Database, Chinese media and web reports. 163 articles were identified using key words including IDA, anemia, IV iron, and oral iron.

Results: 65 articles reported iron use for IDA in CKD patients : 59 in HD-CKD, of which 51 compared IV to oral iron and concluded that IV iron had greater efficacy and fewer AEs. The other 5 in PD-CKD (2 on high vs. low dose) and 1 in ND-CKD came to the same conclusion.

Iron therapies for IDA in CKD patients

IDA Population	Therapy	No. of Publications	Treatment Effects	Adverse Events (AEs)
HD-CKD	IV vs. Oral Iron	51	IV iron: better efficacy (85-90% vs. 40-70%); higher response rate at week 4; faster and greater increase in Hb level at week 8; reduction of rHuEPO	Oral iron: more AEs-GI intolerance; reduced patient compliance; higher discontinuation
HD-CKD	Iron Sucrose vs. Iron Dextran	8	Similar efficacy	Iron sucrose: less and milder AEs, most can be tolerated by patients
PD-CKD	IV vs. Oral Iron	2	IV iron: greater increase in Hb, Hct, SF, TSAT levels	Oral iron: GI intolerance, treatment discontinuation; IV iron: mild allergic reactions without treatment
PD-CKD	Iron Sucrose (Venofer) vs. Iron Dextran (Cosmofer)	1	Iron sucrose: faster increase in Hb to target level; shorter treatment	Iron Sucrose: less allergic reactions
ND-CKD	Iron Sucrose vs. Polysaccharide iron complex	1	IV iron: greater increase in Hb, Hct, SF, TSAT levels	Iron Sucrose: no AEs observed

HD=Hemodialysis; PD=Peritoneal dialysis; ND=Non-Dialysis; SF=Serum Ferritin; TSAT=Transferrin Saturation

Conclusions: Literature generally recommended IV iron as a better therapy than oral iron due to the improved efficacy and less AEs. IV iron was also reported to be more cost saving.

Funding: Pharmaceutical Company Support - Vifor Pharma Ltd.

PUB164

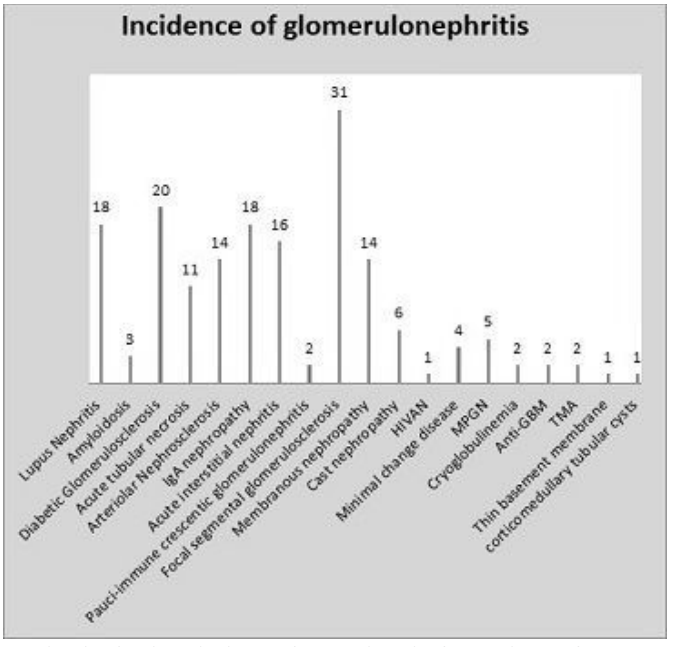
The Incidence of Biopsy-Proven Glomerulonephritis in New York City

Chadi Saifan, Rabih Nasr, Elie El-Charabaty, Suzanne E. El Sayegh, Morton J. Kleiner. *Nephrology, Staten Island University Hospital, Staten Island, NY.*

Background: In the U.S., there is limited epidemiological data that analyzed the trends of glomerular diseases, mostly because of the enormous differences in ethnicities among different locations. New York City is the most representative location of the nation because of the various people backgrounds. This is a descriptive analysis of biopsy-proven glomerulonephritis in NYC.

Methods: A retrospective review of the results of 168 pathology reports of all native renal biopsies performed in a university hospital of 714 beds from 2006 till 2011. Data was collected including age, sex, pathology report. Comparisons were made based on the incidence and the percentage of different age groups.

Results: The three most common glomerulonephritis (GN) are focal segmental glomerulosclerosis (19%), diabetic glomerulosclerosis (11.9%), and lupus nephritis (10.7%).



There is a female predominance of 59 % and a male of 41 %. The most frequent GN according to age groups are: [30-45]: 22%, [45-60]: 25%, and [60-75]: 22.6%.

Different categories of glomerulonephritis according to age-groups

	Age					
	0-15	15-30	30-45	45-60	60-75	75-90
LN	0	7	10	0	0	0
Amyloidosis	0	0	1	0	2	0
Diabetic GN	0	0	2	9	7	2
ATN	0	1	3	3	3	1
Arteriolar nephrosclerosis	0	0	2	7	2	0
IgA	1	2	6	7	2	0
AIN	1	0	0	1	4	1
Pauci-immune GN	0	0	0	1	0	1
FSGS	1	4	5	7	8	6
MN	0	1	3	2	5	3
Cast nephropathy	0	0	2	1	2	1
HIVAN	0	0	0	1	0	0
MCD	1	0	0	1	1	1
MPGN	2	0	0	2	1	0
Cryoglobulinemia	0	0	1	1	0	0
Anti-GBM	1	0	1	0	0	0
TMA	1	0	0	0	1	0
TBM	0	1	0	0	0	0
Corticomedullary tubular cysts	1	1	0	0	0	0

Total: 168 Kidney biposies. %: percentage

Conclusions: This descriptive review reflects the incidence of glomerulonephritis in a diverse population that can be extrapolated nationwide. The frequency of the different types of glomerulonephritis should be compared over time and across different countries to assess the trends of glomerular diseases, identify the risk factors and improve the treatment.

PUB165

Serum Phospholipase A_2 Receptor Autoantibodies (PLA_2R-AB) Predict Changes in Proteinuria in Patients with Membranous Nephropathy

Elion Hoxha,[1] Sigrid Harendza,[1] Ina E. Thiele,[1] Gunther Zahner,[1] Ulf Panzer,[1] Udo Helmchen,[2] Rolf A. Stahl.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Nierenregister, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: PLA_2R-AB are detected in up to 80% of patients with primary membranous nephropathy (MN). Antibody levels could reflect disease activity and may help to manage patients. In order to test whether this hypothesis applies, we performed a multicenter, open, prospective observational study in patients with biopsy proven MN.

Methods: In this study, 201 patients from 145 centers in Germany and Austria with biopsy proven MN were included. No immunosuppressive therapy was allowed before the recruitment. A specific immunofluorescence-test was applied for PLA_2R-AB measurement.

Results: The mean age of the patients (137 males, 64 females) was 54.6±16.3 years. The first PLA_2R-AB measurement was performed 0.94±1.25 months after the time of renal biopsy. In 144 patients (72%) PLA_2R-AB were present. PLA_2R-AB levels neither correlated with the levels of proteinuria nor with serum creatinine at the first measurement. 12-months prospective follow up data are available from 83 patients. 59 of them received immunosuppressive treatment. In 31 of 63 PLA_2R-AB positive patients antibodies were no longer detectable or were significantly reduced after 10.4±3.9 months (mean titer at inclusion 5276 vs. 16). Proteinuria significantly decreased in these patients from 9.9±5.0 g/24h to 3.5±3.9 g/24h at 12 months ($p<0.001$). Reduction of proteinuria significantly correlated with the decrease in PLA_2R-AB levels (Fisher's exact test $p=0.002$).In the remaining 32 PLA_2R-AB positive patients antibody levels did not decrease (inclusion 2471

vs. 4887 at 12 months). Only 12 patients in this group had a reduction of proteinuria, while in the remaining 20 patients proteinuria did not decrease (6.8 g/24h vs 6.7 g/24h). Twenty of the 83 patients were negative for PLA_2R-AB. In these patients proteinuria significantly decreased from 7.7±7.5 g/24h to 1.5±1.3 g/24h at 12 months (p=0.002).

Conclusions: PLA_2R-AB levels predict response or failure of therapy in patients with MN when proteinuria is used as clinical parameter.

PUB166

Predialysis Care and Optimal Hemodialysis Initiation Habib Mawad, Stephanie Raymond-Carrier, Stephan Troyanov, Alexandra Renald, Francois Madore, Josee Bouchard, Jean-Philippe Rioux. *Nephrology Division, Hôpital du Sacré-Coeur de Montréal, Canada.*

Background: A significant proportion of chronic kidney disease (CKD) patients known to nephrologists still undergo suboptimal initiation of renal replacement therapy (RRT) thus increasing morbidity and mortality. We aim to identify risk factors associated with suboptimal hemodialysis (HD) initiation among patients followed at a predialysis clinic.

Methods: We conducted a chart review retrospective study of patients starting HD from 2008 to 2011 in our predialysis clinic. Optimal start implied outpatient setting and the use of an arteriovenous fistula (AVF). Baseline characteristics and predialysis management of patients starting HD were compared on the basis of optimal vs suboptimal start.

Results: A total of 88 patients were included of whom 22 had optimal starts. Suboptimal start was due to inpatient status in 38 cases (32 with central venous catheter (CVC) and 6 with AVF). The remaining 28 patients started HD with a CVC as outpatients. The main characteristics between the two groups are summarized in Table 1.

Comparison of patients with optimal and suboptimal start

	Optimal start (n=22)	Suboptimal start (n=66)	p-value
Age (yrs)	62+/- 14	67 +/- 12	NS
Gender (% male)	68	45	NS
Diabetic nephropathy (%)	32	50	NS
≥ 2 comorbidities (%)	60	74	NS
Predialysis F/U (months)	25 +/- 16	16 +/- 14	0.03
Initial HD refusal (%)	9	29	0.06
Acute event first dialysis (%)	0	42	< 0.001
Vascular surgeon referral (%)	100	65	0.03
AVF creation (%)	100	26	< 0.001
Mortality one year post dialysis initiation (%)	10	20	NS

One year after initiating dialysis, 29% of patients starting with AVF had switched to CVC and 3% of those starting with CVC had switched to AVF.

Conclusions: This study identified modifiable risk factors associated with suboptimal HD start. Early referral to a predialysis clinic among patients with progressive CKD should be promoted. Evaluation by a vascular surgeon need to be encouraged in predialysis patients and in patients starting dialysis with a CVC. A multidisciplinary team should continue to follow patients after initiation of dialysis to increase the proportion of patients with an AVF and potentially improve their outcome.

PUB167

The NEFIGAN Trial: A Phase 2b Multicenter, Randomized, Placebo-Controlled Study to Evaluate the Efficacy and Safety of Two Doses of NEFECON® in Primary IgA Nephropathy Patients at Risk of Developing ESRD: Rationale and Study Design Bengt C. Fellstrom,[1] Rosanna Coppo,[2] John Feehally,[3] Jürgen Floege,[4] Alan G. Jardine,[5] Petri K. Koskinen,[6] Francesco Locatelli,[7] Bart Dirk Maes,[8] Alex Mercer,[9] Manuel Praga,[10] Vladimir Tesar.[11] *[1]Uppsala University Hospital; [2]University Hospital Regina Margherita; [3]University of Leicester; [4]RWTH University of Aachen; [5]University of Glasgow; [6]HUCH; [7]Ospedale A.Manzoni; [8]Heilig Hartziekenhuis; [9]Pharmalink AB; [10]Hospital 12 de Octubre; [11]Charles University.*

Background: NEFECON is a new oral enteric-coated capsule formulation for the treatment of IgAN patients at risk of developing ESRD. NEFECON is designed to release the potent glucocorticosteroid (GCS), budesonide, in the distal ileum and ascending colon where the highly immune-active Peyer's patches reside, providing topical immunosuppression of the intestinal mucosa. Local immunosuppression by NEFECON aims to reduce B-cell activation and proliferation, and the subsequent production of polymeric IgA1, thereby decreasing mesangial deposition. The high first pass metabolism of budesonide (~90%) ensures systemic GCS-related side effects are minimal or absent. An exploratory study on 16 patients showed 6-month treatment with NEFECON (8 mg/day) was well-tolerated and conferred a significant reduction in albuminuria.

Methods: In a randomized, multicenter study, 90 patients at risk of progressive IgAN despite maximized ACEI/ARB therapy to reduce blood pressure and proteinuria, will be treated with placebo or NEFECON (8 mg/day OR 16 mg/day) for 9 months and followed up for a further 3 months. Main eligibility criteria: Biopsy verified IgAN

≥18 years

UPCR ≥0.5 g/g

eGFR ≥45 mL/min/1.73m^2.

Results: The primary efficacy objective is to investigate whether patients on NEFECON achieve a larger mean reduction in UPCR compared with patients on placebo at 9 months. Secondary objectives will evaluate whether other urine protein response criteria and eGFR are in favour of NEFECON.

Conclusions: Results from the study will clarify whether NEFECON is an effective, safe and well-tolerated steroid formulation in reducing risk of IgAN patients developing ESRD.

PUB168

Renal and Patient Survival in More Frequent Biopsied Glomerulonephritis in Castilla la Mancha (Spain) between 1994 and 2008 GLOMANCHA Study Results Jose Luis Conde Olasagasti,[1] Carmen Vozmediano,[3] Luisa Illescas,[2] Acevedo M. Ribó,[1] Serafin Tallon,[4] Francisco Rivera.[3] *[1]Nephrology, Complejo Hospitalario, Toledo, Spain; [2]Nephrology, Complejo Hospitalario Universitario, Albacete, Spain; [3]Nephrology, Hospital General Universitario, Ciudad Real, Spain; [4]Nephrology, Hospital Universitario, Guadalajara, Spain.*

Background: In 2009 a collaborative multihospitalary task-force was established in CLM to know and compare the clinical outcome of the more frequent biopsied GN between 1994 and 2008 followed up to mid 2011.

Methods: We included 765 adults with proven selected GN in four hospitals covering an 1.8 million population area:157 focal and segmental glomerulosclerosis (FSGS),166 membranous nephropathy (MN),170 IgA nephropathy (IgAN),147 lupus nephritis (LN) and 125 crescentic type III glomerulonephritis(CGNIII).The median follow-up was 6,89 years (range 0.01-17,5).

Renal and patient outcome were assesed by survival analysis methods (Kaplan-Meier) comparing crude survival between diagnostic categories (Log rank) and analyzing adjusted influence of age, sex,GN type,initial eGFR(MDRD) and proteinuria (Cox regression).End Stage Renal Disease (ESRD) means dialysis or renal transplant.

Results: Cumulative (%±SE) renal survival (alive and free of ESRD) after 5 and 10 years was:74±4 and 55±5 in FSGS; 83±3 and 75±4 in MN;75±4 and 64±5 in IgAN;94±2 and 91±3 in LN; 55±5 and 45±6 in CGNIII.Log rank test showed significant difference ($p<0.01$) between categories except IgAN vs FSGS. Cox regression shows independent predictive influence of age,sex, initial eGFR and proteinuria,and some histological diagnosis (LN better vs reference category)

Cumulative (%±SE) patient survival (alive in or out of ESRD) after 5 and 10 years was : 96±2-86±4 in FSGS; 91±3 - 83±4 in MN; 95±2 - 92±3 in IgAN; 96±2-93±3 in LN and 69±5-57±6 in CGNIII.Log rank test showed CGNIII as the worse (significant difference vs all categories) followed by MN, being LN the best prognostic diagnosis.After Cox regression adjustement, only age, sex and initial eGFR show independent predictive influence.

Conclusions: The observed figures are not different from those reported in recent medical literature.

Funding: Government Support - Non-U.S.

PUB169

Effects of Irbesartan on Inflammatory Cytokine Concentrations in Patients with Chronic Glomerulonephritis Shuichi Tsuruoka, Kunihiro Yamagata. *Nephrology, University of Tsukuba, Tsukuba, Ibaraki, Japan.*

Background: Some angiotensin receptor blockers including irbesartan increase PPAR-γ activity in vitro. Aim of this study was to evaluate the interaction between obesity and the effects of irbesartan on the inflammatory cytokines (such as adiponectin, hsCRP, IL-6, TNF-α and leptin) in patients with chronic glomerulonephritis.

Methods: Anti-inflammatory effects of irbesartan were evaluated for 26 weeks in 29 hypertensive non-diabetic chronic glomerulonephritis patients by prospective, single-arm study for 26 weeks. Body weight, blood pressure, and urinary protein excretion rate were also evaluated.

Results: After treatments with irbesartan (200 mg daily) for 26 weeks, blood pressure and proteinuria were significantly decreased (blood pressure from 142 ± 1/87 ± 1 to 131 ± 1/81 ± 1 mmHg and urine protein/creatinine ratio from 1030± 143 to 779 ± 121 mg/g Cr, $P<0.01$). BMI was not significantly changed after the study. Among inflammatory parameters, adiponectin concentration was significantly increased (11.1±0.9 to 11.8±0.7 mg/ml, $P<0.05$) and hsCRP concentrations were significantly decreased (1318±207 to 1095 ±147 ng/ml, $P<0.05$) after the treatment; however, changes of IL-6, TNF-α and leptin concentrations did not reach statistical significance. Moreover, the improvements of these five parameters by the treatment were positively correlated with patients' BMI at the initiation of the study, and the improvements were particularly prominent in those with BMI greater than 25. Improvement of proteinuria was significantly correlated with increase of adiponectin concentration but not with BMI. There was also a significant correlation between change of adiponectin and change of insulin concentration.

Conclusions: We concluded that irbesartan improved metabolic parameters in non-diabetic hypertensive chronic glomerulonephritis patients, especially those with high BMI. Improvement of adiponectin concentration may be important for reduction of proteinuria.

Funding: Government Support - Non-U.S.

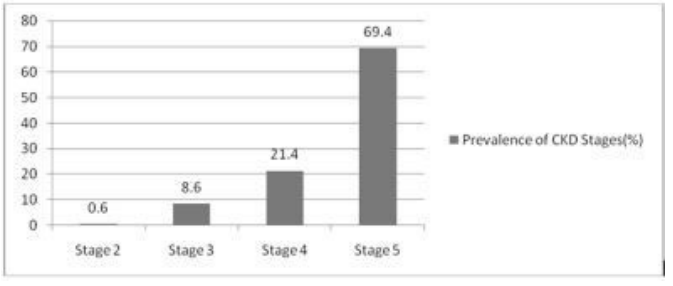
PUB170

Epidemiology of Chronic Kidney Disease in the Philippines: Baseline Data Extract from the Global Renal Information Database Kuyilan Karai Subramanian,[1] Romina Danguilan,[2] Youssef M.K. Farag,[1] Vanier Kethi-reddy,[1] Ajay K. Singh.[1] *[1]Renal Division, Department of Internal Medicine, Brigham and Women's Hospital, Boston, MA; [2]Department of Adult Nephrology, National Kidney and Transplant Institute, Quezon City, Philippines.*

Background: There is very limited information on chronic kidney disease (CKD) practice patterns in the Philippines due to lack of comprehensive prospective studies conducted on management of CKD and ESRD.The Philippine Renal Disease Registry(2009) reports diabetes (42%) as the leading cause of CKD followed by hypertension (25%) and glomerulonephritis (20%).

Methods: We conducted a prospective observational cohort study launched in 2009 in India, Malaysia, Vietnam, Philippines and Indonesia. A specifically designed computer database software application was developed to collect information on management of CKD and ESRD patients on dialysis in a longitudinal follow-up for 3 years.

Results: The sample comprised of 400 patients. Renal function data (serum creatinine levels) was available on 359 (90%) of subjects. The mean age of the cohort was 49.2±14.8 years. There was equal representation of gender, with 53.8% (n=193) males. Mean baseline serum creatinine at the baseline visit was 7.6±5.3 mg/dL, and the mean estimated glomerular filtration rate was 12.7±11.15 ml/min/m2. The most common cause of CKD in our cohort was primary chronic glomerulonephritis (33.1%), type 2 diabetes mellitus (31.2%) and hypertension (19.5%). Most of patients in this cohort were stage 5 CKD (69.4%) and stage 4 (21.4%).



Conclusions: This descriptive analysis report for the baseline data of the GRID project from Philippines revealed that the causes of CKD are similar to those observed in the Philippine Renal Disease Registry. We plan to do further analysis on the practice patterns of CKD in Philippines as well as other countries participating in GRID.

Funding: Pharmaceutical Company Support - JANSSEN-CILAG

PUB171

Outcomes of Patients with Anti-Neutrophil Cytoplasm Antibody (ANCA) Asscoiated Vasculitis (AAV) Following a Change from Oral to Intravenous Cyclophosphamide in a UK Regional Nephrology Unit Anisha Tanna, Suresh Mathavakkannan, Maria Prendecki, Barbara Thompson. *Lister Hospital, East &North Herts NHS Trust, Stevenage, United Kingdom.*

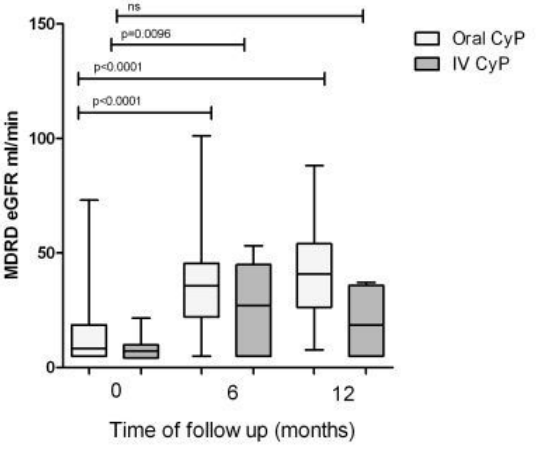
Background: Guidelines recommend using intravenous (IV) over oral Cyclophosphamide (CyP) for remission induction in ANCA associated vasculitis (AAV). Patients presenting dialysis dependent and treated with Plasma Exchange (PEX) have been excluded from large studies comparing these preparations. IV CyP has been used at our centre since 2008. We review outcomes following this change and include patients presenting dialysis dependent and treated with PEX.

Methods: Retrospective analysis was carried out on data from patients presenting to Lister with a new diagnosis of AAV between 01/01/2005 and 31/12/2011.

Results:

	Oral CyP	IV CyP	p=
N	46	9	
Age	64 (23-87)	57 (29-76)	
M:F	27:19	4:7	
Cumulative Dose (mg)	5581(600-20050)	2450 (1200-3750)	
Presenting EGFR(ml/min)	15 (5-73)	8.4 (5-22)	0.197
% Dialysis dependent at presentation	43.5	66.7	0.363
% Plasma exchanged	47.8	88.9	0.0579
% Failed to achieve remission	8.7	0	0.521
Mean total follow up (months)	44.3 (0.25-81.33)	18.8 (4.8-38.0)	0.024
Mean time to first relapse (months)	24.14	21.2	0.7492
% Death	15.2	11.1	0.608
% Significant infections	41.3	22.2	0.204
% leucopenia	34.8	22.2	0.403
Mean improvement in EGFR at 6 months (ml/min)	+ 19	+24	0.5
Mean improvement in EGFR at 12 months (ml/min)	+25	+13	0.2
Mean improvement in EGFR at end of follow up(ml/min)	+14	+13	0.784
% of patients presenting dialysis dependent who recover renal function at 12 months	70	50	0.679

Dialysis dependent analysed as eGFR 5ml/min.



Conclusions: Renal function improved significantly in both groups at the end of the study with a mean improvement in eGFR of 13-14 ml/min although with small numbers in the IV group there was no difference between groups. 46% of patients presenting dialysis dependent and plasma exchanged, recovered independent renal function.

PUB172

Practice Patterns of Treatment of Hyperuricemia and Predictors of Serum Uric Acid Level in Japanese Patients with Chronic Kidney Disease Miho Tagawa,[1] Takayuki Hamano,[2] Naohiko Fujii,[3] Enyu Imai,[4] Tadao Akizawa,[5] Kosaku Nitta,[6] Tsuyoshi Watanabe,[7] Seiichi Matsuo,[4] Hirofumi Makino,[8] Satoshi Iimuro,[9] Yasuo Ohashi,[9] Akira Hishida.[10] *[1]Kyoto Katsura Hospital; [2]Osaka University; [3]Hyogo Prefectural Hospital, Nishinomiya; [4]Nagoya University; [5]Showa University; [6]Tokyo Women's Medical University; [7]Fukushima Medical University; [8]Okayama University; [9]University of Tokyo; [10]Yaizu City Hospital.*

Background: The Japanese guidelines recommend pharmacologic treatment of asymptomatic hyperuricemia for patients with chronic kidney disease (CKD) if uric acid (UA) is > 8.0 mg/dL, which is exceptionally aggressive compared with other guidelines. The aim of the study is to examine practice patterns of treatment of hyperuricemia and predictors of UA level in Japanese CKD patients.

Methods: This is a cross sectional study on Chronic Kidney Disease Japan Cohort study (estimated glomerular filtration rate [eGFR] 10-59 ml/min/1.73m2 and age 20-75 years). Linear regression analysis was used to determine predictors of UA level.

Results: Median (interquartile range) age was 63 (54-70), 62.1 % were male. Mean (SD) eGFR was 28.8 (12.2) ml/min/1.73m2 and UA was 7.2 (1.6) mg/dL (n=2,977). As many as 45.5 %, 6.2 % and 3.2 % of subjects were on allopurinol, probenecid and citrate, respectively. Male sex, eGFR, body mass index, loop and thiazide diuretics, allopurinol, probenecid, alcohol use but not K sparing diuretics or citrate were significant predictors of UA level. UA was significantly higher at age <= 40 years compared with age 51-60 in male though UA did not differ across age categories in female (p for interaction=0.04).

Predictors of UA level by linear regression analysis

	Coefficients	95% CI		p
Male	0.96	0.58	1.34	<0.001
eGFR	-0.029	-0.034	-0.024	<0.001
Log BMI	0.99	0.13	1.85	0.03
K sparing diuretics	0.22	-0.03	0.48	0.09
Loop diuretics	0.48	0.33	0.63	<0.001
Thiazide diuretics	0.78	0.56	1.00	<0.001
Allopurinol	-0.60	-0.73	-0.48	<0.001
Probenecid	-0.62	-0.87	-0.38	<0.001
Citrate	0.18	-0.15	0.51	0.29
Alcohol use	0.13	0.002	0.26	0.046

Conclusions: Large proportion of Japanese CKD patients was on UA lowering agents and they were significant predictors of UA level.

Funding: Pharmaceutical Company Support - Kyowa Hakko Kirin

PUB173

Glycemic Control with Intensive Insulin Treatment Is Fundamental to Renal Protection in Diabetes Anil K. Mandal, Linda M. Hiebert. *[1]University of North Florida, Jacksonville, FL; [2]University of Saskatchewan, Saskatoon, SK, Canada.*

Background: Diabetes is the main cause of end stage renal disease (ESRD). We questioned why diabetes patients develop ESRD. Current preventative measures such as use of angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blocking drugs (ARB) may contribute. We investigated if renal preservation in diabetes is attainable without ACEI/ARB.

Methods: Forty-six office patients (28 female, 18 male), mean age 62.2 years (range 36-86 years), were followed for an average of 14.2 months (1.5-115 months). Diabetes as diagnosed by 2-h postprandial glucose (2hPPG) ≥ 11.1 mmol/L was treated with Glargine or detemir insulin, supplemented with regular insulin based on finger stick 2hPP and at bedtime. Anti-hypertensive therapy excluded ACEI/ARB. Laboratories were: glucose, serum creatinine (Scr), estimated glomerular filtration rate (eGFR), and glycosylated hemoglobin (HBA_{1c}). Values between first and last visits were compared (paired two-tailed t-test, $P<0.05$ significant). Patients were divided by 2hPPG < or > 11.1 mmol/L.

Results: Fasting glucose at last visit (8.4±0.6 mmol/L) was significantly lower than the first visit (10.3±0.7 mmol/L) in the all patients group, with significantly reduced Scr at last (100.3±5.2 µmol/L) compared to first visit (110.9±7.8 µmol/L). Little change was seen in eGFR between first and last visits in all groups (eGFR was consistent with chronic kidney disease (CKD) stage 2 in the majority of patients at the last visit). Less than half of patients achieved glucose control (2hPPG <11.1 mmol/L). HBA_{1c} was significantly reduced (9.14±0.52% vs. 7.60±0.45%, $p<0.0148$) in the <11.1 mmol/L group. Blood pressure was normal in all groups. Diastolic pressure at the last visit in the all patients group was lower than the first visit 81.6±1.9 vs. 77.0±1.5. $p<0.0297$).

Conclusions: Paradigm of therapy for glycemic and blood pressure control in this study, which excludes ACEI/ARB drugs, is proven effective for renal protection in diabetes.

Funding: Clinical Revenue Support

PUB174

Strategic Approach to Decrease Burden of Chronic Kidney Disease in the Texas Panhandle Tetyana L. Vasylyeva,[1] Sudha P. Chennasamudram,[1] Sharma S. Prabhakar,[2] Rodney Young,[1] Roger D. Smalligan.[1] *[1]Texas Tech University Health Sciences Center, Amarillo, TX; [2]Texas Tech University Health Sciences Center, Lubbock, TX.*

Background: The purpose of this study was to identify demographic specifics of chronic kidney disease (CKD) patients in the Texas Panhandle and to develop a strategic approach to improve quality of care.

Methods: To assess undiagnosed CKD among general population eGFR was measured among patients with unknown renal disease, who visited TTUHSC primary care (PCP) clinics. Also, a retrospective chart review of CKD patients attending nephrology clinics was performed with the collection of demographic data, creatinine, urinary protein excretion, lipid panel and HbA1c, etiology, duration of CKD, medication profile and family history. Data was compared to center for disease control (CDC) data.

Results: In PCP clinics 170 patients were recruited (average age 62±17 years; 64% women; 36% men). White were 76%, Hispanic - 7.6%, other races- 16.4%. CKD stage 1 was diagnosed in 23%, stage 2 in 48%, stage 3 in 27%, stage 4/5 in 2%. Risk factors for CKD included HTN (58%), DM (10%), and CVD (6%). Seventy percent patients with DM had been tested for microalbuminuria during the past year. For the retrospective chart review study, a total of 394 CKD patients' charts were reviewed (192 men and 192 women). Patient population was older than in the CDC database (66% over 65 years; 26% age 45-64; and 8% under 45). A majority of the patients had HTN as a cause of CKD, which is different from CDC database. Most patients were referred to the nephrology clinic at CKD stage 3. The average HbA1c in DM patients was 13±2.

Conclusions: This pilot study revealed a remarkably high number of patients who visited their PCP with no previously known renal disease, but decreased renal function. The major risk factor for CKD is age and HTN in the Panhandle in the Panhandle is HTN. The study helped to develop a strategic management approach and promote collaboration between PCPs and nephrologists in the area to improve identification and prevent the progression of CKD.

Funding: Other U.S. Government Support

PUB175

Albuminuria Is an Independent Predictor of All-Cause and Cardiovascular Mortality in the Japanese Population: The Takahata Study Tsuneo Konta, Hiroko Sato, Kosuke Kudo, Kazuko Suzuki, Ami Ikeda, Kazunobu Ichikawa, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

Background: Albuminuria is a known risk for premature deaths. However, the association between urinary albumin excretion and mortality is unknown in Japanese population. To clarify this point we conducted a longitudinal study in community-based subjects.

Methods: The participants of this study were 3446 subjects (male 45%, mean age 63 years) in Takahata town, Japan, that were registered and followed up for 7 years (median 6.2 years). Albuminuria was defined as urine albumin-creatinine ratio ≥30 mg/gCr in morning spot urine.

Results: The subjects with albuminuria (n=514, 14.9%) was older, and showed a higher prevalence of hypertension, obesity, diabetes and a lower values of eGFR than the subjects without albuminuria (n=2932, 85.1%). During the follow-up 138 subjects died. Kaplan-Meier analysis showed that an all-cause mortality was significantly increased along with the increase in urine albumin excretion (Log-rank $P<0.001$). The subjects with albuminuria showed a significantly lower survival rate than those without albuminuria (92.6% vs. 96.6%, Log-rank $P<0.001$). Cox proportional hazard model analysis with the adjustment for possible confounders showed that albuminuria was a significant and independent risk factor for all-cause and cardiovascular mortalities (hazard ratio [HR] 1.73, 95% confidence interval [CI] 1.11-2.70 for all-cause mortality, and HR 2.74, 95%CI 1.15-6.55 for cardiovascular mortality).

Conclusions: Albuminuria was a risk for all-cause and cardiovascular mortalities in Japanese population. For the early detection of high risk subjects and the prevention for premature death, albuminuria could be used.

Funding: Government Support - Non-U.S.

PUB176

The Comparison of the Mortality Predictive Ability of Albuminuria and Dipstick Proteinuria in Local Inhabitants: The Takahata Study Hiroko Sato, Kosuke Kudo, Kazuko Suzuki, Ami Ikeda, Kazunobu Ichikawa, Tsuneo Konta, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

Background: Albuminuria and proteinuria are known risk for premature mortality. This study compared the mortality predictive ability of albuminuria and proteinuria in Japanese local inhabitants.

Methods: We measured urinary albumin creatinine ratio (ACR) and proteinuria by dipstick at baseline survey and compared the predictive abilities for seven-year mortality of three cutoff points (albuminuria [ACR ≥30mg/g], trace proteinuria, 1+ proteinuria) in 3446 subjects at local health checkup in Takahata, Japan.

Results: The prevalence of albuminuria, ≥trace proteinuria and ≥1+ proteinuria were 514 (14.9%), 290 (8.4%) and 151 (4.4%) subjects, respectively. During the follow-up there were 138 deaths (4.0%) including 29 cardiovascular deaths. Kaplan-Meier analysis showed that an all-cause mortality was significantly increased along with the increase in ACR and proteinuria levels by dipstick (Log-rank $P<0.001$). The mortalities in subjects with albuminuria (7.4%), ≥trace proteinuria (7.2%) and ≥1+ proteinuria (9.3%) were higher than that in total subjects (4.0%). In Cox proportional hazard analysis all the three cutoff points were significant predictors for all-cause mortality in unadjusted model, however after the adjustment for possible confounders the significant association was observed only in albuminuria. For cardiovascular mortality, albuminuria, but not proteinuria was the significant predictor both in unadjusted and adjusted models.

Conclusions: The prevalence and predictive ability of albuminuria were higher than those of proteinuria by dipstick, suggesting albuminuria might be a superior predictor for seven-year mortality in local inhabitants.

Funding: Government Support - Non-U.S.

PUB177

KNOW-CKD (KoreaN Cohort Study for Outcome in Patients with Chronic Kidney Disease): A 10-Year Longitudinal Cohort Study of the Chronic Kidney Disease Kook-Hwan Oh,[1] Curie Ahn,[1] Dong Wan Chae,[1] Young-Hwan Hwang,[3] Soo Wan Kim,[4] Kyu Hun Choi,[2] Seung Hyeok Han,[2] Su-ah Sung,[3] Kyu-Beck Lee,[5] Wookyung Chung,[6] Ho Jun Chin,[1] Hayne C. Park,[1] Joongyub Lee,[1] Yong-Soo Kim.[7] *[1]Seoul National Univ.; [2]Yonsei Univ.; [3]Eulji Univ.; [4]Chonnam National Univ.; [5]Sungkyunkwan Univ; [6]Gachon Univ.; [7]Catholic Univ.*.

Background: The goals of the KNOW-CKD (KoreaN cohort study for Outcome in patients With Chronic Kidney Disease) study are 1) to establish a CKD cohort representing Korean CKD population for up to 10-year follow-up, and 2) to investigate the renal progression, mortality, complications, risk factors, and the genetic influence.

Methods: KNOW-CKD Research Group comprises nephrologists, pediatric nephrologists, epidemiologists and statisticians from eleven centers in Korea. KNOW-CKD will enroll 2,850 individuals with CKD stage from 1 to 5 between 2011 and 2015 and follow them up to 10 years. Dialyzed patients or those with allograft kidney are excluded. At enrollment and at pre-specified intervals, laboratory tests will be conducted on the kidney function, biochemical profiles, anemia, cardiovascular complication (echocardiography, coronary CT, arterial stiffness), and mineral bone disorder. A biobank is also established

for the DNA, serum and urine at regular interval. Information on the medical history, health questionnaires, QoL will also be collected. Web-based case-report forms (CRF) is developed for the systemic management of the patient data.

Results: As of the end of 2011, 753 adult CKD patients were enrolled in the cohort study. Patients were 52.2 years old on the average with 58.4% male. The etiologic diseases of CKD were GN (32.2%), diabetic nephropathy (17.2%), hypertensive nephropathy (16.2%), and others. The CKD stages were stages 1 to 2 (27.9%), stage 3 (40.1%), stage 4 (22.9%) and stage 5 (9%).

Conclusions: It is expected that KNOW-CKD cohort, when established, will represent Korean CKD population and provide information on the natural course, clinical manifestation including for various complications, predicting factors for adverse outcomes and treatment tools of CKD.

PUB178

Assessment of Lupus Nephritis in Youth at SLE Presentation Divya Moodalbail,[1] Susan L. Furth,[1,3] Julia R. Maisel,[1] Barbara A. Fivush,[2] Sangeeta D. Sule.[2] *[1]Pediatrics, The Children's Hospital of Philadelphia; [2]Pediatrics, Johns Hopkins Children's Center; [3]Epidemiology, University of Pennsylvania.*

Background: Nephritis is a devastating complication of SLE. Recent American College of Rheumatology (ACR) guidelines recommend that all patients with clinical evidence of active lupus nephritis (LN) undergo renal biopsy for classification of their glomerular disease.

Methods: Retrospective cohort study documenting demographics, serum, urinary investigations for children and adolescents aged 1 to 19 years with SLE, at disease presentation and follow up within 6 months in pediatric rheumatology/nephrology clinics at 2 tertiary care children centers (presentation between 2002 - 2011 at JHCC and 2007 - 2011 at CHOP). We did a systematic assessment for LN per ACR criteria (persistent proteinuria >3+ on dipstick and/or active urinary sediment >5 RBCs/hpf, >5 WBCs/hpf or cellular casts) and compared with biopsy performance and results.

Results: 90% of the 47 subjects in our cohort were female, 55% were African American. Median age of SLE diagnosis was 14 years (IQR 11 - 15 yrs), median serum creatinine at initial presentation was 0.6 mg/dL, and 19 (59%) had dipstick proteinuria (≥1+); 6 (32%) were ≥3+. In 20 (43%) subjects urine protein: creatinine ratio was assessed; 9(45%) had a ratio>2. Twenty three (64%) subjects met ACR criteria for LN. Retrospectively, 17 (36%) underwent kidney biopsy; 11 (64%) had diffuse proliferative LN (Class IV), 4 (24%) had focal proliferative LN (Class III), and 2 (12%) had membranous nephropathy (Class V). Of the 17 biopsied, dipstick protein results at initial visit was noted to be 4+ in 3, 3+ in 2, 2+ in 6, 1+ in 1, negative in 2 and unavailable in 3. Nine subjects with Class III - V LN had ≤2+ proteinuria.

Conclusions: Using ACR criteria, the majority (64%) of children in our SLE cohort had LN; only 36% had undergone kidney biopsy. Systematic assessment for LN including dipstick, microscopic urinalysis and urine protein: creatinine estimation should be standard practice in management of children with SLE. Our retrospective cohort suggests that even low levels of proteinuria (≤2+) rather than >3+ (as per ACR criteria), should be a consideration for kidney biopsy.

Funding: Other NIH Support - NIAMS

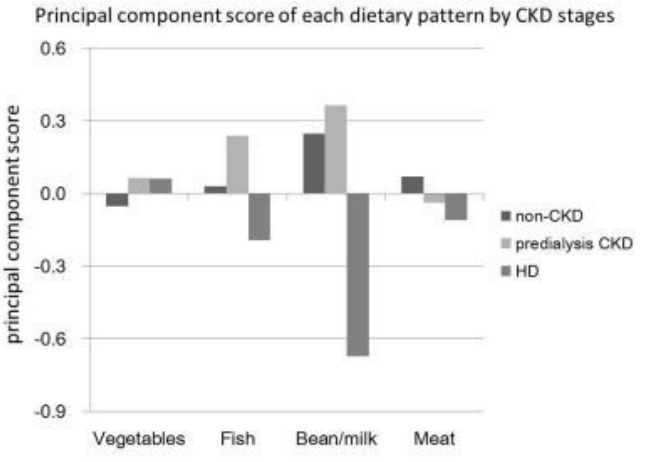
PUB179

Dietary Patterns in Chronic Kidney Disease Patients: Joint Analysis of the Hisayama Study and the Dialysis Outcomes and Practice Study in Japan Kazuhiko Tsuruya,[1] Shingo Fukuma,[2] Takafumi Wakita,[2] Satoru Fujimi,[1] Yutaka Kiyohara,[1] Takanari Kitazono,[1] Tadao Akizawa,[3] Takashi Akiba,[3] Akira Saito,[3] Shunichi Fukuhara.[2] *[1]Kyushu University; [2]Kyoto University; [3]Steering Committee of JDOPPS.*

Background: Little is known on actual dietary intake in chronic kidney disease (CKD) patients. We attempted to identify dietary patterns in CKD patients and examined their differences by CKD stage and associations with laboratory markers.

Methods: In a cross-sectional study, we selected 2,356 non-CKD and 717 predialysis CKD patients from the Hisayama study, a population-based cohort, and 1,510 hemodialysis (HD) patients from the Japan Dialysis Outcomes and Practice Study between 2005 and 2007. Principal component analysis using data from a brief self-administered diet history questionnaire (BDHQ) helped us identify patterns and estimate principal component score by CKD stage. We examined the associations between score and levels of phosphorus and bicarbonate in HD patients by linear regression model, after adjusting for potential confounders. Each beta coefficient indicates mean difference of score, compared with the first tertile group.

Results: We identified 4 dietary patterns (vegetables, fish, bean/milk, and meat)based on 19 food items and found patterns of fish, bean/milk and meat less common in HD patients than in non-CKD and predialysis CKD patients.



Higher score for vegetable and fish were associated with reduced levels of phosphorus (beta -0.18, 95% CI -0.32 to -0.03 for third tertile of vegetable score) and bicarbonate (beta -0.98, 95% CI -1.85 to -0.11 for third tertile of fish score), respectively.

Conclusions: Of the 4 clinically interpretable dietary patterns identified, the vegetable pattern was associated with reduced phosphorus levels and the fish pattern with reduced bicarbonate in HD patients.

Funding: Pharmaceutical Company Support - Kyowa Hakko Kirin

PUB180

Glycohemoglobin Not as Predictive as Fasting Glucose as a Measure of Prediabetes in Predicting Proteinuria Yuji Sato,[1] Yuichiro Yano,[3] Shouichi Fujimoto,[2] Tsuneo Konta,[2] Kunitoshi Iseki,[2] Toshiki Moriyama,[2] Kunihiro Yamagata,[2] Kazuhiko Tsuruya,[2] Hideaki Yoshida,[2] Koichi Asahi,[2] Kazuo Kitamura,[3] Tsuyoshi Watanabe.[2] *[1]Dialysis Division, University of Miyazaki Hospital, Miyazaki, Japan; [2]Steering Committee for the 'Research on the Positioning of Chronic Kidney Disease (CKD) in Specific Health Check and Guidance in Japan'; [3]Department of Internal Medicine, University of Miyazaki, Miyazaki, Japan.*

Background: There is little data on the assessment of prediabetes with proteinuria.

Methods: This is a cross-sectional cohort study assessing prediabetes with proteinuria. Using a nationwide health check-up database of 228,778 Japanese aged >20 years (median 66years; 39.3% were men; none had pre-existing cardiovascular disease), we examined the association between prediabetes and proteinuria (>1+ on dipstick) separately in prediabetes subjects diagnosed with the new HbA1c criterion only (PD-A1c), the impaired fasting plasma glucose only (PD-IFG) and fulfilling both criteria (PD-Both).

Results: According to ADA's criterion of 5.7-6.4% HbA1c and/or 100-125 mg/dl fasting plasma glucose, 43.8% of subjects were judged as having prediabetes. 53.7%, 21.7% and 24.5% of prediabetes subjects were divided into subclasses of PD-A1c, PD-IFG and PD-Both, respectively. Therefore, 21.7% of prediabetes subjects were missed using the new HbA1c criterion only. Compared with subjects with normal glucose tolerance, the odds ratio (OR) (95% confidence interval: CI) for the increased risk of proteinuria (>1+) in diabetes itself was 2.191 (2.081-2.307) and in whole prediabetes was 1.093 (1.046-1.142), when prediabetes was subdivided, OR for proteinuria in PD-IFG was 1.217 (1.140-1.300) and that in PD-Both was 1.249 (1.174-1.329), but that in PD-A1c was not significant, even after adjustment for significant covariates, such as age, sex, BMI, systolic blood pressure, antihypertensive medication, eGFR, lifestyle, lipid profile.

Conclusions: Prediabetes is a significant risk factor for proteinuria compared with completely normal glucose level, and subjects with prediabetes defined using IFG are at significantly higher risk for proteinuria than those defined by HbA1c only.

Funding: Government Support - Non-U.S.

PUB181

Vascular Calcification on Plain Radiographs Is Associated with Carotid Intima Media Thickness, Malnutrition and Cardiovascular Events in Dialysis Patients Young Ki Son,[1] Hyun Kyung Nam,[2] You Jeong Oh,[3] Won Suk An,[1] Ki Hyun Kim,[1] Seong Eun Kim.[1] *[1]Nephrology, Dong-A University Hospital, Busan, Korea; [2]Nephrology, Busan Medical Center, Busan, Korea; [3]Nephrology, Dong-Eui Hospital, Busan, Korea.*

Background: Vascular calcification (VC) and carotid intima media thickness (CIMT) are highly correlated with cardiovascular (CV) disease. We hypothesized that significant VC on plain radiographs is associated with CIMT and CV events in dialysis patietns. In addition, we evaluated risk factors for VCprogression on plain radiographs in dialysis patietns.

Methods: In this two-year observational, prospective study, 67 dialysis patients were included. We checked plain radiographs at baselineand after 2 years.Laboratory tests and malnutritionscore were obtained at baseline, after 1 year, and after 2 years.

Results: The mean age of dialysis patients was 56.3 ± 10.3 years and the duration of dialysis was 41.3 ± 34.5 months. The prevalence of significant VC was 61.2% and the prevalence of carotid artery atheromatous plaque was 36.6% in enrolled dialysis patients. The prevalence of carotid artery atheromatous plaque(p = 0.025), CIMT(right: p = 0.045, left: p = 0.014), malnutrition scores and CRP were significantly higher in patients with significant VC compared to patients without significant VC. Serum albumin and total iron binding capacity were significantly lower in patients with significant VC compared to patients without significant VC. Patients without significant VC showed lower CV events by the Kaplan-Meier method (p = 0.015). VC progression was found in 35.7% among 56 patients followed up. Hemoglobin (Hb) was significantly increased according to elapsed time in patients who did not show VC progression on plain radiographs(10.3 g/dL at baseline, 10.8 g/dL after 1 year, 11.4 g/dL after 2 years). Hb (beta = - 0.458, p = 0.006)after 2 yearswas an independent factor for VC progression on plain radiographs.

Conclusions: Significant VC on plain radiographs was associated with CIMT, malnutrition, inflammation and CV events in dialysis patients. Conditions maintaining adaquate Hb maybe retard VC progression on plain radiographs in dialysis patients.

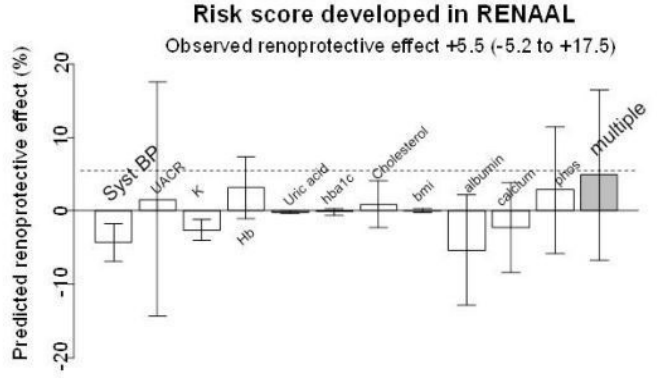
PUB182

Efficacy Estimation of Amlodipine on Hard Renal Outcomes Using Changes in Multiple Biomarkers Paul Smink,[1] Jarno Hoekman,[1] Hans-Henrik Parving,[2] Julia Lewis,[3] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands; [2]Medical Endocrinology, University Hospital of Copenhagen, Copenhagen, Denmark; [3]Division Nephrology, Vanderbilt University, Nashville, TN.*

Background: We have developed and validated a multiple parameter risk response (PRO) score that combines short-term changes in multiple biomarkers to predict ARB drug effects on hard renal outcomes.[1] We questioned whether this PRO score can also be used to estimate the effect of CCB amlodipine on hard renal outcomes. 1 PA Smink et.al. The importance of short-term off-target effects in estimating the long-term renal and cardiovascular protection of ARBs.

Methods: Data from IDNT and RENAAL were used (2,661 type 2 diabetics with nephropathy). The PRO score was created using Cox analysis in the placebo group of RENAAL and subsequently applied to the baseline and month 6 measurements of the amlodipine arm of IDNT to predict long-term renal risk reduction. Predicted risk change was compared with the observed risk change (DSCR or ESRD) to test the accuracy of the PRO score.

Results: The change in blood pressure during amlodipine therapy predicted a -4.3% (95%CI -6.9 to -1.7) renal risk change as compared with placebo, whereas the actual amlodipine effect was a +5.5% (-5.2 to +17.5) renal risk change. As shown in the figure, amlodipine caused other changes in renal risk parameters that could add to the ultimate renal outcome.



The multiple PRO score (taking into account all changes) predicted a renal risk increase of 4.9% (-6.8 to +16.5) which is nearly the same as the observed drug effect.

Conclusions: A PRO score based on month-6 changes in multiple biomarkers performs better in estimating amlodipine's effect on hard renal outcomes than any change in single biomarkers. The PRO score thus works for two completely different antihypertensives.

Funding: Government Support - Non-U.S.

PUB183

Primary Diffuse Mesangioproliferative Glomerulonephritis: Predictors of Renal Survival Mette K.M. Axelsen,[1] James G. Heaf,[2] Robert Smith Pedersen,[3] Torkell Ellingsen.[4] *[1]Nephrology B, Copenhagen University Hospital at Herlev, Herlev, Dominica; [2]Institute of Public Health, Aarhus University, Aaarhus, Denmark; [3]Flexdialysis A/S, Rungsted Kyst, Denmark.*

Background: Diffuse mesangioproliferative glomerulonephritis (MesP), with an incidence of 10.8/mio/year, is the most commonly diagnosed type of glomerulonephritis in Denmark. We describe the natural history of MesP and studied the relation between renal survival and baseline characteristics at the time of renal biopsy in a cohort with a follow-up of up to forty years.

Methods: The study is a historical cohort investigation. 140 patients with biopsy-proven MesP between 1967 and 2006 were included. Immunofluorescence was performed in 100; of these 46 had IgA deposits. Renal death was defined as patient death or need for dialysis or transplantation. Factors influencing renal survival were investigated using univariate and multivariate Cox proportional hazards regression analysis.

Results: Mean follow-up18 years ± 11.2, range 0.01-43.3. Mean age 34.2 years ± 17.9, range 5-80. Male: 62.9 %. Renal survival at 5, 10, 20 and 30 years was 87.1, 78.4, 58.6 and 49.9% Female survival was significantly better than male (70 vs. 40%). Multivariate analysis, adjusted for age, s-creatinine and nephrotic syndrome (NS) was performed for each sex individually, due to non-proportional survival curves for men and women. An increase in s-creatinine of 100 µmol/l was associated with a hazard risk (HR) of 1.88 (p<0.001) in women and 1.18 (p<0.001) in men. Older age was associated with a HR of 1.06 (p<0.001) in women and 1.05 (p<0.001) in men. NS had a poorer prognosis in men (HR 2.27, p<0.02), but not in women. There was no overall difference in prognosis between IgA positive and IgA negative patients, but in men, IgA positivity had a better prognosis.

Conclusions: Increasing age and s-creatinine are adversely associated with renal prognosis. Gender differences were seen: renal prognosis was better for women after 30 years of observation, and the detrimental effect of azotaemia was greater; nephrotic syndrome resulted in a significantly poorer prognosis in men, but not women. These findings suggest that the disease course and prognosis is different between men and women.

Funding: Private Foundation Support

PUB184

Nephrotic Patients with Membranous Nephropathy (MN) Treated with ACTH: Five Year Follow-Up Anna-lena Berg, Anneli Jonsson, Julia Dolinina, Omran Bakoush, Sten-erik Bäck. *Lund University, Lund, Sweden.*

Background: We previously reported that ACTH 1-24 (Synacthen Depot) has a lipid-lowering effect and also an ACTH-specific antiproteinuric effect in patients with Membranous Nephropathy (MN). ACTH might work directly via melanocortin receptors (MC 1-5) in white blood cells and/or the kidney. Clinical and experimental studies support our findings.

Methods: We now report 5 years follow up of 28 patients (10F/18M), median age of 63 years (34-85) with MN treated with ACTH. Six patients had previously been treated with the Ponticelli regimen, 3 patients with prednisolone/Cyclosporine (CsA) and 8 patients with prednisolone alone. Eleven patients had no earlier immunosuppressive treatment. Median time from MN diagnosis to initiation of ACTH treatment was 9.5 months (0.25-252). All patients received standard of care, including ACE-inhibitors/ARB, statins and other symptomatic therapies. Treatment with ACTH was started at 1 mg wk and was increased to a maximum of 1 mg twice weekly. The median duration of ACTH therapy was 10 months (2-24). Follow up data were obtained between 36-58 months after cessation of ACTH.

Results: With ACTH treatment, urinary albumin decreased from a pre ACTH median value of 4381 mg/L (3000-18398) to 270 mg/L (8-1802) with follow up median value 89 mg/L (0-1540; p< 0.0001 at both points), serum albumin increased from a pre ACTH median value of 20 g/L (8-30) to 35.5 g/L (22-49) with follow up median value 37 g/L (31-50; p<0.0001 at both points). e-GFR increased from a pre ACTH median of 56.5 mL/min/1.73m2 (14-104) to 62.5 mL/min/1.73m2 (16-96) post-ACTH (p<0.01). During follow up two patients required retreatment with ACTH for proteinuria relapse during the follow up period, and one patient progressed to ESRD. Five years after the initiation of ACTH therapy, 19 patients had complete remission (proteinuria <200 mg/L) and 8 patients had partial remission (proteinuria reduced by 50% to <2000 mg/L), and median eGFR for the group remains improved compared with baseline (65 mL/min/1.73m20).

Conclusions: Our 5 year observations suggest that ACTH may be useful to durably reduce proteinuria and preserve kidney function in patients with idiopathic MN.

Funding: Pharmaceutical Company Support - Questcor Pharm Inc, Government Support - Non-U.S.

PUB185

Analysis of 368 Non-Dialysis-Patients on Once Monthly C.E.R.A. Demonstrates High Stabilized Hemoglobin Values at Low C.E.R.A. Doses Christoph Wanner,[1] Thomas Rath,[2] Stefan N. Heidenreich,[3] Michael Koch,[4] Dirk Markus Henrich,[5] Frank Leistikow.[6] *[1]University Hospital, Würzburg, Germany; [2]Hospital, Kaiserslautern, Germany; [3]Renal Center, Aachen, Germany; [4]Renal Center, Mettmann, Germany; [5]Renal Center, Saarlouis, Germany; [6]Renal Center, Mannheim, Germany.*

Background: The TREAT-study results initiated a discussion about the interdependence between hemoglobin (Hb) -levels and erythropoiesis stimulating agents (ESA) -doses. The similar protocols of the German studies MERCUR (interventional) and SUPRA (non-interventional) in chronic kidney disease (CKD) stage IV patients (pts) allowed exploring the relation between ESA dose and Hb in pooled data.

Methods: Data were analyzed in ESA naïve pts showing at least one Hb value <10 g/dL followed by a treatment period of ≥3 months of stable Hb-values within defined ranges: <10, 10-11, 11-12, >12 g/dL. The primary and secondary Hb- targets of both studies were 11–12 and 11–13 g/dL. The 4 Hb- strata were further analyzed with respect to duration within the Hb-range, median dose, Nr of C.E.R.A. dose adaptations and incidence of AEs and SAEs (only for MERCUR within this stable period).

Results: 368 pts received at least one C.E.R.A. dose (safety population). The mean age was 68 years (18 – 94), 47.6% were females. The mean BMI was 27.9 kg/m² (16.3 - 48.4) and 44.8% suffered from diabetes. 62 pts displayed Hb-values within the defined ranges ≥3 months. The highest stabilized Hb – levels required the lowest C.E.R.A. doses.

Further results

Hb ranges, g/dl	< 10	10-11	11-12	> 12
Pts	25	12	15	10
Duration (Mo) Mean (SD)	4.54 (1.774)	4.14 (1.289)	3.68 (0.778)	4.88 (2.924)
Dose, µg, Median (Q1,Q3)	120 (75,150)	100 (75, 150)	100 (75, 100)	75 (50, 75)
Nr of dose-adap, Median (Q1, Q3)	2 (1,3)	1 (0.5, 2.5)	2 (0, 3)	0.5 (0,1)
Nr of SAE, MERCUR	5	1	0	0

Conclusions: In CKD non-dialysis pts, low doses of once monthly C.E.R.A. maintained stable high Hb-values (> 12 g/dl) potentially reflecting the background disease of the pts. The number of pts was too small to correlate the AE/SAE incidence with Hb-levels. These results need further evaluation in prospective controlled trials.

PUB186

Clinical and Biological Evolution of the More Frequent Biopsied Glomerulonephritis in Castilla la Mancha (Spain) between 1994 and 2008
Jose Luis Conde Olasagasti,[1] Elena Andres Mompean,[2] Elisa Pereira,[3] Ana Roca Muñoz,[1] Katia Matilde Perez,[2] Francisco Rivera.[3] *[1]Nephrology, Complejo Hospitalario, Toledo, Spain; [2]Nephrology, Hospital Universitario, Guadalajara, Spain; [3]Nephrology, Hospital General Universitario, Ciudad Real, Spain; [4]Nephrology, Complejo Hospitalario Universitario, Albacete, Spain.*

Background: In 2009 a collaborative task-force was established to investigate the progression and outcome of selected biopsied GN in CLM. (1994-2008).

Methods: Patients: 765 adults with histologically selected GN in four hospitals (1.8 million population area):157 focal and segmental glomerulosclerosis (FSGS).166 membranous nephropathy (MN),170 IgA nephropathy (IgAN),147 lupus nephritis (LN),125 crescentic type III glomerulonephritis(CGNIII).The median follow-up was 6,89 years (range 0.01-17,5). **Outcomes**:alive in or out of end stage renal disease (ESRD:dialysis or transplant),died, lost for follow up (LFU). Initial and final (for alive out of ESRD) renal situation was stratified and compared acording eGFR (MDRD) and level of proteinuria (Wilcoxon rank test).Association with outcomes and renal situation was assessed by binary logistic regression(covariates: age,sex,initial eGFRand proteinuria,and histological diagnosis).

Results: 118 died (46CGNIII,27MN,21FSGS 15IgA,9LN) 115 alive in ESRD (46 FSGS,38 IgA,17 RPGNIII,9 NM,5 LN) 483 alive out of ESRD (125 LN,120 NM,107 IgA,78 FSG,53 CGNIII),and 49 LFU accountig respectively for 15,4%,15,1% 63,1% and 6,4% of the cohort. After adjustement, ESRD or death,were significantly ($p<0.01$) associated wih initial eGFR and proteinuria,age,sex (male worse) and FSGS **Functional status**:Among 483 patients alive out of ESRD,50% have a eGFR (ml/min) >60, 34% 30 to 60 and 16% <30, being different ($p<0.00$) from initial.Individual renal situation was better (60,3%),same (17,1%), worse (22,5%) than in onset. In this self-selected group, progression to better renal situation was only significantly ($p<0.05$) associated wih initial eGFR and some diagnosis (FSGS worse).

Conclusions: Our results do not differ from those reported in literature.It's noteworthy the relative good prognosis of CGNIII once patients survive the first year.

Funding: Government Support - Non-U.S.

PUB187

Survey of Physical Activity and Dynamometry in Patients with Advanced Chronic Kidney Disease (ACKD) Guillermina Barril, Fernando F. Hadad, Maria Bernardita Puchulu, Ángel Nogueira Pérez, Jose-Antonio Sanchez-Tomero. *[1]Nephrology, Hospital Universitario de la Princesa, Madrid, Spain; [2]Nephrology, Hospital Clinico San Carlos, Madrid, Spain; [3]Nwphrology, Hospital U. de la Princesa, Madrid, Spain.*

Background: There is evidence of the importance of exercise in preserving muscle mass in patients with advanced chronic kidney disease ACKD.. There are useful tools that can help to assess status and muscle strength.

Methods: Assess muscle strength by dynamometry (Baseline 12-0240)in upper limbs in 98 patients inACKD Unit and correlate with physical activitythrough a basic survey of physical excercise performed. Parameters evaluated: age, gender, employment status, autonomy for functions of daily living, physical exercise ussually and type, difficulty climbing stairs, difficulty getting up from his chair, smoker and former smoker.

Results: 98 patients fronm a ACKD Unit were evaluated.61.5%men,xage 72.94 ± 12.88. no significantl diference between men and women, 72.9% inactive, 30.7% were current smokers, autonomous daily living 86.5%, sedentary49%, 57.3% walk normally, it costs up 28.1% of the chair, difficulty climbing stairs 62, 5%, realyzing regular exercise 44.8% and 55.2% having done exercise before. Dinamometries: xDizda 51.30±22.4, Ddcha 53.42±20.86 lbs. 27% have increased dynamometrtry left arm. There are higher dinamometry values in males than women and under 65 vsover 65years (0.000). We found inverse correlation between dynamometry values and age(0.000).

Patients who usually perform physical exercise have significantly higher dinamometrry than those who do not realize p0, 000 and 0.001 for Dizda Ddcha. In a high % of patients who had high exercise performed previously but they did not now retained higher dinamometrtry values than those who have not ever made.

Conclusions: 1.-The dynamometry is a useful tool for measuring muscle strength and monitor it over time.

2. - The men have larger values than women.

3.-Age was inversely correlated with dynamometry and musclestrength was greater in younger than 65 years

4.-Patients with regular physical exercise showed better muscle strength and have previously done to help preserve muscle strength over time.

PUB188

Underlying Cause of Chronic Kidney Disease (CKD) Could Be a Better Predictor of Stroke than Stage of CKD.(Miyagi Gonryo Study)
Tasuku Nagasawa,[1] Mariko Miyazaki,[1] Masaaki Nakayama,[2] Hiroshi Sato,[3] Sadayoshi Ito.[1] *[1]Nephrology, Hypertension and Endocrine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Nephrology, Fukushima Medical University School of Medicine, Fukushima, Japan; [3]Clinical Pharamacology and Therapeutics, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan.*

Background: Chronic kidney diseases (CKD) are known to be risk factor of cardiovascular disease (CVD). Miyagi Gonryo Study is comprised 2,692 patients recruited from 11 outpatient nephrology clinics, classified into 4 groups by underlying renal disease (Primary renal disease:PRD, hypertensive nephrophathy:HN, diabetic nephropathy:DN, other nephropathies:ONs).We reported induction of renal replacement therapy (RRT) is higher than incidence of CVD.(Hypertens Res. 2011:1106-10). Prevalence of stroke in Japan is higher than that of United States. But relation between stroke and underlying cause of CKD is unclear.

Methods: In this study, we focused on background of stroke patients. During follow up 22.6 months from recruitment, total of 115 CVD events has occurred (stroke in 37 cases.), and 44 has dead.We analysed background of 37 patients.

Results: In 37 cases, 23 are cerebral infarction, 9 are cerebral hemorrhage and 3 are other intracranial event. Incidences of stroke of PRD, HN, DN, and ON were 0.3%, 3.0%, 1.9% and 2.4%, respectively. Level of blood pressure, age, and use of anti-platelet drugs are no difference between groups. 11 of 24 patients were recurrence of cerebral infarction. Incidence of cerebral infarction of general population of Japan is about 0.3%. Taken together, patients with HN and DN are extremely high risk of stroke. And history of cerebral infarction is also predictor of recurrence.

Conclusions: We conclude that underlying cause of CKD is much important than stage of CKD.

PUB189

Referral Pattern of Patients from an Indian Subpopulation in a Standalone Dialysis Setup Topoti Mukherjee, Nagesh Manjunath Rao. *Nephrology, DaVita NephroLife India Pvt. Ltd., Bangalore, Karnataka, India.*

Background: Contrary to the conventional approach of maintenance hemodialysis being a tertiary setup in a hospital in India, our dialysis unit (DaVita NephroLife) primarily focuses on providing quality dialysis services, while ensuring geographic convenience and financial comfort to the End Stage Renal Disease (ESRD) patients. This retrospective, explorative and observational study aims to survey and improve patient referral patterns in order to track morbidity and mortality as part of dialysis outcomes.

Methods: Patient demographics, referral modes and consulted specialities were recorded. Patients were segregated based on their mode of referral.

Results: Between December 2009 and May 2012, 2995 patients registered themselves with NephroLife for renal and allied complications. A total of 958 patients, comprising 32% of the sample size had presented for consultation on account of symptoms. 659 individuals (22%) were found to be comorbid during kidney disease screening camps. The rest 44% accounted for referrals by classifieds and other personal referrals. 30% of the nephrology consult registered for hemodialysis while the rest were for other renal concerns. Among the dialysis patients, 93%, 62% and 80% achieved a Kt/v of >1.2, haemoglobin of 10-12g/dl and albumin of >3.5g/dl respectively.

Conclusions: An encouraging statistic is the significant number of patients that have promptly presented for consultation upon feeling symptoms they felt were characteristic of renal disease. Also important is the substantial number of latent illnesses brought to light during screening camps. Other in–house facilities like patient education, laboratory, dietician, psychologist, vascular surgeon and family physician, in addition to clinical nephrology, have both drawn a steady patient pool and also helped better patient management, as evident from our outcomes. We conclude that standalone dialysis units are a feasible concept in India.

PUB190

The Clinical Presentation of Kidney Disease at Queen Elizabeth Central Hospital, Blantyre, Malawi Gavin Dreyer. *Queen Elizabeth Central Hospital.*

Background: Risk factors for kidney disease are prevalent in Malawi and include HIV infection, diabetes mellitus, hypertension and sepsis. No studies have examined how kidney disease presents to health services in Malawi. There is a pressing need to investigate the clinical presentation, aetiology and risk factors for kidney disease in Malawi to enhance clinical service provision, determine research opportunities and improve patient outcomes.

Methods: A new nephrology service at Queen Elizabeth Central Hospital has been established. Ward referrals and patients from the nephrology out-patient clinic in the first 12 months of the service were included in the study. Routine, fully anonymised, demographic

and clinical data were collected prospectively. Clinical and laboratory parameters were analysed by a nephrologist to determine the likely cause of kidney disease, need for kidney biopsy (although not currently available in Malawi) or dialysis therapy and confirmation of end stage kidney disease based on the best available clinical and laboratory evidence.

Results: 157 patients (83 inpatients) were referred in the first 12 months of the service, 89 male (56.7%), mean age 40.6 years, 53 (33.8%) HIV positive. The mean creatinine at presentation was 8.0 mg/dl (mean eGFR 30.1 ml/min/m^2). 113 (72.0%) patients presented with evidence of chronic kidney disease, 29 (18.5%) with acute kidney injury, and 9 (5.7%) with acute on chronic kidney disease. Glomerular disease was the primary renal disorder in 31.8% of patients with hypertension and diabetes mellitus accounting for the primary renal lesion in 10% of cases each. 47 (29.9%) patients presented with end stage kidney failure (eGFR < 15 ml/min and symptomatic), 40 (25.5%) required a kidney biopsy at presentation and 25 (15.9%) required emergency dialysis on the day of referral.

Conclusions: Patients present to QECH with advanced kidney disease and glomerular pathology is the main aetiological factor in line with findings from other studies in the region but the exact histological subtypes remain undefined. Problem based research combined with enhanced clinical services for identifying and treating early kidney disease could reduce the morbidity and mortality associated with kidney disease in Malawi.

PUB191

Urine Uric Acid Excretion in the Japanese Population: The Takahata Study Kazuko Suzuki, Tsuneo Konta, Hiroko Sato, Kosuke Kudo, Ami Ikeda, Kazunobu Ichikawa, Isao Kubota. *Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.*

Background: Hyperuricemia is an established risk factor for end-stage renal disease and cardiovascular events and the serum levels of uric acid are largely regulated by urinary excretion of uric acid. To clarify the determinants of urinary uric acid excretion in general population, we conducted a community-based study, using the uric acid clearance-creatinine clearance ratio (UACr/CCr) as an index of urinary uric acid excretion.

Methods: The subjects were 2018 Japanese individuals (885 men, 1133 women, mean age 63 years) without history of kidney disease participated in local health checkups. The UACr/CCr was assessed in morning spot samples of urine and blood.The urinary uric acid excretion were classified into low (UACr/CCr <5.5%), normal (5.5-11.1%), and high (>11.1%).

Results: In this population, the proportions of low, normal, and high group of UACr/CCr were 40.4%, 39.0%, and 20.5%, respectively. The proportion of low UACr/CCr group was significantly higher in men (46.7%) than that in women (36.5%) (P <0.001). Multiple linear regression analysis showed that UACr/CCr values were related positively with estimated GFR and negatively with HbA1c and triglycerides in men, and were related positively with body mass index and negatively with age in women, respectively. Multivariate logistic analysis showed that the independent predictors for low UACr/CCr were aging, diabetes and renal insufficiency in men, and aging and high urinary sodium excretion in women, respectively.

Conclusions: This study showed that reduced urinary uric acid excretion was observed in the large part of Japanese population. The renal clearance of uric acid was associated with aging, comorbidities and dietary habit, and its association might be different by gender.

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PUB192

Obesity as Risk Factor for Chronic Kidney Disease in the Moroccan General Population Monique M. Elseviers,[1] Mohammed Benghanem Gharbi,[2] Zamd Mohamed,[2] Abdelali Belghiti Alaoui,[3] Mohammed El Hassane Trabelssi,[3] Naima Benahadi,[3] Benyounès Ramdani,[2] Bayahia Rabia,[2] Marc E. De Broe.[1] *[1]Department of Health Sciences, University of Antwerp, Wilrijk, Belgium; [2]Moroccan Society of Nephrology; [3]Ministry of Health, Morocco.*

Background: The MaReMar study aims to investigate chronic kidney disease and associated risk factors in the general population of Morocco and to treat the population at risk during 5 years. We wondered if BMI and waist-hip ratio are valid parameters to evaluate the risk of obesity in the Moroccan population.

Methods: A random sample of the adult population of 2 middle-sized towns in Morocco was selected, stratified according to gender and age categories 26-40, 41-55 and 56-70. In the local health centre, consented subjects were screened by a structured questionnaire, clinical investigations (with body weight and height, waist and hip circumference) and blood and urine sampling. Obesity was defined has BMI >30 or a waist to hip ratio (WHR) >1 in male and > 0.85 in female.

Results: Baseline screening was performed in 10 524 participants, equally spread over the predefined gender and age strata. Mean bodyweight was 73.8 kg (SD13.9) in women and 74.6 kg (SD13.1) in men, with a mean BMI of 28.6 (SD5.2) and 24.9 (SD4.1) respectively. Obesity (BMI>30) was observed in 36.4% of women and 11.3% of men. Obesity was already present in 27.4% of women of 26-40yr, increasing to >40% in older age groups. Using WHR, more extreme differences were observed with obesity found in 53.5% of women and 4.6% of men. Health outcome parameters showed however, no significant differences in women compared to men for eGFR <60ml/min (2.5 versus 3.0%), blood pressure ≥140/90mmHg (22.5 versus 21.3%) and hyperglycemia >1.26g/L (17.8 versus 16.5%).

Conclusions: In Morocco, obesity is clearly more present in women than in men. Using WHR, more than half of the female population has to be considered as obese. Despite the difference in BMI, no significant gender differences could be observed in the prevalence of CKD, hypertension or diabetes.

Funding: Government Support - Non-U.S.

PUB193

Intraclass Correlation Coefficients for Cluster Randomized Trials in CKD H. Lester Kirchner, Robert M. Perkins. *Geisinger Clinic, Danville, PA.*

Background: Cluster randomization trials (CRT) are a type of pragmatic clinical trial useful for testing new interventions in real-world settings. Large health care systems provide access to robust cohorts in which primary care clinics (PCC) may serve as the cluster unit. CRT requires sample size calculations which incorporate the intraclass correlation coefficient (ICC) and design effect (DEFF).The ICC measures variation between and within clusters and represents the lack of independence. The DEFF quantifies the inflation of sample size necessitated by clustering. The purpose of this study was to determine ICCs and DEFFs for common variables at the PCC level for CKD patients.

Methods: . Adult patients receiving primary care through Geisinger Clinic between January 1, 2001 and December 31, 2011, with stage 3-5 CKD and no history of renal replacement therapy were eligible for the study. Clinical data were obtained from the patients' most recent outpatient encounter prior to December 31, 2011 and before renal replacement therapy to mimic trial enrollment of prevalent CKD patients. ICCs were calculated from a random intercept linear model treating the PCC as the cluster.

Results: 12,215 adults met inclusion criteria from a total of 41 PCCs (median number of CKD patients/PCC was 214). The average age was 74.6 years with mean CKD duration of 5.9 years. 60.5% were male, 46.1% had diabetes, 23.1% had peripheral arterial disease, and 36.0% had proteinuria. The largest ICCs were observed for serum albumin (0.022) and diastolic BP (0.020) yielding DEFFs of 1.3 to >3.0. These results are similar to that seen in general primary care populations.

Variable	Mean (SD)	ICC (95% CI)
Cholesterol	166.3 (41.8)	.011 (.006, .021)
HDL	47.6 (14.7)	.001 (.000, .012)
LDL	89.3 (33.3)	.013 (.007, .023)
Albumin	4.0 (0.5)	.022 (.012, .036)
Serum Creatinine	1.5 (0.7)	.005 (.002, .011)
eGFR	53.2 (16.0)	.004 (.001, .010)
Systolic BP	127.3 (18.7)	.016 (.009, .027)
Diastolic BP	69.2 (10.4)	.020 (.011, .034)
Body Mass Index	30.5 (7.2)	.006 (.003, .014)

Conclusions: These findings imply that trial sample sizes for CRTs enrolling CKD patients will require substantially more subjects than traditional non-clustered designs.

PUB194

Obstacles and Constraints in the Implementation of a Population Based Screening Program of Chronic Kidney Disease in Morocco (MaReMar) Zamd Mohamed,[1] Mohammed Benghanem Gharbi,[1] Monique M. Elseviers,[2] Abdelali Belghiti Alaoui,[3] Mohammed El Hassane Trabelssi,[3] Naima Benahadi,[3] Ramdani Benyounes,[1] Bayahia Rabia,[1] Marc E. De Broe.[4] *[1]Moroccan Society of Nephrology; [2]University of Antwerp, Wilrijk, Belgium; [3]Ministry of Health, Morocco.*

Background: The MaReMar study estimated the prevalence of chronic kidney disease and the associated risk factors in a representative sample of the population of Morocco aged 26-70. We aim to highlight the difficulties faced in the implementation of the survey and in the recruitment of participants.

Methods: A stratified random sample of the population, using official voting lists, was taken. Recruitment was organized at the level of health care area. A trained nurse visited all selected subjects at home offering standardized information using a visual presentation of the planned investigations. An appointment was made for a visit at the local health center to perform the baseline renal screening.

Results: Main difficulties were the access to the electoral list (election period), the elaboration of the Electronic Case Report Form and the related software, training and motivation of the teams and financial issues. Among the selected participants, only 50.56% were found by investigators. The main causes were: incorrect addressesormissing persons (moving, students in other cities, slums which were eradicated in the meantime, travel or deaths). New lists were performed to resolve these difficulties. Among those found, 85.02% accepted to participate in the survey and 82.56% of them came to their first visit to the center. Of the last, 97.73% came the second visit which permit to confirm a possible abnormality detected during the screening.

Conclusions: MaReMar generated strategic informations on the prevalence of chronic kidney disease, hypertension, and diabetes in Morocco. It is also an important source of information on the difficulties encountered in the implementation of such survey in the field, allowing others to benefit from the experience of MaReMar team.

Funding: Government Support - Non-U.S.

PUB195

Low Heart Rate Variability and Risk for Renal and Cardiovascular Outcomes and All-Cause Mortality in CKD: A CRIC Study Paul E. Drawz,[1,2] Denise C. Babineau,[1] Carolyn S. Brecklin,[2] Jiang He,[2] Radhakrishna Reddy Kallem,[2] Elsayed Z. Soliman,[2] Dawei Xie,[2] Amanda Hyre Anderson,[2] Mahboob Rahman.[1,2] *[1]Case Western Reserve University; [2]CRIC Study Group.*

Background: The goal of this study was to evaluate: 1) the characteristics associated with heart rate variability (HRV) and 2) the association between HRV and adverse outcomes in patients with CKD.

Methods: In the Chronic Renal Insufficiency Cohort study, a 10 second ECG was obtained at baseline. The relationships between clinical and demographic characteristics

and HRV, as measured by SDNN (standard deviation of all R-R intervals) and RMSD (root mean square of successive differences between all R-R intervals), were assessed using linear regression. Cox models were used to assess the association between baseline HRV and the relative hazard rate of renal outcomes, cardiovascular (CV) outcomes (composite of myocardial infarction, congestive heart failure, and stroke), and all-cause mortality.

Results: 3276 of the 3939 subjects in CRIC had available data and were included in the present analyses. Lower HRV was associated with older age, congestive heart failure, and diabetes, as well as elevated diastolic BP, phosphorus, FGF23, A1c, and C-reactive protein and marginally associated with low estimated glomerular filtration rate (eGFR) but not proteinuria. Median follow up was 4.2 years. In longitudinal analyses adjusted for age, gender, race, eGFR, and proteinuria, lower HRV was associated with an increased relative hazard ratio for renal outcomes (SDNN HR 1.11, *P*=0.02; RMSD 1.13, *P*=0.01), CV outcomes (SDNN 1.23, *P*<0.001; RMSD 1.14, *P*=0.02), and all-cause mortality (SDNN 1.20, *P*=0.007; RMSD 1.14, *P*=0.06). In fully adjusted models, lower HRV was not associated with renal outcomes but was marginally associated with an increased risk for CV outcomes (SDNN 1.10, *P*=0.08) and all-cause mortality (SDNN 1.13, *P*=0.09; RMSD 1.10, *P*=0.2).

Conclusions: HRV was associated with numerous risk factors for renal and cardiovascular disease. Independent of eGFR and proteinuria, lower HRV was associated with increased risk for renal and cardiovascular outcomes and all-cause mortality, although this effect was attenuated after adjustment for additional covariates.

Funding: NIDDK Support

PUB196

Late Referral of Children Who Develop Established Renal Failure: A 15 Year Renal Registry Study

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Background: Early referral of children with chronic kidney disease (CKD) to a paediatric nephrology centre is recommended to minimise clinical complications related to CKD in childhood, as well as delaying progression to established renal failure (ERF), improving pre-emptive transplantation rates, and optimising renal replacement therapy (RRT) work up. There is currently no published data on late referral rates in the UK paediatric RRT population.

Methods: Using data provided by the UK Renal Registry we analysed all incident patients starting renal replacement therapy (RRT) aged >3months and <16years between 1996 and 2010. Late referral was defined as having seen a paediatric nephrologist less than 3 months from commencing RRT.

Results: Of 1554 eligible patients, analysis was performed on 1347 patients (86.7%) in whom late referral was calculable using registry data. Overall late referral was seen in 25.5% (n=343) of patients. Late referral was significantly lower in males 20.18% (n=156) than females 32.75% (n=188), P<0.0001, and highest in the 3-months-2 years age group 31.61% (n=49) p=0.0005. No significant differences in late referrals were noted amongst different ethnic groups, different UK paediatric centres, or when comparing late referrals over 5-year time intervals 2006-2010, 2001-2005, and1996-2000.

Conclusions: A significant proportion of children who develop ERF are referred late to nephrology units in the UK, with significantly higher rates seen in female patients and those under 2 years old. There is a need to understand these differences and the reasons behind late referral, to reduce its occurrence and the potential exposure to children of clinical complications that accompany late referral.

PUB197

First Report from the Chronic Kidney Disease (CKD) Registry, Queensland, Australia

Sree Krishna Venuthurupalli,[1,2] Wendy E. Hoy,[2] Zaimin Wang,[2] Helen G. Healy,[2,3] Anne Salisbury,[2] Robert G. Fassett.[2,3] *[1]Renal Medicine, Toowoomba Hospital, Toowoomba, Queensland, Australia; [2]Centre for Chronic Disease, University of Queensland, Brisbane, Queensland, Australia; [3]Renal Medicine, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.*

Background: CKD Queensland (CKD.QLD) is the first CKD surveillance system in Australia. Its CKD Registry, established in 2011, includes all CKD patients in QLD public renal services. We describe the profile of the first patients recruited.

Methods: After ethical and governance approval, and with informed consent, recruitment of CKD patients to the to the Registry has begun, with summary information from their practice sites transferred to a central repository.

Results: Over 2300 CKD patients have been recruited, from an estimated 15,000, and data of the first 1000 analysed. Mean age was 64.5 years and included 55% males and 31.6% of age ≥ 75 years, with less gender differential and more elderly patients, compared to QLD incident ESKD patients on Renal Replacement Therapy (RRT) (62% and 22% respectively). The aetiology of CKD was unknown (36%), renovascular disease (10.2%), diabetic nephropathy (5.8%) and hypertension (HT) (5.4%). Most patients had CKD stage 3 (43.5%) or 4 (31%) and 9% were stage 5, not on dialysis.

There were no major differences in age and gender between a large metropolitan/urban site (N=560) and two regional/rural sites (N=265). Renovascular disease and HT were more commonly attributed causes in urban compared with regional areas (18.2% vs. 4.8% and 9.6% vs. 2.5% respectively p<0.05), but an uncertain aetiology was more common in regional areas (44.2% vs. 31%).

Conclusions: The higher number of elderly in the CKD.QLD group than in QLD's RRT population probably reflects many CKD patients who do not ultimately receive RRT. The higher proportion of females suggests different outcomes and/or rates of progression of CKD, and/or selection biases for RRT. The greater proportion of CKD patients in regional areas with an uncertain CKD aetiology may be genuine or reflect lack of diagnostic resources. All will be better understood with an expanded group of CKD patients, and longitudinal surveillance of their course.

PUB198

Co-Prevalent Diabetes and Kidney Disease Does Not Increase Fracture Risk Compared to Diabetes or Kidney Disease Alone

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Background: Chronic Kidney Disease (CKD) and type 2 diabetes mellitus (DM) are both associated with increased fracture (Fx) risk. However, it is not fully clear whether co-prevalent CKD and DM (DM-CKD) is associated with higher Fx risk than CKD or DM alone. We conducted a cross-sectional analysis of the continuous National Health and Nutrition Examination Survey (NHANES) to test the hypothesis that patients with DM-CKD are at higher Fx risk than patients with either DM or CKD alone.

Methods: We included subjects, ≥40 yrs, enrolled in NHANES from 2000 to 2010 (n=26,070). DM status and a reported history of Fx at any site were ascertained from responses to NHANES questionnaires. CKD status was dichotomized based on an eGFR<60 ml/min. Continuous and categorical data are presented as means±SD and percents (%), respectively. Uni- and multivariate logistic regression were used to compare Fx prevalence rates.

Results: DM patients (n=2,240) had a mean age of 60±14 yrs, eGFR of 98±32 ml/min and body mass index (BMI) of 32±7; 25% were black, 49% were female and 12% reported a Fx. CKD patients (n=1,264) had a mean age of 73±12 yrs, eGFR of 47±12 ml/min and BMI of 28±5; 12% were black, 56% female and 16% reported a Fx. DM-CKD patients (n=530) had mean age of 71±10 yrs, eGFR of 44±14 ml/min and BMI of 32±8; 22% were black, 54% female and 13% reported a Fx. Compared to patients with DM or CKD, Fx prevalence was not higher in patients with DM-CKD (OR 1.10, 95%CI 0.83-1.46; OR 0.79, 95%CI 0.59-1.07, respectively for DM-CKD versus DM or CKD). However, prevalent Fxs were 41% more common in patients with CKD than with DM (OR 1.41, 95%CI 1.13-1.70). Adjustment for age, gender, race and BMI did not alter relationships between disease status and Fxs.

Conclusions: In summary, these analyses suggest that Fxs are more common in patients with CKD than with DM, but co-prevalent CKD and DM does not confer additional risk above that of patients with DM alone. Epidemiologic and mechanistic studies are needed to evaluate the effects of DM-CKD on site-specific Fx rates and the biochemical and microarchitectural basis of fragility in these common chronic diseases.

Funding: NIDDK Support

PUB199

Rate of GFR Decline Estimated Using Joint Models of GFR Slope Is Strongly Associated with Long-Term Outcomes in the MDRD Study

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Background: The rate of decline of GFR over time intuitively relates to risk of ESRD and potentially death. However, assessment of this relationship has been limited by short follow-up.

Methods: 839 subjects from the MDRD randomized trial (conducted between 1989-1993 with iothalamate GFR (iGFR) measurements every 4 months; mean baseline iGFR of 32 ml/min/1.73 m^2) were included. Assessment of the relationship between slope of iGFR decline and the composite outcome of ESRD or death prior to ESRD was evaluated using a joint mixed linear model with both survival and rate-of-change components (described by Vonesh et al.) This approach allows use of all data regardless of timing of the endpoint. A hazard ratio was calculated for slope of decline and adjusted for baseline predicted iGFR as well as other risk factors associated with progression of kidney disease.

Results: Median follow-up was 5.9 years (range 0.25-18.6). Slope of iGFR during the trial showed a wide range (mean of -7.7 ml/min/1.73m^2/year in the quartile with most rapid decline and mean of +0.4 ml/min/1.73m^2/year in the quartile with least rapid decline). The endpoint was reached by 86% of subjects during long-term follow-up (ESRD 616, death prior to ESRD 106). There was a strong relationship between the slope of iGFR and risk of the endpoint (Table). This was not appreciably attenuated after multivariable adjustment.

	Hazard ratios (95%CI): unadjusted	Hazard ratios (95%CI): adjusted
iGFR slope, per 1 ml/min/1.73m^2/year more rapid decline	1.52 (1.44, 1.61)	1.48 (1.40, 1.56)
Predicted baseline iGFR, per 1 ml/min/1.73m^2 decrease	1.09 (1.08, 1.10)	1.09 (1.08, 1.10)

All HR were significant with p<0.001. Both models included slope and baseline iGFR; adjusted model included sex, age, race, proteinuria, MAP, transferrin level, cause of kidney disease, and HDL.

Conclusions: Slope of GFR during a short period is strongly associated with long-term outcomes using a joint modeling approach. Further investigation should explore how this relationship varies with time and methods of incorporation into prediction of outcomes in individual patients.

Funding: NIDDK Support

PUB200

Outcome of Late Steroid Resistant Nephrotic Syndrome in Children: A Study by the Midwest Pediatric Nephrology Consortium Caroline E. Straatmann, V. Matti Vehaskari. *Pediatrics, Louisiana State University Health Sciences Center, New Orleans, LA.*

Background: Idiopathic nephrotic syndrome (NS) is classified as steroid-sensitive or steroid-resistant. Some initially steroid-sensitive patients later develop steroid resistance, sometimes with histologic progression to focal segmental glomerulosclerosis (FSGS). Patients who develop late steroid resistance (LSR) often receive intensified immunosuppression (IS) with multiple, potentially toxic drugs. The impact of intensified IS on the long-term prognosis of these children is unknown.

Methods: Retrospective chart review was performed in 29 patients from 8 centers who developed LSRNS from 2002-2009 to evaluate outcome and impact of additional IS therapy. Outcome measures were: response of proteinuria to each drug, proteinuria and eGFR at latest follow-up, and number of adverse events. Role of demographic factors and histology in outcome was also analyzed.

Results: Nineteen males (66%) and 10 females (34%) were enrolled. There was equal distribution among whites and African-Americans. Median duration of follow-up was 75 months. Majority (72%) received treatment with ≤ 2 drugs. Calcineurin-inhibitors (CNIs) and mycophenolate mofetil (MMF) were most frequently used. Sixty-seven percent received steroids ≥50% of the total treatment time. CNIs had highest individual rate of remission (86%.) At final outcome, 70% percent of patients were in complete or partial remission. Three patients developed ESRD, but renal function was overall well-preserved with no significant change in estimated GFR over study period. Minimal adverse events were noted, with infection being most common. Age, ethnicity, or histology was not significantly associated with final outcome. More males remained nephrotic or developed ESRD (42% vs. 10%.) Patients with a shorter median time to development of LSR had significantly better final outcome.

Conclusions: Most LSR patients respond to additional non-steroid IS. Additional IS after documented non-response to 2 drugs may not be beneficial but does not seem to be deleterious either. LSRNS may have a relatively good long term outcome and the risk: benefit of escalating IS therapy in LSRNS does not appear to be highly unfavorable.

PUB201

Kidney Injury Markers in Nicaraguan Adolescents in a Region of an Epidemic of CKD of Unknown Etiology Daniel R. Brooks,[1] Oriana Ramirez-rubio,[1,2] Juan Jose Amador,[1] James S. Kaufman,[3] Chirag R. Parikh,[4] Usman Ahmed Khan,[4] Michael Mcclean,[1] Rebecca L. Laws,[1] Daniel E. Weiner.[5] *[1]Boston University School of Public Health; [2]Universidad Autonoma de Madrid; [3]VA Boston Healthcare System; [4]Yale University School of Medicine; [5]Tufts University School of Medicine.*

Background: An epidemic of CKD is occurring in Central America with over 20,000 deaths, mainly among younger men. Most studies have focused on occupational factors, but the early age at diagnosis suggests that initial injury may begin in childhood.

Methods: We studied markers of kidney injury in 200 students (age 12-18) with no work history from 4 schools in Nicaragua. Schools represented a range of risk based on adult CKD mortality data. Urine was tested by dipstick and analyzed for ACR, NGAL, NAG, and IL-18.

Results: Dipstick proteinuria (3%) or glucosuria (1%) were rare, and 8% had ACR >30 mg/g. The median IL-18 level (pg/ml) was higher than in healthy controls identified from other studies (45, IQR 21-115 vs. 15, IQR 7-28). The ratios of mean NGAL, NAG, and IL-18 levels are shown by sex and school in the table. Males had lower levels than females for all markers. The results by school were consistent with the a priori risk. Among males, the highest mean levels of NAG, NGAL, and IL-18 were at the highest risk school. Females at the two highest risk schools had elevated levels of NAG. Results were the same regardless of whether measurements were normalized for creatinine.

	Mean ratio (95% CI) of markers by sex and school		
	NAG	NGAL	IL-18
Sex			
F	Ref	Ref	Ref
M	0.78 (0.58-1.05)	0.25 (0.20-0.32)	0.29 (0.23-0.37)
School (Males)			
1 (lowest risk)	Ref	Ref	Ref
2	1.43 (0.70-2.92)	1.36 (0.87-2.13)	0.58 (0.35-0.96)
3	2.15 (1.13-4.07)	0.77 (0.52-1.16)	0.85 (0.54-1.33)
4 (highest risk)	3.25 (1.61-6.55)	1.53 (0.98-2.38)	1.62 (0.99-2.66)
Schools (Females)			
1 (lowest risk)	Ref	Ref	Ref
2	1.28 (0.86-1.90)	0.76 (0.45-1.27)	0.65 (0.40-1.05)
3	2.00 (1.36-2.94)	1.28 (0.77-2.13)	0.64 (0.40-1.04)
4 (highest risk)	2.27 (1.46-3.52)	1.40 (0.79-2.50)	0.57 (0.33-0.98)

Conclusions: The results suggest that tubular kidney injury may be present among youth in an area of epidemic CKD. If confirmed, factors in addition to occupational exposure should be studied as possible causes of CKD.

Funding: Private Foundation Support

PUB202

Clinicopatholgical Manifestation in Elder Patients of Idiopathic Membranous Nephropathy Shinji Kitajima,[1] Tadashi Toyama,[1] Kiyoki Kitagawa,[1] Hitoshi Yokoyama,[2] Takashi Wada.[1] *[1]Department of Nephrology, Kanazawa University Hospital, Kanazawa, Japan; [2]Division of Nephrology, Kanazawa Medical University Hospital, Kanazawa, Japan.*

Background: The 20-year renal survival of Idiopathic membranous nephropathy (IMN) in Japanese adults with nephrotic syndrome was reported around 60%. On the other hand, the rate of remission, renal death, and patient death of elder IMN remains unclear so far. In this study, we evaluated the predisposing clinicopathological factors for elder IMN patients.

Methods: One hundred forty seven patients (85 males and 62 females; mean age 46.3 years) with biopsy proven IMN from 1965 to 2008 in Kanazawa Universitiy Hospital were evaluated in this study. The patients were followed for more than three years, or until renal or patient death. The patients were divided into elder group (aged 65 and over, 21 cases), or younger group (less than 65 years old, 126 cases). Clinicopathological factors, which might affect rate of remission, renal death, and patient death were evaluated.

Results: Rate of patients with nephrotic proteinuria were similar between two group (71.4%). Elder group showed higher remission rate (elder ; 73.3%, younger ; 60.0%). Based on the electron microscopic findings, the patients were assigned to two distinct groups, homogeneous type and heterogeneous type (synchronous electron dense deposits or various phases of dense deposits in basement membrane, respectively) as previously published in *Kidney International* in 2004. The rate of homogeneous type was higher in elder group (elder ; 75.0%, younger ; 58.2%). Although patient death rate (elder ; 9.5%, younger ; 5.5%) was higher in elder group, renal death rate was lower in elder group (elder ; 0%, younger ; 6.4%).

Conclusions: Patient death, rate of homogeneous type, and remission were higher in elder IMN patients. Therefore, elder IMN patients should be paid more attention in patient death as well as remission or renal death.

PUB203

Determinants of Serum Uric Acid and Associations with Cardiovascular Risk Factors in Chronic Kidney Disease Stage 3 Determinants of Serum Uric Acid and Associations with Cardiovascular Risk Factors in Chronic Kidney Disease Stage 3 Adam Kirk,[1] Natasha J. Mcintyre,[1] Richard J. Fluck,[1] Chris W. McIntyre,[1,2] Maarten W. Taal.[1] *[1]Renal Medicine, Royal Derby Hospital, Derby, Derbyshire, United Kingdom; [2]School of Graduate Entry Medicine and Health, Nottingham University, Derby, Derbyshire, United Kingdom.*

Background: Chronic kidney disease is an independent risk factor for cardiovascular (CV) events. Elevated serum uric acid (SUA) has been proposed as a mediator of this association. Our aim was to investigate factors influencing SUA and associations between SUA and CV risk factors.

Methods: We studied 1741 volunteers with eGFR 30-59 mls/min/1.73m^2. From each participant, medical history, demographics, blood and urine were analysed.

Results: Median age was 74 years. 60.4% of participants were female. Median eGFR was 53.2mls/min/l.73m^2. Median SUA was 379 µmol/L with 22.5% having hyperuricaemia. Univariate analysis identified positive correlations with age, serum phosphate, total protein, bicarbonate, cholesterol, HDL cholesterol, BMI, waist:hip ratio and pack years of smoking while an inverse correlation was observed with eGFR. Being male, diabetic, socially deprived (lowest quintile) or receiving diuretics was associated with a significant higher SUA. Allopurinol treatment was associated with lower SUA. Multivariable analysis identified eGFR, diuretics, male gender, BMI, allopurinol and serum total protein as independent determinants of SUA (Adjusted $R^2 = 0.39$). Additionally SUA concentration did not correlate with arterial pulsewave velocity but did with other markers of CV risk including diastolic blood pressure, skin autofluorescence, urine albumin:creatinine ratio and high sensitivity CRP and was higher in those with past history of cardiovascular disease.

Conclusions: SUA concentration is elevated in a significant proportion of persons with early CKD. Several potentially modifiable factors determine it. Correlations with several CV risk factors suggest that SUA may itself be a useful marker of CV risk. This will be tested during planned long term follow up of the cohort. Further randomized trials are required to assess the potential benefits of lowering SUA in persons with CKD.

Funding: Clinical Revenue Support

PUB204

Heredity for Myocardial Infarction Demonstrates More than Two-Folded Increased Risk for Significant Renal Dysfunction in Middle-Aged Men Anders G. Christensson. *Nephrology and Transplantation, Clinical Sciences, Malmö, Malmö, Sweden.*

Background: We have evaluated a potential association between family history of MI and renal dysfunction during long-term follow up of a large, population-based cohort.

Methods: The cohort included 33,125 subjects (22 297 males and 10 828 females), aged 33-60 years at baseline, in a representative, population-based study which enrolled subjects from 1974 to 1992, in the city of Malmö. Median follow-up time was 26 years. Females were significantly older (6 years) than males, 49.7 years and 43.7, respectively, at inclusion. Heredity for MI was defined as mother or father having had MI and/or died from MI, and/or brother or sister having had MI. Estimated GFR (eGFR) was calculated from serum creatinine with the Cockroft-Gault (CG) and abbreviated Modified Diet and Renal Disease(MDRD) equations. We have used CG adjusted for body surface area as the method for GFR estimates in the following work. Every participant filled in a self-administered

questionnaire on medical and personal history including family history of cardiovascular disease. The impact of heredity was analysed using binary logistic regression.

Results: Mean GFR for males was 89±16 ml/min/1.73m2 and for females 83 ml/min/1.73 m2, respectively. Males with heredity for MI at the age of 43 years has a 2.5 times higher risk (p=0.02) of belonging to the group with GFR less than 45 ml/min/1.73m^2 compared to those without heredity.

GFR 45 ml/min. Males

	n	HR	p
Heredity for MI	22153	2.5	0.021
Previous MI	22153	9,8	0.004
Hypertension	22153	2.7	0.020
Diabetes	22153	2.9	0.061
BMI	22153	0.92	0.198
Cholesterol	22153	1.172	0.345
Age	22153	1.045	0.142

For the whole cohort the increased risk was 4.6 times (p=0.147). At a cut-off at 60 ml/min/1.73 m2 the HR was 2.4 (p=0.003) for the whole group and for males HR 1.5 (p=0.001). In the whole cohort previous MI sevenfolded (7.5, p=0.008) the risk of belonging to the group with GFR less than 45 ml/min/1.73m2. For males the HR was 9.8 (p=0,004).

Conclusions: These findings suggest that genetic variants may underly predisposition to CKD in patients with heredity for or history of MI. The genetic background remains largely unknown and needs further exploration.

PUB205

Treatment of Focal Segmental Glomerulosclerosis with ACTH Anna-lena Berg, Julia Dolinina, Omran Bakoush. *Lund University, Lund, Sweden.*

Background: We previously reported that synthetic ACTH1-24 (Synacthen Depot) improved proteinuria in patients with membranous nephropathy. Experimental data suggest that ACTH may reduce proteinuria via activation of melanocortin receptors expressed on white blood cells and/or in the kidney, independent of its effects on cortisol production.

Methods: 10 patients with severe Focal Segmental Glomerulosclerosis (FSGS) (median age 45 yr, range 19-66) were treated with ACTH. Prior to starting ACTH, all patients were treated with prednisolone in combination with other immunosuppressants, including cyclosporine, tacrolimus, mycophenolate mofetil and/or cyclophosphamide. Data was collected over a 3 yr period after replacing prednisolone with ACTH, while continuing therapy with a second immunosuppressive agent. Median time from FSGS diagnosis to initiation of ACTH therapy was 30 mo (range 2-108). Treatment with ACTH started at 1 mg/wk and was increased to a maximum of 1 mg 2x/wk. The median duration of ACTH therapy was 18 mo (range 7-48), and follow up data were obtained 0-29 mo after stopping ACTH. All patients were treated with standard of care, including ACEI/ARB, statins, and other symptomatic therapies.

Results: Urinary albumin decreased from a pre-ACTH median of 5517 mg/L (range 386-26061) to 1086 mg/L (range 69-3296) with ACTH, and remained low at last follow up (median 178 mg/L, range:17-4253) (p<0.01 at both time points vs baseline). Serum albumin increased from a pre-ACTH median of 23 g/L (range 15-35) to 35.5 g/L (range 23-44) after ACTH, and remained improved at last follow up (median 35 g/ L, range 21-43) (p<0.0001 at both points vs baseline). eGFR and serum creatinine did not change significantly with ACTH or at last follow up. Eight patients reached complete (proteinuria <200 mg/L; 3 patients) or partial (proteinuria reduced by 50% to <2000 mg/L; 5 patients) remission. At last follow up, 5 patients were in complete and 2 patients in partial remission, 2 patients were off immunosuppression and 7 patients remained on their second immunosuppressive agent, and one patient progressed to ESRD.

Conclusions: ACTH in combination with other immunosuppressive agents may be more effective than prednisolone for reducing proteinuria in patients with severe FSGS.

Funding: Pharmaceutical Company Support - Questcor Pharm Inc, Government Support - Non-U.S.

PUB206

Healthcare Use and Costs before and after Parathyroidectomy in Dialysis Patients Vasily Belozeroff,[1] Kerry Cooper,[1] Gregory P. Hess,[2] Chun-lan Chang.[2] *[1]Amgen Inc., Thousand Oaks, CA; [2]IMS Health.*

Background: Parathyroidectomy (PTX) is often done in dialysis patients when medical treatment fails to control secondary hyperparathyroidism (SHPT). Such patients typically have been treated with drugs that include vitamin D analogs and calcimimetics, and PTX is viewed by many as a cost-containing measure. Nevertheless, information about health resource utilization and costs before and after PTX is limited.

Methods: This retrospective cohort study used professional service and pharmacy claims to identify dialysis patients undergoing PTX from 1/1/2008-12/31/2010. Subjects with primary hyperparathyroidism or kidney transplant were excluded. Only patients with at least 6 months of information before and after PTX were considered. Prescription use, physician encounters, and surgical complications during the 6 months immediately before and after PTX are reported.

Results: The mean (SD) age of the 181 study subjects was 51 (15) years; 59% female; 80% on Medicare. Overall, the percentage of patients receiving medications to manage altered mineral metabolism increased from 67% before to 79% after PTX. Specifically, cinacalcet use decreased and oral vitamin D use increased resulting in mean (SD) monthly medication charges decrease from $486 (507) to $226 (288) (p<0.01). The mean (SD) number of physician encounters rose from 15 (14) before to 21 (22) after PTX (p<0.01) resulting in the corresponding increase in mean (SD) monthly charges from $1531 (2150) to $1965 (3317) (p=0.08). Hypocalcemia was the predominant diagnosis linked to post-surgical physician encounters occurring in 31% of all subjects; 84% of hypocalcemic episodes were managed in acute care facilities.

Conclusions: The cost of medications to manage SHPT decreased after PTX largely due to reduction in cinacalcet use, whereas vitamin D use increased likely to manage hypocalcemia. The frequency and cost of physician encounters, especially in acute care settings, were higher in the 6 months after PTX attributable largely to episodes of severe hypocalcemia. Overall, the reduction in prescription costs during the 6 months after PTX is outweighed by higher costs associated with physician care.

Funding: Pharmaceutical Company Support - Amgen Inc.

PUB207

The Clinical Usefulness of Serum Cystatin C Levels for Early Detection of Renal Impairment in General Population Yan Qin, Ke Zheng, Xiaohong Fan, Jianfang Cai, Xuewang Li, Xuemei Li. *Kidney Division, Peking Union Medical College Hospital, Beijing, China.*

Background: The aim of the study was to evaluate clinical usefulness of serum cystatin C levels for early detection of renal impairment in general population.

Methods: In the epidemiology study of Pinggu District of Beijing, a total of 6287 participants aged over 18 years old were selected by systematic randomized cluster sampling.The questionnaire, blood pressure, serum creatinine(E), cystatin C levels, and albumin-creatinine ratio (ACR) were determined. Anthropometric and body composition data were also collected at the same time.

Results: In this population 6239 participants (99.24%) present with mildly renal impairment (eGFR more than 60 mL/min/1.73m^2 with CKD –EPI eGFR equation) while the prevalence of albuminuria was 10.58%. ROC curve analysis showed that the cut-off value of serum cystatin C for eGFR<90, <75, <60 mL/min/1.73m^2 was 0.93 (sensitivty 80.68%, specificity 82.44%, AUC 0.89), 1.05 (sensitivty 91.78%, specificity 91.54%, AUC 0.957), 1.27mmol/l (sensitivty 91.78%, specificity 91.54%, AUC 0.984), respectively. On the other hand the cut-off value of serum creatinine(E) for eGFR<90, <75, <60 mL/min/1.73m^2 was 65.9 (sensitivty 84.34%, specificity 69.85%, AUC 0.868), 72.3 (sensitivty 97.96%, specificity 81.87%, AUC 0.967), 84.2μmol/l (sensitivty 100%, specificity 95.86%, AUC 0.996), respectively. In multiple regression analysis, serum cystatin C was affected by age, bodyweight, CrE, total cholesterol(TC), waist to hip ratio(WHR) and ACR. After the adjustment of other factors and controlled on confounding factors, the results of logistic regression analysis demonstrated that DM history, smoke history, drink history, and BMI,LDL-C,TC,UA, total fat and WHR may serve as risk factors of eGFR < 60 mL/min/1.73 m2, their odds ratios (OR) were 2.30, 1.79, 2.07, 1.04, 1.17, 1.11, 1.02, 1.03 and 92.47 (P<0.001) respectively. Higher literate level and inorganic weight might serve as protective factors with OR 0.52 and 0.69 (P<0.001), respectively.

Conclusions: In the general population, the detective ability of serum cystatin C is better than the serum creatinine for mildly-impaired renal function.

Funding: Government Support - Non-U.S.

PUB208

Direct Renin Inhibition for IgA Nephropathy Andrew Lazar, Scot Eskestrand, Brandon Lazar. *Nephrology, Case Western Reserve University, Cleveland, OH.*

Background: IgA nephropathy is the most common primary glomerulonephritis throughout most developed countries of the world. Although this disorder was initially thought to follow a benign course, upwards of 30-40% of affected patients will progress to end stage renal disease over 25-30 years. It has been recognized that the intrarenal RAS system can manufacture angiotensin II locally, turning on NADPH oxidase and other proinflammatory pathways. It has been increasingly recognized that renin and prorenin directly, by acting on the Prorenin-renin receptor, can activate the RAS leading to angiotensin II production as well as AngII-independent mechanisms such as ERK-MAP kinase activation. Such receptors have been identified in both the mesangium and podocytes, raising the question of whether direct renin inhibition may be a treatment option for IgA nephropathy patients.

Methods: We identified two such patients with IgA nephropathy and proteinuria, started aliskiren in addition to an angiotensin receptor blocker at maxiumum dose. Both subjects had a normal creatinine. Blood pressure was less than 130/80 mmHg for both subjects prior to the addition on aliskiren. Both patients had microscopic hematuria.

Results: Within 10 weeks, subject one enjoyed a 67% drop in proteinuria, 86% by 4.5 months. Subject two enjoyed a drop of 56% at 6 months, 47% at two years. Blood pressure remained at goal, no symptoms of hypotension, no adverse effects. Both subjects had negative dipsticks for blood post-aliskiren.

Conclusions: As proteinuria reduction largely predicts renoprotection in many proteinuric renal disease outcome studies, results such as these are thought-provoking, and hypothesis-generating. Aliskiren was well-tolerated and was efficaceous in terms of proteinuria reduction, and may be a reasonable treatment option for IgA nephropathy patients, but warrants further evaluation.

PUB209

The Analysis of Prognosis and Risk Factor for CKD in HIV-Infected Japanese Patients Junya Yamamoto,[1] Daigo Nakazawa,[1] Yasunobu Ishikawa,[1] Naoko Matsuoka,[1] Akiko Sato,[1] Tasuku Nakagaki,[1] Sekiya Shibazaki,[1] Katsuya Fujimoto,[2] Tomoyuki Endo,[2] Saori Nishio,[1] Tatsuya Atsumi.[1] *[1]Department Medicine II, Hokkaido University, Sapporo, Hokkaido, Japan; [2]Department of Hematology, Hokkaido University, Sapporo, Hokkaido, Japan.*

Background: As HIV-infected patients live longer while receiving highly active antiretroviral therapy, chronic disease, in particular chronic kidney disease (CKD) has increased. In this study we aimed to evaluate the course and feature of CKD /HIV patients for long time.

Methods: We retrospectively followed 153 HIV patients who were treated at Hokkaido University hospital for more than a year (from 1990 to 2011). We recorded their CKD stage and various factor (age, gender, serum-creatinine, eGFR, serum albumin, proteinuria, hematuria, presence of hemophilia, HCV/HBV infection, diabetes mellitus, hypertension, hyperlipidemia). We evaluated the proportion of CKD stage at the end of visit and risk factor for progressive CKD by the univariate and multivariate regression analysis.

Results: First visit findings were the following; Age 44.5 years, serum-creatinine 0.80 mg/dl, eGFR 99.6 ml/min/1.73m^2, serum-albumin 4.38 g/dl (these data show average), men/women 149/4, proteinuria (≥1+) 11.3%, hematuria (≥1+) 6.1%, hemophilia 18.3%, HCV 22.3%, HBV 12.0%. The average of observation period was 7.1 years. The average of eGFR reduction rate in HIV patients was 2.9 %/year and was significantly higher than in general Japanese (0.36 %/year). The number of CKD stage at the end of visit was non-CKD; 75, CKD2; 66, CKD3; 11, CKD4; 0, CKD5; 1. During this course the following complications occurred; diabetes mellitus 7.8%, hypertension 15.6%, hyperlipidemia 23.8%. In CKD patients, age, the frequency of low eGFR (first visit), the presence of proteinuria and hypertension tended to be higher than in non-CKD patients. By multivariate regression analysis, age, low eGFR (first visit), proteinuria were significantly associated with the progression of CKD.

Conclusions: CKD was prevalent in HIV-infected Japanese patients, The eGFR levels were significantly decreased in HIV patients. Age, eGFR (first visit), presence of proteinuria could possibly be risk factor for the progression of CKD.

PUB210

Medication Adherence to Antihypertensive and Phosphate Lowering Medications in Hemodialysis Patients John Paul Harmon,[1] Martin Glen MacKinnon.[2] *[1]Medical Education, Dalhousie University, Saint John, NB, Canada; [2]Internal Medicine, Division of Nephrology, Horizon Health Network, Saint John, NB, Canada.*

Background: The Saint John Regional Hospital nephrology department dispenses prescription medication to almost all of its hemodialysis patients. This provides a relatively unique opportunity to study medication adherence in this population. Medication possession ration (MPR) is a commonly used formula that estimates medication adherence. It is used to calculate the percentage of time a patient has access to a medication using prescription refill history. Many trials consider an acceptable adherence rate to be greater than or equal to 80%.

Methods: We identified non-acute hemodialysis patients who had prescriptions filled between January 1, 2010 and October 31, 2011. In this retrospective study, we collected patient refill data for phosphate lowering and antihypertensive medications. A total of 37 patients met our inclusion criteria and gave consent. MPR values were calculated and compared to average serum phosphate levels, an average of pre- and post-dialysis blood pressures and average fluid removed on dialysis.

Results: Non-adherence (MPR <0.80) was seen in a significantly greater proportion of patients taking phosphate lowering medication (N=21 of 33, 64%) compared to antihypertensive medications (N=5 of 27, 19%) [$X^2(1, N = 37) = 10.54$, $p = .001$]. Patients who were adherent to phosphate lowering medication had significantly lower serum phosphate levels compared to their non-adherent counterparts.

Phosphate Lowering Medications

Mean combined MPR	Number of patients	Mean serum phosphate
<0.8	21 (64%)	2.07mmol/L
≥0.8	12 (36%)	1.73mmol/L

[$t(28)=2.31$, $p = .029$]

Medication adherence to antihypertensive medication was not a significant factor contributing to mean pre- and post-dialysis blood pressures or average fluid removed on hemodialysis.

Conclusions: Medication adherence is likely an important factor contributing to serum phosphate levels in hemodialysis patients. Clinicians should be aware of lower adherence rates to phosphate lowering medications and develop approaches to improve adherence as part of a comprehensive strategy for better serum phosphate control.

Funding: Government Support - Non-U.S.

PUB211

Predictive Values of PTH[1-84] and of PTH[1-84] to PTH[non-1-84] Ratio for Mortality in Patients with Chronic Kidney Disease Cedric Gauci,[1,2] Benedicte Stengel,[2] Marc Froissart,[1,2] Jean-philippe Haymann,[3] Tilman B. Drueke,[4] Pascal Houillier.[1] *[1]Division of Physiology, HEGP Hospital, Paris-Descartes University, Paris, France; [2]Inserm U1018, CESP, Villejuif, France; [3]Division of Physiology, Tenon Hospital, Pierre and Marie Curie University, Paris, France; [4]Inserm U1088, Picardie University, Amiens, France.*

Background: Excessive serum 'intact' parathyroid hormone (iPTH) levels in dialysis patients are associated with an increase in the relative risk (RR) of mortality. The commonly used iPTH assays measure both PTH[1-84)] and PTH fragments that may exert actions opposite to those of PTH[1-84]. Thus, PTH[1-84] or the ratio of PTH[1-84] to PTH[non-1-84] could be better predictors for mortality than iPTH.

Methods: We tested these hypotheses in 343 CKD stage 2-5 patients from the French NephroTest cohort, age 59 years, men 73%, diabetes 26%. Serum PTH[1-84] and iPTH were measured by the Duo PTH assay (Scantibodies) (normal ranges, 5-36 and 15-66 pg/mL, respectively), and PTH[non-1-84] calculated as the difference between iPTH and PTH[1-84].

Results: Baseline PTH[1-84] was 34.7 pg/mL, and iPTH 49 pg/mL. Patients were subdivided according to baseline PTH[1-84] quartiles: Q1 [< 22 pg/mL], Q2 [22-35], Q3 [35-59], and Q4 [>59]. After a mean follow-up of 6 years, 10, 16, 20, and 29 deaths were observed in Q1, Q2, Q3, and Q4 patient subgroups respectively. Crude hazard ratios (HR) and 95% confidence intervals for all-cause mortality were 1.61 [0.73-3.55], 2.43 [1.14-5.19], and 5.31 [2.58-10.93] for Q2, Q3, and Q4 as compared with Q1 (reference). An adjusting model for age, gender, diabetes and 24hr proteinuria with stratification for baseline mGFR provided HR of 1.6 [0.70 - 3.66], 1.36 [0.60 -3.07], and 2.53 [1.14 – 5.59] for Q2, Q3, and Q4 respectively. Neither PTH[1-84] to PTH[non1-84] ratio nor iPTH alone were associated with mortality risk. Presence of diabetes and proteinuria conferred higher mortality RR.

Conclusions: In patients with CKD stages 2-5 a high PTH[1-84] level, but not iPTH or PTH ratio, is associated with an increased risk of mortality. Distinguishing total PTH from its fragments may be important in terms of patient outcomes.

Funding: Government Support - Non-U.S.

PUB212

Renal-Angiomyolipoma Related Conditions before and after Renal Artery Embolization or Nephrectomy: A US National Retrospective Cohort Study Hearns Charles,[1] Peter Sun,[2] Zhimei Liu,[3] Judith A. Prestifilippo,[3] John C. Hulbert,[4] John J. Bissler.[5] *[1]NYU Langone Medical Center; [2]Kailo Research Group; [3]Novartis; [4]Urologic Physicians PA; [5]University of Cincinnati.*

Background: This study aimed to compare renal angiomyolipoma (AML) related conditions before and after renal artery embolization (RAE) or nephrectomy in patients with AML in the United States.

Methods: We conducted a retrospective cohort study using three US national health claims databases (Years: 2000-2010; Covered population: >60 millions). AML patients with health plan enrollment in the year preceding and following newly observed embolization or nephrectomy were included. Each embolization or nephrectomy cohort was further divided into TSC-AML and sporadic AML subgroups based on patients' TSC claim history. The prevalence rates of AML related clinical conditions were estimated. Repeated measures analysis and bootstrapping methods were used to assess cross-period differences in these prevalence rates.

Results: The embolization cohort (N= 9,901) had a mean age of 50.6 years at the newly observed RAE. AML related conditions with significant post embolization increase (before vs. after) were unspecified disorder of kidney or ureter (17.1% vs. 19.2%, $p<0.05$), end stage renal diseases (1.6% vs. 2.3%, $p<0.05$), anemia in chronic kidney disease (1.8% vs. 2.4%, $p<0.05$), and gross hematuria (0.0% vs. 0.2%, $p<0.05$). Non-acute renal insufficiency increased in the TSC AML sub-cohort (5.8% vs. 6.0%, $p<0.05$), but decreased in the sporadic AML sub-cohort (4.8% vs. 4.0%, $p<0.05$) after RAE. The nephrectomy cohort (N=15,381) had a mean age of 52.2 years at the newly observed nephrectomy. Unspecified disorder of kidney or ureter (15.0% vs. 16.2%, $p<0.05$), anemia in chronic kidney disease (1.7% vs. 2.0%) and end stage renal diseases (1.5% vs. 1.8%, $p<0.05$) increased after surgery. These trends were consistent for TSC AML and sporadic AML. Other AML related conditions remained similar after the procedures.

Conclusions: This study suggested that after RAE or nephrectomy, the prevalence of some AML related clinical conditions in patients with AML seemed to increase. This association warrants further investigation.

Funding: Pharmaceutical Company Support - Novartis Pharmaceuticals Corporation

PUB213

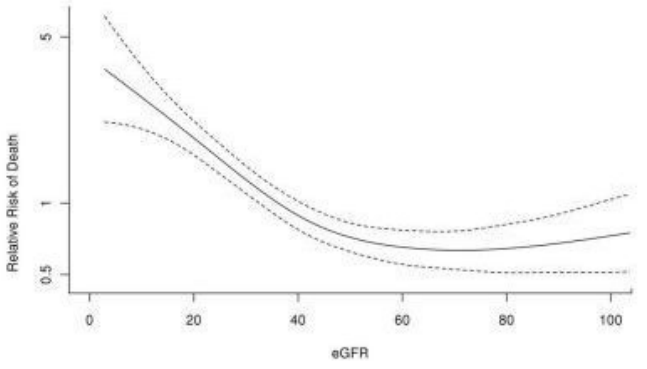
Chronic Kidney Disease Progression and Mortality in Liver Transplant Recipients Alina M. Allen, W. Ray Kim, Terry Therneau, Chun Fan, Andrew D. Rule, Julie Heimbach. *Gastroenterology and Hepatology, Biomedical Statistics, Nephrology, Transplant Surgery, Mayo Clinic, Rochester, MN.*

Background: Chronic kidney disease (CKD) is a common comorbidity in liver transplant (LTx) recipients. It is well known that CKD leads to increased mortality; the exact magnitude of mortality risk as a result of reduced renal function is not defined in LTx recipients.

Methods: A database that prospectively tracks all LTx recipients at this academic transplant program was queried to identify all adult primary LTx recipients. Our post-LTx

protocol incorporates glomerular filtration rate (GFR) at regular intervals. If measured GFR data were not available, it was estimated by using a formula that we previously developed based on age, sex, standardized serum creatinine, albumin and hemoglobin. Time-dependent Cox regression analysis was performed to evaluate the impact of GFR changes on survival.

Results: A total of 1860 liver transplant recipients met the eligibility criteria (median age was 52 years, 58% male and 78% Caucasians). After a mean follow-up of 7.6 years, 612 (32.9%) died and 58 (3.1%) underwent renal transplantation. The figure illustrates the influence of GFR on mortality. Mortality risk does not increase when GFR remained above 60 (CKD stage 2 or less); reduction in GFR below 60 was associated with significant increase in the risk of death. Thus, patients in CKD stage 3 had 1.17 fold increase ([HR]=1.17, 95%[CI]=0.89-1.55) in mortality, whereas CKD stage 4 had 2.5-fold increase (HR=2.5, 95%CI=1.8-3.45). As expected, patients with endstage CKD faced extremely high mortality risk (HR=4.24, 95%CI=2.69-6.67).



Conclusions: While early stage CKD (GFR>60) did not have demonstrable effect on mortality, reduction of GFR beyond CKD stage 3 increased mortality in an exponential fashion, thus prevention and early recognition of CKD in LTx recipients is important.

Funding: Private Foundation Support

PUB214

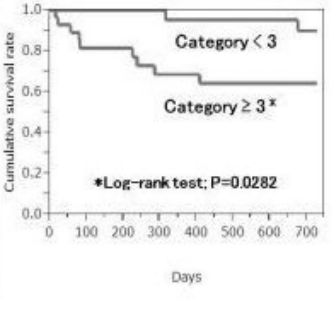
A New Risk Assessment of Myeloma Patients with Chronic Kidney Disease Using Proteinuria and Estimated Glomerular Filtration Rate Minoru Ando,[1] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Department IV of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan.*

Background: A 2009 Kidney Disease: Improving Global Outcomes (KDIGO) conference report proposed a category system adding emphasis on risk stratification for chronic kidney disease (CKD), combining proteinuria and estimated glomerular filtration rate (eGFR). This new category system could be useful to correctly identify myeloma patients at high risk for death.

Methods: A total of 106 patients were diagnosed as myeloma between August 2004 and September 2011 in our hospital. Among them, 48 patients (26 men, 22 women, mean age, 65 years) were prospectively followed-up over 2 years. eGFR at baseline was calculated based on serum creatinine and graded into 5 (≥90, 60-89, 45-59, 30-44, and 15-29 ml/min/1.73m^2). Proteinuria was separated as normal (dipstick test, negative), mild (dipstick test, ± or 1+), and heavy (dipstick test ≥2+). Myeloma patients were classified into 5 risk categories combining both variables. Cumulative survival rate was analyzed by the Kaplan-Meier method, which was stratified by presence and absence of CKD ≥risk category 3.

Results: Classification of all myeloma patients is shown in Table. Seventy-two (68%) had CKD with risk category ≥1, of which 26 (25%) were included in the highest risk category 4. During the 2-year follow-up, 7 out of 43 patients (16%) died, of which 4 (57%) were include in category 4. Cumulative survival rate was significantly lower in patients with CKD ≥risk category 3 (log-rank test; P=0.0282), shown in Figure.

n=106	Proteinuria		
eGFR	Negative	Mild	Heavy
≥90	Risk category 0 No CKD 34 (32.1%)	Risk category 1 18 (17.0%)	Risk category 3 14 (13.2%)
60-89			
45-59	Risk category 1 6 (5.7%)	Risk category 2 3 (2.8%)	Risk category 4 26 (24.5%)
30-44	Risk category 2 2 (1.9%)	Risk category 3 2 (1.9%)	
15-29	Risk category 3 1 (0.9%)		



Conclusions: The new category system may facilitate targeting CKD patients who are at high risk for death among a myeloma population.

PUB215

Outbreak Investigation in Nicaragua: Physician and Pharmacist Perceptions of Chronic Kidney Disease Madeleine Kangsen Scammell,[1] Oriana Ramirez-rubio,[1,2] Daniel R. Brooks,[1] James S. Kaufman,[3] Juan Jose Amador,[1] Daniel E. Weiner.[4] *[1]Boston University School of Public Health, Boston, MA; [2]Universidad Autonoma de Madrid, Madrid, Spain; [3]Boston University School of Medicine, Boston, MA; [4]Tufts Medical Center, Boston, MA.*

Background: Northwestern Nicaragua has a high prevalence of chronic kidney disease (CKD) of unknown etiology among young adult men. A high frequency of urinary tract infections (UTI) is also reported among men along with a syndrome described by manual laborers as "chistata." This study examines health professionals´ perceptions regarding etiology of CKD, UTI and chistata, and their treatment approaches, including use of potentially nephrotoxic medications.

Methods: Nineteen in-person semi-structured interviews were conducted in November 2010 among ten randomly selected physicians and nine pharmacists in the region. Using qualitative methods, responses were coded and analyzed for patterns of agreement or disagreement and the occurrence of themes.

Results: Health professionals perceived CKD as a serious and growing problem in the region, primarily affecting young men working as manual laborers without traditional risk factors for CKD. All interviewees regarded occupational and environmental exposure to sun, heat, and dehydration as critical factors associated with the occurrence of CKD, and noted that hydration practices may be influenced by perceived water contamination. These factors were also considered to play a role in the occurrence of chistata, often treated with non-steroidal anti-inflammatory drugs (NSAIDs), diuretics and antibiotics, including quinolones and aminoglycosides. Physicians acknowledged that the diagnosis of UTI was usually not based on microbial culture, and opined that the use of potentially nephrotoxic medications may be contributing to CKD.

Conclusions: Interviews provided evidence suggesting that diuretics, antibiotics and NSAIDs are widely used and sold over the counter for symptoms that may be related to volume depletion. Acute kidney damage coupled with volume depletion and exposures including medications and should be further evaluated as causal factors for CKD in this region.

Funding: Government Support - Non-U.S.

PUB216

Prevalence of Chronic Kidney Disease in Egypt, Results from the Screening and Early Evaluation of Kidney Disease (SEEK-Egypt Study) Youssef M.K. Farag,[1] Ajay K. Singh,[2] Mohamed Kamel Farag.[3] *[1]Medicine - Renal, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [2]Medicine - Renal, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [3]Epidemiology, Mansoura University Faculty of Medicine, Mansoura, DK, Egypt.*

Background: Chronic kidney disease (CKD) is an inadequately recognized problem in developing countries, including Egypt. The lack of documented large scale population-based research evidence regarding the magnitude of CKD in Egypt is responsible in part for this knowledge gap. This is a health screening research project aimed at estimating the prevalence and risk factors of CKD in Egypt, identifying persons at increased risk for kidney disease, and encouraging at-risk persons to seek further evaluation with appropriate risk management from a healthcare provider.

Methods: We screened 600 apparently healthy subjects as a representative sample of the population of Nile Delta using multi-stage stratified random sampling technique. All subjects were interviewed by a previously trained physician, and data were collected through specifically designed questionnaire used in the SEEK study in other countries. Anthropometric measures and vital signs were recorded. Blood and urine samples were collected.

Results: 600 participants were included in the study. Males comprised 38.5%. Mean age was 42.85 years (±10.86). Mean eGFR was 100.15 ± 27.37 mL/min/1.73 m2. The main finding of this study is that the prevalence of CKD (based on the NKF criteria) is 8%. We found macroalbuminuria in 3% of our sample. When we included participants with abnormal urine albumin/creatinine ratio (UACR), the prevalence of CKD became 15%. CKD was highest among hypertensives ($p<0.001$) and those with high school educational level ($p=0.025$). There was no significant difference in the distribution of CKD as regard to gender or anemia status.

Conclusions: We report a prevalence of CKD of 8-15% from a representative sample from Northern Egypt. Screening programs of individuals at risk of CKD may play an important role in early detection and management of CKD.

PUB217

Stress and Burnout among Nephrology-Dialysis Staff: Results of a 2012 Survey in a Mayo Clinic HD Unit Macaulay A. Onuigbo.[1,2] *[1]College of Medicine, Mayo Clnic, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI.*

Background: The concept of burnout in the workplace was introduced in the late 1970s, mainly in a US context. Healthcare delivery is generally acknowledged to be a stressful industry but few studies in this area are available. Even far less reported is stress or burnout in nephrology practices.

Methods: A preliminary cross-sectional hand-delivered questionnaire-based survey of physicians, nurses, dialysis technicians, social workers and dieticians in a nephrology-

dialysis practice in Mayo Clinic, Eau Claire, WI using the Oldenburg Burnout Inventory (OLBI) (Fig 1) was carried out in January 2012.

The Oldenburg Burnout Inventory

Please provide the following code: The first two letters of your high school (e.g., City High School would be CI), the month and day of your mother's birthday (e.g., July 10 would be 0710), and the last two letters of the city you were born in (e.g., Wausau would be AU). Following the examples above, the code would be CI0710AU.

Please enter your code here: _____

	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
1. I always find new and interesting aspects in my work.	5	4	3	2	1
2. There are days that I feel already tired before I go to work.	5	4	3	2	1
3. It happens more and more often that I talk about my work in a derogatory way.	5	4	3	2	1
4. After my work, I now need more time to relax than in the past to become fit again.	5	4	3	2	1
5. I can stand the pressure of my work very well.	5	4	3	2	1
6. Lately, I tend to think less during my work and just execute it mechanically.	5	4	3	2	1
7. I experience my work as a real challenge.	5	4	3	2	1
8. During my work, I often feel emotionally drained.	5	4	3	2	1
9. With the time, one loses the internal relationship with one's work.	5	4	3	2	1
10. After my work, I usually feel still totally fit for my leisure activities.	5	4	3	2	1
11. Sometimes I feel really sick about my work tasks.	5	4	3	2	1
12. After my work, I usually feel worn out and weary.	5	4	3	2	1
13. I cannot imagine another occupation for myself.	5	4	3	2	1
14. Normally, I can manage the amount of work well.	5	4	3	2	1
15. I get more and more engaged in my work.	5	4	3	2	1
16. When I work, I usually feel energized.	5	4	3	2	1

Results: Eighteen survey questionnaires were distributed across the clinic and sixteen were returned in a completed form, giving a response rate of 89%, mostly female nurses, age range 30-60, average age about 40 years. The average emotional exhaustion score on the OLBI was 2.66, consistent with a low level of emotional exhaustion. Similarly, the average disengagement score was 2.45, again consistent with a low level of disengagement.

Conclusions: The low level of emotional exhaustion and disengagement reported amongst was pleasantly surprising. Higher levels had been anticipated, especially with the inclusion of the dialysis nurses who have often expressed higher levels of anxiety about work-related stressors. The mixture of clerical and clinical staff may have affected the average results obtained from the staff survey. One recurring source of stressors for the staff revolved around the non user-friendliness of the EMR system(s). Further studies focused on specific healthcare professionals are planned in the near future.

PUB218

Radiocontrast Prophylaxis in Patients Receiving Chronic Dialysis Vandana A. Palan, Syed N. Babar, John A. Walker. *Medicine, UMDNJ - Robert Wood Johnson Medical School, New Brunswick, NJ.*

Background: Preserving residual renal function (RRF) in patients receiving chronic dialysis is desirable, since RRF may augment the efficacy of renal replacement therapy and is associated with a lower mortality risk. RRF may be threatened by the intravenous administration of iodinated radiocontrast (IRC) agents. Although radiocontrast prophylaxis (RP) has been studied extensively in the general chronic kidney disease (CKD) population, RP has received little attention in dialysis dependent CKD. The aim of this retrospective cohort study was to investigate the RP prescribing patterns employed in CKD patients receiving chronic dialysis.

Methods: The charts of all adult patients admitted during a six-month period to a single university hospital with ICD codes 585.5 or 585.6 and CPT codes for imaging procedures potentially requiring IRC were identified. Identified charts were reviewed for: 1) type of dialysis and 2) use and modality of RP.

Results: The records of 82 patients meeting the aforementioned criteria were identified; this set included 19 patients with Stage 5 CKD who were not receiving dialysis. Of the the 63 patients receiving chronic dialysis, 13 were not given IRC and were excluded from further analysis, thus yielding a final study cohort of 50 patients.The mean age (± SD) of this cohort was 64.8 ± 14.3 years; 37 were men, and 28 had diabetes mellitus. 42 patients were receiving hemodialysis (HD) and 8 were receiving peritoneal dialysis (PD). 10 of these 50 patients received at least one form of RP:

Types of Imaging Studies and RP Modality

			RP Modality			
Study Type	Total	RP Prescribed	IV Fluid	Acetylcysteine	Withhold ACEI/ARB	Other
Cardiac Catheterization	27	5	3	1	1	1
Fistulagram	13	3	3			
CT Scan	8	0				
Angiogram	2	2			2	

Conclusions: Only 20% of the study cohort received any type of RP; intravenous fluid was the most frequent RP modality. Patients receiving PD were more likely to receive RP than patients receiving HD (75% v. 9.5%, respectively, $p<0.01$). In contrast, 100% of non-dialysis Stage 5 CKD patients received RP. The basis for this relative under-prescription of RP ($p<0.01$) and its impact upon RRF or other parameters in the dialysis population are uncertain and merit further study.

PUB219

Assessment of Dose Relativity: Lanthanum Carbonate versus Sevelamer Rosamund J. Wilson,[1] J. Brian Copley.[2] *[1]Spica Consultants, Marlborough, United Kingdom; [2]Shire Pharmaceuticals, Wayne, PA.*

Background: Knowing the dose relativity of phosphate binders (PBs) may assist clinicians when reviewing a patient's PB regimen. However, the method used to assess dose relativity is critical, because patient type and disease severity may influence the choice and average doses of PBs used. Patient data from a large US dialysis provider that were compared retrospectively based on switches made between common PBs, suggested patients receiving lanthanum carbonate (LC) may have had different characteristics from patients placed on other PBs. Therefore, using such a database to estimate PB dose relativity may be sub-optimal.

Methods: Better estimates of dose relativity may be obtained from observational studies, in which individual patients switch PBs to determine the effectiveness of each, and from randomized clinical studies, in which patients fulfilling similar inclusion criteria are treated to a serum phosphate target and PB dose is reported. We conducted an extensive literature review, evaluated observational studies and reviewed large meta-analyses that assessed the dose relativity of sevelamer (Sev) to LC.

Results: One study in which individuals receiving Sev switched to LC, found that patients who were receiving a mean Sev dose of 7.6 g/day before switching, were receiving LC at 2.8 g/day after 12 weeks (Sev:LC = 2.7). Another study found that patients switching from Sev to LC received a mean Sev dose of 7.3 g/day before switching, and a mean LC dose of 2.3 g/day 1 year after switching (Sev:LC = 3.3). Among patients who switched from any binder, the mean dose of LC was 2.3 g/day, compared with a mean dose of 6.5 g/day for all patients on Sev prior to switching (Sev:LC = 2.8). Two meta-analyses gave dose relativity assessments of 2.7. The largest of these included over 3000 haemodialysis patients and 17 studies. These estimates are generally consistent with the World Health Organization defined daily doses of 6.4 g Sev and 2.25 g LC (Sev:LC = 2.8).

Conclusions: The choice and doses of PBs used in clinical practice that are summarized in large databases may be influenced by patient type and disease severity. Accurate dose relativity assessments may assist management of PB therapy.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

PUB220

Nutritional Status in Haemodialysis Patients Receiving Lanthanum Carbonate Rosamund J. Wilson,[1] Pinggao Zhang,[2] J. Brian Copley.[2] *[1]Spica Consultants, Marlborough, United Kingdom; [2]Shire Pharmaceuticals, Wayne, PA.*

Background: Low serum albumin (alb) can be indicative of poor nutritional status and is a predictor of mortality in haemodialysis (HD) patients. The Dialysis Outcomes and Practice Patterns Study 4 (DOPPS-4) determined the proportion of HD patients achieving the KDOQI guideline alb level of ≥ 4.0 g/dL, and found wide variation between countries (8.5% to 42.5% of patients). This indicates a need to improve alb levels (and possibly outcomes) in HD patients and this may be achievable by increasing protein intake. However, increased protein intake is associated with increased intake of phosphate (P), which can lead to hyperphosphataemia.

Methods: To evaluate and compare the alb and P status of HD patients undergoing lanthanum carbonate (LC) therapy, we conducted post hoc analyses of two large studies: a randomized, controlled clinical study conducted in the EU (n = 510), and a real-world prospective observational study conducted in the USA (n = 2520). Neither study encouraged dietary measures to increase alb levels.

Results: In both studies, the proportion of patients with alb ≥ 4.0 g/dL was essentially unchanged while on treatment. After 24 weeks of treatment in the controlled study, alb ≥ 4.0 g/dL was achieved by 75% (167/223) of patients, and by 80% (97/121) of patients with simultaneously controlled P (3.5–5.5 mg/dL). In the real-world study after 16 weeks of treatment, alb ≥ 4.0 g/dL was achieved by 43% (847/1985) of patients and by 41% (286/694) of patients with simultaneously controlled P.

Conclusions: Most patients in the controlled study met the KDOQI target for alb, implying that patients in clinical trials may not be representative of the overall patient population. The real-world data are consistent with the best countries in DOPPS-4, and highlight that the majority of HD patients are not achieving this target. Measures to increase the proportion of patients achieving alb ≥ 4.0 g/dL, while controlling serum P, may therefore be beneficial. Among patients capable of augmenting their protein intake, increasing P-binder dose, or using high capacity P-binders such as LC, may allow improvements in nutritional status while maintaining simultaneous P control.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

PUB221

Cost-Effectiveness of Lanthanum Carbonate for the Treatment of Hyperphosphatemia in Chronic Kidney Disease Michael S. Keith,[1] Therese M. Conner,[2] Rosamund J. Wilson.[3] *[1]Shire Pharmaceuticals, Wayne, PA; [2]Independent Consultant, Austin, TX; [3]Spica Consultants, Marlborough, United Kingdom.*

Background: Hyperphosphatemia associated with chronic kidney disease (CKD) significantly contributes to patient morbidity and mortality, as well as high treatment cost. Noncalcium phosphate (P)-binders control patients' serum P levels, and are recommended in the presence of hypercalcemia, adynamic bone disease, and vascular calcification. Despite growing interest in costs and outcomes, there have been few cost-effectiveness (CE) analyses of phosphate binder use in CKD. This review summarizes research on the economics of

the noncalcium P-binder lanthanum carbonate (LC) in predialysis and dialysis patients. An additional goal was to summarize cost data between LC and sevelamer hydrochloride (SH).

Methods: On-line databases were used to perform an extensive literature review of publically-available studies published between 2005 and 2011 inclusive. In addition, we evaluated other publically-available studies that reported costs and/or CE associated with LC.

Results: Seven studies of dialysis or predialysis patients were identified and reviewed. Two studies were US-based. Six employed decision analytic modeling methods. Three focused on the use of LC compared with existing treatments, concluding that LC is a cost-effective treatment for hyperphosphataemia. Further, one of these showed that CE ratios were particularly favorable for P > 6.6 mg/dL. Three studies showed cost savings with LC compared to SH, and two of these also demonstrated a lower pill burden with LC. One study provided a head-to-head CE comparison between LC and SH, and reported LC to be the more cost-effective treatment. Patients treated with LC had slightly higher total costs, but had improvements in quality-adjusted life years, survival and greater reductions in serum P levels, than patients treated with SH.

Conclusions: Current evidence indicates LC is a cost-effective treatment for hyperphosphatemia in both predialysis and dialysis patients when compared to calcium-based binders or SH. These findings have been reported in North America and Europe, using different methods.

Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

PUB222

Willingness to Participate in Clinical Research and Perceived Risks of Autosomal Dominant Polycystic Kidney Disease (ADPKD) among African Americans Caitlin Blake, Terry J. Watnick, L. Ebony Boulware, Tariq Shafi, Michael J. Choi, Raquel Greer. *Johns Hopkins Medicine Institutions, Baltimore, MD.*

Background: Despite a similar prevalence of ADPKD in African Americans and Whites, African Americans have been markedly underrepresented in ADPKD research studies. Little is known about African Americans' perceived risks and knowledge of ADPKD or their perceived barriers to participating in ADPKD clinical research.

Methods: We conducted 3 focus groups of African American ADPKD patients (n=9) and 2 focus groups of their family members (n=7) to elicit their knowledge and perceived risks about ADPKD and to identify their self-identified barriers to participating in ADPKD clinical trials. We also solicited participants' input on ways to increase African American research participation. We audiotaped and transcribed focus groups, and 2 independent investigators identified themes raised within groups.

Results: Many participants had poor knowledge of the ADPKD inheritance pattern, the natural history of ADPKD, or how to modify their risk of progression. Participants also poorly understood the potential impact of ADPKD on their health and the health of their family members: "…I never had any symptoms. I never thought about my kids having it…." Participants identified several perceived barriers to participating in ADPKD studies including, potential risk of harm, medical mistrust, fear of being used as a guinea pig, lack of knowledge, lack of time, and lost wages. Additionally, participants cited a low perceived benefit of ADPKD studies: "They keep telling you there's no cure for it, so why are you experimenting on me." To increase the number of African Americans in ADPKD studies, participants suggested increasing ADPKD awareness, utilizing physicians and other ADPKD patients for recruitment, and engaging the community.

Conclusions: African American ADPKD patients and their family members had limited knowledge of ADPKD and identified several perceived barriers to participating in ADPKD clinical research trials. Findings from this study suggest community outreach and ADPKD patient ambassadors will be critical to increasing the recruitment of African Americans in clinical trials.

Funding: NIDDK Support

PUB223

Healthcare Utilization by Medicaid Recipients Pre- and Post-Transfer of Care to an Adult Nephrologist Edward Iglesia,[1] Hannah P. Kim,[1] Kristi Bickford,[2] Maria E. Ferris.[2] *[1]Robert Wood Johnson Medical School, Piscataway, NJ; [2]University of North Carolina Kidney Center, Chapel Hill, NC.*

Background: Studies have shown poor patient outcomes post-transfer from pediatric to adult-focused health services. Healthcare utilization around transition has not been well-characterized. We examined healthcare utilization by young adults pre- and post-transfer to adult nephrology.

Methods: We performed a retrospective study of patients ≥18 years old followed at the UNC Kidney Center pediatric nephrology clinic who were active Medicaid recipients and had transferred care to an adult nephrologist. We defined transfer date as the first visit with adult nephrology after which the patient no longer had visits with pediatric nephrology. Data was extracted from the electronic health record and the Community Care of North Carolina Medicaid Provider Portal. Healthcare utilization outcomes included hospital admissions, length of stay (LOS), emergency department (ED) visits, outpatient (OP) visits, and imaging. Outcomes were determined pre- and post-transfer, and comparative analyses were performed using Wilcoxon signed rank sum tests.

Results: Twenty adult patients had transferred care from pediatric to adult nephrology and had Medicaid data available pre- and post-transfer. Patients transferred at 19.7±1.1 years of age, 55% were female, and 70% African American. Healthcare utilization was determined for a median of 15.5 months (IQR 8-22) pre- and post-transfer. There was an 81% increase in admissions (p=0.04) post-transfer. Changes in all other healthcare utilization outcomes were not statistically significant.

Healthcare utilization pre- and post-transfer to adult-focused health services

	Pre-transfer	Post-transfer	% change	p-value
Admissions	21	38	+81	.04
LOS (days)	76	123	+62	.11
ED visits	55	79	+44	.69
OP visits	224	211	-6	.75
Imaging	232	279	+20	.40

Conclusions: A significant increase in admissions was observed post-transfer. While not significant, there were substantial increases in the number of ED visits and imaging. These results might suggest the need for better systems of care and interventions surrounding the transfer of young adults with kidney disease to adult-focused services.

PUB224

Could We Do a Reallocation of Public Financial Resources in the Maintenance of Nephrology Departments in the National Health Service? Would It Be More Cost-Efficient? Juan Abascal Ruiz, Rafael Alvarez Lipe. *Nephrology and Medicine Preventive, Hospital Lozano Blesa, Zaragoza, Spain.*

Background: Throughout the years 2000-2011 we have studied the whole health activity of our department and the costs it generates. We have done the same with the Hospital general costs and their fraction assigned to our department.

Objectives: The care work developed in nephrology units by our department and its assistance complexity do not require the whole infrastructure which needs for its optimal operation a third level hospital. On the other hand, the labour legislation makes the costs of specialized staff be larger if the work is done within a hospital.

Methods: The established method has been: **For Human Resources the distribution of work loads measured in hours considering the hour as the unit of technical value. For the health assistance we have applied microeconomics techniques (analytical management) and established the doctrine of homogeneous functional group.**

Results: They are exposed for each homogeneous functional group (hospitalization, consultations and its areas, interventionist nephrology, hypertension maps, intravenous iron treatment, haemodialysis and peritoneal haemodialysis within nephrology unit and extra-departmental emergencies. The economic cost of human resources in our department is 47.61%. The functioning costs are 31.32%. The costs of using other services are 17.15% and the structure costs of other services are 3.65% of the Nephrology unit budget. **Discussion:** A more rational use of any resources allocated in budget to unit is sought by means of the application of technical criteria and social management (criteria in the expense of money coming from public origin).

Conclusions: 1. - The ability to negotiate is possible in the 83.85% of our Department's budget.

2. - The possibility of negotiating, the existing work legislation in the 47.61%.of the budget except in a very small margin.

3. - The saving percentage of 10 points is possible on the 10.90% of present budget.

4.- A change in present labour legislation would make possible a thorough negotiation with the 47.61% of the Human resources budget.

Greetings: To Luisa Moya.

PUB225

Economics Costs and Trends in a Hospital: It Is Bearable for a Nephrology Department? Juan Abascal Ruiz,[2] Rafael Alvarez Lipe,[1] Felipe A. Monrroy.[2] *[1]Nephrology, Hospital Lozano Blesa, Zaragoza, Spain; [2]Medicine Preventive and Social, Hospital Lozano Blesa, Zaragoza, Spain.*

Background: Summary: Our Department is located in a reference hospital of the Spanish National Health Service.

Aims: Studying economic alternatives to improve the rational use of the cost of sanitary services paid with money from taxes.

Methods: We studied the costs and the most significant trends of our service. The percentage of costs which means the use of other hospital services and the percentage allocated within the overall budget allocated to our department. We studied our cost structure and each of our services and functional units.

We worked with "microeconomics techniques" of "functional groups" using "homogeneous procedures".

Results: The cost of hospital stay in the year 2011 is 402.91€. The price of the first and second consultation in that year had a cost of €107.62 and € 64.56 respectively.

The costs of AVF, indwelling catheters and percutaneous biopsy were € 254.62, € 114.5 and € 62.46 for these techniques. The single-use equipment, imaging and depreciation and use of premises was € 171.0€, 111.21 and € 130.0.

The staff cost, measured in technical value units was 2 hours to doctor and nurse and nursing assistant in the case of AVF and biopsy and 1 hour for a permanent catheter; ½ hours in the case of catheters no permanent. The estimated office staff time is 1/6 time for all processes and non-health personnel (cleaning and caretaker is 1 hour, 1 / 2 hours in the association of AVF and 1 / 2 hours of cleaning in the case not permanent. The percentage of emergency doctor is not included in the calculation of internal cost.**erall unit of**

Discussion:

We have studied the legal framework of Spanish legislation(Agreement by production/ economic sectors) " Convenios laborales" of all compatible production framework compatible with hospital performance.

Conclusions: The maintenance of "Health care quality" in a global setting of economic crisis makes it necessary to deepen in the microeconomic studies and set alternatives for the rational use of public resources in the Health environment.

Greetings: To the Department of Computer science of the our Hospital and to Dña. Luisa Moya advice on writing in English.

PUB226

Fighting Bloody Diarrhea for HUS Prevention and Mitigation: Lombardy Regional HUS Network: An Update Gianluigi Ardissino,[1] Sara Testa,[1] Fabio Paglialonga,[1] Stefania Salardi,[1] C. Baldioli,[2] N. Borsa,[1] E. Cama,[2] A. Caprioli,[1] L. Cariani,[2] D. Casnaghi,[2] L. Daprai,[2] Rosaria Colombo,[1] F. Minelli,[2] A. Negri,[2] B. Osnaghi,[2] L. Parola,[2] A. Pellegatta,[2] D. Picicco,[2] S. Rampoldi,[2] A. Rosco,[2] R. Rubini,[2] F. Russo,[2] S. Sardini,[2] G. Scavia,[1] M. Seia,[1] Silvana Tedeschi,[1] Francesca Tel,[1] E. Torresani,[1] R. Tozzoli.[1] *[1]Center for HUS Control, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; [2]Rete Regionale SEU.*

Background: Typical hemolytic uremic syndrome (tHUS) still represents a major public health problem caused by VTEC infection often presenting with bloody diarrhea.

Methods: To identify patients at risk for tHUS early in the course of the disease, a network connecting pediatric hospitals in Lombardy (10 millions gp) was developed with 53 units partecipating. Since May 28 2010 children with bloody diarrhea were tested for Shigatoxin (Stx) 1 and 2 with a Reverse Dot Blot commercial kit (EHEC Arnika). The objectives of the project were: 1. to increase the ability of the surveillance system in identifying the sources of VTEC infection; 2. to understand the mechanisms of Stx delivery to target organs; 3. to test the potential role of overhydration to prevent or mitigate renal and CNS involvement.

Results: So far 632 patients have been tested. Hereafter are the preliminary results. 34 (5,4%) were positive for VTEC (80% non-O157 – 65% with Stx 2). Among negative samples Salmonella (25,0%), Campylobacter (14,6%) and EPEC (8,4%) were the most common identified bacteria. Among patients with negative colture (41,0%) 2 patient had Henoch-Schonlein purpura, 2 Meckel diverticulum, 1 acute pancreatitis and 1 ulcerative colitis.

Conclusions: The surveillance system indicates that in Lombardy: bloody diahrrea in children is due to VTEC in a significant portion of cases, non-O157, particularly O26, play an important role and most strains produce Stx2.The role of stx-negative, eae-positive strains deserves further attention.

Acknowledgement: the project is feasible thanks to the members of the Regional HUS Network whose complete list is available at www.centroseu.org. The project has been supported by the " PROGETTO ALICE ONLUS – Associazione per la lotta alla SEU".

Funding: Private Foundation Support

PUB227

A Qualitative Assessment of Patient Educational Materials: The Role of Phenomenology in the Increasing Kidney Awareness Network (IKAN) Transplant Project Clarence Spigner,[1] Bessie A. Young.[1,2,3] *[1]Health Services, School of Public Health, Seattle, WA; [2]VA Puget Sound Health Care System, Nephrology, Health Services Research and Development, Seattle, WA; [3]Kidney Research Institue, University of Washington, Seattle, WA.*

Background: We investigated world views about patient education materials for chronic kidney disease (CKD) from people most affected by the disease. The phenomenological approach was felt to be most appropriate technique.

Methods: We developed and tested our interview guide based on prior key informant interviews and asked about familiarity with existing CKD educational materials. A purposeful sample of 12 African American subjects was recruited from dialysis, transplant recipients, living kidney donors, potential kidney donors and pre-dialysis patients. Interviews were administered by a trained moderator and digitally-recorded and transcribed verbatim for content analysis. A matrix was created listing every relevant quote reflective of the questions asked. To ensure reliability and validity, each member of the research team independently defined and sorted quotes in the matrix into "clusters of meanings" which, in turn, resulted in discerning the themes. Overlapping and repetition of themes was avoided as much possible through consensus between research team members until saturation was obtained.

Results: Subjects suggested an overreliance on information in DVDs, pamphlets, brochures, and the internet. Materials have instilled "awareness" and "confidence" by having increased patient knowledge. While self-education materials are generally "understandable," they are also sometimes "overwhelming," "clinical" and "broad." Educational information "had everything" if patients "knew where to look." Most patients readily identified with people-of-color seen in most materials. Information was generally "clear," "easy to understand," but not always as specific to their particular bio-medical or social issue.

Conclusions: Focus groups provided crucial insights into a seemingly over-reliance on print and electronic self-education media. Materials are highly informative, but can be more effective when developed with direct input of the patient population.

Funding: NIDDK Support, Veterans Administration Support

PUB228

Gene Expression in Postnatal Mouse Kidney Development Bo Wu,[1] Debashis Sahoo,[2] James D. Brooks.[1] *[1]Urology, Stanford University, Stanford, CA; [2]Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA.*

Background: Obstruction and other processes that lead to renal damage can have different effects in adult versus developing kidneys. These differences could influence selection of biomarkers used for detection of renal damage. To date, gene expression changes that occur in mouse kidney development postnatally have been poorly characterized. We describehere a comprehensive gene expression of thedeveloping mouse kidney based on microarray profiling.

Methods: C57BL/6 mice were sacrificed and kidneys were harvested at embryonic day E19.5, and postnatal days P1, P3, P5, P7, P10, P14, P21, P28 and P35. RNA was extracted from kidneys and transcript profiling was performed using Agilent microarrays. Transcripts that undergo abrupt transitions in expression level over the time course were identified using StepMiner analysis. Ingenuity pathway analysis (IPA) was used to analyze the biological function and gene networks of gene expression data.

Results: Transcript levels were modulated significantly over the time course, with 6949 up-regulated and 6696 down-regulated. IPA was used to identify genetic pathways, networks and functions significantly altered over the time course. Interestingly, in days P10 to P14 gene functions significantly altered were up-regulated exclusively with the functions including lipid metabolism, small molecule biochemistry and molecular transport. Between days P5 to P7 down-regulated genes predominated with enriched functions including, gene expression, cell cycle, protein synthesis and embryonic development. For P14 to P21 enriched functions included DNA replication, cell cycle and tissue development. In the late stages (P21 to P28) functional enrichment was seen for cell-to-cell signaling, tissue development, cellular movement.

Conclusions: This study provides the most comprehensive temporal survey of postnatal kidney development to date which provides a framework for interpreting nephropathies, such as those induced by congenital obstruction.

Funding: Other NIH Support - K99-CA151673, Other U.S. Government Support, Private Foundation Support

PUB230

Changes of TRPC 6 and BK_{Ca} Channels in Undifferentiated and Differentiated Cultured Podocytes Pengjuan Xu,[1] Zhuo Yang.[1] *[1]Medical School, University of Nankai, Tianjin, China.*

Background: Podocytes, also called glomerular visceral epithelial cells, form an interdigitating network of foot process, synthesize components of the glomerular basement membrane and are the target of injury in many glomerular diseases. Transient receptor potential canonical (TRPC)6 channels and large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels) are two important ion channels locate on podocytes, which play an essential role in regulating calcium homeostasis cell signaling. In this report, expressions of TRPC 6 and BK_{Ca} channels in undifferentiated and differentiated podocytes were detected respectively by laser scanning confocal microscope.

Methods: In the present study, immunohistochemistry was used to determine the localization and expression of TRPC6 and BK_{Ca} channels in undifferentiated and differentiated podocytes.

Results: Results demonstrated that in undifferentiated podocytes, TRPC 6 was mainly located in nuclear regions. In differentiated podocytes, the expression of TRPC6 in the nuclear regions seemed almost all translocated to other regions, and the expression on the membrane increased. The same situation also occured to BK_{Ca} channels. It was observed that functional BK_{Ca} channels were expressed at a detectable but the low level on the surface of undifferentiated podocytes. And with the maturity of differentiation, the expression of BK_{Ca} channels on the surface of podocytes increased remarkably.

Conclusions: The development changes of the two ion channels may be part of a mechanism to maintain the stability of the glomerular filtration, and TRPC 6 and BK_{Ca} channels may play an important role in the podocyte differentiation. These results have implications for the physiology and development of kidney and will also serve as a baseline for future studies designed to investigate developmental changes of ion channel expression in podocytes.

Funding: Government Support - Non-U.S.

PUB231

Regulation of Prox1 Expression in the Renal Medulla by Osmolality *In Vivo* Yumi Kim, Wan-Young Kim, Sun-ah Nam, Hak Soo Kim, Jin Kim. *Anatomy and MRC for Cell Death Disease Research Center, The Catholic University of Korea, Seoul, Korea.*

Background: The transcription factor Prospero-related homeobox 1 (Prox1) is expressed in various internal organs and is related to their differentiations. In a previous study, we found that Prox1 was transiently expressed in the differentiating ascending thin limb (ATL) of Henle's loop in the developing renal papilla and remained only in the ATL in the initial part of renal papilla in adult mouse kidney. Furthermore, Prox1 was expressed in the osmotic conditions within the optimal range *in vitro*. In the present study, we extend our experiments to the *in vivo* situation. We examined whether osmolality affects the expression and distribution of Prox1 in the renal medulla in both postnatal developing mice and adult mice.

Methods: In the first experiment, adult male C57BL/6 mice were randomly divided into 3 groups: dehydrated mice given minimum water, hydrated mice given 3% sucrose in water, and control mice given free access to water for 7 days before death. In the second experiment, one-day-old pups were administered daily subcutaneous injections of furosemide (30 mg/kg) or vehicle for 4 or 7 days.

Results: In the first experiment, the immunoreactivity for Prox1 in the ATL was weak in the initial part of the renal papilla, but no immunoreactivity was observed in the terminal part of the renal papilla of the normal adult mouse. However, in the hydrated mice, Prox1 was expressed not only in the initial part of the renal papilla but also in the terminal part of the renal papilla. Moreover, the intensity of immunoreactivity for Prox1 was increased in the hydrated mice but markedly decreased in the dehydrated mice compared to the control group. In the second experiment, the immunoreactivity for Prox1 was considerably decreased in the papillae of furosemide-treated group, where the development of the ATL of Henle's loop was delayed, compare to vehicle-treated group.

Conclusions: These results suggested that the expression of Prox1 was regulated by osmolality and was required for the differentiation and maintenance of ATL in the developing and adult mouse kidney, respectively.

PUB232

Hypoxia Inducible Factor-1a Regulates Branching Morphogenesis in Kidney Development Kenji Tsuji, Shinji Kitamura, Hitoshi Sugiyama, Hirofumi Makino. *Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Scineces, Okayama, Japan.*

Background: The Kidneys are exposed under hypoxia condition (O_2 2-9%) in developing stage (Simon et al. Nat Rev Mol Cell Biol, 2008). Hypoxia-inducible factor (HIF), an important mediator for responding to hypoxia, is thought to play an important role in development. Although previous studies indicated that hypoxia enhances ureteric bud branching in vivo on kidney development (Akimoto et al, Landes Bioscience, 2005), they didn't reveal the relationship between HIF and branching morphogenesis. We examined whether the kidney development is regulated with HIF which was induced by hypoxia or not.

Methods: We harvested embryonic 13-days kidneys (E13K) from embryo rats and cultured under normoxia (20% O_2 / 5% CO_2) or hypoxia (5% O_2 / 5% CO_2) for 4 days. After the culture, we evaluated the kidneys on the point of morphology, gene expression and protein expression.

Results: E13Ks under hypoxia were observed significantly more branching than the E13Ks under normoxia (the numbers of end buds under hypoxia vs. normoxia were 57.7±12.5 vs. 40.6±6.6, respectively; $p<0.05$). In addition, HIF-1a and Ret expression increased under hypoxia condition in E13Ks. When we added HIF-1a inhibitors (digoxin or SiRNA of HIF-1a), Ret gene expression was inhibited under hypoxia condition, and we could not observe the increased branching under hypoxia condition (the number of end buds under hypoxia vs. under hypoxia with digoxin and under hypoxia with siRNA-HIF1a were 57.7±12.5 vs. 41.5±7.5 and 39.0±4.76, respectively; $p<0.05$).

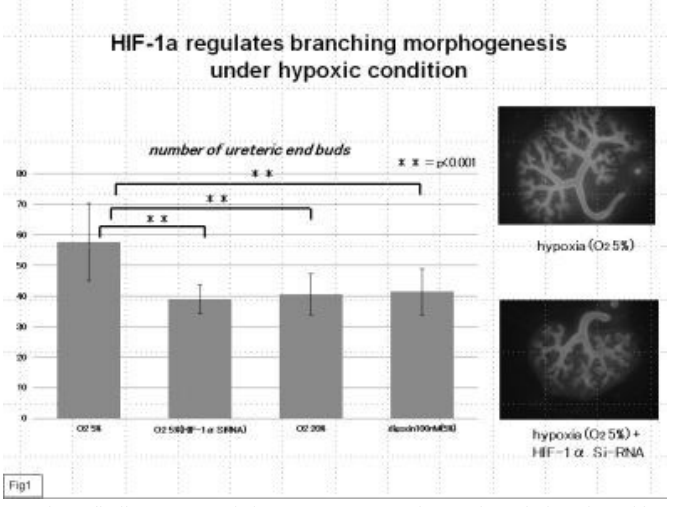


Fig1

These finding suggested that HIF-1a may make a role to induce branching morphogenesis in kidney development with GDNF / Ret signal pass way.

Conclusions: HIF-1a may regulate the branching morphogenesis via the signaling of RET/GDNF.

PUB233

HPSE2, a Gene Mutated in Human Urofacial Syndrome, Directs Neuro-Muscular Differentiation Neil A. Roberts,[1] Raphael Thuret,[2] Edward A. Mckenzie,[2] William G. Newman,[1] Emma N. Hilton,[1] Adrian S. Woolf.[1] *[1]Faculty of Medical and Human Sciences, University of Manchester, Manchester, United Kingdom; [2]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.*

Background: Homozygous *HPSE2* mutations cause urofacial syndrome (UFS). UFS features congenitally dysmorphic and dysfunctional bladders associated with vesicoureteric reflux and renal failure. UFS individuals have a diagnostic grimace when smiling and some have severe constipation. *HPSE2* codes for heparanase-2 which binds heparan sulphate and inhibits endoglycosidase activity of heparanase-1. Normal human fetal bladders express *HPSE2*, with protein immunolocalised in nascent detrusor smooth muscle in a partially overlapping pattern with heparanase-1. Heparanase-2 is also immunodetected in ganglia and nerves in the bladder wall.

Methods: We studied heparanase-2 during *Xenopus tropicalis* development. Transcripts were measured by qPCR. Single cell embryos were injected with splice-variant or ATG morpholinos to knockdown *xhpse2*. Immunostaining was performed using an antibody raised to *Xenopus* heparanase-2.

Results: Wildtype embryos showed an increase in *xhpse2* after gastrulation, with expression maintained through organogenesis. Use of either morpholino caused a phenotype comprising a bent tail and protrusion in the proctodeal/cloacal zone. The latter was associated with absent gut looping; instead the gut remained as an ovoid endodermal mass. A similar defect has been reported after knockdown of *Foxf1*, a BMP4-activated transcription factor expressed in lateral plate mesoderm fated to form gut muscle. In wildtype embryos we detected heparanase-2 in skeletal and smooth muscle cells, and morphants had impaired gut muscularisation. Heparanase-2 was also expressed within the neural tube and hindbrain, and in nerves connected to these structures. Moreover, detailed examination of *xhpse2* morphants revealed poor progress of peripheral nerves in the face and trunk.

Conclusions: Our current and previous results support the conclusion that heparanase-2, which is mutated in patients with UFS, mediates neuro-muscular differentiation in both skeletal muscles and also in smooth muscle within viscera such as gut and urinary bladder.

Funding: Private Foundation Support, Government Support - Non-U.S.

PUB234

Clinical and Genetic Analysis of a North East England Cystinuria Cohort Alice Hartley,[2] Noel Edwards,[1] Ann Marie Hynes,[1] Sarah Rice,[1] David T. Thwaites,[3] John Andrew Sayer.[1] *[1]Institute of Genetic Medicine, Newcastle University, Newcastle, Tyne and Wear, United Kingdom; [2]Department of Urology, Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcastle upon Tyne, Tyne and Wear, United Kingdom; [3]Institute of Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom.*

Background: Cystinuria is an inherited renal stone disorder secondary to tubular transport defects in the proximal tubule. We have examined clinical and biochemical characteristics from a cohort of patients from the North East of England.

Methods: Medical records and family history, together with serum and urine biochemistry data of 20 patients were reviewed. Genetic analysis was undertaken using exon PCR of all coding regions of the genes implicated in cystinuria (SLC3A1 and SLC7A9).

Results: The age of first episode of colic ranged from 2 to 48. Eleven patients (55%) had at least one first degree relative with cystinuria. All patients were diagnosed following stone analysis at presentation, or on urinalysis. Cystine staghorn calculi were seen in 12 patients (60%). Nineteen patients (95%) required surgical intervention and had an average of 6.3 procedures (ESWL, ureteroscopy and stone extraction, PCNL or open lithotomy). One patient reached end stage renal failure as a result of cystinuria. Abnormal baseline renal function was seen in fifteen patients (75%). Medical treatment options included Captopril, Penicillamine, Tiopronin, Potassium Citrate and Sodium Bicarbonate. Fourteen patients (70%) were on two or more of these therapies. Genetic analysis revealed that 3 patients were heterozygous or compound heterozygous for mutations in SLC3A1. Five patients had single or compound heterozygous mutations in the SLC7A9 gene and four patients showed a digenic pattern, with mutations in both SLC3A1 and SLC7A9.

Conclusions: Cystinuria is clinically and genetically a heterogeneous condition. Cystinuria predisposes patients to recurrent stone formation requiring multiple urological interventions. The morbidity associated with these procedures and with the condition itself is high and we advocate close liaison between Urology and Renal specialists to optimise patient management.

Funding: Government Support - Non-U.S.

PUB235

Short-Term Effects on Efficacy Parameters with Tolvaptan in Subjects with ADPKD at Various Levels of Kidney Function Wendy E. Boertien,[1] Esther Meijer,[1] Paul E. de Jong,[1] Frank S. Czerwiec,[2] Dorothee Oberdhan,[2] Holly B. Krasa,[2] Ron T. Gansevoort.[1] *[1]Nephrology, UMC, Groningen, Netherlands; [2]Otsuka Pharmaceutical Development & Commercialization Inc., Rockville.*

Background: Tolvaptan, a vasopressin V2-receptor antagonist, delays disease progression in rodent models of ADPKD. To date, human ADPKD trials have focused in patients with preserved kidney function. In this trial, we investigated responses to tolvaptan in patients with various levels of kidney function.

Methods: ADPKD patients with a wide GFR range (18-148 mL/min) were studied at baseline and after 3 weeks tolvaptan (up to 120 mg/day). GFR was assessed as ^{125}I-iothalamate clearance and total kidney volume (TKV) by MR imaging.

Results: 27 patients (52% male; 46±10 y, GFR 69±39 mL/min) were included. At baseline lower GFR was associated with higher TKV ($p=0.002$), 24hr urinary volume ($p=0.05$), and lower urinary osmolality ($p<0.001$). Baseline GFR was not correlated with plasma tolvaptan concentration ($p=0.9$). The absolute as well as percentage change in 24hr urine volume and urine osmolality with tolvaptan was less in patients with lower GFR (absolute: $p=0.001$ and $p<0.001$; percentage: $p<0.001$ and $p=0.003$, resp.). Final treatment urine osmolality was low (median 139 mOsm/kg) and not associated with GFR ($p=0.7$), suggesting that patients reached maximal urine dilution capacity independent of GFR. TKV decreased after 3 weeks treatment (median -60 mL, $p<0.001$). Baseline GFR was not associated with the absolute change in TKV ($p=0.5$), whereas the association with percentage decrease in TKV reached borderline significance ($p=0.06$). Importantly, final as well as change in fractional free water clearance was higher in subjects with lower kidney function ($p<0.001$ and $p=0.001$, resp.), indicating that subjects with impaired GFR had more response per functioning nephron.

Conclusions: Response to tolvaptan on urinary volume, osmolality and TKV is lower in patients with impaired kidney function. Based on our results with respect to fractional free water clearance, we hypothesize that this is not due to decreased sensitivity for tolvaptan, but could be due to less functioning renal mass or structural renal abnormalities.

Funding: Pharmaceutical Company Support - Otsuka Pharmaceutical Development & Commercialization, Inc., Rockville, Maryland, USA

PUB236

Effect of Dietary Phosphate on the Progression of Polycystic Kidney Disease (PKD) Xiaofang Wang,[1] Hong Ye,[1] Maria V. Irazabal,[1] Rajiv Kumar,[1] Peter C. Harris,[1] Vincenzo Savica,[2] Vicente E. Torres.[1] *[1]Mayo Clinic, Rochester, MN; [2]U of Messina, Messina, Italy.*

Background: Phosphate content of the diet varies widely among individuals and may affect CKD progression.

Methods: To determine whether it affects PKD development we fed male (M) and female (F) PCK rats identical artificial diets except for the phosphorus content (low 0.2% LPD; intermediate 0.6% IPD; high 1.2% HPD) between 3 & 10 weeks of age.

Results: LPD caused growth retardation compared to the IPD, but did not change cyst area or fibrosis (not shown). HPD worsened PKD, more in female than in male rats. There was no effect on the liver cystic disease.

	IPD (M)	HPD (M)	IPD (F)	HPD (F)	Diet#	Gender#
Body wt, g	387±20	345±12‡	245±13	228±8†	<0.001	<0.001
Kid wt, g	3.62±0.50	4.95±1.48*	2.51±0.31	4.72±0.48‡	<0.001	0.015
Cyst area, %	12.1±3.3	16.1±4.8*	11.2±4.4	21.5±4.7‡	<0.001	NS
Fibrosis area, %	3.2±1.6	6.6±2.0‡	3.8±1.5	12.3±2.1‡	<0.001	<0.001
Calcium deposit, %	0.03±0.03	0.33±0.24†	0.66±0.25	1.28±0.59†	<0.001	<0.001
Renal cAMP[a]	9.1±1.7	14.5±3.6†	7.9±2.3	18.0±6.6‡	<0.001	NS
S. FGF23, pg/ml	684±111	773±244	310±44	832±235*	<0.001	0.009
S. PTH, pg/ml	551±230	626±292	390±81	1082±523‡	<0.001	NS
S.BUN, mg/dl	27.8±3.6	30.2±7.6	29.3±3.2	38.8±5.4‡	<0.001	0.004
U.Phosphorus[b]	2045±202	2720±683*	1907±190	2486±463†	<0.001	NS

* P<0.05 † p<0.01 ‡ p<0.001 compared to IPD (unpaired t test) # Two-way ANOVA (p values) [a]pmol/mg protein [b]mg/g Creat

Moderate (IPD) and extensive (HPD) deposits of calcium phosphate were observed in the distal nephron and collecting ducts of female but not male rats. HPD rats had higher serum PTH and FGF23 and renal cAMP, without an increase in serum phosphorus, than IPD rats. Renal dopamine and sFRP4, measured because of reported upregulation by acute phosphate loading, were not increased. Increased susceptibility of female rats to renal calcium phosphate deposition which also occurs in women after phosphate loading may be due to more vigorous PTH and FGF23 responses.

Conclusions: Increased PKD severity in HPD rats is associated with enhanced renal deposition of calcium phosphate and likely due to mitogenic effects of inorganic phosphate or calcium phosphate deposition. These results suggest a possible effect of dietary phosphorus on PKD progression particularly in women.

Funding: NIDDK Support

PUB237

A Canine Autosomal Recessive Model of Collagen Type III Glomerulopathy Runa Rørtveit,[1] Frode Lingaas,[1] Tina Bønsdorff,[1] Anna Vigdis Eggertsdottir,[1] Ann Margaret Grondahl,[1] Ragnar Thomassen,[1] Agnes B. Fogo,[2] Johan Hogset Jansen.[1] *[1]Norwegian School of Veterinary Science, Oslo, Norway; [2]Vanderbilt University Medical Center, Nashville, TN.*

Background: Collagen type III glomerulopathy (Col3GP) is a rare renal disease characterized by massive glomerular accumulations of collagen type III. The disease occurs in both humans and animals, and has been presumed to be heritable with an autosomal recessive inheritance pattern. The pathogenesis is unknown. We have performed detailed investigation of a novel model of canine autosomal recessive Col3GP.

Methods: This spontaneously occurring canine disease was incidentally diagnosed in six mongrel dogs. We then established and studied a pedigree segregating the disease to investigate the genetic nature and inheritance of canine Col3GP, and eight additional affected offspring were produced. The age of the 14 affected dogs at euthanasia ranged from 41-326 days. Kidney autopsy specimens were studied by light microscopy, electron microscopy, immunohistochemistry and *in situ* hybridization. We assessed whether the Col3A1 gene was involved in the disease by performing a simple segregation analysis using polymorphic markers in the gene.

Results: 29 % of offspring (14/48, 5 males and 9 females) showed morphologic evidence of disease, strongly supporting a simple autosomal recessive inheritance pattern. Clinical signs of chronic renal failure including azotemia and/or proteinuria were observed in six of the affected dogs. Characteristic morphological findings of Col3GP were present in all affected dogs, including membranoproliferative pattern injury and massive glomerular collagen type III deposition. In situ hybridization for collagen III showed increased mesangial signal. Our initial genetic studies did not show evidence that the canine Col3A1 gene is mutated in the disease.

Conclusions: We propose that this canine Col3GP model may serve as an animal model of human Col3GP. This is the first animal model of Col3GP. Further studies of this phenotype in these dogs may have the potential to provide information on the pathogenesis and genetics of the disease in both animals and humans, and may thus contribute to the development of novel treatment.

PUB238

High-Resolution Melt as a Screening Method in ADPKD Grazia Maria Virzì,[1,2] Valentina Corradi,[1,2] Fiorella Gastaldon,[1] Maurizio Clementi,[3] Claudio Ronco.[1,2] *[1]Nephrology Dep., St Bortolo Hosp, Italy; [2]International Renal Research Institute Vicenza, IRRIV; [3]Clinical Genetics and Pediatrics, University of Padua, Italy.*

Background: ADPKD is an inherited condition caused by mutations at two genes (PKD1-PKD2). The genetic diagnosis of ADPKD is challenging because of the genetic heterogeneity and marked allelic heterogeneity at ADPKD genes. Hence, complete analysis of both genes is typically required in each patient. Several screening methodologies have been used to characterize the PKD genes.In this study, we explored the utility and robustness of High Resolution Melt (HRM) as a tool for mutation analysis of the PKD2 gene in ADPKD families.

Methods: HRM is a mismatch-detection method based on the difference of fluorescence absorbance behaviour during the melting of the DNA double-strand to denatured single-strands in a mutant sample as compared to a reference normal-control, after PCR amplification in the presence of intercalating fluorescent dye. Our families were previously screened by linkage analysis. Subsequently, HRM was used to characterize PKD2-linked-families. Amplicons that produced an overlapping profile sample vs control were not further evaluated; while those amplicons with profile deviated from the control were consequently sequenced.

Results: We analyzed 15 PKD2-linked families by HRM. We observed 10 different variations: 5 single nucleotide polymorphisms (83G>C, 108G>A, IVS1-16C>T, IVS3-22G>A, 1830G<A), 1 unknown variation (1459T>C) and 4 mutations. The mutations detected by HRM and confirmed by sequencing were: 1158T>A, 1459T>C, 2159delA, 2224C>T, 2533 C>T. In particular, the nonsense mutation 2533 C>T was found in 8/15 families (20 subjects). We had a ADPKD pre-symptomatic diagnosis in 8 subjects (mean age: 29 years) and we excluded ADPKD in 16 subjects (mean age: 28 years) by HRM and linkage analysis.

Conclusions: We have developed a strategy for rapid molecular mutation analysis of the PKD2 gene in ADPKD families, which utilizes a HRM-based pre-screening followed by Direct Sanger Sequencing of amplicons with abnormal melting profiles. This is a simple and good technique for PKD2 genotyping and may significantly reduce the time and the cost for diagnosis in ADPKD.

PUB239

Achievement and Maintenance of Blood Pressure Targets in HALT:PKD Vicente E. Torres,[1] Robert W. Schrier,[2] Arlene B. Chapman,[3] Ronald D. Perrone,[4] D. Miskulin,[4] Theodore I. Steinman,[5] Franz Winklhofer,[6] William E. Braun,[7] Marie C. Hogan,[1] Frederic F. Rahbari-Oskoui,[3] Kaleab Z. Abebe,[8] Michael F. Flessner.[9] *[1]Mayo Clinic, Rochester, MN; [2]U of Colorado, Denver, CO; [3]Emory U, Atlanta, GA; [4]Tufts U, Boston, MA; [5]Beth Israel, Boston, MA; [6]UKMC, Kansas City, KS; [7]Cleveland Clinic, Cleveland, OH; [8]U of Pittsburgh, Pittsburgh, PA; [9]NIH/NIDDK HALT Study Group.*

Background: HALT-PKD seeks to determine whether ACEI/ARB is superior to ACEI alone and low BP (<110/75) to standard BP (120-130/70-80) in delaying cyst progression in patients with eGFR >60 (Study A) and whether ACEI/ARB is superior to ACEI alone (BP target 110-130/70-80) in slowing eGFR decline in patients with eGFR 25-60 mL/min/1.73 m^2 (Study B).

Methods: Stepwise dosing of lisinopril (L) and telmisartan/placebo (T/P) (steps 1-4) followed by other agents (steps 5-10) is used to achieve BP targets. During 2006-2012, 519, 501, 500, 451, 293, 143 A and 458, 447, 437, 411, 232, 97 B patients completed 4, 12, 24, 36, 48, 60-mth follow-up. BP control is assessed by home BP measurements.

Results:

	Mths	Study A Std	Study A Low*a	Study B
MAP †	4	91.5 (5.8)	85.0 (6.5)	91.0 (6.9)
	12	91.7 (6.3)	83.8 (6.7)	91.5 (5.8)
	24	92.1 (6.2)	83.0 (6.5)	91.6 (6.3)
	36	92.6 (6.1)	83.0 (6.9)	91.6 (6.1)
	48	93.3 (6.5)	82.7 (6.6)	91.6 (6.1)
	60	93.2 (6.3)	82.1 (8.4)	91.5 (5.6)
Step; L, T/P mean dose	4	2.2; 15.0, 50.7	3.4; 24.0, 64.3	2.9; 18.2, 55.9
	12	2.2; 15.6, 50.9	3.7; 25.0, 66.6	3.0; 18.7, 56.3
	24	2.3; 15.1, 50.9	3.8; 25.7, 65.7	3.1, 18.0, 56.0
	36	2.4; 15.7, 51.5	3.8; 25.3, 66.2	3.2; 17.3, 55.3
	48	2.5; 15.6, 52.4	4.0; 26.2, 66.4	3.0; 17.0, 54.3
	60	2.4; 14.1, 52.0	4.2; 26.1, 67.3	2.8; 15.4, 52.0

† mean ± SD. *Underlined: P<0.0001 compared to Study A standard. [a] Step and dosage comparisons were made using the Wilcoxon test

Based on random regressions, significantly negative in Study A low and significantly positive BP slopes in Study A standard result in increasing BP separation over time.

Conclusions: ACEI alone or ACEI/ARB achieves BP control and MAPs are within target in most subjects at 4-60 months of follow-up. Excellent BP and LIS and T/P dose separation between study A arms is achieved, without differences in heart rate.

Funding: NIDDK Support

PUB240

Assessment of DNA Variant Databases for Inherited Kidney Diseases for Clinical Relevance, Accessibility, Expertness of Curation and Currency
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Background: The Human Variome Project (HVP) recommends that gene-specific DNA variant databases contain clinical data, and are up-to-date, curated by experts and freely-available. A recent review identified 62 inherited renal diseases caused by mutations in 134 different genes (Hildebrandt, Lancet 2010). This study examined how many of these databases fulfilled the HVP recommendatations.

Methods: We used the disease name, MIM number, and gene name, alone or together with 'mutation' or 'database' to identify websites for gene-specific databases. For each, the total number of variants, and the number that could be accessed without charge, were noted, as of 1 March 2012. The numbers that contained clinical details, had a named or 'expert' curator, and had been updated within the previous 12 months, were also noted.

Results: There were databases for 61 (98%) of these 62 diseases (all excepting Multicystic kidney dysplasia, MIM 602868) and for 128 of the 134 (96%) corresponding genes (excepting CDC5L, USF2, SLIT2, MYOG, DLX5, and MSK3). Altogether there were 352 databases. One hundred and ninety-two (54.5%) databases were accessible without charge. All published variants were freely-available for 37 (27.6%) genes. Two hundred and fifty-one (71%) databases had fewer than 20 mutations, and 8 (6%) genes had no free database at all. One hundred and twenty-nine (37%) databases had no clinical information beyond the diagnosis. Sixty-one (17%) databases, including 4 of the largest, were curated by an individual expert or group of experts. The date of the most recent update was known for 285 databases and was within the previous 12 months for 168 (59%).

Conclusions: Most inherited renal diseases have a database for DNA variants. However, only half of these are free, and where they are, are often incomplete. In addition, about one third of databases have no clinical information, and few are curated by experts. Finally, there is much duplication with frequently more than one database per gene.

PUB241

Mutation Analysis in Japanese Patients with Congenital and Infantile Nephrotic Syndrome
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Background: Mutations in podocyte genes (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*) are associated with congenital (<3 months) and infantile (3-12 months) nephrotic syndrome (NS). However, the frequency of causative mutations in these genes in NS manifesting in the first year of life has not been fully investigated in Japan.

Methods: To clarify the role of mutations in these genes, all exons and exon-intron boundaries were investigated in consecutive, unrelated 33 patients from regional pediatric kidney disease centers, by PCR-direct sequencing.

Results: We detected disease-causing mutations in 60.6% (20 of 33) patients (85% in congenital and 3% in infantile). *NPHS2* mutation was not detected (Table).

	No. of patients	Total Mutations N (%)	*NPHS1* N (%)	*NPHS2* N (%)	*WT* N (%)	*LAMB2* N (%)	Not detected N (%)
All (CNS+INS)	33	20 (60.6)	12 (36.4)	0 (0.0)	7 (21.2)	1 (3.0)	13 (39.4)
CNS	20	17 (85.0)	12 (60.0)	0 (0.0)	4 (20.0)	1 (5.0)	3 (15.0)
INS	13	3 (23.1)	0 (0.0)	0 (0.0)	3 (23.1)	0 (0.0)	10 (76.9)

CNS = congenital nephrotic syndrome; INS = infantile nephrotic syndrome

Of a total 12 different *NPHS1* mutations 8 (3 missense, 3 nonsense, 1 small deletion, and 1 large deletion) were novel. The large deletion of *NPHS1* was detected by semi-quantitative PCR. C.2515delC in *NPHS1* was detected in 8 patients, suggesting a founder effect. All *NPHS1* mutations were associated with a large placenta. Patients with *WT1* mutations had early-onset (<4 months) end-stage renal disease. A patient with *LAMB2* mutations showed Pierson syndrome.

Conclusions: Although a large placenta is thought to be a non-specific feature of CNS, it may be a key to decide the order of mutation analysis. *NPHS2* mutations are reported to have an important role in NS manifesting in the first year of life in Europe and the United States. However, in Japan *NPHS2* mutation was not detected in the present study. *NPHS1*, *WT1* and *LAMB2* mutations are also responsible in a considerable part of CNS and INS in Japan.

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PUB242

Differential Renal Gene Expression in ADPKD Compared to Other CKD Causes
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Background: ADPKD accounts for 4-10% of end-stage renal disease (ESRD) worldwide. The hallmark of ADPKD is the development of multiple renal cysts that replace the normal renal parenchyma. However the exact mechanism and identity of the factors influencing this process remain unclear. While animal models are useful for study of certain aspects of the disease process they also have limitations for study of human disease. Human ADPKD research is limited due to the availability of kidneys for research especially those from patients still in the early stages of disease. The majority of nephrectomies performed on ADPKD patients are those on subjects who have reached ESRD. It has been argued that kidneys from patients with advanced disease are not representative of ADPKD since it is difficult to differentiate changes due to ADPKD or ESRD itself. We hypothesized that ADPKD has a unique pathogenesis different from other causes of ESRD.

Methods: We selected several genes that were differentially expressed between ADPKD and normal kidneys and compared their expression in ADPKD kidneys and in kidneys from patients with ESRD due to other causes (CKD). Real time RT-PCR was used to measure renal mRNA levels. Gene expression was expressed as the ratio of the target gene/GAPDH.

Results: Renal *VEGF* mRNA levels were significantly lower in ADPKD kidneys compared to normal kidneys (0.9±0.2, n=5 vs 1.4±0.4, n=3, $p<0.05$) but significantly higher than those in CKD kidneys (0.9±0.2, n=5 vs 0.5±0.2, n=3, $p<0.05$). *HIF-1*β expression was significantly higher in ADPKD kidneys than in normal controls (4.78±2.34, n=4 vs 1.12±0.51, n=4, $p<0.01$) while there was no difference between the normal controls and CKD kidneys (1.12±0.51, n=4 vs 1.10±0.11, n=4, p=NS). Strikingly, *RUNX3* gene expression was much higher in ADPKD kidneys than in either normal controls or CKD kidneys (14.9±8.0, n=6, vs either 0.64±0.39, n=3 or 1.34±1.4, n=3, $p<0.05$).

Conclusions: Gene expression observed in kidneys from ADPKD patients with advanced disease differs markedly from that seen in kidneys from patients with other renal disease causes. This implies that ADPKD has its unique pathogenesis leading to ESRD.

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PUB243

Mutation Analysis of UMOD and REN Genes in Pathological Diagnosed and Clinical Suspected MCKD2/FJHN Families
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Background: Medullary cystic kidney disease 2 (MCKD2) and familial juvenile hyperuricemic nephropathy (FJHN) characterized by hypertension, hyperuricaemia, and progressive renal insufficiency, are reported to be caused by mutations in the UMOD, HNF-1β or REN genes. In this study we try to identify UMOD and REN gene mutations in pathological diagnosed and clinical suspected MCKD2/FJHN families.

Methods: 9 patients were included in this study, 3 of them were diagnosed as MCKD2 according to renal pathologic changes, and 6 of them were suspected to have MCKD2/FJHN due to clinical features and family histories of hyperuricemia or ESRD. The mean age was 24.2 years (18-40 years), with 5 males (5/9) and 4 females (4/9). 4 patients progressed to ESRD within 2-3 years of diagnosis. UMOD and REN gene mutation analysis were performed in all patients as well as some family members.

Results: Two types of UMOD mutations were identified in the 2 patients and their relatives. A novel heterozygous missense mutation(c.326T/A;p.Val 109 Glu), altering the epidermal growth factor-like domain(cbEGF3) in one family. This patient's mother and elder brother also have this mutaion without any clinical abnormalities. In another family, we found a previously reported UMOD gene mutation(c.744C/G;p.Cys248Trp), affecting D8C domain. This patient's mother and uncle have the same mutations, progressed to ESRD. Among 9 patients with MCKD2/FJHN phenotype, UMOD mutation was found in 2(22.2%). We failed to find any mutations in REN gene in all families.

Conclusions: A novel mutation (p.Val 109 Glu) and a previously reported mutation (p.Cys248Trp) of UMOD gene were detected in 2 cases (22.2%) with UMOD mutations. Genetic testing may be a useful tool in helping diagonosis of MCKD2/FJHN in patients suspecting MCKD2/FJHN without renal biopsy.

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PUB244

Restoring Multidrug-Resistance Associated Protein 3 (MRP3) Reduces Proliferation through ERK/B-Raf Signaling Down-Regulation
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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of common genetic disorder which is characterized by progressive cyst formation and resulting loss of renal function. In previous reports, numerous genes involved in cystogenesis were identified so far, we don't have effective drugs for polycystic kidney. Currently, in most of clinical cases, renal failure occurs.

Methods: We found that MRP3 is related to cyst formation using three-dimensional culture system. To determine the novel function of MRP3 in the kidney, we used RNAi system for knockdown and Abcc3 clone for over-expression. Immunohistochemistry-fluorescence was performed to evaluate MRP3 expression in PKD mouse model and human patients.

Results: MRP3 inhibition stimulates mitogen-activated protein kinases (MAPKs) and it reveals that MRP3 inhibition enhance cell proliferation. We also confirmed MRP expression in vivo level. MRP3 is significantly down-regulated in the kidneys of PKD2 knockout–Rescued mice and human ADPKD patients. Opposite of the results, we found that restored MRP3 reduces cell proliferation in vitro.

Conclusions: The results suggest that novel renal function of MRP3 which is related to cell proliferation. Reversely, MRP3 over-expression represses cell proliferation. Therefore, MRP3 restoring may be one of effective therapeutic approaches for polycystic kidney disease.

PUB245

Modeling of Epithelial Organization in PKD Cystogenesis Julio M. Belmonte,[1] Sherry G. Clendenon,[1] James A. Glazier,[1] Robert L. Bacallao.[2] *[1]Physics, Biocomplexity Institute, Indiana University, Bloomington, IN; [2]Medicine, Division of Nephrology, Indiana University, Indianapolis, IN.*

Background: Polycystic kidney diseases (PKD) are the most common genetic causes of end-stage renal disease. Despite discovery of PKD linked mutations, disease progression is unclear. Renal cysts, but not normal renal epithelia, express cadherin 8. In 3D culture, exogenous cadherin 8 expression, but not N-cadherin expression, causes renal epithelial cells to form cysts. Our hypothesis is that changes in cell-cell adhesion due to cadherin type switching drives cystogenesis.

Methods: Using CompuCell3D, open-source multiscale modeling software developed at the Biocomplexity Institute, we have begun development of a multiscale *in silico* model of cystogenesis based on differential adhesion data from *in vitro* cystogenesis experiments.

Results: Preliminary results show that cadherin type switching is sufficient to disrupt local tubule morphology and initiate the formation of cysts. Further development of the 3D *in silico* model will show how relative strengths and disruptions of cell-matrix and cell-cell interactions contribute to the formation of cysts from a renal tubule.

Conclusions: Despite the complexity of PKD, our mathematical model shows that cystogenesis can be described as an emergent phenomena arising from a single change in cell behavior. Our 3D model will generate testable hypotheses about the underlying processes involved in this disease progression that may lead to PKD treatments based on alteration of cell adhesion.

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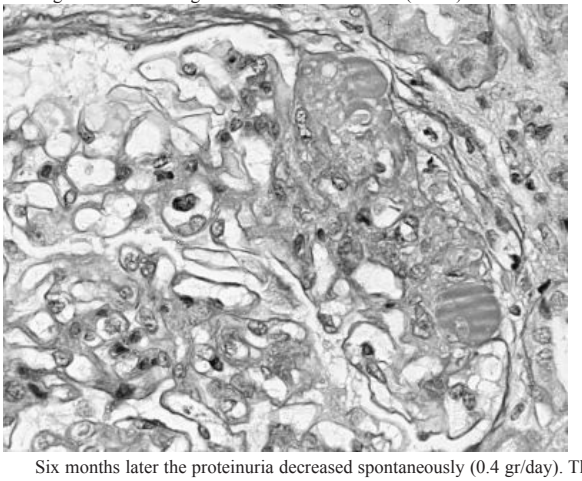
PUB246

Focal Segmental Glomerulosclerosis in Type 1 Gaucher Disease Alessandra Marega,[1] Stefano Pizzolitto,[2] Bruno Bembi,[3] Maria Gropuzzo,[1] Giulio Romano,[1] Clotilde Vallone,[1] Domenico Montanaro.[1] *[1]Department of Nephrology, Dialysis and Transplantation, SMM Hospital, Udine, Italy; [2]Department of Pathology, SMM Hospital, Udine, Italy; [3]Department of Rare Disease, SMM Hospital, Udine, Italy.*

Background: Gaucher disease (GD) is a genetic lysosomal disease characterized by deficiency in glucocerebrosidase. It induces the formation of Gaucher Cells (GC) that infiltrate bone marrow, spleen, liver, lungs and brain and are considered to be mainly responsible for the clinical manifestations. Renal complications do not seem to be part of the spectrum of GD.

Methods: 55 years old Caucasian man was diagnosed GD type 1 [c.1226A>G(N370S)/c.750A>G(Y212-K215del)] heterozygous. Due to progressive thrombocytopenia and splenomegaly he underwent splenectomy at age 56. At 72, the patient was diagnosed JAK2 positive myeloproliferative disorder (MPD). At 75, he was discovered to have heavy proteinuria (6 gr/die), with serum creatinine 1 mg/dl. Ramipril was started as antiproteinuric agent and oncocarbide for MPD. At 77 proteinuria was 8 gr/day and a renal biopsy supported the diagnosis of Focal Segmental Glomerulosclerosis (FSGS).



Six months later the proteinuria decreased spontaneously (0.4 gr/day). Then he died of sudden cardiopulmonary arrest. The autopsy revealed acute myocardial infarction and lung adenocarcinoma.

Results: GD with severe clinical and pathological renal involvement is exceptionally rare. In literature there are only few cases in which the renal involvement is characterized by heavy proteinuria and/or renal failure caused by the accumulation of GC in glomeruli and interstitium of the cortex; in another report a boy was diagnosed GD and mesangiocapillary glomerulonephritis. Here we have described a patient with GD and heavy proteinuria secondary to FSGS.

Conclusions: To our knowledge this is the first combination of FSGS and GD and the second case of JAK2 MDP and GD.

PUB247

Different Mitochondrial DNA Point Mutations in Four Mitochondrial Cytopathy Patients with Renal Involvement Honglang Xie, Shutian Xu, Qunpeng He, Yang Liu, Jinzhou Guo, Zhi-Hong Liu. *Research Institute of Nephrology, Jingling Hospital, Nanjing University Clinical School of Medicine, Nanjing, China.*

Background: Mitochondrial cytopathy is a heterogeneous disease with multiple organ system involvement. We report 4 mitochondrial cytopathy patients with 4 different mitochondrial DNA (mtDNA) point mutation and renal involvement.

Methods: DNA was isolated from peripheral blood leukocytes from four patients. Polymerase chain reaction was performed to amplify the mtDNA. Sequencing analysis was used to detect the presence of any point mutation of mtDNA.

Results: 1)General condition:cases includes a 74-yr male, a 13 yr male, a 18 yr female, and a 20-yr female. Nothing special in family history except for case 2, who has a diabetic family history, both his mother and brother died young. 2) Renal involvement:Case 1, 3 had nephrotic proteinuria (8.6 to 12.7g/d). All had hypoalbuminemia, interstitial-tubular function injury. Renal biopsy proved FSGS in case 2 and IgAN in case 3. 3)Systemic involvements:all of them were malnutrition and diabetes. Epileptic seizure were complicated in case 3 and 4, hearing loss were noticed in cases 1, 2 and 3, muscle weakness in case 1 and 3, arrhythmia in case 2, 3 and 4, heart failure in cases 1, 3, and 4, peripartum myocardiopathy in case 4, and anesthesia in case 1. Both case 2, 3 were preterm infants, complicated with microsomia, athrepsy, hypoevolutism and dysnoesia. Sequencing analysis proved presence of point mutation of mtDNA:8994G>A in case 1, 3243G>A in case 2, 8969 G>A in case 3 and her mother, 8277 T > C in case 4. 4)Prognosis:case 1 died of amyasthenia of respiratory muscle, pulmonary infection and respiratory failure; case 2 died of pulmonary infection; case 4 died of perinatal myocardiopathy and acute heart failure. But case 3 was sensitive to steroid and got complete remission of proteinuria and normal renal function, although 8969 G>A mutation was also demonstated in her mother, but no sign of mitochodrial cytopathy was noticed till now.

Conclusions: mtDNA 8969 G>A is a new point mutation to cause mitochondrial cytopathy. The prognosis of mitochondrial cytopathy with renal, heart and central nerval system involvement is poor.

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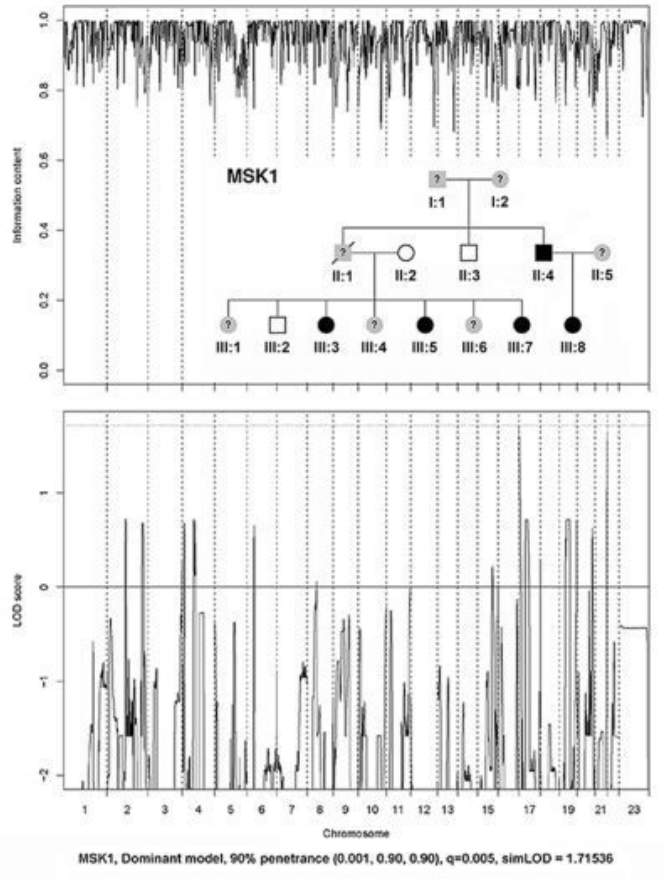
PUB248

Genome-Wide Linkage Scan and Whole Exome Sequencing in a Family with Medullary Sponge Kidney Young-Hwan Hwang,[1] Nicole M. Roslin,[2] Xuewen Song,[1] Moumita Barua,[3] Andrew D. Paterson,[2] York P. Pei.[1] *[1]Division of Nephrology, University Health Network and University of Toronto, Toronto, ON, Canada; [2]Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; [3]Division of Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [4]Division of Nephrology, Catholic University, Rome, Italy.*

Background: Medullary sponge kidney (MSK) is a relatively common but often undiagnosed disorder of renal malformation characterized by dilatation of precalyceal tubules. Patients with MSK have a high risk of nephrocalcinosis and nephrolithiasis. Most patients present sporadically but a few familial cases have been reported.

Methods: Here we report a multiplex family (MSK1) with a dominant segregation pattern for non-syndromic MSK. The proband (III:5) and two relatives (III:3, III:8) had definitive MSK (nephrocalcinosis, calyceal "blushing", and stones) based on ultrasound (US) and CT urogram while two other relatives (II:4, III:7) had probably MSK (nephrocalcinosis and stones) based on US. Two at-risk individuals (II:3, III:2) with a normal renal US >40 years of age were considered unaffected.



We performed a genome-wide linkage scan by genotyping 11 DNA samples from MSK1 using the Illumina Linkage-24 arrays and analyzed the data using Merlin.

Results: Simulations based on the pedigree structure (and 90% penetrance) yielded an max. expected LOD of 1.72. Under a dominant model with an allele frequency of 0.005, phenocopy rate of 0.001, and penetrance of 90%, we identified two suggestive linkage signals on chr. 17 and 21 with max. multipoint LOD score of 1.79 and 1.72, respectively. These results are consistent across a range of penetrances of 70-90%.

Conclusions: Whole exome sequencing (currently in-progress) focusing on the two regions of linkage signals provide a promising approach to identify the disease gene for MSK1.

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PUB249

Phosphorylation of α Actinin 4 Regulates Its Binding Affinity with Actin

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Background: Point mutations in the actin binding domain of the actin crosslinker α actinin-4 (Actn4) cause FSGS and lead to increased affinity to actin filaments. We speculate that post-translational modification, phosphorylation, may regulate the actin binding affinity of Actn4.

Methods: The serine at residue 159 and tyrosine at residue 265, both identified as phosphorylation sites of Actn4, were substituted with a negatively charged amino acid aspartate and glutamate (S159D, Y265E) to mimic the phosphorylated state, or with a neutral amino acid alanine and phenylalanine (S159A, Y265F) to abolish phosphorylation. Actn4-actin binding affinity was studied by an actin co-sedimentation assay. GFP-tagged Actn4 mutants were transfected into murine podocytes and Cos7 cells. The localization of Actn4 was examined by confocal microscope.

Results: S159D showed markedly higher actin-binding affinity than WT, similar to that in disease-causing K255E mutant. S159A control did not significantly alter actin binding affinity. Y265E demonstrated a higher actin-binding affinity than WT, whereas its control Y265F had no effect. Y265E further increased actin binding affinity of K255E while S159D showed no additive effect. WT localized to stress fibers and focal adhesions. K255E was seen as a dense cytoplasmic aggregate. S159D showed a distribution similar to that in K255E. This effect was not seen in S159A control. Neither mutation affected the aggregation of the K255E. Y265E showed a localization similar to that seen in K255E while Y265F control showed a similar distribution as WT. Y265E caused further aggregation of K255E which appeared more punctate. Y265F did not have further effect on K255E. This result is consistent with our finding of co-sedimentation assay.

Conclusions: Our study showed that both S159D and Y265E mutants increase actin binding affinity of Actn4. Y265E further enhances actin binding affinity of K255E. S159D does not show additive effect. Phosphorylation of Actn4 seems to regulate its actin binding activity.

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PUB250

Genome Wide Transcriptome Analysis of Human Hypertensive Chronic Kidney Disease

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Background: Hypertension is one of the most common causes of end stage renal disease in the United States. Hypertensive nephropathy remains an elusive entity, with very few confirmatory biopsies and histological characteristics. In this study, we compared patients with hypertensive chronic kidney disease to those with normal renal function in the presence and absence of hypertension.

Methods: Human kidney tissue samples were obtained from elective nephrectomies. Samples with eGFR<60, and in absence of diabetes were classified as hypertensive nephropathy (HN). Tubular epithelial cells were isolated from glomeruli by manual microdissection. cDNA amplification fragmentation and labeling and finally Affymetrix GeneChip expression arrays (U133A 2.0) were used to analyze transcripts in samples.

Results: Genespring GX software was used for normalization and statistical analysis with Benjamini-Hochberg corrected two-tailed t-test with p value < 0.05 and > 1.5 fold change. We identified 1792 differentially expressed probesets in CKD tubules, with 1372 unique gene transcripts. Among them, the probesets with the highest fold change were albumin, epithermal growth factor, and uromodulin known to play a role in chronic kidney disease. Gene ontology of the differentially expressed genes highlighted metabolic processes, localization, establishment of localization, and immune system responses in statistically enriched terms. Of the metabolic processes, pathways related to fatty acid metabolism showed the strongest enrichment.

Conclusions: This is the first genome wide transcriptome analysis of tubular epithelial cells in human hypertensive nephropathy. These results demonstrate that multiple known and novel genes and pathways are regulated in the HN and they may play a role in the pathogenesis of HN or serve as biomarkers.

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PUB251

The Expression Profiling of Intestinal Nutrient Transporter and Channel Genes in Rats with Renal Failure

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Background: The understanding of intestinal function in chronic kidney disease (CKD) has been important elements in the clinical management of CKD with dietary and drug therapy. Numerous studies have indicated that CKD patients or model rats have enzymatic abnormalities and impairments of absorptive function in the small intestine. However, it is still unclear how the intestinal function, especially digestion and absorption of nutrients, is different from CKD. In order to estimate the functional changes in the intestine of CKD, in this study, we demonstrated the microarray analysis of global gene expression in intestine of adenine-induced CKD rat.

Methods: 8-week-old male Wister rats were fed on 0.75% adenine diet for about five weeks to induce the kidney failure. In addition, 5/6 nephrectomy rats were also prepared. The plasma levels of bood urea nitrogen, creatinine and phosphate were monitored by each specific test kit. Intestinal total RNA was extracted from the mucous from duodenum, DNA microarray analysis were performed using Affymextrix rat gene chip. For quantitative mRNA expression analysis was Real.

Results: DNA microarray analysis revealed that CKD caused great changes in gene expression in the rat duodenum: about 400 genes exhibited more than a two-fold change in expression level. Gene ontology analysis showed that a global regulation of genes by CKD involved in iron ion binding, alcoholic, organic acid and lipid metabolism. Furthermore, we found that the mRNA expression of transporter and channel genes which related to absorption of calcium, phosphate, iron and chloride were markedly reduced in intestine of CKD rats.

Conclusions: These results suggest that CKD may alter some nutrient metabolism in the small intestine by modifying the expression of specific genes. The intestinal transcriptome database of CKD might be useful to develop the novel drugs or functional foods for CKD patients.

Funding: Government Support - Non-U.S.

PUB252

Toll-Like Receptor 9 Gene Polymorphisms Contribute to Development of Proteinuria in Childhood IgA Nephropathy Shin-hee Kim,[1] Jin-soon Suh,[2] Byoung-Soo Cho.[3] *[1]Department of Pediastrics, School of Medicine, Kyng Hee Univerisity, Seoul, Republic of Korea; [2]Department of Pediastrics, School of Medicine, Kyng Hee Univerisity, Seoul, Republic of Korea; [3]Department of Pediastrics, School of Medicine, Kyng Hee Univerisity, Seoul, Republic of Korea.*

Background: Toll-like receptors (TLRs) play important roles in the immune responses of both innate and adaptive systems. There is increasing evidence that TLRs play a role in the development and progression of immune complex-mediated glomerulonephritis. In this study, we investigated the association of *TLR9* polymorphisms with the immunoglobulin A nephropathy (IgAN) in Korean children.

Methods: We analyzed two single nucleotide polymorphisms (SNPs) of *TLR9* gene (rs352140 and rs187084) using direct sequencing in 199 IgAN patients and 290 healthy controls. The IgAN patients were subgrouped by the presence of proteinuria (>4 mg/m^2/h), nephrotic-range proteinuria (>40 mg/m^2/h), gross hematuria, podocyte foot process effacement, and advanced pathological markers in renal biopsy such as interstitial fibrosis, tubular atrophy, or global sclerosis.

Results: In our case-control analysis, no association was found between *TLR9* SNPs and development of IgAN. In an analysis of associations between subgroups of IgAN and genotypes of each *TLR9* SNP, we found that rs352140 was associated with the presence of proteinuria [overdominant model, odds ratio (OR) = 0.55, 95% CI = 0.31–0.99, P = 0.046]. Moreover, both *TLR9* SNPs were associated with the presence of nephrotic-range proteinuria (rs352140: overdominant model, OR=0.21, 95% CI = 0.06–0.75, P = 0.013; rs187084: overdominant model, OR = 0.19, 95% CI = 0.05–0.75, P = 0.008).

Conclusions: Our data indicate that *TLR9* SNPs of rs352140 and rs187084 may contribute to the development of proteinuria in IgAN patients.

Funding: Private Foundation Support

PUB253

A Comprehensive Molecular Network for Congenital Anomalies of the Kidney and Urinary Tract: Integrating Genetic Findings Kirsten Y. Renkema,[1] Albertien M. Van Eerde,[1] Nayia Nicolaou,[1] Loes F.M. Van der Zanden,[2] Iris Van Rooij,[2] Nel Roeleveld,[2] Barbara Franke,[3,4] Ernie M.H.F. Bongers,[3] Geert Poelmans,[3,5,6] Nine V. Knoers.[1] *[1]Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands; [2]Epidemiology, Biostatistics, and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [4]Psychiatry, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [5]Cognitive Neuroscience, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [6]Molecular Animal Physiology, Radboud University Nijmegen, Nijmegen, Netherlands.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT) form a spectrum of developmental malformations. CAKUT are the most common cause of end-stage renal disease in children. Animal and human studies have shown that defects in genes expressed during kidney and urinary tract organogenesis are important causal factors for CAKUT, though the biological mechanisms underlying CAKUT etiology are still poorly understood. Instead of focusing on individual genes, analyses that aim at the identification and integration of functional relationships between these genes are likely to lead to novel insights.

Methods: In this study, 185 candidate genes were selected based on experimental evidence implicating them in CAKUT pathogenesis. By applying a novel and innovative genetic network building approach, combining systematic bioinformatics and literature analyses, we integrated 129 of 185 CAKUT candidate genes into a comprehensive protein network.

Results: Glial cell line-derived neurotrophic factor (GDNF)-dependent signaling was shown to integrate the different signaling cascades. Moreover, three microRNA genes that were known to be differentially expressed in CAKUT patients, as well as their target genes, were integrated in the network.

Conclusions: This analysis provides important clues on the molecular etiology of CAKUT, being instrumental for the development of novel approaches aimed at early diagnosis and counseling of CAKUT patients and their relatives, and the identification of 'druggable targets' for treatment or even prevention of these disorders.

Funding: Private Foundation Support

PUB254

Early Endothelial Outgrowth Cells (eEOCs) and BMP-5 in Chronic Hypertensive and Diabetic Nephropathy Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany.*

Background: Early Endothelial Outgrowth Cells (eEOCs) protect mice from acute kidney injury. These effects can be augmented by eEOC pretreatment with Bone Morphogenetic Protein-5 (BMP-5). Aim of the study was to analyze modulatory effects of BMP-5 in an eEOC-based therapy of chronic hypertensive and diabetic nephropathy.

Methods: Male, 8-12 weeks old C57/Bl6N-mice underwent 5/6-nephrectomy in order to induce chronic hypertensive nephropathy, or they received STZ for inducing chronic diabetic nephropathy. Animals were systemically injected with syngeneic murine eEOCs at 1 and 2 weeks after surgery / the last STZ administration. Animals were analyzed 8 weeks later.

Results: Mice after 5/6-nephrectomy (5/6-N.) and STZ administration (STZ) displayed significantly impaired renal function at week 8 (5/6-N.: 0.24 ±0.01 mg/dl und STZ: 0.2 ±0.01 mg/dl vs. Controls (C.): 0.15 ±0.008 mg/dl, p<0.0001 und p=0.02). Only STZ treatment was associated with significant proteinuria (STZ: 1.4 ±0.16 mg/day vs. C.: 0.85 ±0.13 mg/day, p=0.01). Injection of either untreated or BMP-5 pretreated eEOCs in 5/6-N. was not associated with improved renal function but proteinuria was decreased. The latter effect was abrogated with BMP-5 pretreated cells. In diabetic animals renal function was improved after the administration of untreated and BMP-5 pretreated eEOCs (STZ + eEOCs: 0.125 ±0.008 mg/dl und STZ + eEOCs + BMP-5: 0.14 ±0.01 mg/dl vs. STZ: 0.2 ±0.01 mg/dl, p=0.001 und p=0.03). Proteinuria was reduced as well, this effects was more pronounced after Injections of BMP-5 treated cells (STZ + eEOCs + BMP-5 0.9 ±0.13 mg vs. STZ 1.4 ±0.16 mg, p=0.04).

Conclusions: Systemic eEOC therapy in chronic diabetic nephropathy improves renal function and proteinuria. Antiproteinuric effects are increased after eEOC treatment with BMP-5.

PUB255

Effects of Bone Marrow-Derived Mesenchymal Stem Cells Transfected with Hepotocyte Growth Factor Gene on Renal Fibrosis in Rats Jian-xin Wan. *Nephrology, The First Affiliated Hospital of Fujian Medical University.*

Background: To investigate the effects of bone marrow-derived mesenchymal stem cells (MSC) transfected with adenoviral vector carrying hepotocyte growth factor (HGF, Ad-HGF) on renal fibrosis in unilateral ureteral obstruction (UUO) rats.

Methods: MSC were transfected with Ad-HGF at the optimal gene transduction efficiency of 150 multiplicity of infection (MOI). The efficiency of transfection and the expression of HGF in the suspension were detected by RT-PCR and enzyme linked immunosorbent assay (ELISA) respectively. MSC-modified Ad-HGF marked by DAPI were detected in renal tissue by fluorescent microscope. Deposition of collagenous fibers in renal tissue were observed by Masson stain. The expressions of HGF and α-SMA in renal tissue were detected by immunohistochemistry. The expressions of HGF, α-SMA and fibronectin (FN) mRNA in renal tissue were detected by RT-PCR.

Results: MSC could be transfected efficiently as seed cells to accept target genes by Ad-HGF, and a higher level of expression of HGF in vitro, which persisted 16 days at least. Compared with the control group, the content of HGF in the supernatant after transfection increased time-dependently and peaked at 8 d. The expression of HGF mRNA were detected by RT-PCR, which persisted 11 days at least. DAPI-labeled transplantating cells were found in the obstructed kidney of rats at day 3, 7, 14 after transplantion. Results of Masson stain revealed that the range of collagenous fibers in renal tissue in UUO group was significantly larger than in transplanting groups. Compared with UUO group, the expression of α-SMA mRNA and protein were decreased in transplanting group. The expressions of HGF mRNA and protein in transplantion group were significantly higher than in UUO group. Simultaneously the expression of fibronectin mRNA in transplanting group was significantly lower than in UUO group.

Conclusions: This study suggests that transplantation of MSC modified with Ad-HGF are capable of homing to renal tissue of unilateral ureteral obstruction following renal artery infusion and could attenuate renal fibrosis.

PUB256

Astragaloside IV Promote the Proliferation of Adipose-Derived Stem Cells In Vitro Weiwei Wang, Wei Wang, Yan Jiang, Jinyuan Zhang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: The proliferation ability of stem cells may be affected by microenvironment in the injury tissue. Astragaloside IV(Ast) could promote proliferation of stem cells. In this study, adipose-derived stem cells(ADSCs) were investigated under different concentration of Ast and different time point in vitro so as to select the most suitable intervention conditions and also to evaluate the feasibility for ADSCs cultrued with Ast.

Methods: The human adipose-derived stem cells(hADSCs) at passage 3 were used for the experiments. Cells were cultured in five groups and observed at 24hrs, 48hrs and 72hrs respectively. Control group cells were cultured in 10% FBS complete medium. Experimental group cells were cultured in 10% FBS complete medium with different concentration of Ast(10mg/l, 20mg/l, 30mg/l and 40mg/l). Cell proliferation was determined by Cell-Counting Kit-8 (CCK8) and proliferating cell nuclear antigen (PCNA) assay to select the most suitable intervention conditions (concentration and time point) and then hADSCs (under suitable intervention conditions) were identified by specific surface markers(CD moleculors) by FACS and differentiation abilities into osteoblasts, adipocytes and chondroblasts in vitro under appropriate conditions.

Results: CCK-8 examination showed that absorbanee values of cultured cells were significantly changed at different time points after treating with 10mg/l,20mg/l,30mg/l,40mg/l Ast. At 48hrs, 20mg/l and 30 mg/l Ast had the most significant effect on proliferation of cultured cells. Compared with 20mg/l group, proporition of hADSCs had no statistical difference in 30mg/l group. Expression of PCNA had also no statistical difference in the 20mg/l and 30mg/l groups for 48hrs. Control group and hADSCs cultured with 20mg/l Ast for 48 hours both expressed a high level of typical MSCs markers (CD29, CD44 and CD105).They could also differentiate into osteoblasts, adipocytes and chondroblasts in vitro under appropriate conditions.

Conclusions: The 20mg/l Ast for 48hrs could obviously promote the proliferation of hADSCs and have no influence on differentiation abilities, which may enhance the effect of hADSCs in tissue engineering.

Funding: Government Support - Non-U.S.

PUB257

Interaction between Cyclosporine A and Bone Marrow Mesenchymal Stem Cells, Their Conditioned Medium and Microvesicles in a Mouse Model

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Background: Adult stem cells have been extensively investigated for their regenerative and immunomodulatory properties. This process is orchestrated by cytokines and growth factors secreted by injured renal tissue which mobilize bone marrow mesenchymal stem cells (BM-MSCs) to the affected regions. Recent studies have suggested that this paracrine-endocrine mechanism between differentiated cells and stem cells mediated by microvesicles (MVs). Several studies with MSCs and their MVs treatment have showed acute kidney injury improvement but few studies have been done in chronic kidney disease. The objective is to determine the possible regenerative or preventive effect of BM-MSCs, their conditioned medium (CM) and MVs derived from them in a chronic nephrotoxic mouse model induced by Cyclosporine A (CsA).

Methods: Murine BM-MSCs were isolated from C57BL/6 mice and CM and MVs were obtained *in vitro* for being as part of the different therapies. Chronic nephrotoxic mouse model was induced by CsA 75 mg/Kg or vehicle for 4 weeks in male C57BL/6 being randomized in two groups: 1) Preventive group (PG), where three different therapies where weekly inoculated and 2) Regenerative group (RG), where mice were inoculated at week 2 and 3 after starting CsA treatment with MVs. BUN levels were determined in all animals. Histopathological kidney sections (hematoxylin/eosin and PAS stain) were scored for the percentatge of vacualization, tubular necrosis, casts, cyts and glomerular hialynosis.

Results: Survival rate in PG and RG groups was lower than CsA group. No differences were observed in BUN levels between treatments. RG showed better results than PG in histopathological analysis.

Conclusions: The application of three different therapies in a chronic nephrotoxicity mouse model induced by CsA showed an interaction between BM-MSCs, CM, MVs and CsA that decreases the mouse survival and increases the histopathological renal injury.

Funding: Government Support - Non-U.S.

PUB258

Embryonic Kidney Provides a Niche for Bone Marrow-Derived Mesenchymal Stem Cells Differentiation into Epithelial Cells In Vitro

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Background: Renal tubule injury plays a major role in pathogenesis of acute kidney injury. Recent findings have demonstrated that bone marrow-derived mesenchymal stem cells (MSCs) can be differentiated into renal epithelial cells and promote tissue repair in an in vivo mouse model of acute renal failure, but the rate of cell differentiation is relatively low. The major reason for this lack of success is because developmental programs that differentiate MSCs into renal cells consist of numerous factors, some of which are as yet unknown. Some data showed that MSCs can differentiate into mature cells when supplied with a niche containing factors identical to those in the kidney developmental process.

Methods: In order to trace the MSCs, we used red fluorescent protein(RFP)-labeled murine MSCs. To assess if cultured MSCs were already differentiated into epithelial cells in vitro without embryonic kidney niche, the cells were subjected to RT-PCR analysis for specific epithelial marker genes. To test if a niche for epithelial cells can be provided by disaggregation/reaggregation of embryonic kidney cells, we used disaggregation/reaggregation method. In order to determine if whole embryonic kidney can provide a different niche, we injected MSCs into embryonic kidney and cultured with kidney organ culture system.

Results: No expression of Cadherin6 and E-Cadherin in MSCs were observed before culturing in the niche. When MSCs were mixed with embryonic kidney cells using disaggregation/reaggregation method, they migrated and surrounded ureteric bud, some of which integrated into intrinsic renal cells, not cell fusion. Only a few MSCs expressed E-cadherin which indicated epithelial cells. Compared to the first method, we found more MSCs can be differentiated into epithelial cells, taking part in development of renal tubule with intrinsic embryonic cells.

Conclusions: These results indicate that migration and recruitment of MSCs occurred in embryonic kidney niche. Furthermore, MSCs can be differentiated into renal cells.

Funding: Government Support - Non-U.S.

PUB259

Poor Lysosomal Membrane Integrity in Proximal Tubule Cells of Haptoglobin 2-2 Genotype Mice with Diabetes Mellitus

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Background: The Haptoglobin (Hp) genotype is a major determinant of progression of Diabetic Nephropathy(DN) in DM patients. The major function of the Hp is to bind and modulate the fate of extracorpuscular Hb and its iron cargo. In humans we have 3 different Hp genotypes: Hp 1-1, 2-2, 2-1. Hp 1-1 is potent anti-oxidant and protective against DN than Hp 2-2. We have demonstrated an interaction between Hp genotype and DM on the accumulation of iron in renal proximal tubule cells (PCT) in mice.

Methods: All mice were C57B1/6 genetic background. Mice with Hp -2,2-1,1-1, were sacrified after DM duration of 3 months induced by SZT. We analyzed iron deposition by Transmission Electron Microscopy (TEM), Energy dispersive X-ray spectroscopy (EDX) and electron energy loss spectroscopy (EELS). We Assess lysosomal membrane integrity & lipid peroxides, redox-active chelatable iron, vit.E content in the PCT of different Hp genotypes treated with Vit.E or placebo.

Results: TEM, EDX,EELS: Accumulation of electron dense deposits (iron rich) in the lysosomes of PCT cells from Hp 2-2 (65±4% of all lysosom.) compared with Hp 1-1 DM mice (41±4% of all lysos.) $p<0.05$. A 2-fold increase in the amount of redox-active iron in the lysosomes of Hp 2-2 (0.56 ± 0.07 μM) vs those from Hp 1-1 DM mice (0.23 ± 0.14 μM, $p = 0.036$). Lysosomal membrane integrity was decreased in Hp 2-2 DM PCT l Vs Hp1-1, $P<0.003$. Vit. E supplement. Resulted in a significant 45% reduction in lysosomal redox-active iron in Hp 2-2 vs Hp 1-1 DM mice, decreased lysosomal lipid peroxidase (LP) in Hp 2-2 Vs Hp2-2 DM mice treated with placebo (75.7+/-9 vs 109.2+/-8.8 nmol LP/mg protein) $p=0.03$. A correlation between lysosome membrane α-tocophorol conc. And the degree of lysosomal membrane oxidation in Hp 2-2 DM mice but not in Hp 1-1, $p<0.003$.

Conclusions: Increased Iron deposits in PCT of DM mice with Hp 2-2, were associated with lysosomal membrane lipid peroxidation. Vit. E adm. To Hp 2-2 DM mice resulted in a significant decrease in intralysosomal iron induced oxidation. Iron induced renal PCT injury, may play a role in the development of DN.

Funding: Government Support - Non-U.S.

PUB260

Effects of HP Acthar Gel® in the STZ Rat Model of Diabetic Nephropathy

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Background: H.P. Acthar® Gel (repository corticotropin injection; Questcor) is a long-acting formulation of the full sequence ACTH(1-39) that may include other pro-opiomelanocortin peptides. Prior evidence demonstrated that Acthar had a positive effect on renal function and pathology in animal models of FSGS, diabetic nephropathy and acute kidney injury (Gong, 2009; 2010, 2011; Wang 2010). Clinical evidence in human subjects with diabetic nephropathy also demonstrated Acthar's efficacy in improving renal function (Tumlin, 2011). Here we investigated whether Acthar can slow the progression of renal function loss in the STZ diabetic rat model.

Methods: Diabetes was induced in Wistar rats with a single dose of STZ (55 mg/kg) administered intravenously. At 6 weeks post STZ insulin management (2IU BID) and treatment commenced. The following groups were treated by s.c. injection QOD: placebo with insulin management; Acthar (10IU) with insulin management; Acthar (10IU) no insulin; α-MSH (10 ug) with insulin management and diabetic animals with no treatment or insulin. Treatment was for a total of 8 weeks post STZ and half the animals recovered with no treatment (except for insulin management) for an additional 4 weeks.

Results: Hyperglycemia occurred 4 days post STZ and renal function, measured by creatinine clearance, declined steadily over the 6 weeks prior to treatment. Acthar, with and without insulin management, slowed the progression of diabetic nephropathy compared to placebo and the comparator melancortin peptide, α-MSH, by several measures of renal function including creatinine clearance. Importantly, glycemia was not significantly worsened by Acthar treatment.

Conclusions: Acthar slowed progression of diabetic nephropathy in STZ rats with established disease by multiple measures of renal function. This preclinical evidence adds to the growing body of preclinical and clinical evidence that Acthar has beneficial effects on renal function and supports the clinical development of Acthar as a treatment for diabetic nephropathy, a non approved indication.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals Inc

PUB261

Abelmoschus manihot (L.) Medic, a Compound of Chinese Herbal Medicine, Improves Renal Inflammation and Glomerularsclerosis by Regulating p38MAPK and TGF-beta1/Smad Signaling Pathways in Diabetic Nephropathy

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Background: *Abelmoschus manihot (L.) Medic* (AM), a compound of Chinese herbal medicine has been applied extensively for treatment of patients with diabetic nephropathy (DN) in China. Accumulating evidences demonstrate that renal inflammation and glomerularsclerosis are determinants during glomerular injury progress under high-glucose condition. Among which, p38 mitogen-activated protein kinase (MAPK) and transforming growth factor (TGF)-beta1/Smad signaling pathways play critical roles respectively. We aim to investigate effects and mechanisms of AM on renal inflammation and glomerularsclerosis by regulating p38MAPK and TGF-beta1/Smad signaling pathway activities in streptozotocin (STZ)-induced nephropathy rats.

Methods: Rats were randomly divided into 3 groups, sham-operated group, AM-treated group, and vehicle given group, and sacrificed at weeks 8 after induction of DN induced by 2 consecutive intraperitoneal injections of STZ at 30 mg/kg dose with an interval of 1 week following unilateral nephrectomy. Daily oral administration of AM and vehicle (saline) was started after the second injection of STZ until the day of sacrifice. Proteinuria,

urinary albumin, blood glucose, biochemical indicators, pathological changes, and protein expressions of TGF-beta1, Smad3, Smad7, phosphorylated Smad 2/3 (p-Smad2/3), p38MAPK, and phosphorylated p38MAPK (p-p38MAPK) in glomeruli were examined, respectively.

Results: The p38MAPK and TGF-beta1/Smad signaling pathways were activated in DN rats. AM markedly down-regulated p-p38MAPK, p-Smad2/3, and TGF-beta1 protein expressions, and ameliorated proteinuria, renal function, mesangial lesions, and macrophage accumulation in glomerulus without lowering blood glucose level.

Conclusions: AM could improve renal inflammation and glomerularsclerosis by inhibiting p38MAPK and TGF-beta1/Smad signaling pathways activation in vivo.

Funding: Government Support - Non-U.S.

PUB262

Study on the Relationship between Plasma Activated Protein C and Atherosclerosis in Type 2 Diabetic Nephropathy

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Background: Serum active protein C decreased(APC) obviously in diabetic patients and was reported to be negatively correlated with atherosclerosis, which may be associated with endothelial cell injury or inflammation. Inflammation was reported increased in patients with type 2 diabetic nephrology. So the objectives of this study were to investigate the relationship between plasma APC and atherosclerosis in type 2 diabetic nephropathy, and the mechanism of atherosclerosis in type 2 diabetic nephropathy.

Methods: 30 patients with type 2 diabetic nephrology(non-dialysis)were enrolled from April 2011 to July 2011. The carotid intima-media thickness(IMT) was measured by ultrasonography. APC, interleukin-1β (IL-1β), soluble vascular adhesion molecular-1 (sVCAM-1), soluble endothelial cell protein C receptor (sEPCR), soluble thrombomodulin (sTM) were assayed by ELISA. The relationship between these parameters and IMT were analyzed.

Results: 1) APC significantly decreased in type 2 diabetic nephropathy (2865.99±571.38pg/ml vs. 3227.70±300.44 pg/ml, p=0.005), while IMT(0.18±0.07cm vs. 0.06±0.02cm,p=1.67E-10),sTM(1.96±0.77ng/ml vs. 1.23±0.53ng/ml, p=3.22E-4),sEPCR(365.85±143.75ng/ml vs. 266.16±132.20ng/ml, p=0.010), sVCAM-1(493.55±411.26ng/ml vs. 85.10±76.99 ng/ml,p=2.4E-5), IL-1β(395.82±250.4pg/ml vs. 199.53±80.12 pg/ml,p=0.001)was higher in 2 type 2 diabetic nephropathy. 2) APC negatively correlated with albuminuria (r=0.402, p=0.027), IMT(r=-0.720,p=0.002), sTM(r=-0.505, p=0.005), sEPCR(r=-0.477,p=0.008), sVCAM-1(r=-0.437,p=0.018) and IL-1β(r=-0.564,p=0.002. IMT positively correlated with age(r=0.416, p=0.022), albuminuria (r=0.490, p=0.006), sTM(r=0.575, p=0.001), sEPCR(r=0.567, p=0.001), sVCAM-1(r=0.518, p=0.004), IL-1β(r=0.562, p=0.002), and hs-CRP(r=0.380, p=0.042). 3)APC concentrations were significantly different in different proteinuria groups and different IMT groups by ANOVA analysis(p=0.001,p=0.021).

Conclusions: Plasma APC significantly decreases in type 2 diabetic nephropathy patients, with possible association with inflammation or endothelial cell injury. Decreased plasma APC might be related to atherosclerosis in type 2 diabetic nephropathy.

Funding: Clinical Revenue Support

PUB263

Expression of Organic Anion and Cation Transporters in Experimental Diabetic Nephropathy

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Background: Association between diabetic nephropathy and renal expression of organic anion transporters (Oats) and organic cation transporters (Octs) is unclear. The aim of this study was to identify sex-dependent changes of Oats and Octs in Zucker spontaneously hypertensive fatty rats (ZSF1), a rat model for diabetic nephropathy.

Methods: Kidneys of 18 week old ZSF1 female and male rats, and their lean controls were obtained from Charles River. Total RNA was isolated from kidney cortical slices and genes of interest were analyzed by real-time PCR. Renal sections were stained with periodic acid Schiff stain (PAS) and anti-fibronectin antibody to characterize structural changes induced by diabetes.

Results: Despite similar structure of renal tissue between lean males and females, lean males showed minor changes in tubular basement membrane and a higher accumulation of fibronectin than female rats. Glomerulosclerosis, tubulointerstitial fibrosis and protein casts in Bowman's space and in tubular lumen were stronger in obese male than in obese female rats. Oat1, Oat2, Oat3, the sodium-dependent dicarboxylate transporter 3 (NaDC3), Oct1 and Oct2 were significantly lower expressed in obese females than in lean females, whereas obese and lean ZFS1 male rats showed no difference. Expression of multidrug resistance-associated protein 2 (Mrp2) and Mrp4 remained unchanged in both sexes. Renal vimentin and E-cadherin were higher expressed in obese males compared to lean males. In females, no differences were observed. RNA level of transcription factor hepatocyte nuclear factor 4 (Hnf4a) was decreased in kidneys of obese female and male rats as compared to their lean controls, Hnf1b was decreased in ZSF1 females but not in males.

Conclusions: Diabetic nephropathy was associated with severe structural changes in male rats and milder renal injury with decreased expression of Oats and Octs in females, possibly leading to a decreased secretion of organic anions and cations (e.g. drugs).

Funding: Other NIH Support - Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)

PUB264

Linagliptin Reduces High Glucose Induced Inflammatory and Fibrotic Markers in Human Kidney Proximal Tubular Cells

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Background: Linagliptin is a Dipeptidyl Peptidase (DPP4) inhibitor used to lower blood glucose in type 2 DM. It inhibits cleavage of the endogenous incretin hormones GLP-1 and GIP, raising their levels and thus promoting insulin release and inhibiting glucagon secretion resulting in lowering of blood glucose. DPP4 inhibitors may have benefits other than glucose lowering as DPP4 also cleaves a host of additional peptides (not just GLP-1/GIP), thus regulating their biological function resulting in a range of possible outcomes. It would be desirable to have antidiabetic therapies with additional renoprotective effects beyond glucose lowering. We aimed to evaluate the effect of linagliptin on high glucose induced inflammatory and fibrotic markers in human kidney proximal tubular cells.

Methods: Immortalised Human Proximal Kidney cells (HK2 cells) were exposed to control (5mM) or high glucose (30mM) +/- linagliptin (30nM). Cells were harvested for nuclear binding assays, western blot, flow cytometry and supernatants were collected for ELISA. Inflammatory/fibrotic markers relevant to diabetic nephropathy like High Mobility Group Box Protein 1 (HMGB1), fibronectin, NFKB and TGFB were measured as endpoints.

Results: High glucose downregulated the expression of DPP4. Linagliptin reduced HMGB1 induced NFκB binding, independent of DPP4 cleaving of HMGB1. There was no effect on high glucose induced NFKb binding. High glucose-induced fibronectin expression was also reduced with linagliptin and this was associated with unchanged total TGFB levels.

Conclusions: The downregulation of DPP4 with high glucose may be due to shedding of the surface protein. In summary our data support a role for DPP4 inhibition in limiting the impact of extracellular mediators on renal tubular/tubulointerstitial pathology.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim, Government Support - Non-U.S.

PUB265

The Targeting Design of Recombinant Protein C and Its Protection from Endothelial Cells Injury

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Background: Important relationship between atherosclerosis and vascular endothelial damage in diabetic nephropathy (DN) was reported before, while active protein C (APC) may have a protective effect for endothelial cells. The objectives were to study the influence of these patients' serum on human vascular endothelial cells (HUVEC), to construct adenovirus with specific promoter and protein C targetedly express in endothelial cells and its protect effect for vascular endothelial injury.

Methods: 1) Cultured HUVEC in vitro;2 Construction, appraisal and packaging of restructuring adenovirus Ad-FIT-1/PC, transfected with HUVEC and human aortic smooth muscle cells (HA-vsmc); 3)Grouping HUVE into 4 groups: Control group, Black adenovirus group (AD group)(Ad-GFP transfected+thrombin), PC group (Ad-FLT-1/PC transfected), PC activation group (Ad-FLT-1/PC transfected+thrombin; APC). Apoptosis rate was analyzed;4) APC,sEPCR,sTM,VCAM-1,IL-1β in the supernatant of cell culture was measured by ELISA.

Results: 1)The intercellular space were wider, and the damaged cells were more in the intervention group than in the control. 2) Western blot showed increased PC expression in HUVEC after transfect with Ad-FLT-1/PC. 3)The apoptosis rate of APC group(1.53±0.39)% was lower than control group(5.16±3.53)%(P<0.05). 4)APC of APC group was higher than PC group and AD group. sEPCR of APC group was lower than the control. sTM in APC group was lower than the control, AD group and PC group. The VCAM-1 level of APC group was the lowest. IL-1 β in APC group was lower than the PC group.

Table1 Concentrations of biomarkers

Group	APC(ng/l)	sEPCR(ng/ml)	sTM(ng/ml)	VCAM-1(μg/l)	IL-1β(ng/l)
Control group	77.411±7.609	7.569±1.515	0.405±0.071	5.417±1.180	0.146±0.070
AD group	72.079±5.512	6.630±1.067	0.407±0.066	4.660±0.770	0.149±0.064
PC group	53.776±1.515	6.530±0.755	0.377±0.155	4.559±0.565	0.319±0.059
PC activated group	82.172±4.361	6.127±1.130	0.384±0.150	4.205±0.710	0.043±0.083

Conclusions: Serum of DN patients with atherosclerosis can affect HUVEC. Restructuring adenovirus Ad-FLT-1/PC can targeted transfect HUVEC, and express the PC. APC can protect HUVEC by restrain the apoptosis and reduce the secretion of inflammatory factors caused by DN serum.

Funding: Clinical Revenue Support

PUB266

Serum Retinol Binding Protein 4 Is Associated with Type 2 Diabetic Nephropathy

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Background: Serum retinol binding protein 4(RBP4) is known to be associated with insulin resistance, obesity, and type 2 diabetes. Whether it can be used as a biomarker of diabetic complications remains undeterminable.

Methods: We investigated the association of RBP4 level in type 2 diabetic nephropathy (DN). In vivo study of the 236 patients, 71% were diagnosed as type 2 diabetes. These patients were divided into three groups (normoalbuminuria vs microalbuminuria vs macroalbuminuria). In vitro study we tried to examine the mechanism of RBP4 in renal cells.

Results: Total four groups including non-diabetic controls showed similar basal characteristics in age, sex, BMI, eGFR, Hb, serum albumin, CRP, lipid levels, etc. Diabetic patients had higher blood glucose levels and HOMA-IR index than controls. Interestingly, patients with macroalbuminuria showed higher serum RBP4 level compared to patients without diabetic nephropathy or with normoalbuminuria or with microalbuminuria. It is notable that RBP4 level was also increased in patients with lower creatinine clearance. In univariate and multiple regression analysis, 24-h albuminuria and systolic blood pressure were positively correlated with logRBP4 level. BMI was negatively correlated with that level. In case of other vascular complications of type 2 diabetes, RBP4 level was not increased in the patients with retinopathy and peripheral neuropathy. However, the patients with higher RBP4 had significantly increased risk of cardiovascular, peripheral arterial and cerebrovascular disease. Podocytes expressed their uptake of RBP4 strongly compared to other renal cells including mesangial and tubular cells. Treatment of RBP4 into podocytes induced the activation of insulin receptor substrate-1(IRS-1).

Conclusions: RBP4 may contribute to the pathogenesis of DN and be used as a novel biomarker of DN. It also might be used as an indicator of macrovascular complications in type 2 diabetes.

Funding: Pharmaceutical Company Support - Korean Society of Nephrology

PUB267

Anti-Inflammatory Effects of BMP7 on AGEs-Induced Tubular Injury Ruixi Li,[1] Wai Han Yiu,[1] Miao Lin,[1] Hao-Jia Wu,[1] Loretta Y.Y. Chan,[2] Joseph C.K. Leung,[1] Kar Neng Lai,[1] Sydney C.W. Tang.[1] *[1]University of Hong Kong; [2]Queen Mary Hospital.*

Background: Formation and accumulation of advanced glycation end products (AGEs) are remarkably accelerated in the diabetic kidney. We recently demonstrated that AGEs stimulated various pro-inflammatory and pro-fibrotic responses in cultured human proximal tubular epithelial cells (PTEC). Bone morphogenetic protein (BMP) 7 has been reported to confer renoprotective effects in a variety of cell types and disease models. However, data is lacking in AGEs-induced renal tubular inflammation. Our present study explored the therapeutic potential of BMP7 on AGEs-induced tubular injury and its possible mechanisms.

Methods: Primary human PTEC were growth-arrested and exposed to glycated human serum albumin (AGE-HSA) with or without recombinant human BMP7 (rhBMP7). Pro-inflammatory chemokines and cytokines were detected at both gene and protein levels by real-time PCR and ELISA, respectively. Inhibitors of different signaling pathways were used (SB203580, PD98059 and PDTC for p38 MAPK, p44/42 MAPK and NF-κB respectively) to study the involvement of these pathways.

Results: AGE-HSA induced PTEC expression of pro-inflammatory factors through multiple pathways - IL8 through both p38 and p44/42 MAPK pathways, sICAM-1 through p44/42, and MCP1 through p38 MAPK. Although PDTC suppressed the mRNA and protein expression of IL-6 and TNF-α induced by AGE-HSA, AGE failed to stimulate NF-κB under these experimental conditions, indicating involvement of other pathways. rhBMP7 (5-200 ng/mL) dose-dependently attenuated AGE-HSA (100 ug/mL)-induced expression of sICAM-1, MCP1, IL8, TNF-α and IL6 at both mRNA and protein levels. Moreover, rhBMP7 suppressed phosphorylation of p38, p44/42 which was induced by AGE-HSA.

Conclusions: Our *in vitro* results demonstrated that BMP7 can attenuate pro-inflammatory responses to AGE stimulation in PTEC via suppression of multiple signaling pathways including p38 and p44/42 MAPK. Its potential application as a therapeutic molecule warrants further investigation. This study is supported by a General Research Fund of the Research Grants Council (Grant number: HKU 7770/09M) of Hong Kong.

Funding: Government Support - Non-U.S.

PUB268

Protective Effect of Rhein on Pancreatic β-Cells Jing Liu, Zhao-hong Chen, Cai-hong Zeng, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu.*

Background: Rhein, an anthraquinone compound isolated from rhubarb, is effective in treating diabetic nephropathy (DN). We found Rhein can also lower blood glucose level of diabetic patients, raising the possibility of a protective effect of rhein on pancreatic β-cells. We set out to test this hypothesis.

Methods: Mouse β-cell line, NIT-1, was treated with high glucose in the presence or absence of rhein, followed by examinations of mitochondrial distribution of rhein with MitoTracker RED CMXRos, mitochondrial morphology with electron microscopy, mitochondrial function with JC-1 staining and ATP content, apoptosis by Annexin V staining, and β-cell function by insulin secretion.

Results: The autofluorescence of rhein was detected mainly in the cytoplasma and mostly overlapped with MitoTracker at mitochondria. Rhein restored mitochondrial morphology of β-cells in the treatment of high glucose and prevented the upregulation of Drp1, a protein involved in the establishment of mitochondrial morphology. Rhein also restored mitochondrial membrane potential and increased ATP content in the cells. Moreover, Rhein alleviated high glucose-induced apoptosis as shown by Annexin V staining, as well as its inhibitions of cytochrome C release, bax translocation, and caspase 3 and 9 activation in the cells. Lastly, Rhein was found to increase insulin secretion of the cells.

Conclusions: The rhein uptaken by β cells localizes mainly in mitochondria. Rhein protects β cell against high glucose-induced damage through preserving mitochondrial structure and function, as well as inhibiting apoptosis. Rhein is able to sustain insulin secretion of β-cells. These effects of Rhein on β-cells may explain blood glucose reducion observed in diabetic patients treated with Rhein.

PUB269

Valsartan Prevents High-Glucose-Induced Podocyte Apoptosis via inhibiting Nuclear Factor of Activated T Cells c1 Wei Shi, Ruizhao Li, Wenjian Wang, Juan Ma. *Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Podocyte apoptosis, an early feature of diabetic nephropathy, has been proven to be prevented by angiotensin receptor blockers (ARBs), including valsartan. However, the underlying molecular mechanisms remain poorly understood. Multiple evidences showed the activated nuclear factor of activated T cells c1 (NFATc1) causing podocyte apoptosis. Interestingly, our present study showed that valsartan reduced high-glucose-induced activation of NFATc1 in podocytes and thus prevented podocyte apoptosis.

Methods: Conditionally immortalized mouse podocytes cultured in vitro were divided into 1) normal glucose group, 2) normal glucose plus Ionomycin group, 3) high glucose group, 4) valsartan plus high glucose group,5) 11R-VIVIT plus high glucose group and 6) high glucose plus DMSO group. The activated NFATc1 protein was measured by Western blot, and the cell apoptosis by flow cytometry with Annexin V-FITC/PI double staining.

Results: High glucose activated podocyte NFATc1 in a dose and time-dependent way, as well as ionomycin (NFAT activator). When treated with valsartan, the high-glucose-induced activation of NFATc1 was significantly attenuated. Meanwhile, the high-glucose induced podocyte apoptosis reached the peak at the point of 48 hours when valsartan significantly reduced podocyte apoptosis, as well as 11R-VIVIT (NFAT inhibitor).

Conclusions: Valsartan prevents high-glucose-induced podocyte apoptosis by attenuating the activation of NFATc1, suggesting that there may be a link between angiotensin receptors and activation of NFATc1 induced by high-glucose in podocytes.

PUB270

EP4 Selective Agonist Exacerbate Inflammation and Fibrosis of the Kidney in Streptozotocin Induced Diabetic Mice Riyaz Mohamed, Punithavathi Vilapakkam Ranganathan, Calpurnia Jayakumar, Ganesan Ramesh. *Medicine/Vascular Biology Center, Georgia Health Sciences University, Augusta, GA.*

Background: Diabetic nephropathy is currently the most common cause of end-stage renal disease (ESRD) in the western world. Exacerbated inflammation is known to contribute for the development and acceleration of nephropathy. COX-2 mediated production of inflammatory mediator is increased during diabetes, however their involvement in the progression diabetic kidney disease quite controversial. Here, we show the unexpected role of PGE2-EP4 pathways in inducing inflammation and nephropathy.

Methods: Diabetes was induced using streptozotocin in C57BL/6 mice. EP4 selective agonist ONO-AE1-329 was administered 1 week after confirmation of hyperglycemia at a concentration of 0.1mg/kg BW subcutaneously. Kidney function, fibrosis, albuminuria and inflammation were determined by measuring serum creatinine, Western blot analysis, immunostaining, ELISA and RT-PCR.

Results: Hyperglycemia causes enhanced COX-2 expression and PGE2 production which was associated with increased excretion of pro-inflammatory cytokine (TNFα) and chemokines (MCP-1 and IP-10). Moreover, albumin excretion, collagen and α-smooth muscle actin expression were increased by 12 weeks of diabetes. Increased fibrosis was associated with increased macrophage infiltration in the interstitium. To determine, the receptor that mediates PGE2 inflammatory activity in diabetic kidney, EP4 selective agonist was administered in control and diabetic mice. Administration of EP4 selective agonist exacerbated inflammatory cytokine and chemokine production, collagen I and IV, α-smooth muscle actin expression, glomerular sclerosis and albuminuria (167±25 vs. 71±16 μg/24hr, $p<0.001$) without altering blood glucose level.

Conclusions: Our data suggests that COX-2 mediated PGE2 production is detrimental to diabetic kidney and PGE2 mediates its pro-inflammatory and pro-fibrotic activity through EP4 receptor. Activation of EP4 receptor accelerates diabetic kidney disease.

Funding: NIDDK Support

PUB271

The Role of Toll-Like Receptor Proteins (TLR) 2 and 4 in Mediating Inflammation in Human Proximal Tubular Cells (PTC) Harshini Mudaliar,[1] Steven J. Chadban,[2] Huiling Wu,[2] Carol A. Pollock,[1] Usha Panchapakesan.[1] *[1]Renal, Kolling Institute of Medical Research, University of Sydney, Sydney, NSW, Australia; [2]Renal, Royal Prince Alfred Hospital, Sydney, NSW, Australia.*

Background: Inflammatory responses are central to the pathogenesis of diabetic nephropathy (DN). TLRs are ligand activated membrane-bound receptors which induce inflammatory responses through activation of nuclear factor-kappaB (NF-κB). TLR 2 and 4 are present in PTCs and are activated by high mobility group box-1 (HMGB1), an endogenous ligand pathological in DN.

Methods: HK2 cells (a human kidney PTC line) were exposed to 5mM(control), 11.2mM (approximating the diagnostic threshold for diabetes mellitus) and 30mM (high) glucose for 72h or 7 days. Cells were harvested for protein, mRNA and nuclear extract to assess for TLR 2, 4 and inflammatory markers. HMGB1-mediated NF-κB activation was investigated by silencing TLR 2 (siRNA) and inhibiting TLR 4 (TAK-242).

Results: 11.2mM glucose maximally increased TLR 2 and 4 expression, the release of HMGB1 and NF-κB binding with increased transcription of monocyte chemoattractant

protein-1 (MCP-1) and interleukin (IL)-8. Peroxisome proliferator-activated receptor gamma (PPARγ) transcription was reduced by exposure to 11.2mM glucose and increased with 30mM glucose. Recombinant HMGB1 induced NF-κB binding and this was prevented by TLR 2 silencing and TLR 4 inhibition. However, only TLR2 expression and subsequent NF-kB binding was sustained at 7 days.

Conclusions: 11.2mM glucose levels more significantly induced inflammation via the TLR 2 and 4 pathway compared to 30mM glucose. This may be related to the compensatory increase in PPARγ induced by exposure to 30mM glucose. Furthermore, HMGB1 mediates NF-κB binding in PTCs through both TLR 2 and 4. However, TLR2 is more likely to be the predominant mediator of NF-κB activation in PTCs.

Funding: Government Support - Non-U.S.

PUB272

RAS Blockade Inhibits Bcl-2 Modifying Factor Expression and Prevents Hypertension, Tubulointerstitial Fibrosis and Tubular Apoptosis in Diabetic Akita Mouse Kidneys Hasna Maachi,[1] Chao-Sheng Lo,[1] Shaaban Abdo,[1] Shilin Li,[1] Isabelle Chenier,[1] Shao-Ling Zhang,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] John S.D. Chan.[1] *[1]Res. Ctr., CHUM-Hotel Dieu Hospital, Montreal, QC, Canada; [2]Res. Ctr, Maisonneuve-Rosemont Hosp., Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp, Boston, MA.*

Background: We previously reported that pro-apoptotic protein Bcl-2 modifying factor (Bmf) is highly expressed in diabetic mouse renal proximal tubular cells (RPTCs) and induced RPTC apoptosis *in vitro.* We investigated the impact of renin-angiotensin system (RAS) blockade on Bmf expression and on hypertension and RPTC apoptosis in Akita mice (a model of type 1 diabetes).

Methods: Adult male Akita mice (11 weeks) were treated ± RAS blockers (losartan, 30 $mg.Kg^{-1}.day^{-1}$ and perindopril, 4 $mg.Kg^{-1}.day^{-1}$ in drinking water). Controls were untreated non-Akita littermates. We also examined the effect of angiotensin II (Ang II) on Bmf gene expression in cultured rat RPTCs. Plasma glucose, systolic blood pressure (SBP) and albuminuria were monitored weekly from 10 until 16 weeks. Kidneys were processed for histology and apoptosis studies. Renal proximal tubular angiotensinogen (Agt), Bmf, pro-fibrotic and pro-apoptotic gene and protein expression were evaluated by real time-qPCR and Western blotting, respectively.

Results: Akita mice developed hyperglycemia and significantly higher SBPs, kidney/body weight ratios and albuminuria as compared to non-Akita littermates. Kidneys from Akita mice displayed renal hypertrophy, tubulointerstitial fibrosis and tubular apoptosis as compared to non-Akita littermates. Increased expression of Agt, Bmf, transforming growth factor-beta1, collagen type IV and active caspase-3 were evident in RPTCs of Akita mice. Treatment with RAS blockers inhibited Agt and Bmf expression, prevented hypertension and albuminuria and attenuated kidney abnormalities in Akita mice. Finally, Ang II stimulated Bmf mRNA expression in RPTCs in a dose-dependent manner, and its effect was abolished in the presence of losartan and perindopril.

Conclusions: Ang II up-regulates Bmf expression and RAS blockade effectively inhibits Bmf expression and prevents hypertension and tubular apoptosis in diabetic kidneys.

Funding: Government Support - Non-U.S.

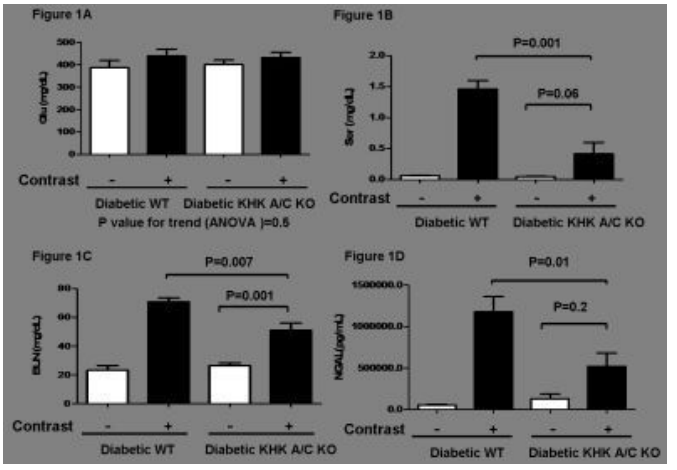
PUB273

Role of *Fructokinase* (KHK) in Contrast Induced Acute Kidney Injury in Diabetic Mice Wei Chen,[1,2] Miguel A. Lanaspa,[1] Katsuyuki Tanabe,[1] Diana I. Jalal,[1] Xueqing Yu,[2] Richard J. Johnson.[1] *[1]Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; [2]Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University, Guangdong, China.*

Background: Renal fructokinase (KHK) may have a novel role in diabetic renal disease. We aimed to elucidate the role of KHK A/C in the pathogenesis of contrast induced acute renal injuryin diabetic mice.

Methods: KHK A/C wild-type (WT) or knockout (KO) diabetic mice were injected with a contrast (isovue, 3 g iodine/kg), with pretreatment of indomethacin and N^G-nitro-L-arginine methyl ester. Serum and tissue samples were collected after 24 hours. CD68 was detected by immunofluorescence. For the *in vitro* studies HK2 and KHK silenced HK2 cells were treated with glucose and varying dosages of isovue. Cell viability was measured. Aldose reductase was detected bywestern blotting.

Results: KHK A/CWT diabetic mice developed significantly worse acute renal injury after isovue treatment compared with the KHK A/C KO diabetic mice, with higher serum creatinine, BUN and NGAL ($P = 0.001$, $P = 0.007$, $P = 0.01$ respectively), (Figure 1) more renal cortex vacuolization ($P < 0.0001$).



CD68 staining, MCP-1 and ICAM-1 mRNA were significantly higher in KHK A/C WT diabetic mice with isovue compared to the KHK A/C KO diabetic mice. *In vitro*, Isovue caused a direct dose-dependent reduction in proximal tubule cell viability. Moreover, lack of KHK A/C likely protects against the isovue toxicity on proximal tubule cells.

Conclusions: These studies provide the first evidence that contrast causes acute renal injury in diabetic mice may via mechanisms dependent on KHK A/C.

Funding: Other NIH Support - ISN Salmasi Family Fellowship

PUB274

In Vitro and In Vivo Competition between Glycation and Carbamylation Christine Pietrement,[1,2] Camille H. Nicolas,[1,2] Laetitia Gorisse,[2] Stephane Jaisson,[2,3] Philippe Gillery.[2,3] *[1]Pediatric Nephrology Unit, University Hospital; [2]FRE CNRS/URCA N° 3481, Faculty of Medicine; [3]Laboratory of Pediatric Biology and Research, University Hospital, Reims, France.*

Background: Glycation and carbamylation are two non-enzymatic post transcriptional protein modifications encountered during aging and diseases as diabetes and renal failure, and both involved in cardiovascular complications of these diseases. These reactions can theoretically compete for the same binding sites, which are often α or ε amino groups, but nothing is known about the real effect of one reaction on the other.

Methods: We studied the competition between carbamylation and glycation of albumin or total plasma proteins *in vivo* and *in vitro* by LC-MS/MS quantification of two specific biomarkers: carboxymethyllysine (CML) for glycation, homocitrulline (Hcit) for carbamylation.

Results: After 3 weeks *in vitro* incubation with 20 mM urea, human albumin carbamylation was enhanced: Hcit content reached 24.2 µmol/g of proteins, whereas it was only 18.8 µmol/g when 20 mM glucose was added to the incubation medium together with urea (22% inhibition, $p<0.05$). After 3 weeks incubation with 20 mM glucose, human albumin was specifically glycated with a CML content of 4.1 µmol/g. A 18% reduction of glycation was observed when 20 mM urea was simultaneously added ($p<0.05$). Seven weeks after subtotal nephrectomy on C57Bl6 mice (CRF) plasma Hcit was 253 nmol/g of proteins, but only 92 nmol/g in control mice and 120 nmol/g in streptozotocin-induced diabetic C57Bl6 mice ($p<0.05$). Nine weeks after streptozotocin injections, plasma CML was 189 nmol/g (+ 35% *vs* controls) and CML in CRF mice was 170 nmol/g.

Conclusions: *In vitro* experiments demonstrated the competition between glycation and carbamylation. Glycation competed more over carbamylation, perhaps because of different kinetics between the two reactions. Our mice model could not clearly evidence the influence of glycation on carbamylation. The development of a new diabetic CRF mice model will help to better characterize the *in vivo* competition between glycation and carbamylation which may have different deleterious effect in diabetes and renal failure.

Funding: Government Support - Non-U.S.

PUB275

Therapeutic Effects of EGCG on Streptozotocin-Induced Diabetic Nephropathy in Mice Byung Chul Shin, Wan Soo Lee. *Internal Medicine, Chosun University Hospital, Gwangju, Republic of Korea.*

Background: Diabetic nephropathy is one of the most serious complications in diabetes mellitus and has been the most common cause of end-stage renal disease. Green tea extracts have antioxidant properties, and (-)-epigallocatechin 3-O-gallate (EGCG) is known to be the most abundant in green tea. Osteopontin (OPN) is a large phosphoglycoprotein adhesion molecule, and has emerged as a potentially key pathophysiologic contributor in diabetic nephropathy. We examined whether EGCG could amelliorate the development of diabetic nephropathy and its role of OPN.

Methods: Mice were injected intraperitoneally streptozotocin (STZ, 200mg/Kg) and induced diabetic nephropathy. After a 8weeks, EGCG were administrated at dose of 50, and 100 mg/Kg body weight. Serum glucose, BUN, creatinine and urine volume, protein, creatinine, Western blot assay of OPN and renal histologic and histochemistry were examinated.

Results: STZ-groups were decreased renal functions and increased urine protein amounts. EGCG-treated groups showed suppressed hyperglycemia, proteinuria and the levels of BUN and serum creatinine. Furthermore, EGCG reduced renal OPN accumulation and its protein expression in the kidney cortex as well as associated pathologic conditions.

Conclusions: These results suggest that EGCG ameliorates STZ-induced diabetic nephropathy by OPN suppression. The potential use of EGCG in the treatment of diabetic nephropathy should be further explored.

PUB276

Metabolic Syndrome Associated with Renal Failure & Cardiovasculopathy due to Deletion of the Gene Encoding the Canonical Transient Receptor Potential 1 (TRPC1) Channel: Role of Gene Dosage & Epigenetics in Phenotypes Bonnie Eby,[1] Meghan Pantalia,[1] Alexander Lau,[1] Richard Matthew Atkins,[1] Leonidas Tsiokas,[2] Kai Lau.[1,3] *[1]Medicine, University of Oklahoma Health Sciences Center, OKC, OK; [2]Cell Biology, University of Oklahoma Health Sciences Center, OKC, OK; [3]Medicine, VA Medical Center, OKC, OK.*

Background: TRPC channels have been implicated in cell proliferation & metabolism. TRPC1 expression is reduced in diabetes, but the relationship is unclear. In TRPC1 -/- mice, we noted obesity, dyslipidemia, insulin resistance, diabetes, hyperphagia, renal failure, cardiovasculopathy & a lower hematocrit (Hct). In the current studies, we excludeed the confounding influence of parental hyperphagia & obesity present in previous -/- breeders by now using only +/- mice as parents.

Methods: We studied the phenotypes of low-Hct, obesity & hyperglycemia in littermates (+/+, +/-, -/-) raised & nursed by identical +/- breeders, aged 12 to 21 weeks, to define the role of epigenetics accrued & transmitted by parents of various weights. Glucose tolerance test (GTT) was done at 29 weeks.

Results: Hct was reduced by 7% in only null mice regardless of litter orders, indicating 1 wild-type allele can keep Hct normal. Obesity was noted in only null mice, but Δ was greater (13 vs 4%) in later litters born to heavier (27 vs 18 g) & older breeders (19 vs 12 weeks). Data suggest obesity is prevented by 1 wild-type allele but expressed if missing both & inheriting enough epigenetic risk factors. In mice born to younger smaller dams with fewer epigenetic risks, abnormal GTT was noted in both +/- & -/- mice, indicating normal glucose tolerance needs 2 copies of the TRPC1 gene. In mice born to older larger dams, similar degrees of glucose intolerance were found in +/+, +/- & -/- mice, showing the epigenetic dominance in the hyperglycemia. GTT showed increases of 35, 20 & 19% for the 3 genotypes between mice born to younger thinner dams & mice born to older heavier dams.

Conclusions: Our data support the key role of the TRPC1 gene in keeping Hct normal, preventing obesity & glucose intolerance, but epigenetic risk factors from +/- breeders exert major impact on the hyperglycemic phenotype.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

PUB277

Vitamin B6 Prevents the Cardiovasculopathy in Akita Mice and the Metabolic Syndrome Induced by Fructose:In-Vivo Correlation with Ex-Vivo Endothelial Functions Meghan Pantalia,[1] Bonnie Eby,[1] Uzma Hajiyani,[1] Alexander Lau,[1] Richard Matthew Atkins,[1] Kai Lau.[1,2] *[1]Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Medicine, OKC VA Medical Center, Oklahoma City, OK.*

Background: Hyperlgycemia stimulates advanced glycation end-products (AGE) contributing to diabetic complications. We recently reported the benefits of deleting the gene encoding the receptor for AGE in diabetic nephropathy. We now tested the hypothesis that inhibition of AGE by vit B6 confers end organ protection in states of insulin resistance & insulin deficiency.

Methods: Normal male mice were randomized to nothing, dextrose, fructose, or fructose + vit B6 in drinking water for 6 mon. Male Akita & control were randomized to vit B6 or nothing at 7 weeks of age. By established methods, we measured blood pressure (BP), blood glucose (BS), heart echo & aorta endothelial relaxation.

Results: Fructose raised fasting insulin, cholesterol, & weights of kidneys, hearts, & livers, all but liver mass normalized by concurrent vit B6. Vit B6 reduced BS in fructose- & dextrose-treated mice. Fructose raised systolic (S)BP (122 vs 113), diastolic (D)BP (96 vs 85), & mean arterial (MA)BP (104 vs 94), effects blocked by vit B6. SBP normalized by vit B6 (119 to 109) noted by tailcuff was confirmed by inta-arterial reading (118 to 105). Relaxation of aorta from fructose-treated mice was impaired but corrected by vit B6. At 5 & 10 mon, Akita mice had reduced left ventricular end-D volume (V), stroke V, & cardiac output, all prevented by vit B6. In Akita diabetics, vit B 6 blocked the increased SBP at 5 & 8 mon & the increased DBP & MABP at 8 mon. It corrected the endothelial dysfunctions seen in untreated diabetics.

Conclusions: Vit B6 confers anti-hypertensive & endothelial relaxing benefits in fructose-induced insulin-resistance & in insulin-deficient Akita diabetes. In fructose-induced metabolic syndrome, vit B6 prevents increases in weights of body, kidney, & heart, & normalized fasting blood glucose, insulin, & cholesterol. In Akita diabetics, vit B6 prevents cardiomyopathy. These data support the hypothesis that vit B6 protects end-organ from dysfunctions in states of increased AGE.

Funding: Veterans Administration Support, Private Foundation Support

PUB278

Knockdown of Sodium-Glucose Cotransporter Type 2 in Human Renal Proximal Tubule Cells Prevents High Glucose-Induced Increase in TGFβ1 Maggie K. Diamond-stanic,[1,3] Michael A. Rose,[1,2] Young H. You,[3] Volker Vallon,[1,3] Kumar Sharma.[1,3] *[1]Veterans Administration San Diego Healthcare System, La Jolla, CA; [2]Veterans Medical Research Foundation, San Diego, CA; [3]University of California, San Diego, La Jolla, CA.*

Background: Sodium-glucose co-transporter type 2 (SGLT2) is located primarily on the apical membrane of renal proximal tubule cells and is responsible for the reabsorption of glucose from the glomerular filtrate. Inhibition of SGLT2 results in glucosuria and lowers blood glucose. Pharmacological inhibitors of SGLT2 are in clinical trials as treatment for diabetic mellitus. However, little is known about the effect of SGLT2 inhibition on proximal tubule cells themselves. We hypothesize that preventing luminal glucose uptake in proximal tubule cells may ameliorate diabetic kidney damage.

Methods: Primary cultures of human renal proximal tubule cells (hRPTC) (ScienCell) were cultured with siRNA directed against SGLT2 for 24 hours then treated with low (5.5 mM) or high glucose (25.5 mM) for 24 hours. SGLT2 and pAMPK were measured by western blot. TGFβ1 was measured by ELISA.

Results: Addition of SGLT2 siRNA resulted in a 34% decrease in expression of SGLT2 protein. High glucose induced a 236% increase in TGFβ1 release into the cell culture media under control (scrambled siRNA) conditions. Addition of SGLT2 siRNA abolished the high glucose-induced increase in TGFβ1. Increased expression of TGFβ by high glucose is associated with decreased activity of phospho-AMP kinase (pAMPK) in several glomerular cell types, and decreased pAMPK activity potentially contributes to diabetic kidney damage. We found no change in expression of pAMPK following 24 hour high glucose treatment in hRPTC, and this was not affected by addition of SGLT2 siRNA.

Conclusions: Our data demonstrate that the reabsorption of glucose in the proximal tubule cells via SGLT2 can stimulates TGFb1 release, potentially contributing to diabetic kidney damage. However, this does not occur in association with decreased expression of pAMPK. These data suggest that inhibition of SGLT2 may have direct beneficial effects on the diabetic kidney in addition to lowering blood glucose levels.

Funding: Pharmaceutical Company Support - Bristol-Myers Squibb

PUB279

High Glucose Induces Glomerular Endothelial-Mesenchymal Transition through TGF-β1 and TGF-β2 Hui Peng, Yuanqing Li, Canming Li, Zengchun Ye, Meirong Zhong, Tan-qi Lou. *Division of Nephrology, Department of Medicine, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.*

Background: In recent years, increasing attention has been paid to the roles of endothelial-mesenchymal transition (EndMT) in tissue fibrosis. Our previous researches demonstrated that high glucose could induce endothelial-mesenchymal transition in renal glomerular endothelial cells, however, the mechanics of which are still unclear. The present study aims to investigate whether TGF-β contributes to EndMT process.

Methods: Primary rat renal glomerular endothelial cells were cultured for days in medium containing either normal glucose (NG: 5.5 mmol/l) or high glucose (HG: 30mmol/l), while cells cultured in medium containing 5.5 mmol/l glucose plus 25.5mmol/l mannitol were set as hyperosmotic control group (M). In some experiments, TGF-βtype 1 receptor inhibitor LY364947(10μmol/l)was added to the medium, while in solvent control group, DMSO was added. Real-time PCR was employed to detect TGF-β1 and TGF-β2 mRNA levels; western blotting, as well as confocal microscopy were employed to detect endothelial marker Claudin5 and mesenchymal marker α-SMA protein expression; and cell phenotype were also observed using confocal microscopy.

Results: It was showed that, compared to control group, high glucose stimulation for either 1 or 3 days led to increasing mRNA levels of both TGF-β1 ($p<0.05$, n=3) and TGF-β2 ($p<0.05$, n=3), while no significant difference was observed in hyperosmotic control group. Compared with control group, 5-day high glucose stimulation resulted in decreasing protein expressions of Claudin5 ($p<0.05$, n=3) and increasing protein expressions of α-SMA ($p<0.05$, n=3), while no significant changes were showed in hyperosmotic group. These changes could be inhibited by LY364947 ($p<0.05$, n=3) but not DMSO. When cells were exposed to high glucose, their phenotypes changed from a cobblestone shape to a spindle-liked one, which could also be attenuated by LY364947.

Conclusions: Our results indicate that high glucose may contribute to the EndMT of glomerular endothelial cells through TGF-β1 and TGF-β2 signaling pathway.

Funding: Other U.S. Government Support

PUB280

Fenofibrate Ameliorates Diabetic Nephropathy though the Activation of PGC-1α-ERR-1α Signaling in *db/db* Mice Myung Hyun Lee, Yu Ah Hong, Cheol Whee Park, Ji Hee Lim, Sungjin Chung, Keunsuk Yang, Yong-Soo Kim. *Nephrology, St. Mary Hospital, Seoul, Korea.*

Background: Peroxisome proliferative-activated receptor gamma coactivator (PGC)-1α is a multifunctional transcriptional protein, acts as a 'molecular switch' in pathways controlling fatty acid oxidation and oxidative stress, and may be a critical link in the pathogenesis of type 2 diabetes and metabolic syndrome associated with estrogen-related receptor (ERR)-1α. We evaluated the renoprotective effect of fenofibrateassociated with improving lipotoxicity and oxidative stress through the change of PGC-1α-ERRα signalingon diabetic nephropathy in *db/db* mice.

Methods: Male C57 BLKS *db/db* mice and *db/m* controls at 8 weeks of age were divided to receive either a regular diet chow or a diet containing fenofibrate (0.2% wt/wt, n=6, respectively). The treated *db/m* mice and *db/db* mice were administrated fenofibrate for 2 months starting at age of 12 weeks, and were evaluated about renal functional and pathologic phenotypes and the PPARα-PGC-1α-ERR-1α pathway.

Results: Fenofibrate treatment dramatically reduced fasting blood glucose (P<0.05) and HbA1c levels (P<0.05) in db/db mice. Fenofibrate also ameliorated albuminuria (P<0.001) and decreased urine volume (P<0.001) in db/db mice. There was no difference serum creatinine level in all each groups. The mesangial area expansion, inflammatory cell infiltration, and the accumulation of intra-renal free fatty acid and triglycerides were significantly observed in db/db mice. A downregulation of PPARα suppressed PGC-1α and ERR-1α expressions and led to increases in oxidative stress and decreases fatty acid oxidation. Treatment of fenofibrate increased the PPARα expression and subsequently activated the PGC-1α-ERR-1α signaling, inactivation of the PI3K-Akt pathway, dephosphorylation of FoxO3a, and this all resulted in ameliorating oxidative stress and fatty acid oxidation.

Conclusions: Our results suggest that PPARα agonist improves lipotoxicity through activation of the PGC-1α-ERR-1α signaling in type 2 diabetic nephropathy and may be a potentially therapeutic modality to modulate PGC-1α-ERR-1α signaling to treat type 2 diabetic nephropathy.

PUB281

Prevalence of Non-Diabetic Nephropathies in Diabetic Patients

Antonello Pani, Andrea Angioi, Floris Matteo, Patrizia Melis, Riccardo Cao, Maura Conti. *Az G.Brotzu, Cagliari.*

Background: Diabetic Nephropathy(DN) is the second most common cause of ESRD in Europe. The natural history is an important clue for its clinical diagnosis. However,renal biopsy(RB) could reveal non diabetic renal lesions(NDRD) in 9-63% of patients(pts). Active urinary findings and rapidly progressive renal failure(RPRF)are suggestive of NDRD. We retrospectively selected 88 pts(S) with diabetes mellitus(DM) from among 1,374 pts who underwent RB between Jan 1994 and Feb 2012 to evaluate its prevalence. A control group(CG) of 26 DM1 pts included in the ESPRIT study to evaluate early DN in microalbuminuria(M) was chosen. Furthermore,we evaluated whether NDRD influences the degree of diabetic glomerulosclerosis(DGS).

Methods: ***Pts****(S/CG):mean age(yrs)*:61.7±13.9/38.6±11.4(p=0.00);*M/F:*1.59/1.84(p=NS);*DM1(%)*:8/100;*DM duration(yrs)*:11.9±9.5/20.2±8.5(p=0.05);*M onset(yrs)*:5.3±5.4/5.5±2.3(p=NS).Negative fundoscopy and urinecolture.***RB criteria(%)***:rapid onset nephrotic proteinuria(3.8) or syndrome(34.6);mild(≥0.5<1g/24h)(5.1) or moderate proteinuria(≥1<3g/24h)(20.5);acute nephritic syndrome(19.3);MH(16.7);granular UC(4.8);acute(2.6),chronic(12.9) or acute on chronic renal failure(6.4);RPRF(21.8).**Stats**:Chi-square,Fisher's exact,Wilcoxon's tests.

Results: NDRD(44.3%);**NDRD+DGS**(13.7%; **DGS**(42%). The most frequent renal diseases were: **membranous GN**(15.7%); **interstitial nephritis**(11.8%); **nephroangiosclerosis**(11.8%); **IgAN**(9.8%); **crescentic GN**(7.8%); **acute tubular necrosis**(7.8%); others(*Table 1*). Diabetic lesions in NDRD were compared to the CG and did not influence progression(*Table 2*).

Table 1: Histological diagnosis in selected patients: number of cases (n), selective relative percentages (%), absolute percentages (%tot).

Histological diagnosis	n	%	%tot
ACUTE TUBULAR NECROSIS	4	7,8	4,5
AMYLOIDOSIS	1	2,0	1,1
CAST NEPHROPATHY	2	3,9	2,3
CRESCENTIC GLOMERULONEPHRITIS	4	7,8	4,5
CRYOGLOBULINEMIA	3	5,9	3,4
FSGS, SECONDARY	3	5,9	3,4
IgAN	5	9,8	5,7
INTERSTITIAL NEPHRITIS	6	11,8	6,8
MEMBRANOUS GN	8	15,7	9,1
MESANGIOPROLIFERATIVE GN	1	2,0	1,1
MINIMAL CHANGE DISEASE	3	5,9	3,4
MPGN1	1	2,0	1,1
NEPHROANGIOSCLEROSIS	6	11,8	6,8
NON HODGKIN LYMPHOMA	1	2,0	1,1
NORMAL KIDNEY	1	2,0	1,1
POSTINFECTIOUS GN	1	2,0	1,1
THROMBOTIC MICROANGIOPATHY	1	2,0	1,1
total	51	100	58,0
NDRD	39		44,3
NDRD+DGS	12		13,7
DIABETIC GLOMERULOSCLEROSIS	37		42,0
Total	88		100

NDRD: non diabetic renal diseases; GN: glomerulonephritis; DGS: diabetic glomerulosclerosis; MPGN: membranoproliferative glomerulonephritis.

Table 2. Diabetic renal lesions compared in NDRD and control group.

Diabetic renal lesions	NDRD(%)	Control(%)	p
GLOMERULOSCLEROSIS SEGMENTAL AND DIFFUSE	10.0	30.0	NS
KIMMELSTIEL-WILSON NODULES	15.7	15.8	NS
MESANGIAL EXPANSION AND THICKENED GBM	84.2	84.2	0.06
ARTERIAL HYALINOSIS	33.3	62.6	NS
FIBRIN CAPS AND CAPSULAR DROPS	29.2	21.1	NS

GBM: Glomerular basement membrane

Conclusions: In our experience, the prevalence of NDRD in diabetic pts was 58%(NDRD=44.3%,NDRD+DGS=13.7%). NDRD should be suspected if clinical and urinary findings (normal fundoscopy, proteinuria,MH) differ from the natural history of DN. Close follow-up of diabetic pts from disease onset is crucial. NDRD does not influence diabetic renal lesions.

PUB282

Morbidity and Mortality in Non Proteinuric Type 2 Diabetics with Chronic Kidney Disease: A Retrospective Study

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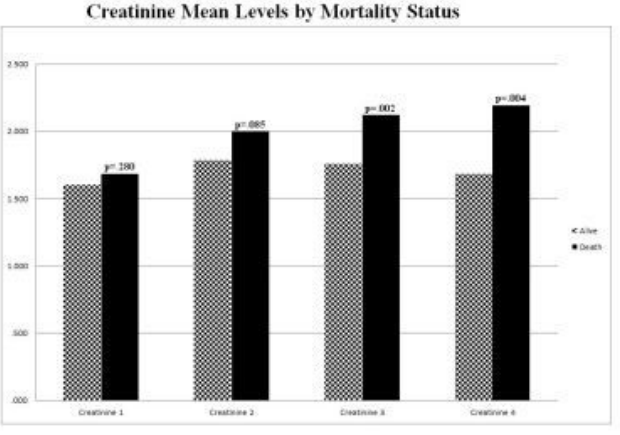
Background: Twenty percent of the diabetics progress to chronic kidney disease without albuminuria. Determinants of chronic kidney disease,natural course of renal dysfunction and mortality in this subgroup of diabetics has not been studied in great detail.

Methods: In a retrospective study design we followed medical records of 121 patients(all males) above the age of 40 years with Type 2 DM and chronic kidney disease in the absence of proteinuria for a mean follow up period of 5.1 years. Primary outcome measured were all cause mortality and requirement for hemodialysis. Secondary outcomes include 1)appearance of proteinuria 2) trend in the kidney function expressed as improvement or worsening of creatinine.

Results: All cause mortality was 33% with mean age of death of 75.9.Mortality was significantly related to higher creatinine at second year,third year, at end of follow up and duration of diabetes(p value 0.002, 0.004,0.027 respectively).



Most common cause of death was cardiovascular disease.5.8% of the patients ended up in hemodialysis.16% of the patients developed proteinuria at end of follow up and theses patients showed higher tendency for progression of renal failure.63% of the patients had improvement in renal function and theses patients showed tendency for better survival compared to patients whose renal function worsened at end of follow up period.

Conclusions: This observation suggests that in Type 2 diabetic patients with chronic kidney disease in absence of proteinuira, functional renal impairment may stem from reversible factors. Careful search for kidney damaging causes that are potentially reversible is recommended,which could subsequently improve mortality.

PUB283

Improved Prognosis in Diabetic Nephropathy

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Background: Blocking the Renin-Angiotensin System (RAS) is thought to benefit kidney function independent of the regulation of blood pressure. However, long term effects of RAS blockade were questioned in register studies. Our aim was to evaluate long term decline in renal function in diabetic nephropathy (DN) during the last decade, compared to historic data in patients with type 1 and type 2 diabetes (DM).

Methods: This was an observational cohort study. Patients with DN at our center have annual measurements of ^{51}Cr-EDTA plasma clearance (GFR). We included all patients with at least 3 measurements during the years 2000-2010 and minimum 3 year follow-up. Since 2001, RAS blocking agents have been recommended to all our patients with DN (prescribed to >95% of our cohort). Results were compared with previously published data on decline in GFR from 1983 to 2000 (2003 for type 2 DM), before RAS blocking became standard treatment.

Results: The study included 315 patients with type 1 DM and 286 with type 2 DM.

Baseline characteristics	Type 1 DM	Type 2 DM
Age, (years)	47(11)	59(9)
Gender, (% M)	61%	76%
GFR, (ml/min/1.73m2)	78(29)	80(29)
HbA1c, (%)	9.2(1.4)	8.5(1.6)
Blood pressure, (mmHg)	140(17)/79(8)	149(17)/82(9
Albuminuria, (mg/24-h)	433[167-950]	414[247-818]
Total cholesterol, (mmol/L)	5.3(1.0)	4.8(1.3)
Follow up, (years)	8.6[5.6-9.9]	6.6[4.6-8.8]
GFR measurements, (n)	7[5-9]	6[4-8]

Values are means (SD) or median [IQ range]

For type 1 DM the rate of decline in GFR was reduced 19% (95%CI 5-34%, p=0.009) from historically 4.0(3.4), n=301, to 3.3(3.1) ml/min/year and for type 2 DM 14% (95% CI 4-28%, p=0.04) from historically 5.2(4.1), n=227, to 4.4(4.0) ml/min/year. Except

for increased diabetes duration for type 1 DM, lower blood pressure for type 2 DM, and lower albuminuria for both types in the present cohort, the historic and present cohorts were similar at baseline.

Conclusions: Modern treatment of diabetic nephropathy, including long term blockade of the RAS, preserves renal function better, not worse, than treatment used in the past where RAS inhibition was not standard treatment.

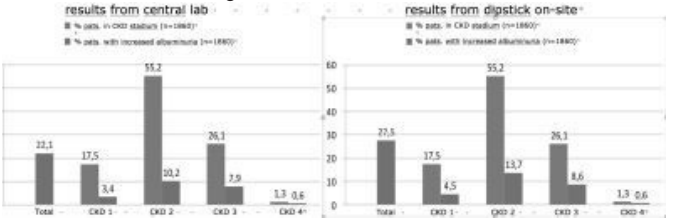
PUB284

CKD Stadium Is Not Associated with Albuminuria in Caucasian Type 2 Diabetics Ludwig F. Merker,[1] Baptist Gallwitz,[2] Katja Schoene,[3] Barbara Waldeck.[3] *[1]Diabetes- und Nierenzentrum, Dormagen, Germany; [2]Med. Klinik 4, Eberhard-Karls-Universität, Tübingen, Germany; [3]Boehringer Ingelheim Pharma GmbH & Co KG, Ingelheim, Germany.*

Background: There is few data available comparing microalbuminuria (MAU) to chronic kidney disease (CKD) stadium in patients with type 2 diabetes (T2DM). Also there are no systematic comparisons between on-site dipstick measurement of MAU resp. albumin-creatinine-ratio (ACR) vs. central lab analysis available.

Methods: We recruited 2541 consecutive patients with T2DM from 245 primary care physicians in a prospective, randomized, cross-sectional epidemiologival study according to GCP standard; pts. with CKD 5 were excluded. The following data was collected by questionnaire: antidiabetic and antihypertensive medication, renal status, hypertension and comorbidities. On site urinanalysis was carried out by dipstick, the same for MAU/ACR. Central lab included HbA_{1c}, serum creatinine, ACR and lipid profile; eGFR (ml/min /1,73m²) was estimated using MDRD formula. For MAU analysis, pts. with signs of urinary tract infection were excluded. We defined ACR < 30 mg/g creatinine as normal, 30-300 mg/g creatinine as MAU and >300 mg/g creatinine as makroalbuminuria (AU). Statistics are descriptive. The study was performed from 01-10/2011.

Results: The results of albuminuria findings central lab vss dipstick on-site in different CKD stadium is seen in this figure:



Independently, there was more albuminuria in men than in women, in higher BMI, in hypertension and in smokers.

Conclusions: Measurement of albuminuria with central lab vs. dip sticks results in a lower proportion of patients with MAU/AU. It is uncertain whether this resulted due to adsorption of albumin to the transport specimen or results from misreadings on-site. There is no correlation between MAU and CKD stage. MAU/AU is positively correlated with gender, BMI, hypertension and smoking.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharma GmbH & Co KG

PUB285

Thiazides and eGFR Decline in Patients with Diabetes Mellitus Type 2 and Hypertension Danny Vo,[1] P.T. T. Pham,[2] P.C. Pham.[1] *[1]UCLA-Olive View Medical Center, Sylmar, CA; [2]UCLA Medical Center, Los Angeles, CA.*

Background: Poor glycemic control and the use of diuretics in DM2 patients can both induce relative volume depletion. The combined effects on volume depletion could potentially induce intermittent acute tubular necrosis and accelerate kidney function decline. The current study examines the effect of hydrochlorothiazide (HCTZ), a mild diuretic, on the annual percentage of change in the estimated (MDRD) glomerular filtration rates (eGFR) in DM2 patients stratified by glycemic and blood pressure (BP) controls.

Methods: This is a retrospective study involving record retrieval of patients with DM2 and hypertension evaluated for any reason at UCLA-OVMC during 2009-2010. Data retrieved include age, gender, use of thiazides and other BP medications, eGFR, hemoglobin A1C (A1C), blood pressure, and albuminuria to creatinine ratio (ACR). Patients were stratified based on (tentative) JNC8 blood pressure goals and A1C<= or >7%. Differences in the annual percentage change in eGFR during follow-up between any 2 groups comparing those receiving thiazides vs. not were based on student's t-test.

Results: 755 patients' records were included. There were 69.3% female, with mean ± standard error age of 57±0.3 years, follow-up duration 628±5 days, systolic blood pressure 134±0.5, diastolic blood pressure 70±0.3 mmHg, A1C 8.3±0.05%, and ACR 119±17 mcg/mg. 49.3% were using thiazides. The percentages of change in the eGFR for the 4 groups are shown. There is a trend for slower eGFR decline with thiazides in patients with above goal SBP, particularly among those with A1C>7%.

SBP ≤ tentative JNC8 goal				SBP > tentative JNC8 goal			
A1C ≤ 7%		A1C > 7		A1C ≤ 7		A1C > 7	
No HCTZ (n=47)	HCTZ (n=48)	No HCTZ (n=255)	HCTZ (n=192)	No HCTZ (n=18)	HCTZ (n=22)	No HCTZ (n=61)	HCTZ (n=112)
1.7±1.9	-1.7±1.9	-0.7±0.8	-0.8±1.0	-1.2±2.4	1.7±2.1	-5.6±1.9	-0.7±2.7

p-values between No hydrochlorothiazides (No HCTZ) and HCTZ groups from left to right: 0.2; 0.9, 0.4, 0.1.

Conclusions: The use of thiazides in DM2 patients with BP above (tentative) JNC8 target does not accelerate but tends to decelerate eGFR decline. The protective effect trends more favorably in those with less well glucose control (A1C>7%).

PUB286

Therapeutic Efficacy of Combination of Angiotensin II Receptor Blockade and Thiazide in Hypertension and Microalbuminuria in Diabetic Subjects Tomoyuki Saito, Atsushi Aoki, San-e Ishikawa. *Department of Medicine, Jichi Medical University Saitama Medical Center, Saitama, Japan.*

Background: The present study was undertaken to determine whether combination therapy of losartan and hydrochlorothiazide (Los/Hyd) controls hypertension and microalbuminuria in type 2 diabetic subjects.

Methods: 47 type 2 diabetic subjects with hypertension were enrolled. They were 24 males and 23 females with the ages of 62.8 ± 8.1 years. They had been treated with normal dose of angiotensin II receptor blockade (ARB), but blood pressure could not be reduced below 130/80 mmHg. The ARB was replaced by Los/Hyd (50mg/12.5mg), and then blood pressure (BP) and microalbuminuria were determined 3 months later. If BP was not decreased to 130/80 mmHg at 3 months, losartan 50 mg was further administered.

Results: The administration of Los/Hyd for 3 months reduced systolic and diastolic BP from 146 ± 12 to 131 ± 13 mmHg and from 79 ± 12 to 75 ± 10 mmHg, respectively ($P < 0.01$). The achievement of lowering BP less than 130/80 mmHg was obtained in 28 subjects (60%). Microalbuminuria was markedly decreased from 100 ± 148 to 65 ± 113 mg/g creatinine during the 3 months period ($P < 0.001$). Because of no achievement of estimated BP, 19 subjects were added losartan 50 mg. 15 of 19 subjects thus obtained BP of less than 130/80 mmHg 3 months later. Finally, 43 of 47 subjects (91%) obtained BP of less than 130/80 mmHg during the 6 months period, namely systolic and diastolic BP to 126 ± 11 and 71 ± 9 mmHg, respectively. Also, microalbuminuria was further reduced to 35 ± 40 mg/g creatinine. Next, we analyzed 32 subjects with early stage of diabetic nephropathy. Systolic and diastolic BP decreased from 145 ± 12 to 125 ± 10 mmHg, and from 80 ± 12 to 71 ± 9 mmHg, respectively ($P < 0.001$). Microalbuminuria reduced from 71 ± 75 to 32 ± 39 mg/g creatinine ($P < 0.001$).

Conclusions: These results clarified that combination therapy of ARB and thiazide reduces BP and microalbuminuria in diabetic subjects who already had been treated with ARB. ARB and thiazide combination is a useful tool for preventing from hypertension and renal impairment in diabetic subjects.

PUB287

Renal Histological Lesions in Patients with Diabetes Mellitus: Clinical Correlations Loyana Teresa Teofilo Lima Silva, Camila Hitomi Nihei, Raquel Maria Maia, Elerson Costalonga, Michell Alves Oliveira, Denise M.A.C. Malheiros, Lectícia Jorge, Cristiane Bitencourt Dias, Rui Toledo Barros, Viktoria Woronik. *Nephrology, Medicine School University of Sao Paulo, Sao Paulo, Brazil.*

Background: The renal biopsy in diabetes mellitus patients with renal disease is controversial in literature. The aim of this study was to evaluate diabetic nephropathy (DN) and non diabetic nephropathy (NDN) in select patients with diabetes mellitus submitted kidney biopsy, and to correlate pathological with clinical and laboratory findings.

Methods: We performed a retrospective study by reviewing clinical and histological data of 77 diabetic patients submitted to renal biopsy at our center from 1999 to 2010. The glomerular filtration rate (GFR) was estimated using modified MDRD simplified equation.

Results: Of the 77 patients studied, 36 were diagnosed with DN (47%), 37 with NDN (48%), while 4 had concurrent DN and NDRD (5%). The most common NDN were membranous nephropathy (16%), igA nephropathy (14%), focal segmental glomerulosclerosis (11%), crescentic glomerulonephritis (11%), other (48%). Clinical features are summarized in table 1.

Baseline Features

	ND group	NDN group	p
n=73	n=36	n=37	
Age(y)	55,8±13	62,2±15,4	NS
Male	21(58%)	21(57%)	NS
Initial eGFR(ml/min)	34,3±23,2	45,9±33,6	0,09
Proteinuria(g/day)	4,9±3,0	4,6±3,9	NS
Hematuria	19(53%)	18(50%)	NS
Hypertension	34(94%)	26(70%)	0,001
Glycated Hemoglobin (%)	9,6±3,1	7,6±1,6	0,001
Nephrotic Syndrome	23(64%)	14(38%)	0.035
Follow up(mo)	27,6±27,7	47,5±42,9	0,03

Results are showed as mean ± SD or n(%)

ND group showed more patients with hypertension and nephrotic syndrome, higher levels of HbA1C as well as tendency lower eGFR. At the end of follow up, in DN group there was a tendency to a lower eGFR (16,1±12,9vs28,3±37,4 p=0,08) and more patients reached a eGFR < 60mlmin (100%vs78%, p=0,005).

Conclusions: Frequency of NDN is very high in our selected population. There was no strong predictor to differentiate DN from NDN by clinical or biochemical data. Our study demonstrated that renal biopsy is is necessary in selected cases. However, DN patients showed a tendency to a lower eGFR at the end of follow up.

Funding: Government Support - Non-U.S.

PUB288

Left Ventricular Hypertrophy and Apelin: Is There a Link? Ana Paula Silva, André Fragoso, Cláudia Silva, Ana Pinho, Nelson Almeida Tavares, Nélio Santos, Fatima Rato, Marilia Faisca, Karina Soto, Pedro Neves. *Nefrologia, Hospital de Faro, Faro, Portugal.*

Background: As cardiovascular disease and renal failure are inextricably intertwined in the so called "cardio-renal syndrome", it is plausible to admit a connection between apelin and the development of cardiovascular disease in patients with renal failure. The aim of this study was to evaluate the link between the left ventricular hypertrophy (LVH) and the adipokines in type 2 diabetics with mild to moderate Kidney disease.

Methods: In this cross-sectional study, we included 78 type 2 diabetic patients (f=30 m=48), with a mean age of 61.9 years and a mean eGRF of 43.5 ml/min. At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed: inflammation (interleukin 6), mineral metabolism (phosphorus (P), calcium, PTH, vitamin D, FGF-23), oxidative stress (oxLDL), adipokines (apelin, resistin, visfatin), haemoglobin and albumin. Our population was divided in two groups. G I (n=54) without LVH and G II (n= 24) with LVH stratified according to gender. In the analysis we for comparison between groups we used the Student's t-test, and a logistic regression analysis was used to determine predictors of risk for LVH.

Results: We found that G=II showed higher levels of P (5.1 *vs* 3.9 mg/dL p=0.0001), PTH (223.1 *vs* 97.6 mg/dL p=0.0001), FGF -23 (346.2 *vs* 90.9 Ru/mL p=0.0001), IL6 (9.4 *vs* 4.5 pg/mL p=0.0001), oxLDL (67.4 *vs* 35.1 U/L p=0.0001), visfatin (118 *vs* 34.6 pg/mL p=0.0001), resistin (9.3 vs4.4 pg/mL p= 0.0001). and G II also showed lower levels of eGRF (31 vs 49 ml/min p=0.002), 25 (OH)D3 (11.7 *vs* 22.8 ng/mL p= 0.0001),albumin (3.8 vs 4.3 g/dL p=0.001), and apelin (78 *vs* 281.8 pg/mL p=0.0001). In multivariate analysis only lower apelin as a predictor independly of left ventricular hypertrophy, the OR= -7.8, 95% CI, -12.9 to -2.4, p=0.005.

Conclusions: In our study the adipokines, and particularly apelin, present themselves as new predictive elements, contributing for the completeness of diversified puzzle of cardiovascular risk biomarkers in type 2 diabetics with mild to moderate kidney disease.

Funding: NIDDK Support

PUB289

Adipokines and Oxidative Stress: Cardiovascular Mortality in Cardio-Renal Axis Dysfunction Ana Paula Silva, André Fragoso, Ana Pinho, Cláudia Silva, Nélio Santos, Nelson Almeida Tavares, Pedro Neves. *Nefrologia, Hospital de Faro, Portugal.*

Background: The interrelation between cardiovascular and chronic kidney disease, with each contributing to the pathogenesis of the other, leads to progression of both diseases. Several substances produced by adipocytes – adipokines (resistin and visfatin) and oxidative stress – have been implicated on the cardio-renal axis dysfunction and plays an important role in the pathogenesis of both cardiovascular and renal diseases. The purpose of this study was to evaluate the relationship of adipokines, and oxidative stress and cardiovascular mortality in type 2 diabetics with mild to moderate kidney disease.

Methods: In this cross-sectional study, we included 78 type 2 diabetic patients (f=30 m=48), mean age 61,9 years, mean eGFR 43,5ml/min. At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed: oxidative stress (oxLDL), adipokines (visfatin, resistin,adiponectin [APN]), inflammation (interleucin 6 (IL6). Our population was divided in two groups. G I (n=65) survivors and G II (n= 13) non survivors (CVD). In the analysis we used the Student's t-test for comparison between groups and the risk factors and their hazard ratio (HR) were calculated using Cox proportion.

Results: We found that G=II was older (73.3 *vs* 59.6 years p=0.0001) showed higher levels of resistin (9.0 *vs* 5.3 pg/mL p=0.0001), visfatin (143.5 *vs* 43.8 pg/mL p=0.0001), LVMI (135.2 *vs* 97.8 g/m^2 p=0.0001), oxLDL (78.9 *vs* 38.3 U/L p=0.0001),IL6 (8.4 *vs* 5.6 pg/mL p=0.013), and G II also showed lower levels of eGRF (23.6 *vs* 45.5 ml/min p=0.001), and APN (7.7 *vs* 33 ng/mL p= 0.0001). In multivariate Cox proportional hazard stepwise LR to identify independent risk factors of cardiovascular mortality.Resistin,visfatin, oxLDL, were found to predict patient survival (HR=12.3, 95% CI, 0.3 to 19.5, p=0.042), visfatin (HR= 5.5, 95% CI, 0.6 to 10.5, p=0.026) and oxLDL (HR= 11.6, 95% CI, 2.4 to 21.5, p=0.012) respectively.

Conclusions: In our study we found that adipokines and oxidative stress are biomarkers predictors of mortality cardiovascular in type 2 diabetic with mild to moderate kidney disease.

Funding: NIDDK Support

PUB290

Resolution of Hyperglycemia without Therapy in Diabetic End Stage Renal Disease Patients Paul G. Jenkins,[1] Piangwarin Phaosawasdi,[2] Fengyi Shen.[2] *[1]Milwaukee Kidney Associates, Milwaukee, WI; [2]Aurora Health Care, Milwaukee, WI.*

Background: The hyperglycemia (HG) of diabetes mellitus (DM) is thought to be responsible for diabetic nephropathy (DN) and thus end stage renal disease (ESRD) in diabetic patients (DP). Conversely, loss of renal function may ameliorate HG in DP. Our interest was to determine the prevalence of resolution of HG and diabetic therapy (DT) in ESRD DP in a single nephrology practice and look for possible contributing factors.

Methods: Records of our current and some recent dialysis patients (pts) were analyzed. Of 119 pts, 46%, or 55 pts, had been treated for DM and most had been clinically diagnosed with DN. Of these 55 DP, 38%, or 21 pts, were able to discontinue DT. The records of these 21 pts were analyzed. All of the 21 pts were treated with hemodialysis, none with peritoneal dialysis.

Results: Of the 21 pts, 52% were women and the mean age was 66.6 years. Insulin was used by 57% and oral agents by 62% of the pts. The duration of DM ranged from 8 years (yrs) to 40 yrs and duration of ESRD ranged from 1 to 13 yrs. A weight history could not be obtained in one pt. Table 1 summarizes patient features.

Patient Features

Yrs of DM	Yrs of ESRD	Yrs of DT	Yrs off DT	Weight loss*	Vision loss	Amputation
24.8	6.8	15.6	5.1	23.5 kg	62%	33%

*Applies to 20 pts

Conclusions: Our experience highlights the frequent development of euglycemia without drugs in diabetic patients with ESRD, most of whom were thought to have diabetic nephropathy and most of whom had other vascular sequelae of diabetes mellitus in the form of retinopathy or amputations. The relationship between renal function loss and hyperglycemia in diabetes mellitus may be multifactorial, but significant weight loss was a prominent feature of most of those pts who appeared "cured" of diabetes while left with the vascular complications. Thus exists the paradox of diabetic hyperglycemia causing kidney failure, but kidney failure, directly or indirectly, possibly curing diabetic hyperglycemia.

PUB291

Pharmacokinetics, Pharmacodynamics, Safety and Tolerability of the CCL2 Antagonist NOX-E36, a Novel Agent Being Investigated for Treatment of Diabetic Nephropathy Grit Landgraf,[1] Dirk Eulberg,[1] Stefan Zoellner,[1] Stephanie Vauleon,[1] Diana Beyer,[1] Frank Fliegert,[1] Matthias Baumann,[1] Carsten Schwenke,[2] Thomas Jax,[3] Thomas Forst,[4] Hermann G. Haller.[5] *[1]NOXXON Pharma AG, Germany; [2]SCO:SSiS Statistical Consulting, Germany; [3]Profil GmbH, Germany; [4]IKFE GmbH, Germany; [5]Clinic of Hypertension and Nephrology, Hannover Medical School, Germany.*

Background: NOX-E36 is a new Spiegelmer®-based therapeutic that specifically binds to and inhibits the pro-inflammatory chemokine CCL2 (MCP-1). Spiegelmers (L-stereoisomer RNA oligonucleotides) are chemically synthesized mirror-image oligonucleotides which are highly selective for their pharmacological target and potent inhibitors of target function.

Methods: The early clinical development program of NOX-E36 consists of a single dose first in human study and a multiple ascending dose Phase Ib study carried out in healthy subjects and type 2 diabetes mellitus patients. Both studies were randomized, double-blind and placebo-controlled. Pharmacodynamic effects were evaluated with mixed linear models.

Results: Single IV doses of up to 2 mg/kg NOX-E36, single SC doses up to 0.5 mg/kg, and 4-week multiple IV doses up to 0.25 mg/kg were safe and well tolerated. NOX-E36 had no clinically relevant effects on vital signs, ECG parameters and laboratory safety parameters. Plasma exposition was dose-linear and PK profiles were similar in healthy subjects and patients. NOX-E36 had a plasma half-life of ca. 50 h and SC bioavailability of ca. 50 %. Steady state was reached after 3-4 IV administrations. A clear and dose-dependent reduction of peripheral CD14+CCR2+ inflammatory monocytes of up to 60 % was observed in the single dose study. This effect was confirmed and certain anti-inflammatory, anti-glycemic and reno-protective effects of NOX-E36 were evaluated in the multiple dose study in patients.

Conclusions: NOX-E36 was safe and well tolerated, no MTD was reached. PK/PD parameters indicate that NOX-E36 effectively antagonizes the CCL2/CCR2 axis. First trends for dose-dependent anti-inflammatory and reno-protective effects were observed. Based on these data, a further study in albuminuric diabetics has been initiated (NCT01547897).

Funding: Pharmaceutical Company Support - NOXXON GmbH Berlin

PUB292

More Severe Secondary Hyperparathyroidism Is Associated with Diabetes Mellitus in Maintenance Hemodialysis Patients Kawin Tangdhanakanond, Sayed A. Kazi, Eric J. Bloom, Rasib Raja. *Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: Bone mineral disorder is a common complication of chronic kidney disease (CKD). Although diabetes mellitus is the leading cause of CKD and is generally associated with poor outcomes, few and conflicting studies have evaluated bone mineral metabolism in CKD including end-stage kidney disease in the presence of diabetes mellitus.

Methods: We conducted a retrospective analysis on 94 hemodialysis patients at an outpatient hemodialysis unit. All patients received maintenance hemodialysis 3 times per week. Demographic and laboratory data including serum calcium, phosphorus, intact parathyroid hormone, and alkaline phosphatase levels were analyzed and compared between diabetic and nondiabetic patients.

Results: Compared with nondiabetic patients (n = 49), diabetic patients (n = 45) had a higher mean serum intact parathyroid hormone level (pg/mL) (433.66 ± 396.37, 282.79 ± 169.85, p=.018), and a lower mean serum calcium level (mg/dL) (8.79 ± 0.60, 9.08 ± 0.71, p=.036). Although there was no statistical significance, a higher mean serum alkaline phosphatase level (IU/L) (113.94 ± 90.09, 91.91 ± 69.85, p=.19) were shown in diabetic patients. Mean serum phosphorus levels were not statistically different. Urea reduction ratio, Kt/V, other biochemical parameters as well as, proportions of patients receiving cinacalcets, vitamin D analogues, and phosphate binders were comparable between the two groups.

Conclusions: Despite some previous small reports suggesting that diabetes mellitus might be associated with adynamic bone disease, our study showed that secondary hyperparathyroidism was more severe among maintenance hemodialysis patients with diabetes mellitus. Further studies exploring mechanisms of these differences and whether they contribute to more adverse clinical outcomes are warranted.

PUB293

What Factors Are Associated to the Progression of Diabetic Nephropathy? Sagrario Soriano, Victoria García, Maria Luisa Agüera, Raquel Ojeda, Alejandro Martin-Malo, Pedro Aljama-Garcia. *Nephrology, University Hospital Reina Sofia, Cordoba, Spain.*

Background: Diabetic nephropathy (DN) is the leading cause of chronic kidney disease (CKD) and needing for renal replacement therapy (RRT). In Spain, there are more than 35% of patients with diabetes mellitus type 2 and CKD.

The studies suggesting the identification of factors associated to the progression of DN are not conclusive. The aim of this study was to determine the factors that may influence in the progression.

Methods: We analyzed patients with CKD secondary to ND followed in the Nephrology (years 2005 to 2011) and collected data of demographic- clinical characteristic and laboratory parameter from the first visit until the end of follow-up (died or RRT).

Results: It were included 113 patients with a median follow up of 26 months. 8 patients died along the follow up. Baseline data (on arrival at Nephrology) were: Estimated glomerular filtration rate (MDRD-4) 21.6 ± 10.6 ml/min, creatinine levels 3.4 ± 2.9 mg/dl, HbA1c levels 7.7 ± 1.5%, and median proteinuria (ratio protein/creatinine) of 1.4 mg / mg.

Along follow up, 54.9% of patients have a decrease in MDRD-4 > 15% per year and 65.5% of patients requiring initiation of RRT after a 18.3±16 months since his arrival.

We compared the group of patients requiring RRT versus those who did not require.The patients who required RRT had higher proteinuria levels at baseline, CRP levels at baseline and more percent of retinopathy. There were not any significant differences between both groups in GFR and creatinine at baseline.

In renal survival study, the baseline levels of creatinine, CRP, proteinuria and BMI had some influence in the univariate analysis. Multivariate Cox proportional regression model showed that the factors really associated with renal survival were: the CRP leves at baseline and ratio protein/creatinine> 1 mg / mg at baseline.

Conclusions: In patients with advanced ND, the independent determinants of the progression were the presence of proteinuria and elevated inflammatory markers such as CRP at the moment of referring to the Nephrology Unit. Classical typical factors, as HbA1c and cardiovascular risk, were not really associated to the progression of ND.

PUB294

Estimation of Glomerular Filtration Rate in Chinese Patients with Type 2 Diabetes Xun Liu,[1] Chenggang Shi,[1] Linsheng Lv,[2] Hua Tang,[1] Cailian Cheng,[1] Tan-qi Lou.[1] *[1]Division of Nephrology, Department of Internal Medicine, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China; [2]Operating Room, The Third Affiliated Hospital of Sun Yet-sun University, Guangzhou, Guangdong, China.*

Background: Type 2 diabetes is a recognized public health problem in the world. In this study, we compared various modified modification of diet in renal disease study equations, which included the reexpressed MDRD equation, the Chinese equation, the previously Japanese equation and the new Japanese equation in Chinese patients with type 2 diabetes.

Methods: 210 type 2 diabetic patients were recruited. Serum creatinine (SC) level was measured by the enzymatic method. Standard glomerular filtration rate (sGFR) was measured by the ^{99m}Tc-DTPA method. The mean age was 62 (range 30-89) years. And the mean sGFR was 47.7 (range 5.9-116.7) ml/min per 1.73 m^2.

Results: The median of difference ranged from -11.3 ml/min/1.73 m^2 to 7.4 ml/min/1.73 m^2. The median percents of the absolute difference ranged from 32.8 ml/min/1.73 m^2 to 43.7 ml/min/1.73 m^2. Accuracy with a deviation less than 15% ranged from 20.0% to 23.8%. Accuracy with a deviation less than 30% ranged from 32.9% to 44.8%. Accuracy with a deviation less than 50% ranged from 57.6% to 71.0%. On the Bland–Altman plot, the precision ranged from 63.1 ml/min/1.73 m^2 to 135.8 ml/min/1.73 m^2. However, none of the equations had accuracies that reached the 70% while differing less than 30% from the sGFR. And the agreement limits of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m^2.

Conclusions: When SC was measured by the enzymatic method, the performances of all the modified modification of diet in renal disease study equationsweredisappointing. Further improved formulas are needed to evaluate renal function in Chinese patients with type 2 diabetes.

Funding: Government Support - Non-U.S.

PUB295

Effects of Renal Function on Canagliflozin (CANA) Pharmacokinetics (PK) and Pharmacodynamics (PD) in Non-Diabetic Subjects Damayanthi Devineni,[1] Thomas C. Marbury,[2] Christopher R. Curtin,[1] Nicole Vaccaro,[1] David Wexler,[1] An Vandebosch,[1] Ewa Wajs,[1] David Polidori.[1] *[1]Janssen Research & Development, LLC, Raritan, NJ; [2]Orlando Clinical Research Center, Orlando, FL.*

Background: CANA is a sodium glucose co-transporter 2 inhibitor in development for treating type 2 diabetes. This study evaluated the PK and PD of CANA in non-diabetic subjects with varying degrees of renal impairment compared with healthy subjects.

Methods: This open-label Phase 1 study enrolled subjects (N=40) with normal renal function as well as mild, moderate, and severe renal impairment (creatinine clearance ≥80, 50 to <80, 30 to <50, and <30 mL/min, respectively), and subjects with end stage renal disease (ESRD) requiring hemodialysis (HD). Subjects received a single dose of CANA 200 mg except those with ESRD, who received 1 dose post-HD and 1 dose pre-HD 10 days later.

Results: CANA area under the plasma concentration-time curve from time 0 to infinity (AUC_∞) and maximum plasma concentration (C_{max}) were slightly higher in subjects with mild renal impairment and modestly higher in subjects with moderate to severe impairment, but not ESRD, than in those with normal function. AUC_∞ and C_{max} for inactive CANA metabolites, M7 and M5, modestly increased in subjects with moderate to severe impairment. Urinary glucose excretion (UGE) after CANA administration decreased as renal function decreased. Following CANA treatment, the renal threshold for glucose (RT_G) was modestly higher in subjects with moderate to severe renal impairment than in subjects with normal function and mild impairment. CANA was well tolerated, with similar adverse event rates and no clinically relevant changes in laboratory parameters across groups.

Conclusions: Subjects with varying degrees of renal impairment had slightly or modestly increased CANA exposure, reduced UGE, and reduced RT_G lowering compared to subjects with normal function.

Table. Summary of PK and PD Parameters

	Renal Function					
	Normal (n = 8)	Mild impairment (n = 8)	Moderate impairment (n = 8)	Severe impairment (n = 8)	ESRD (post-HD) (n = 8)	ESRD (pre-HD) (n = 8)
PK parameters	*Ratio of geometric LS mean relative to normal renal function group (90% CI)*					
CANA						
AUC_∞, ng.h/mL		117.4 (95.3-144.7)	163.1 (132.4-200.9)	150.5 (122.2-185.4)	95.3 (77.0-117.9)	98.9 (78.5-124.6)
C_{max}, ng/mL		112.5 (83.8-151.0)	128.6 (95.8-172.6)	128.9 (96.0-173.0)	94.0 (68.6-128.7)	102.2 (72.7-143.6)
M7						
AUC_∞, ng.h/mL		140.6 (90.9-217.4)	230.4 (149.0-356.3)	203.5 (131.6-314.6)	175.1 (115.7-265.0)	179.9 (111.2-291.1)
C_{max}, ng/mL		156.8 (105.2-233.8)	197.4 (132.4-294.2)	174.6 (117.1-260.3)	164.9 (111.5-244.0)	179.6 (114.1-282.8)
M5						
AUC_∞, ng.h/mL		126.8 (79.4-202.4)	250.5 (156.9-399.9)	260.5 (163.2-415.9)	242.4 (155.1-379.0)	217.1 (128.7-366.2)
C_{max}, ng/mL		142.2 (97.6-207.3)	190.0 (130.4-276.9)	203.8 (139.8-296.9)	236.6 (177.7-315.1)	218.2 (156.8-303.6)
PD parameters						
LS mean UGE_{0-4h}, g	50.7	26.1	10.9	4.7	N/A	N/A
Mean (SD) 24h RT_G, mg/dL	77.5 (7.5)	67.7 (6.6)	97.3 (24.0)	92.7 (9.5)	N/A	N/A

LS: least squares; CI: confidence interval; SE: standard error; SD: standard deviation; N/A: not assessed.

Funding: Pharmaceutical Company Support - Janssen Research & Development, LLC

PUB296

Management of Diabetes in Patients with Chronic Kidney Disease Marguerite Mccloskey, Roy Harper. *Ulster Hospital Dundonald, United Kingdom.*

Background: Management of diabetes in patients with chronic kidney disease (CKD) is challenging. Therapeutic options for patients with type 2 diabetes are limited because a reduced estimated glomerular filtration rate (eGFR) results in the accumulation of drugs and their metabolites, therefore insulin therapy remains the mainstay of treatment. The purpose of this audit was to determine the proportion of diabetic patients with CKD stages 4-5 reaching the glycated haemoglobin (HbA1c) targets in accordance with the National Institute of Clinical Excellence (NICE) guidance, and to make a comparison of glycaemic control with or without insulin therapy.

Methods: A search was performed on a computerised database of all current diabetic patients attending the Ulster Hospital Dundonald with an eGFR <30. We then searched for those on insulin therapy or not, and included the HbA1c level at their most recent hospital attendance, as well as some demographic details. We then analysed the data using an excel spread sheet.

Results: A total of 256 patients were attending with an eGFR <30. Of those 256 patients, there were 45% (116) male, 55% (140) female, 10% (27) type 1 diabetics, 88% (225) type 2 diabetics, and 2% (4) secondary diabetics. Age range was 30-98 with an average age of 72years. Seventy five per cent (193) were on insulin therapy, 25% (48) of which reached the NICE guideline HbA1c target of 6.5-7.5%, 25% (63) were not on insulin therapy, 44% (28) of which reached the target. Of the type 1 diabetic patients on insulin therapy, 15% (4/27) reached the target. Of the type 2 diabetics, 74% (166/225) were on insulin therapy, 27% (44/166) of which reached the target, 26% (59/225) were not on insulin therapy, 46% (27/59) of which reached the target. All of the secondary diabetics were not on insulin therapy, 25% (1/4) of which reached the target.

Conclusions: This data confirms that glycaemic control is difficult in patients with CKD. It also suggests that insulin therapy does not achieve good glycaemic control in this group of patients, and perhaps for type 2 diabetics, switching to insulin therapy may not be beneficial and perhaps they should remain on oral hypoglycaemic agents with appropriate dose adjustment.

PUB297

Is Ventricular Remodeling in ESRD Related to Pulmonary Edema in Diabetic Patients? Oonishi Takahiro. *Nephrology, Ise Red Cross Hospital, Ise, MIe, Japan.*

Background: Diabetic patients are often introduced into the hemodialysis due to pulmonary edema. Echocardiograhic LV hypertrophy(LVH) is probably a more frequent finding in hypertensive daiabetic patients than in hypertensive nondiabetic patients. We assume that there is a difference of the LVH between the diabetic and non-diabetic patients in CKD Stage5.

Methods: 89 patients who had introduced the hemodialysis from January, 2009 to December, 2011 in our hospital were examined, they were divided into 22 diabetic groups: (28 male, 10 female, mean age 68±11 years) and 65 non-diabetic group (male, 22 female, mean age 70±12 years). We measured LV mass index(LVMI) and RWT (relative wall thickness) by echocardiography when they had first time dialysis.

Results: Diabetic group is significant higher than nondiabetic group at RWT (0.45±0.08 vs 0.40±0.07, p<0.05) .No significant differences were found in both group at LVMI(g/m2) (176±32 vs 176±44,p=0.3).RWT was higher 0.45 in the patient of episodes of pulmonary edema than without pulmonary edema.

Conclusions: The diabetic group in CKD stage 5 has been changed from left ventricular remodeling to concentric left ventricular hypertrophy(LVH),however eccentric LVH by the volume overload was seen in non-diabetic group. Diabetic heart had accompany the concentric hypertrophy, because of that, the diabetes patient thought that Pulumonary edema is easy to happen.

PUB298

Cystatin C in Children on Chronic Hemodialysis Olivera Marsenic, Andrea Wierenga, Donna R. Wilson, Tripti Shrivastava, Garfield A. Simon, Anne M. Beck, Tiffany J. Swanson, Nisha S. Singh, Dorit Elberg, Gerard Elberg, Kevin Couloures, Dwayne D. Henry, Martin A. Turman. *Pediatric Nephrology, OUHSC, Oklahoma City, OK.*

Background: Cystatin C (CyC) has been suggested as a marker of middle-molecule (MM) accumulation, hemodialysis (HD) adequacy in adults, and as marker of residual renal function (RRF) in peritoneal dialysis. MMs are poorly removed with standard HD. High CyC is associated with increased cardiovascular disease (CVD) and mortality, and is atherogenic. CyC has not been studied in pediatric HD. This study investigated: 1) MM burden as represented by CyC, 2)CyC kinetics and 3)CyC as a marker of RRF, in pediatric HD.

Methods: 21 HD sessions and 21 interdialytic periods were analyzed in 7 patients (pts), 4 anuric (A), 3 nonanuric (NA), age 14.36±4.54 years (5-19), weight 53.66±28.95 kg (16-102). RRF was 1.63±0.11 ml/min/1.73m^2 (1.54-1.75), urine volume 475-906 ml/1.73m^2/day. CyC was measured before (pre-HD) and after (post-HD) 3 standard HD sessions in 1 week and prior to first session of the following week.

Results: Single-pool (sp) Kt/V was 2.09±0.86, ultrafiltration (UF) 4.61±1.98%, HD duration (t) 218±20 min, blood liters processed 1.04±0.23 l/kg. There was no difference (p>0.05) between pre-HD CyC 9.85±2.15 mg/l (A:9.48±2.33,NA:10.35±1.88,p>0.05) and post-HD CyC 10.04±2.83 mg/l (A:9.45±2.97,NA:10.82±2.58,p>0.05). There was no inter-HD rise in CyC. Weekly average pre-HD CyC per pt was 9.97±1.90 mg/l (A: 9.87±2.68,NA:10.10±0.23,p>0.05), and it correlated with age (r=0.808,p=0.027),Ht (r= 0.799,p=0.03),weight (r=0.471,p>0.05) and body surface area(r=0.583,p>0.05), but not with RRF (r=0.064).

Conclusions: We reached important novel conclusions that require further research:1) CyC does not rise between HD, is not removed by standard HD and remains at steady-state, thus elimination is extrarenal 2) low GFR does not affect CyC elimination, therefore CyC can not be used for estimation of low RRF 3) CyC increases with pt age and size 4) CyC (and likely other MMs) is not removed by standard HD and is very elevated, raising the risk of CVD and all-cause mortality; there is an urgent need to provide intensified HD regimens (routine in adults only) to children.

PUB299

Comparison of Cystatin C and Beta-2-Microglobulin Kinetics in Children on Chronic Hemodialysis Olivera Marsenic, Andrea Wierenga, Donna R. Wilson, Tripti Shrivastava, Garfield A. Simon, Anne M. Beck, Tiffany J. Swanson, Nisha S. Singh, Dorit Elberg, Gerard Elberg, Kevin Couloures, Dwayne D. Henry, Martin A. Turman. *Pediatric Nephrology, OUHSC, Oklahoma City, OK.*

Background: Middle-molecules(MM) are not monitored in children on hemodialysis(HD), but are accumulated and increase risk of cardiovascular disease and mortality. Intensified HD modalities remove MM but are not routinely available to children. Molecular properties of Cystatin C(CyC), 13kDa,potentially make it a preferred MM marker over Beta-2-Microglobulin(B2M), 12kDa. This study is the first to compare CyC and B2M kinetics in children to: 1)determine burden of MM 2) investigate if CyC can be used as marker of MM.

Methods: CyC and B2M were measured in 21 HD sessions during 1 week using cellulose diacetate low-flux high-efficiency dialyzer, in 7 patients(pts), age 14.36±4.54 years(5-19), weight 53.66±28.95kg(16-102). Blood sampling:at HD start(pre),1 and 2 hours(hr) into HD,at end of HD(post) for all sessions(n=21) and at 60min after first HD(eq) in each pt(n=7).

Results: Residual renal function(RRF) (3/7pts,1.54-1.75ml/min/1.73m^2) did not make a difference in pre-HD or post-HD CyC and B2M (p>0.05). Post-HD CyC(mg/l) 10.04±2.83 did not differ(p>0.05) from pre-HD CyC 9.85±2.15. Post-HD B2M(μg/ml) 38.87±7.12 was higher(p=0.006) than pre-HD B2M 33.27±7.41. EqCyC was not different from postCyC (11.07±3.14 vs 10.24±2.08,p>0.05). EqB2M was lower than postB2M (36.48±7.68 vs. 41.09±8.99,p=0.006). There was no change in CyC at 1 and 2 hr into HD. B2M increased by mean 8.72% at 1 hr and 20.72% at 2 hr into HD. CyC or B2M did not significantly correlate with spKt/V(2.09±0.86), ultrafiltration(4.61±1.98%), blood liters processed(1.04±0.23 l/kg) or HD duration(218±20 min).

Conclusions: 1) CyC and B2M are very elevated and not removed by standard HD 2) Low RRF does not have a role in CyC and B2M removal 3) CyC does not rise with dialytic process, and is unchanged 1 hour after 4) B2M is affected by dialytic process (rises during HD independant of UF, and decreases 1 hour after), thus it is not a good marker of MM removal. Intensified HD is needed for MM removal in children, with CyC used as MM marker of its adequacy.

PUB301

Pegloticase and Hemodialysis Marsha Wolfson, Sean Walsh, Peter Clarke. *Savient Pharmaceuticals, Inc., East Brunswick, NJ.*

Background: Pegloticase (KRYSTEXXA) is a recombinant mammalian uricase conjugated to monomethoxypoly (ethylene glycol) (mPEG), developed as an enzymatic treatment for patients with gout refractory to conventional therapy. Clinical trials have excluded dialysis patients, leading to questions regarding pegloticase dialyzability in these patients. The drug manufacturing process can serve as a simulated dialysis treatment since it includes molecular weight cut-off (MWCO) membranes prior to formulation.

Methods: Pegloticase was studied under commercial process conditions using six 100 kDa MWCO ultrafiltration membranes per run. As hemodialysis normally uses membranes with MWCO <70 kDa, the manufacturing process can be considered as an assessment of loss of pegloticase during hemodialysis. Six regenerated cellulose diafiltration cassettes provided 3m^2 of membrane surface area with MWCO of 100 kDa (Millipore-Ultracel PLCHK). Pegloticase was concentrated to approximately 10 mg/mL and diafiltered against 25 volumes of phosphate-buffered saline. Total quantities of protein before and after diafiltration were calculated and percent protein retention was determined.

Results: Retention of protein of 98% (n=19; SD 1.3%; RSD=1.3%) indicates that almost no pegloticase is lost through the filter.

Conclusions: Results suggest pegloticase is unlikely to be removed during hemodialysis. Hence, no dose adjustments are required to treat maintenance hemodialysis patients with pegloticase. A formal pharmacokinetic/pharmacodynamic study is planned to confirm these findings.

Funding: Pharmaceutical Company Support - Savient Pharmaceuticals, Inc.

PUB302

Biocompatibility of Polysulfone Dialyzer Used Newly Developed Hydrophilic Polymer Hideki Kawanishi, Misaki Moriishi, Shinichiro Tsuchiya, Sadanori Shintaku. *Tsuchiya General Hospital, Hiroshima, Japan.*

Background: The polysulfone (PSf) membrane is now becoming the mainstream in the hemodialysis (HD) treatment because PSf membrane has a high membrane performance. Besides, most of PSf membranes hydrophilized by blending polyvinylpyrrolidone (PVP) are well known to have biocompatibility in clinical use. However, in recent years, the improvement in biocompatibility of the HD membrane is needed. Especially, when blood components adhere to the membrane, it is pointed out to generate the oxidative stress. Then, it aimed at creation of the HD membrane that did not adhere blood components based on the PSf membrane. We used a new hydrophilic polymer instead of PVP to the PSf membrane by taking note of the adsorbed water on it.

Methods: PSf dialyzer was developed used new hydrophilic polymer instead of PVP by taking note of the adsorbed water on the internal surface. We report the ex-vivo and clinical studies of biocompatibility of this PSf dialyzer (NV-dialyzers, Toray Medical Co., Japan).

Results: Solute removal capacity: Solutes removals were depended on the pore size distribution of PSf membrane. Reduction rate of beta2-microglobulin and albumin loss of NV-dialyzer on the condition of 4 hour, blood flow 300 mL/min are 74.5±5.2% and 2.5g/session, respectively.

The prevention of platelet adhesion (Ex-vivo,): Eight patients whole blood of DM and non-DM were applied on the internal surface of PSf hollow fibers cut in the longitudinal direction. The platelet adhesion counts on scanning electron microscope findings of NV of all patients' bloods were significant lower than CX-U (Toray), APS-SA (Asahikasei-kurare, Co), PES-S-alfa (Nipro, Co) hydrophilized by PVP.

Comparison of platelet activation marker on the dialysis patients (in vivo) : The platelet factor 4 (PF-4) and beta-thromboglobulin(TG) were compared on 4 weeks, 5 patients crossover studies of NV and APS-SA. The TG and PF-4 were increased on the patients of APS-SA, but it were significant decreased contrary on NV.

Conclusions: Applying the newly develop hydrophilic polymer, NV-dialyzer has superior biocompatibility which had a high performance, retained low elution and dramatically prevented the platelet from adsorbing to the surface.

Funding: Pharmaceutical Company Support - Toray Medical Co., Japan

PUB303

Safe Use of Citric Acid Based Dialysate and Heparin Removal in Post-Dilution Online Hemodiafiltration Julien Aniort,[2] Thierry Petitclerc,[1] Caroline Creput.[1] *[1]Dialysis, Nephrology, AURA, Paris, Paris, France; [2]Nephrology, CHU Clermont Ferrand, Clermont Ferrand, France.*

Background: Anticoagulation of the blood circuit is essential to the success of a hemodialysis session. Heparin is the most used anticoagulant but exposes patients to several potentially serious risks such as bleeding and immuno-allergic thrombocytopenia. The use of a citric acid based dialysate (CitA-D) allows the reduction of heparin in conventional hemodialysis while maintaining dialysis adequacy. We evaluated the possibility of using CitA-D in post-dilution online hemodiafiltration (OL-HDF)and tested the possibility of heparin removal.

Methods: Ten chronic hemodialysed patients treated by post-dilution OL-HDF three times a week were included in a prospective study comparing usual chlorhydric acid based dialysate (ClA-D) versus CitA-D. During the use of the latter, heparin was first reduced by half the dose and, secondly, totally removed.

Results: For all (n=120) sessions using CitA-D, only one clotting episode of the blood circuit was observed during heparin-free period in one patient who was found to have an arterio-venous fistula stenosis. $(Kt/V)_{sp}$ remained the same in all cases. No adverse clinical effect or significant change in pre-dialysis serum bicarbonate, calcium, phosphate, parathormone and b2-m were observed during the study.

Conclusions: Our data indicates that the use of CitA-D in post-dilution OL-HDF is safe and allows heparin removal in most patients but this should be confirmed by larger long-term studies.

PUB304

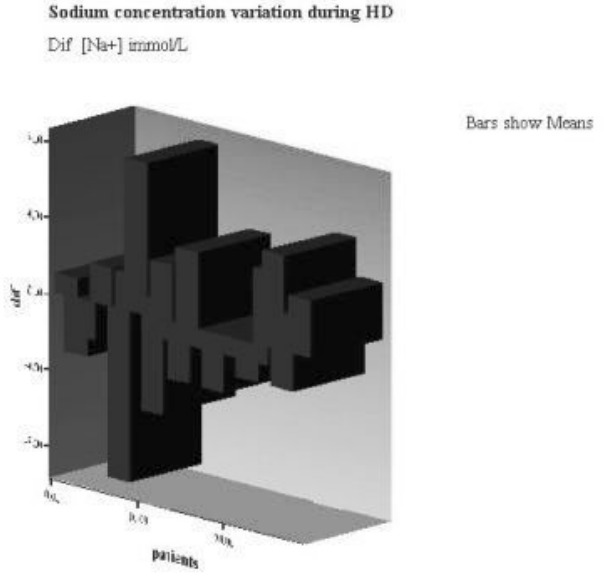
Blood Concentration Sodium Variation during Hemodialysis

Olga R. Carmona. *Nephrology, University of Uruguay, Montevideo, Uruguay.*

Background: During Hemodialysis (HD) most of serum sodium concentration [Na+] is removed by convection with water by ultrafiltration and a small concentration is removed by diffusion for the difference between plasma and dialysate sodium concentration. The purpose of this study is to determinate the serum sodium concentration variation (Δ [Na+]) during HD to adequate the dialysate sodium concentration [Na+]d in each patient.

Methods: Chronic Hemodialysis (HD) patients (n=28)were included measuring plasma sodium concentration,designed as Mean ± St, pre HD [Na+] and post HD[Na+] by potentiometry determination of sodium activity with an ion electrode, corrected by empirical factor. Dialysate sodium was fixed at 140 mmol/L. The error in the stimation due for the dialysis machine may be ± 3mmol/L.Interdialytic weight gain (IDWG)was measure in Kg. Student t test (p<0,05) was used to compare mean pre and post HD.Pearson correlation between Δ[Na+] and IDWG was considered.

Results: Average [Na+] pre HD was 137,75±3,99 mmol/L and the [Na+] post HD was 137,75 ± 2,72mmol/L.. The Δ[Na+] was 0,18±3,52mmol/L(student t test no significant,p<0,05), but with a range of variation from -9 to 8 mmol/L (7,14 ±3,34 mmol/L) at the end of HD. IDWG was 3,68±1,52Kg with a range from a 1 to 7,3 Kg. In the patient were the Δ[Na+] was +8mmol/L the IDWG was +5,70 Kg.There was not significant correlation between Δ[Na+] and IDWG.



Conclusions: Pre HD concentrataion of serum sodium may be used for individualizing the dialysate [Na+] in each patient, avoiding excesive +Δ[Na+] increase post HD with more increase of IDWG.

Funding: Government Support - Non-U.S.

PUB305

Osteocalcin as a Useful Marker of Bone Metabolism, Cardiovascular Risk, and Nutritional Status in Hemodialyzed Patients

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Background: Amongst chronically hemodialyzed (HD) patients, abnormalities of bone metabolism (BM) change due to age and end stage renal disease (ESRD) per se. Osteocalcin (Osc) is a marker of BM and an autocrine bone growth stimulating agent.

Methods: The study was performed on 136 HD pts. Overhydration (OH), calcium-phosphate product (CaP), serum Osc, NT-proBNP, cardiac troponin T (cTnT), blood pressure, and nutritional status were assessed during a 6 month period (at time 0 and 6 mo.). OH was determined using both clinical measures and bioimpedance (BIA), nutritional status was assessed using subjective global assessment (SGA), and the efficacy of HD - Kt/V. The studied group was divided into 2 subgroups according to age: grA (≤60 yo, n=58), grB (>60 yo, n=78). The aim of the study was to determine the effects of age and ESRD on abnormalities of BM and their relation to cardiovascular risk (CVD).

Results: At time 0 there was a tendency toward higher OH in grA, but surprisingly there was a significant decrease in OH (-20.8%; p<0.05) in grA and an opposite tendency in grB (+5.8%). The data shows a statistically significant under-estimation in OH measured clinically in comparison to BIA after 6 mo. in grB (2.76 vs. 3.48%; p<0.05) with higher clinical than BIA OH values in grA (3.42 vs. 2.80%; p<0.05). Improper management after clinical assessment in grB was confirmed with a significant decrease in Hb (11.6 vs. 10.7 g/dl) and slight non-significant decline in NT-proBNP (13200 vs. 13050 pg/ml) and cTnT (0.076 vs. 0.072 ng/ml) at 6 mo. Relations between Osc and other analyzed markers are presented in table 1.

Parameter	0 mo.	6 mo.
PTH	r=0.33; p<0.001	r=0.43; p<0.0001
Kt/V	r=0.33; p<0.02	r=0.16; p=0.06
SGA	r=-0.17; p=0.05	r=-0.15; p=0.08
NT-proBNP	r=0.27; p<0.002	r=0.20; p<0.02
cTnT	r=0.18; p<0.05	ns

Conclusions: Osc seems to be an important marker of BM, CVD and nutritional status in HD patients. However, it should be evaluated for interactions with other markers that measure adequacy of HD and CVD.

PUB306

Images in Hemodialysis

Raymonde Gagnon, Andrea Palumbo. *Medicine, McGill University Health Centre, Montreal, QC, Canada.*

Background: There is an increase in publication of striking photographs of various anatomical abnormalities in ESRD patients used for teaching purposes. These "Images" usually present clinical abnormalities requiring diagnosis and management.

Methods: On routine rounding, we looked for abnormalities visible to the naked eye in our chronic hemodialysis population. Most of the identified abnormalities involved vascular access.

Results: To our surprise, we readily found several significant abnormalities with anatomical features varying from common to the unseen. For instance, these Images include: 1) the scar of a dog's bite on the skin near an aneurysmal forearm A-V fistula (a powerful reminder of the recommended precautions to be taken by hemodialysis patients with companion animals); and 2) an A-V fistula with a buttonhole access located in the forearm of a patient with nephrogenic systemic fibrosis (due to gadolinium exposure) who is needling himself despite marked hand deformities.

Conclusions: Several patients on chronic hemodialysis presented obvious physical abnormalities which had not drawn previous emphasis, essentially because they did not require special attention with regards to further management. Nor did those abnormalities hinder delivery of hemodialysis treatment. Nonetheless, the Images proved to be excellent examples for clinical teaching because of their great visual impact.

PUB307

Prominent Accumulation of Protein-Bound Solutes in Hemodialysis Patients Reflects Limited Clearance by Hemodialysis Compared to Efficient Clearance by the Native Kidneys

Tammy L. Sirich,[1] Natalie Plummer,[1] Thomas H. Hostetter,[2] Timothy W. Meyer.[1] *[1]Medicine, VA Palo Alto HCS and Stanford University, Palo Alto, CA; [2]Medicine, Case Western Reserve University, Cleveland, OH.*

Background: Native kidneys efficiently clear protein-bound solutes by tubular secretion, a process not replicated by hemodialysis (HD). The current study assessed the extent to which this disparity in clearance mechanisms causes disproportionate elevation of the free concentrations of bound solutes in HD patients.

Methods: Clearances of the bound solutes hippurate (HIPP), indoxyl sulfate (IS), p-cresol sulfate (PCS), and the unbound solute creatinine (Cr) were measured in 5 HD and 5 normal (NL) subjects. Calculations were based on the *free levels* in plasma ultrafiltrate as these are the levels to which tissues are exposed. Clearances in NL subjects (k_{NL}) were calculated as the urine excretion rate divided by the plasma free level and for HD subjects (k_{HD}) as the dialytic removal rate divided by the mean of the pre- and post-dialysis plasma free levels.

Results: Results were (mean±sd, [a]p<0.05 vs Cr; [b]NL vs HD):

Solute	k_{HD} (ml/min)	k_{NL} (ml/min)	k_{HD}/k_{NL}	Plasma free level HD/Plasma free level NL
Cr	191 ± 6	154 ± 9[b]	1.2	12
PCS	589 ± 55[a]	1087 ± 196[a,b]	0.54	19
IS	582 ± 28[a]	2123 ± 396[a,b]	0.27	55
HIPP	253 ± 10[a]	1418 ± 134[a,b]	0.18	149

For Cr, k_{HD} was slightly above k_{NL}. With dialysis applied intermittently and the native kidneys functioning continuously, the plasma concentration ratio in HD compared to NL was approximately 12 to 1. Dissociation of solute from binding proteins as blood passes through the dialyzer allowed the k_{HD} of the bound solutes to rise above the k_{HD} of Cr. But k_{HD} of the bound solutes were much below the high native kidney clearances achieved by solute dissociation from binding proteins combined with tubular secretion. Reductions in k_{HD} below k_{NL} for the bound solutes were reflected by greater increases in the plasma free level of these solutes in HD compared to NL.

Conclusions: Increasing the dialytic clearances of bound solutes could prevent the disproportionate elevation in their levels observed with conventional treatment and, to the extent that they are toxic, improve patients' health.

Funding: NIDDK Support

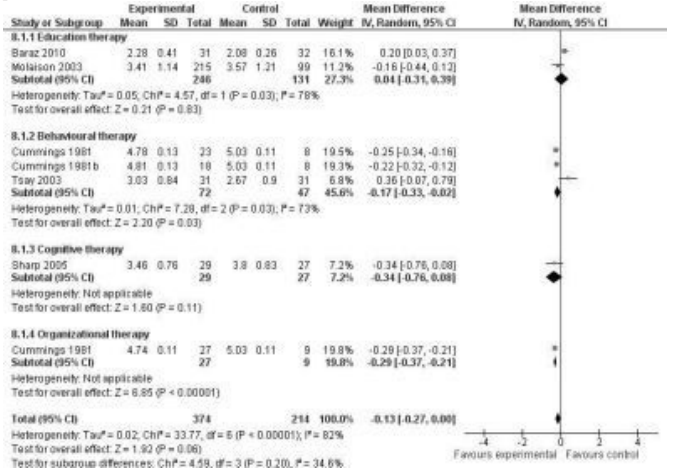
PUB308

Interventions for Promoting Adherence to Fluid Intake and Dietary Salt Restriction in Patients with End-Stage Kidney Disease: A Systematic Review Michelle M.Y. Wong,[1] Jonathan C. Craig,[2] Adeera Levin,[3] Giovanni F.M. Strippoli.[4] *[1]University of British Columbia; [2]Cochrane Renal Group and University of Sydney; [3]St. Paul's Hospital and University of British Columbia; [4]Mario Negri Sud Consortium, Cochrane Renal Group, University of Sydney, and Diaverum AB.*

Background: Volume overload is common in end-stage kidney disease (ESKD), and high interdialytic weight gain (IDWG) is associated with hypertension, cardiovascular events and mortality. Strategies to improve adherence to salt and fluid restriction have not been formally summarized. This was the aim of our systematic review.

Methods: We searched the Cochrane Renal Group specialized registry, Medline and EMBASE to April 2012 for randomized trials of non-pharmacological interventions to improve adherence to salt and fluid restriction, compared head-to-head or versus routine care, in ESKD patients, either not on dialysis, on hemodialysis, or on peritoneal dialysis. Data on mortality, cardiovascular events, quality of life and surrogate endpoints (including IDWG as a volume status measure) were extracted where available. A meta-analysis using random effects model with results expressed as relative risks or weighted mean differences with 95% confidence intervals was done and risk of bias assessed.

Results: Nine trials (n=914) were eligible, 8 in hemo and 1 in peritoneal dialysis. Participant age was 55.6 (SD 7.8) years, dialysis vintage 43.9 (SD 18.4). In hemodialysis patients, education and cognitive therapies did not significantly reduce end of treatment IDWG compared to routine care, while behavioral and organizational therapy (nurse phone calls) did.



There were no data on any hard end points.

Conclusions: Existing data on interventions to promote adherence to dietary salt and fluid restriction are limited and showed minimal or no benefit. Behavioral therapies may be promising but deserve further study.

PUB309

Weather Conditions Influence Interdialytic Weight Gain Only to a Minor Degree Mihaly Tapolyai,[1] Zsuzsa Taborine-Gonczi,[1] Maria Faludi,[1] Virag Reti,[1] Zsolt Lengvarszky,[2] Tibor Szarvas,[2] Klara Berta.[1] *[1]Fresenius Dialysis, Semmelweis University, Budapest, Hungary; [2]Mathematics, Louisiana State University Shreveport, Shreveport, LA.*

Background: Interdialytic weight gain (IDWG) is a measure of compliance as well as it is a predictor of outcome. Patients, however, feel that various weather conditions such as humid or hot weather is to blame for an increased IDWG.

Methods: We prospectively monitored 98 consenting chronic hemodialysis patients for their IDWG. We tabulated their week-end IDWG under 3 conditions in the summer of 2011. Weather data were obtained from the website of the Hungarian Meteorological Service. Weekend_1: humid (93%) warm (24 °C); Weekend_2: dry (38%) but hot (33 °C); and Weekend_3: dry (30%) and warm (24 °C) day. 56% of the patients were men, mean age 60.9 years with a mean residual urine of 362 ±493 mL/day; mean Kt/V: 1.4 ±0.25 mean albumin: 4.0 ± 0.4.

Results: The mean IDWG's did not differ significantly: Weekend_1: 2973 ±1386 mL Weekend_2: 2684 ±1368 mL Weekend_3: 2926 ±1311 mL. The Friedman 1-way ANOVA test could not find a significant difference (p: NS) among the three groups. However, a paired one-sample t test revealed a significant (p: 0.03) difference between Weekend_1 and Weekend_2 by a minor degree (289 mL) though not between the other pairings, indicating that only the ambient hot temperature seems to influence IDWG, not high air humidity.

Conclusions: We conclude that the ambient temperature and not humidity may to a small degree influence IDWG.

PUB310

Describing Nutritionally-At-Risk Hemodialysis (HD) Patients Who Receive Oral Nutrition Supplements (ONS) Rosa Hand,[1] Janeen B. Leon,[1] Lilian Cuppari,[2] Alison Leah Steiber.[1] *[1]Case Western Reserve University; [2]Federal University of Sao Paulo.*

Background: ONS have been shown to be effective at improving the nutrition status of HD patients.

Methods: This was a secondary analysis using data collected through on online nutrition algorithm for nutritionally-at-risk HD patients to determine which patients receive an ONS recommendation (ONS-Rec) from their dietitian and to describe the length of time patients receive the recommendation for. Analysis was performed using JMP 9.0 and significance was set at $p<0.05$.

Results: There were 64 patients at 140 algorithm visits with a dietitian-selected nutrition diagnosis of insufficient energy or insufficient protein, etiology of insufficient intake and barrier of lack of appetite. The median number of visits per patient was 2, range 1-7. At 47 of these visits patients received a new ONS-Rec (defined as not having received an ONS-Rec at the preceding visit). ONS-Recs tended not to carry over from visit to visit-only 19% received an ONS-Rec at 2 consecutive visits and only 1 patient had the ONS-Rec for 3 consecutive visits.

At 81% of the identified visits, patients had a diagnosis of insufficient energy (more likely to receive an ONS-Rec, p=0.05), at 74% of visits patients had a diagnosis of insufficient protein, and at 54% of visits patients had both problems (more likely to receive an ONS-Rec, p=0.0014).

Characteristics (mean ± SD or %) of patients and comparison of visits with and without ONS-Rec

Parameter	Patients (n=64)	Visits with ONS-Rec (n=47)	Visits without ONS-Rec (n=93)	P-value ONS vs no ONS
% Male	55	46	58	0.26
Age (years)	63 ± 16	67±15	64 ± 16	0.35
BMI (kg/m2)	28.4 ±7.4	27±6	28.5±7.4	0.38
Albumin (mg/dL)	3.8 ±0.4	3.67±0.4	3.78±0.4	0.23
% Bothered by a lack of appetite	97	92	63	0.0068
% Subjective Global Assessment score 1-5	57	76	53	0.22
nPNA (g/kg/day)	0.98±0.23	0.78±0.23	0.91±0.22	0.0451
Protein intake (g/kg/day)	0.82±0.36	0.88±0.43	0.82±0.37	0.52
Energy intake (% estimated needs)	77±24	69±31	74±28	0.57

Conclusions: This analysis shows nPNA and appetite are important in dietitian determination of patients who should receive an ONS-Rec in nutritionally-at-risk HD patients. More research should determine why ONS-Recs do not continue for more consecutive visits.

Funding: Pharmaceutical Company Support - Genzyme, Private Foundation Support

PUB311

Single Needle Cross Over with Citrasate: 2-Years Follow-Up Roberto Ervo. *Nephrology and Dialysis, Bordighera Hospital, Ventimiglia, IM, Italy.*

Background: At ASN 2011, we presented the preliminary data of a study on the improvement of the dialysis adequacy of a single needle Cross Over system thanks to the use of Citrasate, an acid concentrate containing citrate and a low amount of acetate. This study involved 6 patients for 10 months. The available literature on single needle dialysis refers to a period of max 6 months.

Methods: We continued our Follow-Up with our patients in single needle dialysis up to 20 months using Citrasate and collecting data of previous studies. In particular, we observed efficiency, performances and safety.

Results: As table shows, data did not change vs last year. Patients are clinically stable, no particular medication was necessary and no relevant change in dialysis adequacy was recorded as for Kt/V, treated blood volume and hemoglobin in compliance with the KDOQI standards. We deem it is due to various factors:

· A low recirculation in the arterovenous fistulae

· A control on Kt/V made by urea withdrawal 20 min before the end of dialysis

· The use of high surface filters with very biocompatibile membranes.

· Single needle therapy is carried out with the continuous operation of blood pumps without any interruption of the flow through the dialyzer (Cross-Over system)

· The dialysis bath with citrate (Citrasate) which increases the depurative performances probably thanks to the higher fiber patency (antifouling effect) with no significant variation in calcemia.

	SN-CO-CITR	SN-CO-CITR-24 MONTHS
Kt/V	1,37±0,39	1,58±0,33
Average Qb	260,25±6,04	254±5,48
Total Blood Volume	57,56±5,82	56,8±1,64
Ca	9,11±1,09	9,82±0,94

Conclusions: Literature reports no recent case of patients undergoing single needle dialysis for more than 6-12 months. We reached a 20 months period respecting the KDOQI standards by increasing, if necessary, the dialysis time within reasonable terms (max 4,30 hours).

Therefore, considering a flexible and tailored treatment which allows reducing the mortality risks in an elderly dialysis population which presents an increase in comorbidities and problems linked to the vascular tree depletion, single needle dialysis may be a valid alternative in the long period and avoids complications linked to an inadequate maturation of the arterovenous fistula and to extravasation.

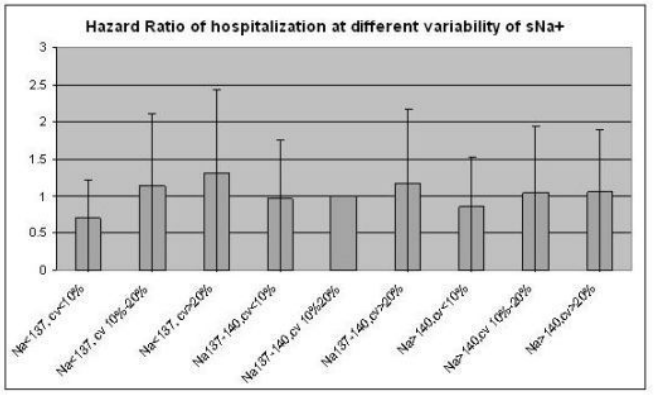
PUB312

Serum Sodium Variability Relates to Hospitalization in Hemodialysis Patients Joselyn Reyes,[1] Jochen G. Raimann,[1] Len A. Usvyat,[1] Stephan Thijssen,[1] Frank van der Sande,[2] Jeroen Kooman,[2] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Nephrology Research, Renal Research Institue/ Beth Israel Medical Center, New York, NY; [2]Nephrology, Maastricht University Medical Centre, Maastricht, Netherlands.*

Background: In hemodialysis (HD) patients recent reports indicated that pre-HD serum sodium (SNa+) concentrations show a certain level of temporal stability ("SNa+ setpoint"; Keen 2007). Increased variability of the pre-HD SNa+ over time has been shown to relate to increased mortality (Raimann 2012). We aimed to investigate the relation between SNa^+ variability and hospitalization in incident HD patients.

Methods: This cross-sectional cohort study included patients who started HD between 1/2001 and 7/2008 in clinics of the Renal Research Institute. Only patients that had at least 3 SNa^+ measurements during the first 3 months on HD were included. Follow-up time was 18 months. Patients were stratified by average SNa^+ of the first three months: (1) <137, (2) 137 to 141, (3) > 141 mEq/L; and by the SNa+ variability during the first year: coefficient of variation (CV) (1) <10%, (2) 10% to 20% and (3) >20%. Cox regression was used to compute hazard ratios (HR) of first hospitalization in months 13 to 18 adjusting for gender, race, age, vascular access, co-morbidities, systolic blood pressure, and eKt/V.

Results: 4451 HD patients (age 61±15.21, 56% male, 56 % diabetic, 43 % Blacks, 22 %) were studied. HR of hospitalization was lower in patients showing a lower degree of SNa+ variability at all levels of SNa+. This relation was particularly pronounced for the group with high variability and low SNa+.



Conclusions: Both SNa+ level and stability are associated with hospitalization in incident HD patients. In our study a lower variability of SNa+ is associated with fewer hospitalizations. Factors causing higher SNa+ variability remain to be elucidated.

PUB313

Isovolumetric Passive Flow Dialysate Delivery System: Proof of Concept Martin C. Gregory. *Division of Nephrology, University of Utah, Salt Lake City, UT.*

Background: Hemodialysis is life-sustaining treatment that is economically beyond the reach of half the people who may benefit from it worldwide. Passive flow dialysis is a concept of providing dialysis without the need for external sources of energy, thus potentially making hemodialysis available at low cost and without electricity (Ethn Dis. 2009;19[Suppl 1]:S1-65–S1-67). Several challenges must be overcome to make this practicable. This project addresses the first two: how to provide a flow of dialysate at constant rate and how to avoid uncontrolled movement of fluid to or from the patient.

Methods: A 1/3 scale model of a dialysate delivery system was constructed from 3 mm acrylic sheet. This consisted of a rectangular box of outer dimensions 12 x 12 x 24 cm, A second box 12 cm high was a loose sliding fit within the first box. A plastic bag was placed in the space within the outer box and below the inner box. This bag was connected by external tubing to a second bag in the space above the inner box. The upper bag was filled with water and the tubing initially clamped.

Results: The volume of water (representing dialysate) in the system is constant, being the difference in the volumes of the two rigid boxes. When the clamp on the tubing between the two bags was released, water flowed from the upper to the lower bag as the inner acrylic box rose (principle of downward displacement of liquid). The rate of delivery was constant during the middle 2/3 of the volume-time cycle but was irregular, and flow incomplete, near the beginning and end of delivery when hindrances from folding of the plastic bags became significant.

Conclusions: This small-scale model demonstrates the feasibility of isovolumetric delivery of dialysate for hemodialysis without external energy. When scaled up to provide 30 - 50 liters of dialysate, the problems with flow hindrance from bag crumpling would be expected to be less, but this remains to be shown.

Before passive flow dialysis is practicable, several further obstacles must be overcome, specifically: adding controlled removal of ultrafiltrate to this isovolumetric system, warming dialysate safely, and above all creating a reliable method for driving blood through the dialyzer without need for a pump.

Funding: Private Foundation Support

PUB314

Heparin-Induced Thrombocytopenia in Hemodialysis Patients Dulce M. Winterdaal,[1] Maria Isabel Gavidia,[2] Bernardo Fargier,[1] Silvia Villarroel,[3] Marquez Carlos,[4] Jesus R. Sierra,[1] Alfredo A. Plata,[1] Abdel J. Fuenmayor.[5] *[1]Nephrology Unit, University Hospital of the Andes, Mérida; [2]Venezuelan Institute of Social Insurances, Mérida; [3]Hemodialysis Unit, Ciudad Bolívar; [4]Metropolitan Hospital, Maturin; [5]Cardiovascular Research Institute, University of the Andes, Mérida, Venezuela.*

Background: Anticoagulation is necessary during hemodialysis (HD). Heparin-induced thrombocytopenia (HIT) should be suspected in patients who develop thrombocytopenia during heparin administration. The aim of this study was to determine the occurrence of HIT during HD sessions.

Methods: We designed a cross-sectional, randomized and multicenter trial. Patients submitted to HD were divided into three groups according to the dose of heparin administered. Group A comprised patients who received 100 UI/Kg of unfractionated heparin not exceeding a total dose of 5000UI. Group B, patients received 50UI/Kg of heparin. Group C patients did not receive heparin. All the patients were dialyzed 4 hours with hollow fiber dialyzers and Qb = 300ml/min, and Qd = 600ml/min. During the second dialysis of the week, the platelet count was measured at baseline, every hour, and at the end of the session. Differences were analyzed with ANOVA for an alpha value of $p< 0.05$.

Results: One hundred and four patients on HD were included. Group A was made up of 37 patients, Group B of 34 patients and Group C of 33 patients. Sixty and a half % of patients were male. Their mean age was 47.75 ± 16.12 years, and they had been on hemodialysis for 32.6 ± 29.71 months. The most common causes of admission to dialysis were diabetic nephropathy (30.7 %), nephroangioesclerosis (24%) and chronic glomerulonephritis (14.4 %). Brachiocephalic fistula (35.3%) and radiocephalic fistula (34,3%) accounted for vascular access. The platelet count measured before hemodialysis initiation was similar in the three groups ($p = 0.3$). Platelet count obtained at the different observation points did not differ either ($p > 0,05$) .

Conclusions: In these patients we did not observe any significant reduction in platelet count related to heparin use during the hemodialysis session.

Funding: Private Foundation Support

PUB315

Comparision between Unipuncture and Bipuncture Tecqnique in Use of Vascular Heterografts: Pros and Cons Oana Rap,[1] Antonio Cabezas,[1] Maria Pilar Ruiz Valverde,[1] Francesc Barbosa,[2] Francesc J. Moreso,[2] Isabel Giménez Torrecilla,[1] Neus Rodriguez Farre,[1] Josep Carrió,[1] Josep Maria Mallafre Anduig.[1] *[1]Nephrology Department, Hospital de Sant Joan Despí "Moisès Broggi"- Fresenius Medical Care "Hospital Dos de Maig, Barcelona, Spain; [2]Nephrology Department, Centro de Diálisis Diagonal-Fresenius Medical Care, Barcelona, Spain.*

Background: Dialysis patients survival depends on duration of their vascular access. Many patients need vascular heterografts,because they exhausted great part of vascular accesses.The vascular grafts viability could be related on the number of punctures that received.

Methods: We analyzed 28 consecutive patients in chronic dialysis program with functional vascular grafts,in which was performed the unipuncture technique and 30 consecutive patients that received the classical bipuncture technique.The patients who left dialysis program(exitus,transplant)with functional graft and patients who continue dialysis currently using vascular graft were excluded for analyze the viability of vascular grafts.

Results: The age and sex distribution of patients was similar in the both groups.The equilibrated Kt/V was 1.37+0.24 in the unipuncture group and 1.56+0.38 in the bipuncture group,with p=0.06. The equilibrated Kt/V was higher in the bipuncture group, probably related by the fact that most of these patients received hemodiafiltration online.The viability of the vascular graft in the unipuncture group was 29.4+32 months and 18.4+12 months in the bipuncture group,with p=0.18.The total number of vascular accesses(catheters, native arteriovenous fistulas, grafts) previous of the actual graft, was 1.9+1.6 in the unipuncture group and 2.89+1.5 in the bipuncture group.Viability of vascular grafts was associated with age of patient at the time of graft collocation and with presence of vasculopathy,although without reaching statistical significance(p= 0.08,p=0.07).

Conclusions: We observed a tendency to higher viability with 11 months in the group receiving unipuncture technique compared with the bipuncture group,although not enough to reach statistical significance. The unipuncture technique was effective and safe for patients, according to current requirements of adequacy of dialysis.

PUB316

Do We Still Need Continuous Hemodiafiltration in Intensive Care? Hugo Mário Silva,[1] Pedro Francisco Azevedo,[1] Pedro V.A. Aguiar,[1] Raquel Gil,[1] Anibal Marinho.[2] *[1]Nephrology, HSA, Porto, Portugal; [2]Intensive Care, HSA, Porto, Portugal.*

Background: Severe acute kidney injury (AKI) is a multisystem clinical problem. Haemodynamically unstable patients are best managed using continuous hemodiafiltration (CVVHDF). Recently, sustained low-efficiency dialysis (SLED) was introduced as a method which theoretically combines the advantages of intermittent dialysis with those of CVVHDF.

Methods: Retrospective analysis, using a data base from the DO-RE-MI study, of 52 patients with AKI treated in our intensive care unit, who underwent either SLED (N=8) or CVVHDF (N=44) for more than three days and didn't have ESRD. We compared clinical

risk scores, volume status and mortality between SLED and CVVHDF subgroups. In a second analysis, intending to eliminate the positive selection bias of patients in SLED we compared only the patients who survived.

Results: Median age 64,3±33,3 years, 58,2% males, 84,4% with sepsis. No differences between subgroups in age, comorbidities and chronic renal disease prevalence. At time 0 SOFA score, PaO2/FiO2, noradrenaline dose(ug/Kg/min) and water balance(mL) in SLED and CVVHDF subgroup were respectively 11,4±3,7 vs. 10,3±4,0; 156,1±73,5 vs. 189,5±96,1; 0,58±0,77 vs. 1,17±1,66(*P*=0,04) and 1395,4±2278,9 vs. 949,3±3491,3. At 72h the same parameters were 12,5±3,9 vs. 15,1±3,5; 209,9±96,4 vs. 191,7±90,8; 0,36±0,82 vs. 1,36±1,99 and 2983±6083,8 vs. 975,7±6115,9. Mortality(%) was 25 vs. 75%(*P*=0,05). Restricting the analysis to patients who survived (SLED N=6, CVVHDF N=11) SOFA score, PaO2/FiO2 and noradrenaline dose at time 0 was 10,5±3,9 vs. 10,4±4,9; 174,9±69,9 vs. 202,0±122,2 and 0,33±0,3 vs. 0,66±0,78. At 72h the same parameters were 11,7±4,2 vs. 12,3±3,0; 227,7±106,7 vs. 209,6±86,7 and 0,08±0,1 vs. 0,38±0,51. On the end of third treatment, total water balance was +2426±4129,2 vs. -3099,0±7125,2mL(*P*=0,02).

Conclusions: CVVHDF is still the therapy of choice for more unstable patients in our hospital. Nevertheless, both techniques are clinically overlapping, which makes the choice of technique more dependent on financial/logistical issues rather than clinical aspects. CVVHDF was better in controlling volume status, rendering it a therapy of choice when hyperhydration is an important clinical issue.

PUB317

Filter Level Analysis of Factors Identifying Successful CRRT Discontinuation in Pediatric Patients Rebecca M. Lombel,[1] Mallika Kommareddi,[2] Theresa Mottes,[1] Neal B. Blatt,[1] Michael Heung,[2] David T. Selewski,[1] Heather A. Lesage-Horton.[1] *[1]Pediatrics, Univ of Michigan, Ann Arbor, MI; [2]Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: There is little consensus regarding timing of initiation or discontinuation (DC) of continuous renal replacement therapy (CRRT). Only two adult studies have specifically examined predictors of successful DC. We evaluated readily available clinical parameters to identify predictors of outcome at each CRRT filter DC over the course of therapy.

Methods: Retrospective single-center study of children requiring CRRT from November 2007 to April 2011. Baseline characteristics at the time of CRRT initiation were collected. Data at time of individual filter DC were collected including urine output (UOP) prior to DC, reason for filter DC (anticipated or not), use of diuretic challenge, and UOP following diuretic challenge/DC. The primary endpoint was re-initiation of CRRT or no return to CRRT at 24 hours post-DC. Univariate analysis and logistic regression models were used to predict outcome.

Results: 68 patients with 271 filters met inclusion criteria. At 24 hours from DC, 192 (71.9%) filters resulted in CRRT re-initiation; 55 (20.6%) did not return to CRRT and 20 (7.5%) ended with patient death. 8-hour UOP prior to DC, albumin and age were not significant predictors of outcome in our model. When a diuretic challenge was performed, the odds ratio of not returning to CRRT compared to re-initiation at 24 hours was 6.27 (CI 3.25, 12.11). For each 1 mL/kg/hr increase in UOP following diuretic challenge/DC, the odds ratio was 1.32 (1.03, 1.70). For each 1 point increase in PELOD score, the odds ratio was 0.95 (0.93, 0.98).

Conclusions: This study is a first step to identify clinical parameters that may predict need for CRRT at 24 hours from filter DC. Decision by the clinician to perform a diuretic challenge and UOP in the 6 hours after diuretic challenge/DC appear to be important factors in predicting outcome. We are analyzing additional variables at time of filter DC that, coupled with these results, may provide a model that can be studied prospectively to predict which pediatric patients display readiness for discontinuation of CRRT.

Funding: Other NIH Support - T-32 DK65517-8

PUB318

Efficacy and Safety of Citrate-Based Anticoagulation in Patients with AKI in the Intensive Care Unit Fabien Stucker,[1] James Ashu,[1] Jérome Pugin,[2] Belen Ponte,[1] Laurent Brochard,[2] Pierre-Yves F. Martin,[1] Patrick Saudan.[1] *[1]Nephrology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [2]Intensive Care Unit, Hôpitaux Universitaires de Genève, Geneva, Switzerland.*

Background: A systemic anticoagulation is often required to prevent clotting of filter in ICU patients undergoing continuous renal replacement therapy (CRRT). A regional citrate anticoagulation (RCA) does not induce a systemic anticoagulation and prolongs the filter lifespan, but is associated with metabolic side-effects. We are conducting a randomized controlled trial with patients requiring CRRT to determine whether a RCA is more effective than heparin in terms of CRRT delivered dose and safety profile.

Methods: Patients: included if aged > 18 yrs with an AKI requiring CRRT. Exclusion criteria: active hemorrhagic disorder, pregnancy, history of heparin-induced thrombopenia, consent form not obtained. Methods: patients randomized to either CRRT with RCA (Prismocitrate, Gambro) or classical heparin anticoagulation. Treatment performed with Prismaflex (HDF mode, overall RRT dose 30ml/kg/h and filter change every 72 hours). Primary endpoints: effective daily RRT dose (% of prescribed dose) and filter lifespan. Secondary endpoints: survival at 28 days, number of hemorrhagical events requiring blood transfusions, severe metabolic complications (metabolic alcalosis with pH >7.55, metabolic acidosis with pH <7.25), ionized calcium < 1mmol/l.

Results: From October 2010 to April 2012, 52 patients were randomized among 120 treated with CRRT. Exclusion criteria were active hemorrhagic disorders or severe thrombocytopenia (21%), terminal liver failure (9%), chronic maintenance dialysis (25%) or others (45 %). Mean age was 60±9 years. Etiology of AKIs were medical (77%), surgical (12%) and posttraumatic (11%). Mean CRRT duration was 5±5 days. Effective daily RRT dose was 96±12% in the RCA group and 85±15% in the heparin group (p=0.057). 28-days mortality was 29% in the RCA group and 27% in the heparin group.

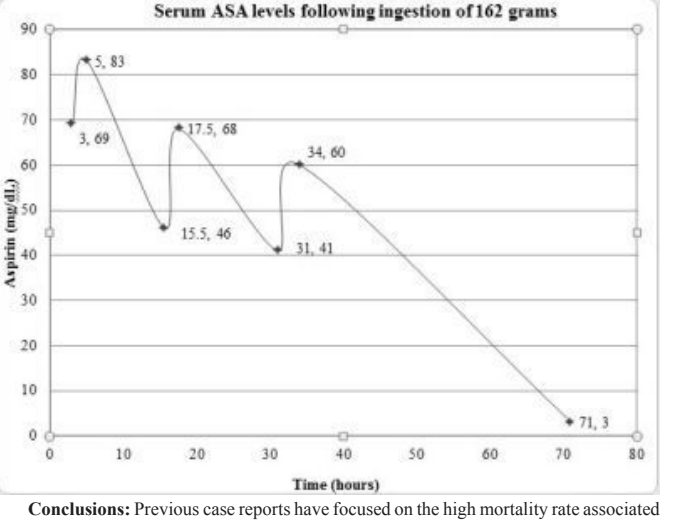
Conclusions: These preliminary results seem to show an advantage of RCA over heparin-based anticoagulation in terms of effective daily delivered RRT dose, which does not translate into a 28-day survival improvement.

PUB319

Survival of a Patient with Large Aspirin Ingestion Using Prolonged Hemodialysis and Continuous Renal Replacement Therapy Mansumeet Singh. *Nephrology, Creighton University, Omaha, NE.*

Background: Although aspirin poisonings are common, optimal management of this toxicological emergency is unclear given the variable pharmacokinetics of acetylsalicylic acid (ASA). We present a patient who survived a significant ASA ingestion (162 grams) after prolonged extracorporeal therapy.

Methods: A 56-year-old woman was transferred to our hospital 4.5 hours after ingesting 500 ASA pills (325 mg each). She was afebrile and hemodynamically stable (RR = 29, BP = 149/80 and HR = 107). Presenting symptoms included nausea, emesis, and tinnitus. 50mg of charcoal was administered. ASA levels were 69 mg/dL and 83 mg/dL at 3 hours and 5 hours post-ingestion, respectively. Given these toxic levels and the upward trend over time, hemodialysis (HD) was initiated. Although the ASA level trended down to 46 mg/dL during five and half hours of HD, a repeat measurement two hours after discontinuation was 68mg/dL. Accordingly, HD was resumed. During this 12-hour HD run, the ASA level trended down to 41 mg/dL. With cessation of HD, the level again rose to 60mg/dL within 3 hours. Given the refractory rebound in ASA levels, continuous renal replacement therapy (CRRT) was started and was continued for more than 36 hours until three consecutive ASA levels were below 3 mg/dL. This time, with the discontinuation of CRRT, the ASA levels remained below 3 mg/dL and the patient recovered without residual effects. In total it took ~18 hours of HD and ~36 hours of CRRT to remove ASA from her system.



Conclusions: Previous case reports have focused on the high mortality rate associated with large ASA ingestions despite the appropriate use of HD. Although unpredictable pharmacokinetics and the potential for rebound complicate the management of such patients, this case suggests that prolonged HD and/or CRRT might be a strategy for managing extremely large ASA poisonings.

PUB320

LVAD and Hemodialysis: Challenges of the New Millennium Imran F. Fatani, Dhiren Kumar. *Nephrology, VCUHS, Richmond, VA.*

Background: The number of patients with LVAD requiring Hemodialysis is rising. However, there is limited information regarding new set of challenges that arise with this patient population. This case series aims to outline our experience with 7 such patients.

Methods: This is a case series of 7 patients. A retrospective chart review was done of 7 patients requiring Hemodialysis after Heartmate II continuous flow LVAD placement. Challenges encountered with respect to Hemodynamic monitoring during dialysis, arrhythmias, infections and nutrition optimization were reviewed.

Results: Case series of 7 patients with Heartmate II LVAD requiring dialysis and their challenges including Hemodynamic monitoring protocol, infection management, nutrition, and arrhythmia management.

Conclusions: The patient population getting Ventricular Assist Devices is growing. LVAD's are more commonly being placed in older patients as destination devices and continue to be placed as bridge to heart transplant. These patients have many risk factors for chronic kidney disease or have pre-existing chronic kidney disease. They are at high risk for developing acute renal failure in the perisurgical period thereby requiring renal replacement therapy. We have done a chart review of 7 such patients at our institution. In this very high risk group of dialysis patients we have examined the challenges we have encountered specifically related to hemodynamic monitoring with dialysis, hypotension, arrhythmias, infections and nutritional optimization.

PUB321

Incidence of Central Venous Stenosis in Patients with Chronic Renal Failure after IJ Catheter Placement Mary S. Hammes, Amishi S. Desai, Brian Funaki, Annette Jean Herlitz. *Medicine, University of Chicago, Chicago, IL.*

Background: The arteriovenous fistula (AVF) is the preferred access for hemodialysis, with the successful creation dependent on presevation of veins. Previous damage to veins from B/P cuffs, phebolomy and peripherally inserted central venous catheters(PICC) limit sites for AVF creation. It is recommended that an alternative to a PICC line, a small bore internal jugular catheter (SBIJ) be used for short term access in patients with chronic renal failure (CRF) to preserve veins. The incidence of stenosis with SBIJ has not been studied. This investigation was performed to determine if complications including stenosis occur with SBIJ catheters.

Methods: Patients were enrolled by written consent if they had evidence of CRF stage 3 or greater (GFR<59 mL/min), end-stage renal disease or a history of renal transplantation and required short-term venous access with a central catheter. Subjects were screened for inclusion by daily review of the Procedure Service consult log by a Nephrologist. If patients agreed, they were scheduled for a SBIJ placement in Interventional Radiology. A venous Doppler was performed during insertion and removal of the SBIJ to evaluate for stenosis, thrombosis as well as the diameter of the IJ vein. Patient demographics and indication for SBIJ were recorded.

Results: 26 patients were enrolled and 28 SBIJ were placed, 2 patients had catheters placed on 2 separate occasions. Demographics included: 11 (42.3%) males and 14 (53.8%) African Americans, 8 (28.6%) had a solid organ transplant, 7 (25%) had stage 3 or greater CRF and 13 (46.4%) ESRD. Indications for catheter placement included: 13 (46.4%) immunosuppression, 11 (39.3%) antibiotics, 1 (3.6%) TPN and 3 (10.7%) a combination. A right SBIJ was placed in 23 (82.1%) subjects and a left in 5 (17.9%). The average number of catheter days was 16.7. The incidence of IJ thrombosis at time of line removal was evident in 1out of 26 subjects or 4%.

Conclusions: Placement of SBIJ catheters in patients with CRF for short term IV access is a safe procedure with a low risk of IJ thrombosis. Efforts to avoid PICC catheters should be made in this population as future permanent access may be required.

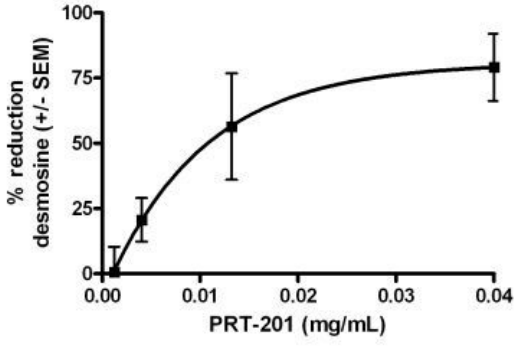
PUB322

Measurement of Elastin Content of Human Cephalic Vein Following Treatment with PRT-201, a Recombinant Human Type I Pancreatic Elastase Steven K. Burke,[1] Marco D. Wong,[1] Karen Macdonald,[2] Emma J. Moss,[2] Kimberly S. Bland,[1] Nicholas Franano.[1] *[1]Proteon Therapeutics, Inc., Waltham, MA; [2]Biopta Ltd., Glasgow, United Kingdom.*

Background: This study explored the effect of PRT-201 on elastin content of human cephalic veins obtained from recently deceased donors. The veins were studied in a perfusion myograph to simulate the pressure in the outflow vein of an arteriovenous fistula.

Methods: Veins were retrieved within 24 hours post-mortem, cut to 2.5 cm segments, and mounted on a myograph. Saline was perfused through the lumen of the segments at a transmural pressure of 25 and 50 mm Hg. PRT-201 0.0012, 0.004, 0.0132, and 0.04 mg/mL or saline was applied in a volume of 2.5 mL drop-wise over 10 minutes on the external surface of the vein followed by rinsing with saline. The vein segments were removed from the myograph and stored in saline for 3 hours at RT then at 4°C overnight before formalin fixation. Vein segments were cut into rings for desmosine (a protein cross-link unique to elastin) content by RIA, histology for the visualization of elastin fibers, and PRT-201 fluorescence. For fluorescence, PRT-201 was fluorescently labeled with Dylight 633 and dosed at a concentration of 0.012 mg/mL. Adventitial imaging was performed using laser scanning confocal microscopy at 633 nm.

Results: Figure 1 shows the percentage reduction in desmosine content by PRT-201 concentration.



Histology confirmed a concentration-related reduction in elastin fiber staining. Confocal miccroscopy demonstrated persistent localization of PRT-201 to elastin fibers following saline rinsing.

Conclusions: PRT-201 caused a concentration-related reduction in elastin content of human cephalic veins. PRT-201 remained bound to elastin in the vein after washing suggesting prolonged PRT-201 activity following a 10 minute-application.

Funding: Pharmaceutical Company Support - Proteon Therapeutics, Inc.

PUB323

Uric Acid and Risk of Catheter Related Bacteremia in Hemodialysis Patients Pavan K. Annamaraju, Seyed-Ali Sadjadi, Navin Jaipaul. *Nephrology, VAMC, Loma Linda, CA.*

Background: In vitro studies demonstrate a paradoxical antioxidant effect of hyperuricemia in the extracellular milieu compared to its pro-oxidant effect in adipocytes. Similarly, previous clinical studies suggest hypouricemia impairs plasma antioxidant capacity in sepsis. It is biologically plausible, therefore, that uric acid (UA) level may modify the risk of catheter related bacteremia (CRB) in hemodialysis (HD) patients. We conducted a single center cohort study to determine if lower UA level may be independently associated with increased risk of HD CRB.

Methods: We identified by CPT code prevalent HD patients over a 2 year period who had tunneled catheter placed. Patients were followed for up to 1 year until they developed CRB or the end of the study period. CRB was determined by chart review and defined as ≥1 positive blood culture with the same organism, clinical signs of infection, and no alternative source for infection. Additional demographic and laboratory variables were collected and averaged over a 3 month period immediately preceding the CRB event. Patients with active malignancy, receiving chemotherapy, or missing UA levels were excluded. Ultimately, 51 patients were included in the final analysis performed using SPSS version 20.

Results: Refer to the table.

Comparison of Patients with and without CRB

Variable	No CRB (N=35)	CRB (N=16)	p-value
Age, years	61.4±12.6	59.0±10.7	0.49
Male sex, n	33	15	0.94
White race, n	23	10	0.26
Hypertension, n	35	16	-
Diabetes, n	28	11	0.38
Coronary artery disease (CAD), n	15	12	0.03*
Allopurinol use, n	2	2	0.40
Body mass index, kg/m²	27.3±5.7	27.5±6.8	0.95
Albumin, mg/dL	3.1±0.5	3.0±0.8	0.67
Calcium x Phosphorus, mg²/dL²	46.9±11.1	42.9±11.2	0.25
Ferritin, ng/mL	375.1±368.7	302.2±200.0	0.38
Uric acid (UA), mg/dL	6.7±1.3	5.8±0.8	0.004*

*p<0.05

The rate of CRB was 0.70/1000 catheter days. Each 1 mg/dL increase in UA level was associated with a 58% reduced risk of CRB (OR= 0.42; 95% CI 0.18-0.95), even after adjustment for CAD, catheter days, and dialysis vintage. Despite the small sample size, our study had 85.7% power to detect a 1 mg/dL difference in UA level between patients with and without CRB.

Conclusions: These findings suggest that lower UA level may be an independent risk factor for HD CRB.

PUB324

Tunnelled Hemodialysis Catheters: A Single Center Report on a Change in Access Delivery Jennifer Palfrey, Iain Moore, Debbie Sweeney, William Hinchliffe, Saeed Ahmed. *Department of Renal Medicine, City Hospitals Sunderland, Sunderland, United Kingdom.*

Background: Our renal unit has established experience of placing size 10 French independent hemodialysis catheters (Tesio®). Following promising data showing reduced infection & catheter dysfunction, we introduced use of a single split 14.5 French catheter with a spiral Z tip design (Palindrome™) for long term hemodialysis access.

Methods: We collected retrospective data from medical records and the renal database system for all patients who had undergone placement of Palindrome catheters from March 2010 until December 2011.

Results: Sixty-six lines were placed in 59 patients of whom 39% of patients had no long-term haemodialysis access prior to insertion of Palindrome. One line was placed into the femoral vein; all others were placed in the internal jugular vein (77% right-sided). No complications were experienced at the time of insertion. Seventy-two percent were placed using real time ultrasound guidance; the remainder also required fluoroscopic screening. We have of 7452 catheter days (excluding data from 5 patients who died with functioning lines insitu). Forty-four percent of lines remained in use at the end of the data collection period. Of the 32 lines removed, 50% were removed electively. Nineteen percent of lines were removed for infection. Catheter dysfunction accounted for 16% of line removals. In addition, 6% were removed for cuff slippage. Vascular compromise necessitated one line removal. Of the 5 patients with line dysfunction, 3 patients had another Palindrome line placed, 2 had a Tesio. Two patients lost central access requiring tunnelled femoral lines.

Conclusions: We have accrued significant experience in using Palindrome and Tesio catheters. We believe this to be the largest data set collected in a UK centre regarding Palindrome use. We have found them to be suitable for the majority of patients and note comparable data to previously reported. However, 28% of Palindrome lines required fluoroscopic assistance for placement, adding to access planning, resource availability and patient inconvenience. We continue using both types of tunnelled lines with individual vascular evaluation prior to selection of line type.

PUB325

Blood Access for an Armband Artificial Kidney through an In Situ DeBranched Vein Fistula Graft Arnold J. Lande'. *Lande' Device, LLC, Minneapolis, MN.*

Background: A wearable artificial kidney will benefit from durable blood access, likely on the forearm or arm, because that's where the blood is. Safety and small size of an Armband Artificial Kidney (AAK) could be furthered by tapping into A/V differential pressure, rather than utilizing a blood pump. Full anticoagulation of an ample 30 ml/min extracorporeal blood flow can be achieved while the patient remains safely merely prophylactically anticoagulated.

Methods: An elective In Situ DeBranched Vein Fistula Graft (VFG), with intermittent transdermal compressions, might serve the purpose. Review records of a common corrective procedure that results in the identical configuration, compulsory accessory vein obliteration by ligation or coil insertion. This procedure is familiar to nephrologists, vascular surgeons and radiologists because, in addition to balloon angioplasty, accessory vein obliteration is frequently employed to rescue a poorly maturing or failing hemodialysis fistula. All the arterial blood is directed through only one rapidly flowing draining vein. Longevity of the rescued vein may be considerably extended. An elective VFG for a wearable kidney is expected to benefit similarly. Intermittent moderated compressions over the VFG promise access to extracorporeal A --> V flowing blood for an AAK through small gauge cannulae inserted in a mature VFG.

Results: The literature is replete with records of excellent long term use of previously endangered fistulae, following accessory vein obliterations that rendered the fistula identical with a VFG. Also note satisfactory early use of pumpless arteriovenous CRRT. Number, length and diameter of hollow fibers, that reflect on resistance and efficiency, remain to be determined. Configuration of sorbent cartridge and means for powering dialysate flow through, remains to be determined.

Conclusions: The forearm or arm are likely locations for a wearable artificial kidney. Durable VFG access is promising for a FAK. The question of when to obliterate accessory veins remains open. Similar to current practice, one will likely observe a fistula for six weeks during maturation, before deciding on the best prospective VFG among the draining veins.

Funding: Other NIH Support - HCFA(CMS)SBIR; University of Kentucky

PUB326

A Single Centre Review of Procedures to Salvage Thrombosed Vascular Access between 2008 and 2012 Linda H. Bisset,[1] Richard O'Neill,[2] Greg Ramjas,[2] Jane Pikett,[1] Heather Ward,[1] Alastair J. Ferraro,[1] Charlotte Bebb.[1] *[1]Renal & Transplant Unit, Nottingham University Hospitals; [2]Department of Radiology, Nottingham University Hospitals, Nottingham, United Kingdom.*

Background: Our unit has a dialysis programme of approximately 400 pts, with <12% dialysing via tunnelled central venous catheters. Our vascular access surveillance programme using monthly measurements by Transonic© ultrasound dilution access flow monitoring, allows early detection of access problems and elective radiological intervention. Despite this, a small proportion of arterio-venous fistulae (AVF) and PTFE grafts (AVG) thrombose.

Methods: We retrospectively studied all vascular access thromboses between January 2008 and April 2012, interrogating our departmental databases and computerised hospital medical records to review outcomes of emergency intervention.

Results: 70 radiological interventions, on 51 AVF (39 brachial, 6 radial and 4 brachiobasilic) and 19 AVG were undertaken in 65 patients; mean age 59 years. The success rate was 84%. 90% underwent radiological thrombectomy with angioplasty; 10% required angioplasty alone. Four interventions occurred whilst patients awaited elective intervention. Five patients had stents inserted. Four patients subsequently underwent surgical intervention. During follow-up, 65% of access required re-intervention with 16% requiring a further emergency thrombectomy. Prior intervention had occurred in 54%. Only 29% had a recorded Transonic© flow rate <500ml/min pre-thrombosis. Pre-and post-access flow rates were available in 60% of cases; mean flow rate increased by 352mls/min. 91% were hospitalised due to their thrombosed access. Mean time from referral to procedure was <1 day (range 0-4). Their mean in-patient stay was 2.5 days. 83% of patients currently remain on the same vascular access.

Conclusions: Previous data from our unit reported that most radiological access intervention is performed electively. Nonetheless, a few of our patients' AVF and AVG clot requiring emergency intervention. Prompt radiological intervention results in an excellent success rate of >80%, minimising the need for central venous catheters and helping to maintain a high usage rate of native AVFs.

PUB327

Effects of Prolonged Ethanol Lock Exposure to Silicone- and Carbothane-Based Hemodialysis Catheters: A 26 Week Study Daniel L. Landry,[1,2] Harry Bermudez,[3] George Lipkowitz,[1,2] Stephen Sweet,[1,2] Gregory Lee Braden.[1,2] *[1]Renal Division, Baystate Medical Center, Springfield, MA; [2]Western New England Renal & Transplant Associates, Springfield, MA; [3]Polymer Science and Engineering, University of Massachusetts, Amherst, MA.*

Background: Antibiotic locks in catheter-dependent chronic hemodialysis patients reduce the rate of catheter-related blood stream infections (CRI), but may be associated with the development of resistant bacteria. Ethanol-based catheter locks may provide a safer alternative, however, there remains limited data on its effects on long-term catheter integrity.

Methods: We performed *in-vitro* testing of two common types of hemodialysis catheter – 9 silicone (SLC) and 9 carbothane (CBT)-based – with a 70% ethanol lock (EL) versus heparin lock (HL) for 26 weeks. Of the 9 catheters tested in each group, one catheter underwent baseline mechanical testing while 4 of the remaining 8 catheters were filled with either HL or EL. Lock solutions were changed thrice weekly to mimic a conventional hemodialysis schedule. We tested mechanical properties of the catheters at 13 and 26 weeks by examining stress at 400% strain and modulus of elasticity at both the upper and lower segments of each catheter. Scanning electron microscopy (SEM) was also performed to examine catheter ultrastructure at 26 weeks.

Results: There was no significant difference in stress/strain relationship or modulus of elasticity when comparing HL versus EL in SLC- or CBT-based catheters at 13 and 26 weeks. SEM examination revealed no gross evidence of damage to EL- versus HL-treated catheter surfaces at 26 weeks.

Conclusions: SLC- or CBT-based hemodialysis catheters exposed to a 70% EL for 26 weeks experienced no adverse effects on catheter integrity. Our data represents the first long-term *in-vitro* EL study to simultaneously examine different catheter polymers and catheter segments while also evaluating catheter ultrastructure using SEM. Given its low cost and potential to avoid antibiotic resistance, the EL appears to be a safe and promising option for the prevention of CRI in catheter-dependent hemodialysis patients.

Funding: Pharmaceutical Company Support - Bard Access Systems

PUB328

Vascular Access and Mortality: A Prospective Follow-Up Study in Two Danish Hemodialysis Centers Rie Io Glerup,[1] My Svensson,[2] Jens K. Madsen,[2] Henrik Carl Schønheyder,[3] Jeppe Hagstrup Christensen.[1] *[1]Department of Nephrology, Aarhus University Hospital, Aalborg, Denmark; [2]Department of Nephrology, Aarhus University Hospital, Aarhus, Denmark; [3]Department of Clinical Microbiologi, Aarhus University Hospital, Aalborg, Denmark.*

Background: Many hemodialysis (HD) patients are still dialyzed using tunneled catheters (TC) despite the use of TCs in these patients is controversial. The aim of the present study was to assess one year mortality in a well described HD population dialyzed on either TCs or arteriovenous fistulas (AVFs).

Methods: From December 2010 to March 2011 307 prevalent HD patients (HD vintage >3 months) from two major dialysis centers were enrolled. During a one year follow-up all-cause mortality was assessed. Patients were divided according to dialysis access, AVF or TC. Mortality rates were calculated using a Cox proportional hazards model, adjusting for age, HD vintage, and cardiovascular disease (CVD).

Results: 273 (89%) had an AVF and 34 (11%) had a TC. Baseline characteristics revealed that more TC patients had CVD, lower mean arterial pressure (MAP), and shorter HD vintage (Table). The crude one year mortality in the AVF group was 15% and 44% in the TC group. The adjusted hazard ratio (HR) comparing patients with TCs to those with AVF´s was 3.62 (95% CI 1.96; 6.71).

Baseline Information by vascular access

	Tunneled catheter (n=34)	AV fistula (n=273)	p
Age (years)	65.7 (±12)	65.1 (±15)	ns
Female sex (n)	16 (47.1)	91 (33.3)	ns
Hemodialysis vintage (months)	27.3 (±31)	52.6 (±53)	0.007
MAP (mmHg)	85.1 (±15)	94.3 (±16)	0.003
Diabetes (n)	12 (35.3)	71 (26.0)	ns
CVD (n)	23 (67.6)	130 (47.6)	0.03
Hemoglobin (g/dl)	11.3 (±1.1)	11.6 (±1.3)	ns
high sensitive C-reactive protein (mg/l)	12.4 (±14)	14.0 (±23)	ns
Procalcitonin (ng/ml)	0.46 (±0.3)	0.71 (±0.9)	ns
Albumin (g/dl)	3.8 (±0.5)	3.9 (±0.4)	ns
Fibrinogen (mg/dl)	490 (±123)	502 (±125)	ns

Values are given as means (±SD) or exact numbers (%)

Conclusions: We confirmed that the use of TCs in HD patients is associated with a high mortality risk. Bias may be a problem in the present design but mortality HR remained high after controlling for potential confounders. Thus, when possible AVF should be used in HD patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

PUB329

Remote Resident Location Increases Likelihood of Catheter Usage in End Stage Renal Disease Lisa M. Miller,[1] Lavern M. Vercaigne,[2] Louise M. Moist,[3] Charmaine E. Lok,[4] Navdeep Tangri,[1] Paul Komenda,[1] Claudio Rigatto,[1] Julie Mojica,[5] Manish M. Sood.[1] *[1]Medicine, Section of Nephrology, University of Manitoba, Winnipeg, MB, Canada; [2]Pharmacy, University of Manitoba, Winnipeg, MB, Canada; [3]Medicine, Division of Nephrology, University of Western Ontario, London, ON, Canada; [4]Medicine, University of Toronto, Toronto, ON, Canada; [5]WRHA Critical Care/Medicine Information System & Research, University of Manitoba, Winnipeg, MB, Canada.*

Background: In Canada, a large proportion of ESRD patients reside in rural areas. We studied the association between resident location and incident vascular access for patients initiating dialysis between 2000-2009 using data from the Canadian Organ Replacement Registry.

Methods: Distance to nearest dialysis centre was calculated as the direct linear distance(km) between patient's primary residence postal code at dialysis initiation and closest dialysis centre, using Vincenty's formula and log transformed for analytic purposes. Multivariate logistic regression analysis was used to assess the association between distance (km) and initiating dialysis with a central venous catheter(CVC).

Results: In 25, 172 incident patients, 80% used a CVC and 20% used an arteriovenous fistula or graft. Patients residing remotely were more likely to initiate dialysis with a CVC (<5km 79.5%, 5-2km 78.6%, >20km 82.8%) and this effect persisted after adjustment for covariates (<5km OR referent, 5-20km OR 0.95 (CI 0.88-1.02, p NS), >20km OR 1.20 (CI 1.10-1.31, p<0.01)) A similar association was seen between distance and prevalent CVC use (>20km OR 1.25, (CI 1.16-1.35 p<0.01)). Duration of pre-dialysis care was a significant effect modifier (interaction p<0.01) as individuals with less pre-dialysis care (<1 yr) who resided remotely had significantly more CVCs than those who resided remotely but >1 yr pre-dialysis care (<1 yr care + reside >20km OR 1.24 (95% CI 1.03-1.48), 1-3 yr care + reside >20km OR 0.58(95%CI 0.49-0.68)). (OR referent <1 yr care + reside <5km).

Conclusions: Increasing distance from a dialysis provider is associated with initiation of dialysis with a CVC, independent of comorbidities and demographic factors. This effect is partially attenuated by longer pre-dialysis care.

PUB330

Prolonged Use of Tunnelled Femoral Arterial Catheter for Haemodialysis in a Patient with Central Venous Access Exhaution and Challenging Vascular Access Sourjya Kar, Paul Warwicker, Suresh Mathavakkannan. *Renal Medicine, Lister Hospital, Stevenage, United Kingdom.*

Background: Advances in practice have led to improved survival rates of our patients many of whom have undergone repeated central venous cannulation for prolonged periods of time. The consequence has been the emergence of central venous exhaution.

Objectives:

We are describing an elderly haemodialysis patient with central venous exhaution. When faced with a lack of viable access, a single tunnelled left femoral arterial teiso was inserted for emergency access. This was the arterial source for haemodialysis and the remaining right common illiac vein teiso was used for venous return to patient.This proved so successful that the arterial access was left in situ and no further access attempted.

Methods: The catheter insertion via the Seldinger technique was suprisingly well tolerated by the patient.The clinician had prior experience of arterial cannulation.

Results: The patient has been dialysing by this route for 2 years. The femoral arterial catheter was changed once (cuff loose) over a wire without complications.He had no infective complications. The average blood flows has been 170mls/min. He has been achieving dialysis adequacies (2 pool KT/V 1.42).

Conclusions: We described an unusual vascular access for a patient with central venous exhaution. The tunnelled arterial access, though being not the priority, can used for vascular access in selected patient population. The arterial line has been used for withdrawl of blood only and that has possibly kept the complication rate low.

Funding: Government Support - Non-U.S.

PUB331

Galway Renal Access Study: Comparison of Thrombosis and Malfunction between Three Types of Permanent Hemodialysis Catheter Gerard M. Healy,[1] Wael F. Hussein,[1] Colm Pascal Keane,[1] Mark Curtin,[1] Sinead Clarke,[1] John Newell,[2] Alberto Alvarez-iglesias,[2] Louise Giblin,[1] David Lappin,[1] Donal N. Reddan.[1] *[1]Department of Nephrology, University College Hospital, Galway, Ireland; [2]HRB Clinical Research Facility, National University of Ireland, Galway, Ireland.*

Background: Tunnelled central venous catheters (TCVC) are used for dialysis patients awaiting formation of arteriovenous fistula (AVF) or those with failed AVF. They are often complicated by thrombosis and poor flow.

Methods: We reviewed flow malfunction in TCVCs inserted at a tertiary hospital from Jan 1st 2009 to July 30th 2011, with follow-up to Aug 31st 2011 or line removal. Kaplan Meier estimates of survivor function, log rank test and a Cox Proportional Hazards model were used to compare three TCVC types: Duraflow (DF) by Angiodynamics, Palindrome (PD) by Covidien and Jetflow (JF) by Jet Medical, while adjusting for patient characteristics. Outcomes were (1) total survival: days until line removal for flow malfunction and (2) tissue plasminogen activator (tPA) free survival: days until first tPA use or removal for flow malfunction.

Results: 92 patients had 145 TCVCs in situ for 34,318 days. TCVC type was unknown in 8 lines. 16%(N=23) of lines were PD, 31%(N=45) DF and 48%(N=69) JF. Except for hypertension (DF: 73%, PD: 43%, JF: 75%, p= 0.02), no difference was noted between groups regarding age, sex, anticoagulant use and comorbidities. Indications for TCVC insertion are listed in table1.

Indication for Insertion				
	DF (N=45)	PD (N=23)	JF (N=69)	P
1st access	8(18%)	9(39%)	36(52%)	
TCVC infected	10(22%)	7(30%)	9(13%)	
TCVC poor flow	17(38%)	6(26%)	7(10%)	0.002
TCVC fell out	3(7%)	0	2(3%)	
Failed AVF/PD/ Transplant	5(11%)	1(4%)	12(17%)	
Unknown	1(2%)	0	0	

21 lines (11.7%) were removed for malfunction. Mean total survival was 780 days (95% CI: 695-865). This was 470 (380-560), 521 (477-565) and 825 (728 – 922) for DF, PD and JF respectively (p = 0.09). tPA-free survival was 458 (365-551) days. Per line type, mean tPA-free survival was DF: 328 (237-420), PD: 292 (184-400), and JF: 509 (389-629) (p=0.23).

Conclusions: TCVC flow malfunction does not seem to be related to type of catheter. Further research is warranted. We will continue to monitor TCVC survival.

Funding: Pharmaceutical Company Support - Covidien

PUB332

Effect of Heparin and Aspirin on Vascular Access Patency Jerzy Glowinski,[1] Jolanta Malyszko,[2] Irena Glowinska,[2] Michal Mysliwiec.[2] *[1]Department of Vascular Surgery and Transplantation, Medical University, Bialystok, Poland; [2]Department of Nephrology and Transplantology, Medical University, Bialystok, Poland.*

Background: Native arteriovenous fistula remains the optimal vascular access for hemodialysis. Unfortunately, early failure occurs in nearly 50% of cases. The important role is played by thrombotic complications. The aim of the study was to assess the usefulness of low molecular weight heparin and aspirin in the prophylaxis of the early and late thrombosis of vascular access.

Methods: The number of 132 operations of vascular access for hemodialysis were performed on 120 consecutive patients. There were 110 native fistulas and 10 grafts, 9 basilic vein elevations and 3 ligations of accessory veins. The study group was composed of 60 patients with LMWH prescribed postoperatively as thrombosis prophylaxis, the control group was composed of 60 patients with no anticoagulative drugs. Additionally, we estimated an impact of aspirin on fistula patency. Twenty five of our patients took 75 mg of acetylosalicylic acid for cardiovascular indications.

Results: There were 3 postoperative bleedings in study group, and only one in control group. Reoperations were not required. One thrombosis of av fistula was noted in the study group, one av fistula and one graft were thrombosed in control group within 30 days. One year follow-up was completed by 102 patients. Four maturation failures required another anastomosis, 2 graft and 4 fistulas thrombosed within 12 months. Primary access 1year functional patency in study group was89.8% (44 from 49 pts), whereas 1year functional patency in non-heparin group was86.8% (46 from 53 pts). The study was completed by 21 patients on aspirin (of 25 enrolled), 1year patency rate was 85,7.

Conclusions: Heparin use in prophylaxis of thrombotic complications of vascular access in early postoperative period should not be recommended. Intake of aspirin does not show any advantage in one year observation period.

PUB333

Should Estimated Glomerular Filtration Rate Be the Sole Criterion to Refer Advanced Chronic Kidney Disease Patients for Pre-Emptive Arteriovenous Fistula? Neeraja Talakanti, Kavitha Potluri, David J. Leehey. *Dept of Renal and Hypertension, Edward Hines Jr. Veterans Hospital, Hines, IL.*

Background: Current KDOQI guidelines recommend referring patients for preparation for renal replacement therapy when estimated GFR is < 30 mL/min/1.73m^2, with arteriovenous fistula (AVF) placement at least 6 months before anticipated need for dialysis. However, the means of anticipating need for dialysis are not well delineated.

Methods: We did a retrospective chart review of CKD patients who underwent preemptive AVF placement during the period of 2004-2009 with a minimum follow-up of 2 years post AVF placement at Hines VA Hospital, IL. We evaluated eGFR at time of AVF placement and rate of eGFR decline over the 3-year period prior to AVF placement. Other data collected included age, cause of ESKD, proteinuria (by urine Pr/Cr), serum albumin, and average clinic blood pressure. We performed multivariate analysis to delineate the factors that best predict use of pre-emptive AVF within one year of placement.

Results: Of 138 CKD patients who underwent AVF placement, 11 died prior to dialysis, 7 had AKI and 3 had insufficient data. Of the 117 patients analyzed, mean eGFR at time of AVF placement was 17.4 ± 5.9 mL/min/1.73m^2, 96% had hypertension, 76% had urine Pr/Cr > 1 g/g, 74% had diabetes, 30% had CHF, and 28% had anemia requiring ESAs. 23 patients never required dialysis and 94 eventually required dialysis, 39 within 1 year of AVF placement and 55 after 1 year. Patients who required dialysis within 1 year of AVF placement had an eGFR of 14.5 ± 5 mL/min/1.73m^2 whereas those who did not require dialysis within 1 year had an eGFR of 20.5±5 mL/min/1.73m^2 (p=<0.001). Rate of decline of eGFR was somewhat higher in the group requiring dialysis than in the group who never required dialysis (mean/SD: 8.8 ± 7.4 vs. 7.4 ± 5.8 mL/min/1.73m^2/yr, p = 0.28). Multivariate analysis revealed that diabetic kidney disease, proteinuria >1 g/g, low serum albumin and eGFR of <15 mL/min/1.73m^2 at the time of AVF placement independently predicted need for dialysis within 1 year.

Conclusions: In most patients, eGFR of < 15 ml/min/1.73 m^2 can be used as sole criterion when considering pre-emptive AVF placement.

PUB335

Clinician Beliefs and Attitudes about Home Haemodialysis Allison Tong,[1,2] Suetonia Palmer,[3] Braden J. Manns,[4] Jonathan C. Craig,[1,2] Marinella Ruospo,[5] Letizia Gargano,[5] David W. Johnson,[6] Giovanni F.M. Strippoli.[1,5,7] *[1]Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, NSW, Australia; [3]Department of Medicine, University of Otago, Christchurch, South Island, New Zealand; [4]Department of Medicine and Community Health Sciences, University of Calgary, Calgary, Canada; [5]Department of Clinical Pharmacology and Epidemiology, Mario Negri Sud Consortium, Bari, Italy; [6]Department of Nephrology, University of Queensland, Brisbane, QLD, Australia; [7]Diaverum Medical-Scientific Office Lund, Diaverum, Lund, Sweden.*

Background: Home hemodialysis (HD) is increasingly advocated as an alternative to in centre HD. This study aims to elicit clinician beliefs and attitudes about home HD.

Methods: Semi-structured interviews were conducted with nephrologists and nurses from dialysis centres within Diaverum AB.

Results: Forty-two clinicians from 15 centres in France, Italy, Portugal, Germany, Sweden and Argentina participated. Four themes were identified: external structural barriers (ready access to dialysis centres, inadequate housing conditions, unstable economic environment); centre capacity (availability of alternative treatments, competing priorities, commercial interests); clinician responsibility and motivation (preserving safety and security, lack of awareness, knowledge and experience, potential to offer lifestyle benefits, professional interest and advancement); and cultural apprehension (an unrelenting imposition, carer burden, attachment to professional healthcare provision, limited awareness).

Conclusions: Clinicians recognised the potential benefits of home HD, such as patient autonomy, but felt apprehensive and doubted its feasibility. Key barriers were commercial interests, financial disadvantage, concerns about patient safety and psychosocial burden, and lack of clinician awareness. Efforts are needed to promote home HD as an important option, improve knowledge about the patient benefits, establish home HD training programs, and to provide reassurance about patients' self-efficacy as demonstrated by international experience. Funding models that offer adequate reimbursement and may be warranted.

Funding: Pharmaceutical Company Support - Part-Funded by an Unrestricted Grant from Diaverum AB

PUB336

High Dose Quotidian Dialysis Is Associated with Improved Outcomes and Low Burnout

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Background: KT/V is an imprecise, infrequently measured, surrogate for HD dose. We report on quotidian home HD in 77 patients prescribed a distinct high HD dose.

Methods: Any patient who initiated the first interview for eligibility was considered if they had the skill set adequate to learn the procedure and a home environment with suitable space, plumbing, and electrics. Patients were monitored remotely electronically and encouraged not to have a trained partner. To provide maximum HD for each patient treated with Fresenius machines, we prescribed 100L of blood to be processed 5-6 nights weekly over 6-8 hours, i.e. 500-600 L/week. NxStage patients were prescribed 30-60L of dialysate over 4-6 hours, 5-6 days or nights weekly, i.e., 300 L/week.

Results: Starting in 2001, 77 patients, average age 50.1 years, were followed to May 2012. Seven had 2 courses of home HD prior to and after failed transplants. In 65 treatment courses patients were monitored remotely and data including blood processed downloaded to the electronic medical record. These patients received 4.49 ± 0.86 treatments/week. Blood processed was 426.9 ± 109.5 L/week (6.35 ± 2.77 L/kg/week, and ~85% of prescribed). This was >2 fold greater than in 328 patients treated by in-center HD (2.84 L/kg/week). NxStage patients were dialyzed 3.83 ± 0.89 times weekly, ~76% of prescribed. Serum albumin was 41.6 ± 3.3 g/L. 11 patients died (mortality 5.46/100 years). 21 received a kidney transplant (10.4/100 years). 18 returned to in center HD. Medical necessity was the cause of return to in center HD in 12 (6/100 years), and patient preference, non-compliance, or non adherence in 6 (3/100 years).

Conclusions: High dose quotidian dialysis improves outcomes and is associated with low return to in-center HD by choice.

PUB337

The Benefits and Harms of Home versus In-Centre Haemodialysis: A Meta-Analysis

Suetonia Palmer,[1] Allison Tong,[2] Braden J. Manns,[3] Jonathan C. Craig,[2] Marinella Ruospo,[4] Letizia Gargano,[4] Giovanni F.M. Strippoli.[2,4] *[1]University of Otago; [2]University of Sydney; [3]University of Calgary; [4]Diaverum Bari.*

Background: Chronic kidney disease requiring dialysis is characterised by a heavy symptom burden, poor quality of life, and excessive risks of cardiovascular events and death. Uncontrolled data suggest dialysis conducted at home (home hemodialysis (HD)) offers improved survival, quality of life, and more flexible treatment schedules. However, data from observational studies are limited by confounding by selection; younger healthier patients are more likely to be offered home HD. Recent randomized trials of longer duration and increased frequency dialysis are suggesting improved survival and cardiovascular function. In this study, we aimed to summarize the available trial data for the effects of home versus in-center HD on mortality, quality of life, and symptom-burden in people with end-stage kidney disease.

Methods: We conducted a systematic review and meta-analysis of randomized controlled trials that included adults with chronic kidney disease and that compared in-center HD with home HD. We considered dialysis of any duration and frequency. Two reviewers systematically searched Cochrane and EMBASE databases to December 2011 without language restriction. We extracted details on participant characteristics, interventions, and risk of bias and summarized treatment effects on mortality, quality of life, and treatment durability (where possible) using random-effects model.

Results: A single randomized cross-over trial including 9 participants and comparing long home HD with short in-centre HD each for 8 weeks was identified by systematic searching. Quality of life was measured using the kidney disease quality of life instrument (KDQOL). Home HD resulted in fewer uremia-related symptoms and less physical suffering but interfered more with social activities and placed a greater treatment burden on families. No deaths or treatment withdrawals occurred.

Conclusions: Randomized trial data comparing home versus in-center HD are few and currently insufficient to guide clinical practice and policy. Given the potential benefits of home HD, a randomized trial is now needed.

PUB338

Extended Nocturnal Hemodialysis: Single Center Experience

Ana Rita Mateus Martins, Lucia Parreira, Ana Sofia Baptista Duque, Ilídio Rodrigues. *Barreiro, Nephrocare, Portugal.*

Background: Despite advances in dialysis, this therapy is still associated with high morbidity, mortality and costs.

Methods: The mortality, morbidity, hypertensive profile, bone mineral disease, anemia, efficacy of dialysis and nutrition were evaluated in 14 patients undergoing extended nocturnal hemodiafiltration (HDF) mean time 18,5 hours/week. Among this patients 5 were male, 1 had diabetes mellitus and 3 had cardiovascular disease. The mean age was 53,1 ± 10,3 years. At admission, average time on renal replacement therapy was 7,4 ± 4,7 years. The follow-up period was 13,6 ± 2,5 months. During the follow up one patient died and one received a kidney allograft. There were no intradialytic complications or failure of vascular access (13 with arteriovenous fistulas and 1 with graft).

Results: In all patients there was an improvement of dialysis efficiency (eKT/v 4,6 ± 6,1vs 0,7 ± 0,8 wk, p 0,000). Although hemoglobin levels (11,5 ± 0,8 vs 11,3 ± 0.7 g/dl, pns) remained unchanged, there was a significant reduction in erythropoiesis stimulating agent (ESA) consumption (121,1 vs 80,7 IU/kg/wk, p 0.000). Iron consumption (40.4 ± 25,3 vs 34,05 ± 37,9 mg/wk, pns) and ferritin values (434,2 ± 305,2 vs 392,9 ± 162,3 ng/ml, p 0,003) significantly decreased and nutritional parameters improved (nPCR 1,1 ± 0,1 vs 1,2 ± 0,7 g/kg/d, p 0,000). Serum calcium levels increased (8,2 ± 0,7 vs 8,9 ± 0,4 mg/dl, p 0.001) and there was a reduction in phosphatemia (4,8 to 4,5 mg/dl) allowing phosphate binder suspension in 4 patients and those reduction in 3; PTH values (688,2 ± 599,2 vs 560,9 ± 310,2 pg/ml, pns) were not affected. Both pulse pressure (67,6 ± 15,8 vs 63,5 ± 17,3 mmHg, p 0,035) and diastolic blood pressure (BP) (71,9 ± 7,3 vs 62,1 ± 13,6 mmHg, p 0,014) were significantly lower; antihypertensive medications consumption decreased (1,9 ± 1,4 vs 0,9 ± 0,8, p 0,008). Left ventricular (LV) mass, posterior wall and LV fractional shortening had a non significant reduction.

Conclusions: Therefore good patient adherence, improvement in dialysis efficacy, BP control, nutritional parameters, inflammatory markers, lower consumption of ESA, antihypertensive and phosphate binder medication were observed with this strategy.

PUB339

Real-World Diagnosis and Treatment Patterns of Iron Deficiency Anemia in Patients with Chronic Kidney Disease in China

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Background: In the past decade, our understanding of impact of iron deficiency anemia (IDA) on human health has advanced significantly. Iron supplementation may avoid, or at least delay, the need for erythropoiesis stimulating agents (ESA) in chronic kidney disease (CKD).

Methods: A comprehensive literature search and a survey with physicians were conducted to collect information on IDA diagnosis and treatment patterns in China. 163 publications were reviewed and 44 senior physicians specialized in nephrology, hematology and obstetrics/gynecology from national hospitals in Beijing, Shanghai, Guangzhou, Shenyang and Chengdu were interviewed. The questionnaire was also validated by information on lab tests and treatments collected from 91 patient cases.

Results: Hemoglobin (Hb), Red Blood Cell (RBC), and Iron Four (serum iron, ferritin, TSAT, and TIBC) are the top three (3) tests ordered by nephrologists. Review of patient cases confirmed this result.

Patient case lab tests review

Test	Observations (%); N=30	Mean	Median	P25	P75
Hemoglobin (g/dL)	29 (96.67)	8.78	9.30	7.60	10.30
Ferritin (ng/mL)	27 (90.00)	189.39	152.00	87.00	261.40
Serum Iron (umol/L)	16 (53.33)	9.91	10.14	7.95	12.50
Transferrin (g/L)	7 (23.33)	2.94	1.76	1.37	1.87
TSAT (%)	19 (63.33)	24.32	18.00	15.00	29.20
TIBC (umol/L)	6 (20.00)	50.03	50.46	40.70	59.30

TSAT = Transferrin Saturation; TIBC = Total Iron Binding Capacity

Nephrologists reported that they usually prescribe intravenous (IV) iron as the first line at a dose of 1000mg per course and targeted Hb levels of 12-13g/dL. Although price was not believed to be the most influential driver for using IV iron, 57% of those surveyed reported the restrictions of IV iron use due to cost.

Conclusions: Prevalence and disease burden of IDA in China is high, especially among CKD patients on dialysis. Appropriate diagnosis and treatment in clinical practice is important. National level guidelines need to be developed and strengthened in China.

Funding: Pharmaceutical Company Support - Vifor Pharma Ltd.

PUB340

Superiority of Darbepoetin Alfa to Epoetin beta by Hepcidin Response
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Background: Hepcidin is the key regulator of iron metabolism and is suppressed by erythropoietic activity (Pak M. et al. Blood 108:3735, 2006). The purpose was to evaluate the efficacy of erythropoiesis stimulating agents by hepcidin response.

Methods: Six hemodialysis patients with stable Hb levels by epoetin beta: EPO (3000 units x 3/week) were enrolled. After 4 weeks, EPO was switched to darbepoetin alfa: DPO (40 mcg weekly). No iron administration was done during the experiment. At each 0, 2nd, 4th, 7th and 28th days, serum Hb, Fe, TIBC, ferritin, Hepcidin-25 (by liquid chromatography tandem mass spectrometry), hs-CRP and IL-6 were measured.

Results: There was no significant change in Hb and ferritin in the EPO administration period. In the DPO period, Hb was increased (11.07±0.84 to 11.68±1.14 g/dL) and ferritin was decreased (105.7±61.8 to 52.5±36.9 ng/mL) significantly after 28 days. No significant change of hs-CRP and IL-6 was found in both period. Area under the percent change in serum hepcidin-time curve in DPO period was significantly greater than that in the EPO period (-348.0±92.4 *vs.* -178.4±131.5 %·day, $p = 0.030$).

Conclusions: Darbepoetin alfa seems to be superior to epoetin beta in erythropoietic activity by hepcidin response.

PUB341

NUTRIPEPA 2: A Prospective Randomized Interventional Study in Hemodialysed Patients with Protein-Energy Wasting
Bernard G.J. Hory. *Service de Nephrologie et de Medecine Interne, Centre Hospitalier, Ales, France.*

Background: Protein-energy wasting (PEW) results in an increase of cardiovascular mortality particularly in the case of Malnutrition Inflammation Syndrome (MIS). In a previous study (Nutripepa 1), we treated 13 cases of MIS for 12 months with PEPA membrane hemodialysis and nutrition intervention. Survival at 12 months was 92%; 66% of leptin was removed without removal of adiponectin. The aims of this study are to confirm our results, and to test the relationship between variation of adipocytokines concentration and mortality.

Methods: This multicenter, single-blind, prospective, randomized study, will compare the 12 month mortality of 2 groups of patients, the first treated with FDX membrane (poly Esther Arylate-PEPA) and the second treated with a non-adsorbing high permeability membrane ("control membrane"). All included patients must present signs of PEW, and will receive nutritional complements. They will be selected by french nephrologists (The Nutripepa Group). Patients and inclusion/exclusion criteria : 100 hemodialysed patients treated for at least 3 months on a non-adsorbing synthetic membrane with an equivalent area to PEPA (Polysulfone, Polyethersulfone, PolyArylethersulfone) will be included if serum albumin average will be less than 35 g /l and PINI score greater than or equal to 1. Exclusion criteria will be : insufficient vascular access, hepatopathy, amyloidosis, cancer, hemopathy, age less than 18y, pregnant women, allergy to PEPA. Methods: BMI, SGA score, albumin, Prognostic Inflamatory and Nutritional Index (PINI score) (Dessi et al), KT/V, nPCR will be determined at inclusion and at 3, 6, 9 and 12 months. Leptin, adiponectin and grhelin concentrations will measured at inclusion, and at 6 and 12 months. Agrements : The study received the agreements of Ethic's Committee of the CHU of Nimes and of the French Drug Agency (AFSSAPS).

Results: The mortality of the two groups will be compared. Correlations will be tested between mortality, BMI, albuminemia, PINI score and adipocytokines ratio.

Conclusions: At the present time, inclusion is in progress and 20 patients has been included. We observed 3 deaths on a 8 months follow-up confirming the high mortality of PEW.

PUB342

Understanding Diet Culture: The Impact of Dietitian Intervention upon Achieving Quality Patient Dialysis Outcomes in the Kingdom of Saudi Arabia
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Background: Nutrition is an important aspect in hemodialysis care. Understanding how the culture of the Middle East influences an individual's diet, eating patterns and norms concerning food choices constantly presents a significant challenge in achieving improved dialysis outcomes. This study investigated the influence of dietitian intervention on mortality, adherence to dialysis prescriptions and Quality Patient Dialysis Outcomes (QPDO).

Methods: 284 hemodialysis patients were assessed using the Subjective Global Assessment tool (SGA) noting progress and regression.

Results: The results revealed SGA ratings of patients with A (86.26%), B (13.38%), and C (0.70%). These ratings were analyzed using cox regression with hazard ratio of 95% confidence interval. Patients who marked significantly as C in the Functional Capacity Items – *Dysfunction* (sp=1.45) showed highest risk to mortality, indicating that these individuals, despite dietitian Interventions had increased mortality related to other co-morbidities. Patients with changes on *eating habits* and had *ankle edema* during physical examination demonstrated a higher mortality risk (sp=1.40) at 3.44%. Results indicated that patients with a higher spKt/V (sp=1.58) than required (spKt/V >1.2) and with SGA rating of A were noted to have lowest risks to mortality and co-morbidities.

Conclusions: This study demonstrated the significant impact of dietitian intervention in reducing the mortality ratio of patients. The findings became the basis for self-made recipes designed for renal patients. Patients and families are invited to participate in cooking classes once every quarter enabling them to become self-reliant in selecting different foods and knowledgeable in identifying nutritional components on food labels.

Funding: Private Foundation Support

PUB343

Serum Soluble-Fas, Inflammation and Anemia in Acute Kidney Injury
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Background: Anemia is common feature in critically ill patients. Serum soluble-Fas (sFas) levels are associated with anemia in chronic kidney disease. It is possible that sFas levels are also associated with anemia in acute kidney injury (AKI) patients. **Objective:** To investigate the relationship between serum levels of sFas, Epo, inflammatory cytokines and hemoglobin (Hgb) concentration in critically ill patients with AKI.

Methods: We studied 72 critically illpatients with AKI (AKI group; n= 53) or without AKI (non-AKI group; n=19), and 18 healthy volunteers. Serum sFas, Epo, TNF-a, IL-6, IL-10, iron status and Hgb concentration were analyzed in all groups. We also investigated the correlation between these variables in the AKI group.

Results: Critically ill patientshad higher serum levels of Epo. Hgb concentration was lower in the AKI group. Serum sFas, IL-6, TNF-a and ferritin levels were higher in the AKI group. Hgb concentration correlated negatively with serum IL-6 (r=-0.37, p=0.008), sFas (r=-0.35, p=0.01) and Epo (r=-0.27, p=0.04) while serum sFas correlated positively with iron levels (r=0.36, p=0.008) and IL-6 (r=0.28, p=0.04) in the AKI group. In multivariate analysis, after adjusting for markers of inflammation and iron store, only serum sFas levels (p=0.03) correlated negatively with Hgb concentration in the AKI group.

Conclusions: Serum Epo and inflammatory cytokines levels are elevated in critically ill patients with or without AKI. Serum levels of sFas are elevated and associated with anemia in critically ill patients with AKI.

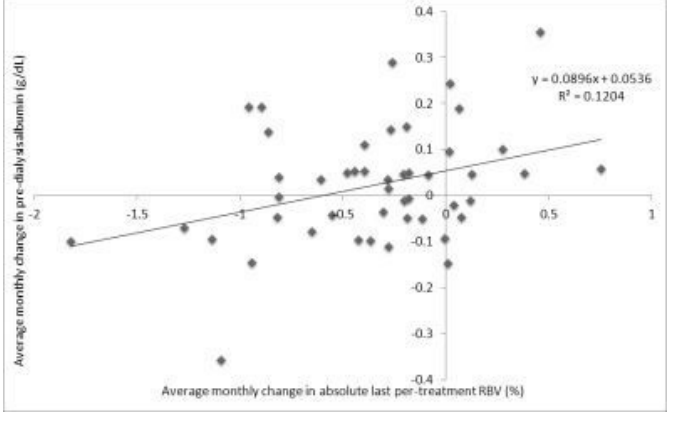
PUB344

Can Optimization of Intravascular Volume Improve Monitoring of Patient Parameters and Clinical Outcomes?
Nancy Ginsberg,[1] Patrice B. Taylor,[1,2] Lisa A. Pacelli,[1] Lynn Saunders,[1,2] Antoinette M. Ordish,[1,2] Len A. Usvyat,[1] Linda H. Ficociello,[3] Michael Black,[3] Claudy Mullon,[3] Jose A. Diaz-Buxo,[3] Mary T. Sullivan,[1] Peter Kotanko,[1] Paul Balter,[1] Paul M. Zabetakis.[1] *[1]Renal Research Institute, NY, NY; [2]University of North Carolina, Chapel Hill, NC; [3]FMC, Waltham, MA.*

Background: An on-going quality improvement (QI) project on fluid management using Crit-Line Blood Volume Monitors (CLM) has been running in 5 RRI clinics since May 2011. CLM measures changes in hematocrit, oxygen saturation and relative blood volume (RBV) during hemodialysis (HD). We aim to understand whether longitudinal changes in the amount of fluid removed during treatment are also associated with changes in serum albumin.

Methods: Data from 47 active hemodialysis (HD) patients at 5 RRI clinics that utilize Crit-line on a routine basis between May 1, 2011 and Apr 30, 2012 ("study period") were analyzed. Using simple linear regression, average monthly change per patient was computed for the following variables: absolute value of the last per-treatment RBV [RBV slope] and pre-dialysis serum albumin.

Results: The majority of patients were male (54%), black (61%), non-diabetic (62%), and had a mean age of 62.0 ±16.0 year. In a bivariate correlation, RBV slope was significantly and positively associated with changes in serum albumin (r=0.35, p<0.05).



Conclusions: This QI project implemented in the RRI clinics showed that higher reduction in blood volume as measured by Crit-Line during HD treatment was associated with increases in pre-dialysis serum albumin concentration. The analysis shows that for every 1% monthly change in RBV, 0.09 g/dL increase in pre-dialysis serum albumin may occur. These findings show the importance of considering intravascular volume parameters when establishing a prescription.

PUB345

Serum Albumin Dynamics in Incident HD Patients: Results of an International Study Rakesh Malhotra,[1] Adrian Marcos Guinsburg,[4] Aileen Grassmann,[3] Adam Tashman,[1] Cristina Marelli,[4] Eric Liu,[2] Frank van der Sande,[7] Inga Bayh,[3] Jeroen Kooman,[7] Laura Scatizzi,[3] Michael Etter,[2] Stephan Thijssen,[1] Edwin B. Toffelmire,[6] Yuedong Wang,[8] Len A. Usvyat,[1] Nathan W. Levin,[1] Franklin W. Maddux,[5] Peter Kotanko,[1] Daniele Marcelli.[3] *[1]Renal Research Institute, NY, NY; [2]Fresenius Asia Pacific Ltd, Hong Kong, Hong Kong; [3]Fresenius Medicare Care, Bad Homburg, Germany; [4]Fresenius Medical Care, Buenos Aires, Argentina; [5]Fresenius Medical Care, Waltham, MA; [6]Fresenius Medical Care Canada, Toronto, Canada; [7]Maastricht University Hospital, Maastricht, Netherlands; [8]University of California, Santa Barbara, CA.*

Background: Albumin is a powerful predictor of outcomes in HD patients (pts). We investigated longitudinal changes of albumin in incident HD pts in diverse worldwide population.

Methods: The MONitoring Dialysis Outcomes (MONDO) consortium consists of HD databases from RRI clinics (USA), FMC clinics in Europe, Asia, Latin America and Canada, Maastricht University (Netherlands) and KfH clinics (Germany). MONDO databases (other than KfH, Maastricht, and Canada) and FMC North America clinics were queried for albumin in incident HD pts who survived >24 months. Pts were stratified: Whites (Australia, Europe (17 countries), Argentina, US); Blacks (US); Chinese (Hong Kong, Taiwan, Singapore, US); and Korean (South Korea, US).

Results: 109281 HD pts from 23 countries were studied. Independent of race and region pts started HD with comparable albumin (mean bn 3.46 and 3.69 g/dL) followed by increase to 3.85 to 3.98 g/dL in the first 24 months after HD initiation.

Figure 1: Albumin levels in White and Black patients

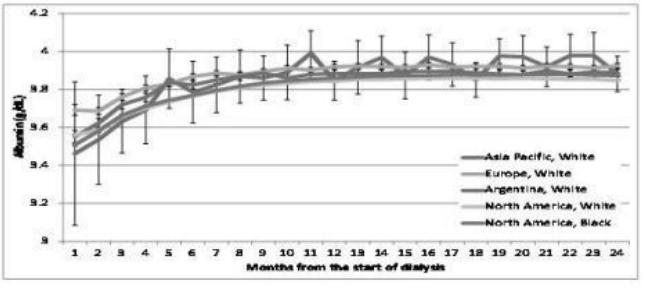
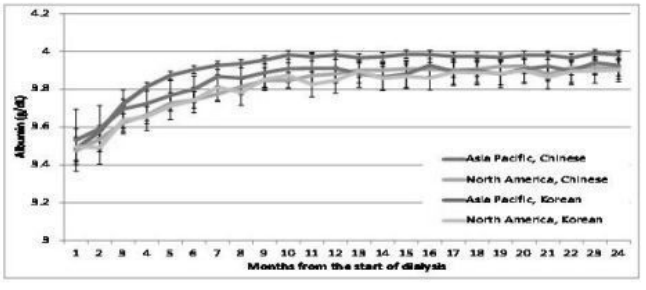


Figure 2: Albumin levels in Chinese and Korean patients



Conclusions: Our international study indicates comparable albumin at start of HD, independent of race and region. While practice patterns differ, pts experience remarkably similar increases in albumin by ~0.4 g/dL in the first 10 months after HD initiation.

PUB346

Cross-Sectional Study about Differences between Hemodialysis Schemes: Standard Intermittent Hemodialysis, Online Hemodiafiltration and Short Daily Dialysis in 57 Patients in HD Guillermina Barril, Fernando F. Hadad, Tania Monzon, Maria Bernardita Puchulu, Martin Giorgi, Jose A. Herrero, Jose-Antonio Sanchez-Tomero. *[1]Nephrology, Hospital U. de la Primcesa, Madrid, Spain; [2]Nephrology, Hospital U. de la Princesa, Madrid, Spain; [3]Nephrology, Hospital Clinico, Madrid, Spain.*

Background: There is evidence that high convective transport or most frequently hemodialysis (HD) can improve some parameters related to it. Objective: To compare demographic, vascular-access (VA), biochemical, blood pressure (BP), need erythropoietic agents(ESA), ESA resistance index and nutritional parameters in different HD schemes.

Methods: : We studied 57 patients from two HD units, with 45.4% men with a mean age of 72.07±11.96 years were 49.1% autologous-prothesis fistula (AVF) and the rest as permanent tunneled catheters (the percentage highest in HD standard). The HD schemes were: 21.1% in online hemodiafiltration (HDF), 28.1% in dailyHDHDD) and 50.9% standard hemodialysis(SHD). Parameters: Lymphocytes, albumin, prealbumin, creatinin, cholesterol, CRP, calcium, bicarbonate, ferritin, transferrin saturation inde (TSI), rate weekly urea reduction(PRU), systolic and diastolic pre HDBP.

Results: No significant differences in blood-flow between AVFvs catheter. Eight patients did not need ESA, two were from online HDF and six HDD. The online HDF showed higher significant difference vs SHD: creatinine (0.027), pre-HD systolic and diastolic BP (0.02), whereas bicarbonate was lower(0.09). By comparing HDE with HDD found significant differences for : systolic BP pre and post-HD (0.002), ESA units (0.006), calcium(0.041), ferritin (0.009) and CRP (0.007). While it was higher for HDD: lymphocytes(0.054), creatinine(0.010), albumin (0.019), prealbumin (0.012), cholesterol (0.018) and PRU(0.000) Significant difference was observed higher for online HDF in relation to HDD: ferritin(0.002), PRU(0.000), systolic BP(0.000) and diastoli(0.003) pre-HD.

Conclusions: The HDD demonstrated better biochemical, nutritional, urea kinetics, blood pressure control, and greater percentage of patients without AEE. The online HDF has advantages over the intermittent SHD. Is necessary to identify the pattern of HD tailored to the needs of patients.

PUB347

Relation between Depression and Anxiety and Daily Physical Activity in Maintenance Hemodialysis Patients Min Zhang,[1,2] Yinan Li,[2] Jun Chul Kim,[2,3] Bryan B. Shapiro,[2] Janos Porszasz,[2] Kamyar Kalantar-Zadeh,[2,4] Joel D. Kopple.[2,4] *[1]Div Nephrol, Tianjin Union Med Ctr, China; [2]Div Nephrol and Htn, LA BioMed Res Inst, Harbor-UCLA Med Ctr; [3]Div Nephrology, CHA Gumi Med Ctr, South Korea; [4]UCLA Schl Med & Pub Hlth, LA, CA.*

Background: Maintenance hemodialysis(MHD) patients have a high prevalence of depression and anxiety and also decreased degree of rehabilitation and daily physical activity(DPA). We examined whether there is a relationship between depression or anxiety and DPA in the MHD patients.

Methods: We measured DPA with the Actigraph GT3X+ Activity Monitor®, in 76 patients receiving MHD 3x/wk for ≥6 months and 40 healthy normals of similar age and gender. Subjects completed the Beck Depression Inventory(BDI), Beck Anxiety Inventory(BAI) and Hospital Anxiety and Depression Scale(HADS-D and HADS-A). Comparisons were adjusted for age and gender.

Results: Patients were 52±12SD years, 33% female, 40% diabetic; dialysis vintage was 55±45 months. Normals were 51±13 years and 40% female. Depression and anxiety by BDI and BAI were identified in 32% and 45% of patients, respectively. Compared to MHD patients, normals had a lower prevalence of depression(5%) and anxiety(2.5%) ($p<0.0001$for each comparison). The average daily vector magnitude for 7 consecutive days of DPA measurements was much lower in MHD patients vs Normals ($p<0.0001$). The average vector magnitude for DPA in MHD patients, measured over these last 7 days, was negatively correlated with the BDI depression score(r= -0.290,p=0.017). DPA on the day of hemodialysis(HD) was also correlated negatively with the degree of depression by BDI (p=0.033), and the DPA 2 days post-HD was correlated negatively with depression by BDI(r= -0.268,p=0.028) and the HADS-D(r= -0.254,p=0.038). DPA on the first day post-HD did not correlate significantly with BDI(p=0.054) or HADS-D(p=0.36) scores. There were no correlations between DPA and either BAI or HADS-A.

Conclusions: These findings indicate that in adult MHD patients the magnitude of depression, but not of anxiety, and DPA are strongly related. Our results confirm previous finding that the prevalence of depression and anxiety are greatly increased in MHD patients.

Funding: Private Foundation Support

PUB348

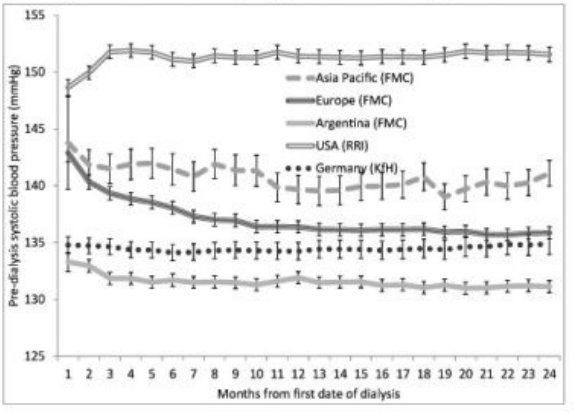
Pre-Dialysis Systolic Blood Pressure (SBP) and Erythropoietin Use: Results from an International Study Adrian Marcos Guinsburg,[4] Aileen Grassmann,[3] Adam Tashman,[1] Cristina Marelli,[4] Daniele Marcelli,[3] Eric Liu,[2] Frank van der Sande,[8] Gero D. von Gersdorff,[6] Inga Bayh,[3] Jeroen Kooman,[8] Laura Scatizzi,[3] Mathias Schaller,[6] Michael Etter,[2] Stephan Thijssen,[1] Edwin B. Toffelmire,[5] Yuedong Wang,[9] Len A. Usvyat,[1] Nathan W. Levin,[1] Peter Kotanko,[1] Claudia Barth.[7] *[1]Renal Research Institute, NY, NY; [2]Fresenius Asia Pacific Ltd, Hong Kong, Hong Kong; [3]Fresenius Medicare Care, Bad Homburg, Germany; [4]Fresenius Medical Care, Buenos Aires, Argentina; [5]Fresenius Medical Care Canada, Toronto, Canada; [6]Cologne University Medical Center, Cologne, Germany; [7]Kuratorium für Dialyse und Nierentransplantation e.V., Neu-Isenburg, Germany; [8]Maastricht University Hospital, Maastricht, Netherlands; [9]University of California, Santa Barbara, CA.*

Background: Given international differences in erythropoietin (EPO) use, we aimed to explore association bn EPO use and SBP patterns in diverse populations of HD pts.

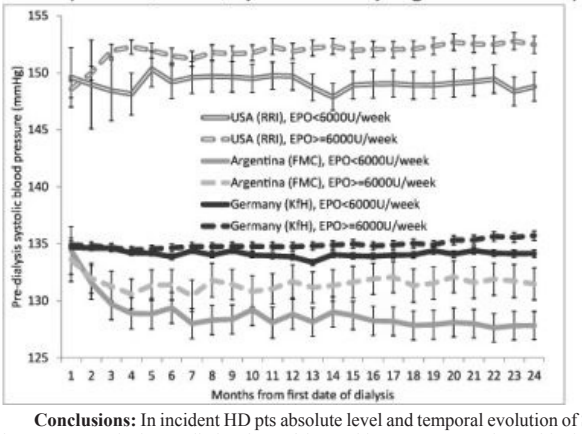
Methods: The MONitoring Dialysis Outcomes (MONDO) consortium consists of HD databases from RRI clinics (USA), FMC clinics in Europe, Asia, Latin America and Canada, Maastricht University (Netherlands) and KfH clinics (Germany). Other than Netherlands and Canada, these databases were queried for incident pts initiating HD bn 1/2000 and 12/2010 who survived >2yrs. In Germany, Argentina and US, pts were stratified based on weekly EPO dose (< or <=6,000U).

Results: We studied 1579 pts from Asia, 5503 from Europe, 25991 from Germany, 1813 from Argentina, 4296 from US. US pts start with highest SBP and experience SBP increase during first 4 mns. After stratification by EPO, SBP patterns were comparable in all databases studied.

Mean preSBP (95% CI) by region in incident patients



Mean preSBP (95% CI) by EPO dose by region in incident pts



Conclusions: In incident HD pts absolute level and temporal evolution of SBP differs between US and other studied regions of the world. SPB appears to be positively associated with EPO dose.

PUB349

Microcytosis Induced by a Larger Dose of Erythropoiesis-Stimulating Agents(ESA) without Iron Supplementation Is Improved Markedly by Oral Iron Administration in Hemodialysis Patients Shigeru Miyazaki,[1] Noriko Saito,[1] Masaaki Shimotori,[1] Kozo Ikarashi,[1] Tetsuo Morioka,[1] Hisaki Shimada,[1] Tadashi Yamamoto.[2] *[1]Shinraku-en Hosp., Niigata, Japan; [2]Inst. Nephrol., Niigata Univ., Japan.*

Background: Erythropoiesis-stimulating agents(ESA) have an ability not only to stimulate erythropoiesis, but also decrease serum level of hepcidin, which disturbs iron utilization for erythropoiesis, resulting in anemia improvement and also increase iron absorption through the proximal intestinal epithelium. Therefore, we examined the effects of administration of ESA with a large dose on the renal anemia and iron metabolism in hemodialysis patients.

Methods: ESA administration was increased from 5200±4300 U/week to 13000±7200U/week (Epoetin beta equivalent, p<0.001) in 30 hemodialysis patients with renal anemia. Complete blood count and iron metabolism marker was examined.

Results: Microcytosis(MVC(fl) 90.2±5.2 vs 81.4±5.0, p<0.0001) was observed 6 month after the high ESA dose administration in 20 out of 30 patients. This group of patients showed no improvement of anemia (Hb(g/dl) 9.6±0.9 vs 8.8±1.5, ns). Serum ferritin (ng/ml) decreased from 40.4±59.8 to 21.8±17.5(p<0.01). The other 10 patients without microcytosis showed the increase of Hb(9.1±1.2 vs 10.5±1.5, p<0.05) with decrease of MCV(95.5±4.6 vs 90.1±6.3, p<0.001), but these values were both in normal range. Serum ferritin decreased from 120.9±109.4 to 40.4±35.6(p<0.01). When 14 out of 20 patients of microcytosis were treated with oral iron supplementation (Fe 100mg/day), microcytic anemia was corrected promptly 3 months after the challenge (Hb 7.9±0.8 vs 11.3±1.9, p<0.0001). MCV was also improved from 78.9±3.8 to 88.1±4.8(p<0.0001). Serum ferritin was increased (16.0±8.4 vs 36.8±22.7, p<0.01), whereas TSAT (%) was unchanged.

Conclusions: Microcytosis induced by the increment of the ESA administration without adequate iron supplementation suggests that dietary iron is not sufficient for erythropoiesis in Japanese hemodialysis patients. In addition, oral iron supplementation dramatically ameliorated the microcytic anemia, suggesting that administration of ESA with a large dose improve the intestinal iron absorption even in hemodialysis patients.

PUB350

Effects of Omega-3 Fatty Acid Supplementation on Plasma Lipids in Hemodialysis Patients Denise Mafra,[1] Magali Monteath,[1] Najla Elias Farage.[2] *[1]Clinical Nutrition, Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil; [2]RenalCor Clinic, Rio de Janeiro, Brazil.*

Background: Hemodialysis (HD) patients present changes in lipid profile and are at high risk for cardiovascular disease. Omega-3 fatty acids are known to have a cardio-protective effect and to reduce plasma triglycerides in subjects with normal renal function. The benefits of omega-3 for a wide range of general populations and a variety with health problems are apparent; however, the reports on the possible benefits for HD patients are still controversial. The aim of this study was to investigate the effect of omega-3 on plasma lipid levels in HD patients.

Methods: Thirty six HD patients from RenalCor Clinic in Rio de Janeiro, Brazil, were studied. Twenty one patients received 2400 mg of fish oil during four weeks (12M/9F; 53.7±12.4 yr, body mass index (BMI) 24.9±5.1Kg/m^2, average dialysis time 60 months) and fifteen patients did not receive supplementation (9M/6F, 46.5±16.6 yr, BMI 22.4±3.4Kg/m^2). The fasting plasma lipid levels were evaluated before and after the use of oil fish.The SPSS for Windows (version 11.0) was used as statistical program.

Results: Analysis of variance revealed no significant differences in age, dialysis time, waist circumference, body mass index and body fat between groups. Additionally, no pretest differences existed between groups for triglyceride, total cholesterol, LDL and HDL. No significant difference in plasma lipids was observed after fish oil supplementation (Table 1).

Plasma lipids levels before and after omega-3 fatty acids supplementation

Parameters	Omega-3 group		Control Group	
	Baseline	After 4 weeks	Baseline	After 4 weeks
Triglycerides (mg/dL)	107.1 (85.8)	107.7 (54.5)	98.8 (54.8)	110.1 (52.8)
Total cholesterol (mg/dL)	143.2 (31.6)	138.8 (38.6)	144.0 (38.2)	139.8 (31.3)
LDL (mg/dL)	101.1 (27.4)	101.4 (36.8)	104.1 (37.9)	101.3 (31.0)
HDL (mg/dL)	42.1 (9.9)	37.4 (8.9)	39.8 (11.8)	38.5 (8.5)

Conclusions: Our data seem to suggest that supplementation with of omega-3 capsules of fish oil content 2400mg during one month was not effective to improve the lipid profile in HD patients.

PUB351

Patterns and Predictors of Blood Transfusion among Hospitalized Hemodialysis Patients Ben C. Wong,[1] Pietro Ravani,[1] Braden J. Manns,[1] Xin Zhang,[1] Rick Chin,[1] Brenda Hemmelgarn,[1] Marcello Tonelli,[2] Matthew J. Oliver,[3] Robert R. Quinn.[1] *[1]University of Calgary, Calgary, AB, Canada; [2]University of Alberta, Edmonton, AB, Canada; [3]University of Toronto, Toronto, ON, Canada.*

Background: Hospitalized hemodialysis (HD) patients may be at increased risk of blood transfusion due to acute illness and relative erythropoietin resistance. The objective of this study was to quantify the risk of transfusion in the 90 days following hospital admission and identify predictors of transfusion risk.

Methods: Adult HD patients admitted to acute care hospitals between June 2004 and March 2008 in Calgary, Canada were identified. Administrative, lab, and blood transfusion records were used to identify baseline patient characteristics and outcomes of interest. Logistic regression was used to model the risk of transfusion in the 90 days following admission to hospital and to identify clinical predictors.

Results: A total of 559 patients experienced 884 hospital admissions. The risk of blood transfusion was 25% at 3 months, with the majority of transfusions occurring within 2 weeks of hospital admission. A lower baseline hemoglobin, a higher baseline transferrin saturation, and a history of peripheral vascular disease were independent predictors of transfusion risk. Age, sex, serum albumin, diabetes mellitus, a history of congestive heart failure, and a history of ischemic heart disease were not independently associated with the risk of transfusion. There was a statistically significant decrease in admission hemoglobin level and erythropoiesis stimulating agent (ESA) dosage over time, but the risk of transfusion remained constant.

Conclusions: Hospitalized HD patients were at a high risk of blood transfusion in the 90 days following admission, particularly those with lower baseline hemoglobin levels, higher baseline transferrin saturations, or a history of peripheral vascular disease. Future work will examine strategies aimed at reducing transfusion requirements in hospitalized HD patients.

PUB352

Relationship between Daily Physical Activity and Body Composition in Maintenance Hemodialysis Patients Yinan Li,[1,2] Min Zhang,[2] Janos Porszasz,[2] Kamyar Kalantar-Zadeh,[2] Joel D. Kopple.[2] *[1]Dept Nephrology, First Affiliated Hospital of Xiamen University, China; [2]Div Nephrology & Hypertension, Harbor-UCLA Medical Center, Los Angeles, CA.*

Background: MHD patients are considered to be physically less active than normal, but the factors affecting diminished physical activity are poorly understood. We assessed the relationship between DPA and body composition in MHD patients and healthy adults.

Methods: 76 clinically stable patients undergoing MHD 3x/week with vintage ≥6 months and 40 Normals were studied. Body composition measured the day after a HD, was assessed by Dual-Energy X-ray Absorptiometry(DEXA), DPA was measured with the Actigraph GT3X+ Activity Monitor®. The average daily vector magnitude for DPA was calculated as the square root of the sum of the squares of the three dimensional axes. All comparisons were adjusted for age and gender.

Results: Patients were 52±12years, 33% female, 40% diabetic; dialysis vintage was 55±45 mos. Normals were 51±13years, 40% female. DPA, averaged for 7 consecutive days, was much lower in MHD patients, 394,032 counts, vs 648,980 counts in Normals, p<0.0001. Body weight and body mass index were 78.8±21.0 kg and 27.8±5.7 kg/m^2 in MHD patients and 75.0±14.4 kg and 26.9±3.9 kg/m^2 in Normals. Average daily DPA and BMI were not different in diabetic vs non-diabetic patients. In MHD patients, DPA was negatively correlated with body weight (r = -0.31, p<0.05)[Fig.1] and body mass index (r= -0.34, p<0.01)[Fig.2]. No other measures of body composition were significantly associated with DPA in MHD patients. In Normals, there were no associations between DPA and body composition.

Conclusions: DPA is markedly reduced in MHD patients compared to normal controls. In MHD patients, DPA was negatively correlated with body weight and body mass index. This may reflect the likelihood that more obese MHD patients are physically less active.

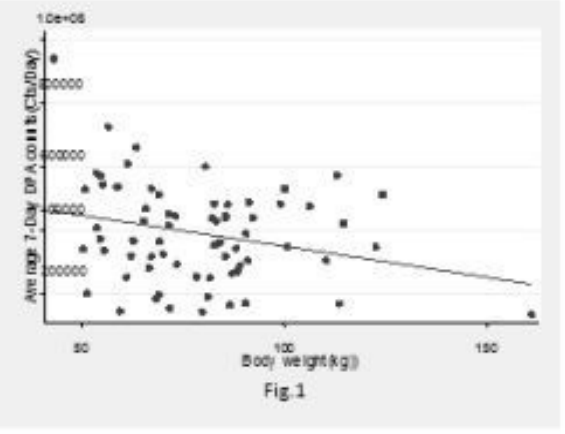


Fig.1

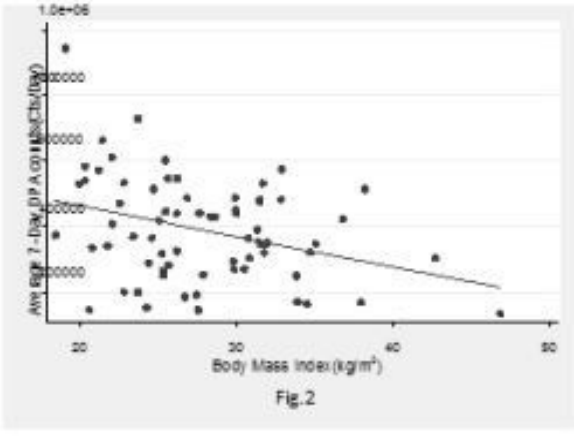


Fig.2

Funding: Private Foundation Support

PUB353

C-Reactive Protein (CRP) and Serum Albumin Levels Are Significant Determinants of Death in Cases of Hemodialysis-Associated Sepsis Hitoshi Iwabuchi, Manabu Asano, Kenichi Oguchi. *Renal Unit, Ikegami General Hospital, Tokyo, Japan.*

Background: Infection remains the second major cause of death in patients undergoing dialysis in Japan. Therefore, strategies for combating sepsis are extremely important in these patients. We investigated cases of sepsis that developed in patients receiving maintenance hemodialysis in our facility and examined the bacteriological etiology and prognosis-related factors.

Methods: A case-control study of 288 patients receiving maintenance hemodialysis between 2001 and 2011 was carried out. All the patients experienced a fever of 38°C or higher and underwent blood culture because of suspicions about sepsis. The primary outcome measure was death, while prognosis-related factors included age, gender, dialysis record, diabetes, catheter placement, CRP, leukocyte count, blood platelet count, albumin value, and methicillin-resistant *Staphylococcus aureus* (MRSA).

Results: Of the 691 blood cultures performed, 19.8% were positive. Twenty-one bacterial species and 143 bacterial strains were identified respectively. Gram-positive cocci accounted for 75.5% of all. Especially Staphylococci accounted for 84.3% of the gram-positive cocci, while MRSA and methicillin-resistant coagulase-negative staphylococci (MRCNS) accounted for 70% of the entire staphylococcus population.

Ninety-one patients (50 men and 41 women) were clinically diagnosed with sepsis. Diabetic complications existed in 60 patients, while 56 patients had an indwelling catheter. Concomitant infections included 21 cases of catheter infection, 12 cases of urinary-tract infection and 9 cases of respiratory infection. Overall, 57 patients died, and approximately 50% of these patients had resistant staphylococci. Multivariate analysis showed that the levels of CRP [Odds ratio (OR) 0.0224, 95% confidence interval (CI) 1.008-1.113] and albumin [OR 0.0016, CI 0.054-0.507] concentrations are associated with an increased risk of mortality.

Conclusions: Managing potential risk factors such as the presence of an indwelling catheter and the evidence of malnutrition should take priority over all else. CRP and albumin values are the most important prognostic factors of hemodialysis-associated sepsis.

PUB354

Clinical Analysis of Malignant Tumors Surgery on Maintenance Hemodialysis Patients Xiaonong Chen, Xiaobo Ma, Tian Xu, Ping Zhu, Ying Qian, Nan Chen. *Ruijin Hospital, Shanghai Jiao Tong University.*

Background: To analyze the clinical characteristics and prognosis of ESRD patients on maintenance hemodialysis undergoing surgery of malignant tumors and to explore peri-operative risk and method of blood purification.

Methods: Patients on maintenance hemodialysis with a history of surgery of malignant tumors were enrolled from Jan. 2006 to Dec. 2011 in center of blood purification in our Hospital and clinical characteristics were collected.

Results: 22 patients on maintenance hemodialysis underwent an operation of malignant tumors during the study period including radical surgery for renal cell carcinoma, bladder cancer, intestinal cancer, breast cancer etc. There were 11 males and 11 females with an average age of 57.18±14.34(30~86) and an average dialysis age of 5.14±3.98(1~15). Types of the malignant tumors consisted of 9 urological tumors (6 cases of bladder cancer, 2 cases of renal carcinoma, 1 case of renal pelvic carcinoma), 6 digestive tumors (3 cases of colonic cancer, 1 rectal cancer, 1 gastric cancer, 1 gallbladder cancer), 6 breast cancer and 1 ovarian cancer. Before surgery, cardiopulmonary function, risk of anesthesia and other routines should be evaluated and serum hemoglobin level should be maintained above 100g/l and serum albumin level above 30g/l. Hemodialysis without heparin should be done one day before surgery, the day of surgery and 24 hours after surgery. The type and dose of anticoagulant can be regulated according to general status of patients. 5 cases (22.73%) were admitted to surgical ICU and underwent continuous renal replacement therapy (CRRT) comprising of CVVH, CVVHD, CVVHDF with an average case-time of 7.4±5.94 and a median dialysis duration of 6~12 hours. 2 patients died during hospitalization in department of surgery and hospitalized mortality rate was 9.09%.

Conclusions: The prevalence of malignant tumors was high in ESRD patients on maintenance hemodialysis and urological, digestive and breast cancer were predominant. Patients on hemodialysis with malignant tumors were eligible to undergo operation and survived in long-term follow-up. ESRD should not be regarded as a contraindication of surgery.

PUB355

Thrombotic Complications in ESRD Patients and Their Relevance to Biomarkers of Inflammation and Thrombogenesis Vinod K. Bansal,[2] Indermohan Thethi,[1] Debra Hoppensteadt,[1] Joesphine Cunanan,[1] Kristiyana Kaneva,[1] Rakesh Wahi,[1] Jawed Fareed.[1] [1]*Pathology, Loyola University Medical Center, Maywood, IL;* [2]*Nephrology, Loyola University Medical Center, Maywood, IL.*

Background: ESRD (End Stage Renal Disease) is kidney failure in the presence of low GFR and occurrence of signs and symptoms of kidney failure necessitating initiation of treatment by replacement therapy such as hemodialysis or transplant. ESRD has been associated with higher cardiovascular mortality including acute coronary syndrome and studies have shown that there may be an increase in inflammation and thrombosis markers in this patient population. In this study, we investigated the levels of biomarkers of inflammation and thrombosis and correlated these with clinical endpoints of acute thrombotic complications (arterial or venous) during a 14 month prospective follow up in ESRD patient population.

Methods: Blood samples were collected from 55 ESRD patients on maintenance hemodialysis and healthy volunteers were analyzed for markers of thrombosis and inflammation – d-Dimer, Thrombomodulin, C-reactive protein (CRP), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Tumor Necrosis Factor Receptor 1 (TNFRI) and Neuron Specific Enolase (NSE). The levels were measured using biochip array technology with the Randox Evidence System (United Kingdom). Clinical endpoints were determined from chart review at the end of 14 months from the day of blood draw.

Results: 12 patients or 21.81 % of ESRD patient population suffered from acute thrombotic events during the 14 month follow up. Biomarkers levels were elevated for all biomarkers ranging from 48.8% - 667.5% in ESRD patients in comparison to healthy volunteers.

Conclusions: A high incidence of acute thrombosis was observed in the ESRD patient population which correlates with the high levels of markers of thrombosis and inflammation, particularly D-dimer and TNFRI, seen in these patients. This simultaneous activation of thrombotic and inflammatory pathways is evident in these patients.

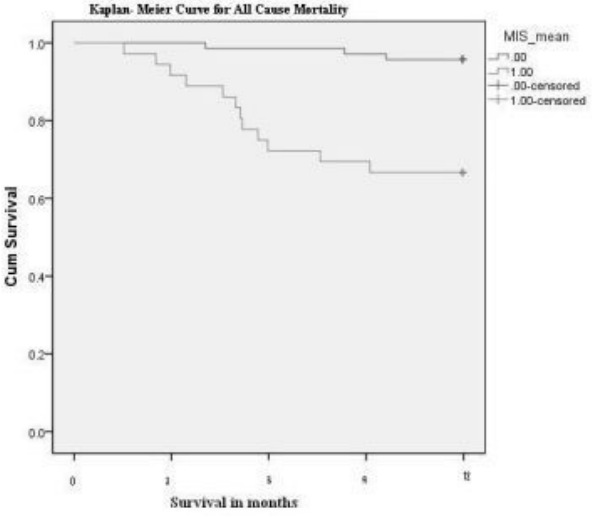
PUB356

Association of Malnutrition-Inflammation Score with Mortality in Haemodialysis Patients: 12months Prospective Study Christopher T. Agbo,[1] Sumith C. Abeygunasekara,[2] Abdelgalil Abdelrahman Ali.[3] *[1]Dept of Renal Medicine, Broomfield Hospital NHS, Chelmsford, Essex, United Kingdom; [2]Dept of Renal Medicine, Broomfield Hospital NHS, Chelmsford, Essex, United Kingdom; [3]Dept of Renal Medicine, Broomfield Hospital NHS, Chelmsford, Essex, United Kingdom.*

Background: There is increasing mortality risk associated with malnutrition and inflammation in haemodialysis patients. Malnutrition-Inflammation Score (MIS) is a comprehensive tool recently validated to assess malnutrition and inflammation in maintenance haemodialysis (MHD) patients. The aim of the study was to establish the association between MIS and mortality in MHD patients.

Methods: A total of 110 patients on MHD for at least 3 months from an East of England District Hospital were enrolled for this study,four patients dropped out. The MIS and variables such as C reactive protein(CRP) of each patient was recorded at the start of the study by two trained physicians,patients were follow up for 12 months and the primary outcome was mortality.

Results: Sixteen patients (15.1%) died during the study period, both logistic regression and Cox proportional hazard analyses showed MIS, CRP, Age and Haemoglobin as predictors of mortality. However, it was only the MIS and Age that had a strong association with mortality; p values 0.0001 and 0.002 respectively.



Cox Regression -Prediction of Short Term Survival

	B	SE	Wald	Sig	Exp(B)
Age(years)	0.120	0.039	9.642	0.002	1.128
MIS	0.374	0.101	13.750	0.000	1.454
Haemoglobin(g/l)	-0.655	0.366	3.205	0.073	0.519
Creatinine(umol/l)	-0.001	0.002	0.402	0.526	0.999
CRP(mg/l)	0.003	0.008	0.165	0.684	1.003
Weight(kg)	-0.026	0.021	1.563	0.211	0.975

Table 1

Conclusions: MIS has a strong association with mortality in maintenance haemodialysis patients and appears to be more superior to CRP. However, there was a significant correlation between MIS and CRP.MIS will be a useful tool in risk stratification of MHD patients in order to improve outcome.

Funding: Government Support - Non-U.S.

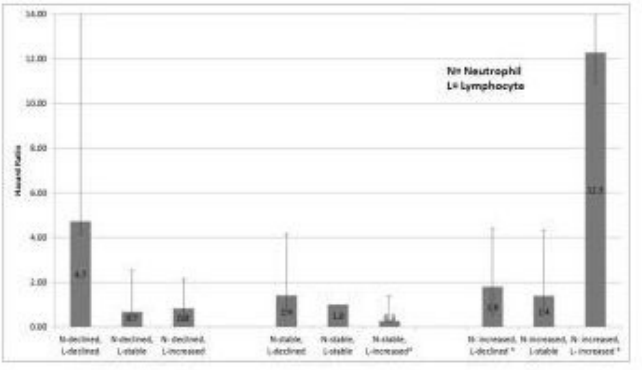
PUB357

Relationship between Trends in Neutrophil and Lymphocyte Counts and Mortality in Incident Hemodialysis Patients Rakesh Malhotra,[1] Len A. Usvyat,[2] Jochen G. Raimann,[2] Stephan Thijssen,[2] Nathan W. Levin,[2] Peter Kotanko.[2] *[1]UMDNJ-Newark; [2]Renal Research Institute.*

Background: The prognostic value of trends (increase or decrease) of neutrophil and lymphocyte counts is unclear. We analyzed the relationship of changes in neutrophils and lymphocytes and mortality in HD patients.

Methods: Incident HD patients treated in RRI clinics between 1/2000 & 12/2010 who survived atleast 12 months were included. Slopes of neutrophil & of lymphocyte counts were computed using linear regression of all available values between months 4 & 12 from the start of HD. Patients were stratified in 9 groups based on:(a)average rate of change in neutrophil % (declined:<-7%points/yr; stable:-7 to 7%points/yr; increased:>7points/yr) & (b)average rate of change in lymphocyte % (declined:<-5.5% points/yr; stable:-5.5 to 5.5%points/yr; increased:>5.5points/yr). Survival was analyzed in months 13 to 18 following HD initiation using a Cox model adjusted for age, gender, race, diabetes, access, BMI, SBP, body temp, nPCR, eKt/V, IDWG, UDV, & slope of neutrophil & lymphocyte counts.

Results: 2809 patients were studied (median(IQR) age at start of dialysis was 63(52-73) yrs, 55% male,& 46% Blacks). The Cox model showed that simultaneous increases in neutrophils & lymphocytes (HR=12.3;P=0.03) or decrease of neutrophils & lymphocytes (HR=4.7;P=0.14) were associated with increased mortality risk vs. reference group (stable neutrophils & lymphocytes). Decline in lymphocytes & increase in neutrophils increased death risk (HR=1.8;P=0.003) whereas increased lymphocytes with stable neutrophils exerted a protective effect on survival (HR=0.3;P=0.07).



Conclusions: Our result indicate survival advantage for patients with stable neutrophil and lymphocyte counts. Further studies are required to understand the roles of neutrophil and lymphocyte counts, their temporal trends and the prognostic significance of these trends.

PUB358

Effect of Shifting from Epoetin α to M-PEG Epoetin β on Hb Levels: A Single Dialysis Center Experience Massimo Torreggiani, Fabrizio Grosjean, Giovanni Montagna, Luca Semeraro, Loredana Picardi, Emanuela Efficace, Vittoria Esposito, Valter Piazza, Gabriella Adamo, Giuseppe Villa, Davide Catucci, Edoardo La Porta, Ciro Esposito. *Nephrology, Fondazione IRCCS S. Maugeri, Pavia, Italy.*

Background: Hemoglobin (Hb) target in hemodialysis patients is still not clear. Often, despite ESA therapy, oscillations of Hb levels are wide. In this study we aimed to evaluate the efficacy of a short-acting erythropoietin (EPO) and a continuous erythropoietin receptor activator (CERA) in a dialysis patient population treated consecutively with the two ESAs for one year.

Methods: We conducted a retrospective study on 10 prevalent patients in a single dialysis center. Inclusion criteria: age>18 years, on hemodialysis from at least two years, treatment with the same ESA for one year. Exclusion criteria: neoplasms, blood transfusions and major infectious episodes during the observation period.

Results: All 10 patients were treated with EPOα for the entire 2010 and then with CERA for 2011. The mean age of patients was 71.4±12.1 years (♂ 80%), 80% on bicarbonate dialysis and 20% on AFB. The average dose of ESA was 8035±4213 UI/week for EPOα and 136.2±72.8 μg/month for CERA. Mean Hb levels were higher during EPOα treatment (11.6±0.9 vs. 10.6±0.8 g/dl, p<0.01) than CERA. Percentage of Hb values≥12.5 or ≤10.5 g/dl was similar between the two treatments.

However, EPOα treatment was associated with a greater number of episodes of Hb≥12.5 g/dl (p<0.01), and a fewer number of episodes of Hb≤10.5 g/dl (p<0.01) than CERA. Iron asset, inflammatory state and dialysis dose were similar during the two treatments. EPOα use was associated with a saving of 84 €/month/patient compared to CERA (p<0.05).

Conclusions: CERA and EPOα have a similar efficacy in maintaining stable Hb values. Hb values were lower during CERA treatment but still in the range suggested by the FDA and the TREAT study. However, CERA treatment was more expensive.

Funding: Government Support - Non-U.S.

PUB359

Both Darbepoetin α and Epoetin β Potentiate Equivalent Effect on Maintaining Hemoglobin Level after Rapid Correction with Blood Transfusion in Hemodialysis Patients Kenji Harada, Hidetoshi Kanai. *Division of Nephrology, Kokura Memorial Hospital, Kitakyusyu, Fukuoka, Japan.*

Background: Majority hemodialysis (HD) patients are administered erythropoiesis stimulating agent (ESA). They often need to be transfused of packed red blood cell (PRBC), on worsening of anemia control, such as bleeding (intestinal bleeding, surgical operation), inflammation, malnutrition. In general initial rise of hemoglobin (Hb) levels tends to higher for epoetin β than darbepoetin α. There are few reports to evaluate the rate of change of Hb levels after transfusion of PRBC and to compare the of ESAs on recovery from anemia.

Methods: We investigated the rate of change of Hb levels in HD patients after transfusion of PRBC(n=54, age 72 year old, male/female=40/16, HD duration 5.9±25years, average transfusion of PRBC 4.05U(1U=140ml), darbepoetin α1.027μg/kg/week; 160 USD, epoetin β149 IU/kg/week; 96 USD). We compared Hb levels before and 4 weeks after transfusion of PRBC, and economical performance of ESAs.

Results: Fifty-four HD patients were subjected. 35 patients were administered of darbepoetin α, 19 patients were administered of epoetin β. Before transfusion of PRBC, in darbepoetin α group average of Hb levels was 7.51(4.4-9.9), meanwhile in epoetin β group was 7.28(5.8-8.5). After four weeks later, darbepoetin α group average of Hb levels was 9.73(7.6-11.3), meanwhile in epoetin β group was 9.53(5.8-8.5). Average rate of change

of Hb levels in darbepoetin α and in epoetin β were 1.92 and 2.38 g/dl, respectively. There was no significant difference (p=0.401).

Conclusions: In short terms, darbepoetinαcan be equivalent to epoetin β for recovery from anemia. Administration of darbepoetin αafter transfusion of PRBC can be effective on correction of anemia, however, epoetin α is 39.9% inexpensive of darbepoetin α to achieve simillary effect. It is necessary to follow longer term the change of Hb levels.

PUB360

Birthweight and Cortisol Levels in Dialysed Patients

Katarzyna Szamotulska,[1] Stanislaw Niemczyk,[2] Joanna Matuszkiewicz-Rowinska,[3] Dorota Szostak-wegierek,[4] Kinga Giers,[2] Katarzyna Romejko-ciepielewska.[2] *[1]Dept. of Epidemiology, National Research Institute of Mother and Child, Warsaw, Poland; [2]Dept. of Nephrology, Military Institute of Medicine, Warsaw, Poland; [3]Dept. of Nephrology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland; [4]Dept. of Preventive Medicine and Hygiene, Medical University of Warsaw, Warsaw, Poland.*

Background: Increase of cortisol levels in chronic kidney disease (CKD) patients is well established. There is also a growing body of evidence that fetal growth restriction is a risk factor for impaired renal function, and that adults born as growth restricted experience elevated cortisol levels.

The aim of the study was to explore cortisol levels in CKD patients in the context of their birthweight.

Methods: An interview regarding early life experience and family history was conducted with 26 hemodialysed (HD) patients and 43 controls with available fasting blood samples. Nineteen HD patients (56 +/- 12 years old, 12 men) and 27 controls (59+/-10 years old, 11 men), were able to give information on birthweight.

Birthweight was expressed in ranks, from <1 kg (rank 1) to >5 kg (rank 11). Three preterm births were excluded. Exact Mann-Whitney test, exact test for Spearman correlation coefficient and multivariate linear regression were applied.

Results: A validity of information on birthweight (BW) was confirmed by the expected associations of mean BW ranks with gender (F vs. M: 6.77 and 7.14), parity (0 vs. 1+: 6.75 and 7.07) and maternal smoking during pregnancy (yes vs.no: 6.50 and 7.00).

Average cortisol levels (mean+/-SD) amounted to 19.6+/-4.5 µg/dL in the HD group and 16.0+/-5.5 µg/dL in controls (p=0.025) and average rank of BW (mean+/-SD) was 7.11+/-1.13 and 6.84+/-0.99 (p=NS), respectively. There was a negative correlation between cortisol concentration and birth weight in the HD group (r=-0.388, p=NS) and in the control group (r=-0.381, p=NS). Multivariate model adjusted for gender revealed significant association of cortisol concentration with chronic kidney disease (beta=3.7, p=0.023) and lower birthweight (beta=-1.6, p=0.031).

Conclusions: Lower birthweight is an independent risk factor for increased cortisol level in dialysed patients.

PUB361

Fluid Overload and Inflammation in Chronic Hemodialysis

Joselyn Reyes,[1] Jochen G. Raimann,[1] Len A. Usvyat,[1] Stephan Thijssen,[1] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Nephrology Research, Renal Research Intitute, New York, NY; [2]Nephrology, Beth Israel Medical Center, New York, NY.*

Background: Chronic inflammation and fluid overload are key issues in hemodialysis (HD). Previously, an association between fluid overload and inflammation has been suggested, possibly related to bowel edema and endotoxin translocation. We aimed to investigate the relation between fluid overload and inflammation in a large cohort of chronic HD patients from the US.

Methods: In this retrospective cross-sectional cohort study we included all patients receiving HD between 1/2001 and 3/2012 in facilities of the Renal Research Institute who did not achieve prescribed target weight. The difference-to-target weight (DTTW; computed as actually achieved post-HD weight minus prescribed target weight) and the neutrophil-to-lymphocyte ratio (NLR) were employed as surrogate markers of fluid overload and inflammation, respectively.

Results: We studied 3565 HD patients (age 61±15 years, 56% male, 51 % diabetic, 50 % Blacks, 22 % with catheter as primary vascular access). In univariable regression analysis DTTW and NLR were positively correlated (R2 = 0.001; P=0.03). This association was corroborated in multivariable analysis with adjustment for age, race, sodium gradient, and vascular access (adjusted R^2=0.06; P=0.03).

Conclusions: It is clinically reasonable that fluid overload is more prevalent in patients who fail to achieve target weight. This reasoning provides the rational for employing DTTW as an indicator of fluid overload in an epidemiologic study. Applying this metric we show that fluid overload is associated with inflammation for reasons yet to be determined. Interventional studies are required to further explore if achieving normal fluid status results in reduced inflammation.

PUB362

Pleural Effusion in Haemodialysis Patients: Diagnostic Investigations and Outcome

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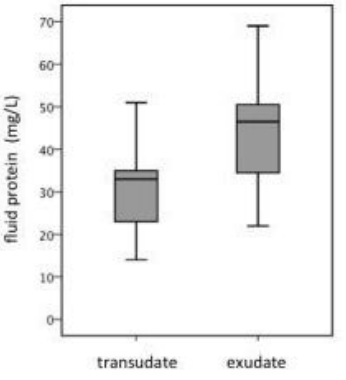
Background: Pleural effusion is common in dialysis patients – although usually transudative, exudative effusions are also frequent. Dialysis treatment often alters the clinical presentation, and the optimum diagnostic investigations are unknown.

Methods: In this retrospective study, all haemodialysis patients undergoing diagnostic aspiration during an 18 month period were identified from electronic records. Diagnostic data were collected and examined in the light of the final clinical diagnosis.

Results: Twenty-two pleural aspirations were identified in 19 patients (aged 30–83, mean 64.6) of which 9 were ultimately diagnosed as transudates. Amongst the remaining 13 cases there were 4 empyemas, and other effusions were parapneumonic (5) or due to malignancy (1), eosinophlia(1), tuberculosis (1) and blunt trauma (1).

Individual components of Light's criteria were poor indicators of effusion type, and in combination had sensitivity and specificity for non-transudative effusion of 92% and 44% respectively. Compared to transudates, exudates did have higher fluid protein levels (44.0 vs 30.2mg/L, p=0.018) and in ROC analysis, fluid protein was reasonably useful as a discriminator, with area under curve 0.782 (p=0.030).



CT imaging was available in all but 2 cases, and proved the most useful test, providing diagnostic accuracy beyond effusion type (for which it was 83% sensitive and 88% specific), but no single test was fully reliable and clinical judgement remained essential. Over a mean follow-up period of 271 days, median survival was 249 days, with no significant difference between effusion types (p=0.196).

Conclusions: Pleural fluid biochemistry is of limited diagnostic value in dialysis patients, in whom CT imaging provides the most useful information. In this group of patients mortality is high, and clinical judgement remains paramount.

Funding: Clinical Revenue Support

PUB363

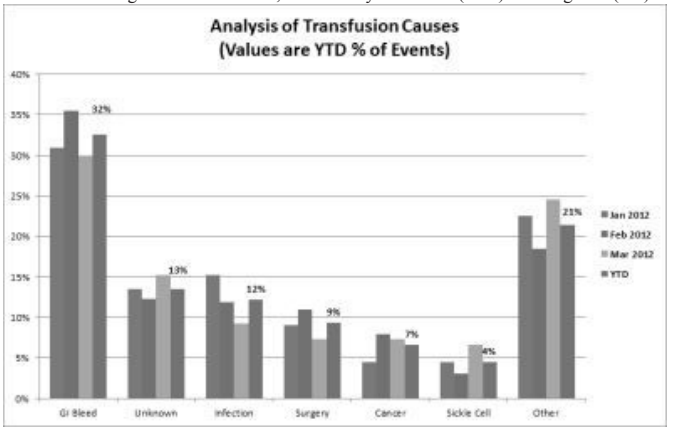
Why Patients Are Transfused: Causes and Hemoglobin Values

Kirk Finchem,[1] Patricia McCarley.[2] *[1]Clinical Operations, Renal Advantage, Inc, Franklin, TN; [2]Clinical Services, Renal Advantage, Inc, Franklin, TN.*

Background: In 2011 anemia protocols and practices were affected by an FDA ruling recommending new lower target hemoglobin (Hb) ranges, reimbursement changes that included ESAs as part of a bundled payment, and Quality Incentive Program (QIP) measures based on selected Hb parameters. The USRDS has reported increases in the monthly transfusion rates beginning in July 2011.

Methods: The study analyzed self-reported transfusion data from a system of 155 dialysis centers in the US over a 3-month period. Transfusion rates (events per 1,000 patient-months) were calculated for patients, grouped by monthly hemoglobin value. Diagnoses related to the reason for subject transfusions are also reported by monthly hemoglobin group, and on a consolidated basis.

Results: Gastrointestinal bleeding was the leading self-reported cause for transfusions, related to nearly one third of transfusions. "Unknown" causes were related to slightly more than one eighth of transfusions, followed by infections (12%) and surgeries (9%).



Transfusion events occurred at the rate of 14.8 per 1,000 patient-months during the three month study, which compared to a rate of 35 per 1,000 patient-month reported by USRDS for the period January thru September of 2011. Transfused patients experienced an average of 1.23 transfusion events during the present study.

Transfusions were more commonly associated with acute clinical factors (e., GI bleeding) than chronically low Hb values.

Transfused and non-transfused groups are compared. Hb levels are reported.

Conclusions: Transfusion events are frequently markers of important clinical changes in patient status. Center-level transfusion-awareness processes, relying on cues from patients and follow-up with hospitals are inadequate to provide actionable data. Clinicians must develop robust processes to identify and categorize transfusuion events.

PUB364

The Effect of Change in Anemia Control Range on System-Wide Hemoglobin Values Kirk Finchem,[1] Patricia McCarley.[2] *[1]Clinical Operations, Renal Advantage, Inc, Franklin, TN; [2]Clinical Services, Renal Advantage, Inc, Franklin, TN.*

Background: With the change in product warnings for erythropoetin stimulating agents (ESAs) (July 2011), some dialysis providers changed target ranges for anemia management.

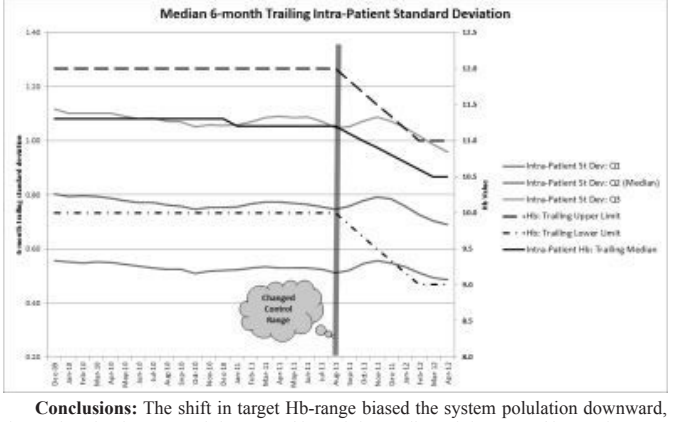
The retrospective study examined the impact on patient hemoglobin (Hb) of shifting target control range from (10 to 12 g/dL) to (9 to 11 g/dL). The study also considered the effect of the shift on the effectiveness of anemia management--i.e., the variability of intra-patient Hb values from month to month--as measure by intra-patient standard deviation of hemoglobin values on a 6 month trailing basis.

Methods: Target Hb range was shifted at all centers (as a change in clinical practice, rather than as a clinical trial) during August 2011.

Study patients (n=9,801 mean monthly patients) received dialysis treatment during the period May 2010 thru April 2012, within a system of 155 dialysis centers in the US, and had been on dialysis for more than 90 days. Further, only patients receiving ESAs during at least 2 of any 3 month period were included.

The fraction of patients with Hb<9 was calculated from the last monthly Hb for each patient. As a measure of control effectiveness, intra-patient Hb standard deviation was calculated for each patient on a trailing 6-month basis; the median value of the intra-patient standard deviation was determined.

Results: The target-range shift increased the faction of ESA patients with monthly hemoglobin values less than 9 g/dL from a mean value of 3.2% prior to the shift, to a mean value of 5.6% following the shift. The shift was associated with less intra-patient variability, as measured by the median intra-patient standard deviation.



Conclusions: The shift in target Hb-range biased the system polulation downward, decreasing the the mean Hb by 0.53 g/dL and the median Hb by 0.60 g/dL. At the same time, control effectiveness increased.

PUB365

Management and Outcome of Patients Receiving Chronic Outpatient Hemodialysis Following LVAD Implantation Judy L. Jang,[1] Anitha Vijayan,[1] Daniel W. Coyne,[1] Susan Joseph,[2] Scott C. Silvestry,[3] Sunil M. Prasad,[3] Tingting Li.[1] *[1]Renal Division, Washington University; [2]Cardiovascular Division, Washington University; [3]Division of Cardiothoracic Surgery, Washington University, St. Louis, MO.*

Background: Renal dysfunction occurs frequently in pts with heart failure (HF). In end-stage HF pts, mechanical circulatory support with LV assist devices (LVADs) is increasingly used as destination therapy or a bridge to heart transplant. No published information describes the management or outcomes of pts receiving chronic outpatient HD following LVAD implantation.

Methods: We conducted a retrospective single-center analysis of all pts who received chronic outpatient HD following LVAD placement. All pts were implanted with HeartMate II continuous-flow devices.

Results: A multidisciplinary team including nephrology, cardiology, and CT surgery was organized to coordinate pt care. Pt, family, and dialysis staff training of the LVAD was implemented prior to HD initiation. Six pts were identified between 2008-2012. 4/6 pts were male, 5 AA, 1 white, with ages between 25-59. 4/6 had CKD prior to initiation of HD, 1 had AKI, and 1 had ESRD which preceded HF. 5/6 received LVADs as a bridge to transplant, but only 1 survived to receive heart/kidney transplant. The pt with AKI recovered renal function. The remainder died on chronic HD at 2, 3, 4 mos, and at 2 yrs. The causes of death were infections(2), cardiac complications(1), and GI bleed(1). During HD, hemodynamic stability was achieved by maintaining LVAD flow rates at 4-6 L/min and MAP between 70-90 mmHg when obtainable, as systolic BPs were not always reliable. A change in symptoms was the most accurate indicator of clinical instability. Dry weight was established after careful assessment of hemodynamics, LVAD flow rates, and pulsatility index. Heparin was utilized without adverse events except in 1 pt with recurrent GI bleeding. All pts had tunneled HD catheters with blood flows of 350-450 ml/min. ESA was administered per standard protocol.

Conclusions: With increasing LVAD implantation and high rates of renal disease in HF, dialysis centers need to be prepared to provide optimal care of LVAD pts. Our description will help guide management of these pts.

PUB366

The Relationship of Cystatin C with Cardiovascular Risk Factors and Inflammatory Marker in Hemodialysis Patients Kyung Mi Park,[1] Hyung-Jong Kim,[1] Dong Ho Yang,[1] Il Park.[2] *[1]Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Republic of Korea; [2]Internal Medicine, South Korea Army Medical Center, Goyang, Republic of Korea.*

Background: Cystatin C is a predictive factor for cardiovascular disease(CVD). In a study for non-dialysis patients, pulse wave velocity(PWV) is related with cystatin C, but there have been limited data for hemodialysis(HD) patients. So, the purpose of this study was to evaluate the relationship among serum cystatin C, inflammatory marker and cardiovascular risk factors in the HD patients.

Methods: We performed a retrospective cross sectional study, enrolling 45 HD patients. We measured CRP, TNF-a, IL-6, insulin and glucose before HD, as well as pre- and post-HD cystatin C at baseline and after 1 month. The PWV was accessed within 1 month. Through the assessment of these laboratory data and the review of medical records, we calculated the Framingham risk score and HOMA-IR.

Results: The mean age was 55 years and 51% were male. The average of cystatin C before HD was 6.57mg/L, and we categorized three groups by Low(<6.49mg/L), intermediate and high(≥7.41mg/L). In these groups, there is no difference in age, BMI, lipid profile, IL-6 (2.87pg/ml, 4.40pg/ml, 4.85pg/ml), TNF-a (4.02pg/ml, 2.06pg/ml, 5.66pg/ml), CRP (0.36mg/dl, 0.09mg/dl, 0.24mg/dl), Framingham risk score(10.22%, 10.83%, 10.78%) and HOMA-IR(2.52, 2.49, 1.92). Also, in the relationship between serum cystatin C and cardiovascular risk factor, it was not associated with PWV, IL-6, TNF-a, CRP, Framingham risk score and HOMA-IR. However, there was a significant correlation between pre- and post-HD cystatin C(0.319, p=0.014) and this value had a strong correlation after the correction of difference in the dialysis membrane(0.596, p<0.001). It assumed that the dialysis membrane affected the concentration of cystatin C.

Conclusions: The serum cystatin C was not associated with cardiovascular risk factors in HD patients. We assumed that the serum cystatin C, with medium molecular weight, was dialyzed by HD, so there was a gap between in vivo production and the remaining amount. Prospective studies involving a longer period concerning the relationship between cystatin C and CVD are needed.

PUB367

Upregulation of Fibroblast Growth Factor and Vascular Endothelial Growth Factor in End Stage Renal Disease Patients May Be Related to Cardiovascular Events Kristiyana Kaneva,[1] Vinod K. Bansal,[2] Debra Hoppensteadt,[1] Joesphine Cunanan,[1] Jawed Fareed.[1] *[1]Pathology, Loyola University Medical Center, Maywood, IL; [2]Nephrology, Loyola University Medical Center, Maywood, IL.*

Background: Fibroblast Growth Factors (FGF) represent families of heparin binding growth factors and Vascular Endothelial Growth Factor (VEGF) are a potent mediator of angiogenesis and vasculogenesis in various diseases. We hypothesized that both of these growth factors may be upregulated in End Stage Renal Disease (ESRD) and may contribute to the cardiovascular events.

Methods: This study included 119 ESRD patients undergoing maintenance hemodialysis after appropriate IRB approval and patient consent. Citrated blood samples were collected prior to and immediately after the dialysis session. The blood samples were centrifuged for 15 minutes at 3000 g at 4°C and platelet poor plasma (PPP) was extracted. Citrated plasma was frozen at -70°C. Samples collected from ESRD patients were analyzed for the circulating levels of FGF-23 by using a sandwich ELISA kit (Millipore, St. Charles, Missouri). The VEGF analysis was carried out using Quantikine sandwich ELISA method (R&D Systems, Minneapolis, Minnesota). Plasma samples collected from normal male and female (n = 80) comprised the normal group.

Results: The FGF-23 levels ranged from 0 to 5934 pg/ml (Mean = 1861 pg/ml with S.E.M = 151) in contrast to the normal levels of 18.4 ± 6.1 pg/ml. Of the 119 patients, 67 (56.3%) had greater than 1000 pg/ml FGF-23 levels. The VEGF levels ranged broadly from 8.2 to 3673 pg/ml (Mean = 141 pg/ml with S.E.M = 50.1) in contrast to the normal levels of 8.7 ± 4.2 pg/ml. Of the 119 patients, 16 (13.4%) had VEGF levels greater than 100 pg/ml of which 4 (3.36%) had levels greater than 1000 pg/ml. No correlation was observed between the VEGF and FGF23 levels.

Conclusions: Measurement of FGF-23 and VEGF provides an additional mean to risk stratify the ESRD patients who may develop cardiovascular and cerebrovascular events. Furthermore, newer targets to modulate these growth factors may be useful in the overall management of these patients.

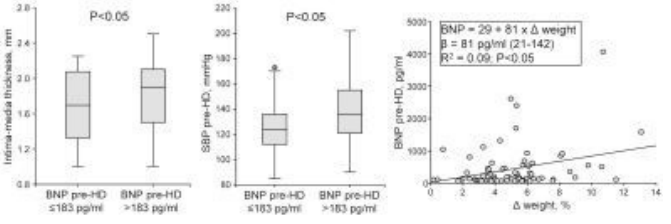
PUB368

Dry Weight, Systolic Blood Pressure and Intima-Media Thickness Predict Brain Natriuretic Peptide Levels in Hemodialysis Patients Paolo Lentini,[1] Luca Zanoli,[1] Valentina Pellanda,[1] Claudio Ronco,[2] Andrea Contestabile,[1] Graziella Berlingò,[1] Anna Basso,[1] Massimo de Cal,[2] Antonio Granata,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, St Bassiano Hospital, Bassano del Grappa, Italy; [2]Nephrology, St Bortolo Hospital, Vicenza, Italy.*

Background: B-type natriuretic peptides (BNP) is an emerging marker of cardiac distress in patients with volume overload. High BNP levels in hemodialysis (HD) patients are correlated with development of pathological cardiovascular findings, including left ventricular hypertrophy, hypertension and increased end-diastolic volume, and with increased cardiovascular and overall mortality. Intima-media thickness (IMT) is a marker of vascular dysfunction well correlated with the cardiovascular risk. We supposed that BNP levels are dependent not only to the interdialytic weight variation (Δ) but also to the vascular dysfunction. Aim: To determine the factors associated with high BNP in chronic HD patients.

Methods: 74 subjects were enrolled. Hematological and biochemical variables were obtained by a pre-HD blood draw. We defined Pre-HD BNP>183 pg/mL as high BNP (were 183 is the median of BNP in our population). Δ weight was defined as [(pre-HD weight - dry weight)/ dry weight]. In all patients IMT of the common carotid artery was measured. Student t-test and chi-square test were used in this analysis.

Results: A total of 74 chronic HD patients were enrolled. The age was 63±13 years, males 54%, BNP 183 pg/mL (range IQ 91-499 pg/mL), IMT 1.76±0.39 mm. Δ weight (1%, OR 1.28, 95%CI 1.01-1.63;P<0.05), Systolic blood pressure (SBP) pre-HD (10mmHg, OR 1.28, 95%CI 1.01-1.61;P<0.05) and IMT (0.1mm, OR 1.22, 95%CI 1.05-1.43;P<0.01) were associated with high BNP pre-HD (Model AUC=0.73, P<0.001).



Conclusions: In chronic HD patients, BNP is influenced not only by the interdialytic weight variation, but also by the vascular function.

PUB369

Validity of the Charlson Comorbidity Index as a Predictor of Mortality in Australian Hemodialysis Patients Asanga Abeyaratne, Kelum Priyadarshana Gunaneththige Don, Kym M. Bannister. *Department of Nephrology, Royal Adelaide Hospital, Adelaide, South Australia, Australia.*

Background: Comorbidity is a significant predictor and confounder needing adjustment, for hemodialysis related mortality. The Charlson comorbidity index(CCI) is used to adjust for comorbidity in general medical patients and has been validated in small cohorts of ESRD patients outside of Australia. This study aimed to assess the CCI as a predictor in our hemodialysis population.

Methods: Demographic and comorbidity data was retrospectively collected on all hemodialysis patients (N=329, male 59%, indigenous 9.7%,dialysis through AV fistula 76.5%) from 12 satellite dialysis centers across South Australia from January 2008 to January 2009. A composite comorbidity index was calculated based on the weights proposed by Charlson. Outcome measure was three year survival.

Results: 140 died during the observation period. Diabetes(41.9%), congestive heart failure(27.6%), and myocardial infarction(14%) were the most prevalent comorbidities. Two cox regression models were evaluated and comprised of individual comorbidities and CCI respectively. Age and albumin were included in both models. Race, gender and vascular access were not significant predictors in multivariate analysis. In the first model age (Hazards ratio [HR] 1.02; 95% confidence interval [CI] 1.01-1.04 p<0.001), serum albumin (HR 0.94; 95% CI 0.92-0.98 p=0.002), diabetes (HR 1.42; 95% CI 1.01-2.03 p=0.04) and congestive heart failure (HR 1.46; 95% CI 1.1-2.11) were independent predictors. In the second model, CCI was the strongest predictor (HR 1.16; 95% CI 1.09-1.24 p<0.001) when adjusted for age and albumin, and had a c statistic of 0.65 and was slightly higher than the first model (c statistic 0.64). The CCI remained a strong predictor for two and one year mortality (HR 1.12 and HR 1.14 p<0.01).

Conclusions: The CCI is a simple linear integer scale easy to calculate and has strong predictive value for mortality in our study population. We recommend its inclusion in data collection models for prognostication.

PUB370

Body Size and Relationship to Outcomes in Asian Pacific Dialysis Patients Michael Etter,[1] Hugh Feidhlim Woods,[1] Len A. Usvyat,[3] Frank van der Sande,[2] Jeroen Kooman,[2] Nathan W. Levin,[1] Peter Kotanko.[1] *[1]Fresenius Asia Pacific, Hong Kong, Hong Kong; [2]Maastricht University Hospital, Maastricht, Netherlands; [3]Renal Research Institute, NY, NY.*

Background: In chronic hemodialysis (HD) patients, larger body size is associated with better outcomes. We aim to understand whether this observation extends to HD patients treated in South-east Asia region.

Methods: We studied incident HD patients treated in the Fresenius Medical Care Asia Pacific region who commenced tx bn 6/1/2002 and 9/30/2011. Only patients who survived >183 days on HD were included. Body mass index (BMI) based on the mean post-dialysis weight in the first 183 days was computed. Patient survival was observed in days 184 to 730 after HD initiation. Patients were stratified into tertiles of BMI and country.

Results: We studied 2854 patients (Australia, 144; South Korea, 185; Taiwan 2525). Patients in Australia start with the highest BMI values (25.3), followed by Taiwan (22.5), with South Korean patients having the lowest BMI of 22.2. Table 1 summarizes ranges of BMI in each tertile in each country.

Table 1. BMI (range; kg/m2) by country

	Tertile 1	Tertile 2	Tertile 3
Australia	14 to 23	23 to 26	26 to 38
Taiwan	16 to 21	21 to 23	23 to 36
South Korea	14 to 21	21 to 24	24 to 40

BMI did not associate with survival in South Korea. In Australia and Taiwan, significant differences were observed bn tertiles 1 and 3, with a longer median survival in tertile 3. Survival advantage was more pronounced in Australia than Taiwan (Australia 48 days; Taiwan 9 days).

	Australia	Korea	Taiwan
Tertile 1	651 (607-695)	716 (697-735)	712 (707-718)
Tertile 2	694 (665-722)	716 (697-735)	715 (710-720)
Tertile 3	699 (670-729)	713 (692-733)	721 (717-724)

Conclusions: Patient size is a predictor of patient outcomes in HD patients. Our analysis suggest that larger patients have better survival in Taiwan and Australia with the survival advantage of large size more pronounced in Australia. Larger patients do not experience a similar degree of survival advantage in South Korea (likely due to small N).

PUB371

Serum Sodium and Its Correlates in Asia Pacific Region Michael Etter,[1] Hugh Feidhlim Woods,[1] Len A. Usvyat,[3] Frank van der Sande,[2] Jeroen Kooman,[2] Peter Kotanko.[3] *[1]Fresenius Asia Pacific, Hong Kong, Hong Kong; [2]Maastricht University Hospital, Maastricht, Netherlands; [3]Renal Research Institute, NY, NY.*

Background: Serum sodium (sNa) is important predictor of outcomes in hemodialysis (HD) patients (pts). In US dialysis population, studies have shown that higher sNa is associated with higher albumin, systolic blood pressure, and better outcomes. We aim to explore these relationships in Australia, Taiwan, and South Korea.

Methods: We studied incident HD pts treated in the FMC Asia Pacific clinics who commenced tx bn Jun 1, 2002 and Nov 30, 2011. Only pts who survived the 183 days on dialysis, had >3 observations, and were >18 years old were included. Mean of patient parameters were computed in first 183 days.

Results: We studied 86 patients from Australia, 182 from South Korea, and 2592 from Taiwan. Pts in Australia start with the highest sNa values [mean±stdev] (138.5 mmol/L±2.5), followed by South Korea (137.7 mmol/L±2.5), with Taiwanese pts having the lowest sNa of 137.2 mmol/L±2.9 (p<0.05 between all 3 groups). Using unpaired t-test, male pts were more likely to have higher sNa in Taiwan but not the other 2 counties. Diabetics were more likely have lower sNa in all 3 countries (p<0.01 for Taiwan). Pearson correlation with sNa showed no significant correlation with age. Postweight and IDWG were positively associated with sNa in all 3 countries. Albumin was positively associated with sNa in Taiwan and Korea but not Australia. Contrary to most other studies, preSBP was negatively and significantly associated with sNa in South Korea and Taiwan. There was no significant association with preSBP in Australia (potentially due to small N).

	Age (yrs)	Albumin (g/dL)	PostWeight (kg)	preSBP (mmHg)	IDWG (kg)	Gender (1=Male)	DM Status (1=Diabetic)
Australia	-0.105	-0.006	.217*	0.033	0.01	0.181	-0.129
Korea	0.054	0.115	0.031	-.151*	0.137	-0.02	-0.15
Taiwan	-0.038	.236**	.108**	-.095**	.181**	.054**	-.219**

* p<0.05; ** p<0.01

Conclusions: Our findings suggest that pts from Australia have the highest sNa. Contrary to most studies in Caucasian and Black pts, our data shows that Chinese and Korean pts have a negative correlation between preSBP and sNa.

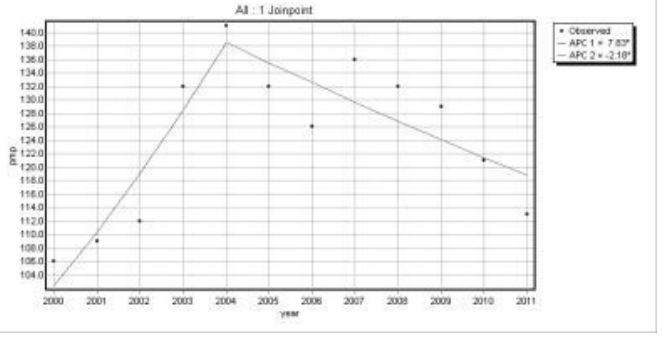
PUB372

Is Chronic Kidney Disease under Control at Last? The Incidence of Renal Replacement Therapy in Croatia Is Decreasing Svjetlana Cala. *Nephrology and Dialysis, Internal Clinic, Clinical Hospital Center Sestre Milosrdnice, Zagreb, Croatia.*

Background: Stabilization in the incidence of renal replacement therapy (RRT) for end-stage renal disease (ESRD) has been observed recently in some European countries, USA, and Japan. An update in Croatia 2000 to 2009 has shown arrest in incidence growth, and slowing the rise in prevalence of RRT. The aim of the present study was to follow on the epidemiological trends in RRT.

Methods: Data from the Croatian Registry of Renal Replacement Therapy (CRRRT) from 2000 to 2011 were analyzed. All patients treated by hemodialysis, peritoneal dialysis and transplantation were covered. Access to RRT was unrestricted. The incidence from day 91 on RRT was adjusted for age and gender of the mid-year population. Time trends were analyzed with Poisson regression and Joinpoint regression methods.

Results: The adjusted incidence of RRT was 106,1 per million population (pmp) in 2000, reached maximum of 140,4 pmp in 2004, and attained 113,3 pmp in 2011.



The year 2004 was identified as breaking point of 2 diverse incidence trends. From 2000 to 2004 incidence was increasing at an annual percentage change (APC) 7,8% (95%CI 2,4; 13,5). From 2004 to 2011 incidence was decreasing by APC -2,2% (95%CI -4,3; -0,0). During the last 5-year period, the decrease was the result of the reduction in the incidence in females: APC -3,2% (95%CI -5,8; -0,5), and stabilization in males: APC -1,5% (95%CI -0,4; 0,9).

Conclusions: After a rapid increase in the incidence of RRT in Croatia from 2000 to 2004, reduction of the incidence succeeded during the 2004-2011 period. The decrease of the incidence, despite unrestricted access to the RRT could be attributed to the prevention and treatment of cardiovascular diseases resulting in decreasing cardiovascular mortality, and simultaneously improving renal survival.

PUB373

RAS Inhibitors Could Modulate the Changes in Left Ventricular Structure after Paricalcitol Therapy in Chronic Hemodialysis Patients Eduardo Baamonde,[1] Elvira Bosch,[1] Carlos Culebras,[2] Bilal El Hayek,[1] German Perez Suarez,[1] Cesar Garcia-canton,[3] Ignacio Ramirez,[1] Maria Dolores Checa.[3] *[1]Nephrology, AVERICUM, Spain; [2]Cardiology, Hospital Universitario Insular de Gran Canaria, Spain; [3]Nephrology, Hospital Universitario Insular de Gran Canaria, Spain.*

Background: Vitamin D may play an important role in reduction of Left Ventricular Hypertrophy (LVH) through modulation of the renin-angiotensin system (RAS). Several studies suggest that treatment with vitamin D may improve cardiac function in hemodialysis patients. Aim: analyze the changes in cardiac structure and function in two group of patients with secondary hyperparathyroidism: treated with and without RAS inhibitors, after 12 months of treatment with paricalcitol.

Methods: 44 chronic hemodialysis patients with secondary hyperparathyroidism (iPTH 300-800 pg/mL) treated with flexible doses of PCT. Baseline, and 12 months after Paricalcitol treatment, echocardiogram was performed and blood pressure, parameters of anemia and bone-mineral metabolism were measured We analyzed the evolution of patients treated with and without RAS inhibitors.

Results: At 12 months, left ventricular mass, left ventricular mass index and left ventricular posterior wall decrease significantly (LVM: 271.2 ± 112.2 vs 243.4 ± 93.1 g, p<0.05 - LVMI: 144.4 ± 50.3 vs 126.6 ± 43.2 g/m2, p=0.026 - LVPW 12.6 ± 2.5 vs 11.89 ± 2.1 mm, p= 0,045) In patients **not treated** with RAS inhibitors, left ventricular diastolic diameter (LVDD: 50.4 ± 4.6 vs 45.6 ± 6.3 mm, p = 0.017), left ventricular systolic diameter (LVSD: 29.3 ± 4.3 vs 24.8 ± 4,1 mm, p = 0.021), LVM (271.6 ± 86.4 vs 212.7 ± 60.9 g, p = 0.000) and LVMI (149.02 ± 36.6 vs 118.48 ± 28.1 g/m^2, p = 0.000) decrease significantly. In patients **treated** with RAS inhibitors only LVSD decrease significantly (p=0.004) We observed significantly decrease in the percentage of patients with LVH (65,9% vs 46,4% p=0.024).

Conclusions: Patients treated with PCT showed an improvement in left ventricular structure. Patients **not treated** with RAS inhibitors showed more evident changes of left ventricular structure, suggesting that the effects of PCT at the cardiovascular level could be less evident when using RAS inhibitors.

Funding: Private Foundation Support

PUB374

Serum Non-High-Density Lipoprotein Cholesterol (Non-HDL-C) Levels and Cardiovascular Mortality in Chronic Hemodialysis Patients Kosaku Nitta. *Department of Medicine, Kidney Center, Tokyo Women's Medical University, Shinjuku-ku, Tokyo, Japan.*

Background: Non-high-density lipoprotein cholesterol (non-HDL-C) has been proposed as a predictor of cardiovascular disease (CVD) in the general population. The aim of this study was to evaluate the utility of non-HDL-C in predicting CV mortality in chronic hemodialysis (HD) patients.

Methods: We calculated the serum non-HDL-C level of 259 HD patients by subtracting their high-density lipoprotein cholesterol (HDL-C) level from their total cholesterol (TC). Cox proportional hazards models were used to estimate the hazards ratio (HR) for CV mortality and the 95% confidence interval (CI). A receiver-operating characteristic (ROC) analysis was performed to estimate the relationship between the sensitivity and specificity of a diagnostic parameter.

Results: There were 44 deaths (17.0%) during the follow-up period, and 33 (12.7%) of the deaths were due to CVD. A multivariate Cox analysis with adjustements for age, diabetes, dialysis vintage, systolic blood pressure, serum albumin and lipid levels showed that non-HDL-C was an independent predictor of CV mortality (HR 1.015, 95% CI 1.004–1.025, P =0.0083). A ROC analysis showed that the plots of the non-HDL-C levels yielded significant specificity and sensitivity for predicting the risk of CVD mortality in HD patients (AUC, 0.62416; *p=0.0366*; cut-off value, 111.0 mg/dL). The Kaplan-Meier survival curves of the HD patients showed significant differences in CV mortality according to their tertiles with respect to serum non-HDL-C levels (*>p=0.0165*).

Conclusions: The results of this study suggest that the serum non-HDL-C level is a significant CV mortality predictor of chronic HD patients.

PUB375

Abdominal Aortic Calcification Score as a Risk Factor for Cardiovascular Disease in Chronic Hemodialysis Patients Myung Jin Choi, Ja-Ryong Koo, Jong-woo Yoon, Jwa-kyung Kim, Sun Ryoung Choi, Min-Gang Kim. *Department of Internal Medicine, Hallym University Medical Center and Hallym Kidney Research Institute, Chuncheon, Kangwon-do, Republic of Korea.*

Background: Vascular calcification (VC) is strongly associated with cardiovascular morbidity and mortality in hemodialysis (HD) patients. Lateral abdominal radiography is a simple and inexpensive tool to detect the presence or severity of VC, compared to the other modalities such as electron bean or multi-slice computed tomography or series of radiographies in pelvis and hands. Using lateral abdominal radiography, we investigate the association between abdominal aortic calcification (AAC) and the development of cardiovascular disease (CVD) in maintenance HD patients.

Methods: Sixty-nine chronic HD patients (52.2 % male; 56.4 ± 1.6 years; median duration of HD 39.2 ± 5.4 months; 60.8 % diabetic) were enrolled. AAC score (range 0-24) were calculated as the sum of calcium deposit score along the anterior and posterior longitudinal walls of the abdominal aorta adjacent to L1-L4 lumbar vertebrae.

Results: The mean AAC score was 3.6 ± 4.1 (range 0-18) and 16 patients (23.3 %) had high AAC score (AAC score ≥ 7). During a mean follow-up period of 92.7 ± 3.2 weeks, there occurred 20 cases (4 fatal, 16 nonfatal) of CVD. Kaplan-Meier survival curve showed that probability of fatal and non-fatal CVD during follow-up period was significantly higher in high AAC score group (Log-Rank test, p=0.029) as compared with low AAC score group (AAC score < 7). In multivariate Cox proportional hazards model, significant independent risk factors for development of CVD were high AAC score (hazard ratio per 1 increase, 1.142; 95% confidence interval (CI), 1.035 to 1.260), high CRP levels (hazard ratio per 1mg/L increase, 1.031; 95% CI, 1.008 to 1.054) and low pre-dialytic diastolic blood pressure (hazard ratio per 1 mmHg increase, 0.927; 95% CI, 0.866-0.992).

Conclusions: AAC score assessed by simple lateral abdominal radiography could be an independent predictor of CVD events in HD patients. Considering easy availability and cost-effectiveness, evaluation of VC by AAC score may be an useful prognostic marker for CVD in chronic HD patients.

PUB376

Serum Uric Acid Is Associated with Arterial Stiffness in Hemodialysis Patients J. Abraham,[1,2] T. Alp Ikizler,[3] G. Wei,[1] R. Filipowicz,[1] Y. Zhang,[1] Nestor E. Almeida,[1] Kalani L. Raphael,[1,2] S. Beddhu.[1,2] *[1]Univ of Utah, Salt Lake City, UT; [2]VA Healthcare System, Salt Lake City, UT; [3]Vanderbilt University, Nashville, TN.*

Background: Serum uric acid (UA) is commonly elevated in hemodialysis (HD) patients but the implications of high UA in HD pts are unclear. Therefore, we examined whether UA is associated with arterial stiffness (a known predictor of cardiovascular events and mortality in HD patients) using the data from the ongoing Protein intake, Cardiovascular disease and Nutrition in CKD stage V (PICNIC) Study.

Methods: Carotid and femoral pulses were used to measure aortic pulse wave velocity (PWV) with a Sphygmacor PVX device. Measurements were conducted at baseline. PWV at baseline was related to baseline serum UA concentration with linear regressions. Adjustments were made for age, gender, race, vascular access, ESRD duration, intra abdominal fat area (IAFA), fasting insulin, hsCRP and study center.

Results: 119 HD patients who had non-missing data at baseline were included in the analysis. The mean age was 52 ±16 years, 59% were men, 82% were Caucasians and 47% had diabetes. Mean UA was 5.5 ± 1.8 mg/dL. The associations of baseline serum UA with baseline PWV are summarized in the table.

Association of uric acid with pulse wave velocity

	Baseline model, beta[95% CI], p-value
Model 1	
Each SD ↑ in Uric acid (mg/dL)	0.84 [0.27, 1.40], p=0.004
Model 2	
Each SD ↑ in Uric acid (mg/dL)	0.77 [0.17,1.38], p=0.013
Model 3	
Each SD ↑ in Uric acid (mg/dL)	0.84 [0.21, 1.47], p=0.009

Model 1: adjusted for age, gender, race, duration of ESRD, vascular access, and study site. Model 2: adjusted for IAFA in addition to model 1. Model 3: adjusted for fasting insulin and hsCRP in addition to model 2.

Conclusions: Serum uric acid is associated with arterial stiffness in hemodialysis patients. Interventions that target UA might decrease arterial stiffness in dialysis patients.

PUB377

All Cause and Cardiovascular Mortality in Dentate and Edentulous Patients in Hemodialysis: A Prospective Multinational Cohort Study
Suetonia Palmer,[1] Marinella Ruospo,[2] Fabio Pellegrini,[3] Jonathan C. Craig,[4] Jorgen B.A. Hegbrant,[2] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Diaverum Medical Scientific Office; [3]Mario Negri Sud Consortium; [4]University of Sydney.*

Background: People who require hemodialysis experience high rates of mortality. Dental disease may be associated with death due to inflammation or as an indicator of general health status. We explored whether total teeth loss predicted 12 month mortality (total and cause-specific) mortality in patients on hemodialysis.

Methods: ORAL-D is an ongoing multinational prospective cohort study of consecutive adults receiving hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Italy, Hungary, Poland, Argentina, Portugal, France and Spain. A dental surgeon evaluated the presence or absence of teeth during a standardized oral examination. We assessed survival at 12 months using centralized mortality data. We conducted analysis using a Cox regression controlling for age, gender, previous cardiovascular events, income status, clinical performance measures, dialysis prescription and performance indicators, and depressive symptoms.

Results: 4720 hemodialysis patients in participating clinics received a complete dental evaluation and completed follow up. Median follow up was 19.9 (17.0 to 28.0) months. 922 patients were edentulous (20%) and 492 (11%) died during follow up. Complete loss of teeth had uncertain associations with risks of all-cause (HR 1.06 [95% CI, 0.86-1.31]) and cardiovascular mortality (HR 0.90 [95% CI 0.66-1.22]) when adjusted for potentially confounding variables.

Conclusions: Dentate status has uncertain associations with all-cause or cardiovascular mortality in patients on hemodialysis. ORALD will be completed by end of 2013.

Other steering group: Stroumza P, Frantzen L, Leal M, Torok M, Benarek A, Dulawa J, Celia E, Gelfman R, Wollheim C, Johnson D, Petruzzi M, De Benedittis M.

PUB378

The Impact of Dialysis Treatment Modality in the Progression of Left Ventricular Hypertrophy in Patients with Chronic Kidney Disease
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Background: Left ventricular hypertrophy(LVH)is highly prevalent in Chronic Kidney Disease(CKD) and has important clinical impact in this population. Patients on hemodialysis(HD) and peritoneal dialysis(PD) have different cardiovascular risk factors which may influence differently the progression of LVH.

Methods: A retrospective study in which we reviewed the medical records of patients followed in chronic HD and DP programs of the Osvaldo Ramos Foundation, which had at least 2 echocardiograms(*E)* registered from Jan 2006 to Jan 2010. We evaluated demographic, clinical and laboratory. The index of left ventricular mass(LVMI) was calculated by dividing LV mass by height. LVH was defined when LVMI was higher than 50g/m^2(men) and 47g/m^2 (women). The progression of LVH was assessed by LVMI difference between the 2nd and 1st *E* corrected by the time interval between the *E*.

Results: At 79 HD and 43 PD were included. HD patients were more hypertensive, received higher doses of erythropoietin, had lower total cholesterol, phosphorus, iPTH and potassium levels. These patients also had higher albumin and ferritin levels. At baseline *E*, HD patients showed higher diastolic(49,13 ± 5,15 x 46,19 ± 5,02 mm; p= 0,003), systolic diameter(32,76 ± 5,43 x30, 14 ± 4,02 mm; p= 0,006) and LV mass(234 ± 84,49 x 204,51 ± 57,98 g; p= 0,04). The progression of LVH[0,94 (0,07; 5,64) in HD vs. 0,97 (0,03; 3,41) in DP patients, g/m^2/months; p= 0,183], as well as the percentage of patients with LVH progression(49,3 vs. 41,8 % HD vs DP respectively; p= 0,307) were similar in both groups. In HD patients, the progression of LVH was positively related with the dose of erythropoietin(r= 0,452; p= 0,004) and negatively with hemoglobin level(r= -0,321; p= 0,046). In PD was positively associated with ionized calcium(r= 0,484; p= 0,042) and negatively with hemoglobin(r= -0,574; p= 0,013). The multivariate analysis, no variable was asociated with the progression of LVH, both groups.

Conclusions: The treatment modality has not influence the progression of LVH in CKD patients on dialysis.

Funding: Government Support - Non-U.S.

PUB379

VAP-1 and Renalase in Regard to Diabetes and Cardiovascular Status in Hemodialyzed Patients
Jolanta Malyszko, Jacek S. Malyszko, Ewa Koc-Zorawska, Edyta Zbroch, Michal Mysliwiec. *Nephrology, Medical University, Bialystok, Poland.*

Background: VAP-1 is a copper-containing SSAO (semi-carbazide sensitive amine oxidase) secreted by vascular smooth muscle cells, adipocytes, and endothelial cells with functional monoamine oxidase activity. Renalase, with possible monoamine oxidase activity, which breaks down catecholamines like SSAO, is also expressed in the endothelium as well as in the kidney

The aim of the study was to assess VAP-1 levels and its correlations renalase in 60 hemodialyzed patients.

Methods: VAP-1 and renalase were assessed with commercially available assays.

Results: The mean VAP-1 was significantly higher in HD when compared to the control group (291.01±94.91 ng/mL vs 158.34±56.89 ng/mL, p<0.01) as well as renalase. Diabetic patients had higher serum VAP-1 than non-diabetic. Patients with blood pressure over 140/90 mm Hg had higher VAP-1 levels when compared to patients with lower blood pressure values. Patients with hemoglobin over 10 g/dL had lower VAP-1 than patients with anemia define as hemoglobin less than 10g/dL. No difference in VAP-1 was found between patients with and without coronary artery disease as well as with and without residual renal function. Males had higher VAP-1 levels when compared to females. Patients treated with beta-blockers or calcium channel blockers had higher VAP-1 than non treated.

In univariate analysis, VAP-1 correlated with presence of diabetes (r=0.27, p<0.05), presence of hypertension (r=0.32, p<0.05), use of calcium channel blockers (r=0.30, p<0.05), use of betablockers (r=0.25, p<0.05), ejection fraction (r=-0.38, p<0.01), systolic blood pressure before (r=0.52, p<0.001) and after hemodialysis (r=0.30, p<0.01), weight gain (r=0.41, p<0.01), MCV (r=-0.29, p<0.05). VAP-1 tended to correlate with fibrinogen (r=0.22, p=0.09) and white blood cell count (r=-0.24, p=0.06).In multiple regression analysis VAP-1 was predicted 77% by serum ejection fraction (beta value -0.66, p=0.000668), and fibrinogen (beta value 0.59, p=0.007).

Conclusions: VAP-1, elevated in patients on renal replacement therapy, is predominantly dependent on blood pressure and diabetes, both factors associated with endothelial damage and promoting cardiovascular complications.

Funding: Government Support - Non-U.S.

PUB380

Better Phosphorus Control May Delay the Need for Heart Valve Replacement in ESRD Patients
Ada Emerenini, Maria Coco. *Renal Division, Montefiore Medical Center, Bronx, NY.*

Background: Chronic Kidney Disease alters metabolism of calcium and phosphate leading to extraosseous calcifications. High phosphorus has been associated with increased mortality in dialysis patients. There is great emphasis on stringent control of mineral metabolism with cinacalcet and phosphate binders, but it is not known whether better control impacts on survival. Reports indicate that as many as 30% of dialysis patients may develop calcified valvular heart disease necessitating valvular replacement surgery. Previous studies report a one year survival approaching 50%.

Methods: Using electronic medical records, we looked at the survival pattern at Montefiore Medical Center during the years 2000 to 2009 of ESRD patients undergoing isolated valvular replacement surgery. We evaluated results pre and post 2005, corresponding to the introduction of cinacalcet and lanthanum in the management of mineral metabolism.

Results: 104 patients underwent isolated valve surgery. They had been on dialysis for 3±1 years. 61% were male; 40% were African American, 32% Hispanic, 18% Caucasian and 10% other. 40% had diabetes, 27% had endocarditis. There were 53 aortic valve replacements (AVR) and 51 mitral valve replacements (MVR). 61% were bioprosthetic.The median survival for AVR was 336 days and 483 days for MVR. There was no difference in survival for sex, mechanical vs bioprosthetic valve, diabetes or dialysis access in the entire cohort. We compared pre-2005 with post-2005 cohorts. There was no difference in survival post valve replacement in pre (763±933 days) vs post (727±600 days) 2005. Phosphorus was significantly lower in the post-2005 cohort (5.9±2.2 v 4.8±1.5 mg/dl, p=0.01, pre vs post 2005). Patients had been on dialysis significantly shorter in the pre-2005 cohort (2±.7 vs 3.7±.5 yrs, p=.0001).

Conclusions: We postulate that mineral metabolism has improved since 2005. The patients undergoing valvular surgery have survived a longer time on dialysis with improved phosphorus control. However, it remains unclear why survival has not improved following heart valve replacement surgery.

Funding: Clinical Revenue Support

PUB381

Carotid Arterial Strain (CAS) Using Speckle Tracking (ST) in Pediatric (ped) Patients (pts) Receiving Maintenance Dialysis (MD)
Poyyapakkam Srivaths,[1] Rajesh Krishnamurthy,[1] Eric Y. Yang,[1] Stuart Goldstein,[2] Gerd Brunner,[1] Vijay Nambi.[1] *[1]Sections of Pediatric Nephrology, Pediatric Radiology, Cardiovascular Research and Nephrology, Baylor College of Medicine, Houston, TX; [2]Center for Acute Care Nephrology, Cincinnati Children's Hospital, Cincinnati, OH.*

Background: Children and young adults receiving MD have very high cardiovascular (CV) mortality. Although arterial stiffness is a potential consequence of end stage renal disease only limited data exist related to the effect of MD on arterial properties in ped pts. Speckle Tracking (ST) tracks the arterial wall, through acoustic fingerprints and gives direct wall information

Aim: We evaluated carotid arterial strain (CAS) using a novel ST approach in ped pts receiving MD and compared them to healthy ped controls (con).

Methods: Bilateral ECG gated ultrasound of the distal common carotid were performed in 3 con and 12 ped pts receiving MD (4 HD, 8PD, vintage 36 ±23 mo). All scans in MD pts performed post dialysis. Peak CAS measured in six equal segments; average of three far wall segments (FWCAS) and global average (GCAS) were used as primary outcome measures based on prior experience in other disease states.

Results: GCAS and FWCAS were significantly lower in pts when compared to controls (see table). There was no correlation of CAS with age or ethinicity in pts or con; there was no difference in CAS between HD and PD, or correlation of CAS with dialysis vintage, Ca, P or PTH in pts.

Table

	Age y *	Ethnicity N	GCAS (%)1 *	FCAS (%)2 *
Cases (n=12)	17 (14.3-18.5)	2 Black, 8 Hispanic, 2 White	5.09 (3.25-5.73)	5.09 (3.06-5.72)
Controls(n=3)	11 (6-14)	2 Hispanic, 1 Asian	7.93 (7.35-9.31)	8.4 (7.85-10.13)

1 p=0.02, 2 p=0.01; * values expressed median (IQR)

Conclusions: CAS measurement with ST is feasible in ped pts. Ped pts receiving maintenance dialysis have lower GCAS and FWCAS implying stiffer arterial wall. More pt and control recruitment is underway to study this novel method in a larger population.

Funding: Private Foundation Support

PUB382

Heart Rate as a Predictor for Adverse Outcomes in Chronic Hemodialysis Patients Arkom Nongnuch, Arisara Khaosabai, Montira Assanatham, Vasant Sumethkul, Prin Vathesatogkit. *Department of Internal Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.*

Background: Elevated heart rate was related to poor cardiovascular outcomes in general population and coronary heart disease (CHD) patients, however information for chronic hemodialysis (HD) patients were limited. This study explored the relationship between heart rate and cardiovascular outcomes and all-cause mortality in chronic hemodialysis patients.

Methods: Data of all chronic hemodialysis patients at Ramathibodi Hospital were retrospectively reviewed from January 2004 to December 2010. Patients with arrhythmia and previous CHD were excluded. Baseline characteristic and heart rate were recorded at the first HD visit of each month for 10 consecutive months and the means were used. These included resting heart rate pre, post HD and the highest value during HD. Primary outcome was combined major adverse cardiovascular events (MACE) and all-cause mortality.

Results: Fifty seven patients were eligible for this study (mean age 53 years, 49% women). Median follow up period was 53 months. Mean dialysis vintage was 66 months. Age, diabetes and hemodialysis vintage were associated with adverse outcomes in univariable models (all $P<0.05$). Average heart rates pre-, post- and during HD were higher in those who developed outcomes compared to who did not (74 vs. 71, 77 vs. 70, and 80 vs. 74 beats per minute (bpm) respectively). In age-adjusted models, every 10 bpm increment in heart rate at pre-, post- and during HD significantly raised the risk of outcome (HR 1.82, 1.76 and 1.51 respectively, all $P<0.05$). In multivariable adjusted model, post hemodialysis heart rate remained an independent predictor for outcome (HR 1.65, 95% confidence interval (CI) 1.03-2.66, P 0.04).

Conclusions: Elevated post hemodialysis heart rate is an independent predictor for adverse outcomes in hemodialysis patient after adjusting for multiple risk factors. Higher heart rate pre- and during hemodialysis also show a trend toward a higher risk in age-adjusted models. This may imply the use of heart rate as a simple and low cost tool for predicting adverse events in chronic hemodialysis patients.

PUB383

Design and Characterization of a Study in Small Dialysis Organizations: STEPPS Steven M. Brunelli,[1] Grace S. Park,[2] Peter J. Neumann,[3] Matthew Gitlin,[2] Brian D. Bradbury,[2] Robert J. Rubin.[4] *[1]Brigham and Women's Hospital, Boston, MA; [2]Amgen, Inc., Thousand Oaks, CA; [3]Tufts Medical Center, Boston, MA; [4]Georgetown University School of Medicine, Washington DC.*

Background: In 2011 the Prospective Payment System (PPS) for ESRD was launched and payment bundling expanded to include independently-reimbursed services. Small dialysis organizations (SDOs) may be particularly susceptible to the financial implications of the PPS. The ongoing prospective observational Study to Evaluate the PPS Impact on SDOs (STEPPS) was designed to describe trends in dialysis treatment and patient outcomes over the PPS implementation period. Herein we report basic study design elements and characterize the STEPPS sample.

Methods: Independents and facilities associated with a chain of ≤50 (n=1020) were contacted to assess interest. 100 expressed interest, 51 were enrolled. A random ~50% of patients within each facility were enrolled. Facility-level data are collected quarterly; patient-level data on demographics, comorbidities, labs, medications and outcomes (death, hospitalization, transfusion, transplantation, withdrawal from dialysis, facility transfer-out) are collected monthly or quarterly.

Results: 1873 hemodialysis and peritoneal dialysis ESRD patients were enrolled during the baseline period (Oct-Dec 2010); total enrollment was 2186 as of May 2012. STEPPS SDOs are representative of the US SDO population circa 2011 although slightly more urban and larger, and over-representative of the Western US. STEPPS patients are generally representative of the US dialysis population but tend to be slightly older, more Hispanic, and with fewer black patients (table).

Mean ± SD; n (%)	STEPPS baseline (N=1873)	US Medicare Dialysis population (%)*
Age (years)	61.2 ± 15.2	58.7
Female	791 (42.2%)	44.7
Race		
White	1021 (54.5%)	55.9%
Black	477 (25.5%)	37.0%
Other/unknown	375 (20.0%)	7.1%
Hispanic ethnicity	551 (29.4%)	16.3%
Dialysis vintage (years)	3.2 ± 3.3	4.1
Primary cause of ESRD		
Diabetes	846 (45.1%)	44.0%
Hypertension	487 (26.0%)	28.4%
Other	451 (24.1%)	23.7%
Unknown	89 (4.8%)	3.9%
Dialysis modality		
Hemodialysis (in center)	1791 (95.6%)	91.9%
Home hemodialysis	11 (0.6%)	1.1%
Peritoneal dialysis	68 (3.6%)	6.9%
Vascular access type		
Arteriovenous fistula	1093 (60.7%)	55.1%
Arteriovenous graft	296 (16.4%)	27.2%
Venous catheter	413 (22.9%)	17.7%

*From USRDS 2011 Annual Data Report (representing 2009 population)

Conclusions: STEPPS captures relevant facility and patient-level data for a representative sample of dialysis patients in US SDOs and will enable timely investigations into the effects of the PPS on patient outcomes in this setting.

Funding: Pharmaceutical Company Support - Amgen, Inc.

PUB384

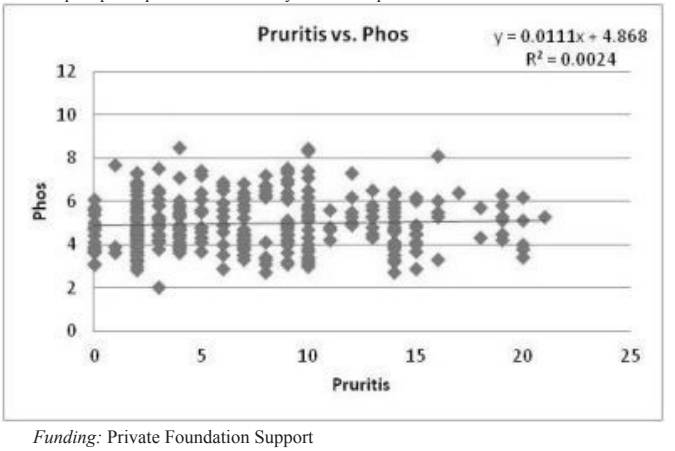
Role of Mineral Disorders in Uremic Pruritis Steven Fishbane,[1] Shayan Shirazian,[2] Mary Schanler,[2] Shubha Shastry.[2] *[1]Division of Nephrology, Hofstra North Shore-LIJ School of Medicine; [2]Division of Nephrology, Winthrop-University Hospital.*

Background: Pruritis is a common problem among hemodialysis patients. There is widespread belief that disorders of phosphate or calcium balance may be an important cause. We sought to explore the relationship.

Methods: This is a post hoc analysis of data from a randomized controlled trial of 3 months of ergocalciferol vs. placebo treatment in 51 hemodialysis patients with uremic pruritis. Ergocalciferol was found not to be superior to placebo. A pruritis survey was administered every two weeks for 3 months. In the current study we have examined changes in pruritis over time, and the relationship between calcium and phosphate to concurrent pruritis scores.

Results: There was significant variation in pruritis survey results, less for calcium (Ca) and phosphate (Phos) for patients during the study, with CV 0.48, 0.03 and 0.15, respectively. For the entire study population we found no correlation between pruritis survey scores and concurrent serum Phos, Ca or Ca/Phos product. Similarly, when subdivided by treatment allocation (ergocalciferol vs. placebo) there were no correlations. When analyzed by quartiles of serum Phos, Ca and Ca/Phos product, there were no significant related differences in pruritis scores. In a sensitivity analysis of weeks when serum phosphate was > 6.5 mg/dL, serum calcium was > 10 mg/dL or Ca/Phos > 65 there was no significant change in concurrent pruritis scores.

Conclusions: We found no relationship between serum calcium, serum phosphate or calcium phosphate product with survey scores for pruritis.



Funding: Private Foundation Support

PUB385

Factors Influencing Regional Differences in the Survival of New Dialysis Patients Satoshi Ogata, Norio Hanafusa, Kunitoshi Iseki, Yoshiharu Tsubakihara. *Committee of the Renal Data Registry, The Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Background: There are regional differences in the survival of new dialysis patients, but few studies have investigated the reasons.

Methods: We investigated 37 clinical factors stratified by gender for patients from 47 prefectures in Japan using the Japanese Society for Dialysis Therapy database (JRDR-09105) of 102,011 patients who commenced dialysis during 2004-06, and 20 institutional factors from data on 3,958 institutions of the 47 prefectures in 2005.

Results: A total of 16 factors were significantly correlated with 1-year survival according to univariate analysis. All of these significant factors were subjected to analysis by the Kaplan-Meier method to compare survival between the upper 23 and lower 23 prefectures (the 24th prefecture from the top/bottom was deleted). As a result, 11 factors (a history of cerebral hemorrhage, protein catabolic rate, creatinine production rate, Kt/V, dialysis time, fluid removal, Ministry of Welfare clinical score at initiation of dialysis, nighttime centers/total dialysis centers ratio, number of full-time dialysis nurses, number of full-time dialysis dieticians (males only), and blood urea nitrogen after dialysis) were significant by the log-rank test.

Conclusions: Various clinical and institutional factors influence the survival of chronic dialysis patients and are associated with regional differences of their outcome.

PUB386

Effects of Intradialysis Resistance Exercise in Patients with Chronic Kidney Disease Elisa M.S. Higa,[1,2] Bruna Lourenco,[1] Marcos A. Nascimento,[1] Thiago S. Rosa,[1] Anderson Sola de Haro,[1] Marco T. Mello,[3] Vicente N. Siqueira,[4] Maria Eugenia F. Canziani.[1] *[1]Medicine, UNIFESP; [2]Emergency Division, UNIFESP; [3]Psychobiology, UNIFESP; [4]Cardiology, UNIFESP.*

Background: The loss of body mass is common in chronic kidney disease (CKD) patients, and it is a predictor of mortality. In Brazil the number of patients utilizing the hemodialysis (HD) in 2010 was 92.091. The decrease the life quality in this population can be attributed for factors as psychological alterations, comorbidities, biological aging, malnutrition, oxidative stress, use of corticosteroids and the HD process itself. Studies show that resistance training (RT) can be efficient on power gain and improvement of life quality in CKD patients analyzed by functional capacity tests. Evaluate the strength values before and after intradialysis RT in CKD patients.

Methods: There were recruited 10 patients in HD clinic Oswaldo Ramos (UNIFESP); we included both male and female patients with age between 20 and 76 yrs and dialysis treatment ≥ 3 months. The average body mass index (BMI) was 25.2± 5.2 characterizing overweight. Previous to RT, the patients were submitted to ergometric test, echocardiography and physical evaluation. Then, it was realized the 1 maximum repetition test (1MR), during the HD, before and after the training, in order to obtain strength values and exercise load estimative for each exercise. The RT was constituted for seven exercises, being: 3 to upper limb and 4 to lower limb, realized on 6 weeks period, 3 times/week during the HD session, with exercise intensity of 40% of 1MR on the first 3 weeks and 60% of 1MR in the other weeks. Each session was composed for 3 series of 12 repetitions with two minutes resting intervals between the exercises. For data analysis (mean ± standard error) it was utilized the t student test with value of $P<0.05$.

Results: On the 1MR tests we found strength increase (kg) after training, on the following exercises: shoulder press 8±0.55 vs 7±0.51; biceps 8±0.47 vs 7±0.61, triceps 5±0.56 vs 4±0.82 and leg extension 10±1.32 vs 7±0.70, $P<0.05$ for all.

Conclusions: Intradialysis resistance exercise promotes power increase in CKD patients which may improve their daily life activities.

PUB388

The Effect of Vitamin D Insufficiency on Uremic Pruritis Shayan Shirazian,[1] Mary Schanler,[1] James Drakakis,[1] Nobuyuki (Bill) Miyawaki,[1] Steven Fishbane.[2] *[1]Division of Nephrology, Winthrop University Hospital, Mineola, NY; [2]Division of Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

Background: Vitamin D insufficiency is highly prevalent in patients on hemodialysis (HD). In addition, uremic pruritis is a common and troubling symptom in HD patients. The objective of this study was determine whether vitamin D insufficiency was more prevalent in patients with uremic pruritis and whether repletion with ergocalceriferol improved itching severity in HD patients with vitamin D insufficiency.

Methods: This is a post hoc analysis of a randomized controlled trial of 3 months of ergocalciferol vs. placebo treatment in 50 HD with uremic pruritis. Ergocalciferol 50,000 IU was given weekly for 12 weeks and was found to be equivalent to placebo for treating uremic pruritis. In the current study we tested vitamin D 25OH levels in a control population of 50 HD patients without uremic itching and compared them to levels in our original study population. We also tested the effect of ergocalciferol supplementation in a subgroup of HD patients with uremic pruritis that were vitamin D insufficient.

Results: Baseline vitamin D 25OH levels were lower in HD patients with uremic pruritis compared to HD patients without uremic pruritis, however this difference did not reach statistical significance (17.4 ng/mL vs. 19.4 ng/mL, p=0.40). 19 out of 25 HD patients from our original study who were randomized to ergocalciferol treatment were vitamin D 25OH insufficient (levels<30ng/mL) at study initiation. In these patients, there was a mean increase in vitamin D 25OH levels of 20.6ng/mL. When compared to placebo, our subgroup had a larger decrease in itching severity scores from the start to end of study, however this did not reach statistical significance (5.6 vs. 4.7, p=0.58).

Conclusions: Ergocalciferol repletion in HD patients with vitamin D insufficiency and uremic pruritis did not lead to a significant improvement in itching when compared to placebo in this small subgroup analysis. Further, larger studies are needed to determine whether ergocalciferol repletion is an effective treatment for uremic pruritis in HD patients with vitamin D insufficiency.

Funding: Private Foundation Support

PUB389

Interactions (Age by Race; Age by Vitamin D Analog) Modify the Relationships between Vitamin D, and Hospitalization in Hemodialysis Patients K. Servilla, O. Myers, E. Bedrick, S. Paine, P. Zager. *Medicine, UNM, Albuquerque, NM.*

Background: The Institute of Medicine has questioned the benefits of vitamin D beyond improving bone health. The present study explores the hypothesis that active vitamin D is associated with decreased hospitalization in hemodialysis (HD) patients.

Methods: We studied black (n=5301), Hispanic (n=633) and non-Hispanic whites (NHW)(n=8727) who began HD between 1/1/99 and 12/31/04 in Dialysis Clinic Inc. facilities. Patients were followed for 3 years or until 12/31/07. Hospitalization was analyzed using Prentice, Williams, Peterson (PWP) gap-time models for recurrent events. Models contained baseline (age, sex, race, ESRD cause, vintage, Ca, PO_4) and time varying (albumin, Hgb, Kt/V, BP, BMI, clinic SMR) covariates. We fitted models with main effects for vitamin D use or type. We added interaction terms to see if relationships with hospitalization were modified by race and age.

Results: PWP (HR1.00, 95%CI 0.98-1.02) and marginal structural models (HR 0.96, 95%CI 0.90-1.01) did not detect an overall association between vitamin D and hospitalization. There was not a significant race x vitamin D interaction (p=0.84). Thus, the relationship of vitamin D to hospitalization was similar across races. There were significant age x vitamin D ($p<0.001$) and age x race ($p<0.01$) interactions. Vitamin D was associated with reduced ($p<0.01$) and increased ($p<0.05$) hospitalization in patients 20 to 39 and 60 to 69, respectively. Among patients over 50, blacks had lower hospitalization than NHW. In PWP models including vitamin D type, paricalcitol (HR 0.94; 95%CI 0.91-0.97) but not calcitriol (HR HR 1.01; 95%CI 0.98-1.04) or doxercalciferol (HR 0.99; 95%CI 0.96-1.02) was associated with an overall decrease in hospitalization. In these models there were significant age x vitamin D (p=0.004) and age x race (p=0.004) interactions. Paricalcitol and doxercalciferol were associated with decreased hospitalization in patients 20 to 39.

Conclusions: Overall, there was no association between vitamin D and hospitalization but results differed across subgroups. It is necessary to test for interactions when assessing relationships of vitamin D to outcomes among HD patients.

PUB390

Endogenous Testosterone Deficiency in Dialysis Patients: Influence of Dialysis Modality Secundino Cigarran,[1] Francisco Coronel,[2] Juan Villa Rincon,[1] Enrique Antonio Florit,[2] Jose A. Herrero,[2] Juan Jesus Carrero.[3] *[1]Nephrology, Hospital Da Costa, Burela, Lugo, Spain; [2]Nephrology, Hospital Clinico Universitario San Carlos, Madrid, Spain; [3]Nephrology, Karolinska Institute, Stockholm, Sweden.*

Background: Testosterone deficiency (TD)is common in men with chronic kidney disease (CKD). Age-related decline in testosterone levels in healthy men is associated with decreased muscle mass, muscle strength and lower extremity strength, decreasing exercise capacity. However, it is unknown if reduced endogenous testosterone associates with dialysis modality and changes in body composition.

Methods: Cross-sectional study of 43 men in dialysis (24 HD & 19 PD) mean age 66.44 ± 13.22 yrs, 41.1% diabetic status.Both groups were compared. Endogenous testosterone was measured by immunolumiscence method (Male normal range 3-11 ng/ml), and anemia, inflammation and nutricional markers as well. Body composition was assessed by bioelectrical impedance (Vectorial in HD and Spectroscopy in PD). Parameters derived from bioelectrical impedance were : lean body and fat mass. Data were processed with software package for windows SPPS 18 (Chicago IL, USA). Variables were processed as appropiate.A p value <0.05 was considered statistically significant.

Results: 17 HD pt (70.4%) and 1 on PD (5.3%) had TD. and was associated significntly with older, higher PCR, anaemia and poor nutritional status by serum markers and BIA, and lower Vit D.

T-Paired test Testosterone levels.

variable	Deficiency (n=18)	Normal (N=25)	P
Age (yr)	71.94±10.9	62.48±13.4	.05
Fat Free Mass (%)	59.64±5.12	57.88±14	.05
Fat mass (%)	33.33±5.3	38.81±12.28	.05
nPNA(gr/kg/d)	1.11±0.48	1.47±0.25	NS
%Hypocromes	8.71±13	3.07± 87	.001
SCr	6.68±2.25	10.1± 4.10	.001
Prealbumin (mg/dl)	29.7±2.25	37.06±10.8	.05
CRP (mg/dl)	1.15±1.7	1.52± .58	.001
Phosphorous (mg/dl)	4.43±1.32	5.0±1.43	.05
25OHD	9.94±4.43	12.96±6.59	.001
iPTH (pg/ml)	272.8±197.5	234.9±141.9	.05

Conclusions: In conclusion, TD in dialysis patients are associated to dialysis modality (HD) and body composition in an independent way. TD is associated to inflammatory, nutritional status associated to age effect, and increased cardiovascular risk. More studies are required to compare TD in both techniques.

Funding: Government Support - Non-U.S.

PUB391

A Single Center UK Experience of Pregnancy in Women Receiving Dialysis
Arvind Ponnusamy, Maharajan Raman, Hayley L. Mcmanus, Daniel J. Hall, Philip A. Kalra, Teresa Kelly, David I. New. *Renal, Salford Royal Hospital, Salford, United Kingdom.*

Background: Patients with end stage renal disease rarely conceive due to anovulatory menstrual cycle.

Methods: We undertook a retrospective review of pregnancy in women on renal replacement therapy from 2007 to 2011. All patients are cared in a multidisciplinary team involving comprising nephrologists, obstetricians, midwives, dietician and dialysis nurses from the antenatal period to delivery and beyond.

Results: There were 4 pregnancies during this period all of which resulted in a live newborn giving an incidence rate of 2 per 1000 dialysis patients/year. They were no reported case unsuccessful pregnancy. However, one of the cases was a twin pregnancy whereby one of twins died. The mean age was 34 years (range 29-42). Three patients were Asian and 1 was Caucasian. All patients were on aspirin and some form of anti-hypertensive medication. All pts were on daily dialysis – or at least 6 times/week with a mean frequency was 5.5 times/week. Our patients tolerated daily dialysis. Average urea levels were less than 17 mmol/L. Mean UF during the dialysis was 1.4 ± 1 litres. Average gestational age at delivery was 32.3 ± 3.3 weeks. The average birth weight was 1827 ± 750 g. Table below shows the laboratory features of these patients during pregnancy.

	1st trimester	2nd trimester	3rd trimester
Hb	104+ 11	99 + 14	93.2 + 19
Uric Acid	0.27 + 0.1	0.31 + 0.1	0.31 + 0.02
Systolic BP	127	122	128

Conclusions: Evidence on pregnancy in dialysis patients is scattered and heterogeneous. Our cohort of patients had good maternal and foetal outcome. This is due to combination of daily dialysis, optimum BP control, dietary input for PO4/fluid gains/essential vitamins(avoiding vitamin A), aspirin as pre eclampsia prophylaxis and careful monitoring foetus.

PUB392

Impact of Late Nephrology Consultation on Mortality in End Stage Renal Disease: A Single Center Experience in Korea
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Background: Late encounter of nephrologists before dialysis initiation on maintenance hemodialysis (HD) or peritoneal dialysis (PD) has been reported to be associated with increased mortality. However, the benefits of timely consultation in patients receiving renal replacement therapy (RRT) including HD, PD, and transplantation are rarely described. We therefore investigated the relationship between timing of nephrology consultation and mortality in those patients. The effect of emergent dialysis including emergent HD, PD, and continuous RRT on mortality was also evaluated.

Methods: Based on end stage renal disease registry (ESRD) databases in a tertiary university hospital from 1995 to 2010, 3499 subjects were identified. Early (EC) and late (LC) consultation were defined by the time of first nephrologists encounter more than or less than 3 month before first ESRD diagnosis, respectively.

Results: Of 3499 subjects (mean age 56.5±15.3 year, male to female ratio 2084:1415), 2237 (63.9%) were qualified for EC group and 1262 (36.1%) were for LC group. The mean baseline creatinine of the subjects was 7.49±3.65 mg/dL and emergent RRT patients were 403 (11.5%). During the periods, 984 patients were reported dead. LR was significantly associated with increased all-cause mortality with a hazard ratio of 1.23 (95% CI 1.05 to 1.44, P=0.011) in cox proportional hazard model adjusted for age at diagnosis, gender, presence of hypertension, diabetes, and baseline glomerular filtration rate. Compared to planned RRT, emergent RRT patients had significantly higher mortality rate adjusted for relevant factors (Hazard ratio 2.39, 95% CI 2.03 to 2.82, P<0.0001).

Conclusions: A comprehensive analysis of RRT subjects demonstrated the significant relationship between late encounter of nephrologists and all-cause mortality. Patients with emergent RRT revealed increased mortality compared to planned RRT. These analytic results suggest that the timely consultation to nephrologists before first RRT is important in determining long term prognosis in ESRD.

PUB393

Association of Pruritus and Quality of Life (QOL) among Patients at a Large Dialysis Provider
T. Christopher Bond, Richard Mutell, Helen M. Wilfehrt, Mahesh Krishnan, Tracy Jack Mayne. *DaVita Clinical Research, Minneapolis, MN.*

Background: Patients on dialysis frequently report pruritus, ie, itchy skin. The Kidney Disease Quality of Life 36 (KDQOL) assesses dialysis-specific, patient-reported health-related QOL for individuals with end-stage renal disease (ESRD) who are undergoing dialysis; it includes the SF-12 physical and mental component scores (PCS and MCS, respectively), subscales for burden of disease, symptoms and problems (including skin itch), and effects on daily life. This survey is administered to all dialysis patients yearly. We hypothesized that patient-reported itch would be associated with other items of the KDQOL.

Methods: We analyzed the association between itchiness score on the KDQOL and component scores and subscales reported on the same test for 71,012 patients who completed the SF-12 portion of the survey (Jan 2009-May 2012). ANOVAs were performed to compare scores on the 4 scales not including the skin itch question by level of itchiness.

Results: Thirty percent of patients reported that they were moderately to extremely bothered by itching; 61% reported any complaint of itching. A significant association was observed between itchiness and both PCS and MCS scores and between itchiness and the burden and effects subscales (not shown). Itchiness was also correlated with other questions in the symptoms subscale.

Table. Association Between Itchiness and KDQOL SF-12 MCS and PCS Scores.

Itchiness score	PCS Score			MCS Score		
	Mean	SD	Median	Mean	SD	Median
Not bothered at all (N=28360)	39.28	10.66	39.22	52.06	10.00	54.59
Somewhat bothered (N=21481)	36.97	10.15	36.39	49.73	10.40	51.60
Moderately bothered (N=10912)	34.91	9.66	33.91	47.68	10.75	48.63
Very much bothered (N=6556)	33.14	9.26	32.28	44.79	11.06	44.42
Extremely bothered (N=3703)	32.18	9.53	30.51	43.42	11.87	42.77
ANOVA p-value	<0.0001			<0.0001		

Conclusions: Self-reported skin itch is highly correlated with validated component and subscale scores on the KDQOL. Skin itch is an independent predictor of other aspects of the quality of life of dialysis patients, suggesting that it is an important condition to address. Acknowledgement: Research funded by Mitsubishi Tanabe Pharma Corporation.
Funding: Pharmaceutical Company Support - Mitsubishi Tanabe Pharma Corporation

PUB394

Risk Factors for Bacteremia in Incident Hemodialysis Patients
Sara Atwater,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] Mufaddal F. Kheda,[1] Puja Chebrolu,[1] Earnest J. Baulkmon,[1] Kristina W. Kintziger,[1] N. Stanley Nahman.[1,2] *[1]Georgia Health Sciences University, Augusta, GA; [2]Charlie Norwood VAMC, Augusta, GA.*

Background: Infection is the second leading cause of mortality in incident hemodialysis (HD) patients. We recently showed that bacteremia (BAC) occurred in ~ 22% of incident HD patients from the USRDS (Cherbrolu, IDSA, submitted). BAC may be access-related or be associated with other co-morbidities. To address this question, we queried the United States Renal Data System (USRDS) for potential access-dependent and access-independent risk factors for BAC in these patients.

Methods: All incident HD cases from the USRDS for calendar years 2005-2008 were queried for a diagnosis of bacteremia and several potential clinical covariates using ICD-9 diagnosis codes submitted for Medicare billing. Descriptive statistics and log-binomial regression analysis were performed. Covariates were stratified by time of occurrence (before BAC, and/or before starting HD) and grouped according to access-related or access-independent factors.

Results: For the 4 year period of study, 362,799 patients were available for analysis. The median age was 65 years. BAC was identified in 79,725 (22%) patients. Among bacteremic patients, vascular access type included AVF in 47,936 (13%), AVG in 14,226 (3.9%) or vascular catheter in 295,688 (82%) patients. When compared to AVF, the relative risk (RR) of catheter access-related BAC was 1.91, 95% confidence interval (CI) 1.86-1.96, and for grafts, RR 1.57, 95% CI 1.51-1.64. Access-independent co-morbidities included other infectious events (candidemia, MRSA colonization and HIV, [RR 3.69, CI 3.55-3.84; RR 3.32, 95% CI 3.26-3.38 and RR 2.12, 95% 2.04-2.21, respectively]), diabetes (RR 2.27, 95% CI 2.24-2.30), and lupus(RR 1.75, 95% CI 1.67-1.83). A decreased risk of BAC was associated with normal and increased BMI, female sex and previous kidney transplant.

Conclusions: BAC occurs in nearly one fourth of incident HD patients. Access type, infectious co-morbidities, diabetes and lupus represent high risk conditions for BAC in incident HD patients and may contribute to mortality in the first year of dialysis.

Funding: Clinical Revenue Support

PUB395

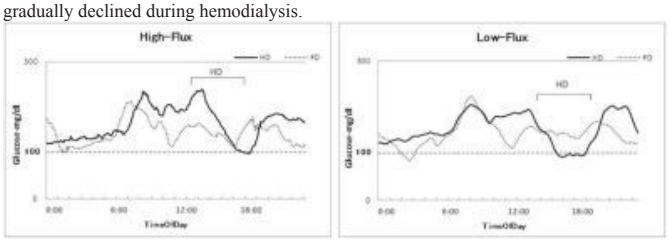
Assessment of Hemodialysis Membranes in Glycemic Control on Diabetic Hemodialysis Patients Using Continuous Glucose Monitoring (CGM)
Jyunichiro Hashiguchi,[1] Satoshi Funakoshi,[1] Yoshiaki Lee,[1] Tomoya Nishino,[2] Yoko Obata,[2] Jyunichiro Hashiguchi,[1] Kenichi Miyazaki,[1] Takashi Harada,[1] Kazunori Utsunomiya,[3] Shigeru Kohno.[2] *[1]Nagasaki Renal Center, Japan; [2]Nagasaki University Graduate School of Medicine, Japan; [3]Jikei University, Japan.*

Background: Although the glycemic control in diabetic hemodialysis patients is considered to be affected by the type of dialyzer used, it is still controversial which membranes to choose. We hereby compared higher and lower flux dialyzers on the glycemic control in diabetic hemodialysis patients monitored by CGM.

Methods: Five relatively well-controlled (HbA1c<7.0) diabetic hemodialysis outpatients were enrolled in this study, then monitored overall 48-hours glycemic control,

on both hemodialysis day (HD) and non- hemodialysis days (free day: FD), by CGM. Polyester-polymer membrane dialyzers with higher insulin-clearance (high-flux) were replaced with EVAL membrane with lower insulin-clearance (low-flux) for a week, and glycemic controls were compared in each patient.

Results: As shown in Figure 1, the average plasma glucose curve in high-flux on HD gradually declined during hemodialysis.



On the other hand, that in low-flux on HD steeply declined followed by relatively hyperglycemic state thereafter. More importantly, the mean amplitude of glycemic excursions (MAGE) in low-flux on HD was significantly higher than in high-flux (64.0±19.5 mg/dL vs 47.9±20.1 mg/dL, p=0.042).

Conclusions: Glycemic variability plays a major role in the development and progression of cardiovascular diseases in diabetic hemodialysis patients. Dialyzers with high performance membrane like polyester-polymer are recommended for glycemic control in relatively well-controlled diabetic hemodialysis patients.

Funding: Private Foundation Support

PUB396

Prevalence of Bacterial Catheter Colonisation Using Different Catheter Lock Strategies in Longterm Hemodialysis Patients Philipp Grosse,[1] Michael Dickenmann,[1] Stefan Erb.[2] *[1]University Hospital Basel, Clinic for Nephrology and Transplantation Immunology, Basel, Switzerland; [2]University Hospital Basel, Division of Infectious Diseases and Hospital Epidemiology, Basel, Switzerland.*

Background: Catheter related bloodstream infections (CRBSI) in longterm hemodialysis patients with permanent venous catheters have been attributed to adverse outcomes in terms of mortality, morbidity and excess costs. To minimize the danger of CRBSI by manipulation of dialysis catheters the needleless luer-lock device TEGO® connector has been FDA-approved in 2006 as an alternative to standard catheter caps (SCC).

Methods: Our prospective, interventional study investigated the prevalence of asymptomatic catheter contamination as a risk factor for CRBSI in three different catheter lock techniques in 39 patients with permanent venous dialysis catheters: (i) TEGO® system with saline locking solution, (ii) SCC with 46.7% citrate locking solution and (iii) SCC with 30% citrate locking solution plus intensive training of dialysis staff in aseptic catheter manipulation technique.

Results: We could demonstrate significantly higher catheter colonisation rates using the TEGO® system with saline locking solution as compared to SCC with 46.7% citrate solution (OR 0.22, p=0.011) or 30% citrate solution (OR 0.07, p=0.001).

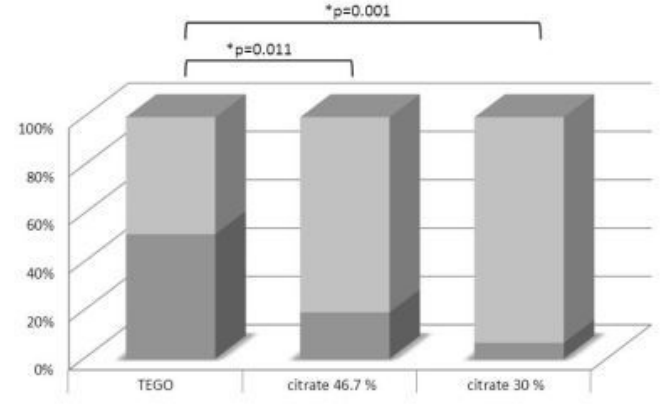


Figure 1: Prevalence of catheter colonisation using different catheter lock strategies. Percentages of sterile and contaminated catheters are indicated. Light green: sterile catheters. Dark green: contaminated catheters. Statistical analysis was performed using the Generalised Estimation Equation GEE. P-values <0.05 are considered statistically significant*.

Conclusions: We recommend cautious use of needlefree connectors for dialysis venous accesses since they might bear the danger of increased rates of CRBSI.

Funding: Clinical Revenue Support

PUB397

QUALITY in Crisis Time: The Value of the Synergies Maria Eva Baro-Salvador, Raul Noguera. *Nephrology, Hospital de Torrevieja/Hospital de Vinalopo, Torrevieja/Elche, Alicante, Spain.*

Background: Though the quality for years is evaluated systematicly in the majority of dialysis units (DU),the quality accreditations in the same ones are long and costly processes that requires time and implication of the whole team.The synergy between two services in protocols, quality, clinical electronic history..can optimize the work,minimize the costs and benefit, later,the improvement of both centers.

Methods: Aim: Take advantage of a common headquarters in the nephrology service of two hospitals from the same company to accredit the welfare quality in both UD.In Valencian Community,DU can realize a quality accreditation with a specific institution for dependent on the Qualit Agency of the government.This is a long process that begins with an autoevaluation from which there is issued a situation-report that will be the base for the preparation of the accreditation visit and his later analysis.All this process it needs a time and dedication from professionals who,in addition, generally are profane to this knowledge area.The same headquarters and identical organization of service improves the performance of the above mentioned process shortening it in time,costs and improving the results of the same one.

Results: In December, 2010 we request to initiate the process of autoevaluation for the qualit accreditation of our DU of Torrevieja's Hospital. In November, 2011 we had the above mentioned accreditation with EXCELLENT qualification and immediately it was requested date evaluation for the UD of the Hospital of the Vinalopó without realizing the previous autoevaluation since the whole documentation, processes, protocols, qualit tools,.. are common. His evaluation is foreseen in June, 2012. The majority of the accreditations are realized with auditors that they were not specifics of the sanitary area.This process needs an implication in whole team, a documentation reaodering, an analysis of the weak points for his modification and, definitively, a new vision of the key processes.

Conclusions: In our experience the effort realized to accredit two related DU, not only optimize efforts but also reduce direct and indirect costs.

PUB398

Comprehensive and Personalized Care of the Hemodialysis Patient in Tassin, France: A Model for the Patient-Centered Medical Home for Subspecialty Patients Evamaria Anvari,[1] Charles Chazot.[2] *[1]Nephrology, University of Arizona, Tucson, AZ; [2]Nephrology, Nephro Care, Tassin-Charcot, Sainte Foy Les Lyon, France.*

Background: End-stage renal disease (ESRD) continues to increase in prevalence and incidence in developed countries. According to the International Study of Health Care Organization and Financing (ISHCOF) the United States has the second highest death rate per year among patients on hemodialysis even though it had the highest annual expenditure. Patient-centered medical homes (PCMH) are being studied as models for health care delivery. Their aim is to have the primary care physician provide all the patient's health care needs and arrange care with other qualified professionals in order to maximize health outcomes.

Methods: ESRD patients may represent the ideal patient population for PCMH for subspecialty patients. The American Society of Nephrology (ASN) appointed a taskforce to assess how nephrologists would interact with PCMH.

Results: The Centre de Rein Artificiel in Tassin, France provides a model for PCMH for patients with chronic renal disease and ESRD. This center is completely dedicated to kidney disease patients. It consists of a hospital, an operating room, a radiology suite, an outpatient clinic, outpatient HD rooms, and a next door Chateau where patients do self-dialysis overnight. Patients are seen by a nephrologist during every dialysis. They have frequent visits to the radiologist, dietitian, social worker, and psychologist if needed. Fistula creations are done by the interventional nephrologist and access dysfunction is also managed by them and their interventional radiologist. Hospitalized patients are also managed by the nephrologist and occasional outside specialist are called for consults. These patients have lower mortality rates and fewer complications.

Conclusions: Patient-centered medical homes based in dialysis centers have the potential to improve the overall quality-of-care, provide a more specific focus on the management of chronic diseases. The Tassin experience in France indicates that this approach is possible and has desirable outcomes.

PUB399

Application of Novel Methicillin-Resistant S. aureus (MRSA) Reduction Practices in Outpatient Dialysis Ioan Cosma,[1] Dottie Lagasse,[3] Lori Travis,[2] Brad M. Schimelman,[1] Mark G. Parker.[1] *[1]Maine Medical Center; [2]Maine Medical Center Research Institute, Portland, ME; [3]Fresenius Medical Care North America, Westbrook, ME.*

Background: MRSA infections are highly prevalent in outpatient hemodialysis units, partly due to high utilization of central vein catheter access, raising healthcare costs and exposing dialysis patients to a 2.5 fold increased risk for all-cause mortality. Systems improvement and positive deviance have been shown to reduce MRSA infections in hospital settings. We studied the use of these techniques in an outpatient dialysis unit.

Methods: Staff from a FMCNA dialysis unit introduced lean methods and social/behavioral change dialogues derived from positive deviance methods to identify effective practice changes for prevention of MRSA transmission. They developed hand hygiene education tools, standardized communication tools, and optimized strategies for information dissemination. Historical infection rates were compared to rates after application of new

strategies. Implementation occurred from July, 2010 through September, 2011, and data collection continued through March, 2012. Outcomes included rates of MRSA bacteremia, all-type MRSA infections, and all-cause bacteremia, and were analyzed with descriptive statistics including t-tests.

Results: The average patient census was 97/month. There was no statistically significant change in Quarterly Average Rates/1000-patient-months of MRSA bacteremia (4.51 vs 3.96, average difference 0.56, 95% CI [-3.45, 4.57], p-value 0.78), all-type of MRSA infections (5.40 vs 7.80, average difference - 2.4, 95% CI [-6.35, 1.55], p-value 0.22), or all-cause bacteremia (12.95 vs 7.45, average difference 5.5, 95% CI [-0.69, 11.68], p-value 0.08).

Conclusions: After implementation of lean and positive deviance interventions in an outpatient dialysis unit, no significant difference was noted in rates of MRSA bacteremia, all-type MRSA infections, or all-cause bacteremia. These results may reflect barriers to effective use of these organizational change strategies in outpatient dialysis, as compared to hospital settings. Study limitations included low baseline event rates and short duration of post-intervention observation.

Funding: Other U.S. Government Support

PUB403

A Randomized Control Trial for Prescribing the Best Dialyzer Choice for Elderly Dialysis Patients

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Background: Several recent reports warned that high efficient hemodialysis with polysulfone (PS) membrane occasionally led to the deterioration of nutritional status in elder patients. Ethylene vinyl alcohol (EVAL) membrane has high biocompatibility and a broad solute removal property. In the prior studies, EVAL membrane improved the deteriolated nutritional status caused by PS membrane. The E-HOPOED-Study Group was established in 2010 to determine the best dialyzer choice for elder dialysis patients.

Methods: The E-HOPED-Study is a randomized control trial registered in UMIN-CTR system (UMIN 000003730) and being performed in 278 dialysis facilities in Japan. The patients who accept the enrollment to the study will be randomly divided into the next two groups; the Group A, treated by EVAL membrane; the Group B, treated by several high flux membrane such as PS. The entry criteria of the patients are the age more than 70 years old, the dialysis vintage less than 180 days. The target number of the entry is 800 in each Group. The 5 year-survival, changes in the nutritional status, occurrence of complications and others are the end points.By the end of April 2012, 362 patients have recruited to the study, and 88 questionarreis about the patients information at 0 and 6month were collected. Interim analysis for the first 88 data set was conducted.

Results: Interim analysis showed that the mean age of the patients was 77.4 years old, 58 (66 %) were male, and 38 (43 %) were diabetes. The mean serum creatinine was 6.67 mg/dL, the mean estimated GFR was 6.97 ml/min. There were no differences in all parameters in cluding outcome parameters between Group A and B at 0 month and 6 months after. In the comparison between 0 and 6 months, the mean level of serum creatinine was increased and the mean level of intact PTH was decreased siginificantly in Group A. The post-dialytic serum UN was siginificantly decreased in Group B.

Conclusions: Based on this preliminary analysis there are no clinically noteworthy differences between EVAL and PS for elder dialysis patients. However, we believe that further data collection can propose a suitable dialyzer choice for elder dialysis patients.

Funding: Pharmaceutical Company Support - Asahikasei Medical Co., Ltd.

PUB405

Clinical Sexology and Quality of Life in Clinical Renal Insufficiency Patients: A Quality Evaluation of Training Program

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Background: Chronic renal insufficiency negatively impacts on patients' affective and relational experiences, thus compromising their quality of life.

The aim of this study was to evaluate the effects of training program to provide a correct sexological background to health professionals in order to prevent the onset of pathological relationships in renal insufficiency patients. The course focuses on counseling and active-selective listening tools to deal with any sexual issue that may be brought up and ensure a comprehensive care of patients.

Methods: 67 members of the dialysis staff participated in the course and gave a qualitative evaluation by answering a series of question. The Sternberg triangle test and a body perception test on " sensitive body zone" were submitted and completed. Besides frontal lessons, the didactical method included interactive discussion after slide presentation, movies or readings. Didactic brochures were provided.

Results: 84% of the staff felt better informed on how to deal with sexual in dialysis patients; 86% appreciated the course design and didactical method.; 82% found the course useful and relevant for their profession; 69% found the duration of the course appropriate and 31% felt the need for more sessions.

Conclusions: Our findings provide evidence that gaining awareness of one's own behaviors and rigidities often greatly improves relationships with patients, ensuring a better quality of life for patients and a better professional life for caregivers.

Funding: Other NIH Support -

PUB406

Relationship between Initial Serum Uric Acid Level and One-Year All-Cause Mortality in Incident Hemodialysis Patients: A Single Center Experience over Five Years

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Background: Serum uric acid level is known as a risk factor for death in chronic hemodialysis patients. However, there are conflicting reports regarding the association of hyperuricemia with mortality. We examined whether the initial serum uric acid level might affect all-cause mortality in incident hemodialysis patients.

Methods: One-year all-cause mortality in which 150 patients initiated chronic hemodialysis in our facility between 2006 and 2010 were retrospectively assessed. The incident hemodialysis patients were assigned to two groups, 32 patients to the fetal group and 118 patients to the survival group. A multiple logistic regression model was used to compare the predictive factors of two groups. The model included known risk factors (e.g. age, gender, diabetes, physician type, serum uric acid, albumin, total cholesterol, creatinine, hemoglobin, C-reactive protein, calcium, phosphate).

Results: The number and causes of death were 11 for infection, 9 for heart failure, 3 for malignant tumor and 9 for others. Initial serum uric acid levels were 8.4±1.9 mg/dl and 9.3±3.3 mg/dl for the survival group and fetal group, respectively. Linear regression analysis showed that serum uric acid correlated with creatinine ($r=0.37$, $p<0.05$) and phosphate ($r=0.44$, $p<0.01$). The odds ratios (95% confidence interval, p-value) for these risk factors were 1.087 (1.031-1.166, 0.003), 5.713 (1.757-18.57, 0.004), 1.150 (1.031-1.284, 0.012) and 1.253 (1.008-1.559, 0.042) for age, whether attended by a nephrologist, C-reactive protein and uric acid.

Conclusions: The present study suggests that a higher level of initial serum uric acid is a risk factor for one-year death in incident hemodialysis patients. Uric acid management before starting hemodialysis therapy may help to improve one-year mortality. Interventional study should be conducted to confirm an association between the serum uric acid level and mortality in prevalent hemodialysis patients.

PUB407

Reliability of the 2014 Quality Incentive Program (QIP) Total Performance Score

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Background: The reliability of the 2014 CMS ESRD QIP total performance score (TPS) for dialysis facilities was evaluated using CMS ESRD claims data. The TPS, which is a composite measure based on three clinical measure scores (hemoglobin>12 g/dL, URR≥65, and the average of fistula use and catheter use) and three reporting measures, was estimated using only the clinical measures due to data availability. The reliability of a facility level measure reflects the extent to which it measures the performance of the facility as opposed to random variation of patient outcomes within the facility. The variance of a measure is the sum of a between-facility variance (Sb) and within-facility variance (Sw). The ratio of Sb to the sum of Sw and Sb is commonly referred to as the inter-unit reliability (IUR).

Methods: The IUR for simple measures can often be calculated using an analysis of variance (ANOVA). A complex composite measure like the TPS cannot be expressed as an average of responses at the patient level and another approach is needed. We present a bootstrap methodology whereby Sw is estimated by selecting a large number of bootstrap samples of individuals from each facility and the TPS is calculated for these samples.

Results: Since the reliability of the TPS may vary with facility size, we divided facilities into deciles based on dialysis patient counts. The IUR ranged from 0.61 in the lowest decile to 0.80 in the highest decile and generally increased as facility size increased.

Reliability of 2014 QIP TPS stratified by facility size

Decile (patient count)	# Facilities	Average facility size	IUR
1 (11-21)	491	16.8	0.61
2 (22-29)	497	25.4	0.65
3 (30-37)	531	33.5	0.66
4 (38-44)	472	41.1	0.64
5 (45-52)	515	48.5	0.64
6 (53-61)	512	57.0	0.68
7 (62-72)	511	66.8	0.68
8 (73-85)	532	78.5	0.69
9 (86-106)	512	94.8	0.68
10 (>=107)	512	137.7	0.80

Conclusions: These results indicate that most of the variation in the TPS is due to differences between facilities as opposed to random variation in patient outcomes within facilities.

Funding: Other U.S. Government Support

PUB408

Risk of Calciphylaxis with Commonly Used Medications in Hemodialysis Patients

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Background: Calciphylaxis, a rare but highly fatal condition seen in hemodialysis (HD) patients, is reportedly on the rise. We investigated the risk of calciphylaxis in relation to commonly used medications that have a potential role in vascular calcification (active vitamin D analogues, erythropoietin, iron, calcium-based phosphate binders and warfarin).

Methods: Cases (n=62) comprised HD subjects with skin-biopsy confirmed calciphylaxis diagnosed between 2000 and 2011. Controls (n=124) were HD subjects without calciphylaxis matched to cases by gender and calendar year. Medications and other covariates were compared between cases and controls. Association with calciphylaxis was determined using unadjusted and adjusted logistic regression models for each medication category.

Results: Utilization of calcitriol (21% vs. 7%, p=0.01) and warfarin (43% vs. 19%, p<0.001) was more common in cases compared to controls. Utilization of paricalcitol (29% vs. 26%, p=0.64), doxerecalciferol (6.5% vs. 6.6%, p=0.99), erythropoietin (42% vs. 34%, p=0.28), iron (7% vs. 11%, p=0.37), and calcium-based phosphate binders (32% vs. 33%, p=0.91) was similar. In unadjusted analyses, calcitriol (OR 5.77, 95% CI 1.75-18.46) and warfarin (OR 9.72, 95% CI 3.00-31.40) were associated with increased odds of calciphylaxis but other medications were not. In analyses adjusted for age, race, diabetes, macrovascular disease, obesity, and laboratory values (serum calcium, phosphorous, alkaline phosphatase, parathyroid hormone, 25-hydroxyvitamin D, and albumin), calcitriol and warfarin continued to confer higher risk for calciphylaxis.

Adjusted odds of calciphylaxis

Medication	OR	95% CI	P
Calcitriol	4.56	1.19-17.48	0.03
Selective vitamin D analogues (paricalcitol and doxerecalciferol)	1.89	0.72-4.99	0.20
Erythropoietin	1.51	0.57-4.05	0.41
Iron	0.45	0.13-1.51	0.20
Calcium-based phosphate binder	0.81	0.30-2.18	0.68
Warfarin	5.66	1.91-16.76	<0.001

Conclusions: Calcitriol and warfarin use are associated with greater calciphylaxis risk. Future studies are needed to establish if these relationships are causal. Considering significant morbidity and mortality of calciphylaxis, HD providers should take these potential risks into account while prescribing medications.

PUB409

Outcome of Chronic Dialysis Patients Admitted to the ICU over a 5-Year Period in a Single Center Hugues Bouchard,[1] Georges Ouellet,[1] Jean-Philippe Lafrance,[1] Lynne Senecal,[1] Denis Ouimet,[1] Martine Leblanc.[1,2] *[1]Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada; [2]Critical Care, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Critical illness in chronic dialysis pts may further increase their high mortality rates. The aim of our study was to describe outcomes of chronic dialysis pts when admitted to ICU.

Methods: By chart review, we identified all pts admitted to ICU (medico-surgical or coronary care) among our chronic dialysis (>90d) cohort from March 2006 to March 2011 (672 on HD, 79 on PD).

Results: 175 pts were admitted 231 times to ICU (mean age 67.4y, 44% women, 44% HD with a catheter, 40.6% HD with an AVF, 15.4% PD, median vintage 37m). For the 35 pts admitted more than once, only the first admission was considered for survival analysis.

Causes of ESRD were DM 36%, HTN 27%, and GN 11%. Main comorbidities were HTN (158 pts), DM (94), CAD (118) and peripheral vascular disease (76). Cardiac insufficiency was previously diagnosed in 45%.

At ICU admission, the median APACHE II and SOFA scores were 23 and 7; main reasons for transfer to the ICU were acute coronary syndrome (32.2%), severe infection (13.9%), urgent surgery (11.3%), cardiorespiratory arrest (9.6%), arrhythmia with hemodynamic instability (8.3%), neurologic cause (5.2%), pulmonary edema (4.8%), and "other" including GI bleeding (10.9%). During the ICU stay, 23% were mechanically ventilated for >24h, over a median time of 55h. Renal replacement therapy was usually performed with intermittent HD (72.2%); CRRT was used in 7.4% of the admissions, much less frequently than for AKI in our center.

The mean delay between hospital admission and transfer to the ICUs was 3.7d, while the mean ICU stay was 4.3±3.3d. During the same hospitalization, readmission rate to ICU was 6.9%. Mean hospital stay was 23.6±27.9d (median 14). Mortality rates were 16.6% and 29.1%, during the first ICU stay and hospital stay, respectively Survival at 30d, 6ths, 1 y and 2 y were 69.1%, 59.4%, 51.1% and 35.6%.

Conclusions: In conclusion, our chronic dialysis pts are admitted relatively often in the ICU for relatively short stays. Although their global mortality rate is high, most were discharged alive from the ICU.

Funding: Clinical Revenue Support

PUB410

Inpatient Hepatitis B Vaccination for Non Immune Hemodialysis Patients: A Critical Need Prabhat Singh, Maureen Lawlor, Raghavesh Pullalarevu, Saba Akhtar, Amit Ladani, Mohamed Hamdy Yassin. *Internal Medicine, UPMC Mercy, Pittsburgh, PA.*

Background: It is the standard of care to confirm that all patients with end stage renal disease (ESRD) are vaccinated for the hepatitis B virus (HBV). A hepatitis B antibody titer (HBsAb) above 10 is considered protective. Despite efforts to vaccinate HD patients, the response to the HBV vaccine is lower and less sustained than healthy individuals. Due to a greater percentage of acute HD patients treated within inpatient units, there is an increased risk of a HBV outbreak if a newly presenting HBV positive patient is introduced into the HD unit.

Methods: A prospective quality improvement initiative conducted over 2 months (10-2011 till 12-2011) that was designed to improve protection against HBV in the HD population treated at UPMC Mercy. All patients treated within the UPMC Mercy inpatient HD unit were included. The following HBV serologies were monitored; HBsAg, HBsAb titer, and HBc Ab. Vaccination with Engerix 40 mcg was offered to all HD with HBsAb titers < 10 IU/L.

Results: Sixty HD inpatients were enrolled in the study over two months period. Among these 60 patients, only 25 (42%) were immune with HBs Ab titer ≥10. Forty five patients had received prior vaccinations and 15 patients had no history of prior vaccination. Out of 45 vaccinated, only 32 patients had completed series of vaccination prior. All vulnerable patients 35 (58%) were offered vaccination.

Conclusions: HBV poses a great risk as a bloodborne pathogen particularly in inpatient HD units. Monitoring HBV serology (HBs Ag, HBs Ab, HBc Ab) on a routine basis is essential to prevent HBV outbreaks within HD units. Monitoring HBs Ag alone is insufficient and should be coupled with additional serology to ensure adequate protection for this particularly vulnerable population. Focus on vaccination especially in the inpatient facilities is a great opportunity to control HBV spread.

PUB411

Thirst and Oral Symptoms in People on Hemodialysis: A Multinational Prospective Cohort Study (Oral-D) Suetonia Palmer,[1] Marinella Ruospo,[2] Michela Sciancalepore,[2] Mariacristina Vecchio,[3] Letizia Gargano,[2] Patrizia Natale,[2] Valeria Maria Saglimbene,[3] Paul Stroumza,[2] Jorgen B.A. Hegbrant,[2] Fabio Pellegrini,[3] Eduardo Jorge Celia,[2] Jonathan C. Craig,[4] Giovanni F.M. Strippoli.[2,3,4] *[1]University of Otago; [2]Diaverum Medical Scientific Office; [3]Mario Negri Sud Consortium; [4]University of Sydney.*

Background: Thirst and xerostomia, the subjective complaint of dry mouth, may be increased in people on hemodialysis due to reduced salivary and lacrimal secretion, intravascular volume changes, fluid-restriction, endocrine hormone abnormalities, and medication use. Existing data for the prevalence of thirst and xerostomia are limited. We evaluated the prevalence of thirst and oral symptoms in adults on hemodialysis.

Methods: ORAL-D is a multinational cohort study of oral diseases in consecutive adults on hemodialysis in 75 outpatient clinics selected randomly from a collaborative dialysis network in Europe and South America. We administered xerostomia and thirst inventories based upon validated methodology. We analyzed prevalence data using descriptive analyses.

Results: 4720 hemodialysis patients in the participating clinics completed a self-administered questionnaire on oral symptoms. 1773 (38%) patients reported occasional use of candies for dry mouth sensation, 1095 (34%) had difficulties swallowing and 2437 (53%) needed to sip to aid swallowing. 2112 (45%) reported waking up during the night to drink, 1700 (36%) reported dry mouth sensation and 2309 (50%) had dry lips. The mean xerostomia inventory score was 21.14 (SD 5.56). Thirst was a reported problem for 2895 (62%) patients (62%); 3585 (78%) were thirsty during the day and 2173 (47%) during the night. Overall, 1169 (26%) patients reported that thirst influenced their social life. The mean dialysis thirst inventory score was 17.64 (SD 5.41).

Conclusions: Oral symptoms are highly prevalent in hemodialysis, with marked interference with daily life. Additional study of the predictors of thirst and xerostomia are now needed.

Other authors: David Johnson; Marietta Torok; Luc Frantzen; Miguel Leal; Jan Dulawa; Ruben Gelfman; Charlotta Wollheim; Anna Bednarek (missing disclosure affidavit).

PUB412

Assessing Overall Dialysis Center Efficiency among Free Standing Dialysis Centers Sanatan Shreay,[1] Mark Stephens,[2] Jill Mccluskey,[3] Matthew Gitlin.[1] *[1]Health Economics, Amgen, Thousand Oaks, CA; [2]Prima Health Analytics, South Weymouth, MA; [3]School of Economics, Washington State University, Pullman, WA.*

Background: Assessment of dialysis center (DC) efficiency has been limited, although research suggests efficiency is affected by multiple factors. Due to the limited research available, the purpose of this study was to assess overall DC efficiency as well as the impact of anemia drug choice on efficiency.

Methods: A data envelopment analysis (DEA) was performed using Medicare Renal Cost Reports 2010 data to model the efficiency of 4343 free-standing DCs relative to one another. Using a linear programming technique, DEA converts multiple inputs (costs, staffing levels) and an output measure (number of dialysis sessions) to a single score between 0 and 1 (low to high efficiency) for each DC. Simulations were conducted to assess changes in score distribution related to using a longer acting anemia drug by adjusting supply costs and staffing levels.

Results: Most DCs were located in the South (47%), 78% were affiliated with a large organization (2 largest chains) and 93% were for-profit. Annual supply costs per DC represented 14% (mean: $270,519; median: $202,287; SD: $230,627) of operating costs and nursing staff levels averaged 4.2 per DC (median: 3; SD: 3.6). The average number of treatments per year was 11,444 (median: 9893; SD: 7078). The DEA indicated 33% of the DCs were most efficient (score 0.9 to 1) followed by 11% (score 0.8 to 0.9); 19% (score 0.7 to 0.8); 22% (score 0.6 to 0.7); 11% (score 0.5 to 0.6) and 4% (scored <0.5). Overall, 26% (n=1055) of the for-profit DCs scored between 0.9 and 1. About 42% (n=104) of small organizations (< 50 DCs) and 23% (n=773) of large organization centers were most efficient. The simulated model when adjusting for longer acting anemia drug administration did not reveal any changes in the distribution of efficiency scores (32% of facilities were most efficient).

Conclusions: About 33% of DCs were deemed most efficient and simulated changes to supply costs and staff time by switching to longer acting anemia drug did not impact the distribution of overall DC efficiency scores.

Funding: Pharmaceutical Company Support - Amgen

PUB413

Randomizing Study Group Labels to Estimate Valid P-Values in Complex Data Sets John Rogus, Shu-Fang Lin, Franklin W. Maddux, Eduardo K. Lacson. *Fresenius Medical Care North America, Waltham, MA.*

Background: Maintenance hemodialysis (HD) allows for collection of repeatedly measured clinical, laboratory, and anthropometric values. Such correlated data complicates statistical analyses, particularly with assignment of valid p-values. Aggregating data into a single summary value per patient offers one option to circumvent the issue, albeit highly inefficient. Other methods such as generalized least squares or linear mixed models can be used, but with large national data sets, become too computationally intensive. We describe an efficient, non-parametric approach to assign valid p-values to effects estimated using ordinary linear regression.

Methods: For a continuous outcome and variable of interest with two labels (e.g., "case" or "control"), we derive regression statistics in terms of sums and sums of squares of the outcome. From these, we can calculate true parameter estimates (by preserving label designations) and also resample under the null (by randomizing label designations). We demonstrated this Monte Carlo procedure with an example based on 629,452 measurements of systolic blood pressure (SBP) in 39,313 patients undergoing HD at Fresenius Medical Care, North America. Cases had dialysate Na+ decreased by 3 mEq/L while controls had no change. The transition period was from Jan-June 2009. We compared the two groups during a 6-month baseline and 24-month follow-up period (excluding transition).

Results: Using the Monte Carlo procedure, we found a significant decrease in SBP for patients with a decrease in dialysate Na+ (p=0.04). Other computationally feasible, but inefficient, approaches such as data aggregation and year-over-year comparisons were unable to demonstrate this significant association.

Conclusions: Monte Carlo simulation is a simple way to assign valid p-values to the complex data sets common in HD studies. This method can accommodate covariates with a 2-step procedure involving an initial regression analysis. Future work is needed to characterize power relative to other methods and to study whether weighting strategies are useful when subjects have varying counts of data points.

PUB414

Routine Monthly ACTs Not Associated with Lower Clotting Events in Hemodialysis Adults on Heparin M. Khaled Shamseddin,[1,2] Sean W. Murphy,[1] Bryan M. Curtis,[1] Brendan J. Barrett.[1] *[1]Nephrology, Memorial University, St. John's, NF, Canada; [2]Nephrology, Queen's University, Kingston, ON, Canada.*

Background: Anticoagulant effect of heparin used during hemodialysis (HD) is usually measured by activated clotting time (ACT) to achieve a specific target.

Methods: A prospective study in 109 HD patients was designed to evaluatewhether a change in practice from monthly ACTs to one in which ACTs are only measured if clotting occurred will lead to any significant increase in clotting. All clotting events on heparin during phase I (4 months with monthly ACTs) were compared with phase II (4 months with ACTs if clotting occurred), using Poisson and Logistic Regression. Clotting event was classified as type 1 (Clot in the dialyzer bottom), 2 (Clotted filter), or 3 (Circuit changed).

Results: Routine ACTs were <150 in more than 50% of cases regardless of clotting occurrence in phase I. Heparin dose was changed in <10% of low ACTs, and dose change was effective (new ACT ≥150) only in 50% of cases. Surprisingly, 87 clotting events occurred in phase I (Type 1 (62), 2 (23), 3 (2)) compared with 58 events in phase II (Type 1 (41), 2 (16), 3 (1)). The incident rate for all clotting events in phase I was 1.5x higher than phase II (Exp (B) 1.5, 95% CI: 1.1-2.1, *P* 0.02). The incident rates for clotting events type 1, 2, and 3 were also higher during phase I compared with phase II, 1.5x (*P* 0.04), 1.4x (*P* 0.27), and 2x (*P* 0.57), respectively. To evaluate intrapersonal effects on clotting, since some patients had more than one event, we compared if any clotting event occurred in phase I vs. II, using binary logistic regression. The Odd Ratio of any clotting event to occur in phase I vs. II was 1.87, 95% CI: 1-3.4, *P* 0.04. Furthermore, the rate of effective heparin dose change, achieving ACT ≥150, was higher in phase I compared with phase II (*P* 0.00). If clotting type 2 or 3 occurred in phase I, the incident rate for heparin dose change was significantly higher (*P* 0.04), but was not more effective compared with phase II (*P* 0.4).

Conclusions: Routine monthly ACTs are not associated with lower clotting events in HD adults on heparin, as heparin dose was not changed or dose change was not effective.

PUB415

Chylous Ascites: Delayed Transient Cloudy Peritoneal Fluid after Laparoscopic Placement of Peritoneal Dialysis Catheter Alvaro A. Ryes. *St. Elizabeth Physicians, Cincinnati, OH.*

Background: Chylous ascites after laparoscopic implantation of a peritoneal dialysis cateheter (PDC) may be a less rare event than previously recognized. We describe the transient nature of this complication in one of our patients.

Methods: A 76 year-old man with end stage renal disease after complications of cholecystitis started hemodialysis via tunneled catheter (TC). After 6 months, he agreed to switch to peritoneal dialysis (PD) to allow TC removal. He underwent uncomplicated laparoscopic placement of a PDC which was flushed weekly with NS over the next 2 weeks. He began trainnig for chronic ambulatory PD and had 4-5 clinic visits with uneventful exhanges, each time obtainng clear PD fluid. He was scheduled to have his TC removed but he presented to the ER for an unrelated injury after falling on a parking lot. He was otherwise asymptomatic. In the ER he had his PDC flushed as per protocol and it was noticed that the PD fluid was cloudy. Vanc was given IP, PD fluid samples were obtained and he was discharged. On follow up his PD cell count was 10 WBC/mm3, RBCs were 43/mm3. No more antibiotics were given. On the following day he came to the PD clinic and had a PD exchange which was cloudy (see photograph). Samples were obtained: cholesterol was 22 mg/dL, amylase was 22 U/L and lipase was 62 U/L. The lab did not run triglycerides. A CT of the abdomen identified a previously noted paraceliac adenopathy but was otherwise negative. After another less cloudy PD exchange the chylous ascites resolved spontaneously without further episodes. There was no time to implement dietary modifications (low fat diet). He will have a follow up CT in 6 months to monitor paraceliac adenopathy.

Conclusions: Chylous ascites may have been the result of unavoidable trauma to the lympathic flow on the abdominal wall during laparoscopic insertion of the PDC. However, the trauma did not result in immediate development of chylous fluid; instead, it was a complication first evident as late as 10-14 days after laparocopic surgery and lasted only a couple of days making the detection more difficult. The development of chylous ascites could have benn missed altogether if the exchanges had not coincide with its transient nature.

PUB416

Icodextrin Preserved Renal Residual Function, Phosphate Clearance and Improved Atherosclerosis Takeyuki Hiramatsu, Takahiro Hayasaki, Akinori Hobo, Shinji Furuta, Yoshiyasu Iida. *Department of Nephrology, Konan Kosei Hospital, Konan, Aichi, Japan.*

Background: We reported that use of icodextrin ameliorates the progression of cardiac hypertrophy and valve calcification in incident peritoneal dialysis patients. Since icodextrin removed more phosphorus than glucose does, this study was aimed to examine whether removal of phosphorus affect the cardiac hypertrophy or not.

Methods: A retrospective analysis was conducted on 20 only glucose patients (Group A) and 20 icodextrin patients (Group B). Data on laboratory and cardiovascular ultrasound examination at the start of PD and every 6 months for 2 years were retrieved and analyzed.

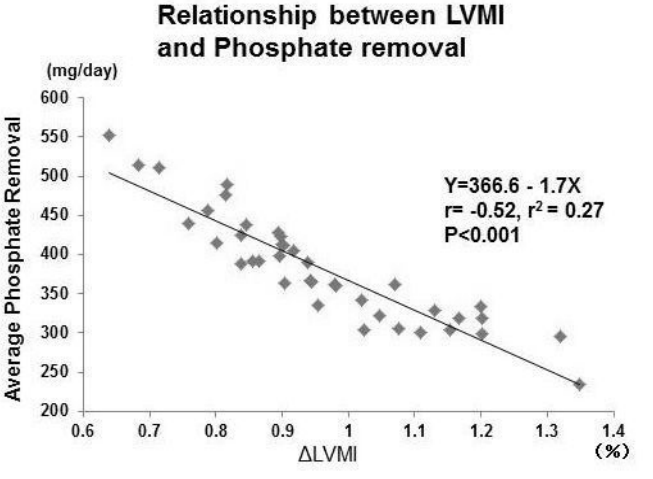
Results: At the start of PD, group B showed significantly higher value in PET, BNP left ventricular mass index(LVMI) and aortic valve calcification score(AVC) and lower urinary volume(UV) than those of group A. During 2 years on PD, phosphate removal and UV were preserved. And improvement of PET, LVMI, and decreased value of BNP were also observed.

Change in laboratory findings

	baseline		after 2 years	
	group A (Glucose)	group B (Icodextrin)	group A (Glucose)	group B (Icodextrin)
phosphate removal(mg/day)	406.7±17.0	369.4±21.8	305.8±16.4	422.4±14.1
urinary volume(ml/day)	1210.0±99.0	762.5±53.5	712.5±109.1	625.5±70.1
LVMI(g/m2)	139.3±7.0	161.9±9.5	155.2±8.5	130.0±8.0

Table 1

The correlation between phosphate removal and progression of LVMI showed statistical significance.



And in the cases with AVC improvement, phosphate removal were more than those without improvement.

Conclusions: These results indicate icodextrin preserved UV and peritoneal membrane function and ameliorated the progression of cardiac hypertrophy and valve calcification. And phosphate clearance by icodextrin was better than that by glucose. More phosphate removal ameliorates the progression of atherosclerosis. So the phosphorus was thought to be the factor of atherosclerosis.

PUB417

Mycobacterium Fortuitum: A Rare Exit Site Infection in a Peritoneal Dialysis Patient James E. Novak,[1] David R. White.[2] *[1]Henry Ford Hospital; [2]Nephron Associates.*

Background: Exit-site infections (ESI) are common in patients undergoing peritoneal dialysis (PD), but < 1% of causative pathogens are mycobacterial.

Methods: We report a case of *Mycobacterium fortuitum* ESI in which aggressive treatment was required for technique salvage.

Results: A 63-year-old man with end-stage renal disease status post recently failed kidney transplant initiated PD. His immunosuppressive regimen of rapamycin,

mycophenolic acid, and prednisone was gradually tapered. One month after starting PD, he noted pain, erythema, and purulent discharge from the PD catheter exit site. He denied fever, chills, abdominal pain, or cloudy dialysate, and did not show signs of peritonitis. He was prescribed trimethoprim-sulfamethoxazole (TMP-SMX) for 2 weeks with complete resolution of signs and symptoms. Discharge from the exit site subsequently grew *M. fortuitum*. After 2 months, purulent discharge recurred. TMP-SMX was resumed and cultures again grew *M. fortuitum*. TMP-SMX was changed to intravenous cefoxitin for 2 weeks and oral clarithromycin for 6 weeks. The patient underwent laparoscopic removal of the infected catheter with simultaneous placement of a new catheter at a different site. His dialysis prescription was temporarily adjusted to low-volume exchanges to allow the new catheter site to heal. The patient has remained on PD with no recurrence of infection. In this case, the tapering immunosuppression from his failed kidney transplant may have predisposed him to ESI with *M. fortuitum*.

Conclusions: *M. fortuitum* is a Gram-positive, acid-fast, rapidly growing, nontuberculous mycobacterium (NTM) with global distribution. It can be found in natural and processed water, sewage, and dirt. It has been associated with nosocomial infection of implanted devices, including catheters. Of the NTM, *M. fortuitum* is the most common pathogen giving rise to ESI, tunnel infections, or peritonitis in patients undergoing PD. In cases of peritonitis with any organism, 30–50% result from inadequately treated ESI, and rates of catheter loss in this setting are as high as 15–57%. In summary, *M. fortuitum* ESI requires aggressive treatment, including prolonged antibiotic treatment and frequently catheter exchange, to allow continuation of PD.

PUB418

Clinical Outcomes of Uncommon Bacterial Peritonitis in Peritoneal Dialysis Patients Omkar U. Vaidya, Ramesh Saxena. *Nephrology, University of Texas Southwestern, Dallas, TX.*

Background: Peritoneal dialysis (PD) patients occasionally get uncommon bacterial peritonitis (UBP).Not much data on prevalence of UBP and their clinical outcomes is available.That led us to perform a retrospective analysis of outcomes of UBP in our PD patients.

Methods: We performed a retrospective evaluation of all the patients initiated on PD between 2001 and 2012 and developed UBP.Patients who developed peritonitis from common bacteria and fungi were excluded.Primary outcomes were defined as resolution of UBP, PD technique failure or death due to peritonitis.

Results: We initiated 421patients on PD between 2001 and 2012. Of these, 31 patients developed 41 episodes of UBP.All patients received appropriate intraperitoneal antibiotics for 14– 21 days as per their sensitivities & response to treatment. Of the 31 patients with UBP, 7 had PD technique failure leading to transition to hemodialysis. Of these 7 patients, one had 2 recurrent Acenetobacter infections, one had three recurrent Corynebacterium infections and 2 had single episodes of Acinetobacter Peritonitis.Additionally one each has UBP from Campylobacter, Acid fast bacilli (AFB) and Neisseria species. All other UBP resolved. All cases of UBP from Acinetobacter, Corynebacterium, Campylobacter, AFB and Neisseria species led to PD failure.No death resulted from UBP.

Patient characteristics of PD techinque failure from UBP

Sr no	Age/Race	Sex	Etiology of End stage renal disease	Medical and Sugical history	Organism
1	44 yr African American	Male	IgA nephropathy	Hypertension(HTN)	Acinteobacter baumanni
2	29 yr Caucasian	Male	Failed living related kidney transplant	HTN	Neisseria species
3	32 yr Hispanic	Male	HTN/DM	Diabetes Mellitus(DM)/HTN	Recurrent Acinetobacter baumannii
4	54 yr Caucasian	Female	HTN	HTN/ Oopherectomy	Acid fast Bacilli
5	32 yr African American	Male	HIV/HTN	HIV/HTN	Recurrent Corynebacterium species
6	46yr Caucasian	Male	DM/HTN	DM/HTN	Campylobater species
7	42 yr Hispanic	Female	Lupus	Cesarean section, Hysterectomy	Acinetobacter baumannii

Table 1

Conclusions: We observed that overall UBP has good outcome. However, UBP from Acinetobacter, Corynebacterium, AFB, Neisseria species and Campylobacter is associated with PD technique failure.

PUB419

Aggravation of Peritoneal Permeability during Long-Term Peritoneal Dialysis Therapy Related to Increase in PD Dropout Yasufumi Takahashi,[1] Mamoru Kobayashi,[1] Koji Hashimoto,[2] Yuji Kamijo,[2] Makoto Higuchi.[2] *[1]Nephrology Internal Medicine, Nagano Red Cross Hospital, Nagano, Japan; [2]Nephrology Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan.*

Background: Insufficient bodily fluid removal is the most serious problem causing dropout from peritoneal dialysis (PD) therapy and in these cases, a transfer to hemodialysis or a combination therapy of PD and hemodialysis is crucial. Therefore, control of the fluid volume is very important for management of PD. The fluid removal capacity in PD is known to be related to peritoneal permeability; however, it is still unclear whether peritoneal permeability status has an influence on the PD dropout rate.

Methods: We investigated 74 patients undergoing PD, retrospectively. Throughout the observation period, we regularly carried out a peritoneal equilibration test (PET) to evaluate their peritoneal permeability status. Using this PET data -- as well as other parameters, including various laboratory examinations, the incidence rate of peritonitis, existence of diabetes, and medication history -- we statistically analyzed prognostic factors responsible for PD dropout. Patients who withdrew premeditatedly from PD to prevent encapsulating peritoneal sclerosis were excluded as dropouts.

Results: During the observation period (average: 49 months), 30 patients (41%) dropped out of PD and 12 patients (16%) died. The dialysate/plasma creatinine (D/P Cr) ratio at the PD starting point for each patient was unrelated to the dropout and mortality rates. The Cox proportional hazards model revealed that an increase in the D/P Cr ratio during long-term PD, the existence of diabetes, and the incidence rate of peritonitis were all significant independent predictors of dropout from PD (HR and 95% CI, 4.83 (1.3-17.6) vs. 4.31 (1.0-23.2) vs. 4.89 (1.7-14.1), respectively).

Conclusions: These findings suggest that the detection of peritoneal permeability aggravation during long-term PD is more important for predicting dropout than a one-point evaluation of PET. Careful monitoring of peritoneal permeability status would be very important for preventing PD dropout.

PUB420

Risk Factors for Recurrent Peritoneal Dialysis Peritonitis in CAPD Patients Sug Kyun Shin,[2] Youngeun Kwon,[1] Kyoung Sook Park,[1] Jae-kyoung Kim,[1] Yong Kyu Lee,[2] Tae Ik Chang,[2] Ea Wha Kang,[2] Seung Hyeok Han,[1] Tae-hyun Yoo,[1] Beom Seok Kim.[1] *[1]Internal Medicine, Yonsei University Medical School, Seoul, Korea; [2]Inernal Medicine, NHIC Ilsan Hospital, Goyang-shi, Kyunggi-do, Korea.*

Background: Recurrent peritoneal dialysis peritonitis(RPDP) is a serious cause in PD patients' drop out. In spite of the advancement of connectology and biocompatibility in peritoneal dialysate, the incidence of RPDP does not seems to be reduced. Assessment of risk factors for RPDP can be helpful to prevent PD complication.

Methods: Total 498 PD peritonitis episodes(PDPE) were included that were developed from Mar. 2000 to Dec. 2011 at NHIC Ilsan hospital, Korea. We conducted the analysis of PDPE that was devided to simple PD peritonitis(SPDP) and RPDP. RPDP was defined as a developed peritonitis within 4 weeks after antibiotic therapy or a peritonitis needed antimicrobial therapy of more than 6 weeks. We compared the basic data, hematologic, biochemical parameters and the results of culture study between RPDP and SPDP.

Results: Total 498 PDPE were measured in 389 PD patients. Univariate analysis showed that Hb., albumin and residual renal function(RRF) were lower, and age, the proportion of diabetes was higher in RPDP than SPDP group. Bicarbonate dialysate and no growth in culture were significantly frequent in RPDP compared to SPDP group. Multivariate analysis showed that RRF, bicarbonate dialysate, plastic bag and fungal peritonitis were significant.

Multivariate Analysis on Risk Factors of Recurrent PD Peritonitis

Factors	O.R.	95% C.I.	p-value
Time to peritonitis	1.027	0.97-1.51	0.387
Serum albumin (g/dL)	0.831	0.45-1.48	0.457
Initial dialysate WBC count on peritonitis (/mm^3)	0.954	0.57-1.58	0.098
RRF on peritonitis (ml/min/1.73mm^2)	1.142	0.95-2.89	0.038
Kinds of dialysates	1.254	0.91-2.88	0.049
Kinds of PD bag's materials	1.313	0.79-1.57	0.045
Fungal peritonitis	8.791	3.69-21.24	0.000

PD: peritoneal dialysis, OR: odds ratio, C.I.: confidence interval, RRF: residual renal function

Conclusions: In this study, not only RRF and fungal peritonitis, but also bicarbonate dialysate and plastic bags were revealed as independent risk factors for RPDP. To verify our results may be needed multi-center analysis with larger size.

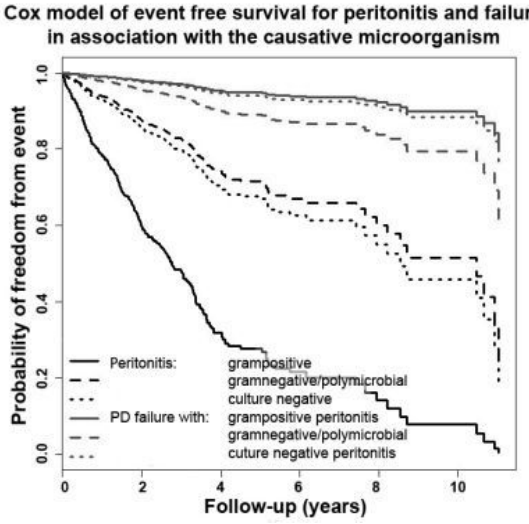
PUB421

Infectious Peritonitis in Peritoneal Dialysis: Germ Spectrum and Its Impact on PD Failure Claudia Praehauser,[1] Bernard Descoeudres,[2] Andreas Schötzau,[3] Michael Mayr.[1,4] *[1]Medical Outpatient Department, University Hospital Basel, Basel, Switzerland; [2]Department of Nephrology, Hôpital du Jura, Porrentruy, Switzerland; [3]Schötzau & Simmen, Basel, Switzerland; [4]Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, Basel, Switzerland.*

Background: Infectious peritonitis (IP) is a major reason for peritoneal dialysis (PD) failure. Aim of the study was to analyze the importance of specific pathogenic microorganisms for clinical outcome.

Methods: Retrospective single centre study of all patients admitted for PD from 1983 to 2011. Combined endpoint was IP related PD failure consisting of technique failure (defined as the inevitable definitive stop of PD) and death in association with the causative microorganism.

Results: 139 of 321 patients (43%) experienced 268 IP episodes. 226 (84%) episodes were culture positive, of them 68% were gram-positive, 19% gram-negative and 13% polymicrobial. Compared to the risk for gram-positive IP, the risk for gram-negative or polymicrobial (HR 0.26, CI 0.20-0.35) and culture negative IP (HR 0.31, CI 0.21-0.44) was significantly lower (figure).



39 patients experienced IP associated PD failure (12 deaths and 27 technique failures). The percentage of fatal episodes and technique failures was 1% (2/154) and 3% (4/154) in gram-positive, 7% (3/42) and 21% (9/42) in gram-negative, 13% (4/30) and 33% (10/30) in polymicrobial and 7% (3/42) and 10% (4/42) in culture negative IP episodes, respectively. The risk of IP associated PD failure was significantly higher for gram-negative/ polymicrobial (HR 8.33, CI 3.62-19.19) and culture negative (HR 3.82, CI 1.36-10.79) IP than for gram-positive IP.

Conclusions: Grampositive bacteria are the most frequent cause of IP in PD. However, the risk of IP associated PD failure is much higher in gram-negative, polymicrobial and culture-negative IP than in gram-positive IP.

Funding: Clinical Revenue Support

PUB422

Serum Response Factor Expedites Epithelial-to-Mesenchymal Transition by Modulating the Expression of Snail in Peritoneal Mesothelial Cells

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Background: Treatment of primary human peritoneal mesothelial cells (HPMCs) with high glucose (HG) induced EMT, characterized by downregulating E-cadherin, upregulating of α-SMA and cell spindle-like morphology. Serum response factor (SRF), a transcription factor activated when translocation in nucleus, can regulate cell metastasis by expediting EMT. However, the mechanism of SRF in PD process hasn't to be elucidated.

Methods: We isolated HPMCs from PD effluents from CAPD patients. All these cells were characterized by phenotype markers and tested the location of SRF by immunofluorescence. We also tested these in immortal HPMCs and PD animal model, and explored the possible pathway regulated by SRF.

Results: With the dialysis time, HPMCs undergo a transition from an epithelial to a mesenchymal phenotype with a loss of epithelial morphology, a decrease E-cadherin, an increase α-SMA and the translocation of SRF from cytoplasm into nucleus. Moreover, blockade of SRF expression could reverse EMT in transdifferentiated HPMCs isolated from PD effluents. In vitro analyses, HG-induced HPMCs were change into fibroblast-like cells, down-regulation of E-cadherin, up-regulation of α-SMA and translocation into nucleus of SRF. Overexpression of SRF demonstrated that active SRF is required in EMT process marking for down-regulation E-cadherin and up-regulation α-SMA. Obtained with SRF inhibitor or infection with SRF-siRNA vectors could reverse EMT by upregulation E-cadherin and downregulation α-SMA. HG-mediated induction of mRNA and protein expression of the transcription factor Snail1, a repressor of E-cadherin expression and a potent inducer of EMT, was inhibited by blockade of SRF. Furthermore, ChIP and reporter assay showed SRF could directly promote the transcription of snail by binding serum responding element 2 (SER2).

Conclusions: The findings suggest that modulation of the SRF/Snail1 pathway may provide a means of enhancing the progressive structural and functional deterioration of the peritoneal membrane during PD.

PUB423

Analyses for Peritoneal Dialysis Withdrawal: Twenty-Seven Years of Experience in a Single PD Center in China

Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: The number of peritoneal dialysis (PD) withdrawal is increasing last ten years. The study investigated causes of PD withdrawal and assessed risk factors of death in 27 years of experience in a single PD center.

Methods: All the Patients undergoing PD at lest 1 months, enrolled this retrospective observational study from January 1985 to December 2011. We investigated causes of PD withdrawal, analyzed demographic characteristics, laboratory data, dialysis adequacy parameters, residual renal function, peritoneal transport characteristics, and nutritional status according their medical records.

Results: A total of 595 patients were enrolled, of whom 292 were females (49.1%). The mean age at the start of PD was 59.9 ± 16.8 years. The primary diseases of ESRD were glomerulonephritis (52.3%), hypertension (16.6%), diabetes (15.3%) respectively. The mean PD duration was 14.7 ± 16.8 months. Until December 2011, 296 (49.8%) died, 152 (25.5%) transferred to hemodialysis, 64 (10.8%) received kidney transplant, and 83 (13.9%) lost in follow up. The first cause of death was cardiovascular events (41.6%); the second was infection (33.0%) in which pulmonary infection (23.2%) and peritonitis (9.8%) was the severe events directly related to death. The cerebral vascular accident (9.5%) was the third cause for death. PD associated infection was first reason for transfer to hemodialysis (59.2%), the followed reason was technique failures (occlusion, drift, leakage, etc. 25.7%) and insufficient PD (7.2%). In the Cox proportional hazards model analysis, the risk factors related to mortality were gender which means female PD patients had a higher risk (RR:1.33, p = 0.014); age (RR: 1.04, p < 0.001), diabetes (RR: 1.65, p = 0.001), hemodialysis transfer to PD (RR: 1.33, p = 0.015), and low serum albumin level (RR: 1.27, p = 0.015).

Conclusions: In our PD center, the major cause of PD withdrawal was death, and then was transferred to hemodialysis. The cardiovascular events was the first cause of death. PD associated infection was the major reason for transferred to hemodialysis. Female, older age, diabetes, malnutrion condition or hemodialysis transfer to PD, predict higher risk for all cause-death.

Funding: Government Support - Non-U.S.

PUB424

Multicultural Dialisys Unit New Opening: Three Years Experience

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Background: New Peritoneal Dialysis Unit opening always represents a challenge for a Nephrology Service.Initial inexperience´s team, sluggish growth in patients number,new tests implementation in other servicies(laboratory,radiology), understanding with the surgical unit,.are reasons of constant effort and learning.If patients have diverse nationalities this is impeded furthermore.In the unit openning besides the previous one, we had to bear in mind a cultural and idiomatic differential fact and that, in addition, was very dispersed.It was worrying us that this idiomatic and cultural barrier can cause implementation and maintenance problems of the technique.For this reason we analyze the three years experience information of prevalence,incidence,complications and cessations of technique,TT and age to reject that the differential(cultural/idiomatic)fact had effect in the above mentioned information.

Methods: Annalisis of three years data of prevalence, incidence,complications and cessations/changes of technique, time of training and age.

Results: Program entries 23 patients(13W/10M),ages from 30 to 80 years (total ages:59; ages spanish:52. 8; ages other languages:69.1);14 Spanish and 9 foreigners (61 and 29 % respectively. Time of training (TT),total: 7.6,TT spanish:7.25,TT others: 8.7, TT <65 years (total): 7.2, TT> 65 y (total): 9.8. Exits of program 5 patients.Complications: peritonitis 6 in 4 patients (2 episodes/year); placements of catheter 28 in 23 patients (5 refills, 2 peritonitis and 3 dysfunction);cuff extrussion 4;escapes of liquid 2; exit site infection 23; tunelitis 1. Results: we have not found any relation between nationality/language and complications or technique maintenance,we found differences in TT between age and idiom, our results shows that TT is higher in the 65-year-old major patients not only in the foreign but also in the spanish speak group.

Conclusions: Putting in march a new unit of peritoneal dialysis is costly but an idiomatic difference in the patients does not imply more complications or diferences on technique maintenance in this group; nevertheless the training time in this group is more difficult and take us more time as it happens also in the most aged patients of our unit.

PUB425

Peritoneal Balances of Mg in Peritoneal Dialysis Patients Treated and Not Treated with Oral Mg Salts Binders

Jesus Montenegro, José Ignacio Cornago, Maria Isabel Gallardo, Paula García, Ainhoa Hernando, Rosa Ines Munoz. *Nephrology, Hospital de Galdakao-Usansolo, Galdacano, Spain.*

Background: Optimun concentration of Mg in Peritoneal Dialysis solutions remains in controversy.With the objective of finding out the peritoneal balances of Mg, a randomized cross over study was done in prevalent CAPD patients.

Methods: The patients were divided into 2 randomized groups. Group 1: the 23 patients started taking Mg salts (Mg carbonate and Ca acetate, Osvaren®, Fresenius Medical Care) during 2 months; after these two months they took Lanthane Carbonate (Fosrenol®, Shire) during two months and at the end they changed to Osvaren during 2 months. In group 2 there were 22 patients and the design: Fosrenol→Osvaren→Fosrenol, changing every 2 months. The peritoneal fluid contained 1,2 mg/dL (0,50 mmol/L) of Mg. Every period lasted 2 months and at the end of this period the following values were measured: Mg, Ca, P, in blood, peritoneal fluid and 24 hours urine. PTH levels were measured as well. The residual renal function was calculated as well ultrafiltration (UF) and peritoneal balances of Mg.

Results: Serum Mg increases signiphicantly during the period they took Mg salts. The peritoneal balances of Mg were negative in every patient and in all periods and the peritoneal losses of Mg were greater significantly during the intake periods with Mg salts, related with the UF and the serum levels of Mg, see.

Peritoneal Balances of Mg

Groups: daily peritoneal losses	Basal	2 months	4 months	6 months
G1: Mg.mg/day	-22.7±20.3	-48.8±22.1	-17.9±9.9	-33.3±13.0
G2: Mg.mg/day	-23.7±17.4	-28.5±28.9	-38.0±13.2	-18.6±10.7
p	0.87	0.003	0.001	0.0002

UF influences peritoneal balances of Mg (p= 0, 02), in other words, the greater the UF the greater the peritoneal loss of Mg. The serum levels of Mg contributed much more (p=0,001), for every increase of 1 mg of serum Mg, the peritoneal losses of Mg increased in 32, 8 mg/day. There was no case of clinic hypermagnesemia.

Conclusions: Therefore Mg salts are safe in patients treated with PD solutions containing a concentration of 0, 50 mmol/L of Mg. After this study, we recommend an increase in the concentration of Mg in the PD fluids, at least in patients that are not taking Mg salts.

PUB426

Peritoneal Dialysis Associated Achromobacter Xylosoxidans Peritonitis, a Rare but Virulent Organism Seyyar Khan,[1] Ashish Kataria,[1] Elvedin Lukovic,[2] Mala Sachdeva.[1] *[1]Department of Medicine-Division of Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY; [2]Downstate Medical Center.*

Background: Peritonitis is a well known complication in patients on peritoneal dialysis (PD). Staphylococcus species and gram negative organisms such as Escherichia Coli and Pseudomonas Aeruginosa remain the most common causative organisms, although fungal, mycobacterial, and polymicrobial peritonitis do occur. Achromobacter Xylosoxidans causing peritonitis has rarely been reported. We report a complicated case of Achromobacter Xylosoxidans associated peritonitis requiring catheter removal.

Methods: A sixty-eight year old Korean female with a history of diabetes mellitus, hypertension, and end stage renal disease on Continuous Ambulatory Peritoneal Dialysis (CAPD) for four years presented with abdominal pain, fever, and cloudy dialysate for two weeks. Intraperitoneal (IP) vancomycin and ceftazidime were empirically begun for suspected peritonitis. Initial peritoneal fluid cell count and culture revealed 3500 WBC's with 73% granulocytes and coagulase negative Staphylococcus and bacillus species, not anthrax. Day two peritoneal fluid culture revealed coagulase negative Staphylococcus and Achromobacter Xylosoxidans. Achromobacter Xylosoxidans was initially sensitive to ceftazidime and ciprofloxacin. Thus, she was maintained on vancomycin IP, ceftazidime IP, and oral ciprofloxacin. Sensitivities five days post treatment revealed that Achromobacter Xylosoxidans was resistant to ceftazidime. Day six culture continued to grow out Achromobacter Xylosoxidans. Multiple CT abdomen and pelvis were negative for abdominal pathology. Given persistent culture positivity despite aggressive antibiotic treatment, a decision was made for catheter removal and hemodialysis was initiated.

Conclusions: Despite breakthroughs in treating PD associated peritonitis, clinicians must keep in mind all organisms responsible. When Achromobacter Xylosoxidans is the causative agent careful monitoring and quick removal of peritoneal catheter should be implemented. This avoids the high morbidity and mortality that has been associated with this organism as it is a highly virulent and resistant organism.

PUB427

Residual Renal Function Is a Crucial Determinant of Blood Pressure of Patients with Peritoneal Dialysis Hideki Yamahara, Takanobu Imada, Hiroko Ueda, Mitsutaka Nakahigashi, Chikara Nakano, Kazunori Someya, Makiko Kusabe, Sanae Kikuchi, Hiroyasu Tsukaguchi, Hiroya Masaki, Mitsushige Nishikawa, Ichiro Shiojima. *Department of Medicine II, Kansai Medical University, Osaka, Japan.*

Background: Continuous ambulatory peritoneal dialysis (CAPD) is an important renal replacement therapy. However, continuous exposure of the peritoneal membrane to unphysiologic PD fluid results in injury of the peritoneum.

Methods: We performed a peritoneal equilibration test every 6 months to 18 outpatients. We checked weekly Kt/V urea as a dose of small-solute clearance. On the same day, we checked their human atrial natriuretic peptide (hANP), brain natriuretic peptide (BNP) and blood pressure.

Results: Total small-solute clearance (weekly Kt/V urea) was suitable (2.31 ± 0.573 (mean ± SD)). However, some CAPD patients had increased hANP (74.1 ± 65.7 pg/ml) and, BNP (166 ± 164 pg/ml), and blood pressure (153 ± 21.2 / 90 ± 12.7 mmHg) than standard value. Blood pressure was negatively correlated with residual renal small-solute clearance (r=-0.319, p=0.009), and urine volume (r=-0.318, p=0.008), but was not significantly associated with dialysis small-solute clearance (r=0.157, p=0.206). BNP and hANP were also negatively correlated with residual renal small-solute clearance and urine volume, but was not associated with dialysis small-solute clearance.

Conclusions: The main finding of this study is that residual renal function contributes to blood pressures of CAPD patients. CAPD patients with low residual renal function tends to cause volume expansion. Thus, combination therapy of hemodialysis and peritoneal dialysis or discontinuation of CAPD is desirable for CAPD patients with low residual renal function and high blood pressure.

PUB428

Peritoneal Balances of Calcium in Peritoneal Dialysis Patients Treated with and without Oral Calcium Salts Binders Jesus Montenegro, José Ignacio Cornago, Maria Isabel Gallardo, Paula García, Ainhoa Hernando, Rosa Ines Munoz. *Nephrology, Hospital Galdakao-Usansolo, Galdacano, Vizcaya, Spain.*

Background: The concentration of Ca 7 mg/dL or 5 mg/dL in Peritoneal Dialysis Solutions (PDS), can induce positive and negative peritoneal balances of Ca (PBCa).

Methods: A randomized cross-over study was designed in prevalent CAPD patients to find out if the BPCa were related with the intake of phosphate Ca binders (Ca acetate and Mg carbonate, Osvaren®, Fresenius Medical Care) or binders without Ca (Lanthanum Carbonate, Fosrenol® ,Shire). After 15 days of wash-out the patients were randonmized into two groups: group 1: Osvaren-Fosrenol-Osvaren, changing every 2 months the phosphate binder. Sequence in group 2: Fosrenol-Osvaren-Fosrenol, changing the phosphate binder every 2 months as well. The P contained 7 mg/dL of Calcium in both groups. At the end of each period, the following values were determined: Ca, Mg, P, in blood, in peritoneal fluid (LP) and in 24 hour urine. PTH levels were measured. The dose of phosphate binders, the proteins intake, the ultrafiltration and the BPCa were calculated.

Results: The BPCa was positive in all patients of both groups and in all periods, independently of the intake of salts with Ca salts or without Ca, see **Table 1**.

Outline of peritoneal balance of calcium in both groups

Groups	Basal	2 months	4 months	6 months
G1 (BPCa, mg/día)	+50.7±27.8	+26.2±38.3	+35.1±25.2	+ 34.2±27.0
G2 (BPCa, mg/día)	+43.4±30.8	+37.6±26.8	+26.5±28.5	+40.7±29.3
p	0.41	0.26	0.29	0.44

The degree of UF influences on the BPCa, p<0,001, therefore, the greater the UF, the greater the peritoneal emoval of Ca. The serum Ca increased with the intake of Ca salts and had a greater contribution in the Calcium peritoneal removal, p<0,001, for every increase of 1 mg of serum Ca, the peritoneal removal of Calcium raised 24,5 mg per day.

Conclusions: All patients gains Ca with this PDS, independently of type of binders, UF and serum Ca. The standard concentration of 7 mg/dL of Ca in PDS is excessive for every patient. We should consider that the concentration of 6 mg/dL of Ca could be the standard SDP. The concentration of Ca of 5 mg/dL in PDS is recommended in patients who take Ca salts in great amounts.

PUB429

Glucose Profile Assessment of Continuous Glucose Monitoring System and Use of Insulin Pumps in Patients with End-Stage Diabetic Nephropathy on Maintenance Continuous Ambulatory Peritoneal Dialysis Shuangxin Liu, Wei Shi, Xinling Liang, Zhiming Ye, Wenjian Wang, Jianchao Ma, Lixia Xu. *Division of Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.*

Background: The glycemic control is known to be an important determinant of the rate of progression of patients with diabetic continuous ambulatory peritoneal dialysis (CAPD) patients. Good glycemic control is often difficult to maintain in diabetic patients with CAPD, because they are continuously exposed to high concentrations of glucose in peritoneal dialysate. However, recent studies have suggested that diabetic patients who use insulin pump has been shown to reduce glycated hemoglobin levels without an increased risk of hypoglycemia, as compared with a regimen of multiple daily insulin injections. The continuous blood glucose monitor (CGMS) has recently offered an opportunity to monitor blood glucose at 5-minute intervals for 72 continuous hours in diabetic patients.

Methods: There were 12 diabetic patients on CAPD using conventional 1.36% or 2.27% glucose PDFs examined in the study. All participants underwent 72-hour CGMS and HbA1c evaluation. Continuous subcutaneous insulin pump insulin intensive therapy group for the ultra-short effect of human insulin analogues - insulin lispro, conventional subcutaneous insulin Humalog before meals program for the Addition 21:00 Novolin N treatment. There was the whole area under the curve (AUC) of each 24-hour glucose profile. Glucoes area above high limit is 10 mmol/L, and glucose area below low limit is 3.9mmol/L.

Results: Diabetic CAPD patients 12 cases with first use of continuous subcutaneous insulin pump therapy to strengthen, then the routine use of subcutaneous insulin program. Duration above high glucose limits are 65% ± 22%, duration within limits are 35 ± 15% in continuous subcutaneous insulin pump therapy group, while duration above high glucose limits are 81% ± 32%, duration within limits are 19% ± 8% in the conventional subcutaneous insulin program, p< 0.05.

Conclusions: In diabetic peritoneal dialysis patients with insulin pump therapy compared with conventional subcutaneous insulin solution, blood glucoes are more stable standard.

Funding: Government Support - Non-U.S.

PUB430

Surgical Complications of Peritoneal Dialysis Catheters Surabhi B. Thakar, Timothy A. Pflederer, Beverley L. Ketel, Timothy P. O'Connor. *Renal Care Associates.*

Background: Peritoneal dialysis catheters (PDC) are associated with complications requiring surgical treatment, including malposition, inflow or outflow occlusion, refractory peritonitis, and ineffective dialysis leading to removal and change of modality. The rate of these events in North American practices is not widely reported.

Methods: We conducted a retrospective review of initial PDC placements over a 4 year period in an ESRD practice with 14% prevalence of PD. PDC's were inserted by 2 surgeons and 1 nephrologist. Data fields collected were prior abdominal surgery, technique used, types and timing of subsequent surgery, PDC complication noted, other dialysis modalities used, reasons for multiple PDC procedures. PDC removals due to kidney transplant were excluded.

Results: Out of 179 PD insertions, 157 (87.7%) were open procedures and 22 (12.2%) laparoscopic. 79 (44.1%) had a subsequent procedure at a median time of 106 days. 33 (18.4%) of insertions had a second procedure within 60 days. 26 (32.9%) of subsequent procedures were repositioning for malposition/adhesions/flow problems, (18 in first 60 days) and 53 were removals (13 in 1st 60 days). 23 removals (43.3%) were for refractory peritonitis, 7 (13.2%) for malfunction, 4 (7.5%) for tunnel/exit site infection, 13 (24.5%) for elective convertion to hemodialysis, 5 (9.4%) for other reasons and 1 for diaphragmatic fistula. 9 (40.9%) of the laparoscopic and 70 (44.3%) of open insertions needed a subsequent procedure. 114 (63.3%) patients had a history of prior abdominal surgery before PDC placement, 55 (44.5%) of these had a subsequent procedure while 59 (51.7%) did not.

5 (22.7%)laparoscopic insertions and 28 (17.7%) open insertions needed a subsequent procedure in 1st 60 days. 9 (5%) PDC insertions underwent >1 subsequent procedure.

Conclusions: PDC insertions are associated with a reasonable number of surgical complications, needing more removals than repositioning, the most common indication for removal being recurrent peritonitis. Prior abdominal surgery or particular method of insertion did not increase the risk of subsequent procedure. Open PDC insertion when compared to laparoscopic placement was not associated with more subsequent procedures in the first 60 days.

PUB431

Study of Long-Term Peritoneal Dialysis Hiroyuki Tamura. *Nephrology, Toshima Central Hospital, Toshima-ku, Tokyo, Japan.*

Background: In Japan, in order to prevent serious complicating disorder such as encapsulating peritoneal sclerosis, at the beginning of treatment, the available period of peritoneal dialysis (PD) has been set, and combination therapy of PD and hemodialysis has been giving priority rather than solo PD treatment in many cases.

Although we have been providing solo PD treatment since 1986 without the available period, the case of encapsulating peritoneal sclerosis has been zero for now, many PD-patients have continued for long periods.

Here we examined the continuation rates of PD at our hospital and factors affecting long-term PD.

Methods: In patients who started PD at our hospital between 1986 and 2012, we examined continuation rates, patient characteristics at the introduction of PD, the peritoneal equilibration test (PET), and reasons for withdrawal of PD.

Results: This study included 113 patients (66men, 47women). The mean age at introduction was 57.94 years (range: 13-90). The mean PD duration was 5.4years (range: 0.1-19). The 5- and 10-year continuous PD rates were 60%, and 38%, respectively. The factors affecting continuation of PD were the presence or absence of diabetes and ischemic heart disease among patient characteristics, as well as ultrafiltration failure among reasons for withdrawal of PD.

Conclusions: In many cases, as the PD-patients accept the available period and combination treatment, it may be difficult to examine the possibility of PD treatment.

Throughout solo PD treatment, we have achieved certain guidelines (routine performance of PET and assessment of dialysis doses, early detection, early treatment, etc. of peritonitis, catheter dysfunction, etc.; and prevention and treatment of complications), allowing examination of long-term ongoing PD. The factors affecting the long-term continuation were diabetes, ischemic heart disease, and ultrafiltration failure. No influence of peritonitis morbidity was observed. PD is a therapeutic approach using biological membranes, and caution is necessary with the continuation of this approach. However, long-term continuation of better PD may be achieved by periodic screening of PD itself and appropriate treatment of complications specific to this form of dialysis.

PUB432

Anuria: A Villain in Quality of Life and Sexual Dysfunction in Peritoneal Dialysis Pedro Francisco Azevedo,[1] Ricardo Santos,[2] Jose Duraes,[1] Olivia Santos,[1] Maria João Carvalho,[1] António Manuel Nunes Cabrita,[1] Anabela S. Rodrigues.[1] *[1]Nephrology, Hospital Santo António, Porto, Portugal; [2]Gynecology and Obstetrics, Centro Hospitalar do Alto Ave, Guimarães, Portugal.*

Background: There are several studies that address the quality of life of patients on peritoneal dialysis (PD). Sexual dysfunction, anxiety and depression are highly prevalent in patients undergoing PD, but studies focusing these particular issues are scarce, especially, in an integrated approach to the patient with anuria.

This study aims to evaluate sexual dysfunction and its predictors among PD patients, particularly in patients without residual renal function.

Methods: In this observational, cross-sectional study, all chronic PD patients in one Center were asked to complete a self-reported questionnaire: the International Index of Erectile Function (IIEF) for men and the Index of Female Sexual Function (IFSF) for women. Both groups answered the Hospital Anxiety and Depression Scale (HADS) to evaluate the prevalence of depression and anxiety and the EuroQol (EQ5D) to measure quality of life (QoL).

Mann Whitney U and Chi-square tests were used for group comparisons. We assessed confounding variables with multivariate regression analysis.

Results: We evaluated 57 PD patients (50,9% males, mean age 53,9±15,7 years, 27,6% diabetes, 24.6% anuric). Anuric patients had higher CRP (P=0,011), lower serum uric acid (P=0,034) and lower nPCR (P=0,002). The prevalence of sexual dysfunction was higher in anuric patients (78,6 vs. 46.5%, P=0,036). Anuric males had worse QoL (P=0,014) and erectile function (P=0,04). On multivariate analysis, anuria, diabetes and smoking were independent predictors of worse IIEF score (P<0.001), in a model that accounts for 56% of sample variation (adjusted R square). The same was not true with the FSFI score in anuric women. Anuria did not have a significant impact in HADS score.

Conclusions: Sexual function and QoL assessment should be part of the integrated care of PD patients. Male patients with anuria, diabetes and smoking habits deserve closer attention in these issues.

PUB433

Our Experience with Encapsulating Peritoneal Sclerosis (EPS) Erzsebet Ladanyi,[1] Otto Arkossy.[2] *[1]Nephrology Center, Fresenius Medical Care, Miskolc, Hungary; [2]Dialysis Center, Fresenius Medical Care, Budapest, Hungary.*

Background: EPS is a rare but serious complication of mainly long-term peritoneal dialysis (PD). The number of long-term PD patients is increasing in our center. The clinical assessment of EPS is difficult due to the insidious development and broad clinical spectrum. The aim of this study was to evaluate the clinical features of our EPS cases and to identify possible risk factors.

Methods: Retrospective review of patients with diagnosis of EPS and the long-term patients (>60 months) over the last 11 years in our center. Demographic data, peritoneal equilibration test (PET), clinical outcomes were collected.

Results: 239 patients were treated with PD between 2000-2011. The mean duration of PD treatment was 36 months. There were 41 long-term patients (17%) with the average of 95 months PD treatment. The average D/P and the peritonitis rate at PD withdrawal were 0,68 and 1,9 /treatment. We identified 4 EPS cases (0,01%). Their mean duration of peritoneal treatment was 121 months. Their mean peritonitis rate was 5/treatment. The reasons of finishing PD were I type UF failure and inadequate dialysis. Their avarage D/P at PD cessation was 0,85 (0,82-0,88). All of EPS cases had been diagnosed after withdrawal from PD and being on HD treatment. Mean time from stopping PD to time of symtomps and diagnosis of EPS was 5-6 months in all cases! The typical symtomps of EPS were abdominal pain, vomiting, bloody ascites, fever, elevated CRP. The diagnosis was confirmed by CT scan and laparoscopy. Three patients had laparatomy, enterolysis and succesful treatment with temporary parenteral nutrition and immunosuppessive regime (prednisolon, azathioprin) for 6 months. One of them needed a second operation after 8 months.

Conclusions: The first 6 months HD treatment of previously long-term PD patient could be critical of development of EPS based our experience. Along with other published cases it can suggest that cessation in itself maybe a risk factor beside of long term PD (>5 ys), high permeabile peritoneal membran (D/P>0,8), and high peritonitis rate (>4-6). To know more about EPS we have already registered our cases in the European EPS Registry.

PUB434

Predictors of Cardiovascular Events in Peritoneal Dialysis Patients and Impact on Kt/V Adequacy Ana Pinho, André Fragoso, Anabela Malho, Pedro Neves. *Nephrology, Faro, Portugal.*

Background: The emerging evidence linking uremia to an increase in oxidative stress and endothelial injury will necessarily lead to new therapies designed to improve the devastating consequences of vascular diseases in renal failure. The aim of this study was to evaluate the predictive factors of cardiovascular events (CVE) in relation to peritoneal dialysis (PD) adequacy.

Methods: 105 consective PD patients were included; Charlson Comorbidity Index adjusted for age (CCI-age score), sCRP and biochemical data (Kt/V,Hb,Albumin,Ca,P,PTH,lipid profile) at baseline were calculated from the average of repeated evaluations on the first three months of stable PD.Time-averaged values of laboratory and dialysis parameters (Kt/V,transporter type,RRF) during PD period were determined, excluding data from acute states. CVE and death were recorded. The 3rd percentile sCRP level at baseline was defined as the high-risk sCRP group.

Results: 36.2% patients had a CVE(1.4 ep/pt-yr). After adjusting for potential confounders (CCI-age score>=5,gender,diabetes,hypertension,pulse pressure,peritonitis), low levels of albumin (HR= 2.7), high levels of sCRP (HR=1.04) and high levels of PTH (≥400pg/ml) (HR=9.8) remained as significant predictors for CVE. There were no differences between high-risk and low-risk sCRP groups regarding follow-up period, as for other laboratory data and dialysis parameters.The high-risk sCRP group showed a significant rise of sCRP during DP period (median high-risk vs low-risk sCRP groups, 7.7 vs 45mg/L;p<0.001). Each mg/L of the time-averaged sCRP value, adjusted for CCI-age score, was associated with a 0.8% increased risk for death (p=0.018) and 0.6% for CVE (p=0.02). There are no significant differences related to other dialysis parameters and biochemical data, with exception to lower time-averaged Kt/V in high-risk sCRP group (2.1 vs 2.8mg/L;p<0.02).

Conclusions: Higher baseline sCRP, PTH and lower levels of albumin appeared as independent predictors for CVE. The higher baseline sCRP values were associated with higher time-averaged sCRP during DP stage and lower time average Kt/V. These results suggest that more intensive dialysis could be indicated for patients with increased inflammation activity.

PUB435

Assessment of Hydration Status in Patients' Undergoing Peritoneal Dialysis in Relation to Gender Dorota Sikorska, Jolanta Kaczmarek, Maria Wanic-Kossowska, Bartlomiej Posnik, Krzysztof Hoppe, Krzysztof Schwermer, Pawel Samborski, Krzysztof Pawlaczyk, Andrzej P. Oko. *Department of Nephrology, Transplantology and Internal Diseases, Poznan University of Medical Sciences, Poznan, Poland.*

Background: Continuous overhydration is a common problem in patients undergoing peritoneal dialysis (PD). Multi-frequency bioimpedance analysis (BIA) offers the potential to accurately determine a patient's dry body weight. The purpose of the study was to use BIA to assess the level of hydration in PD patients with regard to gender. Secondarily, the results were compared with clinical assessment.

Methods: The examination involved 24 PD patients (female-F n=12, age 49.3±17.4; male-M n=12, age 64.8±17.2). The patients were divided according to gender, the level of hydration was evaluated twice (6 months interval) by means of clinical criteria and the use of BIA. The values of BP, nutritional status using Subjective Global Assessment (SGA), and concentration of NT-proBNP in serum were also analysed.

Results: Considerable differences were demonstrated in regard to the level of hydration and BP with BIA as compared to clinical assessment alone. Comparable values of systolic (F 140.33±22.13 vs. M 146.45±24.92 mmHg) and diastolic (F 83.17±13.43 vs. M 81.36±13.45 mmHg) BP were observed, but after 6 months there was a significant difference between genders in systolic (F 129.88±13.09 vs. M 145.36±16.76) but not in diastolic (F 78.00±8.76 vs. M 78.91±12.36) BP. Comparable SGA (F 8.8±3.13 vs. M 8.8±1.14) were observed in both groups, without a significant difference after 6 months. Initially no statistically significant difference between genders was found in NT-proBNP (F 3020.8 vs. M 2736.3 pg/mL) with increased values observed at 6 months (F 6746.6 vs. M 5811.1). The overhydration level was correlated to the concentration of NT-proBNP ($p<0.02$). Clinically observed overhydration was confirmed using BIA, which revealed physically undetectable overhydration.

Conclusions: BIA seems to be a better criterion of assessing hydration in reference to PD patients as compared to clinical assessment alone. The male gender may be a potential factor determining a higher tendency for continuous overhydration.

PUB436

Advance Care Plans: Haemodialysis Patient View Maria Da Silva-gane,[1] David Wellsted,[2] Ken Farrington.[1] *[1]Renal, Lister Hospital, Stevenage, Hertfordshire, United Kingdom; [2]Centre for Lifespan & Chronic Illness Research, University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.*

Background: Many patients on dialysis are elderly and have multiple co-morbidities. Whilst mortality is high the needs of patients who are approaching the end of their life often go unaddressed. We undertook a study to define attitudes and perceptions of haemodialysis (HD) patients regarding future care including end of life (EoL) care planning.

Methods: We administered a self-completion questionnaire to determine levels of concern and willingness to discuss end of life planning amongst our HD population. The questionnaire used a likert scale (1-9). We classified patients who responded positively to each item (response of 7 or more), response was equivocal (4-6), and negative (3 or less).

Results: 240 (75%) agreed to participate. Participation rates were comparable across age, gender, ethnicity and co-morbidity groups. Mean age of respondents was 63 years (range 17-89); 20% were from non-white ethnic groups. 104 (43%) were considered to have severe co-morbidity.

Less than 45% had thought about future options in face of deteriorating health. 58% thought they should have a personal EoL care plan. In the event of deteriorating health 88% wanted involvement in discussing future options. Those with higher symptom burden were more likely to want this ($p = 0.01$). The majority would wish 'everything done' should their health suddenly deteriorate, 82% would wish ITU and 75% were positive towards resuscitation. Patients >75 years had a more negative view in regard to resuscitation ($p=0.001$). There was a significant association between attitudes on the benefits of a personal EoL care plan and the wish for resuscitation ($p=0.012$). Overall, there was little evidence that attitudes towards future treatment options or EoL care planning were significantly influenced by current physical or mental health states.

Conclusions: Patients on HD are not averse to discussing EoL care planning, perhaps to ensure respect for their wishes about treatment escalation in a crisis. Further qualitative research is needed to enable us to have a more in depth understanding of the views of this patient group.

PUB437

Attitudes and Perceptions of Renal Staff to Advance Care Planning Maria Da Silva-gane,[1] David Wellsted,[2] Ken Farrington.[1] *[1]Renal, Lister Hospital, Stevenage, Hertfordshire, United Kingdom; [2]Centre for Lifespan & Chronic Illness Research, University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.*

Background: It is generally acknowledged by clinicians working in the renal field that there is a need for a supportive care approach for dialysis patients as they face difficult decisions towards the end of their life. The aim of this study was to explore the attitudes and perceptions of clinicians involved in the care of haemodialysis patients regarding advance care planning.

Methods: We conducted private one to one interviews using a semi-structured interview schedule. Interviews were audio taped and transcribed verbatim, before Grounded Theory analysis was undertaken.

Results: 12 members of staff; 3 consultant nephrologists, 6 HD nurses and 1 Social Worker were interviewed, time working in renal & haemodialysis ranged from 1.5 – 21 years (mean 13.4). The majority expressed discomfort regarding discussions around future end of life care planning or ACP. Some felt that they avoided the discussions and that even in the face of deteriorating health of the patient dialysis treatment would often continue. Underpinning apprehension were issues of; lack of time, the environment, sensitivity of the subject, lack of communication skills and support network. Many felt other members of the renal team (counsellors) as having the time, training and experience to develop these conversations. Also cited was concern of upsetting patients or destroying hope. Whilst in general ACP was felt to be useful, they were seen to be about withdrawal of dialysis rather than a holistic care plan.

Conclusions: Nursing staff, in particular, expressed discomfort in relation to discussions in regard to ACP. Some of this was centred on the misconception that the focus would be withdrawal of dialysis. This leads us to consider that the focus on 'Advance' introduces, for some, apprehension and pre-conceived ideas. An alternative is to consider a shared care plan and focus discussions on realistic options in the context of current health status and likely future prognosis. If this is accomplished in an unambiguous way it could mean more open and realistic communications between the renal team and patients.

PUB438

Dialysis Staff Attitudes toward Deceased Organ Donation among End Stage Renal Disease Patients Allyce L. Haney,[1] Ann Andrews,[1] Holly Jenkins Riley,[4] Remonia Chapman,[3] Kenneth A. Resnicow,[5] Jerry Yee.[2] *[1]National Kidney Foundation of Michigan, Ann Arbor, MI; [2]Henry Ford Hospital, Detroit, MI; [3]Gift of Life Michigan/MOTTEP, Ann Arbor, MI; [4]Greenfield Health Systems, Bingham Farms, MI; [5]School of Public Health, University of Michigan, Ann Arbor, Mi.*

Background: The organ donor waiting list continues to expand. Patients with end-stage renal disease (ESRD) are typically not viewed, by themselves or their health care team, as potential donors after death. However, ESRD patients are eligible to donate organs. We examine the attitudes of renal team members, excluding physicians, about the potential of ESRD patients to donate organs after death, as part of a larger study that evaluates if peer mentoring increases ESRD patients enrolled in the Michigan Organ Donor Registry (MODR).

Methods: The cluster randomized design, controlled intervention study is conducted in collaboration with the National Kidney Foundation of Michigan (NKFM), Greenfield Health Systems (GHS), Henry Ford Health System, Gift of Life Michigan, and the University of Michigan. Staff at 12 GHS hemodialysis units in Southeast Michigan will receive training in donation. Hemodialysis units are then randomized to an intervention or control group. ESRD patients in intervention units are assigned peer mentors and will meet 7 times during a 4-month interval, utilizing a mix of in-person and phone contacts. Peer mentor-patient meetings teach coping mechanisms for chronic illnesses and discuss leaving a legacy by MODR enrollment. Patients in comparison units receive mailings about donation and the MODR. Renal team staff were surveyed regarding attitudes toward organ donation and the ability of ESRD patients to donate organs after death.

Results: 87 Greenfield staff were surveyed in 4 hemodialysis units. We expect to survey 10 more staff in 2 more units in the next 3 months.

Conclusions: Peer mentoring of ESRD patients is a potential alternative for augmenting the organ donor pool. Evaluations of organ donation knowledge and attitudes and self-reported donation status will be conducted to determine the success of this novel initiative.

Funding: Other U.S. Government Support

PUB439

Metabolic Acidosis in Hemodiafiltration: The Stewart-Figge Approach Sara Mohrbacher, Maria Julia C.L.N. Araujo, Hugo Abensur, Rosa M.A. Moyses, Manuel C. Castro, Rosilene M. Elias. *Nephrology, Universidade de São Paulo, Brazil.*

Background: On-line hemodiafiltration (HDF) represents the most advanced and clinically appropriate renal replacement therapy available. We hypothesized that HDF and high-flux conventional hemodialysis (HD) would be similar regarding metabolic acidosis correction, given that, in HD, there is mass transfer of HCO_3^- into the blood due to diffusion, whereas in HDF, there is a loss of HCO_3^- by convection, which is compensated for by diffusion and fluid replacement. To test that hypothesis, we used the Stewart-Figge approach to compare HDF and HD in the same patients.

Methods: We performed blood gas and biochemical analyses in 7 dialysis patients before and after the midweek dialysis session, collecting blood samples during 1 regular HD session and during 1 post-dilution on-line HDF session, using the same dialysis fluid. The effective strong ion difference (SIDe) was calculated by Figge's formula and the strong ion gap (SIG) was determined by the difference between the apparent SID (SIDa) and SIDe.

Results: Overall, HD and HDF both improved acidosis; reduced K and Cl; and increased HCO_3^- and SIDe. However, the ΔpH, ΔK, ΔHCO_3^-, Δbase excess and ΔSIDe were significantly higher in HDF than in HD. The ΔSIG correlated with Δbase excess in HD and HDF ($r=-0.859$ and $r=-0.755$; $p<0.05$ for both).

	HDF		HD	
Variable	pre	post	pre	post
pH	7.40±0.04	7.46±0.04*†	7.39±0.04	7.43±0.05
K, mEq/L	6.0±0.9	4.0±0.6*	5.1±0.6	3.6±0.3*
Cl, mEq/L	99.1±2.9	95.0±3.8*	99.7±5.3	96.1±3.5*
HCO_3^-, mmol/L	25.6±2.6	30.5±2.0*†	23.7±1.4	28.5±1.9*
Base excess, mmol/L	1.1±2.5	6.5±2.2*†	-0.4±1.9	4.2±2.1*
SIG	9.8± 3.9	4.7± 1.9*	10.1± 1.9	4.6±2.4*
SIDa	47.8±1.6	48.7±1.8*	47.0±2.2	46.7± 2.5
SIDe	37.9±4.8	45.0 ±3.1*†	36.9±1.7	42.1±2.0*

*p<0.05 pre vs. post dialysis; †p<0.05 vs. HD (Δ)

Conclusions: Uremic acidosis was well corrected in HD and HDF, decreasing SIDe and increasing SIG. The Stewart-Figge approach does not improve the characterization of acid-base status in patients on HDF. However, the HCO_3^- gain was much greater in patients on HDF, indicating the potential risk of alkalosis in this modality.

PUB440

Increased Plasma AVP Levels during Severe Hyponatremia: Paradoxical Secretion? Naoko Iwata,[1] Miho Sasaki,[1] Ryo Horibe,[1] Makoto Ikeniwa,[1] Masako Yamauchi,[1] Takashi Murase,[1,2] Ikuo Yamamori.[1] *[1]Endocrinology and Metabolism, Japanese Red Cross Nagoya Daiichi Hospital, Naogya, Aichi, Japan; [2]Endocrinology and Diabetes, Nagoya Medical Center, Nagoya, Aichi, Japan.*

Background: The regulatory mechanisms controlling arginine vasopressin (AVP) secretion remain to be elucidated in patients with the Syndrome of Inappropriate Antidiuretic Hormone secretion (SIADH). Here we examined the relation between serum [Na^+] and plasma AVP levels in patients with hyponatremia.

Methods: Analysis of plasma AVP levels in 264 samples from 171 patients measured in our hospital during 2010.

Results: There were 64 cases of hyponatremia due to various etiologies: SIADH (26), diuretic use (14), hypovolemia (13), and other causes (11). There were no significant differences between serum [Na^+] and plasma AVP levels among the groups. A U-shaped curve best fit the relationship between serum [Na^+] and plasma AVP in the SIADH group, with the nadir of the curve at a serum [Na^+] of approximately 120 mmol/L. For serum [Na^+] ≤120 mmol/L, there was a significant negative linear correlation (R^2=0.6519, p=0.028) between serum [Na^+] and plasma AVP. There were no significant correlations between plasma AVP levels and BUN, uric acid, or percent change in circulating blood volume calculated from the change in hematocrit. No apparent correlations were observed bewtween plasma AVP levels and neurologic symptoms. In contrast, there was a positive correlation between serum [Na^+] and plasma AVP levels with serum [Na^+] levels >120 mmol/L. For serum [Na^+] ≤120 mmol/L, the reglession lines representing the relation between [Na^+] and AVP were almost identical in the SIADH and the diuretic-treated groups, but the slope of the regression line was steeper in the hypovolemic group.

Conclusions: There was an apparently paradoxical increase in AVP secretion associated with the decline in serum [Na^+] during severe hyponatremia. It is possible that higher plasma AVP levels caused more water retention and resulted in further decreases in serum [Na^+], but there were no apparent correlations between plasma AVP levels and measures of volume status. Also, it would be difficult to explain this finding simply by neurologic symptoms such as nausea.

PUB441

Traumatic Encephalopathy Leading to Cerebral Salt Wasting and Hyponatremia with Permanent Brain Damage Allen I. Arieff. *UCSF.*

Background: 1.5 to 2 million USA civilians per year suffer traumatic brain injury. 4.5% of these sustain a chronic and severe disabling condition. It has not been previously noted that cerebral salt wasting could lead to hyponatremia which might result in severe brain damage or death. I was consulted on 14 individuals who suffered acute traumatic encephalopathy {(motor vehicle accident (10), falls (7)}. All patients were admitted via the ER and had closed head injury by neuroimaging studies. Brain lesions consisted of: subdural hematoma (6), diffuse cerebral edema (5), cerebral infarct (2), subarachnoid bleed (1). After admission, all were receiving isotonic fluids and becoming progressively more alert.. All developed hyponatremia after 57±32 hours, with plasma Na falling from 138±4 to 119±7 mmol/L. All 14 patients were initially diagnosed as having SIADH on the basis of urine osmolality > 400 mOsm/kg. All were initially treated with fluid restriction (below 1000 ml/day). Over 24 hours of fluid restriction, the mean BP declined from 135/63 mm Hg to a systolic BP below 100 mm Hg (92/48 mm Hg). Urine Na (184±66 mmol/day) was significantly above control (113±65 mmol/day, p <0.01). The systolic BP was restored to above 100 mm Hg by IV infusion of 0.9% NaCl. Two patients suffered hypoxic brain damage while the other 12 recovered. The 12 surviving patients were eventually maintained on oral NaCl tablets (mean 2.5 gm/day). Conclusions: a) closed head trauma can lead to hyponatremia secondary to a cerebral Na wasting syndrome; b) fluid restriction can lead to shock, worsening of hyponatremia and hypoxic brain damage; c) the Na wasting could eventually be controlled with oral NaCl tablets.

PUB442

Ultrafiltration Does Not Improve Neurohormonal Activation in Acute Decompensated Heart Failure Natallia Maroz, Amir Kazory. *Division of Nephrology, Hypertension, and Renal Transplantation, University of Florida, Gainesville, FL.*

Background: Blood urea nitrogen (BUN) has recently emerged as a marker of neurohormonal activation, which is considered the central pathophysiologic mechanism of acute decompensated heart failure (ADHF). Compared with conventional therapies, extracorporeal ultrafiltration represents a therapeutic strategy that is superior in symptomatic improvement of ADHF. However, there is no data on the impact of ultrafiltration on the underlying pathophysiologic mechanisms of ADHF and its potential effects on outcomes.

Methods: We searched articles cited in PubMed database from 1970 to 2012 using key words: "ultrafiltration" and "heart failure". All types of publications written in English language journals including case reports and case series were identified and relevant articles were selected. We then reviewed and compared the results of those studies which used the novel portable device approved by the Food and Drug Administration (FDA) exclusively for management of volume-overloaded patients with ADHF.

Results: A total of 60 relevant articles were identified on the use of ultrafiltration in the setting of ADHF. Nine studies using the FDA-approved ultrafiltration device were selected; 4 were randomized controlled trials and 5 without a control group. BUN levels were not reported in 2 studies. Four studies reported no significant change in BUN, while 3 studies found an increase in BUN levels following ultrafiltration therapy. None of these studies directly evaluated the impact of ultrafiltration on neurohormonal status (e.g. serum arginine vasopressin levels) or long-term survival of patients with ADHF.

Conclusions: While the higher efficacy of ultrafiltration for rapid decongestion and improvement in symptoms has been demonstrated in patients with ADHF, currently available data does not support its beneficial impact on BUN which is considered a marker of neurohormonal activation in this setting. Further studies are needed to assess whether this observation translates into lack of improvement in long-term outcomes due to inability to modify underlying pathophysiologic mechanisms in this population.

PUB443

Is Sodium Polystyrene Sulfonate (SPS) Effective in Treating Hyperkalemia in Acute Setting? Manoj Bhattarai,[1] Douglas G. Shemin.[2] *[1]Internal Medicine, Memorial Hospital, Pawtucket, RI; [2]Renal, Rhode Island Hospital, Providence, RI.*

Background: With conflicting reports regarding effectiveness and adverse effects of SPS, it is still used as an initial treatment in majority of hyperkalemia cases in emergent conditions. We sought to evaluate its effectiveness.

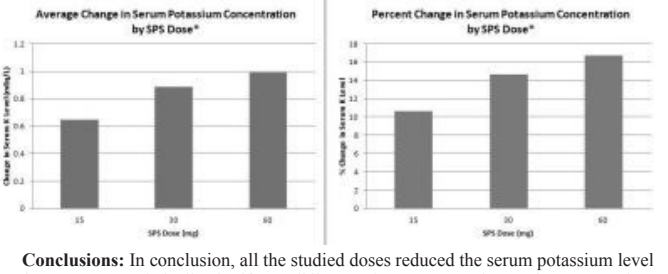
Methods: We carried out a retrospective study of patients who were admitted to Rhode Island Hospital in a six month period and received a single dose of SPS as an initial sole agent for the treatment of hyperkalemia. There were 559 cases of hyperkalemia treated with SPS alone. Patients received one of the three different doses of SPS (15 gms, 30 gms, or 60 gms) orally. Outcomes examined were change in serum potassium level and percent change in serum potassium level while accounting for the potential of repeated observations of the same patient. Outcomes were adjusted for pre-treatment serum potassium concentrations and for creatinine level.

Results: Out of 559 SPS cases that were examined, 179 cases received 15 gms, 295 cases received 30 gms, and 85 cases received 60 gms of SPS. Table 1 depicts the unadjusted mean serum potassium levels before and after SPS for the true dosage groups.

Table 1

SPS dose (Grams)	Number of cases (n)	Mean Potassium level before SPS	Mean Potassium level after SPS	K change
15	179	5.79	5.16	0.62
30	295	5.83	4.97	0.87
60	85	6.25	5.07	1.19

The mean adjusted change in potassium after 15 gms, 30 gms, and 60 gms of SPS are illustrated in figure below. The reductions in serum potassium level between 30 and 60 gms groups were no different (p=0.179). None of the subjects in any group developed symptomatic hypokalemia during the study.



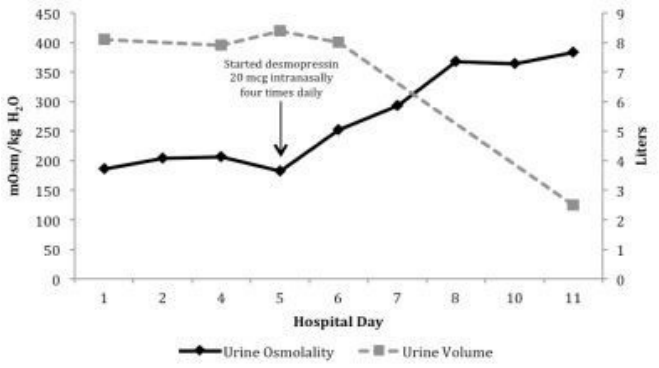
Conclusions: In conclusion, all the studied doses reduced the serum potassium level but there was no statistically significant difference between SPS doses of 30 gms and 60 gms. Thus, cautious use of higher doses is warranted to prevent potential complications.

PUB444

Ifosfamide-Induced Fanconi Syndrome and Concomitant Desmopressin-Responsive Nephrogenic Diabetes Insipidus Sophia C. Kamran,[1] William Franklin Pendergraft,[2] David Harmon,[2] Mario F. Rubin.[2] *[1]Harvard Medical School, Boston, MA; [2]Massachusetts General Hospital, Boston, MA.*

Background: Ifosfamide, an alkylating chemotherapeutic agent, has been reported to cause Fanconi syndrome in cancer patients and can also cause nephrogenic diabetes insipidus (NDI), although this latter complication is rare. The most commonly proposed risk factors for nephrotoxicity include a high cumulative ifosfamide dose, young age at treatment, prior exposure to cisplatin, and a prior reduction in kidney mass.

Methods: We report here a case of a young adult patient who developed Fanconi syndrome and concomitant NDI after only receiving a cumulative dose of 23.4 g/m^2 of ifosfamide for recurrent rhabdomyosarcoma. Remarkably, her concentrating tubular defect responded to repetitive administration of intranasal desmopressin as evidenced by a normalization of urine osmolality.



Conclusions: The pathophysiologic mechanisms responsible for ifosfamide mediated tubular toxicity (proximal and distal) remain elusive. Despite cessation of ifosfamide, our patient has continued to require treatment for the Fanconi syndrome (suggesting irreversible proximal tubular damage) while her polyuric state has resolved. This pattern of residual injury suggests that the distal tubular dysfunction appears to be partial and reversible. Therefore, clinicians should consider repetitive doses of desmopressin as treatment for ifosfamide-induced NDI in order to prevent severe volume depletion.

PUB445

Body Temperature, and Not Disease Specificity, Is the Major Factor Responsible for Hyponatremia in Children with Common Febrile Diseases Hideki Matsumura,[1] Akira Ashida,[2] Akihiko Shirasu,[2] Hyogo Nkakura,[2] Motoshi Hattori,[3] Hiroshi Tamai.[2] *[1]Pediatrics, Hirakata City Hospital, Osaka, Japan; [2]Pediatrics, Osaka Medical College, Osaka, Japan; [3]Pediatric Nephrology, Tokyo Women's Medical University, Tokyo, Japan.*

Background: Hyponatremia is a common electrolyte abnormality in hospitalized patients. We previously reported that the serum sodium level is inversely correlated with body temperature in children with common febrile diseases (ASN 2011, FR-PO1741). However some reports that have pointed out the relationship between clinical diagnosis and hyponatremia, have paid little attention to body temperature. Here we examined pediatric patients with hyponatremia in relation to their clinical diagnoses, excluding the effect of body temperature.

Methods: In this retrospective case study based on chart review, 1,973 children presenting with acute illnesses at Hirakata City Hospital between November 2008 and October 2009, and for whom blood test data were available, were enrolled. The median age of this cohort was 2.7 years. The patients were classified into four groups on the basis of body temperature: <37°C, 37°C, 38°C and ≥39°C. Within each temperature group, serum sodium levels in patients with different clinical diagnoses were compared.

Results: The mean serum sodium levels in the temperature groups were, in ascending order, 138.6 mEq/L, 137.3 mEq/L, 136.1 mEq/L and 134.6 mEq/L, respectively. The mean sodium level in patients with febrile seizure in the 38°C group was 134.9 mEq/L, and that in patients with Kawasaki disease in the ≥39°C group was 132.3 mE1/L, both being significantly lower than the levels in the patients with other clinical diagnoses. However, there were no significant differences in sodium levels between patients with different diagnoses except for these two diseases only at the particular febrile conditions.

Conclusions: There is no specific disease that leads to hyponatremia, regardless of body temperature. Body temperature, rather than disease specificity, appears to be a major factor contributing to the development of hyponatremia in children with common febrile diseases.

PUB446

Association between Extracellular Fluid Status and Autonomic Function in Hemodialysis Patients Yi Shin Chen,[1] Ming-Ju Wu.[2] *[1]Division of Nephrology, Department of Medicine, Taichung Tzu Chi General Hospital, Taichung, Taiwan; [2]Division of Nephrology, Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan.*

Background: Autonomic dysfunction is common in hemodialysis patients and associated with sudden cardiac death. Fluid control affects the outcome especially for cardiovascular mortality and morbidity. Little is known for the association between extracellular fluid status(ECF) and autonomic function.

Methods: In this prospective study, autonomic function was measured by frequency domain analysis of heart rate variability (HRV) from 5 minutes ECGs in 24 hemodialysis patients(DM/non-DM, 9/15). Bioelectrical impedance analysis (BIA) was conducted on all patients and ECF was acquired via BIA. Correlation between ECF and HRV was investigated.

Results: ECF was significantly correlated to variance of RR interval values (r=-0.442, P=0.031) and total power (r=-0.406, P=0.049) both of which represent summation of sympathetic and parasympathetic tone. No significant correlation between gender and ECF. No difference of ECF between DM and non-DM patients.

Conclusions: ECF was negatively related to autonomic function. Poor fluid status control may worsen the autonomic function. Further investigation is needed.

PUB447

Chronic Outpatient Hyponatremia: Reduced Mortality with Thiazide Diuretic Associated Hyponatremia Greg Nieckula,[1] Brittany J. Hale,[2] James A. Tumlin.[3] *[1]Internal Medicine, University of Tennessee College of Medicine, Chattanooga, TN; [2]Clinical Research, Southeast Renal Research Institute, Chattanooga, TN; [3]Internal Medicine Division Nephrology, University of Tennessee College of Medicine, Chattanooga, TN.*

Background: Hyponatremia is a signifcant cause of morbidity and mortality among patients with chronic kidney disease (CKD). While multiple risk factors including heart failure, advanced cirrhosis and other disorders contribute to hyponatremia in hospitalized patients, few studies have investigated hyponatremia in outpatient CKD patients. Moreover, it is unknown whether thiazide diuretics worsen patient mortality in the out patient setting. To investigate this, we evaluated the effect of thiazides on patients with three different levels of hyponatremia.

Methods: We retrospectively reviewed 7287 patient records from an outpatient CKD clinic and found a 14.1% incidence of hyponatremia. Of the 1026 patients with hyponatremia, 898 had mild (126-130), 178 moderate (121-125), and 50 severe (115-120) hyponatremia. The annulaized mortality rate was calculated for each group using date of death. Non-parametric analysis was performed using a Chi-squared test when appropriate.

Results: Mean Na+ for the mild, moderate and severe groups were 128+0.04, 123+0.1, and 118+0.2 meq/L respectively. The annualized mortality was 17.8%, 22.4%, and 19.4%/year for each group respectively. There was no difference in mortality between groups. For patients with severe hyponatremia, there was a trend toward worse mortality but this was not signfican.

Hyponatremia	Severe	Moderate	Mild
	Na+ (115-120) Pt#-50	Na+ (121-125) Pt#-178	Na+ (126-130) Pt#-898
Mean Na+	118.4	123.4	128.4
Total Mortality	19.4%/year	22.4%/year	17.8%/year
Mortality Thiazide	13.5%/year	32.2%/year	16.7%/year
Mortality Non- Thiazide	31.3%/year	18.7%/year	18.4%/year

Conclusions: In conclusion, we find that that out-patient hyponatremia is common and associated with significant mortality. A serum Na+ below 130 meq/l was associated with a 17% annual mortality, but did not worsen with falling sodium. These data suggests that out-patient hyponatremia is common and contributes to the mortality of CKD patients.

Funding: NIDDK Support, Clinical Revenue Support

PUB448

Risk Factors for Developing Significant Hyponatremia in Thyroid Cancer Patients Undergoing Low Iodine Diet and Radioactive Iodine Therapy Hyeong Cheon Park, Seung Kyu Kim, Seung Kyo Park, Kihyun Kim, Gi Young Yun, Jung Eun Lee, Hoon Young Choi, Sung-Kyu Ha. *Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.*

Background: Postoperative management of high risk thyroid carcinoma patients consists of radioactive iodine (RAI) therapy. These high risk patients are given low-iodine diet along with levothyroxine withdrawal to optimize RAI uptake by thyroid tissues and patients experience mild hyponatremia during this short-term hypothyroid status. However, some patients develop life threatening severe hyponatremia. This study aimed to assess risk factors for developing siginificant hyponatremia during low iodine diet and RAI therapy.

Methods: Data for patients with thyroid carcinoma who underwent total thyroidectomy and RAI therapy from July 2009 to February 2012 at Gangnam Severance hospital was retrospectively collected. Clinical and biochemical parameters including serum sodium and thyroid function tests were assessed along with medication history.

Results: Total 2223 patients [female: 1679 (76.3%)] were enrolled and the mean age was 47±11 yrs. The number of patients with serum sodium level less than 130 mEq/L (significant hyponatremia), 131 to 135 mEq/L, and above 136 mEq/L (normonatremia) were 44 (2.0%), 263 (11.8%), and 1916 (86.2%), respectively. Three hundred fifty patients (15.7%) were older than 60 years of age, 44 patients (2.0%) used thiazide agents, and 23 patients (1.0%) had lung metastasis. Patients who developed significant hyponatremia were older (46±11 vs. 61±11, p<0.001) and showed lower mean TSH level (82.8±19.7mcIU/mL vs. 71.3±24.6mcIU/mL, p<0.001) and lower mean serum sodium level measured at the start of RAI therapy (139±2mEq/L vs. 137±4mEq/L, p<0.001). Logistic regression analysis showed that thiazide use (OR 5.4), lung metastasis (OR 5.1), older age (≥ 60 yrs, OR 7.9) were independent risk factors for occurrence of significant hyponatremia in patients undergoing RAI therapy after total thyroidectomy.

Conclusions: Our data suggest that old age, thiazide medication use or presence of lung metastasis are risk factors for developing significant hyponatremia during RAI therapy after total thyroidectomy.

PUB449

Randomized, Double-Blind, Placebo-Controlled Trial of Tolvaptan in Cancer Patients with Hyponatremia: Preliminary Report Najeeba Ali, Shana Palla, Marina George, Amit Lahoti, Abdulla K. Salahudeen. *General Internal Medicine and Biostatistics, University of Texas MD Anderson Cancer Center, Houston, TX.*

Background: Hyponatremia is the most common electrolyte abnormality, yet it is often underdiagnosed and untreated. We recently reported a high rate of hyponatremia in hospitalized cancer patients (Am J Kidney Dis. 2012;59:222-8). Therapy with V2 receptor antagonists that allow renal water excretion is a major breakthrough. Tolvaptan is a newer

orally active antagonist, but data is limited whether it can ameliorate hyponatremia in cancer patients. The objective is to compare the rate of hyponatremia correction between tolvaptan and placebo treated cancer patients over a 14-day period, along with comparision of length of hospital stay and mental test score change.

Methods: This study, based on adaptive randomization, is designed to accrue a minimum of 30 and a maximum of 120 patients. Inclusion criteria: Euvolemic or hypervolemic adult patients with cancer admitted to hospital with serum sodium of 125-130 mEq/L. Exclusion criteria: Critical illness, renal failure, volume depletion- or hormonal deficiecy-related hyponatremia or taking strong Cyp-3A4 modulators.

Results: Over the last 12 months we have identified 8670 patients with hyponatremia, 104 were screened and 45 eligible patients were randomized to receive either tolvaptan 15 mg daily or matching placebo. The dose was titrated aiming for a serum sodium correction of 6-12 mEq/L daily to a target of 136-145 mEq/L. Of the 45 patients randomized, 3 discontinued the study due to polydipsia, 3 were moved to ICU for worsening cancer, 3 were transferred to hospice care, 6 withdrew consent and 5 lost to follow up. Of the 25 subjects completed this ongoing blinded study, 15 were males and the age was 63±12 yr (mean±SD). Serum sodium and urine osmolality (mOsm/kg) on D_0 and D_{14} were 128±1 and 136±5 mEq/L and 400±169 and 351±127 respectively.

Conclusions: Our preliminary review supports the feasibility of the study in hospitalized cancer patients. A higher dropout rates mostly due to advanced illness and poor cancer outcomes is noted. A larger patient sample size than initially estimated may be required to complete the study.

Funding: Pharmaceutical Company Support - Otsuka

PUB450

Hyperkalemia of Angiotensin-Converting Enzyme Inhibitors or Angiotensin Receptor Blockers in Hemodialysis: A Meta-Analysis Qian Zhang, Yan Chen, Lili Liu, Yongman Lv, Zufu Ma. *Tongji Hospital.*

Background: Agiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ABRs) are well accepted for the cardiorenal-protective benefits added to antihypertensive effects in patients with chronic renal insufficiency, but associated with an increased risk of hyperkalemia. However, the effect of ACEIs or ARBs on serum potassium in hemodialysis (HD) patients is unclear. So we conducted a meta-analysis to evaluate the safety of ACEIs or ARBs use on the hyperkalemia in HD patients.

Methods: We searched Medline, Embase, the Cochrane Library, as well as reference lists of eligible and review articles for the randomized clinical trials (RCTs) on comparison of ACEIs/ARBs or placebo in HD patients. 224 citations were identified. Eight met the eligibility criteria and were subjected to Meta analysis by employing the Cochrane Collaboration's RevMan 4.2 software package.

Results: The results showed that there was no difference on hyperkalemia in HD patients between ACEIs or ARBs group and control group (ACEIs vs Control: RD: 0.03; 95% CI: -0.13-0.18; Z=0.34; P=0.73; ARBs vs Control: RD: -0.02; 95% CI: -0.07-0.03; Z=0.75; P=0.45). However, the serum potassium between ACEIs or ARBs group and control group in HD patients has significant difference (ACEIs vs Control: WMD: 0.10; 95% CI: 0.06-0.15; Z=4.64; P<0.00001; ARBs vs Control: WMD: -0.24; 95% CI: -0.37--0.11; Z=3.58; P=0.0003).

Conclusions: It is concluded that the use of ACEIs or ARBs will not cause an increased risk of developing hyperkalemia in HD patients. But the serum potassium will be increased with use of ACEIs in HD patients. So the serum potassium concentration should still be closely monitored when the medication of ACEIs is taken during the maintenance hemodialysis.

PUB451

Blood and Urinary Physicochemical Alterations in the Course of Acute Kidney Injury in Critically Ill Patients Etienne Macedo, Alexandre Toledo Maciel, Marcelo Park. *University of Sao Paulo.*

Background: Metabolic acidosis in acute kidney injury (AKI) is generally attributed to increases in serum unmeasured anions and phosphate. The combination of urinary electrolyte composition and urinary strong ion difference can be a parameter to assess adequate renal handling of metabolic acidosis. The aim of our study was to assess sequential blood and urinary physicochemical parameters and evaluate the potential differences between transient and persistent AKI.

Methods: Blood labs and spot urine sample were daily collected from patients with urinary catheters in a mixed ICU. Serum creatinine, strong ion difference, strong ion gap (SIG), phosphate levels, urinary sodium (NaU), chloride (ClU) and 2-h creatinine clearance were measured daily. AKIN sCr criterion was used to determine AKI diagnosis with daily sCr adjusted for fluid balance. Transient AKI was defined as AKI resolution within 72 hours.

Results: 113 out of 168 patients (67.3%) developed AKI during the follow up period. Of these, 50.4% had a transient AKI, and 49.6% a persistent AKI. AKI development was characterized by increases in SIG and phosphate levels and decreases in both NaU and ClU values. SID value remained stable. In transient AKI, these alterations were also transient, reaching values similar to those of non-AKI patients 2 days after the AKI diagnosis.

Blood and urinary physicochemical parameters in the course of acute kidney injury.

		Day of AKI diagnosis by sCr (n=168)	Day +1 (n=164)	Day +2 (n=117)
SIG (mEq/L)	No-AKI	4.8 [2.9,7.3]	4.8 [3.3,6.4]	5.2 [4.1,7.1]
	transient	5.9 [4.1,7.3]	5.1 [3.5,7.1]	4.3 [2.0,6.6]
	persistent	7.1 [4.7,9.7]	8.7 [5.8,10.8]	7.6 [5.2,10.3]
	P	.198	<.000	<.000
Phosphate (mEq/L)	No-AKI	1.9 [1.3,2.3]	1.4 [1.2,1.9]	1.4 [1.1,1.9]
	transient	2.2 [1.6,2.8]	1.7 [1.3,2.0]	1.3 [1.1,2.0]
	persistent	2.4 [1.7,3.1]	2.2 [1.6,2.8]	2.2 [1.6,2.7]
	P	.122	.007	<.000
Urinary Cl (mEq/L)	No-AKI	113 [39,173]	109 [52,163]	125 [95,184]
	transient	56 [23,101]	68 [31,135]	135 [80,191]
	persistent	39 [17,84]	42 [15,97]	60 [22,103]
	P	.221	.001	<.000

P for difference between transient and persistent AKI.

Conclusions: Though transient and persistent AKI have the same blood and urinary physicochemical characteristics, they differ in severity and duration.

PUB452

Does Bio-Impedance Analysis Predict Volume Overload States and Clinically Relevant Outcomes in Septic Intensive Care Unit Patients? Bram Rochwerg, Jason H. Cheung, Peter Margetts, Scott K. Brimble, Catherine M. Clase, Christine M. Ribic, Deborah Cook, Azim S. Gangji. *Department of Medicine, St. Joseph's Healthcare, Hamilton, ON, Canada.*

Background: The mortality rate in patients with septic shock is as high as 40%. The initial management of sepsis has been well-established through early-goal directed therapy. Beyond the acute setting, persistent hypervolemia results in prolonged mechanical ventilation, the need for renal replacement therapy, and increased mortality.

Clinical assessment of volume status is limited by the absence of a feasible, continuous, non-invasive methods. Bio-impedance analysis (BIA) devices are compact, portable and can be used to make repeated measurements. BIA vector length has been previously shown to correlate with volume status in hemodialysis populations. We hypothesized that the change in BIA measurements is predictive of extubation status in patients who have SIRS due to infection and require mechanical ventilation.

Methods: We conducted a prospective observational study targeting 52 ICU patients. BIA is was measured on ICU admission and then every 4 days until discharge. Patients were followed for 30 days. Secondary outcomes included mortality, rates of acute kidney injury requiring dialysis, and length of stay in the ICU. A correlation between BIA with known measures of volume status was completed.

Results: The baseline vector length correlated significantly with the clinical edema score, proBNP serum level & CVP ($p<0.05$ for all). As far as our primary outcome of ventilator requirement during the first 30 days after enrolment, we did see that for every 50 unit increase in VL (ohms/m), there was a 22% increase in having a "status of extubation" (p=0.126). This makes sense based on our hypothesis that longer vector lengths should be correlated with improved outcomes.

Conclusions: The significant correlation between vector lengths with known clinical measures of volume status strengthens the construct validity of the BIA method in assessing volume status in ICU patients. This pilot study has proved to be feasible and may identify BIA as a safe and easy bedside measure to assess ICU patients' volume status.

Funding: Private Foundation Support

PUB453

Value of Determining Fractional Excretion of Urate in Nonedematous Hyponatremia James Drakakis,[1] Louis J. Imbriano,[1] Shayan Shirazian,[1] Dympna Gallagher,[2] Maria Marotta-kollarus,[1] Maanvi Kumar,[1] John K. Maesaka.[1] [1]*Department of Medicine, Winthrop University Hospital, Mineola, NY;* [2]*Department of Body Composition, St Lukes Hospital.*

Background: FEurate is increased in SIADH and cerebral/renal salt wasting (RSW), but normalizes in SIADH and remains persistently increased in RSW after correction of hyponatremia. We recently reported a normal FEurate to be highly suggestive of reset osmostat (RO) in patients with nonedematous hyponatremia.

Methods: We conducted a prospective study to determine the diagnostic value of determining FEurate in nonedematous hyponatremic patients.

Results: Of 25 consented patients, 13 were found to have increased FEurate > 12%. SIADH could be confidently differentiated from RSW in only 4 patients. Two had SIADH based on an increase in total body (TBW) and extracellular (ECW) water as determined by deuterium and sodium bromide, respectively. Two had RSW; 1 patient with pneumonia without cerebral disease had decreased TBW and ECW and 1 had subarachnoid hemorrhage with increased FEurate and normonatremia. The remaining Twelve patients had normal FEurate of 4-11% that was consistent with RO; TBW and ECW were increased in the one patient studied and 3 had dilute urines on random urine samples. Determinations of plasma renin, aldosterone and BNP, and urine sodium concentrations were generally not helpful and all 25 patients had diverse comorbid conditions.

Conclusions: These studies demonstrate how difficult it is to differentiate SIADH from RSW; RSW was again noted in a patient without cerebral disease, supporting our previous proposal to change cerebral salt wasting to RSW because RSW is being noted in increasing number of patients without cerebral disease. RO is common in hospitalized patients with nonedematous hyponatremia. RO can be readily identified by a normal FEurate and should be considered a separate clinical entity and not designated as type C SIADH, because it is patho-physiologically different from SIADH by virtue of the normal FEurate and predictable ADH response to changes in plasma osmolality.

Funding: Pharmaceutical Company Support - Otsuka

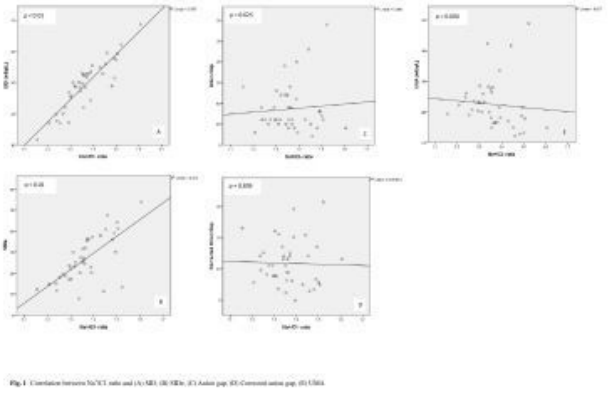
PUB454

The Use of Sodium-Chloride Ratio and Sodium-Chloride Difference in Evaluating the Etiology of Metabolic Acidosis in Critically Ill Patients
Preetika Rao, Prachi Aggarwal, Devjit Roy, Thara Basavaiah, Frantz M. Duffoo, Shitij Arora. *Internal Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.*

Background: Acid-base disorders are a common problem in critically ill patients. Stewart's physiochemical approach is an accurate, reliable method to analyze base deficits, but involves complex mathematical equations. Previous studies suggest using the sodium-chloride ratio (Na:Cl), where a high or low ratio indicates hypochloremia or hyperchloremia respectively as a cause of metabolic acidosis. Our study aims to assess the use of Na:Cl as a simple bedside diagnostic tool and compares it to other existing base deficit equations.

Methods: This retrospective study included 41 ICU patients with evident metabolic acidosis, defined as pH <7.25 and sHCO3 concentration <22 mEq/l. Henderson-Hasselbach and Stewart's physiochemical approach were used to calculate Na:Cl ratio, sodium-chloride difference (DiffNaCl), anion gap (AG), albumin-corrected anion gap (AGcorr), strong ion difference (SID), effective strong ion difference (SIDe), unmeasured anions (UMA), and tissue acids (TA) at each episode of metabolic acidosis. Patients were grouped according to the Na:Cl ratio; group I, Na:Cl< 1.3; group II, Na:Cl 1.3-1.5; group III, Na:Cl> 1.5.

Results: The study shows that both DiffNaCl and the Na:Cl ratio have correlations with SID and SIDe but none with AGcorr, UMA, and TA. This suggests that the chloride ion plays a more important role in metabolic acidosis than an elevation of UMA or TA. We also demonstrate the Na:Cl ratio as the two strongest cations and anions showing a positive correlation with coeffecients of Stewarts theory (SID, SIDe).



Conclusions: Our study suggests that the Na:Cl ratio and DiffNaCl may be a simple, fast alternative method to Stewart's physiochemical approach in identifying raised UMA and TA in critically ill and otherwise acidotic patients.

PUB455

High Rates of Hyperkalemia in Patients with Chronic Kidney Disease Stage 3 or 4, Diabetes, and Hypertension
Jamie Cope,[1] David A. Bushinsky,[2] Lance Berman.[1] *[1]Relypsa, Santa Clara, CA; [2]University of Rochester Medical Center, Rochester, NY.*

Background: Hyperkalemia can cause life-threatening arrhythmias and is associated with increased mortality. The reported incidence of hyperkalemia varies widely, depending on the population studied and the definition of high potassium. Renal impairment, diabetes, advanced age and the use of RAAS blockading agents all increase the risk of hyperkalemia. Hyperkalemia is a barrier to the use of renoprotective and cardioprotective RAAS blockers. Here, we report the rates of hyperkalemia observed during enrollment for AMETHYST-DN (ID: NCT01371747), a Phase-2 study to investigate in >300 patients with CKD, type 2 diabetes mellitus (T2DM) and hypertension (HTN) the effect of RLY5016 (patiromer), a new polymer-based treatment for hyperkalemia.

Methods: Qualifying subjects were 30-80 y.o., with CKD [eGFR 15-60ml/min/m^2), T2DM, HTN, urine albumin to creatinine ratio > 30mg/g and on RAAS blockers. Those with pre-existing hyperkalemia (serum K^+ 5.1-6.0mEq/L) at screening were randomized to the treatment phase directly. Those who were normokalemic (serum K^+ 4.3-5.0mEq/L) at screening discontinued their current RAAS blockers and were run in for up to 4 weeks on maximum-labeled doses of RAAS blocker for additional BP control [100mg losartan, and/or up to 50mg spironolactone].

Results: Thirty-nine percent of screened subjects (225/572) had pre-existing hyperkalemia (central K^+ ≥5.1 mEq/L) and 14% (80/572) had a serum K^+ ≥5.5 mEq/L (table). Of the 72 subjects who entered run-in (average serum K^+: 4.8mEq/L) at the beginning of maximum-dose RAAS blockade, 69% (50/72) became hyperkalemic within an average of two weeks and 25% (18/72) had a serum K^+ ≥5.5 mEq/L. The average serum K^+ increase was 0.4mEq/L.

Hyperkalemia	≥5.1mEq/L	≥5.5mEq/L
Pre-existing	39%	14%
After maximum-dose RAAS blockade	69%	25%

Conclusions: Hyperkalemia is common in patients with compromised renal function (CKD Stages 3/4), hypertension and diabetes, even without the use of maximum-labeled doses of RAAS blockers. Most patients placed on maximum-labeled doses of RAAS blockers develop hyperkalemia rapidly.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

PUB456

Combination of ARB with an L/N-Type CCB Achieves an Excellent Outcome Compared with L-Type CCB on Adriamycin-Induced Cardiomyopathy and Nephropathy in SHRs
Shizuka Aritomi, Kazumi Niinuma, Mai Kawakami, Eri Harada. *Research Center, Ajinomoto Pharmaceuticals Co., Ltd., Kawasaki, Kanagawa, Japan.*

Background: In this study, we investigated the effect of L/N-type CCB cilnidipine on the adriamycin (ADR)-induced cardiomyopathy and nephropathy in SHR comparing with that of L-type CCB when given concomitantly with ARB.

Methods: ADR (1.5 mg/kg) was administered intravenously to SHR once a week for 3 weeks. Control rats were administered saline (unaffected group). One week after the last administration of ADR, rats were divided into 3 groups and administered either the vehicle (untreated group), valsartan (Valgroup), cilnidipine+valsartan (CV group), or amlodipine+valsartan (AV group) for 4 weeks.

Results: There was no difference in antihypertensive effect between CV group and AV group throughout the experimental period. ADR caused an increase in the urinary albumin excretion (UAE). Although CV group showed same antihypertensive effect as AV group, only CV suppressed the increase of UAE. In addition, ADR induced cardiac contractile dysfunction assessed by echocardiography. CV group also showed an improvement of contractile dysfunction, but AV group did not. Furthermore, CV group showed a lower level of plasma aldosterone than untreted group.

Conclusions: The results of the present study indicate that, when given concomitantly with ARB, cilnidipine suppresses the progression of renal and cardiac dysfunction induced by ADR. Since such effect was not observed by L-type CCB amlodipine treatment, blockade of N-type calcium channel might play an important role for protection of both renal and cardiac function. And the mechanism of cilnidipine's effect can be partly explained by its suppressive action in aldosterone through N-type calcium channel.

Funding: Pharmaceutical Company Support - Ajinomoto Pharmaceuticals Co., Ltd.

PUB457

Fractalkine and Its Receptor, CX3CR1, Contribute to Hypertensive Kidney Fibrosis
Kazuaki Shimizu,[1] Kiyoki Kitagawa,[1] Yasunori Iwata,[1] Takashi Wada.[2] *[1]Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan; [2]Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan.*

Background: Fractalkine/CX3CL1 is a unique chemokine, which works as a leukocyte chemoattractant as well as an adhesion molecule. Fractalkine/CX3CL1 was reported to be involved in the progression of human glomerulopathy. The involvement of the fractalkine-CX3CR1 axis in the pathogenesis of hypertensive kidney fibrosis remains unclear.

Methods: The impacts of the fractalkine-CX3CR1 axis on hypertensive kidney fibrosis were investigated in a deoxycorticosterone acetate (DOCA)-salt hypertensive model in CX3CR1deficient mice and wild type mice, which were sacrificed on day 28.

Results: Systolic blood pressure was elevated in all mice 14days and 21days after DOCA treatment. Levels of blood pressure were similarly elevated in both CX3CR1-/-C57BL/6 (119.3mmHg) and wild-type C57BL/6 mice (119.7mmHg). Fractalkine and CX3CR1 were upregulated in damaged kidneys by hypertension in wild-type C57BL/6 mice, expressed in tubular epithelial cells and vascular endothelial cells. The mean interstitial fibrosis was reduced in DOCA administered CX3CR1-/- (2.9%) compared with DOCA administered C57BL/6 mice (8.7%). CX3CR1 deficient mice had been showed downregulation of type I collagen protein and type I procollagen mRNA expression in damaged kidneys. Kidney transforming growth factor (TGF)-β_1 expression was decreased by CX3CR1 deficiency. The number of infiltrating F4/80-positive macrophages in damaged kidneys was also decreased in CX3CR1 deficient mice.

Conclusions: These results suggest that fractalkine-CX3CR1 axis contributes to kidney fibrosis in a hypertensive mouse model.

PUB458

The Bufodienolide, Cinobufatalin, Produces a Preeclampsia-Like Syndrome in a Rat Model
Daad Abi-Ghanem, Cindy J. Balog-alvarez, Luc R. Berghman, Darijana Horvat, Biresh Kumar, Mohammad Nasir Uddin, Jules B. Puschett. *Texas A&M University, College Station, TX.*

Background: The bufodienolides are a group of agents found in the skin and venom of the common toad *Bufo marinus* and in plants. They are members of a family of substances called cardiotonic steroids. Previous studies from this laboratory have demonstrated that one of these substances, marinobufagenin (MBG) causes a syndrome in the rat which includes many of the characteristics of human preeclampsia (PE): hypertension, proteinuria and intrauterine growth restriction (IUGR).

Methods: To determine if the administration of another bufodienolide, cinobufatalin (CINO) could cause a similar disorder, we gave normal pregnant (NP) rats CINO (30 µg/kg/day) daily from early pregnancy by intraperitoneal injection. IUGR was estimated by analyzing the number of pups/litter (#pups) and the percentage of abnormal pups (%abn). Final blood pressure (FBP) data, protein excretion (UVPr), #pups, and %abn were compared to the values obtained in NP rats.

Results: Mean FBP +/- SD in CINO rats of 128+/-6 mmHg exceeded that of NP (90 +/- 6 mmHG), P< 0.05. UVPr in CINO rats was 5.8 +/- 1.8 mg/24hr which exceeded that in NP (2.4 +/-1.2), P< 0.05. The #pups in CINO rats (12.2 +/- 1.8) were less than those in NP (15.0 +/- 1.9), P< 0.05. Furthermore, there were 11% abn pups in CINO but none in NP.

Conclusions: CINO produces a PE-like syndrome in rats similar to that induced by MBG.

Funding: Private Foundation Support

PUB459

Increases in ACE Aggravates the Preeclampsia-Like Phenotype in Mice

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Background: Preeclampsia (PE) is pregnancy-induced hypertension with proteinuria. Excess sFlt-1, an endogenous VEGF inhibitor of placental origin has been implicated to play an important role in hypertension, proteinuria and glomerular endotheliosis, hallmarks of PE. sFlt-1 induces endothelial dysfunction via several pathways including decreased eNOS. We have proven that mice lacking eNOS have aggravated PE-like phenotype. (JASN 2012) ACE inactivates bradykinin, then its down-stream factor, eNOS. Human *ACE* polymorphisms leading to elevated activity of ACE are associated with PE.

Methods: We tested whether an increase in ACE aggravates PE using mice having 4 copies of *Ace* gene.

Results: Their basal levels of blood pressure (BP) do not differ from those of WT mice. Adenoviral mediated sFlt-1 overexpression to the non-pregnant female mice increased BP more in mutant mice than WT mice (from 118.4 ± 6.7 mmHg to 162.4 ± 10.1 mmHg in the mutant mice, from 120.1 ± 3.9 mmHg to 142.4 ± 3.9 mmHg in WT mice). Mutant sFlt-1 mice showed higher daily urinary albumin excretion (288 ± 42 vs. 150 ± 60 mg/day in WT sFlt-1) and lower GFR (170 ± 55 vs. 493 ± 109 ml/min in WT sFlt-1). Mutant sFlt-1 mice had less glomerular open capillary volume (20.5 ± 3.8 vs. 34.7 ± 10.5 % in WT sFlt-1 mice), and lost fenestration of glomerular capillary endothelial cells, suggesting mutant sFlt-1 mice have more severe endotheliosis than WT sFlt-1 mice. Mutant mice also show podocyte foot process effacement in some glomeruli.

Conclusions: We conclude that mice having 4 copies of *Ace* gene develop more severe PE-like phenotype than WT mice. The mechanism underlying this phenomenon is under investigation.

Funding: Other NIH Support - NIH

PUB461

Down-Regulation of Integrin β1 Associated with Focal Adhesion Kinase Signaling Pathway in Renal Glumoruli under Various Hemodynamic Conditions

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Background: The study focuses on the changes of β1 integrin and the associated signal molecular focal adhesion kinase (FAK) under various hemodynamic conditions in podocytes using novel "In vivo cryotechnique". [1, 2]

Methods: "In vivo cryotechnique"group: The "In vivo cryotechnique" was performed on left kidneys of anesthetized C57BL/6 mice, as reported before. [3]. Control group: the kidney tissues were fixed with the conventional immersion and perfusion preparation methods. Their serial sections were stained and observed by light, confocal laser scanning microscopy and immunoelectron microscopy. The expression levels of β1 and FAK proteins and mRNA were measured by western blot and Real Time PCR.

Results: By the "In vivo cryotechnique", the β1 integrin was significantly decreased under the acute hypertensive and cardiac arrest conditions, compared with the control group. Being a potential regulator, the expression of FAK was increased, and it was translocated and accumulated in nuclei of the podocytes under abnomal hemodynamic conditions.

Conclusions: These results indicate that the decreased expression of β1 integrin and associated FAK pathway my play an important role in regulating the functions of podocytes under abnormal hemodynamic conditions. "In vivo cryotechnique" followed by freeze-substitution should be a reliable tool to observe the proteins in situ and capture transient dynamic images of functioning glomeruli in living mice.

Funding: Government Support - Non-U.S.

PUB462

World Kidney Day (WKD): Four Year Analysis of Health Events in UK

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Background: WKD is an annual kidney awareness event. For the past 4 years, health clinics have been held for the staff and shoppers in Liverpool. The aim was to determine the general health: blood pressure, blood glucose, urine abnormality, heart rate and cholesterol.

Methods: Individuals who attended annual WKD events from 2009-2012 came forward for blood pressure (electronic blood pressure monitor), capillary blood glucose monitoring (CBGM), urinalysis (automated urinalysis machine), heart rate (manual radial pulse) and cholesterol readings.

Results: A total of 871 patients were included in the analysis. The median age was 54 (range 12-102) with a M:F ratio of 1:3. The median systolic and diastolic blood pressure was 137 mmHg (range 83-217; SD 22) and 81 mmHg (range 40-128; SD 12) respectively. Defining hypertension as blood pressure >140/90, 361 (51%) patients were identified to be hypertensive. A systolic BP >180 was found in 37 patients. Median heart rate from 395 patients was 76 bpm (SD 13). Of the 585 patients who had a random CBGM test, 5 patients had blood glucose of over 10 mmol/L (mean 5.5; SD 1.5). There were 675 patients who had a urinalysis test. Of these, 125 and 201 showed some degree of haematuria and proteinuria respectively. A combination of at least moderate (++) haematuria and proteinuria was found in 22 patients. There were signs of leucocyturia in 246 patients and 11 patients were found to have glycosuria. Two of these had severe glycosuria (+++). Twelve patients showed mild positive (+) nitrate results suggesting infection. Random cholesterol was measured in 174 patients (median 5.00 mmol/L; range 3.00-7.76; SD 0.87). Twelve patients had high cholesterol >6.5.

Conclusions: The above analysis shows that hypertension, urine abnormalities and hypercholesterolaemia are prevalent throughout the community. Regular events like this help to discover undiagnosed patients and increase confidence in the community thus improving health check-up attendances. It also helps identify and prevent severe consequences of undiagnosed and prevalent risk factors in the community.

PUB463

Telmisartan Increase the Nitric Oxide Production in 5/6 Nephrectomy Rats: Partly Dependent of PPARγ/nNOS Activation

Ying Yao,[1] Rong Zou,[1,2] Yong He,[1,3] Min Han,[1] Zufu Ma,[1] Xiaocheng Liu,[1] Rui Zeng,[1] Gang Xu.[1] *[1]Tongji Hospital; [2]Wuhan Integrated TCM & Western Medicine Hospital; [3]Wuhan No.5 Hospital.*

Background: To study the nitric oxide synthesis and probable mechanisms influenced by telmisartan in 5/6 nephrectomized rats.

Methods: male Wistar rats were randomly divided into sham-operation group, untreated 5/6 Nx rats, telmisartan group, telmisartan + GW9662 group, and troglitazone group. The 12th week rats were killed with decapitation method, and detected in serum urea nitrogen, creatinine, 24h urinary protein, NOx. Remnant kidney tissue were taken pathological examination and immunohistochemistry to observe the PPARγ, nNOS distribution. RT-PCR and Western Blot were used to detect the expression of PPARγ and nNOS.

Results: The degree of glomerular sclerosis and tubulointerstitial damage in the telmisartan group was markedly reduced, compared with untreated 5/6 Nx rats. But the protection effects of telmisartan were markedly abolished by GW9662 (P<0.05). Immunohistochemistry showed that the distribution of PPARγ protein in the glomerulus and renal tubules decreased obviously in 5/6 Nx group, which was recovered by telmisartan or troglitazone. The NO production could be increased by telmisartan, with the abolishment of GW9662. nNOS in renal cortex was mainly localized in dense patches and renal tubular epithelial cells. The expression of PPARγ and nNOS were downregulated in 5/6 Nx group compared to sham group, which were recovered by treatment with Telmisartan and Troglitazone.

Conclusions: 5/6 nephrectomy causes a significant downregulation of intrarenal nNOS and NO. Telmisartan exert a stimulatory effect on nNOS and NO levels via PPARγ activation in the 5/6 nephrectomized rats. The renal protection effects of telmisartan partly due to the regulation of nNOS and NO system.

PUB464

Role of (Pro)renin Receptor in Angiotensin II-Dependent and -Independent EGF Receptor Transactivation

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Background: Prorenin-induced intracellular signaling pathway is not fully elucidated. We investigated whether the (pro)renin receptor mediates epidermal growth factor (EGF) receptor transactivation throughangiotensin (Ang) II-dependent and -independent pathways in human embryo kidney 293 cells.

Results: Prorenin(2 nmol/L) caused biphasic phosphorylation of EGF receptor (Tyr992) and extracellular signal-regulated kinase (ERK) 1/2, peaking at 5 minutes followed by a decrease and a second peak at 60–120 minutes, whereas EGF receptor (Tyr1068) and Src were phosphorylated at only 120 minutes. These prorenin-induced phosphorylation processes were inhibited by (pro)renin receptor siRNA. Similarly, Ang II type 1 (AT_1) receptor blocker (ARB) or AT_1 receptor siRNA completely inhibited prorenin-induced phosphorylation of EGF receptor (Tyr1068) and Src, as well as the late peaks of EGF receptor (Tyr992) and ERK 1/2. However, early peaks of EGF receptor (Tyr992) and ERK 1/2 at 5 minutes were not effectively blocked byARB or AT_1 receptor siRNA. Incubation with prorenin (120 minutes) significantly increased Ang II levels of cell lysate.

Conclusions: These data indicate that the (pro)renin receptor mediates EGF receptor transactivation in both Ang II-dependent and-independent pathways.

Funding: Government Support - Non-U.S.

PUB465

Aldosterone Breakthrough during Aliskiren, Valsartan, and Combination (Aliskiren + Valsartan) Therapy for Proteinuric Kidney Disease Andrew S. Bomback,[1] Yelena Rekhtman,[1] Philip J. Klemmer,[2] Pietro A. Canetta,[1] Jai Radhakrishnan,[1] Gerald B. Appel.[1] *[1]Columbia University; [2]UNC Kidney Center.*

Background: Aldosterone levels increase in 30-40% of patients on ACE-inhibitors and/or ARBs over the long-term. Aldosterone breakthrough may carry important clinical consequences given aldosterone's non-epithelial, pro-fibrotic actions. The renin inhibitor, aliskiren, by suppressing the RAAS proximally, may limit breakthrough compared to conventional RAAS blockade.

Methods: This open-label study (NCT01129557) randomized subjects to aliskiren 300 mg daily, valsartan 320 mg daily, or valsartan 160 mg daily + aliskiren 150 mg daily for 9 months. Eligible subjects had proteinuria >300 mg/day, eGFR>45 ml/min/1.73m2, and SBP>130 or DBP>80 mm Hg. Serum and 24-hr urine aldosterone (indexed to 24-hr urine Na) were checked prior to initiation of therapy and at 3, 6, and 9 months. Aldosterone breakthrough was defined as a sustained increase from baseline aldosterone by study end. The study was intended to enroll 120 subjects but was terminated early by the sponsor. We present here the results of subjects who completed the protocol.

Results: This analysis includes 33 subjects randomized to aliskiren(n=12), valsartan(n=11), or valsartan+aliskiren(n=10). Twenty-five(76%) were male; 20(61%) were white; mean age was 44.7(±14.7) years. Mean baseline eGFR was 75.5(±23.3) ml/min/1.73m^2; baseline proteinuria was 3104(±2943) mg/day; and baseline BP was 134.7(±10.5)/84.8(±8.4) mm Hg. Three(27%) subjects on valsartan, three(25%) subjects on aliskiren, and three(30%) subjects on aliskiren + valsartan had aldosterone breakthrough. Mean proteinuria reduction was 31% from baseline in all subjects – in subgroup analysis, proteinuria reduction was 30% in subjects with breakthrough vs. 32% in subjects without breakthrough. Mean BP reduction was 11.0/8.8 mm Hg in all subjects – in subgroup analysis, BP reduction was 8.4/6.1 in subjects with breakthrough vs. 12.0/9.8 in subjects without breakthrough.

Conclusions: Aliskiren, alone or in combination with valsartan, did not reduce the incidence of aldosterone breakthrough in subjects with hypertension and proteinuria compared to conventional RAAS blockade.

Funding: Pharmaceutical Company Support - Novartis

PUB466

An Analysis of Blood Pressure Management of Patients with Chronic Kidney Disease and Hypertension Steven Arikian,[1,3] Frank A. Corvino,[1,2] David Oliveri,[1] Marko Zivkovic,[1,2] Michael Hagan.[4] *[1]Genesis Life Sciences, Hoboken, NJ; [2]Stevens Institute of Technology, Hoboken, NJ; [3]Columbia University, New York, NY; [4]Takeda Pharmaceuticals International, Inc., Deerfield, IL.*

Background: Hypertensive (HTN) patients with chronic kidney disease (CKD) are at greater risk of hypertension complications, require lower blood pressure (BP) levels, and may have non-standard response to therapy. Since outcomes depend on the treated population, a better understanding of population characteristics, BP management and goal attainment (BP<130/80 mmHg), and adverse event occurrences is important for successful treatment of CKD and HTN patients.

Methods: We performed a retrospective analysis of the General Electric Electronic Medical Records database of 3,348 CKD and HTN patients (January 2007 to May 2011). Patients had 1+ year of database activity prior/post the start of the most recent therapy, and ≥1 quarterly BP reading for the first year of therapy.

Results: The population was 56.7% female and 38.3% white, with a mean age of 68.6 years. Stage 2 hypertension was observed in 69.5% of the population. Mean BP was reduced from baseline 155.5/80.5 mmHg to 134.8/72.8 mmHg after 1 year (p<0.01). The most common therapeutic classes added were angiotensin-converting-enzyme inhibitors (ACEi, 39.2%) and angiotensin receptor blockers (ARB, 18.9%). 7.1% of the population was treated with ARB+thiazide (ARB+HCTZ) and 5.05% with ACEi+thiazide combinations (ACEi+HCTZ). The most common ARB and ARB+HCTZ was valsartan (6.0%) and valsartan+HCTZ (4.2%). ACEi+HCTZ exhibited the greatest systolic BP reduction after 1 year (15.4%). After 1 year of treatment, 43.4% of patients reached BP goal regardless of administered therapy, with the highest goal attainment observed for ACEi+HCTZ (51.5%). In the 1-year follow-up, 16.9% of the population had a CVD event, most often in patients treated with centrally acting antiandrenergics (18.9%).

Conclusions: CKD and HTN patients have a high risk of CVD events. Monotherapies are more broadly administered. BP goal attainments of 40-50% suggest a possible unmet need for more effective therapies in this population.

Funding: Pharmaceutical Company Support - Takeda Pharmaceuticals International, Inc

PUB467

Aliskiren Suppresses Renin-Angiotensin-Aldosterone System Leading to Reduced Blood Pressure and Albuminuria in Elderly Chronic Kidney Disease Patients with Hypertension Akihiko Numata,[1] Yoshiyuki Morishita,[1] Toshihiro Yasui,[2] Akira Onishi,[1] Eiji Kusano.[1] *[1]Division of Nephrology, Department of Internal Medicine, Jichi Medical University, Shimotsuke-city, Tochigi, Japan; [2]Internal Medicine, National Health Insurance Yukawa Clinic, Niimi-city, Okayama, Japan.*

Background: The effects of blockade of the renin-angiotensin-aldosterone system (RAAS) by aliskiren in elderly chronic kidney disease (CKD) patients have not been established. In the present study, we investigated the effects of aliskiren on the suppression of RAAS as well as blood pressure (BP) and renoprotection and cardioprotection in elderly CKD patients with hypertension.

Methods: This study was a 28-week, multicenter study consisting of a 4-week observation period to fix any drugs, including existing antihypertensives, and an 24-week treatment period with 150mg/ day of aliskiren. Twenty three elderly CKD patients were enrolled. Nineteen patients (9 males, 10 females, aged 74±7, estimated glomerular filtration rate (eGFR) 52±29 mL/min/1.73 m^2) completed the study period and analyzed. The changes of plasma renin activity (PRA), plasma renin level, plasma angiotensin I (Ang I) level, plasma angiotensin II (Ang II) level, plasma aldosterone (Ald) level, BP, eGFR and urine albumin/creatinine ratio (UACR) with aliskiren treatment were measured. The echocardiography with aliskiren treatment was also performed.

Results: Aliskiren treatment suppressed RAAS as follows: PRA (1.3±1.0 to 0.3±0.3 ng/ml/h, P<0.05), Ang I (59±32 to 26±17 pg/ml, P<0.05), Ang II (58±62 to 14±9 pg/ml, P<0.05) and Ald (86±38 to 80±53 pg/ml, NS), whereas plasma renin level was increased (4.9±5.0 to 30.9±26.4 pg/ml, P<0.05) by negative feedback. Aliskiren treatment reduced systolic BP (154±15 to 131±16 mmHg, P<0.05) and diastolic BP (77±10 to 72±10 mmHg, P<0.05). It also reduced UACRs (747±112 to 409±146 mg/g, P<0.05), whereas it did not change eGFR (52±29 to 51±29 mL/min/1.73 m^2, NS) and ejection fraction measured by echocardiography (66.8 ±7.9 to 66.5±6.8 %, NS).

Conclusions: Aliskiren suppressed RAAS leading to reduced BP and UACR, whereas it maintained eGFR and ejection fraction in elderly CKD patients with hypertension.

PUB468

Combination Therapy of Angiotensin II Receptor Blocker and Calcium Channel Blocker Exerts Pleiotropic Therapeutic Effects in Addition to Blood Pressure Lowering: Amlodipine and Candesartan Trial in Yokohama (ACTY) Akinobu Maeda, Kouichi Tamura, Yoshiyuki Toya, Satoshi Umemura. *Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan.*

Background: Accumulated evidence showed that strict blood pressure (BP) control is essential to prevent target organ damage and to reduce cardiovascular mortality in hypertensive patients. Although angiotensin II receptor blocker (ARB) and calcium channel blocker (CCB) are the first-line antihypertensive drugs, monotherapy either by ARB or CCB achieves the target BP in only a limited number. Thus, we conducted the ACTY trial to examine effects of combination therapy with ARB and CCB on BP profile and several target organ functions in essential hypertensive patients.

Methods: We recruited the participants aged 26-76 years, who did not achieve the target BP level during the monotherapy period either by candesartan or amlodipine. After the monotherapy period, for the patients already being treated with amlodipine, a once-daily 8 mg dose of candesartan was added-on during the combination therapy period (ARB add-on group), and a once-daily 5 mg dose of amlodipine was added-on for those already being treated with candesartan (CCB add-on group), followed for 12 weeks.

Results: Participants were characterized as middle-aged, obese, mild-to-moderate hypertensive patients with impaired renal function and insulin resistance. Combination therapy with ARB and CCB significantly decreased clinic and home SBP and DBP. In addition, urine albumin excretion was significantly reduced (374±124 vs 105±34mg/g-Cr, *P<0.01)* without decrease in estimated glomerular filtration ratio (73.3±4.6 vs 71.8±4.8ml/min/1.73m^2, N.S.), and resulted in improvements in vascular function, such as baPWV and cSBP, and insulin sensitivity. Furthermore, the CCB add-on group showed a significantly greater decrease in clinic and home DBP than the ARB add-on group, and also exhibited better improvements in vascular functional parameters.

Conclusions: These results suggest that combination therapy with candesartan and amlodipine is an efficient therapeutic strategy for hypertension with pleiotropic benefits.

PUB469

Home Blood Pressure Control after the Great East Japan Earthquake Is Associated with Renin-Angiotensin System Inhibitor Medications in Hemodialysis Patients Makoto Kanno,[1] Kenichi Tanaka,[1,2] Kimio Watanabe,[1] Yoshihiro Tani,[1] Yoshimitsu Hayashi,[1] Koichi Asahi,[1,2] Masaaki Nakayama,[1] Kazuhiro Suzuki,[3] Tsuyoshi Watanabe.[1] *[1]Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan; [2]Chronic Kidney Disease Initiatives, Fukushima, Japan; [3]En-jin-kai Suzuki Clinic, Koriyama, Japan.*

Background: At 14:46 on March 11, 2011, northeastern Japan was struck by a major earthquake measuring 9.0 on the Richter scale. We recently reported poor BP control after the Great East Japan Earthquake in patients with stage 3-4 CKD (Tanaka et al. *Am J Hypertens. in press*). The present study evaluated deteriorations in BP control after this earthquake and clarified factors associated with these changes in patients on chronic hemodialysis.

Methods: Changes in self-measured BPs in the morning at home following the earthquake were investigated in 25 patients on chronic hemodialysis who were residents of Koriyama City (mean age, 65.7 years; median dialysis duration, 27.0 months).

Results: Steep elevations in home BP were observed the day after the earthquake (March 12) compared with baseline (systolic 161±18 vs. 151±13 mmHg, *P*=0.02; diastolic 81±13 vs. 78±11 mmHg, *P*=0.08). Mean home systolic and diastolic BPs were significantly elevated 1 week after the earthquake compared with at baseline (systolic 158±16 vs. 151±13, *P*<0.01; diastolic 81±13 vs. 78±11, *P*=0.01), but these values had returned to baseline by 4 weeks after the earthquake (systolic 151±16, *P*=0.8; diastolic 79±13, *P*=0.5). Mean BP was not significantly changed 1 week after the earthquake compared with at baseline in patients treated with RAS inhibitors (104±9 vs. 102±9, *P*=0.5), but was significantly higher than pre-earthquake levels in patients not treated with RAS inhibitors (112±13 vs. 105±12, *P*=0.01). Moreover, changes in mean BP were significantly greater even 6 weeks after the earthquake in patients who were not treated with RAS inhibitors than in those treated with RAS inhibitors (+4.4±5.6 vs. -2.3±3.9 mmHg, *P*<0.01).

Conclusions: Home BP was significantly increased after a major earthquake, and RAS inhibitor medication was associated with earthquake-induced poor BP control in patients on chronic hemodialysis.

PUB470

Effects of Eplerenone Add-On Therapy on BP and Sodium Excretion in Patients Taking Renin-Angiotensin System Blockers Noritaka Kawada,[1] Toshiki Moriyama,[1] Tomonori Kimura,[2] Harumi Kitamura,[2] Hiromi Rakugi,[2] Yoshitaka Isaka.[2] *[1]Health Care Center, Osaka University, Toyonaka, Osaka, Japan; [2]Division of Nephrology, Osaka University Gradutae School of Medicine, Suita, Osaka, Japan.*

Background: This study determined whether addition of anti-aldosterone agent, eplerenone (Ep) could result in clinical benefits in patients already taking angiotensin-converting enzyme inhibitors (ACEis) or angiotensin receptor blockers (ARBs).

Methods: During the course of the study, patients were treated with a gradual increase of Ep to a final dose of 50 mg/day. Last year, we have reported the data obtained by sitting position (Group A, n=25), and this year, additional data obtained by supine position (Group B, n=12) were included to this study. In Group B, we have also assessed central BP measured by HEM-9000AI, PWI, and 24hours urine analysis.

Results: At baseline, plasma Na, K, and serum osmolarity at sitting position (Group A) were higher than that of supine position (Group B). In the total thirty-seven patients, the administration of 50 mg of Ep significantly reduced SBP and DBP measured by sitting position, but had no significant effects on TTKG or proteinuria measured by spot urine analysis. Ep reduced serum osmolarity at sitting position (Group A), but had no effect on supine position (Group B). Therefore, osmolarity gap between sitting and supine position were minimized by Ep. In Group B, Ep reduced central BP (-8.5%, p<0.05) and baPWV (left: -4.4%, p<0.05. right: -4.5%, p<0.05), and increased FENa, but failed to show effects on proteinuria or albuminuria in 24hours urine analysis.

Conclusions: The Ep add-on therapy in CKD or HT patients taking renin-angiotensin system blockers may have cardioprotective effects by lowering central BP and moderating serum osmolarity gap between sitting and supine position.

PUB471

Anti-Hypertensive Therapy Reverses Microalbuminuria in Children M. Khurram Faizan,[1] Maman Mansoor,[2] Jason M. Kurland.[3] *[1]Pediatrics, Hasbro Children's Hospital, Providence, RI; [2]Aga Khan University, Karachi, Pakistan; [3]Medicine, UMass Memorial Medical Group, Worcester, MA.*

Background: An association between microalbuminuria (MA) and hypertension (HTN) has recently been described in adults. We have previously reported that this important relationship also exists in the pediatric hypertensive population [ASN 2010, PAS 2008, 2012 (abstracts)]. We now studied whether microalbuminuria can be reversed after treatment of hypertension in children.

Methods: Retrospective cohort study from Dec 2006 to Apr 2010, on *non-diabetic* pts between the ages of 0-21 years. All pts had a diagnostic study with 24-hour ambulatory blood pressure monitoring (ABPM) in an urban pediatric nephrology clinic. HTN was defined as ≥ 95th percentile of systolic and/or diastolic blood pressure for age, gender and height. MA was assessed on a morning clinic sample and defined as urine albumin-creatinine ratio of ≥20 mg/g. Pts with HTN were treated with standard anti-hypertensive medications. A follow-up urine was assessed for the presence of MA after treatment.

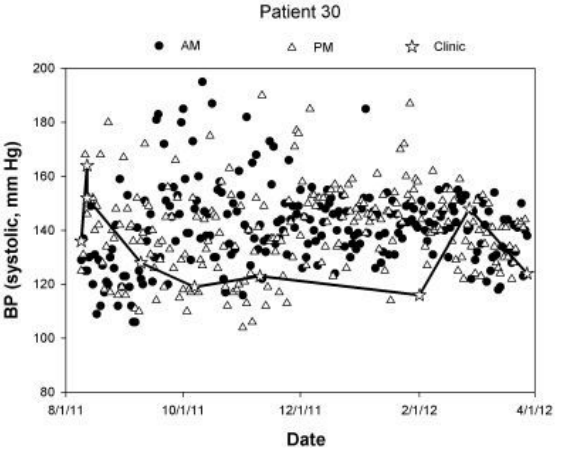
Results: 10 pts were included. Mean age was 12.6 yrs. 4 (40%) were male. Mean BMI was 24.3 kg/m^2 and 50% of pts were obese or overweight. 5 pts received ACEi, 3 β blockers, 1 calcium channel blocker, and 1 diuretic. **MA decreased in *all* pts on anti-hypertensive therapy, and normalized (<20 mg/g) in 9/10 pts on last clinic follow-up.** Mean pre- and post-treatment MA was **65 mg/g** and **19 mg/g**, respectively (***p* = 0.002**). Mean BP decreased from **140/88 mmHg** at baseline to **115/71 mmHg** after treatment. Mean time between performance of ABPM and final MA collection was 11.5 mths. Mean time between start of anti-hypertensive treatment and final MA collection was 10 mths.

Conclusions: Our study shows for the first time in children that MA is readily reversible with treatment of HTN, irrespective of the choice of agent. Recent studies have shown an association between MA and cardiovascular and renal disease in the adult hypertensive population. Microalbuminuria likely represents an important injury marker and risk factor in patients with clinically significant hypertension. This evolving relationship needs to be further explored in large scale prospective trials.

PUB472

Masked Hypertension (MH) Is a Chronic Condition that Is Seen in Both CKD and Non-CKD Patients Christopher Valentine, Ravish Shah, Daniel J. Birmingham, Lee A. Hebert. *Division of Nephrology, The Ohio State Univeristy Medical Center, Columbus, OH.*

Background: MH is defined as normal blood pressure in the office (clinic) but hypertension out of office determined by either ambulatory blood pressure monitoring (ABPM), or home blood pressure monitoring (HPBM). MH is associated with increased cardiovascular (CV) risk. The risk is comparable to that of sustained hypertension (SH, hypertension both in and out of the office). These associations of MH with increased CV risk were found by cross sectional testing, or longitudinal testing in which the status of the MH patients was tested 3-10 years after the diagnosis of MH was made. The patients' blood pressure during this interval was not studied. The mechanism of CV risk of MH is unknown. We tested the hypothesis that MH is chronic condition.



Methods: 33 patients with treated hypertension followed by our Resistant Hypertension Program were selected for study because they agreed to daily HBPM both AM and PM, and they had calibrated HBPM equipment and proper technique.

Results: Follow up was 4-15 months, median 10 months. The number of daily HPBM measurements per patient during follow-up ranged from 103 to 767, median 301. MH was present in 8/33 (24%), White Coat Hypertension (WCH) in 4/33 (12%), neither MH or WCH (NMW) in 64%. The figure is a representative MH patient. In the MH cohort, mean systolic BP (SBP) during follow up was: Clinic 128 +/- 8, HBPM PM, 143 +/- 4.2, P=0.003. In the NMW cohort, mean SBP during follow up was: clinic 125+/-5, HBPM PM 124+/-6, P=0.48. CKD was present was present in 50% of MH and 64% NMW (NS).

Conclusions: HBPM is needed to identify and appropriately treat the chronic hypertension of MH. This might be especially important in CKD and in order to minimize both CV disease and CKD progression.

Funding: NIDDK Support

PUB473

Resistant Hypertension and Vitamin D Deficiency Alexis Payette, Jean-Philippe Lafrance, Vincent Pichette, Michel Vallee. *University of Montreal - HMR, Canada.*

Background: Vitamin D deficiency is thought to play a significant role in cardiovascular disease and hypertension. In animal models, vitamin D regulates the renin-angiotensin system. Several observational studies have reported an association between vitamin D deficiency and hypertension in humans. This study evaluates the association between vitamin D status and BP in patients with resistant or difficult-to-control hypertension. We measured BP levels using 24-hour ambulatory blood pressure monitoring (ABPM) which correlates more closely with cardiovascular complications. We hypothesized that patients with resistant hypertension could have a significant vitamin D deficiency.

Methods: We conducted a retrospective study among patients referred in a tertiary hypertension clinic in Montreal, Canada. Through chart review, we collected clinical and laboratory data from 113 patients. Association between vitamin D and BP was evaluated with linear regression. We performed a subgroup analysis among patients requiring ≥3 antihypertensive drugs.

Results: The average initial BP was 139/79mmHg on ABPM. The mean age was 59.3y and 49.6% of patients were females. On their initial visit, 53.1% required ≥3 antihypertensive drugs and were classified as having resistant hypertension. The most prevalent comorbidity was diabetes (24.8%). Less than 10% of patients had a history of stroke or myocardial infarction. The mean circulating level of 25(OH)D was 27.8 ng/mL. This mean value is similar to that of the general Canadian population (27.1 ng/mL). We did not observe a significant relationship between 25(OH)D circulating level and systolic or diastolic 24-hour mean BP in this population [beta regression coefficient 0.07 (95%CI:-0.03-0.17) for systolic BP and -0.02 (95%CI:-0.11-0.06) for diastolic BP]. This absence of relationship was also observed in patients requiring ≥3 antihypertensive drugs.

Conclusions: We did not observe a significant relationship between circulating levels of 25(OH)D and BP levels in our population of patients with resistant or difficult-to-control hypertension, as measured with ABPM. These results suggest that vitamin D deficiency is not a clinically important risk factor in resistant or difficult-to-control hypertension.

PUB474

Evidence for the Need to Screen Patients before Treatment with Renal Denervation Eva Vink,[1] Willemien Verloop,[2] Evert-jan Vonken,[4] Michiel Voskuil,[2] Wilko Spiering,[3] Peter J. Blankestijn.[1] *[1]Nephrology, UMC Utrecht, Utrecht, Netherlands; [2]Cardiology, UMC Utrecht, Utrecht, Netherlands; [3]Vascular Medicine, UMC Utrecht, Utrecht, Netherlands; [4]Radiology, UMC Utrecht, Utrecht, Netherlands.*

Background: Currently renal denervation(RDN) is only advised for patients with resistant hypertension. We have implemented a standardized stepwise screening protocol to screen patients before treatment with RDN. The aims of this protocol are: confirmation of the diagnosis of hypertension(HT), exclusion of secondary forms of HT and obtaining imaging of the renal arteries. The aim of this study is to evaluate the results of our screening protocol. We hypothesize that the use of a standardised screening protocol allows exclusion of a considerable number of patients from treatment with RDN.

Methods: We have evaluated screening results from all patients referred to the UMC Utrecht for RDN in the period of August 2010 and April 2012. All patients underwent a stepwise protocol, including 24-h ambulatory blood pressure monitoring(ABPM), 24-h urine collection, collection of serum and saliva, tests of the RAAS and MRI/MRA of the renal arteries. Central in our protocol is a multidisciplinary approach.

Results: 126 patients were referred to the UMC Utrecht for treatment with RDN. 79 patients(63%) were excluded from treatment. Excluded patients were slightly younger compared to the eligible patients and had a lower BP. The most important reason for exclusion was a BP-related factor in 38(48%) patients: 18 patients(23%) had an office systolic BP <160mmHg, 19 patients(25%) did have an ABPM below the threshold. This last category of patients did have a clear white coat effect. We have found 8 cases(11%) of secondary HT, the majority being primary hyperaldosteronism(5 patients: 6%).

Conclusions: We have found relevant percentages of patients with a secondary cause of HT or a severe white coat effect, evidenced by the 24-h ABPM. Therefore all patients should be screened before treatment with RDN with the use of a standardized screening protocol. Because of the high percentage of patients with a severe white coat effect, the first step in screening should be 24-h ABPM. Finally a multidisciplinary approach is advised.

PUB475

Haptoglobin Phenotype and Antihypertensive Treatment Response Charlotte Strandhave,[1] My Svensson,[3] Henrik B. Krarup,[2] Jeppe Hagstrup Christensen.[1] *[1]Department of Nephrology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [2]Department of Clinical Biochemistry, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [3]Department of Nephrology, Aarhus University Hospital, Aarhus, Denmark.*

Background: Many hypertensive patients fail to reach blood pressure (BP) goals of 135/85 mmHg despite treatment. Genetic markers may be important in resistant hypertension (RH), among these haptoglobin phenotypes (Hp) (1-1, 2-1, and 2-2). Hp 2-2 has been associated with RH and a need for several drugs to achieve BP goals. The aim was to examine if Hp 2-2 may predict poor response to treatment regarding both BP lowering effect and response to antihypertensive drugs.

Methods: Patients with incident hypertension (n=127) were examined at baseline and one year. We performed 24h ambulatory BP measurements and estimated the difference in daytime systolic BP (SBP) between visits. Hp phenotype was determined by high-performance liquid chromatography.

Results: Patients were divided in two groups: 1-1+2-1 (n=87) and 2-2 (n=40). Baseline characteristics and response to treatment are shown in the table.

	Hp 1-1+2-1	Hp 1-1	p
Age (yrs)	50±11	50±13	ns
Female (n)	49(56%)	20(50%)	ns
Smoking follow-up (n)	15(17%)	10(25%)	ns
ΔBody mass index (kg/m^2)	0.1±1.1	0.3±1.3	0.09
SBP baseline (mmHg)	152±12	148±11	ns
SBP follow-up (mmHg)	133±11	132±11	ns
Antihypertensive treatment (n)	70(87%)	35(88%)	ns
Antihypertensive drugs (n)	1.5±1	1.5±1	ns
Calcium antagonists (n)	22(31%)	10(29%)	ns
ΔSBP, calcium antagonists (mmHg)	26±13	17±10	0.08
ACE inhibitors (n)	15(21%)	10(29%)	ns
ΔSBP, ACE inhibitors (mmHg)	22±14	22±12	ns
Diuretics (n)	26(37%)	16(46%)	ns
ΔSBP, diuretics (mmHg)	29±16	21±10	ns

Mean±SD or numbers(%)

SBP goals was reached in 61% of Hp 1-1+2-1 patients versus 69% in Hp 2-2 patients (p=0.3). Adjusted regression analysis also showed that Hp 2-2 patients reached BP goal to same extend as Hp 1-1+2-1 patients (β- coeff.=-0.04, p=0.7).

Conclusions: In hypertensive patients Hp 2-2 did not predict poor response to antihypertensive treatment. Hp 2-2 patients did not need more intensive treatment to achieve BP goals, nor did BP decrease less sufficient in response to different antihypertensive drugs.

Funding: Private Foundation Support

PUB476

Incidence of Hypokalemia and Acute Kidney Injury with the Use of Triamterene/Hydrochlorothiazide versus Hydrochlorothiazide Amy J. Zwettler, Kevin C. Abbott, David K. Oliver, Robert Nee. *Nephrology Department, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Triamterene/Hydrochlorothiazide (Maxzide) was introduced with the goal of reducing the potential for hypokalemia. This study aims to look at the incidence of hypokalemia comparing use of Maxzide with the separate use of hydrochlorothiazide (HCTZ).

Methods: We queried the institutional electronic medical record for all adults (>18 years of age) with new prescriptions for Maxzide or HCTZ between September 1, 2002 to October 31, 2010. Serum potassium, magnesium, and creatinine values were obtained both prior to initiation and subsequent to therapy. The primary outcomes were: incidence of hypokalemia (serum potassium level <3.5 mEq/L) and acute kidney injury (AKI, defined as a >= 50% increase in serum creatinine from baseline) within one year of prescription. Logistic regression was used to calculate adjusted outcomes.

Results: We identified 940 patients with new Maxzide prescriptions and 3525 patients with new HCTZ prescriptions. Maxzide users were significantly younger and more likely female, compared to HCTZ users. The majority of the study population was also prescribed either an angiotensin converting enzyme-inhibitor or angiotensin-receptor blocker. Overall hypokalemia was significantly more common in the Maxzide group at one year (16.17% compared to 13.23% in HCTZ group, p=0.024 by Chi square). When evaluating potassium at both 30 and 60 days after initial prescription, however, there was no difference in the incidence of hypokalemia. Furthermore, after adjusting for baseline demographics, comorbid conditions, and concomitant medication use, there was no difference in the incidence of hypokalemia at 60 or 90 days. There was also no difference in the incidence of AKI, hyperkalemia, or hypomagnesemia.

Conclusions: These results suggest that Maxzide use, when compared to HCTZ, is not associated with a significantly reduced incidence of hypokalemia. *"The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, the department of the Navy, the Department of Defense, or the United States government."*

PUB477

Can We Stop Using Mercury in Pregnancy? Patrick Lan, Adrian G. Gillin. *Renal Medicine, Royal Prince Alfred Hospital, Sydney, NSW, Australia.*

Background: Mercury sphygmomanomtery remains the gold-standard in measuring blood pressure (BP) in hypertension in pregnancy. However it has a number of limitations and exposure risks that may be overcome by automated BP measuring devices. We sought to determine if such devices are a reliable alternative to mercury sphygmomanometry in an outpatient setting.

Methods: Patients were recruited from the hypertension in pregnancy clinic at Royal Prince Alfred Hospital. Three sequential BP measurements were taken by mercury sphygmomanometry and by an automated device (Omron HEM-7200). The order of the two methods was randomly allocated. Paired t-test was used to determine the presence of a difference between the two methods. The level of intra-observer variability for each method was determined by random effects regression. The number of different clinical decisions that would have been made with the automated reading compared to the manual reading was determined based on our management guidelines.

Results: The study included 51 patients for a total of 110 sets of measurements. 73% of the readings were performed in patients with either pre-eclampsia or gestational hypertension, and 70% were performed in post-partum patients. There was a difference between the automated and manual methods for both systolic BP (mean 2.6mmHg, SD 6.8mmHg, 95%CI 1.3 to 3.9mmHg, p<0.01) and diastolic BP (mean -2.3mmHg, SD 6.2mmHg, 95%CI -3.5 to -1.1mmHg, p<0.01). This difference was similar both antenatally and postnatally. In regards to intra-observer variability, the standard deviation for the manual readings were 4.0mmHg (p<0.01) for systolic BP and 3.1mmHg (p<0.01) for diastolic BP. The corresponding standard deviations for the automated readings were 4.6mmHg (p<0.01) and 3.1mmHg (p<0.01), respectively. Additionally, the automated measurements would have resulted in 4 (3.6%) different clinical decisions being made.

Conclusions: There was a difference between the two methods of BP measurement. However, this was of little clinical significance. In addition, intra-observer variability within either method was comparable. As such, automated BP measuring devices are a reliable alternative to mercury sphygmomanometers in outpatients with hypertension in pregnancy.

PUB478

Combination Antihypertensive Drugs Could Improve Blood Pressure Control and Adherence to Medications Hideki Kato, Masayuki Tanemoto, Shunya Uchida. *Nephrology, Teikyo University, Itabashi, Tokyo, Japan.*

Background: Hypertension constitutes a major risk factor for cardiovascular and chronic kidney disease. Blood pressure control, however, are sometimes difficult to achieve. Recently several combination antihypertensive drugs of angiotensin-receptor blocker (ARB) and calcium-channel blocker (CCB) have become available in clinical medicine. In this study, we examined whether combination antihypertensive drugs could improve blood pressure control and patients' satisfaction using questionnaire forms.

Methods: We retrospectively examined 97 outpatients in our hospital to whom anti-hypertensive treatment was switched from individual drug to combination drugs. Blood pressures (BPs) in clinics were measured and costs for the drugs were calculated. We also asked these patients to fill in a questionnaire about the alteration of home BPs, skip

medications, copayments for the drugs, and satisfaction levels. We assigned titer to each anti-hypertensive drug, and calculated the total titers in each patient.

Results: The average BPs of 97 patients were significantly decreased after the switch (SBP from 143.2 ± 19.1 to 135.7 ± 17.7, p<0.01). We divided patients into three groups according to the changes of the titers (decreased, not changed and increased). The group with the increased-titers had significantly higher BPs before the switch as compared to other groups and this group exhibited the most significant improvement of BPs after the switch. Costs for the anti-hypertensive drugs were decreased by 23% and copayments were also reduced. Adherence to medications was improved and home BPs were decreased. 79 % of patients preferred combination drugs.

Conclusions: Switching to combination drugs exhibit several advantages of BP control, cost reduction, adherence to medications and patients' satisfaction levels.

Funding: Clinical Revenue Support

PUB479

Micro RNA Profile Distinguishes Subjects with Cardiovascular Risk Factors from Healthy Individuals

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Background: Recent studies reported that miRNAs can be detected in the blood and may play a key role in cardiovascular development. Thus, we hypothesize that it may be possible to differentiate patients with cardiovascular disease (CVD) risk factors from healthy participants based on a distinct set of serum miRNAs.

Methods: We performed miRNA profiles on 10 healthy non-Hispanic Caucasian volunteers (controls) and 9 race matched subjects (cases) with cardiovascular risk factors. Subjects were free from clinical CVD and other chronic diseases and not using prescription medication. miRNA profiles was analyzed using a custom PCR array containing 372 miRNAs previously determined to be readily detectable in serum.

Results: Characteristics of the study population are shown in Table 1

Characteristics of study subjects

	cases (n=9)	controls (n=10)
Gender (M/F)	6/3	7/3
Age (y)	58±2	58±2
BMI (kg/m²)	36.3±1.3*	21.5±0.6
Regular aerobic exercise (%)	0*	100
Total cholesterol (mg/ml)	219±10*	174±9
SBP>120 or DBP>80	89*	0
Fasting glucose>100mg/dl (%)	56*	10
CRP (mg/ml)	6.2±1.0*	0.5±0.1

Data are mean±SEM. * p<0.05 vs.control. Regular aerobic exercise is defined as ≥1 hours of vigorous activity≥5days/week

For the arrays cases and controls were analyzed as 3 respective pools. 12 miRNAs were significantly downregulated and 23 miRNAs were upregulated (≥ 2.5 fold) in the cases compared to the controls. Among these 3 miRNAs were downregulated and 5 miRNAs upregulated ≥ 4 fold. These included miR-365 which has been associated with cardiac hypertrophy.

Conclusions: Subjects with CVD risk factors can be distinguished from healthy subjects without CVD risk factors based on a miRNA profile. The utility of this profile for prediction of future cardiovascular events will need validation in a larger population.

Funding: NIDDK Support, Private Foundation Support

PUB480

Ultrasound-Guided Trans-Hepatic Embolization of Renal Artery Pseudoaneurysm

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Background: The standard treatment of pseudoaneurysm is selective angiographic coil embolization. We report a case of an alternative renal artery pseudoaneurysm (RAP) repair in a high-risk pt.

Methods: Female, 67 ys, chronic renal failure (CRF) from phenacetin abuse, sCreat 4 mg/dl, previous left nephrectomy for cancer, prone to develop pseudoaneurysms, mainly after endovascular procedures. After a diagnosis of a RAP, she underwent a selective angiographic stent insertion, via right brachial artery, with the RAP exclusion. The procedure was complicated by a right brachial artery pseudoaneurysm, then surgically corrected. One month later, a ColorDoppler US (CDUS) and Contrast-enhanced US (CEUS) of the treated RAP showed its diameter increase (from 2.5 to 3.6 cm) and a turbulent flow in the site of RAP. Due to CRF and previous pseudoaneurysm development, a novel percutaneous trans-hepatic US guided approach for coil embolization was proposed. The procedure was planned and guided by CEUS and 3D-CEUS.

A iU22 US machine (2007, Philips, WA), with a 5-2 MHz curved-array probe and contrast-specific software, was used. A 2nd generation echo-contrast agent SonoVue(Bracco, IT) was employed.

With the pt in supine position, with a trans-hepatic approach, by US guidance a 18 gauge needle was advanced into the RAP. Through the needle, a fluoroscopy control was performed. When the needle was in the flow lumen, numerous coils (Balt, Montmorency, France) of 6, 8 e 12 mm of diameter were inserted. The flow in the lumen was assessed by CDUS and CEUS for monitoring the development of occlusion. Once the aneurysm was thrombosed, the needle was removed. The characteristics of the main renal artery and intrarenal flow were documented before, during and after the procedure using CDUS and CEUS. The procedure was well tolerated and no worsening in sCreat occurred.

Conclusions: The reported procedure may be an attractive alternative to surgery or traditional percutaneous trans-catheter approach in RAP management in selected high-risk pts.

PUB481

Causes of Hypertension and Prevalence of Target Organ Damage in a Population-Based Sample of 9- to 10-Year-Old Icelandic Children

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Background: The aim of the study was to investigate the causes of hypertension and the prevalence of target organ damage (TOD) in a population-based sample of hypertensive Icelandic children.

Methods: Thirty 9- to 10-year-old children found to have hypertension in a population–based study conducted by our group were invited to participate. Blood and urine biochemical studies were performed for the evaluation of secondary causes of hypertension and TOD. All study subjects underwent ambulatory blood pressure monitoring (ABPM), echocardiography, ultrasound evaluation of carotid intima-media thickness (cIMT) and an ultrasound examination of the kidneys. Controls, one for each study subject, were matched for gender, height and weight underwent echocardiography and cIMT evaluation.

Results: Of the 30 hypertensive children, sustained hypertension was confirmed in 24 children with ABPM. Six children with white-coat hypertension were excluded from further study. The 24 hypertensive subjects were found to have essential hypertension. Eight of these (33%) had signs of TOD, of whom 6 had left ventricular hypertrophy (LVH) defined as LV mass >51 g/m² vs. only one in the control group (p=0.041), 2 had isolated mild microalbuminuria and 1 of the study subjects with LVH did also have microalbuminuria. The hypertensive group had greater septal (p=0.001) and posterior wall (p=0.009) thickness than controls. When controlled for body surface area and height, a significant difference was noted in LV mass between study subjects and controls, 84 vs. 71 g/m² (p=0.044) and 72 vs. 62 g/m(p=0.047). There was no significant difference in the cIMT between the 2 groups.

Conclusions: All children in our population-based study cohort had essential hypertension. Manifestations of TOD, primarily LVH, were observed in a substantial number of study subjects.

PUB482

Thrombotic Microangiopathy Complicating Malignant Hypertension

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Background: TMA is an uncommon complication of malignant hypertension. Though the prognosis for patients with TMA occurring with malignant hypertension has improved over the past decade, the clinical and prognostic features are poorly understood. We describe two cases of severe hypertension complicated by TMA and also highlight the disparate renal outcomes and time for resolution of hemolysis and correlation with BP control.

Methods: Case 1

A 36yr old AAM with h/o HTN and CKD who presented with a 2day h/o of abdominal pain, nausea, vomiting. He stopped taking his BP meds 3 days prior. Admission BP was 225/158.

P/E revealed mildly tender abdomen, neuro exam was normal.

Labs: creatinine of 9.51, plt 42,000, Hb 14.3, LDH 1352, haptoglobin 8, Coags normal, Tbil 2.4.

Working diagnosis were hypertension complicated by acute on chronic kidney failure and/or TMA, Scleroderma renal crisis and TTP/HUS. ADAMTS13 level was normal.

See table 1.

table1

1	42	28
2	29	24
3	37	38
4	78	40
5	82	54
6	151	
7	220	
8	278	
9	299	
10	283	
11	274	

Case 2

A 39yr old AAM with pmhx of HTN and type 2 DM presented with 1wk h/o of abdominal pain, nausea with jaundice but no pruritus. He had no other c/o. On presentation, his BP was 179/116.

P/E was significant for sclera icterus, pallor and epigatric tenderness.

Labs showed Hb 10, plt 28000, LDH 1718, haptoglobin 3, PT 16.7s, INR 1.3 and creatinine 1.05, Tbil was 8.4. CT Abd and renal US were normal. ADAMTS13 levels was not done.

Results: Thrombocytopenia and Creatinine improved with BP control. Kidney biopsy revealed findings consistent with TMA and peripheral smear revealed schistocytes.

Conclusions: Malignant hypertension can be complicated by TMA but severe hypertension is also associated with HUS/TTP. The clinical features of these two entities overlap significantly hence it is sometimes difficult to distinguish them. In our patients, their response to BP control, normal ADAMTS13 levels and resolution of thrombocytopenia

excluded TTP. Renal failure was irreversible in case 1 as repeat renal biopsy 3 months after dialysis initiation showed persistent TMA which highlights that persistent renal TMA may account for the irreversibility of renal failure in cases of malignant HTN.

PUB483

Standing Blood Pressure: A Useful Tool for Risk Stratification in Healthy Young Adults? Hsiao L. Lai, Caitlin Thys, Courtland Winborne, Cynthia R. Christiano. *IM, ECU, Greenville, NC.*

Background: Primary prevention of risk factors for cardiovascular disease (CV) has become an important focus in the past 10 years. A preliminary study conducted between Jun and Oct 2010 in NC undergraduate students demonstrated high prevalence of multiple CV risk factors: 30% had two or more risk factors, 12% had three or more risk factors. Risk factors were defined as hypertension, obese body mass index, dyslipidemia, abnormal fasting blood glucose, smoking and sedentary activity level. This study demonstrated the feasibility of screening young adults utilizing the college setting, existing college forums, and Health curricula. Because of limited space and time use of standing, at rest blood pressure (BP) were taken instead of the standard seated BP.

Methods: Nov 2011 to Dec 2011, 209 undergraduate students participated in CV screening. Standing BP was obtained at rest with arm at heart level using manual aneuroid sphymanometry followed by seated resting BP obtained at rest under standard conditions. Hypertension, a systolic blood pressure (SBP) > 139 mmHg or diastolic blood pressure (DBP) > 89 mmHg.

Results: The mean age was 19 yo with 51% female, 79% white, and 11% Black. There was no difference between average standing and sitting SBP: 115.7 mmHg versus 116 mmHg, (p=0.85); or in the average DBP: 73.9 mmHg versus 73.8 mmHg, (p=0.91). The proportion of participants classified as hypertensive: 5.2% versus 3.8%; prehypertensive: 27.7% versus 27.6%; and normotensive: 67.9% versus 69.3%; based on standing or seating BP did not significantly change.

Conclusions: Young adults without established CVD the standing BP at rest does not significantly differ from seated BP taken under standardized setting. We did not perceive any significant trend towards higher or lower measurements in standing versus seated BP. Use of standing at rest BP taken by experienced clinicians may suffice as well as standard, seated, at rest BP measurement for initial BP screening in settings where space limitations. This single center study of a limited population and cannot be generalized to all young adults. Multicenter studies with larger number and increased proportion of Black and other demographic groups are needed.

PUB484

Changes to Renal Perfusion and Oxygen Bioavailability in Swine when Exposed to Propofol and Isoflurane Anesthesia Zaheer Akhtar,[1,3] Andrew Wentland,[1] Nathan Artz,[1] Sean Fain,[1] Arjang Djamali,[3] Elizabeth Sadowski.[1] *[1]Department of Radiology, University of Wisconsin Madison, Madison, WI; [2]Department of Nephrology, University of Wisconsin - Madison, Madison, WI.*

Background: In this study, we evaluated total renal blood flow, renal perfusion and oxygen bioavailability in swine anesthetized with either propofol or isoflurane.

Methods: Seven swine were evaluated at two time points: (1) under two hours of propofol anesthesia and (2) under two hours of isoflurane anesthesia. For each time point, mean arterial pressure (MAP), mean heart rate (MHR), total renal blood flow (via phase contrast MRI), perfusion (via arterial spin labeling MRI), and oxygen bioavailability (via BOLD MRI) were evaluated. Differences in these measures between the two time points were evaluated with paired Student's t-tests.

Results: MAP (87 versus 57 mmHg; p<0.001) was significantly higher and MHR (68 versus 86 bpm; p<0.04) was significantly lower under propofol anesthesia than under isoflurane anesthesia respectively. Total renal blood flow was significantly higher under propofol than under isoflurane anesthesia (263.7 versus 96.5 mL/min; p<0.0014). Cortical perfusion and oxygen bioavailability were significantly greater with propofol than with isoflurane (ASL perfusion: 208- versus 78 mL/100g/min; p<0.00001; R2*: 9.9 versus 11.9 s^{-1}; p<0.009). Medullary perfusion and oxygen bioavailability were not significantly different between the two time points.

Conclusions: There are significant hemodynamic differences between isoflurane and propofol, which likely affect total renal blood flow, cortical perfusion and oxygenation. Medullary perfusion and oxygenation are not significantly different between the two anesthetics, likely due the kidney's autoregulatory mechanisms which maintain stable medullary oxygenation over a broad range of blood pressures and varying levels of renal blood flow. When conducting studies aimed at assessing the effects of disease or pharmacologic maneuvers on renal blood flow, perfusion, or oxygenation bioavailability, it is important to recognize the baseline effects commonly used anesthetic agents have on the kidney.

Funding: Private Foundation Support

PUB485

What Is the Significant Cut-Off Level of Albuminuria for Diagnosis of Subclinical Atherosclerosis in General Population? Ja Seon Kim, Soon Bae Kim. *Division of Nephrology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.*

Background: The aim of this study was to estimate the cut-off level of microalbuminuria diagnostic for subclinical atherosclerosis. To do this, we evaluated the carotid, coronary and peripheral arteries in subjects recruited from the general population.

Methods: Study population included a total of 1,692 persons (1,069 males and 623 females), who participated in health screening at Asan Medical Center. The study involved performing coronary CT and carotid Doppler ultrasound on all patients, as well as determining their ankle-brachial index, pulse wave velocity and urinary albumin-to-creatinine ratio (UACR). Subjects with at least one of the following were classified as members of atherosclerosis group: 1) a coronary CT: coronary calcium score >400; 2) sonographically visible plaque or intimal thickening detected using carotid Doppler ultrasound; 3) an ankle-brachial index <0.9; or 4) a pulse wave velocity >8.5 m/s.

Results: Among 1,692 participants, 510 persons were assigned to the atherosclerosis group. The respective mean ages in the atherosclerosis and non-atherosclerosis groups were 59.2 ± 8.6 years and 52.6 ± 8.9 years (p < 0.001). The median UACR in the atherosclerosis group was 8.50 mg/g (95% CI: 7.60–9.55 mg/g), which was significantly higher (p = 0.017) than that in the non-atherosclerosis group, where it was 7.05 mg/g (95% CI: 6.60–7.55 mg/g). The use of receiver operating characteristic (ROC) curves for UACR to identify atherosclerosis indicated that the optimal cutoff value of UACR was 7.75 mg/g (47.5% sensitivity; 69% specificity). The gender-specific cutoff points were 6.45 mg/g (50.0% sensitivity; 70.1% specificity) in men and 8.45mg/g (62.7% sensitivity; 63.0% specificity) in women. Using multivariate analysis, male gender was the most powerful independent predictor for atherosclerosis. Smoking, hypertension, HbA1c, age, and body mass index were all independently associated with atherosclerosis, although microalbuminuria was not.

Conclusions: We have shown that the cut-off value of albuminuria for atherosclerosis using ROC curve analysis is much lower than the traditional level of microalbuminuria.

PUB486

Alterations in Cardiac Autonomic Tone in IgA Nephropathy: Baseline and Responses to Angiotensin II Challenge Michelle C. Mann, Derek Exner, Brenda Hemmelgarn, Tanvir Chowdhury Turin, Darlene Y. Sola, Sofia B. Ahmed. *Faculty of Medicine, University of Calgary, Calgary, AB, Canada.*

Background: Cardiac autonomic tone (CAT) dysfunction, particularly loss of cardioprotective vagal activity, and renin-angiotensin sysmte (RAS) upregulation increase cardiovascular disease (CVD) risk in chronic kidney disease (CKD), though whether this applies to those with early kidney disease and normal glomerular filtration rate (GFR) is unknown. We examined the CAT response, as measured by the balance of sympathetic to vagal activity, in response to angiotensin II (AngII) challenge in subjects with IgA nephropathy (IgAN) and normal GFR compared to healthy controls.

Methods: Eight male IgAN subjects (39±4y) and 26 healthy controls (34±2y, 27% male) were studied in high-salt balance during AngII challenge (3ng/kg/min x30min, 6ng/kg/minx30min). Sympathetic (LF), vagal (HF), and total sympathovagal (LF:HF) CAT were measured by heart rate variability (HRV) power analysis at baseline and in response to AngII. Baseline and response variables were adjusted for gender, BMI, and baseline HRV using ANCOVA.

Results: Compared to healthy controls, IgAN subjects demonstrated similar baseline sympathetic activity (LF nu, p=0.4 vs. controls) but significantly decreased vagal tone (HF nu, 26.2±4 vs. 35.8±7, p=0.02 vs. controls), though total sympathovagal CAT did not differ between groups (LF:HF, 1.86±0.3 vs. 1.94±0.6 p=0.8 vs. controls). In response to each AngII dose, both groups maintained similar sympathetic activity (ΔLF nu 30min, p=0.3; ΔLF nu 60min, p=0.4 vs. controls) but IgAN subjects demonstrated an exaggerated vagal response (ΔHF nu 30min, 10.4±3 vs. -2.8±2 nu, p=0.002; ΔHF nu 60min, 5.8±2 vs. -7.0±3 nu, p=0.07 vs. controls). Overall sympathovagal CAT did not differ between the two groups (ΔLF:HF 30min, p=0.4; ΔLF:HF 60min, p=0.2 vs. controls).

Conclusions: Compared to healthy controls, individuals with IgAN and normal GFR demonstrate withdrawal of cardioprotective vagal tone at rest and an augmented vagal response to AngII challenge, suggesting that alterations in CAT, which may influence CVD risk, occur even in patients with the earliest stages of CKD.

Funding: Government Support - Non-U.S.

PUB487

Vitamin D Supplementation Improves Cardiac Autonomic Tone in IgA Nephropathy Michelle C. Mann, Derek Exner, Brenda Hemmelgarn, Tanvir Chowdhury Turin, Darlene Y. Sola, Sofia B. Ahmed. *Faculty of Medicine, University of Calgary, Calgary, AB, Canada.*

Background: Vitamin D (VD) deficiency, increased renin-angiotensin system (RAS) activity, and poor cardiac autonomic tone (CAT) are common in chronic kidney disease (CKD) and are risk factors for cardiovascular disease (CVD). We examined the impact of VD supplementation on CAT in subjects with IgA nephropathy (IgAN) at baseline and in response to angiotensin II (AngII) challenge.

Methods: Eight male IgAN subjects (39±4 yrs, eGFR 91±11 ml/min/1.73m2) with VD-insufficiency (<75nmol/L 25OH VD) were studied in high-salt balance before and after 4 weeks of VD therapy (10,000 IU cholecalciferol/day). Sympathetic (LF), vagal (HF), and overall sympathovagal (LF:HF) CAT was measured by heart rate variability (HRV) power spectral analysis both at baseline and in response to AngII (3ng/kg/minx30min, 6ng/kg/minx30min). Pre- and post-supplementation measures were compared by non-parametric paired analysis.

Results: Both 25OH and 1,25OH VD levels increased in all subjects post-supplementation (25OH 59±9 vs. 137±19nmol/L, p=0.02; 1,25OH 111±12 vs. 139±15pmol/L, p=0.08, all values pre- vs. post-supplementation). VD supplementation did not result in a significant change in baseline CAT (LF nu, 68±7 vs. 70±8, p=0.4; HF nu, 19±6 vs. 27±9, p=0.1; LF:HF, 1.7±0.3 vs. 1.9±0.4, p=0.4), though the increase in 25OH VD with supplementation was positively correlated with baseline vagal tone (r=0.78, p=0.02) while the increase in 1,25OH VD with supplementation was positively correlated

with the change in baseline sympathovagal CAT (r=0.84, p=0.04). Overall sympathovagal CAT response to AngII challenge was improved post-supplementation (ΔLF:HF time 60, -0.02±0.3 vs. -0.43±0.3, p=0.04), which shifted more dramatically post-supplementation due to increased cardioprotective vagal contribution (ΔHF nu, 6±3 vs. 12±6, p=0.07) and an unchanged sympathetic response (ΔLF nu, p=0.5).

Conclusions: VD supplementation in VD-insufficient individuals with IgAN and normal kidney function appears to improve CAT, mainly through enhancing cardioprotective vagal resistance, both at rest and in response to acute RAS upregulation through AngII challenge.

Funding: Government Support - Non-U.S.

PUB488

Effect of Vitamin D Supplementation on the Arterial Response to Angiotensin II Challenge in Individuals with IgA Nephropathy

Ahmed Abdi Ali, Michelle C. Mann, Brenda Hemmelgarn, Jennifer M. MacRae, Tanvir Chowdhury Turin, Darlene Y. Sola, Sofia B. Ahmed. *Department of Medicine, University of Calgary, Calgary, AB, Canada.*

Background: IgA nephropathy (IgAN) is associated with increased cardiovascular (CV) risk. Arterial response to angiotensin II (AngII), a measure of endothelial health and intrinsic RAS tone, is an intermediate measure of CV risk. Low vitamin D (VD), a negative endocrine regulator of the RAS, status is common in kidney disease patients and is associated with increased CV risk. We sought to clarify the influence of vitamin D (VD) supplementation in modulating arterial response to angiotensin (Ang) II challenge in individuals with IgAN.

Methods: Ten normotensive, non-diabetic, non-obese subjects with IgAN (age 43±5, 90% men, eGFR 77.4±9.7 ml/min, 25OH VD 62±8 nmol/l), were studied before and after ingestion of 10000U of cholecalciferol daily x 28 days. All subjects were studied in high salt balance and women were studied in the same phase of their menstrual cycle. Blood pressure and arterial stiffness (aortic augmentation index (AIx)) were measured manually and by tonometry respectively, at baseline and in response to AngII infusion (3ng/kg/min x 30 min then 6ng/kg/min x 30 min). The primary outcome was the effect of VD supplementation on the BP and arterial response to AngII challenge.

Results: Baseline serum 25(OH)-VD levels increased from 628 to 14521 nmol/l after VD supplementation (p=0.04). VD supplementation had no effect on baseline BP (MAP:94±3 vs. 96±6 mmHg, p=0.3) or arterial stiffness (AIx:12±2.7 vs. 13±4 %, p=0.7) (all values pre- vs. post- VD supplementation). However, post-VD supplementation, a decreased in BP sensitivity to the vasoconstrictor effects of AngII challenge was observed (pre- vs. post- supplementation ΔMAP:21±2 vs. 17±3 mmHg, p=0.005). Post-VD supplementation, there was a trend towards decreased arterial sensitivity to AngII challenge (pre- vs. post-supplementation ΔAIx:12±2 vs. 9±5 %, p=0.2).

Conclusions: VD supplementation alters both the hemodynamic and arterial sensitivity to AngII in humans with IgAN. Further studies are needed to determine if VD supplementation ultimately decreases CV disease in individuals with IgAN.

PUB489

A Rare Case of Renal Infarction in a 35 Year Old Female: A Puzzle Unsolved

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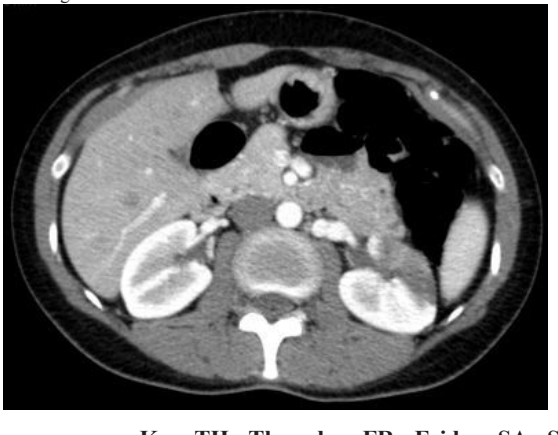
Background: Renal infarction is rare and easily missed diagnosis due to its non-specific symptoms. The exact incidence is not known. Hematuria, leukocytosis, and an elevated serum lactate dehydrogenase (LDH) are associated with renal infarction. Delay in the diagnosis is associated with higher morbidity and mortality. A computed tomography (CT) scan is the initial test of choice but angiography is still the gold standard. Some of the common etiologies are hypercoagulable state, and thromboembolic diseases, while fibromuscular dysplasia (FMD), vasculitis, spontaneous artery dissection, and segmental arterial mediolysis (SAM) are less common.

Methods: A 35 year old non-pregnant female without significant history presented with left flank pain for 3 days. On examination, she was afebrile, normotensive, in normal sinus rhythm, and had left flank tenderness. Her serum creatinine, amylase, lipase and electrolytes were normal. LDH and WBC were mildly elevated. The urinalysis was negative for any hematuria, pyuria or proteinuria. The CT scan of abdomen showed a left renal segmental infarct.



Her autoimmune, vasculitis, and hypercoagulable work-up were negative. The renal angiogram showed mild irregularity and short segment narrowing of the second and third order branches of the left renal artery, respectively, suggestive of questionable FMD versus SAM. These diagnoses are frequently associated with aneurysm, dissection, occlusion or stenosis, which were absent in our case. The normal transesophageal echocardiogram made thromboembolic cause unlikely. In view of inconclusive workup, idiopathic etiology remains a possibility.

Conclusions: This case provided a diagnostic and therapeutic challenge. The renal infarction should be suspected in all patients with flank pain even in the absence of risk factors. Once the diagnosis is made, FMD or SAM should also be considered as etiology.

PUB490

The Administration of Pitavastatin Augments Creatinine Clearance Associated with the Reduction in Oxidative Stress Parameters: Acute and Early Effects

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Background: Chronic kidney disease (CKD) is a serious health problem worldwide. Therapies that can halt the progression of CKD are limited, and the identification of new strategies for CKD treatment is therefore important. Pitavastatin, one of the newest statins introduced to the market, has been shown to exhibit some beneficial effects on renal and endothelial function.

Methods: We enrolled 12 health volunteers for our study. With or without pitavastatin administration, creatinine clearance (Ccr), urine albumin excretion, lipid status and oxidative stress markers are evaluated in acute and early phase after the administration of drug.

Results: A single pitavastatin administration increased Ccr and reduced oxidative stress parameters, such as 8-OHdG levels and isoprostane production, within 6 hours, without altering lipid status in healthy participants. A two-week treatment with pitavastatin lowered total- and LDL-cholesterol and triglycerides but not HDL-cholesterol at 7 and 14 days. This change in lipid profile is associated with enhanced Ccr and the suppression of oxidative stress parameters. Urine albumin excretion was reduced after either acute or chronic administration of pitavastatin, although this effect was not significant yet.

Conclusions: Here, we found that pitavastatin augmented Ccr and reduced oxidative stress parameters in healthy subjects. These data suggest that pitavastatin affects renal outcomes in both lipid status-dependent and -independent manners. Pitavastatin could be potential therapeutic options for CKD patients regardless of lipid status.

PUB491

Uremia-Induced Inflammation Preceeds Myocardiac Fibrosis in an Animal Model of Uremia

Aline Borsato Hauser,[1] Viviane Carvalho,[2] Caroline Gribner,[1] Mateus Justi Luvizotto,[2] Danielle De Paula e Silva Carneiro,[2] Melani Custodio,[3] Lúcia Noronha,[2] Roberto Pecoits-Filho.[2] *[1]Department of Medical Pathology, Universidade Federal do Paraná, Curitiba, Parana, Brazil; [2]School of Medicine, Pontificia Universidade Catolica, Curitiba, Parana, Brazil; [3]School of Medicine, Universidade de Sao Paulo, Sao Paulo, Brazil.*

Background: Chronic kidney disease is a multiplier of cardiovascular disease, through the induction of accelerated vascular disease and myocardiopathy. Uremia-induced inflammation leading to fibrosis may play an important role. It was analyzed the myocardial inflammation and fibrosis in an animal model ofuremia.

Methods: The uremicgroup underwent subtotalnephrectomy and the Control manipulation ofrenal pedicles. Euthanasiawas performed 4 and 8 weeksafter surgery. Histological sections were prepared from the myocardium to detect fibrosis and Tissue Micro Array was performed to detect expression of αTNF.

Results: In Uremic group, the weight of thecardiac masswas higher (p=0.043) andmyocardiac fibrosis increased over time (Fig1). In the 4 week the differences between uremic and control were not that pronounced (p=0.15); however fibrosis was significantly more intense in the Uremic group compared to controls at the 8 week (p=0.042). Regarding the αTNF expression, the highest levels occurred at 4 weeks, declining in the 8 week evaluation (Fig2). The increase in αTNF expression in uremic animals compared to controls was statistically significant at 4 weeks (p=0.001).

Conclusions: The αTNF expression suggesting that the uremia-induced inflammation of the heart decreases over time, unlike the fibrosis that seems to increase linearly until 8 weeks. Our study provides indirect evidence that uremia-induced inflammation preceeds myocardiac fibrosis in animal model.

Funding: Private Foundation Support

PUB492

Are Kidney Volume and Renal Vascular Resistance Associated with Augmentation Index in Early ADPKD? Ankit Chothani,[1] Nitender Goyal,[2] Kevin Heffernan,[3] Mark J. Sarnak,[1] Vandana Menon,[1] D. Miskulin.[1] *[1]Tufts Medical Center; [2]Steward Carney Hospital; [3]Syracuse University.*

Background: Augmentation Index (AIx) is elevated in Autosomal Dominant Polycystic Kidney Disease (ADPKD) before the onset of hypertension or reduced kidney function. Cysts impinging on arteries and arterioles create reflection points that may increase the amplitude of the reflected wave that is produced at bifurcation of resistance vessels. We sought to explore the association of AIx with Total Kidney Volume and Total Liver Volume (TKV+TLV), and Renal Vascular Resistance (RVR), in a subset of HALT PKD Study A Subjects (GFR>60 mls/min/1.73m^{2}).

Methods: Peripheral AIx was measured by pulse wave analysis from finger plethysmography in 44 subjects. TKV, TLV and renal blood flow (RBF) were measured via MRI. RVR was calculated as (MAP/RBF)*80000. Relationships of TKV+TLV and RVR with height adjusted AIx were determined through linear regression.

Results: The mean ±SD age was 43±7 years and the mean eGFR 71±27 mls/min/173 m^2. Factors significantly associated with AIX in unadjusted and multivariable models without RVR (Model 1) and with RVR (Model 2) are shown in the table.

Factors Associated with Augmentation Index

	UNIVARIATE	MULTIVARIATE without RVR	MULTIVARIATE with RVR
	B[95% CI]	B[95% CI]	B[95% CI]
AGE year	0.74[0.38 -1.11]	1.22[0.35 - 2.08]	2.66[1.10 - 4.23]
GLOMERULAR FILTRATION RATE ml/min/1.73m2	-0.21[-0.36 - -0.06]	ns	ns
MEAN ARTERIAL PRESSURE (MAP) mmHg	0.38[0.01 - 0.75]	ns	ns
URINARY ALBUMIN CREATININE RATIO mg/g	0.02[-0.05 - 0.09]	0.13[-0.02 - 0.27]	0.55[0.17 - 0.93]
TKV+TLV ml (per 50% increase in TKV+TLV)	5.9[-4.79 - 16.59]	11.85[2.01 - 21.7]	ns
RVR 1000 dynes second/cm-5	0.50[-0.17- 1.17]	-	-2.24[-4.85 - 0.36]

ns=non significant

Conclusions: AIx is significantly associated with TKV+ TLV after age, height and UACR are accounted for. When RVR is added to the model, TKV +TLV is no longer significant. Contrary to our hypothesis, RVR and AIx are inversely related. RVR measured in the main renal arteries may not capture vascular resistance at smaller arterial beds. An alternate explanation is that the relationship of TKV+TLV with AIx is not mediated through effects of cyst growth on vascular resistance.

Funding: NIDDK Support

PUB493

Efficacy of Cinacalcet for the Treatment of Secondary Hyperparathyroidism in CKD Patients on Peritoneal or Hemo Dialysis: The Middle-East Experience Krishan L. Gupta, Abdullah Al Hwiesh, Ibrahim Saeed, Fahd Muhanna. *Department of Internal Medicine, Nephrology Division, University of Dammam, KFHU, Al-Kobar, Eastern Province, Saudi Arabia.*

Background: Management of secondary hyperparathyroidism (SHPT) is challenging with traditional therapy. Calcimimetics lower parathyroid hormone levels without increasing calcium and phosphorus levels. Our study was aimed at evaluatating effectiveness of calcimimetic, cinacalcet hydrochloride in reducing serum intact PTH (iPTH) levels in patients with end stage renal diseases and SHPT.

Methods: The study included patients who were receiving regular dialysis and had inadequately controlled SHPT despite standard treatment (calcium based phosphorus binders and/or sevlamer carbonate at ceiling doses with or without vitamin D sterols - 1,25(OH)2-vitamin D). They were assigned to receive cinacalcet (Group I, n= 69; 45 on hemodialysis and 24 on automated peritoneal dialysis) or their usual drugs without cinacalcet (Group II, n= 40; 20 each on hemodialysis and peritoneal dialysis) for 12 months. Once-daily doses of cinacalcet hydrochloride was increased from 30 mg to 180 mg to achieve iPTH levels of < 300 pg/ml. Serum calcium, phosphorous and iPTH were monitored before starting cinacalcet, at 3 months, 6 months and 12 months.

Results: Overall the mean iPTH before start of therapy was 1086 ± 84.52 pg/ml in cinacalcet-group I and 644.9 ± 86.58 pg/ml in no-cinacalcet group II [p= 0.60]. At the end of the study these levels changed to 465.1± 46.51 pg/ml and 914± 173.6 pg/ ml respectively [p=0.01]. Serum calcium at 12 months was higher in the cinacalcet group compared to controls. Serum phosphorus was higher in the cinacalcet group at the start of therapy and persisted to remain so till end of study at 12 months.

Conclusions: Cinacalcet effectively lowers parathyroid hormone levels in patients receiving dialysis and having uncontrolled secondary hyperparathyroidism. Frequent monitoring and adequate replacement with calcium and vitamin D sterols prevent hypocalcemia with cinacalcet therapy. Thus, cinacalcet is a goad therapeutic option for controlling SHPT in end-stage renal disease patients on both hemo and peritoneal dialysis.

PUB494

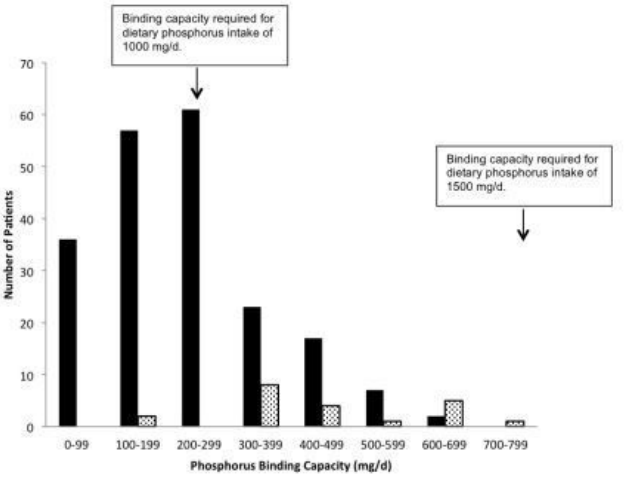
The Adequacy of Phosphorus Binder Prescriptions among Hemodialysis Patients Anne M. Huml, Catherine M. Sullivan, Janeen B. Leon, Ashwini R. Sehgal. *Department of Medicine, Division of Nephrology, MetroHealth Medical Center, Cleveland, OH.*

Background: Because hemodialysis treatment has a limited ability to remove phosphorus, dialysis patients must both restrict dietary phosphorus intake and use phosphorus binding medication. Among patients with restricted dietary phosphorus intake, binders must bind about 250 mg/d of phosphorus to maintain balance. Among patients with more typical phosphorus intake, binders must bind about 750 mg/d. We sought to determine the phosphorus binding capacity of binder prescriptions among hemodialysis patients.

Methods: We randomly selected 300 chronic hemodialysis facilities from *Dialysis Facility Compare.* Study personnel called each facility's dietitian and obtained the following data for one randomly selected patient: phosphorus binder prescription, most recent serum phosphorus level, post-dialysis weight, height, age, gender, race/ethnicity, and years on dialysis. We calculated the phosphorus binding capacity for each binder prescription. To determine the adequacy of binder prescriptions, we used standard estimates of phosphorus intake, gut absorption, and dialytic removal.

Results: Among the 224 patients prescribed binders, the mean phosphorus binding capacity was 256 mg/d (SD 143). 59% of prescriptions had insufficient binding capacity for restricted dietary phosphorus intake, and 100% had insufficient binding capacity for typical dietary phosphorus intake. Patients using two binders had a higher binding capacity than patients using one binder (451 vs. 236 mg/d, p <0.001).

Figure 1: Distribution of PBC. Dark bars represent 203 patients on single binder. Light bars represent 21 patients on two binders.



Conclusions: A majority of binder prescriptions have insufficient binding capacity to maintain phosphorus balance. Use of two binders results in higher binder capacity. Further work is needed to understand the impact of binder prescriptions on mineral balance and metabolism and to determine the value of substantially increasing binder prescriptions.

Funding: NIDDK Support, Other NIH Support - Anne Huml Was Supported by Grant T32DK007470 from the National Institutes of Health. Catherine Sullivan, Janeen Leon and Ashwini Sehgal Were Supported by Grants MD002265 and RR024989 from the National Institutes of Health

PUB495

Efficacy of PA21, a New Iron-Based Phosphate Binder, as Compared to Lanthanum Carbonate and Sevelamer Carbonate on Mineral Metabolism Disorders and Vascular Calcifications in Uremic Rats Olivier Phan,[1] Marc P. Maillard,[1] Felix W. Funk,[2] Bruno Vogt,[1] Michel Burnier.[1] *[1]University of Lausanne CHUV; [2]Vifor (International) Inc.*

Background: The present study compared the efficacy of PA21 with lanthanum carbonate (La) and sevelamer carbonate (Se) on hyperphosphatemia, secondary hyperparathyroidism and vascular calcification in rats with chronic renal failure (CRF).

Methods: CRF was induced by feeding a 0.75% adenine-enriched high phosphorus (P 1.3 %) diet for 4 weeks. Rats were randomized to one of 3 binder treatment groups (PA21, La and Se) or to CRF and non-CRF controls for another 4 week period. The concentration of each binder (% of binder added to the diet) was chosen to deliver approximately the same amount of active pharmaceutical ingredient to each rat: PA21 5% (corresponding to 1% iron), La 2% (1% lanthanum), Se 1.5% (1% sevelamer). A computer-assisted automated quantitative measurement was used to assess the degree of calcification from von Kossa stained vessel sections. Data were expressed as the relative proportion (%) of calcified area to total surface area of each vascular ring.

Results:

	N	Creat (micromol/l)	P (mmol/l)	iPTH (pg/ml)	Abdominal aorta (%)	Inferior Thoracic Aorta (%)	Superior Thoracic Aorta (%)
Non-CRF control	4	48±3	2.09±0.05	275±38[b]	0	0	0
CRF control	20	144±11[a]	3.30±0.29	3567±593	1.25±0.66	3.22±1.31	8.05±2.01
CRF PA21	19	141±10	2.06±0.06[b]	1459±242[b]	0.10±0.06[b]	0.34±0.21[b]	0.14±0.13[b,c]
CRF Se	20	147±11	2.51±0.12[b]	1569±238[b]	0.53±0.40	0.55±0.38[b]	1.59±0.91[b]
CRF La	18	140±8	2.24±0.07[b]	1360±170[b]	0.20±0.15	0.39±0.27[b]	3.93±1.13[b]

Values are shown as mean±SD, [a]p<0.05 vs non-CRF control, [b]p<0.05 vs CRF control, [c]p<0.05 vs CRF La

Conclusions: PA21 is as effective as La and Se to control hyperphosphatemia and secondary hyperparathyroidism. The extent of vascular calcifications in all phosphate binder treated animals was significantly lower than in the CRF control animals. In the upper part of the thoracic aorta, PA21 was even more efficient than Lanthanum carbonate to prevent calcifications.

Funding: Pharmaceutical Company Support - Vifor (International) Inc., Government Support - Non-U.S.

PUB496

Comparison of Efficacy and Equivalent Dose between Osvaren and Fosrenol for the Control of Serum Phosphate in CAPD Patients Jesus Montenegro, José Ignacio Cornago, Maria Isabel Gallardo, Paula García, Ainhoa Hernando, Rosa Ines Munoz. *Nephrology, Hospital de Galdakao, Bilbao, Vizcaya, Spain.*

Background: To find out the capacity and the equivalent dose between two phosphate binders, Mg Carbonate and Ca Acetate (Osvaren®, Fresenius Medical Care) and Lantanum Carbonate (Fosrenol®, Shire) for the control of serum P, a randomized cross over study was done (Fosvaren Study) in CAPD patients.

Methods: After two weeks of wash-out, they were randonmized into 2 groups. Group 1, 23 patients: Osvaren→Fosrenol→Osvaren; in group 2, 22 patients: Fosrenol→Osvaren→Fosrenol. The patients changed the binder every 2 months. By protocol, the equivalent dose was: one pill of Osvaren (435 mg of Ca acetate and 235 mg of Mg carbonate) to 1 pill of Fosrenol 500 mg. It was also established by protocol that for every increase of 1 mg/dL of serum P two pills per day of binder. Every two months before changing the binder the following values were determined in blood: P, total Cal, Ca++, Mg, PTH, alkaline phosphatase and bicarbonate. Daily urinary excretion of P, Ca and Mg were calculated. Daily peritoneal phosphate removal and protein intake were calculated.

Results: The binder dose, vitamin D dose and the protein intake varied very little. The urinary phosphate and the peritoneal phosphate removal were similar in both groups all time. The serum P at the beginning, basal, was similar in both groups, but the serum P decreased significantly with both phosphate binders, as we can observe throughout the study in the table.

Outline of serum phosphate in both groups

Groups and serum P	Basal	2 months	4 months	6 months
G1: P (mg/dL)	6.38 (0.71)	4.85 (0.78)*	4.90 (0.80)	4.76 (0.83)
G2: P (mg/dL)	6.18 (0.64)	4.86 (0.72)*	4.92 (0.81)	4.79 (0.73)

*p<0.05

The serum Ca and Mg increased significantly during the intake periods of Calcium and Magnesium salts and the PTH had a greater descent reaching in some periods a significant decrease.

Conclusions: We can conclude that both phosphate binders, Osvaren and Fosrenol, return to normal the levels of serum phosphate in the majority of PD patients. According to these results, an equivalent dose of 1 tablet of Osvaren/1 tablet of Fosrenol 500 mg has been established for serum phosphate control.

PUB497

Extracorporeal Phosphate Removal with a Packed Bed Adsorber Rebecca J. Desch,[1] Juliann K. Leny,[1] Stephen W. Thiel,[1] Vadim Guliants,[1] Heather Duncan,[2] Kotagal Shashi Kant.[2] *[1]School of Energy, Environmental, Biological and Medical Engineering, University of Cincinnati, Cincinnati, OH; [2]School of Energy, Internal Medicine, University of Cincinnati, Cincinnati, OH.*

Background: Phosphorous (PO4) control continues to be a complex and vexing problem for dialysis patients. PO4 dialysance with conventional dialysis is suboptimal largely because of the multicompartment distribution of phosphate. Effectiveness of oral PO4 binders is limited by issues of cost, efficacy and adherence. We are exploring an alternative approach that incorporates a packed bed for PO4 adsorption into the dialysis circuit to remove excess PO4 during regular dialysis treatments. This will address the multicompartment nature of PO4 distribution and increase total PO4 removal.

Methods: The efficacy of adsorbents incorporating aluminum, lanthanum, and zirconium were evaluated with batch equilibrium (0-25 mg/dL PO4) and kinetic studies (10 mg/dL PO4) in water at pH 8.2 at 37°C.

Results: PO4 adsorption to resin-based adsorbants was slow. Lanthanum activated carbon and aluminum oxide combined relatively fast adsorption with PO4 capacity of 30mg/g adsorbent, at a PO4 concentration of 5mg/dL. The interference of blood components, including salts and proteins, was tested on the adsorption of 20mg/dL PO4. Most of the tested salts and biomolecules enhanced or minimally impacted PO4 loadings, but bovine serum albumin (BSA) reduced the PO4 loading significantly.

Interference effects on PO_4 adsorption onto lanthanum activated carbon

Blood Component	Concentration (mg/dL)	%Phosphate Loading
Water	-	100
NaCl	900	95
$NaHCO_3$	233	88
Urea	50	96
BSA	4300	71
γ-Globulin	2400	92

Finally, the PO4 desorption in the presence of potential regeneration solutions was measured. PO4 was effectively desorbed using concentrated acid or base; however these strong regeneration solutions damaged the adsorbents.

Conclusions: Lanthanum activated carbon and aluminum oxide show promise as selective, high capacity, and cost effective materials for phosphate adsorption. The insights from this research will guide the design of packed bed adsorption units to safely and effectively control PO4 concentrations in dialysis patients.

Funding: Private Foundation Support

PUB498

Magnesium Retards the Progress of the Artery Calcifications in Hemodialysis Patients: A Controlled Prospective Study Ioannis P. Tzanakis, Elisavet E. Stamataki, Antonia N. Papadaki, Vlasios V. Spantidakis, Stella N. Kagia, Styliani-rafaella Poulidaki. *Nephrological, General Hospital of Chania, Chania, Krete, Greece.*

Background: Results from observational studies provide enough evidence that high serum magnesium is related with less calcifications in hemodialysis patients. The aim of the study was to evaluate the impact of magnesium (Mg), dosing as a phosphate binder, on the evolution of artery calcifications in hemodialysis patients.

Methods: Seventy-two stable hemodialysis patients were randomly allocated to two groups: 36 administered the regimen OSVAREN containing Magnesium carbonate plus Calcium acetate as a phosphate binder (Mg group), while the rest 36 received Calcium acetate alone (Ca group). The presence and the progression of artery calcifications were evaluated in plain X-ray of pelvis, femur, abdomen and hands at the begging and at the end of the study using a simple vascular calcification score (SVCS). The duration of the follow up period was 12 months.

Results: Fifty-nine patients completed the sudy: 32 of Mg group and 27 of Ca group. The mean time average values of Calcium, Phospate, Calcium X Phosphate product and parathyroid hormone did not differ between the two groups, whereas there was a significant difference in serum Mg: 2.83±0.38 in Mg group vs 2.52±0.27 mg/dl in Ca group, p=0,001. In 9/32 (28.12%) patients of the Mg group and in 12/27 (44.44%) patients of the Ca group the artery calcifications were worsened within the 1 year period, p=0.276. Moreover in 4/32 (15.6%) patients of Mg group and in 0/27 (0%) patients of Ca group the artery calcifications were improved, p=0.040 Figures 1-4.The multivariate logistic regression analysis revealed that serum magnesium was the only independed predictor for no progression (improvement and stable) of the artery calcifications, p=0.47.

Conclusions: The hemodialysis patients who received the magnesium containing regimen manifested less progression or regression of artery calcifications. Serum magnesium was found to be an independed factor of no progression of the calcifications. Magnesium probably retards or even improves the evolution of the artery calcifications in hemodialysis patients.

PUB499

Association of Serum Magnesium and Mortality in Incident Dialysis Patients Johanna Van den Broek,[1] Christiane Drechsler,[2] Vincent Brandenburg,[3] Friedo W. Dekker,[4] Marc G. Vervloet.[1] *[1]Nephrology, VU Medical Center, Amsterdam, Netherlands; [2]Internal Medicine 1, Division of Nephrology, University of Würzburg, Würzburg, Germany; [3]Cardiology, University Hospital of the RWTH, Aachen, Germany; [4]Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands.*

Background: Although several biomarkers of the chronic kidney disease-mineral and bone disorder (CKD-MBD) syndrome are associated with clinical endpoints, the potential role of magnesium is underexposed. Magnesium appears to have beneficial effects on the process of vascular calcification. We therefore searched for epidemiological evidence to support the assumption of a favorable effect of magnesium on mortality in CKD.

Methods: Magnesium levels were determined in 766 incident hemodialysis and peritoneal dialysis patients from the NECOSAD cohort. Association with all-cause mortality at months 36 from start of dialysis was studied for tertiles of magnesium levels. Multivariate adjustments were made for age, dialysis modality, primary kidney disease and presence of diabetes mellitus.

Results: 766 patients were studied (63 ± 14 years, 59% male, 91% hemodialysis, 93% Caucasian). Diabetes was present in 25%. Magnesium levels were 1.05 ± 0.19 mmol/l. All-cause mortality at month 36 after start of dialysis was 30%. Using univariate analysis mortality was associated with age (B 0.049, p <0.005), dialysis modality (B -1.002, p 0.005), primary kidney disease and diabetes (B 0.673, p <0.005). Using multivariate analysis dialysis modality was no longer significant. The lower magnesium tertile was associated with a non-significant trend with higher mortality in the full-adjusted model (B 0.167, p 0.31 compared with the highest, and B 0.194, p 0.23 compared with intermediate tertile).

Conclusions: Low magnesium levels show a non-significant trend to be associated with higher mortality at month 36 for incident dialysis patients. The inference of a causal relation cannot be made from our data. However, our results do reassure the relative safety of magnesium levels in high-normal range in these subjects.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

PUB500

Predicting Serum Phosphate Concentrations in Patients Undergoing Chronic Hemodialysis Using a Dynamic Model Derrick J. Stobaugh, Parakkal Deepak, Eli D. Ehrenpreis, Stuart M. Sprague. *NorthShore University HealthSystem.*

Background: Managing phosphate overload is a significant clinical problem in patients with End Stage Renal Disease (ESRD). A dynamic model to predict serum phosphate concentrations in ESRD subjects was developed by simulating phosphate intake, absorption and elimination and the effects of diet, phosphate binders and dialysis.

Methods: Using published data, a computer model with STELLA software (ISEE, Hanover, NH) and advanced simulations with the Berkeley Madonna program (Berkeley Madonna Inc., Berkeley, CA) was developed. Simulations of a variety of conditions were performed using multiple adjustable parameters. To test model function, serum phosphate concentrations following standardized thrice weekly chronic hemodialysis (CHD), nocturnal hemodialysis (NHD) for 8 hours daily, short daily (SHD; 2 hours/day, 5 days/week) and peritoneal dialysis (PD) were compared. This simulation included NHD (no phosphate binders, average dietary phosphate intake of 1.4g a day), and CHD, SHD, and PD with the highest recommended doses of lanthanum (4.5g) or sevelamer (4.8g), (average phosphate consumption 0.9 g/ day. Lanthanum or sevelamer were ingested with meals, thrice daily. Graphic display of phosphate concentrations over ninety 24 hour cycles (3 months) were produced.

Results: Simulations demonstrated that patients on NHD had the lowest mean phosphate level (3.53 mg/dl), followed by SHD, PD, and then CHD (mean phosphate 5.06 mg/dl, 5.28 mg/dl, and 6.07 mg/dl respectively). PD had the lowest variability of phosphate levels. Table 1 shows phosphate levels simulated in patients receiving either sevelamer or lanthanum.

Serum phosphate levels with binder and/or dialysis

	NHD	SHD		CHD		PD	
		Sev	Lan	Sev	Lan	Sev	Lan
Mean phos	3.53	4.62	3.93	5.62	4.43	4.86	4.44
SD	2.03	1.23	1.12	0.92	0.70	0.41	0.20
Max phos	7.49	7.15	5.91	8.29	5.71	6.10	5.13

Sevelamer Sev; Lanthanum Lan.

Conclusions: A model has been developed to simulate intake and removal of phosphate in ESRD patients. Inputs are adjustable to develop predictive data. Further validation will require real time patient testing. Potential applications of this model include individualization of patient care and new drug development.

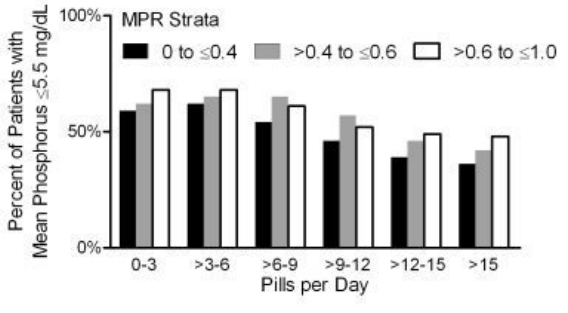
PUB501

Serum Phosphorus Levels Are Inversely Associated with Medication Possession Ratio among Hemodialysis Patients Steven Wang,[1] Thomas Alfieri,[1] Peter G. Braunhofer,[2] Britt B. Newsome.[3] *[1]Davita Inc., Denver, CO; [2]Vifor Pharma, Glattbrugg, Switzerland; [3]Denver Nephrology, Denver, CO.*

Background: Phosphate binders (PB) account for ~1/2 of the high daily pill burden (median=19) for US hemodialysis (HD) patients (pts), which may reduce adherence. Effective adherence is best estimated using the medication possession ratio (MPR) which reflects the proportion of time the pt had sufficient supply to have taken the medication as prescribed. The longer the gaps between fills, the lower the MPR. Using data from a dialysis provider's pharmacy management program, we assessed the effect of PB pill burden and compliance (MPR) on phosphorus goal attainment.

Methods: Monotherapy HD pts were tracked from first PB fill from 1/1/2007–6/30/2011 for 1 year, or until they switched PBs or were censored (at automatic refill program enrollment, 180 day gap between medication fills, 2 consecutive phosphorus levels < 3.0 mg/dL, discontinuation of HD, or death). MPR was calculated as (prescription days filled in period – excess days) / (days in period), where excess days is the days of pills left at the end of the period. Mean MPR scores were weighted by pt-time contributed. Associations were assessed by GLM.

Results: After exclusion of dual therapy pts (28%), 9346 pts were included. Lower mean phosphorus levels and higher % of pts with ≤ 5.5 mg/dL phosphorus were associated with higher MPR; effects were accentuated in higher pill burden strata ($p<0.001$ across strata for both). However, the 6-9 and 9-12 pills/day strata were U-shaped.



Conclusions: MPR was negatively related to phosphorus level and positively related to % of pts in phosphorus range. Within pill burden strata, phosphorus decreased and % of pts in phosphorus range generally increased with increasing MPR suggesting that pts prescribed more PB pills are more likely to have treatment gaps and poorer outcomes.

Funding: Pharmaceutical Company Support - Vifor Pharma

PUB502

Improved Patient Survival with Sevelamer Compared to Calcium-Based Phosphate Binders: A Meta-Analysis of Long-Term Randomized Controlled Trials Nashila AbdulRahim,[1] Donald A. Molony,[1] Shradha Kulkarni,[1] Antonio Bellasi.[2] *[1]Renal Dis & Hypertension, Univ Tx Houston Med Sch, Houston, TX; [2]U.O.C. di Nefrologia, Dialisi, Ospedale Sant'Anna, Como, Italy.*

Background: Epidemiologic studies support the hypothesis that excess oral intake of calcium results in increased cardiovascular events in chronic kidney disease (CKD) and ESRD patients. Conversely, by reducing calcium and PO_4 exposures in CKD patient, sevelamer could potentially improve survival. Demonstrating a survival benefit with sevelamer has been difficult, mainly because of the small size, short duration, or incomplete follow-up of the RCTs conducted to date. Meta-analyses have reported mixed results. The purpose of the current study is to perform a standard meta-analysis comparing the survival benefits of sevelamer versus calcium-based phosphate binders in CKD patients, antecedent to a network meta-analysis.

Methods: A systematic review of the literature was performed using PubMed, EmBase, Trip and the Cochrane trials registry through 6/01/2012. We included only those studies with follow-up of 1 year or greater and transformed the number of deaths reported into a rate expressed in terms of subjects years at risk to account for the variation in drop-out rates and duration of follow-up.

Results: Eleven RCTs (9 for ESRD and 2 pre-dialysis CKD cohorts) were included in the final analysis. For the 9 ESRD studies the combined odds ratio (OR) using a random effects model was 0.498 (95% CI 0.274 – 0.889, I^2 79%) favoring a survival benefit with sevelamer. The survival benefit with sevelamer was no-longer observed when RCTs of less than 1 year duration were included or when incident dialysis patient RCTs were excluded (OR 0.753, 95% CI 0.51-1.119) but was observed when only studies of moderate or higher grade were analyzed. A survival benefit with sevelamer was observed when the incident patients were analyzed separately (OR 0.296 95%CI 0.154 – 0.57, I^2 26%). The pre-dialysis studies demonstrated a relative risk similar to that in incident ESRD patients.

Conclusions: The current meta-analysis demonstrates a survival advantage with sevelamer which can be attributed, in part, to inclusion of higher quality or incident dialysis studies.

Funding: Private Foundation Support

PUB503

Regulation of Type II Sodium Phosphate Cotransporter Trafficking Eleanor D. Lederer,[1,2,3] Rachel Grisham,[2] Syed J. Khundmiri,[2,3] Edward J. Weinman.[4,5] *[1]Medicine, Robley Rex VA Medical Center, Louisville, KY; [2]Medicine, University of Louisville School of Medicine, Louisville, KY; [3]Physiology and Biophysics, University of Louisville School of Medicine, Louisville, KY; [4]Medicine, Baltimore VA Medical Center, Baltimore, MD; [5]Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Background: We have previously shown that the type II sodium phosphate cotransporter Npt2a exists in a multi-protein complex in the brush border membrane (BBM) of proximal tubule cells. The molecular mechanisms responsible for the assembly and trafficking of the complex are unknown. We hypothesized that the complex assembles in intracellular compartments and traffics to the apical membrane by vesicular transport.

Methods: We compared compartment protein expression at 37C and 16°C, to arrest protein trafficking, in opossum kidney (OK) cells in the presence of low phosphate, a stimulus for Npt2a exocytosis. Cell lysates were separated by sucrose gradient centrifugation and analyzed by western blot. To determine if Npt2a trafficked through vesicular transport, we compared low phosphate stimulated Npt2a plasma membrane expression in OK cells where the SNARE protein SNAP 23 was knocked down and in cells transduced with a competitive SNAP23 inhibitory peptide.

Results: Incubation at 16°C resulted in a parallel increase in expression of Npt2a and NHERF1 in Golgi fractions (GM58+) and a comparable decrease in plasma membrane fractions. Western blot of Npt2a in BBM derived from OK cells, vector-transfected (VF), and knockdown (E9) cells showed a 25 kD decrease in the molecular size of Npt2a in E9 cells. F-glycosidase digestion of BBM resulted in the appearance of a band at 75 kDa for all three cell types. PTH decreased Npt2a expression by 50% in VF and 75% in E9. Low phosphate stimulated 25% increase in Npt2a expression in VF and a 75% increase in E9.

Conclusions: We conclude that NHERF and Npt2a traffic in the same temporal pattern, suggesting association prior to apical membrane insertion and that SNAP 23 does not play a role in Npt2a apical membrane trafficking, but may play a role in post-translational modification.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

PUB504

Comparison of Dose Holds for Paricalcitol and Cinacalcet during Treatment of Secondary Hyperparathyroidism (SHPT): Secondary Analysis of the IMPACT Study Markus Ketteler,[1] Kevin J. Martin,[2] Michael Amdahl,[3] Mario Cozzolino,[4] David Goldsmith,[5] Amit Sharma,[6] Samina Khan.[3] *[1]Klinikum Coburg, Coburg, Germany; [2]St. Louis University, St. Louis, MO; [3]Abbott, Abbott Park, IL; [4]University of Milan, Milan, Italy; [5]Guy's Hospital, London, United Kingdom; [6]Pacific Renal Research Institute, Meridian, ID.*

Background: Secondary hyperparathyroidism (SHPT) is a complication of chronic kidney disease (CKD), typified by elevated intact parathyroid hormone (iPTH) and altered mineral metabolism. Most guidelines require dose holds when SHPT medications disrupt calcium (Ca) or phosphorus (P) metabolism beyond specified levels. Such recurrent interruption may jeopardize treatment optimization and possibly lead to non-compliance. Here we report a secondary endpoint examining dose holds during the IMPACT-SHPT study.

Methods: IMPACT-SHPT was a study of hemodialysis subjects receiving IV (IV Stratum) or oral paricalcitol (Oral Stratum) compared with subjects receiving cinacalcet with low-dose vitamin D. Doses of study drugs were adjusted or held based on pre-defined iPTH, Ca and Ca x P product thresholds. A negative binomial regression analysis was used to compare the number of doses held between paricalcitol and cinacalcet groups in each stratum.

Results: There were a total of 126 subjects in the IV Stratum and 142 in the Oral Stratum. There were 268.4% (P=0.003) and 171.6% (P<0.001) more doses held for cinacalcet than paricalcitol in the IV Stratum and Oral Stratum, respectively. Lab results (Table) showed that dose holds in paricalcitol subjects mostly followed high Ca, CaxP and/or low iPTH and dose holds for cinacalcet subjects mostly followed low Ca, CaxP and iPTH.

	IV Stratum		Oral Stratum	
	Paricalcitol	**Cinacalcet**	**Paricalcitol**	**Cinacalcet**
Subject lab results prior to dose hold, n (%)	**(16 total dose holds)**	**(43 total dose holds)**	**(39 total dose holds)**	**(86 total dose holds)**
Ca>11mg/dL, CaxP>75mg²/dL², iPTH<150pg/mL	2 (13)	0	4 (10)	0
Ca>11mg/dL, CaxP>75mg²/dL²	0	0	1 (3)	0
Ca>11mg/dL, iPTH<150pg/mL	5 (31)	0	10 (26)	0
Ca>11mg/dL	1 (6)	0	3 (8)	0
CaxP>75mg²/dL², iPTH<150pg/mL	0	0	2 (5)	0
CaxP>75mg²/dL²	0	0	1 (3)	0
Ca<7.5mg/dL, iPTH<150pg/mL	0	7 (16)	0	0
Ca<7.5mg/dL	0	13 (30)	0	30 (35)
iPTH<150pg/mL	4 (25)	15 (35)	11 (28)	27 (31)
Other	4 (25)	8 (19)	7 (18)	6 (7)

Conclusions: In the IMPACT study, subjects given paricalcitol required fewer dose holds than those given cinacalcet.

Funding: Pharmaceutical Company Support - Abbott Laboratories

PUB505

Acetazolamide-Induced Remission in Severe Tumoral Calcinosis Despite Persistent Hyperphosphatemia Gal Finer,[1] Heather E. Price,[1] Richard M. Shore,[1] Kenneth E. White,[2] Craig B. Langman.[1] *[1]Kidney Diseases, Northwestern University, Chicago, IL; [2]IUPUI, Indianapolis, IN.*

Background: Idiopathic tumoral calcinosis (TC) is an AR disorder characterized by ↑ tubular phosphate (P) resorption, hyperphosphatemia (hiP) and tumor-like extraosseous periarticular calcifications. Despite the hiP, patients have ↑ serum 1,25-dihydroxyvitamin D. TC results from an inactivating mutation in *FGF23*, *GALNT3* or *KLOTHO*. In addition to tumor removal by surgery, medical Rx of low P diet and P-binders aims to prevent new ones but is not effective uniformly. Sporadic reports of alternative medical Rx include acetazolamide (ACTZ).

Results: We describe a 7yo AA boy with severe and protracted TC (multiloculated calcified masses of buttock & elbow), who responded to ACTZ-induced metabolic acidosis despite ↑ serum P (Table). Histopathology of the masses confirmed TC. FGF23 assays revealed ↑c-terminal but ↓intact FGF23 levels for the degree of hiP (Table). DNA sequencing showed compound heterozygosity in *GALNT3*. Rx with sevelamer was initiated, but the patient had recurrence of para-articular mineral deposits in the hip & elbow and progression of bone disease involving tibia, ulna, mandible & maxilla that required surgeries. At 9.5y, ACTZ(40 mg/kg/day) was begun, & resulted in complete cessation of new tumor eruption for 2 years. ACTZ induced mild acidosis but no change in hiP or TRP (Table). Patient had no side effects related to Rx, had normal eGFR and improved linear growth to the 40th%ile for age.

	At presentation	On P Binder Mean (range)	On ACTZ Mean (range)
P (mg/dL)	6.8	6.7 (5.3-7.9)	6.9 (4.9-8.8)
CO2 (mEq/L)	24	25.6 (25.2-27.4)	21.2 (19-25.3)
serum pH	7.42	7.38 ((7.32-7.42)	7.31 (7.29-7.4)
TRP (%)	96.6	97.4 (96.9-97.9)	95.6 (91.7-98.2)
25(OH)D (ng/mL)	22	20.7	20
1,25-D3 (pg/mL)	52	42.4	48
iCa (mM)	1.27	1.30 (1.25-1.4)	1.32 (1.21-1.43)
Total Ca (mg/dL)	9.7	9.8	10.2
PTH (pg/mL)	15	10	9
FGF23 C-terminus RU/mL	759		1584
FGF23 intact pg/mL	26		7

Conclusions: In TC, ACTZ halted disease progression despite no changes in serum P and TRP. This suggests that ACTZ increased the calcium-phosphate complex solubility, likely through pH modifications, and not by promoting phosphaturia as previously thought.

PUB506

25OHVitD Levels and His Association with Demographic, Biochemical and Cardiovascular Factors and Survival in Hemodialysis Patients Yanet Parodis López,[1] Beatriz Quintana Viñau,[1] Francisco Alonso Alman,[1] Juan Francisco Betancor Jiménez,[1] Miguel Angel García Bello,[2] Jose C. Rodriguez-Perez.[1] *[1]Nephrology, Baxter; [2]Investigation Unit Department, Dr Negrín Hospital, Las Palmas, Spain.*

Background: Low vitamin D levels are common in hemodialysis patients and its deficit has been associated with cardiovascular complications and mortality. We conduct this study to know the 25OHVitD levels and correlate these levels with the biochemical, demographic and cardiovascular state as well as assess its impact on survival.

Methods: This is a cross sectional study in the first half of 2011 in 232 hemodialysis patients. Demographic and clinical data including echocardiographic, ankle/brachial index (ABI) and Charlson index were recorded. We correlate these variables with each other as with 25OHVitD levels. Survival (Kaplan-Meier) is assessed by levels of vitamin D at one year of follow up.

Results: The 88.79% of patients had some degree of hypovitaminosis D. Diabetes Mellitus (p=0.003) and older age (p=0.017) were associated with more 25OHVitD deficiency. Female sex (OR:4.78: 95% CI 2.53 to 9.03) and Charlson Index ≥5 (OR 3.84: 95% CI 1.68 to 8.76) were considered independent risk factors for 25OHVitD deficiency. No relationship was found between 25OHVitD and echocardiographic parameters. Those with ABI ≤0.9 (26/73), only 1 patient had normal 25OHVitD levels, the remaining 25 (96.1%) had 25OHVitD hypovitaminosis (p=0,02). Significant differences were seen between the ABI mean levels comparing 25OHVitD deficient patients vs others (p=0.036). There was significant association between Diabetes Mellitus and Charlson ≥5 with ABI ≤0.9 (p=0.01 and p=0.003 respectively). We found worse survival in 25OHVitD deficient patients when compared with others (p=0,03).

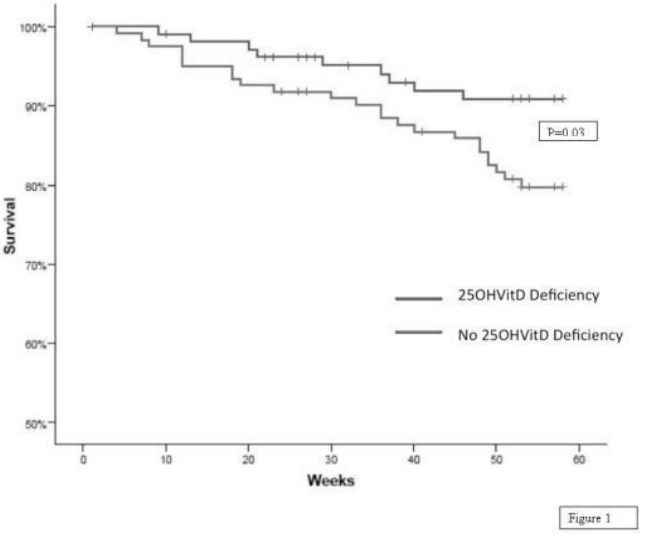


Figure 1

Conclusions: Vitamin D deficiency is common in hemodialysis patients. The higher vitamin D deficiency increased mortality.

PUB507

25 OH Vitamin D Therapeutic Response in the Two Fist Weeks after Calcidiol Administration Emilio E. Gonzalez-parra, Maria Vanessa Perez Gomez, Laura Rodríguez-osorio, Valeria Sainz, Alberto Ortiz, Jesus Egido. *Nephrology, Fundación Jiménez Díaz, Madrid, Spain.*

Background: Recommendations of guidelines, CKD patients must maintain values of 25OHD> 30 ng / ml. In Spain, the most used is Calcidiol supplementation, although other groups advocate the use of Cholecalciferol to avoid adverse effects such as hypercalcemia.

However, there is a lot of factors that can alter the effectiveness or toxicity of treatment. Objectives: 1) Observe 25OHD levels after administration of 16000 u. Calcidiol in patients on hemodialysis (HD) 2) determine whether treatment with Calcidiol affects to calcium (Ca), phosphorus (P) and PTH 3) Detect whether FGF23 affect baseline calcidiol response.

Methods: In 27 HD patients (10 m and 17 w, mean age 72.3 ± 13.58 years) was administered 16000 units Calcidiol in the first HD session of the week (HDb) messurements of 25OHD, Ca, P and PTH before administration and before the following sessions a week (HD1, HD2, HD3). There was also a baseline measurement of FGF23.

Results: 25OHD of 16.49 ± HDb 7.29 ng / dl, HD1 and HD3 18.68± 7.5 vs 16.07 ± 6.71 (ns). 10%, 59% increased levels of 250HD> 10%, 41% had no increased or decreased. The Cab 9.15 ± 0.69, Ca1 = 8.8 ± 0.56 and Ca3 = 8.6 ± 0.63, down 4% from baseline (p = 0.0001). Percentage of patients with Ca> 9.5, HDb = 22.2% = 12% HD1, HD3 = 14.8%. Pb = 3.66 ± 1.55, P1 = 4.36 ± 1.73 and P3 = 4.34 ± 1.92. 4.5, HDb=22,2%, HD1=44%, HD3=40,7. The P increased by 36% (p = 0.0047). Patients with P> 4.5, HDb = 22.2% = 44% HD1, HD3 = 40.7%. The PTHb = 417.19 ± 572.95, PTH1 = 448.41 ± 497.23, PTH3 = 449.05 ± 474.64 (ns). The FGF23b= 3292.59 ± 5101.32. Patients with increased 250HD1> 10% FGF23b = 2375 ± 3550, in which 250HD1 <10%, FGF23b= 5127 ± 6619.77 (ns).

Conclusions: 1) There is a high heterogeneity in the values of 25OHD Calcidiol after supplementation. 2) We found no differences between baseline and 48 hours after treatment 3) Appreciate the decrease in Ca and P increased 4) One week no change in PTH levels 5) FGF23 values have not been shown to significantly influence the response to Calcidiol, although a trend towards better response to supplementation with lower levels of FGF23.

Funding: Private Foundation Support

PUB508

Differentiation of 1-84 Parathyroid Hormone (PTH) and 1-84 Fragments in CKD-BMD Emilio E. Gonzalez-parra, Laura Rodríguez-osorio, Valeria Sainz, Maria Vanessa Perez Gomez, Alberto Ortiz, Jesus Egido. *Nephrology, Fundación Jiménez Díaz, Madrid, Spain.*

Background: PTH is one of the molecules used in the management of CKD-BMD. The methods currently used determine both, whole molecule 1-84, and their degradation fragments not 1-84 (PTH). Both molecules have different and sometimes opposite effects. This ratio is used to determine if the patient has or not low bone remodeling. Will be marketed automated methods 3rd generation, that these measured PTH 1-84 (PTHbio). This change will rethink many of the current approaches. OBJECTIVES: 1. - To determine the values of 1-84, 7-84 and PTH1-84/7-84PTH ratio in HD patients. 2. - To determine the correlations between the ratio 1-84/PTH 7-84 with other clinical and biochemical parameters.

Methods: In 147 hemodialysis patients has made the determination of iPTH, PTHbio, PTH ratio (PTHbio / PTH-PTHbio), Ca, P, FGF23, 25 OH vitamin D, before hemodialysis. IPTH, and PTHbio ratio was determined by the method of Roche Elecsys, the FGF23 (Inmunotopics).

Results: The average age was 66.1 ± 14.59 years, 76 men and 71 women, with a stay at 5.2 ± 4.79 HD years. 13 in online HDF, 134 conventional. Mean values were studied: Ca 9.21 ± 0.74 mg / dl, P 5.34 ± 2.3 mg / dl, PTH 298.04 ± 306.53 pg/ ml, PTH bio 174.94 ± 172.18 pg / ml, PTH1-84/PTH7-84 ratio: 1,723 ± 3285, FGF23: 2855.0 ± 4246.8 RU / ml, 25 OH VitD 35.55 ng / ml. Correlation between FGF23 and PTH i, PTHbio and PTH1-84/ PTH7-84 ratio, but not with the 25-OH-VitD. In the univariate model PTH1-84/PTH7-84 ratio positively correlated with FGF23 (p 0.04) so that a 1% increase in the ratio of an increase of 1.6% of FGF23. PTHbio and iPTH also correlated with FGF23. The ratio does not correlate with either the Ca or P, or years in HD or age.

Conclusions: 1. - We must know the values of 1-84, and 1-84 fragments not of our patients. 2. - 1-84 PTH values are significantly lower than those used now. 3. - All forms of measuring PTH correlate well between them even indicate different aspects. 4. - The ratio of our sample expressed hyperdynamic bone and is correlated with FGF23.

Funding: Private Foundation Support

PUB509

Association between Estrogen Use, Mineral Metabolism Markers and Bone Mineral Density in Women: The MESA Study Nisha Bansal,[1] Ronit Katz,[2] Bryan R. Kestenbaum,[2] Ian H. de Boer,[2] Gail A. Laughlin,[3] Matthew Jay Budoff,[4] Joachim H. Ix.[3] *[1]UCSF; [2]UW; [3]UCSD; [4]UCLA.*

Background: Markers of mineral metabolism (MMM) vary by hormonal status and may be associated with bone mineral density (BMD). We evaluated associations of estrogen therapy (ET) with MMM, as well as associations of ET and MMM with lumbar BMD, in a community-based population of women.

Methods: We studied 3,422 post-menopausal women in the Multi-Ethnic Study of Atherosclerosis (MESA), 946 of whom were on ET. Serum phosphorus (phos), 25(OH) vitamin D (vit D), FGF-23, PTH, serum calcium (Ca), and fractional excretion of phosphorus (FEphos) were measured at baseline. A subset of 894 women had lumbar BMD measured by CT scans. We explored the association between ET and MMM as well as MMM and BMD, adjusting for age, race, education, clinical site, season, tobacco use, physical activity, BMI, eGFRcys, urine ACR, and diuretic use. We examined associations between ET and BMD, adjusting for MMM.

Results: Compared to women not on ET, women on ET were similar in age (62±9 vs 62±11 years), however more likely to be White (55% vs 32%), have lower BMI (28 vs 29 kg/m^2), higher eGFRcys (97 vs 92 ml/min/1.73 m^2) and higher BMD (110 vs 107 mg/cm^3) (all P<.05). In multivariate models, ET was associated with lower phos, higher FEphos, higher vit D, and lower Ca (all P<.05). There was no significant association between ET and PTH or FGF-23. Every 1mg/dL higher Ca was associated with 0.106 (0.033, 0.180) mg/cm^3 higher BMD; there was no significant association between other MMM and BMD. ET was associated with higher BMD, and adjustment for MMM did not change this association.

Table 1. Association between estrogen use (ET) and BMD (mg/cm3)

	Univariate	Multivariate*
ET	0.153 (0.084, 0.222)	0.097 (0.024, 0.170)
ET + mineral metabolism markers†	0.176 (0.104, 0.249)	0.120 (0.043, 0.197)

*adjusted for age, race, education, site, season, tobacco use, physical activity, BMI, eGFRcys, ACR, diuretic use; † phos, vit D, FGF-23, PTH, Ca, FEphos

Conclusions: ET is associated with lower phos, higher FEphos, and higher vit D. Except for serum Ca, we did not observe an association between MMM and BMD, and MMM do not explain the association of ET with lumbar BMD.

Funding: NIDDK Support

PUB510

Linear Functions Relate [PTH]7-84 to [PTH]1-84 in Normal Subjects and Patients with Chronic Kidney Disease Kenneth R. Phelps,[1,2] Roy Mathew,[1,2] Kim Stote,[1,3] Lisa M. Hewson,[1] Deborah A. Hallenbeck.[1] *[1]Stratton VAMC, Albany, NY; [2]Albany Medical College, Albany, NY; [3]SUNY Empire State College, Saratoga Springs, NY.*

Background: Parathyroid cells secrete intact PTH 1-84 and a fragment thought to be PTH 7-84. The fragment may interfere with end-organ effects of the hormone, and that interference is sometimes quantified with the ratio [PTH]1-84/[PTH]7-84. We present evidence that linear functions relate [PTH]7-84 to [PTH]1-84 in normal subjects and patients with CKD. These functions determine the ratio [PTH]1-84/[PTH]7-84 at a given [PTH]1-84.

Methods: We submitted plasma specimens to Scantibodies Laboratories from 30 patients with CKD (eGFR 14-49) and 14 normal subjects (eGFR > 60). Assays for combined [PTH]1-84 and [PTH]7-84 and for [PTH]1-84 ("x") alone were performed on each specimen. [PTH]7-84 ("y") was estimated as the difference between the two results. In each group, we sought linear regressions of y on x and additionally plotted x/y against x. Having found linear relationships between y and x, we employed the equations to derive "ideal" values of y for each x. We calculated x/y from these values of y and plotted the resulting ratios against x to yield idealized curves.

Results: In each group, we found a highly significant linear relationship between [PTH]7-84 (y) and [PTH]1-84 (x) (see Table 1).

Table 1. Linear Regressions*

Group	Equation	R2	p
CKD (n = 30)	y = 0.63x + 7.76	0.71	< 0.001
Controls (n = 14)	y = 0.92x - 9.37	0.78	< 0.001

*y = [PTH]7-84; x = [PTH]1-84

Slopes and y-intercepts of the two equations were dissimilar. Plots of x/y against x suggested curvilinear relationships in which x/y was between 1 and 2 for most values of x. As x increased, idealized curves showed a gradual increase in x/y in the CKD group and a gradual decrease in x/y in controls.

Conclusions: Linear relationships between [PTH]7-84 and [PTH]1-84 suggest that a regulated process links secretion of the fragment to secretion of the intact hormone. The slope and y-intercept of each linear function determine the shape of the curve relating [PTH]1-84/[PTH]7-84 to [PTH]1-84. The ratio changes gradually over the range of [PTH]1-84 in normal subjects and patients with CKD.

Funding: Veterans Administration Support, Pharmaceutical Company Support - Genzyme Corporation

PUB511

Serum Vitamin D Concentration and Estimated Glomerular Filtration Rate (eGFR) in the Polish Elderly Population Andrzej Wiecek,[1] Magdalena Szotowska,[1] Marcin Adamczak,[1] Jerzy Chudek,[1,2] Edward Franek.[3,4] *[1]Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland; [2]Department of Pathophysiology, Medical University of Silesia, Katowice, Poland; [3]Department of Internal Diseases, Endocrinology and Diabetology, Central Clinical Hospital MSWiA, Warsaw, Poland; [4]Medical Research Center, Polish Academy of Sciences, Warsaw, Poland.*

Background: Maintaining an appropriate vitamin D levels seems to be one of the crucial public health issues. The aim of this study was to estimate serum 25-hydroxyvitamin D concentration (25-OHD) and its relationship with eGFR in representative samples of the elderly population in Poland.

Methods: In 4054 subjects (2117 males; 1937 females) aged over 65 years (mean age 79 ± 9 years) and 715 subjects with age 55-64 years (332 males; 383 females) from the "PolSenior" study serum 25-OHD was measured by ELISA method.

Results: Serum 25-OHD concentration was significantly lower and vitamin D deficiency (<30 ng/ml) was found significantly more frequent in the subjects with age over 65 years than in subjects with age 55-64 years (respectively, 39.3±22.4 vs 50.5±24.7 p<0.001 and 39.0 % vs 17.7 % p<0.001). Serum 25-OHD concentration in the elderly subjects was significantly higher in subjects with normal (eGFR>60 ml/min; n=3371) comparing with impaired kidney function (eGFR<60 ml/min n=1186) (42.0±23.4 vs 36.5±20.9; p<0.001). Serum 25-OHD concentration in the females with age over 65 years was significantly lower comparing to males (36.3±20.8 vs 42.0±23.4; p<0.001). Significant, negative correlation was found between serum 25-OHD concentration and age (R=-0.286; p<0.001) and positive correlation between serum 25-OHD concentration and eGFR (R=0.143; p<0.001). Multiple regression analysis with gender, age and eGFR as independent factors showed that only age and gender have significant influence on serum 25-OHD concentration in the elderly Polish population (p<0.001).

Conclusions: 1. 25-OHD concentration decrease with growing age of subjects from the Polish population study. 2. Moderate kidney impairment does not independently influence vitamin D status.

Funding: Government Support - Non-U.S.

PUB512

Prevalence and Determinants of 25-Hydroxyvitamin D Deficiency in the Chronic Kidney Disease in Children (CKiD) Cohort Juhi Kumar,[1,2] Lisa Aronson Friedman,[2] Alison G. Abraham,[2] Valerie L. Johnson,[1] Frederick J. Kaskel,[2] Bradley A. Warady,[2] Susan L. Furth,[2] Michal L. Melamed,[3] Anthony A. Portale.[2] *[1]Pediatrics, Weill Cornell Medical College, New York, NY; [2]CKiD Investigators; [3]Medicine, Albert Einstein College of Medicine, Bronx, NY.*

Background: Deficiency of 25-hydroxyvitamin D (25OHD) is highly prevalent in healthy children. Children with chronic kidney disease (CKD) are at higher risk for 25OHD deficiency.

Methods: We analyzed baseline data of 414 children, with CKD stages 2-4 enrolled in the observational Chronic Kidney Disease in Children (CKiD) study, to determine the associations of demographic, nutritional and behavioral factors with 25OHD deficiency. 25OHD deficiency was defined as levels ≤20 ng/ml and sufficiency defined as levels ≥30 ng/ml. We used multivariate logistic analysis to determine significant predictors of 25OHD deficiency.

Results: 43% of the children had sufficient 25OHD levels and 28% had deficient levels. 53% of African-American children had deficient levels compared to 21% of other races. Unadjusted analyses showed that African-American race, older children, lower maternal education, lower income, higher body mass index (BMI), more time spent watching TV, infrequent milk consumption, nephrotic range proteinuria, lower serum calcium, and lower serum albumin were associated with 25OHD deficiency. Multivariate analysis showed that African-American race (OR 4.4, 95% CI 2.1-9.1, p <0.001), higher BMI (OR 1.1, 95% CI 1.0-1.2, p <0.01), nephrotic range proteinuria (OR 4.3, 95% 1.8-10.2, p<0.01) and less than daily milk intake (OR 2.9, 95% CI 1.6-5.4, p <0.001) were significant predictors of 25OHD deficiency. Season, gender, fish intake and GFR were not significantly associated with 25OHD deficiency. Only 1% of subjects were taking inactive vitamin D supplements. Plasma FGF 23 and iPTH levels were not associated with 25OHD deficiency in the multivariate models.

Conclusions: Deficiency of 25OHD in the CKiD cohort is associated with race and the potentially modifiable risk factors of lower milk intake, higher BMI and proteinuria.

Funding: NIDDK Support

PUB513

Paricalcitol and Left Ventricular Hypertrophy in Young Hemodialysis Patients with Elevated FGF23 Levels Wacharee Seeherunvong, Chryso P. Katsoufis, Carolyn L. Abitbol, Jayanthi Chandar, Gaston E. Zilleruelo, Michael Freundlich. *Pediatric Nephrology, University of Miami, Miami, FL.*

Background: Left ventricular hypertrophy (LVH) in uremia is multifactorial including calcitriol deficiency and ↑FGF23 levels. Studies have demonstrated improved LVH with vitamin D analogs although the recent PRIMO trial in predialysis CKD adults given the analog paricalcitol (Pc) failed to demonstrate regression of the left ventricular mass index (LVMI) (JAMA, 2012). Therefore LVMI changes in hemodialysis (HD) patients on Pc were evaluated.

Methods: In 27 young patients (age 16.8±3.1 yr) on hemodialysis (HD) vintage 23.1±23 months receiving Pc for hyper-PTH and with↑FGF23 levels [381± 1,285 fold>upper limit of normal (ULN)], we studied mineral metabolism markers and cardiac function by echocardiograms (echo) including 14 studied longitudinally after 17±6.8 months.

Results: Baseline LVH (LVMI>95%tile) was observed in 15/27(55%). LVMI correlated with log-C-terminal FGF23(r=0.52, p=0.03), systolic(S) (r=0.58, p=0.01) and diastolic Z-score blood pressure (BP) (r=0.63, p=0.006) but not with phosphorus (P), PTH, proBNP, or Pc dose (6 month time-average 20.4±16.3 mcg/week). Longitudinally, while on Pc treatment (17.8±10 mcg/week), hyper-PTH improved (↓70% to 379 ± 158 pg/ml). On sequential echos LVMI was 45±11g/m$^{2.7}$ with LVH in 9/14 (64%; 7 concentric, 2 eccentric) including 5 with original LVH and 4 de-novo LVH. In 5 remaining patients, 4 had LVMI progression without reaching LVH, and only 1 had LVH regression >30%. In patients with progressive LVMI, intact FGF23 fold-levels > ULN correlated with %change LVMI (r= 0.9, $P < 0.05$). LVMI correlated with SBP Z-score (r=0.65, p=0.01) but not with calcidiol, logFGF23, P, PTH, proBNP or HD vintage. Pc dose was similar in those with or without LVMI progression, and did not correlate with PTH, logFGF23, or LVMI.

Conclusions: In young HD patients with hyperPTH and ↑FGF23 levels, LVMI and LVH continued to progress. Treatment with Pc improved hyper-PTH but did not significantly regress LVMI or improve LVH in most. Longer treatment and higher doses of Pc in a larger cohort may be required to further evaluate the potential salutary effects of Pc on LVH.

Funding: Clinical Revenue Support

PUB514

Vitamin D Status and Its Association with Mineral and Bone Disorders in a Multi-Ethnic Chronic Kidney Disease Population in Southeast Asia Melissa Ngai,[1] Valerie Lin,[2] Hung Chew Wong,[3] Vathsala Anantharaman,[4] Priscilla P. How.[2,4] *[1]Department of Pharmacy, National University Hospital, Singapore; [2]Department of Pharmacy, Faculty of Science, National University of Singapore; [3]Yong Loo Lin School of Medicine, National University of Singapore; [4]Department of Medicine (Nephrology), National University Hospital, Singapore.*

Background: Vitamin D insufficiency/deficiency is associated with elevated parathyroid hormone (PTH) concentration and mineral and bone disorder (MBD) in chronic kidney disease (CKD). Suboptimal vitamin D concentrations have been reported globally.However, the vitamin D status of CKD patients in Singapore has not been well-described. As such, the objectives of this study were to determine the prevalence of vitamin D insufficiency/deficiency, and the association between vitamin D status and MBD in our multiethnic CKD population.

Methods: Predialysis CKD patients from an outpatient renal clinic were included in this single-center cross-sectional study. Patient demographics, medical/medication histories and laboratory parameters [serum 25(OH)D, creatinine, phosphorus, calcium, albumin and intact-PTH (i-PTH) concentrations] were collected and compared among patients in various stages of CKD. Multiple linear regression was used to determine the association between 25(OH)D and these parameters.

Results: A total of 204 patients with a mean(SD) eGFR of 28(13.7)ml/min/1.73m^2 were included. Vitamin D deficiency and insufficiency was found in 28.9% and 57.8% of the patients, respectively. Mean serum 25(OH)D was 20.9(9.3)µg/L. Female patients had significantly lower vitamin D concentrations (17.2µg/L vs 24µg/L; p<0.001). Serum 25(OH)D concentrations were also significantly higher in Chinese (22.4 µg/L) than Malay (17.4 µg/L) and Indian (13.1µg/L) patients (p<0.05). i-PTH was found to be inversely associated with 25(OH)D (p=0.05).

Conclusions: Despite being a sun-rich country with abundant sunshine all year round, majority (86.7%) of predialysis CKD patients in Singapore have suboptimal vitamin D status. Lower vitamin D concentrations were found in females and patients with darker skin tone. Patients with lower serum 25(OH)D concentrations tended to have higher i-PTH values.

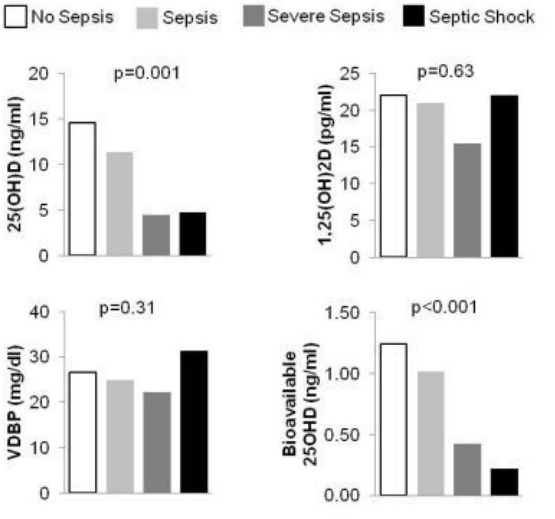
PUB515

Vitamin D and Outcomes in Acute Kidney Injury David E. Leaf,[1] Sushrut S. Waikar,[1] Leonard Stern.[2] *[1]Renal Medicine, Brigham and Women's Hospital, Boston, MA; [2]Renal Division, Columbia University Medical Center, New York, NY.*

Background: Vitamin D deficiency is common among critically ill patients and is associated with adverse outcomes. In contrast, little is known about vitamin D deficiency among patients with acute kidney injury (AKI) and whether lower levels are associated with adverse outcomes.

Methods: We recruited 30 patients with AKI and 30 patients without AKI from the intensive care units and general hospital wards. The following vitamin D metabolites were measured at study enrollment: 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), 24R,25-dihydroxyvitamin D_3 (24R,25$(OH)_2D_3$), and Vitamin D Binding Protein (VDBP). In addition, using previously developed equations, we estimated levels of bioavailable 25(OH)D, defined as the sum of free- and albumin-bound hormone.

Results: Participants with AKI, compared to controls, had a trend toward lower levels of 25(OH)D (p=0.057); significantly lower levels of 1,25$(OH)_2$D (p=0.01), 24R,25$(OH)_2D_3$ (p=0.01), and VDBP (p<0.01); and no difference in levels of bioavailable 25(OH)D. Among the vitamin D metabolites, bioavailable 25(OH)D showed the strongest dose-response relation with severity of sepsis (figure).



In analyses adjusted for age and enrollment creatinine, levels of bioavailable 25(OH) D, but not other vitamin D metabolites, were inversely associated with higher risk of in-hospital mortality (odds ratio 0.16 per SD of natural log-transformed bioavailable 25(OH) D, 95% C.I. 0.03 to 0.85, p=0.032).

Conclusions: Patients with AKI, compared to controls, have lower levels of most vitamin D metabolites. Although bioavailable 25(OH)D was similar between groups, levels were inversely associated with severity of sepsis and mortality, suggesting a potential role as a biomarker or even a mediator of adverse outcomes among patients with AKI.

Funding: Private Foundation Support

PUB516

FGF-23 in Acute Sarcoidosis with Normal Kidney Function Donal John Sexton, Anthony O'Regan. *Department of Respiratory Medicine, University College Hospital Galway, Galway, Ireland.*

Background: Fibroblast growth factor (FGF) 23 regulates serum phosphate and calcitriol. It is a marker of abnormal renal phosphate handling. Sarcoidosis is characterised by granulomatous inflammation, which is associated with extrarenal calcitriol production. This results in increased intestinal calcium and phosphate absorption with hypercalciuria in 30-50% and hypercalcaemia in 10-20% of patients. Little is known about the role of FGF-23 in the adaptation to increased intestinal phosphate absorption.

Methods: We conducted a cross sectional study of 43 patients with a diagnosis of acute sarcoidosis. Ethical committee approval was obtained to conduct this study. We measured serum levels of FGF-23 as well as calcium, phosphate, 24 hour urinary calcium, iPTH and 25-OH vitamin D_3.

Results: 26 patients were male. Mean (st dev) age was 33 (9.6) yrs, iPTH 25.35(12) pmol/L, serum calcium 2.32(0.16)mmol/L, phosphate 1.07(0.21)mmol/L,25-OH Vitamin D_3 38(18.5) nmol/L, and eGFR 117 (4)ml/min/1.73m^2. Median (25th-75th centile) FGF23 levels were 0 (0-129.5)pg/ml and 24 hour urinary calcium excretion was 4.8(2.8, 7.5) mmol/L. FGF-23 was detectable only in those patients with hypercalciuria (14%). On univariate linear regression, FGF-23 was independently associated with serum calcium (β=99 P<0.001), and inversely with iPTH (β =-1.3, P=0.001), but not with 24 hour urinary calcium, serum phosphate or 25-OH Vitamin D_3 (all P>0.05). Using stepwise multivariate linear regression iPTH was no longer independently associated with FGF-23 levels (P=0.09), however serum calcium (β=91, P=0.02), serum phosphate (β=54, P=0.02)and 24 hour urinary calcium (β=3.4 P=0.01)were all independently associated with FGF-23 levels. The final model had an R^2 adjusted of 59.2%.

Conclusions: FGF-23 is detectible in patients with acute Sarcoidosis with normal kidney function, these levels are independently associated with serum calcium, serum phosphate and 24 hour urinary calcium levels but not with eGFR. Whether serum FGF-23 is associated with adverse events in patients with chronic Sarcoidosis warrants further investigation. 1,25 OH_2Vitamin D_3 levels and TmP/GFR phosphate measurements were not available which is a limitation in our study.

PUB517

25-Hydroxy Vitamin D Insufficiency in CKD Stage 3 Maarten W. Taal,[1] Victoria Thurston,[1] Natasha J. Mcintyre,[2] Nigel Lawson,[1] Chris W. McIntyre,[2] Richard J. Fluck.[1] *[1]Department of Nephrology, Royal Derby Hospital, Derby, United Kingdom; [2]School of Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom.*

Background: Deficiency or insufficiency of 25-hydroxy Vitamin D ($25OHD_3$) is common in CKD and is associated with increased mortality in the general population. Uncertainty remains, however, regarding the optimal strategy for screening or treatment. Most previous studies have been conducted in Secondary Care. We aimed to investigate serum $25OHD_3$ concentration and factors related to $25OHD_3$ insufficiency in CKD stage 3 in Primary Care.

Methods: Serum $25OHD_3$ was measured by tandem mass spectometry in 1664 persons with estimated GFR 59-30ml/min/1.73m^2 recruited from 32 Primary Care practices. Detailed medical history and clinical assessment were performed as well as urine and serum biochemistry. $25OHD_3$ deficiency was defined as <25nmol/L (<10pg/L) and insufficiency as 25-49nmol/L (10-20pg/L).

Results: Median values for key variables were: age 74(IQR 67 to 79)y, eGFR 53(46 to 60)ml/min/1.73m^2, PTH 46(34 to 66)pg/ml, $25OHD_3$ 53(38 to 71)nmol/L. Overall 104 (6.2%) persons had $25OHD_3$ deficiency and 648 (38.9%) insufficiency. $25OHD_3$ deficiency or insufficiency was more prevalent in several clinically relevant subgroups (Table). $25OHD_3$ concentration showed no correlation with serum phosphate and only weak correlation with serum calcium (r=0.09; p<0.001) but was more strongly correlated with logPTH (r=-0.35; p<0.001). $25OHD_3$ concentration showed no correlation with arterial pulsewave velocity.

Factor	Present	Absent	p-value
Asian ethnicity	22/28(79%)	730/1636(45%)	<0.001
Female	480/1010(48%)	272/654(42%)	0.02
Age≥75y	382/791(48%)	370/873(42%)	0.02
Socially deprived	80/146(55%)	845/671(44%)	0.02
Diabetes	151/275(55%)	601/1389(43%)	<0.001
Obesity	325/620(52%)	427/1043(41%)	<0.001
CKD 3B	193/388(50%)	559/1276(44%)	0.04
Albuminuria	145/275(53%)	607/1388(44%)	0.006

Conclusions: Whereas $25OHD_3$ deficiency was uncommon, insufficiency was relatively common, though values are similar to those reported for the general population in the UK. These data provide support for measuring $25OHD_3$ in all persons with CKD stage 3 but further randomised studies are required to evaluate the benefit of $25OHD_3$ replacement in this group.

Funding: Pharmaceutical Company Support - Roche; Sanofi

PUB518

Up-Regulation of Fibroblast Growth Factor 23 in Prostate Cancer and Bone Metastatic Lesions of Prostate Cancer-Induced Oncogenic Hypophosphatemic Osteomalacia Akira Iguchi,[1] Hajime Yamazaki,[1] Junichiro J. Kazama,[2] Ichiei Narita.[2] *[1]Nagaoka Red Cross Hospital, Nagaoka, Niigata, Japan; [2]Nephrology and Rheumatology, Niigata University, Niigata, Japan.*

Background: Fibroblast growth factor 23 (FGF23) secreted by mesenchymal benign tumor has been known to induce hypophosphatemic osteomalacia, as a kind of paraneoplastic syndrome. In previous reports, a part of prostate cancer, which is an endodermal origin, with bone matastasis have resulted in oncogenic hypophosphatemic osteomalacia, although FGF23 was secreted from osteocyte of mesenchymal origin. Moreover, patients of prostate cancer with hypophosphatemic osteomalacia have a poor prognosis compared to patients without it. However, whether prostate cancer cells or osteocytes in bone metastatic lesion secrete FGF23 has been unclear to date.

Methods: We investigated the serum level and expression of FGF23 in the tissues in two cases of prostate cancer with bone metastasis accompanied by hypophosphatemia with elevated fractional excretion of phosphate. Immunohistochemical staining for FGF23 was performed in prostate cancer and bone metastatic lesion.

Results: The serum level of FGF23 was elevated and that of 1,25-hydroxyvitamin D was low. In both prostate cancer cells and tumor cells of bone metastatic lesion, immunohistochemical stainings were positive for FGF23, but not in normal prostate cells.

Conclusions: Although, it is well known that almost cases of prostate cancer-induced oncogenic hypophosphatemia had bone metastasis, we have for the first time confirmed that FGF23 was expressed by not only osteocytes in bone metastatic lesions, but also by prostate cancer cells which were an endodermal origin in patients with prostate cancer-induced oncogenic hypophosphatemic osteomalacia.

Funding: Pharmaceutical Company Support - Kyowa Hakko Kirin Co., Ltd.

PUB519

PTH Regulates FGF23 Gene Transcription via Multiple Signaling Pathways Maria Encarnacion Rodriguez Ortiz,[2] Zhousheng Xiao,[1] Leigh Darryl Quarles.[1] *[1]UTHSC, Memphis, TN; [2]University Hospital Reina Sofia, Spain.*

Background: Whether PTH directly stimulates FGF23 gene transcription in osteoblasts (Obs) is uncertain.

Methods: PTH regulation of FGF23 gene expression was assessed by quantitative RT-PCR measurement of endogenous mRNA levels and activity of previously characterized FGF23 promoter/reporter constructs (pFGF23-luc) in UMR-106 Obs.

Results: We observed a dose-dependent stimulation of FGF23 mRNA levels by both PTH and forskolin (FK), achieving a 3 to 6-fold increase at 100 nM PTH and 10 μM FK, respectively. The effect of FK on FGF23 mRNA was detected by 8 hr and was sustained for 48 hrs, whereas PTH transiently stimulated FGF23 expression at 8 but not 24 hrs. IBMX (0.5 mM) significantly augmented the stimulatory effects of PTH, treatment with actinomycin D blocked both PTH and FK stimulation. but cycloheximide (CHX) partially blocked FK- without affecting PTH-stimulated FGF23 mRNA levels in UMR-106 obs. Surprisingly, CHX alone increased FGF23 mRNA levels 3-fold, suggesting suppression of basal FGF23 mRNA levels by a synthesized protein. FK equally but weakly (2-fold) stimulated FGF23 promoter activity in UMR-106 obs transfected with p8Kb-FGF23-luc, p3.5Kb-Fgf23-luc, and p0.6Kb-Fgf23-luc, compared to a 6-fold stimulation of pCRE-luc expressing cells. PTH, in the presence of IBMX, also weakly stimulated luciferase activity by ~2.5 fold in UMR-106 obs transfected with p0.6Kb-FGF23-luc, compared to a 40-fold stimulation in pCRE-luc transfected obs. The PKA inhibitor H89 (1 μM) inhibited both FK and PTH effects on FGF23 gene transcription in UMR-106 cells. In contrast, staurosporine (100 nM), a broadly selective protein kinase inhibitor, augmented PTH stimulation of both FGF23 mRNA and p0.6Kb-FGF23-luc promoter activity, but had no effect on FK stimulation of FGF23 gene transcription in UMR-106 obs.

Conclusions: PTH, especially in the presence of IBMX and staurosporine, stimulates FGF23 gene transcription in UMR-106 obs *in vitro*. Inhibition of protein synthesis increases basal FGF23 gene transcription in these cells. The net effect of positive and negative regulators of FGF23 gene transcription may explain the variable effects of PTH to regulate FGF23 expression *in vivo*.

Funding: Other NIH Support - NIAMS

PUB520

Long-Term Treatment of Cinacalcet with Maxacalcitol Has Limitations to Control Mild to Severe Secondary Hyperparathyroidism with Enlarged Parathyroid Glands Tatsuo Tsukamoto,[1] Motoko Yanagita,[1] Eri Muso.[2] *[1]Nephrology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Nephrology & Dialysis, Kitano Hospital, the Tazuke Kofukai Medical Research Institute, Osaka, Japan.*

Background: We demonstrated the suppressive effect of the protocol therapy of cinacalcet with maxacalcitol, an active vitamin D derivative, on the secondary hyperparathyroidism (SHPT) in chronic hemodialysis patients whose intact parathyroid hormones (iPTH) ranged from 300 to 1000pg/ml in the last ASN meeting (SA-PO2298). In addition, we showed the decrease of the enlarged parathyroid gland volume after one year. However, long-term use of cinacalcet with vitamin D treatment may bring lower turnover of bone leading to hypercalcemia. Here, we show exacerbation of SHPT after the reduction of maxacalcitol, together with the regrowth of parathyroid glands in our extensive cohort study (UMIN 000001793).

Methods: Fifty-three hemodialysis patients with 159 enlarged parathyroid glands were enrolled and treated with the combination therapy for 3 years.

Results: The dose of cinacalcet increased every 2 to 4 weeks until intolerance, and predicted hypocalcemia was dealt with increase of maxacalcitol. The maximum dose of cinacalcet and maxacalcitol were 40mg daily and 18mg per week in the first year, respectively. PTH was successfully controlled from 565.5±32.0pg/mL (mean±SE) to 164.1±2.5pg/mL after one year (n=51), 159.6±3.1pg/mL after 2 years (n=39), and 207.2±6.5pg/mL after 3 years of the treatment (n=28). Calcium increased within normal range along with the decrease of ALP from 323.2±3.3U/L to 224.6±2.6U/L after 2 years. Phosphate did not change significantly. Thus, we eventually reduced maxacalcitol to 10mg per week after 3 years. 85 enlarged parathyroid glands showed significant volume reduction after the one-year treatment, however, 21 of the shrunk glands displayed regrowth by a high-resolution color Doppler ultrasonography.

Conclusions: The combination of cinacalcet with maxacalcitol could be a powerful strategy to suppress SHPT, however, this therapy failed by hypercalcemia along the decrease of ALP in some patients with regrowth of the parathyroid glands, suggesting that those might be the limitation of this therapy.

PUB521

A Better Response to 48-Month Cholecalciferol Supplementation Is Associated with Improvement of Cardiovascular Risk Markers in Hemodialysis Patients Patrícia Matias,[1,2,3] Cristina Jorge,[1,2,3] Marco Mendes,[1,2,3] Carina Ferreira,[1,2,3] Inês Aires,[1,2,3] Tiago Amaral,[1,2,3] Marília Borges,[4] Célia Gil,[1,2,3] José Cortez,[4] Manuel A. Ferreira.[1,2,3] *[1]Nephrocare, Vila Franca de Xira, Portugal; [2]Dialverca, Forte da Casa, Portugal; [3]NIDAN, Lisboa, Portugal; [4]Laboratório Dr. Fernando Teixeira, Lisboa, Portugal.*

Background: There is a wide variation in the response of serum 25-hydroxyvitamin D [25(OH)D] levels to native vitamin D supplementation.

The aim of this prospective study was to evaluate if a better response to cholecalciferol supplementation was associated with improvement of cardiovascular (CV) risk factors in chronic hemodialysis (HD) patients.

Methods: Serum 25(OH)D levels were measured before and 48-months after cholecalciferol supplementation with 7992 units per week (divided in 3 doses given during the HD session). Patients were distributed in tertiles according to their supplementation response. Parameters of inflammation, pulse pressure (PP), left ventricular mass index (LVMI), vascular calcifications (VC) and serum markers of bone metabolism were evaluated before and after supplementation, in 96 HD patients with mean age (± SD) of 61.8±14.2 years, 46% female, 27% diabetics, and mean HD time of 39.8±34.3 months.

Results: At baseline 79.3% of the patients were insufficient/deficient in [25(OH)D] and a significant increase of 25(OH)D levels was observed after 48 months of supplementation, from 23.4±13.3 to 44.5±18.6 ng/mL, ($p<0.001$). Older patients ($p=0.02$), diabetics ($p=0.01$) and females ($p=0.03$) had a worse response to supplementation. Patients with higher C-reactive protein ($p=0.01$) and lower albumin ($p=0.03$) levels were also worse responders. Patients from the third tertile (with the best response to supplementation) had a higher decrease of PP ($p=0.02$), LVMI ($p<0.001$) and less progression of VC ($p=0.03$). This supplementation was well tolerated without adverse effects.

Conclusions: In conclusion, a better response to 48-month cholecalciferol supplementation was associated with improvement of CV risk markers like inflammation, PP, LVMI and VC, in prevalent HD patients.

PUB523

Calcifediol Intoxication Isabel Martínez,[1] Ramon M. Saracho,[2] Adriana S. Dusso.[3] *[1]Nephrology, Hospital de Galdakao, Usansolo, Vizcaya, Spain; [2]Nephrology, Hospital Universitario de Alava, Vitoria, Alava, Spain; [3]Experimental Nephrology Lab, IRBLleida, Lleida, Spain.*

Background: Patients with CKD frequently present vitamin D (VitD) deficiency, and Guidelines recommend VitD supplementation. Correction of VitD deficiency in individuals with no renal disease is well established. However, the extrapolation of VitD supplementation strategies from other pathologies fails in CKD.

Usually, the correction of VitD deficiency is achieved with daily low doses of cholecalciferol, an inactive compound, or with intermittent doses of calcifediol every 1 or 2 weeks. Calcifediol can directly activate the VitD receptor with a

potency much lower (200 times) than calcitriol, but it circulates in concentrations 1,000 times higher than calcitriol. Furthermore, calcifediol has a half life of 18 days vs. 5-7 days for cholecalciferol. In Spain, calcifediol exists in two oral formulations of 0.266 and 3 mg. In order to assess the risk of toxicity using oral calcifediol formulations, we performed a retrospective study.

Methods: We search for all patients receiving oral calcifediol with serum 25(OH) vitaminD levels ≥100 ng/ml. We identified 55 positive subjects from a sample of 469 patients. Complete information for analysis was available in 28 patients. The characteristics were: male/female 11/17; Average age 74 (12)yrs, CrCl adjusted by 1.73m2, Median 43: range 20-78ml/min. Patients were receiving calcifediol at doses of 3mg, 0.266mg or 0.1 mg/ml. Some patients took different doses sequentially.

Results: Serum chemistries during the period of the highest 25(OH)vitaminD levels showed: Increases in plasma total and ionic calcium of 0.6 mg/dl, $p<0.0001$, and 0.5 mg/dl, $p=0.0009$, respectively, with no increase in phosphatemia. Significant

reduction in PTH from 130 to 71pg/ml, $p<0.0001$. An striking increase in urinary calcium from 43 to 76 mg/24, $p=0.001$, with no changes in phosphaturia. Average increases in serum creatinine of 17%, $p=0.0012$, which reached a maximum of 97%, suggesting ARF.

Conclusions: Supplementation therapies with high doses of oral Calcifediol in patients with CKD should be replaced by small dosage and higher frequency and subjected to a strict analytical control to avoid VitD intoxication and ARF.

Funding: Government Support - Non-U.S.

PUB524

Differential Contribution of 25-Hydroxyvitamin D Status to Insulin Resistance According to Degree of Renal Function in Non-Diabetic Korean Population Seong Woo Lee, Kook-Hwan Oh, Kwon Wook Joo, Yon Su Kim, Suhnggwon Kim, Dong Ki Kim. *Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea.*

Background: There have been only few literatures on the interaction between kidney function and 25-hydroxyvitamin D (25OHD) status on insulin resistance (IR).

Methods: Data from the Korea National Health and Nutrition Examination Survey in 2008-2010 were analyzed. The study subjects comprised 16,379 non-diabetic participants aged ≥ 20 years whose glomerular filtration rate (GFR) were ≥ 30 ml/min/1.73m^2. GFR and IR were estimated by Chronic Kidney Disease Epidemiology Collaboration equation and Homeostatic model assessment (HOMA), respectively. The evaluations of unit-change effect and threshold were done by generalized linear model and generalized additive model (GAM), respectively.

Results: No specific relation between HOMA-IR and estimated GFR (eGFR) was observed until eGFR decreased to 90-99.9 ml/min/1.73m^2; thereafter, HOMA-IR increased constantly with decreasing eGFR. With GAM, the threshold value of eGFR for HOMA-IR was 97.2 ml/min/1.73m^2. As for 25OHD, HOMA-IR increased constantly with decreasing 25OHD. Since 25OHD was not fitted to HOMA-IR with GAM, we could not identify the threshold value. The unit-change of 25OHD was associated with 0.005 decrease of HOMA-IR. The unit-change effect of 25OHD tended to decrease from -0.005 in ≥ 120 ml/min/1.73m^2 to -0.008 in 90-99.9 ml/min/1.73m^2; thereafter it increased as eGFR decreased. The statistical significance of unit-change effect was evident in eGFR ≥ 80 ml/min/1.73m^2.

Conclusions: The threshold value of eGFR for HOMA-IR was 97.2 ml/min/1.73m^2. The effect of 25OHD on IR was significant only in eGFR ≥ 80 ml/min/1.73m^2 and the maximum effect was observed in eGFR 90-99.9 ml/min/1.73m^2. Future study needs to be followed.

PUB525

1,25/25D Ratio as an Indirect Tool to Evaluate the Activity of Vitamin D Hydroxylases (D-OHase) Sandro Mazzaferro,[1] Lida Tartaglione,[1] Silverio Rotondi,[1] Cristiana Leonangeli,[1] Martino Marangella,[2] Marzia Pasquali.[1] *[1]Department of Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, Sapienza University of Rome, Rome, Italy; [2]Nephrology and Dialysis Unit, ASO Ordine Mauriziano, Turin, Italy.*

Background: Differently regulated anabolic and/or catabolic D-OH-ases are responsible for variable 1,25D levels in different clinical conditions. The overall balance could also affect sensitivity to replacement therapies: cases with more efficient activity are, predictably, less dependent from vitamin D stores, and more sensitive to storage replacement therapies. We hypothesized that the ratio between the active hormone, 1,25D, and its precursor, 25D, could represent an indirect tool to estimate the overall balance of D-OH-ases activity.

Methods: We considered that two CKD populations, CRF on conservative therapy and renal TX pts, who are developing or, respectively, recovering from secondary hyperparathyroidism, should have different overall D-OH-ase activity. 70 CRF and 80 TX, naïve to vitamin D therapy, were selected to be comparable for age (58±15vs54±10y), renal function (eGRF 45±22vs46±15ml/min) and 25D levels (23±11vs26±11ng/ml);300 normal subjects (N;45±14y;eGFR113±14ml/min;25D 25±13ng/ml) as controls.

Results: 1,25D levels were 24±13, 42±15 and 50±16 pg/ml respectively in CRF, TX and N($p<.001$). The ratio 1,25D/25D was 1,4±1,0 in CRF,2.0±1,4 in TX,and 3.0±2,4 in N($p<.0001$). In cases with 25D≥20 ng/ml the ratio was 0.9±0.5 in CRF; 1.4±0.6 in TX and 2.0±0.5 in N;$p<.0001$; and in cases with 25D<20 ng/ml was 2.2±1.2 in CRF;3.4±1.6 in TX and 4.0±3.3 in N($p<.001$). The ratio was different between the two storage conditions ($p<.001$), in CRF, TX and N. The ratio correlated negatively with 25D in each population ($p<.0001$), with r values definitely increasing (from linear to non-linear models) in N (from 0.556 to 0.774) and in TX (from 0.692 to 0.812), but not in CRF (from 0.648 to 0.692).

Conclusions: As expected in feed-back systems, the ratio 1,25D/25D, is negatively correlated with the substrate, has higher values in case of deficiency, with an exponential increment in N. CRF patients with the lowest values, are presumably less sensitive to storage replacement therapies, as compared to TX.

PUB526

Evolution over 48 h in Levels of 1-84 ("whole") and Non-1-84 Parathyroid Hormone (PTH) after a Single Administration of Cinacalcet in Patients with Secondary Hyperparathyroidism (SHPT) Dolores Arenas,[1] Vanesa De la Fuente,[1] Pablo Delgado,[1] Patricia Gutiérrez,[1] Jorge Ribero,[1] Mariano Rodriguez,[2] Victor Lorenzo,[3] Yolanda Almaden Peña.[2] *[1]H.Perpetuo Socorro, Alicante, Spain; [2]H.Reina Sofía, Córdoba, Spain; [3]H.Canary Islands, La Laguna, Spain.*

Background: "Intact" PTH(iPTH) measures not only PTH 1-84(whole(W)-PTH) but also non-1-84 fragments(non-whole(NW)-PTH), including PTH 7-84, which can cause skeletal resistance to W-PTH. We evaluated whether cinacalcet(CIN) modifies the relative proportion of PTH fragments in hemodialysis(HD) pts with controlled SHPT.

Methods: Single-center, open, exploratory, uncontrolled trial with administration of a single dose of CIN(30–90 mg) and 48-hour(h) follow-up. iPTH, W-PTH and ionic Ca(iCa) were measured at baseline (24h after last CIN dose, trough level in daily regimen), and at 1,3,6,12,24 and 48h. PTHs were determined with the Duo PTH kit (Scantibodies).

Results: 10 pts with SHPT were included (8 men, median diagnostic age 4.5 years(y) (range 2–31), mean age 66 y(39–82), on HD for a median of 5 y(2–31). 6 pts received 30mg of CIN, 3 pts 60mg and 1 pt 90 mg. Mean(SD) baseline levels of iPTH and W-PTH (pre-CIN) were 164(98) and 90(58)pg/ml, respectively. Following CIN, there was a significant and comparable reduction in iPTH and W-PTH at 1h and a return to baseline values at 24h. At 48h(24h after the absence of the daily dose), a significant increase was observed in both iPTH and W-PTH vs baseline. The relative proportion of W-PTH/iPTH was constant between 1-48h. No significant changes were observed in iCa within the dosing intervals of 24h or 48h (intra-patient variability 4.9–18.6%,range 4.3–5.9mg/dl).

Table 1.

	0 hours	1 hour	3 hours	6 hours	12 hours	24 hours	48 hours
iPTH (pg/ml), *average (SD)*	140.1 (96.7)	74.6 (42.5)*	78.4 (35.1)*	131.4 (94.6)*	204.2 (147.9)*	175.5 (118.2)	250.0 (141.7)*
Whole PTH (1-84 PTH) (pg/ml), *average (SD)*	80.8 (54.2)	43.3 (25.6)*	39.5 (16.7)*	64.6 (36.0)*	99.9 (71.0)*	98.0 (59.5)	127.8 (61.9)*
W-PTH/iPTH, % (CI 95%)	61% (48 to 74)	63% (50 to 75)	52% (44 to 56)	55% (41 to 69)	53% (42 to 63)	60% (50 to 70)	54% (44 to 63)
Average percentage change over baseline for iPTH, % (CI 95%)		−42%* (−19 to −64)	−30%* (−34 to −66)	−17%* (−2 to −33)	+26%* (1 to 50)	+14% (−5 to 32)	+51%* (26 to 76)
Average percentage change over baseline for W-PTH, % (CI 95%)		−44%* (−26 to −61)	−51%* (−37 to −66)	−22%* (−11 to −33)	+21%* (3 to 38)	+14%* (−2 to 31)	+53%* (10 to 97).
Ionic calcium (mg/dl), *average (SD)*	4.95 (0.40)	5.05 (0.23)	5.05 (0.25)	5.11 (0.50)	4.87 (0.25)	5.11 (0.30)	5.05 (0.40)
Ratio wholemon-whole PTH, *median*	1.35	1.64	1.13	1.17	1.30	1.37	1.10
Ratio wholeintact iPTH, *median*	0.57	0.61	0.53	0.54	0.57	0.58	0.52

*p<0.05 compared to baseline; SD = standard deviation, CI = confidence interval

Conclusions: In HD pts with controlled SHPT assessed in this study, the chronic administration of cinacalcet seems not to change the relative proportion of 1-84 and non-1-84 fragments(W:NW PTH ratio).

Funding: Pharmaceutical Company Support - This Study Was Supported in Part by a Grant from Amgen

PUB527

Vitamin D: A Marker of Metabolism Mineral or Something More in Type 2 Diabetic? Ana Paula Silva, Ana Pinho, André Fragoso, Cláudia Silva, Nelson Almeida Tavares, Pedro Neves. *Nefrologia, Hospital Faro, Portugal.*

Background: A recent study in type 2 diabetic patients, investigating the mechanism by which vitamin D (25 (OH)D) deficiency mediates increased risk of cardiovascular disease. Found that low vitamin D levels, causing an increased oxidative stress, inflammation, activation of the renin-angiotensin-aldosteren system, speed up the onset of cardiovascular disease in type 2 diabetic compared with non diabetic.

The aim of our study was to evaluate the relationship of 25 (OH) D and cardiovascular mortality in type 2 diabetics with mild to moderate kidney disease.

Methods: In this prospective study, we included 92 type 2 diabetic patients (f=38 m=54), mean age 63 years, mean eTGF 42, 1 ml/min.

At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed: insulin resistance (HOMA-IR), inflammation (TNF α), mineral metabolism (phosphorus (P), PTH, FGF-23), and oxidative stress (oxLDL), adiponectin, eTGF. We evaluated the systolic and diastolic blood pressure, the mean arterial pressure (MAP), the pulse pressure (PP) and the left ventricular mass index (LVMI) was calculated using the Penn convention criteria.

We divided the population in 3 groups, according to the 25 (OH)D tertiles. GI (n=30) 25(OH)D <12.5 ng/mL; GII (n= 30) – 25(OH)D >12.6 and < 24.9 ng/mL; GIII (n=32) 25(OH)D ≥ 25 ng/mL.

Results: We found the G=I are older (p=0.015) and showed higher levels of FGF-23 (p=0.0001), PTH (p=0.0001), P (p= 0.0001), TNFα (p=0.0001), oxLDL (p=0.0001), HOMA-IR (p=0.0001), LVMI (p= 0.0001), systolic blood pressure (p=0.0001), PP (p=0.0001)and lower levels of adiponectin (p=0.0001) and eTGF (p=0.0001) when compared with other groups.

Using the Kaplan Meier analysis we found that the survival of the GI, GII, and GIII at 43 months was respectively: 23.3%, 44.6 %, 100% (Log –rank = 43.8 p=0.0001).

Conclusions: In our study, we observed a relationship between low levels of vitamin D with non-traditional factors of cardiovascular risk in a population at high cardiovascular risk.

Low levels of vitamin D is a factor of cardiovascular mortality in type 2 diabetic patients in stage 3 and 4 of chronic kidney disease.

Funding: NIDDK Support

PUB528

Vitamin D Deficiency in Post Transplant South Asian Patients Priyanka Govindan,[1] Arjun V. Sharma,[1] Georgi Abraham.[2] *[1]Internal Medicine, University of Connecticut, Farmington, CT; [2]Nephrology, PIMS, Pondicherry, India.*

Background: Vitamin D deficiency is prevalent in CKD patients but there is limited data available in vitamin D levels in renal transplant recipients in India. CKD MBD is influenced by multiple factors including pre transplant supplementation, immunospressants, age, gender and Vitamin D. Presence of the type 5 skin predisposes South Asians to vitamin D deficiency compared to Caucasians. This is an ongoing prospective study to study the vitamin D levels in the first month after renal transplant in a tertiary care centre in South India. This would allow us to intervene and follow up these patients.

Methods: The 25-(OH) vitamin D levels were measured in all renal transplant patients in their first month, irrespective of their age, gender, renal parameters and other co morbid conditions. We studied 60 renal transplant patients between 2009 to 2011, and monitored their 25(OH) vitamin D levels in the first month after transplant. Males- 46, females-14, age-30 to 62 yrs, DM-18,HTN -50, The vitamin D level is sufficient if it is > 30 ng /ml. All the patients were on pretransplant 1 alpha or 1, 25 Dihydroxyvitamin D3 therapy.

Results: Vitamin D levels - no of patients.

>30ng/ml – 17
15 -29 ng/ml-25
7 -14 ng/ml-15
1-7 ng/ml- 3.

Conclusions: Vitamin D insufficiency and deficiency is highly prevalent in South Asian Patients. In the studied population it was seen inspite of pre transplant supplementation with 1 alpha or 1,25 dihydroxy vitamin D3. Appropriate replacement therapy has been instituted using oral cholecalciferol. Close surveillance of Vitamin D levels is necessary for the management of these patients.

PUB530

Demographic Factors and Practice Patterns Associated with Facility Level Parathyroid Hormone Control Jamie Heise,[1] David M. Spiegel,[2] J. Brian Copley,[1] Moshe Fridman.[3] *[1]Shire Pharmaceuticals; [2]University of Colorado Denver; [3]AMF Consulting.*

Background: Management of mineral and bone disorders in chronic kidney disease remains difficult and optimal therapeutic targets are controversial due to a lack of randomized outcome trials. It is common to consider hyperparathyroidism as a risk factor for cardiovascular and bone diseases.

Methods: Hemodialysis patients (n=116,489) treated in a large dialysis organization during August 2011, were grouped and assessed by facility. Facilities with ≥ 50 patients (N=972) were ranked according to the proportion of patients with serum parathyroid hormone (PTH) in the range 150–600 pg/mL. The top and bottom 5% of facilities were compared for demographic factors and achievement of targets for other laboratory measures (calcium, Ca; phosphate, P; and albumin), use of vitamin D, vitamin D analogues, P-binders and calcimimetics.

Results: The top (n=48) and bottom (n=58) 5% of facilities treated 4035 and 4949 patients, respectively. Target PTH range was achieved by 88% and 51% of patients, respectively (p<0.0001), and overall 70% of patients had serum PTH in range. Compared with the bottom-ranked facilities, patients in the top facilities were older (63 vs 60 years; p=0.0001), a higher percentage were Hispanic (25% vs 12%; p=0.007) or white/other (42% vs 26%; p=0.001), and a lower percentage were African American (28% vs. 57%; p<0.0001). There were no significant differences in the proportions with serum albumin ≥ 3.5g/dL (89% vs 87%), Ca 8.8–10.2 mg/dL (99% vs 98%), or P ≤ 5.5 mg/dL (79% vs 77%). Patients in top-performing facilities were more likely to use IV paricalcitol (13% vs 3%; p=0.038), lanthanum carbonate (16% vs 10%; p=0.001) or sevelamer (68% vs 62%; p=0.044) and were less likely to use calcium acetate (28% vs 37%; p=0.006) or calcium carbonate (1% vs 3%; p=0.001). There were no other significant differences.

Conclusions: With respect to PTH control, demographic factors were different between the top and bottom facilities and top performing facilities used more non Ca-based P-binders. Causality of these factors and PTH control needs to be investigated.

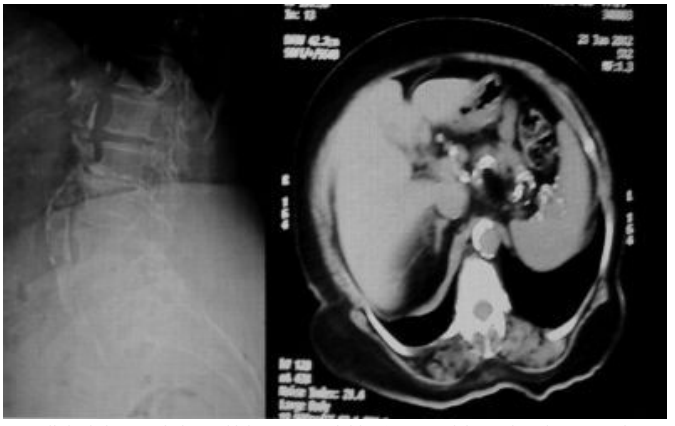
Funding: Pharmaceutical Company Support - Shire Pharmaceuticals

PUB531

Screening for the Prevalence of Vascular Calcification in Pre-ESRD (CKD Stage 4 & 5) Indian Patients: A Single Center Experience Sham Sunder, Himanshu Verma, Venkataramanan K. *Nephrology, PGIMER & Dr. Ram Manohar Lohia Hospital, New Delhi, Delhi, India.*

Background: Vascular calcification (particularly coronary artery and aortic calcification) is a common complication and a predictor of cardiovascular morbidity and mortality in dialysis patients. However, the prevalence of vascular calcification in pre-dialysis chronic kidney disease (CKD) is poorly understood.Currently in India, we do not have epidemiological data on the extent of vascular calcification existing in pre-ESRD (stage 4 and 5 CKD) patients. Abdominal aortic calcification (AAC) can be recorded using plain radiographs (digital X-Ray lumbar spine), multi-slice CT scan and EBCT-scan.

Methods: A total of 90 CKD patients were screened for AAC in the study. Inclusion criteria were age ≥18 years, CKD stage 4 & 5 not on dialysis ,hyperphosphatemia (serum phosphate > 5.5mg/dL).Digital X-Ray lumbar spine (lateral view) and non contrast 40-slice CT scan of the abdominal aorta was used to determine abdominal aortic calcification.



Clinical characteristics and laboratory variables (serum calcium, phosphate, ALP, iPTH, 25,OH,vit D_3) were also assessed. The duration of study was 1 year.

Results: 90 patients (mean age 56.13 ± 11.9 years old, 46 male and 44 female) with pre – dialysis CKD in stage 4 and 5 (66 and 24 in stages 4 and 5, respectively) participated in the study.The primary kidney diseases were diabetic nephropathy (34), chronic interstitial nephritis (35), chronic glomerulonephritis (9), hypertensive nephropathy (7), chronic pyelonephritis (5). Among the patients,75% had abdominal aortic calcification (73.6% and 81.8% for CKD stages 4 and 5, respectively).

Conclusions: The prevalence of vascular calcification (AAC) in pre – ESRD (CKD stage 4 and 5) Indian patients is 75%.Decreased GFR and serum CaxPo4 product correlates with the extent of abdominal aortic calcification in pre-dialysis CKD stages 4 & 5.

Funding: Government Support - Non-U.S.

PUB532

Introduction of a Protocol for Management of Hungry Bone Syndrome Following Parathyroidectomy Sandesh Parajuli, Jacob Moalem, David A. Bushinsky, Scott E. Liebman, Byron T. Slaton, Rebeca D. Monk. *University of Rochester Medical Center, Rochester, NY.*

Background: There is no standard approach to treating patients with secondary hyperpara-thyroidism (SPT) who require parathyroidectomy (PTX) and then develop hungry bone syndrome (HBS). In January 2010 we established a protocol to manage HBS in post-PTX dialysis pts with the goal of standardizing care, providing adequate calcium repletion and reducing complications.

Methods: Retrospective chart review of patients with SPT who underwent PTX in the last five years. Pts treated on or after 1/1/ 2010 were treated by protocol (Prot) and those treated previously were not (No Prot). Prot pts were transferred to the Nephrology service on POD1. Pre- and post-op PTH, serum ionized calcium (iCa), total calcium (Ca) and phosphorus (P) levels were monitored until discharge. Rate of Ca infusion, P repletion and other medications were adjusted according to Prot. Ca infusion was started at ~1 amp/ hr with goal iCa of 4.5 – 5.1 mg/dl. Oral calcitriol and Ca were started on day 1. IV Vit D was started at dialysis. Complications, demographics, length of hospital stay (LOS), year of PTX, and readmission data were reviewed. Ca drip was tapered off as the iCa remained > 4.5. Pts were discharged when iCa remained ≥4.5 x 24 hours off iv Ca.

Results: There were 10 pts in Prot and 10 in No Prot group. Pts on Prot had a trend toward higher PTH (2010 vs. 1400), higher mean iCa during admission and less readmission (1 vs. 3). LOS was mean 24 (9 – 49) days in prot vs 13 (2 – 44) with No Prot. 2 pts in No Prot group had seizure/arrhythmia vs.0 in protocol group.

Conclusions: Use of a protocol for management of HBS simplified care despite a trend toward extended LOS. Results of Prot use suggest that very high dose parenteral infusion is needed to achieve pt safety and adequate Ca repletion. This is often only permissible in the inpatient setting. Higher PTH levels by the time pts are referred for PTX may contribute to worse HBS, greater Ca deficit and longer LOS. Pts referred for PTX with very high PTH levels should anticipate a long LOS. Protocols may assist in increasing safety in the management of these complex pts.

PUB533

Relationship of Activated Vitamin D and Parathyorid Hormone Levels in Hemodialysis Patients Gregor Paronian,[1] Ming Yuan,[2] K. Edmund Tse.[2] *[1]Huntington Hospital, Pasadena, CA; [2]Pasadena Nephrology, Pasadena, CA.*

Background: The SEEK study revealed that as kidney function declined, parathyroid levels (PTH) increased while 1,25 dihydroxyvitamin D (1,25D) levels decreased. Often, esculating dose of activated vitamin D is used for this treatment. However, overuse of activated vitamin D can induce many side effects. Our goal of this study is examine the relationship of iPTH and 1,25D levels in ESRD patients on hemodialysis (HD).

Methods: This is a retrospective review of 67 ESRD patients treated with HD from April 2008 to March 2010. Patients on cinacalcet or paricalcitol were excluded. Calcium bath on dialysis was constant at 2.5mEq/L. 23 patients had iPTH levels measured by both the Nichols and Scantibodies assays. CAP (1-84 PTH) to CIP (7-84 PTH) ratio was calculated from the Scantibodies method. High and low iPTH was defined as iPTH > 300 and < 150 pg/mL, respectively. Normal 1,25D levels was 15-75 ng/mL.

Results: 52% patients' iPTH levels were found to be at 150-300 pg/mL in which 26% had normal 1,25D levels. Only 7% of patients were found to have both high iPTH and low 1,25D. 76% patients with high iPTH had normal 1,25D levels instead of low; and yet, 44% patients had both low iPTH and low 1,25D levels. The mean serum calcium, phosphate, and 25D levels were 8.3 ± 0.7 mg/dL, 5.3 ± 0.6 mg/dL, and 31.2 ± 4.2 ng/mL respectively. No patient was found to have high phosphate levels (>5.5 mg/dL), high iPTH, and low 1,25D togather. 29% of patients with normal 1,25D had both high phosphate levels and high iPTH. No difference in the iPTH levels between the Nichols and Scantibodies assays (p=0.49) was found. There was no difference between the number of low 1,25D patients with low CAP/CIP ratio (<1.4) by the Scantibodies method and that with low iPTH by the Nichols assay (p=0.29).

Conclusions: iPTH levels correlate poorly with 1,25D levels in ESRD patients. Caution should be made to avoid excessive amount of activated vitamin D to be given to patients who may not have low 1,25D. Low CAP/CIP was found in patients with low 1,25D making Scantibodies assay confusing in determinating appropiate vitamin D therapy.

Funding: Clinical Revenue Support

PUB534

Parathyroidectomy: Good for the Bones and Good for the Heart? Maria Julia C.L.N. Araujo, Sara Mohrbacher, Vivian Lumi Onusic, Patricia T. Goldenstein, Hugo Abensur, Fabiana G. Graciolli, Luciene M. dos Reis, Rosilene M. Elias, Rosa M.A. Moyses. *Nephrology, Universidade de Sao Paulo, Brazil.*

Background: Parathyroidectomy (PTX) has been associated with better outcomes in chronic kidney disease (CKD) patients, who usually present an improvement in their clinical status, as well as a decrease in mortality risk, after PTX. However, little is known about the effects of PTX on cardiac function.

Methods: We prospectively studied 10 patients who underwent PTX, evaluating weight, body mass index (BMI), blood pressure (BP), and use of antihypertensive drugs, as well as serum parathyroid hormone (PTH), calcium (Ca), phosphate (P), alkaline phosphatase (AP), fibroblast growth factor 23 (FGF23), potassium (K), aldosterone and pro-brain natriuretic peptide (proBNP).

Results: During one year of follow-up, no symptoms or signs of cardiac failure were observed. As expected, a significant decrease was seen in Ca, P, AP, PTH, and FGF23 (10.1 ± 0.9 vs. 8.8 ± 1.0 mg/dl; 6.8 ± 1.8 vs. 4.8 ± 1.6 mg/dl; 442 ± 324 vs. 89 ± 35 U/L; 1,590 ± 517 vs. 66 ± 71 pg/ml; and 16,341 vs. 850 pg/ml, respectively; $p < 0.05$ for all). There was also a reduction in the need for hypertensive drugs, with no change in BP, accompanied by a consistent increase in BMI. No significant differences were seen in aldosterone or K. However, a significant increase in proBNP was observed (1,070 vs. 1,322 pg/ml; $p < 0.05$). Higher serum proBNP was seen in patients with hypocalcemia (2,861 vs. 1,219 pg/ml; $p< 0.05$). Log proBNP correlated negatively with BMI and Ca ($r = -0.56$ and -0.47, respectively; $p < 0.05$). Stepwise multiple regression analysis revealed that log proBNP was dependent on both BMI and Ca (adjusted $R^2 = 0.4$; $p < 0.05$).

Conclusions: Despite improving clinical status, PTX is associated with myocardial dysfunction or hypervolemia, as indicated by elevated proBNP, either of which could be related to hypocalcemia. Better control of BP, initially interpreted only as a consequence of an improvement in nutritional status, can also be an indicator of such impairment. This information should be taken into account mainly in patients with a history of cardiac disease who are to be submitted to PTX.

PUB535

No Interaction of PA21 with Micro- and Macronutrients under Physiologically Relevant Conditions Michael Löpfe,[1] Maria Wilhelm,[1] Sylvain Gaillard,[2] Felix W. Funk.[1] *[1]Vifor (International) Inc., St. Gallen, Switzerland; [2]Vifor Pharma, Glattbrugg, Switzerland.*

Background: Hyperphosphatemia, a common and serious complication of chronic kidney disease, often necessitates the use of phosphate binders. A possible undesirable effect of phosphate binders is adsorption or degradation of micro- or macronutrients, requiring supplementation of these elements. This *in vitro* study investigated the interaction of the novel iron-based phosphate binder PA21 with physiologically relevant concentrations of water-soluble B vitamins, amino acids, fluoride, sulphate and oxalate.

Methods: *Method 1:* Each nutrient was incubated with PA21 active pharmaceutical ingredient at pH 3.0, 5.5 and 8.0 for 2 hrs at 37°C. The supernatant was filtered and the nutrient concentration assessed using high-performance liquid chromatography with diode array and mass spectroscopy detectors, and ion chromatography. A recovery range of 80–125% was defined as bioequivalence limits. *Method 2:* As Method 1, but with addition of phosphate buffer. *Method 3 (simulated gastrointestinal [GI] tract conditions):* As Method 2, but for each nutrient, pH was increased stepwise from 3.0 to 5.5 to 8.0 after each incubation (total incubation time: 6 hrs).

Results: In the absence of phosphate (Method 1; Figure 1), only amino acids and sulphate showed a recovery rate of >80% across pH 3.0–8.0. In the presence of phosphate (Method 2), the vitamins B3, B5 and B7 were recovered at >80% at pH 3.0–8.0, B9 and oxalate at pH 8.0, B9 and fluoride at pH 5.5 and B6 at pH 3.0. Under simulated GI conditions (Method 3), vitamins B6, B9 and fluoride were recovered at >80%. The minor interaction of oxalate did not affect the binding capacity of PA21.

Conclusions: PA21 did not show a biologically relevant interaction with the investigated nutrients under physiologically relevant conditions.

Figure 1: Nutrient recovery rate

	pH	Vitamins	Amino acids	Fluoride	Sulphate	Oxalate
Method 1	3.0 and 5.5	<80%	>92%	<80%	>91%	<80%
	8.0	B3: 97.9% B5: 91.9% B7: 93.7%		83.7%		
Method 2	3.0–8.0	>94.1% for B3, B5, B7				
	3.0	B6: 95.9% B9: <80%		<80%		<80%
	5.5	B6: 76.6% B9: 104.4%		92.0%		<80%
	8.0	B6: 75.5% B9: 100.7%				102.1%
Method 3	3.0–8.0	B6: 95.8% B9: 96.4%		92.1%		79.4%

Funding: Pharmaceutical Company Support - Vifor (International) Inc.

PUB536

A Prospective Audit of Dietician-Led Management of Chronic Kidney Disease-Mineral Bone Disorder Parameters in Haemodialysis Patients

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Background: Haemodialysis(HD) patients with chronically elevated serum inorganic phosphate(Pi) are at increased risk of vascular calcification, a marker for adverse events. An expanded role for dieticians(RD), to include adjustment of phosphate binder therapy, and its integration with dietary support, may improve Pi management in HD patients.

Methods: The aim was to prospectively audit the difference between RD vs physician(PL) management of serum Pi, corrected calcium(Ca) and parathyroid hormone(PTH), use of binders, active vitamin D(alfacalcidol), calcimimetics and patient experience in HD patients. The PL group(n=131) had the medications adjusted by the nephrologist and RD input bi-annually. The RD group(n=144) were seen monthly for dietary counselling & review of these medications which were adjusted as required following agreed protocol. Data were collected at baseline and 12 months with an experience questionnaire distributed.

Results: There was no statistically significant difference in the adjusted mean serum Pi, Ca or PTH between baseline and 12 months (Adjusted means for Pi were:PL 1.57mmol/l & 1.56mmol/l, RD 1.45mmol/l & 1.44mmol/l baseline and 12 months respectively). There was NS difference in alfacalcidol dose, % taking a calcimimetic, or in dose or type of binder used (calcium vs.non-calcium) at baseline or 12 months between groups. There was a trend towards using fewer binders in the RD group although this was NS (4.92 & 4.95 PL and 4.13 & 3.91 RD). 85 individuals (n=42 RD,n=43 PL) responded to the questionnaire. Individuals in the RD group self reported to be more confident in managing diet and bone medications and felt more involved in making changes vs those in the PL group.

Conclusions: In regard to biochemical control of bone & mineral parameters, there is no difference in the quality of care delivered by dieticians or nephrologists. Despite their greater experience in the management of CKD-MBD, nephrologists may be disadvantaged by poorer knowledge of the dietetic issues. RD's may be a more cost effective strategy for CKD-MBD management.

PUB537

Serum C-Reactive Protein and K/DOQI Guidelines for Bone Metabolism and Disease in Predialysis Patients: Can We Achieve Them?

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Background: Mineral metabolism abnormalities and several inflammatory parameters, mainly C-reactive protein (CRP) and IL-6 are concerns in chronic kidney disease (CKD). In spite of the important prevalence and relevance of alterations of mineral metabolism and inflammation in CKD patients, the achieving K/DOQI guidelines have been scarcely analyzed, especially in predialysis patients. The **aim** of this study was to examine the achieving among the main parameters of mineral metabolism (Ca, P, CaxP, and PTH) in the presence/absence of an inflammatory state in predialysis patients.

Methods: A cross sectional study was carried out. The study included patients with CKD not on dialysis and not receiving calcium supplements, phosphorus binders, or vitamin D. The following parameters were determined and analyzed: calcium (Ca), phosphorus (P), Ca-P product, parathyroid hormone (PTH) and CRP. The presence of an inflammatory state defined as CRP>3mg/l and the absence as CRP<3mg/l. Continuous data are presented as mean± standard deviation and categorical data as absolute and relative frequencies.

Results: In the study participated 144 patients (86 males / 58 females, mean age 63±14yearsand mean estimated GFR 34.6±9.5 ml/min/1.73m^2). The percentage of the patients who met the criteria for Ca, P, CaxP, PTH and CRP<3mg/l were 45.1% (n=65), 61.1% (n=88), 88.9% (n=128) 32.6% (n=47) and 36.8% (n=53) respectively. Only 3.5% (n=5) of the patients met all four criteria simultaneously. The percentage of the patients who met the criteria for Ca, P, CaxP, PTH and CRP>3mg/l were 5.6% (n=8). The correlation between P and CRP was not statistically significant (r=0.149, p=0.07).

Conclusions: These data indicate that current practice for the management of mineral metabolism in patients with CKD not on dialysis falls far short of meeting the K/DOQI guidelines both in the presence and in the absence of an inflammatory state.

Funding: Other U.S. Government Support

PUB538

Racial Impact on Bone Metabolism Parameters: Retrospective Review

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Background: Bone metabolism is deranged in end stage renal disease. Race may contribute to the differences observed in the bone metabolism profiles. We explored the impact of Asian race versus other races on the bone metabolism parameters at the initiation of chronic dialysis.

Methods: Medical records of new admissions at Trude Weishaupt Dialysis Center from 2009-2011 were reviewed. Race and parameters of bone metabolism were extracted for every new admission.

Results: There were 181 new admissions between 2009 and 2011. Fifty seven patients did not have lab data from within 14 days of initiation of chronic dialysis, 5 had failed renal transplant before initiation of chronic dialysis, 2 had parathyroidectomy before initiation of chronic dialysis, and 3 were on cinacalcet at the time of chronic dialysis initiation leaving 114 patients for analysis.

Table 1: Demographics

	Asians n=38	Other Races n=76
Age (years)	70±13	67±16
Gender (M / F)	20 (53%) / 18 (47%)	52 (68%) / 24 (32%)
HD / PD	31 (82%) / 7 (18%)	65 (86%) / 11 (14%)
Diabetes	28 (74%)	35 (46%)
Not Taking Phosphate Binder or Vitamin D[¥]	23 (61%)	40 (53%)

[¥] at chronic dialysis initiation or within 14 days

Table 2: Parameters of Bone Metabolism

	Asians (n=38)	Other Races (n=76)
Albumin (g/dL)	2.8 ± 0.6	2.8 ± 0.7
Calcium (mg/dL)	8.1 ± 0.9	8.1 ± 0.9
Phosphorus (mg/dL)	6.2 ± 2.4	6.3 ± 2.4
Alkaline Phosphatase (U/L)	70 ± 25	92 ± 99
PTH [intact] (pg/mL)	306 ± 169*	417 ± 369*

*p<0.05

PTH levels were significantly lower in the Asian population compared to other races even when adjusted for diabetes. There were no significant differences in other parameters of bone metabolism in Asian versus other races group.

Conclusions: Our study shows that PTH levels in the Asian population at chronic dialysis initiation were significantly lower compared to other races. There were no significant differences in albumin, calcium, phosphorus, and alkaline phosphatase, Vitamin D or binder prescription between the two groups. Difference in dietary intake in different racial groups could explain these findings and should be studied to better understand the impact of race/diet on the parameters of bone metabolism.

PUB539

Low-Grade Inflammation and Achieving Laboratory Target Values for Bone and Mineral Metabolism in Hemodialysis Patients

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Background: Inflammation is recognized in up to 50% of Chronic Kidney Disease patients being a common feature of advanced renal disease. Serum C-reactive protein (CRP) had been found to be significantly elevated in hemodialysis (HD) patients and reflects chronic inflammation. Most studies have been performed in hemodialysis on the achievement of more recent and stringent guidelines. The K/DOQI guidelines for HD patients define therapeutic targets for renal osteodystrophy: iPTH: 150-300 pg/ml P=3,5-5,5mg/dl Ca=8,4-9,5mg/dl and product Ca x P ≤ 55mg^2/dl^2. **Aim**: To evaluate our ability to meet the laboratory target values for bone and mineral metabolism in HD patients and the status of inflammation (CRP<3mg/l or CRP>3mg/l).

Methods: We have retrospectively investigated the laboratory parameters for bone and mineral metabolism and the serum levels of CRP of 52 HD patients over a 12 months period in our Hemodialysis Unit. Serum calcium (Ca) and phosphorus (P) levels were determined using standard assay, PTH and CRP levels were determined using chemiluminescence and nephelometry immune assay respectively.

Results: A total of 52 patients (32M/20F, mean age 65±15 years, mean period on dialysis 8±5years, mean Kt/V 1,5±0,3)were included in the study. A total of 104 determinations for CRP and each bone metabolism parameters assessed. Percentage of laboratory determinations that were within the recommended range: 82% for Ca and 71% for CaxP product. Regarding the P and PTH, only 46 (44%) and 35 (33,6%) of the values met the target range respectively. Notably, only 13 determinations (12, 5%) met all four requirements simultaneously. However, 73% of the values for CRP were above 3mg/l and only 27% were below 3mg/l.

Conclusions: Our data suggest that while achieving laboratory values within the target range for bone and mineral metabolism is difficult, it is even more difficult to sustain low-grade inflammation in HD patients.

Funding: Other U.S. Government Support

PUB540

Reversibility of Medial Vascular Calcification in Renal Failure: A Case of Osteoclast Deficiency? Na'Da Abouhassan,[1] W. Charles O'Neill.[2] *[1]Medical University of South Carolina, SC; [2]Nephrology, Emory University, Atlanta, GA.*

Background: Dissolution of hydroxyapatite requires a low pH which, in bone, is achieved by binding of osteoclasts and secretion of protons. Whether this process is active in the arterial wall and can reverse vascular calcifications is unknown.

Methods: Calcified arteries in breast tissue from 19 patients with chronic kidney disease (10 with ESRD) and in amputated lower limbs of 10 additional ESRD patients were examined histologically for osteoclasts. To determine reversibility of medial calcification, breast artery calcification was compared in a blinded fashion by two observers in sequential mammograms at least one year apart from 15 women with end-stage renal disease (ESRD) after successful renal transplantation (mean serum creatinine 1.04 ± 0.05 mg/dl, range 0.7 – 1.29 mg/dl).

Results: Arterial calcification was present in breast tissue from 18 of 19 women but no osteoclasts were observed. Occasional osteoclasts were observed in 3 of 22 heavily calcified arteries from amputated lower limbs of 10 ESRD patients. Of the 8 women with arterial calcification on the earliest mammogram after transplantation (age 58 ± 4 y; 4 with diabetes), 1 showed slight regression on the second mammogram while 2 showed no change and 4 showed progression (mean interval: 3.4 ± 0.65 y; range: 1.0-5.2 y). Of the 7 patients with no arterial calcification on the first mammogram (age 54.5 ± 3.9 y; 4 with diabetes), none had calcification on the second mammogram (mean interval: 3.4 ± 0.5 y; range: 2-6 y).

Conclusions: Osteoclasts are rare in medial arterial calcification and this is reflected by the lack of regression of calcification in most of the patients after successful renal transplantation. In addition to impeding the resolution of vascular calcification, this failure to mobilize osteoclasts may also exacerbate ongoing vascular calcification.

Funding: Clinical Revenue Support

PUB541

Diastolic Dysfunction Is Common in CKD-5D Patients with Coronary Artery Calcifications Paul Anaya,[1] Hartmut H. Malluche,[2] Anas Al Rifai,[3] Gustav A. Blomquist,[4] Daniel Davenport,[5] Marie-Claude M. Faugere,[2] Vincent L. Sorrell.[1,4] *[1]Division of Cardiology, University of Kentucky; [2]Division of Nephrology, University of Kentucky; [3]Department of Internal Medicine, University of Kentucky; [4]Department of Radiology, University of Kentucky; [5]Department of Surgery, University of Kentucky, Lexington, KY.*

Background: In CKD 5D patients, coronary artery calcification (CAC) is common and cardiovascular disease is the leading cause of death. This study was designed to investigate cardiac morphologic and functional implications of CAC in patients with CKD-5D who are often excluded from cardiovascular clinical trials.

Methods: As part of an ongoing IRB approved study, echocardiograms were performed in 37 subjects (54±15yrs) with CKD-5D. Concurrently, CAC was measured by 64-slice multidetector computed tomography (MDCT) and quantified by Agatston scores. Echocardiograms adhered to American Society of Echocardiography guidelines and comprehensive measures were analyzed by expert cardiologists blinded to CAC results.

Results: The median CAC score was 518, a value conferring high risk for cardiovascular events. The range of CAC scores was wide (0 to 4628). Left ventricular (LV) global systolic function was normal (LV ejection fraction [LVEF] = 60±9%), however, sensitive markers of LV myocardial performance (LVMP index 0.80±0.25) and diastolic function (left atrial volume index=39±14) were abnormal. LV elastance and ventricular-arterial coupling were also abnormal. CAC was associated with concentric LV hypertrophy (p=0.06) even in the small cohort enrolled to date. Additionally, cardiac calcification was positively associated with CAC score.

Conclusions: CAC is associated with structural and functional cardiac abnormalities in patients with CKD-5D. These findings imply a link between CKD, subclinical atherosclerosis, adverse cardiac remodeling, and myocardial stiffness (diastolic dysfunction) emphasizing the need for continued investigation.

Funding: NIDDK Support

PUB542

Usefulness of Radiologic Evaluation to Predict the Response of Cinacalcet Treatment in Secondary Hyperparathyroidism of Hemodialysis Patients Gang Jee Ko,[1] Yoo Sun Cho,[1] Mi-yeon Jung,[1] Soo Young Oh,[1] Jae Hee Seo,[1] Heui Jung Pyo,[1] Sangil Suh,[2] Young-Joo Kwon.[1] *[1]Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea; [2]Department of Radiology, Korea University College of Medicine, Seoul, Korea.*

Background: Cinacalcet, a currently available calcimimetics was supposed to have a role in treatment of secondary hyperparathyroidism (SHPT) refractory to previous medical treatment. However, although resistance to cinacalcet therapy was reported in advanced nodular hyperplasia, the method elucidating cinacalcet response was not established yet. We aimed to investigate whether radiologic examinations would be helpful to determine the response of cinacalcet treatment.

Methods: Twenty six patients who received hemodialysis more than 3 months were treated with cinacalcet for 20 weeks. Before administration of cinacalcet, radiologic evaluations for parathyroid glands were done with sonographic measurement for diameter and volume of each gland by 3 dimentional reconstruction by one expert. Computed tomography (CT) was also taken to measure the diameters of glands by one expert. Patients with median PTH <300 pg/mL during the period of therapy and follow-up were considered responders.

Results: Among 26 patients, 17 patients were responders. Serum calcium and PTH before treatment, and ALP and PTH in follow-up period were lower in responder group. Diameter in sonography and CT, and gland volume were tended to be lower in responder, though it was not significant (responder vs nonresponder: 10.3±5.6 vs 12.5±6.3 mm, 14.0±4.0 vs 17.3±5.0 mm, 700.0±726.8 vs 1015.6±1331.6 mm^3, p>0.05). When cutoff value for each radiologic parameter was calculated to maximize sensitivity and specificity with ROC curve, the number of glands bigger than the cutoff in CT scan (>11.2mm) was significantly more in non-responder group (p=0.021).

Conclusions: Measurement of parathyroid gland diameters with CT scan was useful to predict the response of cinacalcet therapy. Further studies with larger population should be needed.

PUB543

The Predictors of Progression of Vascular Calcification in Hemodialysis Patients Sun Ryoung Choi, Youngki Lee, Min-Gang Kim, Myung Jin Choi, Jwa-kyung Kim, Soo Jin Kim, Taejin Park, Young Rim Song, Sung Gyun Kim, Ja-Ryong Koo, Hyung Jik Kim, Jung-woo Noh. *Internal Medicine, College of Medicine, Hallym University, Seoul, Korea.*

Background: Vascular calcification is thought to be associated with a significant mortality and morbidity in patients with chronic kidney disease (CKD). Although the KDIGO recommended that a lateral abdominal radiograph be used to detect the presence or absence of vascular calcification, the risk factors for progression of calcification are not clearly elucidated. Therefore, we investigate the predictors of progression in vascular calcification.

Methods: This study was prospective observational study. Lateral lumbar radiography of the abdominal aorta was used to evaluate the overall abdominal aorta calcification (AAC) score, which is related to the severity of calcific deposits at lumbar vertebral segments L1–L4. Lumbar radiography was performed at baseline and after 1 year, respectively. The progression of AAC score was defined by any increase in Δcalcification (the change of AAC score).

Results: The subjects were 124 patients on maintenance hemodialysis. 68 (58.1%) were female. The mean age was 57.2±10.9 years. The vintage of dialysis was 56.7 ± 53.8 months. The underlying renal diseases were DM in 66(56.4%) patients. The mean baseline AAC score of the study population was 6.2±6. The risk factors of AAC were age, presence of cardiovascular diseases, and dialysis vintage. 65 patients (53%) showed AAC progression, and 59 patients (47%) showed no change of AAC score after 1 year. In a multivariate analysis, the presence of AAC at baseline (p = 0.017) were independent risk factors for AAC progression. No significant association with AAC progression was found between the baseline and follow up clinical parameters, including gender, obesity, diabetes, hypertension, and dialysis vintage.

Conclusions: This study identified that the risk factor related with AAC progression in hemodialysis patients was the presence of AAC at baseline. Furthermore, considerable number of patients had progression in AAC even after 1 year. Patients should be carefully evaluated and managed from early stage to prevent development and progression of AAC.

PUB544

Differences of CKD-MBD Characteristics and Treatment Practices among Dialyzed Patient's Groups in Hungary Istvan Kiss, Zoltan Kiss, Andras Szabo, Dr.szegedi János, Jozsef Balla, Erzsebet Ladanyi, Botond Csiky, Otto Arkossy, Marietta Török, Sándor Túri, Imre Kulcsar. *Working Group of CKD-MBD, Hungarian Society of Nephrology, Budapest, Hungary.*

Background: Different age groups have different health conditions demanding different treatment approches. Our aim was to survey epidemiology, disease characteristics and treatment practice of CKD-MBD in Hungarian dyalized patients according to age groups.

Methods: It is a multicentre, non-interventional, retrospective, cross-sectional study. We collected CKD-MBD related data: gender, age, BMI, cause of ESRD, se-PTH, se-Ca, se-P, comorbidities and treatment practice. 5008 dialized patient were enrolled into the study. The patients were allocated by their ages (years) into three groups (I:<55; II: 55-74; III: 74+).

Results: Mean age: 63,4±14,2 years old, male proportion: (n=2644) 52,8% (I: 60 %, II: 53,6 %, II: 43,8 %), hemodialysis: 88,6% (I: 85 %, II: 88,6 %, III: 92,4 %). The most frequent cause of ESRD were in 22,9 % hypertension, in 22,1 % diabetes mellitus, in 16,2 % glomerulonephritis. Serum median iPTH level was 178,0 pg/ml (IR: 75,8-361,5). iPTH level was in group I: 223,5 pg/ml (IR: 93,5-514,0), in group II: 181,9 pg/ml (IR: 78,0-348,8), in group III: 141,0 pg/ml (62,2-280,5) (p<0,001). The higher the age was the lower the PTH level was found (p<0,001). Significant differences were among the age-groups in use of calcimimetics (I: 11,3 %, II: 4,1 %, III: 1,3 %), non-Ca-based phosphate binder (I: 29,6 %, II: 18,4 %, III: 8,2%). Among older patients there were significantly higher prevalence of bone disease (BD / I: 30,4 %, II: 39,7 %, III: 44,9 %) and soft tissue calcification (STC / I: 37,1 %, II: 53,6 %, III: 58,5 %).

Conclusions: There are significant differences in epidemiology, laboratory, treatment practice, BD and STC related data in groups divided by ages. Serum iPTH level significantly decreased as the patients ages increased. Further research of this topics and incorporation of current age speciifc findings into clinical practice and also guidelines are needed in the near future.

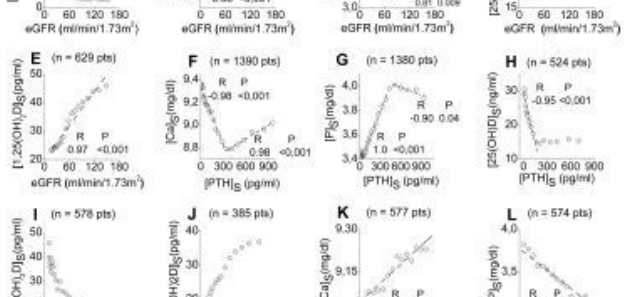
PUB545

Chronic Kidney Disease: Development of Secondary Hyperparathyroidism in 3 Phases Ana Pires,[1] Hugo G. Ferreira.[2] *[1]Nephrology, Professor Doutor Fernando Fonseca Hospital, Portugal; [2]REQUIMTE, Chemistry, New University of Lisbon, Portugal.*

Background: Regulation of the phosphorus-calcium metabolism changes in the course of chronic kidney disease (CKD) but always relying on well known physiological mechanisms.

Methods: Retrospective data from 2507 CKD patients (pts) not on vitamin D therapy, dialysis or submitted to a renal transplant. Each pt was represented by a set of serum concentrations of creatinine, calcium ($[Ca]_S$), phosphorus ($[P]_S$), $[PTH]_S$, $[25(OH)D]_S$ and $[1,25(OH)_2D]_S$. Glomerular filtration rate (eGFR) was estimated by MDRD formula. Functional relations were obtained by pairing variables measured, filtering with a moving average of 3 to 5 points, followed by binning into frequency classes represented by their means and respective standard errors.

Results: Pts were 64±17 years old, 52% male, 97% caucasian. Mean eGFR was 57±34 [15-160ml/min/1,73m^2].



eGFR was negatively correlated with $[PTH]_S$ (Panel A) and $[Ca]_S$ for eGFR above 60 ml/min/1.73m^2 (Panel B) and $[P]_S$ for eGFR under 60 ml/min/1.73m^2 (Panel C) and was positively correlated with $[25(OH)D]_S$ (Panel D), $[1,25(OH)_2D]_S$ (Panel E), $[Ca]_S$ for eGFR under 60 ml/min/1.73m^2 (Panel B) and $[P]_S$ for eGFR above 60 ml/min/1.73m^2 (Panel C). $[PTH]_S$ was negatively correlated with $[Ca]_S$ for PTH > 300 pg/ml (Panel F), $[P]_S$ for PTH < 460 pg/ml (Panel G), $[25(OH)D]_S$ for PTH < 200 pg/ml (Panel H) and $1,25(OH)_2D$ (Panel I) and positively correlated with $[Ca]_S$ for PTH > 300 pg/ml (Panel F) and $[P]_S$ for PTH < 460 pg/ml (Panel G).

Conclusions: For eGFR above 60ml/min/1.73m^2 there was a decline of $[1,25(OH)_2D]_S$ and $[P]_S$ and an increase of $[Ca]_S$ (First Phase). As eGFR reduces $[1,25(OH)_2D]_S$ continues to decrease and $[25(OH)D]_S$ and $[Ca]_S$ diminish and $[P]_S$ increases, suggesting that parathyroid glands are driven by their regulatory actions (Second Phase). In a later phase the parathyroid cells become partially or totally insensitive (Third Phase).

PUB546

The Italian Multicentric Study on the Prevalence of Vascular Calcifications and Vertebral Fractures in Parathyroidectomised (PTX) Dialysis Patients (Cave PTX Study): Phase I Results Sandro Mazzaferro. *Cardiovacular, Respiratory, Nephrology and Geriatric Sciences, Sapienza University, Rome, Italy.*

Background: With a low prevalence of cases, data of clinical outcomes of PTX are limited. The CAVE PTX study of dialysis patients who received PTX, aims to evaluate the control of divalent ions and pertinent medical therapies (phase I), and the prevalence of aortic calcifications and vertebral fractures (phase II).

Methods: Biochemistries (Ca, P, PTH) and therapies of PTX patients were collected by means of an electronic data sheet from 149 Italian dialysis Units. With a computerized procedure, a control group ©, comparable for age, sex and dialysis duration, was selected from the whole cohort. We report on the preliminary data of biochemistries and therapies.

Results: From a total of 12515 patients (HD = 87.7%; PD = 12.3%), 528 (4.22%) received PTX. Prevalence of PTX was definitely higher in HD (4.5%) compared to PD (1.9%). Cases and C (n=320) characteristics are compared in table 1.

TABLE 1	Age, y	M/F, %	Dialysis, y	Ca, mg/dl	P, mg/dl	PTH, pg/ml
PTX	58±13	56/44	15±8	8,8±0,8	4,9±1,3	182±292
C	60±14♦	56/44	12±13♣	9,0±0,6♣	5,1±1,3♦	335±300♣
♦p<.05, ♣p<.001 vs PTX						

TABLE 2	PTH < 150		PTH 150 - 300		PTH > 300	
Ca, mg/dl	8,6±0,8	9,0±0,8♠	8,9±0,7	9,0±0,3	9,2±0,8	8,9±0,3♠
P, mg/dl	4,8±1,3	4,7±1,5	4,9±1,3	4,5±0,6♠	5,1±1,3	4,6±0,6♠
PTH, ng/ml	40±30	87±40♠	216±40	217±41	630±417	567±337
♠p<.0001 PTX vs C						

Ca, P and PTH values in the three K/DOQI PTH range groups are in table 2. Respectively in PTX and C, PTH was low (<150) in 64% vs 20%; optimal (150-300) in 17% vs 40%; and high (>300) in 19% vs 40%. Prescribed drugs, respectively in PTX and C, were: Vitamin D (61 vs 69%); Phosphate binders (88 vs 80%) and Calcimimetic (13 and 37%). Notably, Calcitriol and Ca based binders, and Paricalcitol and Sevelamer were the most frequently prescribed drugs respectively in PTX and C.

Conclusions: PTX has a low prevalence in Italy, and is confirmed to mainly involve relatively young, females and long-term haemodialysis patients. In these patients PTH values are mostly low or high while therapeutic choices may be challenging.

Funding: Pharmaceutical Company Support - Abbott, Amgen, Government Support - Non-U.S.

PUB547

Cinacalcet and Paricalcitol Flexible Doses Combination in the Treatment of Moderate to Severe Secondary Hyperparathyroidism in Hemodialysis Patients German Perez Suarez,[1] Bilal El Hayek,[1] Elvira Bosch,[1] Ignacio Ramirez,[1] Eduardo Baamonde,[1] Cesar Garcia-canton,[2] Maria Dolores Checa.[2] *[1]Nephrology, AVERICUM, Las Palmas, Spain; [2]Nephrology, Hospital Universitario Insular de Gran Canaria, Las Palmas, Spain.*

Background: Combined therapy with Cinacalcet and low doses of Paricalcitol has shown improved achievement of the biochemical targets for bone and mineral disorder (MBD) in dialysis patients with moderate to severe secondary hyperparathyroidism (HPTMS). Aim: Determine the long time effect of flexible doses of Paricalcitol and Cinacalcet combination in the parathyroid hormone (iPTH) and calcium-phosphorus product (CaxP) in hemodialysis patients with HPTMS.

Methods: Forty eight patients (male 60%, mean age:58±16 years) with HPTMS(mean iPTH (727± 352pg/mL) and CaxP<55, received combined treatment with flexible dose of Paricalcitol (mean dose: 9.4 mg/week) and Cinacalcet (mean dose of 38.5 mg/day). Clinical and demographic data and biochemical parameters of MBD were recorded (iPTH, calcium, phosphorus and Ca x P). In addition, we recorded all attached medication at the beginning and during the follow-up (mean 17±11month).

Results: Three months after treatment started a reduction in iPTH level were observed (727±352pg/mL to 509±281pg/mL, P<0.000) and this decline persisted at first year of combined treatment, being the reduction of 40.7% (727 ± 352pg/mL to 431±144pg/mL, P <0,000). Also, at 1 year Phosphorus decreased (-9%, 5.2±1.2mg/dl to 4.7±0.9 mg/dl, P=0,048), Calcium (-1%, 9.1±0.7mg/dl to 9.0 ± 0.6mg/dl, P=0.270) and Ca x P (-3%, 47.1±10 mg/dl to 45.9±13mg/dl, P=0.600). Non calcium-containing phosphate-binding doses increased (Sevelamer hydrochloride (P=0.550), lanthanum carbonate (P=0.621), while calcium-containing phosphate-binding doses decreased, (P=0.188). Finally, we observed a reduction of erythropoiesis stimulating agents doses (P=0.08). One patient need a parathyroidectomy and an episode of symptomatic hypocalcemia was recorded.

Conclusions: Combined therapy with flexible doses of Paricalcitol and Cinacalcet in dialysis patients with HPTMS is capable of producing a sustained decline in iPTH and serum phosphorus without increasing others biochemical parameters of MBD.

PUB548

Hip DXA and QCT Scans Are Useful Screening Tests for Low Bone Volume in CKD-5D Patients Gustav A. Blomquist,[1] Hanna W. Mawad,[2] Daniel Davenport,[3] Marie-Claude M. Faugere,[2] Hartmut H. Malluche.[2] *[1]Department of Radiology, University of Kentucky; [2]Division of Nephrology, University of Kentucky; [3]Department of Surgery, University of Kentucky, Lexington, KY.*

Background: Non-invasive radiologic techniques for the measurement of bone mass by dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) are used to screen patients for osteoporosis, defined as t-scores ≤ -2.5. However, in CKD-5D patients, previous reports have shown that osteoporosis by DXA and QCT does not accurately identify patients with low bone volume as determined by bone biopsy. Low bone volume, the invasive measurement of low bone mass, is a risk factor for fractures and vascular calcifications.

Methods: In this study, 20 men (mean age 52) and 19 women (mean age 50) with a mean dialysis vintage of 51 months underwent DXA and QCT measurement of bone mass. Concurrently, iliac crest bone biopsies were done for assessment of cancellous bone volume, cortical thickness and porosity to identify low bone volume.

Results: Low bone volume was detected by histology in 33/39 patients (85%). Sensitivity and specificity of t-scores from QCT and DXA at hip and spine for prediction

of low bone volume are shown (Table). A t-score cutoff of ≤-2.5 at the hip missed ¾ of CKD-5D patients with low bone volume (sensitivity 23-25%). A hip t-score cutoff value of ≤0 increased the sensitivity of DXA and QCT for identifying low bone volume to 81-94%. This cutoff level did not reach clinically acceptable specificity (67% vs. the required >80%). Spine sensitivity and specificity were insufficient for screening.

	Spine		Hip	
t-score	QCT	DXA	QCT	DXA
	Sensitivity			
≤0	58%	61%	81%	94%
≤-2.5	18%	12%	25%	23%
	Specificity			
≤;0	67%	67%	67%	67%
≤-2.5	83%	100%	100%	100%

Conclusions: DXA or QCT should be used to screen for low bone volume. The recommended cutoff of t-score ≤ -2.5 for treatment of osteoporosis in the general population should be extended to ≤ 0 in CKD-5D patients.

Funding: NIDDK Support

PUB549

Dietary Zinc Intake and Kidney Stone Disease: The National Health and Nutrition Examination Survey III, 1988-1994 Jie Tang, Kim Mcfann, M. Chonchol. *Medicine/Nephrology, University of Colorado Sch of Med, Aurora, CO.*

Background: Trace elements are thought to affect calcium-based crystal formation. However, role of zinc intake on kidney stone formation is unclear, as existing studies have shown inconsistent results.

Methods: We used The Third National Health and Nutrition Examination Survey, to determine the independent association between dietary zinc intake, obtained from 24h dietary recall, with kidney stone disease defined as self-report of any previous episode of kidney stone.

Results: A total of 762 among 16456 eligible participants reported a history of kidney stone.

Table 1. Characteristics of the study population

	Stone formers (N = 762)	Non-stone formers (N = 15694)	P value
Age (years)	53.65 ± 0.79	43.12 ± 0.19	< 0.0001
Men	453 (59%)	7211 (46%)	< 0.0001
Race: white	649 (85%)	10433 (66%)	< 0.0001
Hypertension	302 (40%)	4091 (26%)	< 0.0001
Diabetes	86 (11%)	1225 (8%)	< 0.0001
BMI (kg/m2)	27.90 ± 0.30	26.33 ± 0.07	< 0.0001
Zinc use (mg per day)	13.28 ± 0.83	11.91 ± 0.11	0.1
HCTZ use	22 (3%)	266 (2%)	<0.01
Serum calcium (mg/dl)	414.81 ± 73.17	452.29 ± 22.67	0.6

Table 1 shows the characteristics of the study population. Among stone formers, dietary zinc intake increased with advancing age in both men and women, though did not reach statistical significance (p = 0.95). Men had a significantly higher dietary zinc intake than women, regardless of kidney stone history (p<0.0001). After adjustment for age, sex, race, histories of diabetes and hypertension, BMI, dietary protein, water and salt intake, total calorie intake, usage of HCTZ and serum calcium concentration, higher dietary zinc intake was significantly associated with prevalent kidney stone disease when zinc intake (log transformed) was modeled as a continuous variable, OR=1.36, 95% CI 1.07-1.75, p=0.01. In addition, we divided daily zinc intake into three categories using clinically significant cut-offs (< 7 mg, 7-15 mg, > 15 mg). The adjusted odds for stone formation was 1.63 (95% CI 1.08-2.46, p=0.02) when comparing zinc consumption >15 mg/day to <7mg/day.

Conclusions: Our study suggests that higher dietary zinc intake is associated with increased risk of kidney stone disease. Future prospective studies are needed to clarify the causal relationship.

Funding: Clinical Revenue Support

PUB550

Determination of Ureter Stent Appearance on Dual-Energy Computed Tomography Scan Maria Jepperson,[2] William E. Haley,[1] Joseph Cernigliaro,[2] David D. Thiel.[3] *[1]Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL; [2]Department of Radiology, Mayo Clinic, Jacksonville, FL; [3]Department of Urology, Mayo Clinic, Jacksonville, FL.*

Background: Dual-enery computed tomography (DECT) is useful to evaluate renal and ureteral calculi. DECT assigns a color based on density and chemical composition and accurately distinguishes uric acid (UA) calculi from other types. Some ureteral stents are characterized as more closely resembling the dual-energy characteristics (DEC) of UA calculi (red); others the DEC of non-UA calculi (blue). This study examined the DECT properties of commonly used ureteral stents to optimize stent selection for calculi being followed by DECT imaging.

Methods: Seven stents were individually placed in a phantom and imaged by a Siemans SOMATOM Definition Flash CT scanner. DECT peak tube potentials (kVp) of 80/140 and 100/140 were used to reflect our current protocols. These were compared to 31 in-vivo ureteral stents in 27 patients. The data were reconstructed on a Multimodality Work Place (Siemans) using CT Syngo Post Processing Suite Software, version VE 36A.

Results: Average patient age 64 years (range 27-90). Four patients were being treated for UA stones, 22 for calcium-based stones. There was no difference in the DEC of stents from the same manufacturer. All imaged Cook and Bard stents had DEC that approached those of calcium stones (blue). All Boston Scientific and Gyrus ACMI stents had DEC resembling UA stones (red). Patient age, BMI, gender, type of stone disease, time duration since stent placement, and stent location had no effect. There was no discrepancy between in vivo and in vitro findings. Varying kVp had no effect on stent characterization.

Conclusions: Stents from different manufacturers are characterized by the dual-energy algorithm on DECT imaging and, therefore have color appearances that mimic UA or non-UA calculi. This study provides, to our knowledge, the first data on DECT stent characterization for various stent manufacturers. This information will aid in optimal stent selection for stent/calculi color contrasting and improved visualization of stone material in patients being treated for obstructing ureteral calculi and followed with DECT imaging.

Funding: Other NIH Support - NIH P50DK083007

PUB551

Are Healthy Subjects at Risk to Become Stone Formers? Oriane Dohein,[1] Inmaculada Buendia,[1] Ivan A. Tack,[2] Michel Daudon,[3] Picard Pascaline.[1] *[1]Danone Research RD128, Palaiseau, France; [2]Dpt of Clinical Physiology, Université Paul Sabatier, Toulouse, France; [3]Dpt of Clinical Physiology, Tenon Hospital, APHP, Paris, France.*

Background: Nephrolithiasis is a common disease often related to dietary habits and low diuresis. Several urinary markers, including urine osmolality, calculated risk index such as Tiselius index (TI), and crystalluria have been proposed to assess the risk of stone recurrence in patients. The relevance of these markers to predict first stone occurrence in general population have not yet been assessed. The aim of this study was to describe the status of normal subjects as regard to the risk of nephrolithiasis.

Methods: We enrolled 144 healthy volunteers (96 males and 48 females) aged 37.9 years (16-67 years) who provided a first or second fasting morning urine sample for urine biochemistry and research of crystalluria. The presence of crystals was compared to urine biochemistry and theorical risk of stone formation assessed by TI.

Results: We enrolled 144 healthy volunteers (96 males and 48 females) aged 37.9 years (16-67 years) who provided a first or second fasting morning urine sample for urine biochemistry and research of crystalluria. The presence of crystals was compared to urine biochemistry and theorical risk of stone formation assessed by TI.

Table

	Q1 (TI<0.6)	Q4 (TI>2)	p
Presence of crystals (% yes)	3.4	48.6	<0.001
Osmolality (mean mOsm/kg±SD)	366±156	827±145	<0.001

Conclusions: In stone formers, we previously found that TI > 2.1 was related to a high occurrence of crystals (> 50% of urine samples) and a high occurrence of stone formation. Our data suggest that this relationship applies also in general population, with more than 50% of subjects with TI > 2 presenting crystals in urine and a higher osmolality. As TI or crystalluria are not often assessed in general population, it would be of interest to determine if urine osmolality can identify subjects at risk in further studies.

Based on crystalluria studies, we suggest that a significant part of the general population who exhibit high Tiselius index and osmolality values could be at risk to form urinary stones.

PUB552

Nephrolithiasis, Obesity, and Dietary Factors Laura Soldati,[1] Simona Bertoli,[2] Annalisa Terranegra,[1] Alessandra Mingione,[1] Elena Dogliotti,[1] Francesca Frau,[1] Alessandro Leone,[2] Laila Vignati,[2] Daniele Cusi,[1] Alberto Battezzati.[2] *[1]Department of Health Sciences, University of Milan, Italy; [2]International Center for the Assessment of Nutritional Status (ICANS), Dep. DEFENS, University of Milan, Italy.*

Background: This study investigated the nephrolithiasis (NL) frequency in 621 Caucasian subjects recruited from ICANS outpatient facility and divided in: normal weight (NW; BMI 20-25, n=125), overweight (OW; BMI 25-30, n=235) and obese (OB; BMI >30, n=261).

Methods: Nutritional assessment included: Mediterranean diet questionnaire, anthropometry and biochemistry. The presence of NL, osteoporosis, hypertension, diabetes mellitus and metabolic syndrome (MS) were noted.

Results: The frequency of NL was 9.91%. NSF were younger than SF (47.8±10.1 yrs. vs 51.9±11 yrs [p=0.001]. In particular, in the decade 60-70 yrs the frequency of NL doubled in comparison with the decade 50-60 yrs (18.2% vs 8.97%), in parallel with the increase up of subjects with a BMI > 25 (40% vs 20%).The percentage of subjects with osteoporosis was higher in SF than in NSF (16.9% vs 6.7%, p=0.007). SF had higher hepatic GGT (51.9±124.5 vs 27.9±30.4 U/I, p=0.0006), AST (24.3±12.3 vs 20.3±7.8 U/I, p=0.0008), ALT (29.7±20.4 vs 24.4±15.0 U/I, p=0.0186), triglycerides (147.8±150.6 vs 111.5±68.8 mg/dl, p=0.0016) and higher fasting serum glucose (100.1±20.7 vs 94.4±14.2 mg/dL, p=0.0078). Moreover, SF with a BMI>25 had higher transaminases, serum glucose level, waist circumference and umbilical visceral adipose tissue than SF with a BMI < 25. Not differences were found about the adherence to the Mediterranean diet between SF and NSF.

Conclusions: In conclusion, SF had an increased risk of osteoporosis, altered glucose metabolism and MS than NSF. OW and OB subjects with NL had higher risk of MS than NW subjects with NL. The cohort of OW and OB subjects presented an increase of prevalence in NL mainly in the decade 60-70 yrs linked to a BMI >25 in comparison with general Italian population (18% vs 5%). This increase does not seem linked to dietary habits, but probably to metabolic disorders developed in the long term.

PUB553

Descriptive Analysis of Clinical and Nutritional Status of Patients with Nephrolithiasis Carmen B. Tzanno-martins,[1] Bárbara Margareth Menardi Biavo,[2] Camila Machado de Barros,[2] Jacqueline Santos,[2] Elzo R. Junior.[1] *[1]Director, CINE-HDC-RENALCLASS Group, Sao Paulo, Brazil; [2]Nutritionist, CINE-HDC-RENALCLASS Group, Sao Paulo, Brazil.*

Background: Nephrolithiasis is a high incidence and high recurrence condition and, if untreated, it progresses with high rate of complications. The objective of this study is to describe the nutritional and feeding status in patients with diagnostic imaging of Nephrolithiasis.

Methods: 30 adult patients in regular follow-up outpatient clinic. Data evaluated were gender, age, anthropometry and dietary survey (Food Frequency Questionnaire - FFQ adapted to highlight food sources of oxalate, calcium, sodium and purines).

Results: Mean age was 46.6 ± 13 years, predominantly female (51.7%). About 41.4% of the patients underwent metabolic investigation, main disorders detected were hypercalciuria, hypocitraturia, hyperoxaluria and hyperuricosuria. Most patients showed overweight (25.9%) and obesity (41.8%). According to the FFQ, 35% of patients ingested foods with excessive oxalate content (> 99mg oxalate per serving),while 59.4% consumed foods with high oxalate content (26 to 99 mg). Regarding purines, 20% of patients consumed food with excessive (150-1000 mg of purines in 100 g of food) while 75.8% ingested foods with high amount (75-100 mg of purines per 100 g of food). Daily consumption of high sodium content foods proved to be elevated in 70%. 96.7% consumed food which were calcium sources, while fluid intake was inadequate (<2.5 L / day) in 93.3% of them.

Conclusions: Most patients were overweight or obese. In spite of calcium intake proved to be appropriate, there were factors that contribute to bladder stone formation such as alarming frequency of foods with high levels of oxalate, purine and sodium in the diet, associated with an inadequate fluid intake.

Funding: Private Foundation Support

PUB554

Systematic Review of Dietary and Pharmacological Treatment to Prevent Recurrent Kidney Stones Keith E. Eidman,[1] Manoj Monga,[2] Timothy J. Wilt,[4] Pranav S. Garimella,[3] Roderick Macdonald,[4] Indulis R. Rutks,[4] Michelle Brasure,[4] Robert Kane,[4] Jeannine Marie Ouellette,[4] Howard Fink.[4] *[1]Hennepin County Medical Center, Minneapolis, MN; [2]Cleveland Clinic; [3]John H. Stroger, Jr. Hospital of Cook County; [4]Minneapolis Evidence Based Practice Center.*

Background: Optimal treatment to prevent recurrent kidney stones in adults is uncertain. We systematically reviewed the evidence on benefits and harms of dietary and pharmacological treatment to prevent recurrent kidney stones and on whether baseline or follow up biochemistries predicted treatment outcomes.

Methods: We searched MEDLINE®, Cochrane and other databases through November 2011 and reference lists from systematic reviews and eligible randomized controlled trials (RCT's). Eligible studies must have been English-language RCT's of dietary or pharmacological treatment to prevent recurrent kidney stones in adults that reported stone outcomes and/or adverse events. Two reviewers assessed study design, participant characteristics, efficacy outcomes and adverse events and rated quality and strength of evidence.

Results: There were 28 eligible RCT's. We found low strenth of evidence (SOE) that compared to no treatment, both increased fluid intake (RR=0.45 95% CI=0.24-0.84) and reducing high soft drink consumption (RR=0.83 CI=0.71-0.98) cut risk of recurrent stones. Other dietary interventions had mixed results. In patients with past calcium stones, we found moderate SOE that thiazides (RR=0.59 CI=0.41-0.68), citrates (RR=0.25 CI=0.14-0.44) and allopurinol (RR=0.59 CI=0.42-0.84) reduced risk of recurrence versus control. We found low SOE that addition of citrate or allopurinol to thiazides added no further benefit. Allopurinol benefit appeared limited to individuals with hyperuricemia or hyperuricosuria. Other baseline biochemistries did not predict treatment outcomes. Adverse event reporting was poor. Withdrawals were low with fluid intake trials, high for other dietary interventions, and increased for thiazide and citrate therapy.

Conclusions: Increased fluid intake and reducing high soft drink consumption (low SOE); and thiazides, citrates and allopurinol (moderate SOE) reduce risk of recurrent kidney stones.

Funding: Other NIH Support - Primary Funding Source: Agency for Healthcare Research and Quality

PUB555

Urinary Citrate Protects Rats from Oxalate-Dependent Whewellite Nephrolithiasis Françoise Praddaude,[1] Marie Buleon,[1] Julien Allard,[1] Acil Jaafar,[1] Michel Daudon,[2] Ivan A. Tack.[1] *[1]INSERM UMR1048, CHU Toulouse, Toulouse, France; [2]Dpt of Clinical Physiology, Tenon Hospital, APHP, Paris, France.*

Background: Whewellite (Wh), the oxalate-dependent form of calcium oxalate (CaOx) lithiasis, is difficult to prevent otherwise than by diet. Relevance of the experimental models of CaOx lithiasis in rodents is controversial since they use either a tubulotoxic precursor of oxalate (Ethylene Glycol) and L-Hydroxyproline (HP) or oxalate alone do not induce lithiasis. We assumed that rodents do not develop urinary stone spontaneously because their high urinary oxalate excretion and concentration are balanced by a high excretion of citrate, a powerful inhibitor of crystallization. Therefore, a new model of lithiasis based on citraturia reduction was developed.

Methods: Adult, 20 male Sprague-Dawley rats were divided in two groups: group I received a lithogenic diet containing 3% of HP and 3% sodium oxalate and 0.21M NH4Cl in drinking water for 10 weeks whereas group II (controls) received standard diet and tap water. Urinary parameters (osmolality, citrate, oxalate, urinary acidification and dipstick) were analyzed weekly and creatinin clearance was performed every 3 weeks. After 10 weeks, arterial blood was collected, X-ray and photographs of uro-genital apparatus were performed. Stone composition was analysed using infrared spectrometry (IRS).

Results: All rats from group I developed millimetric calculi and four had migrating stones in the ureter (no lithiasis in group II). IRS analysis was typical of Wh.

Despite a lower body weight, lithiasic rats developed neither renal failure nor metabolic acidosis. Treatment resulted in a marked hypocitraturia whereas oxaluria was increased. Calciuria remained unchanged. Urine and blood osmolality were unaffected. Finally in group I, urine pH was decreased, titrable acidity and ammonuria were increased and haematuria was present since the third week ($p<0.05$ for all data).

Conclusions: In conclusion, hypocitraturia in rats that excrete a large amount of oxalate induces bilateral nephrolithiasis closely mimicking human Wh kidney stones. This model sustains the critical role of citrate for the protection of Wh kidney stones in rat, but also possibly in human.

PUB556

Genome-Wide Analysis of Differentially Expressed Genes in Human Kidney Epithelial Cells (HK-2 Cells) in Response to Elevated Oxalate Sweaty Koul, Lakshmipathi Khandrika, Hari K. Koul. *Urology, University of Colorado School of Medicine, Aurora, CO.*

Background: Nephrolithiasis is a multi-factorial disease, which in majority of cases involves renal deposition calcium oxalate. Oxalate a metabolic end product excreted primarily by the kidney and is the driving force for renal damage and nephrolithiasis in subsets of patients suffering from hyperoxaluria. In previous studies others and we have shown that oxalate is injurious to the renal epithelial cells, however oxalate renal epithelial cell interactions are not completely understood.

Methods: In this study, we utilized an unbiased approach of gene expression profiling using Affymetrix HG_U133_plus2 gene chip to understand global gene expression changes in human renal epithelial cells (HK-2) after exposure to oxalate. We analyzed the expression of 47000 transcripts and variant including 38,500 well characterized human genes in the HK2 cells after 4h and 24h of oxalate exposure. Gene expression was statistically compared among replicates as per the Affymetrix statistical program. Gene expression among various groups was compared using various analytical tools and differentially expressed genes wereclassified according to the Gene Ontology Functional Category.

Results: Results from this study show that oxalate exposure induces significant expression changes in many genes. We show for the first time that, oxalate exposure induced as well as shuts off genes differentially. During this time course, we found 750 up-regulated and 2276 down-regulated genes, which have not been reported before. Our results also show for the first time that oxalate exposure to the renal cells results in regulation of genes that are associated with specific molecular function, biological processes as well as cellular components. In addition we have identified for the first time a set of 20 transcripts that is differentially regulated by oxalate irrespective of duration of exposure and may be useful in monitoring oxalate nephrotoxicity.

Conclusions: Taken together our studies profile for the first time genome wide gene expression changes in response to oxalate and provides a unique insight in oxalate renal cell interactions and oxalate nephrotoxicity.

Funding: NIDDK Support

PUB557

Novel Report on Practice Patterns among Pediatric Nephrologists Managing Nephrolithiasis David J. Sas, Kimberly L. Hays. *Pediatrics, Medical University of South Carolina, Charleston, SC.*

Background: Pediatric kidney stones are increasing in incidence and are a significant burden for affected children and their families. Little is documented regarding practice patterns of pediatric nephrologists caring for these patients. Recent studies suggest examination of different practice patterns may be beneficial in developing more consistent and effective standards of care. In this study, we surveyed pediatric nephrologists in an effort to describe practice characteristics and identify areas for future research.

Methods: We conducted a survey using an online pediatric nephrology list serve (pedneph@lists.uchicago.edu) to collect data regarding practice characteristics of pediatric nephrologists caring for patients with nephrolithiasis.

Results: Responses were received from 52 institutions across the United States and Canada. Results revealed 57% of responders see >15 pediatric stone patients per year, 96% have access to a full-time pediatric urologist and 22% have a dedicated stone clinic, of which 70% are jointly managed by nephrology and urology. Of the responders, 45% have >20 years of experience. In regards to evaluation, 58% use Litholink exclusively for 24-hour urine profiles, while 17% utilize their home lab, 2% use Urorisk (or Stonerisk) and 23% use a combination of Litholink, their home lab, and/or another lab. In reference to imaging, 71% choose ultrasound for initial imaging while 29% use CT. For calcium phosphate stone formers who are hypocitraturic, responders differ regarding urine pH values at which it is appropriate to start citrate supplementation (for example, 50% supplement at pH 5.8, 42% at 6.5 and 25% at 7.0). Only 8% of respondents supplement citrate if urine citrate is normal.

Conclusions: Our results reveal significant differences in the way pediatric nephrologists care for their nephrolithiasis patients. It is clear from the results there is no consensus regarding optimal practice setting, evaluation, and treatment. The number of responses to this survey is encouraging and suggests a multicenter registry for the prospective collection of data on nephrolithiasis patients would provide critical information for improving future standards of care.

PUB558

Increasing Trends in Uric Acid Stone Formation in the Setting of the Growing Obesity Epidemic Cindy T. Pynadath, Xiaobo Liu, Surafel K. Gebreselassie. *Glickman Urological and Kidney Institute, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Kidney stone occurrences are increasing in the United States with a lifetime prevalence of 10% in men and 5% in women. Calcium stones predominate, followed by uric acid and struvite. The purpose of this study was to evaluate the effects of BMI and urine pH on recent patterns in uric acid stone formation.

Methods: Our retrospective analysis included 1905 adult subjects seen in the Cleveland Clinic between January 2006 and December 2010 who had kidney stone analysis and corresponding 24 hour urinalysis including volume, creatinine, citrate, calcium, sodium, oxalate, uric acid and pH.

Results: Ninety-one percent of our patients were Caucasian, 58.6% were male, and the mean age was 53.2 years. Similar to other reports, calcium stones predominated (70.7%) followed by uric acid (both mixed 17.1% & pure 6.4%), struvite (4.2%) and cystine stones (0.42%). A two sample T test to compare BMI between calcium and uric acid stone formers found a significantly higher mean BMI of 35.53 among the pure uric acid group when compared with a mean BMI of 28.88 among the calcium stone group ($p<0.001$), while mixed stones showed a mean BMI of 30, as depicted in Table 1. Using the Wilcoxon rank sum, urine pH was found to be lower among uric acid stone formers when compared to those with calcium stones, with the mean urine pH 5.5 and 6.0 respectively ($p<0.001$), as depicted in Table 1.

Table 1: Comparison of Mean BMI & Mean Urine pH Among Calcium, Mixed (Calcium + Uric Acid), & Pure Uric Acid Stone Formers

Stone Type	Calcium	Mixed (Calcium + Uric Acid)	Uric Acid
Mean BMI	28.88	30.8	35.53
Mean Urine pH	6	5.5	5.5
p-value	<0.001	<0.001	<0.001

Conclusions: In conclusion, our study shows that higher BMI and lower urine pH is prevalent among uric acid stone formers when compared with calcium stone formers, which is consistent with previous studies. However, it is notable that our study found an increase in uric acid (both pure and mixed) stones when compared with previous reports. This increasing trend may be explained by the worsening obesity epidemic. Further studies can evaluate whether lowering BMI will decrease the occurrences of uric acid stone formation.

PUB559

Electrolyte Club: A Proposal for a Novel Curriculum to Enhance Understanding of Fluid, Electrolyte, and Acid-Base Disorders Helbert Rondon-Berrios. *Nephrology, University of New Mexico Health Sciences Center, Albuquerque, NM.*

Background: In an internet-based survey between 2004 and 2008 among US nephrology fellows, 1.5% of them indicated that their fellowship program offered little or no training in electrolyte disorders and 13.5% indicated that they received some training but not enough to feel competent. This has reflected in the ASN-In Training exam scores. In 2009, the overall mean percent correct scores in the areas of sodium/water and acid-base/potassium were 76% and 59% respectively. Delivery of content in this area is usually in the way of a lecture that do not favor either learning acquisition or long-term retention. Multiple RCTs have shown that spaced education is an effective way to increase the amount and durability of learning. Will the implementation of a novel curriculum in fluid, electrolytes and acid-base disorders that utilizes spaced education for a group of nephrology fellows lead to improvement in MCQ examination scores compared to usual training opportunities?

Methods: The electrolyte club features a curriculum across 5 modules: solution stoichiometry, sodium, water, potassium, and acid-base. A 40-item validated test will be administered before the course. Nephrology fellows will be block randomized to 2 cohorts: Cohort 1 receives instruction in solution stoichiometry, sodium and water. Cohort 2 receives instruction in potassium and acid-base. The method of instruction will consist of prior reading, case discussions and adaptive spaced education. Each module consists of two 1-hour sessions per week for 8 weeks. Fellows will be given review articles and 2 clinical cases prior to every session. Sessions will consists of case discussions facilitated by a tutor. After completion of each module, adaptive spaced education items will be distributed via email. Based on a validated curriculum, an spaced education course was structured for each module so fellows will be sent 2 items via email every other day. At the completion of all modules, fellows will be asked to take the same validated test and fill a survey to assess satisfaction. Fellows will be asked to take the validated test again after 3 months to assess durability of learning.

PUB560

Changing Needs for Information and Support in an Online System for Parents of Children with Kidney Disease Maury N. Pinsk,[1] David B. Nicholas.[2] *[1]Department of Pediatrics, Division of Nephrology, University of Alberta, Edmonton, AB, Canada; [2]Department of Social Work, University of Calgary, Calgary, AB, Canada.*

Background: Paediatric chronic kidney disease is psychologically, financially and physically demanding on parents providing care. Many parents feel isolated because of the rarity of the condition; geographic isolation confounds this perception in Canada. Many parents seek assistance online for both information and social support. This study examines an online portal "Ability Online" that provides these functions to a diverse group of parents.

Methods: Transcripts were reviewed from participants in the online system to generate semi-structured interviews focused on technology and social support. Telephone interviews were then conducted with an experienced user group. Participant experiences were evaluated using Descriptive Interpretation as a qualitative methodology, enriched with assessments of demography, social support quality, stress and satisfaction as a caregiver.

Results: Many parents experience a progression through which their needs for knowledge and support change over time. Technology parallels to provide those resources are discussed.

Conclusions: Web developers should account for the changing needs of participants in designing such online support networks, and minimize the reasons that participants fail to adopt, or terminate their online experiences.

Funding: Government Support - Non-U.S.

PUB561

Hemodialysis Nurses' Knowledge of Dietary Control of Potassium and Phosphorus Is Insufficient and Can Be Improved by Education Margo Laute, Renee Bultynck, Marijn M. Speeckaert, Annemieke Dhondt, Raymond C. Vanholder. *Nephrology, Ghent University Hospital, Ghent, Belgium.*

Background: Dietary non-adherence is a common and serious problem among patients on hemodialysis. Patient coaching and support by trained nurses improves their adherence and quality of life. This study evaluates nurses' knowledge on dietary control measures influencing serum levels of potassium (K) and phosphorus (P) before and after a 2h theoretical educational program given by a dietician.

Methods: This prospective self-controlled study was organized in a dialysis unit with a full-time dietician linked to the unit (A) and in a unit without a dietician (B). Initial knowledge was assessed using a questionnaire consisting of 17 dietary control questions (DCQ) and 10 medical questions (MQ), assessing K/P-content of food, use of K/P-binders and complications associated with hyperkalemia and hypophosphatemia. Subsequently, a nephrology dietician conducted a 2h educational program, herewith instructing 25 dialysis nurses (11 of unit A, 14 of unit B). To evaluate the change of knowledge associated with the program, the same questionnaire was applied 3 weeks after the program. A correct answer was attributed 1 point, an incorrect answer 0. The mean percentage of correct answers was calculated (mean score) and the ANOVA repeated measures test was used for comparing change of knowledge, with a significance level of <0.05.

Results: At baseline the mean DCQ score was significantly different between both units ($p = 0.017$). Overall, the DCQ score was only 52% in unit A and even lower in unit B (42%). No significant difference was observed in mean MQ score (71% vs. 61%, $p = 0.8$). Three weeks after the program, the mean score for both units combined had significantly improved for both DCQ and MQ (DCQ: 44% vs. 56%, $p = 0.009$ and MQ: 69% vs. 79%, $p < 0.001$). There was no longer a significant difference observed after the program between both units concerning DCQ and MQ scores.

Conclusions: A short educational program significantly improves nurses' knowledge on dietary control measures and its medical importance. The presence of a full-time dietician in a dialysis unit positively impacts nurses' basic dietary knowledge.

PUB562

Information Needs of Patients with Early Stage Chronic Kidney Disease: Focus Group Study Pamela Andrea Lopez-Vargas,[1,2] Allison Tong,[1,2] Richard K.S. Phoon,[3] Steven J. Chadban,[4] Yvonne Shen,[5] Jonathan C. Craig.[1,2] *[1]Centre for Kidney Research, the Children's Hospital at Westmead, NSW, Australia; [2]Sydney School of Public Health, University of Sydney, NSW, Australia; [3]Department of Renal Medicine, Centre for Transplant and Renal Research, Westmead Hospital, NSW, Australia; [4]Royal Prince Alfred Hospital, University of Sydney, NSW, Australia; [5]Royal North Shore Hospital, University of Sydney, NSW, Australia.*

Background: The key risks of early-stage chronic kidney disease (CKD) are premature cardiovascular mortality and progression to end-stage kidney disease. Education for prevention of CKD progression in patients with early stage kidney disease may be fragmented and inadequate. The aim of this study was to identify the information and education needs of patients with CKD.

Methods: Patients with CKD Stages 1-3 were purposively sampled from three major hospitals in Sydney, Australia to participate in focus groups. Transcripts were thematically analysed.

Results: From nine focus groups including 38 participants, seven major themes were identified: disease management (medication safety, treatment options, pathology results); lifestyle changes (dietary advice, alcohol consumption, physical activity, smoking cessation); understanding causes and risk factors of CKD (diabetes, high blood pressure,

medication); hopelessness (no cure, genetic predisposition, unknown future); motivation (avoid dialysis, find a cure, prevent progression); lack of medical support (education, follow up); and access to multi-modal education (pamphlets, booklets, DVDs, internet, health care professionals).

Conclusions: Patients believe that their capacity to slow the progression of CKD is limited by the lack of knowledge of risk factors, treatment, lifestyle modifications and poor access to education. Development of multi-modal educational resources including practical lifestyle recommendations, such as adequate diet according to CKD stage and types of appropriate physical activities, combined with active physician engagement in prevention, are likely to promote patients' ability and motivation to make lifestyle modifications for prevention of CKD progression.

Funding: Government Support - Non-U.S.

PUB563

Conservative Management of Sever Hyperkalemia due to Oral Potassium Overdose

Meteb M. AlBugami, David J. Hirsch. *Internal Medicine, Division of Nephrology, Dalhousie University, Halifax, NS, Canada.*

Background: Elevated serum potassium (K) is a common problem. However, hyperkalemia due to oral potassium overdose is rarely encountered. The management of severe hyperkalemia with normal renal function is controversial, with no evidence based guidelines. We report a case of intentional oral potassium overdose resulting in severe hyperkalemia with normal kidney function and minor electrocardiogram (ECG) changes, that was managed without hemodialysis (HD).

Methods: A 48-year-old female presented to our Emergency Department after ingesting 30 tablets of 20 mg sustained release potassium chloride. Her main symptoms were peripheral paraesthesia and mild distal weakness. She was hemodynamically stable, with peaked T waves seen on her initial ECG. The maximum K level was 10.1 mEq/L. She was treated initially with calcium gluconate, insulin, salbutamol, and calcium resonium. later on, she was started on insulin drip, and furosemide drip along with intravenous fluids, and small dose PEG to enhance K excretion via the renal and GI routes were added. She was kept in ICU for close monitoring with no significant arrhythmia. K level went to normal level in 10 hours, during which she was hemodynamically stable. her urine output was more than 3 L during the first 24 hours, and she was transferred to psychiatry floor.

Conclusions: This patient with severe hyperkalemia on suicide attempt was managed successfully without HD. The main reasons to go conservatively were absence of significant ECG changes and having normal renal function, otherwise HD was to be utilized for prompt removal of K. We highlighted the importance of volume expansion and forced diuresis as rapid K excretion is expected with normal kidney function. Using PEG as whole bowel irrigation was reported before in such case but needs to be given early. In the literature, cases of severe hyperkalemia (K >8), some of them had significant arrhythmia, were managed without HD and few cases had bad outcomes including death.

Severe hyperkalemia with normal renal function could be managed successfully with forced diuresis as long as there is no hemodynamic instability due to arrhythmia.

PUB564

Acute Relapse of MELAS Syndrome in Dialysis Patient Induced by Infection

Pietro Claudio Dattolo, Stefano Michelassi, Giulia Sansavini, Alma Mehmetaj, Marco Amidone, Giuseppe Ferro, Francesco Pizzarelli. *Nephrology, S M Annunziata Hospital, Florence, Italy.*

Background: MELAS syndrome is a maternally inherited multisystemic disorder caused by mutations of mitochondrial DNA. Frequently it is associated with hypertrophic cardiomyopathy and diabetes, while association with nephropathies is less common. We describe the first case of MELAS relapse following an acute infectious disease in a hemodialysis patient.

Methods: A 42-years-old Caucasian man was admitted to our hospital because of progressive weakness and lethargy. The patient was on hemodialisys since 2008. At the age of 30 he was diagnosticated with MELAS (besides characteristic clinical features, muscular biopsy presented ragged red fibers and DNA analysis showed C3243A>G mutation); he presented congenital bilateral hypoacusia, recurring generalized seizures and encephalopathy,progressive bradyphrenia and bradykinesia, restrictive cardiomyopathy, diabetes and CKD due to FSGS. At the time of hospitalization, he was febrile and drowsy and he was suffering from malaise, anorexia, vomiting, dyspnea, and lower limbs paresthesias. Laboratory tests showed severe lactic acidosys, systemic cytolysis and increased inflammation markers.

table1

Laboratory Tests	Admission	Nadir	Discharge
Lactic Acyd (mg/dL)	9	22	2
AST (U/L)	735	1628	29
ALT (U/L)	1331	2384	40
Ammonemia (µg/dL)	63	108	
PT ratio (%)	46	31	94
CK (U/L)	507	507	64
LDH (U/L)	1236	1420	285
Troponin-I (ng/mL)	2.84	3.53	0.24
CRP (mg/L)	12.94	16	0.17
Procalcitonin (pg/mL)	6.78	14.93	2.5
White blood count /µL)	12000	18500	7300

Abdominal US showed hepatomegaly and gallbladder wall edema, while EEG, cranial TC, and echocardiography had no significant variations. He was treated with intensive dialysis (CVHDF), broad-spectrum antibiotics and supporting medical therapy, which produced an improvement of his conditions. When discharged, the patient's clinical picture was completely solved.

Conclusions: We are not aware of any case of relapsing MELAS syndrome in HD patients. Our patient had been stable during the previous 4 years of dialysis. Following an acute infectious episode, he ostensibly suffered from a relapse of MELAS, with appearance of several of the disease's archetypal symptoms.

PUB565

Splenic Laceration in a Peritoneal Dialysis Patient with Recurrent Peritonitis

Sandesh Parajuli, Scott E. Liebman, Sai Subhodhini Reddy. *Department of Medicine, Div of Nephrology, University of Rochester Medical Center, Rochester, NY.*

Background: Introduction: Hemoperitoneum is not an uncommon occurrence in peritoneal dialysis (PD) patients. Rarely hemoperitoneum may signify serious intra-abdominal pathology. Here we report a case of hemoperitoneum related to a splenic laceration.

Methods: Case: A 28 year old woman with diabetes mellitus, hypertension, and ESRD on PD for 23 months presented to an outside hospital with pneumonia and *Enterobacter cloacae* peritonitis (her third episode of peritonitis). At that time, her PD fluid had >90,000 WBC and <3,000 RBC / milliliter (ml) and frank blood clots. She was treated with oral moxifoxacin and intra-peritoneal (IP) cefepime. Two days after discharge she presented to our hospital with nausea, vomiting and severe abdominal pain. On exam, she was hypertensive to 231/149. Her exit site was benign. She had left lower quadrant abdominal tenderness and rebound. Laboratory evaluation showed hemoglobin of 11.8. The PD fluid was clear, with 59 nucleated cells and 20 RBC / ml. She was treated with antihypertensive, antiemetic and analgesics. CCPD with IP cefepime was continued. Despite antibiotics and a negative cell count, her severe abdominal pain persisted and her hemoglobin dropped to 7.1. A CT scan showed a splenic laceration, mesenteric edema and colitis. She denied any trauma, and was managed non-operatively. PD was discontinued, the hemoglobin stabilized, her abdominal pain gradually improved and she was discharged after one week on hemodialysis.

Conclusions: A potential explanation of the splenic laceration is that adhesions from multiple episodes of peritonitis resulted in decreased splenic mobility with traction related injury due to fluctuating abdominal pressures. A similar mechanism has been postulated for splenic injury in inflammatory bowel disease patients undergoing colonoscopy. In this case, inflammatory adhesions render the spleen less mobile with traction injury induced by the increased intra-abdominal pressure during the procedure. Splenic injury in PD patients is uncommon, but should be considered in those with recurrent peritonitis, hemoperitoneum and unremitting abdominal pain.

PUB566

Antineutrophil Cytoplasmic Antibody Associated Vasculitis Following Interferon Treatment for Hepatitis C

Reginald Ifeanyi Obi, Karlene O. Hewan-Lowe, Courtland Winborne. *Internal Medicine/Nephrology, East Carolina Brody School of Medicine, Greenville, NC.*

Background: ANCA associated vasculitis is a multisystem autoimmune syndrome characterized by vasculitis predominantly affecting microscopic vessels and circulating autoantibodies to neutrophil cytoplasmic antigens. Renal involvement occurs in 70% of affected patients and is manifested as rapidly progressive glomerulonephritis (GN) with pauci-immune necrotizing, crescentic GN on biopsy. This case study illustrates the rare occurrence of ANCA vasculitis following interferon therapy for treatment of Hepatitis C.

Methods: 49 yo African American male with history of stage 2 Chronic Kidney Disease due to hypertension and Hepatitis C associated with positive cryoglobulins, completed a 24-week course for Hepatitis C with triple therapy of Pegylated Interferon, Ribavirin and Telaprevir. He responded with an undetectable viral load at the end of treatment. In the final week of therapy, he developed generalized malaise, muscle aches, joint pain, stiffness, swelling and episodic mild hemoptysis. He was seen 2 weeks later and found to have an elevated creatinine at 5.26 mg/dL up from his baseline of 1 mg/dL. He also had significant anemia with hemoglobin of 6.4 g/dL. His chest X-ray was normal. The workup revealed normal complement levels, a positive P–ANCA at 1:160 titer, and renal biopsy confirmed pauci-immune GN. Patient was treated with pulse steroids and prednisone taper. He responded well with a decline in creatinine to 2 mg/dL and complete resolution of symptoms. His Hepatitis C viral load remains undetectable.

Conclusions: Pauci-immune GN arising from interferon therapy is a rare complication. This should be suspected if a rapidly progressive renal failure occurs while receiving this medication. Interferon should be included in the list of drugs inducing ANCA vasculitides. Treatment of ANCA vasculitis traditionally involves cyclophosphamide in conjunction with steroids. We opted to treat conservatively with steroids due to concerns of Hepatitis C reactivation. The patient's creatinine stabilized at 2 mg/dL and his Hepatitis C viral load remains undetectable post steroid treatment.

PUB567

Pet Ownership and Automated Peritoneal Dialysis, Can They Coexist?

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Background: Automated peritoneal dialysis (APD) is more popular option than continuous ambulatory peritoneal dialysis (CAPD) since it offers more flexibility for patients during the daytime. However, in either option, aseptic technique including proper

handling of the dialysis tubing and connector in a safe and clean environment are the key to prevent infectious complication such as peritonitis.

Methods: A 65-year-old Caucasian male with end stage renal disease (ESRD) who started hemodialysis at age 62 and later switched to APD 7 months prior to his presentation with abdominal cramping and discomfort associated with cloudy peritoneal fluid. He was hemodynamically stable and afebrile. His abdominal examination revealed a soft, non-distended abdomen with diffuse tenderness but no guarding. His peritoneal fluid cell count showed: WBC 11480 /uL with 93% Neutrophils. Peritoneal fluid culture revealed Pasteurella Multocida. The patient admitted there are two cats at home. He also admitted that they have chewed on the tubing system. He was started on intra-peritoneal Ceftazidime (1.5 grams daily) and oral Ciprofloxacin (500 mg daily) for a total duration of 3 weeks. His symptoms improved significantly and his peritoneal fluid cell count and differential normalized. The patient was discharged after 5 days of hospital stay.

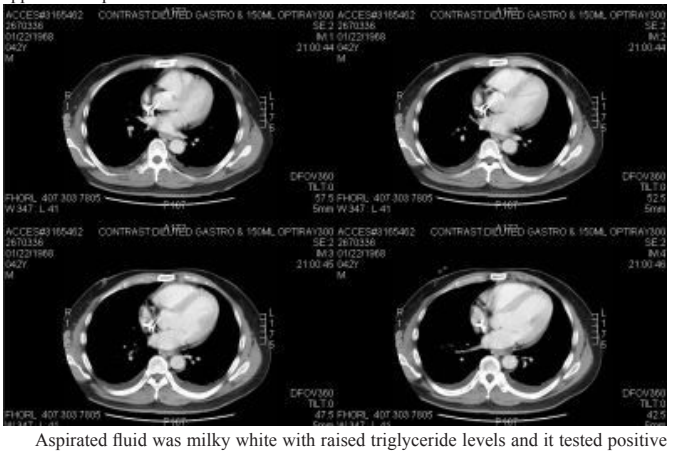
Conclusions: Pasteurella Multocida is a rare form of PD-related peritonitis and is closely associated with pets namely cats. Patients on APD are more affected than those on CAPD since the patient performing the CAPD fluid exchange is in control of his or her surrounding as opposite to the patient on APD where the exposure to hazards such as a pet chewing on dialysis tubing may not be controlled. Our patient's wife refused to remove the cats from her household regardless the consequences. The patient himself refused to go on CAPD or in-center hemodialysis as an alternative. His wife then arranged for building gates in their house. Hence, the issue of safety and prevention should be discussed thoroughly with patients before their discharge with follow up home visit to assure the application of these measures.

PUB568

An Unusual Presentation of Abdominal Pain Pran M. Kar,[1] Shaan E. Alam.[2] *[1]Nephrology, Florida Hospital, Orlando, FL; [2]University College of Medical Sciences, New Delhi, India.*

Background: Chylous ascites consists of chyle accumulation in the peritoneal cavity. It rarely develops following unrecognized iatrogenic injury to lymphatic vessel. We present a patient with chylous ascites following bilateral nephrectomy for polycystic kidney disease (PKD).

Methods: A 42-year-old Filipino man with PKD presented with left flank pain, vomiting and hematuria. On examination he had costo-vertebral angle tenderness and trace bilateral pedal edema. CT scan of abdomen revealed multiple cysts in both kidneys consistent of PKD. He underwent bilateral open nephrectomy. The post operative period was uneventful except mild abdominal discomfort and he was discharged on antibiotics and a repeat CT scan was advised in a week. After 10 days, he presented with vomiting, abdominal pain and poor appetite. A repeat CT scan of abdomen revealed fluid collection in bilateral renal fossae.



Aspirated fluid was milky white with raised triglyceride levels and it tested positive for Sudan III. A diagnosis of chylous ascitis was made with likelihood of iatrogenic lymphatic duct injury. He was managed conservatively with dietary modifications to which he responded poorly. Oral diet was then with held and the patient was managed with total parenteral nutrition, anti secretory drugs and supportive treatment with repeated aspiration of ascites fluid. To this he improved significantly with complete resolution of ascites.

Conclusions: Although rare, chylous ascites is a possibility in patients with nephrectomy. This must be ruled out when such patients present with abdominal pain and intra abdominal fluid collection in the post operative period.

PUB569

Hypercalcemia and Acute Kidney Injury due to Panhypopituitarism and Adrenal Insufficiency Julia Lichtnekert, Hans J. Anders, Wolfgang Neuhofer. *Division of Nephrology, Medizinische Klinik und Poliklinik IV, Munich, Germany.*

Background: Uncontrolled hypercalcemia is a frequent cause of AKI. In the present report we describe a rare case of hypercalcemia-induced AKI associated with hypopituitarism following brain injury with severe lesions in the hypothalamic-pituitary area.

Methods: We report a 24-year old man with a known history of traumatic brain injury 7 months ago. Due to AKI the patient was referred. He reported a 4-month history of increasing fatigue, depression, absence of libido, nausea and emesis, increased thirst (5l/d), polyuria and loss of weight. The medical history before the accident was unremarkable. The medication consisted of thyroxine. In the physical examination an anorectic body habitus, left incomplete hemiparesis and amaurosis of the right eye was noted. Blood and urinary parameters showed elevated levels of creatinine, severe hypercalcemia and hypercalciuria, low urine osmolality with normal serum osmolality, elevated alpha1-microglobulin excretion, and normochrome anemia. TSH was suppressed. Renal sonography was unremarkable. There was no evidence of malignancy, hyperparathyroidism, vitamin D intoxication and sarcoidosis. However, in endocrinological tests, panhypopituitarism was detected: Secondary adrenal insufficiency, hypogonadotropic hypogonadism, secondary hypothyroidism, growth hormone deficiency, and central diabetes insipidus. The MRI revealed traumatic ischemic lesions in the hypothalamic-pituitary region. Initial treatment consisted of bisphosphonates and forced diuresis. After correction of serum calcium, renal function improved, suggesting AKI was associated with hypercalcemia. Further treatment included substitution of hydrocortisone, testosterone, thyroxine and desmopressin. Under this medication, the patients' general condition improved within a week: Polydipsia and polyuria ceased, fatigue disappeared and the mental status stabilized. At discharge from the hospital, serum calcium had returned to normal levels and renal function was completely restored.

Conclusions: Therefore, in the absence of usual conditions associated with hypercalcemia, endocrinological abnormalities should be considered in cases of otherwise unexplained AKI.

PUB570

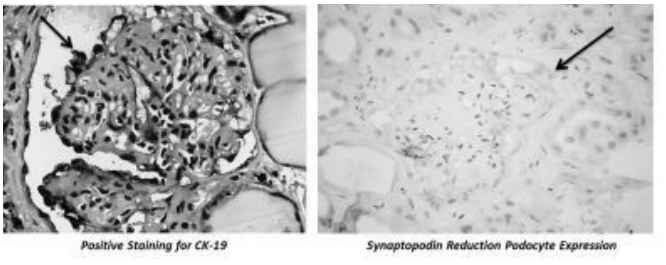
Collapsing Glomerulopathy Associated with Pamidronate: Case Report and Immunohistochemical Analysis Maria Carolina N.R. Neves,[1] Andrea C.E.P. Valenca,[1] Luis H.B.C. Sette,[1] Maria Alina G.M. Cavalcante,[1] Denise M.A.C. Malheiros,[2] Lucila Maria Valente.[1] *[1]Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil; [2]Patologia, Universidade de Sao Paulo, Sao Paulo, Brazil.*

Background: Pamidronate has been described as a cause of Collapsing Glomerulopathy (CG). This is a distinct clinicopathologic entity seen in patients with HIV infection and has its main feature as renal insufficiency and nephrotic syndrome (NS).

Methods: A 60 years old woman with multiple myeloma (MM), diagnosed in 2004, was submitted to autologous stem cell transplantation. Pamidronate therapy was initiated in 2007, at a dose of 90mg IV monthly. In 2011, she presented with impairment of renal function and NS (Proteinuria 3.8g/d, albumin 1.7g/dl and edema). MM was in remission (urinary immunoelectrophoresis and bone marrow examination were without abnormalities). Pamidronate therapy was stopped and a renal biopsy was performed, which was consistent with CG with moderate interstitial fibrosis and tubular atrophy with microcystic degeneration. The immunohistochemical (IHC) analysis showed positive staining for CD-10, WT-1 and CK-19 (arrow) and negative staining for Ki67 and synaptopodin (arrow).

Immunohistochemical Markers

Markers of Podocyte Differentiation	Markers of Podocyte Dedifferentiation
WT-1 (+)	CK19 (+)
CD10 (+)	Ki67 (-)
Synaptopodin (-)	



Two months later, she started hemodialysis.

Conclusions: We described a case of CG associated with the use of pamidronate and its IHC profile.

PUB571

Purpuric Rash: A Benign Pediatric Disease Can Be Extremely Rare and Critical in an Adult Subhasish Bose, Joseph Benjamin. *Department of Nephrology, Temple University Hospital, Philadelphia, PA.*

Background: We present a 30 year old previously healthy Caucasian female with a purpuric rash. The final diagnosis represents a primarily childhood vasculitis which is extremely rare in adults.

Methods: Our patient presented with limb swelling, polyarthralgia and crampy diffuse abdominal pain with loose stool for 4 days. One week prior to this, patient had URI symptoms. On admission, patient was noted be afebrile, distressed with abdominal pain and had purpuric rash on her lower extremities. Lab data included a normal chemistry panel (serum creatinine 0.69 mg/dl) and normal CBC except total WCC of 19.4 X 10*3/uL with no bandemia. Pregnancy test was negative. CT scan of abdomen and pelvis revealed small bowel wall thickening with mesenteric lymphadenopathy. Colonoscopy showed scarring of the ileocecal valve and ulcerations in transverse colon. ANA, Anti-ds DNA, ANCA, Anti-Smith, Anti-RNP, RF, C3&C4, Hepatitis serologies, respiratory viral panel, Monospot, blood & stool culture, hemolysis screen were all normal. Echocardiogram showed normal EF, no endocarditis. Over next 5 days, patient's renal function rapidly deteriorated with significant proteinuria and microscopic hematuria. Her purpuric rash spread to all extremeties. Biopsy

samples from skin and kidney were obtained and patient was started on high dose steroid. At this point, patient developed dyspnea and seizure needing intubation. Subsequently, patient was started on hemodialysis(HD) and plasmapharesis. Patient's ASO titre came back as high. Colonic and skin biopsies were inconclusive. Kidney biopsy was consistent with IgA mediated glomerular disease strongly suggestive of Henoch-Schonlein Purpura(HSP). Patient's clinical condition progressively improved, she was extubated successfully but needed routine HD with hope of regaining significant kidney function.

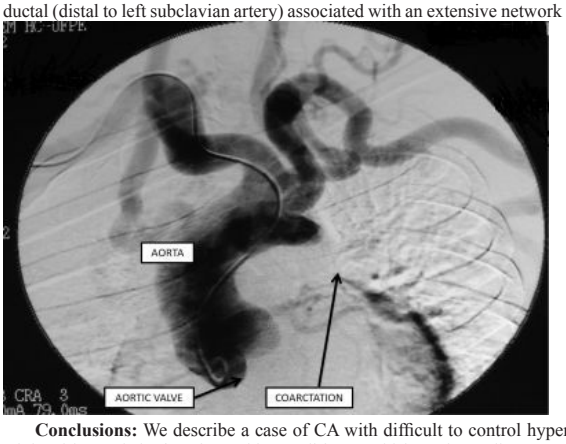
Conclusions: HSP is a systemic disease where antigen-antibody (IgA) complexes activate the alternate complement pathway, causing small vessel vasculitis. It is often triggered by streptococcal URI. Early recognition and appropriate timely intervention is extremely crucial to manage the disease and limit organ damage.

PUB572

Coarctation of the Aorta in a 46 Years-Old Man as a Cause of Severe Hypertension and Renal Dysfunction: The Importance of Physical Examination in Nephrology Marclebio M.C. Dourado, Camila B.l. Oliveira, Edmir R.B. Dias, Maria Carolina N.R. Neves, Andrea C.E.P. Valenca, Luis H.B.C. Sette, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil.*

Background: Coarctation of the aorta (CA) is a rare cause of secondary hypertension in adults and leads to a poor cardiovascular prognosis.

Methods: A 46-years old patient presented to consultation with difficult to control hypertension and renal dysfunction (CrCl 48mL/min/1.73m^2). He has been using his medications irregularly for the last 25 years, until 4 years ago, when he had an episode of pulmonary edema requiring hospitalization. At that time, brachial access coronary angiography showed mild coronary atherosclerosis. Echocardiography revealed moderate concentric left ventricular hypertrophy, slight enlargement of the aortic root and ascending aorta, without valvular abnormalities. Physical examination revealed asymmetric pulse in upper and lower limbs and asymmetric blood pressure (BP) in all four limbs. BP (mmHg) in the left and right upper limbs were 240x120 and 230x110; left and right lower limbs were 120x80, 130x80. There was no bruit over subclavian arteries or abdominal aorta. Patient reported no history of claudication. CT angiography showed severe stenosis of the descending aorta after the left subclavian artery branch and hypertrophy of the intercostal, mammary and epigastric arteries. Angiography showed arterial occlusion after aortic ductal (distal to left subclavian artery) associated with an extensive network of collaterals.



Conclusions: We describe a case of CA with difficult to control hypertension in an adult with renal dysfunction. This condition could have been discovered earlier with physical examination and prevent kidney and heart failure. The patient is now awaiting surgical procedure.

PUB573

Extreme Pseudohyperkalemia in a Patient with Acute Myelogenous Leukemia Nadear A. Elmahi, Kenneth E. Kokko. *Department of Medicine, Division of Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: Pseudohyperkalemia occurs in some patients with extreme leukocytosis and the diagnosis is often difficult to establish. We describe a challenging case of possible pseudohyperkalemia associated with extreme leukocytosis.

Methods: 55 year old African American male was seen in the oncology clinic on 07/28/11 for follow up of acute myelogenous leukemia for which he refused chemotherapy. During that visit his potassium (K) was normal and WBC was moderately elevated. Subsequent visits revealed progressive hyperkalemia and worsening leukocytosis. On August 18, he refused hospital admission for K of 8.8 mmol/l with normal ECG. On August 22 he came to the hospital for evaluation of shortness of breath. Initial work up was significant for K of 15.3 mmol/l, WBC of 403 th/cmm and Creatinine of 1.6 mg/dl. Repeat K the next day was 15.5 mmol/l.

Lab Results

Date	WBC (th/cmm)	Potassium (mmol/l)
7/28/11	23	Normal
08/04/11	139	5.6
08/18/11	240	8.8
08/22/11	403	15.3

Potassium and WBC count

Initial and repeat ECGs showed atrial flutter with no findings suggestive of hyperkalemia. Careful handling of the sample, measurement of plasma potassium and measurement of potassium in arterial blood gas (ABG) were not helpful in establishing the diagnosis of possible pseudohyperkalemia. The patient and family refused aggressive interventions (dialysis and leukophresis) and chose comfort care. Diagnosis of pseudohyperkalemia was assumed and no further treatment was offered. The patient expired 48 hours later after major stroke due to leukocytosis.

Conclusions: The lessons learned from the case include: 1) Pseudohyperkalemia should be considered as cause of elevated potassium in patients with substantial leucocytosis who do not have clinical or ECG changes suggestive of hyperkalemia, 2) Comparison of serum and plasma potassium and measurement of potassium in ABG may help in establishing the diagnosis of pseudohyperkalemia. However, the diagnosis should not fully ruled out based on negative results 3) The diagnosis should be considered in the right clinical setting even in the absence of confirmatory tests since aggressive treatment can lead to life-threatening hypokalemia.

PUB574

Acute Renal Failure Complicating Preeclampsia/HELLP Khin S. Yee. *Renal Department, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Acute renal failure complicating preeclampsia is rare but serious. It is often a diagnostic challenge as it carries overlapped manifestations with TTP where plasma exchange is the mainstay of treatment. This case demonstrates a rare presentation of acute renal failure secondary to preeclampisa/HELLP.

Methods: Medical record review of a a previously healthy woman for clinical course, diagnostic studies, treatment strategies and overall outcome.

Results: 34 year old female G1P0 with gestational hypertension presented to ER @ 40 weeks. On admission, patient's BPs were 130-150/70-80, asymptomatic except labor pain with normal lab values. She was treated with cytotec for induction of labor followed by epidural four hour later. At that time her BPs were significantly increased with sign of fetal distress. Patient started complaining of epigastric discomfort. Repeat labs showed elevated liver enzymes, coagulopathy, thrombocytopenia and hemolytic anemia consistent with HELLP/preeclampsia. Primary cesarean section was performed. Patient received 2 units FFP, 1 unit platelet and magnesium. Creatinine started to rise post op day 1 and peaked to 6.14 over 6 days. Due to relentlessly rising creatinine while LFT and coagulation profile were improving, question of whether renal biopsy should be pursued arose. Peripheral smear showed occasional schistocytes seen average 1 per 3-5 hpf. Given patient's asymptomatic nature, decision was made to defer renal biopsy. Renal function improved after 2 sessions of dialysis and back to baseline in a week.

Conclusions: Preeclampsia/HELLP complicated with acute renal failure is a rare entity with only few cases reported in literature. Vasoconstriction that characterizes preeclampsia,leads to mild functional renal impairment and improves completely after delivery of the fetus. Early prophylactic dialysis of acute renal failure is associated with rapid renal recovery. However, no data are available on long-term renal outcome due to paucity of such cases. Bilateral renal cortical necrosis seen most commonly in women with underlying chronic hypertension and superimposed preeclampsia is associated with more severe adverse maternal and fetal outcomes.

PUB575

Pseudoacidosis due to the Presence of a Plasma Inhibitor Naveen S. Sandhu,[1] Michael Shoemaker-Moyle,[1] Son G. Lam,[2] Vikram R. Beemidi,[1] Luis A. Juncos.[1] *[1]Division of Nephrology, University of Mississippi Medical Center, Jackson, MS; [2]Oxford Nephrology Associates, LLC, Oxford, MS.*

Background: A patient presented to an outside hospital with a low serum bicarbonate (HCO3-) and high anion gap (AG). She had no signs or symptoms of acidosis despite a HCO3- level of 1.6 mmol/L with an AG of 33. She received a HCO3- infusion, but her serum HCO3- remained low and her AG persisted. A workup for a high AG acidosis was negative. She was then referred to our hospital where our lab confirmed the low measured HCO3- level using Cobas bicarbonate liquid test. Her blood gas showed a pH of 7.39 and pCO2 of 41. With the normal blood gas and the negative work up for acidosis the validity of the lab measurement for serum HCO3- came into question.

Methods: To assess for a substance interfering with the lab test, we performed serial dilutions of the sample. The HCO3- level measured by the Cobas assay increased with dilution but remained below the predicted HCO3- level which is consistent with the presence of an interfering substance. An alternative method to measure serum HCO3- is the Vitros ECO2 slide method. In this test a drop of serum is placed on a multilayered slide where it diffuses from the initial spreading layer to the gel layer where the same enzymatic reactions occur as in Cobas assay. Theoretically a large enough interfering substance would not be able to diffuse through the spreading layer and interfere with the enzymatic reactions. When the patient's serum was analyzed using this method her HCO3- was 27. This implies that an interfering compound was too large to diffuse through the spreading layer and her serum HCO3- could undergo the requisite enzymatic reactions to yield an accurate result.

Conclusions: The spuriously low HCO3- value in this case results in a high AG without a detectable endogenous or exogenous acid. A similar case was reported in NDT (Navaneethan, et al) in 2008. To our knowledge no other examples of this phenomena have been reported. Reduction in the serum HCO3- due to an interfering substance should be considered in an asymptomatic patient presenting with a high AG acidosis without detectable exogenous or endogenous acids.

PUB576

Use of Citrasate Dialysate in Extended Dialysis Kunal Malhotra, Venkatesh Kumar Ariyamuthu, Vibhu Dhawan, Preethi Yerram. *Department of Nephrology, University of Missouri, Columbia, MO.*

Background: Anticoagulation for continuous renal replacement therapy(CRRT) can be challenging in acutely ill patients due to the risk of bleeding with heparin, and the risk of citrate toxicity with regional citrate anticoagulation, especially in patients with decompensated liver failure. We describe our experience in an acutely ill patient who successfully underwent extended dialysis with citrasate dialysate.

Methods: 53-year-old male with end stage renal disease on chronic hemodialysis three times a week via arterio-venous fistula presented with delirium, abdominal pain, jaundice and septic shock, and was initiated on broad-spectrum antibiotics, vasopressors and intubated for respiratory failure. He was found to have pancytopenia, ischemic liver injury, metabolic acidosis and worsened rapidly to develop disseminated intravascular coagulation (DIC) and bleeding from IV lines and other orifices. He received multiple transfusions of red blood cells and fresh frozen plasma and developed abdominal compartment syndrome and anasarca. He required CRRT for acidosis and attempted fluid removal. However, heparin couldn't be used because of ongoing bleeding, while citrate use was undesirable given the risk of citrate toxicity. The patient was started on CRRT with citrasate dialysate (CD) that continued for 20 hours before clotting.

Relevant labs

	Serum bicarbonate meq/L	Serum sodium meq/L	Anion gap	Ionized Calcium
pre CRRT	15	141	30	1.09
post CRRT	28	138	14	1.04

Conclusions: CD contains a low concentration of citric acid (2.4meq/L) that can provide anti-coagulation in the extra-corporeal circuit, without any systemic anticoagulant effects. When faced with a therapeutic dilemma of choosing an anticoagulant to prevent clotting of the CRRT circuit in patients with bleeding diatheses, dialysis with CD is a viable option that seems to be safe and effective.

PUB577

Pregnancy & Renopancreas Transplant: Is Patient Satisfaction Enough? Amelia Rita Bernasconi,[1] Ricardo M. Heguilen,[1] Rosa Alejandra Waisman,[1] Jorgelina Petroni,[3] Roland C. Blantz.[2] *[1]Medicine, Hospital J.A.Fernandez, Bs As, Caba, Argentina; [2]Medicine, University of California, San Diego, CA; [3]Nephrology, Instituto de Nefrología de Bs As, Bs As, Caba, Argentina.*

Background: The major benefit of a pancreas-kidney graft (KPT) is an improvement of QOL due to freedom from both insulin & dialysis, along with the prevention of progressive DBT-associated organ damage. Better organ preservation and immunosuppressive therapies improved graft-survival. Prenatal counselling must address maternal & fetal complications. We communicate the outcome of pregnancy in a (DKPT) recipient.

Methods: A 30 year-old, DKPT recipient in 2008, underwent successful pregnancy after a complex medical history of diabetes in her childhood with retinopathy & polyneuropathy, hypothyroidism, and ESRD afterwards. She became pregnant in 2011, MMF was withdrew and switched into a pregnancy-friendly schedule (AZA, TAC, prednisone). Scr was 1.7 mg/dl and remained initially stable; increased near delivery (36.2 wks) and remained at 2.5 mg /dl postpartum. Her BP increased near term without need of medication, serum uric acid rose to 6.52 mg/dl and proteinuria developed. She was normoglycemic, with normal pancreatic and hepatic function throughout pregnancy and postpartum. Epo and iron (H^{to} 24.7%, HB 8.2 g/dl) were prescribed. Betamethasone was given for fetal lung maturity. She developed preterm premature rupture of membranes, with clear amniotic fluid; methylprednisone pulse was given peripartum to avoid intra and postpartum stress. She delivered vaginally a healthy baby (BW: 2100 g., intrauterine growth retardation) with no congenital anomalies & Apgar 9/10. Both were discharged home 3 days later without complications. Breastfeeding was inhibited. There was no postpartum hypertension and she continued with increased but stable renal parameters. Three months later her Scr returned to baseline.

Conclusions: Pts should be advised of the high risk of graft loss, and adverse pregnancy outcome. CIN should be used with caution; adequate levels of immunosuppressive drugs must be maintained to avoid acute rejection. Preterm delivery, IUGR and low birth weight are common probably due to adverse effects of drugs.

PUB578

Hyponatremia due to Reset Osmoreceptor in HIV Positive Patient Devang V. Lodhavia, Zohreh S. Soltani. *Nephrology, LSUHSC, New Orleans, LA.*

Background: Hyponatremia is a common manifestation in HIV infection and AIDS. Most of this patients have SIADH but in some cases may be due to intravascular volume depletion, adrenal insufficiency and CNS infection. Here we present a case of SIADH variant of reset osmoreceptor in a patient with HIV and CNS infection.

Methods: A 31 year old African American male with past medical history of HIV presented with altered mental status. Initial serum work up was normal. CT Head showed a brain mass with midline shift. The patient was underwent an open brain biopsy with final diagnosis of toxoplasmosis. However the patient had his sodium dropped from 136 to 127 mEq/L after his cranial surgery. At this point, he was started on 3% normal saline (NS) by primary team but sodium remained stable at 131mmol/l after 3 days. Nephrology was consulted due to persistent hyponatremia. Further workup revealed urine osmolality of 751 moms/kg (while he was on hypertonic fluid), urine sodium of 204 mol/l, serum osmolality of 264 mosm/l, uric acid of 3.9 mg/dl and normal cortisol and TSH level. Despite continuous 3% NS for two more days sodium level remained in range of 128-131 meq/L. Based on the available clinical and laboratory data, we made a presumptive diagnosis of the syndrome of inappropriate antidiuretic hormone (SIADH). After 5 days of fluid restriction and tolivaptan therapy, his sodium levels failed to rise above a high of 131 mEq/L. As patient remained persistently hyponatremic, the diagnosis of reset osmostat secondary to neurological infection and surgery was implied. In our patient we confirmed the reset osmostat variation of SIADH by giving a free-water challenge. He was discharged home with follow-up upon which his sodium level continued to be stable at 131 mEq/L.

Conclusions: Hyponatremia due to downward resetting of osmostat is one form of the SIADH. Because it has been suggested that one third of the SIADH might be due to the reset osmostat, we recommend that physicians consider investigating this possibility when appropriate. Identification of a reset osmostat is important because the therapeutic recommendations for the SIADH may not apply for this variant.

PUB579

Renal Cell Carcinoma in the Transplanted Kidney Sayed Husain, Noori Al-Waili, Anita K. Patel, Mariella Goggins, K.K. Venkat. *Nephrology/Transplant, Henry Ford Health System, Detroit, MI.*

Background: Immunosuppression increases the risk of malignancies. Renal cell carcinoma (RCC) develops mainly in native kidneys and it is rare in the allograft. We report 3 recipients who developed RCC in their allograft.

Methods: The first patient was a 57 year old female with end stage renal disease (ESRD) secondary to lupus nephritis who received a living related kidney transplant (LRKT) with zero haplotype match in 1994. She was treated with azathioprine (AZA), cyclosporine (CyA). MRI in 2011 due to chronic abdominal pain showed a new 1.3 cm mass in the allograft and biopsy showed conventional clear cell RCC. The second case was a 45 year old female with ESRD due to unknown eiology and underwent a LRKT with one haplotype match in 2001. Her regimen was CyA and MMF. Ultrasonography in 2011 for microscopic hematuria, showed a complex cyst and mass of 1.3 cm in the allograft. Biopsy revealed papillary RCC. The third patient was 46 year old male with ESRD associated with IgA nephropathy that underwent LRKT with zero haplotype match in 1997 and was given CyA and MMF**.** In 2011, due to increasing serum creatinine ultrasound and CT scan showed a solid and vascular lesion 3.1 cm the allograft. Nephrectomy specimen showed RCC. Total allograft nephrectomy was performed and dialysis was reinstituted in all of them.

Conclusions: The uniqueness of this case series is that in all 3 patients RCC developed in the allograft following LRKT suggesting the possibility of an inherited susceptibility aggravated by immunosuppression. Also of interest is the development of RCC after a span of many years suggesting that the tumor was not transplanted with the allograft. The donors have been advised regular monitoring for the development RCC in their remnant native kidney**.** Allograft RCC cases have been reported to be very rare (0.32% to 0.45% incidence). Genetic analysis using fluorescence in situ hybridization (FISH) and DNA microsatellite analysis of the tumor have generally revealed donor-cell origin of the tumor. Partial nephrectomy and percutaneuos ablation of the tumor to preserve renal function has been performed though our patients were treated by total allograft nephrectomy.

PUB580

IgA Nephropathy and Amyloidosis Associated with Ankylosing Spondylitis Ikuyo Narita, Michiko Shimada, Takeshi Fujita, Yuko Shimaya, Reiichi Murakami, Norio Nakamura, Hideaki Yamabe, Ken Okumura. *Nephrology, Hirosaki University, Hirosaki, Japan.*

Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease of the vertebral joints and soft tissues. Although the kidneys can be affected sometimes, the precise etiology and causal relationship remain to be elucidated. A patient with IgA nephropathy and amyloidosis associated with AS is presented.

Methods: A 58-year-old male with a three-year history of hypertension, anemia and micro-hematuria was admitted with fever, rapid onset of edema, weight gain, gross hematuria, proteinuria and pleural effusion. Last doctor's visit was 3 years ago. Blood pressure was 170/85mmHg, physical examination was compatible with presenting symptoms. Laboratory data were as follows: Hb 9.1 g/dl, total protein 6.0 g/dl, albumin 3.0 g/dl, BUN 18 mg/dl, serum creatinine (s-Cre) 0.96 mg/dl, CRP 13 mg/dl, IgA 573 mg/dl, serum protein electrophoresis: negative, antinuclear antibody:negative, C3/C4 :normal, urinary protein 1.1 g/24hr. Renal biopsy revealed mild mesangial proliferation in Periodic acid-Schiff staining, Congo Red negative on light microscopy (LM), IgA positive staining on immunofluorescence microscopy (IF) and electron dense mesangial deposits were seen on electron microscopy (EM), compatible with diagnosis of IgA nephropathy. After exclusion of other etiologies, treatment with corticosteroid resulted in normalization of CRP and resolution of fever. The patient was re-admitted a year later with low back pain, massive lower extremity edema, low grade fever, s-Cre 1.35 mg/dL, CRP 13.6 mg/dL and urinary protein 4.0 g/24hr. Skeletal x-rays showed typical bamboo-spine which is diagnostic feature for AS. Repeat renal biopsy now revealed disappearance of IgA deposition on IF and EM, but with appearance of Congo Red positive deposits on LM, and amyloid fibrils on EM, compatible with amyloidosis.

Conclusions: We experienced a case of AS complicated with IgA and renal amyloidosis. One year steroid therapy was effective for his IgA nephropathy, whereas it was not effective to prevent the progression to amyloidosis.

Funding: Government Support - Non-U.S.

PUB581

Tolvaptan Is a New Therapeutic Tool for Patients with Congestive Heart Failure Who Underwent Peritoneal Dialysis Noriaki Iino, Ichiei Narita. *Division of Nephrology and Rheumatology, Niigata University, Niigata, Japan.*

Background: Peritoneal dialysis is one of the blood purification therapies for end-stage renal diseases. Some PD patients occasionally developed congestive heart failure due to ultrafiltration failure of peritoneal dialysis. Patients suffered from congestive heart failure; they received some medical interventions such as prescription of increased doses of diuretics or modification of peritoneal dialysis regimens. Patients did not respond to these therapies; they required more intensive interventions such as ultrafiltration using extracorporeal circulation. Recently, we can use vasopressin V2 receptor antagonist, tolvaptan for the treatment of loop-diuretics resistant congestive heart failure in Japan. We tried to verify the capability of tolvaptan for the PD patients with congestive heart failure.

Methods: Case was 59 years old male. He developed end-stage renal disease because of progression of diabetic nephropathy. Peritoneal dialysis started since August 2010. His peritoneal equilibrium test was high. Thus, It was difficult to maintain optimal extracellular body fluid by conventional peritoneal dialysis. In September 2011, he felt dyspnea on exertion. The chest x-ray reveled moderate cardiomegaly and lung congestion. He had diagnosed congestive heart failure. On laboratory tests, a value of B-type natriuretic peptide elevated at 1778 pg/ml. After he had admitted a hospital, he prescribed tolvaptan 15mg once daily. One day after admission, his body weight decreased by 3kg. Tolvaptan effectively increased his urine output. Moreover, tolvaptan also increased his peritoneal drain volume. Thus, his symptom of congestive heart failure gradually improved. Six days after admission, a value of B-type natriuretic peptide decreased to 380 pg/ml.

Conclusions: According to this experience, we believe tolvaptan is a promising drug for patients with congestive heart failure who received peritoneal dialysis. Futhermore,if tolvaptan can substitute for ultrafiltration therapy using extracorporeal circulation, patients with severe congestive heart failure will receive safety benefits because of not requiring central venous catheter insertion.

PUB582

An Unusual Case of Membranous Nephropathy Antoine Azar, Apurv Khanna. *Nephrology, Suny Upstate Medical University, Syracuse, NY.*

Background: Currently accepted treatment for high risk idiopathic membranous nephropathy is combined immunosuppressive treatment. Here we present an unusual case in which patient responded to steroids alone.

Methods: A 52 year old man with DM 2,previous history of cocaine abuse,and a normal serum creatinine,presented with shortness of breath,and anasarca,he denied any non-steroidal anti-inflammatory drugs use.A spot urine protein creatinine ratio of >20 gram protein per gram creatinine.Subsequently he rapidly developed worsening kidney function and hyperkalemia necessitating dialysis,serologic work up was negative,Kidney biopsy showed subepithelial deposits(IgG and C3),thickening of glomerular basement membrane,secondary focal segmental glomerulosclerosis with foot process effacement,and tubulitis,consistent with membranous nephropathy. Due to a history of irregular adherence to treatment he was started on Prednisone alone(Dose mg/kg)with the intent to initiate further immunosuppression on outpatient followup.His kidney function improved within 10 days after starting steroids,he did not require further dialysis any more,his serum creatinine improved to 1.5 mg/dl,a follow-up spot urine protein creatinine ratio showed a decreased proteinuria to 3 gram protein per gram creatinine,patient was discharged home. He was then lost to follow up and presented months later with a similar presentation.During this hospitalization,kidney function again deteriorated significantly necessitating dialysis;He was restarted on prednisone,within 7 days his serum creatinine normalized;his proteinuria improved to 3 from 10 in a spot urine sample.

Conclusions: UK research Council studied 52 patients with idiopathic membranous nephropathy with heavy proteinuria (10 g/day) and normal renal function treated with steroids,and compared them with 51 patients with same characteristics treated conservatively,study showed steroids alone did NOT show better results. High risk patient are those with proteinuria of >8 grams/day,renal function that is either below normal or declines over three months despite maximum conservative therapy. Our case is unique because it represents a high risk membranous nephropathy patient who responded to steroids alone on two occasions.

PUB583

An Unusual Case of Hyponatremia Varun Malhotra,[1] Medha Joshi,[2] Pooja Singh,[1] Rakesh Gulati.[1] *[1]Nephrology, Thomas Jefferson University, Philadelphia, PA; [2]Internal Medicine, Mercy Catholic Medical Center, PA.*

Background: Differentiating Syndrome of inappropriate antidiuretic hormone hypersecretion (SIADH) from renal salt wasting (RSW) rests on evaluation of the patient's "effective arterial blood volume (EABV)." We present a case which illustrates that EABV assessment based solely on clinical exam can often be misleading.

Methods: 68-year old woman with past history of breast cancer and failure to thrive; presented with productive cough. Physical examination was remarkable for a cachectic female, afebrile, pulse of 70/min, BP 118/74, with no orthostasis, respiratory rate of 16/min, crackles over the left lung base but no jugular venous distention or peripheral edema. CXR showed a left lower lobe infiltrate. Initial labs were impressive for a serum osmolality of 260 mosm/L, serum sodium of 122 meq/L, creatinine of 0.5 mg/dL, Uosm of 426 mosm/kg, urine sodium (Una) of 101 mmol/L and urate of 0.9 mg/dL. TSH and cortisol were normal. A diagnosis of SIADH secondary to the lung process (pneumonia) was made. Over the next two days, her serum sodium dropped to 112 meq/L despite strict fluid restriction and protein supplementation. Symptoms of nausea and headache prompted treatment with hypertonic saline. While repeated clinical and laboratory evaluations showed no evidence of volume depletion, echocardiogram revealed a small LV cavity and collapsed IVC consistent with low central venous pressure. Una and Uosm were persistently elevated. Further questioning revealed a history of nocturia for several years in the setting of chronic analgesic use and past cisplatin use for breast cancer. Possibility of RSW was entertained. Her serum sodium improved slowly to >130 meq/l over the next 4 days with IV normal saline, salt tablets and fludrocortisone. We concluded that her hyponatremia was a result of RSW from chronic tubulointerstitial disease.

Conclusions: A valid diagnosis of "salt wasting" requires evidence of inappropriate urinary salt losses and a reduced "EABV." The clinical assessment of EABV, is fraught with errors unless invasive procedures, like determination of blood volume by radioisotope dilution methodology, pulmonary wedge, or central venous pressures are used.

PUB584

Adult Minimal Change Disease with Acute Kidney Injury Elise J. Barney, James Wilson. *Nephrology, Olive View-UCLA Medical Center, Sylmar, CA.*

Background: Introduction: Minimal change disease (MCD) is responsible for 10-15% of primary nephrotic syndrome in adults. Acute kidney injury (AKI) in the setting of MCD is uncommon; pathology shows foot process effacement and focal proximal tubular epithelial flattening. The following is a case in which the severe tubular injury led to a pathological misclassification as ATN rather than MCD.

Methods: Case: A 43 year-old Hispanic man was admitted for lower extremity edema following recent discharge from an outside hospital, where he had kidney biopsy done for nephrotic syndrome. At the time of initial presentation, labs noted serum creatinine of 5.5 mg/dL, urea nitrogen of 54 mg/dL, serum albumin of 1.1 g/dL, and 6.7% eosinophilia. Proteinuria workup was unremarkable. Renal ultrasound noted normal echogenicity and minimal fluid anterior to right kidney. History was significant for food-associated urticaria 2 months prior and NSAID use over the past 6 weeks. Physical exam noted blood pressure 125/81 mm Hg and anarsaca; 24-hour urine revealed 66 gm of protein. Biopsy pathology reported severe diffuse ATN. However, given significant proteinuria, a pathology second opinion was obtained. Light microscopy review noted irregular flattening of many tubular cells, debris and granular casts in tubules with mild diffuse edema of interstitium. Immunofluorescence demonstrated IgG pseudolinear staining; electron microscopy revealed effacement of foot processes and no electron dense deposits. The patient was diagnosed with MCD. After 9 weeks of prednisone, the serum creatinine was 0.95 mg/dL and urine protein/creatinine ratio was less than 0.140.

Conclusions: Discussion: This case illustrates the importance of obtaining a second opinion if the initial biopsy read is not consistent with the clinical picture. In this case, the biopsy was incorrectly read as ATN, but upon second opinion, was correctly diagnosed as MCD. Potential mechanisms for ATN coexisting with MCD may be secondary to renal interstitial edema and/or ischemic tubular injury. Associated risk factors are older age, hypertension, vascular disease, high protein excretion, and very low serum albumin. The AKI is usually reversible with complete recovery of function.

PUB585

Tenofovir (TDF) Induced Acute Kidney Injury Joseph Zhao, Louis A. Carrera, Maria V. DeVita, Michael F. Michelis. *Division of Nephrology, Lenox Hill Hospital, New York City, NY.*

Background: Tenofovir (TDF) is a commonly used to treat human immunodeficiency virus infection and has been only rarely reported to cause acute renal injury. Since its use in 2001, less than 40 cases have been reported with last series done in 2010. We report a case of TDF nephrotoxicity in a woman on the medication for 18 months.

Methods: A 57 year old Hispanic woman with CKD 3 (creatinine 1.7mg/dL and proteinuria 500mg); DMII and HTN, with HIV since 1996, on anti-retroviral therapy. To reduce pill burden, she was changed to Atripla (efavirenz, emtricitabine, and tenofovir) in 2010. She developed AKI with an increase in creatinine to 3.5 mg/dL and proteinuria of 2195 mg/g creatinine. Her viral load was undetectable; CD4 count 828; urinalysis revealed blood, 4-20 rbc, no wbc, 1+ protein, no glucose or casts. Since Nexium and alendronate were possible nephrotoxic agents, both were discontinued; despite this, her creatinine continue to increase.BP 138/68 mmHg; she was clinically euvolemic without edema or localizing findings. HbSAg, Hep C AB, anti-ds DNA, myeloperoxidase AB, proteinase-3, and immunofixation were all negative. C3 and C4 were normal. With worsening in her condition, a kidney biopsy was performed which revealed a distinctive pattern of proximal tubular injury characterized by severe mitochondrial damage due to TDF induced renal injury. Her medication was changed to Combivir and Efavirenz in May 2012 with a decrease in her creatinine to 2.4 mg/dL within 4 weeks.

Conclusions: AKI is a known adverse effect of TDF, however, empiric discontinuation of her anti-retroviral therapy was fraught with concern since her HIV was controlled. Most cases occur within a median of 8 months after initiating therapy. In addition, most cases also display Fanconi's Syndrome. Despite the presence of severe renal failure, our patient showed significant improvement after TDF cessation. It is important to emphasize that TDF nephrotoxicity does not occur immediately and has been reported as late as 2 years after initiation. Kidney biopsies are critical to establish a diagnosis prior to empiric change in anti-retroviral treatment in this rare but potentially serious complication.

Funding: Clinical Revenue Support

PUB586

43-Year-Old Woman with Systemic Lupus, Abdominal Pain, New-Onset Severe Hypertension, and Bilateral Perinephric Hemorrhage Ivan E. Porter, William E. Haley. *Internal Medicine, Mayo Clinic, Jacksonville, FL.*

Background: Connective tissue disease can manifest with the onset of renal failure suggesting lupus nephritis. Definitive diagnosis requires biopsy as well as broad differential and consideration of associated conditions as illustrated in this case.

Methods: A 43-year-old female with history of systemic lupus and no renal involvement presented with 5 days of worsening bilateral lower abdominal pain radiating to the back. There was history of treatment for chronic refractory autoimmune thrombocytopenia with low dose prednisone, danazol, eltrombopag and intermittent IVIG. On PE, BP 164/108, HR 105; she was in acute distress. Abd was soft, nondistended, with no organomegaly, but tenderness to palpation diffusely. Tests revealed Hbg 10.6 g/dL, WBC count 24,700, plts 134,000, INR 2.1, creatinine 2.1 mg/dL, and UA positive for moderate occult blood and microhematuria. Complement levels normal, negative c-ANCA, PR3, MPO, anti-DNA and cryoglobulins, positive p-ANCA and IgG phospholipid antibody, and high titer ANA and SS-A. Abd CT scan showed bilateral renal subcapsular hematoma and retroperitoneal hemorrhage. Angiogram showed innumerable areas of well-contained extravasation (pseudoaneurysms) throughout the renal parenchyma. Renal biopsy showed focal medium artery transmural disruption with perivascular fibrin extravasation, zonal cortical necrosis with ATN and GBM ischemic corrugation but no evidence of immune complex processes on IF or EM. Renal failure worsened requiring dialysis and she also required coil embolization of branch arteries on two separate occasions to control hemorrhage. She was treated with glucocorticoid and cyclophosphamide. Three months following presentation she experienced sudden death following acute onset abdominal pain.

Conclusions: This patient had polyarteritis nodosa (PAN) associated with systemic lupus and autoimmune thrombocytopenia, and manifesting severe new-onset hypertension, renal failure, intrarenal and perinephric hemorrhage. Poor prognosis despite aggressive treatment was demonstrated with death occurring within 3 months of presentation.

PUB587

Reversible Fanconi Syndrome Associated with Human Immunodeficiency Virus Alex J. Hernandez, Zohreh S. Soltani. *Department of Medicine/Section of Nephrology, LSUHSC-NO, New Orleans, LA.*

Background: Renal disease is a common complication of patients infected with the human immunodeficiency virus (HIV); it can be directly related to the virus, or associated to the highly active antiretroviral therapy (HAART). Since the introduction of HAART, a variety of renal side effects have been recognized and vary from proteinuria to acute renal failure.

Methods: A 45-year-old Caucasian man with HIV infection for five years, was referred to the renal clinic for proteinuria work-up. He noticed increase thirst, polyuria, as well as fatigue for the last six weeks. He was taking lisinopril, ritonavir, atazanavir, and emtricitabine/tenofovir. He was compliant with his medications, does not use tobacco, alcohol or drugs. He denied use of NSAIDs or herbal supplements. Physical examination was unremarkable, except for trace edema. The laboratory data was normal except creatinine 1.54 mg/dL, and phosphorus 1.8 mg/dL. A urine analysis showed a pH of 5.0, protein of 150 mg/dL, glucose of 1000mg/dL and blood of 25/uL. protein creatinine ratio 1719 mg/g. Hemoglobin A1c was 5.1%. Renal ultrasound was normal. Serum and urine protein electrophoresis did not reveal gammopathy.

Conclusions: Fanconi syndrome was suspected secondary to antiretroviral tenofovir. Therapy was changed to darunavir and lamivudine/zidovudine. Phosphorus was replaced orally. A follow-up appointment showed a decreased proteinuria of 654 mg/day, MACR 85 mcg/mg, phosphorus within normal limits and no glucosuria on urine dipstick. Fanconi syndrome consists of a generalized defect of membrane transporters in the proximal tubule, leading to renal loss of glucose, as well as a loss of phosphate, calcium, uric acid, amino acids, bicarbonate and tubular proteins. This case confirms the previously reported association of tenofovir with Fanconi syndrome. It is recommended that patients on this drug should be followed closely after initiation of therapy and should have a urinalysis, serum creatinine, and electrolytes performed on a regular basis. It is important for clinicians to recognize this potential side effect, so that patients with similar scenarios may be discovered early and be switched to an alternate antiretroviral therapy.

PUB588

Membranous Lupus Nephritis in the Absence of Serum Anti-Nuclear Auto-Antibodies Hanni Menn-Josephy, Ramon G. Bonegio, Jean M. Francis, Ian R. Rifkin. *Nephrology, Boston University Medical Center, Boston, MA.*

Background: Membranous lupus nephritis and nephrotic syndrome can occasionally present in patients with negative ANA. Most of these patients will become ANA positive later in the course of the disease.

Methods: A 36 year old Hispanic female presented with a 4 month history of weight loss, diffuse lymphadenopathy and intermittent fever. Two months later she developed acute pulmonary emboli from a left leg DVT. One month thereafter, she was hospitalized for abdominal pain. She had cervical and axillary lymphadenopathy but no other abnormalities on exam. Serum creatinine and electrolytes were normal but she had hypoalbuminemia, microcytic anemia and lymphopenia. Urinalysis revealed 3+ protein with a protein: creatinine ratio of 11.3 but no cellular casts. Chest and abdominal CT revealed lymphadenopathy and hepatosplenomegaly with a large spleen infarct. Comprehensive infectious disease work-up was negative. ANA, anti-dsDNA and cryoglobulins were not detected. Serum complement levels were normal. Coagulopathy work-up was negative, with no anti-cardiolipin antibody or lupus anti coagulant detected. Free light chains were not increased and no monoclonal bands were seen on serum or urine immunoelectrophoresis. Bone marrow, lymph node and small bowel biopsies all showed reactive follicular hyperplasia. Kidney biopsy showed many subepithelial, subendothelial and mesangial immune complex deposits on EM, and strong immunofluorescence staining for IgG, C3, kappa and lambda with weaker staining for IgM, IgA and C1q. Notably, immune deposits were also seen in the tubular basement membrane. The patient subsequently developed alopecia and autoimmune hemolytic anemia. The clinical and pathological presentation was highly suggestive of lupus and membranous lupus nephritis, despite the repeatedly negative ANA. Treatment with prednisone and mycophenolate was started with reduction in lymphadenopathy and proteinuria and resolution of constitutional symptoms.

Conclusions: Membranous lupus nephritis should be considered in the differential diagnosis of patients with nephrotic range proteinuria even in the absence of systemic or serologic features diagnostic of lupus.

PUB589

Rapid Progressive Glomerulonephritis in Diabetic Patient Presenting with Urinary Tract Infection and Acute Kidney Injury Arksarapuk Jittirat,[1] Natthavat Tanphaichitr.[2] *[1]Olive View Medical Center- UCLA; [2]Northeast Ohio Medical University.*

Background: Diabetic nephropathy occurs in approximately 40% of diabetic patients. Renal biopsy is not routinely performed in diabetic patients with proteinuria. However, non-diabetic renal disease(NDRD) may coexist in up to one third of diabetic patients. It is important that NDRD is diagnosed early since its treatment and outcome are different from diabetic renal disease. The delay in treatment can cause worsening renal function and may progress to ESRD.

Methods: A 56 year old woman with DM, HTN, CKD with baseline creatinine(Cr) 1.2 presented to the hospital with fever, nausea, vomiting, dysuria and flank pain for 3 days. Urine culture grew ESBL E.coli. She was also found to have acute kidney injury(AKI) with Cr of 2.8 and urine protein to creatinine(UPC) ratio of 3.1.Work up revealed positive myeloperoxidase ANCA. Renal biopsy showed an active crescentic glomerulonephritis(GN) in 80% of glomeruli with a mild degree of interstitial fibrosis and tubular atrophy consistent with pauci-immune GN. Treatment was initiated with cyclophosphamide and steroid. The renal function and proteinuria continued to improve during subsequent follow up. Her Cr 4 months after therapy was 1.2 and UPC ratio was 0.8.

Conclusions: Many studies have been done to determine the role of renal biopsy in diabetic patients with renal involvement. Chong et al. reviewed the clinical predictors of NDRD from 2004-2008 and concluded that the useful clinical markers are AKI and microscopic hematuria. The prevalence of NDRD is high in patients with short duration of DM, absence of diabetic retinopathy and nephrotic range proteinuria.

In this case, the patient had DM, AKI and UTI which are associated with proteinuria. Treatment of symptomatic UTI reduces proteinuria. Many experts recommend repeating UPC two weeks after the treatment of UTI. However, early proteinuria work up in this patient led to the diagnosis of rapidly progressive GN. The treatment was started immediately and the renal function had recovered. It is important to make a diagnosis of NDRD in diabetic patients with variable presentations and initiate the treatment in a timely manner.

PUB590

Mitochondrial Disease and Renal Transplant Matthew Miller,[1] Mohan Cooray,[2] Catherine M. Clase,[1] Chidam Yegappan,[3] Azim S. Gangji.[1] *[1]Div. of Nephrology, McMaster University, Hamilton, ON, Canada; [2]Dept. of Medicine, McMaster University, Hamilton, ON, Canada; [3]Div. of Neurology, McMaster University, Hamilton, ON, Canada.*

Background: Mitochondria are responsible for ATP generation and have their own DNA (mtDNA) that is maternally inherited and present in multiple copies per cell. Prevalence of mitochondrial disease is 1-2:10000 and usually presents in childhood but diagnosis can occur in adulthood.

Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes (MELAS) is the most common mitochondrial disease. It can be accompanied by seizures, diabetes, deafness, and weakness. 80% of MELAS is associated with mtDNA A3243G mutation; 5% have nephropathy. Tubular dysfunction and proteinuria are the most common manifestations. Pathology shows FSGS or interstitial nephritis with proliferation of large, dysmorphic mitochondria. 50% develop ESRD.

Six transplants in mitochondrial disease have been described. One had pre-existing cognitive impairment and stroke. Three others developed strokes within two years of transplant. Two had pre-existing diabetes, whereas three developed it post-transplant. Transplant function remained normal in all.

It is difficult to determine if transplant worsens mitochondrial disease or if these represent natural disease progression. Nephropathy does not recur after transplant. Given the development of complications shortly after transplant, nephrologists should be cautious about increased risks of complications when considering transplant for patients with mitochondrial disease.

Methods: GW was a 58-year-old male with ESRD from chronic interstitial nephritis, interstitial fibrosis, and tubular atrophy. Other history included deafness, ataxia, and chronic fatigue. He received a transplant in August 2010. Post-transplant he developed severe weakness, a CK of 1700, and NODAT. In May 2011, he developed progressively worsening multifocal strokes and seizures.

He had elevated lactates throughout his final admission. A muscle biopsy was consistent with MELAS. Despite therapy, he suffered further cognitive decline and died in September 2011. His transplant functioned well until death.

PUB591

Non-Cryoglobulinemic Hepatitis C with Renal Thrombotic Microangiopathy Sara Husain, Dilip Samarapungavan, Raviprasenna K. Parasuraman, Gampala Harish Reddy. *Internal Medicine, Wiliam Beaumont Hospital, Royal Oak, MI.*

Background: Hepatitis C (**HepC**) associated renal disease manifests typically as MPGN, often with mixed cryoglobulinemia(**Cryo**), membranous nephropathy and rarely fibrillary glomerulonephritis. We present an unusual case of HepC associated isolated renal thrombotic microangiopathy (**r-TMA**).

Methods: A 57 year-old male with untreated HepC presented with worsening dyspnea. He had no diarrhea. Examination showed 3+ leg edema, but no fever, skin rash or neurological deficits.

Laboratory tests: platelets 75 bil/L, hemoglobin 8.2 g/dL, BUN 22 mg/dL, creatinine 1.1 mg/dL (rising to 2.0 mg/dL, with a baseline of 0.45 mg/dL), 24 hour urine protein 2530 mg, LDH 612 U/L; haptoglobin < 8 mg/dL. Peripheral smear showed schistocytes. Bone marrow biopsy, C3, C4, and ADAMTS 13 activity were normal. ADAMTS inhibitor, ANA, anti-dsDNA, anticardiolipin antibodies (**aCL**), RF, Cryo and drug screen were negative.

Renal biopsy showed several small arteries with thrombi formation on light microscopy. By electron microscopy the glomerular basement membrane had scattered subendothelial electron-lucent areas with fibrin material mixed with lucent ischemic changes characteristic of r-TMA. There were no features of MPGN or immune-complex disease.

Conclusions: TMA is associated with several causes. Endothelial injury is the common primary event in its pathogenesis. HepC associated r-TMA has been described. There are a few cases in renal transplant recipients with HepC in association with aCL. It has also been occasionally reported in native kidneys in combination with MPGN and Cryo, aCL, as well as in conjunction with interferon therapy. The mechanism is yet to be established. If present, aCL may initiate endothelial damage. Cacoub et al have demonstrated that HepC with mixed Cryo may induce r-TMA by the developement of anti-endothelial cell antibodies. Our patient had no detectable aCL or Cryo and the only renal lesion was the TMA. It is possible that current laboratory assays do not detect all possible aCL antibodies that exist. It is also unclear if HepC may cause direct endothelial injury. We are awaiting results of immunohistochemical staining for HepC in the biopsy sample.

PUB592

Calciphylaxis and Leukocytoclastic Vasculitis in End Stage Renal Disease Patient on Warfarin: Is There a Synergy? Karthik Karanam. *Indiana University Hospital, Indianapolis, IN.*

Background: Calciphylaxis is a well-known complication in end stage renal disease (ESRD) patients and warfarin puts them at increased risk of it. Warfarin is also known very rarely to cause leukocytoclastic vasculitis. We report a first case of calciphylaxis and leukocytoclastic vasculitis in an ESRD patient probably exacerbated by warfarin.

Methods: A 41 year old female with past medical history of blindness secondary to retinitis pigmentosa, ESRD on hemodialysis, hypertension, cardiomyopathy, and history of thrombosis on warfarin was referred to the hospital for painful lesions on the buttocks and thighs. Patient was also on calcium containing phosphate binders and calcitriol. She apparently was seen by dermatology at an outside clinic and received a biopsy of the lesion before she presented here. The lesions on the buttocks showed diffuse erythema and black eschar in the middle. The lesion on the left thigh was also similar and the one on the right was erythematous. The skin biopsy showed perivascular and interstitial neutrophilic infiltrate. Microcalcification was present in the wall of the small blood vessels. These changes were consistent with leukocytoclastic vasculitis and calciphylaxis with early epidermal necrosis. All the potentiating factors such as calcium containing binders and calcitriol were stopped. Patient was optimized on dialysis and sodium thiosulfate was started. Warfarin was stopped as there was no proper indication for it after appropriate work up was done. As vasculitis was noted on the biopsy, appropriate serology was sent for analysis. Antiphospholipid antibodies were negative, C3 and C4 were normal and ANA was negative. Patient later underwent total parathyroidectomy during the hospitalization. Patient had a good recovery later.

Conclusions: Warfarin is known to potentiate calciphylaxis by inhibiting the vitamin K dependent carboxylation of matrix G-1a protein, which inhibits calcification locally. Warfarin is also known to cause leukocytoclastic vasculitis, but is there a synergistic effect between its ability to potentiate calciphylaxis and cause vasculitis needs to be ascertained more at the histological and molecular level.

PUB593

Haemolysis, Elevated Liver Enzymes and Low Platelets (HELLP) Syndrome Complicated by Acute Kidney Injury: Is Therapeutic Plasma Exchange the Answer? Konstantinos Koutroutsos,[1] Louise E. Ross,[1] Aris Papageorghiou,[2] Roberto Stasi,[3] Debasish Banerjee.[1] *[1]Renal Unit, St George's Healthcare NHS Trust, London, United Kingdom; [2]Obstetrics Unit, St George's Healthcare NHS Trust, United Kingdom; [3]Haematology Department, St George's Healthcare NHS Trust, London, United Kingdom.*

Background: Differential diagnosis of microangiopathic disorders in pregnancy is difficult, due to overlapping clinical and laboratory findings.Limited case series suggest that plasma exchange may be successful in patients with HELLP syndrome complicated by organ failure.

Methods: A 38 year old pregnant woman (Gestational age:39+6 weeks) presented to the obstetric unit following a tonic-clonic seizure. She had an uncomplicated pregnancy and no past medical history. On arrival, she was hypertensive (BP: 177/125mmHg) with proteinuria. The seizure was considered an eclamptic fit and the patient was started on MgSO4 and Hydralazine infusion. She underwent emergency caesarean section and delivered a healthy baby girl(Weight: 3190g, Apgar score 9). Postpartum, the patient was found to have microangiopathic haemolytic anaemia (Hb:7.7g/dl,PLT:42000/ml,LDH:1647u/l), elevated liver function tests (ALT:1538U/L) and acute kidney injury (AKI, cr:286umol/L).She was transferred to the Intensive Care Unit, received blood transfusions and underwent haemofiltration. On the 2nd day postpartum and in view of persistent haemolysis, she was started on daily Plasma Exchange with fresh frozen plasma (1,5x plasma volume). The patient had 10 Plasma Exchange sessions in total. Complement factors B(293,reference range(ref):295-400) H(517,ref:345-590) I(66,ref:38-58) and ADAMTS13 activity (61%,ref:60-123) were found to be normal and a diagnosis of HELLP was established.The patient was discharged 10 days after admission and recovered completely from HELLP and AKI.

Conclusions: This case underlines the complexity in the diagnosis and management of pregnancy-related thrombotic microangiopathies. In our case plasma exchange was initiated early, based on a mixed clinical presentation of severe HELLP and atypical Haemolytic-Uremic syndrome. It appears that plasma exchange may be a successful treatment in persistent, life-threatening HELLP syndrome.

PUB594

Solitary Kidney Autotransplantation: An Alternative to Dialysis Commitment in a Patient with Significant Vascular Disease Nadear A. Elmahi, Kenneth E. Kokko, Tibor Fulop, Mehrdad Hamrahian. *Department of Medicine, Division of Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: Renal autotransplantation (RAT) refers to the relocation of the kidney within the body. Although RAT is indicated in a variety of renal and ureteral diseases, it remains underutilized procedure. We describe a unique case of solitary RAT in a patient with significant vascular disease (VD) and hypercoagulable state.

Methods: 41 yr old African American female presented to the hospital with bilateral lower extremities and mesenteric ischemia due to extensive VD and thrombosis of the abdominal aorta (AA). Computer topographic angiography showed an isolated patent segment of the AA at the level of renal arteries. She underwent a complex thoraco-abdominal bypass surgery with a four-limb graft; 1st limb to the superior mesenteric artery, 2nd limb to the isolated patent segment of AA, and 3rd and 4th limbs to femoral arteries. Further work up revealed low levels of protein C, protein S and antithrombin III. Patient was discharged on long-term anticoagulation, but unfortunately she lost the follow up and stopped her anticoagulation. Two years later she presented to the hospital with acute kidney injury (AKI) and volume overload that required intermittent hemodialysis (HD). Her blood pressure (BP) remained elevated despite ultrafiltration and multiple antihypertensive medication use. Extensive work up without use of contrast revealed inoperable occluded graft limb to AA and a non-functional atrophic left kidney with inadequate blood flow to the right kidney. Renal biopsy of the right kidney showed severe tubular necrosis and viable tissue. Based on the tissue viability, she underwent RAT of the right kidney after which she recovered from her AKI and had BP well controlled on a single agent. Her creatinine was 0.75 mg/dl 3 months post RAT.

Conclusions: RAT is an alternative therapeutic approach to lifelong HD commitment and to resistant hypertension in a patient with solitary kidney and inoperable renal vascular perfusion disorder. Multidisciplinary team approach is the key factor for such a challenging surgery.

PUB595

Should Nephrologists Be Aware of Posterior Reversible Encephalopathy Syndrome? Javier A. Neyra,[1] Carlos Calle-muller,[2] James E. Novak.[3] *[1]Division of Nephrology, UT Southwestern Medical Center, Dallas, TX; [2]Department of Internal Medicine, Henry Ford Health System, Detroit, MI; [3]Division of Nephrology, Henry Ford Health System, Detroit, MI.*

Background: Posterior Reversible Encephalopathy Syndrome (PRES) is a clinicoradiologic entity characterized by altered mental status, headache, visual disturbances, or seizures in combination with classic neuroimaging changes that are frequently reversible. Patients with kidney disease are uniquely at risk for developing this syndrome, since they may be uremic, hypertensive, and exposed to immunosuppressants.

Methods: A 25 year-old man with history of heroin abuse presented to the emergency department with 1 week of diffuse abdominal pain, myalgias, nausea, and vomiting. The patient was afebrile with blood pressure 160/100 mmHg. Evaluation revealed acute kidney injury (AKI) with creatinine 7.1 mg/dL, blood urea nitrogen 58 mg/dL, phosphorus 5.1 mg/dL, and creatine phosphokinase 1,642 IU/L. After a few hours, the patient became anuric and developed tonic-clonic seizures, for which he required emergent hemodialysis and antiepileptic drugs. Brain MRI revealed extensive bilateral cortical and subcortical white matter hyperintensity involving the cerebral and cerebellar hemispheres. Following hemodialysis, the patient's seizure activity ceased, as confirmed by continuous electroencephalography. The patient was initially dialysis-dependent but achieved adequate solute control and remained seizure-free without the need for additional antiepileptic drugs. Subsequent neuroimaging revealed interval improvement in brain white matter signal abnormalities.

Conclusions: Our patient represents the first known case of PRES secondary to severe AKI attributed to heroin-induced rhabdomyolysis. Additionally, our patient exhibited the classic clinical and radiographic interval improvement and remission of seizure activity without the need for continued antiepileptic therapy. Therapeutic intervention in PRES should be targeted to symptom control and identification of the underlying cause. PRES may result in significant long-term neurologic deficits or death, and prompt recognition may be life-saving.

PUB596

Acute IgG4 Interstitial Nephritis and Cholestatic Jaundice: Metformin as a Possible Trigger Swati Arora, Katherine M. Jasnosz, Barbara A. Clark. *Nephrology and Pathology, Allegheny General Hospital, Pittsburgh, PA.*

Background: Acute interstitial nephritis (AIN) with IgG4 positive plasma cell infiltrates is a recently recognized entity most often associated with inflammation in the pancreas or intrahepatic bile ducts. Though etiology is unclear, autoimmunity, allergen or drug induced triggers remain a possibility. Metformin has been rarely associated with cholestatic hepatitis and post marketing analysis suggests a 0.07% incidence of AIN.

Methods: We report a case of a 75 y/o male with a history of DM-II, hypertension and hyperlipidemia maintained on metformin, lantus, quinapril, metoprolol, asa, rosuvastatin presenting with jaundice and AKI. MRCP and ERCP were negative. CRP was 21.8. Total IgG was 1959 mg/dl (nl 70-1600) but IgG4 wnl. Hep B and C; ANA, ANCA; anti smooth muscle ab all negative; SPEP, kappa/lambda ratio wnl. Enlarged kidneys on renal US (14.8; 15.4cm); liver bx with small bile duct cholangitis (IgG4 negative); no active infectious process; kidney bx with diffuse plasma cell infiltrate in interstitium (up to 20 IgG4 positive cells/hpf) suggesting IgG4 AIN. Upon admission, all meds except insulin were held. Despite this, liver and kidney disease progressed (peak cr 9.35mg/dl; bili 7.2mg/dl; AP 673 u/L). Following the kidney bx, he received solumedrol 250mg IV x 1 and prednisone 60 mg daily was started. Within a few days both the liver and kidney function began improving. Within 3 weeks, his creatinine returned to his prior baseline of 1.1 mg/dl and LFTs normalized. Prednisone was tapered to zero over the next ten weeks. His metoprolol had been resumed prior to discharge from the hospital but the metformin, quinapril and rosuvastatin were held. Quinapril was resumed after 6 weeks because of persistent HTN and statin was resumed after 12 weeks for hyperlipidemia, without any adverse effects on kidney or liver function. Metformin remained a possible suspect inciting medication and was not resumed.

Conclusions: While this case has some features of IgG4 nephritis, it did not meet all the criteria. We postulate that some cases with IgG4 positive AIN may be drug induced and implicate metformin as a possible trigger in this case of cholestatic jaundice and AIN.

PUB597

N-Acetyl Cysteine in Management of Methotrexate Induced Kidney Damage Rikin Kartikbhai Shah, Osvaldo Regueira, Curtis W. Turner, Tetyana L. Vasylyeva. *Department of Pediatrics, Texas Tech University Health Science Center, Amarillo, TX.*

Background: High Dose Methotrexate (HD MTX) /Leucovorin Rescue (LCV) alternating with Doxorubicin/Cisplatinum is the current therapy for Osteosarcoma in the Pediatric Population. NAC has been demonstrated to decrease MTX induced oxidative renal damage in preclinical models (Cetinkaya, 2007). We describe the use of NAC to reverse MTX induced nephrotoxicity in an adolescent treated with MTX/LCV for osteosarcoma.

Methods: 18-year old women initiated chemotherapy for osteogenic sarcoma of the left proximal humerus with alternating cycles of Doxorubicin/Cisplatinum (Doxo/CDDP) and High Dose Methotrexate (HD MTX) /Leucovorin Rescue (LCV). Pretreatment serum creatinine level was 0.57 mg/dl and received prehydration with IV fluids at 3000 cc/m^2. The patient was admitted for a sixth overall systemic chemotherapy encounter (Doxo/CDDP - cycles 1 and 3) and specifically the fourth admission for intravenous HD MTX which was administered at a dose of 12 grams/m^2 over 4 hours. She received post hydration intravenous fluids and LCV 15mg/ m^2 IV /PO every 6 hours beginning at hour 0. Serum MTX levels at 24, 48 and 72 hours after MTX infusion demonstrated toxic range. Serial serum creatinine levels increased from baseline 0.57 mg/dl to 0.87 and 1.43 mg/dl at 24 and 48 hours respectively.

Sixty hours after the MTX infusion, we began NAC 600 mg orally every 8 hours. Subsequent creatinine levels decreased to 1.01 and 0.80 mg/dl on day 4 and day 5 respectively. For subsequent admissions for HD MTX/LCV therapy, we coadministered prophylactic NAC and creatinine level remained between 0.65 to 0.95 mg/dl. MTX levels have remained in therapeutic levels.

Conclusions: NAC may have ameliorated the MTX induced nephrotoxicity in our patient. NAC may act as an antioxidant and reverse free-radical mediated cell damage and mitochondrial injury in the renal cells.

Further prospective clinical trials for oncology patients receiving nephrotoxic drugs may be warranted. Additional preclinical studies may clarify the mechanisms of action.

PUB598

Non-Dilated Urinary Tract Obstruction and Oliguric as Well as Non-Oliguric Acute Kidney Injury Layla Kamal,[2] Ilya Glezerman,[1] Carlos D. Flombaum.[1] *[1]Nephrology, Memorial Sloan Kettering Centre; [2]Nephrology, New York Presbyterian Hospital, Weill Cornell Medical College.*

Background: Urinary tract obstruction is a significant cause of acute kidney injury (AKI), accounting for about 9.5% of causes of AKI. In approximately 4% of patients with obstructive uropathy imaging studies do not reveal any evidence of dilatation of the collecting system. We report one patient who developed AKI as a result of non-dilated obstructive uropathy.

Methods: Patient is a 66 year old female with a large pelvic mass secondary to endometrial cancer who is being evaluated for a SCr of 3.8 mg/dl from a baseline of 1.0 mg/dl, confusion and fever secondary to Serratia Marcescens in blood and urine. A renal ultrasound (US) was negative for hydroureteronephrosis. The next day, Scr was 5, with daily UOP of 1.2l and persistent bacteremia. The patient underwent a left sided antegrade pyelogram that demonstrated a minimally dilated left collecting system with a moderately to severely dilated proximal and mid ureter. She underwent bilateral percutaneous nephrostomy which resulted in increase in UOP and decrease in SCr to 0.9 mg/dl.

Conclusions: Conventional imaging techniques such as CT and US are not diagnostic in non dilated obstructive uropathy. In a case series of 166 patients with obstructive uropathy requiring antegrade percutaneous nephrostomy placement, 7 patients had no dilatation on either CT or US. 6 of these patients had intrapelvic neoplasm. Other causes include retroperitoneal fibrosis, urolithiasis and pelvic surgery. Encasement of ureters in neoplastic or fibrous tissue, defect of ureteral peristalsis and intraureteral debris as well as edema have been proposed as possible mechanisms of this phenomenon. In our case, obstruction was secondary to the large intrapelvic malignant mass likely causing encasement of the ureters. What was unusual is the lack of oliguria that is typical of non dilated obstructive uropathy, which could be explained by the partial nature of the obstruction and the associated concentrating defect. The recognition of non dilated obstructive uropathy requires a high index of clinical suspicion as radiologic studies are non diagnostic in these cases.

PUB599

End Stage Renal Disease due to Fibrillary Glomerulonephritis in a Four Year Old Child M. Mandava, Senthilkumar Sankararaman, Sabeen Y. Habib. *Pediatrics, LSU Health Sciences Center, Shreveport, LA.*

Background: Fibrillary glomerulonephritis (FGN) is a very rare disease and only few pediatric cases have been reported so far. Most of the patients had persistent proteinuria and hematuria without progression to renal failure. This is an atypical case of FGN with rapid progression to ESRD with a coincidental family history of IgA nephropathy.

Methods: Four-year-old African-American male with no significant past medical history presented with a two weeks history of cold symptoms and cola colored urine. He has a strong family history of IgA nephropathy. On evaluation, he had macroscopic hematuria, severe proteinuria and a serum creatinine of 1.2mg/dl. Two days later his renal functions worsened with a serum creatinine of 3.3mg/dl and serum potassium of 6meq/L. Further labs showed ASO 1659, low C3, normal C4, normal ADAMTS 13, normal Factor H, negative serology and urine protein of 1.6gm/day. Renal biopsy showed coarsely granular capillary loops and mesangial deposits with IgG, C3 and fibrin in all glomeruli. Electron microscopy showed proliferative glomerulonephritis with fibrillary deposits. He did not improve with steroids and required peritoneal dialysis. Patient was then started on cytoxan. He demonstrated transient improvement in renal function and despite treatment he developed end stage renal disease within a month. He developed membrane failure and switched to hemodialysis.

PUB600

A Case of Fibrosing Cholestatic Hepatitis Following Renal Transplant Umbar Ghaffar, Nitin Relia, Mandeep Singh, Sundararaman Swaminathan. *Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Background: This case describes a novel diagnosis for acute hepatic failure in renal transplant population.

Methods: 64 year old male with history of IgA nephropathy s/p living donor kidney transplant, Gout, HTN, and CAD was admitted for jaundice and altered sensorium.His medications included Tacrolimus, Mycophenolate, Prednisone, Valganciclovir and Bactrim. Vitals were Temp: 97°F, HR: 48/min, BP: 131/77 mm Hg. Examination was significant for somnolence, scleral icterus, absence of asterixis and spider nevi. Abdomen: non-tender with good bowel sounds. Significant labs :Total bilirubin:12mg/dl, Direct bilirubin:7.4 mg/dl, AST:447 IU, ALT:548 IU, ALP:220 IU,Calcium:10.8 mg/dl, Albumin: 2.6 g/dl, PT:14.7 seconds, INR:1.2, serum amylase and lipase: normal, serum creatinine:0.9 mg/dl. All infectious and autoimmune workup was negative. No recent change in medications was reported. Hepatitis panel and HIV antibody were negative. Serum acetaminophen level was normal. Ultrasound showed fatty infiltration of the liver. Liver biopsy showed cholestatic hepatitis with neutrophilic cholangitis with negative CMV immunostain and no viral inclusions. Patient was discharged with improvement in liver functions. He was readmitted 5 months later with similar complaints. HIDA scan showed acute cholecystits leading to cholecystectomy. Repeat liver biopsy showed cholestatic injury, now with bridging fibrosis and early nodularity. Repeat hepatitis panel for Hep B and C and autoimmune work up was negative.CMV, EBV, HSV, Parvo and BK virus PCRs were also negative. Literature search revealed similar reported cases of cholestatic injury with fibrosis described in transplant population, an entity called Fibrosing Cholestatic hepatitis (FCH), attributable to overwhelming Hepatitis B or C virus infection following solid-organ transplantation,c hemotherapy,and immunocompromised states like HIV. Our patient's HCV PCR returned positive at >69 million copies.The donor tested negative for HCV. It is possible that patient had acquired Hepatitis C infection prior to transplant, but demonstrated a negative HCV antibody. He is currently awaiting liver transplant.

PUB601

Uretero-Colonic Diversion Causing Colonic Cancer and Obstructive Nephropathy in a 38 Year Old Man Ricardo Salas, Arksarapuk Jittirat, Ramy Magdy Hanna, Eduardo A. Lopez, Rajendra P. Niraula. *Olive View - UCLA Medical Center.*

Background: There are several complications associated with utero-colic diversions(UCD). Approximately 10% of patients with congenital bladder exstrophy(CBE) status post UCD develop renal failure and death. The most common complication is obstructive nephropathy secondary to anastomotic site stenosis. However, malignant tumors at the anastomosis have also been reported. The risk of malignancy is increased

by 100–200 times after 20-30 years of prolonged exposure to urine. Strict follow up and screening with sigmoidoscopy is important in early recognition of tumor and treatment to prevent further renal complications.

Methods: A 38 year old man with CBE who had a reconstruction surgery and UCD at 13 month old presented to the hospital with acute kidney injury(AKI) and bilateral hydronephrosis secondary to obstruction, requiring hemodialysis. He never had screening sigmoidoscopy in the past. Further evaluation revealed a mass at the site of UCD anastomosis. Biopsy showed poorly differentiated epithelial mesenchymal neoplasm–carcino sarcoma.

Three months later the patient was readmitted for severe anemia secondary to bleeding from the tumor. CT indicated an interval increase in the size of the mass with mesenteric lymphadenopathy. He was submitted to surgery, followed by chemotherapy. His hospitalization was complicated by infection, respiratory failure leading to his death.

Conclusions: Malignancies near the site of UCD anastamosis have been reported to be as high as 24% with half of these being adenocarcinomas. The pathogenesis of the tumor is thought to be secondary to the production of nitrosamines from nitrates and endogenous amines in the urine by bacteria in the colon and due to increased expression of CYP 450 isoenzyme 2E1. Complications of the tumor include obstructive nephropathy, AKI, nephrolithiasis and pyelonephritis.

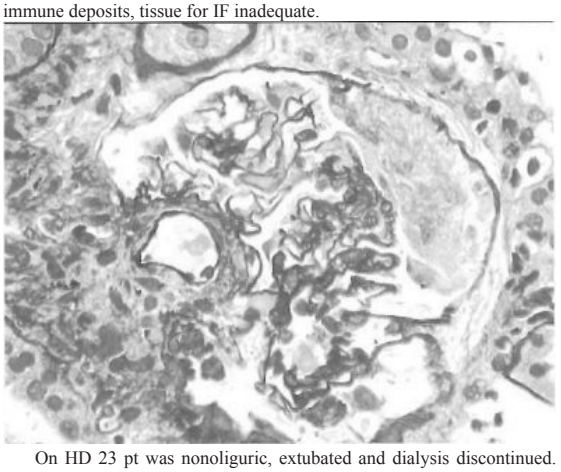
However, tumors at UCD anastomotic site are often overlooked and screening is not routinely done. Studies have shown that time of surgical diversion to the onset of tumor has a mean of 20 years. Screening with flexible sigmoidoscopy should begin 10 years after the initial operation and yearly thereafter.

PUB602

A Case of Dual Anti-Glomerular Basement Membrane Antibody and Myeloperoxidase-ANCA Crescentic Glomerulonephritis Hilda E. Fernandez, Brenda B. Hoffman, Raphael M. Cohen. *Univ of Pennsylvania, Philadelphia, PA.*

Background: Rapidly progressive glomerulonephritis (RPGN) in pts with both anti-glomerular basement membrane (GBM) antibodies and antineutrophil cytoplasmic antibodies (ANCAs) specific for myeloperoxidase (MPO-ANCA) have a better prognosis than anti-GBM glomerulonephritis.

Methods: 76 yo AAF with a h/o hypertension presented w/ acute shortness of breath. Given bloody secretions from ET tube and white-out of R side on CXR, emergent bronchoscopy performed, w/ no acute bleed or lesion. CT Chest consistent with diffuse alveolar hemorrhage. Acute kidney injury (AKI) was also noted w/ a Cr of 6.2 (baseline Cr 1.5) w/ gross hematuria, no dysmorphic RBCs. Pt on maximal ventilatory support and anuric within 48 hours. Due to concern for RPGN with pulmonary hemorrhage, pt was pulsed steroids and plasmapheresis initiated. Dialysis required for anuric AKI, volume overload, hyperkalemia. Serologic testing showed positive anti-GBM antibody, ANA, and pANCA/MPO. Plasmapharesis was continued daily for 7 days, then every other day for 7 more days. Repeat anti-GBM antibody negative after plasmapharesis. Cytoxan given on hospital day (HD) 7. Renal biopsy demonstrated a necrotizing crescentic RPGN, no EM immune deposits, tissue for IF inadequate.



On HD 23 pt was nonoliguric, extubated and dialysis discontinued. On HD 30 pt discharged w/ Cr of 3.7. Five days after discharge pt had further decrease in Cr to 3.2 w/ plan to continue prednisone taper and IV cytoxan therapy monthly for 6 months.

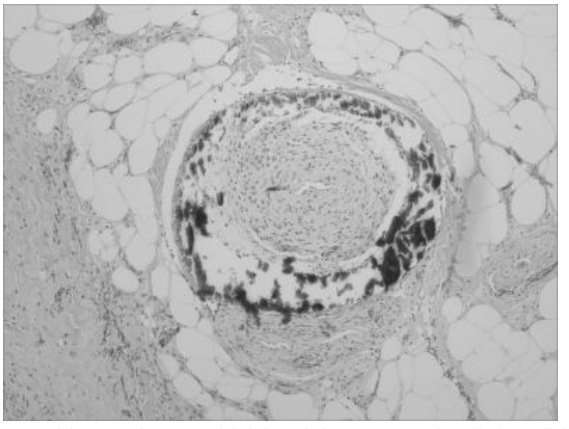
Conclusions: The literature suggests that pts w/ anti-GBM RPGN requiring dialysis do not recover renal function, but pts with dual positive anti-GBM and ANCA positive RPGN tend to have a better prognosis than w/ anti-GBM RPGN, though worse than w/ ANCA positive RPGN. The rapid initiation of therapy prior to diagnosis w/ serologic testing may have had a significant impact on this outcome.

PUB603

Between the Devil and the Deep Blue Sea Evamaria Anvari, Bijin Thajudeen, Preethi William, Mordecai M. Popovtzer. *Nephrology, Southern Arizona VA Health Care System, Tucson, AZ.*

Background: Calciphylaxis is a poorly understood disease with high mortality causing vascular calcification and skin necrosis.It occurs 1-4% of ESRD patients annually.

Methods: 63 yr old male evaluated for ulcerated, infected skin lesions of extremities .He was diagnosed with atherosclerotic disease one year ago and underwent angiography and endovascular intervention.Despite intervention skin lesions progressively worsened. Warfarin was initiated 10 months back for DVT.Past medical history include type 2 DM,hypertension and ESRD on PD.Medications include calcium acetate,calcitriol.Physcial exam showed dry gangrene of fingers and toes,multiple ulcers coated with dark eschar on hands, forearm, knee ,dorsum of foot and heel with feeble peripheral pulses in upper and lower extremities .Labs showed calcium 10.5 mg/dL, phosphorus 6.4 mg/dL,PTH 390pg/ml.CTA left upper extremity showed diffuse atherosclerotic calcifications and stensosis of brachial ,ulnar and radail arteries.He underwent ulnar artery exploration, patch angioplasty and finger amputations. Due to predisposing factors and distribution of ulcers, possibility of calciphylaxis was considered.A skin biopsy showed gangrenous necrosis of soft tissue with calcification of the subcutaneous arterioles.



Calcium supplements,calcitriol,warfarin were stopped .Daily hemodialysis with low calcium bath,non calcium based phosphate binders and thrice weekly intravenous sodium thiosulphate were initiated with some improvement in the skin lesions.Eventually he died of sepsis.

Conclusions: Diagnosis of calciphylaxis could be missed in presence of atherosclerotic peripheral vascular disease. A high index of suspicion is required especially in the back ground of predisposing factors.Simultanous management of calciphylaxis and peripheral vascular disease is essential.Initiation of warfarin could have precipitated the calciphylaxis.

PUB604

Fluid Removal in Hypertensive Pregnant Female on Dialysis Avoided Early Termination of Pregnancy Hiral V. Desai, Alekhya Potluri, Jacques Abi Rached, Ziauddin Ahmed. *Nephrology, Hahnemann Hospital, Philadelphia, PA.*

Background: Hypertension is a common complication in pregnant dialysis females leading to a high percentage of early termination of pregnancy due to possible preeclampsia. One of the reasons could be gradual fluid accumulation as fluid removal is done judiciously during pregnancy to avoid maternal hypotension for the fear of fetal compromise and to allow for the 1 lb weight gain a week. Here, we present case of a difficult to treat hypertension in a pregnant dialysis patient which was managed by aggressive fluid removal.

Methods: A 22 year old female with h/o of HTN, ESRD on HD was admitted at 26 wks gestation with complaints of shortness of breath and hypertensive urgency with BP of 170/90. Her outpatient dialysis prescription was 6 days/wk, 3 hrs each .She was allowed to gain 1 lb/wk in her 2nd trimester to account for the normal pregnancy weight gain. During the hospitalization she remained hypertensive with systolic BP ranging in the 150's-160 despite 2 maximally titrated BP medications. She had no edema and was at her dry weight. Lab studies were normal. Chest x-ray showed bilateral pulmonary congestion. Emergent control of her BP was necessary at this point to avoid early termination of pregnancy due to suspected preeclampsia. At this point a key decision was made to dialyze her with aggressive fluid removal challenging her dry weight. A fetal monitor was placed during HD to monitor for fetal distress and she was dialyzed daily with 3-4 liters ultrafiltrate per session. Her BP improved after 3 dialysis sessions and she was 6 kg below her dry weight. During aggressive fluid removal no sign of fetal distress was observed. She continued to be dialyzed throughout her pregnancy with fluid removal to target a systolic BP of 130's. She delivered a healthy baby at term.

Conclusions: We believe that fluid removal in this hypertensive pregnant woman to control BP even in the absence of edema did not harm the fetus. This may be due to effective auto regulation by the placenta. Aggressive volume removal to improve hypertension may have prevented early termination of pregnancy improving fetal outcome. A pilot study is required to substantiate this finding.

PUB605

Rhabdomyolysis Post Streptococcal Pharyngitis Neelima Chilukuri. *Nephrology, Richmond University Medical Center, Staten Island, NY.*

Background: We describe here the case of a severe rhabdomyolysis post streptococcal pharyngitis which has been reported as only two case reports in the past with group C streptococcal infection.

Methods: This is a case of a 23year old male who presented with generalized body pain and brown urine, he reported to have taken amoxicillin for five days for streptococcal infection as outpatient and started to feel body pains since then and so came to ER, only past

medical history was left shoulder surgery for recurrent dislocation two weeks ago, no h/o trauma,denies any illicit drug use, physical exam was essentially normal, his labs were wbc 17.3, Hgb/Hct 14.6/44, Na 136,K 6.0,BUN 12.6, Cr 1.0, Total CPK 843535.00,AST,2925, ALT 806, Urine analysis showed specific gravity 1.026, urine ph 5.5 protein >300,large blood,positive nitrate,large bilirubin,moderate leukocyte esterase, RBC 2-4, WBC 12-14, sediments moderate,C ANCA negative, P ANCA negative,C3-114, C4-31, ASO 261, LDH 18480, CRP 3.1,Hepatitis B and C non reactive, HIV non reactive, Urine drug screen Negative, Renal ultrasound was normal, patient was hemodynamically stable, patient was treated with normal saline @250cc/hr and 150meq of sodium bicarbonate and 20mg of furosemide, during the hospital course the total CK trended down to 13863 over 9 days and his creatinine remained stable at 0.7 to 0.8.

Conclusions: It is rare and interesting to see rhabdomyolysis after a streptoccoal pharyngitis with a very high total CK of 843535, in literature only few cases were reported with streptococcal C infection and the total CK was 35506.

PUB606

HIV Nephropathy: A Minimal Change Disease Perspective, and Glucocorticoid Treatment Dilemma in Immunocompromised States

Leonard Gyebi, Zohreh S. Soltani. *Nephrology, Lousiana State University, New Orleans, LA.*

Background: A 20-year-old transgender male with a one year history human immunodeficiency virus (HIV) was seen by the Nephrology consult for acute renal failure, proteinuria, and edema.

Methods: The patient was in his usual state of health up until 2 weeks prior to his admission, when he suddenly developed nausea, vomiting and abdominal pain. His review of symptoms was noted for penile and rectal cultures positive for human herpes simplex 2 (HSV2). RPR was negative. A day prior to admission Gonorrhea and Chlamydia cultures were positive in urine and rectum so, he had been treated with Rocephin IM and azithromycin and asked to follow up the next day. On follow up his creatinine had increased to 5.34 mg/dL from baseline 0.5mg/dl. At the time of consult his blood pressure was 148/86 with a heart rate of 100. He weighed 72kg and 173cm. He had generalized edema. Additional laboratory data included a creatinine of 11mg/dL and BUN 125 mg/dL, positive hepatitis B core and surface antibodies, negative surface antigen, CD4 count of 27 per cubic millimeter, and the viral load of 3,980,000 copies per cubic millimeter. His urine microscopy revealed hematuria, along with muddy brown cast. Urine protein to creatinine ratio was 10,000 mg/g. Renal ultrasound showed right kidney 12 cm and left kidney 12.4 cm. Serum electrophoresis and urine protein electrophoresis was unrevealing. Serum levels of IgA, IgM, complement levels of C3 and C4 were all within normal limits. Test for antinuclear antibodies, c-ANCA, p-ANCA and anti- GBM antibodies was negative. A renal biopsy demonstrated complete foot process effacement with microvillous transformation of the epithelial cells. Final diagnosis of minimal change disease, and acute tubular necrosis was made. Dialysis was initiated and tentative use of steroids after initiation of HAART out patient was favored.

Conclusions: While the most common HIV associated glomerular injury is focal segmental glomerular sclerosis this case is a reminder that the differential diagnosis related to common comorbid conditions is broad. This case also exposes some of the treatment dilemmas in use of steroids in the immunocompromised patients.

PUB607

Colchicine Induced Nephrogenic Diabetes Insipidus

Keith R. Bartolomei,[1] Abraham Cohen-Bucay,[2] Suzanne Saindon,[3] Geetha Narayan.[4] *[1]Nephrology, Tufts Medical Center, Boston, MA; [2]Medicine, St. Elizabeth's Medical Center, Boston, MA; [3]Medicine, St. Elizabeth's Medical Center, Boston, MA; [4]Nephrology, St. Elizabeth's Medical Center, Boston, MA.*

Background: Nephrogenic diabetes insipidus (NDI) is the resistance to antidiuretic hormone (ADH) resulting in a urinary concentrating defect. We present a case of NDI induced by colchicine.

Methods: 65-year-old male admitted for drainage of an idiopathic pericardial effusion. His medical history included a traumatic brain injury, bipolar disorder and seizures. He was on valproic acid, olanzapine, metoprolol and doxepin. He was started on colchicine 0.6 mg twice daily to prevent re-accumulation of pericardial fluid. Five days after starting colchicine he abruptly developed polyuria of 5-6 L/day. Initial serum creatinine (SCr), glucose and electrolytes were normal and obstruction was ruled out by ultrasound. Over 36 hours, his serum sodium (SNa) rose from 138 to 151 mEq/L. While hypernatremic, urine osmolality (Uosm) was 164 mOsm/Kg and plasma ADH was 0.9 pg/ml (normal <0.7 pg/ml). Administration of DDAVP did not change the Uosm or output. Simultaneously, SCr rose from 1.0 to 2.1 mg/dl as he became volume depleted. Repletion with hypotonic fluids resulted in normalization of SNa and SCr. Colchicine was stopped and within days his urine output decreased to 2 L/day, Uosm increased to 406 mOsm/Kg and SNa remained normal.

Conclusions: A diagnosis of NDI was made based on a low Uosm despite hypernatremia, a high plasma ADH and a dose of DDAVP. Acquired NDI is caused by infiltrating diseases, obstruction, metabolic disturbances and drugs. In our patient, all well-known causes of NDI were ruled out. Colchicine has been linked to NDI in two case reports. One case was reported after ingestion of a large dose of colchicine in a suicide attempt. A second case was seen in a patient taking colchicine for gout. Both patients improved after cessation of the drug. Colchicine has been found to inhibit ADH- and cAMP-mediated water reabsorption in the distal nephron in *in situ* studies, possibly leading to increased free water losses. Despite the absence of other causes, a chance association cannot be excluded.

PUB608

Progression towards End Stage Renal Disease in Primary Sjogren's Syndrome Complicated by Thrombotic Microangiopathy

Karthik Karanam. *Indiana University Hospital, Indianapolis, IN.*

Background: Kidney involvement in Sjogren's syndrome is characterized by interstitial nephritis, mild elevation in creatinine, distal RTA type I or nephrogenic diabetes insipidus. We present a case of primary Sjogren's syndrome presenting at CKD stage III and progressing towards ESRD secondary to the development of thrombotic microangiopathy not explained by other causes.

Methods: A 54 year old female with past medical history of Sjogren's syndrome (ANA +, SSA +, SSB +), B cell lymphoma 11 years back status post parotidectomy and radiotherapy, GERD, migraines, hypothyroidism and hypertension presented to the hospital with complaints of intractable nausea and emesis, associated with non blanchable purpuric spots on the hands and thighs. She was in oliguric acute renal failure, thrombocytopenia and had hemolytic anemia. Blood pressure was also high initially. Plasmapheresis was initiated as the initial thought was TTP/HUS. Later the ADAMTS 13 came back as normal, platelet counts were stabilized and the hemolytic picture resolved. The purpuric lesions were still present and the skin biopsy showed leucocytoclastic vasculitis. The patient's kidney function continued to deteriorate as she became more uremic and urine output was low. A kidney biopsy was done as the possibility of vasculitis was high. The biopsy showed acute and chronic thrombotic microangiopathy with mesangial sclerosis, moderately severe chronic tubulointerstitial nephritis, acute tubular necrosis and arteriosclerosis. High dose of furosemide was used to improve the urine output but in vain. Patient was started on hemodialysis because of uremia and increasing confusion. After 3 sessions of hemodialysis patient's condition improved and creatinine was back to the baseline. Other laboratory tests showed normal C3, low C4 which was chronic, negative ANCA, negative dsDNA and negative Smith antibodies. Cryoglobulins and SPEP were negative. After a year the patient again presented with same symptoms and high creatinine. This time she was started on hemodialysis and being presently continued on that.

Conclusions: This represents a rare case of thrombotic microangiopathy complicating the course of kidney involvement in a patient with Sjogren's syndrome.

PUB609

Beta Blocker Use in End Stage Renal Disease: Time to Consider One Agent over Another

Karthik Karanam. *Indiana University Hospital, Indianapolis, IN.*

Background: Many patients with ESRD on hemodialysis are on beta blocker therapy either to control hypertension or as a treatment for other co-morbid conditions such as congestive heart failure and myocardial infarction. The choice of the agent to use has not been extensively studied before. We report an ESRD case on hemodialysis about that particular choice and discussion about the further consideration.

Methods: A 67 year old white male with past medical history of hypertension, diabetes mellitus, ESRD secondary to diabetic nephropathy, diabetic neuropathy, stroke, depression and GERD came in with lower extremity redness and pain associated with diabetic ulcers. Patient was diagnosed with cellulitis and started on IV antibiotics. During the hospitalization it was found that his heart rate was in 50 – 60's at the time of admission and started to drop further. Patient got symptomatic with dizziness when his heart rate finally went to low 40's and reached 35 during the night. Review of his medications showed that he was on atenolol at 75 mg daily. The recommended dose of atenolol in ESRD patients on hemodialysis is 50 mg every other day with dose being given post dialysis. Because of the prolonged effects seen with atenolol and patient's blood pressure being normal and well managed with HD, the atenolol was stopped with gradual tapering. Patient was finally discharged to the extended care facility after his cellulitis started clearing. A week after discharge, patient again got readmitted for new onset atrial fibrillation. Patient was stabilized on cardizem drip and then anti coagulated. Later he was started on metoprolol twice a day and finally discharged. Patient to this day has been stable on metoprolol.

Conclusions: Atenolol is moderately dialyzable and has to be used cautiously in ESRD patients as its half-life is significantly prolonged in them from 6 hours to nearly 35 hours. Metoprolol on the other hand does not need to be adjusted according to renal function and undergoes significant hepatic metabolism with half-life being the same irrespective of kidney functions. Large size studies may be needed to exclusively study these both drugs in ESRD patients but theoretically at least metoprolol may have an edge over atenolol here.

PUB610

Unusual Facial Swelling as an Early Manifestation of Lupus Nephritis: Case Report

Abubakar Ibrahim. *Medicine, Nephrology Unit, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna, Nigeria.*

Background: Systemic lupus erythematosis is easily recognizable when it manifests classically. Nevertheless, confusion may arise when it presents atypically which can delay diagnosis. To highlight this issue, a young Nigerian woman was referred by her family practitioner with massive facial puffiness which was considered to be due to angioneurotic oedema or nephrotic syndrome.

Methods: A 23 year-old unmarried nigerian woman presented with isolated massive facial puffiness with no urinary or uremic symptoms. Her Family Practitioner had treated her for angioneurotic oedema to no avail and referred her with suspected nephrotic syndrome. Aside experiencing fatigue and joint pains, she offered no other relevant symptoms.

Pertinent findings on examination included facial swelling involving the lips and periorbital areas and mild 'malar' rash. Laboratory results revealed numerous dysmorphic red blood cells and red cell casts in her urine sediments, with no proteinuria. Full blood count showed ESR, 122 mm/hour (W-G method), positive 'LE cells', serum creatinine

98 umol/L. She commenced prednisolone therapy; within a few days there was complete disappearance of the facial swelling leaving behind exuberant malar rash. Kidney biopsy showed a histological pattern consistent with diffuse proliferative glomerulonephritis. She received azathioprine and prednisolone and Neoral. She developed neuropsychiatric symptoms, episodes of convulsions with progressive kidney dysfunction and died within 3 years of diagnosis.

Conclusions: Practitioners in the West African sub region should familiarize with varied features of lupus to facilitate early intervention.

This case typifies the challenges of evaluation and management of SLE in a resource-poor setting and highlights the implications on patients survival. Athough several studies indicate high prevalence of lupus in Blacks in USA and UK, data on the native populations of West Africa are inconsistent, with some studies reporting rarity of lupus while others showing high prevalence. It is conceivable that complementary tests for diagnosis and therapy of SLE are lacking due to their cost-intensive nature (human and material) and may have impacted on the disease course.

PUB611

Acute Membranoproliferative Glomerulonephritis in a Patient with Hepatitis B and Autoimmune Hemolytic Anemia Katharine Cheung,[1] Alan C. Pao.[1,2] *[1]Nephrology, Stanford University, Stanford, CA; [2]Nephrology, Palo Alto Veterans Hospital, Palo Alto, CA.*

Background: 57 year old male with a history of chronic hepatitis B infection, and schizophrenia presented with two weeks of fevers, nausea, emesis, abdominal pain, arthralgias and dark colored urine.

Methods: Six months prior he was diagnosed with autoimmune hemolytic anemia and treated with steroids and rituximab, which resulted in a partial response. Two years prior he had a lower leg skin biopsy that demonstrated leukocytoclastic vasculitis. Physical exam revealed hypertension, somnolence, abdominal distention, asterixis and sacral edema. Laboratory exam demonstrated a rise in serum creatinine to 3.26mg/dL from 1-1.5mg/dL and blood urea nitrogen 132g/dL. Urinalysis showed 2+ proteinuria and 3+ blood with red blood cell casts on microscopy. A spot urine protein/creatinine ratio was 2.37. Serologies were negative for ANA, ANCA, Anti-GBM, HCV and HIV. Hepatitis B viral load was greater than 110 million, complements were low, rheumatoid factor was greater than 100U/mL and cryoglobulin positive with 4% cryocrit. A renal biopsy revealed membranoproliferative glomerulonephritis. The patient was started on hemodialysis and tenofovir. He received high dose steroids and two weeks of plasmapheresis for the cryoglobulinemia, with subsequent decrease in his cryocrit percent to 1%, although without effect on his rheumatoid factor. His renal function recovered to a point where he no longer required dialysis.

Conclusions: Although this case illustrates a classic presentation of cryoglobulinemia, it is unique in that chronic hepatitis B infection was the underlying cause for the MPGN and cryoglobulinemia. Most viral cases of MPGN are related to hepatitis C, and there is limited published literature on the treatment of hepatitis B associated MPGN. It is notable that our patient presented with acute glomerulonephritis just a few months after steroids and rituximab. While rituximab is a treatment option for cryoglobulinemia, it has also been reported to accelerate immune complex deposition. We present a case of hepatitis B associated MPGN and cryoglobulinemia that was successfully treated with high dose steroids and plasmapheresis.

PUB612

Desmopressin, an Effective Adjunct Treatment, for Reversing Rapid Correction of Hyponatremia and Prevention of Osmotic Demyelination Syndrome Nadear A. Elmahi, Mehrdad Hamrahian, Bereket Alemu, Tibor Fulop. *Division of Nephrology, University of Mississippi Medical Center, Jackson, MS.*

Background: Osmotic demyelination syndrome (ODS) is a devastating consequence of rapid correction of chronic hyponatremia. Hypokalemia at presentation increases the risk of ODS further. Uncontrolled large free water diuresis could complicate correction of hyponatremia, once underlying etiology (e.g. nausea) is controlled. We present a unique case illustrating the added value of ADH analog in eliminating erratic polyuria and simplifying management of these patients.

Methods: A 50-year-old male was admitted to the intensive care unit after 3 episodes of seizure. He had nausea and vomiting for two weeks prior to admission and was drinking large amount of water and carbonated beverages in an attempt to control his chronic hiccups. Laboratory results on admission showed sodium 107 mmolL, potassium < 1.5 mmol/L, chloride < 60 mmol/L, bicarbonate 38 mmol/L, PH of 7.60 and a serum osmolality 217 mOsm/kg. He was treated with 3% saline and then maintained on 0.9% saline. After successful medical management of his nausea, he developed polyuria of 6 L over 8 hours with low urine osmolality of 60 mOsm/kg. His serum Na^+ increased to 126 mmol/L within 12 hours after admission. His free water deficit was replaced over 10 hours with target Na^+ of 120 mmol/L. Desmopressin was initiated at 1 mcg IV and then increased to 2 mcg IV twice daily. Few hours after initiation of desmopressin, his Na^+ dropped to 118 mmol/L and his urine output decreased to around 2 L/day. His sodium was then corrected by 2-3 mmol/L/day. His potassium was then corrected slowly, which helped in the correction of his metabolic alkalosis. At the time of discharge, the serum Na^+ was 128-130 mmol/L, hypokalemia was corrected and he had fully recovered without neurologic sequelae.

Conclusions: Overcorrection of hyponatremia is often blamed on overzealous correction with hypertonic IV fluid. The condition is often complicated by uncontrolled large free water diuresis. Use of ADH analog could provide a controlled correction of hyponatremia by limiting urine output and control unpredictable free water losses.

PUB613

Sporadic Gitelman Syndrome Complicated by Severe Vitamin D Deficiency and Hypocalcemia Noori Al-Waili, Mark D. Faber. *Nephrology, Henry Ford Hospital, Detroit, MI.*

Background: Gitelman syndrome is a rare autosomal recessive kidney disease. We report a case of sporadic Gitelman Syndrome. This 42 year old African American woman demonstrated all of the classical diagnostic criteria (recurrent severe hypomagnesemia, hypokalemia, hypocalciuria, volume depletion, and chondrocalcinosis) but without any family history. She also had severe hypocalcemia likely related to superimposed vitamin D deficiency.

Methods: The patient was admitted for syncope, generalized weakness and muscle cramps. BP was 130/80, and HR 116 bpm. Extremities were spastic with cog wheeling. She had carpopedal spasm. Lab showed severe hypomagnesemia (<0.4 mg/dl), hypocalcemia (4.6 mg/dl) and hypokalemia (2.2 mEq/L). EKG showed SVT. Serum creatinine was 1.85 mg/dl. Uric acid and CPK were normal. PMH was remarkable for frequent episodes of severe symptomatic hypomagnesemia since age 12 despite taking Mg supplements. FH in parents, sibling, and children (ages 16 and 22) was negative. Hypomagnesemia was often accompanied by hypokalemia and hypocalcemia. She experienced multiple episodes of volume depletion and hypotension. She developed stage 3 CKD (creatinine baseline 1.3-1.6 mg/dL) following an episode of NSAID related AKI, and had been diagnosed with gout and normocytic anemia. Hospital Course- Vitamin D was 5 ng/ml. Intact PTH was 409 pg/ml. Urinary calcium excretion was < 100 mg/day. She responded to electrolyte replacement, vitamin D, calcitriol, lisinopril, and amiloride. Wrist pain and swelling developed and responded to oral prednisone. X- ray showed chondrocalcinosis. At discharge she was asymptomatic and serum Ca, Mg, and K were normal.

Conclusions: Gitelman syndrome is a clinical diagnosis typically established by history and supporting laboratory findings. Genetic testing is not usually available. Establishing the correct diagnosis is important for proper management of various complications (e.g. CPP crystal arthropathy or pseudogout would not respond to xanthine oxidase inhibition), recognition of co-morbid conditions and possibly for genetic counseling. Hypocalcemia is not a hallmark of this disorder but was likely a manifestation of severe vitamin D deficiency.

PUB614

Membranous Nephropathy Associated with Sjögren's Syndrome Sabine Karam, Kevin A. Sterling. *Medicine, George Washington University Hospital, Washington, DC.*

Background: Tubulointerstitial nephritis with defects in tubular function is the most common renal manifestation of Sjögren's syndrome. However, glomerular disease can be rarely seen in this condition.

Methods: We present the case of a 34-year-old woman with history of allergic rhinitis who presented with increased weight gain, fatigue and lower extremity edema which worsened over a three month period. She also complained of both oral and vaginal dryness. She was found to have nephrotic range proteinuria with serological tests positive for ANA, RNP, and SS-A antibodies. Subsequent renal biopsy showed thickened capillary loops with moderately expanded mesangium on light microscopy (LM). Foamy histiocytes and a moderate lymphoplasmacytic infiltrate were also seen in the interstitium on LM. On immunofluorescence, capillary loop and possible mesangial granular staining patterns were seen with antisera specific for IgG (3+), IgA (2+), IgM (3+), C1q (1+), C3 (3+), kappa and lambda light chains (both 3+). Electron microscopy showed superficial subepithelial and large mesangial deposits with severe visceral epithelial cell foot process effacement. The patient was given the diagnosis of Sjögren's syndrome associated membranous glomerulonephritis. She was managed in collaboration with the Rheumatology service and was treated with steroids with reduction in her proteinuria.

Conclusions: Therefore, as is the case for other auto-immune conditions characterized by B cell hyperactivity and subsequent production of autoantibodies and immune complexes, glomerular involvement rarely occurs in Sjögren's syndrome. Routine screening of patients with Sjögren's syndrome for proteinuria might be appropriate.

PUB615

Treatment of Fatal Pulmonary Embolism in Hepatitis B Virus-Associated Membranous Nephropathy Guochun Chen, Yinghong Liu, You-ming Peng, Lin Sun, Fu-You Liu. *Renal Division, Kidney Research Institute of Central South University, Changsha, Hunan, China.*

Background: Patients with nephrotic syndrome (NS) are at increased risk of developing venous thrombosis. Embolism occurs when all or a portion of the thrombus breaks free and flows downstream in the circulation, blocking flow to vital organs. Although modern anticoagulant and thrombolytic therapies have been widely developed since 1960s, thromboembolism remains a life-threatening complication in NS. Here we report a case of NS with severe pulmonary embolism (PE) that was completely resolved by urokinase (UK) thrombolytic therapy.

Methods: A 39-year-old man presented with 2-day chest pain, hemoptysis and progressive dyspnea. He has been diagnosed 2 months earlier with nephrotic syndrome following intermittent generalized edema. Hepatitis B virus-associated membranous nephropathy was subsequently confirmed by renal biopsy. Ultrasonography revealed diffuse thrombi in his left superficial femoral vein and popliteal vein. On admission, the patient had marked respiratory difficulty, with a respiratory rate of 30 breaths per minute. Urgent chest X-ray showed decreased lung markings in the right lung hilum. CT angiogram confirmed the diagnosis of PE, showing filling defect in both left and right inferior pulmonary artery. Intravenous infusion of UK (20,000U/kg) for 2 hours was administered to the patient right

after the diagnosis of PE. The patient's respiratory status dramatically improved and his respiratory rate normalized to 20 breaths per min 24 hours after thrombolytic treatment. Repeated chest X-ray and computed tomography demonstrated resolution of PE 10 days later. The patient was discharged in good clinical condition 4 weeks later followed with continuous warfarin treatment. Prednisolone combined with tacrolimus was administered for nephrotic syndrome. A 3-month follow-up visit showed remission of nephrotic syndrome and no recurrence of PE.

Conclusions: Our case suggests that body weight adjusted UK-2 h regimen could be safe and effective for acute PE in NS. Further studies of randomised controlled trials are required to evaluate the short and long-term outcomes of UK thrombolytic regimens in NS.

Funding: Clinical Revenue Support

PUB616

Normokalemic, Normotensive Primary Aldosteronism Associated with Supra Physiologic Aldosterone Levels and Nephrocalcinosis Neha Garg, Jiwan K. Thapa, Surafel K. Gebreselassie, Emmanuel L. Bravo. *Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Primary Aldosteronism is an important cause of secondary hypertension and usually associated with hypokalemia, metabolic alkalosis and often times with resistant hypertension.

Methods: We report a case of 61 year old Caucasian woman with long standing history of medullary sponge kidney, bilateral nephrolithiasis and osteopenia who was found to have a 2.5 cm left adrenal mass on CT imaging in 2003. Three years later she developed new onset hypertension that was well controlled with triamterene and hydrochlorothiazide. More recently she had worsening diastolic blood pressure that prompted workup for secondary causes and subsequent referral to our center when found to have serum aldosterone > 400 ng/dl. Her initial serum studies showed: potassium 3.9 mmol/L, HCO3 28 mmol/L, creatinine 1.28 mg/dl and eGFR 48 ml/min. After an oral salt load, the 24 hour urine collection showed a urine sodium level of 296 mmol/24 hours with a blunted kaliuretic response (potassium 93 mmol/24 hours) despite high urine aldosterone level (231 ug/24 hours). Plasma renin activity was 5.7 ng/dl and plasma aldosterone 475.5 ng/dl. We evaluated the renal tubular function with 80 mg intravenous lasix challenge which showed only a 2 fold increase in fractional excretion of potassium despite a 7 fold increase in fractional excretion of sodium. Serum potassium before (3.9 mmol/L) and after (3.7 mmol/L) lasix were similar.

Conclusions: Regardless of high aldosterone levels, this patient had normokalemia and normotension at the time of initial diagnosis. Given her age and underlying kidney disease it is possible that subsequent onset of hypertension could have been from essential hypertension and possibly exacerbated by aldosteronism. We concluded that resistance to aldosterone action was at the level of distal tubules secondary to tubulointerstitial disease from nephrocalcinosis. This was supported by lack of adequate kaliuresis after salt load and lasix challenge. This illustrates that patients with primary hyperaldosteronism can have normal blood pressure and normal potassium if there is an underlying renal tubular disease.

PUB617

Merkel Cell Carcinoma and Squamous Cell Carcinoma in a Kidney Transplant Recipient Neil D. Ybanez,[1] Pamela C. Gibson,[2] Shirley Shwu-Shiow Chang.[1] *[1]Nephrology, University of Vermont, Burlington, VT; [2]Pathology, University of Vermont, Burlington, VT; [3]Hematology, University of Vermont, Burlington, VT; [4]Surgery, University of Vermont, Burlington, VT.*

Background: Merkel cell carcinoma (MCC) is a rare & aggressive neuroendocrine (NE) skin cancer. It occurs in 0.9% of transplant (tx) recipients with denovo skin cancers. MCC occurs from 5-286 months after the tx, affects older adults, usually involves the head & neck, and may be associated with other skin cancers. Risk of MCC is increased in kidney(K) tx recipients in whom it occurs at a younger age with a more aggressive clinical course.

Methods: A 56 yo man had living unrelated kidney tx in 1/2011 from ADPCKD. Patient (pt) was induced with thymoglobulin and maintained on Mycophenolic acid & Tacrolimus. BK viruria was dectected three months (mo)after his tx, progressed to BK viremia 10 mo post-tx, leflunomide was started and mycophenolic acid reduced. Pt noted a golf-sized lump in his left axilla 12 months post-tx, reported to tx center 2 mo later. 14 mo post-tx, left axillary mass bx showed mixed NE carcinoma (stained postively for CK20 conisistent with MCC), squamous cell carcinoma (SCC); tx k bx showed BK/polyoma tubulointerstitial nephritis. Work-up for MCC included CT of the chest, abdomen & pelvis without a primary neoplasm source. Mycophenolic acid was discontinued and renal function remained stable. Left axillary node dissection at 16 mo post-tx showed all 15 nodes + for metastatic MCC & SCC. Initial PET scan 15 mo post-tx showed left axilla lymph nodes uptake, numerous skeletal lesions; repeated PET scan 2 months later showed bone marrow metastatic disease progression. Pt received 15 radiation treatments, with plans for Imatinib instead of the usual regimen for high-grade NE tumors (platin & etoposide) due to potential toxicity to K allograft and CD117 + (c-kit expression). Pt was started on carboplatin & etoposide at 17 mo post-tx, died 11 days later.

Conclusions: MCC tends to be more aggressive in tx recipients, majority of whom have lymph node metastases, and about half die of their malignancy. MCC occurs in association with SCC or basal cell skin cancers or malignant melanoma.

PUB618

Complication after Embolization of a Renal Vascular Malformation Myung Hyun Lee, Hyun Chul Whang, Gun Hee An, Jeong Gwan Kim, Yul Hee Cho, Yong-Soo Kim, Chul Woo Yang. *Nephrology, St. Mary Hospital, Seoul, Korea.*

Background: A renal artery aneurysm (RAA) combined with arteriovenous fistula(AVF) in native kidney is so rare. Here, we present a case of left RAA with AVF, in which massive thrombosis developed after transarterial embolization(TAE) for the treatment of RAA with AVF, and therefore left nephrectomy was done.

Methods: A 60-year-old woman was referred for management of a left renal artery aneurysm diagnosed incidentally in regular medical checkups. She was asymptomatic and didn't have any concomitant morbidities and chronic diseases. Her physical examination and laboratory analysis were unremarkable except cardiomegaly on chest x-ray. Contrast-enhanced computer tomography(CT) showed a 2.4 cm diameter arterial aneurysm with AVF in upper pole of the left kidney and an associated 4.4 cm diameter aneurysm of the renal vein. We performed an embolization of renal vascular malformation.The next day, the patient had syncope and was pulseless. Her echocardiography showed right ventricular dilation and D-shaped left ventricle. Chest and abdomen enhanced CT showed a pulmonary thromboembolism(PTE) in right pulmonary artery and thrombosis in left renal artery and vein near embolization site. We planned a heparinization with IVC filter but we could not use an IVC filter because of mega IVC (3.2 cm). For the prevention of the development of recurrent thrombosis around embolized site in left kidney, we performed a left nephrectomy and started an anticoagulation with enoxaparine and warfarin. Three weeks after anticoagulation, she didn't complain of any chest discomfort and improved cardiomegaly compared to previous study was detected on follow-up chest x-ray and echocardiography. In follow-up chest CT performed after 3 weeks from the start of anti-coagulation, PTE was not observed any more.

Conclusions: In case of RAA combined with AVF, performance of TAE needs caution, because it can induce massive thrombosis around embolized site due to the development of venous stasis after procedure.

PUB619

Vancomycin Resistant Staph Aureus Niama Huda, Adepeju A. Jinadu, Sayed Husain, Vivek Soi. *Nephrology Department, Henry Ford Hospital, Detroit, MI.*

Background: Only a few cases of infection by *Staphylococcus aureus* with reduced susceptibility to vancomycin (vancomycin-intermediate *S. aureus* (VISA) or glycopeptideintermediate *S. aureus* (GISA) have been reported in the United States. We report a case of VISA infection.

Methods: The patient is an 83 year old female who presented after her hemodialysis session with fevers. Blood cultures were checked. Vancomycin and gentamycin were started empirically. The blood cultures were positive for Staphylococcus Aureus and endocarditis was diagnosed on echocardiogram. The source of the patients bacteremia was an infected arterio-venous graft (AVG).The patient was discharged per infectious diseases recommendations on intravenous vancomycin for two weeks after resection of the AVG.

Two months later, the patient presented with chills and back pain. Imaging revealed that the patient had L1-L2 discitis and osteomyelitis. Repeat blood cultures were drawn yielding VISA. Diagnosis of VISA infection was based on vancomycin breakpoints (vancomycin minimum inhibitory concentration 4–8 μg/ml) established by the Clinical and Laboratory Standards Institute (CLSI). The patient was then started on Ceftaroline and Bactrim for a total of 6 weeks.

Conclusions: VISA or/and VRSA (vancomycin resistant S.aureus) isolates remain rare and represent less than 0.3% of >300 000 *S. aureus* isolates in the United States.In the first four cases of VISA documented world wide three patients were dialysis patients. The three had poor clinical response to vancomycin therapy. These findings suggest that monitoring for colonization or infection with *S. aureus* with intermediate glycopeptide resistance may be warranted among patients who are often treated with vancomycin, such as patients on dialysis.

The widespread use of vancomycin resulted in the dramatic increase in the prevalence of vancomycin-resistant enterococci in U.S. hospitals may cause a similar increase in the prevalence VISA and GISA. VISA infection should be suspected in MRSA-infected patients who fail to respond to vancomycin therapy.

Infection-control and laboratory personnel should implement active surveillance for VISA and GISA in populations at high risk, such as patients on hemodialysis.

PUB620

Intractable Pain in Loin Pain Hematuria Syndrome from Thin Basement Membrane Disease Karthik Karanam, Richard N. Hellman. *Indiana University Hospital, Indianapolis, IN.*

Background: Loin pain hematuria syndrome is a poorly characterized disorder seen as a primary or secondary disorder. It is considered primary when it is associated with abnormally thin or thick glomerular basement disorder and secondary when it is associated with acquired glomerular disorders such as IgA nephropathy. We report a case of debilitating flank pain in primary loin pain hematuria syndrome.

Methods: A 40 year old female with past medical history of thin GBM disorder, chronic flank pain, CKD stage 2, hypertension and asthma was referred to the nephrology clinic for further management. Patient has chronic flank pain bilaterally with exacerbations of the pain on the left side more than the right. She is incapacitated with pain and cannot sit at work in her job as secretary. Significant family history included her son, diagnosed with the same disorder of thin GBM disorder. Physical exam was remarkable for hyperalgesia in her left

flank area. Work up in the past for recurrent pain was unrevealing for any musculoskeletal etiology or sciatica. Urine analysis showed microscopic hematuria with dysmorphic RBC. Ultrasound showed a 3 cm Bosniak class 2 cyst which has been stable for several years. Her kidney functions were stable for the past 10 years. She was on opiod, non –opiod analgesics, lidocaine patch, pregabalin and amitriptyline over many outpatient visits without improvement. She was on Lisinopril. She was followed at pain clinic; had a nerve block with partial improvement in pain and recurrence again. Later she was considered for a spine stimulator. She did not notice any improvement and had worsening of pain over the next couple of days. Work up for UTI was negative. Patient continues to stay in severe pain even to this day without effect from medications and interventions, significantly affecting her quality of life and work.

Conclusions: Thin GBM disorder has been rarely associated with loin pain hematuria syndrome and the intractable nature of the pain is rarely reported. The hypothesis is thought to be the occlusion of renal tubules with RBC's and back leak of filtrate causing stretching of the kidney and its capsule. Further treatment strategies for pain needs to be defined in this condition.

PUB621

A Case of Membranous Nephropathy Secondary to a Small Mucinous Cystic Neoplasm of the Pancreas Daniel J. Soberon,[1] Fernando E. Pedraza,[1] Ivonne Hernandez Schulman.[2] *[1]Division of Nephrology, University of Miami Miller School of Medicine, Miami, FL; [2]Division of Nephrology, Miami Veterans Administration Medical Center, Miami, FL.*

Background: A 73 year old male with hypertension and benign prostatic hypertrophy presented with worsening lower extremity edema, hypoalbuminemia, and intermittent hemoptysis.

Methods: CT scan incidentally found left renal vein thrombosis, right lower lobe pulmonary embolus, and pancreatic mass. MRI described a 1.6 cm T2 hyperintense nonenhancing cystic structure in the uncinate process of the pancreas. Laboratory studies revealed a serum creatinine of 1.3 mg/dL, and proteinuria of 5.8g per gram creatinine. Serum complements, serum and urine protein electrophoreses were normal. ANA, anti-dsDNA, ANCA, hepatitis B, hepatitis C and HIV serologies were negative. A CT guided kidney biopsy was performed. Light microscopy showed glomerular capillary wall thickening. Immunofluorecense was positive for +3 IgG, +1 IgA, +3 IgM, +3 C3, +1 C4, +2 C1q. Electron microscopy showed severe effacement of glomerular visceral epithelial cell foot processes and numerous subepithelial deposits. A diagnosis of secondary Membranous Nephropathy was made. Whipple procedure was performed and the uncinate lesion was resected with clean margins. The mass was a 2 x 2 x 1.5 cm mucinous cystic neoplasm consisting of predominantly pancreatic intraepithelial neoplasia with focal areas of micropapillary architecture (IPMN).

Conclusions: In this case of secondary membranous nephropathy, the patient endured a prolonged hospital course including refractory volume overload, acute on chronic kidney injury due to hemorrhagic shock, and eventual dialysis dependence. The patient has now been on dialysis for greater than 6 months. This is a dramatic disease course ultimately due to such a small tumor.

PUB622

Acute Renal Failure Associated with Hemophagocytic Lymphohistiocytosis Julie Ann T. Linatoc, Todd W. Gehr. *Virginia Commonwealth University.*

Background: Hemophagocytic Lymphohistiocytosis (HLH) is a rare but distinct condition caused by dysregulated activation of the immune system with infiltration of bone marrow and various organs by activated T-lymphocytes and histiocytes (macrophages). HLH could be due to inherited immune dysregulation, or secondary to infectious, neoplastic disease or immune deficiency state such as post transplantation.

Methods: A previously healthy 38-year-old woman was admitted to the hospital because of fever. On physical examination, blood pressure was 110/60 and febrile at 39.2. Heart and lung exam were normal. She had hepatosplenomegaly. There were no rashes. Laboratory studies showed white cell count 3,100 mcL, hemoglobin 8.2g/dL, platelet 32, 000/mcL, BUN 54 mg/dL, creatinine 3.4 mg/dL, AST 778 u/L, ALT 135 u/L. Urinalysis showed granular cast. Coagulation profile showed a prothrombin time (PT) of 27 s (normal: 9-11) and activated partial thromboplastin time (APTT) of 41 s (normal: 24–35). Fibrinogen level was <60 mg/dL.There was no evidence of hemolysis on peripheral smear. Cultures and serologic tests for infectious and autoimmune diseases failed to reveal any obvious source of fever. Both kidneys appeared normal and no hydronephrosis on ultrasound. She became anuric and was started on intermittent hemodialysis. Renal biopsy was not performed due to coagulopathy. Further evaluation showed elevated ferritin of 107,323 ng/mL. Bone marrow biopsy revealed frequent histiocytes with leukophagocytosis and erythrophagocytosis. She was initially treated with corticosteroids and cyclosporine which was later switched to IL-1 blockade with anakinra after she developed leukopenia from cyclosporine. Her kidney function recovered and has been on remission.

Conclusions: Our patient had fever, liver dysfunction, hepatosplenomegaly, pancytopenia, hypofibrinogenemia, hyperferritinemia and bone marrow morphology suggestive of HLH. Multi-organ failure can complicate this life-threatening condition and renal involvement has been reported with acute kidney injury due to tubular necrosis as common renal presentation. The possibility of HLH should be considered in febrile pancytopenia accompanied by acute kidney in the setting of malignancy, infection or immunosuppression.

PUB623

No Clear Consensus Regarding the Timing for Renal Biopsies Post Pre-Eclamptic Pregnancy Oyije S. Iheagwara, Kevin A. Sterling. *Division of Nephrology, George Washington University Hospital, Washington, DC.*

Background: Introduction: Persistent proteinuria after delivery in a preeclamptic patient is not uncommon. Watchful waiting for resolution is typical. Unfortunately, underlying renal disease may be present. Evidence based guidelines about the timing of biopsies for the evaluation of proteinuria post pre-eclampsia are lacking. Some argue that renal biopsies should be performed if proteinuria persists for more than 6 weeks to 3 months post-partum to allow for complete resolution of the renal lesions associated with preeclampsia. **Clinical Case:** We present a 42 year old Hispanic female with no known history of renal disease who was evaluated for persistent nephrotic range proteinuria 6 weeks after a pre-eclamptic pregnancy. A renal biopsy was performed at 4 months post-partum. Laboratory Studies: sCrt was 0.6mg/dl, hemoglobin of 12.1g/dl and a platelet count of 330,000.Serum albumin was 2.5g/dl, AST and ALT was 25 IU/L and 22 IU/L respectively. Urinalysis showed 3+ proteinuria but no blood. The urine sediment had no dysmorphic RBC or casts. UPC ratio was 16.1. HBsAg and HCV antibodies were negative. ANA was positive, but dsDNA antibodies were absent. Anti-centromere and anti-SSA antibodies were positive. C4 was normal, but C3 low. **Renal Biopsy:** Light microscopy revealed moderately thickened capillary loops. Tubular atrophy and interstitial fibrosis was absent. Immunofluorescence showed capillary loop staining for IgG(3+), IgA(2+), IgM(2+), kappa (2+) and lambda(3+). Staining was equivocal for c3 but absent for C1q. EM revealed numerous capillary loop deposits. Mesangial deposits were present with tubulo-reticular inclusions(TRI). Near full house staining, TRI and positive anti-centromere and SSA antibodies suggested Lupus Nephritis class 5 though other features suggestive of SLE were absent. Management: She initially received an ACEi with no improvement in proteinuria, then Prednisone and MMF were started.

PUB624

Successful Remission of Dense Deposit Disease with Plasmapheresis Sheena Sharma, Katarina Supe-Markovina. *Department of Pediatrics, Stony Brook Long Island Children's Hospital, Stony Brook, NY.*

Background: Dense Deposit Disease (DDD) is an extremely rare condition affecting 2-3 persons per million in the general population. The most common etiology is due to C3 nephritic factor (C3NF), an IgG autoantibody which stabilizes C3 convertase, resulting in uncontrolled activation of the alternative complement pathway. Remnants of C3 are deposited in the glomerular basement membrane causing injury and approximately 50% of patients develop end-stage renal disease within 10 years. Due to its rarity, there are no evidence based guidelines for the treatment of DDD. We present the case of a 15 year old Caucasian male with DDD secondary to C3NF who achieved remission with short-term plasmapheresis therapy.

Methods: Our patient presented with gross hematuria and proteinuria. A native kidney biopsy revealed DDD. Components of the complement cascade were measured. C3 was found to be low and C3NF was elevated. In order to remove the pathologic antibody, pheresis treatments were initiated. Treatments were performed with the Cobe Spectra Collect Flow Path using citrate dextrose containing heparin for anticoagulation and 5% albumin as replacement fluid. Our patient had a total of 18 treatments over 6 months; weekly for 3 months then bimonthly for an additional 3 months. C3 levels and proteinuria were monitored bimonthly, and C3NF was monitored prior to the initiation of treatments, mid-way through treatments, and following cessation of treatments.

Results: C3 levels and proteinuria normalized after 6 months of plasmapheresis. C3NF initially decreased while receiving treatments, but increased once stopped. Despite this, he remains in clinical remission defined as urine protein/creatinine <0.5 on first morning void, normal estimated GFR, normotensive, and normal serum C3.

Conclusions: We have shown that plasmapheresis is a safe and effective method for inducing remission in patients with DDD secondary to C3NF. The efficacy may be due to removal of the pathologic antibody. The persistent elevation of C3NF after pheresis could possibly be explained by change in epitope or anti-idiotypic antibodies which should be explored in the future.

PUB625

Temporary Depletion of Regulatory T Cells Exacerbates Cytokine Release and Progression of Spontaneous Autoimmune Nephritis in BIM Knockout Mice Yuan Min Wang,[1] Geoff Yu Zhang,[1] Ya Wang,[2] Min Hu,[2] Jimmy Jianheng Zhou,[1] Andrew Sawyer,[1] Mitsuru Saito,[1] Tania Polhill,[1] Qi Cao,[2] Yiping Wang,[2] Guoping Zheng,[2] Vincent W.S. Lee,[2] David C. Harris,[2] Stephen Alexander.[1] *[1]Centre for Kidney Research, Children's Hospital at Westmead, University of Sydney, Sydney, NSW, Australia; [2]Centre for Transplant and Renal Research, University of Sydney at Westmead Millennium Institute, Sydney, NSW, Australia.*

Background: Bim is a pro-apoptotic member of the B-cell lymphoma 2 (Bcl-2) family, and is an inducer of programmed cell death and stress-induced apoptosis. Bim knockout (Bim-/-) mice fail to delete autoreactive T cells in the thymus, leading to spontaneous autoimmune renal disease. The aim of the study is to evaluate the role of regulatory T cells (Tregs) in spontaneous immune nephritis.

Methods: We evaluated the role of Tregs in glomerulonephritis in Bim-/- mice by depleting Tregs in two month old mice using PC61, an anti-CD25 monoclonal antibody. At six months of age, renal function, histologic injury, serum cytokines and in vitro Treg functional assessment were performed.

Results: Treg depletion was associated with increased proteinuria, worse renal function, weight loss and worse histological injury characterised by glomerular scarring, antibody deposition and tubular damage. There was a marked increase in interstitial infiltrate comprising T cells and macrophages. Serum cytokines IL-2, IL-4, IL-6, IL-10, IL-17a, IFN-γ and TNF were significantly increased after Treg depletion in Bim-/- mice. Tregs from Bim-/- mice demonstrated less suppressive function than C57BL/6 wild-type Tregs in vitro.

Conclusions: Temporary loss of Tregs allows effector T cell activation of all classes leading to worsening of nephritis in this spontaneous model.

Funding: Government Support - Non-U.S.

PUB626

Activation of Innate Immunity in Children with Henoch-Shoenlein Purpura: Toll-Like Receptors in Circulating Mononuclear Cells

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Background: Henoch-Schoenlein purpura (HSP) in children is often triggered by mucosal infections and presents with a benign course with rapid resolution of purpura and transient hematuria and proteinuria. We aimed at investigating the activation of innate immunity and the relationships with the regulatory T (Treg) cells and proinflammatory Th17 cells.

Methods: In 42 children with HPS (aged 3-14 years) and 35 healthy controls peripheral mononuclear cells (PBMC) were isolated by centrifugation gradients. Real time PRC (Taqman) was used to to measure in PBMC mRNA expression of Toll-like receptors (TLR) TLR2, TLR3, TLR4, TLR9 and of regulation-associated genes of Treg including forkhead box P3 (Foxp3), Th17-related factors (IL-17), retinoid orphan nuclear receptor (RORc), and TFG-β1 which modulates the differentiation of Th17. Values were normalized using Abelson housekeeping gene mRNA and expressed as fold changes.

Results: PBMC ofchildren with HSP had, in comparison to healthy controls (HC), significantly increased expression of mRNA encoding for TLR 2 (2.83 ± 2.57 vs 1.42± 0.77 in HC, P=0.042) and TLR4 (2.17 ± 1.63 vs 1.37± 0.73 in HC, P= 0.011), while showed a significantly decreased expression of mRNA encoding for TGFβ1 (0.89 ± 0.58 vs 1.44 ± 0.5 in HC, <0.0001). A significant correlation was found between TLR4 and TGFβ1 mRNAs (p=0.033) and between TLR4 and foxp3 mRNAs (P< 0.05). No differences were observed in cases with systemic purpura with or without renal involvement.

Conclusions: In children with HSPwe observed a significant activation of innate immunity, with particular engagement of TLR4, which was correlated with down modulation of factors favouring the differentiation of Th17. This changes are coincident with the benign course of HSP in the children investigated.

PUB627

Regulatory T Cells (TREG) and Tryptophan/Kynurenine Pathway in IgA Nephropathy

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Background: In IgA nephropathy (IgAN) a dysregulation of the immune system is likely to lead to persistent T cell response after exposure to common mucosally encountered antigens. In these patients we aimed at investigating regulatory T-cells (Treg), which pay an essential role in the negative regulation of immune response. We assessed also the activity of the ancestral metabolic enzyme indoleamine 2,-dioxygenase (IDO), an inducer and amplifier of Treg functions.

Methods: PBMC were isolated from 54 patients with IgAN (28.4±21 y.o.), with e-GFR 96.8±42 ml/min and proteinuria 0.62 ±1.19 g/day, and from 30 healthy controls (HC). We measured by Taqman the mRNA expression of the Treg regulation-associated gene forkhead box P3 (Foxp3) and we assessed IDO activity effects as change in the ratio between tryptophan (Trp) and its catabolic product kynurenine (Kyn) (isocratic RP HPLC with UV detection).

Results: The transcriptional level of Foxp3 was significantly lower in patients with IgAN versus HC (0.90±0.51 vs 1.26±0.65, P= 0.0014). Trp levels were similar in IgAN patients and HC, but Kyn, its catabolic product, resulted significantly increased (2.47 ± 0.60 vs 2.02 ± 0.32, P= 0.0012) and so was the Kyn/Trp ratio (5.03±1.65 vs 3.72±0.55 in HC, P= 0.0016). No correlation was found with proteinuria levels, nor with eGFR values. No correlation was found with possible TReg modulator factors, including Toll-Like Receptors (TLR 3,4 and 9).

Conclusions: In patients with IgAN there is a defective transcription of mRNA encoding for Foxp3 hence a likely defective Treg activity, in spite of hyperactivity of the IDO enzyme which is expected to trigger Tregs. Environmental cues have been reported to induce Foxp3 Treg to undergo phenotypic changes, including conversion, plasticity or reprogramming to rapidly adopt a pro-inflammatory phenotype. The mechanisms inhibiting Tregs in IgAN in spite of IDO activation deserve further investigation.

PUB628

Renal Cell Snail Expression Determines Vitamin D Receptor Status in HIV Milieu

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Background: HIV transgenic mice have been demonstrated down regulation of renal tissue vitamin D receptor (VDR) and activation of the renin angiotensin system (RAS). However, the involved mechanism of down regulation of VDR is not clear. Since VDR is a negative regulator of the RAS it may be playing an important role in the development and progression of HIV-associated nephropathy (HIVAN). Therefore, it may be important to explore the involved mechanism of HIV-induced down regulation of VDR. We hypothesized that Snail family of proteins could be down regulating renal cell VDR in HIVAN.

Methods: Renal tissues of four weeks old, age and sex matched control and Tg26 (HIV transgenic mice) in groups of six were evaluated for protein and mRNA expression of Snail, VDR, renin and angiotensinogen. Renal cortical sections of control and Tg26 mice were labeled for Snail and VDR and examined under an immunofluorescence microscope. In *in vitro* studies, human podocytes (HPs) and human proximal tubular cells (HPTCs) were transduced with either empty vector or NL4-3 (HIV) constructs and assayed for expressions (protein and mRNA) of Snail and VDR. Both HPs and HPTCs were also transfected with siRNA-Snail and scrambled siRNA and then probed for Snail and VDR expression.

Results: Renal tissues of control mice showed only minimal expression of Snail, whereas, renal tissues of Tg26 mice displayed moderate expression of Snail. In renal cortical sections of Tg26 both podocytes and tubular cells displayed expression of Snail but attenuated expression of VDR. Renal cells in control mice displayed robust expression of VDR but attenuated expression of Snail. Renal cortical sections of Tg26 mice also displayed enhanced expression of renin. In *in vitro* studies, both HIV/HPs and HIV/HPTCs displayed higher expression of Snail and attenuated expression of VDR when compared to EV/HPs and EV/HPTCs. HIV/HPs and HIV/HPTC silenced for Snail displaced enhanced expression of VDR.

Conclusions: These findings indicate that HIV may be downregulating VDR expression in HIVAN mice by upregulating renal cell expression of Snail.

Funding: NIDDK Support

PUB629

Hyperglycemia Up Regulates Snail and Epithelial Mesenchymal Transition in HIV-Associated Nephropathy

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Background: Epithelial mesenchymal transition (EMT) has been reported to contribute to the progression of renal lesions in HIV-associated nephropathy (HIVAN) as well as in diabetic nephropathy. We hypothesized that HIVAN patients with hyperglycemia are likely to have accelerated EMT. We further hypothesized that hyperglycemia may be inducing EMT in kidney cells of HIVAN patients by enhancing renal cell expression of Snail, a repressor of E-cadherin transcriptor and negative regulator of vitamin D receptor (VDR). Conversely, VDR being a negative regulator of renin-angiotensin system (RAS), would further enhance EMT in HIVAN patients.

Methods: Four weeks old control (C) and Tg26 (HIV) mice (n=4) were either administered vehicle or streptozotocin (STZ, 150 mg/Kg, intraperitoneal, single dose). At the end of two weeks, mice were sacrificed. Kidneys were harvested for renal histology, imunohistochemical, and immunoblotting studies for Snail, proliferating cell nuclear antigen (PCNA), a-SMA, fibroblast specific protein (FSP1), and E/P-cadherin, and VDR. In *in vitro* studies, empty vector (EV) and HIV (NL4-3) constructs transduced human podocytes (EV/HP and HIV/HP) and tubular cells (EV/HPTC and HIV/HPTC) were incubated in media containing either 5 mM or 30 mM glucose for 24 hours. Immunoblots were probed for Snail, PCNA, a-SMA, FSP1 and E/P-cadherin and VDR.

Results: Renal cells in HIV alone displayed enhanced expression of Snail, PCNA, a-SMA, and FSP1 and down regulation of E/P-cadherin; Interestingly, renal cells of HIV-STZ displayed higher expression of Snail, PCNA, "-SMA, and FSP1 and more down regulation of E/P-cadherin and VDR when compared to HIV alone. Western Blot studies and real time PCR studies further confirmed these findings. HIV/HPs and HIV/HPTCs treated with high glucose also displayed enhanced EMT markers and downregulation of VDR when compared to HIV/HPs and HIV/HPTCs treated with normal glucose.

Conclusions: These findings indicate that hypeglycemia accelerates the progression of HIVAN by enhancing EMT via upregulation of Snail and down regulation of VDR.

Funding: NIDDK Support

PUB630

The Accumulated VH Replacement Products Contribute to the Generation of Auto/Polyreactive Antibodies in Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is characterized by the over-production of high affinity autoantibodies. However, it is not clear how these autoantibodies are generated and selected in SLE patients. V_H replacement is originally considered as a receptor editing process to change unwanted non-functional immunoglobulin heavy chain (IgH) genes or IgH genes encoding self reactive BCRs. Our sequence analysis show that V_H replacement products are highly enriched in IgH genes derived from different autoimmune diseases, including SLE.

Methods: To gain insight into the mechanisms responsible for autoantibody generation in SLE, we performed single cell RT-PCR analysis to study the functional antibody repertoires in active SLE patients.

Results: Analysis of 180 recombinant antibodies derived from SLE patients showed that 19.0% of the antibodies derived from naïve B cells and 56.9% of the antibodies derived from plasmablasts are autoreactive. In contrast, only 7.7% of the antibodies derived from naïve B cells and 17.4% of the antibodies from plasmablast B cells are autoreactive in healthy donors. Detailed sequence analysis revealed that V_H replacement products are significantly increased in IgH genes obtained from SLE patients (20%) compared to that in healthy controls. Importantly, the accumulated V_H replacement products in SLE patients have long CDR3 regions enriched with positively charged amino acids; 74% of them directly encode anti-nuclear antigen or polyreactive antibodies.

Conclusions: Based on these results, we conclude that the accumulated V_H replacement products in SLE patients contribute to the generation of auto/polyreactive antibodies in SLE.

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PUB631

Combined C3b and Factor B Autoantibodies and MPGN II Peter F. Zipfel,[1] Qian Chen,[1] Christine Skerka,[1] Dominik Müller.[2] *[1]Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Thueringen, Germany; [2]Pediatric Nephrology, Charite Berlin, Berlin, Germany.*

Background: Membranoproliferative glomerulonephritis type II, dense deposit disease is a rare glomerular disease. MPGN II is complement-associated, due to systemic C3 activation and C3 deposition along the GBM. Genetic causes include mutations in the *factor H* and *C3* genes, which cause C3 convertase deregulation. Also autoantibodies, such as C3 nephritic factor (C3Nef), which stabilize C3 convertase have been identified in 50-80% of MPGN2 patients. Single cases with autoantibodies to Factor B and Factor H were described. There is currently no effective treatment for MPGN II, and the prognosis for survival of a kidney transplant is poor.

Methods: We now report combined C3b and Factor B autoantibodies in two unrelated patients, an eight-year-old girl with MPGNII and ESRD and a 20-year-old man with MPGN. Both patients lacked C3Nef but presented autoantibodies to the two individual components of the C3 convertase, i.e. C3b and Factor B. These autoantibodies enhance C3 convertase activity.

Results: Based on the diagnosis of autoimmune MPGN2 weekly plasma exchanges and immunosuppressive therapy (rituximab (375 mg), tacrolimus (10 ng/l), mycophenolate mofetil (2.0 ng/l) and glucocorticoids (1 mg/kg)) was administered to the 8-year-old patient, and her C3b autoantibody levels declined (0.8 to 0.4 arbitrary units (AU)), and her Factor B autoantibody levels decreased Her Ba activation fragment dropped to background levels, and C3 plasma levels increased to normal values. She was transplanted and plasma-exchange was performed every other day prior to and for three weeks after transplantation, then was continued weekly for eight weeks after transplantation (week 4-12), and then every other week for four more weeks (week 13-16). Serum-creatinine was 0.7 mg/dl, and proteinuria was absent one week after transplantation.

Conclusions: 30 months after transplant, she is stable and without disease recurrence. This autoimmune form of MPGN shows that in addition to C3Nef further autoantibodies develop in MPGN2 that lead to complement deregulation that requires targeted treatment.

Qian Chen New England J Medicine 2011, 365, 2340.

PUB632

The CCL1-CCR8 Axis Drives the Recruitment of Inflammatory Mononuclear Phagocytes and Tissue Injury in Murine Experimental Glomerulonephritis Erik M. Disteldorf,[1] Hans-Joachim Paust,[1] Oliver M. Steinmetz,[1] Anett Peters,[1] Sabrina Bianca Bennstein,[1] Hans-willi Mittrücker,[2] Rolf A. Stahl,[1] Ulf Panzer.[1] *[1]Nephrology, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; [2]Immunology, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany.*

Background: Human and experimental forms of crescentic glomerulonephritis are characterized by the recruitment of leukocyte subsets that cause renal tissue damage. Infiltration of leukocytes upon renal injury is substantially affected by chemokines. CCR8 is expressed on T cells and mononuclear phagocytes, which are found to accumulate within the inflamed tissue in large amounts. However the functional role of CCR8 and its ligand CCL1 during crescentic glomerulonephritis remains currently unclear.

Methods: Experimental crescentic glomerulonephritis (nephrotoxic nephritis - NTN) was induced in CCR8-deficient and WT mice.

Results: Induction of nephritis resulted in an up-regulation of renal CCL1 and CCR8 mRNA expression. CCR8-deficient mice developed an ameliorated course of disease in terms of lower BUN levels and significantly reduced glomerular and tubulointerstitial damage. Analysis of cellular infiltrates by flow-cytometry revealed a markedly reduced number of mononuclear phagocytes ($CD11b^+F4/80^+$) to be present in the inflamed tissue compared to WT nephritic mice. In line, immunohistochemical examination clearly showed less $F4/80^+$ mononuclear cells within the renal cortex of nephritic CCR8 deficient mice. Intracellular staining showed a considerable positivity for TNF-α, underscoring a proinflammatory phenotype of the renal $CD11b^+F4/80^+$ mononuclear phagocytes. Moreover, renal damage and infiltration of mononuclear phagocytes could be restored by adoptivley transferring CCR8 expressing, bone-marrow derived CD115+ monocytes during experimental injury.

Conclusions: In conclusion, our study shows that the CCL1- CCR8 orchestrates renal recruitment of pro-inflammatory mononuclear phagocytes and tissue injury during experimental glomerulonephritis. Thus, CCR8 might be a promising new therapeutic target in human proliferative and crescentic glomerulonephritis.

PUB633

LPS-Contamination May Contribute to FGF-23 Induced *In Vitro* Monocyte Activation Adam M. Zawada, Aurelia Luthe, Sarah Seiler, Kyrill S. Rogacev, Danilo Fliser, Gunnar H. Heine. *Dept. of Internal Medicine IV, Saarland University Medical Center, Homburg, Saarland, Germany.*

Background: CKD patients display proinflammatory activation of monocytic cells. Moreover, monocyte subset distribution is skewed towards proinflammatory subsets (CD14++CD16+ and CD14+CD16++ monocytes). This phenomenon is unexplained so far. Recently, FGF-23 has been reported to influence monocyte function, e.g. their anti-microbial capacity; however the effect on monocyte subsets has not previously been reported.

Methods: Human monocyte subsets were stimulated for 5 h *in vitro* with rFGF-23 (0; 0.25; 0.5; 1; 10; 50 ng/ml) which was either derived from *E.coli* (Alexis Biochemicals), or from mouse-myeloma-(NS0)-cells (R&D). Formation of proinflammatory CD14++CD16+ and CD14+CD16++ monocytes and their activation status – i.e. CD86 expression – was analysed flow-cytometrically; the production of ROS was measured by electron-spin-resonance.

Results: Stimulation of monocytes with rFGF-23 from *E.coli* significantly activated proinflammatory monocyte subsets and increased their CD86 expression ($p < 0.001$), whereas rFGF-23 from NS0-cells did not. LPS-stimulation of monocytes had a comparable effect as rFGF-23 from *E.coli*.

Conclusions: Activation of monocytes after short-term (≤ 5 h) *E.coli*-rFGF-23 stimulation is likely to be an artifact due to LPS-contamination. Since CKD-patients are chronically subjected to elevated FGF-23 levels, future studies should evaluate potential long-term effects. For such studies, we advocate the use of non-*E.coli*-rFGF-23 as monocytes are extremely sensitive to LPS-contamination.

PUB634

Human Factor H-Related Protein 2 (CFHR2) Regulates Complement and Is Affected in a Patient with MPGN I Hannes Uwe Eberhardt,[1] Qian Chen,[1] Markus J. Kemper,[2] Teresia Hallström,[1] Peter F. Zipfel,[1] Christine Skerka.[1] *[1]Infection Biology, Leibniz Institue for Natural Product Research and Infection Biology, Jena, Germany; [2]University Hospital Hamburg, University Hospital, Hamburg, Germany.*

Background: Membranoproliferative glomerulonephritis type I (MPGN) is a severe kidney disease characterized by subendothelial and glomerular basement membrane deposits containing immune complexes and complement activation products. Previous studies in knock out mice demonstrated a critical role of iC3b, a C3 cleavage product, for MPGN development.

Results: Here we describe a MPGN I patient with a stop mutation in exon 4 of the *CFHR2* gene and severely reduced CFHR2 plasma levels. This CFHR2 deficiency suggests to be linked to disease pathology as we identify CFHR2 as novel human complement regulator, that inhibits C3 convertase activity, which is continuously activated in this patient.

Conclusions: Thus deficiency of the novel human regulator CFHR2 under conditions of enhanced complement activation likely leads to accumulation of inflammatory iC3b at the glomerular basement membrane and pathology. These results shed light on the underlying pathomechanism of MPGN I and may have implications for therapy.

PUB635

Effects of L-Carnitine on Interstitial Fibrosis and Inflammation in Chronic Cyclosporine Nephrotoxicity in Rats Can Li,[1] Ying Xiang,[1] Jing Hao Cui,[2] Hong Bin Zou,[3] Ying Shun Jin,[1] Shang Guo Piao,[4] Bi Hu Gao,[5] Chul Woo Yang.[4] *[1]Nephrology, YanBian University Hospital, YanJi, JiLin, China; [2]Control Release of Drug Delivery System, SuZhou University, SuZhou, China; [3]Nephrology, The First Affiliated Hospital of JiLin University, ChangChun, JiLin, China; [4]Nephrology, The Catholic University of Korea, Seoul, Korea; [5]Nephrology, The Affiliated Zhong-Shan Hospital of DaLian University, DaLian, China.*

Background: L-carnitine has been shown to prevent allograft dysfunction and cyclosporine induced lipid peroxidation; however, its effects on the kidney with chronic cyclosporine nephrotoxicity are undetermined. The aim of this study was to investigate whether concomitant treatment with l-carnitine attenuates interstitial inflammation and fibrosis in an animal model of chronic cyclosporine nephrotoxicity.

Methods: Male adult Sprague-Dawley rats kept on a slat-depleted diet (0.05% sodium) were treated daily for 28 days with vehicle (olive oil 1 mL/kg s.c), cyclosporine (15 mg/kg s.c), l-carnitine (50 mg/kg or 200 mg/kg i.v), and both cyclosporine and l-carnitine. Body weight, renal function, histomorphology (tubulointerstitial fibrosis and ED-1-positive cells), and the expression of transforming growth factor (TGF)-β1 and apoptotic cell death were compared for different treatment groups.

Results: CsA-treated rats showed decreased renal function and increased histomorphological parameters compared with VH-treated rats. Concomitant administration of l-carnitine significantly improved renal function and histomorphological parameters (all $P < 0.05$). At the molecular level, the increased TGF-β1 expression and TUNEL-positive cells seen in CsA-treated rat kidneys were markedly decreased after l-carnitine treatment in a dose-dependent manner.

Conclusions: These findings suggest that concomitant treatment with l-carnitine prevents CsA-induced renal interstitial inflammation and fibrosis through suppressing apoptotic cell death and TGF-β1 expression in chronic cyclosporine nephrotoxicity.

PUB636

Abelmoschus Manihot (L.) Medic, a Compound of Chinese Herbal Medicine, Ameliorates Adriamycin-Induced Glomerular Inflammatory Damage via Down-Regulating p38MAPK Expression in Rats Yue Tu, Wei Sun, Yigang Wan. *[1]Department of Graduate School, Nanjing University of Chinese Medicine, Nanjing, China; [2]Jiangsu Provincial Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China; [3]Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China.*

Background: *Abelmoschus manihot (L.) Medic* (AM) is widely used for chronic kidney disease (CKD) in China. Increasing evidence suggests that AM can significantly reduce proteinuria and improve glomerulosclerosis. Given a rich literature identifying glomerular inflammatory injury as an important step in the pathophysiological progression to glomerulosclerosis, we hypothesized that AM ameliorates glomerular lesions from undergoing inflammatory injury.

Methods: A rat model of glomerulosclerosis created by unilateral nephrectomy and twice intravenous injections of adriamycin. Rats in sham-operation and vehicle group received distilled water, while the ones in AM-treated group received AM for 4 weeks. Proteinuria, immunofluorescence (IF) staining of macrophages (ED1+ and ED3+ cells), α-smooth muscle actin (α-SMA) and collagen type I, glomerular morphological changes, and the protein expressions of TGF-beta1, p38MAPK and phosphorylated-p38MAPK (p-p38MAPK) were evaluated, respectively.

Results: ADR induced massive proteinuria, intense staining of α-SMA and collagen type I elevation, as well as glomerulosclerosis in ADR-nephropathy (ADRN) rats, which were associated with up-regulation of TGF-beta1, an essential molecule for glomerulosclerosis. Moreover, ADR induced glomerular inflammatory damage, as a result of infiltrated ED1+ and ED3+ cells, which was correlated with activation of p-p38MAPK, a key molecule in the inflammation-related p38MAPK-signaling pathway. Compared with ADRN rats, AM significantly prevented proteinuria elevation, alleviated glomerulosclerosis and glomerular inflammatory damage via down-regulating the expressions of TGF-beta1 and p-p38MAPK.

Conclusions: AM ameliorates ADR-induced glomerular inflammatory damage in ADRN model. The anti-inflammatory action of AM may be partly attributable to the depression of TGF-beta1 and p-p38MAPK protein expression.

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PUB637

Increased Maternal IL-6 during Gestation Alters Fetal Kidney Development Mukut Sharma,[1] Jianping Zhou,[1] Junming Luo,[1] Pramod B. Mahajan,[2] Tarak Srivastava,[3] Ram Sharma,[1] Thomas Wiegmann,[1] Ellen T. McCarthy,[4] Virginia J. Savin,[1] Jean-francois Gauchat.[5] *[1]Research, KC VA Medical Center, Kansas City, MO; [2]PBA Sciences, Drake University, Des Moines, IA; [3]Nephrology, CMH, UMKC, Kansas City, MO; [4]Kidney Institute, KU Medical Center, Kansas City, KS; [5]Pharmacology, Université de Montréal, Montreal, QC, Canada.*

Background: The effect of increased interleukin 6 (IL6) in obese pregnant women on fetal development and on the susceptibility to chronic disease in the offspring is not known. Recently, we showed that podocytes express IL6 receptor-alpha (IL6Ra) and that exogenous IL6 causes derangement of the podocyte actin cytoskeleton and increases *in vitro* glomerular albumin permeability. We hypothesize that maternal IL6 adversely affects fetal renal development.

Methods: We injected recombinant IL6 to timed pregnant C57BL6 mice (10 pg/g body weight, i.p.) on embryonic days (E) 13.5, 15.5 and 17.5 and observed its effect on E19.5 fetal renal histology using light microscopy and morphometry, components of JAK/STAT, SHP2-MAPK/ERK and PI3K/Akt pathways using Western blotting. Methylation of genomic DNA was determined by measuring 5-methylcytosine (5mC) levels using LC-MS.

Results: Midgestation injection of IL6 to pregnant mice altered fetal renal structure. Light microscopy showed glomerulopenia, poorly defined corticomedullary junction and approximately 35% increase in tubular lumen diameter ($P<0.01$) in fetal kidneys from IL6-injected mothers. IL6 injection resulted in increased phosphorylation of JAK2 and STAT3 in fetal kidney tissue confirming activated JAK/STAT pathway ($P<0.001$). We also noted significantly down-regulated phosphorylation of STAT5, ERK1/2 and Akt ($P<0.001$). Levels of 5mC were significantly increased ($P<0.05$). In summary, midgestational increase in maternal IL6 altered the renal development, key signaling pathways and DNA methylation in the fetal kidney by late gestation.

Conclusions: Elevated maternal IL6 may have trans-generational effect on renal function and cause increased susceptibility to chronic kidney disease in the offspring through epigenetic mechanisms.

PUB638

NTS Is Aggravated in CD73/Ecto-5'-Nucleotidase Deficient Mice by Increased Leucocyte Invasion into the Renal Tissue Nelli Shushakova,[1] Kathrin Eller,[3] Alexander R. Rosenkranz,[3] Hermann G. Haller,[1] Christine S. Falk,[4] Jurgen Schrader,[2] Cornelia Anneliese Blume.[1] *[1]Nephrology and Hypertensiology, Medical School Hannover, Hannover, Germany; [2]Molecular Cardiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany; [3]Internal Med., Nephrology, Graz Medical University, Graz, Austria; [4]Transplant Immunology, Medical School Hannover, Hannover, Germany.*

Background: We previously have shown that a lack of extracellular adenosine formed by the ectoenzyme CD73 leads to an autoimmune phenotype with proteinuria and renal injury in older animals. These mutant mice exhibit glomerular endotheliosis with elevated levels of the chemoattractant IL-18 and fibrosis [Plos One2012].

Methods: Now we induced a nephrotoxic serum nephritis (NTS) in 6-10 CD73-deficient ($CD73^{-/-}$)mice and wild-type (WT) controls and monitored proteinuria and renla function over 2 weeks, before kidneys were harvested for further histological analysis. Histomorphometry, immune staining of the kidneys, analysis of cytokines and chemokines in the plasma and a 19 F magnet resonance imaging method to track the monocytes were performed.

Results: In NTS, proteinuria was elevated in the $CD73^{-/-}$ mutant (13.8 mg/24 h ± 0.5 vs. 5.5 mg/24h ± 0.4 or 0.31 ± 0.01g/g creatinine versus 0.19 ± 0.03 g/g creatinine), and this phenotype was not abrogated by a continuous i.p. application of the A2A-agonist CGS-21680 twice a day. Histomorphometry revealed accelerated tubule-interstitial injury as well as vaso-occlusion in $CD73^{-/-}$mice with more crescents and perivascular fibrosis. Using a 19F MRI for noninvasive inflammation imaging, we observed a significant shift of monocytes into renal tissue in mutant mice compared to WT-controls (figure 1), which is likely to be mediated by the adenosine A2B-receptor as it was previously shown for ischemia. The increased plasma levels of IL-18 were down-regulated in CD73-/-mice in NTS.

Conclusions: Due to the disturbed barrier function of the endothelium together with immune cells without adenosinergic feedback, lack of CD73-derived extracellular adenosine aggravates the course of NTS. Analysis of immune cell subtypes and cytokine levels in the renal tissues is in support of this interpretation.

Funding: Private Foundation Support

PUB639

1,25$(OH)_2$- Vitamin D3 Prevents Interleukin-6 Induced Muscle Atrophy by Stimulating Differentiation and Inhibiting Myostatin Expression in C2C12 Muscle Cells Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: The 1,25-dihydroxyvitamin D (1,25-D3) deficiency is highly prevalent among patients with chronic kidney disease (CKD). Although 1,25-D3 was administrated wildly for bone mineral disorder in CKD, and the mechanism or the role of 1,25-D3 regulation of calcium and phosphate homeostasis is well known, there is little information about vitamin D in muscle wasting, a main aspect in CKD with protein energy wasting.

Methods: Cultured mouse C2C12 myogenous cells, treated with interleukin-6(IL-6 20ng/ml) and 1,25-D3 (100nM). We observed myotube's morphology by light microscopy, measured the expression of myogenic and proliferation molecular by quantitative RT-PCR and Western blotting.

Results: After chronic exposure to IL-6(20ng/ml) from day 1 to day 8, the size of muscle myotube which differentiated from cultured C2C12 myoblast was reduced markedly, the mRNA expressions of Atrogin-1 and myostatin are elevated 3-6 folds, the myoD and myogenin down-regulated 3-5 folds. After treated with 1,25-D3(100nM/day), there resulted in an increase in the diameters in muscle size, indicating that the muscle atrophic responses stimulating by IL-6 were inhibited. As expected, with the myotubes expressing VDR, the expression of Atrogin-1 and myostatin significantly blocked, and the expression of myoD and myogenin were increased. In addition, we found the VDR activated enhanced the Insulin growth factor-II (IGF2) expression.

Conclusions: 1,25-dihydroxyvitamin D prevents interleukin-6 induced muscle atrophy by promoting myogenic differentiation, up-regulating the expression of myoD and myogenin, and inhibits the expression of atrogin1 and myostatin.

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PUB640

HIV-Induced Down Regulation of Tubular Cell Vitamin D Receptor Is Mediated through Tumor Necrosis Factor-α Divya Salhan, Nirupama Chandel, Tejinder Singh, Gautam Kishore Valecha, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Tumor necrosis factor (TNF)-α is an important mediator of inflammation. Recently, we demonstrated that HIV-induced down regulation of vitamin D receptor (VDR) contributed to the activation of the tubular cell renin angiotensin system (RAS) in HIV transgenic (Tg26) mice. We hypothesize that HIV down regulates tubular cell VDR expression through the generation of TNF-α in Tg26 mice.

Methods: Renal tissues of four weeks old, age and sex matched control and Tg26 mice in groups of six were prepared for Western blotting and real time PCR studies for the expression of TNF-α, VDR, and renin. The immunoblots were stripped and probed for actin. Renal cortical sections of control and Tg26 mice were immunolabeled for TNF-α, VDR, and renin and scored for intensity of staining. To determine temporo-spatial relationship

between VDR and renin, renal cortical sections of control and Tg26 mice were co-labeled for VDR and renin and examined under an immunofluorescence microscope. Human renal proximal tubular cells (HRPTC) were transduced with either empty vector or HIV (NL4-3) constructs. EV/HRPTC and HIV/HRPTCs were incubated in media containing either buffer, neutralizing anti-TN-α antibody (5mM) or mouse IgG (5mM) for 24 hours, followed by probing of immunoblots for VDR and actin. Cellular lysates and supernatants of EV/HRPTCs and HIV/HRPTCs were also assayed for TNF- α by Western blotting and ELISA respectively. HRPTCs were treated with variable concentration of TNF- α. Subsequently, immunoblots were probed for VDR and actin.

Results: Renal cortical sections of Tg26 mice displayed higher expression of TNF-α-and renin but diminished expression of VDR. HIV/HRPTCs displayed down regulation of VDR, however, anti-TNF-α antibody enhanced VDR expression by HRPTCs both under basal and HIV-stimulated states. HIV also stimulated two-fold increase in TNF-α production by HRPTCs. Moreover, TNF- α directly attenuated tubular cell VDR expression.

Conclusions: These findings indicate that HIV-induced down regulated VDR expression is mediated though tubular cell TNF-α generation.

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PUB641

Impact of Fructose on Markers of Kidney Injury in Animal Model

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Background: It is has been shown that fructose (FR) induces pro-inflammatory mediators as MCP-1 in proximal tubules, which lead to macrophage infiltration of tubulointersitial tissue. FR elevates uric acid which decreases NO and then alters renal autoregulation and results in glomerular hypertension [1]. This study was designed to evaluate the impact of 60% FR diet on markers of kidney injury and inflammation in subtotally nephrectomized and control rats.

Methods: Male Wistar rats (386±40 g) underwent sham operation (CON=10) or 5/6 nephrectomy (SNx=12). Animals were further assigned to 2 diets protocol: regular – RD or 60% FR – F60. After 8 weeks serum concentration of creatinine (Cr), uric acid (UA), soluble intercellular adhesion molecule (sICAM) and homocysteine (HCY) was measured. On the basis of a 24-hour urine collection protein to creatinine ratio (PCR), N-Acetyl-Glucosaminidase (NAG) to urinary creatinine (NAG/Cr), MCP-1 to urinary creatinine (MCP-1/Cr), and urinary uric acid excretion (UAE) were done. Creatinine clearance (CrCl) was calculated upon Cockroft-Gault formula.

Results:

Table 1

	CON+RD (I)	CON+F60 (II)	p (I vs II)	SNx+RD (III)	SNx+F60 (IV)	p (III vs IV)
Fructose [mg/dl]	0,59±0,05	0,94±0,47	=0,08	0,87±0,39	1,15±0,51	NS
CrCl [ml/min]	3,11±0,42	2,58±0,66	NS	1,61±0,64*	1,14±0,58*	=0,08
UA [mg/dl]	1,7±0,4	1,6±0,1	NS	1,59±0,3	2,04±1,3	NS
UAE [mg/24]	4,1±2,1	2,2±0,5	=0,08	1,1±0,4*	0,9±0,4*	NS
HCY [µmol/l]	4,5±0,2	6,4±1,5	<0,05	5,2±1,3	10,1±2,8*	<0,01
sICAM [ng/ml]	15,1±2,4	16,7±4,5	NS	37,8±16,8*	23,9±8,3*	<0,05
PCR [mg/mg creatinine]	3±1,6	13,5±1,3	<0,01	34,5±1,8*	49±2,2*	<0,01
MCP-1 / Cr [ng/mg creatinine]	3,5±0,5	11,9±2,3	<0,01	8,7±6,4*	7,6±8,3	NS
NAG/Cr [U/g creatinine]	7,9±5,4	21,4±5,5	<0,05	43,9±28,3*	28,4±17,5*	NS

* p<0,05 SNx vs CON

Conclusions: In CON animals F60 diet is associated with induction of a local (MCP-1) and systemic (HCY) inflammatory response which leads to proximal tubular injury (NAG) and proteinuria. In SNx rats F60 diet, through these factors, contribute to deterioration of renal function.

[1] doi:10.1152/ajprenal.00433.2009.

PUB642

Spleen Tyrosine Kinase (Syk) Is Involved in the Mesangial Cell Proliferation Following Stimulation with IgA1 Isolated from IgA Nephropathy (IgAN) Patients

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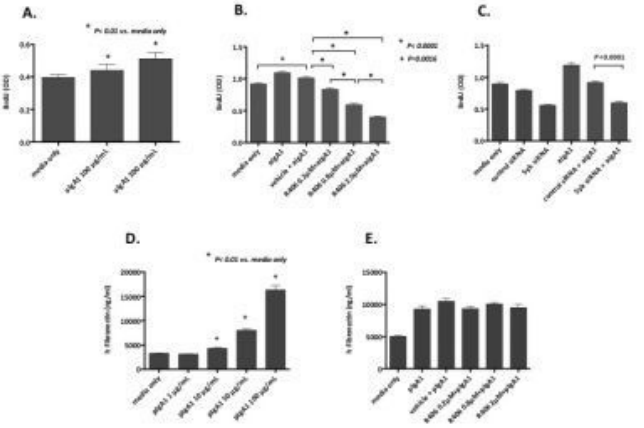
Background: We previously reported that the inhibition of Syk by Syk inhibitor, R406 or Syk siRNA reduces the synthesis of various cytokines by human mesangial cells (HMC) upon stimulation with IgA1 isolated from serum of IgAN patients (pIgA1). We examined if Syk is involved in the HMC proliferation and the production of fibronectin following stimulation with pIgA1.

Methods: IgA1, purified from serum of IgAN patients, was aggregated at 63°C for 150 min (aIgA1). HMC were incubated with aIgA1 for 24h and cell proliferation assay with BrdU was performed. We incubated HMC with R406, 1h before stimulation with aIgA1 (200µg/mL). HMC were transfected with Syk- or control siRNA, 72h before stimulation. Human fibronectin produced by HMC upon stimulation with pIgA1 for 24h was examined in culture supernatants by ELISA. HMC were incubated with R406, 1h before stimulation with pIgA1 (50µg/mL).

Results: HMC proliferation increased upon stimulation with aIgA1 and was inhibited by R406 in a dose dependent manner. HMC transfected with Syk siRNA proliferated significantly less than the cells transfected with negative control siRNA (A-C). The concentration of human fibronectin increased significantly following stimulation with pIgA1. The preincubation with R406 did not reduce fibronectin (D-E).



Conclusions: Our data suggest the involvement of Syk in HMC in the production of various cytokines and HMC proliferation, but not in the synthesis of fibronectin, upon stimulation with pIgA1. Syk may be considered as a potential target in the treatment of IgAN.

PUB643

Activation of Protease-Activated Receptor 4 (PAR-4) in Tubular Epithelial Cells Contributes to Diabetic Nephropathy

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Background: Protease-Activated Receptors (PARs) have been implicated in the pathogenesis of many inflammation-associated disorders, however their roles in diabetic nephropathy (DN) have not yet been explored. Further to our findings on the up-regulation of PAR-4 expression in human renal proximal tubular epithelial cells (PTEC) in response to high glucose stimulation, herein we investigated the pro-inflammatory potential of this receptor in tubular cells and examined its expression in renal tissues of DN.

Methods: Renal biopsies from patients with proven DN were immunohistochemically examined for the expression of PAR-4. To elucidate the role of PAR-4, human PTEC were cultured with 1) selective PAR-4 activating peptide or 2) high glucose medium in the presence of PAR-4 antagonist, and the effects on the expression of pro-inflammatory genes were studied.

Results: High PAR-4 expression was detected in tubular cells of all DN biopsies whereas very low expression was found in normal control subjects. *In vitro*, PAR-4 activating peptide induced chemokine (C-C motif) ligand 2 (CCL-2) expression and to a lesser extent, interleukin 6 (IL-6) expression in PTEC in a time- and dose-dependent manner. Inhibition of PAR-4 by a specific antagonist partially suppressed high glucose-induced mitogen-activated protein kinase (MAPK) p42/p44 signaling and the subsequent CCL-2 and IL-6 production.

Conclusions: Our data demonstrated an increased expression of tubular PAR-4 in human DN biopsies and an enhanced secretion of PAR-4 stimulated inflammatory cytokines from PTEC. These findings suggest a novel role for PAR-4 in mediating diabetic tubular injury. This study is supported by the General Research Fund of the Research Grants Council of Hong Kong (project number 7796/11M).

PUB644

Increased Granulocyte Heparanase Activity: The New Specific Marker of Neutrophils Activation in Patients with Lupus Nephritis and Idiopathic Membranous Nephropathy

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Background: Heparanase is a beta-glucuronidase, that cleaves sugar chains of heparan sulfate proteoglycans. It is supposed that heparanase may be involved in proteinuria pathogenesis. The aim of the study was to assess the heparanase activity in the proteinuric pts with different glomerulonephritis types.

Methods: The evaluation of heparanase activity in serum, urine, granulocytes and superoxide dismutase activity in granulocytes of patients with: lupus nephritis (n=17), membranous nephropathy (n=11), IgA nephropathy (n=12), focal and segmental glomerulosclerosis (n=18), mesangiocapillary glomerulonephritis (n=12) and in 19 healthy

volunteers was processed. Immunohistochemical heparanase staining of kidney biopsy specimens from 6 patients with lupus nephritis was also processed.

Results: The heparanase activity in granulocytes of patients with lupus nephritis and membranous nephropathy was higher than heparanase activity in granulocytes in control group (p=0,02 in both cases). There was no difference between superoxide dismutase activity in granulocytes of patients with all investigated types of glomerulonephritis and control group. Correlations between heparanase activity in urine and the dsDNA titer r=0,51 (p=0,04), and between heparanase in urine and hemolytic activity of the complement r=-0,57 (p=0,03) in the lupus nephritis group, and between heparanase activity in granulocytes and serum total protein level r=-0,69 (p=0,02) in membranous nephropathy was observed. Evaluation of biopsy specimens from lupus pts revealed the correlation between the heparanase intensity staining in loop Henle's tubule and percent of the glomeruli with sclerosis r=0,92 (p=0,009).

Conclusions: In active lupus nephritis and in the nephritic phase of idiopathic membranous nephropathy heparanase appears as the novel marker of granulocyte activation.

Funding: Clinical Revenue Support

PUB645

The Role of Macrophage Stimulating Protein and Its Receptor, Recepteur d'Origine Nantais, RON in Gentamicin Induced Nephropathy

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Background: Macrophage stimulating protein (MSP) was reported to mediate inhibition of apoptosis and inflammation responses, suggesting that it may play a role in tubular regeneration in acute kidney injury. We investigated whether MSP ameliorates tubular dysfunction and inflammation in gentamicin (GM) induced kidney injury.

Methods: Human renal proximal tubular (HK-2) cells were incubated with 2mg/mL GM for 24h at different concentrations of MSP, and cell viability was measured by MTT assay. The protein expressions of RON, COX-2, Bcl2, Bax, caspase3, nuclear factor-kappa B (NF-κB), IκB-α, Akt and mitogen-activated protein kinases (MAPKs) were analyzed by immunoblotting. To examine the changes of MSP and RON in GM-induced kidney injury, rats were injected with GM (150mg/kg, i.m.) for 7 days.

Results: GM (2 mg/ml) treatment decreased the cell viability in HK-2 cells which was counteracted by MSP treatment (30 ng/ml). GM decreased the expression of Bcl-2, while it increased that of Bax and cleaved-caspase 3, which were attenuated by the treatment of MSP. Additionally, MSP attenuated GM-induced increased expression of COX-2 and phosphorylated Akt. GM treatment increased phosphorylation of ERK, JNK and P38, however, MSP attenuated expression of p-P38, but not that of p-ERK and p-JNK. In GM-induced nephropathy rats, the expression of MSP and RON was markedly upregulated compared with controls.

Conclusions: In conclusion, MSP attenuates GM-induced nephropathy by suppression of inflammatory and apoptotic factors through the inhibition of Akt/P38/NF-κB signaling pathways.

PUB646

The Effect of Statin on Epithelial-Mesenchymal Transition in Peritoneal Mesothelial Cells

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Background: Statin has recently been highlighted due to its pleiotropic actions besides cholesterol-lowering effect. However, it is currently unknown whether statin therapy may inhibit peritoneal dialysis-related epithelial-mesenchymal transition (EMT).

Methods: *In vitro*, human peritoneal mesothelial cells (HPMCs) were exposed to 5.6 mM glucose (NG), NG+1 μM simvastatin (SV), or 4.25% peritoneal dialysis fluid (PDF) with or without SV. *In vivo*, PD catheters were inserted into 32 Sprague-Dawley rats, and saline (C, n=16) or PDF (PD, n=16) was infused for 4 weeks. Eight rats from each group were treated with 5 mg/kg/day of simvastatin intraperitoneally. EMT markers in HPMCs and the peritoneum were evaluated by Western blot analysis. We also examined the protein expression of RhoGTPases in HMPCs exposed to PDF with or without SV.

Results: Compared to the NG group, E-cadherin expression was significantly decreased, while α-SMA and fibronectin expression were significantly increased in HPMCs exposed to PDF, and these changes were significantly abrogated by SV ($p<0.05$). In addition, the cobblestone-like appearance of HPMCs was converted into a fibroblast-like morphology after PDF treatment, which was reversed by SV. This EMT-like change was also observed in HPMCs treated with 5 μM geranyl-geranyl pyrophosphate. Moreover, PDF significantly increased the protein expression of RhoA and Rac1 in the membrane fractions, and these changes were ameliorated by SV ($p<0.05$). In PD rats, E-cadherin/α-SMA ratios in the peritoneum were significantly decreased, whereas fibronectin expression was significantly increased compared to C rats ($p<0.05$). Furthermore, the thickness of the submesothelial layer was significantly greater and the intensity of Masson's trichrome staining was significantly higher in PD rats than in C rats ($p<0.05$). These changes in PD rats were significantly attenuated by SV ($p<0.05$).

Conclusions: PD-related EMT was mediated through the activation of mevalonate pathway and statin treatment inhibited EMT changes in PDF-stimulated HPMCs and PD rats.

PUB647

Renal Infiltration of T Lymphocytes in Radiation Nephropathy

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Background: The role of renal inflammation in the pathogenesis of hypertension and renal disease is intensely studied. Prevention of infiltration of T lymphocytes into the kidneys attenuates hypertension and renal damage (Am J Physiol 298:R1136-42, 2010). The role of the immune system in the kidney damage associated with radiation treatment is uncertain.

Methods: We tested for T cells ($CD3^+$), B cells ($CD45R^+$), and macrophages ($CD11b^+$) in a radiation nephropathy model. Rats underwent 10 Gy total body irradiation (TBI), without or with captopril treatment (300 mg/L in drinking water starting on day 9 after TBI), and were sacrificed 22 weeks after TBI. Peripheral blood samples and kidneys were extracted from three groups (non-irradiated, irradiated, and irradiated + captopril). The mononuclear cell populations were isolated from the peripheral blood and the kidneys of each rat as described (Am J Physiol 298:R1136-42, 2010). Flow cytometry was performed for T cells (CD3), B cells (CD45R), and macrophages (CD11b).

Results: There were no significant differences between groups in the total number of mononuclear cells or any of the cells assessed via flow cytometry in the peripheral blood samples. The total kidney cell counts for T cells, B cells, and macrophages were:

Rat Group	BUN (mg/dL)	Total CD3+ cells/2 kidneys (10^6)	Total CD45R+ cells/2 kidneys (10^6)	total CD11b+ cells/2kidneys (10^6)
Control (non-irradiated) (n=4)	17.0±1.7	1.06±0.32	0.06±0.02	1.00±0.37
Irradiated (n=4)	96.3±17.5*‡	4.47±1.3*	0.52±0.25	6.15±1.98
Irradiated + captopril (n=4)	34.5±0.6	4.07±0.48*	0.33±0.07	4.47±0.93

*significant compared to control; ‡significant compared to iraddiated + captopril

Conclusions: Both irradiated groups had significantly increased renal infiltration of CD3+ cells. There is a trend for an increase of CD11b+ in the kidneys of the irradiated rats. These results indicate a role for T cells and macrophages in radiation nephropathy. This may clarify the hypertension and salt sensitivity in this model, and may be relevant for development of new mitigators of radiation injury.

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PUB648

Specific Deletion of the IL-6 Cytokine Family Receptor gp130 in Podocytes

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Background: Gp130 is a ubiquitously expressed co-receptor for cytokines of the IL-6 family. So far, the role of gp130 or IL-6 signaling in podocytes has not been defined.

Methods: Podocyte-specific gp130 knockout (KO) animals were generated by breeding mice expressing cre-recombinase under the control of the podocin promotor (pod-cre+) with mice bearing loxP sites surrounding exon 16 of gp130, yielding pod-gp130 KO mice. Pod-cre-, gp130 f/f animals served as controls (WT). In these mice lipopolysaccharide (LPS)-induced nephritis (1x LPS 10mg/kg body weight) was studied in age-matched 15~22 week old female animals. Additionally, nephrotoxic nephritis was induced in 12 week old male mice.

Results: Successful generation of pod-gp130-KO was confirmed by RT-PCR, Western blot, and immunofluorescence. As expected, gp130 downstream signaling was also abrogated in the KO mice: the number of phospho-STAT3-positive glomerular cells following LPS injection was significantly reduced (WT 5.3±0.13 vs KO 0.84±0.11 cells/glom cross section, means±SEM, $p<0.01$). Pod-gp130-KO mice exhibited no spontaneous functional or histological renal phenotype at 10, 20, and 40 weeks of age. Following LPS injection no significant difference in albuminuria was found between WT and KO at 24h (mean urinary albumin/creatinine ratio: WT 0.232±0.049 vs KO 0.210±0.040 mg/mg, $p=0.73$). Following the induction of nephrotoxic nephritis KO animals tended to exhibit milder disease but all differences at day 14 remained non-significant: crescents WT 15.6±4.2 vs KO 11.8±3.1 %, $p=0.47$; fibrinoid necrosis score (0~4/glom) WT 1.0±0.23 vs KO 0.86±0.17, $p=0.75$. Day 3 urinary albumin/creatinine ratio was WT 45±6.5 vs KO 34±2.8 mg/mg, $p=0.14$.

Conclusions: Our data suggest that gp130 is not a critical component of the podocyte in the normal or ageing kidney. Similarly, in two inflammatory models no statistically significant effects of podocytic gp130 deletion could be identified.

Funding: Government Support - Non-U.S.

PUB649

C3a/C3ar Was Involved in the Recruitment of Renal Mast Cells in Patients with Renal Diseases

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Background: Increased renal mast cells (MCs) have been reported and thought to contribute to renal injury. However, mechanisms about the recruitment of renal MCs are still unclear. The aim of the present study was to evaluate the possible role of C3a/C3aR in the recruitment of renal MCs.

Methods: 100 patients with renal diseases (including diabetic nephropathy, IgA nephropathy, membranous nephropathy, lupus nephritis, acute interstitial nephritis and chronic interstitial nephritis) and 10 normal controls (kidney donors) were recruited. Immunohistochemical staining for tryptase, C3aR and C3c was done on the sections of

renal biopsy. For each specimen, the number of MCs was counted, the immuno-staining intensity of C3c was measured by using Image Plus 2 software, and the correlations between MCs number and C3c level was analyzed in each disease.

Results: C3aR was found highly expressed by renal MCs. Increased C3c was found in the renal tubular interstitium and correlated with the increases of MCs number in patients with renal diseases. MCs were found distributed in the areas where increased C3c was found.

Conclusions: These findings suggested that C3a/C3aR was involved in the recruitment of MCs in patients with renal diseases. As it has been reported that C3 can also be activated through a MCs dependent way, a vicious cycle between complement activation and renal MCs recruitment might exist and contribute to renal injury.

Funding: Government Support - Non-U.S.

PUB650

Evaluation of High-Density Lipoprotein-Associated Proteins as Novel Biomarkers in Chronic Kidney Disease Chantal Maureen Kopecky,[1] Georg Michlits,[1] Philipp Eller,[2] Walter Hoerl,[1] Marcus Saemann.[1] *[1]Dept. of Internal Medicine III, Clinical Division of Nephrology and Dialysis, Medical University of Vienna, Vienna, Austria; [2]Dept. of Angiology, Medical University of Graz, Graz, Austria.*

Background: Cardiovascular events are the main cause of death in individuals with chronic kidney disease (CKD) and end-stage renal disease (ESRD).Patients are further characterized by increased atherosclerosis and dyslipidemia affecting quantity and quality of high-density lipoproteins (HDL). By proteomic analysis, we identified disease-specific proteins including serum amyloid A (SAA), surfactant protein B (SP-B) and Apo-CII enriched in ESRD-HDL compared to healthy HDL and found that the altered protein composition of HDL influenced its anti-inflammatory function and reversed the molecule's atheroprotective action.

Methods: Blood samples were drawn from a total of 20 ESRD patients and 16 healthy controls and immediately centrifuged to obtain plasma.

HDL-bound proteins were detected by enzyme-linked immunosorbent assay. We used an HDL catching antibody to capture HDL directly from plasma samples. Detection antibodies were directed against SAA, SP-B and Apo-CII. Protein amount was measured by a colorimetric reaction using a biotinylated secondary antibody and streptavidin-HRP.

Results: We developed an easy applicable assay using plasma samples for quantitative detection of HDL-bound proteins without the requirement of a preliminary extensive isolation of HDL from plasma. HDL-associated SAA, SP-B and Apo-CII can be measured individually for ESRD patients in considerably higher levels than controls. Consistent results with high reproducibility and low inter-assay variation are obtained for different patient-control groups which were recruited at different time points. We are currently testing biobank samples for subsequent correlation of HDL-bound proteins with several laboratory parameters, disease progression and mortality.

Conclusions: We demonstrate a defective anti-inflammatory function of ESRD-HDL caused by specific protein alterations that may provide a molecular explanation for the cardiovascular mortality in ESRD and can be used as novel cardiovascular biomarkers.

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PUB651

S100A12 Induces the Proliferation and the Osteochondrogenic Differentiation in Vascular Smooth Muscle Cells(VSMCs) Eiko Matsuoka,[1] Yasukiyo Mori,[1] Yayoi Shiotsu,[1] Atsushi Kosaki.[2] *[1]Division of Nephrology, Department of Medicine, Kyoto Prefectual University of Medicine, Kyoto, Japan; [2]Faculty of Nursing, Setsunan University, Osaka, Japan.*

Background: S100A12 is an endogenous ligand for the receptor for advanced glycation end products (RAGE). We have reported that plasma S100A12 level was an independent factor associated with the prevalence of cardiovascular disease(CVD)in the patients with end-stage renal disease (ESRD)(Shiotsu Y and Mori Y et al. Clin J Am SocNephrol 2011; 6: 718-723). However, the detailed mechanism of the association of S100A12 in ESRDwith CVD remains uncertain.In this study,we evaluate the effect of S100A12 on the phenotypical changes in VSMCs.

Methods: Because the mice lacks the S100A12 expression, we have generated the human S100A12 overexpressing mice (C57BL6/J) using serum amyloid P promoter (S100A12Tg mice) to maintain the plasma levels of circulating S100A12. Subtotal nephrectomy (5/6NTx) followed by AngiotensinII (AngII) infusion (1000ng/kg/min) for 3 weeks was performed.We evaluated the histological changes of VSMCs in the thoracic aorta. As the *in vitro* experiment, we prepared cultured VSMCs from WT mice. First, we determined the proliferation of VSMCs using the Ki-67 staining. Authentic S100A12 (1μg/mL; Circulex, Japan) was added to serum-free culture medium, and the changes in Ki-67 positivity was observed. Furthermore, we determined the mRNA expression of osteochondrogenic marker (Runx2) and smooth muscle markers (SM22α) by real-time PCR.

Results: In 5/6NTx-S100A12Tg mice with AngII, the density of VSMCs in thoracic aorta was higher than that in WT mice. In cultured VSMCs, the treatment with S100A12 for 24hr increased the positivity of Ki67 significantly (1.6-fold vs no-additives, P<0.01). The stimulating effect was abolished by the pretreatment with RAGE neutralizing antibody. Furthermore, the addition of S100A12increased the Runx2 mRNA level for 24hr (1.9-fold). In contrast, the accumulation of SM22α mRNA was decreased for 4days (0.5-fold).

Conclusions: These findings suggest that S100A12 induce the cell proliferation via the RAGE pathway and the osteochondrogenic differentiation in VSMCs.

PUB652

1,25-Dihydroxyvitamin D3 Suppresses High Glucose Activated Macrophage Driven Tubular Epithelial to Mesenchymal Transition in Proximal Tubular Epithelial Cells Xiaoliang Zhang, Yansheng Jin, Bi-Cheng Liu. *Nephrology, Zhong Da Hospital, Southeast University, Nanjing, Jiangsu, China.*

Background: 1,25-dihydroxyvitamin associates with amelioration of renal fibrotic lesions in patients of diabetic nephropathy (DN). Classically activated macrophage may promote fibrosis process, while alternatively activated macrophage play a role in repairing process. The aim of this study is to investigate the effect of $1,25(OH)_2D_3$ on glucose induced macrophage activation and subsequent influence to EMT.

Methods: U937 cells were incubated with high glucose. Activity of intracellular iNOS and cytokines of IL-6, IL-12 and TNF-α in supernatant were measured. The high glucose incubated U937 cells were removed from supernatant and put into HK2 cells followed by co-culturing for 24 hours. α-SMA, fibronectin and E-cadherin in HK2 cells were examined. $1,25(OH)_2D_3$. pre-treatment with macrophages was done before high glucose treated macrophage being put into HK2 cells for co-culture. EMT Markers and pro-inflammatory cytokines in macrophages were examined.

Results: After high glucose stimulation, iNOS activity was increased as same as in the IFNγ/LPS group. Meanwhile, IL-6, IL-12 and TNF-α were increased. After co-culture with high glucose incubated U937 cells, HK-2 cells developed an EMT phenomena and protein and mRNA levels of E-cadherin decreased, while a-SMA and fibronectin increased. In subsequent experiments, $1,25(OH)_2D_3$ caused fibronectin and mannose receptor C increased and cytokines of IL-6, IL-12, TNF-a and iNOS decreased. In co-culture experiment, $1,25(OH)_2D_3$ reversed the phenotype changes of HK-2 cells and also revealed an inversion changes in the expression of E-cadherin, a-SMA, and fibronectin expression.

Conclusions: High glucose inducedclassically activated macrophage facilitates the process of EMT in renal proximal tubular epithelial cell. 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced classically activated macrophage and may contribute to blocking EMT process.

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PUB653

Gene Expression of *Pattern Recognition Receptor* Signaling in Patients of Chronic Kidney Disease Yasutaka Kamikawa, Shinji Kitajima, Tadashi Toyama, Kiyoki Kitagawa, Kengo Furuichi, Takashi Wada. *Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan.*

Background: Pattern recognition receptors (PRRs) play a critical role in innate immunity. However, few studies have been revealed on PRR expression in chronic kidney disease (CKD) patients. In this study, we evaluated the gene expression of PRRs in CKD patients, by analyses using RNA microarray of peripheral blood leukocyte.

Methods: Nineteen gender- and age-matched hypertension patients without diabetes were evaluated in this study.The patients were divided into three groups: HT group (8 hypertension patients with eGFR > 60 mL/min/1.73m2), CKD group (7 patients with eGFR < 60 mL/min/1.73m2), and HD group (4 patients on hemodialysis). All CKD and HD patients were clinically diagnosed benign nephrosclerosis. Blood samples were collected in PaxGene RNA collection tubes, and genesof innate immunitywas evaluated by RNA microarray.

Results: The ratio of neutrophils in peripheral blood was higher in HD group than HT group (HT ; 55.2±12.5%, HD ; 68.4±4.4%, p<0.05). Reversely, the lymphocytes ration in peripheral blood was lower in HD group than HT group (HT ; 35.4±12.1%, HD ; 21.1±4.9%, p<0.05). Gene array data indicated that some PRR genes of Toll like receptor (*TLR) signaling pathway and Nod like receptor (NLR)* signaling pathway were varied in CKD and HD group. Among the gene expression of PRRs, TLR4 expression was down-regulated in accordance with decline of eGFR. Moreover, some genes in NLR signaling pathway, including IPAF, RICK, and cardinal, were also down-regulated in the similar way to that of TLR4.

Conclusions: Some genes of PRR signaling pathways were affected by kidney function. These results suggested that these genes might play a role in innate immunity involved in the progressing of CKD.

Funding: Government Support - Non-U.S.

PUB655

Crude *Cecropia pachystachya* Extract Effect in the Development of Renal Lesions in 5/6 Nephrectomized Rats Claudia Maquiaveli,[1] Heloisa Francescato,[1] Cleonice G. Da Silva,[1] Edson Roberto Da Silva,[2] Terezila Machado Coimbra.[1] *[1]Physiology, University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazil; [2]Basic Sciences, University of Sao Paulo, Pirassununga, Sao Paulo, Brazil.*

Background: Angiotensin II has an important role in the renal lesions observed in 5/6 nephrectomized rats. Recent studies showed that molecules present in the *Cecropia pachystachya* (CP) extract can inhibit the angiotensin converting enzyme (ACE) *in vitro*. This study evaluated the effect of this extract in the renal lesions in 5/6 nephrectomized rats.

Methods: Male Wistar 5/6 nephrectomized rats were treated (NE, n=10) or not (NW, n=7) with CP extract (6 g/kg/day), from 15 to 90 days after the surgery. The control groups were subjected to a sham operation and treated (SE, n=7) or not (SW, n=8) with the extract. Albuminuria and systolic blood pressure (SBP) were evaluated during the treatment. The glomerular filtration rate (GFR) was determined 75 days after starting treatment. The animals were killed and the kidneys removed for histological and imunohistochemical studies.

Results: The results showed that nefrectomized animals presented progressive albuminuria (mg/24h) [96.1 (64.9;238.5) and 194.0 (104.0;264.9)], 60 and 90 days

after surgery, respectively, that was less intense in NE group [16.8 (7.5;83.0) and 113.7 (31.1;226.2)], respectively, p<0.05. SBP (mmHg) was lower at the end of treatment in NE (180.5±11.0) than in NW group (217.0±12.7), p<0.05. Nefrectomized animals also showed reduction of GFR (mL/min/100g) (0.12±0.03), compared to SW group (0.44±0.04), which was attenuated by treatment with CP extract (0.27±0.04). Nephrectomized rats presented glomerulosclerosis and tubulointerstitial lesions, associated with increased ED1+ cells (macrophages/monocytes) in renal cortex. All these alterations were less intense in the NE group (p<0.05).

Conclusions: Treatment with CP extract attenuated the functional and structural changes, as well as SBP increase and inflammatory process in the renal cortex of 5/6 nephrectomized rats. The inhibitory effect of the components present in the CP extract on ACE might be contributing to the renal protective effect of this extract. Financial support: CAPES, FAPESP.

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PUB656

Erlotinib Preserves Renal Function and Prevents Salt Retention in Doxorubicin Treated Nephrotic Rats Raed Bou Matar,[2] Janet D. Klein,[1] Jeff M. Sands.[1] *[1]Department of Medicine, Renal Division, Emory University, Atlanta, GA; [2]Department of Pediatrics, Emory University, Atlanta, GA.*

Background: Nephrotic syndrome is associated with an up-regulation of the heparin-binding epidermal growth factor-like growth factor (HB-EGF). Erlotinib blocks the activation the epidermal growth factor receptor (EGFR) in response to HB-EGF. This study investigates the effect of Erlotinib on the progression of proteinuria, renal dysfunction and salt retention in doxorubicin treated nephrotic rats.

Methods: Male Sprague Dawley rats were divided into 3 weight matched groups (n = 13/group) as follows: Controls (Ctrl), IV doxorubicin 7.5 mg/kg once (Dox), IV doxorubicin followed by oral Erlotinib 10 mg/kg daily starting at day 6 (Erl). The animals were pair fed throughout the observation period. Weight, urine output and urine protein excretion were serially monitored. Upon establishment of high grade proteinuria (defined as a 4 fold increase in urine protein), urine sodium and creatinine clearance were measured. Kidney tissue was dissected and analyzed for the abundances of γ-ENaC, NKCC2, NCC and AQP2 using western blot.

Results: Creatinine clearance was preserved in rats treated with Erlotinib as compared to untreated nephrotic animals (in ml/min: Ctrl: 5.2 ± 0.5, Dox: 1.9 ± 0.3, Erl: 3.6 ± 0.5, p < 0.01). Despite a minimal effect on the degree of proteinuria (in mg/d: Ctrl: 240 ± 35, Dox: 1177 ± 229, Erl: 1082 ± 252), Erlotinib prevented salt retention (Urinary Na in mEq/d: Ctrl: 2.2 ± 0.2, Dox: 1.8 ± 2.3, Erl: 2.2 ± 0.2, p < 0.05). Cortex tissue abundance of uncleaved γ-ENaC was reduced by 40 ± 7% in the Dox group when compared to controls but unchanged in the Erl group. Cleaved γ-ENaC was unchanged in the groups tested. NKCC2 in the outer medulla was reduced by 76 ± 5% in the Dox group and by 59 ± 7 % in the Erl group when compared to control levels. Inner medullary AQP2 was reduced by 68 ± 12% in the Dox group but was unchanged in the Erl group compared with controls.

Conclusions: Erlotinib therapy, started 6 days following IV doxorubicin, preserves renal function and prevents salt retention in nephrotic rats. The observed effects are likely mediated by a reversible blockade of EGFR.

Funding: NIDDK Support

PUB657

Dose-Dependent and Temporal Effects Enalapril in ZSF1 Model of Diabetic Nephropathy: Insights from Renal Functional and Histopathological Endpoints and Metabolomics Assessment Ryan M. Fryer, Xiaomei Zhang, Suzanne Nodop Mazurek, Akalushi C. Muthukumarana, Kathleen A. Lincoln, Paul Harrison, Glenn A. Reinhart, Hu Sheng Qian, Steven M. Weldon. *Cardiometabolic Disease Research, Boehringer-Ingelheim Pharmaceuticals Inc., Ridgefield, CT.*

Background: The effects of enalapril were characterized temporally on multiple renal functional and histopathological endpoints, including metabolomic profile, in a pre-clinical model of diabetic nephropathy.

Methods: Enalapril was tested in telemetered ZSF1 rats over 15-wks of treatment. Metabolic profiling was used to assess biochemical components in urine for identification of disease and treatment biomarkers.

Results: Enalapril had no effect on glucose or HbA1C and no effect on urine or plasma creatinine and electrolytes. Enalapril (1, 3, 10, 30 mg/kg/d in water) produced dose-dependent and sustained reductions in blood pressure compared to vehicle (mean study values = -7, -16, -17, and -31 mmHg below baseline, respectively). In the 30 mg/kg group decreases in MAP were accompanied by an increase in BUN suggestive of renal hypoperfusion. Enalapril also elicited dose-dependent reductions in urinary protein excretion in the 3, 10, and 30 mg/kg dose groups beginning at weeks 8-9 of treatment that were sustained throughout the study. After 15-weeks of treatment and based on semi-quantitative scoring, enalapril produced dose-dependent decreases in the incidence and severity of glomerulosclerosis in all treatment groups (-75% and -41% below vehicle controls, respectively, in the 30 mg/kg dose group). Enalapril also elicited reductions in the severity of interstitial lesions, KIM-1, and a-SMA expression in the 3, 10, and 30 mg/kg dose groups. Samples for metabolomic analysis were assayed at baseline, 5, 10 and 15 weeks of treatment. Several metabolites were identified from a variety of biochemical classes (fatty acids, acetylated amino acids, uremic toxins and sugars) that correlated to disease progression.

Conclusions: The dose-responsive effects of enalapril in ZSF1 rats were characterized. Temporal changes in urinary metabolites were able to successfully classify treatment groups with up to 80% accuracy over the 15 week study.

PUB658

P-Cresyl Glucuronide-Induced Cell Stress Affects Xenobiotic Elimination Pathways in Human Renal Proximal Tubule Cells Henricus A.M. Mutsaers,[1,2] Anita C.A. Dankers,[1] Andries Seegers,[1] Martijn J. Wilmer,[1] Lambertus Vd Heuvel,[3] Joost G. Hoenderop,[2] Rosalinde Masereeuw.[1] *[1]Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [2]Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [3]Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: During chronic kidney disease (CKD), toxic solutes accumulate due to inadequate renal clearance. Here, the impact of p-cresyl glucuronide (pCG), on the phenotype and functionality of conditionally immortalized renal proximal tubule epithelial cells (ciPTEC) was studied.

Methods: HPLC was used to determine plasma pCG concentration and to study cellular metabolic activity (7-hydroxycoumarin and p-cresol glucuronidation). ciPTEC phenotype was analyzed by flow cytometry and qPCR. Membrane vesicles isolated from multidrug resistance protein 4 (MRP4) or breast cancer resistance protein (BCRP) overexpressing cells were used to study the impact of pCG on substrate specific uptake.

Results: Plasma of end-stage CKD patients showed 160-fold accumulation of pCG (mean concentration: 46 ± 30 μM), compared to controls. Moreover, ciPTEC actively metabolized p-cresol into pCG (Km: 33 ± 13 μM, Vmax: 266 ± 25 pmol/min.mg). Exposure of ciPTEC to 2 mM pCG for 48h caused a 1.6 ± 0.3 fold increased expression of the mesenchymal marker vimentin, compared to untreated cells, suggesting epithelial-to-mesenchymal transition (EMT). Also, mRNA levels of BCRP, Bcl-2 and iNOS were elevated by pCG with a fold change of 2.2 ± 0.3, 1.5 ± 0.3 and 2.0 ± 1.5, respectively. In contrast, MRP4 expression was unaltered. Furthermore, pCG decreased 7-OHC glucuronidation with 23 ± 7% in ciPTEC. In addition, pCG inhibited MRP4-mediated [^{3}H]-methotrexate uptake in concentrations ranging from 10 μM to 1 mM, whereas transport by BCRP was unaffected.

Conclusions: Thus, pCG induces a stress-response in ciPTEC. Consequently, cellular phenotype and functioning are influenced, as shown by the inhibition of glucuronidation and altered efflux transporter function and expression. Together with the increased pCG levels in CKD patients, this indicates that this solute could contribute to the progression of CKD.

Funding: Private Foundation Support

PUB659

Proximal Tubular Expression of Megalin Is Down-Regulated in Human Proteinuriic Nephropathies Mohamed Hussein Chunara,[1] Richard J. Baines,[2] Nigel J. Brunskill.[1,2] *[1]Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom; [2]John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom.*

Background: Proteinuria is a hallmark of progressive renal disease. Filtered macromolecules including albumin activate proximal tubule cell (PTC) signalling pathways to induce an aberrant PTC phenotype characterised by inflammation, fibrosis and apoptosis. Low-density lipoprotein receptor-type megalin is an important PTC receptor for albumin. Whilst the megalin-albumin interaction is a recognised PTC albumin retrieval pathway, it may also activate intracellular signalling cascades and have gene regulatory capacity via the megalin cytoplasmic tail. Cellular abundance of megalin is crucial in this regard. In vitro, PTC expression of megalin is down-regulated by high concentrations of albumin and in people with diabetes urinary losses of megalin are increased possibly as a consequence of cell shedding. However whether PTC expression of megalin is altered in human proteinuric disease is unknown.

Methods: Patients with nephrotic syndrome as a result of minimal change disease (MCD, n=9), membranous nephropathy (MN, n=10) and focal segmental glomerulosclerosis (FSGS, n=9) were identified from the renal tissue biobank. Normal renal tissues taken from the unaffected pole of renal cell cancer nephrectomy specimens were used as healthy controls (HC, n=11). Immunostaining using a primary antibody directed against the megalin cytoplasmic tail was performed. 6 blinded scorers studied 6 fields of view along the length of a stained biopsy section and scored each slide on a scale of 0 to 5, where 0 represented minimum staining of megalin and 5 - maximum staining.

Results: PTC staining for megalin was substantial in healthy renal tissue with a significant reduction of megalin expression in proteinuric renal disease (HC 4.56±0.032 vs MCD 2.29±0.074, MN 3.14±0.064 and FSGS 2.31±0.060, p<0.0001).

Conclusions: In health there is substantial PTC staining of megalin. Proteinuria associates with a reduction in such expression, the pattern of which relates to the underlying glomerular lesion. For the first time, these date report changes in megalin PTC expression in human disease.

Funding: Private Foundation Support

PUB660

C4d Staining Impact on Renal Outcome in Lupus Nephritis Mamdouh N. Albaqumi, Lutfi Alkorbi. *Medicine, KFSHRC.*

Background: A recent study has shown a strong association of C4d staining and the presence of micro thrombi in lupus nephritis renal biopsies, suggesting a role for complement activation.

Whether activating lectin complement pathway and subsequent C4d deposition play a role in lupus nephritis remains unclear. Herein, we study a population of lupus nephritis (Class IV and V) to look at C4d staining impact on renal and patient outcome.

Methods: We retrospectively reviewed all renal biopsies with lupus nephritis and reclassified them according to ISN/RPS 2003 classifications. Medical records reviewed to include their demographics, co-morbidities, laboratory, and patient and renal survival.

Results: A total of 86 patients included in this analysis. 24 (28%) patients stained positive for C4d while 42 (72%) stained negative. Both groups were similar in terms of co-morbidities.

Majority of patients in the C4d+ group were class V (58.3%) while C4d – group had more patients with IV global (51.6%). Estimated GFR and proteinuria at baseline showed no statistical difference between the two groups. More patients in the C4d – group were treated with cyclophosphomide as induction therapy in comparison to more patients in the C4d + group who were treated with CsA as an induction therapy. Complete remission, partial remission, and treatment failure were not significantly different among the two groups. Probability of renal survival and patient survival showed no statistical difference between the two groups.

Conclusions: C4d staining might not have an impact on clinical outcome in lupus nephritis.

PUB661

Disruption of Gastric, Jejunal, and Ileal Epithelial Tight Junction in Uremi

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Background: Inflammation is a constant feature and a major mediator of progression of chronic kidney disease (CKD) and its numerous complications. There is increasing evidence pointing to the intestinal barrier dysfunction and its contribution to the prevailing inflammation in advanced CKD. In a recent study we found marked depletion of the key trans-cellular [claudin-1 and occludin) and intracellular [ZO1] constituents of colonic epithelial tight junction (TJ) in animals with chronic renal disease induced by either subtotal nephrectomy or adenine-induced tubulo-interstitial nephropathy [Vaziri et al, NDT, 2012]. The present study was designed to determine whether uremia-induced disruption of epithelial TJ is confined to colon or extends to other segments of the gastrointestinal tract.

Methods: Sprague–Dawley rats were randomized to undergo 5/6 nephrectomy (CKD) or sham-operation (control) and observed for 12 weeks at which point they were euthanized, ileum, jejunum, and stomach were removed and processed for expression of the TJ proteins by Western blot and immunohistological analysis.

Results: The CKD group showed hypertension, proteinuria, azotemia, and reduced GFR. As can be seen in the table below the TJ proteins were significantly reduced in stomach, jejunum and ileum in the CKD animals when compared with the control group.

Protein abundance of ZO-1, Occludin and Claudin-1measured by Western blot and expressed as relative optical densities normalized against beta actin in the given tissues of CKD and control rats

Tissues	Stomach		Jejunum		Ileum	
Groupss	CTL	CRF	CTL	CRF	CTL	CRF
ZO-1	100 ±4	69 ±2*	100 ±6	75 ±3*	100 ±5	61 ±5*
Occludin	100 ±1	34 ±4*	100 ±5	60 ±2*	100 ±3	59 ±2*
Claudin-1	100 ±2	58 ±4*	100 ±3	70 ±4*	100 ±2	81 ±1*

*P< 0.05

Conclusions: The results of the present study revealed that uremia-induced depletion of the epithelial TJ is not confined to the colon as shown in our earlier study and extends throughout the gastrointestinal tract.

PUB662

Red Propolis Reduces Proteinuria and Glomerular Damage in the Remnant Kidney Model

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Background: The pathogenic role of inflammation and oxidative stress in chronic kidney disease (CKD) is well established and pharmacological inhibition of these pathways has shown potential renoprotection in experimental CKD models. Brazilian Red Propolis (RP), resinous glue produced by honeybees through the collection of plant exudates, has been demonstrated to possess antioxidant and anti-inflammatory effects.The present study was designed to evaluate the renal effects of RP on remnant kidney model (Nx).

Methods: In order to obtain the Nx model, adult male Wistar rats underwent a surgical procedure performed in two steps: Removal of the left kidney poles, followed by right nephrectomy (performed 7 days after polectomy). The animals were distributed among 4 groups: Sham-operated rats (S, n=6), Sham rats treated orally with RP, 150mg/kg/day (S+RP, n=6), untreated Nx rats (Nx, n=6) and Nx rats treated orally with RP, 150mg/kg/day (Nx+RP, n=6). All groups were followed for 3 months. At this time, body weight, BW (g), tail-cuff pressure (TCP, mmHg), serum creatinine (SCr, mg/dL), urinary protein excretion (Uprot, mg/dL), percent glomerular esclerosis (%GS), percent renal cortical interstitial area (%INT) and renal macrophage infiltration (MØ, cells/mm^2) were determined.

Results: (Mean ± SD, one-way ANOVA, †p <0.05 vs CN, §p <0.05 vs CN+PV, *p <0.05 vs Nx+PV).

Table 1

GROUPS	TCP	SCr	Uprot	%GS	%INT	Mø
CN	117±4§	0.63±0.06	28±7	0.4±0,3	0.8±0.5	18±7
CN+PV	105±5,0	0.53±0.06	23±5	0.6±0,4	0.8±0.2	13±8
Nx	128±7†§	0.8±0.14†§	190±10†§*	23±14†§*	9.7±6.2†§	95±23†§
Nx+PV	129±8†§	0.82±0.07†§	91±8†§	9±6†§	7.0±0.2†§	69±31†§

Treatment with RP reduced Uprot levels, glomerular damage, interstitial expansion and macrophage infiltration in Nx rats; independently of blood pressure lowering.

Conclusions: These partial results suggest that RP treatment attenuates renal damage in the remnant kidney model.

PUB663

Angiotensin II/DOCA Salt Model of Chronic Kidney Disease in the C57BL/6 Mouse

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Background: C57BL/6 mice are resistant to Chronic Kidney Disease (CKD) by standard techniques such as reduced renal mass and angiotensin II (Ang II) infusion, thereby limiting the use of knockout mice to study the progression of CKD. We developed a model of CKD in the C57BL/6 mouse using Ang II infusion and DOCA treatment with high sodium intake. Our study characterizes the model.

Methods: All the mice underwent a uninephrectomy (Unx) or a sham operation 2-weeks before the start of the experiment. The Unx mice were placed on 1% sodium chloride in the drinking water and received a 50 mg 21-day release deoxycorticosterone acetate (DOCA) pellet implant and Ang II infusion (1.5 ng Ang II/minute/g) using osmotic mini pumps. Blood pressure was measured using tail cuff plethysmography and 24 h urine was collected for protein analysis. On day 21, the animals were sacrificed. Cardiac and renal tissues were weighed and evaluated by periodic acid-schiff (PAS), α-smooth muscle actin and TUNEL staining to assess kidney injury.

Results: This model of CKD involves various physiological changes in Unx versus sham operated animals. A significant increase in systolic and diastolic blood pressure and total protein in 24 h urine was observed post-CKD development. Kidney sections from Unx animals exhibited an increase in PAS-positive intratubular protein casts indicative of renal pathology. Proteinuria was highly predictive of total protein cast area (R^2=73%). *De novo* α-smooth muscle actin staining showed renal interstitial fibrosis and T cell infiltration further confirmed a neo-antigen mediated immune response in CKD. A significantly higher level of apoptosis occurred in the tubular epithelium of Unx animals. Electron microscopy showed glomerular capillary dilation and disruption of the podocyte filtration barrier.

Conclusions: The Ang II/DOCA salt model of CKD developed in the C57BL/6 mouse resembles the pathological features found in many patients with late stage CKD, including proteinuria, hypertension, cardiac hypertrophy and renal pathology. This model is highly applicable to the study of various genetic knockouts in CKD development.

Funding: Government Support - Non-U.S.

PUB664

Establishment of New High Functional Culture System for Renal Cells and In Vitro Model of Diabetic Nephropathy Using Radial Flow Bioreactor

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Background: It is not possible that we could apply monolayer culture system as in vivo three dimensional condition. A radial-flow bioreactor (RFB) can be characterized as a system in which high perfusion rate at the center would allow adequate supply of oxygen and nutrients to the cells at the center. We have established three dimensional and high functional renal cell culture system by using RFB in order to create in vivo condition on in vitro. We also carried out experiments to make sure how renal cells characterize in the conditions of several glucose and oxygen concentration by using culture control system in closed culture system of RFB.

Methods: Two to 5 ×10^7 renal cells (LLC-PK1 or human mesangial cell) were injected in the RFB. Isolated cells were loaded in the RFB column and became trapped and adhered to the porous culture beads under the condition of several glucose and oxygen concentration. For each data point, RNA was obtained from the cells cultured in the RFB or monolayer dish and we performed real-time quantitative PCR. Furthermore, we stained the cells with antibodies to connective tissue growth factor (CTGF) and fibronectin (FN) so as to investigate of renal fibrosis.

Results: Renal cells were able to be cultured in the RFB. Especially, we could culture LLC-PK1 for longer than 100 days without passage. Then the mRNA expression of the 1-alpha-hydroxylase (CYP27B1) in LLC-PK1 cells were higher than those in a monolayer culture. Furthermore, FN expression of LLC-PK1 cells under high glucose and low oxygen were higher than those another condition. In terms of CTGF, the condition of high glucose and low oxygen led to the opposite results.

Conclusions: RFB system composed of proximal tubular cells could be useful as high functional culture system similar to in vivo, and we can detect CYP27B1 mRNA anytime as their function. We also observed the expression of FN and CTGF associated with renal fibrosis under high glucose and hypoxic condition in this system were changed. Based on these results, it could be useful for the in vitro model of diabetic nephropathy.

PUB665

Epidermal Growth Factor Inhibits Basal and Transforming Growth Factor Beta-Induced Collagen and Smooth Muscle Alpha-Actin Expression in Renal Tubular Epithelial Cells

Xiaoying Liu, H. William Schnaper. *Department of Pediatrics, Northwestern University, Chicago, IL.*

Background: Transforming growth factor beta (TGFβ) signaling plays an important and complex role in renal fibrogenesis. The seemingly simple TGFβ/Smad cascade is intensively regulated at different levels including crosstalk with other signaling pathways. Epidermal growth factor (EGF) is a potent mitogen for epithelial cells and is elevated in

diseased kidneys, but its effect on TGFβ-induced fibrotic changes is unclear and will be examined in this study.

Methods: Human proximal tubular epithelial cells (HKCs) were treated with TGFβ (1 ng/ml) and/or EGF (25 ng/ml) for different time periods. EGFR inhibitor AG1478 (10 uM), JNK inhibitor SP600125 (20 uM), PI3K inhibitor wortmannin (10 uM) or MEK inhibitor PD0325901 (100 nM) were added 1 hour prior to TGFβ or EGF treatment. Actinomycin D (10 ug/ml) was used to determine mRNA half-life. qPCR and western blotting were used to examine mRNA and protein level, respectively.

Results: EGF specifically inhibited basal and TGFβ-induced type-I collagen and smooth muscle α-actin (αSMA) expression at both the mRNA and the protein level. Inhibitors AG1478 and PD0325901 prevented the inhibition by EGF, but SP600125 or wortmannin did not. Further, EGF did not block Smad2 or Smad3 phosphorylation by TGFβ, or Smad2/3 nuclear import. However EGF reduced the activation of ARE-luc (Smad2 readout) and SBE-luc (Smad3 readout). Both reductions were prevented by PD0325901. EGF also diminished TGFβ induction of both collagen I and αSMA promoters. Collagen I mRNA had a half-life of ~4 hr whereas the half-life for αSMA mRNA was longer than 24 hr. EGF had little effect on collagen mRNA stability, suggesting that regulation was solely transcriptional, whereas EGF accelerated degradation of αSMA mRNA.

Conclusions: These results suggest EGF inhibits two markers of EMT through EGF receptor tyrosine kinase and downstream ERK activation, but not through PI3K or JNK. The inhibition results from both reduced basal mRNA expression and effector mechanisms downstream of Smads. Thus EGF stimulates multiple, complementary systems to regulate TGFβ/Smad signals mediating renal fibrogenesis.

Funding: NIDDK Support

PUB666

Thromboxane A_2 Receptor Stimulation Suppresses ICAM-1 Expression on Renal Tubular Epithelial Cells that Results in Inhibition of Leukocyte Adhesion Yoshiyuki Morishita, Minami Watanabe, Hiromichi Yoshizawa, Akira Onishi, Eiji Kusano. *Division of Nephrology, Department of Internal Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan.*

Background: Thromboxane A_2 receptor signaling was reported to increase ICAM-1 expression on vascular endothelial cells; however, little is known about its effects on renal tubular cells. In the present study, we investigated the effects of TXA_2 receptor stimulation on ICAM-1 expression and adhesion with leukocytes for renal tubular epithelial cells.

Methods: The subcellular localization of TXA_2 receptor on HK-2 cells (immortalized human renal proximal tubular epithelial cells) was investigated by immunohistochemistry. HK-2 cells were stimulated with U46619 (9,11-dideoxy-9α,11α-methano-epoxyprostaglandin F2α) (10^{-6}-10^{-10} M), an agonist of TXA_2 receptor, with or without pretreatment with SQ29548 ([1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino) carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (10^{-5} M), an antagonist of TXA_2 receptor, for 24 hrs. Then, the change of ICAM-1 expression on HK-2 cells was analyzed by qRT-PCR and flow cytometry at 24, 48, 72, 96 and 120 hr after stimulation with U46619. HK-2 cells stimulated with U46619 (10^{-6} M) for 72 hrs were co-cultured with peripheral blood mononuclear cells (PBMCs) obtained from healthy donors for a further 2 hrs to investigate the change of their adhesion by cell adhesion assay.

Results: TXA_2 receptor was a localized intracellular structure on HK-2 cells. U46619 decreased ICAM-1 expression on HK-2 after 72 hrs in a dose-dependent manner. This decrease of ICAM-1 expression on HK-2 cells was inhibited by pretreatment with SQ29548. The adhesion between PBMCs and HK-2 cells was significantly decreased to 60% ($p<0.05$) by stimulation of HK-2 cells with U46619 (10^{-6} M) compared with that of HK-2 cells without stimulation. Pretreatment of HK-2 cells with SQ29548 (10^{-5} M) inhibited this decreased adhesion between PBMCs and HK-2 cells.

Conclusions: The results of the present study suggested that TXA_2 receptor stimulation suppresses ICAM-1 expression on renal tubular epithelial cells, which results in inhibition of leukocyte adhesion.

PUB667

Cordyceps Sinensis Ameliorates Renal Fibrosis in the Remnant Kidney Model by Down-Regulating Mitochondrial Oxidative Stress Ming-hui Zhang, Haifeng Ni, Junfeng Chen, Bi-Cheng Liu. *Institute of Nephrology, Zhong Da Hospital, Southeast University, Nanjing, Jiangsu, China.*

Background: Cordyceps sinensis(CS) is widely used in traditional Chinese medicine for nearly 2,000 years, and it has potential renoprotective effects. but the mechanisms by which CS improves renal function and fibrosis still not be fully ascertained. Our study is aimed to investigate the effects of CS on the mitochondrial oxidative stress level and find the mechanisms of the Chinese herbal as a nephroprotective agent in the remnant kidney model.

Methods: Male SD rats were allocated to the following groups: Sham surgery+vehicle (S) group, 5⁄6 nephrectomy+vehicle (N) group, and 5⁄6 nephrectomy+CS(2g/kg bw·d)(C) group. Rats were killed on week 12 after surgery. Pathological and ultrastructural changes of the renal tissues were observed. Activity of MnSOD, GSH-Px,and MDA in the kidney cortex, and the level of mitochondrial membrane potential(MMP), ROS by chromatometry. And the expression of CytoC and Prohibitin in both mitochondria and cytoplasm were tested by western blot.

Results: Compared with the S group, BW, SBP, 24hUTPro, NAG, SCr and BUN were significantly increased ($P<0.01$) in the N group, while they all appeared significantly decrease in C group compared with the N group ($P<0.01$); Glomerular sclerosis, tubulointerstitial fibrosis, the infiltration of inflammatory cells and the mitochondrial swelling and the diminution of cristae were observed in the N group. The changes described above were obviously improved in the C group; The activities of MnSOD, GSH-Px and MMP were significantly reduced in the renal cortex of the N group. ($P<0.01$), and there were significantly elevated in the C group. While the MDA and ROS levels appeared crosscurrent; the expression of CytoC and Prohibitin in mitochondria were significantly reduced ($P<0.01$) in the N group. And there were significantly elevated in the C group.

Conclusions: Our findings indicate that the CS exerts effective antioxidation and plays a protective role against the development of renal fibrosis in experimental animal model, which might be related with its down-regulating mitochondrial oxidative stress.

PUB668

Cross Talk between Cardiotonic Steroids (CTS) and 20-HETE in Cardiac Fibroblasts Vinai Kumar Katragadda,[1] Imad Hariri,[1] Steven T. Haller,[1] Yanling Yan,[1] Deepak K. Malhotra,[1] Zi-jian Xie,[2,1] Michal L. Schwartzman,[3] Nader Abraham,[2] Joseph I. Shapiro,[1,2,4] Jiang Liu.[1,4] *[1]Medicine, University of Toledo College of Medicine, Toledo, OH; [2]Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, OH; [3]Pharmacology, New York Medical College, Valhalla, NY; [4]Pharmacology and Physiology, Marshall University JCE School of Medicine, Huntington, WV.*

Background: 20-HETE has been characterized as a second messenger in signal transduction pathways participating in the regulation of vascular tone, sodium excretion, cell proliferation, and angiogenesis. 20-HETE has been shown to inhibit Na/K-ATPase but its effect on fibrosis is unclear. We investigate if 20-HETE can interact with cardiotonic steroids to stimulate collagen production in isolated rat cardiac fibroblasts.

Methods: Primary culture of rat cardiac fibroblast, c-Src phosphorylation, collagen production.

Results: We have reported that cardiotonic steroid marinobufegenin (MBG) stimulates collagen (type 1) production and cardiac fibrosis both *in vitro* and *in vivo*, through Na/K-ATPase signaling. In primary culture of rat cardiac fibroblasts isolated from Sprague Dawley rats, both MBG (1 and 10nM, 24 h) and 20-HETE (10 and 100nM, 24h) stimulated collagen production. Interestingly, the selective inhibitor of 20-HETE biosynthesis HET-0016 significantly attenuated the stimulatory effect of MBG, whereas anti-MBG antibody has no significant effect on 20-HETE-induced collagen production. It appears that 20-HETE production may interplay with MBG and enhance the effect of MBG in collagen production. While low dose of MBG (0.1nM) and 20-HETE (0.1 and 1nM) alone did not stimulated collagen production, co-administration of MBG (0.1nM) and 20-HETE (0.1 and 1nM) had synergistic effect and significantly stimulated collagen production. Furthermore, both MBG and 20-HETE (10nM, 15min) activated c-Src.

Conclusions: Like MBG, 20-HETE stimulated collagen (type 1) production in cardiac fibroblasts. Inhibition of 20-HETE might attenuate MBG-induced collagen production. The data also suggest that MBG and 20-HETE might have substantial cross-talk.

Funding: Other NIH Support - HL-109015 GM-78565, Clinical Revenue Support

PUB669

The Role of the Urokinase Plasminogen Activator Receptor and Complement Factor H in Neutrophil Adhesion: Relevance for the Atypical Hemolytic Uremic Syndrome? Judith Gras,[1] Fred G. Pluthero,[1] Christoph Licht.[1,2] *[1]Program for Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada; [2]Division of Nephrology, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: In the pathogenesis of the atypical haemolytic uremic syndrome (aHUS), an interplay of complement and coagulation can be observed: Through dysfunctional regulation of the alternative complement pathway (e.g. mutations in complement factor H (CFH)) on the surface of the glomerular basement membrane endothelial cells are damaged, which leads to an activation of the coagulation system. Many aspects of the disease also suggest an involvement of polymorphonuclear leucocytes (PMN). As PMNs are known to interact with CFH through complement receptor 3 (CR3) during adhesion and CR3-mediated adhesion in general is positively regulated by the coagulation protein urokinase plasminogen activator receptor (uPAR), this project focused on the investigation of a putative connection between CFH and uPAR in neutrophil adhesion.

Methods: The potential consequence of an interaction between CFH and uPAR was investigated with a microfluidic flowchamber system (BioFlux). Human PMNs were preincubated with αuPAR, αCR3 antibodies or nonspecific IgG as a control. Cells were then under constant flow introduced into BioFlux channels that were covered with a monolayer of Human umbilical vein endothelial cells (HUVECs), HUVECs bound with additional CFH or CFH alone. PMN adhesion was then quantified and compared with the control.

Results: Whereas PMNs incubated with αCR3 in all approaches adhered less to the surface, the use of αuPAR lead to different results: As expected, neutrophils blocked with αuPAR compared to the control adhered less to a surface covered with a HUVEC monolayer. However, if the surface was coated with additional CFH, the effect of αuPAR could partly be reversed. If the surface was coated solely with CFH, PMNs incubated with uPAR adhered more.

Conclusions: With uPAR we have been able to identify a protein of the coagulation pathway that is able to negatively regulate the interaction of PMNs with CFH. Whether these findings have consequences for the pathogenesis of aHUS has to be established in further experiments.

PUB670

17β-Estradiol Attenuates Renal Fibrosis in Mice with Obstructive Uropathy Min Hyun Cho,[1] Youngju Hwang,[1] Hee-Seong Jang,[2] Kwon Moo Park.[2] *[1]Pediatrics, Kyungpook National University; [2]Anatomy, Kyungpook National University.*

Background: Men are generally more prone to chronic kidney disease and to progress to end stage renal disease than are women. The purpose of this study is to prove the effect of gender and sex hormone on the renal fibrosis in mice with unilateral ureteral obstruction (UUO) and to elucidate the specific mechanisms.

Methods: We compared the expression of α-smooth muscle actin (α-SMA) in female and male mice with complete UUO (day 7). After this, we estimated the changes of renal fibrosis in the female mice with ovariectomy and in the female mice with ovariectomy and replacement of 17β-estradiol, respectively.

Results: The level of α-SMA in the female kidney with UUO was significantly lower than that in the male kidney with UUO. Ovariectomy and replacement of 17β-estradiol did not change the expression of angiotensin II type 1 (AT_1) receptor in the female kidney with UUO, whereas the expression of angiotensin II type 2 (AT_2) receptor was significantly more elevated in the intact female (IF) and the ovariectomized female with estrogen (OF+E) than that in the ovariectomized female (OF). The expressions of inducible nitric oxide synthase (iNOS) in the IF and OF+E mice were significantly more elevated than that in the OF mice, which was similar to the expression of AT_2 receptor.

Conclusions: The female gender is associated with resistance to renal fibrosis in obstructive uropathy and this gender difference may originate from the existence of 17β-estradiol, which has an anti-fibrotic effect via upregulation of the AT_2 receptor and iNOS.

PUB671

Effect of Notoginseng on Renal ICAM-1 Expression in Unilateral Ureteral Obstruction Rats Xia Liu, Yanjie Huang, Xiaoqing Yang. *Pediatrics, The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, China.*

Background: Notoginseng is a anti-coagulant drug of traditional chinese medicine (TCM). The effect of Notoginseng on the expression of P-selectin, intercellular cell adhesion molecule-1 (ICAM-1), transforming growth factor-1 (TGF-β1), and the infiltration of macrophage were observed in kidney of unilateral ureteral obstruction (UUO) rats.

Methods: 52 male SD rats were randomly divided into: 1) sham group (n=12); 2) UUO model group (n=20); 3) notoginseng group (n=20). Half rats in each group were sacrificed respectively on day 7 and day 14. Renal P-selectin, ICAM-1, CD68, TGF-β1 expression and localization were detected by immmunohistochemical analysis. Rat kidney tissue sections were stained with Masson's trichrome and periodic-acid Schiff for histopathological analysis.

Results: Compared with the sham group, the levels of renal tissue ICAM-1, P-selectin, CD68, TGF-β1 expression were significantly upregulated in UUO model group on day 7 and day 14 ($P<0.05$). Notoginseng decreased tubulointerstitial injury score and the renal interstitial inflammatory cell count, and suppressed significantly the levels of renal ICAM-1, CD68, TGF-β1 expression on day 7 and day 14, compared with the UUO model group ($P<0.05$).

Conclusions: Notoginseng meliorates development of tubulointerstitial fibrosis via decreased ICAM-1-mediated inflammatory cell infiltration, then lowed TGF-β1 level in UUO rats.

PUB672

The Impact of Reversible Unilateral Ureter Obstruction on Epithelial-Myofibroblast Transdifferentiation Hong Li Lin, Shen Nan, Yan Ling Sun. *The First Affiliated Hospital of Dalian Medical University.*

Background: The aim of this present study is to investigate the process of epithelial-myofibroblast transdifferentiation or myofibroblast-epithelial transdifferentiation in reversible unilateral ureter obstruction rats.

Methods: Ninety male Sprague-Dawley rats(20 to 25 g) were randomly divided into four groups, i.e, normal control group (CON, n = 10), sham operation group (SOR, n = 10), unilateral ureteral obstruction group (UUO, n = 24) and reversible unilateral ureteral obstruction group (RUUO, n = 36). RUUO was induced by clamping the left ureter using a hemostatic ligating clip. The obstruction was reversed three days after the operation by removing the clip. Six animals in each group were killed at weeks 1, 2, 4, 8 and 12. Firstly, we used Real time-PCR Arrays to detect the mRNAs expressions of TGF-β1 and α-SMA among the above-mentioned groups, then we further detected and confirmed the expression changes of N-cadherin, collogen-1, FSP-1, TGF-β1 and α-SMA by Western blot or immunofluorescence. Serum creatine (Scr) and blood urea nitrogen (BUN) was used to assess renal function. Interstitial fibrosis and renal morphology were assessed by examination of PAS and PASM staining.

Results: Untreated obstructed rats showed marked tubular atrophy and apoptosis, tubulointerstitial macrophage infiltration and fibrosis. Following the relief of obstruction, however, there was a significant restoration. Compared with CON and SHAM groups, the mRNA levels of TGF-β1 and α-SMA were significantly increased in UUO rats detected by Real time-PCR ($P<0.05$). They were significantly higher than baseline from 3 to 7 days after relief of UUO, and then gradually reduced to normal. Western blot showed that the expression of TGF-β1 and α-SMA significantly increased in UUO group compared with CON group($P<0.05$), whereas, the expressions were obviously decreased after the release of ureter.

Conclusions: Our study described a model of renal injury and repair in the adult mouse after RUUO. Following the relief of obstruction, the indicators of inflammation, EMT and fibrosis were attenuated significantly.

Funding: Private Foundation Support

PUB673

Cobalt Chloride Deteriorates Renal Fibrosis after Ischemia Reperfusion Injury Associated with Decreased Hypoxic Renal Tubular Cells Yoichiro Ikeda, Tetsuhiro Tanaka, Reiko Inagi, Masaomi Nangaku. *Division of Nephrology and Endocrinology, University of Tokyo, School of Medicine, Tokyo, Japan.*

Background: Chronic hypoxia exists in chronic kidney disease in general. Renal ischemia reperfusion injury (IRI) devastates renal architecture especially in tubules with maximal magnitude in one day and within some days, kidney restores histologically and functionally with some traces of the insult, namely tubulointerstitial fibroses according to the magnitude of the insult. Cobalt chloride (Co), hypoxia mimetics, upregulates hypoxia inducible factors (HIFs) and its administration before IRI ameliorate IRI, but its effect on chronic kidney diseases is controversial and may be dependent on cases. HIF-1a is predominant in tubules. Pimonidazole is a chemical indicating the histological low oxigen tension. Here we demonstrate Co administered at recovering phase of IRI deteriorates renal function and fibrosis.

Methods: Wistar male rats were subjected to IRI by clamping bilateral renal arteries for 45min. After 24h, BUN and Cre were measured to confirm the same insult, and Co was injected intraperitoneally (30mg/kg) once and continuously administered by osmotic pump (80mg/rat). Pimonidazole was injected (60mg/kg) and 1h later they were sacrificed 15days after IRI with PFA perfusion fixation.

Results: Renal function and fibrosis were deteriorated by Co administration at restoring phases of IRI for 14days (BUN 23±1.5 vs 39±2.2 mg/dL, Cre 0.71 ± 0.08 vs 1.0±0.08 mg/dL; IR-Co(-) vs IR+Co(+), mean±SEM). Immunohistochemical staining of pimonidazole revealed sustained hypoxia especially in cortical tubules even weeks after IRI, and Co decreased the area of hypoxic tubules in cortex due to dropout of tubules. Elevation of HIF1a protein level sustained elevated after IRI for weeks, which was enhanced by Co administration.

Conclusions: Hypoxia sustained in cortical tubules long after IRI with enhancement of HIF. Cobalt chloride administered at the restoring phase of IRI deteriorated renal function and fibrosis. HIF activation by cobalt chloride during the development of renal fibrosis is dependent on the timing of the administration.

PUB674

Protein Loading Mediates Epithelial-to-Mesenchymal Transition in Biomimetic Proximal Tubular Microenvironment Hong Li Lin. *Tthe First Affiliated Hospital of Dalian Medical University.*

Background: Leaked protein-loading has been implicated in the development of tubulointerstitial injury in the clinical and basic researches when glomerulonephritis existed. Yet we lack an in vitro model that would allow exploration which type of the leaked proteins damaged renal interstitial and further our ability to elucidate the underlying mechanisms that govern the progression of tubulointerstitial fibrosis.

Methods: Here, we introduced a simple microfluidic and compartmental cell-culture system in which simulate proximal tubular microenvironment and recapitulated the process of epithelial to mesanchymal transition (EMT) in vitro. The system consisted of two micro scale devices, one for exploration of EMT inductors of multiple protein loading, the other for assay of the behavior of cells migration directional trans-tubular basement membrane (TBM) the process of EMT.

Results: The results showed that, on the microfluidic rapid evaluating of multi-inductors device, tubular epithelial cells exposed to normal serum proteins presented phenotypic and functional characteristics of mesenchymal cells. The expression of E-cadherin was reduced and the expression ofα-SMA and FSP-1 was increased. Exposure of the cells to the complement anaphylotoxin C3a induced similar features. On the compartmental microscale decice, cells migrated significantly, mediated by TGF-β1 and C3a, along with the matrix around destroyed during the process of EMT. And cells migrated accompanied with morphological changing from typical "flagstone" to obvious spindle shape.

Conclusions: In summary, this microscale model enables control of both spatial and temporal aspects within the microenvironment, allowing recapitulation of the environment *in vivo* in ways not practical with existing experimental models. We found that lower concentration of active serum proteins can induce EMT on proximal tubular epithelia cells (PTECs) on the microfluidic devices, and the complement anaphylotoxin C3a is an important mediator of proximal tubular injury and can induce tubular EMT. Meanwhile, we proved PTECs through EMT migrate trans-TBM directionally *in vitro.*

Funding: Private Foundation Support

PUB675

Targeting Kirsten Ras Antisense in the Murine Chronic Folic Acid Nephropathy Model Lucy Jade Newbury, Bruce M. Hendry, Claire C. Sharpe. *Department of Renal Medicine, Kings College London, London, United Kingdom.*

Background: Renal fibrosis is thought to occur via different signaling pathways, many of which converge on Ras GTPases. We used antisense oligonucleotides (ASO) to silence Kras expression to observe its effect on interstitial fibrosis in a novel chronic folic acid nephropathy (CFAN) model.

Methods: ASO were screened in CD1 mice. 20mg/kg ASO were injected SC on alternate days for 6 days. The animals were killed on day 7.

The CFAN model: Male CD1 mice were given folic acid (FA) 125mg/kg IV in $NaHCO_3$ at day 0 and at day 21 (n=24). Controls received $NaHC0_3$ alone(n=8). FA-treated animals were divided into three equal groups. Each group received 3x SC injections/week from day 35 reducing to 2/week from day 49 of saline, ASO166 or ASO143 (20mg/kg). Animals were killed on day 85. Control groups were killed on day 0 (n=4) and day 85 (n=4).

Kras mRNA knockdown was analyzed by RT-QPCR. Fibrosis was analyzed by quantifying total collagen using picrosirius red staining (PSR).

Results: In normal mice we achieved 43% and 44% knockdown of Kras with ASO166 and 143, respectively. FA-treated mice gained weight more slowly than vehicle controls, demonstrating acute weight loss post FA injections ($p> 0.05$). The FA placebo group showed a trend increase in Kras expression when compared with aged controls. The FA-ASO143 group showed a significant Kras mean knockdown, of 43% when compared with placebo-treated animals ($p<0.05$). The FA-ASO166 demonstrated a non-significant mean knockdown of 29%.

FA-treated animals demonstrated a 3.4 fold increase in collagen deposition at day 85 when compared with vehicle. ASO-166 did not appear to affect fibrosis. ASO-143 was associated with a 27.5% reduction in fibrosis compared to placebo-treated animals. Although this was not statistically significant, the fibrosis score of each animal showed a positive correlation with Kras mRNA expression.

Conclusions: This novel chronic folic acid model achieved a 3.4 fold increase in collagen deposition (PSR) compared with controls. Expression of Kras mRNA positively correlates with amount of collagen deposition in the kidneys - This highlights Kras as a potential therapeutic target.

PUB676

Immunization against Marinobufagenin Inhibits Renal Fibrosis in Experimental Uremic Cardiomyopathy Steven T. Haller,[1] Jiang Liu,[1,2] Yanling Yan,[1,2] Anna P. Shapiro,[1] Olga Fedorova,[4] Alexei Bagrov,[4] Joseph I. Shapiro,[1,3] Deepak K. Malhotra.[1] *[1]Medicine, University of Toledo, Toledo, OH; [2]Pharmacology, Marshal University, Huntington, WV; [3]Medicine, Marshal University, Huntington, WV; [4]National Institute on Aging, Baltimore, MD.*

Background: Experimental uremic cardiomyopathy causes cardiac fibrosis and is associated with increased levels of the cardiotonic steroid marinobufagenin (MBG), a ligand of the Na/K-ATPase. We have shown that treatment with a monoclonal antibody against MBG (3E9 mAb), and Digibind (the Fab fragments of ovine digoxin antibody), which binds to endogenous cardiotonic steroids, attenuated cardiac fibrosis and improved creatinine clearance in experimental renal failure. In the present study, we tested the effects of the 3E9 mAb and Digibind treatment on the development of renal fibrosis.

Methods: Male Sprague Dawley rats weighing between 250-300 gms were used for these studies. At four weeks following partial nephrectomy (PNx,) these rats were administered vehicle (n=6), 10 mg/kg of Digibind (n=6), or 50 µg/kg of the 3E9 anti-MBG mAb (n=6). One week following treatment (five weeks post PNx), animals were sacrificed and tissues were harvested for biochemical and histological analysis.

Results: The 3E9 mAb treatment group demonstrated a substantial decrease in renal fibrosis (assessed by trichrome staining, sirius red/fast green staining, and Western blot) compared to PNx. Digibind treatment had a similar, yet less pronounced effect.

Conclusions: Our results suggest that immunization against MBG may offer a potential therapy for uremic cardiomyopathy by inhibiting the development of renal fibrosis.

Funding: Clinical Revenue Support

PUB678

Mesenchymal Stromal Cells and Lisinopril in Combination Therapy Reduce Renal Fibrosis Inhibiting Renin Angiotensin System in Unilateral Ureteral Obstruction Model Giulia Bedino, Marilena Gregorini, Teresa Valsania, Chiara Rocca, Valeria Corradetti, Francesca Bosio, Eleonora Francesca Pattonieri, Pasquale Esposito, Carmelo Libetta, Teresa Rampino, Antonio Dal Canton. *Unit of Nephrology, Dialysis & Transplantation, Fondazione IRCCS Policlinico San Matteo & University, Pavia, Italy.*

Background: Renin-angiotensin system plays a pivotal role in renal fibrosis and angiotensin-converting enzyme inhibitors(ACEi)are the most effective therapy to reduce progression of chronic kidney disease.It is known that ACEi induce a compensatory increase in plasma renin levels and renin promotes renal injury by stimulating angiotensinII(ANG II) generation and up-regulating pro-fibrotic genes.We have proved that ACEi and mesenchymal stromal cells(MSC)reduce renal fibrosis in unilateral ureteral obstruction model(UUO). Aim of this study was to understand the mechanism underlying the protective effects of ACEi and MSC inUUO.

Methods: We studied 5 groups of SD rats.MSC were isolated by bone marrow.A:5 rats sham operated.B:8 rats UUO received saline solution d0.C:8 rats UUO received MSC d0 via tail vein.D:8 rats UUO received lisinopril(ACEi),d1-21.E:8 rats UUO received MSC d0, and ACEi d1-21.Rats were sacrified on d7 and 21.Serum ANGII levels,renin mRNA expression,ED1 positive cells,interstitial fibrosis(Masson's trichrome staining),%of broken tubules/HPF were evaluated.

Results: Serum ANGII levels increased in B compared to A,monotherapy with MSC reduced ANGII levels, but ACEi and combination therapy determined a further suppression effect($p<0.05$)on d7.On d21 serum ANGII levels were lower in all rats.Renin mRNA expression did not increase in B and C, compared to A,while increased in D.Rats receiving combination therapy showed lower renin mRNA levels compared to D.ED1 positive cells number in B was greater than in C and D.In E it was further reduced.The fibrosis was less severe in C,D and even more inE than inB($p<0.05$).% of broken tubules/HPF was reduced in D compared to B and C($p<0.05$),but further in E.

Conclusions: Our results show that MSC in UUO model prevent renin increase,reduce angiotensinII generation but combination therapy suppress further ANGII block leading to a synergistic effect.

PUB679

Mesenchymal Stromal Cells and Lisinopril in Combination Therapy Modulate Scatter Factors in Unilateral Ureteral Obstruction Model Valeria Corradetti, Marilena Gregorini, Teresa Valsania, Chiara Rocca, Francesca Bosio, Eleonora Francesca Pattonieri, Giulia Bedino, Pasquale Esposito, Carmelo Libetta, Teresa Rampino, Antonio Dal Canton. *Nephrology, IRCCS Policlinico S.Matteo and University of Pavia, Pavia, Italy.*

Background: Hepatocyte Growth Factor (HGF)/Met and Macrophage Stimulating Protein (MSP)/Ron systems are Scatter Factors that regulate tissue remodelling and inflammatory cells response in renal diseases, including unilateral ureteral obstruction (UUO). We have proved that angiotensin-converting enzyme inhibitors (lisinopril, ACEi) and MSC accelerate renal repair in UUO. The aim of this study was to evaluate whether ACE and MSC in mono and combination therapy induce renoprotective effect modulating Scatter Factors.

Methods: We studied 5 groups of Sprague-Dawley rats. MSC were isolated from bone marrow. Group A: 5 sham-operated rats. Group B: 8 rats undergone UUO received saline solution on day 0, Group C: 8 rats UUO received MSC ($3x10^6$) on day 0 via tail vein. Group D: 8 rats UUO received ACEi in water, days 1-21. Group E: 8 rats UUO received MSC on day 0 and ACEi, days 1- 21. Rats were sacrified on days 7 and 21. HGF and MSP mRNA (RT- PCR), HGF, Met, MSP, Ron proteins (Western blot) were analysed in renal tissue. Ron and Met expression were evaluated by immunohistochemistry.

Results: HGF and MSP mRNA were not significantly different in all rats. HGF and Met proteins were similar in A and B, increased in rats treated respectively with MSC (C) and ACEi (D)($p<0.001$ vs A and B), and rose further in E ($p<0.0001$vs A and B). MSP and Ron proteins were similar in A and B, but increased significantly only in C ($p<0.005$ vs A, B, D, E). Compared to sham operated rats (A) and controls (B) monotherapy with ACEi (D) and MSC (C) increased the extent of tubular Met staining ($p<0.005$), but combination therapy induced additional increase of positive tubular area ($p<0.001$). The combination therapy induced an increase of Ron tubular expression in E ($p<0.001$ vs B, C, D).

Conclusions: Our results showed that both ACEi and MSC may promote renal repair modulating Scatter Factors in UUO, yet the combination therapy lead to additive upregulation of HGF/Met and MSP/Ron signalling.

PUB680

A Short Isoform of Podocin Is Expressed in Human Kidney Linus A. Völker,[1] Eva-maria Schurek,[1] Tobias Lamkemeyer,[2] Denise Ungrue,[2] Christine E. Kurschat,[1,2] Bernhard Schermer,[1,2,3] Thomas Benzing,[1,2,3] Martin Höhne.[1,3] *[1]Renal Division, Department of Medicine and Center for Molecular Medicine, University of Cologne, Cologne, NRW, Germany; [2]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, NRW, Germany; [3]Sybacol - Systems Biology of Ageing Cologne, University of Cologne, Cologne, NRW, Germany.*

Background: Podocin, the protein encoded by the NPHS2 gene, is an essential and integral part of a functional kidney filtration barrier. Database searches and recent publications suggest the presence of a short 315 amino acid isoform of human podocin that lacks exon 5 and a central part of the PHB-domain. Given the importance of a functional PHB-domain in recruiting a specialized lipid microenvironment, we sought to elucidate the effect of this structural alteration on protein function and localization. Additionally, as all evidence of expression hitherto has been based on antibody-mediated detection or RT-PCR, conclusive proof of expression was required. We employed a bottom-up mass spectrometric approach to identify a peptide unique to the short isoform in human glomerular lysates. After tryptic digestion, the presence of a peptide bridging the gap between exon 4 and 6 was indicated by nanoLC-ESI-MS/MS. Upon biochemical analysis by sucrose gradient density centrifugation, we found this short isoform still to be associated with detergent-resistant membrane fractions. In overexpression, the short isoform was mainly retained in the endoplasmic reticulum while the full-length protein localized to the plasma membrane, indicative of divergent functions. An additional band of higher molecular weight in western blot could be attributed to post-translational modification: Removal of N-glycosylation by PNGase F treatment consistently abrogated the formation of the additional band, and site-directed mutagenesis of a potentially N-glycosylated residue mapped this effect to an asparagin at position 287 (355 in the full length protein). We present for the first time proof of presence of an additional human isoform of podocin on protein level, which adds new aspects to the molecular function of podocin in human podocytes.

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Albumin Exposure Induces an Inflammatory Response In Vitro and In Vivo
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Background: Albuminuria is associated with an increased risk of kidney disease progression. While the injurious effects of albumin on renal proximal tubule cells are well documented, the effects of albumin on podocytes are less well studied.

Methods: We studied previously characterized human podocytes isolated from urine (Sakairi et al., 2010). Podocytes were exposed to medium supplemented with fetal bovine serum plus either 5 mg/ml low endotoxin recombinant human albumin or dextran of a similar molecular mass as an oncotic control. Cell death as measured using the trypan blue exclusion assay. The inflammatory response to albumin was assessed by measuring cytokine mRNA levels using quantitative RT-PCR and cytokine release by ELISA. To examine the effects of albuminuria *in vivo* we used the murine albumin overload nephropathy model.

Results: After 24 hrs the percentage of dead cells was 23 +/- 5% in podocytes exposed to albumin versus 11 +/- 3 % in dextran treated cells (P<0.0001) and after 48 hrs the percentage of dead cells was 25 +/- 5 % versus 10 +/- 1 % for controls, P = 0.0009. Albumin exposure significantly increased caspase 3 and 7 activity (P<0.0001) and increased the number of apoptotic cells as measured by the TUNEL assay. Albumin exposure increased expression of interleukin-1beta (IL-β) expression (peak mRNA increase ~ 8 fold at 3 hours, peak protein increase, P=0.005 compared to control), increased tumor necrosis factor (TNF) expression (peak mRNA increase ~3 fold at 3 hours, peak protein increase ~15 fold at 6 hours, P=0.003 compared to control), increased interleukin-6 expression (peak mRNA increase ~ 7 fold and peak protein increase ~8 fold at 48 hours, P<0.0001 compared to control). After 6 sequential bovine serum albumin (BSA) injections glomeruli isolated from BSA-injected mice had a 2-fold increase in IL-1β and TNF expression compared to saline injected animals (P=0.02).

Conclusions: These results suggest that podocyte exposure to albumin induces a pro-inflammatory response and increases apoptosis. Thus albumin exposure may contribute to podocyte loss in proteinuric states.

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PUB682

Cholesterol Loading Activates Intracellular Renin-Angiotensin Systemin Human Renal Mesangial Cells: A Potential Mechanism for Glomerulosclerosis
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Background: Dyslipidemia and local activation of renin-angiotensin system (RAS) play crucial roles in the progression of chronic kidney disease (CKD). The present study was undertaken to investigate possible effects of dyslipidemia on intracellular RAS activation and its underlying mechanism in glomerulosclerosis using human renal mesangial cells (HMCs).

Methods: HMCs were cultured and divided into Control group (treated with serum free medium) and cholesterol loading group (treated with serum free medium plus 30 μg/ml cholesterol and 1μg/ml 25-hydroxycholesterol). The lipid accumulation in HMCs was examined by Oil red O staining. The production of extracellular matrix in HMCs was examined by immunohistochemical staining and immunofluorescent staining. The gene and protein expressions of molecules involved in RAS components and the pathway of mammalian target of rapamycin (mTOR) were examined by real-time PCR and Western blot.

Results: Cholesterol and 25-hydroxycholesterol loading increased lipid accumulation in HMCs, and inhibited protein expression of α-SMA and collagen IV. Further analysis showed that cholesterol loading upregulated mRNA and protein expression of RAS components (angiotensinogen, angiotensin II, rennin, angiotensin-converting enzyme, agiotensin II type 1 receptor and type 2 receptor) in HMCs. Interestingly, lipid accumulation activated mTOR pathway, characterized by increased mRNA and protein expression, and protein phosphorylation level of mTOR, eukaryotic translation initiation factor 4E binding protein 1(4EBP1), ribosomal protein S6 kinase 1(S6K1), which were closely correlated with intracellular RAS activation.

Conclusions: Cholesterol loading affected mesangial cell functions and stimulated extracellular matrix excretion through intracellular RAS activation, which was correlated with the upregulation of mTOR pathway, suggesting a potential mechanism in the progression of glomerulosclerosis.

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Podocytes Are Detached by PAI-1 Coupling with uPA and uPAR through the Internalization Mechanism
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Background: Mouse model of collapsing FSGS (NEP25 mice) with podocyte specific injury revealed thrombotic microangiopathy (TMA) with PAI-1 gene activation, and PAI-1 inhibitor ameliorates both proteinuria and disease progression with preservation of podocyte number (ASN 2011). Furthermore, in vivo, elevation of mRNA uPAR, which triggers internalization and degradation of PAI-1 with uPA, was observed in kidney from NEP mice. We hypothesized PAI-1 binding to uPA coupling with uPAR was associated with detachment of podocyte through the internalization mechanism.

To elucidate the mechanism whereby PAI-1 inhibition reserved podocyte number, we tested the role of PAI-1 on podocyte detachment *in vitro*.

Methods: Immortalized cultured podocytes were incubated either by uPA or PAI-1 alone (Group P, Group U), uPA+PAI-1 (Group U+P), uPA+PAI-1 with uPAR antibody or PAI-1 inhibitor (Group R, Group I), and remnant cell number was counted after incubation to assess cell detachment by effect of PAI-1. Immunofluorescent staining of uPAR on podocytes incubated by PAI-1 with uPA at 4°C (to prevent endocytosis and keep the protein at the cell surface)and 37°C (to promote endocytosis) were performed to analyze the localization.

Results: Most cells remained attached in Group U (100 ± 0.1%) and Group P (93.4 ± 1.5%).

Notably, when incubated with uPA+PAI-1, significant cells were detached, resulting in decreased attachment (60.6 ± 4.4%, $p < 0.05$ vs Group U). Furthermore, addition of uPAR antibody or PAI-1 inhibitor to uPA+PAI-1 restored attachment of podocytes (92.7 ±7.2, 110.9 ± 1.9%, respectively).

Confocal microscopy revealed that mostly uPAR with PAI-1 and uPA were on cell membrane at 4°C. However, after the incubation at 37°C for 5min, uPAR was decreasing from the cell membrane, and internalized in cell cytoplasm.

Conclusions: This suggests that PAI-1 involved in podocyte detachment by coupling with uPA and uPAR through the internalization mechanism, and potential role of PAI-1 inhibition to prevent podocyte from detachment.

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Mice Lacking Expression of the PI3K-C2β Enzyme Develop Mild Hyperglycaemia and Glomerular Abnormalities
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Background: In mammals phosphoinositide 3-Kinase (PI3K) enzymes form a family of 8 intracellular signalling molecules that regulate a wide spectrum of cellular processes. Class I PI3K isoforms (p110α, p110β, p110γ and p110δ) have been implicated in cancer, autoimmunity, chronic inflammation, allergy, cardiovascular and metabolic disease. We have recently shown that mice null for the class II PI3K enzyme PI3K-C2α develop focal segmental glomerulosclerosis, affecting podocyte morphology and function (Harris et al 2011). In contrast, the precise physiological role of the class II PI3K enzyme PI3K-C2β remains unclear. In this study, we investigate whether the PI3K-C2β enzyme is also required for maintaining normal renal architecture and function.

Methods: Mice null for PI3K-C2β (PI3K-C2β$^{-/-}$) were re-derived as previously described (Harada et al 2005). All experiments were performed using sex and age matched controls, and animals of both sexes were used in this study. Assessment of basal level parameters was performed in control and PI3K-C2β$^{-/-}$ mice. 50 glomeruli were counted/measured in each section for histological analysis. Renal histology was performed to quantify glomerular volume, number of nuclei inside glomerulus and mesangial matrix deposition using morphometric analysis.

Results: PI3K-C2β$^{-/-}$ mice developed mild proteinuria (24 hours urine) compared to WT mice. The lack of PI3K-C2β expression also resulted in elevated blood glucose concentrations compared to control animals. Histological examination of PAS stained kidney sections from PI3K-C2β$^{-/-}$ mice revealed an increased number of nuclei inside the glomerulus, mesangial matrix deposition and a greater glomerular volume.

Conclusions: Mice deficient in PI3K-C2β expression exhibit renal abnormalities demonstrating that the PI3K-C2β enzyme plays a critical role in the maintenance of normal kidney function. PI3K-C2β$^{-/-}$ mice also have mild hyperglycaemia and show glomerular abnormalities, supporting its role in insulin signalling.

PUB685

Extraglomerular C3 Deposition and Metabolic Impacts in Patients with IgA Nephropathy
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Background: Although extraglomerular (Bowman's capsule and/or arteriole) deposition of C3 is encountered in the results of routine histological studies in IgA nephropathy (IgAN), these findings have received little attention. The objective of the present study then was to explore the clinical significance of extraglomerular C3 (ex-C3) deposits in IgAN patients.

Methods: 170 patients with IgAN were divided into two groups: Group A (n = 79), patients who did not have ex-C3 deposits and Group B (n = 91), patients who had ex-C3 deposits. Anthropometric, histological and laboratory data were evaluated in a cross-sectional as well as in a prospective manner.

Results: At the time of renal biopsy, Group B was characterized by a significant increase in diastolic blood pressure, as well as total cholesterol, triglyceride and lower density lipoprotein-cholesterol levels. After 4 years, the estimated glomerular filtration rate (eGFR) in Group B was significantly decreased compared with that in Group A. In electron microscopy, arteriolar dense deposits in Group B were significantly higher than those in Group A. 134 patients underwent a 3 years follow-up study after the intervention and were re-divided into the following 2 therapeutic factors: "conventional therapy", i.e.

treatment with anti-hypertensive drugs and/or anti-platelet drugs, and "aggressive therapy", i.e. additional treatment with either tonsillectomy and/or corticosteroid. Patients treated with conventional therapy in Group B showed significantly higher levels of body mass index, C3 and CH50 compared with other groups. Aggressive therapy was significantly effective in urinary protein reduction in both groups A and B. Except for the patients who received aggressive therapy in Group A, the levels of eGFR gradually declined.

Conclusions: It is necessary to pay particular attention to IgAN patients who have ex-C3 deposits and to develop a new therapeutic approach.

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Long-Term Observation of Prognostic Factors and Outcomes of Japanese Patients with Pauci-Immune Crescentic Glomerulonephritis

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Background: Prognostic factor and outcome of patients with pauci-immune crescentic glomerulonephriris (CreGN) are presumed to vary among periods. We examined characteristics and prognostic factors of Japanese patients with CreGN according to the periods.

Methods: From 1968 to 2011, a total of 102 patients diagnosed as CreGN by renal biopsy in Kanazawa University Hospital and collaborative group was examined in this study. The patients were divided into three groups by the treated periods: Group I (1968-1988, 18 cases), group II (1989-2001, 37 cases) and group III (2002-2011, 47 cases). Mean follow-up period was 1625±1387 (mean ± SD) days. To evaluate prognostic factors at the diagnosis of RPGN, we selected age, sex, levels of proteinuria, serum creatinine (Cr), C-reactive protein and MPO-ANCA for clinical features, and degree of crescentic formation, interstitial inflammatory cell infiltration, interstitial fibrosis, vasculitis and atherosclerosis for pathological features. The Hazard ratios (HR) for renal and life outcome were estimated using a Cox regression model.

Results: The serum Cr (<3.0, 3.0-6.0, >6.0 mg/dl) and crescentic formation (<30, 30-50, 50-80, >80%) were a significant renal prognostic factor in group III (Serum Cr : HR 5.37, CI 1.84 – 15.6, $p < 0.01$. Crescentic formation : HR 2.86, CI 1.06 – 7.73, $p = 0.04$). Furthermore, renal survival rate of patients with less than 50% of crescentic formation and patient survival rate of patients with less than 3.0mg/dl of serum Cr improved in group III. On the other hand, neither renal nor patient survival rate of cases with more than 3.0mg/dl of serum Cr and 50% of crescentic formation improved in group III.

Conclusions: In conclusion, outcomes of the patients diagnosed with less than 3.0mg/dl of serum Cr associated with less than 50% of crescentic formationwas improved in recent years. However, prognosis of the cases diagnosed with serum Cr ≥ 3 mg/dL and rate of crescentic formation ≥ 50% was not improved.

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Immunoenzymatic and Biochip Array Profiling of the Biomarkers of Inflammation and Hemostatic Activation Processes

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Background: Considerably higher cardiovascular events occur in ESRD patients on hemodialysis. The objective of this study was to utilize newly introduced cardiac biomarker chips and immunoenzymatic methods, profiling various biomarkers of inflammation and thrombogenesis.

Methods: Seventy-two patients on maintenance hemodialysis were recruited for this study. The normal group comprised of 25 to 50 healthy male and female adults. Blood samples from the ESRD patients were drawn prior to maintenance hemodialysis. ELISA methods were used for Tissue Plasminogen Activator – Plasminogen Activator Inhibitor Type-1 complex (tPA-PAI-1 complex), Plasminogen Activator Inhibitor Type-1 (PAI-1), Myeloperoxidase, (MPO), Thrombomodulin, (TM), Interleukin-1 beta (IL-1β), Anti-Annexin V, human sL-selectin, and Inter-Cellular Adhesion Molecule 1 (ICAM-1). Functional methods were used for measuring Antithrombin and Von Willebrand factor (vWF) activity. The biochip arrays (Randox, Evidence System, United Kingdom)for cardiac markers, included creatine kinase-MB (CK-MB), Myoglobin (Myo), Heart-type fatty acid binding protein (hFABP) and cardiac troponin I (cTn1). The cerebral array included C-reactive protein (CRP), D-Dimer (DDMER), Neuron Specific Enolase (NSE), Neutrophil Gelatinase-Associated Lipocalin (NGAL), soluble Tumor Necrosis Factor Receptor 1 (TNFRI) and Thrombodulin (TM).

Results: ESRD patients showed assay dependent decrease in markers such as tPA-PAI-1, Anti-Annexin V, L-selectin and Antithrombin activity (ranging from 18 to 46%). The levels for MPO, TM, CK-MB, MYO, FABP, CRP, DDMER, NSE, NGAL, TNFR1, and vWF activity all showed elevation in the ESRD patients (ranging from 25%-5587%). No changes were observed in the IL-1B, ICAM1, or cTn1 in ESRD patients.

Conclusions: The cardiac biochip array revealed a remarkable elevation of the FABP and as well as a notable increase in MYO. The cerebral array revealed a remarkable elevation of the TNFR1. Taken together these results indicate that ESRD represents a complex pathophysiologic syndrome resulting in the generation of various prognostic biomarkers.

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Urinary Dipstick pH Accuracy: Better than You Thought

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Background: Urine pH measurement is a basic step to assess distal urinary acidification. The "gold standard" measurement is by pH meter determination in a morning sample collected under oil or in a sealed syringe. Dipstick urine pH measurement is utilized in multiple clinical circumstances such as urinary alkalinization, but its accuracy has been questioned. To determine dipstick pH accuracy, we compared it to the "gold standard."

Methods: pH of 87 urine specimens was determined by Ames Multistix and a pH meter. Dipstick accuracy was defined as the percentage of time the dipstick value was consistent with the metered value of each specimen. The dipstick pH was reported in 0.5 pH unit increments and was considered accurate if within ± 0.5 pH units of the metered value. The dipstick pH was accurate at pH 7.0 if the metered level was 7.00 to 7.50.

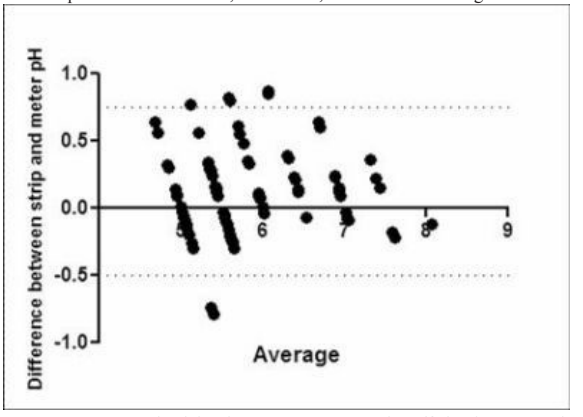
Results:

Patient's demographic

Age	18 – 94 (60.14 ±16.26)
Gender (M/F)	46/41
Urinalysis	69/87 (79.31%)
Proteinuria	
Microscopic Hematuria	59/87 (66.67%)
Blood chemistry panel (* mMol/L)	
Sodium*	127 – 145 (140.56 ± 4.24)
Potassium*	3.1 – 6.2 (4.38 ± 0.42)
Bicarbonate*	17 – 34 (29.09 ± 0.71)
Chloride*	93 – 117 (103.76 ± 7.07)
SCr (mg/dL)	0.5 – 6.28 (2.21 ± 1.81)

1. Dipstick pH accuracy was 88.50% in all specimens; 91.83%, 81.82%, 87.50% in urine pH of 5.0–5.5; 6.0–6.5; 7.0–8.0, respectively.

2. Bland-Altman plot demonstrated significant correlation between dipstick and metered pH values: bias 0.122, SD 0.3190, and 95% limits of agreement -0.503 to 0.747.



Conclusions: Dipstick urine pH measurement is sufficiently accurate in the clinical, diagnostic setting. It may be applicable to evaluate type I RTA, obviating cumbersome metered pH measurement; or function as a monitoring tool for specific therapies. Urine dipstick pH accuracy must be confirmed at each laboratory before utilization in this manner.

PUB689

Baseline Cohort Description of the Randomized Intervention in Children with Vesicoureteral Reflux (RIVUR) Trial

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Background: Vesicoureteral reflux (VUR) is a common congenital urological abnormality in children, which is believed to predispose to UTI and renal scarring.

Methods: RIVUR is a double-blind placebo-controlled trial of TMP/SMZ prophylaxis in children < 6 years old with grade I-IV vesicoureteral reflux (VUR) diagnosed following a 1st or 2nd UTI. The study involved central reading of renal imaging by expert radiologists, two each for VCUGs and dimercaptosuccinic acid (DMSA) renal scans. Patient enrollment started in June 2007 and was completed in May 2011. A total of 607 children were randomized at 19 participating sites. Primary referral sources for patient enrollment were urology, radiology, primary care practices, emergency departments, laboratories and inpatient.

Results: 49% of the randomized patients are aged 2-11 months (300), 92% (558) are females, 81% (482) are white and 5% (27) black; 87% (527) participants have non-Hispanic and 13% (77) have Hispanic ethnicity. Male participants (49) were younger (median age 5 mo) than female participants (median age 12 mo), p<0.0001. The distribution of VUR grades in randomized children is 61 (10%) grade I, 254 (42%) grade II, 230 (38%) grade III, 49 (8%) grade IV, and 5 (< 1%) missing grade, and did not differ by sex. Seven participants have grade 0 VUR and one has grade V VUR; these children were included

in the study because eligibility was based on local readings that were interpreted as grade I-IV. Baseline DMSA was done in 582 (96%) patients, and showed a variable degree of renal scarring in 21 (4%) of assessments.

Conclusions: Majority of the patients included in the study are caucasian females with grade II and III VUR and the number of patietns with renal scarring on baseline DMSA is lower than anticipated.

Funding: NIDDK Support

PUB690

Plasma CystatinC and Monocyte Chemotactic Protein-1 in ADPKD Progression Grazia Maria Virzi,[1,2] Fiorella Gastaldon,[1] Valentina Corradi,[1,2] Dinna N. Cruz,[1,2] Massimo de Cal,[1,2] Maurizio Clementi,[3] Claudio Ronco.[1,2] *[1]Nephrology Dep., St Bortolo Hospital, Vicenza, Italy; [2]International Renal Research Institute Vicenza, IRRIV, Italy; [3]Clinical Genetics, Univ. of Padua, Italy.*

Background: Autosomal polycystic kidney disease (ADPKD) is a hereditary cause of end-stage renal disease in the world. It is a genetically heterogeneous condition with a variable rate of progression and it is typically characterized by renal cystic changes paralleled by interstitial inflammation and gradual fibrotic changes. The aim of the study was to evaluate whether CystatinC (CystC) and Monocyte Chemotactic Protein-1 (MCP-1) could predict the progression of the kidney failure in ADPKD patients in stage II-III CKD, as defined in NKF-K/DOQI CKD Guidelines.

Methods: Patients with ADPKD, based on ultrasound criteria and genetic diagnosis, were enrolled and followed prospectively. Creatinine (sCr), CystC and MCP-1 levels were measured at baseline and followed up in plasma. eGFR was calculated with 4-variable standardized-MDRD formula. CystC and MCP-1 were measured by ELISA tests. Survival data were analyzed by the Kaplan-Meier curve using the median values as a cut-off value (CystC: 0,842 mg/L; MCP-1: 203,73 pg/ml).

Results: We enrolled 24 ADPKD pts (18M/6F; mean age 41±9 yrs); mean sCr was 1,3±0,7mg/dl and median eGFR was 62 mL/min/1.73m^2(range:36-75.5). After a median follow-up of 19months (range:12-27.5), 4 patients (17%) progressed to a worse stage of CKD and 83% were stable. No statistically significant relationship between higher levels of CysC and MCP-1 and ADPKD progression was observed by Kaplan-Meier survival curves. Although, CysC and MCP-1 levels were inversely correlated with eGFR (CysC r= -0,706; MCP-1 r= -0,472, both for p<.005). eGFR was higher in subjects with these values below medians.

Conclusions: We did not observe a relationship between CystC/MCP-1 and progression of CKD in ADPKD patients in stage II-III CKD. The present study has some limitations: it was a single-center study and the cohort of patients was very small. It is necessary to increase the sample size of ADPKD subjects enrolled to validate our hypothesis and to examine the relationship between proinflammatory cytokines and ADPKD.

PUB691

Effect of Paricalcitol on Renin and Albuminuria in Non-Diabetic Stage III-IV Chronic Kidney Disease: A Randomized Placebo-Controlled Trial Thomas Larsen, Frank H. Mose, Jesper N. Bech, Erling B. Pedersen. *Department of Medical Research, Holstebro Hospital, Holstebro, Denmark.*

Background: Vitamin D receptor activators reduce albuminuria and may improve survival in chronic kidney disease (CKD). Animal studies suggest that the reno-protection associated with vitamin D treatment is mediated by suppression of renin. However, randomized trials in humans remain to establish this relationship.

Methods: In a randomized, placebo-controlled, double-blinded crossover study, the effect of oral paricalcitol (2μg/day) was investigated in 26 patients with non-diabetic, albuminuric stage III-IV CKD. At the end of each six-week treatment period we determined plasma concentrations of renin (PRC), angiotensin II, aldosterone, brain natriuretic peptide, vasopressin and FGF23. BP was assessed by 24-h ambulatory BP monitoring, GFR by ^{51}Cr-EDTA clearance and assessment of renal NO dependency was performed by infusion of NG-monomethyl-L-arginine (L-NMMA). Albumin excretion was analyzed in both in 24-h urine and during the clearance experiment.

Results: Paricalcitol did not alter PRC, other hormones measured, 24-h BP or sodium and potassium excretion. FGF23 increased by 46% (p=0.001) without any major changes in plasma levels of calcium and phosphate. Paricalcitol reduced albumin excretion rate by 19% (p=0.003) and the albuminuric response to L-NMMA seemed abrogated by paricalcitol.

Conclusions: The assumption that paricalcitol reduces albuminuria by suppressing renin release was not confirmed in this study. The abrogation of the rise in albumin excretion during NOS blockade by paricalcitol may indicate that a novel pathway involving a favourable modulation of renal NO dependency could be involved in mediating reno-protection and survival benefits in CKD.

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PUB692

Normotensive and Hypertensive Scleroderma Renal Crisis Cases Compared by Renal Biopsy Yasunori Suzuki,[1] Fae Kim,[1] Ichiro Mizushima,[1] Hiroshi Fujii,[1] Masami Matsumura,[2] Mitsuhiro Kawano,[1] Kazunori Yamada.[1] *[1]Division of Rheumatology, Department of Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa, Japan; [2]Research Center of Medical Education, Kanazawa University Graduate School of Medicine, Japan.*

Background: Scleroderma renal crisis (SRC) is characterized by acute kidney injury, malignant hypertension and hyperreninemia. About 10% of SRC patients are normotensive, but its pathogenesis has not been well documented. The purpose of this study is to assess clinicopathological differences between hypertensive and normotensive SRC by renal biopsy.

Methods: We evaluated 4 patients with SRC, excluding ANCA positive cases, between 2007 and 2011. Three female and one male, mean age 60 years. All cases had diffuse cutaneous type and two were normotensive. Mean duration from onset of systemic sclerosis to SRC was 24 months. Three cases had taken steroid at the diagnosis of SRC. Anti RNA polymerase I/III was positive in 3 cases. All patients were treated with ACE inhibitors, 2 normotensive cases with plasma exchange, 2 with temporary dialysis and 1 with permanent dialysis. Renal biopsy was performed in all patients when the platelet count became normal.

Results: One of two normotensive patient had heart failure and might not be able to raise blood pressure. Another normotensive patient showed severe hemolytic anemia, thrombocytopenia and relatively low plasma renin activity (4.0 ng/ml/hr vs 11.8, 15.0 and 70.8). Renal biopsy of this patient showed mesangiolysis, subendothelial expansion and thrombi in glomeruli which were compatible with thrombotic microangiopathy (TMA). Glomerular lesions other than collapse of capillary tufts were shown only in this case. In this case, smaller arteries were affected than in the other 3 cases (mean size of arteries: 28.9 μm vs 67.5, 51.6 and 89.5).

Conclusions: In normotensive SRC, endothelial cell injury in relatively small artery may cause TMA.

PUB693

Immunohistochemical Evaluation of Collapsing Glomerulopathy in Non-HIV Patients: Series of Cases Andrea C.E.P. Valenca, Maria Carolina N.R. Neves, Luis H.B.C. Sette, Maria Alina G.M. Cavalcante, Gisele Vajgel Fernandes, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil.*

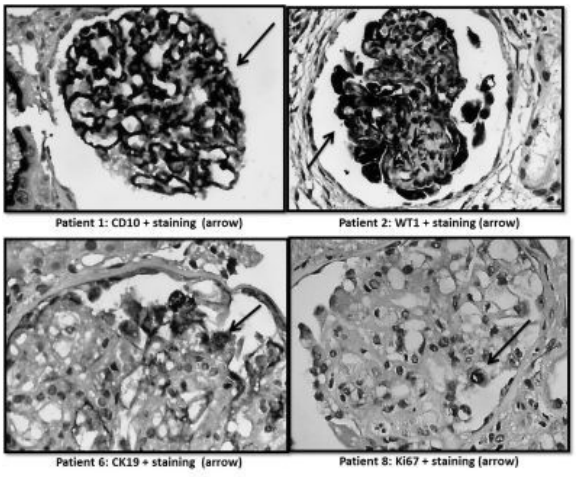
Background: Collapsing Glomerulopathy (CG) is characterized by severe podocyte injury and glomerular epithelial cell proliferation. It is clinically marked by proteinuria and renal insufficiency. First described as associated with HIV infection, it is now related with various etiologic factors. Collapsing variant of FSGS presents molecular changes that differs from others and can be demonstrated by immunohistochemistry (IHC).

Methods: We reviewed files of 11 patients wtih CG, according to Columbia classification, from 2005-2012. Renal biopsy specimens were submitted to IHC reactions with the following antibodies: CD10, WT-1, Ki67 and CK19.

Results: Patients characteristics were: mean age, 38.7 yrs; female, 54.5%; non-white, 54.5%. Nephrotic syndrome (NS) was the clinical presentation in 54.5% of the patients followed by rapidly progressive glomerulonephritis (RPGN) with 45.5%. None cause were associated in 72.7%, classified as idiopathic. Associated factors were pamidronate, tacrolimus (FK) and lymphangioleiomyomatosis (LAM). Laboratory revealed: mean proteinuria, 12.4g/day; albumin, 1.75g/dL; sCr, 3.18mg/dL. IHC did not show the lost of the expression of podocyte differentiation's markers (WT-1, CD10). Staining for CK19 was positive only in FK, LAM and pamidronate associated CG. Ki67 was positive in 2 other patients. Both are podocyte dedifferentiation's markers.

Epidemiological, Clinical and IHC Characteristics

Patient	Age	Gender	Clinical Presentation	WT-1	CD10	CK19	Ki67
1	49	M	RPGN	+	+	-	-
2	19	M	NS	+	+	-	-
3	69	F	RPGN	+	+	-	-
4	60	F	NS	+	+	+	-
5	27	F	NS	+	+	+	-
6	55	F	RPGN	+	+	+	-
7	14	M	NS	+	+	-	-
8	21	M	RPGN	+	+	-	+
9	33	F	NS	+	+	-	+
10	34	M	RPGN	+	+	-	-
11	45	F	NS	+	+	-	-



Conclusions: We described a serie of cases of 11 patients with non-HIV CG and its IHC analysis.

PUB694

Morphological Characteristics of Kidney Involvement in Plasma Cell Dyscrasias and Lymphoproliferative Disorders: One Center Experience
Elena Zakharova,[1] Ekaterina Stolyarevich.[2] *[1]Nephrology, Clinical Hospital n.a. Botkin, Moscow, Russian Federation; [2]Patology, Nephrology Center, Moscow, Russian Federation.*

Background: Kidney damage in plasma cell dyscrasias and lymphoproliferative disorders (PCD/LPD) include specific infiltration, organized and non-organized paraprotein deposition, paraneoplastic glomerulonephritis, treatment complications and others. We aimed to evaluate incidence of different kidney lesions in patients, admitted to nephrology unit and diagnosed with (PCD/LPD).

Methods: Using electronic database for 1994-1012 we searched 204 patients with "primary" amyloidosis, multiple myeloma (MM), Waldenström's macroglobulinemia (WM), non-Hodgkin lymphoma/leukemia (NHL/CLL), Hodgkin's lymphoma (HL) and monoclonal gammapathy of undetermined significance (MGUS). Work-up included serum and urine immunochemistry, kidney biopsy with light microscopy and immunofluorescent study, and occasionally electron microscopy, bone marrow aspiration and/or biopsy with immunohistochemistry and lymph node biopsy. Patients with non-verified morphologicaly kidney damage were excluded from analysis.

Results: 89 patients, 50 (56.2%) male/39 (43.8%) female, median age 56 [17; 78] had biopsy-proven kidney damage. Pathology and clinical correlations shown in table.

Paraprotein deposition and non-paraproteinemic lesions

Organized paraprotein deposits (N 66)				Non-organized paraprotein deposits (N 5)			
AL-amyloidosis	**AH-amyloidosis**	**Cryoglobulinemic GN**	**Cast-nephropathy**	**Light Chain Deposition Disease**	**IgM deposition GN**	**Monoclonal proliferative IgG/IgA GN**	
46							Primary amyloidosis
15			1	1			MM
1		1			2		WM
1							Castleman disease
	1						Franklin disease
						1	NHL/CLL
						1	MGUS
63 (70.7%)	**1**	**1**	**1**	**1**	**2**	**2**	**Total**
Non-paraproteinemic damage (N 11)				**Complications of treatment (N7)**			
Lymphoid infiltration	**Membranous nephropathy**	**Minimal changes**	**Focal segmental glomerulosclerosis**	**Tubulointerstitial nephritis**		**AA-amyloidosis**	
4	1			3		1	NHL/CLL
		2	1			2	HL
			1				WM
	1		1	1			MGUS
4 (4.5%)	**2**	**2**	**3**	**4 (4.5%)**		**3**	**Total**

Conclusions: In our cohort dominated AL-amyloidosis, constituting 71% of all biopsy-proven cases with more than 2/3 of "primary" amyloidosis. Specific lymphoid infiltration and drug-induced tubulo-interstitial nephritis were rare, about 4.5% each, other variants - miscellaneous.

PUB695

Detection of Urinary Trefoil Factor Family Peptides in Association with Renal Dysfunction and Proteinuria in Patients with Chronic Kidney Disease
Hiroshi Morinaga,[1] Hitoshi Sugiyama,[1] Masashi Kitagawa,[1] Ayu Ogawa,[1] Toshio Yamanari,[1] Keiichi Takiue,[1] Yoko Kikumoto,[1] Tatsuyuki Inoue,[1] Shinji Kitamura,[1] Yohei Maeshima,[1] Yasukazu Ohmoto,[2] Daisuke Ogawa,[1] Kenichi Shikata,[3] Hirofumi Makino.[1] *[1]Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan; [2]Otsuka Pharmaceutical Co., Ltd., Japan; [3]Center for Innovative Clinical Medicine, Okayama University Hospital, Japan.*

Background: The Trefoil factor family (TFF) peptides 1, 2 and 3 are small peptide hormones secreted by mucus-producing cells, and by epithelial cells from multiple tissues. TFF3 plays essential functions in both mucosal surface maintenance and restitution in the intestines, colon and kidney. A decrease in the urinary levels of TFF3 is associated with acute kidney injury in animal models. However, whether or not the urinary levels are associated with proteinuria and renal dysfunction in patients with chronic kidney disease (CKD) remains to be elucidated.

Methods: This study determined urinary TFF levels by peptide-specific ELISAs and investigated their association with clinical parameters in 219 CKD patients (mean age 53.6 years, female 47.9%, chronic glomerulonephritis 46.6%, nephrosclerosis 10.5%, mean eGFR 57.5 ml/min/1.73m^2).

Results: All TFF peptides were detectable in the urine of CKD patients (Concentration, TFF2 > TFF3 > TFF1). Significant correlations were identified between the urinary levels of TFF1 and TFF2 ($p < 0.0001$) or TFF3 ($p < 0.0001$), but not between urinary TFF2 and TFF3. The urinary TFF1 and TFF3 levels inversely correlated with eGFR, but TFF3 alone positively correlated with the excretion of urinary protein ($p = 0.0051$). The levels of urinary TFF2 decreased along with the degree of renal dysfunction and also inversely correlated with daily proteinuria ($p = 0.0165$).

Conclusions: These data suggested that the urinary excretion of TFF peptides are differentially regulated in CKD patients. TFF3 may be associated with renal dysfunction and proteinuria. Further examination is therefore required to elucidate the role of urinary TFF in CKD patients.

PUB696

Altered Vitamin D Receptor Expression in the Renal Tissues with IgA Nephropathy
Huiling Wang. *Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: Local inflammation is thought to contribute to the progression of IgA nephropathy (IgAN). Recently, vitamin D receptor (VDR) activated has reported ameliorate proteinuria and kidney injury with inhibited expression of proinflammatory cytokines, reduced renal infiltration of monocytes/macrophages, and suppressed expression of the fibrogenic factor. But these effects still need confirm in patients renal biopsy. The aim of study was to investigate the change of VDR in renal tissues of patients withIgAN, cooperative expression of Transforming growth factor-β(TGF-β1),Monocyte chemoattractant factor (MCP-1), endo-Nitric oxide synthase (eNOS) and Angiotensin II (AngII) and its association with changes of renal injury.

Methods: Fifty patients diagnosed as having IgAN by renal biopsy were enrolled as IgAN group(IgAN), and 5 normal renal biopsy specimens were used as controls(CTL). Expression of VDR,TGF-β1,MCP-1,eNOS and AngII in renal tissues was observed by immunohistostaining. The relationships between these factors and degree of renal pathological lesions in IgAN were assessed by Spearman correlation.

Results: Immunohistostaining analysis showed that VDR protein was present in epithelial cells of renal tubules, mesangial cell, andin normal and IgAN renal tissues. With more severe renal pathological lesions, the expression of VDR in IgAN was decreased(21.5±6.63 IgAN vs 36.8±18.71CTL, $p<0.05$);and the inflammatory cytokines increased respectively, TGF-β1(35.7±19.57 IgAN vs 18.3±10.34 CTL, $p<0.05$), MCP-1(19.7±13.08 IgAN vs 10.1±5.25 CTL, $p<0.05$), eNOS(17.2±8.78 IgAN vs 8.8±10.95 CTL, $p<0.05$);AngII(8.5±3.62 IgAN vs 4.4±3.73 CTL, $p<0.05$). The expression of VDR was negatively correlated with the degree of pathological lesions, including of mesenchymal cell proliferation, tubular atrophy, interstitial fibrosis, and inflammatory cell filtration. A single factor analysis showed that VDR was negatively correlated to the expression of TGF-β, MCP-1, and AngII, but no relationship with eNOS.

Conclusions: The expression of VDR showed down-regulation in renal tissue of patients with IgA nephropathy, and negatively correlated with the severity of pathological lesions and inflammatory factors.

Funding: Government Support - Non-U.S.

PUB698

Minimal Change Disease with IgA Deposition Might Not Be a Phenotype of IgA Nephropathy
Yu Yan, Wei Chen, Bao Dong, Mei Wang. *Renal Division, Peking University People's Hospital, Beijing, China.*

Background: Some IgAN patients with nephrotic syndrome (NS), who mostly have no hematuria, no hypertension and normal renal function were named MCD with IgA deposition (MCD-IgA). Whether it is a special phenotype of IgAN or MCD was in debate. The current study was to investigate the relationship among MCD-IgA, MCD and typical IgAN through clinical and pathological analysis.

Methods: Patients diagnosed as IgAN presenting NS without hematuria, hypertension and renal dysfunction were enrolled in the study. Those with NS and hematuria but without

hypertension and renal dysfunction (NS-IgAN) and those with nephrotic proteinuria without hypoalbuminemia (HP-IgAN) were used as controls. Clinical features were collected. Pathological slides were studied using two scoring systems (Katafuchi's IgAN scoring and OXFORD-MEST). Numbers of glomerular inherent cells were counted using image acquisition. Percentages of each cell were calculated.

Results: Clinically, comparing with HP-IgAN and NS-IgAN, patients with MCD-IgA and MCD had lower SBP, albumin, serum IgG, less urine RBC, TCHO and LDL-C ($P<0.05$). No significant differences were found between MCD-IgA and MCD. No significant differences were seen comparing with HP-IgAN. There were only less urine RBC and lower UP in NS-IgAN group than in HP-IgAN ($P<0.05$). In pathological analysis, MCD-IgA had lower scores in every item and total score except vascular hyaline score according to Katafuchi's IgAN scoring system. The same profile was obtained using OXFORT-MEST scoring system ($P<0.01$). 38 patients who had at least one glomerulus with both urinary pole and vascular pole in the slides were used for cells counting. Similar with MCD, MCD-IgA had more podocytes but less mesangial cells than HP-IgAN and NS-IgAN. All inherent cells percentages were similar between HP-IgAN and NS-IgAN ($P<0.05$).

Conclusions: MCD-IgA may be MCD with non-specific deposition rather than a phenotype of IgAN. More podocytes with less mesangial proliferation might help to differentiate MCD-IgA from typical IgAN.

Funding: Government Support - Non-U.S.

PUB699

Proximal Tubule Expression of Klotho in Healthy Kidney Transplant Donors Eileen W. Tsai,[1] Sharon E. Joo,[1] R.C. Pereira,[1] Miguel Fernando Palma Diaz,[2] Robert B. Ettenger,[1] Isidro B. Salusky,[1] Katherine Wesseling-Perry.[1] *[1]Pediatrics, Division of Nephrology, Mattel Children's Hospital UCLA, Los Angeles, CA; [2]Pathology and Laboratory Medicine, UCLA, Los Angeles, CA.*

Background: FGF23 requires the presence of Klotho to induce renal phosphate wasting; however, membrane-bound Klotho has not previously been identified in human proximal tubular cells.

Methods: Immunohistochemical staining using a monoclonal anti-goat antibody against human Klotho (Immuotopics) was performed in wedge biopsies from 16 healthy adult kidney transplant donors. Protein expression was classified as present or absent by two blinded observers and demographic data were compared between groups.

Results: Klotho expression was detected exclusively in proximal tubular cells in 10 biopsy specimens (63%). Specimens with Klotho staining were derived exclusively from deceased donors who, as a group, were younger than their living donor counterparts. Additionally, living donor kidneys were preserved with heparinized solution.

Donor Kidney Characteristics	Klotho positive (n=10)	Klotho negative (n=6)
Age (years)	22 ±3.9*	38 ±8.9*
White	3 (30%)	4 (66.7%)
Male	5 (50%)	3 (50%)
Deceased Donor	6 (100%)	0 (0%)*
Heparinized Preservation Solution	0 (0%)	6 (100%)*
Cold Ischemia Time (hours)	15.8 ±5	1.3 ±0.7*

*p<0.05 between groups

Conclusions: Klotho protein expression localizes to the proximal renal tubule in kidneys from deceased donors. The absence of Klotho in living donor kidneys may be secondary to the use of heparinized solution and/or increased donor age.

Funding: NIDDK Support

PUB700

Validation of Point of Care Module (Nitto Denko Portable Urinalysis Device) for Detection of Urinary Microalbuminuria Vijay K. Kher,[1] Manish Jain,[1] Puneet Sodhi.[1] *[1]Medanta-The Medicity, Gurgaon, India; [2]Nitto Denko Asia Technical Centre, Singapore.*

Background: Microalbuminuria (MAU) is an early marker for detection of renal and cardiovascular disease.Current urine analysis for MAU is restricted to laboratories. Measuring MAU at the point of care helps to screen large population in countries like India.

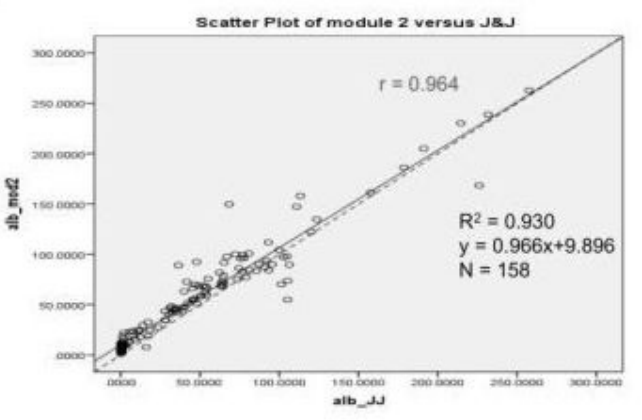
Nitto Denko Asia Technical Center,Singapore has devised a portable hand held device (150mm x 75mm x 25mm) by which urine ACR could be rapidly detected bed side.

The preliminary study was to test the efficacy of the device with urine of patients as well as normal subjects and comparing it with the standard laboratory test (Johnson and Johnson Vitros 5.1-Turbidometric immunoassay) at Medanta.

Methods: A total of 164 urine samples were collected and analysed for albumin and creatinine by Nitto Denko Device in comparison with standard test performed in Medanta Laboratary.

Results: The concentration correlation for urine albumin between both the methods is 0.964.

Figure 1 shows the scatter plot of urine albumin obtained by Nitto Device(Module 2) vs Medanta Laboratory (J&J).R2 value of0.930 suggests 93% of variability of data could be explained by linear regression.



The concentration correlation for urine creatinine is 0.981.The R2 is 0.962 suggesting 96.2% of variability of data could be explained by linear regression.

Conclusions: The Nitto Urinalysis Device was found to be comparable with the standard laboratory method in terms of the determination of urine albumin and creatinine concentrations.

This is a preliminary study to validate the Point of Care module to measure Microalbuminuria.We plan to confirm the results across a larger set of samples across multiple centres.

The authors acknowledges the members from Nitto Denko Asia Technical Centre (Visit Thaveeprungsriporn, Yingsong Wang, Hui Yee Sin, Juliana Chua) and Drs.A.Raizada,Deepti Gupta & K.Vinayak for their contributions.

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PUB701

Analysis of Relative Expression Level of VEGF, HIF-1alpha, and CTGF Genes in Renal Tissue from Renal Biopsies of Chronic Glomerulonephritis Patients Rafal Donderski,[1] Ilona Miskowiec-wisniewska,[1,2] Jacek Manitius,[1] Andrzej Marszalek,[3] Andrzej Tretyn.[4] *[1]Dept. of Nephrology, Hypertension and Internal Diseases, CM UMK; [2]Dept. of Hygiene and Epidemiology, CM UMK; [3]Dept. of Clinical Pathology, CM UMK; [4]Dept. of Biotechnology, Nicolaus Copernicus University, Torun, Poland.*

Background: Gene expression analysis of renal tissue is a valuable diagnostic tool in renal diseases. We wanted to evaluate a relative gene expression level of VEGF, CTGF and HIF-1α and to evaluate relationship with eGFR and daily proteinuria (DP) in chronic glomerulonephritis (CGN) patients (pts).

Methods: 28 pts with CGN. Type of CGN confirmed by kidney biopsy: MCD-3pts, IgA nephropathy-5pts, FSGS-3pts, MN-4pts, MPGN-4pts, lupus nephritis-6pts, Wegener's Granulomatosis-2 pts; hypertensive nephropathy-3pts. Control-renal tissue from 3 pts with normal eGFR and histology. eGFR and DP were assessed at the time of biopsy and at 6months intervals. RT-PCR was used to determine relative gene expression. The *GAPDH* gene served as normalization control.

Results: At the time of the biopsy relative expression of 3 genes were diminished in comparison to control.

Table 1. Results of relative gene expression level

GENE	TYPE	Reaction efficiency	Expression	Std.Error	95% C.I.	P(H1)
VEGF	TRG	1,06	0,300	0,163-0,485	0,107-1,445	0,003
GAPDH	REF	0,99	1,000			
CTGF	TRG	1,06	0,571	0,271-1,474	0,133-2,005	0,211
GAPGH	REF	0,99	1,000			
HIF1alfa	TGR	0,95	0,614	0,106-1,559	0,048-22,535	0,542
GAPDH	REF	0,99	1,000			

Legend: P(H1)- Probability of alternate hypothesis that difference between sample and control is due only to chance, TRG - Target, REF – Reference.

Pts with GFR≤60ml/min (at the onset of study) showed significantly lower VEGF relative expression in compare to pts with GFR≥60ml/min ($p<0,022$), we found higher VEGF expression in pts with DPE≥3,5g in compare to subjects with DPE≤3,5g ($p<0,034$) at the onset of study. There were no statistically significant relationship between VEGF, CTGF and HIF-1α relative gene expression and ΔeGFR and ΔDP.

Conclusions: Overexpression of VEGF gene in pts with DP≥3,5g may indicate oxygen supply deficiency in renal tissue which may result in tubulointerstitial fibrosis with further renal function impairment and eGFR decline.

PUB702

Sarcoidosis: Atypical Renal Presentation Fernando Caeiro Pereira, Fernanda Carvalho, Dulce Carvalho, Manuel A. Ferreira, Fernando Barbosa Nolasco. *Nephrology, Hospital Curry Cabral, Lisbon, Portugal.*

Background: Sarcoidosis is a systemic disease associated with derangement of the immune system, with upregulation of the TH1 response. Renal involvement is albeit rare (5-10% of cases) and is usually manifested has nephrocalcinosis and nephrolitisis, however some cases of acute kidney injury (AKI) due to acute interstitial nephritis with or without granulomas have been described.

Methods: We retrospectively analyzed all the biopsies performed at our institution between January 1992 and Septembre 2011, and identified five patients with the diagnosis of acute kidney injury due to sacoidosis. We performed imunohistochemistry analysis of these specimens.

Results: Median age at time of presentation was 53,4 years and median creatinine was 5,54mg/dL. All patients were started on prednisolone (40-60mg/day). Two patients required hemodialyis but only one recovered. All other patients had a remarkable recovery of GFR (average 55ml/min) and normalization of calcium levels. Median follow-up is 8,6 months (2 to 16).

Morphological analysis of all biopsies and identification of the cells present by imunohistochemistry reveled interstitial infiltrate has the predominating feature with no glomerular involvement whatsoever. Only one biopsy had granulomas. The infiltrate was characterized by lymphocytes, particularly CD4.

Kidney biopsies morphological analysis

Patient	Glomerulus	Cellular Infiltrate	Tubular atrophy	Interstitial fibrosis	Sarcoid angiitis	Granuloma	Asteroid bodies
1	0	+++	+++	++	0	0	0
2	0	+++	ATN	0	0	0	0
3	0	++	++	+++	0	+	+
4	0	++	+++	+++	+	+	+
5	0	++	++	+	0	0	0

ATN: acute tubular necrosis

Conclusions: Even if sarcoidosis is a rare cause of AKI its timely diagnosis is important since it seems to respond fairly well to treatment, however because diagnostic criteria have not been firmly established a large amount of clinical suspicion associated with all available data are required. Finally, granulomas may not be present in the biopsy specimen, as they are rare and sparse, but fibroedema with a large cellular infiltrate of lymphocytes is highly characteristic.

PUB703

Lectin-Based Flow Cytometry for Glycoprofiling of Urinary Extracellular Vesicles and Tamm-Horsfall Protein Anja Krüger,[1,2] Jared Q. Gerlach,[2] Shirley Hanley,[1] Christopher James Ward,[3] Marie C. Hogan,[3] Lokesh Joshi,[2] Matthew D. Griffin.[1] *[1]Regenerative Medicine Institute, National University of Ireland, Galway, Ireland; [2]Glycosciences Group, National University of Ireland, Galway, Ireland; [3]Dept of Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Detection of alterations to urinary extracellular vesicles (uEVs) may be a novel, non-invasive means to diagnose and monitor kidney health. In this study, flow cytometry (FCM)-based analysis of lectin binding was adapted to profile glycosylation of uEVs as well as purified Tamm-Horsfall protein (THP).

Methods: uEVs were isolated by ultracentrifugation (UC) or by centrifugal filter concentration (CON) from multiple healthy volunteers and were labelled with PKH26. For FCM analysis, 5 µg of uEVs or purified THP were attached to 4 µm aldehyde/sulfate beads and were incubated with 6 distinct biotinylated lectins followed by streptavidin-PE-Cy7 or -APC. A FACSCanto cytometer and FlowJo software were used for detection and analysis of lectin binding. uEV proteins and THP were also immunoblotted on PVDF membranes with biotinylated lectins followed by Avidin-AP.

Results: Binding of PKH26-labelled uEVs prepared by UC and CON methods to latex beads was confirmed by FCM. uEV- and THP-coated beads produced distinct patterns of lectin binding. PNA (Galβ(1,3)GalNAc) bound selectively to uEV- but not to THP-coated beads. Other lectins - AAA (α-Fuc), SNA-I (NeuAcα2,6Gal/GalNAc) and MAA (NeuAcα2,3Gal) – also bound consistently to uEVs from multiple donors, albeit with lower intensity, indicating a complex profile of surface glycosylation. In contrast, PHA-E (biantennary N-glycans) and WFA (sulfated GalNAc/GalNAc) bound at high to medium intensity to THP but only weakly or variably to uEVs. Immunoblots of uEV-derived protein lysates with PNA, AAA and SNA-1 demonstrated distinctive band patterns consistent with a broad repertoire of differentially-glycosylated proteins.

Conclusions: uEVs contain differentially-glycosylated surface proteins which are detectable by lectin-based FCM. Lectin-binding profiles of uEVs are distinct from THP and may serve as diagnostic/prognostic markers of renal diseases.

Funding: Government Support - Non-U.S.

PUB704

Glomerulonephritis Combined with Nutcracker Syndrome in Children with Isolated Proteinuria Jin-soon Suh,[1] Yumi Choi,[2] Byoung-Soo Cho.[2] *[1]Department of Pediatrics, Bucheon St. Mary's Hospital, Catholic University, Bucheon-si, Gyeonggi-do, Korea; [2]Department of Pediatrics, School of Medicine, Kyung Hee University, Seoul.*

Background: Nutcracker syndrome (NS) refers to the compression of the left renal vein between the aorta and the superior mesenteric artery, resulting in renal venous hypertension. It is known that NS is the frequent cause of hematuria and/or low-grade proteinuria and is a benign condition. However, some reports have suggested that NS can be combined with glomerulonephritis (GN) such as IgA nephropathy (IgAN). In this study, we evaluated the characteristics of patients with GN in combination with NS in children.

Methods: This study included 8 children with isolated proteinruria who were diagnosed of NS by renal Doppler sonogram and evaluated for GN by renal biopsy between 2002 and 2012. The indication of renal biopsy were based on the following criteria: persistent and increasing proteinuria (spot urine protein to creatinine ratio more than 1) during follow up period or persistent proteinuria (spot urine P/Cr more than 0.5) after disappearance of NS in follow-up Doppler sonogram.

Results: Subjects included male 4 and female 4, and their age ranged between 9 and 15 years. The mean spot urine protein to creatinine ratio at initial diagnosis of NS and renal biopsy were 0.60 and 1.16, respectively. Mean period between the point of time for diagnosis of NS and the time for renal biopsy was 25 months. The renal biopsy findings were as follows; IgAN 1, focal segmental glomerulosclerosis 1, minor glomerular abnormalities such as focal GBM thinning or focal foot process effacement and/or mild mesangial proliferation 5 and no abnormality 1.

Conclusions: Renal biopsy findings were almost mild as previous studies reported. However, some patients had significant abnormalities such as FSGS and IgAN. Therefore, long term follow-up are needed for proteinuria with NS and renal biopsy is indicated when they show persistent proteinuria and/or newly developed hematuria.

PUB705

Failure of Soluble CD30 Decent at Day 3 to 5 after Renal Transplantation Rather than Its Absolute Level Is a Predictor of Increased Risk of Rejection Ahmed G. Adam,[1] Rasha Shaefi,[1] Nahed Baddour,[2] Wesam Gendy.[3] *[1]Renal, Dialysis & Transplantation Unit, Faculty of Medicine; [2]Pathology Department, Faculty of Medicine; [3]Clinical Pathology Department, Faculty of Medicine, University of Alexandria.*

Background: The impact of sCD30 on renal graft survival in renal transplant patients with the follow up and its serial measurement over a period of one year in comparison to CKD & normal.

Methods: sCD30 by ELISA – for at least 9 times during follow up, urine albumin creatinine ratio, resistive index (RI) & histo pathological study were done.

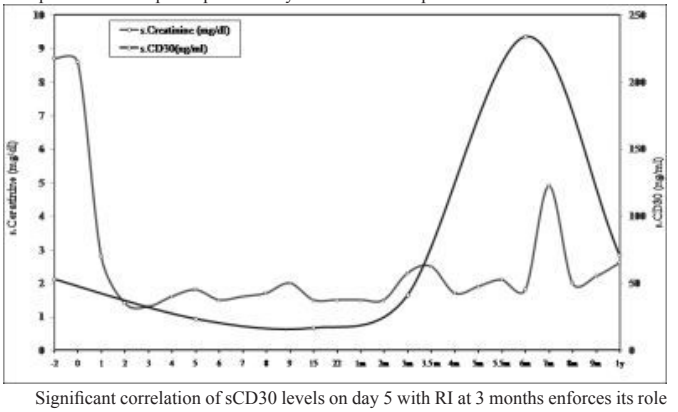
Results: sCD30 level was found normal within normal population increased in CKD patients, return to normal level post transplant at day 5/ day 15/ 1m.

Significant correlation between cyclosporine trough at 3m with sCD30 on the same day adds a benefit for use of sCD30 in adjustment of immunosuppressant dose.

Correlations between urine albumin creatinine ratios at 6m and the degree of HLA mismatch with sCD30 on the same day, at 3m respectively suggesting its role in long term renal graft survival.

As predictive value of sCD30 in renal graft survival a significant correlation was found with ischemia time on day 0 & levels at 3 months contributes to its role in detection of renal graft survival.

Relationship between s.creatinine (mg/dl) & sCD30 (ng/ml) from day -2 up to 1y post transplant as an exmple of predicitivity value of sCD30 prior to s-creatinine.



Significant correlation of sCD30 levels on day 5 with RI at 3 months enforces its role as prognostic marker to aid risk stratification for future transplant dysfunction.

Correlation between soluble CD30 levels on day 5 with RI on 5d and RI at 3m

	Soluble CD30	Soluble CD30
	r (Pearson coefficient)	p
On Day 5	-0.101	0.767
At 3 months	0.719	0.029

Conclusions: Pre transplant levels of sCD30 over 100 U/mL and a failure of soluble CD30 to decrease at day 3 to 5 predicts increased risk of rejection.

PUB706

Corticosteroids Significantly Improve Renal Dysfunction and the Clinical Prognosis in Patients with Tubulointerstitial Nephritis Yoko Kikumoto,[1] Hitoshi Sugiyama,[2] Toshio Yamanari,[1] Ayu Ogawa,[1] Masashi Kitagawa,[1] Keiichi Takiue,[1] Hiroshi Morinaga,[2] Shinji Kitamura,[1] Yohei Maeshima,[1] Hirofumi Makino.[1] *[1]Department of Medicine and Clinical Science, Okayama University; [2]Department of CKD and Peritoneal Dialysis, Okayama University.*

Background: The etiological and clinicopathologic features and prognosis of tubulointerstitial nephritis (TIN) tend to vary greatly. Controversy persists regarding the role of corticosteroids in the treatment of TIN.

Methods: We retrospectively analyzed 16 patients with TIN identified by reviewing the records of 411 renal biopsy specimens received at our hospital during the period from 2007-2011. TIN was classified as acute or chronic based on the clinicopathologic features. The clinical, laboratory and histological parameters at the start of the study were analyzed. The degree of cell infiltration ranged from 0 and 3 (absent, mild, moderate, severe). The endpoint of the study was the recovery of the renal function expressed by a more than 30% increase in the estimated GFR (eGFR), and the effectiveness of corticosteroid/conservative therapy for improving the renal function were evaluated.

Results: The baseline serum creatinine level and the interstitial cell infiltration grade were significantly higher in the patients showing a greater recovery of the renal function as expressed by a >30% increase in eGFR (group 1, n=9) than the remaining patients (group 2, n=7). A ratio of steroid-treated patients was significantly higher in group 1 (p=0.048). Steroid treatment improved the recovery of the renal function, which was shown by an increase in eGFR, at 3 months following the initial diagnosis (p=0.047) and the effect continued during the follow-up period (mean follow-up period was 27.2± 4.9 months, p=0.036). However, no beneficial effect of ACE inhibitor/ARB administration was seen regarding the rate of renal recovery during the follow-up period.

Conclusions: In conclusion, these results suggest that corticosteroid treatment could significantly improve both renal dysfunction and the clinical prognosis of TIN, particularly in cases exhibiting a higher grade of interstitial cell infiltration.

PUB707

Lifting the Veil on Complementary and Alternative Medicine: Prevalence in the Vasculitis Population Elisabeth Berg, JulieAnne G. McGregor, Madelyn Burkart, Caroline Jennette Poulton, Ronald J. Falk, Susan L. Hogan. *Internal Medicine, UNC Kidney Center, Chapel Hill, NC.*

Background: Complementary and alternative medicine (CAM) has been explored in managing various chronic diseases. This study aimed to discern the prevalence of CAM use in a cohort of small vessel vasculitis (SVV) patients. Included patients participate in the Glomerular Disease Collaborative Network (GDCN).

Methods: Structured CAM questionnaires were administered at UNC medical appointments (7/11-4/12). CAM treatments (acupuncture, massage, reflexology, etc.) and self-help practices (meditation, yoga, qigong, etc.) were reported separately.

Results: Of 102 patients surveyed, 85% were Caucasian and 62% were female. Within the last year, 81% received CAM treatments or used self-help practices. Further, 46% received ≥1 CAM treatments while 74% (33% excluding prayer) indicated use of ≥1 self-help practices. The highest reported treatments were exercise promotion (22%), nutritional supplements (20%) and massage (15%). Top self-help practices included prayer (65%), relaxation techniques (20%) and meditation (13%). Among specified treatment and self-help users, perceived CAM helpfulness was ascertained. Exercise promotion was helpful to 64%, nutritional supplements (55%), and massage (87%). Helpful self-help practices included prayer (67%), relaxation techniques (60%) and meditation (69%). Among CAM users, 23% used CAM to manage symptoms, 11% to tolerate treatment side effects or 9% for both. Overall, 67% of patients were comfortable sharing CAM practices with their physician, while only 21% stated their physician has talked about CAM. The primary reason for not using CAM was due to not receiving information (49%).

Conclusions: SVV patients commonly report some form of CAM treatment and/or self-help practices, but discussion surrounding CAM usage with their physician is limited.

Funding: NIDDK Support, Other NIH Support - ANCA Glomerulonephritis: From Molecules to Man; Principal Investigator: Ronald J. Falk, M.D.; Grant Number: 2P01DK058335-06 (9/1/2000-8/30/2015)

PUB708

Amyloidosis Secondary to Hidradenitis Suppurativa: Exceptional Response to Infliximab Adoración Martin,[1] Francisco Javier Gonzalez,[2] Secundino Cigarran,[3] Eugenia Palacios.[1] *[1]Nephrology, Poniente Hospital, Almeria, Spain; [2]Nephrology, Poniente Hospital, Almeria, Spain; [3]Nephrology, Costa Hospital, Burela, Lugo, Spain; [4]Nephrology, Poniente Hospital, Almeria, Spain.*

Background: Secondary (AA) amyloidosis (SAAA) seldom complicates the course of hidradenitis suppurativa (HS), a chronic skin condition which is notoriously difficult to treat.Both conditions have been reported independently to respond to anti-TNF agents.The course of renal amyloidosis may lead to chronic renal failure.Therefore,management must be focused on ensuring a strict control of the underlying disease to prevent amyloid deposits.

Methods: A 39-year-old male with a 9-year history of relapsing HS.No sustained response could be yielded from medical measures (antiandrogenic agents,antibiotics,isotretinoin), having multiple surgical interventions. He was assessed due multiple scars inaxillae, groins and perineum. There were no oedema.Proteinuria (3.7 g/24 h) and microhematuria were detected.Plasma creatinine was 0.83 mg/dl;albumin 2.9 g/dl;cholesterol 245 mg/dl;triglycerides 156 mg/dl.Abdominal ultrasound showed no abnormalities.kidney biopsy revealed abundant type AA amyloid deposits.Echocardiography yielded no further abnormal findings.Treatment with infliximab infusions (5 mg/kg) at weeks 0,2, 6 and 14 was then started.Significant reduction in HS activity and gradual decrease in proteinuria, without any adverse reactions was seen.From week 10 onwards, proteinuria has been undetectable on follow-up. This favourable response has been sustained throughout the 24 months of follow-up.

Results: SAAA is due to amyloid formed from serum amyloid A, produced in response to inflammation.The use of anti-TNF therapies in the latter may reduce the development of SAAA, as well as stabilise or revert established one, since they seem to interfere with fibril formation.To our knowledge, this is the first report of both entities concurring on the same patient ever being successfully treated with an anti-TNF agent.

Conclusions: We´d like to emphasise the possible role of anti-TNF therapy in the treatment of both SAAA and HS. Prospective, controlled trials are needed to assess the actual role and security profile of these agents in both entities.

Funding: Pharmaceutical Company Support - I Don´t Know Which Company Is Going to Support Me Yet. Maybe Amgen

PUB709

Higher Incidence of AKI Contributed to Longer Time to Remission in Older Patients with Adult-Onset Minimal Change Nephrotic Syndrome, Compared with Younger Patients Daisuke Mori,[1] Maki Shinzawa,[2] Tomoko Namba,[2] Ikue Nagayama,[1] Yoshito Yamaguchi,[1] Seiji Itano,[1] Natsuko Imakita,[1] Masanobu Takeji,[1] Ryohei Yamamoto,[2] Yoshitaka Isaka,[2] Atsushi Yamauchi.[1] *[1]Division of Nephrology, Osaka Rosai Hospital, Osaka, Japan; [2]Geriatric Medicineand Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan.*

Background: Little information is available about the predictor of the time to remission in patients with adult-onset minimal change nephrotic syndrome (MCNS), including acute kidney injury (AKI). The aim of the present study was to identify these predictors in younger and older patients with adult-onset MCNS.

Methods: The present retrospective cohort study included 40 patients aged at least 18 years who were diagnosed as idiopathic MCNS at Osaka Rosai hospital between 1994 and 2009. All except 1 patient received steroid pulse therapy. The outcomes was time to remission defined as urinary protein <0.3 g/day. Associations of age (younger group (18-50 years) vs. older group (>50 years)) and AKI during follow-up period, defined as 50% increase in serum creatinine level according to the RIFLE, with remission were assessed using Log-rank test.

Results: Baseline characteristics of the 40 patients were as follows; age median 42 (interquartile range 28-63) years, male 70%, serum creatinine 0.9 (0.7-1.0) mg/dL and urinary protein 7.8 (3.9-10.4) g/day. The time to remission is 12 (8-21) days. Compared with younger patients, 3-week cumulative probability of remission of older patients were significantly lower (0.50 vs. 0.91, P = 0.014 in log-rank test). AKI during follow-up was observed in 11 (61%) and 3 (14%) patients in 18 older and 22 younger patients, respectively. Interestingly, 11 older patients with AKI significantly had higher 3-week probability of remission, compared with 22 younger patients (0.27 vs. 0.91, P = 0.003), whereas 7 older patients without AKI did not (0.86 vs. 0.91, P = 0.88).

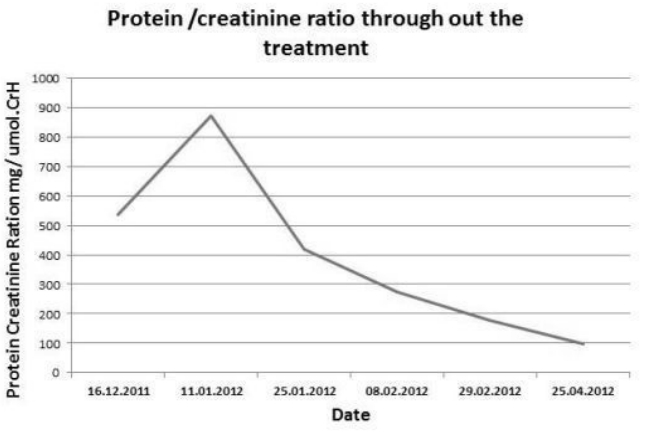
Conclusions: Higher incidence rate of AKI contributed to longer time to remission in older patients with MCNS, compared with younger patients.

PUB710

Successful Use of Angiotensin Converting Enzyme Inhibitor, and Angiotensin Receptor Blocker for Nephrotic Syndrome in Patient with Solitary Kidney Ihab El Madhoun. *Renal Unit, Al Wakra Hospital- HMC, Qatar.*

Background: Hypertension and proteinuria if not treated are associated with long term morbidity and mortality. The management of patients with solitary kidney can be challenging.

Methods: 41 years old female referred because of hypertension for 12 years and proteinuria from late childhood. She also has congenital solitary kidney. Her past history includes preeclampsia required caesarean section twice about 20 and 10 years ago and bronchial asthma. She was maintained on Losartan 50 mg once a day and Amlodipine 5 mg once a day. BP 166/103 mmHg, had pedal oedema and otherwise unremarkable. Serum creatinine 64 umol/l, Urea 4.5 mmol/l, Albumin 31 g/l, total protein 61g/l, potassium 4.4 umol/l and total cholesterol 6.53 mmol/l. Immunology screen was negative. 24 hour urine collection for protein was 5.3 gram. There was 1+ of microscopic haematuria on urine dipstick. Ultrasound scan confirmed normal size solitary right kidney. She declined renal biopsy and was started on Atorvastatin 20mg once daily, ACE-I Lisinopril 2.5 mg/ day and gradually increased to 30mg/ day. The ARB Losartan dose was increased to 100mg/ day with careful and regular monitoring of serum creatinine. The BP came down to 113/75 mmHg. Proteinuria improved to 1.1gram/24 hour urine, Serum Albumin 36 g/l and serum cholesterol lowered to 4.6 mmol/l.

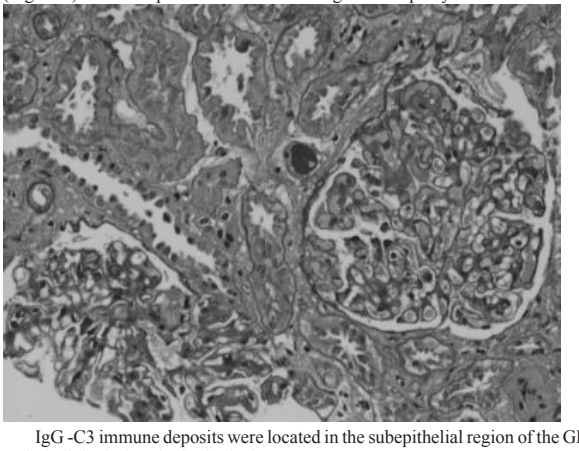


Conclusions: A previously published systematic review and meta-analysis showed that combination use of ACE-I and ARB in treating proteinuric kidney disease is safe and efficacious. In patient with single kidney, the use of high doses of dual blockers could be challenging. This case, shows that carefully introduced and monitored ACE-I and ARB proven to be highly successful in controlling the blood pressure and also massively reducing the proteinuria form nephrotic range down to almost 1 gram/day.

PUB711

Mixed Membranous and Focal Proliferative Crescentic Glomerulopathy in a Patient with Past Hepatitis B Exposure Heino R. Anto, Catalin Mihai Perju, Robenson Jean Marie. *Nephrology, St. John's Episcopal Hospital, Far Rockaway, NY.*

Background: A 64 y/o female presented with nephrotic syndrome (urine protein of 8.3 gms/24 hrs.,serum albumin 1.6 g/L, serum cholesterol 261 mg/dL), BUN 14 mg/dL, and serum creatinine 1.34 mg/dL. ANA, double stranded DNA antibody, and ENA antibodies were all negative. Serum C3, C4, ANCA and anti-GBM antibodies were all normal or negative.Hepatitis B surface, core and e antigens were all negative; however hepatitis B surface, core, and e antibodies were all positive. Renal biopsy revealed a membranous (Figure 1) and focal proliferative crescentic glomerulopathy.



IgG -C3 immune deposits were located in the subepithelial region of the GBM,without endothelial tubuloreticular inclusions on EM.

Conclusions: The rare association of membranous and crescentic gn has been reported in patients who are ANCA or anti-GBM antibody positive and who are carriers of hepatitis B antigens. While our patient had exposure to hepatitis B she was no longer a carrier of the viral antigens. In the abscence of any observable cause of crescentic gn it is concievable that prior exposure to hepatitis B may have primed the glomerulus, leading to a mixed membranous and crescentic gn.

PUB712

Adrenocorticotropic Hormone Therapy for Idiopathic Glomerulonephritis: 6-Week Interim Data from an Ongoing, 6-Month Trial Robert A. Welik. *12502 Willow Brook Rd, Suite 450, Cumberland, MD.*

Background: Previous research has shown treatment with adrenocorticotropic hormone (ACTH) can provide benefits for patients with idiopathic glomerulonephritis, but as of yet no long-term or prospective studies have evaluated whether natural source ACTH gel (H.P. Acthar Gel®; Questcor Pharmaceuticals, Inc.) can offer a complete or partial remission of nephropathy. This prospective study examines ACTH treatment in 7 patients, all of whom have previously been treated with standard immunosuppressive therapies according to their etiologies, including IgA nephropathy, idiopathic membranous glomerulonephritis, acute interstitial nephritis, and focal segmental glomerulosclerosis, without adequate response.

Methods: This is an ongoing, 6-month study in which patients are receiving deep intramuscular injections of ACTH 80 units (1 ml) twice weekly. The data presented here are the comparisons of glomerular filtration rate (GFR) calculated by using the Modification of Diet in Renal Disease formula at baseline and Week 6.

Results: Mean GFR of the patients (n=7) at baseline was 38.0 mL/min per 1.73 m^2. After 6 weeks of treatment with ACTH, mean GFR improved to 44.1 mL/min per 1.73 m^2, and those increases approached significance (P<0.0526). The GFR for one patient who has completed 8 weeks of treatment increased from 15.0 to 27.0 mL/min per 1.73 m^2, and creatinine decreased from 3.2 to 2.3 mg/dL. Once this 6-month study is complete (expected shortly), analyses will reveal the effects of ACTH therapy on other outcomes, including creatinine clearance, serum chemistries, 24-hr urine samples to assess protein, lipid levels, glucose concentrations, and blood urea nitrogen.

Conclusions: Although this study is still ongoing, the data obtained thus far at 6 weeks (as well as 8 weeks for one patient) and presented in this interim analysis indicate that ACTH therapy may be effective for improving GFR and creatinine, is well tolerated, and may be preferable in terms of administration and side effect profile. Therefore, ACTH may provide several benefits for patients with nephropathy that have not responded adequately to other types of treatment.

PUB713

Collapsing Glomerulopathy Occurring in a Patient with Pheochromocytoma: Chance Occurrence or a New Pathogenic Association? Joseph P. DeJonckheere,[1] Hammad Arshad.[2] *[1]Three Rivers Nephrology and Hypertension Associates, Pittsburgh, PA; [2]Internal Medicine, Mercy Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: Collapsing glomerulopathy (CG) involves podocyte transformation to a more immature and proliferative phenotype. Pheochromocytomas (PHEOs) are rare tumors that are known to secrete a number of biologically active peptides and, based on variations in hormones the tumor releases, present with a wide variety of signs and symptoms. Case reports have shown CG to occur in association with Parvovirus B19, Pamidronate, and interferon gamma. Here, we report a case of CG occurring in association with PHEO and explore whether this is a chance occurrence or if a pathogenic mechanism of PHEO induced CG exists.

Methods: 53-year-African American female presenting with nausea and fatigue was found to have nephrotic range proteinuria and a PHEO found incidentally. Kidney biopsy revealed CG. HIV, Parvovirus B19, and other serologic tests were negative. Prednisone was started with improvement in proteinuria and this was followed by a sustained steroid free remission after tumor removal.

Conclusions: Secretory products from PHEOs are well established to have effects on the proliferation, structure, or function of various cell types, including podocytes. This report and other published cases suggest that biologically active products from PHEOs have direct effects on glomerular permeability resulting in proteinuria that is not due to hypertension and which resolves upon removal of the tumor. Our case is the first demonstrating biopsy proven CG in association with PHEO. The patient's response to steroids suggests a potential moderating effect on an unspecified factor released by the tumor which caused CG and allowed for steroid withdrawal shortly after tumor removal. Based on the clinical evidence presented and the unlikely occurrence of CG and PHEO occurring together as isolated events, it is likely that this case is the first description of a new pathogenic association.

PUB714

Human Immunodeficiency Virus-Associated Nephropathy with Undetectable Viral Load: A Departure from the Usual Shweta Punj,[1] Mark A. Kraus.[2] *[1]Medicine, John H Stroger Hospital, Cook County, Chicago, IL; [2]Nephrology, John H Stroger Hospital/ Rush Univ, Chicago, IL.*

Background: HIV infection is a widespread infectious disease with progressive immunosuppression mediated by lysis of CD4 T helper cells. It's most common renal manifestation, HIV-associated nephropathy (HIVAN), is an AIDS-defining illness typically a consequence of advanced untreated HIV/AIDS with reduced CD4 count and detectible viremia. The current standard of care of HIVAN is immune reconstitution by administration of highly active antiretroviral therapy (HAART) with a clinical goal of increase in CD4 count and undetectable viral load. We describe a patient who developed the clinical syndrome of HIVAN despite ongoing HAART administration, undetectable viral load and an intact CD4 count.

Methods: The patient, a 55 year old African American male with hypertension, type 2 diabetes, hepatitis C, and HIV, presented to his provider with anasarca for 2 weeks. He was noted to have a blood pressure of 119/81 mmHg, to be alert and oriented and in no distress. There was 2+ edema in the legs and 1+ edema up to the shoulders. Systemic exam was otherwise unremarkable. Laboratory analysis revealed AKI with a serum creatinine of 2.9 mg/dL, a urinalysis with 3+ proteinuria, glucosuria, and bland sediment, and a spot urine protein to creatinine ratio of 16 g/g. His serum albumin was 1.8g/dL and total cholesterol was 329mg/dl. CD4 count was 629 cells/uL and serum HIV RNA level was undetectable. The renal biopsy revealed focal (10 of 23 glomeruli) and segmental scars with collapsing features and proliferation and hypertrophy of overlying podocytes. Immunofluorescence revealed nonspecific staining of a segmental scar for IgM and C3. Tubular casts were positive for IgA, IgM, and light chains. A rapid loss of renal function resulted in ESRD over 3 months despite the addition of steroids and ACE inhibitor therapy.

Conclusions: This case is unique in that HIVAN developed despite clinically effective HAART for over 2 years previously. It suggests that the occurrence of HIVAN may not depend wholly on viral mediated immunosuppression and highlights the need for continued vigilance for diagnosing this potentially reversible glomerulopathy.

PUB715

Epidemiologic and Clinical Presentation of 215 Cases of Lupus Nephritis: A Single Center Experience Maria Carolina N.R. Neves, Andrea C.E.P. Valenca, Marclebio M.C. Dourado, Edmir R.B. Dias, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas-UFPE, Recife, Pernambuco, Brazil.*

Background: Lupus nephritis (LN) is a common and is the most important predictor of morbidity and mortality in patients with systemic lupus erythematous. Clinical presentation of LN is variable, ranging from asymptomatic proteinuria to rapidly progressive glomerulonephritis. We describe a case series of 215 patients attended in our center.

Methods: We conducted a retrospective study evaluating medical records of patient at initial presentation. We evaluated ambulatory patients in a tertiary hospital at the northeast of Brazil, from 1989-2012. Epidemiological, clinical and laboratory data were analyzed.

Results: The table below describes epidemiological, clinical and laboratory data.

Epidemiologic, Laboratory and Clinical Characteristics

	N=215 (100%)
Age (range)	32.4 (14-60)
Female, n (%)	97.6 (210)
Race	
White, n (%)	86 (40)
Non-white, n (%)	129 (60)
Presentation, n (%)	207 (96.2)
Acute renal failure	47 (22.7)
Nephrotic syndrome	39 (18.9)
Nephritic and Nephrotic syndrome	23 (11.1)
Hematuria and/or non-nephrotic proteinuria	98 (47.3)
Renal Biopsy according to WHO class, n (%)	137 (63.7)
II	4 (3.0)
III	17 (12.4)
IV	34 (61.3)
V	14 (10.2)
Mixed membranoproliferative	15 (13.1)
Laboratory	
Serum creatinine (mg/dl) (mean/±SD)	1.54 ± 1.56
CrCl (mean/±SD)	77.5 ± 40.6
CrCl < 30ml/min/1.73m² (%)	14.5
Serum albumin (g/dl) (mean/±SD)	3.0 ± 2.9
Hematuria (%)	62.6
Proteinuria (g/day) (mean/±SD)	3.48 ± 3.7
ANA positive (%)	94

ANA= anti-nuclear antibody; CrCl=Creatinine Clearance

Conclusions: We reported a large series of LN cases attended in our center. Like other reports in the literature, there was a high prevalence of women and non-white patients. The most prevalent clinical presentation was hematuria and/or non-nephrotic proteinuria. Histological classification revealed a predominance of Class IV.

PUB716

Induction Therapy in Proliferative Lupus Nephritis: Single Center Retrospective Cohort Analysis Andrea C.E.P. Valenca, Maria Carolina N.R. Neves, Marclebio M.C. Dourado, Edmir R.B. Dias, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrology, Hospital das Clínicas - UFPE, Recife, Pernambuco, Brazil.*

Background: Lupus Nephritis (LN) is common in patients with systemic lupus erythematosus and is the most important predictor of morbimortality in these patients. The aim of this study was to evaluate the treatment success in patients followed in our center.

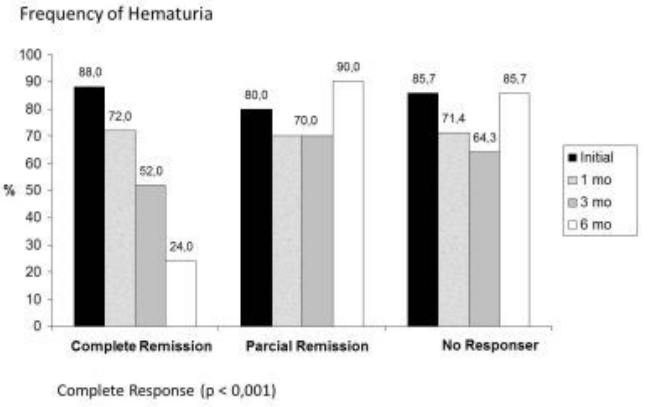
Methods: We included patients that underwent renal biopsy that had proliferative LN. They were classified as complete remission (CR), defined as 2 or more of the following: proteinuria <0.5g; absence of hematuria (HT) or cellular casts; stability or decrease in 25% of sCr. Partial response (PR), defined as improvement of 50% of all CR criteria, and non-responders (NR) neither CR or PR.

Results: Main findings are detailed in table below.

General Characteristics

Characteristics	All (N = 49)	CR	PR	NR	p-value
Total Patients (%)	49 (100)	25 (51.0)	10 (20.4)	14 (28.6)	-
Age: mean ± SD	31.7 ± 9.9	33.8 ± 10.1	27.9 ±9.4	30.7 ±9.4	0.257
Induction Therapy (%)					
CYC	47 (96)	24 (96)	10 (100)	13 (92.9)	0.683
MMF	02 (4)	01 (4)	00	01 (7)	
Biopsy (WHO class) (%)					
III	08 (16.3)	07 (87.5)	01 (12.5)	00	0.152
IV	33 (67.3)	14 (42)	07 (21)	12 (36)	

Class III LN had a higher CR rate while PR and NR were mostly observed in class IV. Albumin significantly increased in CR, PR and NR. In the group that reached CR or PR this increase was observed in the first month (CR: p = 0,01; PR: p = 0,026) while in the NR the albumin raised only in the third month (p = 0,006). In patients with CR the percentage of HT significantly reduced after 6 months of treatment (p < 0,001), while in the groups with PR and NR this did not occur.



Conclusions: This study suggests that first month serum albumin could be an early surrogate marker of therapeutic response. HT is also a relevant parameter during induction therapy regarding complete remission.

PUB717

Clinical Follow-Up of 45 Patients Treated for Proliferative Lupus Nephritis: Retrospective Cohort Maria Carolina N.R. Neves, Andrea C.E.P. Valenca, Luis H.B.C. Sette, Marclebio M.C. Dourado, Edmir R.B. Dias, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *Nefrologia, Hospital das Clinicas - UFPE, Recife, Pernambuco, Brazil.*

Background: Lupus Nephritis (LN) is a major contributor to morbimortality in patients with systemic lupus erythematosus (SLE) and impacts in clinical outcomes. We performed a retrospective cohort study with 45 patients with proliferative LN.

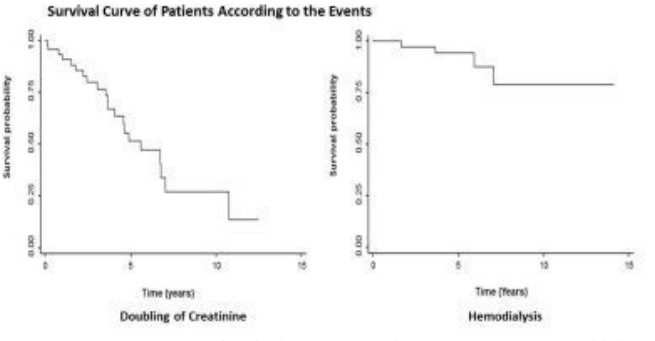
Methods: We reviewed files of patients with SLE who had underwent kidney biopsy and had proliferative lupus nephritis. Exclusion criteria were prior immunosuppressive treatment within the past 10 months. Patients were evaluated separately in creatinine (sCr) and hemodialysis (HD) cohorts.

Results: Forty five patients were included. Mean age was 31.6 yrs, sCr 2.7 mg/dL and proteinuria 4.6g/d. Most common presentation was rapidly progressive glomerulonephritis (RPGN). Analysis showed class III in 17% and class IV in 70.3% of patients. Nephritis flare was seen in 29.3%. Cyclophosphamide (CYC) was the only induction therapy. Maintenance therapy was: azathioprine 76.7%; CYC 14%;MMF 9.3%; non available 8.5%. Mean follow-up time was 3.8 yrs and 5.3 yrs in the HD and sCr cohort, respectively.

Descriptive Analysis and Hazard Ratio According to Doubling sCr and ESRD

Description of Cohort	Doubling sCr	ESRD
No of Events	21	4
Incidence (100 persons/yr)	12 (7.9-18.6)	1.6 (0.6-4.4)
Occurrence of events (%)		
1 yr	7	0.0
3 yrs	20.5	2.6
5 yrs	48.3	5.9
10 yrs	72.6	20.9
Maintenance Therapy		
Yes	1.0	1.0
No	2.16 (0.28 - 17)	¥
Flare		
No	1.0	1.0
Yes	1.93 (0.78 - 4.8)	3.71 (0.37 - 36.7)
Therapeutic Evaluation		
NR	1,0	1,0
CR/PR	1.22 (0.43 - 3.44)	¥

¥ No ESRD in this group



Conclusions: We reported a single center experience on LN treatment with long term follow up. After ten years, most patients doubled creatinine and 20% were in ESRD. These data are similar to those found in literature and shows a poor overall renal outcome.

PUB718

The Renal Pathological Characteristics and Clinical Features in Elder Patients with Kidney Disease Huiling Wang.[4] [1]*Division of Nephrology, Jimin Hospital, Shanghai, China;* [2]*Division of Nephrology, Jimin Hospital, Shanghai, China.*

Background: With the elderly population increased to more than 20% in Shanghai and other district of China, the composition of elder patients with kidney disease was increased too. The number of renal biopsy in elderly patients goes up to more than 10% recent years. This study was to investigate the clinical features and renal pathological characteristics in elder patients in our division.

Methods: It was a retrospective study, 56 patients over age 60 were enrolled, and we investigated the original causes of renal biopsy, analyzed clinic feature including blood pressure, GFR, proteinuria, biochemistry meters, and renal pathological change. We compared the results with 280 patients under age 60.

Results: In the elderly patients, the original causes of renal biopsy were nephrotic syndrome, hypertension, acute Kidney Injury, renal function insufficiency, which accounts for 32.1%, 28.6%, 14.3%, and 14.3% respectively; the incidence of anemia was increased(32.1% vs 18.2%,P<0.05), but the plasma albumin and glomerular filtration rate were decreased compared with the young patients(p<0.05).The Systolic blood pressure, cholesterol, triglyceride were significant higher in elder patients than that in young patients(P<0.05). The renal pathology showed Membranous nephropathy (27.5%) and IgA nephropathy (25.0%) was the major primary glomerulonephritis(71.4%), the arteriolonephrosclerosis was the major secondary kidney disease (41.7%). We also found that the acute tubular necrosis (37.5%) and crescentic glomerulonephritis(37.5%) were the mostly histopathologic features in the elderly patients undergoing renal disease with acute kidney injury. However, the lupus nephritis was the major secondary kidney disease in non-elderly patients (54.8%).

Conclusions: The original causes of renal biopsy and pathologic feature were difference in the elderly patients from that of the non-elderly patients. Membranous nephropathy and IgA nephropathy were two major primary glomerulonephritis; and the arteriolonephrosclerosis was the major secondary kidney disease. We should confirm these data in a large number of elderly patients.

Funding: Government Support - Non-U.S.

PUB719

Primary Gastrointestinal Involvement in ANCA-Associated Vasculitis: Poor Outcome and Possible Alternative Treatment Targets Joerg Latus,[1] Peter Fritz,[2] Martin Kimmel,[1] Dagmar Biegger,[3] German Ott,[4] Kerstin U. Amann,[5] Mark Dominik Alscher,[1] Niko Braun.[1] [1]*Nephrology, Robert-Bosch Hospital, Stuttgart, Germany;* [2]*Division of Pathology, Diagnostic Medicine, Stuttgart, Germany;* [3]*Margarete Fischer-Bosch Institute, Clinical Pharmacology, Stuttgart;* [4]*Department of Diagnostic Medicine, Devision of Pathology, Robert-Bosch Hospital, Stuttgart;* [5]*Nephropathology, University Erlangen, Erlangen.*

Background: Patients with granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) often present with ear, nose and throat manifestations, pulmonary disease and renal disease. Primary gastrointestinal involvement is rare, however it is associated with poor outcome and high mortality.

Methods: We retrospectively reviewed the medical charts of 7 patients with (ANCA)-associated vasculitis (AAV) and primary GI involvement regarding clinical presentation, outcome, diagnostic tools and therapy. Second, we studied the cellular composition of the inflammatory infiltrate associated with the vascular lesions to detect possible treatment targets using antibodies against CD20, CD3 and CD34 and compared them with a healthy control group.

Results: Mean age at onset of the first symptoms of vasculitis was 48 ± 21.3 year. All patients suffered from abdominal pain, 4 of 7 (57%) had an acute kidney injury and 3 patients required hemodialysis. At the time of diagnosis 5 of 7 patients (71%) required surgery, mean Birmingham Vasculitis Activity Score (BVAS) on admission was high (26.3 ± 7.7) and remained high after induction therapy. All patients were treated with i.v. steroids, i.v. cyclophosphamide. The expression of CD3 was significantly higher in patients with AAV with GI involvement compared to the control group (p=0.02). The analysis of the expression of CD20 and CD34 showed no statistically significant differences between patients with AAV with GI involvement compared to the control group.

Conclusions: Primary GI involvement in AAV is associated with poor outcome and treatment response to established therapies is insufficient. Target related therapy with CD20 antibodies might be a treatment option. Other targets like CD3 need further evaluation.

PUB720

Long Term Follow Up of Once Daily Dose versus Twice Daily Dose Cyclosporine in Primary Membranous Glomerulopathy with the Nephrotic Syndrome Frieder Keller. *Internal Medicine I, Nephrology, University Hospital, Ulm, Germany.*

Background: Cyclosporine (CsA) in combination with corticosteroids has shown efficacy in primary membranous glomerulopathy in achieving remission of the nephrotic syndrome. Our hypothesis was that cyclosporine-associated nephrotoxicity could be avoided by monitoring trough concentrations (Co) and by reducing the total daily dose using single daily dose regimes (Clin Nephrol. 2007 May;67(5):285-92.). In this retrospective long-term analysis we compared patients with the nephrotic syndrome and membranous nephropathy treated with once daily dose (ODD) or twice daily dose (TDD) cyclosporine regimes.

Methods: Twelve patients (6 in each group, mean age 65 SD 15 years) were treated with CsA (target through level 50-100 ng/ml) and an average total dose of 200 mg per day (150-300) that is 2.74 mg/kg body weight (1.69-4.44) over more than 60 months.

Results: After 60 months, a complete remission of the nephrotic syndrome was achieved in both groups. With ODD proteinuria decreased from 8.6 g/l (4.2-10.9) to 0.4 g/l (0.1-0.7), p=0.008, and with TDD from 3.3 g/l (1.2-2.5) to 0.3 g/l (0.1- 3.2), p = 0.029, Friedman-test. There was no difference in final proteinuria between ODD 0.43 g/l (0.06-0.07) and TDD 0.27 g/l (0.0-3.19); Mann-Whitney-U-Test p=0.394. Serum protein was significantly increased in both groups after two years. The increase of serum creatinine was significant in both groups: ODD from 84 µmol/l (62-106) to 147 µmol/l (92-182), p = 0.002, and TDD from 93 µmol/l (70-103) to 105 µmol/l (71-138), p = 0.045, Friedman-test. However and contrasting to our hypothesis, after60 months the MDRD-2 GFR was significantly less in the ODD as compared to the TDD group (39 ml/min (25-77) versus 69 ml/min (47-105), p = 0.026 Mann-Whitney-U-test).

Conclusions: ODD and TDD CsA is an effective treatment of the nephrotic syndrome in patients with membranous glomerulopathy. However, for long term therapy twice daily CsA dosing (TDD) should be preferred due to less nephrotoxicity.

Funding: Clinical Revenue Support

PUB721

Prospective Therapy with Adequate Steroids Inducement in Adult Minimal Change like Iga Nephropathy Jinquan Wang, Chenxia Juan, Qian Huang, Cai-hong Zeng, Zhi-Hong Liu. *Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu.*

Background: There is no high-quality evidence to recommend using adequate steroids in patients with minimal change like-IgA nephropathy (MCD-IgAN). This study is to prospectively evaluate its efficacy and safety.

Methods: Thirty biopsy-proved adult MCD-IgAN patients volunteered in the prospective treatment. Daily single doses of 1mg/kg (maximum 60mg) prednisone were given until complete remission (CR). Then the doses were tapered slowly. The clinical data were collected from baseline up to 12 weeks.

Results: (1). General situation: The patients consisted of 18 males and 12 females, aged 28.4±11.3y (18-60y) at renal biopsy. The baseline data were seen in table 1. (2). Efficacy and duration of remission: One (3.03%), 12 (40%), 14 (46.7%) and 8 (10%) patients achieved CR (proteinuria< 0.4g /24h) at 1, 2, 4 and 8 weeks after the treatment, respectively. (3) Relapse: Two cases relapsed at the 6th and 8th week respectively, one for rapid withdrawing of steroids, the other for upper respiratory infection.(4) Side effect: Infection, alanine aminotransferase elevation (>twice the normal value), fasting blood sugar elevation (> 6.2mmol/L) and hypokalemia (< 3.5mmol/L) occurred in 2, 5, 2 and 5 cases respectively. All recovered after treatment modulation.

The main laboratory parameters at renal biopsy

Parameter		n	%	χ±S
Blood	Albumin (g/L)	30	100	23.0±3.44
	Serum creatinine (mg/dl)	30	100	0.817±0.45
	Hightercholesterolemia (>6mmol/L)	30	100	10.0±2.22
	Hypertriglyceridemia (>2.2mmol/L)	17	56.7	2.93±1.62
Urine	Proteiuria(g/24h)	30	100	8.03±3.64
	Red blood cells of urinary sediment (million /ml)	13	43.3	23.7±66.52
	Ascending of NAG (>17 u/g.cr)	19	63.3	38.0±11.2
	Ascending of RBP (>0.5mg/L)	10	33.3	1.47±1.02
	Ascending of lysozyme (>0.5mg/L)	5	16.7	0.77±0.32
	Ascending of C3 (>2.76mg/L)	11	36.7	3.69±1.46
	Ascending of α2 macroglobulin (>2.87mg/L)	16	53.3	3.25±0.78

Note:NAG- N-acetyl-β-D-gluco Saminidase, RBP- retinol binding protein

Conclusions: Adequate steroids induction therapy is effective and safe for adult MCD-IgAN patients.

Funding: Government Support - Non-U.S.

PUB722

Clinical Observation of Low-Dose Mycophenolate Mofetil in the Treatment of IgA Nephropathy Ping Fu, Tao Ye, Jin Qiaoling, Xiao Xiao Yu, Mian Wei. *Division of Nephrology, Department of Medicine, Chengdu, Sichuan, China.*

Background: IgA nephropathy (IgAN) is the most common form of glomerulonephritis worldwide.Up to 40% progress to end-stage renal disease (ESRD) over 10–20 years. Currently, treatment is limited. We investigate the efficacy and safety of steroid and low-dose mycophenolate mofetil (MMF) in the treatment of IgA nephropathy retrospectively.

Methods: 23 patients with IgA nephropathy from may 2009 to may 2011 in our hospital were treated with steroid and low-dose mycophenolate mofetil (MMF). The initial dose of prednisone was 0.6 mg/kg/d,after 4 to 8 weeks,gradually reduced to 10mg/d maintenance. MMF was started at 0.5-1.5g/d and target blood concentration was 30-60mg.h/L. Clinical parameters such as serum creatinine,urine protein,serum albumin and side-effect were recorded and analyzed each follow up. Induction course of the treatment was initially set to 6 months,if subjects did not achieve complete remission (PR) within 6 months,it would be extended to 9 months. The primary end point was complete and part remission(CR) during induction therapy.

Results: All of the 23 patients had been observed for 6 months or above. Among the 23 cases, CR 4 cases, PR 16 cases, NR 3 cases, the totel effective rate was 87.0%.2 patients experienced respiratory infection, one of them was hospitalized;2 patients suffered from urinary tract infection,1 patient had diarrhea.

Conclusions: Low-dose MMF therapy was an effective and safety therapy with low medical cost for IgA.

PUB723

Antineutrophil Cytoplasmic Autoantibody-Negative Small Vessel Vasculitis with Renal Involvement: A 24-Year Retrospective Study Helena Marco,[1] Emilia Corica,[2] Montserrat Picazo,[1] Yolanda Arce,[3] Silvia Gracia,[4] Jm Llobet,[2] Montserrat M. Diaz Encarnacion,[1] Jose Ballarin.[1] *[1]Nephrology, Fundacio Puigvert, Spain; [2]Rheumatology, Hospital de Sant Pau, Spain; [3]Pathological Anatomy, Fundacio Puigvert, Spain; [4]Haematology, Fundacio Puigvert, Spain.*

Background: Pauci-immune renal vasculitis with focal glomerular necrosis and crescent formation is usually associated with antineutrophil cytoplasmic antibodies (ANCAs). However, ANCA´s are absent in 10 to 33% of cases. Prevalence, clinical manifestations, histopathology and outcomes of ANCA-negative SVV remain controversial.

Methods: We performed a single center retrospective review of 105 cases of SVV with renal involvement (1985-2009). We evaluated clinical and laboratory variables, presence and type of ANCA, kidney biopsy, immunosuppression therapy and renal/patient survival.

Proportions of patients were compared using Chi-Squared tests and serum creatinine by a t-student test.

Results: 13/105(12%) patients diagnosed with renal vasculitis were ANCA-negative. The median age was 69(57-89) years in ANCA-negative and 68(12-84) years in ANCA-positive. Creatinine at diagnosis was 453(71-1668) µmol/L in ANCA-negative and 386(49-1101) µmol/L in ANCA-positive (p=0.044). There was not a statistically significant difference regarding clinical variables at diagnosis except for the presence of oliguria, 4/13(31%) in the ANCA-negative and 6/86(7%) in the ANCA-positive (p=0.02). 10/12(83%) of the ANCA-negative received immunosuppressive therapy and 89/90(99%) of the ANCA-positive (p=0.03). The amount of patients that received dialysis at diagnosis was 8/12(67%) of the ANCA-negative and 34/91(37%) of the ANCA-positive (p=0.06). Mortality within 6 months of diagnosis was 6/11(54%) of the ANCA-negative and 12/88(14%) of the ANCA-positive (p = 0.004).

Conclusions: 12% of the patients were ANCA-negative, they had higher creatinine levels with higher tendency to be dialyzed at diagnosis. A greater number of patients did not receive immunosuppressive therapy.

This increased mortality highlights the importance of early diagnosis and treatment of this disease despite the ANCA-negative to improve renal and patient survival.

Funding: Private Foundation Support

PUB724

Nation-Wide Consciousness Investigation on the Clinical Remission of IgA Nephropathy in Japan Keiichi Matsuzaki,[1,5] Yusuke Suzuki,[1,5] Tetsuya Kawamura,[2,5] Hitoshi Suzuki,[1,5] Naoko Sakamoto,[3,5] Hiroyuki Yanagawa,[1,5] Satoshi Horikoshi,[1,5] Seiichi Matsuo,[4,5] Yasuhiko Tomino.[1,5] *[1]Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; [2]Jikei University School of Medicine, Tokyo, Japan; [3]National Research Institute for Child Health & Development, Tokyo, Japan; [4]Nagoya University Graduate School of Medicine, Aichi, Japan; [5]Progressive Renal Diseases Research, Research on Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan.*

Background: Since annual check-ups with urinalysis are routine in Japan, various stages of IgA nephropathy (IgAN) are diagnosed and managed. Because of recent disease specific therapies, we frequently experience the clinical remission. However, the clinical remission has not been clearly defined, and thus the definitions are largely different among the Japanese nephrologists. The present study aimed to investigate the consciousness on the clinical remission for IgAN in Japan.

Methods: The Special IgAN Study Group of the Progressive Renal Diseases Study Committee organized by the Minister of Health, Labor and Welfare conducted the questionnaire survey to nephrologists in 312 teaching hospitals in Japan.

Results: Valid answers were returned from 193 hospitals (59.2%). 95 hospitals (50.2%) had an original criteria. 126 respondents (65.3%) answered that the clinical remission is defined as complete disappearance of urinary abnormalities. 43 (22.3%) and 10 (5.2%) respondents choose proteinuria alone or hematuria alone as critical definition criteria, respectively. Most common answer of clinical remission criteria was as below; hematuria; (-) in dipstick (59.0%) or less than 5 erythrocytes/HPF in urinary sediment (77.2%) and proteinuria; (-) in dipstick (50.2%) or less than 0.2g/day (73.6%). More than half respondents needed three consecutive times in 6 months to determine the clinical remission.

Conclusions: Although there were various definitions of clinical remission for IgAN in Japan, we may find the consensus criteria for the definition of the clinical remission.

PUB725

Secondary Amyloidosis in the Setting of Periodic Fever Syndromes: Two Mutations in the Same Patient Anna Clementi,[1] Dinna N. Cruz,[2] Giovanni Giorgio Battaglia.[1] *[1]Nephrology and Dialysis, SS Marta-Venera, Acireale, Italy; [2]Nephrology, Dialysis and Transplantation, San Bortolo Hospital, Vicenza, Italy.*

Background: Secondary amyloidosis (AA) may complicate inflammatory diseases, neoplasms and several periodic fever syndromes, such as familial Mediterranean fever (FMF) and tumor necrosis factor receptor-1 syndrome (TRAPS), where specific genetic mutations correlates with the severity of amyloidosis. We report the first case in literature of AA amyloidosis where mutations involving the gene of FMF and the gene of TRAPS coexist.

Methods: A 21-year-old Romanian woman at the 35th week of gestation developed acute abdominal pain, nausea, vomiting and inability to pass flatus. Pitting edema was present in the lower limbs. Past medical history was significant for hepatitis C(HCV). Laboratory workup performed after delivery showed heavy proteinuria (3.6g/24h) and increased serum amyloid A protein (591.3ng/ml). ANA,ENA,ANCA,C3,C4 and Ig levels were normal. No monoclonal band was present on serum protein electrophoresis. Hepatitis B virus infection markers were negative, and apart from a slight increase in HCV antibody titre, there were no signs of active hepatitis. Kidney ultrasound showed hyperechogenic cortex. Echocardiography revealed only mild mitral regurgitation. Electromyography detected initial signs of lower limb sensory neuropathy. Kidney biopsy revealed diffuse glomerular and tubular deposition of amorphous hyaline material which stained weakly with PAS and bound Congo red and thioflavine T. Rectal biopsy showed the presence of A protein and the absence of immunoglobulin light chains. Genetic testing for AA amyloidosis detected two mutations; the first involved the FMF gene (MEFV), and it was p.M694I on chromosome 16. The second involved the tumor necrosis factor receptor-1 gene (TNFRSF1A), and it was p.R92Q on chromosome 12p13.2.

Results: The diagnosis of AA amyloidosis secondary to periodic fever syndromes was then confirmed.

Conclusions: Although the two mutations identified in our patient are not usually associated with amyloidosis, the presence of both together might have been determinant for the development of the disease.

PUB726

Mycophenolate Mofetil or Combined Corticosteroids in Progressive Immunoglobulin A Nephropathy Suya Wang, Heng Li, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.*

Background: Progressive IgA nephropathy (IgAN) is a common cause of ESRD. Whether mycophenolate mofetil (MMF) or combined corticosteroidsis necessary and effective in progressive IgAN has not been clarified.

Methods: Twenty-two Chinese patients (male 12, mean years 46.32±13.9, range 27-78)with hematuria (434.48red cells per high-power microscopic field, range 5-2455.8), and proteinuria (mean 3.23±1.81g/day, range 0.27- 6.45)were enrolled. All patients have biopsy-proven IgAN, 20 patients (90.91%) had diffuse mesangial proliferation with 2.63-42.61% florid crescents, 21 patients (95.45%) had mild to moderate degrees of glomerular sclerosis (4.17- 76.6%) and interstitial changes. Seven patients accepted MMF (1.0/d)monotherapy, ten patients adopted MMF(1.0/d)combined prednisone (0.5mg/kg/d) withtapered maintain until discontinuation. Five of 12 patients with florid crescents more than 10% treated with 3 pulses of methylprednisolone (MP)(10mg/kg) followed by MMF/ prednisone (MP-MMF/prednisone).

Results: Two patients stop using MMF at 2 and 3 months due to pulmonary infection (one with MMF/prednisone, and another one with MP-MMF/prednisone schedule). Six of 20 patients treated with MMF up to 6 months, and 8 of 20 patients were up to 12 months. The mean serum creatinine level significantly decreased from 209.82±113.47 (range 124-660) µmol/L before therapy to 126.5±34.85 (range 58-206)µmol/L at 6 months, p =0.002, and to 117.9±27.99 (range 87-188)µmol/L at 12 months, p = 0.001. The mean values of eGFR had improved significantly from 33.6±10.39 (range 6.32-51.88)mL/min/1.73m^2 before therapy to 54.77±16.35 (range 29.37–102.29) mL/min/1.73m^2 at 6 months, p< 0.001, and to 58.1±14.88 (range 39.04-77.26)mL/min/1.73m^2 at 12 months, p=0.001. The mean proteinuria excretion decreased from 3.23±1.81 (range: 0.27-6.45) g/day at baseline to 1.29±1.26 (range 0.1-4.88) g/day at 6 months(p=0.002), and to 0.59±0.7(range 0.1-3.1) g/dayat 12 months(p=0.001).

Conclusions: MMF or MMF/corticosteroids therapies may be an effective proposal for reversal renal function lesion in progressive IgAN patients with acute inflammatory histologic changes.

Funding: Clinical Revenue Support

PUB727

C3 Glomerulonephrtis in Children: Presentation as Atypical Post-Infectious Glomerulonephritis(PIGN) Sang Taek Lee,[1] Yo Han Ahn,[2] Su Jung Park,[1] Hye Jin Chang,[1] Jiwon L. Lee,[1] Hyewon Park,[3] Hee Gyung Kang,[1] IL-Soo Ha,[1] Hae Il Cheong.[1] *[1]Departs of Pediatrics, Seoul National University Children's Hospital; [2]Center for Pediatric Oncology, National Cancer Center; [3]Health Promotion Center, Seoul National University Bundang Hospital, Republic of Korea.*

Background: C3 glomerulonephropathy (C3GN) is characterized by mesangial (± subendothelial) C3 deposition without other immnunoglobulin or complement. Pathogenesis is assumed to be related to disregulation of alternative complement pathway (ACP). Clinical manifestaions have not been clarified yet; we reviewed our previous renal biopsy reports to investigate characteristics of C3GN.

Methods: Among more than two thousand biopsy cases performed in our center, five cases were compatible to C3GN. Initial pathologic diagnoses were postinfectious glomerulonephritis (PIGN); Renal function was normal. Serum complement 3 (C3) levels were within normal range in four cases but decreased in case 1. Case 1 and 5 had fluctuation of C3 level but others had normal range.

Results: One patient presented with gross hematuria and proteinuria (case 1), two patients with nephrotic syndrome (case 2, 4), and two patients with asymptomatic proteinuria and hematuria (case 3, 5). Case 1 and 5 showed fluctuation of their hematuria, proteinuria and hypocomplementemia despite treatment. Follow-up biopsy of case 5 revealed membranoproliferative glomerulonephritis (MPGN) pattern. Case 2 and 4 were resistant to steroid and calcineurin-inhibitor and persistent for seven and two years without deterioration of renal function, respectively. Re-biopsy revealed MPGN pattern in case 4 and disappearance of C3 deposit with focal segmental glomerulosclerosis in case 2. In case 3, C3 deposit was lost in 2 years with newly found mesangial IgA deposition. She lost her renal function in five years and got kidney transplantation.

Conclusions: While the entity of C3GN is relatively new and not universally applied yet, our cases showed diverse presentation and outcome, which were quite distinctive from typical PIGN. For better understanding this entity, further study on regulatory system of ACP is required, as well as other markers which can predict clinical course of C3GN.

PUB728

Tachyphylaxis to Cyclosporin A in Children with Refractory Nephrotic Syndrome

Yoshitsugu Kaku. *Nephrology, Fukuoka Children's Hospital, Fukuoka, Japan.*

Background: Cyclosporin A (CYA) is an essential drug for children with steroid dependent (SD) or steroid resistant (SR) nephritic syndrome (NS). CYA has excellent effects to prevent the relapse of SDNS and to introduce the remission of SRNS. However, these patients often have considerable dependency to CYA. Recently, as long-term use of CYA increases, the tachyphylaxis to CYA is becoming the considerable problem. Tachyphylaxis often makes the disease return to steroid dependent or frequent relapser again.

Methods: 134 idiopathic NS patients had been treated in our department from 2004 to 2010. They had various clinical courses and 31 were SRNS. CYA was used in 70, and combined with or without other immunosuppressants. In almost of them, CYA was effective initially.

Results: 4 of 70 patients, however, showed the tachyphylaxis to CYA subsequently.

[Case 1] At the age of 2 years old, her SRNS developed. mPSL pulse therapy and CYA could introduced the remission. CYA and small dose PSL could maintain the remission for 18 months, however NS became SD and SR thereafter.

[Case 2] His NS developed at 8 years old and relapsed frequently. Between 15 to 30 months from the onset, relapse was infrequent by CYA. However, the relapse became frequent again in spite of using CYA.

[Case 3] Her NS developed at 8 years old and was SR. We could obtain the remission with chlorambucil. After 4 years, relapse was found and became frequent. From 15 years old, CYA could maintain the remission and after 3 years CYA was discontinued. However, NS shows secondary resistance to CYA now.

[Case 4] His FRNS developed at 4 years old. Although various therapies were performed, he suffered relapses. At the age of 21 years old, CYA was started and the relapse did not develop for 15 months. However relapse became frequent again subsequently.

Conclusions: CYA is very useful and essential for refractory NS. However, since many patients have the dependency to CYA, the duration of treatment tends to be prolonged. It is necessary to hurry measures to the tachyphylaxis.

PUB729

The Effects and Safety of Tacrolimus for the Rescue Therapy of Refractory Lupus Nephritis

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Background: Systemic lupus erythematosus (systemic lupus erythematosus, SLE) is a multisystem autoimmune disease. The kidney is a common systemic lupus erythematosus involving parts. Tacrolimus may be a promising alternative for the patients with refractory lupus nephrits (LN). It was used as a conversion alternative or combined therapy with mycophenolate mofetil (MMF) as reported.

Methods: Twelve LN patients did not respond to prednisone (1.0 mg/kg/d)combined with MMF(1.5-2.0g/d)therapy were retrospectively analyzed. Oral tacrolimus was administered as conversion alternative for MMF (n =6) or combined therapy with MMF (n=6). The initial dose of tacrolimus was 0.05 mg/kg/d ,the target trough levels was 3 to 8 ng/mL for 24 weeks, then reduced to 3 to 6 ng/mL for another 24 weeks. Outcome variables included mean remission rate, average remission time.

Results: Eight of 12 patients (66.7%) experienced remission (either complete or partial), and 4 of 12 patients (25%) experienced complete remission. There were no significant difference in remission rate between the two groups (remission rate:66.6% vs 83.3%, p=0.221;remission time:38.0±16.49 weeks vs 33.8±17.6 weeks, p=0.71). The decrease of proteinuria in 24 weeks and 48 weeks were all statistically significant (p=0.04, p=0.00, respectively). The increase of serum albumin in 24weeks and 48 weeks were all also statistically significant (p=0.003, p=0.001, respectively). There were no difference between the two groups in proteinuria (24w, 48w), but the tacrolimus combined group indicated higher serum albumin than the alterntive therapy group, it was significantly in 24 weeks, and in 48 weeks (41.52±4.70mg/dL vs 32.43±10.96mg/dL p=0.003, 46.9±2.37mg/dL vs 39.88±9.32 mg/ dL, p=0.001). The renal function during observe was stable. No patient experienced renal flair in 1 year.

Conclusions: Tacrolimus may be an effective and safety rescue therapy for refractory lupus nephritis. Tacrlimus combined with MMF therapy probably had better result than tacrolimus monotherapy.

Funding: Clinical Revenue Support

PUB730

Dense Deposit Disease: What Can We Learn with Long Term Follow-Up of Three Atypical Cases?

Marie-lucile Figuères,[1] Veronique Fremeaux-bacchi,[2] Marion Rabant,[1] Louise Galmiche,[1] Philippe H. Lesavre,[1] Laure-Helene Noel,[1] Aude Servais.[1] *[1]Necker, Paris, France; [2]HEGP.*

Background: Dense deposit disease (DDD) is a severe nephropathy, associated with uncontrolled activation of the complement alternative pathway. We report 3 atypical cases with long term evolution and various histological patterns on iterative biopsies.

Methods: Clinical, biological, immunological and histological data of the 3 patients (P1, P2 and P3) were collected between 1976 and 2012.

Results: Ages at the first manifestations were respectively 6 (P1), 11 (P2) and 23 (P3) years. These manifestations were different in the 3 cases: mild proteinuria with macroscopic hematuria (P1) or nephrotic syndrome (P2 and P3). Estimated GFR was initially normal in all cases. P1 and P2 had a low C3 level and the three patients had a C3Nef. Genetic analysis found a rare variant of factor I in P1. P1 had 3 biopsies during a 34 years follow-up. They showed a thickening of glomerular (GBM) and tubular basement membranes with a stable mild mesangial proliferation, but a progressive accumulation of mesangial deposits and " humps " (C3 and C5b-9 positive). However, eGFR remained normal and proteinuria disappeared. P2 had also three biopsies (22 years of follow-up). There was a constant thickening of GBM but mesangial proliferation and C3 deposits decreased. However, he developed a mild kidney failure (eGFR 54 mL/min/1.73 m2) and proteinuria increased (2.4g/day).The 2 biopsies of P3 were peculiar with GBM thickening, without mesangial proliferation, but rare C3 and C5b-9 epimembranous deposits. Renal function remained normal despite of persisting proteinuria (2.7g/L) after 5 years of follow-up.

Conclusions: These three observations of DDD show heterogeneous histological patterns: C3 deposits accumulation (P1), decreasing C3 deposits and proliferation (P2), or isolated dense deposits with few C3 deposits (P3). Despite of the detection of a C3Nef in all patients, one did not have any C3 peripheral consumption and had only few C3 deposits (P3). The relation between the activation of the complement alternative pathway and dense deposits remains partially unknown. The factors involved in clinical progression are not yet elucidated.

PUB731

Outcomes in Patients with Acute Interstitial Nephritis: A Large Single-Center Experience

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Background: Acute interstitial nephritis (AIN) is a common cause of acute kidney injury. However the evidence for treatment with steroids is anecdotal. In our centre decision to treat with steroids is based on the renal biopsy.

Methods: We performed retrospective analysis of all biopsy proven AIN's between 2007 and 2012. We identified 50 patients with mean age of 64.7±1.6 years. Demographic data and clinical outcomes at one year were collected from electronic records. Patients with disease associated AIN like sarcoid, vasculitis, SLE and transplants were excluded from analysis. These patients had a baseline eGFR of 58.7±3.1 ml/min more than 3 months prior to biopsy.

Results: 39 patients were treated with steroids for a mean duration of 20.6±2.6 months. 11 patients were not given steroids due to minimal inflammation and/or spontaneous improvement in creatinine. Patients in the steroid group had a 3 fold decline in eGFR to 15.1±1.5 ml/min from baseline compared to only a one fold decline to 26.4±4.7 ml/min in the un-treated group. Not surprisingly patients in the treatment group failed to reach their baseline creatinine at one year (43.5±3.6 vs 60.5±3.5 ml/min). However the un-treated group was at its baseline within one year (53.6±3.7 vs 52.4±6.4 ml/min). In 13 patients the cause of interstitial nephritis was not clear; 12 received steroids, 4 were on steroids at one year and 3 patients required Azathioprine. In this series the steroid group appears to have florid interstitial infiltrate compared to the un-treated group.

Conclusions: Our retrospective analysis suggests that treatment with steroids is beneficial in the presence of florid inflammatory infiltrate on renal biopsy. Despite treatment these patients fail to reach their baseline creatinine. This emphasizes the importance of early renal biopsy in suspected cases. Patients with unknown offending agents are more likely to need long term steroids or immunosuppressants. A randomized control trial is needed to evaluate treatment in varying severity of inflammation on biopsy. An AIN grading system based on clinical and biopsy findings may help to evaluate treatment strategies.

PUB732

CD147 Is a Prognostic Predictor of Renal Survival in Patients with IgA Nephropathy

Huang Chen. *Nephrology, Xijing Hospital, Xi'an, China.*

Background: Renal fibrosis is a final common process that leads to the progression of various types of kidney disease. CD147, a multifunctional molecule, plays a key role in the development and progression of several tumor tissues. Previous study indicated that CD147 promotes renal fibrosis after unilateral ureteral obstruction(UUO). However, the relationship between CD147 expression and clinical significance in patients with IgA nephropathy(IgAN) is not known. We examined whether the degree of expression of CD147 in renal biopsies could be a novel marker for predicting IgAN progression.

Methods: We obtained the renal biopsy samples from IgAN patients who had received renal biopsy and evaluated the correlation between immunohistochemical staining scores and clinical parameters. We also studied whether the expression of CD147 in the renal samples was correlated with renal survival in IgAN patients.

Results: Increased CD147 expression was found in tubular epithelial cell from the kidneys of patients with IgAN, however, in normal kidneys, little positive staining for CD147 was found. Our data showed a negative association between CD147 protein and estimated glomerular filtration rate (eGFR) and a positive association with serum creatinine and the percentage of tubulointerstitial fibrosis. Kaplan-Meier survival curves showed that the CD147 overexpression was relevant with decreased renal survival.

Conclusions: Our results demonstrated the involvement of CD147 in the pathophysiology of IgAN and suggested its potential role as a surrogate marker for renal survival in IgAN patients.

PUB733

Variation in EPO Response to Dopamine Identifies Differences in Tubulointerstitial Function in Patients with IgA Nephropathy

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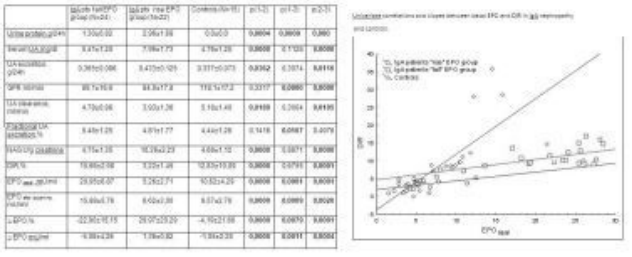
Background: Clinical course of IgA nephropathy depends on progression and irreversibility of tubulointerstitial renal changes. Hypoxia is one of the mechanisms leading to irreversible interstitial changes. A number of interdependencies between the marker of renal tubular dysfunction (NAG), progression of changes in biopsied kidneys, and EPO plasma levels was observed in patients with IgA nephropathy. The aim of study: to establish the relationship between EPO plasma level changes, and intrarenal vascular function in pts with IgA nephropathy.

Methods: 46 non-nephrotic IgA pts, 15 controls with GFR 86.7±17.4 and 118.1±17.2 ml/min respectively, renal vascular function estimation based on DIR. DIR was measured using two 120-min creatinine clearances (before and after i.v. administration 2µg/kg/min dopamine) and at the same points EPO was measured. UA, urinary UA, urine protein and NAG were measured.

Results: Dopamine-induced changes in EPO plasma levels using dose of dopamine in all pts, were different for different patients.



Conclusions: In IgA nephropathy with relatively preserved glomerular filtration and proteinuria, patients with the same GFR present different vascular and interstitial functional status. Vascular functional status expressed by blunted response to vasodilating effect of dopamine accompanies higher proteinuria and lower uric acid clearance and lower EPO levels.

PUB734

Cyclophosphamide for Nephrotic Associated Idiopathic Membranous Nephropathy (IMN)

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Background: Idiopathic Membranous nephropathy (IMN) is a common cause of nephrotic syndrome in adults. There is a paucity of data regarding the role of cyclophosphamide in IMN. We report our centre's experience of treating IMN with cyclophosphamide.

Methods: A single centre retrospective 5-year study of nephrotic associated IMN. Patients included in the study had biopsy proven MN and clinical evidence of nephrotic syndrome. Patients were treated with either pulsed IV or oral cyclophosphamide (1-2mg/kg/day) along with oral prednisolone 1mg/kg tapered over 6 months. The primary outcome was remission of nephrotic syndrome (defined as normalisation of serum albumin). Untreated matched patients served as controls. Chi squared and unpaired T test were used.

Results: 39 patients with IMN met study inclusion criteria. 21 were treated with Cyclophosphamide (n=16 IV, n=5 oral) and 18 patients had no immunosuppressive treatment (control). Median time from biopsy to treatment was 5.39 months. Use of ACE inhibitors/ARBs/diuretics were similar between both groups. There was no statistical difference in treated and untreated groups in terms of age (P=0.76), gender (p=0.3), proteinuria (p=0.28) and serum albumin (p=0.71) at baseline. There was a significant difference in eGFR at the time of diagnosis in treated and untreated groups (mean eGFR 45ml/min,eGFR 79ml/min, respectively, P=0.007). In the treated group 38% of patients achieved remission by 6 months, 61% by 12 months and 75% by 24 months. Remission rates were similar in the oral and IV therapy groups. Only 6% of untreated patients achieved remission by 12 months and 25% by 24 months. Mean increase in eGFR at 12 months was similar in both groups 6ml/min (treated) vs. 5ml/min (untreated). Leucopenia, infection and hospital admission rates were higher in oral as compared to IV cyclophosphamide group.

Conclusions: Cyclophosphamide is an effective therapy for nephrotic associated IMN. Complete remission rates and time to remission are significantly improved following therapy. Given the lower rate of treatment related side effects, we would advocate an IV pulsed Cyclophosphamide regimen.

PUB735

Prognostic Factors and Treatment Outcomes in Idiopathic Membranous Nephropathy: A Single Center Experience

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Background: This study was aimed to investigate the prognostic factors and outcomes in patients with idiopathic membranous nephropathy (IMN).

Methods: Data from a total of 76 patients with biopsy-proven IMN (male=37, median age=39 yrs [range 19-84 yrs]) between January 2002 to June 2011 were analyzed. Outcomes included remission (complete and partial) (complete remission [CR], proteinuria <0.02g/day and serum albumin >3.5g/dl; partial remission [PR], decrease of proteinuria >50% between baseline and last follow-up) and progression (increase of serum creatinine [s-Cr] >50% vs. initial s-Cr). Multivariate logistic regression analysis was used.

Results: At presentation, 41 patients (53.9%) had a nephrotic range of proteinuria. Mean protein excretion was 4.6 g/day and s-Cr was 0.84±0.36 mg/dl. Thirty-nine patients (51.3%) were treated only with ACEi and/or ARBs, whereas 35 patients (46.1%) with immunosuppressants. Remission occurred in 57 (75.0%) patients (CR 26 [34.2%] and PR 31 [40.8%]) during mean follow-up of 52±33 months. On multivariate logistic regression, uric acid level (OR 1.367, CI 1.014-1.844, p=0.041) was an independent risk factor for persistent proteinuria. Twenty-two patients (28.9%) were progressed and 4 of them (5.3%) needed renal replacement therapy. On univariate analysis, age (OR 1.067, CI 1.019-1.117, p=0.005), hypercholesterolemia (OR 7.857, CI 1.656-37.283 p=0.009), proteinuria >6g/day (OR 2.974, CI 1.181-10.371, p=0.024), use of immunosuppressants (OR 0.227, CI 0.078-0.659, p=0.006) and remission induction (OR 0.218 CI 0.071-0.674, p=0.008) were associated with progression. Multivariate logistic regression showed age (OR 1.095, CI 1.029-1.166, p=0.004) and remission induction (OR 0.190, CI 0.042-0.854, p=0.030) were independent prognostic factors for progression.

Conclusions: Uric acid level at presentation was an independent risk factor for persistent proteinuria in IMN. In addition, old age and persistent proteinuria without remission were independent risk factors for deterioration of renal function in IMN.

PUB736

Rituximab in Idiopathic Membranous Glomerulonephritis

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Background: Rituximab represents a new therapeutic option in idiopathic membranous GN (IMGN). Although several studies have proven its efficacy in improving outcomes of this disease, its role in treatment regimens is still unclear. The purpose of this study was to evaluate the clinical course of IMGN patients treated with Rituximab.

Methods: We retrospectively studied 13 IMGN patients with frequent relapses of nephrotic syndrome despite standard immunosuppression therapy. Median age was 65 years (IQR 46-76); all patients had nephrotic proteinuria (median 5.78 g/24h, IQR 4.6–9,93). Median eGFR was 56 ml/min/m² (IQR 41.9-95). Median CD20+ B lymphocytes count before treatment was 9% (IQR 7-10). Rituximab was administered at a dose of 375 mg/m² for a total of 4 weekly cycles. A quarterly follow-up for an average observation period of 12 months was performed by monitoring proteinuria, renal function and lymphocyte subpopulations.

Results: Five patients (38%) achieved a complete remission of nephrotic syndrome, with significant reduction in proteinuria (<0.5 g/24h) in the first 3 months and maintained stable values of proteinuria and renal function during the observation period. Five patients (38%) achieved a partial remission, with reduction of proteinuria from 52 to 76% compared to baseline values. Three patients (24%) did not respond to therapy, with persistence of high levels of proteinuria and, in two cases, a reduction of eGFR(from 75 to 56 and from 61 to 25 ml/min/m²). In 11 patients, eGFR remained stable in the period of observation. Median eGFR and proteinuria at last follow-up were 54 ml/min/m² (IQR 33.2-64.2) and 1.04 g/24h (IQR 0.71-3.65; p<0.45, Wilcoxon test). CD20+ B lymphocytes remained cleared in the first six post-treatment months; four patients had an increase of CD20+ lymphocytes in the seventh month (median percentage of CD20+: 0.2 %,IQR 0-5). Treatment with Rituximab was well tolerated by all patients.

Conclusions: In conclusion, our experience suggests that Rituximab may represent a therapeutic option for patients with membranous nephropathy, resistent to conventional immunosuppressive therapies. The drug is well tolerated and may have a role in slowing the progression to ESKD.

Funding: Government Support - Non-U.S.

PUB737

Clinicopathologic Correlates for Activity and Damage of Lupus Nephritis in Childhood-Onset Systemic Lupus Erythematosus Michael R. Bennett, Rina Mina, Shannen Nelson, Jessica Hummel, David P. Witte, Prasad Devarajan, Hermine Brunner. *Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: High AI activity (AI), tubulointerstitial (TI), and chronicity index (CI) scores from renal biopsy may predict poor renal outcomes in lupus nephritis (LN) in childhood-onset systemic lupus erythematosus (cSLE). Our aim is to evaluate the relationship between histologic evidence of renal disease activity and damage of LN with conventionally used biomarkers in cSLE.

Methods: Biopsy specimens of 18 cSLE patients were rated by a single nephropathologist for the AI, TI, and CI. Using logistic regression, the relationships between the biomarkers and high scores for AI (≥ 4), high CI (≥ 7), and high TI (≥ 4) were evaluated. Biomarkers evaluated include serum creatinine, creatinine clearance, urine sediment, proteinuria, albumin, blood pressure, anti-ds DNA antibody, C3, C4, sedimentation rate, and blood urea nitrogen (BUN); these were obtained on the day of renal biopsy to 30 days after.

Results: Patient's mean age ± SD was 14.1 ± 2.7 years. LN class distribution was as follows: II (28%), III (17%), IV (50%), and III plus IV (6%). All were positive for anti-ds DNA antibody. Only elevated BUN (odds ratio=14, 95% CI=1.2-156, p-value=0.04) was significantly associated with high TI score. None of the biomarkers were significantly associated with high AI and high CI scores.

Conclusions: Commonly used biomarkers are poorly associated with histological features for activity and damage of LN in cSLE highlighting the need for better biomarkers that can be used in clinical care.

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PUB738

Increased Risk for Type 2 Diabetes Mellitus and Renal Injury in a Low Level Cadmium Exposure Population Ernesto Sabath,[1,2] Ma. Ludivina Robles-osorio.[2] *[1]Unidad Estatal de Hemodialisis, SESEQ, Queretaro, Mexico; [2]Endocrinologia y Enfermedades Metabolicas, Universidad Autonoma de Queretaro, Queretaro, Mexico.*

Background: Environmental pollution by heavy metals has been recognized as major risk factor for chronic diseases in developed and non-developed countries and is likely that cadmium may play a role in the high prevalence of renal disease and type 2 diabetes mellitus seen in low-income areas. The aim of this study was to know the association between Cd urinary excretion (Cd U), renal injury and T2DM in an open population in Central Mexico.

Methods: Cross-sectional study done in 7 communities of Central Mexico with diabetic and non-diabetic people. Pregnant women and those patients with uncontrolled blood pressure were excluded. Urine was collected in cadmium-free flasks for albumin, α1-microglobulin (α1M) and cadmium measurements; α1-microglobulin was measured by ELISA and cadmium by cell-mass spectrometry.

Results: We enrolled 185 participants with a mean age of 46.9 years old. Non diabetic participants with Cd levels higher than 0.7 µg/grCr had a higher urinary α1M excretion (12.9 vs 2.1 µg/gCr p=0.002), and albuminuria (0.03 vs 0.08 gr/gCr p=0.04) than those with with Cd < 0.7 µg/grCr. Multivariate analysis showed that Cd was the most important factor associated with α1M urinary excretion (p=0.03) and albuminuria (p=0.001).

In patients with T2DM, CdU excretion higher than 0.7 µg/grCr was associated with decrease in GFR (71.6 vs 88.4 ml/min p=0.003) but not differences in α1M or albuminuria were found.

Median Cd U excretion was higher in patients with T2DM (0.48 vs 0.37 µg/gCr p=0.01) and the OR associated with a higher risk for T2DM for patients with Cd ≥1.0 µg/grCr) was 5.6 (IC95% 1.2-26.3 p=0.03).

Conclusions: In this study we found in non-diabetic people an association between Cd U levels with albuminuria and α1-microglobulin excretion. In T2DM patients, Cd was associated with less GFR but not with proteinuria. Also, this study suggest a role of Cd in the physiopathology T2DM.

Funding: Government Support - Non-U.S.

PUB739

Observational Pilot Study to Assess the Efficacy and Safety of Mannitol in Reducing Severe Edema in Children with Nephrotic Syndrome Indira Agarwal, Nalini Aswathaman, Leni Kumar Joseph, Rajiv Sinha. *Pediatric Nephrology Divison, Christian Medical College, Vellore, India.*

Background: The potent diuretic effect of Mannitol in management of severe edema in children with nephrotic syndrome has not been adequately studied though literature reports it to be safe, effective and a possible inexpensive alternative to 20%Albumin.

Methods: Aims: To study the efficacy and safety of mannitol in reducing severe edema in childhood onset nephrotic syndrome.

Methodology: A prospective pilot study was conducted in the Pediatric nephrology division of Christian Medical College, Vellore,from March - November 2011. Children between 1-15yrs old with proven nephrotic syndrome and persistent edema despite 3 days of frusemide, with none of the exclusion criteria (secondary nephrotic syndrome, low GFR,abnormal blood pressure, cardiac failure, coexisting severe infection) were recruited. Mannitol (20%) 1gm/kg infusion with frusemide 2mg/kg/dose midway was given twice daily for 3 days. Children were uniformly assessed for edema based on a devised grading scale, weight changes, abdominal girth, urine output and adverse events. Results were compared with similar data while on frusemide. Statistical analysis using SPSS and Epidata was done and Student t test was applied.

Results: 20 children were recruited.Reduction in edema by 2.1 grades after mannitol and 0.2 grades after frusemide was statistically significant (p=0.00). At 72 hours, mean percentage weight loss was higher after mannitol compared to frusemide (9.3% vs. 4.83%- p=0.07).Mean abdominal girth reduction by 3.8cms after mannitol was statistically significant when compared to 0.9cms after frusemide(p=0.05). Mean increase in urine output was higher after frusemide(0.9 vs. 0.5 ml/kg/hour-p=0.5). Side effects of mannitol noted were hyponatremia, hypokalemia and hyperkalemia in 3/20, reduced creatinine clearance (2/20) and hypernatremia and hypotension in 1/20 each. Four children with these effects needed withdrawal.

Conclusions: Mannitol shows promise as a diuretic in reducing edema in Nephrotic Syndrome. A Randomised control trial comparing its efficacy against the more expensive albumin may be helpful in promoting its use in resource poor countries.

PUB740

MCP-1 Tissue Expression in Renal Biopsy of Patients with Proliferative Lupus Nephritis Correlates with Chronicity Index Cristiane Bitencourt Dias,[1] Patrícia Malafronte,[1] Aline Lázara Resende,[1] Cilene Carlos Pinheiro,[1] Denise M.A.C. Malheiros,[2] Rui Toledo Barros,[1] Viktoria Woronik.[1] *[1]Nephrology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; [2]Pathology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.*

Background: The aim of this study was to describe correlations of renal outcomes with tecidual MCP-1 expressed in renal biopsy specimens obtained on the diagnosis of proliferative lupus nephritis.

Methods: Thirty four female newly diagnosed patients with proliferative LN were prospectively followed-up during 3.5 (3.2 - 4.0) years. Conventional laboratory tests were collected on diagnosis and on last follow-up. Renal biopsy was done on diagnosis and immunohistochemical study was performed with monoclonal antibody anti-MCP-1, and results expressed as cells/microscopic fields. Patients were stratified in two groups according to renal outcome: GFR ≤ 60 mL/min/1.73m^2 (n=15) and GFR > 60mL/min/1.73m^2 (n=19). Considering treatment, all patients received prednisone and 6 pulses of cyclophosphamide (CYA) on induction. Maintenance treatment was conventional.

Results: There were no difference between group with GFR > 60mL/min/1.73m^2 and GFR ≤ 60 mL/min/1.73m^2 in relation to age 24.0 ± 7.0 vs 28.8 ± 11.7 years, activity index 5.0 ± 1.6 vs 4.3 ± 2.5, initial MDRD 49.4 ± 28.0 vs 32.0 ± 23.2, initial proteinuria 3.8 ± 2.2 vs 5.4 ± 2.7 g/day, systemic SLEDAI 25.3 ± 5.6 vs 25.0 ± 6.6, glomerular MCP-1 expression 0.5 ± 0.5 vs 0.8 ± 0.9 cells/field, interstitial MCP-1 expression 5.2 ± 4.9 vs 7.4 ± 7.6 cells/field and tubule MCP-1 expression 0.2 ± 0.3 vs 0.5 ± 0.7 cells/field. The only difference was in the chronicity index that was higher in GFR ≤ 60 mL/min/1.73m^2 group, 3.6 ± 1.8 vs 1.8 ± 2.1.

Considering all patients (n=34) there was positive correlation between interstitial MCP-1 and chronicity index (r=0.56, p=0.001) and negative correlation between tubule MCP-1 and final MDRD (r= -0.3, p=0.04).

Conclusions: Interstitial MCP-1 showed significant correlation with chronicity index. Chronicity index was the only parameter correlated with worse renal function on follow up.

PUB741

A Rare Case of Idiopathic Collapsing Focal Segmental Glomerulosclerosis that Responded to Treatment Alaa Abu-sayf, Sulaiman Alhassan, Omid Bakhtar, David R. White. *Department of Medicine, Detroit Medical Center, Detroit, MI.*

Background: Out of the five histological types of FSGS, the Collapsing variant is characterized with the worst prognosis, frequent association with HIV infection, predominance of blacks, massive proteinuria (>10g/day), resistance to treatment and rapid progression to End Stage Renal Disease. We report a case of idiopathic collapsing FSGS successfully treated with steroids despite advanced renal failure and lack of evidence-based guidance.

Methods: A 51-year-old Jamaican-American woman with a history of hypertension, presented with fever and flu-like symptoms for one month. She denied any recent usage of NSAIDS, interferon, bisphosphonates or intravenous drugs. She had a temperature of 38.5 Celsius, tachycardia and bilateral lower extremity pitting edema. Initial creatinine (Cr) was as high as 5.4 mg/dl with no known baseline kidney function. She was diagnosed with nephrotic syndrome with 17 grams/day of proteinuria. Our workup ruled out SLE, ANCA-vasculitis, multiple myeloma, HIV (nuclear amplification test also done), viral hepatitis and other infections associated with this diagnosis. The renal biopsy demonstrated collapsing variant FSGS, with tubuloreticular inclusions (TRIs) identified in 2 out of 13 glomeruli. Treatment was started with pulsed methylprednisolone of 1 gram for 3 days followed by 40 mg of prednisone daily. The constitutional symptoms resolved after one week. Six weeks into treatment, Cr fell to 2.6 mg/dl and proteinuria was down to 4.7 grams. Biopsy was repeated and confirmed the initial diagnosis. Partial remission was achieved after 20 weeks with a Cr of 1.8 mg/dl and proteinuria of 1.7 grams. The prednisone is currently being tapered down to complete a six month course.

Conclusions: This case provides organization of evidence of a rare case of idiopathic collapsing FSGS, diagnosed despite the presence of TRIs. The rare response to steroids was dramatic and we are now contemplating the next course of treatment. It is important to consider this therapeutic intervention as we move forward to potentially conduct controlled studies to coagulate its significance.

Funding: NIDDK Support

PUB742

Quantitative Assessment of Serum Free Light Chains in Patients with Immunoglobulin A Nephropathy Byeong Yun Yang,[1] Sang Heon Song,[1] Min Ji Shin,[1] Harin Rhee,[1] Il Young Kim,[2] Eun Young Seong,[1] Dong Won Lee,[2] Soo Bong Lee,[2] Ihm Soo Kwak.[1] *[1]Division of Nephrology, Pusan National University Hospital, Busan, Republic of Korea; [2]Division of Nephrology, Pusan National University Yangsan Hospital.*

Background: Polyclonal free light chains have recently been assessed in chronic kidney disease (CKD), diabetic nephropathy, and renal transplantation patients. Serum free light chain (sFLC) is suspected as a novel biomarker reflecting renal function of these patients. So we evaluated sFLCs in patients with Immunoglobulin A nephropathy (IgAN), the most common primary glomerulonephritis.

Methods: We studied 56 patients with primary IgAN. sFLCs were assessed with the immunoassay Freelite.

Results: sFLC κ and λ levels increased progressively with KDOQI stages (both $p<0.001$) and significantly correlated with estimated glomerular filtration rate (eGFR), cystatin C, and β2-micoglobulin (Table 1). Mean arterial pressure and urine protein to creatinine ratio (UPCR) had significant associations with λ level, not κ level (Table 1). In multivariate regression analysis, independently contributing factors to eGFR were log cystatin C (standardized $\beta=-0.588$, $p<0.001$) and log κ (standardized $\beta=-0.197$, $p=0.003$). Gradual increments in the variables according to UPCR grades (0: < 0.5 g/g, 1: 0.5 to 1.0 g/g, 2: 1.0 to 2.0 g/g, 3: > 2.0 g/g) were observed in eGFR ($p=0.009$) and λ level ($p=0.043$). In UPCR grade 0 group, eGFR was 107.1±29.0 ml/min/1.73m^2, while λ level was 21.0±6.6 mg/L, higher than the reference (reference average: 13.4 mg/L).

Conclusions: sFLCs accounts for renal function in patients with IgAN. sFLC λ may be a new biomarker of isolated hematuria originated from renal parenchymal disease such as IgAN, which should be validated in further studies.

Table 1: Spearman correlation coefficients among the variables

	MAP	κ	λ	B2M	Cystatin C	eGFR	UPCR
MAP	1	0.219	0.352*	0.106	0.291*	-0.295*	0.444*
κ	0.219	1	0.674*	0.512*	0.525*	-0.626*	0.214
λ	0.352*	0.674*	1	0.570*	0.514*	-0.641*	0.378*
B2M	0.106	0.512*	0.570*	1	0.616*	-0.651*	0.159
Cystatin C	0.291*	0.525*	0.514*	0.616*	1	-0.785*	0.281*
eGFR	-0.295*	-0.626*	-0.641*	-0.651*	-0.785*	1	-0.394*
UPCR	0.444*	0.214	0.378*	0.159	0.281*	-0.394*	1

* means $p<0.05$

PUB743

Epidemiologic Study on the Relations between HBV Infection and Proteinuria in South China Yimin Zhang. *Division of Nephrology, The 6th Affiliated Hospital, Sun Yatsen University, Guangzhou, Guangdong, China.*

Background: Hepatitis B virus(HBV) infection is a serious threat in Chinese population, it is estimated that about 170 million Chinese are chronically infected with HBV, about 20 million of whom are chronic hepatitis B patients. Although a major target organ of HBV infection is the liver, extrahepatic manifestations are also frequently observed in patients with HBV infection. Glomerulonephritis is an important extrahepatic manifestation of chronic HBV infection, which are clinically characterized by hematuria and proteinuria. In the present study, we investigated whether HBV infection, diagnosed by HBsAg and HBeAg positivity,, were associated with CKD components in South China individuals who underwent general health screening.

Methods: Between March 2007 and June 2010, 6816 people (3199 women and 3617 men) underwent a general health screen at Sun Yatsen Memorial Hospital, including an estimation of urinary excretion of albumin, haematuria, various markers of HBV, and were enrolled in the study. Blood and urine samples were taken from the subjects after an overnight fast. The levels of HBsAg and HBeAg in the sera were determined using commercially enzyme immunoassay kits. The data in this study were analyzed by χ^2 test and Mann-Whitney U test using the computer software SPSS 15.0.

Results: 903(13.25%)of the 6816 subjects were HBV carriers and 390(5.72%) had abnormal proteinuria. The frequency of abnormal proteinuria was not significantly different in those with[57 of 903 cases(6.31%)] or without[333 of 5913 cases(5.63%)] HBV carriage($P>0.05$). This lack of association remained when carriers were classified into those who were HBsAg positive and those with active viral replication (HBeAg positive)($P>0.05$). Haematuria was not significantly different either in those with [199 of 903(22.04%)] or without [1419 of 5913(23.30%)]HBV carriage($P>0.05$).

Conclusions: In conclusion, HBV infection does not uniformly lead to development of renal disease. There was no association between HBV infenction and CKD components (haematuria and albuminuria) in south China. Further studies will need to identify the incidence, risk factors and the natural history of renal involvement among patients with HBV infection.

Funding: Government Support - Non-U.S.

PUB744

"How Well Is RPGN Doing?" Rapidly Progressive Glomerulonephritis, an Institutional Experience in Disease Outcome Kamal Shemisa, Lavinia A. Negrea. *Nephrology, University Hospitals Case Medical Center, Cleveland, OH.*

Background: Rapidly Progressive Glomerulonephritis, RPGN, is characterized by the development of renal insufficiency within a short period, of weeks to months, and left untreated progresses to ESRD and death. RPGN is characterized by >50% crescents on renal biopsy. The known etiologies for RPGN vary form PAN, Wegeners', Churg Strauss, micro-PAN, Good-Pasture's, Lupus, and HSP. The majority are pauci-immune types (55%), ANCA associated. Despite the numerous causes, the prevalence is rare 7/1,000,000; and constitutes 1% of renal biopsy diagnoses. RPGN reports describe rapid progression to uremia and death. Outcomes for RPGN however are improving with combined treatment of corticosteroids, cyclophosphamide, or rituximab. We sought to assess clinical outcomes at UHCMC to verify this claim.

Methods: Standard computations for statistical analysis calculated mean, standard deviation and range were reported for mortality, hospital length of stay, ICU admission, rehospitalization, and rates of remission.

Results: In biopsy proven RPGN, the overall patient mortality at 18 months was (0%± 0). The mean age was 44 years ± 27 with equal gender representation. In our series, pauci-immune RPGN was diagnosed in 50% of patients. Treatments consisted of corticosteroid and cyclophosphamide 87% or rituximab 13%. Remission was characterized as being hemodialysis free or without renal transplant after treatment. The mean rate for remission was 56%. Patients with pauci-immune RPGN who received rituximab achieved a 100% remission rate. The time for remission ranged from 3 to 8 months. The most common reason for hospital readmission was renal replacement therapy, 38% and treatment for symptomatic anemia, 13%. Adverse side affects of therapy were neutropenia 19%.

Conclusions: The results are similar to prior estimates of prevalence and rates of remission. Higher rates of remission were achieved in pauci-immune RPGN with rituximab. Time to remission may be as long as 8 months. Consideration for long-term hemodialysis or renal transplantation should be reserved in patients who do not remit beyond this time. Awareness of potential adverse hemotologic side effects including neutropenia is crucial.

PUB745

Patterns of Glomerular Diseases from a Tertiary Care Center in Pakistan Nauman Tarif,[1] Omer Sabir,[1] Imrana Tanvir,[2] Rizwan Akhtar,[2] Haroon Younas,[1] Noman Hameed.[1] *[1]Medicine, Division of Nephrology, Fatima Memorial Hospital, Lahore, Pakistan; [2]Medicine, Division of Nephrology, Fatima Memorial Hospital, Lahore, Pakistan; [3]Pathology, Fatima Memorial Hospital, Lahore, Pakistan; [4]Pathology, Fatima Memorial Hospital, Lahore, Pakistan.*

Background: The data regarding the patterns of glomerular disease in Pakistan remains scanty. We present data from two years of our recently established nephrology unit.

Methods: Renal biopsies performed under real time ultrasound guidance using semiautomatic biopsy needle. The samples were routinely stained with: H&E, PAS, Silver and congo red. Immunoperoxidase staining for IgG, IgA, IgM, C3, C1q and C4 were initiated after one year. Electron microscopy is not available in Pakistan except a single center that is not accessible to everyone. The final diagnosis was approved in the nephropathology conference held every week. Retrospective review and descriptive data is presented.

Results: 148 biopsies were performed from March 2010 till May 2012. 73 were males (49.32%,) and 75 were females (50.67%). The mean age was: males 34.09±13.6 years and females 30.18 ±10.98. Missed or non-informative biopsies were 9 (6.0%). Being a referral centre for Rheumatology, we had 49 cases of Lupus Nephritis (33.1%). Among the idiopathic glomerular diseases, Focal Segmental Glomerulosclerosis was the most common diagnosis in 29 patients (19.59%). The other diagnoses included Membranous Nephropathy 6(4.0%), Membranoproliferative GN 6 (4.0%), Mesangioproliferative GN 4 (2.7%) Minimal Change Disease 5 (3.37%), secondary Amyloidosis 6 (4.0%), primary amyloidosis 2 (1.35%), Diabetic Nephropathy 5 (3.37%), Rapidly Progressive GN 12 (8.1%), Post infectious GN 2 (1.35%), Acute interstitial Nephritis/Tubulointerstitial Nephritis 2 (1.35%), IgA Nephropathy 2 (1.35%) were the other common biopsy findings. One patient had Chronic Kidney Disease (0.67%). 6 patients had biopsy of transplanted kidney (4.0%). One patient had vasculitis (0.67%) and other patient had scleroderma renal crises (0.67%).

Conclusions: Pattern of glomerular disease in our patients is consistent with the international data revealing FSGS as the commonest finding for idiopathic glomerular diseases.

PUB746

Increased Body Mass Index May Be a Risk Factor for the Development of Lupus Nephritis Abhijeet S. Danve, Nripesh Pradhan, Supriya Kulkarni, Donald I. Baumstein. *Nephrology, Metropolitan Hospital/New York Medical College, New York, NY.*

Background: Increased body mass index (BMI) is a risk factor for development of Chronic Kidney Disease (CKD). Obesity is low grade inflammatory state which leads to CKD by lipotoxicity, increased leptin levels, cytokine mediated glomerular injury and fibrosis. Systemic Lupus Erythematosus (SLE) is autoimmune inflammatory disease associated with high leptin levels and dyslipidemia. This study was designed to assess whether BMI is a predictor for development of lupus nephritis in SLE.

Methods: A retrospective cross sectional case control study was conducted at a municipal hospital serving mostly Hispanics and Blacks in East Harlem, New York. Data for 172 SLE patients identified by ICD code and further confirmed by American College of Rheumatology criteria was retrieved from electronic medical records for years 2002-2011. All 22 cases of biopsy proven lupus nephritis were included. We then randomly selected 25 controls who had SLE without nephritis. Age, sex, and race were compared between the two groups. To avoid treatment related alterations, BMI was calculated for all patients at diagnosis of SLE or first clinic contact. Cases and controls were grouped into normal weight (0-24.9), over weight (25-29.9) and obese (>30) based on their BMI. Mean BMI was compared between the two groups using independent-samples *t test.* BMI subgroups were compared in cases and controls by chi square test.

Results: There is no significant difference between both groups across age, sex and race. Mean BMI was significantly higher in lupus nephritis cases compared to SLE controls. There are significantly more overweight and obese patients in cases than in controls.

Results

	Lupus Nephritis (Cases) N=22	SLE without nephritis (Controls) N=25	p
Mean Age +/- SD (years)	34.5 ±10	40.9±14	0.10
Female	91%	96%	0.51
Race: Hispanic/ black	81% / 9%	60% / 40%	0.1
Mean BMI (kg/m²)	27.4	24.3	0.032
BMI (0-24.9)	18%	56%	0.029
BMI (25-29.9)	36%	24%	0.029
BMI (>30)	46%	20%	0.029

Conclusions: Increased BMI may be associated with increased risk for developing lupus nephritis in patients with SLE. Prospective studies involving broader population samples are required to substantiate this finding.

PUB747

Incidence of Lupus Nephropathy in the Colombian Caribbean Region during the Years 2008 to 2012 Using Data from NEFRORED Gustavo Jose Aroca Martinez, Andres A. Cadena. *Atlantico, Clinica de la Costa, Barranquilla, Atlantico, Colombia.*

Background: There is no reliable information in Colombia about the incidence of SLE and Lupus Nephritis, using date derived from NEFRORED we evaluated the incidence of biopsy diagnosed lupus nephropathy in the caribbean region of Colombia. All the biopsies were performed at the Clinica de la Costa in Barranquilla Colombia, this is a referral center for kidney biopsies in all the region.

Methods: Using data collected from NEFRORED, 805 kidney biopsies were documented in the database from Jan 2008 to April 2012, 194 biopsies were lupus nephritis,28 biopsies were insufficient for a proper diagnosis, 166 biopsies were studied under light microscopy and immunofluoresence.

Number of biopsies per region.

Results: Lupus Nephritis was the diagnosis in 20,6% of all the biopsies performed in this time period. 81% of the patients were female with a female to male ratio of 4:1, the race was latino in 95% of the cases with a high school education in 66,2% of the cases. Family history of SLE was found in 5.6% of the patients and 50% of the patients had a preexisting diagnosis of lupus at least for the past 3 years before the biopsy was performed.

The most common clinical finding was nephrotic syndrome in 58% of cases followed by asymptomatic non nephrotic range proteiniuria + microscopic hematuria in 33% of the cases.

The most common age group was 30 to 39 y/o. Class IV LN was the most common histologic type (69,5%), class III (16,2%), Class II (8,6%), Class V (4,8%) and type I (0,9%) and Class VI (0%).

Conclusions: This is the first attempt to describe the demographics and pathology findings of patients diagnosed with lupus nephritis in the Caribbean region of Colombia. Lupus nephritis is more commong in young women with class IV being the most common finding on biopsy.

Data on the long term progression to worsening kidney disease and ESRD is not yet available.

PUB748

A 3 Years Prospective Comparison of Safety of Bedside Insertion of Permanent Hemodialysis Catheter by Nephrologist and the Hemodialysis Permanent Catheters Inserted by Vascular Surgeons, and Interventional Radiologist in Riyadh Military Hospital Ebadur Rahman,[1] Habib Rahman,[2] Raees Farhan Mushtaq.[1] *[1]Nephrology, Riyadh Military Hospital, Saudi Arabia; [2]Nephrology, King Khaled University, Riyadh, Saudi Arabia.*

Background: This is a prospective comparative study on the safety and cost effectiveness of bedside Permanent catheters insertion. Comparison was between nephrologist inserting Permanent catheters (PC) using vascular ultra sound(USG) at bed side and PC inserted by vascular surgeons in theater.

Methods: 441 procedures were included from October 2009 to October 2011. Catheter related bacteremia within 2 weeks was defined as acute infection in this study. Nephrologist inserted bed side PC using USG or in fluoroscopy using both (USG) and fluoroscopy. Vascular surgeons used both ultrasound and fluoroscopy for their procedure. All of them had utilized Bird precurved PC. SPSS software version 19 was used for the data analysis. Blood flow rate (BFR), morbidity; cost of procedure, minimum number of consultations needed for the insertion of PC were analyzed. BFR was measured by same type of HD machine. Cost of procedure was derived from the number of days in hospital multiplying with cost of stay per day in hospital.

Results: Total number of procedure included in this study was 441. Gender distribution and mean average age was not significant among the different groups. The average costs per procedure in Saudi Riyals (S.R.) were:

I.) 1525.42 SR for Bedside insertion by nephrologist: and ii). 9653.63 S.R.for Vascular Surgeons. The mean BFR in the two groups were:

i). Bedside 457.29ml/min, ii).Vascular Surgeons: 380.22 ml/min;

BFR was significantly ($p < 0.0001$) better in PC inserted by nephrologist at bedside than those inserted by vascular surgeon. Nephrologist needed significantly low number of consultations when compared with vascular surgeons ($p < 0.0001$). Infection rates and morbidity among the groups did not show any significant difference (p value 0.06 and 0.164 respectively).There was no difference in BFR of PC inserted bedside or in flouroscopy by nephrologist or PC inserted by intervention radiologist during this observation period.

Conclusions: Bed side PC insertion by trained nephrologist using (USG) is safe and cost effective.

Funding: Other NIH Support - Riyadh Military Hospital , Nephrology Department

PUB749

Delayed Catheter Removal and Fatal Outcome in Catheter Dependent Hemodialysis Patients with Candida Parapsilosis Infection Ebadur Rahman,[1] Habib Rahman,[2] Raees Farhan Mushtaq.[1] *[1]Nephrology, Riyadh Military Hospital, Saudi Arabia; [2]Nephrology, King Khaled University Hospital, Saudi Arabia.*

Background: This is the largest series of CP infection in Hemodialysis in Saudiarabia dueto out break of Candida parapsilosis infection occurred due to colonization of distribution tubings of treated water in Hemodialysis unit of Riyadh Military Hospital in October 2009. This lead to infection of 15 patients dialyzing with permanetcathter (permcath).

Methods: The diagnosis was made either by positive culture from 2 separate peripheral Intravenous (IV) sites, or one peripheral IV site and perm cath or 2 positive cultures from permcath where samples were taken 15 minutes apart. Trans thoracic Echocardiography was done in all patients to rule out infective endocarditis. Trans esophageal echocardiogram was performed in selected patients with high clinical suspicion of infective endocarditis. Fundoscopy examination was made in all cases. All patients were initially treated with imperic IV Fluconazole. Antifungal therapy was upgraded to IV Caspofungin despite having sensitivity to Fluconazole in patients with infective endocarditis.

Results: The observation revealed that all the cases were sensitive to Fluconazole. Fatality was seen in 4 out 15 (26.7%) patients. These 4 patients refused to remove the infected perm cath early on. Moreover, 50% (2 out of 4) of the fatal cases developed Infective Endocarditis. These patients antifungal therapy was upgraded to IV Caspofungin despite having sensitivity to Fluconazole. The other 11 infected (73.3%) patients had their perm catheter removed within 72 hours of detection of the infection. They received Fluconazole for additional 2 weeks. These patients did not have re-infection after discontinuation of the drug. Survelence Echo in this patients were negetive for infectine endocarditis. Fundoscopic examinations were negetive in all cases.

Conclusions: Candida Parapsilosis is potentially fatal infection if infected catheter removal is delayed.Fluconazole is an effective treatment if catheter is removed early.

PUB750

A Challenge of Safety in Hemodialysis Practice: Advancing Anticoagulation Therapy in Saudi Arabia Ali Mohammed Allehbi,[1] Ayesha Noor,[2] Archie Dumdum Bunani.[3] *[1]Medicine - Nephrology, DaVita Lehbi Care - Saudi Arabia, Riyadh, Saudi Arabia; [2]Pharmacy, DaVita Lehbi Care - Saudi Arabia, Riyadh, Saudi Arabia; [3]Clinical Services, DaVita Lehbi Care, Riyadh, Central Province, Saudi Arabia.*

Background: Effective heparinization during dialysis is vital since it allows blood to flow into the extracorporeal circuit. This study aimed to develop a relationship between errors in Heparin administration and the study of Partial Thromboplastin Time (PTT), Hemoglobin (Hgb), Hematocrit (Hct), and Platelet (Plt) levels of hemodialysis (HD) patients.

Methods: 255 HD patient records were examined for compliance and errors in heparin administration practices. With multiple tendencies, cox regression was used to analyze trends whilst Pearson rho moment correlation determined relationships.

Results: The results indicated that heparin was administered via three routes namely bolus (90.47%), maintenance (100% via machine, 19.04% via manual approach) preparation and administration. It was significant that only 8% of nurses followed the *Independent Double Check* method of heparin preparation and administration which was a required standard within the unit through Clinical Pharmacist's and Nurse's protocol. Data showing both medication administration practices and extent of errors versus the mean scores of the PTT, Hct, Hgb and Plt were analyzed individually showing a significant regression of PTT (r=1.38, 1.50), Hgb (r=0.80, 1.03), Hct (r=1.11, 1.07), and Plt (r=1.22, 1.27). Results were summed and revealed strong correlation between the errors versus the mean values of the PTT (p=+0.77), Hct (p==0.55), Plt (p=+0.67) with the exception of Hgb which did not show any correlation at all p=(+0.04).

Conclusions: The results of this study led to the development of a standardized protocol minimizing errors relating to heparin administration during dialysis. Additionally, the study provided a *Process Map* when untoward incidences relating to use of Low Molecular Weight Heparins occurred. Further, the study has led to a significant decline in errors in medication administration practices in general within the unit.

Funding: Private Foundation Support

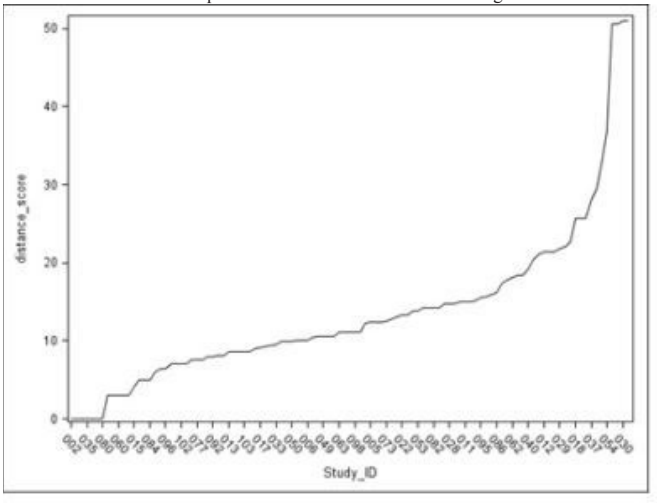
PUB751

A Composite Measure of Medication Hazard in Pre-Dialysis Chronic Kidney Disease (CKD) Min Zhan,[1] Clarissa Jonas Diamantidis,[3] Corinne Woods,[2] Jeffrey C. Fink.[3] *[1]Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD; [2]Pharmaceutical Research Computing, School of Pharmacy, University of Maryland, Baltimore, MD; [3]Department of Medicine, University of Maryland, Baltimore, MD; [4]University of Maryland, Baltimore, MD.*

Background: Many medications require dosing modifications/special considerations in CKD. FDA guidance on medication precautions with low GFR vary and CKD patients have poly-pharmacy making it difficult to quantify the extent of their ***medication hazard***. We recorded the frequency of medications with precautions in the Safe Kidney Care (SKC) cohort, developed a composite measure of medication hazard, and identified factors associated with this measure.

Methods: SKC phase 1 participants (n=107) had baseline medications (prescribed and OTC) and last eGFR recorded. The SKC drug cross-walk flagged medications with dose precautions based on GFR and established FDA/literature-based recommendations. Unique flags included: excessive dose/frequency, avoid use, use with caution, reduce dose, individualize dose, and NSAID use. A distance procedure input the type and frequency of each precaution flag into a hazard measure using a Euclidian distance matrix of flags weighted by severity (SAS 9.2 User's Guide). Multiple regression identified factors associated with the hazard score.

Results: 1293 medications were recorded at baseline SKC visit. 94 (88%) participants had at least one hazard flag, and 684 (52%) medications were flagged with a precaution. The distribution of the composite hazard measure are shown in Figure 1.



Key factors predicting a higher hazard score (after adjusting for age, GFR, and race) included gender, diabetes, and cancer.

Conclusions: Medication precautions are common and diverse in CKD patients and single score can be used to characterize the burden of their medication hazard.

Funding: NIDDK Support

PUB752

Delayed Removal of of Chronic Candida Parapsilosis Infected Hemodialysis Permanent Catheter and Subsequent Adhesion of Catheter to the Vascular Tree and Atrium in a Noncompliant Patient Ebadur Rahman,[1] Raees Farhan Mushtaq,[1] Habib Rahman.[2] *[1]Nephrology, Riyadh Military Hospital, Riyadh, Saudi Arabia; [2]Nephrology, King Khaled University Hospital, Riyadh, Saudi Arabia.*

Background: To the best of our knowledge this is the first case of chronic candida parapsilosis fungemia complicated by catheter adhesion to the vascular tree and with grave outcome.

Methods: This is a case report of a 29-year-old Saudi male patient on maintenance Hemodialysis with history of poor compliance who was offered fistula creation several times but insisted on remaining in catheter for dialysis and had several hospital admissions for treatment of line related sepsis. During the last one year with the exposure of multiple antibiotics and the outbreak of candida parapsilosis (CP) infection in Hemodialysis unit, he acquired CP infection. Immediate removal of the infected line and anti fungal treatment was offered, but he refused. The blood culture remained positive for CP for one year although he was asymptomatic and he persistently refused to remove the catheter. Finally he agreed to remove the catheter as it was thrombosed. The vascular surgeons tried to remove but unfortunately they failed. Urgent CT Angio was done, which showed calcification in right atrium and the catheter tip was seen adherent to atrial wall. Patient was offered high-risk cardiac surgery for removal of adherent Permanentcatheter, but he declined any sort of intervention. A right jugular permanent catheter was inserted for hemodialysis as he refused femoral route. The patient left the hospital without any medical advice and was dialysing in other hospital. After 3-months he was admitted in ICU with massive left middle cerebral artery territory stroke and found to have vegetations in both aortic and mitral valves. Patient remained intubated and the treating consultants decided to only continue supportive management for him.

Conclusions: Chronic CP infection in hemodialysis catheter can cause catheter ahesion to the vascular tree with grave consequences.

PUB753

Chronic Kidney Disease Awareness in Primary Care: A Cross-Sectional Study in a Central European Country Yuki Tomonaga,[1] Thomas D. Szucs,[2] Lorenz Risch,[3] Patrice M. Ambuehl.[4] *[1]Institute of Social and Preventive Medicine, University of Zurich, Zurich, Switzerland; [2]European Center of Pharmaceutical Medicine, University of Basel, Basel, Switzerland; [3]Labormedizinisches Zentrum Dr. Risch, Schaan, Liechtenstein; [4]Renal Division, Stadtspital Waid, Zurich, Switzerland.*

Background: As severe chronic kidney disease (CKD) is usually identified based to typical symptoms, mild to moderate CKD in primary care remains mostly underdiagnosed. Our aim was to investigate the CKD-awareness in a primary care setting in Switzerland.

Methods: A multicenter, cross-sectional study with randomly selected general practitioners (GP) was performed. Adults visiting the GPs during defined periods were asked to participate. Demographic and social variables were collected. Urine and blood samples were analyzed in a central laboratory. Renal status was assessed calculating the estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR), as suggested in the KDIGO classification [1]. For each patient, the GP had to indicate, based on the subject's personal clinical history and demographics, which laboratory analyses he deemed appropriate.

Results: 1000 individuals with mean age of 57±17 years were included. For subjects with either a substantial reduction in renal function (eGFR<60 ml/min/1.73m^2) or high levels of proteinuria (ACR>30 mg/g creatinine), plasma creatinine (PCr) or urinary albumin (UAlb) tests were considered appropriate for 39% and 22% of the cases, respectively. For patients without CKD, both analyses were considered significantly less useful (PCr: 29%, p=0.003; UAlb: 10%, p<0.001).

		eGFR<60 and/or ACR>30	
Test	Test appropriate?	Yes	No
Serum creatinine	Yes	9%	22%
	No	14%	55%
Urinary albumin	Yes	5%	8%
	No	18%	69%

Conclusions: Although GPs performed more analyses for patients with abnormal renal status, creatinine or albumin tests were considered necessary for less than 40% of the CKD patients. This indicates that the majority of the CKD patients may remain undetected due to a lack of CKD awareness in primary care.

Reference: 1 Levey AS et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney international (2011).

Funding: Pharmaceutical Company Support - Abbott AG, Switzerland

PUB754

Baking Soda Pica in ESRD Patient with Severe Metabolic Alkalosis and Recurrent Hospitalizations Neha Garg, Isaiarasi Gnanasekaran. *Internal Medicine, Lincoln Medical and Mental Health Center, Bronx, NY.*

Background: Pica has been frequently reported in ESRD patients. Pica of alkali/ alkali precursors can lead to severe metabolic alkalosis with increased risk for cardiac arrhythmia, seizures, hypercapnia, hypoxemia, and cardio-pulmonary arrest. A pH >7.65 carries a high risk of mortality (up to 80%). Prompt recognition of pica is critical for patient safety. To our knowledge, this is the first reported case of life threatening metabolic alkalosis with peak pH of 7.71 in an ESRD patient secondary to pica.

Methods: 41 year old black male patient on maintenance hemodialysis for 17 months, requiring >4hrs of dialysis, 4-5 days a week for severe fluid overload with usual interdialytic weight gain of > 10kgs and weekly hospitalization. Patient was admitted to our hospital for acute respiratory failure due to fluid overload and severe metabolic alkalosis. Medical history was significant for diabetes mellitus, hypertension, right parietal skull fracture with subdural hematoma and subarachnoid hemorrhage. Patient was extubated after 5days of daily dialysis. Hospital course was significant for unexplained severe metabolic alkalosis with peak pH of 7.71, peak pCO2 of 77 mmHg, hypoxemia (pO2 of 54 mmHg), peak serum sodium and bicarbonate of 153 mEq/L and 56 mEq/L respectively, nadir serum chloride of 85 mEq/L and interdialytic weight gain of 12kgs (post-extubation). Patient denied GI symptoms. Hemoglobin was low (7.7gm/dL) with normal iron indices. Severe metabolic alkalosis and hypernatremia with excessive weight gain raised the concern for pica. On repeated questioning, patient acknowledged chewing rubber and plastic only. Later patient was witnessed to be consuming baking soda brought from home. Patient admits hiding this information for fear of criticism. Patient was evaluated by psychiatry service and extensive counseling was done with improvement in metabolic alkalosis and weight gain.

Conclusions: Though pica is highly prevalent in ESRD patients, it is under recognized. Besides GI acid loss, high bicarbonate dialysate use and regional citrate dialysis, pica should be suspected as a cause of metabolic alkalosis in ESRD. Failure to recognize pica may jeopardize patient safety.

PUB755

Causative Organisms and Antibiotics Susceptibility in Patients with Community Acquired Acute Pyelonephritis Who Adimitted to Emergency Room Dae-hong Jeon,[1] Dong Jun Park,[2] Se-Ho Chang,[2] Yeojin Kang,[2] Hyejung Ha.[3] *[1]Internal Medicine, Republic of Korea Airforce Eduction & Training Command Aerospace Medical Group; [2]Division of Nephrology, Gyeongsang National University Hospital; [3]Division of Nephrology, Jinju Korea Hospital.*

Background: Over the 10 years, ciprofloxacin has been used as one of antibiotics of choice for the empirical treatment of community-acquired acute pyelonephritis (APN), of which *Escherichia coli (E. coli)* is the main causative bacterium. Recently, however, some studies show that ciprofloxacin-resistant *E. coli* is increasing in APN. This study aimed to re-evaluate ciprofloxacin as one of initial antibiotics for APN according to the survey of the patients' clinical and microbiologic characteristics.

Methods: The records of 356 patients who visited Gyeongsang National University Hospital emergency room from Jan 01, 2000 to Dec 31, 2009 and diagnosed as APN were reviewed retrospectively. This study investigated the clinical characteristics of patients, causative organisms and annual change of antibiotics susceptibility to each antibiotics, associated factors that influence the ciprofloxacin resistance.

Results: According to the urine or blood cultures, the patients with isolated microorganism reached up to 60.4% (215/356) and *E. coli* occupied 84.2% (181/215) out of the isolates. Among the antibiotics chosen initially, ciprofloxacin was most common (75.0%, 267/356) and the third generation cephalosporin was next (23.0%, 82/356). The analysis of antibiotics sensitivity among 181 *E. coli* isolates, demonstrated that ciprofloxacin susceptibility was 81.8%. And in the recent 3 years, from 2007 to 2009, ciprofloxacin susceptibility was 87.7% and this was higher than the years before 2007 (77.8%). When the ciprofloxacin-resistant bacterium was isolated, the APNs had high tendency to be complicated by diabetes mellitus or urinary anatomical abnormalities.

Conclusions: For the community acquired APNs, among the *E. coli* isolates, there were no significant change in ciprofloxacin resistance by years. However, ciprofloxacin should be carefully used as initial antibiotics especially in case of the patients having diabetes mellitus or urinary anatomical abnormalities.

PUB756

Preclinical Safety of Baxter's Anti-MIF Antibody Barbara Dietrich, Frank Horling, Michael Thiele, Margit Spatzenegger, Randolf J. Kerschbaumer, Hans Peter Schwarz, Friedrich Scheiflinger, Eva-Maria Muchitsch. *Baxter Innovations GmbH, Vienna, Austria.*

Background: Baxter developed a fully human monoclonal antibody (BAX B01) directed against the human proinflammatory cytokine macrophage migration inhibitory factor (MIF). The antibody was expressed in a Chinese Hamster Ovary cell line using a plasma protein-free production process including two virus inactivation steps. BAX B01 exerts significant anti-inflammatory properties by neutralizing MIF. BAX B01 is currently being studied in patients with lupus nephritis.

Methods: The in-vivo safety evaluation of the anti-MIF antibody consisted of a safety pharmacology study in telemetered macaques and repeated doses toxicity studies in rats and macaques. In addition, tissue cross reactivity, cytokine release and compatibility with human blood was assessed in vitro.

Results: Treatment with BAX B01 did not result in any toxicologically relevant adverse effect on the cardiovascular or respiratory system at doses up to 200 mg/kg. The anti-MIF antibody was well tolerated in rats at weekly doses up to 250 mg/kg over a period of one month. No adverse effects could be detected in this species. Repeated administration of the anti-MIF antibody to macaques was well tolerated after weekly doses up to 200 mg/kg and no signs indicative of severe toxicity were observed. Dose-dependent, inflammatory signs in knee joints were evident at doses ≥50 mg/kg with partial recovery after a 4-week treatment-free period while similar findings were observed in elbow joints after a single dose of 25 mg/kg. In-vitro tissue cross reactivity on human and macaque tissues showed that the staining pattern was very much comparable. Furthermore, cross-reactivity to other antigenic sites was evaluated as unlikely. The anti-MIF antibody was very well compatible with human blood.

Conclusions: In summary, the results obtained from preclinical studies were basis for a safety profile which supported the initiation of human trials.

Funding: Pharmaceutical Company Support - Baxter Innovations GmbH

PUB757

CTP-499, a Novel Drug for the Potential Treatment of Chronic Kidney Disease, Possesses a Unique Metabolite Profile Lijun Wu, Kristine Hogan, Gary W. Bridson, Julie Fields Liu, Sophia Nguyen, Vinita Uttamsingh, Ara Aslanian. *Concert Pharmaceuticals, Lexington, MA.*

Background: CTP-499 is a novel agent in phase II clinical development for the treatment of diabetic nephropathy, a common cause of chronic kidney disease (CKD). It is structurally identical to the primary active metabolite M1 from pentoxifylline (PTX), except that several key deuterium atoms are present in place of hydrogen. Both M1 and PTX form common metabolites M2-M5 and share the pharmacological properties of inhibiting inflammation and oxidation. Their common downstream metabolites M5, and possibly M4, possess hemorrheologic effects. To date, CTP-499 (deuterated M1) has demonstrated anti-inflammatory, anti-oxidative, anti-fibrotic and reno-protective activities pre-clinically.

Results: In this study, CTP-499, PTX, and the metabolites M2-M5 were characterized for their activity in inhibiting the release of pro-inflammatory cytokines from stimulated human blood as well as inhibiting the formation of reactive oxygen species (ROS) from stimulated human neutrophils. Besides PTX and CTP-499, M2 was found to be the most active species, achieving 70% and 89% of the activity of PTX for TNF-α and ROS inhibition, respectively. In CTP-499-dosed rats, CTP-499 and M2 showed preferential distribution to the kidney, with a kidney to plasma exposure ratio of 2.3- and 8.3-fold (N=4), respectively, 4 hours post dosing.

In a human phase I study, CTP-499-dosed subjects had a significantly higher plasma exposure of M2 (increase of 138%, p= 0.00015) compared to PTX-dosed subjects, whereas similar exposures were found for CTP-499 vs. M1 formed from PTX, as well as for PTX vs. deuterated PTX formed from CTP-499 (D-PTX).

Conclusions: These data suggest that the M2 metabolite of CTP-499 and PTX is biologically active in inhibiting pro-inflammatory cytokines and ROS generation. Its preferential distribution to kidney in rats makes it a potentially very important contributor to the efficacy of CTP-499 in CKD. Since CTP-499-dosed subjects had higher exposures to M2 in comparison to PTX-dosed subjects, CTP-499 may offer potential advantages over PTX in treating CKD.

Funding: Pharmaceutical Company Support - Concert Pharmaceuticals

PUB758

Assessment of Cyclohexanone Levels in Hemodialysis and Continuous Renal Replacement Therapy Systems and Patients: A Pilot Study Joshua D. King, Derek M. Fine. *Division of Nephrology, Johns Hopkins University, Baltimore, MD.*

Background: Hemodialysis (HD) is known to depress cardiac output, although the mechanisms behind this effect are somewhat unclear. Cyclohexanone (CHX), a solvent used in the synthesis of polyvinyl chloride medical devices such as intravenous (IV) fluid bags and tubing used in HD, has been shown to depress cardiac output and cause hypotension, and has been suggested as a possible mediator of these effects in dialysis. Previous studies have revealed the presence of CHX in IV medications and dialysate used for continuous renal replacement therapy (CRRT) in our hospital. We performed a pilot study to see if CHX is removed by several modalities of dialysis in dialysis circuits with and without patients.

Methods: We measured CHX levels via gas chromatography in saline samples and whole blood samples from HD patients. We also measured CHX levels in dialysate and saline used in sham models of HD and CRRT systems, as well as CHX levels in peritoneal dialysate.

Results: CHX was found to be present at detectable levels in saline and dialysate used for CRRT. CHX was undetectable in HD patients' blood before and after initiation of HD.

Cyclohexanone levels in hemodialysis patients

Patient	Saline	Initial blood	1.5 hr into HD	3 hr into HD
1	1.02	0	0	0
2	1.88	0	0	0
3	1.66	0		
4	1.42	0	0	0
5	3.2	0	0	0
6	1.72	0	0	0

All levels in mg/L

In sham models using saline run through dialysis systems, CHX was decreased in saline samples post-HD, but not in saline samples post-CRRT.

Cyclohexanone levels in sham hemodialysis systems (mg/L)

Sample	Saline	Pre-dialyzer	Post-dialyzer
1	4.4	5.0	1.08
2	2.6	2.4	1.74

Cyclohexanone levels in sham CRRT systems (mg/L)

Sample	Saline	Pre-dialyzer	Post-dialyzer	Dialysate
CVVHD	5.2	6.2	5.6	3.0
CVVH	3.4	5.6	4.2	

Further data regarding CHX levels in HD, CRRT and peritoneal dialysate are pending at this time.

Conclusions: Cyclohexanone is effectively removed by HD; however, it does not appear to be significantly removed by CRRT, possibly due to the presence of CHX in dialysate used for CRRT. Further human studies are needed to assess the clinical significance of this finding in patients undergoing CRRT.

Funding: Private Foundation Support

PUB759

Differential Expressions of miRNAs in Kidney in Puromycin Aminonucleoside Nephropathy Model and Intervened Effects of Leizhi Capsule Wei Sun. *Nephrology, Jiangsu Provincial Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China.*

Background: Leizhi capsule (LZC) is widely used for reducing proteinuria. The aim of this study was to investigate the mechanism of LZC for reducing proteinuria and ameliorating podocyte in puromycin aminonucleoside (PAN) nephropathy rat.

Methods: Fifty male Wistar rats were randomly subdivided into five groups, control group (A), model group (B), LZC-treated group(C), multi-glycoside of *Tripterygium wilfordii* Hook. f. (GTW)-treated group (D), and valsartan-treated group (E). Except the rats in A group, PAN nephropathy model was induced by a single jugular vein injection of PAN at the dose of 100 mg/kg in B, C, D, and E groups. At the 2nd day after PAN nephropathy model was established, daily oral administration of LZC in C group, GTW in D group, valsartan in E group, and physiological saline in A and B groups as a control was started and lasted for 10 days. Proteinuria, glomerular ultrastructure, and glomerular immunofluorescence staining of dicer enayme, nephrin, podocin and synaptopodin were examined. The differential expressional characteristics of miRNAs in renal cortex were analyzed through biochip assay. Moreover, the differential expressional volumes of no-miR-23a, rno-miR-300-3p, rno-miR-24, and rno-miR-30c were measured by real-time PCR, respectively.

Results: In PAN-induced rats, proteinuria and podocyte foot processes effacement were investigated, as well as the expressions of nephrin, podocin, and synodopotodin were affected by dicer enayme. The differential expressional miRNAs in kidney included rno-miR-24, rno-miR-30c, rno-miR-23a, and rno-miR-300-3p. LZC could improve proteinuria, alleviate podocyte foot processes effacement, reduce the expression of dicer enayme, increase the expressions of nephrin, podocin, and synodopotodin, and regulate the differential expressional miRNAs as well in PAN nephropathy model.

Conclusions: In PAN nephropathy rat, with the treatment of LZC, proteinuria can be reduced and the expressions of nephrin, podocin, and synaptopodin can be regulated, the mechanism of which is probably due to intervening dicer enayme and differential expressions of miRNAs in kidney.

Funding: Government Support - Non-U.S.

PUB760

The Role of Rosuvastatin in the Cyclosporine Induced Nephropathy in Rats Young Ki Son,[1] Hyun Kyung Nam,[2] Won Suk An,[1] Ki Hyun Kim,[1] Seong Eun Kim.[1] *[1]Division of Nephrology, Dong-A University Hospital, Busan, Korea; [2]Divisin of Nephrology, Busan Medical Center, Busan, Korea.*

Background: Cyclosporine (CsA) – induced kidney injury is characterized by renal dysfunction with interstitial fibrosis, inflammatory cell infiltrations and glomerular sclerosis. Pleiotropic effects of statins may exert anti-inflammatory and anti-arteriosclerotic actions beyond lipid control. The aim of this study was to investigate the effect of rosuvastatin (RUS), which has anti-inflammatory and anti-fibrotic effects, on chronic CsA-induced nephropathy in a rat model.

Methods: Male Sprague Dawley rats fed with low sodium diet were divided into four treatment groups: control (0.9% saline injection), CsA (15mg/kg/day by s.c.), CsA + rosuvastatin (CsA plus rosuvastatin 10mg/kg/day by p.o.). Renal function, cyclosporine level and lipid level were measured at the end of 4 weeks. The expression of ED-1, transforming growth factor (TGF-β1) and α-smooth muscle actin (α-SMA) were examined by western blot analysis. The expression levels of apoptosis-associated factors were examined by western blot analysis. Apoptosis was detected using TUNEL method.

Results: Kidney function was decreased in CsA-treated rats compared with controls, which was attenuated by RUS. RUS did not affect lipid level and blood CsA level. Tubular atrophy, interstitial fibrosis, and glomerular change in CsA-treated rats were attenuated by RUS supplementation. The expression of ED-1, α-SMA, fibronectin, TGF-β1, Smad2/3, and Smad4 was increased in the CsA-treated rats, which was attenuated by RUS. In addition, RUS prevented CsA-induced increased expression of Bax/Bcl-2 ratio. TUNEL staining showed that RUS inhibits CsA-induced tubular apoptosis.

Conclusions: RUS supplementation can reduce or reverse CsA-induced nephropathy through suppression of proinflammatory pathways, profibrotic pathways and apoptotic factors.

PUB761

The Study of the Induction of AGEs on Transdifferentiation of Rat Aortic Smooth Muscle Cells Ruoyun Tan, Min Gu, Chao Liu, Pei Lu. *Department of Urology, The First Affiliated Hospital Nanjing Medical University, Nanjing, Jiangsu, China.*

Background: Objective To investigate the mechanism of advanced glycosylation end products(AGEs) involved in the progression of artherosclerosis after kidney transplantion.

Methods: The fifth SD rat aortic VSMCs was an *in vitro* culture system. The VSMCs were incubated with 200mg/L AGEs or BSA for 12h~12d, then the cells were collected and detected the expressions of alpha-smooth muscle actin(a-SMA), RUNX2 and Osteopontin(OPN) by Western blot and indirect immunofluorescence staining assays.

Results: The abundance of a-SMA was very high in the second to fifth SD rat aortic VSMCs. Compared with normal control or the cells treated with BSA, AGEs can reduce a-SMA protein expression (P<0.05) and the percent of a-SMA positive cells in rat aortic VSMCs in a time-dependent manner(P<0.05). Meanwhile, the expression of RUNX2 protein was rapidly induced after AGEs treatment for 12h(P<0.05), and followed up, the expression of OPN was also increased in rat aortic VSMCs cells(P<0.05).

Conclusions: These results suggest that AGEs maybe act as a key role in the procession of artherosclerosis after kidney transplantion through inducing transdifferentiation of VSMCs to osteoblast-like cells by upregulation of RUNX2.

Funding: Government Support - Non-U.S.

PUB762

Tacrolimus Induces Glomerular Injury via Endothelial Dysfunction Caused by Reactive Oxygen Species and Inflammatory Change Kengo Kidokoro, Minoru Satoh, Hajime Nagasu, Yuko Nishi, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: The calcineurin inhibitor (CNI) tacrolimus (FK506) is used clinically to reduce the rejection rate in patients with kidney transplantation; however, the resultant nephrotoxicity remains a serious problem. Multiple factors have been reported to mediate the pathogenic mechanisms underlying CNI nephrotoxicity including activation of the renin angiotensin system. In the present study we have attempted to elucidate the mechanisms of glomerular injury induced by FK506 and the renoprotective effects of the angiotensin II receptor blocker telmisartan.

Methods: Seven-week-old male Wistar rats were divided into three groups: vehicle group, FK506 group, and FK506 + telmisartan group. After 8 weeks, we assessed kidney function and renal morphological changes including oxidative stress. We also assessed the effect of FK506 in human glomerular endothelial cells (hGECs) with regard to reactive oxygen species (ROS).

Results: Systolic blood pressure did not differ between groups. Serum creatinine and urinary protein excretion were significantly higher in the FK506 group compared with the other two groups. FK506 induced ROS production via activation of NAD(P)H oxidase in the glomeruli. Expressions of p47*phox* and p67*phox* mRNA were significantly increased in isolated glomeruli from the FK506 group. Expression of ICAM mRNA was increased in glomeruli from the FK506 group. These effects resulted in macrophage infiltration into the glomeruli. FK506 directly promoted NAD(P)H oxidase activity and accelerated production of ROS in hGECs. Conversely, co-treatment with telmisartan inhibited both NAD(P)H oxidase activity and production of ROS.

Conclusions: The present findings indicate that oxidative stress induced by FK506 in glomerular endothelial cells is involved mainly in NAD(P)H oxidase activity and subsequently induces inflammatory changes. This action could play a role in vascular injury and in complications associated with the long term use of FK506.

PUB763

Increased HLA Mismatches Promote Clinically Significant De Novo Donor-Specific Antibody Formation Rajat Joshi, Danielle Ladie, Kristina Krecko, Robert Scott, Kenneth Bogdanovich, Harold Yang, Mary Waybill, Seth Narins. *Transplantation, PinnacleHealth.*

Background: Antibody formation against human leukocyte antigens(HLA) is associated with graft dysfunction and loss in renal transplant recipients. We have shown that recipients with positive leukocyte antibody panel(LABB) tests demonstrate higher total numbers of HLA mismatches as compared to patients with negative LABB, and that positive-LABB recipients demonstrate donor and class-specific antibody against mismatched HLA loci with increased serum creatinine levels. It is unknown if differences exist in class identity of HLA mismatches observed.

Methods: This is a retrospective analysis of 106 renal transplant patients undergoing LABB testing October 2010-June 2011. LABB was positive in 49 patients and negative in 57 patients. There were no significant demographic differences between the two groups. Two LABB-negative patients were excluded due to insufficient HLA documentation(*n*=55). Recipient and donor HLA were compared for mismatches at A,B,DR and DQ loci. Student's T-Test was used to compare the differences in mean total, class-specific, and loci-specific HLA mismatches.

Results: LABB-positive patients demonstrated significantly more total HLA mismatches and mismatches in both Class I & Class II HLA than LABB-negative patients. Increasingly significant differences were seen at the A(p=0.0315), B(p=0.0025) and DR loci(p<0.0001), respectively.

HLA Mismatches	Negative LABB(Mean+/-SEM)	Positive LABB(Mean+/-SEM)	p-Value
Total	3.07+/-0.29	4.49+/-0.31	0.0013
Class I	1.87+/-0.18	2.51+/-0.18	0.0141
Class II	1.20+/-0.15	1.98+/-0.18	0.0013
HLA-A	0.82+/-0.11	1.15+/-0.10	0.0315
HLA-B	1.05+/-0.10	1.52+/-0.10	0.0025
HLA-DR	0.69+/-0.10	1.31+/-0.10	<0.0001
HLA-DQ	0.67+/-0.10	0.88+/-0.11	0.1520

Conclusions: Significantly more HLA mismatches were observed in LABB-positive kidney transplant recipients at both Class I & II HLA loci and may promote donor-specific antibody production and subsequent renal dysfunction. The greater statistical significance that exists for HLA-DR and HLA-B mismatches supports previous data demonstrating increased risk for graft loss with mismatches at these loci, and may warrant more rigorous antibody monitoring in post-transplant HLA-mismatched patients.

PUB764

Zinc-N-Acetylcysteine Inhibits Cell Death during Cold Storage of Kidneys
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Background: Cold storage of the kidneys is a major problem in USA. Of all the donated kidneys 16% are discarded due to limited viability because of the cold storage induced cell death.

Methods: Zinc-N-acetylcysteine (ZnNAC) was synthesized in our laboratory. Its antioxidant and anti-endonuclease activities were tested by Trolox equivalent antioxidant capacity assay and plasmid incision assay respectively. The pattern of DNA degradation in male rat kidneys stored in the University of Wisconsin solution (UWS) at 0ºC, 22ºC and 37ºC was studied. To study the effects of ZnNAC on cell death, rat kidney cells were stored at 0 degree Celsius in UWS or ZnNAC (0.3-30 mM in UWS) for 24 hours (in vitro) and the rat kidneys were flushed with pre-cooled UWS or ZnNAC (0.3-30 mM in UWS), and then stored in the same solutions for 24 hours (ex vivo). Propidium iodide assay was used to assess the rat kidney cell death in vitro. TUNEL assay was used to quantify DNA fragmentation indicating irreversible cell death ex vivo. The quantification of cleaved caspase-3 and endonuclease G was done by immunohistostaining of the rat kidney tissue section slides from the 10 mM ZnNAC experiment and control groups. Rat kidney cells stored in 10 mM ZnNAC group were stained with flourophobe, TFL ZN to stain intracellular zinc.

Results: DNA fragmentation (cell death) was significantly inhibited but at 0ºC as compared to 22ºC and 37ºC. Tubules were affected more than glomeruli. The cell death was significantly inhibited both in the cortex and medulla by ZnNAC at concentrations 1-30 mM, with the maximum effect reaching at 10 mM. Immunohistostaining showed that the expression of cleaved caspase 3 and endonucleases were also markedly inhibited by ZnNAC at 10 mM. TFL Zn stain showed that NAC delivers zinc inside the cells.

Conclusions: ZnNAC in UWS can inhibit caspase 3 and endonucleases, and cell death in kidneys during cold storage.

Funding: Veterans Administration Support

PUB765

The Nature of Cylcosporine Induce Tubular Vacuolization
Chi-Hung Cheng, Kuo-hsiung Shu. *Nephrology Section, Taichung Veterans General Hospital, Taichung, Taiwan.*

Background: Cyclosporine (CsA) induce characteristic tubular vacuolization (TV) in renal tubular cell. Previous study showed tha TV was endoplasmic reticulum (ER) in orgin. However the nature CsA induced TV and the relationship between CsA induce ER stress remain unknwon.

Methods: Proximal tubule NRK52E cells was used as an in vitro model and acute CsA nephrotoxicity in Sprague Dawley rats was used as an in vivo study.

Results: In our in vitro study of NRK52 cell line, we showed that CsA-induced TV was ER in origin and was a reversible process. CsA-induced nephrotoxicity overexpressed chaperones mainly within the endoplasmic reticulum of the integrated stress response (ISR) related proteins -Bip/Grp78, ATF6, IRE1 and CHOP. Cytoplasmic ER stress related chaperones-HSP70, HSP 40, HSP27, HSP90 and HSP60 was not overexpressed after CsA treatment. Among the ISR chaperones, Bip/Grp78 was overexpressed on the membrane of TV. Blocking Bip/Grp78 by shRNA inhibited CsA induced TV formation and enhanced CsA induced cell death.

In our in vivo study of acute CsA nephrotoxicity in rat, we found that CsA induced TV had characteristic striped pattern in tubular interstitial. Moreover Bip/Grp78 was overexpressed within TV which was similar to our obervation in our in vitro study.

Conclusions: In summary, we demonstrated that CsA induced TV was a reversible process in which Bip/Grp78 over-expression is essential for TV formation. Inhibition of Bip/Grp78 inhibit TV formation and enhanced CsA toxicity. It is possible that Bip/Grp78 expression and TV formation may be involved in cellular defense mechanism against CsA nephrotoxicity.

PUB766

Outcome of the Kidney Transplantation (KT) in Patients with Liver Cirrhosis (LC)
Kyeong Woo Nho, Su-Kil Park, Soon Bae Kim. *Nephrology, Division of Nephrology, Department of Internal Medicine, College of Medicine, University of Ulsan, Asan Medical Center, Seoul, Korea.*

Background: Patients with established LC on biopsy are at risk for frank hepatic decompensation after transplantation, and KT alone without simultaneous liver transplantation is contraindicated [Textbook of KT, 5th ed.]. We retrospectively reviewed outcome of the KT in patients with liver cirrhosis.

Methods: We performed 13 KT (10 males, age 43±10year) in patients with LC during 1995~2011 and followed-up for median 55 months (range 13-202). The causes of LC are hepatitis B virus in 10, hepatitis C virus in 1, alcohol in 1 and unknown in 1. Diagnosis of LC was based on liver biopsy in 9, on imaging study in 4. One patient biopsied after 16 months after KT, because of the elevation of liver enzymes, AST increased to 144 and ALT to 238. Liver enzymes returned to normal at 6 months after biopsy.

Results: Serum creatinine increased by 2 fold in 3 patients (23%) during follow-up. Two patients received hemodialysis (HD). One patient who started HD at 196 months after KT showed recurrent IgA nephropathy on biopsy. The other patient received HD 56 months after KT. Child class before KT was A in 8 and B in 5. There was no class C. HCC was found in 2 patients (15%) at 31 and 58 months after KT, respectively. When HCC was found, liver enzymes were within normal limit. In 1 patient, who was diagnosed as HCC at 58 months after KT, radiofrequency ablation (RFA) was done 4 times and trans arterial chemo embolization (TACE) was done 3 times. He started HD at 58 months after KT. His Child class was A before KT, however, he died of hepatic decompensation at 33 months after HCC. He was the only mortality. Other patient was diagnosed HCC 31 months after KT. TACE was done 4 times. Pulmonary metastatic nodules were found 21 months after HCC was confirmed. He received Sorafenib chemotherapy. At last follow up, this April, there was no recurred HCC, however, pulmonary metastatic nodules were increased. His kidney function did not change 59 months after KT. His CPS was B before KT and didn't change.

Conclusions: In conclusion, KT may be performed in patients with LC.

PUB767

No Association between Day One Renal Allograft Resistive and Perfusion Indices and Twelve Month Glomerular Filteration Rate
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Background: Renal function within the first year after transplantation has been reported to be an important factor affecting graft survival.However there is no reliable method to predict 1 year allograft function in stable renal transplant recipients. Doppler ultrasonography and MAG3 scan has been the corner stone for assessing immediate graft function post renal transplant.The present study aims to explore the association between early post renal transplant parameters and 12 month graft function.

Methods: We performed a retrospective, observational study and included both live and deceased donor renal transplant recipients between 2008 and 2009 at Sir Charles Gardner Hospital,WA. Early post transplant parameters measured were day 7 GFR, Day 1 Resistive Index and Day 1 Perfusion Index. The outcome assessed was 12 month MDRD-derived glomerular filtration rate (GFR).

Results: In this transplant cohort OF 68 patients, 13% and 21% had abnormal resistive (>0.8) and perfusion (>250) indices respectively. In patients with RI>0.8, the mean Day 7 and Year 1 GFR were 38.2 and 39.8 respectively. However, in patients with RI 250, the mean Day 7 and year 1 GFR were 32.6 and 45.6 respectively while those with perfusion index<150 the mean Day 7 and year 1 GFR were 55.2 and 56.8 respectively attaining statistical significance(p=0.002 and 0.10 respectively).On the univariate regression analysis, Day 7 GFR was closely associated with Year 1 GFR (P<0.001) which was maintained on the multivariate regression analysis after adjusting for the confounding variables.(P=0.01) .The Resistivity index had no association with year 1 GFR in the univariate regression analysis.

Conclusions: There was no association between Day 1 resistive and perfusion indices in the transplant cohort and Day 7 graft function however we found a strong correlation between Day 7 GFR and year 1 graft function.

PUB768

Prevention and Treatment of Polyomavirus-Associated Nephropathy: Clinical Approach Including the Association of Sirolimus and Leflunomide
Caroline Lamarche,[1] Anne Boucher,[2] Raymond Dandavino,[2] Lynne Senecal,[2] Suzon Collette,[2] Duy Tran,[2] Michel Vallee.[2] *[1]Internal Medicine, Hopital Maisonneuve-Rosemont, Université de Montréal, Montreal, QC, Canada; [2]Nephrology, Hopital Maisonneuve-Rosemont, Université de Montréal, Montreal, QC, Canada.*

Background: Polyomavirus-associated nephropathy (PVAN) is an important cause of graft failure, affecting 1-10% of renal transplant patients. Systematic screening and diminution of immunosuppression is now a standard of care, but no specific protocol has been proven superior. Sirolimus and leflunomide are two potential therapeutic agents, but they have never been evaluated in combination.

Methods: In this retrospective cohort study, we reviewed the clinical data of 173 renal allograft recipients transplanted from June 2006 toDecember 2010. We routinely screened for BK viremia (BKV) by real time PCR and performed graft biopsy if viremia was significant ($\geq 10^4$ copies/mL) and sustained or if there was renal dysfunction.

Results: 43 patients (24,9%) developed a BKV-viremia of more the 100 copies/mL. Patients were separated into three groups: (i) definitive PVAN defined by biopsy (n=17), (ii) presumptive PVAN defined by plasma BKV-viremia of $\geq 10^4$ (n = 8) and (iii) low BKV-load defined by BK-viremia $< 10^4$(n = 18). MMF dose has been decreased by 50% in 31 patients, 12 patients have been switched to sirolimus and 9 received leflunomide. 11/43 patients (25.6%) have not cleared viremia and 3/43 (7,0%) had a clinical rejection, all in the PVAN group. In the PVAN group, 8/17 received the combination of sirolimus and leflunomide;7/8 (87.5%) have persistent positive viremia after an average of 23 months of follow-up, but 6/8 have stable renal function.

Conclusions: MMF reduction alone is effective to treat most of low BKV-viremia or presumptive PVAN. Acombination of sirolimus and leflunomide for PVAN patients is not an effective therapy to clear viremia.

PUB769

Minimizing Exposure to Calcineurin Inhibitors in Kidney Transplant: Should We Use Only Tacrolimus Raquel Vaz, Maria Francisca Barros, Ana Teresa Nunes, Isabel Tavares. *Serviço Nefrologia, HSJ, Porto, Portugal.*

Background: Protocols to minimize exposure to calcineurin inhibitors (CNI) intend to be a safe way to maintain graft function and avoid some risks of excessive immunosuppression, without increasing rejection. Our aim was to evaluate the impact of minimizing exposure to CNI in acute rejection rate and graft function after 1 year of follow-up; and to identify differences between two CNI used in minimization protocols.

Methods: Retrospective evaluation of 52 consecutive deceased kidney transplants performed at our institution in 2010 and followed for 1 year. 14 patients received induction with basiliximab and maintenance with mycophenolate mofetil(MMF), prednisolone(PDN) and low-dose tacrolimus (TAC)(trough levels 3-7ng/mL) or cyclosporine (CSA)(trough levels 50-100ng/mL). 29 patients were subjected to basiliximab, MMF, PDN and standard dose TAC or CSA. 9 patients receive MMF, PDN and standard dose TAC or CSA, without basiliximab. Graft function was evaluated by serial serum creatinine and renal biopsy was performed if dysfunction.

Results: Patients of low-dose protocol were older (p=0.002) and more frequently received kidneys from expanded criteria donors (ECD) (p=0.019), but didn't had differences in PRA or number of mismatchs. Initial graft function and serum creatinine at discharge were similar between groups (p=0,893). At 1-year 28,6% of CNI minimization patients had acute rejection versus 17,2% in basiliximab+standard dose CNI and 22.2% in standard dose CNI patients, but it wasn't statistically significant (p=0,427). This high rejection rate in minimization group stemmed mainly from patients taking CSA, who had 42,8% of rejection compared to 14,3% in TAC (but not statistically significant, p=0.559). Renal function at 1-year was similar, regardless the type of CNI used(p=0,233).

Conclusions: Minimization protocol with TAC allowed good graft function and rejection rates similar to standard protocols. However, we found a prohibitive incidence of rejection in low-dose CSA which, even not being statistically significant (probably because of the low number of patients enrolled), shows a trend and must compel us to revise the strategy of immunosuppression at our unit.

PUB770

Sympathetic Overactivity after Renal Transplantation and Calcineurin Antagonists Administration Dan Sapoznikov, Michal Elhalel, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.*

Background: The arterial blood pressure is believed to be controlled by both baroreflex and feedforward centrally activated sympathetic (non-baroreflex) mechanisms. The latter are characterized by an inverse relationship between systolic blood pressure (SBP) and the interbeat interval (IBI).This study was undertaken to define non-baroreflex episodes in patients with end stage renal disease on chronic hemodialysis (HD) and after renal transplantation (TX).

Methods: Continuous IBI and SBP were monitored in chronic HD (n=73), in TX patients with normal renal function (n=53) treated with calcineurin antagonists (CAN) and in control (C) individuals (n=50).Overlapping one minute time windows at one beat steps were selected for analysis of beat-to-beat IBI-SBP relationship.

Results: The percentage (%) of epochs (*Ep*) with inverse SBP-IBI relationship (median and interquartile range) was 20 (57) in C, 13(52) in HD and 73(72) in TX (p< 0.01 vs. C and HD). The SBP-IBI regression coefficients (*b*) were correlated with age, the variability of interbeat intervals (IBI) in the low frequency (LF) range (LF IBI, LF SBP), LF α (square root of LF IBI and LF SBP ratio) and baroreceptor slope (Table 1). LF oscillations are believed to be representative of sympathetic nervous system activation.

Table 1.

	C		HD		TX	
	r	p	r	p	r	p
Age	-0.524	0.001	-0.337	0.005	-0.379	0.007
LF IBI	0.577	0.001	0.679	0.001	0.598	0.001
LFα	0.772	0.001	0.757	0.001	0.858	0.001
sd IBI	0.528	0.001	0.680	0.001	0.634	0.001
Slope	0.733	0.001	0.671	0.001	0.685	0.001

b was enhanced in TX patients treated with cyclosporine as compared with those treated with tacrolimus. % *Ep* and *b* were not affected by antihypertensive drugs administration.

Conclusions: Our data show: 1.In all patients, *b*, representative of the magnitude of SBP-IBI relationship, is significantly correlated to markers of sympathetic activation and baroreflex slope, but decreases with age ;2. % *Ep* is markedly increased in transplanted patients as compared with both C and HD; 3. *b* is increased in patients treated with cyclosporine. Our results support the hypothesis that non-baroreflex episodes are representative of sympathetic overactivity, which is significantly enhanced after renal TX and chronic CAN exposure.

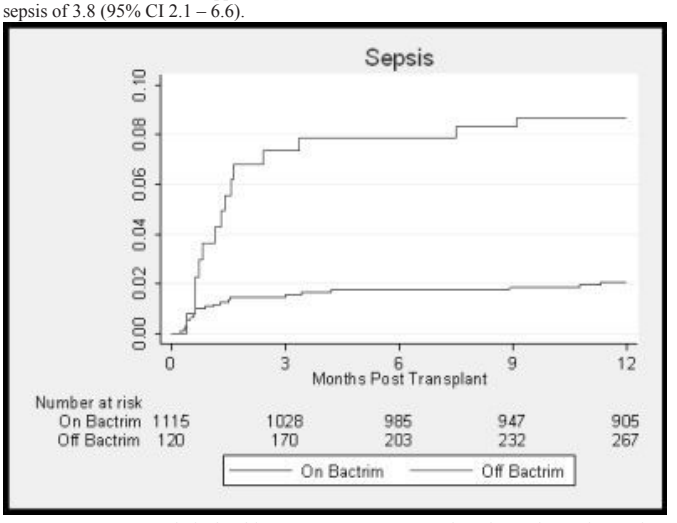
PUB771

Sulfamethoxazole-Trimethoprim Reduces the Rate of Sepsis after Kidney Transplan Timothy A. Horwedel,[1] Lyndsey Bowman,[1] Daniel C. Brennan,[2] Georges Saab.[2] *[1]Pharmacy, Barnes-Jewish Hospital, St. Louis, MO; [2]Nephrology, Washington University in St. Louis, St. Louis, MO.*

Background: Sepsis is associated with significant costs, morbidity, and mortality in renal transplant recipients. SMX-TMP is used for prophylaxis of *Pneumocyctis jirovecii* Pneumonia. SMX-TMP may prevent other infectious complications, such as urinary tract infections (UTI), post-transplant and thus prevent sepsis.

Methods: We included renal transplant recipients who were transplanted between 1/2002 – 12/2010, allowing for >1 year of follow-up. Data were extracted from electronic patient records. The primary outcome was time to first septic episode. Secondary outcomes included UTI and pneumonia. Time to sepsis was analyzed using Cox-regression with time-dependent covariates.

Results: Data were collected on 1235 patients: 61% were male; 76.7% where Caucasian; and mean age was 50.2 years. Overall, 5.2% of patients experienced sepsis which occurred earlier (within 3 months) and was more frequent among those not receiving SMX-TMP (16.2 vs. 4.2%, p <0.001). More UTIs occurred in the non- SMX-TMP group (44.4 vs 23.7%, p <0.001). A genitourinary source was found in 61.9% of sepsis cases. There was a higher rate of SMX-TMP resistant infection in the patients who received SMX-TMP (74.1 vs 21.4%, p = 0.002). After multivariate adjustment for age, induction, and maintenance immunosuppression, lack of SMX-TMP was associated with a HR for sepsis of 3.8 (95% CI 2.1 – 6.6).



Conclusions: Prophylaxis with SMX-TMP prevents sepsis and UTIs in renal transplant recipients, but is associated with SMX-TMP resistance. Alternative UTI prophylaxis during the first three months after transplant should be considered in those intolerant of SMX-TMP.

PUB772

Immunosuppression and Outcomes for Expanded Criteria Donor (ECD) Kidney Transplants: Four-Year Data from the Mycophenolic Acid Observational Renal Transplant (MORE) Registry Kimi Ueda Stevenson,[1] Cataldo Doria,[2] A. Wiland,[3] Kevin M. Mccague,[3] V. Ram Peddi.[1] *[1]California Pacific Medical Center, CA; [2]Thomas Jefferson University Hospital, PA; [3]Novartis, NJ.*

Background: The literature indicates a higher rate of failure with ECD than standard criteria donor (SCD) grafts, with a demonstrable survival difference as early as one year post-transplant.

Methods: MORE is a prospective, observational registry of *de novo* kidney transplant recipients receiving mycophenolic acid (MPA) as mycophenolate mofetil (MMF) or enteric-coated mycophenolate sodium (EC-MPS), managed according to local practice. Data were analyzed for ECD vs SCD grafts. ECD was defined as donor ≥60 years, or 50-59 years with two of the following: hypertension, serum creatinine >1.5mg/dL, or cerebrovascular accident death.

Results: 103 ECD (29 MMF/74 EC-MPS) and 838 SCD (277 MMF/561 EC-MPS) patients were analyzed. ECD vs SCD transplants had older donors (mean 61 vs 39 years, p<0.01) and older recipients (62 vs 50 years, p=0.02). Other characteristics did not differ significantly. ECD and SCD groups were similar regarding use of induction (100% and 99%), tacrolimus (97% and 95%) and steroids (74% and 74%). Compared to the SCD group, fewer ECD patients received full MPA dose (2.0g MMF, 1.44g EC-MPS) at months 3, 24 and 36 (all p<0.03). At 4 years, biopsy-proven acute rejection (BPAR) (11% vs 13%, p=0.66) and all-cause graft survival (91 vs 94%, p=0.12) were similar for ECD vs SCD transplants; patient survival was 85% vs 96% (p<0.01). Efficacy was similar in both groups for MMF- vs EC-MPS-treated patients, except for patient survival in the SCD group (93% vs 97%, p=0.01). Mean (SD) serum creatinine was 1.9 (1.0)mg/dL vs 1.5 (1.3)mg/dL for ECD vs SCD patients, respectively (p=0.05). Reported adverse events were similar (i) between ECD and SCD groups except cardiovascular events (15% vs 7%, p=0.01) and (ii) for MMF vs EC-MPS groups except for infections among ECD patients (45% vs 22%, p=0.03).

Conclusions: In contrast to published data, graft survival was similar in recipients of ECD or SCD kidneys at four years post-transplant. Additionally, despite receiving less MPA, the incidence of BPAR was similar in ECD and SCD patients.

Funding: Pharmaceutical Company Support - Novartis

PUB773

Corticosteroid Withdrawal (CSW) Following Kidney Transplantation: 4-Year Data from the Mycophenolic Acid Observational Renal Transplant (MORE) Registry Kimi Ueda Stevenson,[1] A. Wiland,[2] Kevin M. Mccague,[2] V. Ram Peddi.[1] *[1]California Pacific Medical Center, CA; [2]Novartis, NJ.*

Background: CSW may reduce steroid-related complications but results are conflicting. Long-term evidence from real-life practice is sparse.

Methods: The MORE registry is a prospective, observational study of *de novo* adult kidney transplant patients receiving mycophenolic acid (MPA) at 40 US centers, managed according to local practice. Four-year data from tacrolimus-treated patients were analyzed. CSW was defined as steroid withdrawal by month 3.

Results: The CSW (n=363) and steroid continuation (CSC, n=509) groups were similar except more CSW patients had panel reactive antibodies <30% (90% vs CSC 77%, p<0.01) with a trend to more living donors (46% vs CSC 41%, p=0.09). More CSW vs CSC patients received induction with rabbit ATG (62% vs 59%, p=0.02) or alemtuzumab (24% vs 55%, p<0.01). Tacrolimus trough levels were similar. Fewer CSW patients received the full daily MPA dose (1.44g EC-MPS, 2.0g MMF) vs CSC patients at all timepoints (all p<0.01). In the CSC group, but not the CSW group, more patients were maintained on full MPA dose in year 1 with EC-MPS vs MMF (month 1 p=0.05; month 3, p=0.03; month 6, p=0.03; month 12, p=0.03). Biopsy-proven acute rejection was similar with CSW vs CSC (10% vs 14%, p=0.12) but graft survival was higher (97% vs 94%, p=0.03); patient survival was similar (96% vs 95%, p=0.65). Final mean serum creatinine was 1.6g/dL with CSW vs 1.5g/dL with CSC (p=0.38). Adverse events were similar except for neutropenia (CSW 17%, CSC 11%; p=0.01) and leukopenia (CSW 61%, CSC 30%; p<0.01), with a trend to fewer infections in the CSW group (25% vs CSC 31%, p=0.06).

Conclusions: In this real-life population of patients receiving tacrolimus and MPA, CSW patients received more aggressive induction therapy and were less likely to receive the full dose of MPA than CSC patients. Greater use of lymphocyte-depleting induction may have led to lower MPA tolerability and higher hematological toxicity in CSW patients. Despite a similar rate of BPAR, long-term graft survival was superior with CSW than CSC, possibly due to lower immunological risk and numerically more living donors.

Funding: Pharmaceutical Company Support - Novartis

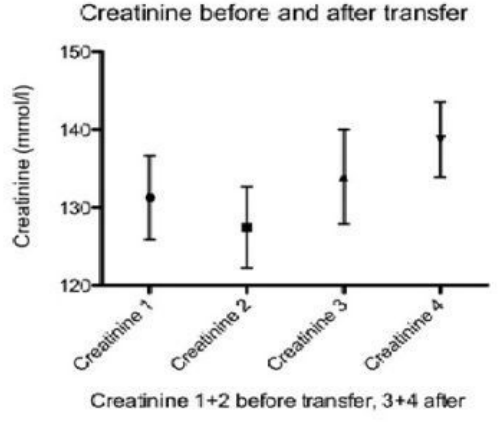
PUB774

Creatinine in Renal Transplant Recipients Following Transfer Back to Referring Centre Adarsh Babu. *Department of Nephrology, Gloucestershire Royal Hospitals NHS Trust, Gloucester, United Kingdom.*

Background: Renal transplant recipients are followed up at transplant centres for six months, before being transferred back to referral centres. In paediatrics transition to adult care is associated with an increase in creatinine, reduced compliance with immunosuppression and increased graft loss. There is limited data on changes in renal function following change of follow-up centre in adults prompting us to this study.

Methods: All patients who were transferred back to the referring centre following renal transplant in the time period between 2004 to 2010. Demographics, transplant and referral back dates were collected.Creatinine values were collected prior to and after transfer using the electronic patient records. Creatinine values were corrected to IDMS standardisation at both centres.Last two creatinine values prior to transferand first two creatinine results after transfer, were analysed using repeated measure ANOVA.

Results: 38 patients were transferred back to the referring centre during the period studied. We compared the last two creatinine values in transplanting centre prior to transfer (labelled as creatinine 1 and 2) and first two creatinine values post transfer (labelled as creatinine 3 and 4). The mean creatinine values were: creatinine 1 131.3mmol/l, creatinine 2 127.4mmol/l, creatinine 3 133.99mmol/l, creatinine 4 138.76mmol/l.



Repeated measure ANOVA shows a significant difference between the mean creatinine before and after transfer (P=0.0064).

Conclusions: The reasons for rise in creatinine are complex and not made clear in this study. It could be related to expertise, reduced intensity of follow-up and review. Non-compliance and psychological factors may also play a role. In our opinion, a useful next step would be replication of these results in a larger group of patients from multiple centres, and comparison with controls remaining at transplanting centres.

PUB775

Effects of Calcineurin Inhibitor Reduction/Withdrawal on Renal Allograft Function and Lipid Control: A Retrospective Analysis Husham Rasheed, Mark J. Andrews, Mahzuz Karim. *Renal Medicine, Norfolk and Norwich University Hospital, Norwich, Norfolk, United Kingdom.*

Background: Calcineurin inhibitors (CNI) have played a central role in immunosuppressive protocols. However, their long term use can contribute to progressive chronic allograft dysfunction and eventual graft failure. The aim was to establish whether CNI reduction or elimination had any effect on progressive graft dysfunction or lipid control in patients in a single centre experience.

Methods: All renal transplant patients in our centre who had undergone immunosuppressive drug substitution (to reduce ongoing CNI exposure) were retrospectively identified. For each patient the serum creatinine at the time of switch and time points closest to one year before and one year after were collated, and the rates of change of creatinine over time pre- and post-switch were compared, together with changes in requirements for lipid-lowering therapy.

Results: 39 patients were identified: 21 switched from CNI to sirolimus and 18 switched from azathioprine to MMF with reduction in CNI dose. Indications for switches were: biopsy-proven CNI toxicity (11/21 in sirolimus group, 6/18 in MMF group), progressive chronic allograft dysfunction without biopsy (8/21, 7/18), and other indications (2/21, 5/8). Adverse events included 1 patient in the MMF group and 5 patients who had to stop sirolimus (2 excluded from further analysis). In the sirolimus group the median rise in creatinine was 28 mmol/L/year pre-switch and -24.8 mmol/L/year post switch, with 17/19 patients showing a reduction in the rate of rise in creatinine; in the MMF group the median rise was 13.2 mmol/L/year pre-switch and -4.3 mmol/L/year post-switch, with 11/18 patients showing a reduction in the rate of rise. 7/19 patients in the sirolimus group required an increase in lipid-lowering therapy post-switch compared to 3/18 in the MMF group.

Conclusions: Outside the setting of a tight protocol of a clinical trial, reduction in long term CNI exposure in renal transplant patients selected on clinical and / or pathological grounds can have a significant beneficial effect on graft function. However, this may be at the expense of worsening hyperlipidaemia, particularly with the use of sirolimus.

PUB776

Urinary Monocyte Chemoattractant Protein-1 Levels and Their Relationship with Interstitial Alterations in the Renal Cortex and Loss of Renal Function in Post-Renal Transplantation Miguel Moyses-Neto, Elen A. Romao, Gyl E.B. Silva, Roberto S. Costa, Marcio Dantas, Silvio Tucci, Terezila Machado Coimbra. *Faculty of Medicine-USP, Ribeirao Preto, SP, Brazil.*

Background: Monocyte chemoattractant protein-1 (MCP-1) is a potent proinflammatory chemokine that has a strong chemotactic action on macrophages. The aim of this study was to determine the urinary MCP-1(uMCP-1) levels and their possible correlation with the relative cortical interstitial area (RCIA), macrophage renal infiltration and the outcome of the graft in post kidney transplantation patients.

Methods: Twenty two patients submitted to cadaveric transplantation were followed up for one year and had their renal function evaluated by plasma creatinine levels. A total of 55 biopsies were examined with a median of 2.5 for each patient. The glomeruli number of each biopsy was 14.3±9.0.

Results: Light microscopy showed morphological features characteristic of acute tubular necrosis in 22 biopsies from 9 patients and chronic nephropathy of the graft in 18 biopsies from 14 patients. The RCIA from renal cortex was 7.1% (6.4; 9.2) in the control and 37.1% (28.1; 43.7) in patients with kidney transplantation (p<0.0003).There was also a significant increase in uMCP-1 of transplanted patients (p<0.0001) compared to controls. Patients who presented in the first biopsy higher RCIA showed higher levels of plasma creatinine one year after transplantation (r=0.44; p<0.05). There was also a correlation between the RCIA and the number of macrophages in the interstitial area (r=0.49; p<0.0002). However, there was no correlation between uMCP-1 and creatinine plasma levels one year after the transplant (p=0.26). The number of macrophages per grid field of 0.245 mm^2 was higher [19.4 (9.0;47.1)] in the TI area from renal cortex of transplanted patients compared to control [2.5 (1.8;3.4)] (p<0.001) and correlated with the creatinine plasma level at one year after transplantation.

Conclusions: In conclusion, our results demonstrated increased levels of uMCP-1 in the transplanted patients and that macrophages interstitial infiltration and RCIA could predict the outcome of renal function in these patients.

Funding: Government Support - Non-U.S.

PUB777

Outcomes of Thrimethoprim-Sulphamethoxazole Use as Chemoprophylaxis Following an Outbreak of Pneumocystis Pneumonia in a Renal Transplant Population Nicos Mitsides,[1] Darren Green,[1] Rachel Middleton,[1] Kerry Greenan,[2] Elizabeth H. Lamerton,[1] Jude Allen,[1] Jane Redshaw,[1] Paul Chadwick,[1] Chinari Pradeep Kumar Subudhi,[1] Grahame Wood.[1] *[1]Salford Royal Hospital, Salford, United Kingdom; [2]University of Manchester, Manchester, United Kingdom.*

Background: An increase in Pneumocystis pneumonia (PJP) clusters has been reported in renal transplant recipients. In response to such an outbreak affecting 19 patients in our tertiary nephrology unit, all transplant patients were offered chemoprophylaxis, except for females of child-bearing potential. Trimethoprim-sulphamethoxazole (T-S) 480mg daily was used as first line agent but a high rate of complications was noted. The aim of this study was to quantify the adverse events associated with T-S, and whether the prophylactic benefit outweighed the complications.

Methods: This was an observational study of outcomes in 300 transplant recipients commenced on T-S prophylaxis in the 12 months from February 2011. End-points were adverse events due to the T-S, the additional medical burden as a result of these events, and diagnoses of PJP.

Results: Of 300 patients commenced on T-S, 121 (40%) developed compilations. Of these, the most common were: rise in serum creatinine >15% from baseline (79%), gastrointestinal symptoms (11%), leukopenia (6%). 24 (20%) complications led to hospital admission (median time in hospital 4 nights, range 1-13 nights). Because most patients with intolerance required unplanned medical review, a dedicated review clinic had to be set up. Many patients required extra investigations to rule out other causes of allograft dysfunction including renal ultrasound imaging (18%) and renal biopsy (6%). Few patients responded to desensitisation (3%) or dose reduction (5%). The remainder had to stop the T-S. Despite this PJP incidence fell. Prior to use of prophylaxis there were 19 cases in 19 months. During the study period there were 2 cases in 12 months.

Conclusions: Although use of chemoprophylaxis reduced the incidence of PJP we faced an unexpectedly high number of intolerant patients. The fact that this was mainly in the form of raised serum creatinine caused significant anxiety and let to a significant increase in investigations.

PUB778

Risk Factors and Outcomes of Asymptomatic Bacteriuria in the First Year after Renal Transplantation Syed Hassan,[1] Fatima Khalid,[1] Sarim Rashid,[1] Hasan Zahid,[1] Chetan Mittal,[1] Anita K. Patel,[2] Mayur S. Ramesh,[3] George J. Alangaden.[3] *[1]Internal Medicine, Henry Ford Hospital; [2]Nephrology, Henry Ford Hospital; [3]Infectious Disease, Henry Ford Hospital.*

Background: The optimal management of asymptomatic bacteriuria (ASB) in renal transplant (RT) patients is poorly defined. We examined the risk factors and outcomes of ASB in 436 RT patients in the first year after RT.

Methods: All patients who underwent RT at Henry Ford Hospital,detroit from 2008-2011 were enrolled in this retrospective study. A total of 490 patients underwent RT,436 had complete follow up data for 1 yr and were included in the analysis. Patient demographics, clinical and laboratory data were collected. ASB was defined as: isolation of $\geq 10^5$ cfu/ml of bacteria in an appropriately collected urine specimen in the absence of symptoms or signs of urinary infection (UTI). UTI was defined as: presence of signs and symptoms of infection with $\geq 10^5$ cfu/ml of bacteria. Results were computed using univariate and multiple logisitic regression analysis.

Results: 102/436 (23%) of patients developed ASB in the first year post-RT. Median time to ASB was 78 days.Significant factors associated with ASB were female gender, UTI prior to RT, prior hospitalization less than 30 days of ASB (all p value <0.001),and CMV seropositive recipient (p =0.019). On multivariate logistic regression, female gender OR 2.2 (CI 1.8-2.6),CMV seropositive recipient OR 2.32 (CI 1.22-4.64), prior hospitalization OR 6.0 (CI 3.22-11.51) were independent risk factors for ASB. Of the 102 RT patients with ASB, 93 (91%) were treated with antibiotics, in those patients 65 (70%) had recurrent ASB and 8 (9%) developed UTI during follow up. Out of the 9 patients who were not treated, 3 cleared ASB, 4 had recurrence of ASB and 2 developed UTI. 1-year graft and patient survival in patients with ASB was 100% and 98.1% respectively.

Conclusions: ASB occurs in about a quarter of RT patients.Female gender, CMV seropositivity, and prior hospitalization appear to be significant risk factors for ASB. Antibiotic therapy of ASB does not appear to significantly impact outcomes. Prospective studies on the natural history and optimal therapy of ASB in the RT patients are warranted.

PUB779

Evaluation of Irbesartan and Losartan on Renal Protection in Renal Transplant Recipients (I-LORD Study) Hitoshi Yokoyama, Hideki Yamaya, Hiroshi Okuyama. *Division of Nephrology, Kanazawa Medical University School of Medicine, Uchinada-Kanazawa, Ishikawa, Japan.*

Background: The beneficial effect of angiotensin II receptor blockers (ARB), especially the differential effects of its subclasses of drugs, in renal transplant recipients is not yet well established. Our objective was to investigate the impact of the use of irbesartan and losartan on graft function and urinary surrogate markers in a single center cohort.

Methods: Sixty-seven Japanese recipients with hypertension were enrolled in this study. Patients were randomly divided into two groups based on demographic factors and were given either irbesartan (Group I, n=35) or losartan (Group L, n=32). During the study period, the dosage was adjusted to control blood pressure levels (BP, <=130/80 mmHg). Physiological and biochemical parameters including urinary albumin and MCP-1 were measured before and after the 12-month study period.

Results: We successfully continued administration of ARB on 28 patients of Group I (80%) and 32 patients of Group L (86.4%) over the one-year study period Mean BP was significantly decreased from 94.69 to 91.24 mmHg in Group I and from 96.60 to 91.25mmHg in Group L (p=0.047 and p=0.003 by paired t-test, respectively). Changes inestimated glomerular filtration rate (ΔeGFR)were observed in both groups (-2.8 and -1.7 mL/min/1.73m^2/year, respectively). No significant differences in albuminuria, serum creatinine, and urinary MCP-1 levels among the groups were detected. In multivariate regression analysis, urinary albumin and MCP-1 levels were correlated with ΔeGFR (beta index, -0.358, p=0.007; -0.283, p=0.03, respectively). Higher urinary albumin levels and body mass index before study, and decreased BP during study, were selected as predictive factors for the reduction of albuminuria by ARB (beta index, -0.542, p=0.001; -0.235, p=0.024; 0.289, p=0.004, respectively). Improvement of urinary MCP-1 level by ARB was positively correlated with the changes in albuminuria (beta index, 0.251, p=0.047).

Conclusions: Both irbesartan and losartan improved BP in Japanese renal transplant recipients with hypertension. However, no significant differences in ΔeGFR, urinary albumin and MCP-1 levels were detected between the groups.

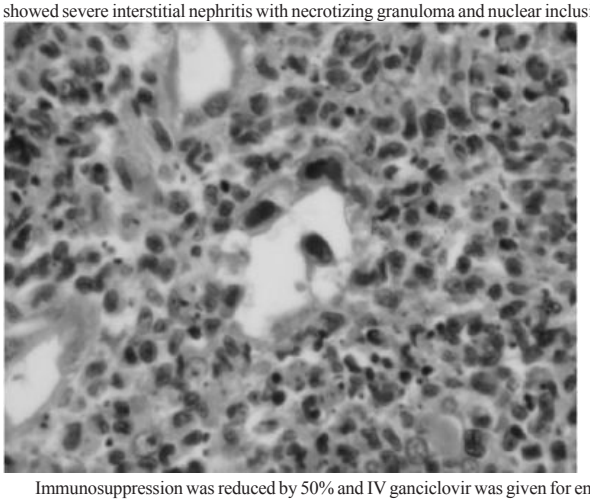
Funding: Private Foundation Support, Government Support - Non-U.S.

PUB780

Severe Necrotizing Adenovirus Nephritis in a Renal Transplant Recipient: A Case Report and Literature Review Steven Schmidt,[1] Ping L. Zhang,[2] Dilip Samarapungavan,[2] Gampala Harish Reddy,[2] Francis Dumler,[2] Leslie L. Rocher,[2] Alan Koffron,[2] Raviprasenna K. Parasuraman.[2] *[1]Internal Medicine, William Beaumont Hospital, Royal Oak, MI; [2]Division of Transplant, William Beaumont Hospital, Royal Oak, MI.*

Background: Viral pathogens remain one of the principal causes of mobidity and mortality in transplant recipients. Vigilance for emerging pathogens is important in the modern era of potent immunosuppression. We present a case of severe adenovirus (ADV) necrotizing granulomatous tubulo-interstitial nephritis.

Methods: A 44-year-old male with ESRD secondary to hypertension underwent a deceased donor renal transplantation with basiliximab for induction and tacrolimus, mycophenalate mofetil and prednisone for maintenance therapy. Post transplant he developed slow graft function with a serum creatinine (SCr) level decreasing to 2.3 mg/dL on day 19. SCr increased to 2.8 mg/dL on day 22 resulting in an allograft biopsy which showed severe interstitial nephritis with necrotizing granuloma and nuclear inclusion bodies.



Immunosuppression was reduced by 50% and IV ganciclovir was given for empiric viral nephritis pending further evaluation. Subsequently, the patient developed fever, hematuria and mild interstitial pneumonitis. Results of urine and serum quantitative PCR for ADV returned with >2,000,000 and 649,000 copies/mL respectively. Subsequently the patient received 3 doses of 240 mg of cidofovir every other week in addition to IVIG 500 mg/kg. The patient had an excellent response with complete clinical and virological resolution. SCr decreased from 2.85 mg/dL to 1.54 mg/dL 3 weeks after initiation of therapy.

Conclusions: ADV induced tubulo-interstitial nephritis is extremely rare with approximately 20 reported cases in the literature. Our case highlights the need for vigilance of emerging infections such as ADV in renal allograft recipients.

PUB781

BK-Viremia and Polyomavirus Nephropathy (PyVAN) Following Kidney Transplantation: Single Center Experience in 352 Transplant Recipients

Johannes Jacobi,[1] Antonina Prignitz,[2] Alexander Weidemann,[1] Klaus Korn,[3] Maike Julia Buettner,[4] Karl F. Hilgers,[1] Kai-Uwe Eckardt,[1] Kerstin U. Amann.[4] *[1]Nephrology & Hypertension, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany; [2]Gemeinschaftspraxis Nuremberg, Nuremberg, Bavaria, Germany; [3]Clinical & Molecular Virology, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany; [4]Nephropathology, University of Erlangen-Nuremberg, Erlangen, Bavaria, Germany.*

Background: To evaluate the incidence and outcome of BK-viremia and PyVAN in n=352 kidney transplants performed between 2008-2011.

Methods: Retrospective analysis of all kidney transplants performed between 2008-2011. Screening for BK-viremia was recommended 3,6,9 and 12 months post transplantation. Protocol biopsies were offered at 3 and 12 months, in addition all patients with BK-viremia underwent transplant biopsies. In patients with PyVAN switch of immunosuppression to mTORi + low dose cyclosporine (CyA) was implemented whenever possible.

Results: The overall incidence of BK-viremia was 12.8%. Transplant recipients >65 years of age (ESP) showed a significantly higher incidence (26.8%). In logistic regression analyses the following risk factors for BK-viremia were determined: ESP-recipient, recipient age, BPAR, previous transplantation, mode of renal replacement therapy, recipient CMV-status (IgG+) and 25-hydroxyvitamin D3 levels. In two-third of the patients viremia occurred between 2-6 months following transplantation. A second peak was noted one year after transplantation in patients with missed screening intervals. In 21 patients (46.7%) BK-viremia was associated with biopsy proven PyVAN. Initial and peak viral load were 1-log scale higher in patients with PyVAN. While renal function remained stable in patients without PyVAN, it significantly deteriorated in patients with PyVAN. Switch of immunosuppression to mTORi + low dose CyA was safe and well tolerated.

Conclusions: Our data highlight the importance of screening for BK-viremia, especially in aged transplant recipients. Without specific antiviral strategies the mainstay of therapy remains reduction of immunosuppression. In patients with PyVAN combination therapy with mTORi + low dose CyA appears to be safe and well tolerated.

Funding: Pharmaceutical Company Support - Novartis Pharma

PUB782

The Early Reduction of Mycophenolate Mofetil Is Safe in Kidney Transplant Recipients Who Need Desensitization by Rituximab

Ja Seon Kim,[1] Won Seok Yang,[1] Duck Jong Han,[2] Su-Kil Park.[1] *[1]Division of Nephrology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine; [2]Department of Surgery, Asan Medical Center.*

Background: For ABO incompatible (ABOi) and/or T cell flow cytometry crossmatch positive (TFCP) kidney transplantation (KT), desensitization is necessary for successful outcome of transplantation under the therapy with rituximab, plasmapheresis, or IVIG. Some people reported increased risk of infection, and others reported no increased risk of infection after rituximab therapy. Our study is aimed to prospectively evaluate the outcome of 1.0g use of mycophenolate mofetil (MMF) in kidney recipients who need desensitization by rituximab at postoperative 1 week.

Methods: We evaluated the outcome of 28 patients who underwent HLA-sensitized or ABOi living donor KT after one dose of rituximab (200mg ABOi, 500mg TFCP) (group 1). Fifty-three living kidney transplant recipients who did not require rituximab served as a control (group 2). The desensitization was performed by plasmapheresis and ritixumab ± IVIG. Maintenance immunosuppression was tacrolimus, MMF (1.5g/day) and methylprednisolone, which was initiated 7 days (group 1) or 3 days (group 2) prior to operation. Basiliximab was administered in both. MMF was reduced to 1.0g/day from 7 days after surgery in group 1.

Results: There was no difference in demographic data. Postoperative infections occurred in 6 patients (21.4 %) of group 1 and 11 patients (20.8 %) of group 2 during the follow-up period (4.3±1.2 mo), which was not different (p=0.579). Renal function was same during follow-up; serum creatinine (mg/dl): 1mo, 1.06±0.32 vs.1.22±0.47, p=0.368; 3 mo, 1.12±0.30 vs. 1.21±0.32, p=0.198. Acute rejection developed in 2 patients (7.1%) of group 1 and 4 patients (7.5%) of group 2, which was not different (p=0.659). The dose of MMF (g/day) at the following times postoperatively was lower in group 1 than group 2: 7 days, 1.02±0.09 vs. 1.46±0.45, p<0.001; 1mo, 0.98±0.09 vs. 1.46±0.45, p<0.001; 3mo, 1.00±0.22 vs. 1.24±0.35, p=0.001.

Conclusions: The early reduction of MMF regimen was safe and might be acceptable in rituximab-treated kidney transplant recipients.

PUB783

Factors Affecting eGFR 5 Year Post Deceased Donor Renal Transplant: Analysis and Predictive Model

Abdalla Elbadri, John T. Veitch, Carol A. Traynor, Patrick O'Kelly, Colm Magee, Mark D. Denton, Peter J. Conlon. *Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: Long-term survival of renal allografts has improved consistently over the last twenty years. However, less is known about factors which accurately predict long-term allograft function as determined by eGFR. The aim of this study was to investigate factors which affect allograft function (eGFR) at 5 years after renal transplantation. The statistically relevant factors were then used to construct a predictive model for expected eGFR at five years post transplant.

Methods: We retrospectively reviewed all adult patients who received a renal transplant in Beaumont Hospital between 1990 and 2004 (Beaumont Hospital is the national renal transplant centre for the Republic of Ireland).Data collected included era of transplantation (1990-1994, 1995-1999, 2000-2004), donor and recipient age and gender, number of HLA mismatches, cold ischaemic time, number of prior renal transplants, immunosuppressive regimen used and acute rejection episodes. eGFR was calculated at 5 years after transplantation using the MDRD equation. This population was then divided into two equal unbiased groups of 489 patients, the first group (hypothesis cohort) being used to construct a predictive model for eGFR five years post transplantation, the second (validation cohort) to test this model.

Results: Nine hundred and seventy eight patients were analysed. The mean age at transplantation was 42.7 years; 620 (63.4%) were male. Factors found to have a statistically relevant (P value <0.05) effect on eGFR in the hypothesis cohort at 5 years were donor age, recipient sex, CMV disease, acute rejection and exposure to tacrolimus. The derived equation was then tested in the validation cohort with results in the Table below.

table:1

Median eGFR after 5 years	Era 1 (1990-1994)	Era 2 (1995-1999)	Era 3 (2000 - 2004)
Predicted	45.1	44.7	50.6
Actual	42.6	47.4	55.3

Conclusions: The predictive model we have developed shows good correllation between predicted and actual median eGFR at five years. Applications of this model include comparison of current and future therapy options.

PUB784

Ureteral Stent Placement and the Risk for Development BK Viruria and Viremia in Kidney Transplant Recipients

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Background: Polyomavirus (BKV) infection is one of the causes of allograft dysfunction following kidney transplant. Placement of a ureteral stent (US) at time of surgery was associated with 4-fold increase in the risk for developing BKV nephropathy (BKVN).

Methods: Retrospective secondary data base analysis of all kidney transplants that performed from July 1, 2007 and December 31, 2010 at the University of Florida with at least 6 months follow up.

Results: From 640 recipients, 621 were eligible for the study. BK viruria was seen in 137/529 (26%) and BK viremia 115/606 (19%). A ureteral stent (US) was placed in 295 (46%) of recipients. BK viruria was seen in 75/250 (30%) of stented patients. In contrast, only 62/279 (22%) of the recipients with BK viruria were without US, p = 0.04. BK viremia was seen in 63/289 (22 %) if stented versus 52/317(16%) without stent, p = 0.05. Patient age, gender, ethnicity and type of induction therapy were not statistically significant factors in the development of BK infection. However BK viremia was significant in recipients with immediate graft function. In a multivariate logistic regression model that included an interaction term between graft function and US placement, graft function fell out and only US placement remained significant for BK viruria (p = 0.03, odds 1.83 with 95% CI 1.29–2.61) and BK viremia (P=0.04, odds 1.65, CI 1.23-1.74).

Univariate and Multivariate analyses

BK virus status	Stent 295 (46%)	No Stent 326 (54%)	P Value
BK Viruria 26 %	30%	22%	0.04
BK Viremia 19%	22%	16%	0.05
Predictor Variable	BKVU P value	BKVM P value	
Stent	0.03	0.04	
Age Group	0.18	0.11	
Gender	0.49	0.43	
Cold Ischemia	0.41	0.42	
Induction Medication	0.91	0.82	
Graft Function	0.41	0.76	

Conclusions: Ureteral stent placement significantly increased the risk for BK viruria in univariate model and both BK viruria and viremia in multivariate model. Future studies should asses the risks and benefits of US placement, including consideration of the cost of BK testing as well as the potential for a nephritis that can affect the long time graft survival.

PUB785

Switching Study of Kidney Transplant Patients with Tremor to LCP-Tacro (STRATO): An Exploratory Study

Anthony J. Langone,[1] Christine Culkin,[2] John C. Morgan,[3] Vincenza Nigro.[4] *[1]Vanderbilt University, TN; [2]Hahnemann University Hospital, PA; [3]Georgia Health Sciences University, GA; [4]Veloxis Pharmaceuticals, NJ.*

Background: Neurotoxicity occurs in up to 50% of renal transplant recipients (RTR). Tremor is the most common manifestation of neurotoxicity. Tacrolimus (TAC) is implicated as the cause of tremor with 2 hour (peak) serum concentrations correlated with toxicity. Reduction or cessation of TAC may reduce tremor symptoms but increases the risk for rejection, especially in patients that are symptomatic despite goal TAC serum concentrations.

Methods: This study is designed to determine if conversion of RTR who have tremor with a standard TAC formulation to LCP-Tacro (extended-release once-daily MeltDose formulation, Veloxis Pharma) leads to measurable improvement in tremor. The LCP-Tacro formulation has smaller and more homogenous molecular components that result in consistent and predictable absorption across the gastrointestinal tract. Peak concentration is reduced but total AUC exposure is comparable to standard dose TAC. We hypothesize that a reduction in peak concentration with preservation of AUC will ameliorate symptoms while maintaining clinical efficacy. The Fahn-Tolosa-Marin (FTM) tremor rating scale is used

to enroll and assess RTR with TAC-related tremors. Eligible subjects have TAC-induced moderate to severe hand tremor and ≥1 complaint of tremor with postural or action tremor on finger-to-nose. Subjects remain on their pre-enrollment Prograf/generic TAC twice-daily treatment for the first week of the study before switching to LCP-Tacro once-daily for a week. The primary analysis is mean change of FTM score from Day 7 to Day 14. The FTM scale is administered 2 hours postdose and evaluated by independent blinded neurologists. Additional endpoints include tremorometer, quality of life, and safety.

Conclusions: We believe this is the first trial in RTR to utilize a sophisticated and reproducible measurement of tremor. This study will determine if RTR who currently experience a minimal threshold of tremor by FTM measurements have a significant reduction in symptoms after conversion to a novel (LCP-Tacro) formulation while maintaining comparable drug exposure.

Funding: Pharmaceutical Company Support - Veloxis Pharmaceuticals

PUB786

A Single Center UK Experience of Pregnancy in Women with Kidney Transplant Maharajan Raman, Arvind Ponnusamy, Daniel J. Hall, Hayley L. Mcmanus, Teresa Kelly, Philip A. Kalra, David I. New. *Renal, Salford Royal NHS Trust Foundation, Salfoird, United Kingdom.*

Background: Renal transplantation improves fertility. Studies report that pregnancy is common in 12 % of women of childbearing age.

Methods: We undertook a retrospective review of pregnancy in women with renal transplant from 2007 to 2011. All patients are cared in a multidisciplinary team involving comprising nephrologists, obstetricians, midwives, dietician and transplant nurses from the antenatal period to delivery and beyond.

Results: There were 5 patients with confirmed pregnancy during this period. Average age of patients were 28.4 years. 3 had live donor and 2 had cadaveric transplants. Average time to pregnancy post transplantation was 24 months (range (25-36) and Two patients were on ACE and ARB at time conception which was subsequently stopped. Average creatinine was 118 ± 30 and proteinuria was 0.28 gm (range 0.6-0.88).

	Medication	Renal function(Pre-Pregancy	Renal function(3 months Post pregnancy)	Outcome
1	Tac/Aza	126	102	Miscarriage at 17 weeks
2	Ciclo/Aza	165	155	Birth at 33 weeks
3	Tac/MMF	85	99	Birth at 38 weeks
4	Tac	106	99	Birth at 38 weeks
5	Tac/Pred	108	88	Birth at 33 weeks

Average tacrolimus level was 1st trimester was 6.7, 2nd was 5.1 and 3rd was 3.9. MMF was stopped and replaced with azathioprine. Average booking BP was 131± 10.1 and 3rd trimester BP was 128 ± 11. There was no statistically significant reduction in creatinine in at 3 months post pregnancy. Average proteinuria was post 3 months was 0.55 gms. Although, one patient had miscarriage at 17 weeks but she is pregnant again. Average birth weight was 2854 gm.

Conclusions: Pregnancy in transplants is associated better foetal and maternal outcome due to careful monitoring of BP, urine protein, renal function and drug therapy.

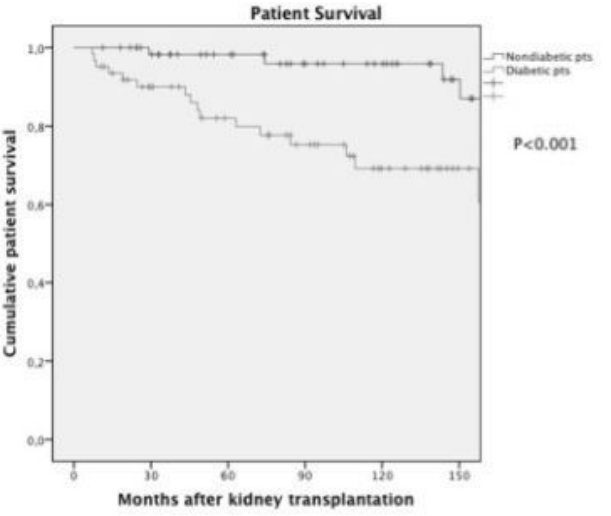
PUB787

Kidney Transplantation in Type 2 Diabetic Patients: A Matched Survival Analysis Ana Rocha, Jorge Malheiro, Isabel Fonseca, La Salete Martins, Antonio Andresen Henriques. *Centro Hospitalar do Porto.*

Background: Diabetes mellitus (DM) is the most prevalent cause of kidney failure. Some concerns have been raised about the kidney transplantation (KT) results in diabetic patients. Therefore we compared outcomes between diabetic and non-diabetic patients after KT.

Methods: All kidney transplants performed in type 2 diabetic patients, from July 1983 to December 2009 in our centre, with a graft survival over 3 months, were included. Non-diabetic controls were individually matched with diabetic patients with respect to gender, age, year of transplantation, number of donor HLA mismatches and dialysis vintage. The two groups were compared concerning patient and graft survival, delayed graft function (DGF) and prevalence of acute rejection (AR).

Results: We included 62 type 2 diabetics and 62 non-diabetic patients who were followed for a mean period of 102±64 months after KT. Diabetic patients and controls were similar for the matched variables. Graft survival censored for patient death for diabetics and non-diabetics was 70 and 83% at 5 years and 54 and 71% at 10 years, respectively (log rank test p=0.13). Patient survival at 5 and 10 years was 69 and 50% for diabetic patients and 96 and 84% for non-diabetic patients, respectively (log rank test p<0.001).



The prevalence of AR was 24.2% in diabetic and 17.7% in non-diabetic patients (X^2 test p=0.38). Occurrence of DGF did not differ (X^2 test p=0.12). Using multivariate Cox's proportional hazards analysis, DM (HR=7.72; p=0.001) and hepatitis (HR=4.18; p=0.02) correlated with reduced patient survival.

Conclusions: Diabetic patients' survival after KT was reduced when compared with non-diabetic matched patients. However, censored graft failure was similar between the two groups. Concerns about graft survival should not prevent KT in diabetic patients with kidney failure.

PUB788

Changes of Serum Bilirubin after Kidney Transplantation and UGT1A1*28 Polymorphism Affect the Graft Survival in Kidney Transplantation Do Hyoung Kim,[1] Jung Pyo Lee,[2,3] Seung Hee Yang,[3] Jeonghwan Lee,[1] Yun Jung Oh,[1] Ho Jun Chin,[4] Dong Ki Kim,[1] Yun Kyu Oh,[2] Chun Soo Lim,[2] Yon Su Kim.[1,3] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Seoul National University Boramaen Medical Center, Seoul, Korea; [3]Kidney Research Institute, Seoul National University College of Medicine, Seoul, Korea; [4]Department of Internal Medicine, Seoul National University Bundang Hospital, Seong Nam, Korea.*

Background: In kidney transplantation patients, oxidative stress is a major mediator of adverse outcome including acute rejection, and is balanced by various anti-oxidants such as bilirubin. Bilirubin degradation is mainly determined by the activity of hepatic bilirubin uridine diphosphate-glucuronosyltransferase (UGT1A1), which is significantly influenced by a TA-repeat polymorphism in the gene's promoter, an allele designated UGT1A1*28. In this study, we evaluated the role of bilirubin in kidney transplantation patients, and the relationship between serum bilirubin levels and UGT1A1*28 polymorphism related with bilirubin degradation.

Methods: We collected clinical data from 229 Korean kidney transplantation patients from 1999 to 2008. Serum bilirubin levels and biopsy proven acute rejection were traced. Genotyping of the UGT1A1 TA-repeat polymorphism was performed. The endpoints were acute rejection and graft survival.

Results: The UGT1A1*28 genotype frequencies were 79.0%, 18.8%, 1.3%, and 0.9% for 6/6, 6/7, 7/7, and 6/8 for kidney transplantation patients. Post-transplant 1 year bilirubin levels of the 6/6 genotypes were significantly lower than those of the 6/7, 7/7, and 6/8 genotypes (0.73±0.29 versus 0.91±0.50 mg/dL, P=0.043). In a Kaplan-Meier model, the 6/6 genotypes showed poor graft survival compared to the 6/7, 7/7, and 6/8 genotypes (P=0.039). The acute rejection incidence in the 6/6 genotypes was higher compared to the 6/7, 7/7, and 6/8 genotypes (19.9% versus 8.3%, P=0.085).

Conclusions: Elevation of serum bilirubin after kidney transplantation was associated with UGT1A1*28 polymorphism. Lower serum bilirubin level and the 6/6 UGT1A1*28 genotype might have a negative effect on graft survival.

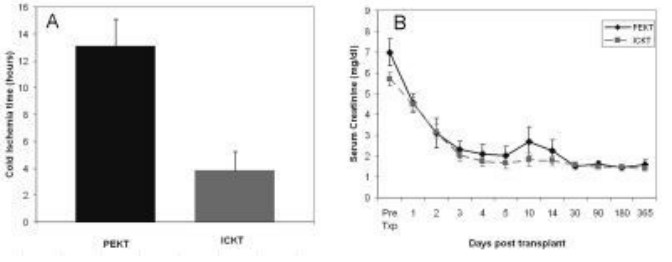
PUB789

Paired Exchange versus In-Center Living Donor Kidney Transplantation: A Single Center Experience Arpit Bhargava, Swati Arora, Richard J. Marcus, Kalathil K. Sureshkumar. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: Paired exchange kidney transplantation (PEKT) is one of the newer strategies aimed at expanding the living donor pool. Recent practices involve transporting the kidneys (with resultant increase in cold ischemia time [CIT]) rather than have the donors travel. It would be interesting to know the outcomes of PEKT vs. the traditional in-center live-donor kidney transplants (ICKT).

Methods: Retrospective chart review of adult patients who undewent PEKT and ICKT from 01/2009 to 02/2012 at our institution was done. CIT, delayed graft function (DGF) incidence, rates of acute rejection (AR), trend of serum creatinine (S.Cr), proteinuria, patient and graft survival were compared between the groups.

Results: The demographics of PEKT (n=15) vs. ICKT (n=30) were similar: recipient age 51±15 vs.52±10 years; donor age 46±13 vs. 43±12 years, pre-transplant dialysis duration 23±15 vs. 17±14 months and HLA mismatch 4±2 vs. 4±2. CIT was 13.1 hours in PEKT group and 3.8 hours in ICKT group ((p <0.001). (Fig 1A). None of the patients in either group developed DGF. At a median follow- up of 12.4 months (range 2-27.5 months), graft and patient survival rates were 100% in both groups. Serial S.Cr levels were similar between the groups (Fig 1B). AR rates (3/15 vs. 3/30, p=0.35) and prevelance of proteinuria post-transplantation (8/15 vs. 22/30, p=0.18) did not show any significant differences for PEKT vs. ICKT groups.



Conclusions: Our study showed similar allograft function, AR as well as graft and patient survivals between PEKT and ICKT groups despite involving organ transportation and higher CIT in the PEKT group. These findings support the current practice of PEKT, which should be encouraged in patients with incompatible living donors in order to increase the chances of a successful living donor kidney transplantation.

PUB790

Urinary Tract Infections during First Year after Cadaveric Renal Transplantation Justyna E. Golebiewska, Alicja Debska-Slizien, Boleslaw Rutkowski. *Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland.*

Background: Urinary tract infections (UTIs) are most common infections in renal transplant recipients and are considered a potential risk factor for poorer graft outcomes. There are conflicting data on risk factors and the influence of UTIs on long-term kidney allograft function in RTx recipients.

Methods: We analyzed urine cultures performed within first 12 months after RTx with reference to clinical data of patients who received a cadaveric RTx at Gdansk Transplantation Centre between January 2007 and December 2009. Renal function assessed by creatinine concentration and eGFR was recorded 24 months after RTx.

Results: We studied 206 cadaveric renal transplant recipients, including 59% of male gender, with mean age of 46,6±14 years. We observed 320 episodes in 109 patients, consisting of asymptomatic bacteriuria (53%, n=169), lower UTIs (25%, n=81) and upper UTIs (22%, n=57) including 13 cases of bacteremia. Over 38% of UTIs were diagnosed during the first month posttransplant and the most frequently isolated uropathogen was *Enterococcus faecium* (36%, n=44). Beginning from the second month *Escherichia coli* predominated (57%, n=113). Risk factors for posttransplant UTIs were: female gender, induction, a history of acute rejection (AR) and/or cytomegalovirus (CMV) infection, recurrent UTIs before RTx, comorbidity measured by Charlson Comorbidity Index. All patients with confirmed vesico-ureteral reflux or strictures (n=19) suffered from recurrent UTIs. eGFRwas significantly worse in patients suffering from UTIs from baseline. However the evolution of renal graft function did not differ significantly between patients with and without UTIs.

Conclusions: UTIs are a frequent problem after RTx, with asymptomatic bacteriuria as most common form. *Escherichia coli* and *Enterococcus faecium* are predominant pathogens. Exposition to greater immunosuppression due to induction, episodes of AR or as a result of CMV infection is a risk factor for UTIs. Recurrent UTIs may be considered either an effect or a marker of underlying dysfunction of urinary tract or comorbid conditions. UTIs do not impair graft function in 24month observation.

PUB791

Prevalence and Predictor Factors of Graft Dysfunction (GD) a Year Postransplant Gerardo Gilberto Azúa Díaz,[1] Jorge Andrade-Sierra.[1] *[1]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico; [2]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico; [3]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico.*

Background: In renal transplant (RT) minimal changes in serum creatinine (SCr) since 6 months predicts worse graft outcomes, an increase of 0.5 to 1.0 mg/dl from 6-12 months increase the risk of GD 2.26 times. On the other hand SCr> 1.5 mg/dl, at one year of RT predict graft loss at 3 years in almost 20%. To determine the prevalence of GD at 1 yr post-RT and associated factors in a RT Mexican population.

Methods: This is a retrospective cohort study performed between Jan-Dec/2010 in 209 RT receptors; GD was defined as SCr≥1.5mg/dl at 1 year.

Results: Main results are show in table.

Comparison of variables in patients with and without graft dysfunction.

Variables	GD (≥8805;1.5 mg/dl)	No-GD (< 1.5 mg/dl)	p value
Receptor age (years)	28.7 ± 13.8	27.2 ± 10.7	0.44
Donor age (years)	40.0 ± 11.5	35.2 ± 10.4	0.01
Time on dyalisis (months)	24.2 ± 18.6	27.9 ± 25.9	0.29
HLA antigens matched N(%)	3.1 ± 1.2	2.8 ± 1.7	0.20
Cold ischemia (min)	70 ± 154	113 ± 232	0.15
Immunosupression (%)	98%	99%	ns
Acute Rejection (%)	64%	25%	<0.0001
Baseline Creatinine	1.30 ± 0.30	1.00 ± 0.26	<0.0001
7 days creatinine	1.30 ± 0.34	1.07 ± 0.43	0.002
1 m creatinine	1.34 ± 0.47	1.04 ± 0.33	<0.0001
3 m creatinine	1.35 ± 0.45	1.07 ± 0.27	<0.0001
6 m creatinine	1.64 ± 0.95	1.07 ± 0.27	<0.0001
9 m creatinine	2.96 ± 2.67	1.04 ± 0.21	<0.0001
12 m creatinine	3.05 ± 2.6	1.04 ± 0.21	<0.0001

Conclusions: Donor age, baseline and 6 month serum creatinine significanty predicts GD at 1 year. (X^2 62.4; p < 0.0001).

PUB792

Body Mass Index of Donor or Recipient Are Not Associated with Delayed Graft Function in Deceased Donor Kidney Transplantation Mahendra V. Govani, Alvin Wee, Islam A. Ghoneim. *Kidney Transplantation, St. Vincent Hospital, Indianapolis, IN.*

Background: Recent reports suggest that high donor and recipient body mass index (BMI) are significantly associated with increased incidence of delayed graft function (DGF), defined as need of dialysis within a week of kidney transplantation. We conducted a retrospective review of our deceased donor (DD) kidney transplant records to confirm this association.

Methods: Records of all 112 DD kidney transplants performed at our center between 1/1/2009 and 4/30/2009 were reviewed for parameters including DGF, demographics and BMI of donor and recipient, SCD/ECD/DCD status, cold ischemia time (CIT), diabetes, PRA, retransplant status, patient and graft survival. and serum creatinine. Four patients were excluded from analysis: 1 with simultaneous heart and kidney transplant, 1 with vascular thrombosis, 1 with primary non-function, and 1 patient who died within a few days of transplant due to presumed pulmonary embolis with normal graft function. Median follow-up at 5/31/2012 was 18.9±11.4 (range 1.4-41.3) months.

Demographics and Outcomes

Variable	N=108
Age in yrs, mean ± SD	55.7 ± 13.4
Male, n (%)	72 (66.7)
AA, n (%)	44 (40.7)
SCD/ECD/DCD	60/23/26*
Recipient BMI, mean ± SD	30.7 ± 6.2
Donor BMI, mean ± SD	28.8 ± 6.3
CIT, hrs mean ± SD	13.7 ± 5.0
PS, n (%)	106 (98.1)
GS, n (%)	103 (95.4)
DGF, n (%)	6 (5.6)
ARR, n (%)	13 (12)

*1 DCD donor was ECD as well.

Thymoglobulin induction was used at standard dosage in all patients, and tacrolimus was introduced after renal function was well established. Mycophenolate and prednisone were used as adjuvant agents.

Results: Six of 108 (5.6%) patients developed DGF. Patient and graft survival (PS & GS) at 1 year (71 patients) were 100% and 99.1% respectively. Overall, PS and GS were 98.1% and 95.4% at follow-up; both patients who died had functioning grafts leading to actual GS 97.2%. Acute rejection rate (ARR) was 12%. No significant association was found between DGF and Donor BMI (p=0.86) or between DGF and recipient BMI (p=0.38).

Conclusions: DGF was not associated with donor or recipient BMI. However, DGF rate in our study was low reducing power of our study. Further studies are necessary.

Funding: Clinical Revenue Support

PUB793

Successful Withdrawal of Antiviral Treatment in Kidney Transplant Recipients with Chronic Hepatitis B Virus Infection Jang-Hee Cho,[1,2] Jun-Seop Kim,[1,2] Yunjeong Kang,[1,2] Ji-Young Choi,[1,2] Sun-Hee Park,[1,2] Yong-Lim Kim,[1,2] Chan-Duck Kim.[1,2] *[1]Kyungpook National University Hospital, Republic of Korea; [2]CRC for ESRD in Korea.*

Background: The optimal duration of antiviral therapy for kidney transplant recipients (KTRs) with chronic hepatitis B virus (HBV) infection remains unclear. We examined the results of withdrawals of antiviral treatment and the factors associated with successful withdrawal in KTRs with chronic HBV infection.

Methods: We investigated the hepatitis B surface antigen (HBsAg)-positive KTRs with antiviral agents between January 2000 and January 2012. Antiviral treatments were withdrawn in patients who met all of the following criteria: (1) normal liver biochemistry, (2) negative for both HBV DNA and hepatitis B e antigen (HBeAg), (3) immunosuppressants at maintenance dosage for more than 3 months, (4) duration of antiviral therapy at least 9 months, and (5) no resistance to antiviral agent.

Results: Fourteen HBsAg-positive patients among a total of 424 KTRs were included in this study (mean follow up period: 4.0±2.5 years). Antiviral agents were used in 11 patients with lamivudine and 3 patients with adefovir, telbivudine and entecavir respectively.

Discontinuation of antiviral agent was attempted in 6 patients (42.9%) who satisfied the criteria, except 8 patients (positive HBV DNA in 2 patients, lamivudine resistance in 2 patients, short duration of therapy in 2 patients, and unwillingness in 2 patients). The duration of antiviral therapy before withdrawal was 1.3±0.5 years. Four (66.7%) of 6 patients were successfully withdrawn and remained negative for HBV DNA for 5.1±1.2 years. The baseline HBV DNA titer and HBeAg status was not related to maintenance of remission after withdrawal. However, the duration of antiviral therapy before withdrawal showed longer tendency in patients maintaining remission compared to relapsed patients after withdrawal (485.0±193.9 and 283.5±91.2 days, P=0.165). During the follow up, patient death and graft failure were not reported for all HBsAg-positive KTRs.

Conclusions: Antiviral treatment can be successfully discontinued in selected KTRs with chronic HBV infection after complete suppression of HBV and sufficient duration of antiviral therapy.

Funding: Government Support - Non-U.S.

PUB794

Mycophenolic Acid Withdrawal in the First 12 Months Predicts Graft Outcome at 24 Months in Renal Transplant Recipients Natalie L. Borman,[1] Scott Harris,[2] Gopalakrishnan Venkat-Raman.[1] *[1]Wessex Renal and Transplantation Service, Portsmouth Hospitals NHS Trust, Portsmouth, United Kingdom; [2]Department of Medical Statistics, University of Southampton, Southampton, United Kingdom.*

Background: Mycophenolic acid precursors (MPAP) are widely used in transplantation. Many patients cannot tolerate MPAP, with reduction or cessation usually reversing side effects. Long term graft survival has not significantly improved despite these drugs and early graft factors influence this. Despite increasing knowledge about MPAP it is unclear if dose alteration independently affects graft function. **AIM:** To establish whether alteration/cessation of MPAP in the first 12 months post transplantation is an independent predictor of graft function at 24 months.

Methods: A retrospective analysis of 153 renal transplant recipients looking at graft function, MPAP dose alteration, clinical and graft factors in the first 24 months post transplantation. Primary outcome measure was change in creatinine from 12 to 24 months.

Results: All participants received CNI (76.5% Cyclosporine A, 23.5% Tacrolimus) and MPAP at the time of transplant. 60.8% were male, 93.5% Caucasian, mean age at transplantation 47 years (range 17-76). Participants were categorized into 3 groups: those remaining on full dose MPAP, those with a dose-reduction and those who discontinued MPAP in the first 12 months. Groups were analysed according to the primary outcome measure. Statistical analysis used ANOVA with $P<0.05$ as significant. Stopping MPAP was found to be significantly associated with a deterioration in creatinine from 12 to 24 months when compared to dose reduction or no change [P= 0.015 (95% CI 6.82 to 63.06) and 0.01 (95% CI 8.83 to 65.08) respectively]. Data was analysed for multiple potential confounding variables including biopsy proven rejection, type of transplant and CNI agent. None were significantly different between the groups and combined adjustment for all the variables made no difference to the significance observed.

Conclusions: Early MPAP withdrawal post renal transplantation is an independent predictor of graft function at 24 months. This may result from chronic / subclinical rejection due to under immunosuppression in these individuals.

Funding: Private Foundation Support

PUB795

Eculizumab Rescue and Bridging in Severe Antibody Mediated Rejection in Kidney Transplantation Martina Guthoff,[1] Barbara Schmid-Horch,[2] Silvio Nadalin,[3] Hans-Ulrich Haering,[1] Nils Heyne.[1] *[1]Dept. of Endocrinology and Diabetology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Tuebingen, Germany; [2]University of Tuebingen, Center of Clinical Transfusion Medicine, Tuebingen, Germany; [3]Dept. of General, Visceral and Transplantation Surgery, University of Tuebingen, Tuebingen, Germany.*

Background: Acute antibody mediated rejection (AMR) is a severe complication following kidney transplantation with profound impact on allograft survival. Human leukocyte antigen (HLA) antibody mediated cytotoxicity involves terminal complement activation and subsequent cell lysis. The humanized C5b-antibody eculizumab is a novel therapeutic option, blocking terminal complement activation. We report eculizumab to effectively interrupt AMR in kidney transplantation.

Methods: A 48-year old sensitized patient underwent HLA-incompatible living donor kidney transplantation from his brother. Preconditioning included rituximab, bortezomib and immunoadsorption, alemtuzumab was used for induction. After excellent primary function, a rise in serum creatinine occurred after two weeks and *de novo* donor-specific antibodies (DSA) were highly detectable. Histopathology revealed C4d-positive AMR.

Results: Despite continued plasma cell therapy and immunoadsorption, renal function deteriorated and DSA remained high with profound rebound after extracorporeal therapy. In consideration of therapeutic options, eculizumab was administered. Urinary output normalized rapidly and renal function stabilized despite unchanged DSA titres. Eculizumab was continued bi-weekly, with subsequent prolongation of intervals. 9 months after transplantation, continued plasma cell therapy effectively decreased DSA levels and eculizumab was tapered. Renal function is stable with a serum creatinine of 1.9 mg/dl and an eGFR of 38 ml/min/1.73 m².

Conclusions: Complement C5b blockade by eculizumab is highly effective in interrupting HLA antibody mediated cell injury in AMR. Eculizumab bridging creates a therapeutic window for antibody-directed B- or plasma cell therapy to become effective, allowing subsequent taper of eculizumab. As novel concept and treatment option, eculizumab may improve outcome of AMR in kidney transplantation.

PUB796

Looking Into the Crystal Ball: Can We Foresee Late-Onset Cytomegalovirus Infection or Disease in Kidney Transplant Recipients? Alainna Jamal, Shahid Husain, Yanhong Li, Olusegun Famure, Joseph Kim. *Multi-Organ Transplant Program, Toronto General Hospital, University Health Network, Toronto, ON, Canada.*

Background: Risk factors for late-onset CMV, apart from D+/R- serostatus, have not been clearly defined. This study aimed to explore other potential risk factors for late-onset CMV infection/disease in kidney transplant recipients.

Methods: A retrospective cohort study was conducted with 641 patients who underwent kidney transplants from 1 Jan 2003 to 31 Dec 2010 at a single Canadian center. A Cox proportional hazards model was used for risk factor analysis. American Society of Transplantation definitions were used for late-onset CMV infection/disease.

Results: This cohort included 110 D+/R- and 531 R+ patients. D+/R- patients were younger (47.4 vs. 52.7 years), more likely White (85.5% vs. 52.7%), had larger BMI (26.8 vs. 25.1 kg/m^2), and more likely living donor transplants (57.3% vs. 41.2%). They also had a shorter dialysis vintage (2.3 vs. 3.7 years), a longer period of prophylaxis (97 vs. 91 days), and a lower neutrophil count at prophylaxis cessation (3.4 vs. 4.0 x 10^3 cells/L). Cumulative incidence estimates for late-onset CMV infection/disease after prophylaxis cessation in D+/R- vs. R+ patients were 27.2% vs. 5.5% at 6-months and 31.3% vs. 5.9% at 1-year, respectively. The Cox model showed that D+/R- serostatus (HR 4.0 [95% CI, 2.4-6.8]) and estimated glomerular filtration rate (eGFR) by the CKD-EPI formula (HR 2.1 [95% CI, 1.1-4.1], for < 45 ml/min and 1.5 [95% CI, 0.8-2.9], for 45 to 60 ml/min) were the most important predictors of late-onset CMV infection/disease. Similar results were observed when the cohort was restricted to patients receiving ≤ 6 months of prophylaxis (HR 2.3 [95% CI, 1.1-4.3]) and those only receiving valganciclovir (HR 2.0 [95% CI, 0.9-4.1]).

Conclusions: In this cohort, D+/R- serostatus and low eGFR at the cessation of prophylaxis were most predictive of late-onset CMV infection/disease. Patients with lower eGFR at prophylaxis cessation should be considered for more intensive CMV surveillance, particularly within the first-year after prophylaxis cessation.

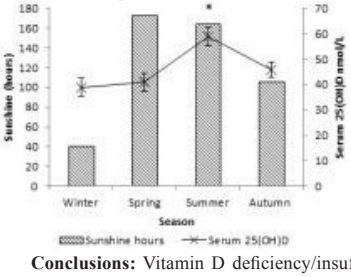
PUB797

Are We Inducing Harmful Vitamin D Deficiency in Our Renal Transplant Patients by Promoting Sun Avoidance? Hugo Penny, Sharon Frame, Francis L.F. Dickinson, Giorgia Louise Garrett, Antony R. Young, Robert Sarkany, Nihil Chitalia, Geeta Hampson, David Goldsmith. *Guy's &St Thomas' NHS Trust.*

Background: Renal transplant (RTx) patients are advised to avoid direct sunlight due to their increased risk of developing non-melanoma skin cancer (NMSC) with cumulative UV light exposure, and immunosuppression. However, sun-avoidance puts these patients at particular risk of vitamin D (25(OH)D) deficiency, which is linked to fractures, cardiovascular disease, and solid-organ cancer risk. In this study, we aimed to characterise the degree of vitamin D deficiency/insufficiency in a cohort of ambulant long-term RTx patients who were all well-educated about sun-avoidance.

Methods: We analysed the serum 25(OH)D and PTH concentrations from 266 RTx patients presenting to our clinic over the period of a year.

Results: The mean age of the cohort was 54±13years, 82% were Caucasian, mean eGFR was 50±19, median allograft age was 194 (149-277) months. 45 (17%) patients had NMSC. During the Winter, 9% of patients were vitamin D sufficient (>75nmol/L), 31% insufficient (75-37.5nmol/L) and 58% deficient (<37.5nmol/L). In Summer, 32% were vitamin D sufficient, 37% insufficient and 31% deficient. Serum 25(OH)D concentrations followed the recorded sunlight hours (figure 1). Patients reporting no/mild activity (72%) had lower 25(OH)D concentrations ($p<0.05$) than patients reporting moderate/vigorous activity (28%). There was an inverse correlation between serum 25(OH)D and PTH concentrations ($p<0.05$).



*[Figure 1. Serum 25(OH)D concentrations in patients presenting in Winter (n=54), Spring (n=59), Summer (n=67), Autumn (n=86). Error bars represent standard error of the mean. Bar charts represent mean seasonal sunshine hours falling on the South of England. *Denotes that Summer 25(OH)D concentration is significantly higher than 25(OH)D concentrations during the other seasons ($p<0.001$; all other values $p>0.05$).]*

Conclusions: Vitamin D deficiency/insufficiency was common (83%). This was particularly marked during the Winter and in patients who had low activity levels. Sun-avoidance is important, but leaves these patients susceptible to the secquelae of chronic vitamin D deficiency. Oral vitamin D supplementation may be the ideal treatment approach, but this needs a randomised controlled trial to confirm.

PUB798

Clinical Outcome of BK Virus Associated Nephropathy in Renal Transplant Recipients: Single Center Experience Hae Min Lee, Keunsuk Yang, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang, Byung Ha Chung. *Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Korea.*

Background: BK polyomavirus nephropathy (BKVN) is an important cause of allograft dysfunction in kidney transplant recipients. Once develop, its clinical course is known to be unfavorable and definite treatment guideline has not been established. In this study, we reported our center's experience of biopsy proven BKVN and their clinical course.

Methods: From Jan 2004 to Mar 2012, 17 cases of biopsy proven BKVN developed in Seoul St. Mary's hospital. Eight cases were deceased donor transplantation and nine were living donor transplantation. Two cases were retransplantation. Main immune suppressant was cyclosporine in five patients and tacrolimus in twelve patients.

Results: BKVN was observed at a mean duration 27.3±24.8 months after transplantation and serum creatinine at biopsy was 2.6±1.9 mg/dL. In five patients, BKVN was combined with acute rejection, and in one patient, it was combined with calcineurine inhibitor toxicity. In ten patients, monitoring for BKV replication using BKV real time PCR was available and the DNA copy number was 1.2 x 10^7 / mL (1.6 x 10^4 / mL -5.0 x 10^7/mL) in plasma and 1.1 x 10^{11} / mL (1.7 x 10^9/mL – 3.9 x 10^{11}/mL) in urine. Immunosuppression modification or reduction were done in fifteen patients. One of two patients who did not reduce immunosuppressant, were treated with steroid pulse due to combined acute cellular rejection. In addition, leflunomide was applied in seven, intravenous immunoglobulin in two out of fifteen patients had immunosuppression modification. Allograft loss developed in 23.5% (4 / 17) during follow up period (median 38.1 months (0.7 – 93.9)). Allograft loss developed at 0.7, 17.1, 21.8, 93.9 months from the diagnosis of BKVN respectively. The serum creatinine at diagnosis was higher in patients who suffered allograft loss compared to patients who did not.

Conclusions: As shown in this report, the clinical outcome of biopsy-proven BKVN is unfavorable, especially in cases diagnosed at later stage. Therefore, surveillance of BKV replication for earlier diagnosis of BKVN is recommended to prevent allograft loss due to BKVN.

PUB799

Optimizing Oral Glucose Tolerance Test for the Prediction of Prediabetes in Renal Transplantation Esteban Porrini,[1] Joan Manuel Diaz,[2] Irene Silva,[2] Meritxell Ibernon,[3] Patricia Delgado Mallen,[1] Francesc J. Moreso,[3] Benitez Rocio,[4] Jose Manuel Osorio,[5] Armando Torres.[1] *[1]Hospital Universitario de Canarias; [2]Fundación Puigvert; [3]Hospital Vall d´Hebron; [4]Hospital de Cruces; [5]Hospital Virgen de las Nieves.*

Background: Prediabetes: impaired fasting glucose (IFG: ≥100<126mg/dL) or impaired glucose tolerance (IGT: 2-h glucose ≥140<200mg/dL), is frequent in renal transplantation (RT), with IGT being more common. Diagnosis is made by oral glucose tolerance test (OGTT), a time-consuming non-standard practice.

Methods: We wished to optimize the use of OGTT by identifying patients with prediabetes. Eight Spanish centers each contributed 50-100 non-diabetics patients. After RT, OGTT was done at 3months and annually for 5 years. Stable patients beyond 12 months without new-onset diabetes were studied. ROC curves were used to analyze the goodness of fit of markers: fasting glucose (FG), HbA1c, triglycerides (TG), BMI or their combination to predict prediabetes.

Results: A total of 427 (144 prediabetic) patients were studied. Areas under the curve (AUC) were 0.713 (FG), 0.694 (HbA1c), 0.557 (TG) and 0.599 (BMI). With FG >or< 90 mg/dL, AUC was 0.657 and 0.493. With TG >or<150 mg/dL AUC was 0.574 and 0.511. The best predictor of prediabetes was a combination of both; **group A:** FG >90 mg/dL and **group B:** TG>150 mg/dL in patients with FG < 90 mg/dL. This strategy yielded overall AUC 0.61 (95%CI: 0.58-0.65), p<0.001, sensitivity 79.17 (71.6-85.5), specificity 42.66 (37.9-47.5), +likelihood ratio 1.38 (1.2-1.6) and-likelihood ratio 0.49 (0.4-0.7). Other analyses with HbA1c or BMI did not improve the prediction. This strategy detected 114 of 144 (79.16%) prediabetic patients.

Figure 1

		PREDIABETES	Cases
Fasting Glucose	Triglycerides		
< 90 mg/dL	< 150 mg/dL	YES	30
		NO	183
	> 150 mg/dL	YES	8*
		NO	49
> 90 mg/dL	-----	YES	106*
		NO	151

* cases detected, 114 of 144 (79.16%), OGTT done to detect these patients in grey

Conclusions: The use of OGTT to detect prediabetes in stable RT patients can be optimized by using the thresholds of FG >90 mg/dL and of TG >150 mg/dL.

Funding: Government Support - Non-U.S.

PUB800

Serum Aldosterone in Prednisolone-Treated Renal Transplant Recipients Laura V. de Vries, Michiel N. Kerstens, Anna Muller Kobold, Ido P. Kema, Jan-Stephan Sanders, Gerjan Navis, Stephan J.L. Bakker. *Department of Medicine, UMCG, Netherlands.*

Background: Short-term outcome after kidney transplantation is excellent, but long-term survival is limited by chronic transplant dysfunction. The mineralocorticoid (MC) aldosterone is essential for sodium and potassium homeostasis, but it is also known for its adverse profibrotic and pro-inflammatory effects on the kidney. We aimed to investigate the associations of serum aldosterone with graft function and graft survival in prednisolone-treated renal transplant recipients (RTR).

Methods: Baseline measurements were performed between 2001 and 2003 in outpatient RTR with a functioning graft >1 yr. Follow-up was recorded until May 2009. Graft failure was defined as return to dialysis or re-transplantation. Serum aldosterone concentration was measured by Siemens Coat-A-Count® Radioimmunoassay, which has no cross-reactivity with prednisolone.

Results: A total of 566 RTR (age 51±12 yrs, 55% men) were studied. Median [IQR] aldosterone concentration was 0.28 [0.16-0.44] nmol/l. Aldosterone was higher in females than in males (P<0.001). It was positively associated with proteinuria (r=0.16, P=0.002) and use of antihypertensive drugs (r=0.13, P=0.002), and inversely associated with serum sodium (r=-0.08, p=0.04), 24h sodium excretion (r=-0.12, P=0.005), and creatinine clearance (r=-0.22, P<0.001). No association with blood pressure, serum potassium, or prednisolone dose was found. During follow-up for 6.9 [6.1-7.4] years, 51 (9.2%) RTR experienced graft failure. In univariate Cox-regression analysis, serum aldosterone was positively associated with graft failure (HR=1.76 [95%CI 1.32-2.35], P=0.001). This association remained significant after adjustment for age, sex, CV risk factors, and sodium status (HR=1.48 [95%CI 1.08-2.03], P=0.01).

Conclusions: In this prospective cohort of RTR, despite MC effects of prednisolone treatment, serum aldosterone was associated with sodium and blood pressure homeostasis, and renal function. Findings are consistent with suppression by high sodium availability, and adverse effects on blood pressure and graft function on the long-term. Intervention with aldosterone blockade might be beneficial in prednisolone-treated RTR.

PUB801

Indoleamine 2,3-Dioxygenase Activity Following Renal Transplantation Rashmi Karanth,[1] Rachel Harbarger,[2] Rokshana R. Thanadar,[1] Julie J. Kim,[1] Todd D. Merchen,[3] Calpurnia Jayakumar,[1] Ganesan Ramesh,[1] Andrew L. Mellor,[2] John White,[1] N. Stanley Nahman.[1,4] *[1]Medicine, Georgia Health Sciences University, Augusta, GA; [2]Immunotherapy Center, Georgia Health Sciences University, Augusta, GA; [3]Surgery, Georgia Health Sciences University, Augusta, GA; [4]Medicine, Charlie Norwood VAMC, Augusta, GA.*

Background: Indoleamine 2,3-dioxygenase (IDO) degrades tryptophan (Trp) to kynurenine (Kyn). Through depletion of lymphocyte Trp, IDO may contribute to the development of host tolerance to alloantigens and thus be of clinical use in kidney transplantation (KTx). Harnessing the immunomodulatory effects of IDO requires the ability to non-invasively monitor its activity in vivo. To address this question, we assessed serum and urine Trp and Kyn levels from individuals with normal renal function (NL), hemodialysis patients (HD), and following KTx.

Methods: NL (N=8) or HD (N=10) had a single sampling of blood and urine (NL only). KTx (N=4) were sampled pre- (blood only), and post-transplant (blood and urine). Serum and urine Kyn and Trp levels were measured using HPLC. The ratio of Kyn/Trp is an accepted marker for IDO enzyme activity. All Kyn/Trp ratios were corrected for level of renal function by dividing by the respective serum or urine creatinine concentration.

Results: KTx patients had 4.3±1.7 (mean±SD) samples obtained on post-operative days ranging from 6 to 58. Serum creatinine was higher in HD (N=10 samples) when compared to NL (N=8 samples) and KTx (N=15 samples) (5.44±2.1 vs 0.8±0.17 and 1.14±0.48 mg/dl, p<0.001, respectively). Serum Kyn/Trp ratios were not different between NL, HD and KTx (0.05±0.007, 0.03±0.015 and 0.04±0.028, respectively). When corrected urinary Kyn/Trp values were compared between NL (N=7 samples) and KTx (N=13 samples), there was a trend toward higher levels in KTx (0.04±0.02 and 0.35±0.44 for NL vs KTx, respectively).

Conclusions: These data indicate a trend toward higher corrected urinary Kyn/Trp ratios following KTx and suggest that allograft IDO activity is increased in these patients. We would theorize that corrected urinary Kyn/Trp activity may be a useful biomarker of kidney IDO activity following KTx.

Funding: Clinical Revenue Support

PUB802

Multimodal Tacrolimus Adherence Measurement: The Role of Depression Daniel Cukor,[1] Nisha Ver Halen,[1] Ankita B. Patel.[2] *[1]Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY; [2]Medicine, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Non-adherence to immunosuppressant medication following kidney transplant is associated with graft loss, increased hospitalization and death, yet is quite prevalent. Accurate assessment of medication adherence is essential to clinical care and

predicting rejection. Despite the importance placed on medication adherence there is little data on the reliability or validity of the various assessment strategies.

Methods: Thirty kidney transplant recipients who were more than 6 months post transplant were followed for their Tacrolimus adherence over a 3 month assessment window. Adherence to Tacrolimus was assessed through 3 different modalities: self-report questionnaire (Immunosuppressant Therapy Adherence Scale - ITAS), pill count (both phone and in-office), and serum Tacrolimus levels.

Results: Over the course of the three-month assessment window, average serum Tacrolimus levels were within target (5-15) range for 62% of the sample. Pill count data indicated 68% of the sample took at least 95% of their pills, and 79% of the sample indicated perfect adherence through the self-report questionnaire. While there was reasonable internal consistency for each of the measurement styles (the monthly values were each highly correlated with one another (Cronbach's alpha -ITAS=.66, lab test=.63, pill count = .60) there was low levels of agreement across the different modalities (.34). Interestingly, subjects self reporting substantial non adherence reported significantly higher depression scores (t=3.3,p<.01) but no difference in actual pill counts (p>.05) or serum Tacrolimus levels (p>.05).

Conclusions: Immunosuppressant non-adherence is common in the kidney transplant community and more rigorous exploration into the methodologies utilized for assessment is an essential first step in improving the quality of the science in this area.

Funding: NIDDK Support

PUB803

Effect of Early Corticosteroid Minimization versus Early Corticosteroid Withdrawal on Graft Function and Cardiovascular Risks in Simultaneous Kidney Pancreas Transplantation Carolyne J. Jepkorir, Basma Sadaka, Jaime A. Pineda, R. Alloway, E. Steve Woodle, Amit Govil. *Nephrology/Transplant Division, Univ of Cincinnati, Cincinnati, OH.*

Background: Early corticosteroid (CS) withdrawal (ECSWD) and early CS minimization (ECSM) in SKP transplantation have been evaluated at single centers with no direct comparison. We report a sequential comparison of graft function and cardiovascular (CV) risks/events between ECSWD and ECSM.

Methods: 110 consecutive SKP transplants receiving rATG induction, FK, MMF and sequential regimens of chronic corticosteroids (CCS) (n=23), ECSWD(n=42), and ECSM with a rapid taper to 5mg/day (n=45) were retrospectively reviewed. CV risk factors were assessed along with graft function. Stats were performed using ANOVA and KM.

Results: Patient characteristics, FK level/MMF dose, SrCr, MDRD and fasting glucose were similar. ECSWD had greater improvement in BP with less meds while CCS had significantly high total cholesterol and LDL levels with no significant difference in TG, HDL and lipid therapy. CV events to date are similar between groups with no significant differences in rejection, patient, allograft function/survival.

	CCS (n=23)	ECSWD (n=42)	ECSM (n=45)	p-value
Median Follow-up (days)	3355	2261	802	
Steroid doses (mg/day) (mean ± SD)				
Month 3	10.2 ± 1.6	1.2 ± 2.2	5.4 ± 1.3	<0.0001
Year 1	7.1 ± 2.1	2 ± 3.1	4.7 ± 1.6	<0.0001
Year 2	3.9 ± 3.1	2.3 ± 3.1	4.2 ± 2.4	0.02
First acute rejection incidence, n (%)				
6-12 months	0	1 (2.4)	1(2.2)	NS
>24 months	3 (13.0)	3 (7.1)	0	0.02
Estimated MDRD (ml/min) (mean ±SD)				
Year 1	66.59 ± 30.54	68.89 ±20.83	65.6 ± 18.75	NS
Year 2	65.52 ± 22.42	65.72 ± 22.24	74.79 ±47.78	NS
Fasting Blood glucose				
Year 1	95 ± 18	93 ± 11	100 ± 41	0.53
Year 2	94 ± 37	93 ± 11	100 ± 36	0.65
Post-transplant LDL (mean ± SD)				
Year 1	108 ± 45.5	77.9 ± 27.6	68.3 ± 19.1	<0.0009
Year 2	97.9 ± 26.4	73.8 ± 24.4	83.6 ± 29.6	0.02
Kidney Graft Survival n (%)				
Year 1	21 (91.3)	41 (97.6)	43 (95.6)	NS
Year 3	19 (82.6	39 (92.9)	43 (95.6	NS
Pancreas Graft Survival n (%)				
Year 1	22 (96.6)	39 (92.9)	41 (91.1)	NS
Year 3	20 (87)	38 (90.5)	38 (84.4)	NS

Conclusions: Long-term follow up is required to better delineate differences in CV events, rejection, patient and graft survival in ECSWD vs. ECSM vs. CCS.

PUB804

Efficacy and Safety of Administering Renin-Angiotensin-Aldosteron System Blockers Very Early after Renal Transplantation Christos D. Chatzikyrkou,[1] Jenny Eichler,[1] Christian Clajus,[1] Jan Menne,[1] F. Lehner,[2] Fabian A. Helfritz,[2] Lampros Kousoulas,[2] Wolf Ramackers,[2] Hermann G. Haller,[1] Mario Schiffer.[1] *[1]Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Clinic for General, Abdominal and Transplant Surgery, Hannover Medical School, Hannover, Germany.*

Background: Renal transplant physicians are reluctant to use renin-angiotensin-aldosteron system (RAAS) blockers, due to the risk of renal function decline and hyperkalemia. The aim of this study was to investigate the safety and antihypertensive efficacy of RAAS blocker in the very early postoperative period after renal transplantation.

Methods: We analyzed the charts of 130 kidney transplant recipients, who received a RAAS blocker in the very early postoperative period 130 kidney transplant patients who did not receive a RAAS blocker served as controls. Cases and controls were matched by age, sex, year of transplantation and serum creatinine at the time of administration of the RAAS blocker. Clinical and laboratory variables, which were monitored closely before and after initiation of therapy, were compared between the two groups. Paired t-test was performed to compare blood pressure, serum creatinine, eGFR and serum potassium levels at therapy initiation and discharge.

Results: 105 (80.8%) patients were treated with an ACE inhibitor, and 25 (19.2%) with an ARB. Most patients received the RAAS blocker at postoperative day 8 and were discharged from hospital at postoperative day 13. Systolic, diastolic and mean arterial blood pressure were significantly reduced from 148 ± 17, 87 ± 11 and 108 ± 11mmHg at treatment initiation to 136 ± 17, 81 ± 10 and 99 ± 11 mmHg at discharge (p<0.001 for all variables). Transplant function was not affected. Serum creatinine decreased and eGFR increased (from 162 ± 79 µmol/l to 132 ± 40 µmol/l and from 48 ml/min ± 21 to 55 ± 18 ml/min). Serum potassium levels increased significantly (4.35 ± 0.46 mmol/l to 4.63 ± 0.58 mmol/l), but remained within the normal range.

Conclusions: RAAS blockers can effectively reduce blood pressure in the early postoperative period after kidney transplantation, without jeopardizing graft function and without increasing the risk of hyperkalemia.

PUB807

The Role of Pre-Dialysis Education on the Choice of Kidney Transplantation among Dialysis Patients Tarek Alhamad, Bruck Yemenu, Nasrollah Ghahramani. *Internal Medicine, Penn State College of Medicine, Hershey, PA.*

Background: End-stage renal disease (ESRD) is associated with significant morbidity and mortality. Among various therapies for ESRD, kidney transplantation (KT) is associated with the greatest longevity and the highest quality of life. The patient's understanding of the process of transplantation is a major determinant of preference for KT.

Objective: To investigate the impact of pre-dialysis education about transplantation on the preference for KT among dialysis patients.

Methods: We conducted a cross sectional self-administered survey among a potential pool of 80 dialysis patients. Participants were recruited during in-center hemodialysis (HD) session, or at home dialysis clinic.

Results: Fifty five dialysis patients (36 male; 38 white) provided informed consent to participate in the study (in-center HD: 43; peritoneal dialysis: 10; home HD: 2). Mean age was 59.27 ± 16.92, time since initiation of dialysis was more than a year for 68% of the patients and 29% had seen a nephrologist for the first time within 3 months of dialysis initiation. Twenty-four patients (44%) believed they had received adequate information about KT prior to dialysis initiation, while 19 patients (35%) considered the information about KT to have been adequate only after dialysis initiation. Ten patients (18%) acknowledged inadequate information about KT. When asked if they were offered the option of continuing current dialysis, switching the dialysis modality or opting for KT, 24 patients (44%) indicated that they would select KT. Using a stepwise logistic regression model which included demographic and treatment-related covariates (age, gender, race, employment status, education, income, current modality of therapy, time since initiation of dialysis), the perceived adequacy of information about KT received prior to initiation of dialysis was an independent predictor of selecting KT as the therapy of choice (OR=4.07; 95% CI: 1.31 to 12.65; p=0.015).

Conclusions: This pilot study highlights the importance of providing chronic kidney disease patients with adequate information about transplantation and other available treatment options before initiation of dialysis.

Funding: NIDDK Support

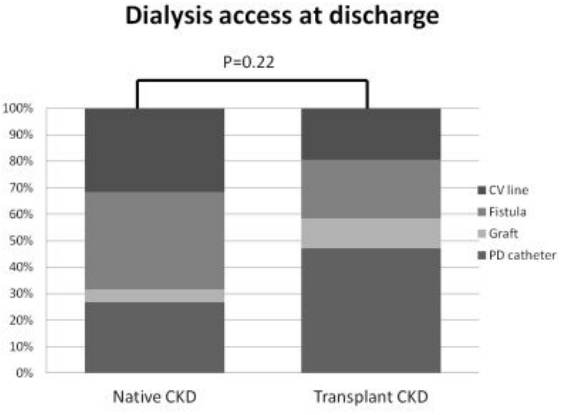
PUB808

Impact of Multidisciplinary Predialysis Care on Outcomes after Kidney Transplant Failure Brendan Boyd,[1,2] Mimi Cheng,[1] Niki Dacouris,[1] G.V. Ramesh Prasad,[1] Jeffrey Perl.[1] *[1]St. Michael's Hospital; [2]University of Toronto.*

Background: Reduced survival of kidney transplant failure (TF) patients relative to native kidney function decline (NKFD) patients initiating dialysis may relate to poor quality and fragmentation of predialysis care. Multidisciplinary predialysis care (MPC) has been associated with improved dialysis initiation outcomes for NKFD patients. We compared dialysis initiation outcomes, as well as the rate of kidney function decline, for TF and NFKD patients attending our MPC clinic.

Methods: Demographic, comorbidity and laboratory data were extracted retrospectively for patients discharged from the MPC clinic, using medical records. Continuous variables were compared via t-test or Mann-Whitney analysis where appropriate. Categorical variables were compared using chi-square analysis.

Results: 54 TF and 66 NKFD patients were identified. TF patients were younger (53.0±11.0 vs. 66.6±16.3, p <0.001), with a lower Davies comorbidity index (0(0-1) vs. 1(0-2), p=0.002), and a lower eGFR (15.0(12.9-18.7) vs. 18.6(14.2-23.0), p=0.002) at MPC referral. At the time of discharge, rates of optimal dialysis initiation (permanent surgical vascular access or the initiation of peritoneal dialysis) were similar between the groups(**Figure**). TF patients also experienced more rapid decline of eGFR(**table**) over the course of follow-up.



Temporal mean eGFRs (±SD)

Days attending MPC	TF eGFR (mL/min/1.73m2)	NKFD eGFR (mL/min/1.73m2)	P-value
0-99	14.8±4.17	18.3±7.32	<0.001
100-199	14.6±5.47	22.4±11.2	<0.001
200-299	12.4±4.56	20.4±10.9	<0.001

Conclusions: Although TF patients are referred at a lower eGFR, they initiate dialysis optimally at a similar rate to NKFD patients. This preliminary data indicates that MPC may confer similar benefits to both NKFD and TF patient highlighting its potential impact on improving the survival of TF patients, which warrants further prospective study.

PUB810

Operative Time and Outcome after Kidney Transplantation: Does the Time of Day or the Day of Week Matter? Seema Baid-Agrawal,[1] Holly J. Kramer,[2] Harold I. Feldman.[3] *[1]Department of Nephrology and Medical Intensive Care, Campus Virchow Clinic, Charite Medical University, Berlin, Germany; [2]Department of Preventive Medicine, Loyola Medical Center, Maywood, IL; [3]Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania Medical Center, Philadelphia, PA.*

Background: Medical care during off-hours i.e. at night-time or during weekends has been associated with increased morbidity and mortality compared with care delivered in regular hours among patients with multiple medical and surgical conditions. We compared outcomes of off-hour kidney transplantation (KTx) to those performed during regular hours.

Methods: Using the United Network for Organ Sharing database, we analzyed 181,925 adults aged >20 years who received a deceased donor KTx between January 1, 1990 and September 1, 2010 and who had complete information on time and date of surgery. Cox proportional hazards models were fit to evaluate the association between KTx during daytime (6 AM -9:59 PM) vs. night-time (10 PM -5:59 AM) and weekend (Saturday/Sunday) vs. weekday (Monday-Friday), and patient and graft survival at 1, 3, 5 and 10 years post-Tx, while adjusting for multiple covariates. All models were adjusted for both time of day and weekend vs. weekday.

Results: The average age was 47.8 years ± 12.7; 60.3% were male, 42.3% and 27.3% received Tx at night-time, and on the weekend, respectively. No significant association was noted between time of day or day of week and allograft failure including death or patient mortality at 1, 3, 5 and 10 years (Table 1).

Table 1: Outcome

	Night vs. Day, HR (95% CI)		Weekend vs. Weekday, HR (95% CI)	
	Allograft Failure	Mortality	Allograft Failure	Mortality
1-y	1.01 (0.98-1.04)	0.99 (0.94-1.04)	1.05 (0.98-1.13)	0.98 (0.84-1.15)
3-y	1.01 (0.99-1.04)	1.04 (0.98-1.06)	1.02 (0.96-1.07)	1.02 (0.91-1.14)
5-y	1.01 (0.99-1.03)	1.00 (0.99-1.03)	1.01 (0.96-1.06)	1.01 (0.95-1.04)
10-y	1.01 (1.00-1.02)	1.07 (0.98-1.16)	1.01 (0.99-1.03)	1.00 (0.98-1.16)

HR, hazard ratio; CI, confidence interval; y, year

Conclusions: The relatively stable condition of KTx recipients before Tx and protocolized nature of care may make this group less susceptible to adverse factors associated with off-hour care.

PUB811

Bone and Mineral Metabolism (BMM) in a Representative Sample of Spanish TR Patents (EMITRAL Study) Armando Torres, Daniel Dorado, Manuel Arias, Josep Maria Campistol Plana. *ICNU, Hospital Clinic, Barcelona, Spain.*

Background: The prevalence and treatment of bone and mineral metabolism disorders in renal transplantation (RT) are little known. The present cross-over study involved 729 recipients from 29 Units, with over one year from RT (69.2±30.6 months).

Methods: The estimated GFR was < 40 and ≥ 40 ml/min/1.73m2 in 55% and 45% of the cases. Centralized biochemical and radiological studies were made (Genant score for dorsolumbar vertebral fracture, and Kauppila score for abdominal aortic calcification). The proportion of patients with values within range for each stage of chronic kidney disease according to the guides (KDOQI and SEN) was 49.7% for Ca (only 6.5% with hypercalcemia), 86.2% for PO4, and 99.3% for Ca x PO4. The PTH levels were inversely correlated to estimated GFR (r=-0.33; p<0.001), and in 71.9% of the cases were above the established range for the corresponding chronic kidney disease stage.

Results: 39.8% of the cases with PTH above the range for the corresponding disease stage had elevated bone alkaline phosphatase. The 25OHD3 levels were insufficient (10-30 ng/ml) in 69.4% of the cases, and deficient (<10 ng/ml) in 13.85%. However, most of the patients presented adequate 1,25(OH)2D3 levels (>25 ng/ml). The 25OHD3 levels were inversely correlated to PTH (r=-0.23; p<0.001). 10% of the patients were receiving calcimimetic treatment, and 37.2% were administered vitamin D metabolites (nutritional, calcitriol, or paricalcitol). Although only 3% had known fracture antecedents following RT, 27% presented some prevalent vertebral fracture (FxV). A total of 67.3% showed aortic calcifications and the patients with FxV had a higher pulse pressure and CaAo score. Only 58% of the known fractures had received treatment.

Conclusions: Among stable RT recipients:a) hypercalcemia is infrequent, and the PO4 levels stay in the recommended range until stage V is reached; b) in most subjects PTH is above and 25OHD3 below the recommended range for the corresponding chronic kidney disease stage; c) asymptomatic FxV and CaAo are frequent in this population and are inter-related; and d) there is room for improvement in the management of BMM disorders after RT.

Funding: Pharmaceutical Company Support - Abbott

PUB812

Possible Predictors for Rituximab Responsiveness in Patients with Standard-Therapy Resistant Kidney Allograft Rejection Maximilian Ernst Daemmrich,[1] Verena Broecker,[2] Clemens L. Bockmeyer,[1] Wilfried Gwinner,[3] Anke Schwarz,[3] Hermann G. Haller,[3] Hans H. Kreipe,[1] Cornelia Anneliese Blume.[3] *[1]Pathology, Medical School Hannover, Hannover, Germany; [2]Histopathology, University Hospital Cambridge, Cambridge, United Kingdom; [3]Nephrology and Hypertensiology, Medical School Hannover, Hannover, Germany.*

Background: Rituximab serves as immunosuppressive rescue to treat standard therapy resistant rejections. Due to possible severe side effects, criteria to predict Rituximab responsiveness are most desirable. In this retrospective analysis, we compared Rituximab responders and non-responders (R and NR).

Methods: 20 renal transplant recipients were analysed with their last biopsy previous to rituximab therapy (375 g/qm2 b.s., 1-2 courses, 15 x combined with plasmapheresis). 10 patients were identified as NR returning to dialysis 5.7 ± 1.5 months after therapy. In 10 patients (R), kidney graft function was preserved after therapy (20.7±7.3 months; eGFR: 28.3 ± 2.6 ml/min; p<0.0001). Clinical, serological and histomorphological parameters were compared between both cohorts by Wilcoxon-or chi-square tests.

Results: NR had a significant lower eGFR (MDRD) at the time of biopsy, higher max. proteinuria and significant more biopsies with acute cellular rejections in their history. Immunohistochemical analysis of CD3, 20, 68 was not different between both groups, but interestingly the infiltrate of CD138 positive plasma cells was higher in the R-group. Histomorphological Banff parameters for acute cellular, acute and chronic humoral as well as glomerular, vascular and tubulointerstitial lesions in the last biopsy before rituximab and serological findings (HLA-mismatches, HLA-antibodies class I and II, DSA) or clinical parameters (recipients' age, sex, deaths, infections, living or cadaveric donor, previous renal transplants, time after transplantation) were not different between groups.

Conclusions: Transplant function, max. proteinuria and the number of historic rejections were predictive for therapy outcome. These criteria and particulary the infiltrate of plasma cells in biopsies of rituximab treated patients need validation in future prospective randomized studies.

Funding: Clinical Revenue Support

PUB813

Endothelial Dysfunction Assessment in Kidney Transplant Recipients Converted from Calcineurin Inhibitors to Everolimus: Preliminary Analysis Ruben O. Schiavelli,[1] Fernando Margulis,[1] Carlos E. Cuevas,[1] Esteban Alvarenga,[1] Alcira B. Otero,[2] Marcelo Ponte.[1] *[1]Nephrology and Transplantation, Hospital Argerich, Buenos Aires, Argentina; [2]Novartis Arg SA, Buenos Aires, Argentina.*

Background: Pulse wave velocity is considered an independent predictor of cardiovascular mortality. Stiffness index (SI) and reflection index (RI) reflect the stiffness of large arteries and vascular tone (endothelial function) of small-size arteries. The objective

of this study has been to assess the effect of the conversion from calcineurin inhibitor (CNI) to everolimus (EVR) on endothelial dysfunction and arterial wall stiffness.

Methods: Open, prospective, one year study with a control arm in kidney transplant patients (KTP). Twenty KTP (3 to 12 months after transplant) were converted from a CNI to EVR. Ten patients remained on CNI (control arm). To assess endothelium dependent arterial vasodilation, 400 ug of salbutamol (Salb) were given by inhalation.The vasodilation, dependent on endothelial function, was defined as the maximum difference in ΔRI between baseline and the post-Salb. SI and ΔRIb/RIs, 24 ambulatory MAP (mean arterial pressure); LVMI (Left Ventricular Mass index) were performed baseline and post conversion. We report a descriptive analysis of the 6 months after conversion period.

Results: At month six 5 patients discontinued: one control and 4 EVR. 95% CI values for main variables: Baseline: 1- 24 Amb MAP Control (90.7,101.3); EVR (97,113); 2-SI Control (6.6,10.6), EVR (9,11); 3- ΔRIb/RIs Control (-0.9,7.1); EVR: (1.5,15.5) and LVMI Control (124,166); EVR (158,190). At month six: 1- 24 Amb MAP Control (94,102); EVR (91,106); 2-SI Control (8.3,10.3), EVR (8.4,10.4); 3- ΔRIb/RIs Control (2.2,8.2); EVR: (0.7,6.7) and LVMI Control (102,152); EVR (144,182). P values were non statistically significant in any case. No changes were registered in endothelial function as per ΔRIb/RIs. A numerical trend to improve figures in variables representing arterial wall stiffness was observed in EVR arm.

Conclusions: In this group of stable KTP and in this time frame, conversion to EVR appears to produce a numerical improvement in arterial wall stiffness markers. No valid changes in endothelial function have been observed.

Funding: Pharmaceutical Company Support - Novartis

PUB814

Association between One-Year Serum Albumin and Patient Outcomes after Kidney Transplantation Il Hwan Oh, Joon-sung Park, Gheun-Ho Kim. *Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Republic of Korea.*

Background: Hypoalbuminemia are associated with increased risk of mortality in patients with kidney failure. There are limited data evaluating the relationship between serum albumin and patient outcomes in kidney transplantation (KT). We investigated whether serum albumin level is associated with patient outcomes after KT.

Methods: All recipients were divided into two groups according to serum albumin level at 1yr posttransplant: Group I (n = 188), >4.5 and 5.0 g/dL; Group II (n = 191), ≥ 4.0 and 4.5g/dL. Multiple linear regression analysis was used to find contributing factors to serum albumin level at 1yr posttransplant, and Kaplan-Meier (KM) analysis was used to compare patient outcomes between the Groups. The relationship between patient outcomes and factors including serum albumin was evaluated by Cox proportional hazard model.

Results: Three hundred and seventy-nine allograft recipients were followed for 121 ± 55 months. At 1 yr posttransplant, Group I had younger age (36.4 ± 9.7 vs. 41.9 ± 10.1 years, P<0.01), shorter dialysis vintage (27.6 ± 31.7 vs. 40.8 ± 46.6 months, P<0.01), higher serum hemoglobin level (13.3 ± 2.2 vs. 12.5 ± 2.0 mg/dL, P<0.01), and higher serum protein level (7.3 ± 0.4 vs.7.0 ± 0.4 g/dL, P<0.01) compared with Group II. Multiple linear regression revealed that serum albumin level at 1yr posttransplnat was significantly associated with age and serum protein level. KM analysis revealed that Group II had a worse patient survival, graft survival, and cardiovascular (CV) event-free survival as compared with Group I (P's<0.05 by the log-rank test). In Cox proportional hazard model, the serum albumin level was significantly associated with patient survival (HR = 0.222; 95% CI = 0.081-0.608), graft survival (HR = 0.403; 95% CI = 0.211-0.773), and CV event-free survival (HR = 0.321; 95% CI = 0.151-0.682).

Conclusions: These results suggest that the one-year serum albumin level may predict patient outcomes after KT. Such finding may have implications for early identification of vulnerable patients and for the development for targeted interventions to reduce the risk of mortality in this patient population.

PUB815

The Impact of Type 2 Diabetes on the Outcome of Patients on the Kidney Transplant Waiting List in France Frank Géoffroy Azimafoussé Assogba, Cécile Couchoud, Christian Jacquelinet. *Agence de la Biomédecine, Saint Denis la Plaine, France.*

Background: End stage of renal disease (ESRD) patients with diabetes have been shown to be less likely wait-listed or transplanted and more likely to have a longer waiting time. We aim to evaluate the proper effect of type 2 diabetes (T2D) on dead or too sick patients delisting (DTSPD) among kidney transplant (KTx) candidates.

Methods: The cohort of all 2785 ESRD adult patients (type 1 diabetes excluded) registered on the French national waiting list between January 2006 and December 2008 for first kidney transplantation was enrolled in the study and followed until 13 July 2011. DTSPD defined the event of interest and KTx a competing event. We used competing risks regression analyses with sequential adjustment to identify factors associated with DTSPD and evaluate the proper effect of T2D.

Results: Mean age was 51±12 years. The 3-year cumulative incidence was 4% for DTSPD and 76% for KTx. In univariate analysis, patients with T2D had an increased risk of DTSPD (Hazard ratio, HR, 1.85; 95% CI, 1.19-2.88) and lower access to KTx (HR, 0.87; 95% CI, 0.76–0.99) when compared to non diabetic. After adjusting for potential confounders such as age and sex, matched donor potential, immunisation status and number of cardiovascular disease the proper effect of T2D on both DTSPD (p=0.7) and KTx (p=0.3) was no more observed. Furthermore, increased age (HR, 2.40; 95% CI, 1.10-5.24 for 50-59 years; HR, 3.91;1.78-8.59 for 60 years) was independently associated with DTSPD and has no influence on KTx. Patients with two and more cardiovascular comorbidities have 30% less likely to access to KTx and 3.49 times to dead or be delisted compared to those with none.

Conclusions: T2D per se is not associated with both DTSPD and KTx when the other factors are taken into account for patients registered on the waiting list. Despite a higher selection of patients before registration on the waiting list, cardiovascular comorbidities are an important barrier to access to KTx and remain with older age the factors leading to either death or delisting from waiting list in France. Should we be more selective before registration on waiting list or need to transplant them faster?

PUB816

Hypertension in Post Renal Transplant Patients: A Pilot Study Anita Saxena, Raj K. Sharma, Amit Gupta. *Nephrology, SGPGIMS, Lucknow, Uttar Pradesh, India.*

Background: Hypertension following renal transplantation being a major risk factor for graft loss and patient survival, must be aggressively treated. Objective: Relationship of body water and body composition with hypertension in renal transplant patients.

Methods: 45 post transplant patients were divided into two groups. Group 1: patients on one antihypertensive drug and Group 2: patients on more than one antihypertensive drug. Nutritional status of the patients was assessed using subjective global assessment. Blood pressure (BP) was labeled controlled if BP was ≤120/80 mmHg and not under good control if BP was ≥ 120/80 mmHg. Body composition (water compartments, body fat and lean mass) was assessed using bioelectrical impedance analysis. Controls were 30 healthy volunteers. All the biochemical parameters were tested.

Results: Patients had normal nutritional status. In patients, systolic blood pressure was associated with TBW (p=0.016), extracellular water (ECW Lt; r =0.99), ECW% (r = 0.78) and diastolic BP with TBW% (p=0.003), dry weight (r= 0.76) ECW% (r= 0.95), and ICW% (r=0.79). Compared to controls, ECW and ECW% was higher in patients and ICW% was less in patients. There was significant difference in the actual weight of the patients and BIA derived dry weight although patients were clinically not edematous. Study shows significant increase in diastolic BP with increase in dry weight. Significant difference in the total body water (TBW) was observed when the patients were grouped on the basis antihypertensive medication patient was taking (1 antihypertensive drug versus more than 1 antihypertensive drug).

Conclusions: Study shows association between hypertension and overhydration. BIA may be a useful tool for clinical assessment of overhydration in nonedematous post transplant patients.

PUB817

Provider–Patient Visit Frequency on Hemodialysis and Kidney Transplantation Yelena Slinin,[1] Haifeng Guo,[2] Suying Li,[2] Jiannong Liu,[2] David T. Gilbertson,[2] Allan J. Collins,[2] Areef Ishani.[1,2] *[1]Veterans Administration Health Care System, Minneapolis, MN; [2]Chronic Disease Research Group, Minneapolis, MN.*

Background: Centers for Medicare & Medicaid Services tied provider reimbursement for outpatient hemodialysis services to a number of times per month providers see their patients on hemodialysis, resulting in increased frequency of provider-patient visits on dialysis. We aimed to determine whether greater provider-patient visit frequency is associated with a greater hazards of transplant waiting list placement and transplantation compared to fewer visits among incident hemodialysis patients.

Methods: Using United States Renal Data System data for patients who initiated in-center hemodialysis between 10/1/2003, and 9/30/2006, we defined patient characteristics and provider visit frequency on dialysis during months 4-6 of hemodialysis. Patients (N=87931) who were not on the kidney transplant waiting list and patients (N=3345) who were on the transplant waiting list at the start of follow-up were followed for 1 year for placement on the transplant waiting list and transplantation, respectively. We used Cox proportional hazards modeling to determine the association between provider-patient visit frequency and the outcomes.

Results: Of the 87,931 patients not on the waiting list and of the 3,345 patients on the waiting list at the start of follow-up, 60.8% and 58.5%, respectively, were seen by their providers at least 4 times a month. After multivariate adjustment for patient characteristics, greater provider visit frequency (4+ times/month compared to <4) was not associated with shorter time to placement on the kidney transplant waiting list: Adjusted Hazards Ratios (AHR) (95% Confidence Interval (CI)) 1.02 (0.96-1.08), or to kidney transplantation AHR (95% CI) 1.05 (0.91-1.22).

Conclusions: Greater frequency of provider visits to hemodialysis patients is not independently associated with either a greater rate of placement on the kidney transplant waiting list or kidney transplantation in hemodialysis patients.

Funding: NIDDK Support

PUB818

Burden of Kidney Disease Post Transplantation Jeanne Dreier, Sridhar K. Reddy, Avrum Gillespie. *Nephrology, Temple University Hospital, Philadelphia, PA.*

Background: Allograft dysfunction, common in an inner city population, may erode both patient satisfaction with transplantation and self-reported health. The purpose of this study is to examine the association between self-reported health, disease burden, treatment satisfaction and clinical markers in an inner city population of kidney transplant recipients.

Methods: Fifty-four patients completed a survey specifically designed for this study. The survey data was merged with common laboratory test data and analyzed in SPSS, version 18. The mean age of participants was 56.7 years, males and females were equally represented and mean serum creatinine was 1.65 mg/dl. Respondents were 64.1% African American, 18.9% Hispanic and 17% Caucasian.

Results: Most patients (77.4%) were satisfied with their transplantation experience. Yet, only 49.1% reported that they were not at all burdened by their current kidney disease. Serum creatinine was not correlated with disease burden, self-reported health or satisfaction with treatment. Patients indicating some level of disease burden were more likely to report poorer health (Pearson .395, p=.003), less likely to be satisfied with their current treatment (Pearson .475, p<.001), and they also had lower serum albumin (Pearson -.304, p=.027) compared to those who reported no burden. Patients who reported more disease burden had shorter allograft vintage (Pearson -.468, p=.001). Interestingly, burden-free patients were more likely to be African American or Hispanic (Chi-Square 7.711, p=0.021).

Conclusions: In conclusion, post-kidney transplantation self-reported health, disease burden, and satisfaction with treatment are not correlated with allograft function. Patients who reported any kidney disease burden have poorer self-reported general health, less satisfaction with current treatment, shorter allograft vintage, and lower serum albumin. Lastly, African Americans and Hispanics are more likely to be burden-free from their kidney disease.

Funding: Clinical Revenue Support

PUB819

Adult Preemptive Kidney Transplantation: A Paired Kidney Analysis
Boleslaw Rutkowski,[1] Alicja Debska-Slizien,[1] Beata Bzoma,[1] Andrzej Chamienia,[2] Grazyna Moszkowska,[3] Dariusz Zadrozny,[4] Anna Milecka.[4] *[1]Department of Nephrology, Transplantology and Internal Diseases, Gdansk Medical University, Gdansk, Poland; [2]Department of Nursing Science, Gdansk Medical University, Gdansk, Poland; [3]Department of Immunology, Gdansk Medical University, Gdansk, Poland; [4]Department of General, Endocrine and Transplant Surgery, Gdansk Medical University, Gdansk, Poland.*

Background: From November 2003 to December 2012, in GdanskCenter, 58 patients received preemptive transplantation (PET). PET consisted 8% of all 703 kidney transplantations performed during this time. The benefits for individual patients and for the health care system are discussed.

Methods: The present study compares the outcomes of these PET patients who had their kidney donor pairs transplanted after variable duration of dialysis (PTD).

The mean age was 41.8 and 45.9 years in 45 PET and 45 PTD, respectively. PTD patients remained on dialysis for 3.5-180 (mean 37,08) months before transplantation.

Results: Mean transplantation waiting time was 3.7 *vs.* 24,3 months (U Test, $p<0.05$) and the mean Charlson co-morbidity index was 2.49 *vs.* 2.96 (U Test, $p>0.05$) for the PET and PTD groups, respectively. Both groups did not differ significantly with respect to one-year patient and graft survival and incidences of acute rejection. Four (9%) PET patients and 14 (35%) PTD patients experienced delayed graft function (U Test, $p<0.05$). The graft function (GFR) one year after transplantation was similar in both groups. A comparison of serum creatinine concentration indicated significantly lower levels in the PET group and this difference was stable throughout our observation (ANCOVA Test, $p<0.05$). More PET patients led normal professional activities or continued education before and after transplantation (Fisher test, $p<0.05$).

Conclusions: Our single-center results confirmed that PET is an optimal mode of renal replacement therapy for both medical and socioeconomic reasons. Long-lasting medical care allows appropriate and optimal treatment of end stage renal disease.

PUB820

Permanent Vascular Access and Mortality among Failed Kidney Transplant Patients Who Initiate Maintenance Hemodialysis (MHD)
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Background: In the US, 1,700 patients return to MHD each year after kidney transplant failure. Several studies suggest these patients have higher mortality compared to wait-listed dialysis patients. It is unknown if this observation is related to differences in hemodialysis access attainment.

Methods: A retrospective USRDS cohort was generated of adult patients who initiated MHD between 2005-2007 after failure of their first kidney transplant. Baseline characteristics and vascular access type were determined from the core files and medical evidence report. Mortality risk was examined using multivariable Cox models.

Results: A total of 4,624 patients with primary kidney transplant failure returned to MHD. Median transplant survival was 7.6 years. Pre-transplant dialysis exposure occurred in 88% (HD only 66%, PD only 10%, mixture HD and PD 12%). Permanent hemodialysis access (AVF or AVG) was present in 36% at re-initiation, but only 16% of those treated with pre-transplant PD. The unadjusted mortality rate after initiation of MHD was 16.7 events per 100 person-years. Pre-transplant PD associated with increased hemodialysis mortality (HR 1.18 CI 1.04-1.34) when compared to pre-transplant HD. After adjustment for permanent dialysis access (HR 0.73 CI 0.65-0.82), pre-transplant PD was no longer independently associated with hemodialysis mortality (HR 1.11 CI 0.97-1.26).

Conclusions: Pre-transplant PD associated with higher mortality after transplant failure and re-initiation of hemodialysis, but is largely attributable to differences in vascular access attainment. Greater attention should be placed at improving fistula rates prior to allograft failure.

Funding: NIDDK Support

PUB821

Renal Transplatation Outcomes in C3 Glomerulopathy: The Irish Experience
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Background: "C3 glomerulopathy" (C3G) describes a heterogenous group of glomerular disorders defined by the presence of glomerular deposits of complement C3, in the absence of significant amounts of immunoglobulin, on immunofluorescence microscopy. Outcomes following renal transplantation in C3G are not well described and therefore we reviewed the experience of renal transplantation in C3G patients.

Methods: All renal biopsies reported between January 1995 and December 2011 were reviewed for evidence of C3G and confirmed by a second renal pathologist with expertise in this field. Patients within this cohort who received at least one renal transplant are described. Clinical data were obtained through retrospective review of medical records.

Results: Ten patients with a pathological diagnosis of C3G were identified (6 dense deposit disease [DDD], 4 non-DDD). Eight patients (80%) developed biopsy-proven C3G recurrence in their allograft (mean time to recurrence 46.5mths, range 2-124mths). Of these, 3 experienced allograft loss due purely to C3G (all DDD, mean allograft survival 84.3mths), 3 experienced allograft loss due to C3G combined with other factors (1 DDD, allograft survival 60.0mths; 2 non-DDD, mean allograft survival 53.5mths), and 2 continue to have a functioning allograft (1 DDD, follow-up 176.0mths; 1 non-DDD, follow-up 41.0mths). Two patients had no evidence of disease recurrence at time of last follow-up (1 DDD, follow-up 65.0mths; 1 non-DDD, follow-up 6.0mths).

Conclusions: This is the largest series describing outcomes following renal transplantation in patients with C3G (with or without features of DDD). The majority of patients in this cohort developed biopsy-proven C3G recurrence in the allograft but this did not universally lead to allograft loss.

PUB822

Transmission of HTLV-1 by Kidney Transplantation and Post-Transplant Manifestation of Cutaneous T-Cell Lymphoma
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Background: Kidney transplant recipients are at risk of post-transplant lymphoproliferative disease (PTLD). PTLD commonly occurs as B-cell non-Hodgkin-lymphoma. Primary cutaneous lymphomas are uncommon. Human T-lymphotropic virus type 1 (HTLV-1) is the first retrovirus discovered in humans (prevalence <1% in most western countries). HTLV-1 infects CD4+ T-cells and persists during life time. Two HTLV-1 associated diseases are known: adult T-cell lymphoma and HTLV-1-associated myelopathy. 1-2 % of infected individuals develop a HTLV-1 associated disease.

Methods: Case report:

Results: A male (born 1984) received a kidney transplant in 2006 (standard immunosuppression: tacrolimus, MMF and low dose steroids). In 2009 the patient developed painful skin lesions (hands/feet). Cutaneous T-cell lymphoma was diagnosed with predominant infiltration of T-cells in biopsies. Systemic evaluation of an extra cutaneous manifestation was negative. The patient was set on high dose steroid therapy (prednisolone 250mg/day for 5 days, tapering during follow up), on which skin lesions healed quickly without residues. In 2011, we became aware of the HTLV-1 positive status of the donor and a possible HTLV-1 transmission during transplantation in 2006. It was confirmed that our patient was HTLV-1 positive and pre-transplant serum of him was HTLV-1 negative. A liver recipient from the same donor developed similar skin lesions with T-cell infiltration. Skin biopsies from this liver recipient showed increased HTLV-1 viral load compared to non-infiltrated tissue controls. Evaluation of viral load in our patient's skin biopsies showed similar results.

Conclusions: Our patient developed a HTLV-1 associated cutaneous T-cell lymphoma after transmission of HTLV-1 during kidney transplantation. An exclusive cutaneous manifestation of HTLV-1 associated T-cell lymphoma has not been described previously. Continuous immunosuppression might be a reason for this uncommon and early manifestation of this HTLV-1 associated disease.

PUB823

Role of Plasmapheresis, Intraveinous Immunoglobulin and Rituximab in Renal Allografts Manifesting Antibody Mediated Change
Salim Baghli,[1] Christin M. Spatz,[1] Osun Kwon,[1] Bishal B. Rawal,[1] Tarek Alhamad,[1] Haresh Mani,[2] Hiroko Shike,[2] Bruck Yemenu.[3] *[1]Nephrology, Penn State University, Hershey, PA; [2]Pathology, Penn State University, Hershey, PA; [3]Internal Medicine, Penn State University, Hershey, PA.*

Background: Antibody-mediated rejection (AMR) has been increasingly recognized as a poor prognosticator of kidney transplant outcome. It is reported that antibody mediated changes (AMC) such as circulating donor specific anti-HLA antibodies (DSA) and C4d

positivity in graft tissues are associated with shortening of renal graft survival; however, no standard therapeutic modality is established yet.

Methods: Twenty five adult renal transplant recipients who received PPIGR for DSA and/or positive C4d after kidney transplant were followed for 6 months to 3 years. Subjects were analyzed for change in graft function as well as circulating DSA and C4d in graft tissues. The control group included 13 kidney transplant recipients with positive DSA who did not receive PPIGR. DSA was monitored by Luminex assay, C4d by immunohistochemistry and graft function by change in serum creatinine concentration.

Results: DSA disappeared in 6 out of 14 DSA positive subjects during follow-up compared to only 2 of 13 controls. Disappearance of DSA was noted within 5 days after PPIGR for 4 out of 6 subjects. Among 5 controls with newly formed DSA, spontaneous disappearance occurred in 2 cases at 84 and 353 days after transplantation. Positive C4d staining in graft tissues disappeared in 94% (17/18) of subjects within one week after PPIGR. No subject nor control developed acute AMR during follow up. Acute cellular rejection coexisted with antibody mediated change in 13 subjects and 2 controls. No significant change in graft function was detected in subjects compared to controls during follow-up even after adjustment for acute cellular rejection. Four subjects and one control had graft failure within one year.

Conclusions: Our findings suggest that PPIGR modulates AMC, but does not have a beneficial effect on long term kidney graft function.

PUB824

Factors Associated with Transplant Glomerulopathy Emma Romo,[IMSS] Jorge Andrade-Sierra.[IMSS] *[1]Nefrología, IMSS, Guadalajara, Jalisco, Mexico; [2]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico; [3]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico; [4]Nefrologia, IMSS, Guadalajara, Jalisco, Mexico.*

Background: Transplant glomerulopathy (TG) is a disease of poor prognosis for graft survival. The general characteristics and factors associated with this entity have not been described in our population.

Methods: We included 35 patients diagnosed with TG from 2008 to 2011. We collected demographic data, clinical and biochemical one month prior to diagnosis and the time of diagnosis. In all cases histopathological report was obtained through allograft biopsy.

Results: 63% male, 54% had a history of cellular rejection and / or humoral confirmed by biopsy. The average TG was introduced 75 months after transplantation. At diagnosis 80% were with sirolimus and 20% calcineurin inhibitor. The average creatinine was 1.8 mg / dl one month prior and at diagnosis 2.3 mg / dl. Proteinuria in 24 hours urine was of 4.45 gr. We compared groups according to C4d positivity with immunohistochemistry in renal biopsy.

Factors associated with transplant glomerulopathy

Characteristic	All cases (n=35)	C4d + (n=13)	C4d - (n=6)
Age	28	30	25
Months of diagnosis of TG	75	69	98
Number of antigens	3	3	3
CD25 induction (n) (%)	31 (88)	9 (67)	6 (100)
Severe glomerulitis (n) (%)	21 (60(	8 (61)	5 (83)
Fibrosis more 25% (n) (%)	30 (85)	10 (77)	5 (83)
Capilaritis (n) (%)	16 (45)	6 (50)	0 (0)
GFR before TG (ml/min)	53.71	52.8	39.2
GFR al diagnosis TG (ml/min)	48.31	46.6	30.2
Loss of GFR (ml/min)	-21.5	-19.1	-13.02
Sirolimus (n) (%)	28 (80)	8 (61)	6 (100)

TG = Transplant glomerulopathy, GFR = Rate filtration glomerular

Conclusions: The TG was presented at 6 years after transplantation, no clinical or histopathological baseline characteristic was associated with the presentation of this entity, but 80% of patients receiving sirolimus at diagnosis. C4d + was associated with the presence of capillaritis and greater loss of glomerular filtration at the end of the study. The outcome in this group of patients confirms the poor prognosis of this disease reported a loss of glomerular filtration rate of 21.5 ml / ml.

PUB825

Cardiovascular Risk in Non-Dipper Pediatric Renal Transplant Recipients Christine Sethna,[1] Cathy H. Laney,[2] Nina Laney,[2] Howard Trachtman,[3] Rachel Frank,[1] Lulette Infante,[1] Kevin E.C. Meyers,[2] Nandita Patnaik.[1] *[1]Pediatric Nephrology, Cohen Children's Medical Center of New York, New Hyde Park, NY; [2]Pediatric Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA; [3]Pediatric Nephrology, NYU Langone Medical Center, NY, NY.*

Background: An abnormal decline in nocturnal blood pressure (non-dipping) is commonly found during ambulatory blood pressure monitoring (ABPM) in pediatric renal transplant recipients (Txp). Non-dipping is independently associated with increased cardiovascular risk in the adult population. The objective was to examine aortic stiffness, left ventricular dimensions and lipids in non-dipper vs. dipper Txp.

Methods: 24-h ABPM, echocardiography for left ventricular mass index (LVMI), and carotid-radial pulse wave velocity (PWV) were performed in 53 Txp at two tertiary centers. Non-dipping was defined as a decline of <10% in blood pressure (BP) from day to night. Differences between non-dipper and dipper groups were assessed by Student's t-test and linear regression.

Results: Non-dipper subjects included 9 F/11 M (38%), age 12.8 ± 4.6 yr. Dipper subjects consisted of 13 F/20 M (62%), age 14±4.7 yr. The two groups showed no difference in age, gender, BMI z-score and GFR as well as cholesterol, HDL, LDL, triglycerides and uric acid levels. The nocturnal dip was 3.7%/7.6% (systolic/diastolic) in the non-dippers and 11.1%/19.5% in the dippers. Non-dippers had significantly higher nocturnal BP compared to dippers (116/70 vs. 105/60 mmHg, p<0.001). Clinic, average 24-h, and daytime 24-h BP were similar between groups. PWV did not significantly differ between non-dippers and dippers (8.27±1.94 vs. 8.34±4.1 ms, p=0.96) with no alteration after adjustment for age, gender and BMI (p = 0.98). LVMI did not significantly differ between non-dippers and dippers (54.4±20.3 vs. 55.2±20.8 $g/m^{2.7}$, p=0.88) with no alteration after adjustment for age, gender and BMI (p=0.67).

Conclusions: The non-dipping pattern in pediatric renal transplant recipients does not appear to be associated with measures of arterial stiffness, left ventricular mass and lipids. Future studies are needed to delineate the effect of non-dipping in these subjects at risk for cardiovascular disease.

Funding: Private Foundation Support

PUB826

Impact of Pre-Transplant Dialysis Modality on Clinical Outcome in Kidney Transplant Recipients Hyun Gyung Kim,[1] Byung Soo Kim,[1] Chul Woo Yang.[2] *[1]Internal Medicine, The Catholic University of Korea, St. Paul's Hospital, Seoul, Korea; [2]Internal Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital, Seoul, Korea.*

Background: We performed this study to evaluate the effect of pre-transplant dialysis modality on graft and patient survival in living donor kidney transplant recipients (KTR).

Methods: This study included 365 living donor KTR receiving dialysis treatment for at least three months prior to KT. We compared the baseline characteristics including age at KT, gender, primary renal disease, and the number of HLA mismatch and clinical outcomes such as graft and patient survival according to the pre-transplant dialysis modality. The mean age at KT was 40.1±10.7 (16-73) years and 60.5% (n=221) were men. The mean duration of follow-up was 92.3±53.7 months. Seventy five percent (n=274) of patients were followed pre-transplant hemodialysis (HD) and 25% (n=91) peritoneal dialysis (PD).

Results: The mean dialysis duration was 26.2±32.5 (3-225) months and it was shorter in the PD group compared to the HD group (24.9±25.0 vs. 26.6±34.7 months, p=0.026). DM was more common in the PD group (8.4% vs. 15.5%, p=0.045). However, there were no significant differences in baseline clinical parameters such as gender (male 62.7% vs. 53.8%, p=NS), age at renal transplantation (40.5±10.8 vs. 39.0±10.4, p=NS), HLA mismatch number (3.0±1.5 vs. 3.0±1.4, p=NS), donor age (37.4±10.7 vs. 38.4±11.1 years, p=NS) and donor gender (male 57.6% vs. 53.8%, p=NS) between HD and PD groups. The 5-year graft survival rate was not significantly different between two groups (90.7% in the HD group vs. 82.9% in the PD group, p=NS). A subanalysis of our data showed no difference in the frequency of rejection (63.4% in the HD group vs. 60% in the PD group, p=NS) or patient death (19.5% in the HD group vs. 25% in the PD group, p=NS) as a cause of graft failure. The 5-year patient survival rate of 96.9% in the HD group was similar to that of the PD group (93.4%, p=NS). There was no association between pre-transplant dialysis modality and cardiovascular mortality rate.

Conclusions: Pre-transplant dialysis modality had no significant influence on graft and patient survival in living donor kidney transplant recipients.

PUB827

Clinical Features of Simultaneous Pancreas-Kidney Transplantation Hee Jung Jeon,[1] Yoonjung Kim,[2] Hyuk Yong Kwon,[2] Myung-gyu Kim,[2,3] Tai Yeon Koo,[4] Jong Cheol Jeong,[1] Jaeseok Yang,[2,4] Curie Ahn.[1,2,4] *[1]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea; [2]Transplantation Center, Seoul National University Hospital, Seoul, Republic of Korea; [3]Department of Internal Medicine, Korea University Medical Center, Seoul, Republic of Korea; [4]Transplantation Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea.*

Background: This study examined the clinical features of simultaneous pancreas-kidney (SPK) transplantation in diabetic end stage renal disease and evaluated the results.

Methods: We retrospectively reviewed SPK transplantation from 2002 to 2010 in Seoul National University Hospital in Korea. 20 patients (11 males and 9 females) underwent SPK transplantation from deceased donors. 8 patients suffered type 1, whereas 12 patients suffered type 2 diabetes mellitus.

Results: The mean recipient age was 42.9 years (range, 29.6-70.7) in SPK. The mean donor age was 28.5 years (range, 16.5-41.9). Immunosuppressive treatment consisted of basiliximab induction followed by tacrolimus, mycophenolate mofetil, and prednisone. Pancreas graft management for the exocrine drainage involved enteric drainage in all cases. The mean duration of hospital stay was 43.5 days (range, 15-121 days). After a mean follow-up of 45.2 months (range, 15-121), patient survival rate, kidney allograft survival rate, and pancreas allograft survival rate were 95%, 90%, and 80%, respectively. One patient died of cerebral infarction and small bowel perforation. Two pancreas allografts were lost due to CMV pancreatitis and small bowel perforation. Other early complications associated with the pancreas transplantation included 4 cases of perigraft fluid collection, 4 of hematoma in abdomen, 3 of small bowel perforation, and 2 of venous thrombosis in pancreas allograft; Beyond the early postoperative period, allograft loss of pancreas and kidney was limited to 1 case of noncompliance to the immunosuppressive medications.

Conclusions: SPK is a well-established therapeutic option with tolerable outcomes for patients with multiple organ failure. However, we should be careful about the complications in the immediate postoperative period of SPK.

PUB828

Trough to Target Immunosuppression Is the Risk Factor for Polyoma Virus Infection in Renal Transplantation Ahmed G. Adam,[1] Nagwa Farouk,[1] Mona Salem,[2] Doaa Hashad,[3] Hala S. Elwakil.[1] *[1]Kidney, Dialysis and Transplantation Unit, Internal Medicine Department, Faculty of Medicine; [2]Pathology Department, Faculty of Medicine; [3]Clincial Pathology Department, Faculty of Medicine, University of Alexandria, Egypt.*

Background: BK virus infection after kidney transplantation is common but usually asymptomatic; however may be life threatening. More potent immunosuppression poses threat to improving transplant graft survival by reactivating BK.

Methods: All kidney transplant recipients in our university clinic were included. Urine decoy cells, PCR for BKV in blood and Transplant biopsy were done. Studied population was divided: Group I: was negative for decoy cells in urine and undetectable BKV load in blood. Group II (reactivation group): was positive for decoy cells and /or viremia and /or nephropathy. Follow up for 6 months.

Results: Among the 75 kidney transplant recipients who consented, 8 were positive for decoy cells (11%), 3 viremia (4%), 2 nephropathy proved by biopsy as intranuclear inclusions in tubular cells. A significant difference as regards acute rejection episodes (p =0.012); more than one methylprednisolone pulse (p =0.012). No difference regarding the immunosuppressive protocol, drug, or the anti-metabolite drug used. However a significant difference in the percentage of patients with immunosuppressive level above the target (p =0.004). Logistic regression analysis revealed that high trough to target ratio was significantly associated with polyoma virus reactivation and increase risk by 12.71 times.

Factors associated with polyoma virus reactivation, results of multivariate logistic regression analysis.

	Regression Coefficient	p	Odds Ratio	95.0 % CI - Lower	95.0 % CI - Upper level
Number of transplantation 1 vs. 2	0.320	0.841	1.377	0.061	31.207
Duration of Transplantation	0.015	0.256	1.015	0.989	1.041
Number of rejections espisodes 0 vs >1	0.575	0.498	1.778	0.336	9.405
Trough above Target vs. within Target	2.542	0.008*	12.710	1.963	82.289

Kaplan- Meier analysis showed 77.8% recovered spontaneously with a mean time 120.8 ±31.302 days.

Conclusions: The Trough to Target immune suppression is a main factor influencing Polyoma. Incomplete response to anti-rejection raises the suspicion of PVAN.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

PUB829

Usefulness of Liver Biopsy in Anti-Hepatitis C Virus Antibody (Anti-HCV Ab) Positive and HCV RNA Negative Kidney Transplantation (KT) Recipient Kyeong Woo Nho,[1] So Mi Kim,[2] Su-Kil Park,[1] Soon Bae Kim.[1] *[1]Nephrology, Department of Internal Medicine, College of Medicine, University of Ulsan, Asan Medical Center, Seoul, Korea; [2]Nephrology, Department of Internal Medicine, College of Medicine, University of Jeju, Jeju, Korea.*

Background: Liver biopsy is recommended to all Anti-HCV Ab(+) KT recipients [Textbook of KT, 5th ed.]. However, the benefit of liver biopsy is unclear in HCV RNA(-) KT recipients. We investigated usefulness of liver biopsy in Anti-HCV Ab(+) and HCV RNA(-) patients.

Methods: A total of 30 Anti-HCV Ab(+) patients underwent liver biopsy before KT in the Asan Medical Center during 1995-2011 were reviewed. The patients were divided into two groups based on HCV RNA positivity; seventeen patients were positive and thirteen negative. Hepatic histological findings, inflammation, and fibrosis were analyzed by using the METAVIR scoring system, and clinical outcomes including mortality, graft loss, progression of advanced liver disease of the two groups were compared.

Results: Age, gender, DM, duration of dialysis, duration of HCV infection, AST and Child-Pugh score were not different between the two groups. Only ALT was higher in HCV RNA(+) group. Ultrasound of liver showed abnormal findings in three HCV RNA(+) patients [one cirrhotic change, two chronic parenchymal change]. Both activity and fibrosis scores were higher in HCV RNA(+) group [Activity score : 1.11±0.85 vs 0.46±0.51, p=0.01, Fibrosis score : 1.05±1.24 vs 0.15±0.37, p=0.01]. Four patients in HCV RNA(+) group received antiviral therapy and 3 showed sustained viral response. One patient in HCV RNA(+) group with fibrosis 4 received liver and kidney co-transplantation. Two patients died of sepsis in HCV RNA(+) group and one patient died due to sudden cardiac arrest in HCV RNA(-) group [p=0.65]. The loss of graft was one patient in both RNA(+) and RNA(-), respectively [p=0.68]. Advanced liver disease was found in 3 patients in the RNA(+) group only [p=0.16].

Conclusions: There was no case of changing management by histological finding before KT or showing advanced liver disease after KT in HCV RNA(-) group. Therefore, we concluded that liver biopsy is not necessary in anti HCV Ab(+) and HCV RNA(-) KT recipient.

PUB830

Incidence of Infection in Patients Continued on Immunosuppression after Allograft Failure Claire T. Kassakian, George P. Bayliss. *Medicine, Rhode Island Hospital, Providence, RI.*

Background: There is little data to guide decisions on whether to continue immunosuppression medications in renal transplant patients whose grafts have failed. Continuation of such regimens can leave patients open to infection, one of the most common causes of mortality in this population. We sought to evaluate the risk of infection from continuing immunosuppression (IS) or stopping it (no IS) in renal transplant patients from a single center whose allografts have failed as well as the types of infections that occurred.

Methods: We reviewed the electronic medical record of 100 renal transplant recipients between 1999 to the present. We included patients described as either on hemodialysis or deceased after restarting dialysis. Patients were excluded if no allograft failure was present prior to death. The primary outcome is the incidence of infections requiring hospitalization.

Results: We identified 18 patients in the IS group and 11 patients in the no IS group. Age at transplant was similar in both groups; there were more men than women in the IS group than in the no IS group. The incidence of any infection was greater in the IS group (72%) than in the no IS group (45%), although not significantly (p=0.24). The incidence of line infections was greater in the IS group than in the no IS group (14% vs 0%, p=0.13), as were incidences of Clostridium difficile infection (7% vs 0%) infectious endocarditis (3% vs 0%), pyelonephritis (7% vs 0%), aspirgilloma (3% vs 0%) and septic arthritis (3% vs 0%). There was no difference in the incidence of urinary tract infections or pneumonia between the two groups.

Conclusions: We found a greater incidence of infections, particularly line infections, in renal allograft failure patients who are continued on immunosuppression after graft failure versus those who are not. Our data also demonstrates that these patients have a higher incidence of other infections as well, although the incidence of pneumonia and urinary tract infections were similar. While not reaching statistical significance, our data point to the need for further study to help define risks and the optimal regimen for discontinuing immunosuppression in patients with failed allografts.

Funding: Clinical Revenue Support

PUB831

Effect of Mycophenolate Mofetil (MMF) on Progression of Chronic Allograft Dysfunction Karlo Mihovilovic,[1] Bojana Maksimovic,[1] Branislav Kocman,[2] Zeljko Vidas,[2] Danica Galesic Ljubanovic,[3] Mladen Knotek.[1] *[1]Division of Nephrology, University Hospital Merkur, Zagreb, Croatia; [2]Division of Surgery, University Hospital Merkur, Zagreb, Croatia; [3]Division of Pathology, University Hospital Dubrava, Zagreb, Croatia.*

Background: Main histological changes that determine chronic transplant dysfunction are interstitial fibrosis and tubular atrophy (IF/TA). Evidence from animal models suggests that MMF may exert a positive effect on renal damage by direct antifibrotic properties due to antiproliferative action on both immune and nonimmune cells. The aim of our study was to investigate role of MMF dose on progression of chronic allograft dysfunction.

Methods: A cohort of 80 patients included patients with kidney, kidney-pancreas and kidney-liver transplantation.Protocol kidney biopsies were scored according to the Banff 07 classification. Interstitial fibrosis (ci) and tubular atrophy (ct) progression was determined by calculating Δci and Δct after subtracting 0 biopsy scores from 1 year chronic scores. Estimated GFR (eGFR) was calculated using Cockroft Gault formula. MMF exposure was determined as average MMF dose over 1 year post transplant, calculated from dose at month 1, 3, 6 and 12. Univariate and multiple regression analyses were done to test relationship between independent variables and eGFR, Δci and Δct.

Results: Average MMF dose during 1 year posttransplant was 2151mg ± 610 (range 1062.5 – 4000). eGFR at 1 year post transplant positively correlated with average MMF dose (p=0.046) while donor and recipient age(p<0.05) negatively correlated with eGFR. Allograft function was also negatively correlated with Δci and Δct (p<0.05). In multivariate analysis average MMF dose remained significant for eGFR at 1 year post transplant (b=0.27±0.1, p=0.01). In univariate analyses both Δci and Δct significantly negatively correlated with average MMF dose (p <0.01). In a multiple regression analyses Δci (b=-0,36 ± 0.11, p<0.01) and Δct (b=-0,40 ± 0,11, p<0.01) were independently only associated with MMF dose.

Conclusions: Higher MMF dose over 1 year is associated with better renal function and slower progression of IF/TA.

Funding: Government Support - Non-U.S.

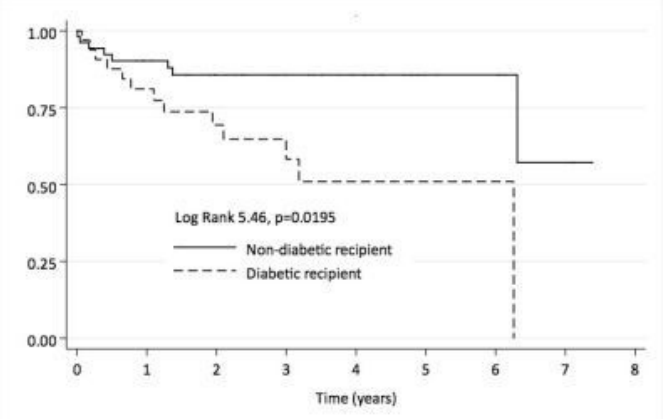
PUB832

Mild Diabetic Changes in Reperfusion Biopsies Do Not Influence Graft Outcomes Eric J. Lai, Heather K. Morris, Sumit Mohan, Michael B. Stokes, Jai Radhakrishnan. *Internal Medicine, Columbia University Medical Center, New York, NY.*

Background: The increasing prevalence of obesity and diabetes mellitus and the greater incentive to expand the donor pool has led to more frequent use of organs from patients with these co-morbidities. We evaluated outcomes in patients who received renal allografts from diabetic donors (DMD).

Methods: We examined 90 patients who received kidney transplants from DMD at Columbia University Medical Center from 2004 onwards. All post-reperfusion biopsies from a DMD were scored and assigned a diabetic nephropathy class. Recipients were then followed to determine whether the findings on the initial post-reperfusion biopsy were predictive of graft outcome.

Results: 86/90 patients in our cohort had adequate follow-up data in which the status of patient and graft survival was known. Among these, 75.6% were alive with a functioning graft at time of last follow-up. Outcomes in diabetic recipients were significantly worse than those in non-diabetic recipients.



The majority of donor kidneys had relatively minor diabetic changes (class I-IIa glomerular lesions and zero or < 25% interstitial fibrosis/tubular atrophy) on the post-reperfusion biopsy. The pathology findings on these biopsies did not influence graft survival.

Conclusions: Our recipients of deceased DMD kidneys fared well with respect to graft and patient survival, although diabetic recipients had significantly worse graft survival than non-diabetic recipients. Pathology findings on the post-reperfusion kidney biopsies did not influence graft survival – probably due to the relatively minor diabetic changes in both the glomerular and tubulointerstitial compartments in the majority of cases. Our data indicate that good outcomes can be achieved with DMD kidneys in non-diabetic recipients, particularly when the glomerular and tubulointerstitial lesions are not advanced.

PUB833

Timely Listing for Kidney Transplantation in England: A Quality Improvement Programme Partha Das,[1] Donal O'Donoghue,[2] Beverley Matthews.[1] *[1]NHS Kidney Care, London, United Kingdom; [2]National Clinical Director for Kidney Care, Department of Health, London, United Kingdom.*

Background: Renal transplantation is the "Gold Standard" modality of renal replacement therapy (RRT). Barriers exist within the National Health Service (NHS) preventing patient access to transplantation and pre-emptive transplantation is only performed in a minority of incident patients. NHS Kidney Care present here the results of a nationally funded and locally led program to improve access to renal transplantation across England.

Methods: All renal units in England were invited to submit a project work profile detailing strategies to overcome barriers to transplantation and measure quality improvement. The successful bids were provided with a grant to assist their projects and for the recruitment of a specialist nurse to act as project lead. Projects began in September 2011 and completed in April 2012.

Results: 20 transplant units in England successfully applied for funding for their projects. Common themes in all projects included a need to refine data collection, formalise care pathways, streamline investigation requesting, educate frontline staff and engage with patients more effectively. NHS Kidney Care supported the projects by providing a regular e-seminar programme, quality improvement workshops and disseminating information on strategies to improve listing for transplantation. An online discussion forum enabled units to share ideas. Most units had difficulties recruiting staff into post due to the fiscal climate. All noted improvements in their transplantation pathways including formation of dedicated multiprofessional assessment clinics, increased numbers of patients with a documented transplant decision, faster completion of investigations and better patient/prospective donor education materials.

Conclusions: This national programme has enabled renal units to improve access to transplantation. The locally led/centrally co-ordinated approach has been successful in terms of implementing change. Though the project period is too short to assess whether there has been an increase in the number of transplants performed, all centres will be followed up in 1 year's time to assess the long term impact of this programme.

PUB834

Safe and Effective Treatment of Persistent Hypercalcemia and Hyperparathyroidism with Cinacalcet in Renal Transplant Recipients Prasad Madhavan Nair, Narayanan Nampoory, Torki Al Otaibi, Osama Gheith, Medhat Abdul Halim, Tarek Said. *Department of Nephrology, Organ Transplant Centre, Hamad Al Essa, Kuwait, Kuwait.*

Background: The calcimimetic, cinacalcet offers an attractive alternative to parathyroidectomy for treating hypercalcemia with persistent hyperparathyroidism in renal transplant recipients (RTR). The objective of this study is to evaluate the efficacy of cinacalcet in RTR with hypercalcemia and persistent hyperparathyroidism and its safety after long term use.

Methods: Cinacalcet at a dose of 30 to 90 mg was prescribed to 15 RTR (8 women, 7 men) with a mean age of of 46.6 years (range = 23 to 68) and hypercalcemia with hyperparathyroidism. Cinacalcet therapy was started at a mean of 35.4 (range=4 to 153) months post transplant and period of follow up after treatment was 20.5 (range = 6 to 54) months.

Results: Treatment with cinnacalcet effectively reduced levels of, serum calcium from 2.70 ± 0.07 to 2.33 ± 0.22 mmol/L in 6 months (P< 0.001) and 2.31 ±0.17 mmol/L in 12 months (P< 0.001); intact parathyroid hormone (iPTH) from 74.8 ± 34.82 to 22.2 ± 12.34 pmol/L in 6 months (P< 0.001) and 19.28 ± 8.08 pmol/L in 12 months (P< 0.001) and raised levels of serum phosphate from 0.92 ± 0.22 to 1.14 ± 0.29 mmol/L in 6 months (P<0.001) and to 1.11 ± 0.26 mmol/L in 12 months (P= 0.001). Renal function remained stable with pretreatment, 6 month and 12 month post treatment serum creatinine levels of 127 ± 72.4, 130 ± 80.45 (P= 0.381), 131.2 ± 95.75 (P= 0.331) umol/L and estimated glomerular filtration rates (eGFR) of 72.6 ± 29.23, 74.6 ± 32.39 (P = 0.406) and 75.82 ± 36.34 (P = 0.816) ml/mt. Immunosuppressant drug levels remained unchanged and there were no rejection episodes or any significant adverse effects described.

Conclusions: Cinacalcet was safe and effective in renal transplant recipients with hypercalcemia secondary to hyperparathyroidism with no evidence of declining renal function or limiting side effects.

PUB835

Impact of the Determination of HLA Antibodies in Kidney Transplant over Ten Years of Monitoring Jacqueline Apaza, Esther Gonzalez Monte, Enrique Morales, Natalia I. Polanco Fernandez, Julie Hinostroza Yanahuaya, Laura Garcia-puente Suarez, Ignacio Bengoa, Amado Andres, J. Morales. *Nephrology, Hospital Universitario 12 de Octubre, Madrid, Spain.*

Background: The goal of this study was to determine the prevalence of anti-HLA antibodies and the clinical outcome in patients with functioning renal graft over ten years.

Methods: We measured the presence and the levels of class I and II anti-HLA antibodies by microbead technology (Luminex) in 120 patients with functioning renal graft over 10 years.

Results: The average time of transplantation was 15, 4±3, 7 years. 25% of the patients studied, had anti-HLA antibodies. The presence of anti-HLA antibodies was associated with worse serum creatinine levels (p 0,03), lower glomerular filtration rate (p 0,02) and increased proteinuria (p 0,00). Antibodies were measured one year after the first determination in patients with positive anti- HLA antibodies. The elevation of these antibodies did not influence the evolution of renal function or degree of proteinuria. (Cr in patients with increased antibody titers compared with those who remained stable was 1, 53 mg/dl vs. 1, 9 mg/dl - p 0.33).

Conclusions: Our study show that in patients with functioning renal graft over ten years, the presence of anti-HLA antibodies was 25%, and this was associated with the development of chronic rejection, worse renal function and greater proteinuria levels, regardless of the evolution of antibody titers at one year. The determination of HLA antibodies could be useful in the management of transplant patients.

PUB836

Effects of Donor-Recipient Gender Mismatch on Intact Parathyroid Hormone (iPTH) Level, Urine Protein/Creatinine Ratio (UPr/Cr) and Kidney Function Sima Terebelo, Ajibola Monsur Adedayo, Ankita B. Patel, Mariana S. Markell. *Medicine/Neprhology, SUNY Downstate Medical Center, Brooklyn, NY.*

Background: Size mismatch between kidney donor and recipient has been variously implicated in causing poorer allograft outcome, or having no effect due to post-transplant adaptation, however studies have looked primarily at size disparities and gender effects have not been carefully studied.

Methods: A retrospective chart review was performed in a random cross-sectional sample of 59 deceased donor kidney transplant recipients attending transplant clinic for whom donor characteristics were available.

Results: Of the recipients 86.4% (51) were black vs 13.6% (8) donors, 64.4% (38) donors were male vs 55.9% (33) recipients. Donor/recipient gender was both male 39% (23), both female 16.9% (10), male donor/female recipient 16.9% (10) female donor/male recipient 24.9% (15). Mean months since transplant was 35 ± 29.5 (range 3 to 183), eGFR by MDRD was 53.87 ml/min ± 19.2 (range 20 to 105). By Kruskal-Wallis test, comparing 3 categories of donor vs. recipient gender, differences were found for creatinine (MM-1.91±0.81 mg/dl, FF-1.32+0.30, MF-1.12+0.22 mg/dl**,** FM-1.80+0.46 mg/dl, p=0.001), iPTH (MM-301.9+215.4, FF-181.7+99.6, MF-114.5+50.3, FM-209.8+151.6, p=0.025) and UPr/Cr (MM-0.80+1.13, FF-0.25+0.12, MF-0.12+0.07 FM-0.26+0.13, p=0.007). There was no significant difference for eGFR by MDRD estimate, recipient age, BUN, albumin, dosage of prograf, hemoglobin level, serum calcium, magnesium, phosphorus, total cholesterol, tacrolimus levels, 25-OH vit D, or donor age. There was no impact of donor race on any of the findings.

Conclusions: In our population, 1. Although males with either gender donor had higher creat values, when calculated as eGFR, which corrects for gender, kidney function did not differ 2. Male to Male recipients had the highest creat, iPTH and UPr/Cr, while male to female had the lowest. 3. The observation that female to male recipients had better values than male to male, suggests that donor/recipient size mismatch does not explain the findings 4. As gender mismatched kidneys had the lowest values for creat, future investigation of the impact of donor gender should be performed.

PUB837

Early Graft Function Is Associated with Long-Term Graft Survival of Kidneys from Expanded Criteria Donors Nassima Smail,[1] Jean Tchervenkov,[2] Steven Paraskevas,[2] Prosanto Chaudhury,[2] Istvan Mucsi,[1] Mazen Hassanain,[2] Dana Baran,[1] Marcelo Cantarovich.[1] *[1]Medicine, McGill University Health Center, Montreal, QC, Canada; [2]Surgery, McGill University Health Center, Montreal, QC, Canada.*

Background: The use of kidneys from expanded criteria donors (ECD) has increased over the past decade. However, ECD transplants are associated with an increased risk of graft loss.

Methods: We studied 280 recipients of KTx from standard criteria donors (SCD), and 243 from ECD, transplanted between January 1990 and December 2006. We analyzed donor and recipient variables.

Results: 10-yr patient survival was similar in recipients of KTx from SCD (81.8%) and ECD (80.8%, p=0.54) with immediate graft function (IGF, defined as a decline in serum creatinine ≥20% on day 1 post-KTx). As well, 10-yr DCGS did not significantly differ in recipients of KTx from SCD (83%) and ECD (76.3%, p=0.17) with IGF. Multivariate analyses showed that predictors of DCGS were serum creatinine at 1 yr (HR: 1.027, CI: 1.02-1.36, p<0.0001), and estimated glomerular filtration rate (eGFR) drop >30% between 1 and 12 months post-KTx (HR: 2.165, CI: 1.12-4.24, p=0.02). There was no difference in 10-yr DCGS in recipients of KTx from SCD or ECD when stratified by IGF (SCD: 87%, ECD: 83.4%, P=0.69) vs. delayed graft function (DGF, defined as the need for dialysis during the first week post-KTx) (SCD: 72.9%, ECD: 74.1%, p=0.61), and by eGFR drop ≤30% between 1 and 12 months post-KTx.

Conclusions: Recipients of KTx from SCD or ECD with IGF have similar long-term patient and DCGS. In addition to serum creatinine, eGFR drop between 1 and 12 months post-KTx is a strong predictor of DCGS. Recipients of KTx from ECD with IGF or DGF whose eGFR decreased ≤30% between 1 and 12 months post-KTx have similar 10-yr DCGS compared to recipients of KTx from SCD.

PUB838

Modelling the Allocation of Deceased Donor Kidneys in Australia Philip A. Clayton,[1] Blair S. Grace,[1] Keith McCullough,[2] Robert Merion,[2] Alan B. Leichtman,[2] Scott Campbell,[3] Jenni Wright,[4] Jeremy Chapman,[4] Stephen P. McDonald,[1] Steven J. Chadban.[1] *[1]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, SA, Australia; [2]Arbor Research Collaborative for Health, Ann Arbor, MI; [3]Princess Alexandra Hospital, Brisbane, QLD, Australia; [4]National Organ Matching System, Sydney, NSW, Australia.*

Background: Donors, their families and the general community expect that scarce deceased donor (DD) kidneys will be allocated fairly and to the best advantage of patients awaiting transplantation. Generation of a model able to simulate current allocation outcomes is an essential first step in exploring alternative allocation systems.

Methods: Data were sourced from the Australia and New Zealand Dialysis and Transplant (ANZDATA) and Organ Donor (ANZOD) Registries and Australia's National Organ Matching System. We adapted the US Scientific Registry of Transplant Recipients Kidney-Pancreas Simulated Allocation Model (KPSAM) software to simulate the allocation of deceased donor kidneys based on current Australian allocation rules which primarily consider waiting time, HLA match and peak PRA.

Results: The simulation included 4146 wait-listed patients and 1882 kidneys from 1043 donors over 06/28/06-12/31/10. There was excellent agreement with actual allocation with no significant differences in recipient age (simulated mean 51.9 vs actual 51.8 years), sex (62% vs 64% male), race, co-morbidity, waiting time (42.0 vs 42.7 months), HLA mismatch or peak PRA. The proportions of kidneys shipped interstate (12.8% vs 14.1%) and allocated to patients previously transplanted (16.1% vs 15.6%) were also well matched. Of the wait-listed patients not allocated a DD kidney the proportion who received a living donor transplant, died or remained alive on dialysis were similar, and the characteristics of those patients at the time of the outcome were very well matched between the simulation and the actual outcomes.

Conclusions: Although KPSAM was developed in the United States it can be adapted to simulate DD kidney allocation in a different country. This provides a valuable tool to explore the potential effects of any changes in allocation rules.

Funding: Government Support - Non-U.S.

PUB839

Screening for Inherited and Acquired Thrombophilia Prior to Renal Transplantation Raquel Melo Silva, Igor Marques, Cinthia Esbrile Moraes, Flavio De Paula, Elias David-Neto. *Renal Transplant Service, University of Sao Paulo - School of Medicine, Sao Paulo, Brazil.*

Background: Renal allograft recipients with thrombophilia are at higher risk for early allograft loss, microvascular occlusion and acute rejection with major consequences for allograft survival.The aim of the present study was to evaluate the prevalence of prothrombotic risk factors in patients awaiting renal transplantation and its contribution to patient and transplant outcomes.

Methods: All patients with a history of a thromboembolic event underwent laboratory screening for thrombophilia.

Results: Since the introduction of the screening for hypercoagulable risk factors, 156 candidates for renal transplantation underwent laboratory evaluation. Eighty-eight patients (56%) exhibited at least one prothrombotic laboratory parameter, besides of isolated hyperhomocysteinemia, which confirmed a thrombophilic state.Among the 156 patients, 30 underwent renal transplantation and were followed for a median of 199 days (range, 9 – 418). Thrombophilia was identified in 16 (53%). Seventeen (57%) received perioperative anticoagulation with unfractionated heparin (9 patients with thrombophilia and 8 without laboratory confirmed thrombophilia). Five (30%) of these patients developed perinephric hematomas. Three patients with thrombophilia developed thrombotic complications (2 upper limbs deep-vein thrombosis and 1 allograft artery thrombosis) and 1 patient without thrombophilia developed allograft vein thrombosis, p=0.35. Nine patients developed acute rejection (5 in the group with thrombophilia and 4 in the group without thrombophilia, p=0.87). Mean glomerular filtration rate was similar between thrombophilic and non-thrombophilic patients in the last follow-up (54±27 vs. 47±22 mL/min/1.73m², p=0.35). One graft loss and 1 patient death were observed in each group.

Conclusions: Prothrombotic risk factors, are highly prevalent in patients awaiting renal transplantation with a clinical or familial history suggestive of thrombophilia. Despite pre-transplant screening and perioperative treatment and/or monitoring, thrombotic and bleeding complications are still frequent and severe.

Funding: Government Support - Non-U.S.

PUB840

Preemptive Living Donor Kidney Transplantation: An Opportunity for the Elderly Amna Daud, Raymond Kang, Anton I. Skaro, Pamela H. Sharaf, John Joseph, Kathleen R. Hoke, Ed Wang, Lee A. Lindquist, Michael Abecassis, Daniela Ladner. *Northwestern University, Chicago, IL.*

Background: Living donor kidney transplantation (LDKT) and especially preemptive kidney transplantation, which is kidney transplantation prior to initiation of dialysis, leads to improved outcomes, better quality of life and greater cost savings. Despite proven benefit of kidney transplantation for elderly who meets operative clearance, surveys have shown that there is reluctance to pursue transplantation and especially LDKT.

Methods: Single center data were analyzed between January 2006 and May 2009. Demographics and donor information were extracted from the electronic medical records.

Results: Between January 2006 – May 2009, 731 kidney transplantations were performed, 451 (62%) were LDKT and 159 (35%) were preemptive. Fifty three recipients were >65 years of age, 40 (75%) received a LDKT and 18 (45%) received it preemptively. Only 14 recipients were > 70 years of age, all but one (93%) received a LDKT and 7 (53%) were preemptive. For preemptive transplants, median days between the first encounter at the center and transplant date was 203 for age >65 vs. 256 days for younger recipients (<65).

Among patients > 65 years of age vs. < 65 years, recipients were predominantly male (72%) vs. (59%), on Medicare (72%) vs. (40%), and non-Hispanic white (55%) vs. (50%). The living donors for elderly recipients (>65 yrs) were predominately children of recipient (35%) vs. sibling (25%) and other non-related donors (30%), while younger recipients primarily received their organs from non-related donors (30%). The most prevalent cause for chronic kidney disease in elderly was hypertension (45%) compared to diabetes (33%) for younger patients.

Conclusions: At our center LDKT rates are high compared to the national average. Our analysis shows that the likelihood of receiving a LDKT and a preemptive kidney transplant increases with age. This suggests timely referral of nephrologists and expeditious work-up at transplant center after referral. LDKT and particularly preemptive kidney transplantation offer an expeditious path to kidney transplant for the elderly sparing them morbid sequelae of prolonged waiting.

PUB841

Intermediate Early Graft Function Is Associated with Increased Incidence of Graft Loss and Worse Long-Term Graft Function in Kidney Transplantation Mário Raimundo, José Guerra, Catarina Teixeira, Alice Santana, Antonio Gomes da Costa. *Nephrology and Kidney Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: Delayed graft function (DGF) is associated with undesired outcomes in kidney transplantation. Regarding early intermediate graft function (IGF) these prognostic observations have not been clearly made. Our objective was to investigate the impact of IGF, as compared to excellent graft function (EGF), on relevant renal allograft outcomes.

Methods: Retrospective observational study. Definitions: DGF-need for dialysis in the first 7 days post-transplantation; EGF-serum creatinine (sCr)<3 mg/dl at 5 days post transplantation; IGF-absence of dialysis need but with a sCr>3 mg/dl at 5 days post-transplantation. Univariate analysis was performed with the Student's t-test, the Mann-Whitney test or the Chi-square test, as appropriate. Kaplan-Meier method was used to determine survival curves and log-rank test for comparison. Multivariate logistic regression analysis was used to determine independent predictors of IGF and of graft survival.

Results: 570 patients were included in the analysis: 69.0% had EGF, 22.6% IGF and 8.4% DGF. Patients with IGF had worse graft survival at 5 and 10 years post-transplantation (75%vs92% and 69%vs85%, respectively;p<0.001 for both comparisons) and higher incidence of acute rejections (AR) (27% vs 41%;p=0.001), compared to patients with EGF. In multivariate analysis, IGF was independently associated with an increased risk of graft loss [Odds ratio (OR) compared to EGF: 2.40;95%CI 1.32–4.35;p=0.004] after adjustment for the occurrence of AR and other covariates. Donor age (OR 1.03 per year; 95%CI 1.02–1.05;p<0.001) was the strongest predictor of the occurrence of IGF. IGF was associated with worse long-term graft function until 7 years post-transplantation (mean GFR 48.3 ± 18.9 vs 57.4 ± 20.4 ml/min/1.73m²;p=0.008).

Conclusions: IGF, as DGF, is associated with increased rate of graft loss and AR and worse long-term graft function. Efforts should be undertaken to minimize the incidence of IGF. Donor age was the strongest risk factor for the occurrence of IGF. This is especially relevant regarding the increasing use of extended donor criteria.

PUB842

Are There Real Advantages of Induction Therapy with Basiliximab in Kidney Transplantation? Catarina Teixeira, Mário Raimundo, José Guerra, Alice Santana, Antonio Gomes da Costa. *Nephrology and Kidney Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: Randomized clinical trials (RCT) have supported the use of interleukin-2 receptor (IL-2R) antagonists as induction therapy in patients with low-moderate immunologic risk. Despite reducing the incidence of acute rejections (AR) this advantage has not been translated into improved graft survival, maybe due to the limited follow-up time of RCT. Our objective was to investigate the impact of induction therapy with basiliximab, as compared to no induction therapy, on relevant clinical outcomes – initial length of stay, incidence of AR, long term graft function and graft survival – in a "real life" clinical setting.

Methods: Retrospective observational study of a 15-year period. Patients who received triple immunosuppression with cyclosporine, mycophenolate mofetil and prednisolone were selected for analysis (n=334) and classified into two groups: G1-no induction therapy (n=131); G2-induction with basiliximab (n=203). Univariate analysis was performed with the Student's t-test, the Mann-Whitney or the Chi-square test, as appropriate. Kaplan-Meier method was used to determine survival curves and log-rank test for comparison.

Results: Mean follow-up time was 72.7±35.4 months. Baseline characteristics were similar between groups. Group 2 had a shorter hospital stay (19.2vs22.5 days;p=0.02), lower incidence of AR (10.8%vs23.7%;p=0.02) and better graft function at 12 months (mean eGFR 59.4±18.4vs54.8±18.7 ml/min/1.73m^2; p=0.015) and 5 years (mean eGFR 64.1±21.5vs55.4±19.6 ml/min/1.73m^2; p=0.009) post-transplantation. There was no difference in graft survival (log-rank: p=0.54).

Conclusions: This study confirms that induction therapy with basiliximab is associated with a reduced incidence of AR and a better long-term graft function. Despite the relatively long follow-up, these advantages have not translated into improved graft survival maybe due to the increasing effectiveness of rescue therapies for AR and the influence of non-immunologic factors. Considering the substantial difference in eGFR at 5 years post-transplantation, a longer follow-up would eventually entail considerable difference in graft survival.

PUB843

Lower Serum Magnesium 1-Year Posttranplantation Is Associated with Decreased Graft Survival in Non-Diabetic Renal Transplant Recipients Camila Hitomi Nihei,[1] Igor Marques,[2] Loyana Teresa Teofilo Lima Silva,[1] Raquel Maria Maia,[1] Bernadete M.C. Ferreira,[1] Elias David-Neto.[2] *[1]Renal Transplant Service, University of Sao Paulo School of Medicine, Sao Paulo, Brazil; [2]Nephrology Division, University of Sao Paulo School of Medicine, Sao Paulo, Brazil.*

Background: Hypomagnesaemia is a known side effect of immunosuppressive regimen, especially calcineurin inhibitors, and has been associated with new onset diabetes after transplantation (NODAT) and decreased graft survival in chronic cyclosporine nephrotoxicity. Proton pump inhibitors-induced hypomagnesaemia has been described recently, although its relevance in renal transplant recipients is still unknown.

Methods: We conducted a single center cross-sectional retrospective study of renal transplantations performed between 2006 and 2011 in order to evaluate the impact of low serum magnesium (Mg) levels in patient and graft outcomes. Serum Mg levels 1-year after transplantation were available for 227 patients. Those with previous diabetes were excluded in order to evaluate the association of hypomagnesaemia and NODAT.

Results: The median follow-up was 1149 days (range, 524–2347). Patients were divided into four groups, based in quartiles of serum Mg levels, and no significant differences were found regarding sex, age, pretransplantation cholesterol, albumin, triglycerides, body mass index, donor age and type, immunosuppressive regimen, calcineurin inhibitors and mTOR-inhibitors trough levels, use of Mg supplements, delayed graft function, acute rejection, HCV infection or NODAT development. Patients with Mg < 1.6 mg/dL (n=56) had a higher frequency of prolonged (> 1 year) PPI use (91% vs. 80%, p=0,05) and a higher glomerular filtration rate in the last follow-up (60±22 vs. 48±22 mL/min/1.73m², p=0.007), when compared to patients with Mg > 2 mg/dL (n=57). Using Cox proportional hazards analyses, the adjusted graft survival was significantly reduced in the low Mg group after 3.4 years posttransplant (50% vs. 55% in the higher Mg group, HR 3.3; p=0.008).

Conclusions: Hypomagnesaemia 1-year posttransplantation, possibly related to prolonged use of PPI, is associated with decreased graft survival independently of the development of NODAT.

PUB844

Evaluating Changes in Kidney Transplantation and Geographic Inequity Since the Final Rule Ashley E. Davis, Sanjay Mehrotra, John J. Friedewald, Anton I. Skaro, Michael Abecassis, Daniela Ladner. *NU Transplant Outcomes Research Collaborative, Northwestern University, Chicago, IL.*

Background: In 1998, the Department of Health and Human Services' Final Rule mandated organ allocation to be geographically equitable. Despite this mandate, geographic inequity persists in kidney allocation. This study assesses the predictive factors of geographic inequity, by examining the kidney transplant system during 2000-2009.

Methods: Retrospective study of US adult End-Stage Renal Disease (ESRD) patients, kidney transplant candidates, and transplant recipients was performed and analyzed by Donor Service Area (DSA) during 2000-2009. Multivariate regression was used to determine significant predictive factors of long waiting times.

Results: During 2000-2009 1,158,928 adults suffered from ESRD, 331,270 listed for kidney transplantation, and 142,549 received a kidney transplant.

58 DSAs were divided into three groups of 19 short (1.3 years), 20 medium (1.7 years) and 19 long (2.5 years) median waiting times. Significant predictors ($p < 0.05$) of long-waiting times were high: ESRD prevalence, waitlisting rates within DSA of residence, and ESRD patient ethnic diversity.

Consequently, long-waiting time DSAs conducted more marginal DDKTs, more living donor kidney transplants, and used more non-locally procured kidneys. These DSAs have higher waitlist mortality and DDKT recipients are at increased risk for graft failure and mortality.

Long waiting time DSAs often (50%) geographically neighbor short waiting time DSAs. In fact, long-waiting time DSAs more frequently educate patients about transplantation and list a higher percentage of ESRD patients. Further, ESRD patients living in long-waiting time DSAs regularly list within their home DSA (94%) while ESRD patients living in short waiting time DSAs tend to list in long-waiting time DSAs (12%).

Conclusions: Geographic inequity continues to exist and affects all regions of the country. Most prominent predictors of long-waiting time were high: ESRD prevalence, waitlisting rates within DSA of residence, and ESRD patient ethnic diversity. Number of transplant centers was not a predictor of long-waiting time.

PUB845

High-Dose I.V. Immunoglobulin (i.v.-Ig) Treatment of Severe Anemia due to Parvovirus Infection in Renal Transplant (R-Tx): Case Report Antonio Di Felice,[1] Emiliana Ferramosca,[1] Giorgio Gallinella,[2] Antonio Santoro.[1] *[1]Nephrology Diaysis Hypertension, S.Orsola-Malpighi University Hospital, Bologna, Italy; [2]Dept of Haematology and Oncological Sciences, University of Bologna, Bologna, Italy.*

Background: Parvovirus is a rare infection, which occasionally involves renal transplantation causing severe anaemia. Few cases are still reported in literature.

Methods: CASE REPORT. A 51 y/o male, with ESRD for polycystic kidney disease, underwent living donor R-Tx in July 7, 2010. No rejection occurred after surgery; immunosuppressive treatment included steroid, Tacrolimus (FK), and Mycophenolic Acid (MPA). Serum creatinine (S-Cr) was stable around 1,3-1,6 mg/dl; Hb (12,5 gr/dl), was normal; FK-levels were 8,0-10,0 ng/ml, with peaks up to 13 ng/ml. After 1 year, the patient was admitted to our hospital for weakness and severe anemia (Hb 5.4 gr/dl). S-Cr at the admission was around 1,2 mg/dl. Haemorrhage was excluded. Viral study showed a rise of Parvovirus-B19 DNA up to 1*10^11 UI/ml, with normal viral IgG/IgM. Bone-Marrow Biopsy was suggestive for Parvovirus infection. The treatment was: RBC-transfusion, erythropoietin (rHu-EPO), MPA withdrawal and Tacrolimus dose reduction. After 2 weeks, persisted anaemia (Hb 5,7) and B19 DNA was still elevated, 3*10^11 UI/ml, with no significant rise of B19-IgM. The patient then started treatment with high-dose i.v.-Ig, 400 mg/Kg/day for 4 days. Few side-effects were recorded: mild fever only for few hours, and a transient slight rise of S-Cr. Afterwards, a slow progressive improvement of anaemia and reduction of rHu-EPO dose, up to complete withdrawal after 5 months, while B19 DNA persisted elevated. Only after 6 months we recorded a significant reduction of B19-DNA (2*10^5 UI/ml) and the appearance of anti-Parvovirus IgM (5.5). To date, after 8 months, Hb is 11.9 gr/dl, S-Cr 1.44 mg/dl, FK-level 6.9 ng/ml, Parvovirus B19 DNA 4*10^4 UI/ml.

Conclusions: B19 infection must be suspected when a R-Tx patient presents an otherwise inexplicable anaemia. High-dose i.v.-Ig can be useful and safe in the treatment of this infection, but virus disappearance is slow. More cases and a longer follow-up are needed to clarify viral transmission, and outcome.

PUB846

Urine Neutrophil Gelatinase-Associated Lipocalin as a Predictor of Recovery from Delayed Graft Function after Kidney Transplantation Kazuhide Saito,[1] Noriko Saito.[2] *[1]Division of Urology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan; [2]Department of Nephrology, Shinraku-en Hospital, Niigata, Japan.*

Background: Urine neutrophil gelatinase-associated lipocalin(UNGAL) is reported to be a sensitive and useful biomarker for acute kidney injury. We have tested its advantage for perioperative management both in living and deceased kidney transplantation.

Methods: 26 living kidney transplant recipient(LKR) and 26 living kidney donor(LKD), 5 deceased kidney transplant recipient(DKR) and 2 deceased donor(DKD) were enrolled in this study. Urine samples were collected and UNGAL level was measured

by chemiluminescence immunoassay(CLIA) using ARCHITECT Urine NGAL (Abbot Japan). Other standard clinical parameters were also evaluated.

Results: Preoperative UNGAL(ng/ml) in LTD was 9.8±14.6, it increased to 19.0±25.0 on POD 1 and 16.5±25.0 on POD7. Postoperative UNGAL in LKR without acute rejection or surgical/urological complication was 59.3±43.8 on POD2, 39.1±26.0 on POD3 and remain stable during 4 weeks. UNGAL in cases with urological complication or acute rejection was elevated than that of other patients. Elevated UNGAL was also observed in patients with residual urine secretion from native kidney within a few days after transplantation.

In 4 of 5 DKR, DGF occurred. 3 were from non-heart beating donors(NHBD) and one from brain dead donor. UNGAL of DGF patients were significantly elevated with maximum level over 10,000, however, they all could leave HD from 11 to 18 POD, when UNGAL decreased less than 1,000. In the patient with immediate function in DKR, UNGAL level achieved less than 100 ng/ml within a few days after transplantation. ROC decision plot curve analysis showed cut off value of 60.3ng/ml was the point that both sensitivity and specificity achieved 100%.

Conclusions: UNGAL is an effective biomarker for DGF and AKI after kidney transplantation. It is useful not only to predict recovery from DGF in DKR, but also to evaluate postoperative renal function in LKD as well as in LKR.

Funding: Government Support - Non-U.S.

PUB847

Incidence of Metabolic Syndrome in the Second Year of Kidney Transplant and Its Effect on Graft Function Alejandro Concepcion Orozco Jimenez, Jorge Andrade-Sierra. *Nephrology and Transplant, IMSS, Gudalajara, Jalisco, Mexico.*

Background: Metabolic Syndrome (MS) is a risk factor to kidney disease. The MS during the second year of kidney transplant (KT) has not been completely evaluated and the possible impacts on graft function.**Objective:** Describe the incidence of MS in three different groups (MS free, One evaluation and Persistent), 24 months after KT and its impact on graft function according to time of development.

Methods: Prospective cohorts (Mar/2009-Apr/2011) of 197 kidney recipients, diabetic patients before transplant were excluded. MS was defined using the ATP III criteria adjusted to Hispanic population.

Results: The accumulated incidence of MS at 6, 12, 24 months were 20, 22, 34% repectively. Abdominal size, CRP and tryglicerides were significantly different among groups. 121 (61%) were MS free during the follow up; 10 patients had MS during the follow. Other results are shown in table.

Comparison of clinical and biochemical variables at 24 months post-KT according to exposure to MS

Variable	MS Free	One Evaluation	Persistent
MS N(%)	39 (61)	25 (39)	133 (68)
Mean age (yr)	38.4 ± 10.2	36 ± 11.3	39.5 ± 9.8
Size (cm)	163.44 ± 8.9 <<	165.07 ± 8.9 *	175.1 ± 9.9*<<
BMI	22.61 ± 3.4	29.71 ± 4.9	27.77 ± 2.96
Waist (cm)	81.37 ± 10.39 << >>	91.8 ± 10.5>>	101.55 ± 9.2 <<
Sistolic blood pressure (mmHG)	125.75 ± 15.03<<	133.15 ± 15.75	149.3 ± 20.21<<
Diastolic blood pressure (mmHG)	78.44 ± 11.89	81.58 ± 11.37	88.6 ± 9.38
Medium blood pressure (mmHG	51.09 ± 47.98<< >>	79.27 ± 40.90>>	108.9 ± 11.69<<
Triglycerides (mg/dl)	123.19 ± 70.05 << >>	184.63 ±99.22>>	189.72 ± 98.38<<
HDL (mg/dl)	52.43 ±12.82 <<	42.53 ± 9.25<<	35.5 ± 5.6<< >>
CRP (mg/l)	5.3 ± 8 <<	8 ± 13 <<	6.2 ± 7.6 <<>>
GFR (ml/min/ 1.73m2)	73.3 ± 27.5	81.2 ± 19.6	68.8 ± 11.7

P<0.05, p significant when compared with an assessment against persistent SM; << p significant when comparing never against persistent; >>p significant when compared against an evaluation never.

Conclusions: 5% of our patients persisted with MS and 61% were MS free at 2 year follow up, the latter is similar with the current literature in MS. Time of exposure to MS could affect the result in renal function.

PUB848

Predictors of Donor Kidney Discard: A Single DSA Experience Edward Wang, Anton I. Skaro. *Comprehensive Transplant Center, Northwestern University, Chicago, IL.*

Background: There is a severe donor organ shortage in the US resulting in high kidney transplant waitlist mortality. Augmentation of the donor pool should entail minimizing kidney discards. However there are limited data on kidneys that are discarded prior to transplant. We sought to examine risk factors for kidney discard in a single donor service area (DSA).

Methods: We examined data prospectively collected by the Gift of Hope from 1996-2011 on all donors considered for kidney procurement (n=7,933). We conducted a comparative analysis of donor characteristics for kidneys that were discarded and those that were transplanted. Data were analyzed using univariate and multivariate logistic regressions in SAS 9.2.

Results: A total of 1,090 kidneys (13.7%) were discarded during the time period studied. The leading reason for organ discard was biopsy findings (66.0%) at the time of procurement. Compared to organs that were not discarded, the donors of kidneys discarded were older (54.4 ±15.8 vs. 37.1 ± 18.3; p<0.001), more likely to be female (48.9% vs. 38.4%, p<.001), had a greater BMI (19.8 ±5.3 vs. 18.6 ± 4.9; p<0.001) and consisted of a higher proportion of African-American (26.9% vs. 23.6%; p<0.001) and Asian race (2.2% vs. 1.3%, p<.05). Moreover, a higher proportion of donors with organ discards had cerebrovascular/stroke (64.1% vs. 35.3%; p<0.001) as a cause of death and DCD (13.2% vs. 9.5%; p<0.001). In the univariate analysis, older age (≥65) (OR=5.39, 95% CI 4.56-6.36), higher BMI (OR=1.04, 95% CI 1.03-1.06), female (OR=1.53, 95% CI 1.35-1.74), Asian or African-American (OR=1.45, 95% CI 1.02-2.88) donors, DCD (OR=1.45, 95% CI 1.19-1.76), and stroke (OR=5.03, 95%CI 4.22-5.98) were associated with greater odds of discard. In the multivariate model, all remained significant predictors of kidney discard.

Conclusions: Donor age, BMI, gender, race, and circulatory status all appear to influence kidney utilization. Despite perceived lower organ quality, broader sharing of rare kidney resources with competitive DSAs might reduce kidney discard. These data may have implications on organ availability for kidney transplantation as the US population increases in age and accumulates associated comorbidity.

PUB849

Is Living Donor Nephrectomy an Acute Kidney Injury? José Guerra, Mário Raimundo, Catarina Teixeira, Alice Santana, Antonio Gomes da Costa. *Nephrology and Renal Transplantation, Hospital de Santa Maria, Lisbon, Portugal.*

Background: Acute Kidney Injury (AKI), defined and stratified by the Risk, Injury, Failure, Loss and End-Stage (RIFLE) criteria, has been associated with increased risk of cardiovascular disease, development of chronic kidney disease and short and long-term mortality. Living donor nephrectomy results in an "acute" loss of about 50% of the functional renal mass. We applied the concept of AKI to the living donor nephrectomy in order to quantify its incidence and impact on long-term kidney function.

Methods: We evaluated sequential renal function in 50 living donors without perioperative complications. Serum creatinine (sCr) was collected at three time points: before nephrectomy (T0), early post-nephrectomy (T1) - the highest value in the first 7 days - and one year after nephrectomy (T2). Glomerular Filtration Rate (GFR) was estimated by the CKD-EPI equation. AKI was defined and stratified by the RIFLE criteria.

Results: The 50 living donors had a mean age of 46.42 ± 11.1 years, 90% were Caucasian and 70% female. Mean length-of-stay was 7.1 ±1.6 days. Using the GFR criteria 76% of patients developed AKI Risk, 14% AKI Injury and none developed AKI Failure. eGFR was significantly lower 1 year after transplantation compared to pre-nephrectomy eGFR.

Serum creatinine and eGFR at T0, T1 and T2

	T0	T1	T2	p value*
sCr (mg/dl)	0.75±0.14	1.13±0.24	1.08±0.22	<0.001
eGFR (ml/min/1.73m2)	110.9±18.3	67.4±13.2	71.6±15.9	<0.001

* Friedman Test

Conclusions: Living donor nephrectomy leads to an early deterioration of renal function that fits the currently accepted AKI criteria. Kidney function improves during the first year post-nephrectomy but does not return to baseline values, representing, approximately, an average loss of 30 ml/min/1.73m^2. This may eventually lead to a progressive loss of function on the long-term and the criteria for living donor transplantation should be revised in a more restricted perspective.

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KEYWORD INDEX

The number refers to the location of the body of the abstract in the publication section.

HS-OR01

The Pathological Role of FGF23 in the Cardiorenal Syndrome Is Mediated by the Activation of Calcineurin/NFAT Signaling in the Heart Ansel P. Amaral,[1] Alexis J. Sloan,[1] Saurav Singh,[1] Ming Chang Hu,[2] Orson W. Moe,[2] Makoto Kuroo,[2] Giovana S. Di Marco,[3] Marcus Brand,[3] Myles S. Wolf,[1] Christian Faul.[1] *[1]Nephrology and Hypertension, Medicine, University of Miami Miller School of Medicine, Miami, FL; [2]Internal Medicine and Pathology, University of Texas Southwestern Medical Center, Dallas, TX; [3]Internal Medicine, University of Münster, Münster, Germany.*

Background: Cardiovascular disease is the primary cause of death in chronic kidney disease (CKD), and left ventricular hypertrophy (LVH) is an important mechanism in this context. Patients with CKD develop marked elevations in circulating levels of the phosphorus-regulating hormone, fibroblast growth factor (FGF) 23, and work from our group has shown that rates of LVH are elevated in CKD, and that elevated FGF23 is independently associated with LVH.

Methods: In a recent experimental study, we demonstrated a direct causal role for elevated FGF23 in the pathogenesis of LVH. We show that FGF23 directly induces pathological hypertrophy of isolated cardiac myocytes, and that multiple mouse models for elevated serum FGF23 levels develop LVH. Most importantly, administering an FGF receptor blocker to the 5/6 nephrectomy rat model of CKD attenuated the severity of LVH without reducing the animals' markedly elevated blood pressure. This data suggests a novel mechanism to explain the high rates of LVH in patients with CKD.

Results: Our ongoing experiments aim to identify the precise signal transduction pathways in the heart that are activated by FGF23, in order to screen for novel drug targets to interfere with LVH in the Cardiorenal Syndrome. Our novel data indicate, that FGF23 activates nuclear factor of activated T-cells (NFAT) in isolated cardiac myocytes as well as in the mouse heart upon i.v. injection. In contrast, FGF23 does not cause an activation of Ca^{2+}/calmodulin dependent kinase II or protein kinase C, two other major branches of Ca^{2+}-mediated pro-hypertrophic signaling.

Conclusions: Since cyclosporine A treatment of 5/6 nephrectomized rats inhibits the development of LVH, the calcineurin/NFAT signaling axis appears to function as the major mediator of FGF23's cardiac effects.

Funding: NIDDK Support

HS-OR02

Molecular Mechanisms of Cadmium (Cd) Dependent Fibroblast Growth Factor 23 Secretion in Bone Shinsuke Kido, Marina Fujihara, Kengo Nomura, Shohei Sasaki, Yuji Shiozaki, Hiroko Segawa, Sawako Tatsumi, Ken-ichi Miyamoto. *Department of Mocular Nutrition, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan.*

Background: Fibroblast growth factor 23 (FGF23) is a bone-derived phosphaturic factor and is known to regulate blood inorganic phosphate (Pi). Medication such as infusion of select iron-containing compounds increases serum FGF23 concentrations, with patients developing hypophosphartemia. In autosomal dominant hypophosphatemic rickets (ADHR) patients, low serum iron was correlated with elevated serum FGF23 concentration. In animal study, cadmium (Cd) administration increases serum FGF23 concentration. Thus, a relationship between metal ion and FGF23 metabolic pathway has been proposed.

Methods: In this study, we investigated the molecular mechanism of FGF23 production by Cd.

Results: Cd injection to mice increased plasma FGF23 concentrations, but FGF23 mRNA expression in bone was not changed. Further studies indicate that increased plasma FGF23 levels in the Cd-injected mice are caused by the post-translational events of the FGF23 protein. GalNAc-T3 is known to be involved in secretion of intact FGF23. To determine potential roles of GalNAc-T3 in Cd-induced FGF23 production, we examined the effect of Cd on the GalNAc-T3 mRNA expression in vivo and in vitro. In Cd-injected mice, GalNAc-T3 gene expression was significantly increased in bone. Cd also enhanced the expression of GalNAc-T3 to cultured osteosarcoma UMR106 cells. Interestingly, we found that Cd activates aryl hydrocarbon receptor (AhR), suggesting the requirement of AhR for the induction of the GalNAc-T3 gene expression by Cd. In addition, Cd enhanced the transcriptional activity of the GalNAc-T3 gene through AhR. AhR siRNA markedly suppressed the stimulation of transcriptional activity by Cd. Moreover, Cd-dependent FGF23 production was inhibited by AhR antagonist. Thus, Cd stimulates GalNAc-T3 gene transcription via an enhanced AhR binding to the GalNAc-T3 promoter.

Conclusions: The present study suggests that the elevation GalNAc-T3 by Cd suppress proteolytic processing of FGF23 and stimulates the elevation of serum FGF23 concentrations.

HS-OR03

T-Cell Autophagy Is Required for Transplantation Tolerance Divya Anna Verghese, Peyman Bizargity, Anju Yadav, Barbara T. Murphy, Bernd Schroppel. *Nephrology, Mount Sinai School of Medicine, New York, NY.*

Background: In order to be effective, tolerance induction regimens require the removal by cell death of potentially destructive alloreactive T cell clones. We investigated the role of autophagy, a pathway utilized by cells during nutrient or growth factor deprivation that can also contribute to cell death, in the induction of transplant tolerance.

Methods: Peripheral transplant tolerance was induced using costimulation blockade with anti-CD154 (MR1) and donor specific transfusion (DST). Chemical and genetic strategies were used to inhibit autophagy.

Results: We found that autophagy is required for peripheral transplantation tolerance induced by costimulation blockade with DST-MR1. Additionally, we observed that tolerance induction with DST-MR1 led to the induction of autophagy in graft infiltrating immune cells. Upon chemical inhibition of autophagy using the phosphoinositide 3-kinase (PI3K) inhibitor 3-methyladenine (3-MA), transplant tolerance was abrogated. The administration of 3-MA at a later time after transplant had no effect, indicating that autophagy was important in the induction of tolerance but not in its maintenance. We confirmed the importance of autophagy in the induction of tolerance using a mouse model with the genetic disruption of the autophagy-related gene (Atg) *beclin*1. We found that *beclin*$1^{+/-}$ recipients displayed high anti-donor immune responses reflected by increased intragraft $CD4^+$ T-cell infiltration and elevated IFNγ production. Adoptive transfer of $Rag^{-/-}$ bone marrow into *beclin*$1^{+/-}$ recipients demonstrated that immune cell-derived autophagy was required to induce tolerance. Furthermore, activated T cells with *beclin*1 deficiency had enhanced survival. Using conditional knockout models where Atg7, a critical protein for autophagy, was specifically deleted in T or B cells, we found that autophagy-sufficiency in T cells but not in B cells was required to achieve peripheral tolerance.

Conclusions: These findings highlight the importance of autophagy as a key mechanism to reduce the allospecific T-cell pool. We postulate that autophagy presents a putative target to facilitate transplant tolerance.

HS-OR04

Absence of Adenylyl Cyclase 6 Is Markedly Protective in Polycystic Kidney Disease in Mice Donald E. Kohan, Karl P. Roos, Kevin A. Strait, Sara Rees. *Division of Nephrology, University of Utah, Salt Lake City, UT.*

Background: Cyclic AMP is an important mediator of renal cyst formation and fluid secretion in autosomal dominant polycystic kidney disease (PKD), however it is unknown which adenylyl cyclase (AC) isoforms are involved in PKD.

Methods: Mice were generated with collecting duct-specific knockout (CD KO) of either the *KD1* gene, the Adcy6 gene, or both using mice with loxP-flanked target alleles bred with mice transgenic for the aquaporin-2 promoter driving Cre recombinase expression.

Results: CD PKD1 KO results in polycystic kidneys that are first evident at 1-2 weeks postnatal. Mice with CD PKD1 KO lived an average of 48 ± 6 days (N=42). Coincident heterozygous deletion of AC6 increased life span to 94 ± 11 days (N=24), while coincident homozygous deletion of AC6 (double CD PKD1 and AC6 KO) further increased life span to at least 147 ± 20 days (N=21) (18 double KO mice remain alive beyond 200 days of age). As compared to PKD1 KO, double PKD1/AC6 KO mice had reduced renal cyst size, cyst number, renal cell proliferation and apoptosis, kidney size and weight, and BUN when all mice were analyzed at 4 weeks of age. Coincident AC6 KO mice had increased urine volumes. Interestingly, double PKD1/AC6 KO did not reduce 24-hr urinary cAMP excretion as compared to PKD1 KO alone. Western analysis of whole kidneys revealed that phosphorylated B-Raf and MEK1/2 protein levels were reduced by coincident AC6/PKD1 KO as compared to PKD1 KO alone.

Conclusions: AC6 is an important mediator of renal disease progression in a mouse model of PKD. This pathogenic effect of AC6 may relate more to AC isoform activation of specific signaling pathways rather than simply a reduction in renal cell cAMP production.

Funding: Veterans Administration Support

HS-OR05

Wnt Signaling Is Required for Kidney Regeneration from Adult Zebrafish Nephron Progenitor Cells Caramai Nanae Kamei,[1,2] Yan Liu,[1,2] Iain A. Drummond.[1,2] *[1]Nephrology, Massachusetts General Hospital, Charlestown, MA; [2]Genetics, Harvard Medical School, Boston, MA.*

Background: In contrast to mammalian kidney regeneration, zebrafish can not only repair existing nephrons but are also capable of de novo generation of new nephrons from adult organ progenitor cells. Activation of both repair mechanisms is likely to be signaled by positive and negative growth regulators that ultimately restore normal kidney function. We report here that Wnt signaling plays a role in adult zebrafish kidney regeneration.

Methods: In situ hybridization as well as a transgenic reporter line expressing gfp in regenerating lhx1a-positive nephron progenitors was used to visualize regeneration in response to acute gentamicin injury. Wnt signaling was perturbed using the chemical activator BIO and the inhibitor XAV939.

Results: Both individual lhx1a-positive adult kidney progenitor cells and differentiating cell condensates are specifically marked by expression of the Wnt receptor frizzled9b (fzd9b). Active Wnt signaling was detected in differentiating nephron progenitors by expression of the canonical Wnt target lef1. Acute injury to the adult zebrafish kidney by gentamicin injection expanded the population of fzd9b-positive nephron progenitors. Pharmacological blockade of Wnt signaling reduced progenitor cell expansion after injury while activation of Wnt signaling was sufficient to expand fzd9b/lhx1a-positive progenitors in the absence of injury.

Conclusions: Our results demonstrate a role for Wnt signaling in adult zebrafish kidney regeneration and identify fzd9b as a new marker of adult kidney progenitor cells. Our results open new avenues to investigate the developmental origins and regenerative potential of nephrogenic stem cells.

Funding: NIDDK Support

Underline represents presenting author/disclosure.

HS-OR06

Missense Mutation in Cell Cycle Gene FSGS7 Is a Cause of Autosomal Dominant FSGS Rasheed A. Gbadegesin,[1] Gentzon Hall,[2] Guanghong Wu,[2] David Howell,[5] Mario Schiffer,[3] Michelle P. Winn.[2] *[1]Pediatrics, Duke University, Durham, NC; [2]Medicine, Duke University, Durham, NC; [3]Nephrology, Hannover Medical School, Hannover, Germany; [4]Pathology, Duke University, Durham, NC.*

Background: Focal and segmental glomerulosclerosis, a condition that is characterized by segmental scarring of the kidney, is a leading cause of kidney failure. Identification of genes causing familial FSGS has improved our understanding of the disease mechanisms and points to defects in the glomerular epithelial cell, the podocyte, as a major factor in the pathogenesis of the disease.

Methods: We identified a family with autosomal dominant FSGS with nine affected individuals and carried out genome-wide linkage analysis (GWLA) using the Illumina Infinum II HumanLinkage-24 beadchip genotyping assay, performed whole-exome sequencing and confirmed all identified novel variants by Sanger sequencing.

Results: We identified a missense mutation *R431C* in *FSGS7* an F-actin binding cell cycle gene as a new cause of FSGS. The mutation segregates with the disease in the family and we did not find the change in 1600 control chromosomes. The change is conserved in evolution and it is predicted to be deleterious by *in-silico simulation*. *FSGS7* is upregulated in proliferating podocytes in kidney biopsies from individuals with collapsing FSGS. Knockdown of *FSGS7* in zebrafish recapitulated the human phenotype. Additionally, *FSGS7* gene deficiency in a human immortalized podocyte cell line resulted in reduced podocyte motility.

Conclusions: Collectively, these findings suggest that *FSGS7* is important in maintaining the podocyte actin cytoskeleton and emphasizes the importance of cell proliferation, cell migration and filopodia formation in the pathogenesis of FSGS. Molecular dissection of the pathways by which *FSGS7* cause FSGS will provide insight into the mechanisms of podocyte renewal in health and disease.

Funding: NIDDK Support, Private Foundation Support

HS-OR07

Endophilin, a Functional Partner of Dynamin and Synaptojanin 1 at Synapses, Is Required for Podocyte Function in the Kidney Keita Soda, Xuefei Tian, Sung Hyun Son, Shuta Ishibe. *Internal Medicine, Section of Nephrology, Yale University, New Haven, CT.*

Background: The importance of clathrin-coated pits and vesicles that are observed in podocyte foot processes, is currently unknown. We have initially identified synaptic proteins, dynamin and synpatojanin1, which when lost in mice, result in severe proteinuria and foot process effacement. These two proteins also interact with Myo1e, CD2AP, and Nck, which have been shown to be causal for nephrotic syndrome in humans and or mice. Here, we identified endophilin, an interacting protein of dynamin, synaptojanin1, and CD2AP, that is recruited to the necks of endocytic clathrin-coated pits, and is necessary for podocyte function in the kidney.

Methods: Wild type and constitutive *endophilin 1,2,3* triple knock out (TKO) mice were analyzed to deteremine the role of endophilin *in vivo*. To assess the temporal and spatial dynamics in podocytes, fluorescently tagged endophilin in relation to clathrin or actin was visualized, using time lapsed spinning disc confocal microscopy.

Results: Immunofluorescence staining of kidney sections from mice revealed endophilin expression in podocytes, which colocalized with nephrin. Histological examination of the *endophilin* TKO kidney cortex demonstrated mesangial matrix accumulation in the glomeruli and dilated tubules with proteinaceous casts. Urine obtained from these mice revealed robust proteinuria (Albumin/creatinine ratio (μg/mg); control 15±7 vs. TKO 1045±220, n=5, p<0.001). Severe foot process effacement with a thickened glomerular basement membrane was also observed by EM at birth. *In vitro*, we observed endophilin arriving at late stage clathrin-coated pits along with CD2AP in podocytes. Moreover, endophilin, as well as dynamin and synaptojanin1, colocalized with F-actin at clathrin-coated pits in podocytes.

Conclusions: Our results emphasize a striking recapitulation of a fundamentally conserved mechanism linking a protein network at neuronal synapses that operates at the interface of the endocytic machinery and the actin cytoskleleton, to also form and maintain the kidney glomerular filtration barrier.

Funding: NIDDK Support

HS-OR08

Feasibility of Repairing GBM Defects in Alport Syndrome Jeffrey H. Miner, Xiaobo Lin. *Renal Division, Washington University Sch. of Medicine, St. Louis, MO.*

Background: The glomerular basement membrane (GBM) is a crucial component of the glomerular filtration barrier (GFB). The GBM's major components are the collagen IV α3/4/5 network, laminin-521, nidogen-1 and -2, and agrin. Of these 9 components, mutations that affect 4 of them cause human kidney disease: *COL4A3*/*A4*/*A5* mutations cause Alport syndrome, and *LAMB2* mutations cause Pierson syndrome/congenital nephrotic syndrome. The existence of well-characterized mouse models make these diseases especially attractive for investigating therapies. Several groups have explored the potential for cell-based therapies aimed at replacing the missing collagen IV network in Alport mice. Although the positive effects of bone marrow and other cell transplants or infusions on progression of kidney disease are promising, the fate of the infused cells and the reported effects of these procedures on the GBM's collagen IV network have been disparate, difficult to interpret, and controversial.

Methods: We have used podocyte-specific and ubiquitously expressed reverse tetracycline transactivator (rtTA) transgenic mice and a novel $(tetO)_7$-regulated collagen IV α3 transgene in *Col4a3*$^{-/-}$ Alport mice to attempt to induce collagen IV network restoration either before or after GBM maturation. We have generated novel nephrin-rtTA-3G transgenic mice with improved podocyte-specific doxycycline-induced gene activation.

Results: Prenatal induction of Col4a3 expression by doxycycline in *Col4a3*$^{-/-}$ Alport mice decreased proteinuria and BUN and greatly extended life span by promoting collagen α3/4/5(IV) deposition and assembly in the GBM. Postnatal induction of Col4a3 expression at P14 also promoted collagen α3/4/5(IV) network assembly, with significant improvements in proteinuria, BUN, and life span. Even induction starting as late as P18 improved kidney function by reducing BUN.

Conclusions: 1) Postnatal incorporation of collagen α3/4/5(IV) into the mutant Alport GBM is feasible. 2) Even partial restoration of the collagen IV network slows progression to ESRD. The results of these feasibility studies have implications not only for treating human kidney disease, but also for our basic understanding of BM biology, GBM plasticity, and cell/matrix interactions.

Funding: NIDDK Support

HS-OR09

Disruption of PHD2 in the Kidney Results in HIF-2 Dependent Polycythemia Pinelopi P. Kapitsinou, Volker H. Haase. *Nephrology, Vanderbilt University Medical Center, Nashville, TN.*

Background: Polycythemia is often associated with increased erythropoietin (EPO) synthesis and defective oxygen sensing. Among the three prolyl hydroxylase domain containing proteins (PHD1-3), which serve as oxygen sensors and regulate Hypoxia Inducible Factors (HIFs), PHD2 has been identified as the critical enzyme controlling renal EPO production. We have previously demonstrated that the induction of renal Epo under low oxygen conditions is mediated by Hif-2. To what degree Hif-2 mediates the effects of PHD2 on Epo synthesis remains to be defined however. Here, we used a genetic approach to dissect the contribution of HIF-2 in a novel model of renal Epo-driven erythrocytosis that results from PHD2 inactivation in renal Epo producing (REPO) cells.

Methods: To delete Phd2 and/or Hif-2 in the kidney, P3ProCre transgenics were crossed to mice carrying conditional Phd2 and/or Hif-2 alleles (P3-Phd2$^{-/-}$, P3-Phd2$^{-/-}$Hif2$^{-/-}$).

Results: While mice lacking one copy of Phd2 (P3-Phd2$^{+/-}$) appeared normal, deletion of both Phd2 alleles (P3-Phd2$^{-/-}$) resulted in a) premature mortality (60% mortality at 10wks, P=0.001), b) decreased body weight (14g vs 24g, n=6-7, P<0.001) and c) excessive erythrocytosis (Hct; 75±1.6% vs 50±1.2 %, n=11-12, P<0.0001). Serum Epo levels were increased by ~60-fold in P3-Phd2$^{-/-}$ mutants compared to controls (36332±6853pg/ml vs 573±136pg/ml, n=11-12, P<0.0001), which associated with a ~60-fold increase in renal *Epo* mRNA (n=3-5, P=0.04). FACS analysis revealed a significant increase of CD71+TER119+ erythroid progenitors specifically in the spleen (4.6-fold, P<0.001) but not bone marrow. Furthermore, kidneys of P3-Phd2$^{-/-}$ mutants showed stabilization of Hif-1 and Hif-2 proteins and increased expression of Hif target genes such as *Glut1*. Simultaneous inactivation of Hif-2 (P3-Phd2$^{-/-}$Hif2$^{-/-}$) resulted in anemia associated with deficient Epo production and augmented Hif-1 signaling as previously described for P3-Hif2$^{-/-}$ (*Kapitsinou, Blood 2010*).

Conclusions: Our data establish HIF-2 as the critical HIF isoform regulating EPO synthesis in Phd2 deficient REPO cells. Our findings identify the PHD2/HIF-2 axis as a specific target for therapy aiming at increasing endogenous renal EPO production.

Funding: NIDDK Support

HS-OR10

Insights into ANCA-Associated Vasculitis Provided by the First Genome-Wide Association Study Kenneth G.C. Smith. *Cambridge Institute for Medical Research and the Department of Medicine, University of Cambridge, Cambridge, United Kingdom.*

Background: Presented on behalf of the European Vasculitis Genetics Consortium.

Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) encompasses two major syndromes – Granulomatosis with Polyangiitis (Wegener's) (GPA) and Microscopic Polyangiitis (MPA) – both of which are significant causes of renal failure. We examined their genetic underpinning by performing the first genome-wide association study (GWAS) in AAV.

Methods: Discovery cohort 1,233 UK AAV patients, 5,884 controls. Replication cohort 1,454 Northern European cases, 1,666 controls. Quality control, population stratification and statistical analysis used standard criteria. Further analysis of the discovery cohort used the Immunochip (Cortes A, Brown MA. Arth Res Ther. 2011;13:101).

Results: MHC and non-MHC associations with AAV were found, and GPA and MPA were genetically distinct. The strongest genetic associations were not with clinical syndrome but with the antigenic specificity of ANCA. Anti-proteinase 3 (PR3) AAV associated with *HLA-DP, SERPINA1 and PRTN3*. Anti-myeloperoxidase AAV associated with HLA-DQ (Lyons PA et al. NEJM 2012;367:214-223). We used the Immunochip to analyse the discovery cohort, allowing the imputation of classical MHC alleles. 3 independent alleles contribute to the risk of anti-PR3 AAV at the HLA-DP locus, one conferring susceptibility and two resistance. Two alleles contribute to the risk of anti-MPO AAV at HLA-DQ, one conferring susceptibility and one resistance. Novel SNP associations outside the MHC were also identified by the Immunochip, and are being replicated.

Conclusions: This study has confirmed a genetic component to AAV , demonstrated that GPA and MPA are genetically distinct diseases better defined by ANCA specificity than clinical syndrome, and shown that the response to the autoantigen PR3 (encoded by

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PRTN3) is a central aetiological feature of PR3-AAV. Further detailed analysis of the MHC association of both PR3- and MPO-AAV has been possible using the Immunochip, which has also uncovered further susceptibility loci. Thus, from a genetic standpoint, PR3- and MPO-AAV must be considered as distinct autoimmune syndromes.

Funding: Government Support - Non-U.S.

HI-OR01

The Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints (ALTITUDE) Hans-Henrik Parving,[1] Barry M. Brenner,[2] John Mcmurray,[3] Dick de Zeeuw,[4] Steven Mark' Haffner,[5] Scott D. Solomon,[2] Nish Chaturvedi,[6] Frederik I. Persson,[7] Akshay Suvas Desai,[2] Maria Nicolaides,[8] Marc A. Pfeffer.[2] *[1]Rigshospitalet, University of Copenhagen, Denmark; [2]Harvard Medical School; [3]University of Glasgow, United Kingdom; [4]University of Groningen, Netherlands; [5]University of Texas, TX; [6]Imperial College London, United Kingdom; [7]Steno Diabetes Center, Denmark; [8]Novartis Pharma AG, Switzerland.*

Background: The macro- and microvascular complications of type 2 diabetes are augmented in those with concomitant kidney and/or cardiovascular disease. ALTITUDE was undertaken to determine whether use of the direct renin inhibitor aliskiren would improve prognosis by reducing fatal and non-fatal cardiovascular and renal events in type 2 diabetic patients at high risk for these complications.

Methods: ALTITUDE was an international randomized double-blind study in 8561 subjects randomized to aliskiren (A) 300 mg once daily or placebo (P) on top of single RAAS blockade. The primary outcome measure was time to first event for the composite endpoint of CV death, resuscitated death, MI, stroke, unplanned hospitalization for HF, onset of ESRD or doubling of baseline creatinine.

Results: Baseline characteristics of the two groups were similar, eGFR 56.9 mL/min/1.73m^2 in both groups and albuminuria (mg/g) 200 vs. 201. Time averaged mean BP was lower in the A group (1.3/0.6 mmHg). At a median follow-up of 32.9 months the primary composite endpoint had occurred in 767 patients (17.9%) assigned to A and 721 (16.8%) assigned to P, HR for A vs. P 1.08 (95% CI 0.98, 1.20) p=0.14. Stroke occurred in 146 (3.4%) of the A and 118 (2.7%) in P, HR 1.25 (0.98, 1.60) p=0.070. Doubling of serum creatinine or ESRD was similar in the two groups and the mean reduction in albuminuria was 14% (CI 11-17) lower in A. Patients on A experienced significantly increased serum potassium ≥6 mmol/L (8.8% vs. 5.6%), and reported hypotension (12.1% vs. 8.0%).

Conclusions: The trial does not support administration of aliskiren on top of standard therapy with RAAS blockade in type 2 diabetic patients at high risk for cardiovascular and renal events, and may even be harmful.

Funding: Pharmaceutical Company Support - Novartis

HI-OR02

Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease: The TEMPO 3:4 Trial Vicente E. Torres,[1] Arlene B. Chapman,[2] Olivier Devuyst,[3,4] Ron T. Gansevoort,[5] Jared J. Grantham,[6] Eiji Higashihara,[7] Ronald D. Perrone,[8] Holly B. Krasa,[9] John Ouyang,[9] Osamu Sato,[10] Frank S. Czerwiec.[9,11] *[1]Mayo Clinic; [2]Emory Univ.; [3]Univ. Catholique de Louvain; [4]Univ. of Zurich; [5]Univ. of Groningen; [6]Kansas Univ.; [7]Kyorin Univ., Mitaka; [8]Tufts Univ.; [9]Otsuka Pharmaceutical Development & Commercialization, Inc., Rockville; [10]Otsuka Pharmaceutical Co., Ltd., Tokyo; [11]for the TEMPO 3:4 Trial Investigators.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) causes kidney cysts often associated with pain, hypertension, and kidney failure. Preclinical studies justify testing vasopressin V_2 receptor antagonists to inhibit cyst growth and slow kidney function decline.

Methods: In a phase 3, multi-center, double-blind, placebo-controlled, 3-year trial, 1445 ADPKD subjects, 18-50 year-old, with total kidney volume (TKV) ≥750 ml and estimated creatinine clearance ≥60 ml/min, were randomized 2:1 to split dose tolvaptan (45/15, 60/30 or 90/30 mg daily as tolerated) or placebo. Primary outcome was annual rate of TKV change. Sequential secondary endpoints included composite of time to clinical progression events (worsening kidney function, kidney pain requiring intervention, hypertension or albuminuria) and rate of kidney function decline.

Results: TKV increase over 3 years was halved in subjects treated with tolvaptan compared to placebo (2.80%/year versus 5.51%/year, P <0.001). The composite endpoint favored tolvaptan (hazard ratio [HR] 0.87, P <0.01) with fewer events of worsening kidney function (HR 0.39, P <0.001) and kidney pain requiring intervention (HR 0.64, P=0.007). Tolvaptan reduced the slope of kidney function decline (reciprocal serum creatinine, -2.61 versus -3.81 (mg/ml)$^{-1}$/year, P <0.001). ADPKD-related adverse events were less common in tolvaptan treated subjects. Aquaretic- and non-ADPKD hepatic-related adverse events were more common in tolvaptan treated subjects contributing to higher rates of discontinuation, 23% with tolvaptan versus 14% with placebo.

Conclusions: Tolvaptan demonstrated clinically meaningful disease-specific benefits for ADPKD patients.

Funding: Pharmaceutical Company Support - Otsuka Pharmaceuticals. Co., Ltd. Tokyo, Japan and Otsuka Pharmaceutical Development & Commercialization, Inc., Rockville, Maryland

HI-OR03

Evaluation of Cinacalcet Therapy To Lower Cardiovascular Events (EVOLVE) Trial Glenn M. Chertow,[1] Geoffrey A. Block,[2] Ricardo Correa-Rotter,[3] Tilman B. Drueke,[4] Jürgen Floege,[5] William G. Goodman,[6] Christian Mix,[6] Marie-Louise Trotman,[6] Yumi Kubo,[6] Charles A. Herzog,[7] Gerard M. London,[8] Kenneth Mahaffey,[9] Sharon M. Moe,[10] David C. Wheeler,[11] Patrick S. Parfrey.[12] *[1]Stanford Univ SOM; [2]Denver Neph; [3]Instituto Nacional de Ciencias Méd y Nutrición, Mexico; [4]Picardie Univ SOMP, France; [5]RWTH Aachen, Germany; [6]Amgen; [7]Univ of Minnesota; [8]Hôpital Manhès, France; [9]Duke Univ Med Ctr; [10]Indiana University SOM, United States; [11]Univ College London, United Kingdom; [12]Health Sciences Ctr, Canada.*

Background: Secondary HPT (sHPT) and other abnormalities associated with chronic kidney disease – mineral bone disorder (CKD-MBD) may contribute to increased cardiovascular (CV) mortality and morbidity. Dietary modification and a variety of medications are used to attenuate the severity of sHPT.

Methods: EVOLVE was designed to test the hypothesis that tx with cinacalcet compared with placebo (on a background of PB +/- vit D sterols) reduces the risk of death or non-fatal CV events among pts on hemodialysis (HD) with sHPT. Sample size was calculated based on these assumptions: primary composite event rate of 23.2%/yr in the placebo group, 20% effect size, 1.5-yr enrollment period, 4-yr total study duration, lost to follow up rate 1%/yr, drop-out rate 10%/yr in the cinacalcet and drop-in rate 10% in the placebo group. With an overall α=0.05, we calculated that 1882 primary composite endpoints are needed to get a power of 90%.

Results: 3883 pts were randomized from 22 countries. The burden of overt CV disease at baseline (BL) was high (e.g. MI 12.4%, HF 23.3%). The median PTH at BL was 693 pg/mL (10%, 90% range, 363 to 1694 pg/mL). 87.2% of patients were prescribed PB and 57.5% were prescribed vit D derivatives at BL.

Conclusions: Using an unadjusted intent-to-treat (ITT) analysis, cinacalcet did not significantly reduce the risk of death or major CV events in patients on HD with moderate to severe sHPT. Results from ITT analyses using multivariable adjustment and censoring data 6 months after cessation of study drug will be presented along with safety data. NCT00345839

Funding: Pharmaceutical Company Support - Amgen

HI-OR04

A Randomised Trial of Mycophenolate Mofetil Versus Cyclophosphamide for Remission Induction in ANCA-Associated Vasculitis: MYCYC. On Behalf of the European Vasculitis Study Group Rachel B. Jones,[1] Michael Walsh.[2] *[1]Department of Medicine, University of Cambridge, United Kingdom; [2]Department of Clinical Epidemiology & Biostatistics, McMaster University, Canada.*

Background: Cyclophosphamide (CYC) induction regimens are standard therapy for ANCA-associated vasculitis (AAV) with major organ involvement. However CYC is associated with considerable toxicity. Mycophenolate mofetil (MMF) is a potential alternative to CYC. We performed an international, multi-centre non-inferiority randomised controlled trial comparing MMF to CYC for remission induction of AAV.

Methods: Eligible patients had newly diagnosed AAV and were randomised to receive up to 6 months induction with either MMF 2-3mg/day (n=70) or 6-10 pulses of IV CYC 15mg/kg (n=70). Both groups received the same tapering oral prednisolone regimen and azathioprine maintenance therapy. The primary outcome was remission (absence of disease activity for ≥4 weeks while adhering to the glucocorticoid regimen). We hypothesized that MMF treatment would result in no more than 12% fewer remissions.

Results: The groups were similar at trial entry. The primary remission endpoint occurred in 46/70 (66%) MMF vs 48/70 (69%) CYC (risk difference -3%, 90% CI -16 to 10%; p=0.06 for non-inferiority). Remission induction irrespective of steroid compliance occurred in 61/70 (87%) MMF vs 54/70 (77%) CYC (risk difference 10%, 90% CI -1 to 21%; p=0.01 for non-inferiority). However, glucocorticoid dosing did not differ significantly between groups overall (p=0.96). Key safety outcomes did not differ significantly (Table 1).

Conclusions: In the primary analysis we were unable to demonstrate that MMF is non-inferior to IV CYC for remission induction at six months in newly diagnosed AAV. How, glucocorticoid treatment affects remission induction with MMF requires further study. **Longer term safety outcomes and relapse data are required to fully understand the role of MMF as induction therapy for AAV.**

Table 1. Key safety outcomes

Safety Outcome	MMF	CYC	Risk Difference (95% CI)	p-value
Any SAE	32 (46%)	27 (39%)	7% (-9 to 23%)	0.39
Serious Infection	18 (26%)	11 (16%)	10% (-3 to 23%)	0.14
Dialysis	2 (3%)	3 (4%)	-1% (-8% to 5%)	0.99
Death	5 (7%)	4 (6%)	1% (-7 to 10%)	0.99

Funding: Pharmaceutical Company Support - Vifor Pharma

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HI-OR05

Survival of Dialysis Patients in the US after Surgical Versus Percutaneous Coronary Intervention Charles A. Herzog,[1,2] Craig Solid.[1] *[1]CVSSC, United States Renal Data System, MMRF, Minneapolis, MN; [2]University of Minnesota, Minneapolis.*

Background: There are few published data on the comparative survival of dialysis pts undergoing surgical versus percutaneous coronary revascularization in the era of drug-eluting stents (DES). Preliminary data have suggested superior long-term outcomes after coronary artery bypass surgery (CAB) vs percutaneous coronary intervention (PCI).

Methods: We searched the records of the United States Renal Data System database to identify 23,038 dialysis pts having CAB, bare-metal stent (BMS) or DES in 2004-2009. Long-term survival was estimated by Kaplan-Meier method and independent predictors of death were examined in a comorbidity-adjusted Cox model.

Results: There were 6,178 CAB pts, 5,013 BMS pts and 11,847 DES patients. The tables show survival and predictors of death, (age < 65, male, white, hemodialysis, time on dialysis < 2 years, no comorbidity, CAB is reference) with hazard ratio (HR). DES patients have the best survival at 12 months, but after 18 months CAB patients have the best survival. CAB pts receiving internal mammary grafts (IMG) (75% of CAB pts) do significantly better than those without (Log-Rank p-value = 0.0005).

Conclusions: Our data suggest that DES provide the best short term survival, but unadjusted long-term survival is best in CAB patients. In the most recent treatment era, CAB and DES have similar overall outcomes in US dialysis pts, but the best survival is associated with CAB utilizing IMG.

SURVIVAL %				
2004-2009 PTS				
Months	CAB(IMG+)	CAB(IMG-)	DES	BMS
1	90	87	94	90
6	79	72	83	75
12	72	64	71	63
24	60	51	53	48
36	48	40	40	35
48	38	30	31	26
60	30	24	23	19
72	24	18	19	15

PREDICTORS OF DEATH		
	2004-2006 pts	**2007-2009 pts**
Variable	HR (95% CI)	HR (95% CI)
Age 65-74	1.29 (1.22, 1.35)	1.35 (1.25, 1.45)
Age 75+	1.74 (1.65, 1.83)	1.95 (1.80, 2.10)
Female	0.98 (0.94, 1.02)	1.02 (0.96, 1.09)
Black	0.88 (0.84, 0.93)	0.84 (0.79, 0.90)
CHF	1.35 (1.28, 1.41)	1.38 (1.28, 1.47)
Peritoneal Dialysis	1.21 (1.10, 1.34)	1.43 (1.25, 1.63)
Diabetes	1.07 (1.01, 1.04)	1.08 (0.99, 1.18)
DES (vs. CAB)	**1.08 (1.03, 1.14)**	**1.00 (0.93, 1.08)**
BMS (vs. CAB)	**1.27 (1.19, 1.36)**	**1.25 (1.15, 1.35)**

Funding: NIDDK Support

HI-OR06

Ten-Year Safety of Prediabetic Living Kidney Donors Sindhu Chandran, Umesh Masharani, Allison B. Webber, David Wojciechowski. *Medicine, UCSF, San Francisco, CA.*

Background: Potential living kidney donors with prediabetes are often excluded due to concerns about the development of diabetes and progression to ESRD in the setting of a solitary kidney. This strategy may be unnecessarily restrictive.

Methods: An electronic database of all living kidney donors at UCSF was queried to identify donors from 1996-2005 with serum glucose >100 mg/dl (impaired fasting glucose or IFG). A medical history questionnaire was administered and blood and urine specimens obtained from donors who were successfully contacted and agreed to participate.

Results: 35 donors were enrolled. At the time of donation, mean fasting glucose was 108.6 mg/dl (SD 6.9) and a 2-hour glucose tolerance test was negative (<140 mg/dl) in all tested (n=10). HgA1c was checked in 15 patients with a mean value of 5.5% (SD 0.4). Other pre-donation characteristics are listed in table 1. At study enrollment, mean time from donation was 10.2 years (SD 3.1). Medical history and laboratory data are listed in table 1. 4 donors had developed diabetes. Except for 2 donors with known diabetes, none had albuminuria >30 mg/g.

PRE-DONATION	N=35
Male (%)	14 (40)
Age yrs (SD)	48.3 (11)
Race (%)	
-Caucasian	26 (74.3)
-African-American	1 (2.9)
-Other	7 (20)
BMI kg/m2	28.1 (3.9)
Related to recipient (%)	19 (54.3)
Urine Pr:Cr mg/g	0.04 (0 to 0.13)
Blood pressure mm Hg, N=21	
-Systolic	131 (12.2)
-Diastolic	76.6 (8.6)
MDRD eGFR 30 d post-donation	59.4 (9.9)
AT ENROLLMENT	N=35
Age yrs (SD)	58.4 (12)
BMI kg/m2 (SD)	27.9 (4.9)
History	
(1) High blood sugar (%) yes	11 (31.4)
(2) Diabetes (%) yes	4 (11.4)
(3) Diet controlled vs. oral meds	1 vs. 3
(4) High BP (%) yes	15 (42.9)
-On BP meds?	12 (34.3)
Mean fasting glucose mg/dl (SD)	102.7 (29.9)
<100 mg/dl	24 (68.6)
100-125 mg/dl	10 (28.6)
>125 mg/dl	1 (2.8)
HgbA1C % (SD)	5.9 (1.3)
<5.7	13 (37.1)
5.7-6.4	19 (54.3)
>6.4	3 (8.6)
MDRD eGFR (SD)	68.9 (14.3)
Urine Alb:Cr mg/g (SD)	9.14 (26.1)
>30 mg/g	N=2
Triglycerides mg/dl	123.4 (64.8)

Conclusions: Only a minority (11.4%) of living donors with IFG had developed diabetes 10 years post-donation. Kidney function remained well-preserved in all. Modest albuminuria was present in only 2 patients, both diabetic. Renal outcomes appear to be excellent in kidney donors with IFG.

Funding: NIDDK Support

SA-PO1097

Amplification of Albuminuria in Remote-Living Australian Aboriginal Adults by Early Life Risk Factors: The Multideterminant or Multihit Model of Renal Disease Wendy E. Hoy,[1] Andrew V. White,[2] Gurmeet R. Singh,[3] David A. Mccredie.[4] *[1]School of Medicine, Univ Queensland; [2]James Cook Univ; [3]Menzies School of Health Research; [4]Royal Children's Hosp and Univ Melbourne, Australia.*

Background: Albuminuria marks renal disease in remote-living Aboriginal people and predicts renal failure and nonrenal deaths. Levels increase markedly with age and over modest ranges of BMI and are higher in females. We describe albuminuria in the context of birthweight (Bwt) and episodes of childhood poststreptococcal glomerulonephritis (PSGN), singly and together.

Methods: 800 members of one tribal group were 10-39 yr during community health screens (>80% participation), and had satisfactory chart reviews for histories of PSGN and recorded Bwt. Urine ACR was the outcome of interest.

Results: Bwts were <2.5 kg in 28% of females & 22% of males, while 23% & 20% had "remote" episodes of PSGN (≥3 years prior). Bwt (inversely), a PSGN history, BMI and age all correlated independently with ACR. ACR levels were lowest in those with Bwts ≥2.81kg (the group median) and no PSGN history, intermediate in those with either lower Bwts or past PSGN, and highest in those with both, and with significant interaction terms between Bwt and PSGN. ACR levels were higher in females, and more sensitive to a single risk factor. ACR ≥3.4 (microalbuminuria threshold) was increased 1.9-fold and 2.7-fold in females with 1 and 2 risk factors respectively; rates were not higher in males with one risk factor, but were increased 3.7-fold with two risk factors.

Conclusions: A "multideterminant" model of renal disease is evident in relation to early life risk factors. Nephron deficiency established in early life, through underendowment (lower Bwts) and/or childhood loss (PSGN) establishes susceptibility and propagates higher ACRs with increasing age or BMI. Greater susceptibility in females is probably due to their lower nephron endowment, a feature of females generally, exacerbated by their lower Bwts, while slightly higher BMIs propagate their adult ACR levels. Potential for primary prevention is excellent.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO1098

Prolonged Use of Sulfamethoxazole-Trimethoprim at DS Dose Decreases Post-Transplant Urinary Tract Infection Timothy A. Horwedel,[1] Lyndsey Bowman,[1] Daniel C. Brennan.[2] *[1]Pharmacy, Barnes-Jewish Hospital; [2]Medicine, Washington University, St. Louis, MO.*

Background: Urinary tract infections (UTI) are common after renal transplant (RT), and guidelines do not address standardized UTI prophylaxis. We sought to examine our policy of indefinite use of sulfamethoxazole-trimethoprim (SMX/TMP) on the rates of UTI after RT.

Methods: We included RT recipients transplanted 1/2002 – 12/2010, allowing for >1 year of follow-up. Demographics and transplant characteristics were extracted from medical

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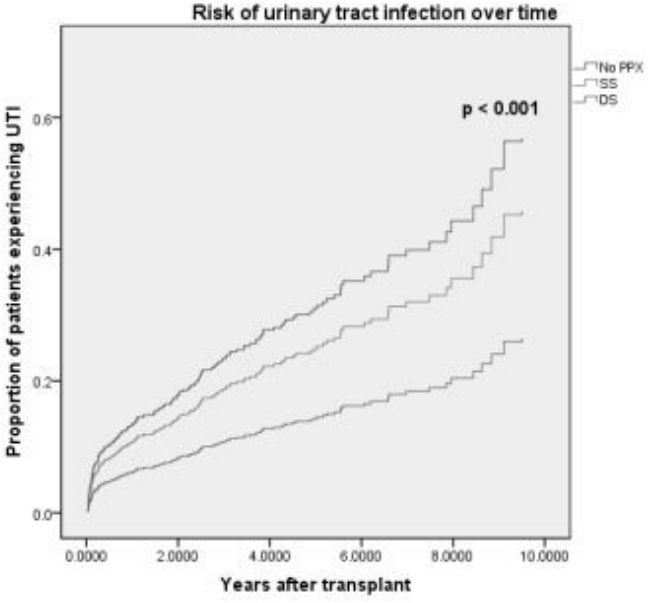
records, and all outcome data were manually reviewed. The primary outcome was time to first UTI. Secondary outcomes included culture data. Time to UTI was analyzed using Cox-regression with time-dependent covariates.

Results: 1235 RT recipients were included, 130 received no SMX-TMP (no PPX), 300 on 400/80 mg (SS) daily and 805 on 800/160 mg (DS) daily. There were more females in the no PPX group; other characteristics were well matched. 198 (16%) experienced a UTI. UTI was associated with a >60% increased risk of graft failure (HR 1.62, 95% CI 1.16 – 2.28). By multivariate analysis, lower SMX/TMP dose ($p < 0.001$), increased age (p 0.03), and female sex ($p < 0.001$) predicted UTI. For each dosing increment there was a 32.8% relative risk reduction.



The benefit for females was particularly robust ($p < 0.001$). The time to UTI was 10 months in the no PPX group, 9 months in SS, and 28 months in DS. There were no differences in pathogens between groups, with coliforms cultured in 65.6% of UTI. There was 76.4% SMX/TMP resistance rate in the groups receiving drug, and 26.9% resistance with no PPX ($p < 0.001$). There was an increase in penicillin resistant organisms ($p < 0.001$), but susceptibility to other drugs was not different.

Conclusions: Prolonged SMX-TMP at the DS level should be used for indefinite UTI prophylaxis after RT to prevent UTIs, which are associated with graft loss.

SA-PO1099

Cardiovascular Risk in Diabetic Kidney and Kidney-Pancreas Transplant Recipients with Persistent Hyperparathyroidism

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Background: Hyperparathyroidism (HPT) has been associated with cardiovascular mortality and morbidity in patients with chronic kidney disease. HPT may persist in kidney transplant recipients. Kidney transplantation may disclose the severity of a functional alteration in the parathyroid glands. Therefore we studied the association between major cardiovascular events (CVEs) and HPT in kidney transplant recipients (KTR) and kidney-pancreas transplant recipients (KPTR).

Methods: We analyzed the association of major CVEs with HPT in 63 diabetic KPTR, 25 diabetic KTR and 61 non-diabetic KTR.

Results: 117 patients (78.5%) had persistent HPT (iPTH > 63 pg/ml, one year after transplantation): 100 of them (67.1%) had HPT and normal serum calcium; 17 (11.4%) had HPT and high serum calcium. The proportion of patients with persistent HPT was lower in diabetic than in non-diabetic transplanted patients (n=64 vs 53, OR=0.4, 95%CI 0.2-1, p=0.039), but not different in diabetic KTR (n=16) or KPTR (n=48).

Considering together pre and posttransplantation CVEs in diabetic KTR or KPTR, a Cox proportional regression model showed that the risk of major CVEs was significantly increased in patients with hypercalcemic (OR=11, 95%CI 1.6-64, p=0.014) and normocalcemic (OR=6, 95%CI 1.5-24, p=0.013) HPT and in patients in the lowest (OR=8.2, 95%CI 2-35, p=0.004) and the highest (OR=3.8, 95%CI 1.1-13, p=0.037) tertiles of pretransplantation serum phosphate or kidney-pancreas transplantation (OR=4.0, p=0.006). Similar findings were obtained considering tertiles of serum iPTH and calcium in the Cox regression model. CVEs were not associated with HPT in non-diabetic KTR. The risk of postransplantation major events was increased in diabetic KTR (n=9, OR=2.5, 95%CI 1-6, p=0.05), but not KPTR (n=12). It was associated with HPT (OR=8.6, 95%CI 1.4-51, p=0.018) in diabetic patients.

Conclusions: Persistent HPT increases cardiovascular risk in diabetic transplanted patients already predisposed to CVEs. The association with cardiovascular risk is proportional to the severity of parathyroid alterations in diabetic CKD patients.

Funding: Private Foundation Support

SA-PO1100

Prolonged Hemodialysis and Low-Salt Diet Reduce Skin Sodium Content

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Background: In Tassin, France, the dialysis center is renowned for its prolonged, slow 8 h thrice-weekly dialysis treatments and rigorous attention to diet. We have developed sodium magnetic resonance imaging (Na-MRI) to measure sodium proteoglycan skin storage. We had occasion to study dialysis patients from Tassin and to compare them with matched dialysis patients undergoing trice-weekly 4 h treatments in Erlangen, Germany.

Methods: We used ^{23}Na magnetic resonance imaging at 3Tesla (T) to quantify sodium and water content in skeletal muscle and skin of the left lower leg. Nine men and 2 women from Tassin (aged 56±13 years), were compared to 9 men and 2 women of similar age from Erlangen. Blood sample were drawn before dialysis and BCM measurements were performed before Na MRI.

Results: Tassin and Erlangen patients had similar muscle sodium contents (18.62±3.29 vs. 17.34±3.21 mmol/l), but Tassin patients had lower skin sodium values (18.13±4.49 vs. 21.76±4.76 mmol/l, $p<0.05$). Water content values were not significantly different. Changes in interdialytic weight gain (2.6±1,2 vs. 1.5±0.8 kg, $p<0,05$) were not reflected by changes in estimated total muscle sodium content or total body water.

Conclusions: We have developed a non-invasive MRI technique to reflect sodium and water content in tissues. The method allowed us to detect differences in two dialysis populations managed with divergent treatment philosophies. We suggest that this method could have utility in predicting outcomes and reflecting treatment quality of dialysis patients.

Funding: Pharmaceutical Company Support - Fresenius Medical Care, Germany

SA-PO1101

Sustained Improvement of Hemoglobin A1c Reduces Risk of End-Stage Renal Disease in Patients with Type 1 Diabetes and Proteinuria

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Background: No strong evidence exists that in patients with type 1 diabetes (T1D) and proteinuria improved glycemic control can reduce the risk of end-stage renal disease (ESRD).

Methods: 350 patients with T1D and proteinuria were followed for 5-18 years to ascertain ESRD (n=112, incidence rate 3.9/100 person-years) or death (n=25). HbA1c before study entry (5-year pre-baseline period) was compared with follow-up values (median 3.4 years after baseline), and changes were examined for effects on risk of ESRD during follow-up. We used follow-up serum creatinines to assess trajectories of estimated glomerular filtration rate (eGFR).

Results: Median HbA1c was 9.3% in pre-baseline period and decreased during follow-up to 8.8%. HbA1c was categorized into good, fair and poor (≤8, >8-≤9.5, and >9.5%, respectively). 220 patients (63%) remained in the same category (Table diagonal, bold), in 35 (10%) post-baseline HbA1c category worsened (below diagonal) and in 95 (27%) it improved (above diagonal). The incidence rate of ESRD decreased dramatically with improving HbA1c (Table; for example, with improvement from poor pre-baseline to fair post-baseline HbA1c, the incidence of ESRD dropped from 7.5 to 3.3/100 patient-years).

Incidence rates of ESRD (per 100 patient-years) according to pre- and post-baseline category of HbA1c

	Pre-baseline HbA1c		
Post-baseline HbA1c	good	fair	poor
good	1.8 (n=59)	3.0 (n=35)	0.0 (n=14)
fair	1.9 (n=12)	3.7 (n=78)	3.3 (n=46)
poor	(n=0)	5.7 (n=23)	7.5 (n=83)

In Cox regression relative hazard of ESRD per 1% post-baseline HbA1c improvement was 0.72 (95% CI: 0.61, 0.89; $p<0.001$). This effect was independent from pre-baseline HbA1c and anti-hypertensive treatment. It was not confounded by sex, age, albumin/creatinine ratio, eGFR or blood pressure. Improvement in post-baseline Hb1Ac was strongly associated with decelerating eGFR decline ($p=0.002$), while trajectories were linear if HbA1c was constant.

Conclusions: In T1D patients with proteinuria sustained improvement of glycemic control is an effective treatment that reduces long-term risk of ESRD.

Funding: NIDDK Support, Private Foundation Support

SA-PO1102

Multicenter Double-Blind, Randomized, Placebo-Controlled Trial of Rituximab for the Treatment of Childhood-Onset Refractory Nephrotic Syndrome

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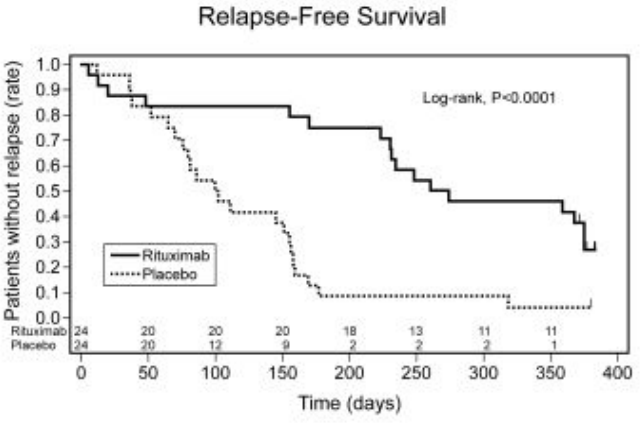
Background: Some studies reported the efficacy of rituximab for the refractory frequent–relapsing nephrotic syndrome/steroid–dependent nephrotic syndrome (refractory FRNS/SDNS); however, the efficacy and the safety of the therapy have not been established.

Underline represents presenting author/disclosure.

Methods: We conducted a multicenter double-blind, randomized, placebo-controlled trial to investigate the efficacy and the safety of rituximab for refractory FRNS/SDNS. Eligible patients were over 2 years old, and diagnosed as refractory FRNS/SDNS at one to 18 years. They received standard steroid therapy at the relapse, and enrolled after confirming remission. Twenty-four patients each were randomized to rituximab group (375 mg/m^2, once a week for four weeks) and placebo group. Both group stopped immunosuppressive agents such as cyclosporine before Day 169, and followed for one year. Primary endpoint is the duration of relapse-free period. Treatment failure is defined as relapse within Day 85, frequent relapse during follow-up, or develops steroid dependence or steroid-resistant nephrotic syndrome.

Results: Relapse-free period of rituximab group was significantly longer compared to the placebo group (50% relapse-free period: 267 vs. 101; HR=0.27, 95%CI: 0.14-0.53; log-rank test, p<0.0001).



Rituximab group had significantly lower rate of treatment failure (log-rank test, p=0.0010) and relapse rate taking multiple recurrences into account (person-year method; 1.09 vs. 3.77; permutation test, p<0.0001). No difference in adverse events, including frequency and severity of infection, was observed between the groups.

Conclusions: Rituximab is effective and safe for the treatment of refractory FRNS/SDNS.

Funding: Government Support - Non-U.S.

SA-PO1103

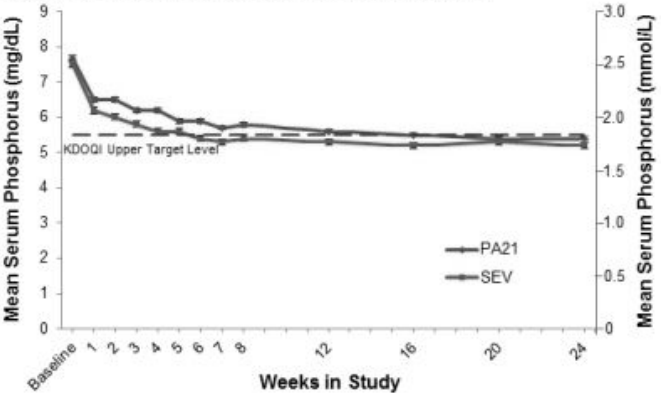
Efficacy and Safety of PA21 in Hyperphosphatemic CKD Patients on Dialysis Jürgen Floege,[1] Markus Ketteler,[2] Anjay Rastogi,[3] Adrian Covic,[4] Edward M.F. Chong,[5] Laura J. Lisk,[5] Sylvain Gaillard,[5] Stuart M. Sprague.[6] *[1]RWTH Univ Hosp Aachen; [2]Coburg Clinic and KfH-Dialysis Center; [3]Univ of California; [4]Gr.T. Popa Univ of Med and Pharm; [5]Vifor Pharma; [6]NorthShore Univ HealthSystem.*

Background: A randomized, open-label, active-controlled, two-stage, phase 3 study investigated efficacy and safety of a new, iron-based phosphate binder (PA21) in lowering and maintaining serum phosphorus (sP) levels, and non-inferiority (NI) vs sevelamer carbonate (SEV).

Methods: 1,059 patients with hyperphosphatemia were randomized 2:1 to PA21 (1.0–3.0 g/day; starting dose 1.0 g/day; n=710) or SEV (2.4–14.4 g/day; starting dose 4.8 g/day; n=349) for 24 weeks. During the first 8 weeks, doses were titrated to reach predefined sP levels (2.5–5.5 mg/dL). Doses were unchanged in weeks 8–12. NI (2° endpoint) was assessed after 12 weeks. In the subsequent maintenance phase, the aim was to maintain sP in the predefined range. Of patients receiving PA21, 99 were re-randomized after 24 weeks of treatment and entered a 3-week superiority assessment of maintenance-dose (n=50) vs low-dose (250 mg/day; n=49) PA21 (1° endpoint).

Results: 1° and 2° endpoints were met. PA21 maintenance dose was superior to low-dose PA21 in maintaining sP levels within the predefined range. NI of PA21 vs SEV was established (Fig.1), with a lower pill burden than SEV (3.1 [SD: 1.14] vs 8.1 [SD: 3.15] pills/day).

Figure 1: Effect of PA21 and SEV on sP over 24 weeks



No differences were observed between PA21 (83%) and SEV (76%) in the frequency of treatment-emergent adverse events (TEAEs) and serious TEAEs. Incidence of gastrointestinal TEAEs was higher with PA21 vs SEV (45% vs 34%), mainly due to discolored feces (15.4% vs 0.3%) and diarrhea (20.1% vs 7.5%). Diarrhea was mostly mild, occurred in the first week of PA21 treatment and abated with continued dosing.

Conclusions: PA21 is efficacious and well tolerated, with a lower pill burden vs SEV.

Funding: Pharmaceutical Company Support - Vifor Pharma

SA-PO1104

Use of Everolimus as Rescue Therapy in Kidney Transplant Recipients Anil Bhalla,[1] Anshul Bhalla.[2] *[1]Institute of Renal Sciences, Sir Ganga Ram Hospital, New Delhi, Delhi, India; [2]Department of Critical Care Medicine, Sir Ganga Ram Hospital, New Delhi, Delhi, India.*

Background: With the availability of newer immunosuppressive agents, there are various innovative strategies that reduce exposure to Calcineurin Inhibitors(CNI) to help preserve long term renal functions. Chronic Allograft Nephropathy(CAN) is also a major cause of progressive renal failure in kidney transplant recipients. Most of the time patient presents with creeping creatinine. As a rescue therapy, conversion from CNI to Tac based regimen has been tried. Recently, interest has generated over Everolimus as a Proliferative Signal Inhibitor(PSI) and as a rescue therapy to prevent further rejection and avoid nephrotoxicity. The objective of this study is to evaluate the effect of reduction of CNI doses in kidney transplant patients with CAN and at the onset of acute rejection.

Methods: 490 kidney transplants were performed in the last two years. All were on triple drug immunosuppresion. In 30 patients with creeping creatinine and those who developed acute rejection, therapy was changed to an Everolimus based regime. Biopsy proven acute rejection was treated with IV Solumedrol for 3 days. Cyclosporine(CsA) or Tacrolimus doses were reduced by 50-80% in 24 patients(Group 1) and discontinued in 6 patients(Group 2). Mycophenolate or Azathiprine were withdrawn in 5 patients in group 1. All patients received prednisolone.

Results: 30 renal allograft recipients were switched to Everolimus based regimen and were followed up for a mean period of 12 months (range 1-24 months). Mean(SD) baseline serum creatinine was 2.6 mg/dl(0.4) and mean serum creatinine at the end of study was 1.8 mg/dl(0.3) (p value<0.01). Graft biopsy showed 23 patients with CAN. 7 patients had acute rejection(Banff grade 2A and 2B). Patient and graft survival was 100%. Mean trough level of Everolimus was 5.2 +- 1.5 ng/ml.

Conclusions: Use of Everolimus as rescue therapy for CAN and after onset, of acute rejection and synchronised with reduction of CNI doses or discontinuance was associated with good patient and graft survival in kidney transplant recipients. This intervention can lead to 80% reduction in CsA and Tac dosage and thus, preservation of GFR.

SA-PO1105

Extended Treatment with RP103 (Procysbi) in Patients with Nephropathic Cystinosis Craig B. Langman,[1] Laurence A. Greenbaum,[3] Georges Deschenes,[4] Heather E. Price,[1] Patrice Rioux.[2] *[1]Kidney Diseases, Lurie Childrens Hosp of Chicago, Chicago, IL; [2]Raptor Pharmaceuticals, Novato, CA; [3]Peds Kidney, Emory Univ, Atlanta, GA; [4]Kidney, Hopital Robert Debre, Paris, France.*

Background: Cystinosis is a ultra-orphan disease for which Rx with Cystagon® is effective but difficult to use long-term. RP103 (Procysbi) is used q12h, & offers better long-term adherence strategy.

Methods: After we showed short term non-inferiority for optimal control of WBC [cystine] < 1 nmol ½ cystine/mg protein with RP103 vs Cystagon® in a randominzed X-over study of 41 patients (CJASN, 2012), we enrolled 40 of these patients in a prospective, ongoing study with RP103 of up to 20mos (m) to determine control of WBC[cystine], its relationship to plasma[cysteamine], eGFR, quality of Life (QOL), and use of antacids.

Results: At study onset on RP103, WBC[cystine]=0.58±0.70 (mean±SD) and remained at this optimal level for up to 19m in all. The dose of RP103 at study onset = 83% of total daily (TD) Cystagon® dose. TD dose for 13(33.3%) subjects has been unchanged; ↑ for 15(38.4%); ↓ for 11(28.2%). We modeled the relation for plasma[cysteamine] and WBC[cystine] using an inhibitory E_{max} PD model, and found that, 0.5h after RP103 dose,91.0 % of WBC[cystine] with blood [cysteamine] > 0.1 mg/L was ≤1;94.8% had WBC[cystine] ≤1 when [cysteamine] was >0.2 mg/L . For 16 patients with >12m follow-up, eGFR at

Underline represents presenting author/disclosure.

study onset = 65±28 ml/min/1.73m^2 and 15m later was unchanged, 61±21, p=NS. From the study onset, the PedsQL4.0 scores improved in all patients, p<10^{-3}, especially in social performance, p<10^{-3}. 9/40 patients began this study on PPI+H2 blockers, and 31/40 did not use them during the entire study. WBC[cystine] on these 9 did not differ for up to 20m from the non-users, & RP103 dose ↓ in 5/9; ↑ in only 1/9.

Conclusions: Optimal WBC[cystine] can be achieved safely and effectively long-term in patients with nephropathic cystinosis using twice daily RP103, with preservation of eGFR and improved QOL. Plasma [cysteamine] post-dose provides an index for optimal WBC[cystine] and represents a new way to monitor patients. RP103, unlike Cystagon®, does not require use of concomitant antacid medications in most.

Funding: Other NIH Support - NIH UL1 RR025741, Pharmaceutical Company Support - Raptor Pharmaceutical

SA-PO1106

EPPIC (Evaluating Prevention of Progression In Chronic Kidney Disease): Results from 2 Phase III, Randomized, Placebo-Controlled, Double-Blind Trials of AST-120 in Adults with CKD Gerald Schulman,[1] Tomas Berl,[2] Gerald J. Beck,[3] Giuseppe Remuzzi,[4] Eberhard Ritz.[5] *[1]Vanderbilt Univ., Nashville, TN, United States; [2]Univ. of Colorado, Denver, CO, United States; [3]Cleveland Clinic, Cleveland, OH, United States; [4]Mario Negri Institute, Bergamo, Italy; [5]Univ. of Heidelberg, Heidelberg, Germany.*

Background: To evaluate the efficacy of AST-120, spherical carbon adsorbent, when administered orally with standard therapy for slowing CKD progression.

Methods: The 2 similar EPPIC trials enrolled pts with CKD III-V (mean eGFR 21-22 mL/min). The primary (1°) endpoint (endpt) was time to initiation of dialysis, transplant, or 2x baseline sCr. The secondary (2°) endpt was the 1° endpt + death. Pooled placebo data were used to identify baseline factors associated with fast CKD progression based on median eGFR decline (-0.292 mL/min/month). Fast progressors exhibited UP:UCr≥1.0 and hematuria+ at baseline.

Results: 2035 pts were randomized in the 2 trials. AST-120 did not show a benefit (Table) in pts with or without use of ACEs/ARBs. The post-hoc subgroup with baseline UP:UCr≥1.0, hematuria+, and AST-120 compliance≥80% had a reduction in CKD progression with AST-120.

	AST-120		Placebo		AST-120 vs Placebo	
	N	n (%)	N	n (%)	HR (95% CI)	P value
EPPIC-1						
1° endpt	500	178 (35.6)	502	177 (35.3)	1.03 (0.84-1.27)	0.777
2° endpt	500	213 (42.6)	502	201 (40.0)	1.08 (0.89-1.31)	0.418
EPPIC-2						
1° endpt	500	172 (34.4)	497	183 (36.8)	0.91 (0.74-1.12)	0.371
2° endpt	500	204 (40.8)	497	217 (43.7)	0.90 (0.74-1.09)	0.281
Pooled Analysis						
1° endpt	1000	350 (35.0)	999	360 (36.0)	0.97 (0.83-1.12)	0.639
2° endpt	1000	417 (41.7)	999	418 (41.8)	0.99 (0.86-1.13)	0.860
Pooled Analysis – UP:UCr≥1.0/hematuria+/compliance≥80%						
1° endpt	239	101 (42.3)	223	116 (52.0)	0.74 (0.56-0.98)	0.035

Conclusions: Results from the 2 EPPIC trials do not support the efficacy of AST-120 for slowing CKD progression; however, the variables used to enrich the population with pts likely to progress did not result in the expected degree of progression in this population. A subgroup analysis indicated a trend that AST-120 may reduce CKD progression in compliant pts with factors associated with fast progressing CKD. Further trials are needed to ascertain whether AST-120 is of benefit in fast progressing CKD pts.

Funding: Pharmaceutical Company Support - Mitsubishi

SA-PO1107

Urine Exosome mRNA as Biomarkers of Diabetic Nephropathy Natalie Sweiss,[1] Masato Mitsuhashi,[2] Loki Natarajan,[3] Kumar Sharma.[1] *[1]Center for Renal Translational Medicine, University of California, San Diego, San Diego, CA; [2]Hitachi Chemical Research Center, Inc, Irvine, CA; [3]Division of Biostatistics & Bioinformatics, University of California, San Diego, San Diego, CA.*

Background: Current tests to assess diabetic kidney disease are limited to evaluation of the degree of kidney dysfunction and are not specific to the disease state. In order to develop a non-invasive diagnostic system to evaluate diabetic kidney disease, urine exosome mRNA was analyzed in patients with diabetic kidney disease and healthy controls.

Methods: Urine samples were obtained from healthy donors (CTL) (n=23) and patients with diabetic nephropathy (DM) (n=23) at the University of California, San Diego Medical Center. Each urine sample was centrifuged at 1,000 x*g* for 15 min, and 10 mL supernatant was applied to 96-well exosome-capture filterplate (Hitachi Chemical Research Center (HCR)). The captured exosome was lysed on the filterplate, and the resultant lysates were transferred to oligo(dT)-immobilized microplate (HCR) for mRNA purification, followed by cDNA synthesis and real time SYBR green PCR. A total of 21 candidate mRNAs were quantified.

Results: Using the Benjamini Hochberg method to control for the false discovery rate, a 2-sample (unequal variance) t-test revealed statistically significant differences between DM and CTL in mean values of PGC1alpha (*P*=0.002), Uromodulin (*P*=0.002), and SMAD1 (*P*=0.002). Patients with DM and CKD Stage 3 (eGFR 30-59 ml/min) compared to controls also had a statistically significant difference in cycle threshold of PGC1alpha (*P*=0.004), Uromodulin (*P*=0.001), and SMAD1 (*P*=0.005).

Conclusions: These results indicate that mRNA is preserved in the encapsulated urine exosomes from normal and diabetic individuals. Use of urine exosomal mRNA gene expression using this method may provide new insight into kidney tissue gene expression. Urine exosomal mRNA levels of PGC1 alpha, Uromodulin and SMAD1 are significantly different in patients with diabetic kidney disease vs healthy controls. Using this technology, these three biomarkers may aid in diagnosis of diabetic nephropathy.

Funding: NIDDK Support, Pharmaceutical Company Support - Hitachi Chemical Research Center

SA-PO1108

TLR9 Gene Polymorphism (rs187084, rs352140): Association with Acute Rejection and Estimated Glomerular Filtration Rate in Renal Transplant Recipients Tae hee Kim,[1] Ho seok Koo,[2] Sunwoo Kang,[1] Yang Wook Kim.[1] *[1]Internal medicine, Inje University, Busan, Republic of Korea; [2]Internal Medicine, Inje University, Seoul, Republic of Korea.*

Background: The Toll-like receptors (TLRs) are related to innate immunity. *TLR9*, a member of TLRs, is expressed in immune cell rich tissues and mediates cellular response. We investigated the association between *TLR9* polymorphisms and kidney allograft outcomes.

Methods: To investigate whether *TLR9* polymorphisms are associated with acute rejection after renal transplantation, two single nucleotide polymorphisms (SNPs) of *TLR9* gene (rs187084, -1486; rs352140, G2848A) were selected and genotyped by direct sequencing in 342 renal transplant recipients. SNPStats, SNPAnalyzer, Helixtree, and Haploview version 4.2 were used to analyze genetic data. Multiple logistic regression models (codominant, dominant, recessive, and log-additive) were used to evaluate odds ratios (ORs), 95% confidence intervals (CIs), and p values.

Results: Both SNPs, *TLR9* rs187084 -1486 and rs352140 G2848A, of recipients were associated with the risk of acute rejection in renal transplantation. C allele of rs187084 -1486 and A allele of rs352140 G2848A were protective genotype for acute rejection (OR 0.6, 95% CI 0.40-0.92; P=0.018, OR 0.64, 95% CI 0.42-0.98; P=0.04, respectively). rs187084 -1486 CT and rs352140 G2848A GA genotype were associated with a lower eGFR after a year of renal transplantation.

Conclusions: *TLR9* polymorphisms, rs187084 and rs352140, of recipients were associated with the risk of acute rejection in renal transplantation. The patients with rs187084 -1486 CT and rs352140 G2848A GA genotype showed a lower eGFR after a year of renal transplantation.

SA-PO1109

Long-Term Outcomes for Vitamin D Treatment on Patients with Chronic Kidney Disease Hector Tamez. *Massachusetts General Hospital. On Behalf of the PRIMO Steering Committee, Boston, MA.*

Background: Paricalcitol did not reduce left ventricular mass in the PRIMO randomized trial (NCT00497146), but it decreased cardiovascular (CV) hospitalizations, left atrial volume and attenuated the rise of brain natriuretic peptide compared to placebo in patients with chronic kidney disease (CKD).

Methods: Patients who completed study were offered to enroll in an 18-month follow up study. Laboratory values are summarized in medians (interquartile range [IQR]) and compared with Wilcoxon Rank Sum test. Frequency data was compared using χ^2-tests.

Results: 64/88 patients in the paricalcitol group and 66/91 in the placebo group enrolled in the follow up study. At baseline patients in the paricalcitol group had lower diastolic blood pressure (74±9 mmHg vs. 78±10 mmHg; P=0.03), parathyroid hormone ([PTH]; 27 pg/mL [IQR: 10, 70 pg/mL] vs. 92 pg/mL [IQR: 65, 149 pg/mL]; P<0.001), estimated glomerular filtration rate (26 mL/min [IQR: 18, 37 mL/min] vs. 34 mL/min [IQR: 23, 46 mL/min]; P=0.01) and higher calcium (9.8 mg/dL [IQR: 9.3, 10.2 mg/dL] vs. 9.2 mg/dL [IQR: 9, 9.6 mg/dL]; P<0.0001) compared to placebo. At the end of the follow up period PTH increased (paricalcitol 95 pg/mL [IQR: 62, 127 pg/mL]; placebo 23 pg/mL [IQR: -5, 52 pg/mL]; P=0.01). Similarly, calcium decreased in paricalcitol compared to placebo (-0.38 mg/dL [IQR: -0.54, -0.22 mg/dL] vs. -0.13 mg/dL [IQR: -0.26, 0.01]; P=0.01) as well as eGFR (-0.1 mL/min [IQR: -1.9, 1.7 mL/min] vs. -3.3 mL/min[IQR: -4.9, -1.8 mL/min]; P=0.002). The percentage of patients with all-cause hospitalizations was similar between groups (27.7% in paricalcitol vs. 15.3% in placebo; P=0.08) with similar findings for CV hospitalizations (1.5% in paricalcitol vs. 4.2% in placebo; P=0.36). Similarly, 1.5% of patients in the paricalcitol group and 2.8% of patients in the placebo group were hospitalized for renal failure or dialysis initiation (P=0.62). One patient died in the paricalcitol group compared to 3 in the placebo group (P=0.36).

Conclusions: All-cause, CV and renal failure hospitalizations did not differ after 18 months in patients with CKD who had received either paricalcitol or placebo for 48 weeks as part of the PRIMO randomized trial.

Funding: Pharmaceutical Company Support - Abbott Laboratories

Underline represents presenting author/disclosure.

SA-PO1110

Patiromer Safely and Predictably Lowers Serum Potassium in Subjects with Chronic Kidney Disease and Type 2 Diabetes: Results of the AMETHYST-DN Study

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Background: Patients with advanced CKD are at high risk of hyperkalemia (HK), especially when guideline-recommended renin angiotensin system inhibitors (RASi) are used. HK limits appropriate dosing of RASi, thus attenuating cardio-renal protective benefits. In the PEARL-HF study, patiromer, a non-absorbed potassium-binding polymer, prevented HK after 4 weeks of increased spironolactone dosing in a placebo-controlled heart failure trial.

Methods: AMETHYST-DN tests the ability of patiromer to reduce potassium (K) in HK subjects (K>5.0 mEq/L). Due to the inherent risks of HK and treatment daily for up to 1 year in the long-term maintenance phase, an active polymer control or placebo was unethical. The study enrolled 306 CKD subjects with T2DM into two K strata (S1 and S2). Mean eGFR was 42ml/min/m^2, 49% of subjects had ACR>300 mg/g and 34% had heart failure.

Results: The primary outcome, mean change from baseline in serum K (mEq/L) at week 4 or first patiromer dose titration analyzed using a parallel lines ANCOVA model, was -0.47±0.038 (p<0.001) in S1 and -0.90±0.076 (p<0.001) in S2. Mean K reduction after a median 2 days of treatment was -0.29±0.03 (S1) and -0.55±0.05 mEq/L (S2). Table 1 summarizes the means and changes from baseline, allowing titration. No treatment-related SAEs occurred and a low incidence of hypokalemia was observed (2% during the 8 weeks).

	Stratum 1 ([S1], BL K >5.0-5.5 mEq/L)			Stratum 2 ([S2], BL K >5.5-<6.0 mEq/L)		
	Baseline (n=217)	Week 4 (n=197)	Week 8 (n=185)	Baseline (N=84)	Week 4 (n=70)	Week 8 (n=70)
Mean K (SE) (mEq/L)	5.15 (0.02)	4.54 (0.03)	4.59 (0.03)	5.64 (0.04)	4.65 (0.06)	4.52 (0.06)
LS Mean change (SE) (mEq/L)	-	-0.61 (0.03)	-0.55 (0.03)	-	-0.97 (0.06)	-1.10 (0.06)

BL=Baseline; K=serum potassium; SE=Standard Error; LS=Least Squares Pub #: SA-PO1112 Edit_id:

Conclusions: Patiromer reduced serum K within days of treatment initiation, an effect sustained over two months without significant adverse effects, including hypokalemia.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

SA-PO1111

Sevelamer is Cost-Effective Versus Calcium Carbonate for the Treatment of Hyperphosphatemia in Chronic Kidney Disease Patients Not on Dialysis in the UK

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Background: The randomized, controlled INDEPENDENT study (Di Iorio et al., 2012) is the first study to show that sevelamer (SV) significantly increases overall survival (OS) (p<0.01) versus calcium carbonate (CC) in CKD patients not on dialysis (CKD-ND). The objective of this study was to conduct a cost-effectiveness analysis (CEA) of SV versus CC from the perspective of the NHS in the UK.

Methods: A Markov model was developed to estimate life years (LYs), incremental cost/LY gained and incremental cost/quality-adjusted LY (QALY) gained. Health states were 'Alive without Dialysis', 'Alive with Dialysis', and 'Dead' and transitions between health states were captured as survival curves. Three-year treatment-specific OS and time to dialysis curves from the INDEPENDENT study were extrapolated to lifetime using Weibull regression analysis. Phosphate binder doses were also from the INDEPENDENT study. Health utilities and unit costs were derived from the literature. Costs (in £2011) and outcomes were discounted at 3.5%. A probabilistic sensitivity analysis (PSA) was performed to characterize the uncertainty around the cost-effectiveness of SV given the variability around all key input variables.

Results: Over a lifetime horizon, treatment with SV resulted in incremental LYs (2.0493), QALYs (1.5613), and costs (£37,282) per patient versus CC.Incremental cost/QALY gained was £23,878; incremental cost/LY gained was £18,193. Results were most sensitive to daily dose of SV, utility, and cost of dialysis; results were not sensitive to a 20% increase/decrease in the cost of SV. At a willingness-to-pay threshold of £30,000/QALY gained, the PSA revealed that SV was cost-effective compared with CC in ~93% of simulations.

Conclusions: SV represents a cost-effective alternative to CC for the treatment of hyperphosphatemia in CKD-ND patients in the UK.

Funding: Pharmaceutical Company Support - Genzyme, a Sanofi Company

SA-PO1112

Relationship between Baseline Serum Uric Acid Levels and CKD Progression in Patients Attending Tertiary Care Centers in Italy

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Background: Evidence from the literature suggests that levels of serum uric acid (SUA) are associated with cardiovascular events and Chronic Kidney Disease (CKD). This study aims to analyze the relationship between baseline levels of SUA and progression of CKD in a population of 2,222 outpatients followed in Prevention of Progressive Renal Insufficiency program (PIRP). Data are drawm from PIRP registry. CKD progression was defined as all-cause mortality+dialysis, dialysis alone, estimated annual decrease in GFR.

Methods: The mean age was 71.3±12.8 years, 65.1% males and 34.9% females, with a mean SUA of 6.46±1.79 mg/dl and 6.27±1.79 mg/dl respectively. Baseline GFR was 29.1±12.4 ml/min per 1.73 m^2. The percentage of patients in CKD stages were: 2 (2%), 3A (7.2%), 3B (32.2%), 4 (49.4%), 5 (9.2%). Linear and logistic regression models were carried out to examine the relationship between baseline SUA levels and each of the three outcomes. All the analyses were adjusted for sex, age and baseline serum creatinine levels.

Results: All-cause mortality+dialysis and dialysis alone were unrelated to baseline SUA levels in logistic regression models after adjusting for covariates. On the contrary, the estimated annual decrease in GFR was significantly associated (p=0.021) with SUA levels. Specifically, the decrease in GFR was 0.123 ml/min per 1.73 m^2 for a 1 mg/dl increase in baseline SUA after adjusting for covariates.

Table1. SUA levels and annual progression in CKD

	b	SE (b)	beta	t	P
Age (yrs)	0.054	0.07	0.153	7.311	< 0.001
Gender (M vs F)	-0.642	0.201	-0.068	-3.198	0.001
S-Creatinine (mg/dl)	0.292	0.104	0.159	2.819	0.005
S-Uric acid (mg/dl)	0.123	0.053	0.048	2.313	0.021
costant	-6.478	0.680		-9.519	< 0.001

Conclusions: In conclusion, this study suggests that SUA levels are associated with the estimated annual decrease in GFR but not with the renal failure and mortality.

SA-PO1113

Minor Elevations of Postoperative Serum Creatinine Are Independently Associated with Higher Mortality and Longer Hospital Length of Stay in Patients Undergoing Non-Cardiac Surgery

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Background: Acute kidney injury (AKI) is a serious complication in hospitalized patients with impact on morbidity and mortality. Here, we hypothesized that already minor elevations of serum creatinine (Scr) levels have profound consequences on postoperative outcomes in patients undergoing non-cardiac surgery.

Methods: The local ethics committee of the Charité – University Medicine Berlin approved the study (EA1/303/11) and waived the requirement of informed consent; the study has been registered at ClinicalTrials.org (NCT01522313). In this retrospective analysis, all patients undergoing non-cardiac surgery at a single tertiary care center between 2006 and 2012 were included. Perioperative renal function was evaluated by Scr level.

Results: The data of 27,616 patients were analyzed. After multivariate adjustment for age, comorbidities, renal function, high-risk surgery, and postoperative ICU admission, we found that already minor changes in Scr (0.25–0.50mg/dl) were independently associated with a prolonged HLOS (hazard ratio for early discharge 0.79 [95%CI 0.71 – 0.88], p<0.01). Furthermore, this increase of serum creatinine was associated with a 2-fold increase in the risk of death during the postoperative hospital stay (odds ratio for all-cause in-hospital death OR 2.17 [95CI% 1.36 – 3.47], p<0.01). Increases in postoperative Scr greater than 0.50mg/dl were independently associated with longer HLOS and higher mortality.

Conclusions: This study demonstrates for the first time, that even minor elevations of Scr in patients undergoing non-cardiac surgery are independently associated with dramatic increases of severe adverse outcomes. These findings implicate that therapeutic approaches to prevent mild postoperative AKI would have a major impact on overall outcomes in surgical patients.

Underline represents presenting author/disclosure.

SA-PO1114

Randomized Trial of Short Prehydration with Sodium Bicarbonate Versus Pre- and Posthydration with Sodium Chloride To Prevent Contrast Induced Acute Kidney Injury: The Salina Trial

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Background: Yearly, millions of patients with renal failure worldwide receive intravascular contrast media, requiring hospitalization for hydration.

Aim: To analyze whether 1 hour (hr) prehydration with 250ml 1.4% sodium bicarbonate (Na-bic) is non-inferior to 4-12 hrs pre- and posthydration with 1000ml 0.9% sodium chloride (NaCl) in preventing contrast induced acute kidney injury (CI-AKI) in patients with a GFR<60ml/min undergoing intravenous contrast-enhanced CT.

Methods: From 2010-2012, 571 patients were randomized. Primary outcome was the relative increase in serum creatinine 48-96 hrs post CT. Secondary outcomes were the incidence of CI-AKI (serum creatinine increase>25%/>0.5mg/dl), recovery of renal function in CI-AKI patients, the need for dialysis, and 2-month hospital costs. Na-bic was considered non-inferior if the mean relative serum creatinine increase was at most 15% higher compared with NaCl.

Results: Patient characteristics were comparable in both groups (mean GFR 45ml/min, range 14-59). Mean relative increase in serum creatinine for Na-bic was 1.2%(SD13.2) and 1.5%(SD14.1) for NaCl (mean difference -0.33%; 95%CI-2.7-2.0). CI-AKI occurred in 22 patients (4.1%); 8 (3%) were treated with Na-bic, 14 (5.1%) with NaCl (P=0.23). Renal function recovered after 2 months in 75% and 70% of CI-AKI patients, respectively (p=0.81). No patient developed a need for dialysis. Mean hydration costs per patient were €224 ($266) for Na-bic and €683 ($811) for NaCl (p<0.001). Other costs were comparable.

Conclusions: Prehydration with Na-bic was non-inferior to pre- and posthydration with NaCl. Incidences of CI-AKI were similar in both groups. Thus, 1 hr Na-bic prehydration can be safely used, and may lead to substantial reductions in healthcare costs. This study is important for efficiently protecting patients from CI-AKI.

Funding: Government Support - Non-U.S.

SA-PO1115

Effects of Aleglitazar on Renal Function in Patients with Stage 3 Chronic Kidney Disease and Type 2 Diabetes

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Background: Aleglitazar (ALE) is a balanced PPAR-α/γ agonist in Phase 3 for CV risk reduction in patients following an acute coronary syndrome who have T2D. This phase 2b study (AleNephro) evaluated renal effects of ALE in stage 3 CKD patients with T2D.

Methods: Patients with stage 3 CKD and T2D were randomized to 52 weeks double-blind treatment with ALE 150µg/d or pioglitazone (PIO) 45mg/d, followed by an 8 week off-treatment period. The primary endpoint was non-inferiority for the difference between ALE and PIO in % change in eGFR from baseline (BL) to end of follow-up (EOF; 8 weeks after end of treatment [EOT]). A pre-specified exploratory analysis evaluated change in UACR in a subgroup with BL macroalbuminuria.

Results: The trial included 302 patients (ALE n=150; PIO n=152) with mean eGFR 47 mL/min/1.73m^2 and 81% on ACEi/ARB at BL. Mean eGFR change at EOT with ALE was –15% (95% CI: –19, –11) vs –5.4% (95% CI: –9.6, –1.2) with PIO and was non-progressive for both. Mean eGFR change from BL to EOF was –2.7% (95% CI: –7.7, 2.7) with ALE vs –3.5% (95% CI: –8.5, 1.8) with PIO, establishing non-inferiority (0.77%; 95% CI: –4.5, 6.0). In patients (N=48) with BL macroalbuminuria (>90% on ACEi/ARB), change in UACR at EOT was –59% (95% CI:–76, –29) with ALE and -51% with PIO (95% CI: –70, –20). The change in UACR at EOF was –54% (95% CI: –74, –20) with ALE and –33% (95%CI: –59, 9) with PIO. No major safety concerns or new toxicities were identified.

Conclusions: The primary endpoint in AleNephro was met, indicating that in stage 3 CKD patients with T2D, the eGFR decrease after 52 weeks treatment with ALE plus 8 weeks off-treatment was comparable (non-inferior) to PIO, implying reversibility. Mean on-treatment decrease in eGFR was mild, not progressive and accompanied by significant UACR reduction in patients with BL macroalbuminuria.

Funding: Pharmaceutical Company Support - F. Hoffmann-La Roche

SA-PO1116

Prevention of Contrast Induced Acute Kidney Injury – A Systemic Review and Network Meta-Analysis

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Background: Contrast induced acute kidney injury (CI-AKI) is among the most severe adverse outcome in patients undergoing coronary angiography. Treatments have been proposed to prevent this potential devastating complication, but relative preventive efficacy was not clear. Therefore, we conducted a systemic review and meta-analysis to assess the current evidence of treatments to prevent renal injury, including N-acetylcysteine, statins, isotonic sodium bicarbonate, ascorbic acid, fenoldopam, and theophylline.

Methods: We performed traditional meta-analyses for head-to-head comparisons with placebos. Furthermore, a network meta-analysis was performed by applying Bayesian mixed treatment comparisons to estimate the odds ratio (OR) and 95% credible intervals (CrI) for the relative effects.

Results: We identified a total of 90 RCTs including 90 with 15676 participants, of which 5684 (36%) developed CI-AKI. Traditional meta-analyses reported that N-acetylcysteine (OR = 0.69, 95% confidence interval [CI] = 0.53 – 0.82), statins (OR = 0.43, 95% CI = 0.19 – 0.98), and Sodium bicarbonate (OR = 0.46, 95% CI = 0.28 - 0.75) were significantly protective; while ascorbic acid (OR = 0.59, 95% CI = 0.28 - 1.23), fenoldopam (OR = 1.01, 95% CI = 0.60 - 1.70), and theophylline (OR = 0.58, 95% CI = 0.23 - 1.47) did not prove their efficacies over placebo. Network meta-analysis identified sodium bicarbonate to be the most effective one to prevent CI-AKI (OR = 0.39, 95% CrI = 0.07 - 6.29). Both high dose (OR = 0.52, 95% CrI = 0.28 - 0.86) and low dose (OR = 0.63, 95% CrI = 0.45 - 0.84) were protective. No specific adverse events were noted.

Conclusions: Our findings suggested the use of Sodium bicarbonate to prevent CI-AKI. in patients undergoing coronary angiography. N-acetylcysteine is protective in this setting and higher dose (more than 1000mg/dose) seems protective for developing CI-AKI.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO1117

Sevelamer Dominates Calcium Carbonate in a Patient-Level Cost-Effectiveness Analysis of the Randomized, Open-Label INDEPENDENT-HD Study

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Background: In the randomized, open-label INDEPENDENT-HD study sevelamer (SV) significantly increased overall survival (OS) (p<0.001) versus calcium salts (CS) in incident dialysis patients. A patient-level cost-effectiveness analysis (CEA) was conducted to determine if improved OS with SV translates into good value for money from an NHS perspective.

Methods: The CEA used OS and concomitant medication use data from 199 (SV) and 198 (CS) completer patients of the INDEPENDENT-HD study. Hospitalizations and length of stay (LOS) were collected retrospectively from medical charts. A clinician (BDI) estimated the frequency of use and dosage of concomitant medications. DRG tariffs and hospital acquisition costs informed hospitalization and drug costs, respectively. CEA outcomes were reported as incremental cost/life year gained (LYG) over 36 months. A probabilistic sensitivity analysis (PSA) was performed by bootstrapping.

Results: SV patients lived 4.75 months (95% CI 3.27–6.23) longer on average than CS patients. SV patients also had 76% fewer hospital admissions and shorter LOS (-0.06 d, p>0.05) than CS patients, resulting in reduced total hospitalization costs (-€455,704 vs. CS). Considering drug acquisition and concomitant medication costs, both higher for SV, SV patients had higher total average costs (€35,280) vs. CS patients (€29,235), resulting in an incremental cost/LYG of €15,272 (without dialysis costs) and €44,078 (with dialysis costs). The PSA revealed that 1) SV provides more life years in 99.4% of simulations, 2) SV dominates CS (i.e., more effective and less costly) in 17% of simulations, and 3) SV is cost effective in >90% of remaining simulations at the €50,000 willingness-to-pay threshold.

Conclusions: SV is cost effective versus CS for the treatment of hyperphosphatemia in incident dialysis patients.

Underline represents presenting author/disclosure.

SA-PO1118

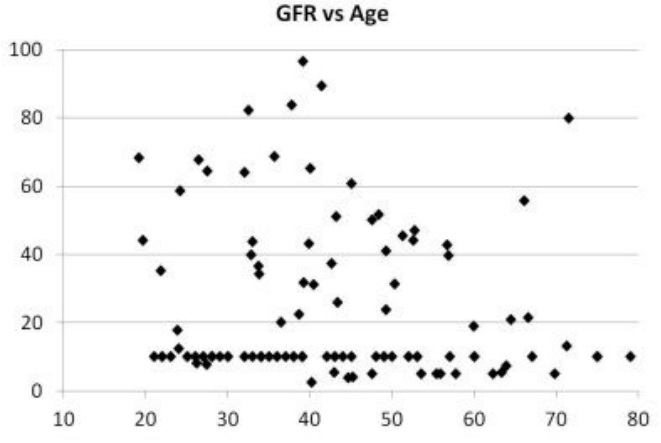
Clinical Characterization of Medullary Cystic Kidney Disease Type 1 (MCKD1) Families with *MUC-1* Gene Mutations Anthony J. Bleyer,[1] Corinne Antignac,[2] Steven J. Scheinman,[3] Stephen J. Knohl,[4] Gerald A. Hladik,[5] Philip J. Klemmer,[5] Stanislav Kmoch.[6] *[1]Wake Forest Medical School, NC; [2]Hopital Necker Enfants Malades, Paris, France; [3]Commonwealth Medical College, Scranton, PA; [4]State University of NY Upstate, Syracuse, NY; [5]Univ North Carolina, NC; [6]First Faculty of Medicine, Charles University, Praga, Czech Republic.*

Background: The very recent identification of mutations in the MUC1 gene as a cause of medullary cystic kidney disease type 1 (MCKD1) allowed us to perform the first clinical characterization of affected individuals and families and perform immunostaining of kidney biopsy material.

Methods: MCKD1 was found to result from a heterozygous mutation resulting in an extra cytosine within a run of seven cytosines in the variable number tandem repeat (VNTR) sequence of the mucin 1 (MUC1) gene in 6 families. Mutational analysis and clinical characterization was carried out in an additional 21 families without UMOD mutations.

Results: 13 of the 21 additional families had the MUC1 mutation. The Figure shows the latest estimated GFR for 114 affected individuals. Marked heterogeneity in GFR decline was seen within and between families, with age of ESRD ranging from 23 to over 70. While MUC1 is expressed in many tissues, there was no evidence of organ pathology in any other tissues besides the kidney. Immunostaining with antibodies to the mutant MUC1 protein revealed intracellular accumulation in affected individuals. The presence of medullary cysts on renal ultrasound was uncommon. While gout is common in kidney disease from UMOD mutations, there was not a significant family history of gout prior to kidney failure in any of the 19 identified families.

Conclusions: MUC1 mutations are common in hereditary interstitial kidney disease families that do not have UMOD mutations. Age of onset of ESRD is highly variable within and between families.



Funding: Private Foundation Support

SA-PO1119

Long Term Pathologic Changes in Kidney Allografts of Highly Sensitized Recipients Serena M. Bagnasco,[1] Andrea A. Zachary,[2] Lorraine C. Racusen,[1] Lois J. Arend,[1] Robert Avery Montgomery,[3] Edward S. Kraus.[2] *[1]Pathology, Johns Hopkins University, Baltimore, MD; [2]Medicine, Johns Hopkins University, Baltimore, MD; [3]Surgery, Johns Hopkins University, Baltimore, MD.*

Background: We studied the development of kidney graft injury over 1-9 years post-transplant follow up (FU) in 218 patients transplanted with an HLA-incompatible kidney between 2000 and 2010.

Results: Protocol and "for cause" biopsies were available in 120 pts: 38 M, 82 F; 98 whites, 16 blacks, 6 of other race; median age 45 years; median FU 3.2 years. Median PRA was 95. DSA at transplant were present in 81 pts: 31 class I only, 27 class II only, 23 both. Five pts died (4 with functioning graft), 14 lost their graft. Biopsy proven rejections were detected in 83 pts: antibody mediated rejection (AMR) in 19%, cell mediated rejection (CMR) in 31%, both in 18%, none in 38 pts, 9 pts had BK nephropathy. Rejections were most frequent in the first month (20% CMR, 16% AMR). The average eGFR (ml/min/1.73 m^2) declined significantly (P<0.001) from 63 at 3 months to 54 at 1 year, and was 48 at 4 year.

After the first year, 56 pts required graft biopsies, revealing CMR in 9 pts, AMR in 11 pts, both CMR and AMR in 3 pts, mixed CMR and AMR in 2 pts, glomerulitis (g≥1) and capillaritis (ptc≥1) but negative C4d in 8 pts, borderline inflammation in 5 pts. There was progressively higher average tubulointerstitial scarring (ci+ct) from 3 to 6 to 12 months (P<0.001), and from 1 to 2 to 3 years (P<0.05) post transplant. Transplant glomerulopathy (cg ≥1) developed in 54 pts, 14 by 6 months, and 23 by year 1, with proteinuria in 31, and was preceded by capillaritis (g≥1, ptc≥1) with positive C4d in 38%, and with negative C4d in 29%. Capillaritis (g≥1 and ptc≥1) with negative C4d was detected in the biopsies of 25 pts, of which at least 8 had moderate to high strength DSA, potentially indicating episodes of C4d-negative AMR.

Conclusions: Our observations support a role for capillaritis, even with negative C4d, in the development of transplant glomerulopathy. Despite good graft survival, acute and chronic allograft injury due to both AMR and CMR develop over time in incompatible allografts.

Funding: Clinical Revenue Support

SA-PO1120

The Effect of Cinacalcet on Mineral and Bone Disorder in Hemodialysis Patrick S. Parfrey,[1] Geoffrey A. Block,[2] Ricardo Correa-Rotter,[3] Tilman B. Drueke,[4] Jürgen Floege,[5] Christian Mix,[6] Bastian Dehmel,[6] Marie-Louise Trotman,[6] Dennis Modafferi,[6] Yumi Kubo,[6] Charles A. Herzog,[7] Gerard M. London,[8] Kenneth Mahaffey,[9] Sharon M. Moe,[10] David C. Wheeler,[11] Glenn M. Chertow.[12] *[1]Health Sciences Ctr, Canada; [2]Denver Neph; [3]Inst Nacional de Ciencias Méd y Nutrición, Mexico; [4]Picardie Univ SOMP, France; [5]RWTH Aachen, Germany; [6]Amgen; [7]Univ of Minnesota; [8]Hôpital Manhès, France; [9]Duke Univ Med Ctr; [10]Indiana Univ SOM; [11]Univ College London, United Kingdom; [12]Stanford Univ SOM.*

Background: Treatment of secondary hyperparathyroidism (sHPT) in patients on dialysis with cinacalcet may reduce parathyroidectomy (PTX) and fracture (Fx) rates, but the extent of this benefit is uncertain.

Methods: We designed the EVOLVE trial to primarily test the hypothesis that treatment with cinacalcet reduces the risk of death or non-fatal cardiovascular events among patients on hemodialysis with sHPT. Adjudicated secondary endpoints included PTX and clinical Fx. 3883 patients on hemodialysis with moderate to severe sHPT (median PTH concentration 693 pg/mL [10%, 90% range, 363 to 1694 pg/mL]) were randomized to cinacalcet versus placebo on a background of conventional therapy and followed for up to 64 months. 4.6% and 19.8% of the study population at baseline presented with a history of PTX and Fx, respectively. We defined an endpoint of 'severe unremitting HPT' as time to first of any of the following three criteria: 1) plasma PTH >1000 pg/mL with serum total calcium >10.5 mg/dL (2.6 mmol/L) on two consecutive occasions; or 2) plasma PTH >1000 pg/mL and total calcium >10.5 mg/L (2.6 mmol/L) on one occasion with prescription of commercial cinacalcet within 2 months, or 3) surgical PTX.

Results:

Conclusions: We will present the treatment effect of cinacalcet on mineral and bone disease, with a focus on PTX, severe unremitting HPT and Fx rates, using the pre-specified intent-to–treat analysis, multivariate adjustment for baseline imbalances and censoring of data 6 months after study drug cessation. (NCT00345839)

Funding: Pharmaceutical Company Support - Amgen Inc.

SA-PO1121

Arteriovenous Fistula Survival and Needling Technique: Results from a Randomized Buttonhole Trial. Jennifer M. MacRae, Sofia B. Ahmed, Brenda Hemmelgarn. *Medicine, University of Calgary, Calgary, Alberta, Canada.*

Background: We have previously shown in a randomized trial that buttonhole (BN) vs standard needling (SN), was associated with significant reduction in the hematoma rate. Given that hematoma is associated with increased risk of thrombosis, BN may increase survival of arteriovenous fistula (AVF). The purpose of this study was to compare AVF survival and AVF complications in BN and SN pts.

Methods: 140 conventional HD pts were randomized to BN or SN to explore the impact of needling on pain score at 8 weeks. Patients were followed until they transferred modality (peritoneal dialysis, transplant) or died. Their AVF was followed until abandonment, removal or thrombosis or until July 30 2011. Complications of infection and stenosis requiring a fistuloplasty (FP) were tracked. In BN pts time to first infection, time to abandonment of buttonholes and reason for abandonment was determined.

Results: Baseline characteristics were similar. The median follow up time was 521(360 – 1149) and 584 days (381-1245) for SN and BN; p=0.24. The median time to abandonment or loss of AVF from study start (censored for death, transplant, PD) was 397 (151- 687) in SN and 674 days (300 – 1216) in BN; p=0.04. AVF thrombosis were similar: 7 SN (20.6%) and 6 BN (17.4%).

Over the follow-up period the majority of pts abandoned their buttonhole (46/70); median time to abandonment 345 days (15 – 554). The time to first infection for BN from study start is 338 days (150 – 912). There are no infections to date in SN.

Conclusions: AVF needled with BN appeared to have longer survival although this is not explained by a reduced thrombotic or F/P rate. The possible increased survival must be weighed against the higher risk of infection.

Rates of secondary outcomes, for standard and buttonhole needling. Rate expressed as time to first event

Outcomes	Standard Needling Rate per 10, 000 patient days at risk	Buttonhole needling Rate per 10, 000 patient days at risk	p value
Infection requiring antibiotics	0	0.029 (0.013 - 0.0.046)	0.99
Thrombosis	0.021 (0.007 - 0.035)	0.014 (0.004-0.025)	0.6
Fistulogram/plasty	0.31 (0.21 - 0.41)	0.37 (0.26 - 0.47)	0.16
Surgical intervention	0.046 (0.018 - 0.075)	0.032 (0.012 - 0.051)	0.59

Values expressed as median (25th - 75th percentile)

Underline represents presenting author/disclosure.

SA-PO1122

A Randomized 5-Year Trial of Angiotensin II Blockade vs. Placebo in Kidney Transplant Recipients Hassan N. Ibrahim,[1] Bertram L. Kasiske,[2] Michael Mauer.[3] *[1]Department of Medicine, University of Minnesota, Minneapolis, MN; [2]Department of Medicine, Hennepin County Medical Center, Minneapolis, MN; [3]Department of Pediatrics and Medicine, University of Minnesota, Minneapolis, MN.*

Background: Interstitial fibrosis/tubular atrophy, not otherwise specified, (IF/TA) is a major cause of kidney transplant loss that lacks treatment or preventive options.

Methods: We conducted a 5-year, double-blind, randomized placebo controlled trial in 153 kidney transplant recipients who were randomly assigned to receive losartan 100mg/d (n=77), or matching placebo (n=76). The main outcome measure was a composite of doubling of the fraction of renal cortical volume occupied by interstitium from baseline to 5 year biopsies or ESRD from IF/TA at any time during the trial.

Results: Both systolic and diastolic blood pressures were highly comparable between the two groups and consistently <130mmHg and <80mmHg, respectively. Annual measured GFR and urine protein were also similar. Twelve participants ended follow-up early by developing ESRD from IF/TA, 4 by developing non IF/TA related ESRD, 12 by death and 16 by withdrawal. In the intention to treat analysis, using only those with complete structured data, the primary endpoint occurred in 6/47 subjects receiving losartan and 12/44 receiving placebo (OR 0.39, 95% CI 0.13-1.15, p =0.08). For doubling of interstitial volume and all cause ESRD, losartan was protective; O.R. 0.36 (95% C.I. 0.13-0.99), p=.049. An increase of 10% in interstitial volume over the 5-year period was associated with a 3 ml/min decline in GFR in the losartan group and 18 ml/min in the placebo group. Losartan treated patients had, on average, serum potassium that was 0.2-0.3 mEq/L higher than placebo treated subjects. Uric acid and hemoglobin, on the other hand, were lower in the losartan group.

Conclusions: These results suggest that losartan, when used preemptively before the development of proteinuria or allograft dysfunction, is not associated with a statistically significant benefit in prevention of cortical interstitial volume. Whether its use in established allograft dysfunction would be beneficial remains to be studied.

Funding: NIDDK Support

SA-PO1123

Tubulointerstitial Inflammation in Lupus Nephritis Gia J. Oh,[1] Paul C. Grimm,[1] Neeraja Kambham.[2] *[1]Pediatrics, Stanford University Medical Center, Stanford, CA; [2]Pathology, Stanford University Medical Center, Stanford, CA.*

Background: The renal biopsy diagnosis including the RPS/ISN class, activity (AI) and chronicity (CI) indices determine the management of lupus nephritis (LN). The glomerular disease may be largely antibody-dependent, while the tubulointerstitial (TI) disease may be antibody-independent and influenced by T cells. Recent studies have suggested that TI inflammation and scarring, rather than glomerular inflammation are better predictors of outcome.

Methods: Our Pathology database was searched for adequate renal biopsies with active lupus nephritis (criteria: class III/IV; AI>3) from 2004-08. 41 biopsies from 41 adult and pediatric subjects with at least one follow-up creatinine value were identified and we perfomed histological and immunophenotypic analysis. Of these, 7 were lost to follow up and hence excluded In addition to determining T cell subsets (CD3, CD4, CD8, GATA3, T-bet, ROR γt, Foxp3, IL-17), B cell (CD20) and monocyte (CD14) infiltrates were evaluated in both glomeruli and TI. On follow-up, the adverse endpoint was a composite outcome of dialysis, transplant or 50% decrease in GFR or proteinuria by ANOVA method.

Results: The mean follow-up time of the study subjects was 3.8 years +/- 750 days. In addition to chronicity index, the individual histological parameters of TI inflammation, tubular atrophy, interstitial fibrosis, glomerulosclerosis and crescents predicted adverse outcome. On immunophenotypic analysis, the density of TI T lymphocytes (CD3; p=0.042), glomerular and TI monocytes (CD14; both p=0.02) and TI IL-17 (p=0.04) were significantly correlated with the endpoints.

Conclusions: The TI infiltrates, especially T lymphocytes and monocytes influence the progression of the renal disease in LN. IL-17 producing cells such as Th17 subset of T cells may be an important driver of kidney disease in systemic lupus erythematosus.

SA-PO1124

A Randomized Controlled Trial To Evaluate the Effect of Ages 3 - 5 Chronic Kidney Disease: The OPERA Study Angela Yee Moon Wang,[1] Fang Fang,[2] John Chan,[3] Qing Shang,[2] Gladys G. Lo,[3] Iris Chan,[5] Kar Neng Lai,[1] Christopher W. Lam,[6] Cheuk-man Yu.[2] *[1]Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong; [2]Medicine & Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong; [3]Diagnostic Radiology, Hong Kong Sanatorium Hospital, Hong Kong, Hong Kong; [4]Medicine, Tung Wah Hospital, Hong Kong, Hong Kong; [5]Pathology, United Christian Hospital, Hong Kong, Hong Kong; [6]Macau Institute for Applied Research in Medicine and Health, Macau.*

Background: Vitamin D has been suggested to play a role in cardiovascular disease through interaction with vitamin D receptor.

Methods: We conducted a prospective double-blind randomized placebo-controlled trial in 60 stages 3-5 chronic kidney disease (CKD) subjects with echocardiographic evidence of left ventricular (LV) hypertrophy who were randomly assigned to receive either oral paricalcitol, 1μg/d (N=30) or matching placebo (n=30). Primary endpoint was change in LV mass index over 52 weeks by cardiac magnetic resonance imaging. Secondary endpoints included echocardiographic changes in LV volumes, systolic & diastolic function, biochemical parameters of CKD-mineral bone disease (MBD), arterial stiffness, 24-hour urine protein & estimated GFR.

Results: After 52-week, the change in LV mass index did not differ significantly between treatment [-2.97 ± 6.97g/m^2] and placebo group [-3.73 ±10.04g/m^2]. Changes in LV end-systolic, end-diastolic volumes, ejection fraction, tissue doppler derived measure of diastolic mitral annular velocity (E'), systolic mitral annular velocity (S') & early mitral inflow velocity to diastolic mitral annular velocity (E/E') did not differ between the two groups. Paricalcitrol treatment significantly lowered intact PTH [-10.31 ± 10.76 vs 6.33 ± 12.41 pmol/L; P<0.0001] and alkaline phosphatase [-14 ± 21.66 vs 2.13 ± 17.95 U/L; P=0.001] in treatment vs placebo group.

Conclusions: Fifty-two weeks treatment with oral paricalcitol significantly improved biochemical parameters of CKD-MBD but did not alter LV mass, volumes and function in stage 3-5 CKD patients.

Clinical Trials.gov NCT00796679

Funding: Pharmaceutical Company Support - Abbott Lab (but this is an investigator initiated study)

SA-PO1125

The Soluble Triggering Receptor Expresed on Myeloid Cells-1 (STREM-1) as Inflammation Marker in Hemodialysis Patients Montañez F. Joseluis,[1] Enrique Rojas-Campos,[2] Alfonso M. Cueto-Manzano.[2] *[1]Nefrologia-Hemodialisis, Hospital General Regional No.110. I.M.S.S, Guadalajara, Jalisco, Mexico; [2]Unidad de Investigaciones Medicas en Enfermedades Renales, Hospital de Especialidades, CMNO, Guadalajara, Jalisco, Mexico.*

Background: Inflammation predicts lower survival in ESRD patients with/without dialysis. sTREM-1 is a new inflammation marker associated to worse prognosis in the intensive care and surgery settings. Aim: To determine the serum sTREM-1 concentrations and compare them with other inflammation markers in hemodialysis (HD) patients.

Methods: In a cross-sectional evaluation, 264 chronic HD patients and 148 healthy subjects were evaluated. Age, gender, cause of ESRD, time on dialysis, type of vascular access, presence of DM and/or hypertension, serum glucose, urea, creatinine, total, HDL- and LDL-cholesterol, triglycerides, albumin, electrolytes, and inflammation markers [interleukin 6 (IL-6), C-reactive protein (CRP), tumor necrosis factor alpha receptor (TNFaR) and sTREM-1] were measured/recorded.

Results: Main results are shown in Table. Serum sTREM-1 concentrations were correlated with IL-6 (r=0.56; p<0.05), TNFαR (r=0.76; p<0.05), and CRP (r= 0.56; p<0.05).

Variable	Hemodialysis N = 264	Healthy subjects N=148
Age (yrs)	47 ± 17*	39 ± 12
Weigth (Kg)	65 ± 16*	73 ± 15
SBP (mmHg)	152 ± 26*	119 ± 15
DBP (mmHg)	86 ± 14*	73 ± 11
Time on HD (months)	10.3 ± 18.2	
Vascular access, N (%)	45% 8% 47%	
Hemoglobin (g/dl) *	9.7 ± 3.1	14.4 ± 2.0
Leucocytes (mm/μL) *	6650 ± 2660	7750 ± 1826
Glucose (mg/dl) *	111 ± 50	87 ± 19
Albumin (g/dl) *	3.7 ± 0.6	4.5 ± 6.4
Creatinine (mg/dl) *	8.7 ± 3.3	0.8 ± 0.2
Uric acid (mg/dl)	5.8 ± 1.8	5.2 ± 1.41
sTREM-1 (pg/mL) *	1006 (613-1650)	0
IL-6 (pg/mL) *	7.9 ± 4.8	1.7 ± 1.0
TNFα (pg/mL) *	35.3 ± 21.7	1.7 ± 1.0
CRP (mg/L) *	6.8 (2.4-16.4)	1.9 (0.9-5.2)

*P<0.05 vs healthy subjects

Conclusions: This is the first study that evaluate sTREM-1 in ESRD. HD patients had higher serum concentrations of sTREM-1, IL-6, TNFαR and CRP than healthy subjects. sTREM-1 was also positively correlated with IL-6, TNFα and CRP.

It is possible that sTREM-1 may have a relevant role as an earlier marker of inflammation than traditional ones.

Funding: Government Support - Non-U.S.

SA-PO1126

COUP-TFII Is Essential for Metanephric Mesenchyme Formation and Kidney Progenitor Cell Renewal Chengtai Yu,[1] Akio Kobayashi,[3] Sophia Y. Tsai,[1,2] Ming-jer Tsai.[1,2] *[1]Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; [2]Program in Developmental Biology, Baylor College of Medicine, Houston, TX; [3]Department of Medicine, Harvard Medical School and Harvard Stem Cell Institute, Boston, MA.*

Background: Development of the metanephric kidney in mammals requires complex reciprocal interactions between the ureteric epithelium and the mesenchyme. Previous studies indicated that loss of *Osr1*, *Eya1*, *Pax2* or *Wt1* gene function in the metanephric mesenchyme compromises the formation of the kidney. Moreover, it has been shown that the Hox11-Eya1-Pax2 complex activates the expression of *Six2* and *Gdnf* in the metanephric mesenchyme to drive nephrogenesis.

Methods: We using the inducible knockout mice to delete COUP-TFII at E7.5 and collect embryos at E10.5 to study COUP-TFII functions in metanephric mesenchyme formation. At later stages of kidney development, the kidney specific Six2-Cre was used to ablate COUP-TFII in kidney progenitor cells.

Results: Here, we demonstrate that the COUP-TFII is required for the specification of the metanephric mesenchyme. Deletion of *COUP-TFII* at E7.5 results in improper

Underline represents presenting author/disclosure.

differentiation of the metanephric mesenchyme. COUP-TFII lies upstream of essential developmental regulators, such as *Eya1*, *Six2,* Pax2 and *Gdnf* to exert its function. Importantly, COUP-TFII directly regulates the transcription of both *Eya1* and *Wt1* in the metanephric mesenchyme. We also observed that the *Six2*$^{Cre/+}$*:COUP-TFII*$^{flox/flox}$ mutant mice kidney showed severe hypoplastic phenotypes and loss of the nephrogenic zone at P0 and cause neonatal death. We also found that Six2 expression in the cap mesenchyme is lost in the E13.5 mutant embryo, resulting in an increase of ectopic pretubule aggregates. Our results shows COUP-TFII is the key factor to regulate Six2 and maintaining kidney progenitor cell renewal.

Conclusions: Our findings reveal that COUP-TFII plays a central role in the specification of metanephric fate and in the maintenance of metanephric mesenchyme proliferation and survival through regulating the expression of *Eya1* and *Wt1*. In the later stage COUP-TFII is also indispensible for kidney progenitor cell renewal by regulate *Six2* expression.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-PO1127

Inhibition of Hyaluronan Synthesis Protects from Renal Function Impairment and Inflammation in a Mouse Model of Renal Ischemia-Reperfusion Injury Vanessa Colombaro,[1] Inès Jadot,[1] Virginie Voisin,[1] Laetitia Giordano,[1] Anne-Emilie Decleves,[2] Isabelle Habsch,[1] Bruno Flamion,[1] Nathalie Caron.[1] *[1]Molecular Physiology Research Unit - URPHYM, University of Namur - FUNDP, Namur, Belgium; [2]Experimental Nephrology Unit, Université Libre de Bruxelles - ULB, Brussels, Belgium.*

Background: Ischemia-reperfusion (IR) injury (IRI) to the kidney is a complex patho-physiological process that may lead to acute renal failure and chronic dysfunction in renal allografts. It was previously demonstrated that during IRI, hyaluronan (HA) accumulates in the cortical and external medullary interstitium together with an increased expression of its main receptor, CD44, on inflammatory and tubular cells. In mice, CD44 deficiency protects against short term IRI. The HA-CD44 pair may be involved in persistent postischemic inflammation.

Methods: Male C57BL/6 mice received a Western diet containing 4-methylumbelliferone (4-MU; 10 mg/g body weight/day), a potent HA synthesis inhibitor, for 14 weeks beginning at 4 weeks of age. At the end of the treatment, unilateral renal IR was performed by clamping the left artery for 28 min before removing the right kidney. Mice were euthanized 48 h post-IR, blood was collected and the kidney was harvested for analytical purposes.

Results: 4-MU treatment for 14 weeks reduced plasma HA level but was otherwise well tolerated. Forty-eight hours after IR in 4-MU treated mice, intrarenal HA content significantly decreased in the cortex (from 75.7 ± 14.8 to 20.6 ± 5.2 ng/mg, P< 0,05 ; n=8) and in the inner medulla (from 167,2 ± 37 to 41,5 ± 9,5 ng/mg, P< 0,05 ; n=8), as were also CD44 expression, creatininemia and histopathological lesions, as demonstrated by semi-quantitative methods. Moreover, inflammation was significantly attenuated as attested by markedly reduced macrophages and lymphocytes infiltration. Finally, proliferation, assessed by immunohistochemical staining, was reduced in animals treated with 4-MU.

Conclusions: Our results demonstrate that HA plays a significant role in the pathogenesis of renal IR injury, at least in part through reduced expression of CD44. Suppression of HA synthesis and/or accumulation during IR injury may protect renal function against ischemic insults.

Funding: Government Support - Non-U.S.

SA-PO1128

Phosphate Binder Comparison: Sevelamer Carbonate & CaSuccinate/MgLipoate Deanna J. Nelson,[1] Keith A. Hruska.[2] *[1]BioLink Life Sciences, Inc., Cary, NC, United States; [2]Washington University, St. Louis, MO.*

Background: Hyperphosphatemia is causally related to atherosclerotic cardiovascular disease, the most important cause of death in all stages of renal failure and the single greatest threat to survival among ESRD patients undergoing dialysis. Patients use cationic binders to bind phosphate (Pi) in the GI tract and prevent its uptake. FDA-approved phosphate binders include calcium acetate or carbonate, lanthanum salts, or sevelamer, a cationic polymer. The combination of calcium succinate (CS) and magnesium lipoate (ML) has the potential both for phosphate binding with reduced calcium load and for reduction in oxidative stress, vascular calcification, and bone dysfunction.

Methods: LDLR -/- mice were fed high fat/high cholesterol diets supplemented with 1% or 3% (w/w) sevelamer carbonate (SC) or 1% or 3% (w/w) CS/ML. The actions of CS/ML were compared with those of SC using multiple criteria, including: (1) Pi binding; (2) effects on vascular calcification; and (3) effects on bone remodeling disorder as described in Hruska et al., *J Am Soc Nephrol 2007;18:122-30.*

Results: The untreated CKD mice were hyperphosphatemic, and significantly, CS/ML treatment dose-dependently decreased the serum Pi at least as effectively as SC treatment, if not more effectively. Similar effects on vascular calcification and bone remodeling were observed after treatment with SC or CS/ML. Four unexpected results related to CS/ML were obtained: (i) A significant reduction in serum glucose was observed in animals treated with 1% and 3% CS/ML in the diet. (ii) A 30% reduction in serum cholesterol, comparable to the reductions typically caused by SC treatment of CKD patients, was observed in animals treated with 1% CS/ML. (iii) Animals treated with 1% and 3% CS/ML in the diet showed an absence of weight gain. (iv) Animals in the 1% and 3% CS/ML treatment groups exhibited high BUN, hair loss, and skin disorders that were likely related to treatment.

Conclusions: These preliminary data suggest that CS/ML has the potential to provide pleiotropic benefits similar to those provided by SC. Additional preclinical studies are underway to confirm that CS/ML can be effectively and safely administered to ESRD patients.

Funding: NIDDK Support

SA-PO1129

Recombinant Human Erythropoietin Improves Rhabdomyolysis-Induced Acute Kidney Injury in Rats Regardless of Dose Yang Wook Kim,[1] Bongsoo Park,[1] Tae Hee Kim,[2] Kyu-Bok Jin,[1] Mi Seon Kang.[3] *[1]Nephrology, Internal Medicine, Haeundae Paik Hospital, Inje University; [2]Nephrology, Internal Medicine, Pusan Paik Hospital, Inje University; [3]Pathology, Pusan Paik Hospital, Inje University, Busan, Republic of Korea.*

Background: Rhabdomyolysis is one of the causes of acute kidney injury. Erythropoietin has cytoprotective effects on various non-erythroid cell as well as erythroid cells.

We studied he preventive and therapeutic effects of recombinant human EPO(rhEPO) on glycerol-induced rhabdomyolysis with acute kidney injury in rats.

Methods: Sprague-Dawley rats were divided into 6 groups of Control(C) , Glycerol(G1), EPO+Glycerol[(G2:1000U/kg(G2-L) or 5000U/kg(G2-H)], Glycerol+EPO(1000U/kg: G3-L), and Glycerol+EPO(5000U/kg:G3-H). Rhabdomyolysis was induced by intramuscular injectionof 50% glycerol (10 ml/ kg) in rats. For EPO+Glycerol group, the rats received intraperitoneal injection (IP) of rhEPO 24 hours prior to Glycerol injection. On the other hand, Glycerol+EPO group received rhEPO daily 24 hours after the injection of Glycerol. Hemoglobin, blood urea nitrogen (BUN), creatinine (Cr), and creatine phosphokinase(CPK) were measured at 0, 24, 48, 72, hr. When rats were sacrificed 5 days later, the same parameter was measure, and the kidneys were removed immediately for pathology and immunohistochemistry.

Results: Intramuscular injection of glycerol increased blood BUN, Cr, and CPK level significantly and induced severe histopathologic damages in the kidneys. In G2, Cr level was not different compared to G1 regardless of the rhEPO dosage . In G3, Cr level decreased subsequently after EPO injections. However, the Cr levels between two groups(G3-L vs G3-H) were not different. Histologic findings revealed that tubular necroses were resolved progressively in G3. The serum concentration of TGF-β increased in G1 & G2, but decreased in G3. The immunohistochemical expressions of TGF-β were correlated with the serum TGF- β level.

Conclusions: This study suggests that rhEPO improved the acute renal injury caused by rhabdomyolysis . It, however, did not have the preventive effect. It may also ameliorate the rhabdomyolysis-induced acute kidney injury through TGF-β suppression regardless of dose.

SA-PO1130

SOCS3-Independent Src Signaling Is Enhanced by cAMP and PC1 p30 in ADPKD Jeffrey Talbot,[1] Xuewen Song,[2] York P. Pei,[2] Thomas Weimbs.[1] *[1]Molecular Cellular & Developmental Biology, University of California Santa Barbara, Santa Barbara, CA; [2]Divisions of Nephrology & Genomic Medicine, University Health Network & University of Toronto, Toronto, ON, Canada.*

Background: Elevated levels of activated STAT3 are present in cyst-lining renal epithelial cells in ADPKD patients. Increased cAMP and decreased HNF1-b induce SOCS3 expression, and are present in ADPKD. Here we look at SOCS3 and its effect on STAT3 signaling in ADPKD.

Methods: SOCS3 mRNA from micro-dissected human cyst-lining renal epithelial cells was quantified by microarray & qPCR. Total/phospho- JAK2, STAT3 & Src were determined by western blot from whole cell lysates of cultured cells or PKD mouse models. Luciferase transcriptional reporter assays were run in HEK293T cells transfected w/a STAT1/3 lucif. reporter & the genes of interest.Forskolin stimulated cAMP levels.

Results: SOCS3 transcripts were significantly elevated in cyst-lining renal epithelial cells of human patient kidney samples at all stages of cystic progression.

JAK2 is a regulatory target of SOCS3, we found slight up-regulation of total protein levels in cystic animals, but no disease-related activation of JAK2.

Luciferase assays revealed that the disease-associated PC1 p30 cleavage product enhances STAT3 signaling by Src in a SOCS3-independent manner. This Src/p30 complex enhances EGFR-STAT3 signaling. Forskolin increased cAMP levels, and enhanced all Src-dependent signaling while inhibiting all JAK2-dependent signaling.

The ability of SOCS3 to regulate JAK2-dependent pathways was tested using Polycystin-1 and IL6. Both were unable to activate STAT3 in the presence of SOCS3.

Conclusions: We investigated the newly discovered paradox of high phospho-STAT3 and high SOCS3 in cyst-lining epithelial cells in ADPKD.

Src/p30 activates STAT3 in a SOCS3- and JAK2-independent manner. In vitro and possibly in ADPKD, the cAMP-induced expression of SOCS3 prevents JAK2-dependent signaling, preventing activation of STAT3 by PC1 and IL6.

High cAMP levels activate Src signaling and in doing so enhance the Src/EGFR positive feedback loop. The PC1 p30 fragment further enhances this feedback loop, creating an environment of high STAT3 activity while in the presence of high SOCS3.

Funding: Other NIH Support - RO1

Underline represents presenting author/disclosure.

SA-PO1131

The Function and Mechanism Study of Curcumin:Loaded Nanoparticles Against Renal Ischemia:Reperfusion Injury Yong Xu,[1] Donghui Zheng.[1] *[1]Nephrology Department, Huai'an Second Hospital, Huai'an, JiangSu, China.*

Background: To study the effects of curcumin-loaded nanoparticles against ischemia-reperfusion renal injury, and to investigate its mechanism.

Methods: Synthetize controlled releasing curcumin-loaded nanoparticles by amphilic mPEG-PCL block copolymers, and cultivated renal tubular epithelial cells (cells line HK-2) in vitro. HK-2 cells were divided into four groups: Control group; Ischenmia reperfusion injurygroup (IRI group); Curcumin group(Cur group); Curcumin nanoparticle group(CurNP group). In each group, HK-2 cells viability was assessed by dimethylthiazol-diphenyltetrazoliumbromide (MTT) tests. Apoptotic Cells were measured by Flow Cytometry. H2DCF-DA was used to detect intracellular generation of ROS. BCA was used to detect SOD activation and the Concentration of MDA. Protein levels of procaspase-3 were analyzed by Western Blot.

Results: We successfully constructed curcumin-loaded nanoparticles by amphilic mPEG-PCL block copolymers. In IRI group, cell viability gradually decreased, cell apoptosis had an obvious increased, SOD activity declined, the level of ROS and MDA activation significantly increased , the expression of Caspase-3 increased. In Cur group and CurNP group, cell viability and SOD activity significantly increased. A great reduction in the level of cells apoptosis was observed. The level of ROS and MDA activation were inhibited, the expression of Caspase-3 decreased. Compared with Cur group, showed marked changes in CurNP group($P<0.05$).

Conclusions: 1.Efficiently produce controlled releasing Curcumin-loaded nanoparticles by amphilicmPEG-PCL block copolymers.Drug loading content(DLS) and encapsulation efficiency(EE) were more than other reports.Data indicated that incorporated Curcould be slow released from the core–shellstructureof polymericnanoparticles. 2.CurNP can ameliorate ischemia-reperfusionrenal injury in vitro. 3.The protective effects of Cur-NP against ischemia-reperfusion renal injury were involved in the suppression of oxidative stress reaction.

Funding: NIDDK Support, Other U.S. Government Support, Government Support - Non-U.S.

SA-PO1132

Tweak Levels Are elevated in Diabetic Nephropathy and Stimulate Cytokine, Chemokine and Fibrotic Genes in Renal Cells Priya S Chockalingam, Michael Luong, Gregory J. Carven, Stephane Olland, Edward R. Lavallie, Nick Pullen, Lih-ling Lin. *Pfizer, Cambridge, MA.*

Background: Tumor necrosis factor-like weak inducer of apoptosis (Tweak, Apo3L, TNFSF12), a member of the TNF super family, activates the Fn14 receptor, regulates cell proliferation, death, differentiation, and inflammation, and plays a role in acute and chronic renal injury. We analyzed expression levels of Tweak and Fn14 in human diabetic nephropathy (DN) kidneys and correlated with animal and in-vitro models of DN/fibrosis. We also studied the effect of Tweak on primary human renal cells.

Methods: Snap frozen human DN (n=17) and non-DN (n=11) kidneys were obtained from various sources. Kidneys were harvested from C57BL6 mice (Taconic; 8-9 weeks old) that underwent a unilateral urethral obstruction (UUO) procedure and sacrificed at different times post-surgery (3, 7, or 10 days; n=6 per group). Human primary renal mesangial (HRMes, Lonza) and proximal tubuloepithelial cells (HRPTec, ScienCell) were treated with 100mM D-glucose or 0.2-2nM recombinant Tweak for 24-72 hrs followed by mRNA analysis and/or assays for cytokine and chemokine release.

Results: Tweak mRNA was elevated 4-fold in human DN kidneys compared with non-DN kidneys ($p=0.00008$); Fn14 mRNA showed a trend towards up-regulation but did not reach significance. Tweak and Fn14 mRNA showed a significant 2-3 and 5-16 fold increase, respectively, in the mouse UUO kidneys as compared with sham and naïve kidneys. Induction of HRMes with Tweak resulted in a dose-dependent increase in chemokine/cytokine release (CCL2, CCL5, CXCL1, IL6 and IL8). Tweak stimulation of HRPTec showed mRNA up-regulation of the same set of cytokines and chemokines as observed for HRMes, and in addition, fibrotic genes such as Col1A1, Col4A1, CTGF, ACTA2, TIMP1, and MMP9. High glucose treatment of HRMes cells induced mRNA expression of Tweak and fibrotic markers (Col4A1, CTGF, FN1, and TIMP1).

Conclusions: Our findings add further evidence for involvement of Tweak-Fn14 in human renal diseases and suggest a novel role for this pathway in diabetic nephropathy. These data provide new insights into potentially preventing pathological remodeling in chronic renal indications including DN.

SA-PO1133

CCL5 Protects from Chronic Hypertensive Kidney Injury Via Blood Pressure-Independent Mechanisms Mehul B. Patel,[1] Jiandong Zhang,[1] Bob Griffiths,[1] Phillip Ruiz,[2] Steven D. Crowley.[1] *[1]Duke University, Durham, NC; [2]Jackson Memorial Hospital, Miami, FL.*

Background: T lymphocytes are critically involved in the pathogenesis of hypertensive kidney disease. In chronic hypertension, the chemokine CCL5 is highly expressed in the kidney and is thought to recruit T cells to peri-vascular tissues. However, the precise role of this chemokine in the progression of hypertensive kidney damage is unknown. Here we study the influence of CCL5 on blood pressure (BP) and kidney injury during angiotensin II (Ang II) induced hypertension.

Methods: We subjected uni-nephrectomized, 129SVE wild-type (WT) and $CCL5^{-/-}$ (KO) mice to chronic Ang II infusion (1000ng/kg/min) for four weeks while measuring BP by radiotelemetry. To gauge the extent of kidney injury, we measured urinary albumin excretion, kidney pathology, and renal gene expression after four weeks of Ang II.

Results: Ang II caused a robust and similar BP elevation in the WT and KO groups (average MAP during third week of Ang II 192±4 vs.190±3 mm Hg). Despite these similar blood pressures, KO mice had significantly increased albuminuria compared to WT controls (21.1±1.9 vs 13.9±2.2 mg albumin/mg Cr; $p=0.02$). Furthermore, compared to WTs, KOs had significantly greater renal gene expression of the injury markers NGAL [0.38±0.06 vs. 1.69±0.37 arbitrary units (au); $p=0.002$], PAI-1 (0.58±0.11 vs. 1.42±0.20 au; $p=0.002$), and TNF-alpha (0.57±0.12 vs. 1.48±0.27 au; $p=0.006$). In examining differences in the immune response to explain the dissimilarities in kidney damage, we found that compared to WT mice, KOs had dramatically increased dense peri-vascular T cell infiltrates in the kidney (28 vs. 57% of vessels surrounded by > 50 T cells; $p<0.03$). To ascertain a possible mechanism of increased renal T cell infiltration in the KO group, we measured gene expression of the other known ligands to CCR5, CCL3 and CCL4; however, renal levels of these chemokines were similar in the two groups.

Conclusions: In chronic hypertension, the T cell chemokine CCL5 mitigates the progression of renal damage by paradoxically suppressing the infiltration of T cells into the kidney.

Funding: NIDDK Support, Private Foundation Support

SA-PO1134

Anti-miR21 Protects Collagen 4A3 Deficient Mice from Progression of Alport Disease Ivan G. Gomez,[2] Deidre Mackenna,[1] Geoffrey Richard Linn,[2] Bryce Gordon Johnson,[2] Xinqiang Huang,[1] Dorin-Bogdan Borza,[3] Jie Zhang,[4] Philip Janiak,[5] B. Nelson Chau,[1] Jeremy Stuart Duffield.[2] *[1]Regulus Therapeutics, San Diego, CA; [2]Division of Nephrology, and Lung Biology Institute for Stem Cell & Regenerative Medicine, University of Washington, Seattle, WA; [3]Vanderbilt University, Nashville, TE; [4]R&D Center, Sanofi-Genzyme, Framingham, MA; [5]R&D Center, Sanofi-Genzyme, Chilly-Mazarin, France.*

Background: Alport syndrome in humans is an inherited form of kidney disease caused by a mutation in the gene coding the capillary basement membrane collagen IV. The disorder is characterized by progressive glomerulonephritis, leading to glomerulosclerosis, tubulo-interstitial disease and organ failure. $Col4a3^{-/-}$ mice also spontaneously develop severe kidney disease highly similar to human disease. Recent studies have shown that miR-21 promotes interstitial kidney disease with fibrosis by silencing metabolic pathways, particularly fatty acid metabolism, and by promoting ROS formation. We hypothesized that treating $Col4a3^{-/-}$ mice with silencing anti-miR21 oligonucleotides would prevent progression of disease.

Methods: $Col4a3^{-/-}$ mice were given anti-miR21 (25mg/kg q4d) or control from wk3 to wk9. Urine, plasma and kidneys were harvested at the end of wk9. All samples were analyzed to determine changes in kidney function and fibrosis.

Results: Anti-miR-21 treatment attenuated the loss of kidney function and development of albuminuria. Glomerulosclerosis and interstitial fibrosis were markedly attenuated and proximal tubules were preserved. Furthermore, anti-miR21 reduced infiltrating macrophages, and myofibroblast appearance. Ppara activity and fatty acid metabolism were preserved by anti-miR21 and cell activation signal transduction pathways including P42/P44 MAPK were inhibited by anti-miR21.

Conclusions: Anti-miR21 prevents progressive loss of kidney function in the $Col4a3^{-/-}$ mouse, attenuates both glomerular and tubulo-interstitial disease. Anti-miR21 is a potential new therapy for human kidney disease.

Funding: NIDDK Support, Pharmaceutical Company Support - Regulus Therapeutics

SA-PO1135

Activation of Soluble Guanylate Cyclase Ameliorates Renal Extracellular Matrix Overproduction in Experimental Diabetes Gabor Kokeny,[1,3] Lilla Fang,[1,3] Sina Abhari,[1] Arash Samarghandi,[1] Laszlo Rosivall,[1,3] Tamas Radovits,[2] Miklos M. Mozes.[1,3] *[1]Dept. of Pathophysiology, Semmelweis University; [2]Heart Center, Semmelweis University; [3]International Nephrology Research and Training Center, Semmelweis University, Budapest, Hungary.*

Background: The pathogenesis of diabetic nephropathy is associated with abnormalities of the NO-cGMP axis and functional imbalance of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). The PDE-5 inhibitor sildenafil has been reported to reduce extracellular matrix (ECM) production in type-2 diabetic rats. We investigated whether restoring cGMP levels by soluble guanylate cyclase (sGC) activator cinaciguat may attenuate renal ECM overproduction in type-1 diabetic rats.

Methods: Diabetes was induced in male Sprague-Dawley rats with single dose of streptozotocin (60 mg/kg). Experimental groups were (n=8/group): 1) Non-treated diabetic (DM); 2) Diabetic + cinaciguat (treated with 10 mg/kg/day per os for 8 weeks, DM-Cin); 3) Non diabetic controls (Co). Kidneys were analyzed after 8 weeks of treatment for histology and mRNA expression levels.

Results: In diabetic rats, cinaciguat treatment elevated serum cGMP levels (Co: 18±2, DM: 15±3, DM-Cin: 36±19 pmol/ml, $p<0.05$), reduced glomerulosclerosis (score: Co: 0.1±0.1, DM: 0.6±0.2, DM-Cin: 0.2±0.1, $p<0.05$) and collagen-IV expression (score: Co: 2.0±0.3, DM: 3.6±0.2, DM-Cin: 2.6±0.2, $p<0.05$). CTGF mRNA expression was also reduced in DM-Cin rats (relative expression, Co: 0.9±0.1, DM: 1.9±0.5, DM-Cin: 0.8±0.3, $p<0.05$). MMP2 expression was reduced in DM rats but restored in DM-Cin rats (Co: 0.7±0.2, DM: 0.3±0.1, DM-Cin: 0.8±0.4, $p<0.05$). TIMP-1 was markedly elevated in DM rats but almost normalized in DM-Cin rats (Co: 1.0±0.1, DM: 4.6±2.4, DM-Cin: 1.4±0.6,

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p<0.05). In contrast, MMP9 expression was reduced in both diabetic (DM and in DM-Cin) rats, regardless of treatment (Co: 1.0±0.1, DM: 0.2±0.1, DM-Cin: 0.2±0.2, p<0.05).

Conclusions: We conclude that NO-independent activation of sGC might attenuate the progression of renal ECM accumulation in type-1 diabetic rats by direct effects on MMP2 and TIMP-1 expression, indicating the potential clinical use of cinaciguat in diabetic nephropathy.

SA-PO1136

Role of EGFR, p53 and ROS Mediated Signaling Networks in Renal Fibrosis and Induction of Fibrotic Effectors by TGF-ß1 Rohan Samarakoon,[1] Amy D. Dobberfuhl,[2] Jessica Overstreet,[1] Paul J. Higgins.[1] *[1]Center for Cell Biology and Cancer Research, Albany Medical College, Albany, NY; [2]Division of Urology, Albany Medical Colllege, Albany, NY.*

Background: TGF-β1 is one of the most potent inducers of kidney fibrosis regardless of etiology (e.g., renal obstruction, diabetes and hypertension). While SMAD3 initiated signaling networks downstream of TGF-ß1 have been extensively studied, the role of non-SMAD mechanisms in the kidney disease progression and orchestration of profibrotic gene changes remains largely unexplored.

Methods: We utilized unilateral ureteral obstruction (UUO) in mice and renal cell lines to investigate the role of non-SMAD signaling in kidney fibrosis.

Results: SMAD3 activation in the obstructed kidney correlated with EGFR and p53 phosphorylation suggestive of potential crosstalk among EGFR, p53 and SMAD3 transduction networks. TGF-ß1 stimulated EGFR activation and p53 signaling were required for induced expression of profibrotic PAI-1 and CTGF genes. Both PAI-1 and CTGF are causatively linked to the progression of obstructive and diabetic renal disease. ROS dependent mechanisms initiated by TGF-ß1 are crucial for both $p53^{Ser15}$ and $EGFR^{Y845}$ phosphorylation and subsequent PAI-1 and CTGF expression. EGFR, p53 and SMAD3 induction, readily evident in the dilated tubules of the fibrotic kidney, correlated with PAI-1 and CTGF induction suggestive of interplay among these transcription factors in profibrotic gene induction.

Conclusions: Our studies establish interplay between non-SMAD (e.g., EGFR, p53 and ROS) elements and SMAD3 in the induction of profibrotic genes by TGF-ß1 suggesting the potential benefit of combinational therapeutic targeting of non-SMAD and SMAD3 pathways to suppress fibrotic gene induction associated with renal fibrosis and CKD.

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SA-PO1137

Soluble Hemojuvelin – An Early Biomarker Promotes Iron Deposition during Acute Kidney Injury Guang-Huar Young,[1] Tao-Min Huang.[2,3] *[1]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan; [2]Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan; [3]Department of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Dou-Liou, Taiwan.*

Background: Free iron plays an important role in the pathogenesis of acute kidney injury (AKI) via the formation of hydroxyl radicals. Systemic iron homeostasis is controlled by the hemojuvelin/hepcidin axis ratio in the liver, but less is known about this process in AKI.

Methods: Proteomic method is used to identify a 42 kDa soluble hemojuvelin (sHJV) from urine during post cardiac surgery AKI. We use several animal models including renal ischemia/reperfusion (I/R) injury, glycerol-induced rhabdomyolysis and folic acid-induced acute tubular necrosisin order to assess the changes of HJV and to identify potential factors associated with iron homeostasis during AKI. Further, we evaluate the effect of iron deposition on cultured renal tubular epithelial cells (HK2).

Results: The biopsies from human and mouse specimens with AKI confirm that extensive HJV ispredominant induced in the proximal renal tubules after I/R injury. Urinary HJV levels could be used to specifically predict AKI compare to healthy volunteers (265.8±109.6 vs. 41.5±36.7ng/ml, P<0.01). In the human renal proximal tubule cells (HK-2), iron overload induces the expression of sHJV and results in iron deposition. Pretreatment of HK2 cells with furin inhibitor significantly decreases the generation of sHJVunder iron overload, induces the expression of ferroportin and prevent iron deposition. Furin inhibitorcould also reduce the degradation of membrane bound HJV, prevent the accumulation of iron in the kidney compare to vehicle group (43.2±17.2vs. 76.1±15.1 mg/kg at 24 hours, P=0.03) and further ameliorate I/R injury during AKI.

Conclusions: Our findings link HJV inextricably with renal iron homeostasis for the first time, add new significance to early predict AKI, and identify novel therapeutic targets to reduce the severity of AKI by furin inhibitor.

SA-PO1138

Redox Control of p53 in the Transcriptional Regulation of TGF-β Target Genes through SMAD Cooperativity Jessica Overstreet, Rohan Samarakoon, Paul J. Higgins. *Center for Cell Biology and Cancer Research, Albany Medical College, Albany, NY.*

Background: SMADs cooperate with non-SMAD co-factors to achieve optimal TGF-β1 target gene expression. TGF-β1/SMAD3 and p53 promote insulin resistance, diabetic nephropathy and renal fibrosis. Our identification of SMAD3 and p53 activation in the obstructed kidney prompted us to investigate the potential cooperation of these transcription factors in TGF-β1-orchestrated pro-fibrotic gene changes, focusing on a major target gene PAI-1 given its causative role in renal diabetic and obstructive fibrosis.

Methods: Molecular approaches (i.e., RNA interference, genetic ablation, pharmacological inhibition) implicate p53 and SMADs as mediators of TGF-β1 signaling in multiple cell lines. Transcriptional cooperation between SMADs and p53 in renal cells were established using immunoprecipitation (IP), chromatin immunoprecipitation (ChIP), and immunohistochemistry.

Results: p53 genetic ablation and gene silencing suppressed, while expression of p53 in $p53^{-/-}$ MEFs rescued TGF-β1-stimulated PAI-1 induction. Furthermore, pifithrin-α, a p53 inhibitor eliminated TGF-β1-mediated expression of pro-fibrotic genes (i.e., PAI-1, CTGF) in HK-2 human tubular epithelial cells. TGF-β1-stimulated p53 and SMAD interactions occurred prior to promoter occupancy of these transcription factors to the PAI-1 gene confirmed by ChIP analysis. Consistent with the emerging role of oxidative stress as a causative element in fibrosis, TGF-β1-initiated phosphorylation of $p53^{Ser15}$ was ROS-dependent. Prominent co-expression of SMAD3, p53 and PAI-1 in the tubular epithelium of the obstructed kidney compared to the contralateral controls highlighted *in vivo* correlation of p53 and SMADs in TGF-β1-driven renal fibrosis.

Conclusions: TGF-β1 regulates the transcriptional activity of p53 promoting interactions with SMADs and subsequent binding of both molecules to the PAI-1 promoter establishing a multi-transcriptional complex involving p300. Collectively, TGF-β1-mediated pro-fibrotic gene expression involves p53, SMAD, and ROS providing attractive targets downstream of aberrant TGF-β1 signaling leading to renal failure.

SA-PO1139

KP-2326, a Peptide Agonist of the Calcium Sensing Receptor, Prevents Vascular Calcification in a Rodent Model of Uremia Sarah Walter, Jin Dong, Shawn T. Alexander, James Tomlinson, Qun K. Yin, Tom Hunter, Derek Maclean, Randolph M. Johnson. *Research, Amgen, South San Francisco, CA.*

Background: Chronic Kidney Disease (CKD) is linked to an increased risk for vascular calcification and deaths from cardiovascular disease. KP-2326 is a novel agonist peptide of the calcium sensing receptor (CaSR) that was evaluated in the adenine model for its ability to prevent vascular calcification.

Methods: Male Wistar rats were fed a low protein (2.5%), high phosphorus (0.92%) diet containing 0.75% adenine and randomly assigned to receive daily subcutaneous doses of placebo or KP-2326 (0.3 or 1 mg/kg) for 4 weeks (15 animals/group). A control group (9 animals) was fed the identical diet without adenine. 24 hours following the last dose, animals were sacrificed, blood samples taken for biochemical determinations and tissues removed for analysis. Aortic tissue was processed and stained both by Alizarin Red and von Kossa methods to visualize vascular mineralization.

Results: Using a scoring system from 0-5, identical sections of von Kossa-stained aorta from all animals were scored in a blinded fashion. 10/15 animals in the vehicle group showed evidence of some calcification; 9 of these animals had evidence of at least 40-60% calcification based upon the intensity and extent of the staining. In contrast, 0/15 rats in the 1 mg/kg group and 1/15 in the 0.3 mg/kg groups had detectable mineralization. One animal in the 0.3 mg/kg group received a score of 1 (0-20% calcification). Plasma PTH and serum creatinine were dose-dependently reduced in the KP-2326 groups, compared with the vehicle control animals (PTH: 295, 492, 1656 pg/mL for 1, 0.3 mg/kg and vehicle, respectively). Parathyroid glands in the KP-2326 groups were also significantly reduced in size compared with controls, normalized to body weight (group means of 3.38 and 7.52 mg/kg for the 1 mg/kg and vehicle groups, respectively).

Conclusions: KP-2326 is an agonist peptide of the CaSR, prevents vascular calcification, reduces PTH and prevents increases in parathyroid gland weight in a rodent model of uremia.

SA-PO1140

Production of Erythropoietin by Renal Human $CD133^+$ Progenitors under Hypoxia and Prolyl Hydroxylase Inhibition Benedetta Bussolati,[1] Massimiliano Mazzone,[2] Giovanni Camussi.[1] *[1]Internal Medicine, University of Torino, Torino, Italy; [2]VIB Vesalius Research Center, University of Leuven, Leuven, Belgium.*

Background: A peritubular population of cells with mesenchymal phenotype is considered to be responsible for the production of erythropoietin. However, in the human tissue, their precise identification is unclear. Using CD133 as marker, a population of renal resident progenitor has been localized in different segments of the nephron. In the present study, we investigated whether $CD133^+$ renal progenitors could be a source of EPO, and its modulation by HIF stabilization through prolyl hydroxylases (PHD) inhibitors.

Methods: CD133+ were isolated from human specimens, cultured and characterized as renal progenitors expressing mesenchymal markers by FACS analysis and real time quantitative PCR. Epithelial differentiation was induced by culture with 10% serum, 10 ng/ml Hepatocyte Growth Factor and 10 ng/ml Fibroblast Growth Factor-4. When cultured in hypoxic conditions, cells were placed in hypoxic chambers with 1% O_2. EPO mRNA was detected by real time quantitative PCR and EPO release into the supernatant using an ELISA. Generation of negative progenitors for HIF-2a or for PHD2 was obtained by infection with specific shRNA lentiviruses.

Results: CD133+ progenitor cells released EPO under hypoxia at levels comparable with those previously reported in EPO releasing human cells in vitro (18 ±3 mU/ml after 24 hours hypoxia). Erythropoietin synthesis was lost when CD133+ progenitors differentiated and acquired an epithelial phenotype. Inhibition of PHD2 induced EPO transcription, but not its release. Indeed, hypoxic conditions were required for the release of the intracellular pool of EPO after PHD2 knock down. Finally, blockade of HIF-2alpha impaired EPO synthesis.

Conclusions: These results indicate that human renal CD133+ progenitor cells via the PHD2-HIF2 alpha axis synthesize and release EPO under hypoxia and provide a new

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rationale for the use of PHD inhibitors in clinical setting of acute or chronic renal injury. In addition, renal CD133+ progenitor cells represent a model for the study of the mechanisms of EPO synthesis and release.

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SA-PO1141

Effects of Preterm Birth and Ventilation on Glomerular Capillary Growth in the Neonatal Lamb Kidney Megan R. Sutherland,[1] Danica Vojisavljevic,[1] Mar Janna Dahl,[2] Kurt Albertine,[2] Mary Jane Black.[1] *[1]Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia; [2]Pediatrics, University of Utah, Salt Lake City, UT.*

Background: Preterm neonates are born with developmentally immature kidneys, with vascularisation of the glomeruli likely ongoing after birth. Preterm birth, and the related increase in blood oxygen concentration, leads to impaired vascular development in a number of diseases of prematurity (such as bronchopulmonary dysplasia). Hence, it is likely that glomerular capillary development will also be impaired. The aim of this study was to determine the effects of preterm birth and ventilation on glomerular capillary growth in a neonatal lamb model.

Methods: Four experimental groups were analysed: Lambs delivered preterm at 130d gestation (term=147d), and ventilated +3 days after birth (Preterm: n=7); 133d gestational controls (Fetal Control: n=5); Term controls born naturally, unassisted breathing +3 days after birth (Term Control: n=8); Term lambs ventilated +3 days after birth (Term Ventilated: n=8). Epon-araldite sections of inner, mid, and outer renal cortex from perfusion-fixed kidneys were assessed using stereological techniques to calculate total capillary length and surface area per renal corpuscle. Nephron number was assessed in glycolmethacrylate sections using the physical disector/fractionator approach, and total renal filtration surface area (TRFSA) calculated for each kidney.

Results: Preterm birth resulted in significantly increased capillary length, capillary surface area, and TRFSA compared to fetal controls. In comparison to term controls, however, preterm lambs had significantly reduced capillary length and TRFSA, but equivalent capillary surface area which may be indicative of capillary dilation. Importantly, term lambs ventilated for 3 days after birth exhibited significantly reduced capillary length, surface area and TRFSA compared to term controls.

Conclusions: The findings demonstrate that ventilation impairs glomerular capillary growth independently of preterm birth; capillary dilation in the preterm kidney may predispose to the development of glomerulosclerosis, and thus pose an additional risk to long-term renal health.

Funding: Government Support - Non-U.S.

SA-PO1142

Pathomechanisms of Vascular Calcification: Urokinase Receptor Directs Osteogenic Differentiation of Mesenchymal Stem Cells Via Regulation of Complement Anaphylatoxin 5a Receptor Expression Margret Patecki, Parnian Kalbasianaraki, Hermann G. Haller, Inna Dumler. *Nephrology, Medical School Hannover, Hannover, Germany.*

Background: The vascular calcification process follows developmental programs that recapitulate embryonic ossification, with modulations by inflammatory and metabolic phenomena; mesenchymal stem cells (MSCs) contribute to this process. In our study we aimed to reveal the role of the multifunctional fibrinolytic urokinase (uPA) / uPA receptor (uPAR) system in induction and propagation of osteogenic differentiation of MSCs and a probable connection to the complement system.

Methods: Osteogenic differentiation of human bone marrow derived MSCs was induced by dexamethason, ß-glycerophosphate and ascorbic acid. Alkaline phosphatase activity and calcium deposition were monitored. Upregulation and silencing of uPAR were achieved by means of lentiviral transfection methods. Changes in expression levels were analyzed by RT-PCR, western blotting and flow cytometry.

Results: We examined the effect of uPAR silencing (uPARsi) and upregulation (uPARup) on osteogenic differentiation of MSCs. The osteogenic differentiation was impaired in uPARsi-MSCs and upregulated in uPARup-MSCs. Interestingly, we detected an increased expression of the complement anaphylatoxin C5a receptor (C5aR) mRNA in MSCs during osteogenic differentiation, whereas no change in C5aR level could be seen in uPARsi-MSCs. Furthermore, use of a specific C5aR antagonist blocked osteogenic differentiation of MSCs. These findings are in agreement with our previous data pointing to uPAR as an important mediator of the C5aR expression and of related signaling and functional effects in other cell types. They further suggest a general role for uPAR in C5aR regulation.

Conclusions: These results clearly show that the fibrinolytic and the complement systems functionally interfere during osteogenic differentiation. uPAR seems to play a regulatory role in this process leading to C5aR expression changes. To clarify the relevance of these findings in vivo, we use a mouse model with uPAR$^{-/-}$/ LDL-R$^{-/-}$ double knockout and LDL-R$^{-/-}$ mice on a fatty diet. Analyzing differences of arterial calcification and C5aR levels is in progress.

SA-PO1143

Effect of Recombinant Human Erythropoietin on Iron Metabolism Gene Expression João Fernandes,[1] Patricia Garrido,[2] Sandra Ribeiro,[3] José Sereno,[2] Joanna Sajkowska,[3] Henrique Nascimento,[3] Susana Rocha,[3] Luis Belo,[3] Elísio Costa,[3] Alice Santos-Silva,[3] Flávio Reis.[2] *[1]IBILI, UC and IBMC, UP; [2]IBILI, UC; [3]FF and IBMC, UP.*

Background: In vitro studies have shown that rhEPO has an inhibitory effect upon hepcidin gene expression. Therefore, rhEPO may interfere with iron metabolism through hepcidin. Our aim was to evaluate the effect of high rhEPO in the expression of hepcidin and other iron metabolism associated genes.

Methods: Three groups of Wistar rats were studied: i) control, ii) rats administrated with 50 IU/kg/week, iii) rats administrated with 200 IU/kg/week, during a 12 weeks period. Several putatively relevant genes involved in iron metabolism (hepcidin- Hamp, ferroportin – Slc40A1; transferrin – TFR; transferrin receptor 1 and 2 – TRFr1 and TRFr2; erythropoietin and erythropoietin receptor – EPO and EPOr; bone morphogenic proteins – BMP2, BMP4 and BMP6; hemojuvelin and hemochromatosis factor – HJV and HFE1; matriptase2 – TMPRSS6; and divalent metal transporter 1 – DMT1) and IL-6 gene expression were studied in the liver; iron serum levels were also evaluated.

Results: We found a down-regulation in EPO gene for both groups under rhEPO administration, suggesting a negative feedback mechanism. No other changes were found for rats under 50 IU/kg/week, when compared to control group. Concerning rats under 200 IU/kg/week we found over-expression of Hamp, TRFr2, Hfe, TRF, EPOr and IL-6 genes in liver, as compared with rats under 50 IU/kg/week. In accordance, iron levels were significantly increased in this group.

Conclusions: Our data suggest that an inflammatory environment might have developed, Hamp and IL-6 genes were overexpressed. Strengthening this hypothesis, we found an overexpression of BMP2 and BMP6, known to trigger the overexpression of Hamp gene. Moreover, due to the high rhEPO levels several iron metabolism associated genes were overexpressed. These results could explain the changes in iron metabolism described for hemodialysis patients requiring high rhEPO doses. Furthermore, Hamp expression seems to be primarily regulated by iron levels and inflammation than by rhEPO administration.

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SA-PO1144

Deletion of the Proton-Sensing Receptor GPR4 Leads to Glomerulosclerosis in Old Mice Xuming Sun,[1] Doris P. Molina,[1] Lois J. Arend,[3] Juan Codina,[2] Thomas D. DuBose,[2] Snezana Petrovic.[1,2] *[1]Department of Physiology&Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC; [2]Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC; [3]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.*

Background: Proton-sensing receptors are members of a unique family of G protein-coupled receptors that function as pH sensors in the blood vessels, bone, kidney, airway smooth muscle, lung cancer, and possibly brain. Of the three receptors characterized so far, GPR4, is the one most abundant in kidney. We have characterized the phenotype of GPR4-/- to include a non-gap metabolic acidosis with features similar to classical distal renal tubular acidosis (RTA).

Methods: Since chronic metabolic acidosis affects progression of chronic kidney disease, the presence of chronic low grade metabolic acidosis in GPR4-/- prompted comparison of kidney histology of GPR4-/- and +/+ at different ages, using both light and electron microscopy.

Results: We analyzed young (<6 months; n=8 of each) and old mice (12 months; GPR4 +/+, n= 18 and GPR4-/-, n= 30). As we reported previously, kidney histology was unremarkable in young animals, but in contrast, ~30% of GPR4-/- developed severe glomerulolerosis by 12 months. Older GPR4-/- glomeruli showed expanded mesangium and excessive deposition of extracellular matrix and distended capillary loops. Mesangial deposits were confirmed by electron microscopy. Glomerular changes in GPR4-/- were accompanied by mild to moderate proteinuria. Interestingly, histological analysis did not reveal significant interstitial infiltration or fibrosis in the affected GPR4-/-. Histologic changes or proteinuria were not apparent in younger GPR4-/- or GPR4+/+, regardless of age.

Conclusions: In conclusion, we report that deletion of the pH sensor GPR4, accompanied by low grade chronic metabolic acidosis, is also associated with the development of glomerulosclerosis with advancing age. Since spontaneous, chronic acidosis in GPR4-/- precedes the development of kidney disease, GPR4-/- may be a useful experimental model in which to investigate the interaction of metabolic acidosis and kidney disease.

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SA-PO1145

BRP39 Expression in Cystic Kidney Disease Mouse Models of PC1 and PC2 Deficient Background Seung H. Lee,[1] Chang-Min Lee,[2] Xin Tian,[1] Sohan Lal,[1] Sung Hyun Son,[3] Stefan Somlo.[1] *[1]Internal Medicine, Section of Nephrolgy, Yale School of Medicine, New Haven, CT; [2]Internal Medicine, Section of Pulmonology, Yale School of Medicine, New Haven, CT; [3]Internal Medicine, Section of Nephrolgy, BHS Han Seo Hospital, Busan, Korea.*

Background: Cystic kidney disease carries an inflammatory component, but the contribution of macrophage to cell proliferation is not clear. *As a chitinase-like protein, BRP-39 (Breast regression protein 39) is strongly induced in the macrophages in a number of inflammatory conditions*. In addition, recent studies indicated that BRP39 induces cellular

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proliferation which contributes to the development and progression of tissue-remodeling responses such as pulmonary or renal fibrosis. To determine BRP39 contribution in the proliferation of cyst-lining cells in kidney, we evaluated the expression of BRP39 in cystic kidney mouse model of polycystin1 (PC1) and polycystin 2(PC2) deficient background.

Methods: Western blot and qPCR were used to evaluate the expression of BRP-39 in the kidney samples from p10, p16, p24, and p35of the mice with early onset cystic kidney model with PC1 deficient background and wild type controls. We also evaluated the levels of BRP39 expressions in the mice of adult onset cystic kidney model with PC2 deficient background by qPCR. Immunofluorescent stain of macrophage and BRP were performed in p10, p16, p24, and p35of the mice with early onset cystic kidney model with PC1 and PC2 deficient background.

Results: We found BRP39 expression was markedly increased along with cystic cell proliferations in early onset cystic kidney model. BRP-39 expression was more than 10 folds higher induced in the mice with PC1 and PC2 null mutation compared to controls (P=0.022) by qPCR. Immunofluorescent stain showed macrophages and BRP expressions are markedly increased in early onset cystic kidney disease models compared to control animals.

Conclusions: These data suggest that BRP39 may play a certain role in cystic cell proliferation through macrophage infiltrations in early and adult onset cystic kidney disease mouse models of PC1 and PC2 deficient background.

Funding: Private Foundation Support

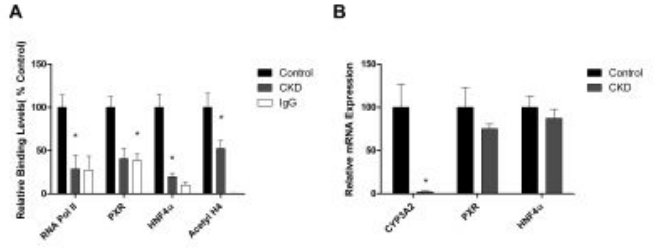
SA-PO1146

Decreased Nuclear Receptor Activity Mediates Down-Regulation of Drug Metabolizing Enzymes in Chronic Kidney Disease. Thomas Velenosi,[1] David A. Feere,[1] Gurjeev Sohi,[1] Daniel B. Hardy,[1] Angel Yi Nam Fu,[1] Brad Urquhart.[1,2] *[1]Physiology and Pharmacology, University of Western Ontario, London, ON, Canada; [2]Medicine - Division of Nephrology, University of Western Ontario, London, ON, Canada.*

Background: Patients with Chronic Kidney Disease (CKD) require many medications, the majority of which are metabolized by the drug metabolizing enzyme, CYP3A. Drug metabolizing enzymes are regulated by nuclear receptors and expression of these enzymes is decreased in CKD; however the mechanism by which this occurs is unknown.

Methods: CKD in rats was surgically induced by 5/6 nephrectomy. Rats were sacrificed on day 42 and hepatic CYP3A1, CYP3A2 and CYP2C11 mRNA expression were determined. Chromatin Immunoprecipitation (ChIP) was performed on the pregnane X receptor (PXR) and hepatocyte nuclear factor 4α (HNF4α) to determine nuclear receptor mediated differences in the transcriptional activation of these enzymes. Epigenetic modifications were also assessed by ChIP.

Results: Hepatic CYP3A and CYP2C11 mRNA expression was significantly decreased in CKD rats compared to controls ($P<0.05$). RNA polymerase II binding to the CYP3A and CYP2C11 promoter regions was decreased in CKD rats ($P<0.05$)(Figure 1). ChIP also revealed a decreased PXR binding to the CYP3A2 promoter in CKD rats ($P<0.05$). HNF4α binding to the CYP3A and CYP2C11 promoter regions was also decreased compared to controls ($P<0.05$); however, no change in nuclear receptor expression occurred (Figure 1). The decrease in PXR and HNF4α binding was concurrent with diminished histone 4 acetylation in the CYP3A2 promoter locus for nuclear receptor activation.



Conclusions: We demonstrate a novel mechanism of drug metabolizing enzyme regulation in CKD. Our results show that decreased CYP3A and CYP2C11 mRNA expression is secondary to decreased PXR and HNF4α binding as a result of histone modulation in CKD.

Funding: Government Support - Non-U.S.

SA-PO1147

A Protective Role of Indoleamine 2, 3-Dioxygenase in Antibody Mediated Nephritis Kapil Chaudhary,[1] Lei Huang,[2] Michael P. Madaio,[3] Tracy L .McGaha.[4] *[1]Molecular Medicine, Georgia Health Sciences University, Augusta, GA; [2]Immunotherapy Center, Georgia Health Sciences University, Augusta, GA; [3]Medicine, Georgia Health Sciences Center, Augusta, GA; [4]Immunotherapy Center, Georgia Health Sciences Center, Augusta, GA.*

Background: Antibody mediated glomerulonephritis is a significant cause of chronic, progressive kidney disease; however, effective therapy remains elusive. Indoleamine 2,3-dioxygenase (IDO) drives immunologic tolerance and restricts inflammation by Trp depletion and generation of Trp catabolities. The aim of the present study is to determine the mechanism(s) by which IDO activity protects the kidney from antibody mediated nephritis.

Methods: Nephritis was induced by i.p. 12µl/g sheep anti-mouse nephrotoxic serum (NTS) to IDO1-KO, GCN2-KO (Trp depletion-responsive pathway), aryl hydrocarbon receptor (AHR) deficient (AHRd, tryptophan catabolite-responsive pathway) and WT (C57BL/6) mice. Renal function was monitored from day 3 to 21. Co-staining of IDO was preformed with markers for endothelial cells, podocytes, macrophages and dendritic cells on frozen sections 3 days after nephritis induction. To induce IDO prior to nephritis, DNA nano-particles (DNPs) were administered i.v.

Results: While IDO1-KO and AHRd mice developed severe form of nephritis with significant increase in BUN, albuminuria, body weight and kidney NGAL expression on day 15, GCN2-KO mice had no change in disease characteristics compared to WT mice. Moreover, lack of IDO1 or AHR enhanced pathologic alteration of the glomerular architecture with increased basement membrane thickening, glomerular and interstitial infiltrates, tubular cell necrosis and crescent formation. IDO expression was primarily in podocytes with some expression in macrophages and interstitial cells. Finally, IDO induction by DNPs prior to NTS administration did not prevent nephritis in IDO1-KO mice.

Conclusions: The data suggests that IDO limits renal pathology by a mechanism dependent on Trp catabolite driven activation of AHR transduction pathway. Moreover, results indicate methodologies to induce IDO expression that may have utility in the treatment of kidney inflammation.

Funding: Other U.S. Government Support

SA-PO1148

Dual Regulation of Cadmium-Induced Apoptosis by mTORC1 through Induction of Selective Unfolded Protein Response Hironori Kato, Shotaro Nakajima, Masanori Kitamura. *Department of Molecular Signaling, University of Yamanashi, Chuo, Japan.*

Background: Cadmium (Cd) causes generation of reactive oxygen species (ROS) and consequent ER stress that triggers renal tubular injury. We found that rapamycin, an inhibitor of mTORC1, attenuated Cd-induced apoptosis in renal tubular cells. In this report, we investigated the link between mTORC1 and individual branches of the unfolded protein response (UPR) in Cd-triggered apoptosis of tubular cells.

Methods: Roles of mTORC1 in the induction of apoptosis and regulation of the UPR were tested using rapamycin, siRaptor and siTSC2. Involvement of IRE1a pathways in the Cd-triggered, mTORC1-mediated apoptosis was investigated using dominant-negative inhibition of IRE1a (kinase or endonuclease domain), XBP1 and JNK. Activity of mTORC1 and UPR pathways was evaluated by Western blot analysis of relevant indicators.

Results: Rapamycin, an inhibitor of mTORC1, attenuated Cd-induced apoptosis in renal tubular cells. Knockdown of Raptor, a positive regulator of mTORC1, also had the similar effect. Rapamycin did not alter generation of ROS, suggesting that mTORC1 is a target downstream of ROS. Indeed, ROS caused activation of mTORC1, and it contributed to activation of the selective UPR; i.e., the IRE1apathway. Although Cd triggered three major UPR pathways, activation of mTORC1 by Cd did not contribute to induction of the PERK–eIF2a and ATF6 pathways.Consistently, knockdown of Raptor caused suppression of JNK without affecting the PERK–eIF2a pathway in Cd-exposed cells. Knockdown of TSC2, a negative regulator of mTORC1, caused activation of mTORC1 and enhanced induction of the IRE1a–JNK pathway and apoptosis by Cd without affecting other UPR branches. Inhibition of IRE1akinase led to suppression of JNK activity and apoptosis in Cd-exposed cells. Dominant-negative inhibition of JNK also suppressed Cd-induced apoptosis. In contrast, inhibition of IRE1aendonuclease activity or downstream XBP1 modestly enhanced Cd-induced apoptosis.

Conclusions: These results disclosed dual regulation of Cd-induced apoptosis in renal tubular cells by mTORC1 through selective induction of the IRE1a kinase and endonuclease signaling.

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APOL1 Is an Innate Immunity Effector That Induces Stress Autophagy by Interacting with the SNARE Protein, VAMP8 Sethu M. Madhavan,[1] John F. O'Toole,[1] Martha Konieczkowski,[1] Santhi Ganesan,[1] David B. Thomas,[2] Laura M.C. Barisoni,[2] Leslie A. Bruggeman,[1] John R. Sedor.[1] *[1]Case Western Reserve University; [2]University of Miami.*

Background: Apolipoprotein L1 (APOL1) prevents African Sleeping Sickness unless it is bound by a trypanosomal SRA resistance protein. Genetic variants in the *APOL1* SRA-binding domain circumvent parasitic resistance, but associate with non-diabetic kidney diseases through unknown mechanisms. We hypothesized that the SRA-binding domain of APOL1 would have human binding partners, which would highlight candidate pathways in disease pathogenesis.

Methods: APOL1:VAMP8 interaction was evaluated with immunoblotting, immunoprecipitation (IP) and pull-downs. Confocal microscopy evaluated colocalization of APOL1 and VAMP8 *in vitro* and *in vivo*.

Results: A BLAST search with amino acids 31-79 of SRA protein sequence yielded no mammalian ortholog. Since structural homology may exist with divergent primary sequences, we performed a secondary structure search with a Hidden Markov Model algorithm. A SNARE protein, Vesicle Associated Membrane Protein 8 (VAMP8) was identified as a structural ortholog of the SRA domain that binds APOL1. VAMP8 mediates membrane fusion required for vesicular trafficking and has been associated with selective autophagy pathways. By IP and pulldowns, VAMP8 bound wild type APOL1 but this interaction was decreased with disease-associated APOL1 variants. Transfected APOL1 and VAMP8 colocalized in HeLa cells. In normal kidney sections, VAMP8 and APOL1 colocalized in podocytes but not in proximal tubules. In FSGS and HIVAN biopsies with *APOL1* risk alleles, APOL1 and VAMP8 partially dissociated. Using immuno-EM, APOL1 was rarely associated with double membranes vesicles in normal kidney, but in diseased kidney, APOL1 was near podocyte vacuoles and in cytoskeleton abutting the GBM. WT APOL1 expression stimulated autophagy, as demonstrated by LC3-II. Autophagy induction by variant APOL1 was attenuated.

Underline represents presenting author/disclosure.

Conclusions: APOL1 interacts with VAMP8, a structural homolog of trypanosomal SRA protein. We propose that variant APOL1 proteins reduce VAMP8 binding and attenuate stress-induced autophagy that leads to kidney injury with relevant environmental challenges.

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